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Original Research

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The availability and validity of safety information of over the counter herbal products for use in diabetes in Sri Lanka: A cross sectional study

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ABSTRACT

Aims: There is an increase of over-the-counter (OTC) herbal products for use in diabetes mellitus. The aim of this study is to evaluate the safety information provided with OTC herbal remedies intended for diabetic patients in Sri Lanka and to assess the completeness of the information provided. **Methods:** Inclusion criteria consisted of OTC herbal remedies meant for use in diabetes. They were bought from local Sri Lankan supermarkets and non-ayurvedic pharmacies and product information regarding the risk of hypoglycemia, precautions for use, adverse events, dose, and interactions were assessed using a scoring system. The accuracy of the information was then compared against published data. **Results:** 11 products fulfilled the inclusion criteria. Five products contained a single constituent and five contained more than one. None had complete and accurate safety information according to our criteria. None specifically warned against the risk of hypoglycemia. 9 out of 11 products (81.8%) carried ≤3 items of the five essential factual information we expected. Hypoglycemic coma, gastrointestinal symptoms, hepatotoxicity, carcinogenesis, and interactions causing elevated drug levels of Carbamazepine were some of the safety information that was missing. **Conclusions:** Key safety information was absent in most products. Regulation of sale, provision of key safety information and adverse event reporting should be a priority.

KEY WORDS: Complementary and alternative medicine, diabetes mellitus, herbal remedies, safety information

INTRODUCTION

Diabetes mellitus is a major cause of morbidity and mortality worldwide with an increasing prevalence. The WHO estimates a prevalence of 347 million people with diabetes worldwide in 2013 [1].

There is an emerging trend worldwide for patients with chronic illnesses such as diabetes to use complementary and alternative medicine (CAM) in an attempt to improve the outcomes of their illnesses. Biologic therapies such as herbal remedies are popular [2], and can broadly be divided into commercialized over-the-counter (OTC) preparations and preparations that are locally sourced and prepared.

Consumers of commercially available herbal products need access to reliable and accessible information to ensure safe and appropriate use. This should preferably be in the form of printed material supplied alongside the product, similar to what's available with conventional medications [3]. This is particularly important, as it has been shown that the staff knowledge on the products sold in community pharmacies and health food shops is sub-optimal elsewhere [4]. In addition, there appears to be reluctance on the patient's part in informing about herbal remedies to their respective physicians. One UK study found that a vast majority did not inform their physicians regarding herbal use [5]. In India, 59.9% of CAM users were not willing to disclose CAM use to their physician [6]. In Taiwan, 75.4% did not disclose CAM use [7]. This is of critical importance as many unrecognized interactions may occur between herbal remedies and conventional medicines. Similarly, many patients believe that herbal products are safe and devoid of adverse effects [8].

Although there is an absence of data for Sri Lanka, these observations may be valid locally as well.

To enable the appropriate and safe use of OTC herbal products, availability of relavant information to the consumer is essential. Particularly information on precautions, adverse events and interactions with conventional medications is critical. Although sale and use of conventional medicine are well regularized globally, herbal medicines (HM) are still largely sold without registration or regulatory supervision.

The European Medicines Agency (EMA) Committee on HM products implemented the development of community

herbal monographs for herbal products in 2004. Community herbal monographs collected scientific data on two aspects of a product, namely the well-established use and traditional use. For some plants, the monograph covers the well-established use, as well as traditional use. Traditional use indication gives credentials to a plant based on its long history of traditional use, well-established use indication also implies innovation and research on a plant [9].

This key directive adopted by the European Parliament in (Directive 2004/24/EC 2004) 2004 enabled the non-prescription use of traditional medicines following assessment of suitability. An important outcome of this was a sharp increase of the HM registrations from 2 in 2005 to 265 in 2012 [9]. The existence of such a legal framework is critical in registering products based on scientific evidence and well-established safety. However, at consumer level there should be legislation or industry concurrence in making available information to the consumer that enables its safe and effective use.

In most instances, herbal products tend to be registered with their respective regulatory authorities under the "traditional use" category as opposed to the "well-established" category, which is more evidence-based. Although there is some evidence of efficacy for few HM, most lack readily reproducible evidence of efficacy, sufficient to meet regulatory standards [3,9]. Further, insufficient research is generated within the use of these medications to make them more likely of obtaining regulatory standards [9].

Nevertheless, the UK sets a valuable example by requiring products registered as traditional herbal registration (THR) to carry essential safety information in the form of a leaflet [10]. In 2011, Raynor *et al*. reported that in the UK the majority (93%) of OTC herbal products were unlicensed [3].

The World Health Organization traditional medicines strategy of 2002 [11] also highlighted the need for reliable information as a key item to enable the safe and effective use of traditional medicines. In 2003, the WHO addressing the use of HM in developing countries strongly recommended the implementation of national advisory committees and guidelines on herbal remedies. It further emphasized the need of a body for monitoring adverse drug reactions for HM [12].

At present, there is no legislative framework for registering or regulating the use of OTC herbal products in Sri Lanka.

Essential information that would enable safe use of herbal products includes (1) precautions, (2) interactions, and (3) adverse events such as allergic reactions.

Very few published research studies are available on the safety related issues in dispensing and use of HM.

In a previous publication, we reported a high prevalence of herbal remedy use among Sri Lanka diabetic patients [13]. These were locally sourced plants that were prepared at home. However, at present, herbal remedies are commercially marketed as capsules, tea bags and syrups, targeting the Sri Lankan diabetic population. There is no published data on the type of products and whether they provide essential information on safe use to consumers.

The aim of this study is to critically evaluate the safety information provided with OTC herbal remedies intended for use among diabetic patients in Sri Lanka and to assess the completeness of the information provided.

MATERIALS AND METHODS

We performed a cross-sectional survey between 20th June and 31st July 2014, using the printed and internet-based information provided with the OTC herbal products in two major cities in Sri Lanka.

Obtaining the Products

Samples of commercially available OTC herbal remedies intended for use by diabetic patients fulfilling the inclusion criteria were bought from supermarkets and pharmacies in the cities of Colombo and Kandy, Sri Lanka. The 2 cities were selected on convenience of access. Six supermarkets were visited ensuring that at least one outlet from each chain of supermarkets in these cities was included. The 10 pharmacies were randomly selected from the city centers of both cities, from a total of 96 that dispensed western medicines and OTC herbal products. When products were bought from supermarkets, those fulfilling the inclusion criteria were picked off the shelves. When products were procured from pharmacies, the authors requested for OTC products intended for use by diabetes patients for glycemic control to the pharmacy staff and those that fulfilled the inclusion criteria were selected. Inclusion criteria included labeling stating, use for diabetes, being of herbal origin and availability as an OTC product.

These products were then re-scrutinized to confirm adherence to inclusion criteria once they were purchased. Herbal products that stated diabetes as the sole indication, as well as those that mentioned diabetes as an indication among others were also included.

In Sri Lanka, ayurvedic medicine is a separately registered stream of practice with its own regulatory authority for practice and sale of medication. Pharmacies that dispense ayurvedic medicines are distinct from those dispensing conventional medications. We intentionally did not include products available at the ayurvedic pharmacies, as it was not our intention to scrutinize the safety of herbal products dispensed in these, but rather the safety of those available to the general public as OTC herbal products.

Evaluation Criteria

The labeling, the package insert and where available websites appearing on the label were scrutinized to gather information about the product. The manufacturer, the active ingredient (s), precautions for use, adverse effects, interactions, indication for use, dose and duration, preparation of the product (tablet, powder, liquid, tea bag, etc.) were recorded as stated by the manufacturer.

We assessed each product to see if they carried information on (1) warning of possible hypoglycemia, (2) Information on adverse events, (3) precautions for use, (4) interactions and, and (5) dose recommendations. A point was awarded for each category of information provided and a score out of 5 was used to assess the completeness of the safety information.

Each category of information (e.g., adverse events) provided for each individual product was then compared against published data to assess if the information provided was complete and accurate.

At present, there is no complete and authoritative reference available to gather information on herbal products. Natural Medicines Comprehensive Database [14], Stockley's HM interactions [15], The EMA [16], the US National Centre for CAM [17] and online searches on PUBMED using constituent names were used for cross-referencing the information. However, it has to be borne in mind that natural products do not conform to the same stringent scrutiny in relation to adverse event reporting, interactions, etc., as conventional medications and many clinically significant events, therefore, may not be on record at present. We limited our search for adverse events and interactions to those reported in humans.

Accuracy was checked by cross-referencing the stated information against the published data stated above. If all parameters were supplied and were accurate, the product was categorized as complete and accurate.

RESULTS

Product Analysis

Eleven products that fulfilled the inclusion criteria were bought from the local supermarkets and pharmacies and analyzed. The manufacturer was mentioned in all the products. Five products contained a single constituent and five more contained more than one. One product failed to mention its constituent(s). Five were marketed as an herbal drink, 3 as herbal tea, 1 as herbal syrup and 2 products were in capsule form. All products stated in the labeling that they were intended for glycemic control. In addition to glucose control, 7 products mentioned additional indications for use; these included diabetic neuropathy, enhancement of memory and cure of circulatory problems.

Package inserts were available in 5/11 (45%) products. Nine products (81.8%) carried telephone numbers or a website for help regarding the product.

Utilizing our scoring system, 9/11 (81.8%) products scored ≤3 on the presence of essential safety information. 2 (18.1%) products scored 4/6.

Table 1 illustrates the summary of the products and the completeness of the safety information.

A complete list of the constituents and their frequency in the products are tabulated in Table 2.

Assessment of Safety Information

None of the products had complete and accurate safety information according to our criteria. None specifically stated the risk of hypoglycemia. However, 2 products containing bitter gourd advised patients on regular blood glucose monitoring. Information regarding adverse events was available in only 3 (27%) of the products. Interactions were mentioned in only 1 (8.1%) product. Precautions for use were mentioned by 5 (45%) products. Pregnancy and lactation, heartburn and age <18 years were some mentioned. The dose was mentioned in all the products, but the duration for use was not mentioned in any. One product contained nutritional information of its contents. The amount of each constituent in weight or volume was mentioned in only 2 products. Details of adverse events and precautions for use as given by the manufacturer are given in Table 1.

When the information provided under different categories were assessed for accuracy against published data, none of the products carried information that was accurate and complete. Reported adverse events and interactions of the key constituents of the products are summarized in Table 3.

DISCUSSION

Diabetic patients use CAM with the expectation of improving their blood glucose levels [2]. Patients should be able to expect full disclosure of safety information on purchasing herbal products [18]. Most patients perceive less adverse events from herbal products than from conventional medicines [8]. However, most diabetic patients use herbal therapies in conjunction with conventional medications raising the possibility of interactions [13].

The products we examined, which were specifically marketed for use among diabetic patients contained little or no information on precautions for use, the risk of developing hypoglycemia, interactions or adverse events.

Adverse event information was available in only 3 (27.2%) products. Since most of the products studied contained more than one constituent [Table 1], the situation is complex in adverse event reporting. Ideally, the manufacturer should mention adverse events for any of the constituents contained in the product. Cinnamon, which was a constituent of two products is known to contain Coumarins [19]. Coumarins are hepatotoxic and carcinogenic, and Cinnamon already carries a caution from health authorities against prolonged and continued use [20]. Fenugreek taken orally can cause mild gastrointestinal disturbances like diarrhea, dyspepsia, abdominal bloating and flatulence [21]. Black seed (*Nigella sativa*) may cause hepatotoxicity in animals, but clinical evidence is lacking [22]. *In vitro* studies have shown inhibition of platelet aggregation, making bleeding a possibility with black seed oil [23]. However, no clinical evidence is available at present. Bitter gourd is known to cause abdominal cramps in some [24]. Dans *et al*. reported the incidence of hypoglycemic coma in children given bitter melon tea [25].

Table 1: Summary of the products, product information provided and the score depicting completeness of the safety information

Product number	Type of product	Number of constituents (n)	Hypoglycemic potential mentioned	Adverse events mentioned (captioned from product)	Interactions (captioned from product)	Precautions mentioned	Dose mentioned (yes=Y, no=N)	Score out of 5
1	Herbal drink	7	No	Yes (devoid of adverse events)	Not mentioned	None	Yes	2
2	Capsules	1	No	No	Not mentioned	Caution in pregnancy gastritis	Yes	2
3	Herbal drink	8	No	No	No mentioned	Caution in pregnancy, lactating mothers, people <18 years	Yes	2
4	Herbal syrup	NA	No	No	Not mentioned	None	Yes	1
5	Herbal tea	2	No	No	Available (none)	None	Yes	2
6	Herbal tea	1	No	No	Not mentioned	None	Yes	1
7	Herbal drink	1	No	No	Not mentioned	None	Yes	1
8	Herbal drink	1	No	No	Not mentioned	None	Yes	1
9	Herbal drink	13	Blood glucose monitoring advised	Yes (Heart burn)	Not mentioned	Caution in non-diabetics, pregnancy, age <12 years, presence of heart burn	Yes	4
10	Capsules	2	No	No	Not mentioned	Caution in pregnancy	Yes	2
11	Herbal tea	1	Blood glucose monitoring advised	Yes (devoid of adverse events)	Not mentioned	Caution in pregnancy	Yes	4

Table 2: Complete list of the constituents and their frequency of inclusion in the products

Constituents		Number of products
Common name	Scientific name	
Tanner's cassia	*Cassia auriculata*	4
Kothala himbutu	*Salacia reticulata*	3
Black tea	*Camellia sinensis*	3
Ceylon cinnamon	*Cinnamomum zeylanicum*	2
Black seed oil	*Nigella sativa*	2
Black plum	*Eugenia jambolana*	2
Indian gooseberry	*Phyllanthus emblica*	2
Bitter gourd	*Momordica charantia*	2
Guduchi	*Tinospora cordifolia*	1
Yellow vine	*Coscinium fenestratum*	1
Java grass/nut grass	*Cyperus rotundus*	1
Wood apple	*Aegle marmelos*	1
Lemon grass	*Cymbopogon citratus*	1
Indian sarsaparilla	*Hemidesmus indicus*	1
Ivy gourd	*Coccinia grandis*	1
Bush passion fruit	*Passiflora foetida*	1
Beleric	*Terminella berelica*	1
Indian fig	*Ficus racemosa*	1
Weeping fig	*Ficus benjamina*	1
Devil's thorn	*Tribulus terrestris*	1
Sickle wild sensitive plant	*Cassia tora*	1
Indian lilac	*Azadirachta indica*	1
Arjuna (tree)	*Terminalia arjuna*	1
Balloon plant	*Cardiospermum halicacabum*	1
Fenugreek	*Trigonella foenum-graecum*	1

Some clinically relevant adverse events and interactions of selected herbal constituents are given in Table 3. Unfortunately, none of these published adverse events were included in the manufacturer's product information. The absence of a formal method for reporting adverse events, non-recognition of adverse events by patients and clinicians and the small sample sizes of previous studies further confound the situation.

None of the products specifically cautioned the user against the possibility of hypoglycemia. Two products, however, recommended frequent blood glucose monitoring. Most of the individual constituents of each product had published evidence of their potential to lower blood glucose. Cinnamon has the largest pool of evidence to date [26]. *Selacia reticulata* has evidence for inhibition of intestinal alpha glucosidase and may have clinical significance in reducing postprandial and fasting plasma glucose (FPG) values [27-29]. Evidence of efficacy for bitter melon is similar with some studies showing improvements in FPG and postprandial plasma glucose [30,31]. Although a common constituent of many anti-diabetic herbal products, Tanners cassia (*Cassia auriculata*) has not been studied widely in humans. Although it has hypoglycemic effects in experimental rat models [32] evidence for safety and efficacy in humans is not available.

As the hypoglycemic potential of the commonly used herbal constituents is evidence backed, we believe an appropriate warning on hypoglycemia is essential with these products. Previous studies have demonstrated that CAMs are often used alongside conventional hypoglycemic agents [7,13,33] probably increasing the risk of a serious hypoglycemic event.

Only 1 (8.1%) product had information on interactions. Interactions may occur with the conventional medications or within herbal constituents where more than one constituent is present. Only 4 products had a single constituent whereas 6 products contained at least 2 constituents. Tanners Cassia the constituent in 4 of the products studied is known to cause significant elevation of Carbamazepine levels with continued use [34].

The commonest precautions stated were use during pregnancy and in individuals less than 18 years.

Using the scoring system we devised, 9/11 products (81.8%) had ≤3 items of the essential items of safety information. Only 2 products (18.1%) had 4 items of information, and none had complete and accurate information.

Table 3: Reported adverse events and interactions of commonly used selected constituents

Constituent		Number of products	Reported adverse events/interactions	Mentioned in product-(Y) or not- (N)
Common name	Scientific name			
Tanner's cassia	*Cassia auriculata*	4	Elevated levels of carbamazepine [34]	N
Ceylon cinnamon	*Cinnamomum zeylanicum*	2	Potential for hepatotoxicity and carcinogenesis based on coumarin levels [19]	N
Black seed oil	*Nigella sativa*	2	*In vitro*: Inhibition of platelet aggregation: No reported clinical evidence [23]	N
Bitter gourd		2	Hypoglycemic coma [24] Abdominal pain [25] Diarrhoea [25]	N
Fenugreek	*Trigonella foenum-graecum*	1	Dizziness [25] Increased urinary frequency [24] Diarrhoea [21] Dyspepsia [21] Hypokalaemia [24]	N

Raynor *et al*., studying 68 preparations of 5 commonly purchased OTC herbal products in the UK found that three-quarters of these preparations contained no safety information and only 3 preparations had complete information [3]. Two-thirds of the products failed to mention interactions.

A study performed in Canada revealed that consumers expect pharmacy staff to be knowledgeable about herbal products [35]. However, others have recognized that pharmacists where herbal remedies are commonly sold lack knowledge regarding herbal products. There is also evidence that most consumers need help from the pharmacy staff to select a suitable herbal product [3]. Therefore, the presence of crucial product information is essential to ensure safe dispensing and use.

Most of the databases or texts cited as references [14-17] contained little or no information on the types of herbal products available in the Sri Lankan market. This study provides the evidence supporting to strengthen the regulation of HM locally. The need for developing a sound database, establishing regulatory authority for products that target a specific population of patients should, therefore, take priority considering the number of individuals who consume these products. In Sri Lanka, complementary remedies were used by 76% [13], in India by 67% [36] and in Malaysia by 48% [2] of diabetes patients. In the western countries the prevalence of use varied between 30% and 57% [37]. In Sri Lanka, all the patients studied continued to use their conventional medications together with herbal use.

The implementation of the "Directive 2004/24/EC" - So called, "traditional HM products directive" resulted in most member countries accepting it with a sharp increase in the registration of herbal products from 2 in 2005 to 265 in 2012 [9].

There seems to be a welcome trend globally toward uniform registration and safe use of traditional herbal remedies. In Brazil, proposed legislation seeks to separate HM into two categories: HM and traditional herbal product. In 2004, the UK created the new category of THR with a 7-year grace period for all the herbal remedies to be registered under this scheme. This scheme required the consumer to be presented with a leaflet similar to that found on conventional registered products [10] detailing product information that would enable safe use. A similar strategy needs to be adopted by Sri Lanka if safe practice of herbal therapies were to be implemented.

European directive of 2004 allows a non-EU product to be registered if an HM has been in use for a minimum period of 15-year in the EU. Alternately, generating scientific evidence favoring a product may be used to obtain registration [38]. Creation of herbal product monographs in the EU has largely simplified the registration of products enabling them to be registered under "well-established use" or traditional use. A similar method of product registration can be utilized in Sri Lanka for products with long-standing history of traditional use, thereby simplifying the process of registration through which legislation can be implemented to promote safe use. In the United Kingdom where herbal medications needed to be registered as THR, 85% of the expected safety information was found to be included within the product [3]. While these legislative measures would regularize registration, sale, and consumer issues to a large extent, still there would be natural remedies that would not fall within the purview of licensed or regulated products such as garlic, which have been traditionally called food supplements [3].

Strengths and Limitations

The strength of this study is that it opens up a new field of research regarding the provision of safety information together with herbal products. Limitations include the limited number of herbal products available to us through supermarkets and pharmacies allowing only 11 products to be analyzed. Although there is no data on the purchasing patterns of this community with regard to herbal products, we believe our mode of sampling reflects the pattern of the community studied.

CONCLUSION

There is a definite deficiency in providing key safety information of the products we studied. At present, Sri Lanka lacks a sound system for OTC herbal product registration and regulation. Since the use of these medications in Sri Lanka is common,

it is essential that legislation be enacted to make essential safety information be available in printed form and to set up a regulatory body for product registration and monitoring. Education of the public and conventional medical practitioners may also enable safe use of OTC herbal products.

AUTHORS' CONTRIBUTIONS

AM conceptualized the study, collected the samples, analyzed the data and wrote the manuscript.
HW: Collected the samples and analyzed the data.
TP: Collected the samples and analyzed the data.

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Ethnomedicinal plants used by yak herders for management of health disorders

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ABSTRACT

Aim: The aim of the study was to document the indigenous ethno-botanical knowledge of the transhumant nomads of Mustang, Nepal, a representative settlement in the Himalayan highland. **Methodology:** A study was carried out during a direct field visit to collect plants, consisting of a semi-structured questionnaire and personal interviews. Both fresh and dried herbs, plants parts, and fungus were collected as far as possible. **Results:** The present study identified 51 medicinal plants and 2 funguses that were used for 47 different ailments in the medicinal practices of the nomadic tribes of Lower Mustang, Nepal. Most of the medicines were prepared as juice (22.64%) or powder (49.05%) and administered orally. Roots (23%) and leaves (28%) were the most frequently used parts of the plants while prayer-laced ties were commonly applied in sheds and housing areas. **Conclusion:** This study has shown that the transhumant pastoralist nomadic communities have their own traditional ethno-botanical medicines that remain cost effective and the method of choice for management of health disorders and is passed down through oral traditions under the guidance of an herbal practitioner. There is a need for such practices to be scientifically validated, with respect and inclusion into sustainable veterinary medicine to support these remotely located communities.

KEY WORDS: Animal husbandry, ethno-medicine, ethno-veterinary practices, Himalayan highland

INTRODUCTION

Nepal is a settlement in the Himalayan lap that has a rich biodiversity. The rough terrains have given rise to some hardy tribes with interesting ways of life. Nature can still exist in a somewhat conserved state, making it a tourist destination for both humans and various species of birds. Nepal is a bio diversity rich country with 1600-1900 plant species commonly used in traditional practices from ancient times [1-3]. High altitude rangelands are highly rich in herbal and aromatic plants, and they are the rich sources of medicines and value products [4]. Cultural healing through traditional knowledge of herbal medicine, including complementary and alternative medicine provides the basis for problem-solving strategies for economically marginalized communities in any nation. Moreover, the remote areas of Nepal are particularly rich in ethno-medical knowledge and practices and are the major collectors and exporters of crude forms. Although many studies have been conducted to document medicinal plants of Nepal [5-13], only three studies have been made to document the medicinal plants in this area [4,8,14], but the ethno-botanical knowledge of yak herders has not been documented until date.

The rugged topography, aridity, and poor soils in the Mustang district make it unfavorable for agriculture; thus, nomadic pastoralism is critically important for the economy of the Mustang District. These nomadic communities are underprivileged communities in the nation and are dependent on ethno-medicine for both humans and animals. Almost every nomad who lives in alpine areas away from villages with yak and chauri rely solely on herbal and traditional practices as medicine. The reason behind this is the lack of reliable and sufficient health facilities in these areas. Over time, generations of these families in Mustang district have generated an immense amount of ethno-botanical knowledge to facilitate in curing diseases. The rich knowledge of herbal medicine in this community is totally oral, and little of it has been documented; however, it has been passed down from generation to generation [15]. Their knowledge regarding the use of plants and plants parts such as leaves, fruits, rhizomes, or bark, and also the method of processing for medicinal purposes needs to be well-documented and preserved, this is not only for the effective and cheap sources of medicine but also for the conservation of indigenous ethno-botanical knowledge and sustainable use of this knowledge. The lack of proper documentation, uncontrolled exploitation, and

also the shortage of effective conservation efforts have caused many medicinal plants to become either extinct or replaced by chemotherapeutic agents [16]. Thus, there is an urgent need to document the indigenous ethno-botanical knowledge, especially in times when natural tragedies such as earthquakes, floods, landslides, glacial lake bursts, and droughts threaten survival in the high mountainous terrains. Thus, the documentation, conservation, and sustainable use of these resources based on indigenous technological knowledge is a present need within the country.

METHODOLOGY

Study Area

The Mustang district covers 3,639 km^2 and is located in the trans-Himalayan Arid Zone [17] in the Midwestern Development region of north-central Nepal, which is bounded by Myagdi to the South, by Dolpa to the West, by Manang to the East, and by the Tibetan Autonomous region of the People's Republic of China to the North [4].

The study area is comprised of the alpine pastures and temperate forests of Lete, Kowang, Marpha, and the Tukuche VDCs of Lower Mustang, Nepal. All the areas are located above 1500 meter altitude, and extend up to 6800 meter altitude. The major ethnic inhabitants of the area are Thakali, Gurung, Bishwakarma, and Sherpa. They have roots with Tibeto Burmese and Indo-Aryan cultures speaking Thakali, Nepali, and Tibetian Dialects. Their economy relies on livestock farming, agriculture, and tourism. Owing to the low productivity of the soil, they are engaged in the collection and trade of medicinal plants and livestock farming [Figure 1].

Ethno-botanical Survey

All the 32 yak herders of lower Mustang, including the Local healers known as *Aamchi*, were surveyed with a set of pre-tested semi-structured questionnaires. The age of informants ranged between 24 and 56 years.

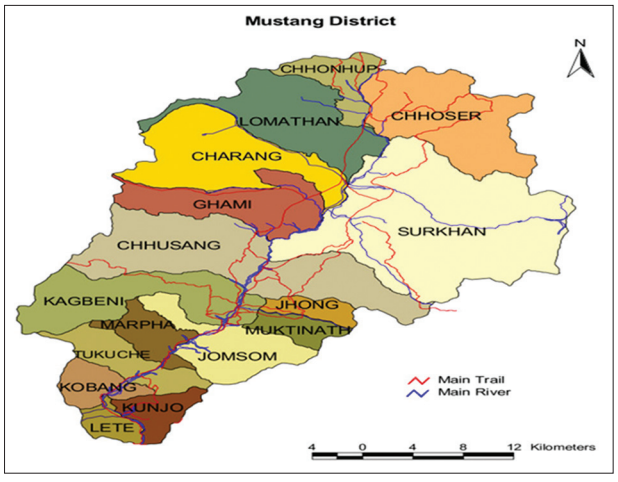

Figure 1: Map of study area (Mustang district) (Source: Adapted from Bhattarai *et al.* [4])

Prior informed consent was obtained verbally before they were interviewed and all agreed to be involved in this survey. Two interview methods were followed, and walks were taken around the grazing land and forest for plant collection and information gathering during February-June 2014. The data were compiled and interpreted in the form of Table 1.

Total Key Informants

During the survey, we discussed with 32 yak herders including local healers called "Aamchi." Among these all of the informants were male (100%), this is because females are confined to the household works and males are only involved in transhuman animal husbandry. The age of informants ranged between 24 and 56 years only two informants were below 30 years of age and rest above 30. The obtained informations were subjected to the other informants to check their precise knowledge of ethno-medicines.

Data Analysis

The obtained informations were put in a Microsoft office excel 2007 and analyzed using descriptive statistics.

RESULTS

Plants Used

The total of 51 plants and 2 fungal species belonging to 32 families and 44 genera were found to be commonly used in treating 47 ailments in the communities studied. The largest number of plant species were recorded from families *Compositae* (4 species), *Gentianaceae* (4 species) followed by *Asparagaceae* (3 species), and *Rutaceae* (3 species). Three families *Pinaceae*, *Rosaceae*, and *Ericaceae* represent 2 plant species each and rest of the families represented 1 species each.

Although Bhattarai *et al.* [4] reported 121 species belonging to 49 vascular plant and 2 fungal families and 92 genera, 8 plant species namely *Acorus calamus*, *Prunus armeniaca*, *Artemisia vulgaris*, *Chlorophytum nepalense*, *Swertia multicaulis*, *Rhodiola rosea*, *Pedicularis siphonantha*, *Taraxacum officinale* were added by the present research. When compared to ancient Tibetian literature, 1 plant species (*R. rosea*) was recorded, and medicinal plants such as *Rhododendron lepidotum*, *Rumex nepalensis*, *P. armeniaca*, *Dactylorhiza hatagirea* were reported with same ethno-medicinal values.

Parts of Plants Used and Modes of Preparation

Various parts of plants were used in the preparation of remedies. The most frequently used were leaves (28%), followed by roots/rhizomes (23%), and fruits/flowers (18%) [Figure 2].

Several types of medicinal plants were used; the most common were climbers and the least common were trees [Figure 3].

The largest numbers of medicinal plants (19 species) were used for respiratory tract infections (cold, cough, headache,

Table 1: Traditional herbal medicine for treatment of disease and ailments by yak herders of Mustang district in Nepal

Scientific name	Family	Vernacular name	Parts used	Conditions	Method of application	References
**Abies spectabilis* D. Don	*Pinaceae*	Kye (Gurung) Talispatra, Gobre salla (Nepali)	Fresh leaves and cones	Bone fracture	About 20 g of pulverized fresh leaves and cones drunk two times a day until recovery. Paste of pulverized roots and cones are applied around the site of fracture	32
Aconitum naviculare (Bruhl) Stapf	*Ranunculaceae*	Bhalaponar (Gurung)	Whole plant	Fever, jaundice	About 15 g of decoction is mixed with a cup of hot water and drunk BID after meal	28
Aconitum orochryseum Stapf	*Ranunculaceae*	Nirmasi (Gurung)	Roots	Altitude sickness, diarrhea, dysentery, cough, fever	5-10 g is taken with a cup of luke warm water BID-TID until recovery	30
Acorus calamus L.	*Acoraceae*	Bojho (Nepali)	Rhizome	Cold, anthelminthics, fever	About spoonful powder of rhizome is taken with hot water for worms. A piece of rhizome is chewed to tear fever and cold	32
**Allium fasciculatum* Rendle	*Amaryllidaceae*	Jimmu (Nepali) Nosyante (Gurung)	Whole plant	Plant poisoining, gastritis, purification of blood	10 g of the whole plant is pounded and boiled with 2 cups of water, and half cup of decoction is drunk twice a day	32
**Artemisia gmelinii* Weber ex Stechm.	*Compositae*	Titepati (Nepali) Bajha (Gurung)	Leaves	Gastritis, scabies, indigestion	10-15 g of plant parts is boiled with 2 cups of water and taken BID-TID to cure Fever, Sore throat, Indigestion, Gastritis	32
Artemisia vulgaris L.	*Compositae*	Titepati (Nepali)	Leaves	Fever, Indigestion Roundworms	About a spoonful of leaves powder is taken with honey or gur or a cup of hot water BID until recovery. About 1-2 spoonful of leaves extract is given for three alternate days to kill roundworms	28
**Asparagus filicinus* Buch.-Ham. ex D. Don	*Asparagaceae*	Kurilo (Nepali) Nirshing (Gurung)	Roots	Mastitis, Menstrual disorders Scabies, Ringworm	10 g of root powder is taken with a cup of hot water once a day after having meal. Root paste applied topically	32
**Asparagus racemosus* Willd.	*Asparagaceae*	Kurilo (Nepali)	Root, tuber, fruit, stem	Tonic Kidney and liver problem Sore throat	2-3 spoonful of root powder is taken with a cup of milk BID until recovery. Paste of roots is applied topically in treatment of mastitis	32
Benincasa hispida (Thunb.) Cogn.	*Cucurbitaceae*	Kubhindo (Nepali)	Fruit, leaves	Alcohol poisoining, Tuberculosis, Colic	About 10 teaspoonful of juice of fruit is used BID as an antidote of alcohol poisoining	32
Berberis aristata DC.	*Berberiaceae*	Chutro (Nepali)	Root, bark	Fever, dysentery, skin troubles	5 teaspoonful of root juice is taken BID until recovery for fever, dysentery, skin troubles and purification of blood	30
**Betula utilis* D. Don	*Betulaceae*	Bhojpatra (Nepali) Buspath (Gurung, Thakali)	Bark, Leaves	Fever	Pulverized powder of bark and leaves is mixed with other plants, and a half spoonful is taken with cow ghee BID-TID until recovery	21
**Cannabis sativa* L.	*Cannabaceae*	Bhang (Nepali) Kantsya (Gurung, Thakali)	Leaves	Diarrhea and Dysentery Clairvoyance, Psychoactive	5-10 g of leaves powder is taken once a day with hot water until recovery. Powder of leaves smoked with tobacco	32
**Chlorophytum nepalense* Lindley	*Asparagaceae*	Ban pyaj (Nepali)	Root	Gout	Root is crushed on stone slab and paste is made. Root paste is mixed with mustard oil and applied topically to care gout	32
Clematis barbellata Edgew.	*Ranunculaceae*	Laharejhar (Nepali) Kramay (Gurung, Thakali)	Leaves, stem, flowers	Jaundice	1 cup of water decoction is taken BID orally until cure	25
Cinnamomum zeylanicum Garcin ex Blume	*Lauraceae*	Dalchini (Nepali)	Barks	Colic, diarrhea, indigestion Throat allergy	1-2 spoonful of powder of bark is taken with Tea or hot water BID-TID until recovery. Green leaves chewed to cure throat allergy	32
Cordyceps sinensis (Berk.) Sacc	*Clavicipitaceae*	Jibanbuti, Yartsagumba (Nepali, Gurung, Thakali)	Whole part	Tonic Sex stimulant	A half spoonful of yartsaghumba powder is taken with milk or honey when enervated. ½ spoonful yartsaghumba powder+½ spoonful Dactylorhiza powder+a cup of milk-honey during lethargic periods. One piece of Yartsaghumba is taken with either alcohol or milk BID to increase sex vigor	18
**Dactylorhiza hatagirea* D. Don	*Orchidaceae*	Panch aaunle (Nepali), Soo (Gurung)	Roots	Snake bite, scorpion stings, cuts, wounds, boils	Paste of root is usually applied around the site of snake bite, scorpion stings, cuts, wounds, boils once a day until recovery	32

(*Contd...*)

Table 1: ***(Continued...)***

Scientific name	Family	Vernacular name	Parts used	Conditions	Method of application	References
**Ephedra gerardiana* Wall. ex Stapf	*Ephedraceae*	Somlata, (Gurung) Chaya (Aamchi)		Chest pain, wounds, gastritis, Respiratory disease, nasal bleeding	Root paste is applied in cuts and wound twice a day until recovery. One spoonful root powder is taken once a day for the cure of asthma, cold, cough, altitude sickness, and dysuria until recovery	32
Girardinia diversifolia (Link) Friis	*Urticaceae*	Chanle sisno (Nepali) Ghyo (Thakali, Gurung)	Leaves and roots	Headache, Joint ache	Leaves are crushed on the stone slab and juice of leaves is applied topically to treat a headache and joint ache	30
Indigofera bracteata Baker	*Fabaceae*	Sakhino (Nepali)	Leaves	Leprosy Menstrual disorder Muscular swelling	About 5 teaspoonful of juice of leaves is taken BID until recovery. Paste of leaves is used to relieve muscular swellings	19
Juniperus communis L.	*Cupressaceae*	Phar, Chuksar (Gurung, Thakali)	Fruits and Leaves	Kidney diseases	2 spoonful of paste of leaves and flowers is taken with hot water or milk TID orally until cure	32
**Lyonia ovalifolia* (Wall)	*Ericaceae*	Angeri (Nepali)	Leaves	Ticks, Lice	About 15-20 g of leaves is pounded on a stone slab and squeezed through a muslin cloth, and liquid is applied on the body OD until recovery	32
Maharanga bicolor A. DC	*Boraginaceae*	Maharangi (Nepali)	Root	Ear pain	Liquid from pounded root extract is taken with 2 spoonful of boiled mustard oil. 1-5 drops of pounded root extract is put in ear BID-TID until recovery	30
Mentha longifolia (L.)	*Lamiaceae*	Patina (Nepali)	Leaves	Tonsilitis, headache, cold cough	10 g of leaves is boiled with 2 cups of water, and a half cup of decoction is drunk in the morning	29
**Mirabilis himalaica* (Edgew.) Heimerl	*Nyctaginaceae*	Nigghibulug, Khemba (Gurung)	Leaves and flowers	Fracture	25 g of leaves and flowers are crushed on the stone slab and paste is applied around fractured part once a day until recovery	30
Morchella esculenta (L.) Pers.	*Morchellaceae*	Guchichaue (Gurung, Thakali)	Whole plant	Heart disease	3 spoonful of dried powder taken with hot water SID until recovery. Taken as vegetables	32
**Nardostachys grandiflora*	*Caprifoliaceae*	Jatamasi (Nepali) Panghphoie (Gurung).	Roots	Diarrhea Conjunctivitis Gastritis Headache Chest pain	½ spoon of root powder+½ *Aconitum* naviculare plus Betula utilis+3 spoonful of Chauri ghee BID until recovery for diarrhea. A spoonful is poured on red coal fire and fragrance at night before sleeping until recovery. ½ spoonful root powder+a cup of hot water BID after meal until recovery	32
**Neolitsea pallens D. Don*	*Lauraceae*	Pya pya (Nepali)	Fruit, seed	Eczema Poisoining	Juice obtained from fruit is applied to treat scabies and eczema. Seeds are crushed and oil obtained is used 2 spoonful BID as an antidote of alcohol poisoining	32
**Neopicrorhiza scrophulariiflora* Hong.	*Plantaginaceae*	Kutki (Gurung, Thakali)	Roots	Diarrhea, Paralysis, Indigestion Scorpion and snake bite Scabies, Ringworm	10 g of root powder is boiled in a cup of water and 30-40 ml of filtered decoction is taken with a cup of milk BID-TID until recovery. Half spoonful of powder is mixed with two to three spoonful of Chauri ghee BID-TID until recovery. Paste of roots	32
**Notochaete hamosa Benth*	*Lamiaceae*	Kuro (Nepali)	Leaves	Snakebite Indigestion	About 5 teaspoonful of juice of leaves taken BID as an antidote to cure until recovery	32
**Paris polyphylla* Sm.	*Melanthiaceae*	Satuwa (Gurung)	Leaves, Flowers, Roots	Indigestion, Diarrhea	About 5 g of stems, leaves and flowers is taken with luke warm water once a day until recovery. About 5 teaspoonful of juice of rhizome is given twice a day in the treatment of Gastritis and menstrual pain	32
**Pedicularis siphonantha* D. Don	*Orobanchaceae*	Halhale (Nepali)	Roots	Plant poisoining		32
Piper nigrum L.	*Piperaceae*	Marich (Nepali)	Seeds	Indigestion, poisoining, mastitis	A spoonful of pulverized powder of is drunk with a cup of hot water BID until recovery	32

(*Contd...*)

Table 1: ***(Continued...)***

Scientific name	Family	Vernacular name	Parts used	Conditions	Method of application	References
**Pinus wallichiana* A.B. Jacks.	*Pinaceae*	Sallo (Nepali) Thansin (Gurung)	Resins	Wounds Fracture Tuberculosis	Paste of leaves and resins are applied topically at the site of injury. Bark cut into smaller parts and applied on fractured site until recovery. Half spoonful of bark powder is drunk BID after meal for 2 years	32
Prunus armeniaca L.	*Rosaceae*	Khurpani (Nepali) Khamba (Thakali, Gurung)	Fruit Seeds	Vitamin deficiency	Seeds are eaten raw 3 time a day until recovery. Sauce is made from seeds and fruits and eaten with meal	24
**Prunus persica* L.	*Rosaceae*	Aaru (Nepali)	Leaves	Maggoted wound	Juice of leaves when pounded on stone slab is poured on maggoted area	22
Rhodiola rosea L.	*Crassulaceae*	Solo (Gurung, Thakali) Sanjjevani, Jivanbuti (Nepali)	Whole plant	Cognitive improvement, Anti- aging, Altitude sickness	Leaves of plants taken as vegetables. About 20 g of the whole plant is pounded on stone slab, and a spoonful of powder is taken with a cup of hot water OD until recovery	1
Rhododendron anthopogon D. Don	*Ericaceae*	Palu (Gurung), Sangalin (Amchi)	Leaves and flowers	High BP	Leaves and flowers are ground to make powder, and a half spoonful of powder is drunk with a cup of hot water or milk BID after meal until recovery	26
Rhododendron lepidotum Wall. ex G. Don	*Ericaceae*	Bhale sunpate (Nepali) Bhaiunako (Gurung)	Plant paste (flower and leaves)	Blood purification	About 2.5-5 g is taken with a cup of hot water until recovery	29
Rumex nepalensis Spreng.	*Polygonaceae*	Somang (Gurung, Thakali)	Whole plants, roots	Fracture, joint pain, edema	A spoonful of powdered plants/roots is taken BID with a cup of hot water or milk until recovery	22
Swertia angustifolia Buch.-Ham. ex D. Don	*Gentianaceae*	Chiraito (Nepali) Tento (Gurung, Thakali)	Whole plant	Fever, indigestion, diarrhea, scabies	10 g of whole plant is boiled with 2 cups of water and half of the decoction is drunk OD-BID until recovery. Whole plant is pounded on stone slab, water extract is made and applied on the site of scabies until recovery	32
**Swertia chiraytia* Rob. ex Flem	*Gentianaceae*	Chiraito (Nepali)	Whole plant	Fever, indigestion	A spoonful of plant powder is taken with a cup of hot water BID until recovery. About 10 g of the plant is boiled with 2 cups of water, and a half cup of decoction is taken BID until recovery	32
Swertia multicaulis D. Don	*Gentianaceae*	Bhale chiraito (Nepali)	Plant and root paste	Cuts and wounds	Paste of plant is applied topically on the wound and cuts until recovery	32
Swertia racemosa C.B. Clarke	*Gentianaceae*	Lakhetikta (Gurung)	Whole plant	Fever, malaria, jaundice, diabetes, cold, cough, headache	About 5 gs of pulverized powder of whole plants is mixed is drunk with a cup of hot water BID-TID until recovery	31
Taraxacum officinale aggr.	*Compositae*	Tuki phool (Nepali)	Plant paste	As an emetics and treatment of altitude sickness	About 5 g of plant paste is drunk with hot water as emetics for the management of altitude sickness	15
Taraxacum tibetanum Hand.-Mazz.	*Compositae*	Khurmang (Thakali, Gurung)	Leaves, stem, and flowers	Vertigo, jaundice, gastritis, fever	A half spoonful of powder is taken with a cup of hot water BID until recovery	22
Taxus wallichiana Zucc.	*Taxaceae*	Silingi (Gurung)	Stem and leaves	Cancer	Plant powder is taken with cup of hot water until recovery	
**Triticum aestivum* L.	*Poaceae*	Gahun (Nepali)	Seeds	Regualation of oestrus cycle, bone fracture, constipation	About 20 g of young leaves powder is taken with hot water BID-TID. Paste of plant is applied topically over the skin at site of fracture and immobilized	32
**Valeriana jatamasi* Jones	*Caprifoliaceae*	Napu, Ghyapo (Thakali, Gurung)	Roots, leaves	Cuts, wounds, headache, fever	Paste of the roots and leaves is applied on cuts and wounds until recovery. ½-1 cup of decoction is taken orally BID until recovery for the headache and fever	32
**Zanthoxylum acanthopodium* DC.	*Rutaceae*	Aaankhe timur, Bhote timur (Nepali)	Fruit, leaves	Fever, cold, respiratory distress	Decoction of leaves used externally to cure abdominal pain. Paste of leaves is used topically to relieve a toothache	32
**Zanthoxylum armatum* DC.	*Rutaceae*	Prumo (Gurung, Thakali)	Fruits	Altitude sickness, vertigo, cold, cough, dysentery, diarrhea	One-fourth spoonful powder of fruits taken with a cup of water for diarrhea	28
**Zanthoxylum oxyphyllum* Edgew.	*Rutaceae*	Siltimur (Nepali)	Fruits	Indigestion, poisoining, tympany	5-10 g of powder of fruit is taken with water TID-QID until recovery	32

Figure (Number) indicates the frequency of citation of each species by the informants, *Are also used in Yaks in addition to humans, OD: Once a day, BID: Two times a day, TID: Three times a day

nasal bleeding, dizziness, altitude sickness, etc.), whereas, gastrointestinal disorders (diarrhea, indigestion, dysentery, gastritis, colic, etc.) treated with 17 species and musculoskeletal disorders (Joint pain, muscular swelling, fracture, etc.) were cured with 8 species. The form of remedies was primarily powder (49.05%), juice (22.64%), or decoction (18.87%), tablets, pills, and infusion were rare [Figure 4].

Tablets, pills, and infusions were usually made only by traditional healers, "Aamchi," as cited in previous studies [4,8]. Per oral use predominated topical use. Plants were generally prepared using cold or hot water, but occasionally other methods of preparation, such as alcohol, milk, ghee, or oils, were used [Table 1]. Medicinal preparations were found to be administered through various routes-oral was the most predominant route followed by topical, nasal, and other routes. While in animals intended oral formulations were found to be drenched by means of drenching tube from *Bambusa indica* (Bans). This was followed in only in young and debilitated animals, and adult animals were given medications either mixed with salt or mixed with oat flour.

DISCUSSION

Notably, a mixture of different plant products rather than a single one was used in the treatment of most diseases. Many nomads believed that combination of plant species increased the potency of medicines owing their synergistic actions unlikely that of Paliyar communities of Tamilnadu, India who selectively used single plant for specific ailments [18]. Similar combined formulations were reported from Kani communities in India [19]. Almost all the plant species were collected directly from their wild state during various seasons and thus were in different stages of growth and development. Without a doubt, the future practice of medicine must take into account traditional healing arts while adopting new scientific discoveries [20], that respects, documents and advocates these traditional healing arts. The yak herders are the major collectors of high altitude medicinal plants from the alpine meadow as mentioned by Oli and Nepal [21]. Though, the herders do not have traditional scientific knowledge which advocates sustainable harvesting of medicinal plants as, they are familiar with the nature of plants and their distribution [22,23]. They collect the medicinal plants in fresh and dried form, especially in spring and autumn when the climate is favorable for collection. Transhumant migrating nomads, they partly collect medicinal plants from grassland and forest and partly purchase from the traditional healers "Aamchi." They follow the rotational grazing system; seasonal and selective harvesting, which is the only management approach and had some contribution to sustainable management of herbal resources of high mountains. North and South trade to India and the Far East through China (now China is in itself a major market) have created huge demands for priced medicinal herbs of Nepalese highland [24]. However, greed is slowly creeping in as highland medicinal plants and materials find premium price leading to over harvesting and social ills which are having an eroding effect in social and ecological harmony.

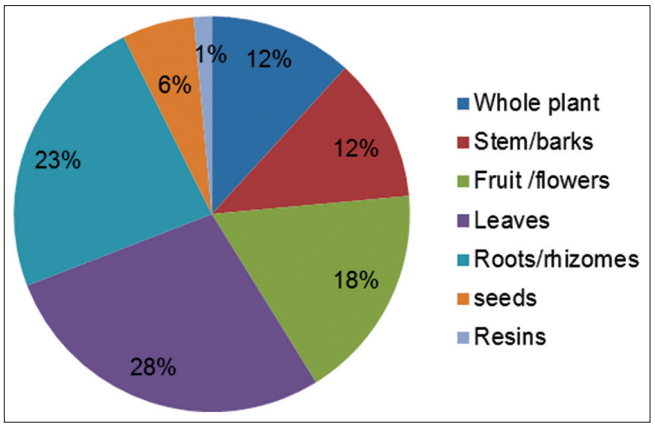

Figure 2: Different parts of plants used in preparation of medicine

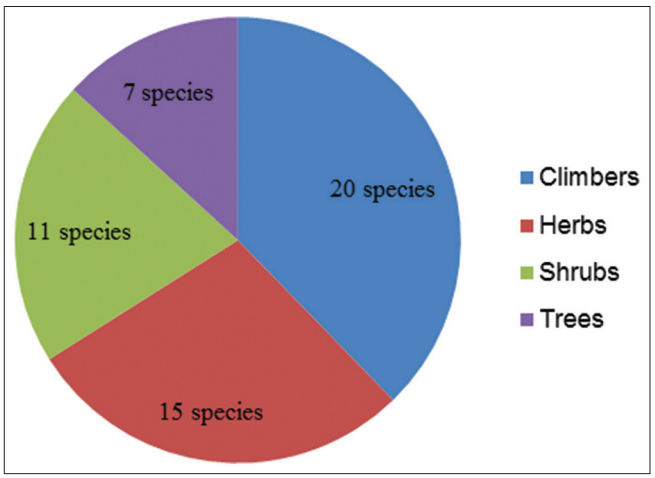

Figure 3: Life form (medicinal plants used by yak herders of Mustang, Nepal)

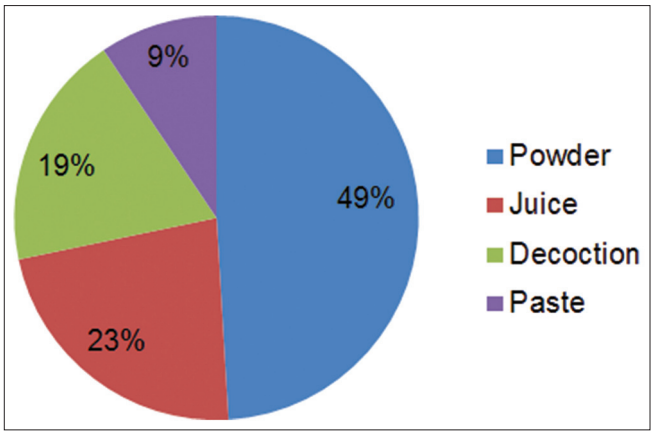

Figure 4: Forms used (Medicinal plants used by yak herders of Mustang, Nepal)

CONCLUSION

It is concluded that transhumant pastoralist nomadic communities have their own traditional ethno-botanical medicines that remains cost effective. Furthermore, method of choice for management of health disorders is passed down to

next generation usually by oral traditions. These communities have detailed and extensive knowledge regarding medicinal plants and their utility. They have their own way of collecting medicinal plants, method of preparation, dose and application. The lack of modern health facilities, coupled with rugged topography, and a strong belief towards herbal medicines, substantiate the preference for herbal medicines for health care. However, the long-term use of herbal medicinal plants, over-harvesting is risking many valuable medicinal plant species to the extent of becoming extinct. Thus, necessary steps towards conservation of these resources are needed. Continuous training of traditional healers and transfer of this knowledge to the younger generation is necessary. Although their traditional medicine is partially effective for management of ailments, they should be further strengthened by the scientific management of health.

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Original Research

ScopeMed

Long lasting preventive effects of piperlongumine and a *Piper longum* extract against stress triggered pathologies in mice

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ABSTRACT

Aim: To compare doxycycline (DOX) such as oral efficacies of piperlongumine (PL) and a *Piper longum* fruits extract (PLE) as stress resistance inducers. **Materials and Methods:** Efficacies of oral pretreatments with 5 mg/kg PL or PLE or of 50 mg/kg DOX for 10 consecutive days against stress resistance were compared. Mice in treated groups were subjected to a stress induced hyperthermia on the 1st, 5th, 7th, and 10th day. Treated mice were then subjected to tail suspension test on the 11th day. Alteration in body weights, core temperatures, and gastric ulcers triggered by occasional exposures to foot shocks were determined. **Results:** DOX like long-lasting protective effects of PL and PLE against gradual alterations in body weights, basal temperatures and transient hyperthermic responses triggered by foot shocks during the post-treatment days were observed. Altered responses of stressed mice in tail suspension test observed 1 day after the last foot-shock exposures and gastric ulcers and other pathologies quantified 1 day after the test were also suppressed in PL or PLE or DOX pretreated groups. **Conclusion:** PL and crude PLE are DOX like long-acting desensitizers of stress triggered co-morbidities. Reported observations add further experimental evidences justifying traditionally known medicinal uses of *P. longum* and other plants of the Piperaceae family, and reveal that PL is also another very long acting and orally active inducer of stress resistance. Efforts to confirm stress preventive potentials of low dose plant-derived products enriched in PL or piperine like amide alkaloids in volunteers and patients can be warranted.

KEY WORDS: Foot-shock stress, hyperthermia, gastric ulcer, *Piper longum*, piperlongumine, piperine, prophylaxes

INTRODUCTION

Piper longum L. is a plant of the Piperaceae family, the roots and fruits of which are often used in Ayurvedic and other traditionally known systems of medicine for prevention and cure of chronic diseases commonly associated with or caused by mental health problems [1,2]. The plant is native in Indo-Malesian region and in Sri Lanka. In India, it is widely distributed in northeastern regions such as Assam, Arunachal Pradesh, Meghalaya, Manipur, and in some parts of the lower hills of West Bengal, Tamil Nadu and evergreen forests of Western Ghats [1]. Traditionally, the roots and fruits of *P. longum* are used as carminative, tonic to the liver, stomachic, emmenagogue, abortifacient, and aphrodisiac [1,2]. Like numerous other plants of this family, *P. longum* is also a rich source of structurally diverse amide-alkaloids with pungent taste and broad spectrums of therapeutically interesting bioactivities [3]. Piperlongumine (PL)is one such amide-alkaloid now attracting considerable attention of modern researchers interested in identifying novel therapeutic leads from secondary plant metabolites [4]. However, most *P. longum* extracts (PLE)now widely used in modernized versions of Ayurvedic formulations are still analytically standardized, or characterized, by their contents of another quantitatively major amide-alkaloid piperine [5,6].

Although piperine was first isolated from *P. nigrum* (black and white piper)[7], and it is also the quantitatively major pungent tasting amide alkaloid of *P. longum* fruits [2].

PL and piperine are structurally analogous molecules [Figure 1], and both of them possess therapeutically interesting anti-mitotic and antimicrobial activities [8,9]. It is now evident that gut microbiota play a crucial role in regulating physiological stress responses [10,11], and that appropriate doses and treatment regimen of antibiotics and other agents with modulating effects on gut microbial ecology can have health benefits [12,13]. Doxycycline (DOX)is one such extensively studied and clinically used antibiotic with stress and neurohormonal status regulating [14], and gastric ulcer protective, anticonvulsant, antidepressant, neuroprotective, and other therapeutically interesting bioactivities [15-18]. Therefore, efforts are now being made in our laboratories to compare DOX like stress response suppressing efficacies of PL a medicinally used PLE.

Results of the very first experiments (under publication)have revealed that ten daily oral doses of 5 mg/kg/day PL or of a medicinally used PLEstandardizes on its piperine contents (1.75%)are high enough for observing their DOX like stress response suppressing effects after their 10 daily doses. Results of a further experiment conducted to verify their longer lasting preventive potentials against chronic mild stress triggered gastric ulcers and other pathologies will be described and discussed in this study.

MATERIALS AND METHODS

Animals

Adult male swiss albino mice (25 ± 5 g)were from Central Animal House of Institute of Medical Sciences, Banaras Hindu University (Registration Number: 542/AB/CPCSEA). They were acclimatized to laboratory conditions for 1 week before starting the experiment. Six animals were used in each group, and all experimental groups were housed in polypropylene cages (28 cm × 19 cm × 12.5 cm)with saw dust beddings and free access to standard rodent diet and tap water. They were maintained at 25°C ± 1°C ambient temperature and relative humidity of 50% ± 10% with 12:12 h light and dark cycle (light on at 06:00 and off at 18:00)and were acclimatized to the laboratory conditions for a week before performing the experiment. Principles and guidelines of laboratory animal care (NIH publication 85-23, revised in 1985)were always followed, and before start of the experiments an approval from Central Animal Ethical Committee of the University was obtained (Dean/2014/CAEC/729, dated August 07, 2014).

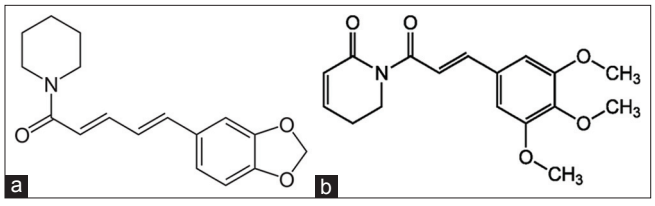

Figure 1: Structure of (a) Piperine and (b) Piperlongumine

Plant Extracts, Drugs and Chemicals

The methanolic extract of *P. longum* fruits analytically characterized to contain 1.75% piperine and almost pure PL (99.33%)isolated from *P. longum* roots used in this study and analytical data on them were generously supplied by Sami Labs Limited Bangalore, India. PLE is a methanolic extract of dried *P. longum* fruits, and purity of PL and piperine contents of the PLE sample used were established by high performance liquid chromatography equipped with ultraviolet/photodiode array detector and using acetonitrile and water as mobile phase.

DOX was acquired from Sigma Aldrich, Bengaluru, India; carboxymethyl cellulose (CMC)from Central Drug House, Delhi, India. All other chemicals and reagents used in this study were of highest purity commercially available in India.

Animal Grouping and Drug Administration

Six randomly selected mice groups were used in this study. Except for the animals of the one of the groups serving as reference (Group: REF), all others were subjected to foot shock stress triggered hyperthermia test. The REF groups and a control group (Group: CON-CMC)were not given any oral treatments. The other four groups were treated daily only on the first 10 days of the experiment either with 0.3% CMC (Group: CON + CMC), or 50 mg/kg/day DOX (Group: DOX), or 5 g/kg daily doses of PL (Group: PL)or PLE (Group: PLE), and on days 10, 15, 17, and 20 of the experiment all animals of these groups were subject to the foot shock stress triggered hyperthermia test describe later. For oral administrations, the test substances were suspended in 0.3% CMC, and oral application volumes were always 10 mg/kg/day, and basal core temperatures and body weights of all groups were recorded on the 1 h before the tests on all observational days. Further details of the experiment are graphically summarized in Figure 2.

Foot Shock Stress Induced Hyperthermia Test

This test was conducted by placing an individual mouse of a group in a black box (24 cm × 29 cm × 40 cm)with a grid floor for 1 min, when foot shocks through the grid floor (2 mA, 50 Hz of 2 ms duration)was delivered. Five consecutive foot shocks of 2 mA at 10 s intervals were given after the animals had stayed in the cage for 10 s. At the end of the minute, the animals were placed back in their home cages, and 10 min thereafter their rectal temperatures were recorded again by using a digital thermometer and a digital probe [19]. Calculated differences between this and the basal core temperature of a mouse recorded one hour before was used as an index for stress induced hyperthermic response of the animal. The animals of the reference group were not subjected to foot shock stress, but were also placed in the black box for a min, and their

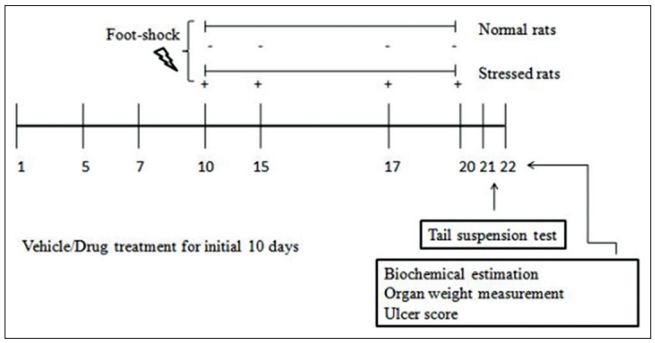

Figure 2: Summary of the experimental methods used

rectal temperatures were recorded again 10 min after they were returned to their home cage.

Tail Suspension Test

The test procedure described elsewhere was used [20]. In short, an individual mouse of a group was hung by tail, 50 cm above the floor by an adhesive tapes placed 1 cm from the tip of the tail on a wire in an upside down posture. After initial vigorous movements, the mouse assumed an immobile posture and the period of immobility during a 5 min observation period was noted. All animals of all experimental groups were subjected to this test on day 21 of the experiment.

Plasma Glucose, Insulin and Cortisol Level, Organs Weights and Stomach Ulcer Scoring

Immediately after last temperature measurements on the 22nd day of the experiment, all animals were sacrificed by decapitation. Their blood samples from eye orbital puncture was collected in ethylenediaminetetraacetic acid coated tubes kept in ice and centrifuged at 1000 × g for 20 min at 4°C to separate plasma (Compufuge CPR-30 Plus, with Rotor No. 8; REMI, India). Plasma glucose levels were quantified by an enzymatic test kit (ERBA diagnostics Mannheim GmbH, Germany). Plasma insulin levels were quantified by using Enzyme-Linked Immunosorbent Assay (ELISA)test kit (Chemux BioSciences, Inc., USA), and plasma cortisol by using ELISA kit (DSI S.r.l., Italy). All biochemical estimations were done in a absorbance micro-plate reader (iMarkTM- Bio-Rad Laboratories, California, USA)according to instructions manual of respective enzyme test kits. Immediately after blood collections, adrenal glands, and spleen of the animals were dissected out and washed under slowly running tap water. After removing adhered water using filter papers and both the organs were weighed [21].

For stomach ulcer scoring, cardiac end of the stomach was dissected out and the contents were drained out. Thereafter, the stomach was cut and opened along with its greater curvature, and washed slowly under running tap water. After washing, stomachs were spread and fixed on a glass slide for scoring ulcers (under ×10 magnification). The ulcer index was evaluated according to their severity and scored as follows: 0 = normal colored stomach, 0.5 = red coloration, 1 = spot ulcers, 1.5 = hemorrhagic streaks, 2 = ulcer > 3 mm but < 5 mm, 3 = ulcers >5 mm [22].

Statistical Analysis

Means ± standard errors of means were calculated for the observed values in each experimental group. Statistical analysis was done by one-way Analysis of Variances (ANOVA)followed by Student Newman Keuls multiple comparison tests. When stated, two-way ANOVA followed by Bonferroni *post-hoc* test and *t*-test were performed. GraphPad Prism-5 (GraphPad Software, Inc. La Jolla, California, USA)and Origin-Pro 8 (OriginLab Corporation, Massachusetts, USA)software were used for statistical analysis and drawing graph. $P < 0.05$ were considered as statistically significant.

RESULTS

Body Weight and Basal Rectal Temperature

Mean body weights of all experimental groups increased slightly during first 10 days of the experiment [Figure 3a]. However, from the 15th experimental days onward, animals of both the control groups (CON + CMC and CON-CMC) continuously lost their body weights, which were not observed in the reference group [Figure 3b]. Mean body weights of the DOX, or PL of PLE treated groups remained almost constant until they were subjected to three foot shock stress sessions on the 10th, 15th and 17th day of the experiment, and thereafter the mean body weights of all these three groups continued to increase steadily.

Results summarized in the Figure 4a and b revealed that the mean basal core temperatures of the reference groups remained almost constant on all observational days, with a tendency to increase slightly during the course of the experiment. Until the 17th day observational day, basal core temperatures of all other groups also remained within the normal range of the mice colony used in the experiment (36.3-36.6°C), but also tented to increase continuously. Mean basal core temperatures of both the control groups (CON + CMC and CON-CMC)continued to increase further (but still remained within physiological range)till the last day of the experiment, whereas from the 17th day onward, mean basal core temperatures of the DOX, PL and PLE treated groups continued to decrease steadily toward the mean vales of all groups observed on the 1st day of the experiment.

Mean ratios of body weight and basal core temperature of the reference group continued to increase steadily till the 21st observational day, i.e. until, they were subjected to tail suspension test for anti-depressants. However on the next day, this mean value of the group was almost equal to that calculated for the group on the 10th day of the experiment [Figure 5a and b]. During the ten oral treatment days, mean ratios of body weight and basal temperature of the reference and both control groups followed similar increasing trend [Figure 5a]. From the 10th day onward [Figure 5b], i.e., after the animals were first subjected to foot shock stress, this mean value steadily decreased in the both

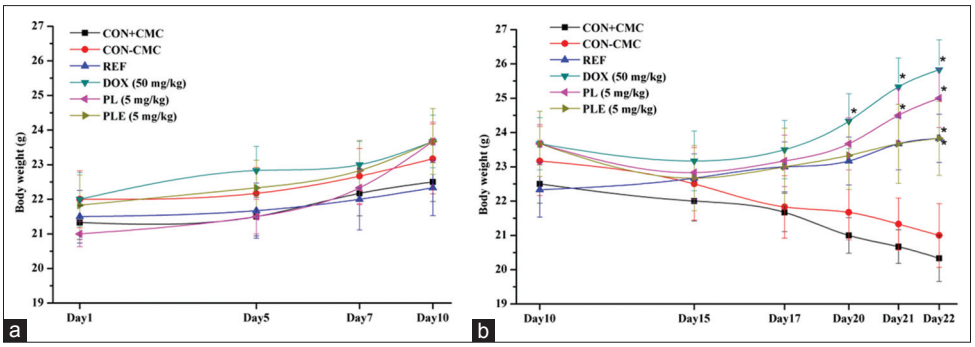

Figure 3: Effect of occasional stress on body weight of male mice treated with piperlongumine and Piper longum fruits extract on day 1-10 (a) and day 10-22 (b) of experiment. Abbreviations: PL: Piperlongumine, PLE: Piper longum fruits extract, DOX: Doxycycline, CMC: Carboxymethyl cellulose suspension and REF: Reference group. Values are mean ± standard error of mean (n = 6). * denotes statistically significant difference (two-way Analysis of Variance followed by Bonferroni post-hoc test) relative to CON + CMC group (*P < 0.05).

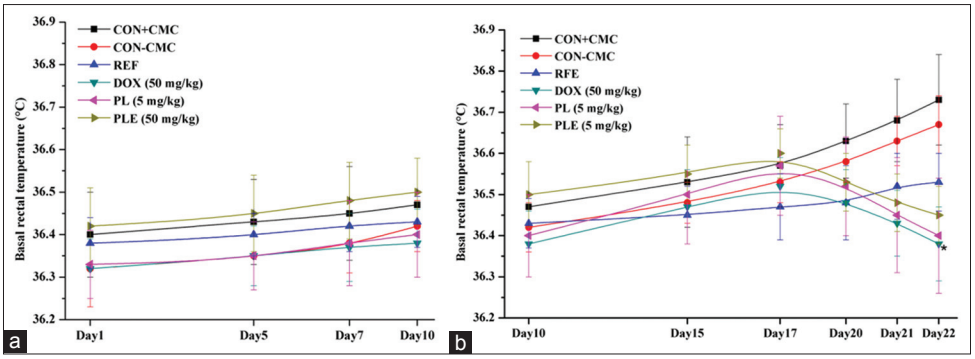

Figure 4: Effect of occasional stress on basal rectal temperatures of male mice treated with piperlongumine and Piper longum fruits extract on day 1-10 (a) and day 10-22 (b) of experiment. Values are mean ± standard error of mean (n = 6). *denotes statistically significant difference (two-way Analysis of Variance followed by Bonferroni post-hoc test) relative to CON + carboxymethyl cellulose group (*P < 0.05).

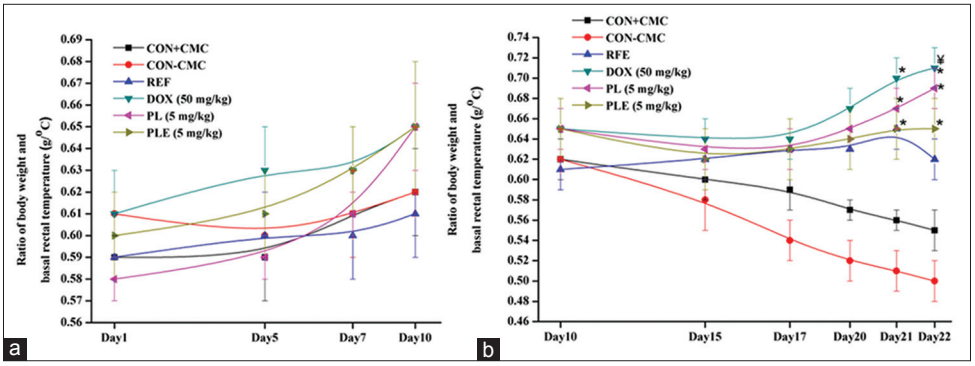

Figure 5: Effect of stress on ratio of body weight and basal rectal temperature of male mice treated with and Piper longum fruits extract on day 1-10 (a) and day 10-22 (b) of experiment. Values are mean ± standard error of mean (n = 6). *denotes statistically significant difference (two-way ANOVA followed by Bonferroni post-hoc test) relative to CON + carboxymethyl cellulose group (*P < 0.05). ¥denotes statistically significant difference (two-way ANOVA followed by Bonferroni post-hoc test) relative to reference (¥P < 0.05)

the control group, whereupon the decrease rate of the CMC treated one was less steeper than the other one not receiving any oral treatments. This ratio of the DOX or PLE treated groups decreased somewhat or remained almost constant till 17th day of the experiment. There after they continued to increase until the last day of the experiment. This slightly elevated energy balance toward higher growth rates observed in the DOX treated group during the 10 treatment days was quite analogous to those of the PLE or PL treated groups, and the protective effects of DOX against stress triggered alterations in growth rate were also somewhat higher than those of PL or PLE.

Foot Shock Stress Induced Transient Hyperthermia

The magnitude of transient hyperthermic response in the REF group observed on day 10th (i.e. the last treatment day) and subsequent observational days remained almost constant and within normal physiological range. It is apparent from the

Figure 6 that there was constant elevation in stress induced hyperthermic response in both the CON + CMC and CON-CMC groups, whereas the magnitude of this response in the groups treated with PL, PLE, or DOX tended to decrease on the 15th and subsequent observational days. Quantitatively, these preventive effects of ten 5 mg/kg/day PLE or PL oral doses were almost identical to that of similar treatments with 50th mg/kg/day DOX.

Tail Suspension Test

Mean immobility period of both the control groups (CON + CMC and CON-CMC)were almost identical and higher than that of the reference group not subjected to foot shock stress. These mean values of the PLE, PL, or DOX treated groups were statistically significantly lower than that of the reference group, whereupon that of the DOX treated one was the lowest. Although the mean value of the PLE pretreated group was numerically slightly higher than the PL pretreated one, these two values were not statistically significantly different from each other. The results are summarized in Figure 7.

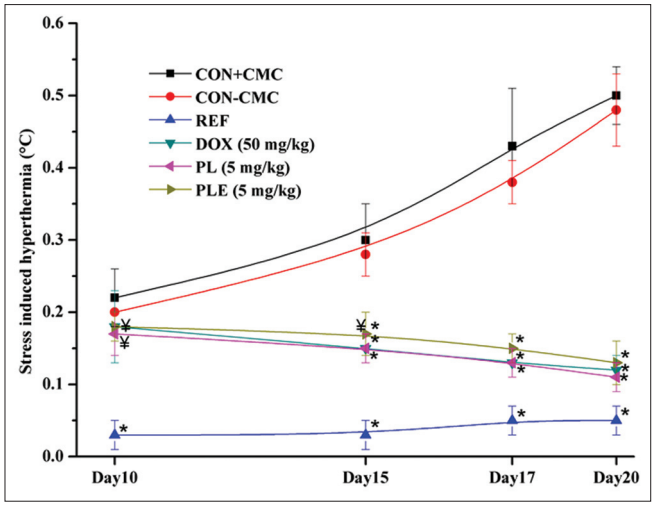

Figure 6: Stress induced hyperthermia of male mice treated with piperlongumine and Piper longum fruits extract. Values are mean ± standard error of mean (n = 6). *denotes statistically significant difference (two-way Analysis of Variance [ANOVA] followed by Bonferroni post-hoc test) relative to CON + carboxymethyl cellulose group (*P < 0.05). ¥denotes statistically significant difference (two-way ANOVA followed by Bonferroni post-hoc test) relative to Reference (¥P < 0.05)

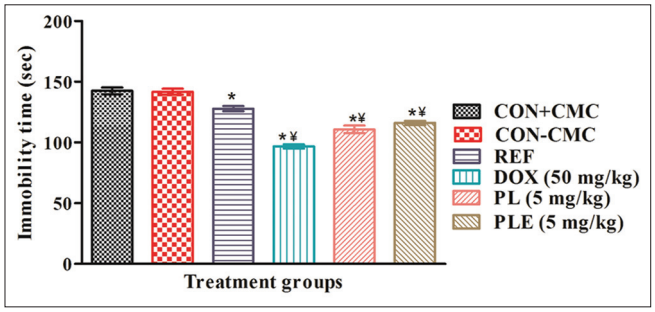

Figure 7: Effect of piperlongumine and Piper longum fruits extract on tail suspension test in male mice. Abbreviations: Values are mean ± standard error of mean (n = 6). * denotes statistically significant difference (One-way Analysis of Variance [ANOVA] followed by Student's t-test) relative to CON + carboxymethyl cellulose group (*P < 0.05). ¥denotes statistically significant difference (One-way ANOVA followed by Student's t-test) relative to REF (¥P < 0.05)

Plasma Glucose, Insulin, and Cortisol Levels

Mean plasma glucose and cortisol levels of both the control groups were significantly higher than those of the reference group [Table 1]. These values of the PLE, PL, or DOX treated ones were significantly lower than that of the CMC treated stressed control group, but were also higher than those of the unstressed reference group. Although mean plasma insulin levels of the reference and the three drugs treated groups were higher than both the control groups, there were no statistically significant differences between these and the mean values of the control groups.

Organ Weights and Gastric Ulcers

These results are summarized in Table 2. As compared to the corresponding mean values of the reference group, the absolute as well as the relative mean weights of the adrenal glands of the both the control groups were significantly higher, whereas those of the spleen significantly lower [Table 2]. Such adrenal gland hypertrophy and spleen hypotrophy observed in control groups were less pronounced in all drugs treated groups. Gastric ulcers observed in both the stressed control groups were not observed in the reference or the DOX pretreated groups, and the mean ulcer index of the PL or PLE treated groups were much lower than the CMC treated control group (ca. 90% protection).

DISCUSSION

The bioassay procedure used in the experiment is a slightly modified version of the one now often used in our laboratories for estimating pharmacologically interesting doses ranges of stress response suppressing herbal extracts and their bioactive constituents [23-25]. Using this and analogous bioassays in our laboratories and elsewhere, it has often been observed that pharmacological observations made after acute doses of plant

Table 1: Effect of PL and PLE on plasma glucose level in male mice plasma cortisol level and plasma insulin level in male mice

Treatment groups	Glucose	Insulin	Cortisol
CON+CMC	113.08±2.07¥	10.82±2.56	105.09±2.36¥
CON-CMC	110.88±0.93¥	9.52±2.28	104.00±3.24¥
REF	85.68±2.04*	16.95±1.68	83.49±1.76*
DOX (50 mg/kg)	92.41±2.01*¥	12.78±1.63	94.95±1.84*¥
PL (5 mg/kg)	97.74±1.57*¥	14.03±1.25	102.70±1.66¥
PLE (5 mg/kg)	103.24±1.61*¥	16.21±1.34	102.77±1.91¥

Values are mean±SEM (n=6). *Denotes statistically significant difference (One-way ANOVA followed by Student's t-test) relative to CON+CMC group (*P<0.05). ¥denotes statistically significant difference (One-way ANOVA followed by Student's t-test) relative to REF group (¥P<0.05), REF: Reference, ANOVA: Analysis of Variance, CMC: Carboxymethyl cellulose, SEM: Standard error of mean, PLE: *Piper longum* fruits extract, PL: Piperlongumine, DOX: Doxycycline

Table 2: Effect of PL and PLE on the weights of adrenal glands, spleen and gastric ulceration index in mice

Treatment groups	Absolute organ weight (mg)		Relative organ weight (mg/g of body weight)		Mean ulcer index	% inhibition
	Adrenal glands	Spleen	Adrenal glands	Spleen		
CON+CMC	23.67±0.99$^{¥}$	64.17±1.17$^{¥}$	1.16±0.03$^{¥}$	3.16±0.67$^{¥}$	2.33±0.21$^{¥}$	-
CON-CMC	22.17±0.70$^{¥}$	62.67±0.92$^{¥}$	1.06±0.04$^{¥}$	2.98±0.25$^{¥}$	2.50±0.22$^{¥}$	-
REF	13.83±0.48*	143.33±1.14*	0.58±0.05*	6.01±0.62*	0.00±0.00*	100
DOX (50 mg/kg)	15.50±0.43*	144.50±1.34*	0.60±0.03*	5.59±0.34*	0.00±0.00*	100
PL (5 mg/kg)	17.33±0.42*$^{¥}$	125.17±1.01*$^{¥}$	0.69±0.04*	5.01±0.46*	0.17±0.11*	92.7
PLE (5 mg/kg)	19.17±0.31*$^{¥}$	112.00±1.29*$^{¥}$	0.80±0.03*$^{¥}$	4.70±0.16	0.25±0.11*	89.27

Values are mean±SEM (n=6). *Denotes statistically significant difference (One-way ANOVA followed by Student's t-test) relative to CON+CMC group (*P<0.05). $^{¥}$denotes statistically significant difference (One-way ANOVA followed by Student's t-test) relative to REF group ($^{¥}P$<0.05), REF: Reference, ANOVA: Analysis of Variance, CMC: Carboxymethyl cellulose, SEM: Standard error of mean, PLE: *Piper longum* fruits extract, PL: Piperlongumine, DOX: Doxycycline

extracts and their bioactive constituents are not very predictive of their medicinal values traditionally known to the scholars and practitioners of traditionally known systems of medicine [26,27]. However, the therapeutically important question concerning their treatment regimen and durations of actions still remains unanswered. Since like aspirin and numerous other covalently binding drugs [28], PL, piperine and other α, β-unsaturated alkyl amides bind covalently to their biological targets [29], we speculated that durations of actions of PLE and pure PL should be longer than predictable from their biological half-lives. However, the results of the reported experiment revealed that 1 h after their 10 daily oral doses neither of them had any significant effects in the stress induced hyperthermia test and also had no significant effects on body weight gains or on basal core temperatures of the animals during the treatments. However, several days after pretreatments, both of them afforded protections against body weights losses and slight elevation in basal core temperature triggered by repeated exposures to foot shock stress, as well as stress induced transient hyperthermia. These observations strongly suggest that both PLE and PL are DOX like very long acting stress resistance inducers with growth promoting effects in stressed animals only. Although these observations could also indicate that PL is somewhat more effective than PLE as growth promoter or stress response desensitizers, further dose response studies with PL, PLE and other types of PLE will be necessary to reconfirm this possibility.

It was interesting to note though that in the tail suspension test for antidepressants conducted 11 days after the pretreatments both PLE and PL had DOX such as effects in stressed mice, and that protective effects of their 10 fairly low doses (5 mg/kg/day)against stress triggered adrenal gland hypertrophy, spleen hypotrophy, gastric ulcers, as well as plasma glucose, and cortisol levels persisted 12 days after their last oral dose. These observations strongly suggest that their observed stress resistance promoting effects are most probably due to their very longer lasting effects on glucose and cortisol homeostasis, and that like DOX both of them are desensitizers of stress triggered physiological responses regulating not only body weights and core temperatures, but also the functions of the central nervous system involved in thermoregulation and depressive state of male mice.

Stress affects food intake in a bidirectional way in both animals and human, and depending on stress intensity and environmental factors stress triggered responses can induce body weight changes accompanying metabolic disorders and co-morbid mental health problems [30-33]. Abnormal body weight gains or losses are the most apparent symptoms of mal- or over-nutrition triggered health problems, and abnormal thermoregulation is a common symptom of almost all systemic inflammatory diseases [34]. Medicinal uses of *P. longum* fruits and roots for prevention and cure of such diseases have since long been known to the scholars and practitioners of traditionally known systems of medicine and for such purposes regular intake of their relatively low oral doses are recommended. Our observations not only justify such medicinal uses of the plant, but also strongly suggest that traditionally known medicinal uses of numerous plant derived products enriched in PL and structurally analogous alkyl amides is mainly due to their ability to promote resistance against chronic unavoidable stress.

CONCLUSION

Appropriate uses of the stress biomarkers quantified in this study are easily quantifiable ones not only for estimating pharmacologically interesting dose ranges of adaptogenic herbs and their bioactive constituents, but also for estimating their durations of actions. PL is another such bioactive secondary plant metabolite of the Piperaceae family.

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Original Research

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Polyphenol-rich extract of *Vernonia amygdalina* (Del.) leaves ameliorated cadmium-induced alterations in feeding pattern and urine volume of male Wistar rats

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ABSTRACT

Aim: To determine the effects of polyphenol-rich extract of the leaves of *Vernonia amygdalina* (PEVA) on the feeding pattern of rats that were exposed to cadmium (Cd) toxicity. **Materials and Methods:** Thirty male Wistar rats, weighing 160-180 g, were divided into 6 groups of 5 rats each as follows; Group 1 received distilled water orally (0.2 ml/100 g), daily, throughout the period of study. Group 2 received Cd alone (in the form of CdSO4) at 5 mg/kg/day via intraperitoneal route for 5 consecutive days. Group 3 were pre-treated with Cd as Group 2 and thereafter left untreated for a period of 4-week. After the oral lethal dose of PEVA was determined, Groups 4, 5, and 6 were pre-treated with Cd as Group 2 after which they received graded doses of PEVA at 100, 200 and 400 mg/kg/day (0.2 ml/100 g), respectively via oral route for 4 weeks. Blood samples were collected for some plasma biochemical assays while urine samples were collected using metabolic cages. **Results:** PEVA administration significantly increased ($P < 0.05$) the body weight and feeding patterns that were significantly reduced ($P < 0.05$) by Cd toxicity. PEVA also significantly reinstated the plasma antioxidant status, as well as glucose and urine volume of the rats toward control values ($P < 0.05$). **Conclusion:** PEVA can be an herbal alternative in the treatment or management of subjects manifesting alterations in feeding pattern and urine volume that is Cd-induced.

KEY WORDS: Cadmium, feeding pattern, oral lethal dose, rats, polyphenol-rich extract of *Vernonia amygdalina*

INTRODUCTION

Cadmium (Cd) is a heavy metal that has found its relevance in various industries. It remains a source of both occupational and environmental hazard, especially in underdeveloped and developing countries where the containment of its emission is inadequate. It is readily absorbed by the body via oral route or inhalation [1]. However, it bioaccumulate once it is cleared from the blood after its absorption. This is regardless of the route of exposure. Hence, it is reputed to be a cumulative toxin [1,2]. Its ability to readily bioaccumulate in the food chain makes food consumption the main source of its exposure. This is true in the most non-smoking population [3,4].

This metal is poorly excreted from the body because it cannot be metabolically degraded to less toxic species [5]. This results in the generation of reactive oxygen species (ROS), which is known to be produced in direct proportion to the body's inability to produce metallothionein (a Cd carrier-protein) [2,6-8]. Consequently, Cd induces a deleterious alteration in the

functionality of biological systems. Therefore, scientific interest is stimulated toward studying its biohazardous effects and possible ways of ameliorating and or preventing its toxic effects.

Polyphenols are potent naturally occurring antioxidants of dietary sources, e.g., vegetables, cereals and dry legumes [9,10]. Although antioxidant vitamins, minerals and carotenoids were the most studied antioxidants by nutritionists, the health benefits of dietary polyphenols have become an interesting area of scientific exploration in recent times due to their health-promoting and chronic degenerative disease-preventing potentials [11]. It is not unlikely that the biological effects of polyphenols may extend well beyond the modulation of oxidative stress [12]. Vernonia amygdalina is reputed for its many medicinal benefits some of which are anti-inflammatory, antimicrobial and immune system-strengthening potentials [13] as well as its use in the treatment of several gastrointestinal tract disorders in patients with emesis, dysentery and loss of appetite-induced Ambrosia [14]. Most literature attributes these health benefits to the presence of polyphenols in its extract. Due to dearth of literature on the effects of the polyphenol-rich extract on Cd-induced toxicity; it was of interest to study the effects of the extract on Cd-induced alterations in feeding pattern using rat model.

MATERIALS AND METHODS

Materials

Fresh leaves of *V. amygdalina* were harvested from a garden in Ile-Ife, Osun State, Nigeria and certified by a Taxonomist in the Department of Botany, Obafemi Awolowo University (OAU), Ile-Ife, Osun State, Nigeria.

$CdSO_4$ was purchased from Guangzhou Fischer Chemical Co., Ltd, Guangdong, China. Acetone used for this study was purchased from Crescent Chemical Co., Inc, New York, United States. Metabolic cages used was Ohaus R Model; Ohaus, Pine Brook, New Jersey, USA. Standard Laboratory kit for glucose assay was purchased from Randox Laboratories Limited, United Kingdom.

Extraction of Polyphenols

The procedure for obtaining polyphenol-rich extract of leaves of *V. amygdalina* (PEVA) was carried out using standard protocol and as described by Mutiu *et al.* [15] and Comfort *et al.* [16]; *V. amygdalina* leaves were air-dried and pulverized with an electric pulverizer (DIK-2910, Daiki Rika Kogyo Co. Ltd, Tokyo-Japan). The pulverized leaves were weighed, and the value was recorded. This was further crushed in 80% acetone (1:2 w/v) using a Waring blender (Waring Commercial, Torrington, CT). The sample was homogenized in a Polytron Homogenizer (Glen Mills Inc., Clifton, NJ) for 3 min, and the homogenates were filtered under vacuum using Buchner funnel and Whatman number 2 filter paper (Whatman PLC, Middlesex, UK). The filtrate was concentrated under vacuum using a rotary evaporator (HahnShin Scientific, HS-2005-N) and freeze-dried in a Lyophilizer (Ilshin Lab. Co. Ltd, Seoul, Republic of Korea). The powdered yield that was obtained (PEVA) was weighed and kept in a desiccator until when needed. The percentage (%) yield of PEVA was calculated as shown below;

$$\% \text{ yield of PEVA} = \text{yield of PEVA} \div \text{weight of pulverized leaves} \times 100\% \text{ [17]}$$

The extraction process was repeated for three different samples and the final % yield of PEVA was expressed as mean ± standard error of the mean (SEM) ($n = 3$).

Determination of Total Phenol and Total Flavonoids Content

The total phenol and total flavonoids in the leaf extract were determined using the procedures described below:

The total phenols content of the leaf extract was determined by the method of Singleton and Rossi [18] and as described by Gulcin *et al.* [19] using Folin–Ciocalteu's phenol reagent which is an oxidizing reagent. 0.2 ml of Folin–Ciocalteu's phenol reagent was added to a mixture of 0.1 ml of the sample and 0.9 ml of distilled water (DW). The resulting mixture was voltexed. After 5 min of standing, 1.00 ml of 7 % (w/w) Na_2CO_3 solution was added and thereafter made up to 2.5 ml with DW before incubation for 90 min at room temperature. Using an ultraviolet (UV)-Vis spectrophotometer (Labtronics, India; Model LT-290), the absorbance was read at a wavelength of 750 nm against a negative control containing 1 ml of DW. The gallic acid equivalent (GAE) of the extract was determined using gallic acid at 0.1 mg/ml as a standard, after preparing a calibration curve.

Total flavonoids content of the leaf extract was determined using aluminum chloride colorimetric assay method according to Zhilen *et al.* [20] and as described by Miliauskas *et al.* [21]. Standard quercetin with varying concentrations 0.1, 0.2, 0.3, 0.4 and 0.5 mg/ml was used as standard in comparison to the sample extract. 0.4 ml of DW was added to 0.1 ml of the extract/standard, followed by 0.1 ml of 5% sodium nitrate solution. After 5 min, 0.1 ml of 10% aluminum chloride, and 0.2 ml of sodium hydroxide solutions were added to the resulting mixture after which the volume was made up to 2.5 ml with DW. Against blank, the absorbance, at a wavelength of 510 nm, was read using a UV-Vis spectrophotometer (Labtronics, India; Model LT-290).

The tests to determine the aforementioned phytochemicals were performed in triplicate, and the final results were expressed as mg quercetin/GAE a gram of the leaf extract using the formula below;

$$X = q\ (V/w)$$

X = total content of flavonoids or phenolic compound in quercetin or GAE, respectively; q = concentration of quercetin or gallic acid established from the standard curve; V = volume of the extract (ml); and **w** = weight of the sample extract [19,21].

Determination of Oral Lethal Dose (LD_{50}) of PEVA

The oral LD_{50} of PEVA was determined by a modification of the procedure outlined by Lorke, 1983 [22]. Lorke's method proposes a total of 13 animals; 9 animals for the first phase and 4 animals for the second phase. However, a total of 17 adult Wistar rats were used for this study. In the initial phase of the experiment, 9 rats were divided into 3 groups of 3 rats each and were treated with PEVA at graded doses of 10, 100 and 1000 mg/kg, orally. The rats were observed for 24 h. In the second phase, 8 rats were divided into 4 groups of 2 rats each and were treated with PEVA at 750, 1500, 3000, and 6000 mg/kg, orally. They were also examined for 24 h, and the LD_{50} was determined using the formula;

$$LD_{50} = \sqrt{a \times b}$$

Where, a= least dose that killed a rat; and b = highest dose that did not kill any rat.

Solutions of PEVA and Cd Salt

The choice of therapeutic doses of PEVA was guided by the predetermined oral LD_{50} of PEVA; these were taken to be s10% of oral LD_{50}. Thus, doses of 100, 200 and 400 mg/kg of PEVA were prepared as follows; 1 g of PEVA was dissolved in 20 ml of DW to prepare a stock solution of 100 mg/kg of PEVA. Stock solutions of 200 and 400 mg of PEVA were prepared by each dissolving 2 g and 4 g of PEVA in 20 ml of DW, respectively. The rats received 0.2 ml/100 g of PEVA, orally. Samples were stored in a deep-freezer after use while fresh samples were prepared every 48 h.

A 50 mg of Cd sulfate salt was dissolved in 20 ml of DW and was administered to the rats at 0.2 ml/100 g. Therefore, each rat received 5 mg/kg/day of Cd solution for 5 consecutive days, via intraperitoneal route (i.p.).

Animal Management and Experimental Design

A total of 30 male Wistar rats, weighing 160-180 g, were used in this study. They were purchased from the Animal Holdings of the College of Health Sciences, OAU, Ile-Ife, Osun State, Nigeria where the study was carried out. Each rat was housed in a separate metabolic cage (to assess their food consumption, water intake, and urine volume) under natural light/dark cycle and allowed to have access to standard laboratory rat chow (Caps Feed PLC Osogbo, Nigeria) and water *ad libitum*. The rats were allowed to acclimatize in the metabolic cage for 2 weeks before the commencement of this study, to allow for adaptation to life in a metabolic cage. All experimental protocols were in strict compliance with the guidelines for animal research, as detailed in the NIH Guidelines for the Care and Use of Laboratory Animals (National Academy of Sciences and National Institutes of Health Publications, 2011) and approved by local Institutional Research Committee.

The rats were divided into six groups of 5 rats each as follows; Group 1 (Control group) received DW orally (0.2 ml per 100 g rats), daily, throughout the course of the study (4 weeks). Group 2 (toxic control) received Cd alone at 5 mg/kg/day via intraperitoneal route for 5 consecutive days. Group 3 (toxic recovery group) were pre-treated with Cd as Group 2 and thereafter left untreated for a period of 4-week. Groups 4, 5, and 6 were also pre-treated with Cd as Group 2 and, thereafter, received graded doses of PEVA at 100, 200 and 400 mg/kg/day, respectively, via oral route for a period of 4-consecutive weeks. 24 h after last administration of Cd (in Group 2), PEVA (in Groups 4, 5, and 6) and after the recovery period (in Group 3), rats were euthanized, and blood samples were collected by cardiac puncture into separate ethylenediaminetetraacetic acid bottles. These were centrifuged at 4000 rpm for 15 min at –4°C, using cold centrifuge (Centurium Scientific, Model 8881). Plasma obtained was collected into separate plain bottles for the assessment of biochemical assays such as activities of thiobarbituric acid reactive substances (TBARS), levels of reduced glutathione (GSH), as well as glucose determination in both plasma and urine of the rats. The experimental design is as depicted in Table 1.

Measurement of Body Weight

Weekly body weight of the rats was determined with the aid of a digital weighing balance (Hanson, China) to assess weekly weight gain or loss.

Measurement of Food Consumption, Water Intake, and Urine Volume

With the aid of metabolic cages, the food consumption, water intake and urine volume for each rat in the groups were determined. Water intake and urine volumes were measured with the aid of a measuring cylinder (Volac, Great Britain) while the food consumption was measured with the aid of a digital weighing balance (Hanson, China). Urine volumes were read off directly with the aid of the measuring cylinder while both water intake and food consumptions were measured by subtracting the final amount (of food or water) obtained from the initial amount that was measured a day before. The value obtained was taken to be the amount consumed by each rat.

Table 1: Experimental design

Groups	5 days	Week 1	Week 2	Week 3	Week 4
Group 1	DW	DW	DW	DW	DW*
Group 2	Cd*				
Group 3	Cd	RP	RP	RP	RP*
Group 4	Cd	100 P	100 P	100 P	100 P*
Group 5	Cd	200 P	200 P	200 P	200 P*
Group 6	Cd	400 P	400 P	400 P	400 P*

DW: Distilled water, Cd: Cadmium (5 mg/kg bw), RP: Recovery period, 100 P: 100 mg/kg PEVA, 200 P: 200 mg/kg PEVA, 400 P: 400 mg/kg PEVA, Group 1: Control, Group 2: Cd, Group 3: Cd+recovery, Group 4: Cd+100 mg/kg PEVA, Group 5: Cd+200 mg/kg PEVA, Group 6: Cd+400 mg/kg PEVA, *point at which rats were euthanized, PEVA: Polyphenol-rich extract of the leaves of *Vernonia amygdalina*

Biochemical Assay

Both plasma and urine glucose levels were estimated using standard laboratory protocols, as provided by Randox Laboratories Limited, United Kingdom.

Non-enzymatic Antioxidant Assay

GSH levels were measured by the method of Beutler and Kelly [23]. 1 ml of plasma was added to 0.5 ml of Ellman's reagent (10 mM). 2 ml of phosphate buffer (0.2 M, pH 8.0) was, thereafter, added. The yellow color developed was read at 412 nm against blank containing 3.5 ml of phosphate buffer. A series of standards were also treated similarly, and the amount of GSH was expressed in μg/mg tissue.

Lipid Peroxidation Assay

TBARS levels were determined by the method of Ohkawa *et al.*, 1979 [24]. To each 0.5 ml of plasma was added 0.5 ml of phosphate buffer (0.1 M, pH 8.0) and 0.5 ml of 24% tricyclic antidepressant. The resulting mixture was incubated at room temperature for 10 min, followed by centrifugation at 2000 rpm for 20 min. To 1 ml of resulting supernatant was added 0.25 ml of 0.33% TBA in 20% acetic acid and the resulting mixture was boiled at 95°C for 1 h. The resulting pink color product was cooled, and absorbance was read at 532 nm (extinction coefficient of TBARS; $\varepsilon 532 = 1.53 \times 105\ M^{-1}\ cm^{-1}$).

Statistical Analysis

The results obtained were collated and expressed as mean ± SEM and subjected to one-way Analysis of Variance. The data were further subjected to a *post-hoc* test using Student Neumann Keuls' method, and differences with probability values of $P < 0.05$ were considered statistically significant. The statistical analysis was carried out with the aid of GraphPad Prism 5.03 (GraphPad Software Inc., CA, USA) and Microsoft Office Excel, 2007 package.

RESULTS

Percentage (%) Yield of PEVA

The result obtained showed that 300 g of air-dried and pulverized leaves of *V. amygdalina* produced a percentage (%) yield of 8.74 ± 0.40 of PEVA [Table 2].

Total Phenolics and Total Flavonoids Content of the Leaf Extract

These were determined to be 681.70 ± 4.70 mg of GAE/g of the leaf extract and 23.70 ± 1.80 mg of quercetin equivalent/g of the leaf extract [Table 3].

Acute Oral Toxicity Test (LD_{50}) of PEVA

The oral LD_{50} of PEVA was determined to be ≥ 4242.64 mg/kg body weight in adult Wistar rats [Table 4].

Table 2: Percentage yield of PEVA

Extraction process	Weight of air-dried and pulverized leaves (g)	Yield of PEVA (g)	Percentage yield
1st	300	24.15	8.05
2nd	300	28.27	9.42
3rd	300	26.23	8.74

The % yield of PEVA that was obtained=8.74±0.40% (n=3), PEVA: Polyphenol-rich extract of the leaves of *Vernonia amygdalina*

Table 3: Total phenol and total flavonoids content of the leaf extract

Total phenol content (mg of GAE/g of the leaf extract)	Total flavonoids content (mg of quercetin equivalent/g of the leaf extract)
681.70±47.36	23.70±1.78

The tests for the stated phytochemicals were performed in triplicates (n=3), GAE: Gallic acid equivalent

Table 4: Acute oral toxicity test (LD_{50}) of PEVA

No. of rats	Dose (mg/kg)	Mortality
1st phase		
3	10	0/3
3	100	0/3
3	1000	0/3
2nd phase		
2	750	0/2
2	1500	0/2
2	3000	0/2
2	6000	2/2

LD_{50} of PEVA=($\sqrt{6000 \times 3000}$) mg/kg=($\sqrt{18000000}$) mg/kg= 4242.64 mg/kg body weight. Therefore, LD_{50} of PEVA≥4242.64 mg/kg body weight in adult Wistar rats, PEVA: Polyphenol-rich extract of the leaves of *Vernonia amygdalina*, LD_{50}: Lethal dose

Water Intake and Urine Volume (ml)

During 5 days of Cd intoxication, the experimental groups recorded a significant decrease ($P < 0.05$) in water intake when compared with their respective baseline and control group [Figure 1]. At week 1 of the study, there was a significant decrease in water intake in the Cd + recovery group (–2.90 ± 0.58 ml) when compared with the groups that were treated with graded doses of PEVA (Group 4 = 8.00 ± 0.07 ml; Group 5 = 5.60 ± 0.80 ml; Group 6 = 7.90 ± 0.12 ml), with reference to the level of alteration that was recorded after Cd toxicity. The Cd + recovery group recorded water intake of over five-fold lower than groups that were treated with PEVA. However, with reference to the alteration that was recorded from baseline levels, the PEVA treated groups recorded significant increase in water intake (Group 4 = –0.40 ± 0.07; Group 5 = –0.20 ± 0.03; Group 6 = –5.0 ± 0.4) when compared with the Cd + recovery group (–6.8 ± 0.10) ($P > 0.05$) at week 4 post-Cd intoxication.

There was a non-corresponding and significant increase ($P < 0.05$) in the urine volume of rats during the period of Cd intoxication in the experimental groups when compared with the control group and the respective baselines [Figure 2]. There was a significant increase in the urine volume of rats in the Cd + recovery group (1.67 ± 0.12 ml) when compared

with the PEVA-treated groups (Group 4 = 0.7 ± 0.14 ml; Group 5 = –0.81 ± 0.14 ml; Group 6 = 0.8 ± 0.13 ml) over the 4-week study period. At weeks 1 and 4 post-Cd toxicity, PEVA treated groups showed significant degree ($P < 0.05$) of reversal in the alterations in urine volume of the rats when compared with the Cd + recovery group [Figure 2].

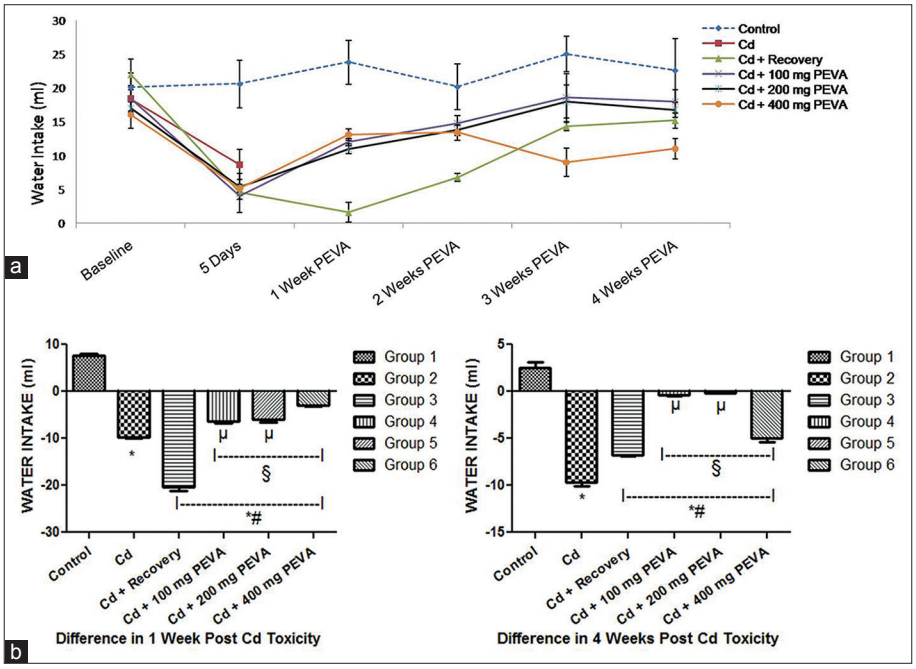

Figure 1: (a) Effect of polyphenol-rich extract of the leaves of *Vernonia amygdalina* (PEVA) on the water intake of rats with Cd-induced toxicity. (b) Differences in the water intake (ml) of control and PEVA-treated groups, during weeks 1 and 4 post-Cd toxicity with reference to baseline values. Each value represents mean ± standard error of mean ($n = 5$); *significantly different from Control Group ($P < 0.05$); #significantly different from Cd group; §significantly different from Cd + recovery group ($P < 0.05$); µ: significantly different from Cd + 400 mg PEVA group ($P < 0.05$)

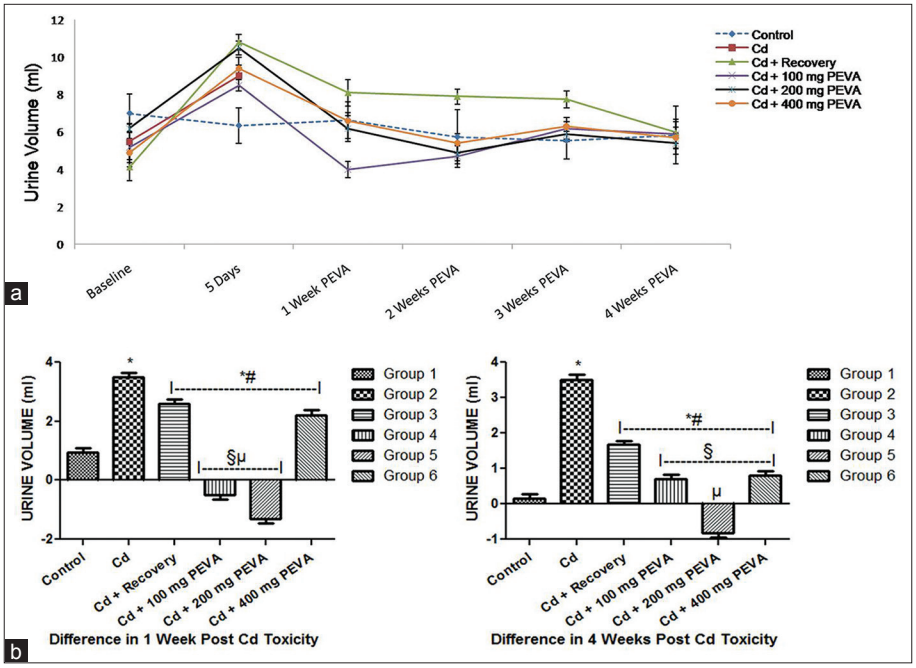

Figure 2: (a) Effect of polyphenol-rich extract of the leaves of *Vernonia amygdalina* (PEVA) on the urine volume of rats with Cd-induced toxicity. (b) Differences in the urine volume (ml) of control and PEVA-treated groups, during weeks 1 and 4 post-Cd toxicity with reference to baseline values. Each value represents mean ± standard error of mean ($n = 5$); *significantly different from Control Group ($P < 0.05$); #significantly different from Cd group; §significantly different from Cd + recovery group ($P < 0.05$); µ: significantly different from Cd + 400 mg PEVA group ($P < 0.05$)

Food Consumption and Body Weight (g)

There was a significant decrease ($P < 0.05$) in food consumption (about –67.72 ± 3.76%) in the Cd-treated groups during the period of exposure to Cd toxicity when compared with the control group and their respective baselines [Figure 3]. This was marked by a corresponding and significant decrease in body weight (about –19.55 ± 0.83%) ($P < 0.05$) in the Cd-treated groups when compared with their respective baselines at day 5 of the study [Figure 4]. PEVA administration was found to

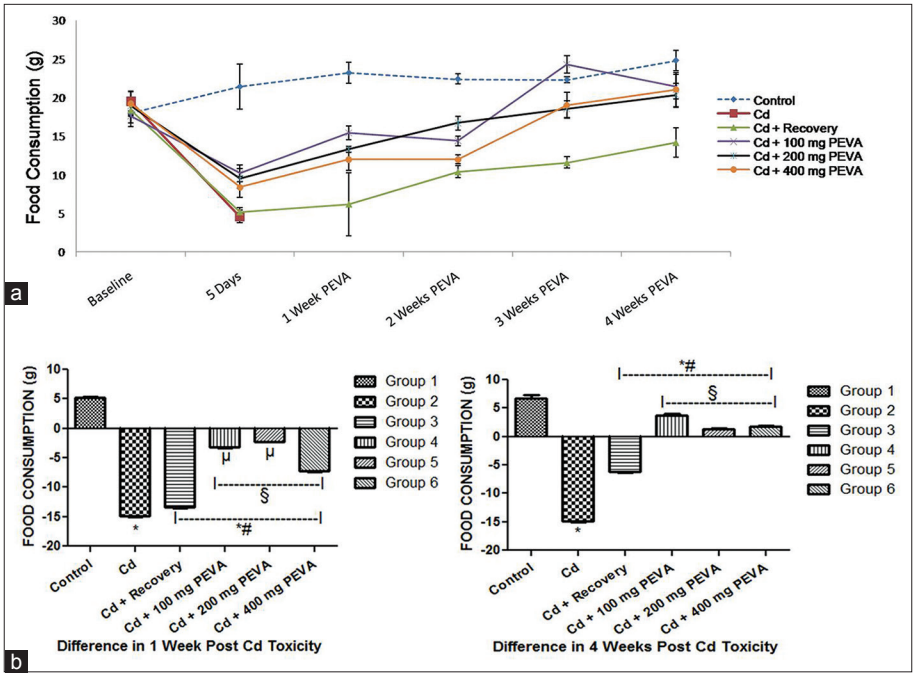

Figure 3: (a) Effect of polyphenol-rich extract of the leaves of *Vernonia amygdalina* (PEVA) on the food consumption of rats with Cd-induced toxicity. (b) Differences in the food consumption (g) of control and PEVA-treated groups, during weeks 1 and 4 post-Cd toxicity with reference to baseline values. Each value represents mean ± standard error of mean ($n = 5$); *significantly different from control group ($P < 0.05$); #significantly different from Cd group; §significantly different from Cd + recovery group ($P < 0.05$); µ: significantly different from Cd + 400 mg PEVA group ($P < 0.05$)

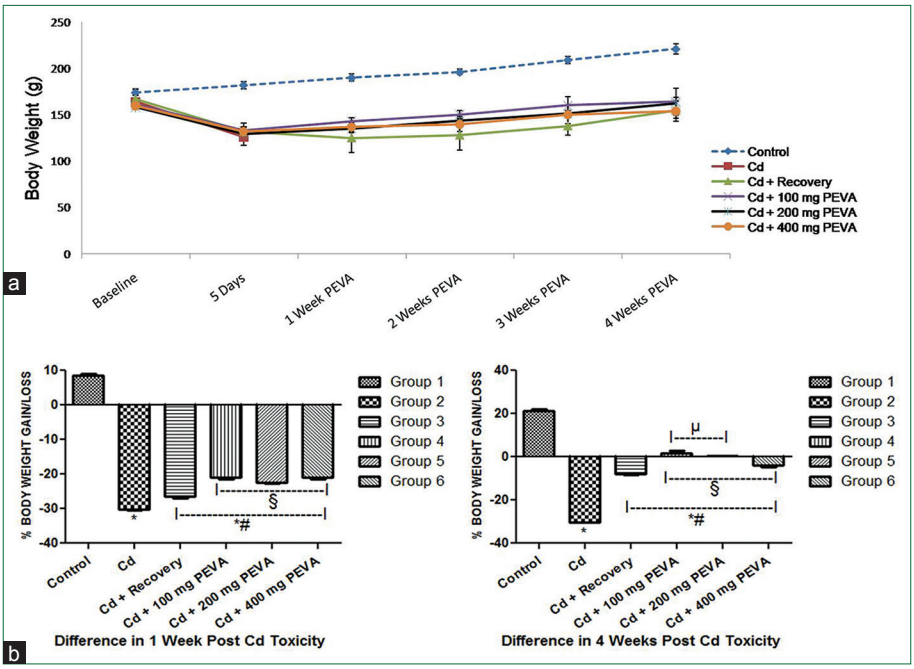

Figure 4: (a) Effect of polyphenol-rich extract of the leaves of *Vernonia amygdalina* (PEVA) on the body weight of rats with Cd-induced toxicity. (b) Differences in the % body weight gain/loss of control and PEVA-treated groups, during weeks 1 and 4 post-Cd toxicity with reference to baseline values. Each value represents mean ± standard error of mean ($n = 5$); *significantly different from control group ($P < 0.05$); #significantly different from Cd group; §significantly different from Cd + recovery group ($P < 0.05$); µ: significantly different from Cd + 400 mg PEVA group ($P < 0.05$)

correct these Cd-induced aberrations toward baseline values in the order 200 mg > 100 mg > 400 mg per kg body weight over the 4-week study period.

PEVA treated groups recorded a significant increase ($P < 0.05$) in total food consumption (Group 4 = 3.80 ± 0.17; Group 5 = 1.40 ± 0.15; Group 6 = 1.80 ± 0.16) when compared with Cd group (–14.90 ± 0.15) and Cd + recovery group (–6.20 ± 0.13). Furthermore, there was a significant increase in total food consumption in Cd + recovery group (–7.74 ± 0.60) when compared with Cd group (–30.15 ± 0.20). However, the experimental groups recorded a significant decrease in total food consumption when compared with the control group (6.80 ± 0.60) [Figure 3].

PEVA treated groups recorded a significant increase ($P < 0.05$) in the % body weight gain of the rats (Group 4 = 1.83 ± 0.90; Group 5 = 0.40 ± 0.07; Group 6 = –3.89 ± 0.70) when compared with Cd group (–30.15 ± 0.20) and Cd + recovery group (–7.74 ± 0.60) at week 4 post-Cd toxicity [Figure 4]. Furthermore, there was significant increase in the % weight gain that was recorded in the Cd + recovery group (–6.20 ± 0.13) when compared with Cd group (–14.90 ± 0.15) during the same period. However, the rats in the experimental groups recorded a significant decrease in total food consumption at the end of the study period when compared with the control group (21.27 ± 0.60).

Plasma and Urine Glucose (mg/dl)

Plasma glucose level in Cd group (311.87 ± 1.36) was significantly increased ($P < 0.05$) when compared with that of the control group (115.31 ± 1.86). Although Cd + recovery group recorded a significant decrease (219.65 ± 2.85) when compared with Cd group (311.87 ± 1.36), this was found to be significantly higher than that of the control group (115.31 ± 1.86). The PEVA-treated groups (Group 4 = 110.10 ± 2.02; Group 5 = 101.28 ± 2.09; Group 6 = 100.97 ± 2.08) recorded significant a significant decrease in plasma levels of glucose when compared with the Cd + recovery group at the end of the study period [Table 5].

Significant increase in urine glucose level was recorded in Cd group (38.19 ± 1.39) when compared with the control group (20.10 ± 1.21) ($P < 0.05$). Cd + recovery group (27.96 ± 1.22) recorded no significant difference when compared with the control group (20.10 ± 1.21). The data obtained showed significant increase in urine glucose level in the Cd + recovery group (27.96 ± 1.22) when compared with the groups that received graded doses of PEVA (Group 4 = 21.93 ± 1.13); Group 5 = 21.48 ± 1.19; Group 6 = 19.77 ± 1.15) at the end of the study period. There was no significant difference ($P > 0.05$) between the control group and groups that received graded doses of PEVA [Table 5].

Non-enzymatic Antioxidant Status (GSH) (μg/mg Protein)

There was a significant decrease ($P < 0.05$) in plasma GSH levels in Cd group (0.49 ± 0.08) when compared with the control group (1.61 ± 0.11). Cd + recovery group (1.18 ± 0.07) recorded significant increase in plasma GSH level when compared with Cd group. Also, a significant increase in plasma GSH level was recorded in the PEVA-treated groups (Group 4 = 1.58 ± 0.02; Group 5 = 1.64 ± 0.09; Group 6 = 1.56 ± 0.02) when compared with Cd + recovery group (1.18 ± 0.07). There was no significant difference ($P > 0.05$) in the plasma GSH level of groups 4 to 6 when compared with the control group (1.61 ± 0.11) [Table 6].

Measurement of Lipid Peroxidation (TBARS) (nmol/mg Protein)

The plasma TBARS level was significantly increased ($P < 0.05$) by Cd toxicity. Cd group (67.20 ± 3.38) recorded significant increase in plasma TBARS level when compared with the control group (22.53 ± 1.09). Cd + recovery group (39.28 ± 2.76) recorded a significant decrease in plasma TBARS level when compared with Cd group, and significant increase when compared with the control group (22.53 ± 1.09). There was no significant difference ($P > 0.05$) in plasma TBARS level of the PEVA-treated groups (Group 4 = 28.53 ± 1.93; Group 5 = 25.13 ± 1.30; Group 6 = 30.16 ± 1.29) when compared with the control group [Table 6].

DISCUSSION

The study demonstrated that Cd toxicity induced significant deleterious alteration in the feeding patterns, urine volume as

Table 5: Changes in plasma and urine glucose level in rats exposed to Cd toxicity

Groups	Plasma glucose (mg/dl)	Urine glucose (mg/dl)
Control	115.31±1.86	20.10±1.21
Cd	311.87±5.36*	38.19±3.39*
Cd+recovery	219.65±2.85*$^{\delta}$	27.96±2.22*$^{\delta}$
Cd+100 mg/kg PEVA	110.10±2.02$^{\delta\#}$	21.93±1.13$^{\delta}$
Cd+200 mg/kg PEVA	101.28±2.09*$^{\delta\#}$	21.48±1.19$^{\delta}$
Cd+400 mg/kg PEVA	100.97±2.08*$^{\delta\#}$	19.77±1.15$^{\delta\#}$

Each value represents mean±SEM (n=5); *significantly different from control group (P<0.05); $^{\delta}$significantly different from Cd group (P<0.05); $^{\#}$significantly different from toxic recovery group (P<0.05) (P<0.05), SEM: Standard error mean, PEVA: Polyphenol-rich extract of the leaves of Vernonia amygdalina, Cd: Cadmium

Table 6: Changes in plasma levels of reduced GSH and levels of in rats exposed to Cd toxicity

Groups	GSH (μg/mg protein)	TBARS (nmol/mg protein)
Control	1.61±0.11	22.53±1.09
Cd	0.49±0.08*	67.20±3.38*
Cd+recovery	1.18±0.07*$^{\delta}$	39.28±2.76*$^{\delta}$
Cd+100 mg/kg PEVA	1.58±0.02$^{\delta\#}$	28.53±1.93$^{\delta\#}$
Cd+200 mg/kg PEVA	1.64±0.09$^{\delta\#}$	25.13±1.30$^{\delta\#}$
Cd+400 mg/kg PEVA	1.56±0.02$^{\delta\#}$	30.16±1.29$^{\delta\#}$

Each value represents mean±SEM (n=5); *significantly different from control group (P<0.05); $^{\delta}$significantly different from Cd group (P<0.05); $^{\#}$significantly different from toxic recovery group (P<0.05) (P<0.05), SEM: Standard error mean, GSH: Glutathione, TBARS: Thiobarbituric acid reactive substances, PEVA: Polyphenol-rich extract of the leaves of *Vernonia amygdalina*, Cd: Cadmium

well as significant disturbance of plasma antioxidant status and glucose homeostasis in a rat model. This is the first report on *in vivo* biological effects of PEVA.

The significant reduction in food consumption and water intake can be attributed to the lethargy that was observed (physical examination) during the period of exposure to Cd toxicity. Furthermore, the desire for food reduces with increasing blood glucose levels [25-27]. It is unknown whether certain intermediary factors or sensing of declining blood glucose by glycostat neurons in the brain is responsible for hunger [28]. The hyperglycemia that was observed suggests that Cd toxicity is associated with reduced body glucose tolerance. This, possibly, could have created a false sensation of satisfaction in the rats; the mechanism of which is subject to further investigation. This observation is similar to the findings of Merali and Singhal [29] on sub-acute Cd treatment in rats. They reported that Cd intoxication potentiates significant disturbance in glucose homeostasis. This was found to be associated with the suppression of insulin release, decrease in hepatic glycogen content and enhancement of hepatic gluconeogenic enzymes, with consequent decrease in glucose tolerance. The decrease in body weight can be attributed to the decrease in food consumption that was observed; since a balance between dietary intake and energy expenditure is the determinant for weight gain or loss [28]. Administration of PEVA for the study period significantly reversed the alteration in the aforementioned indices. The study, therefore, demonstrates the potential of PEVA to (both) increase the body's glucose tolerance and restore body glucose homeostasis.

Solute load and osmolality of medullary interstitium are some basic determinants of urine volume or flow rate [30]. Significantly increased volumes of urine output during Cd toxicity can be attributed to the corresponding hyperglycemia that was recorded. Since glucose is an osmotically active substance [31], there may have been osmotic diuresis during this period. It is evident from the study that one of the mechanisms by which Cd-induced glycosuria was by reducing the body's glucose tolerance with consequent hyperglycemia. Subject to further investigation and verification, other mechanisms could be saturation of glucose transporters (particularly in the kidney) and or reduced sensitivity of these transporters to the available glucose in urine; since targeting the baso-lateral membranes and brush border transporters of the kidneys are characteristic of Cd toxicity [32]. It is noteworthy to state that the Cd-induced hyperglycemia and glycosuria were significantly reversed in the PEVA-treated groups at a dose level of 100 mg/kg when compared with the control group while an increased risk profile of PEVA was observed to be associated with higher doses which recorded a dose-dependent decline in both plasma and urine glucose levels below control values. PEVA administration was found to have significantly ameliorated the Cd-induced glycosuria in a dose-dependent fashion. This further suggests that PEVA administration has a significant effect on body glucose homeostasis.

GSH is a non-enzymatic antioxidant index while TBARS is an index of lipid peroxidation and oxidative stress [33]. The findings on plasma GSH levels and TBARS activities support the reports of Tariq, 2014; Karabulut *et al.*, 2008; Pari and Murugavel, 2005 [34-36]. The significant reduction in GSH levels following Cd intoxication could be attributed to the increased use of GSH (by the body tissues) to mop up ROS that may have been generated following Cd intoxication, possible-decreased tissue production of GSH, and/or direct binding of Cd to the peptide's (GSH) active site. The increased activities of TBARS that were observed in the plasma of rats during Cd toxicity indicated a high degree of lipid peroxidation and oxidative stress. Although indirectly involved in the generation of free radicals, lipid peroxidation is considered a primary mechanism for Cd-induced toxicity [37-40]. The toxic effects of Cd are exerted through oxidative damage to cellular organelles by inducing the generation of ROS [41], which consist mainly of O_2^+, $H_2O_2^-$ and OH^+ [42]. Altered antioxidant system, lipid peroxidation, damage to membrane proteins, alterations to DNA and gene expression as well as apoptosis are some of the reactions of cellular biomolecules to these ROS [41,43]. The ability of Cd to potentiate the generation of free radicals gives a clue to the possibility of ameliorating its toxicity with potent antioxidants. PEVA administration to Cd-intoxicated rats reinstated the GSH levels along with the attenuation of the significantly altered TBARS activities in the plasma. This can be attributed to the ability of PEVA to counteract the aforementioned mechanism of Cd interaction with cellular biomolecules. Hence, the result of this study demonstrated PEVA as a potent antioxidant.

Although there are apparent benefits associated with PEVA administration, a high-risk profile is not unlikely at higher doses, as depicted by some of the indices such as total weight gain/loss as well as both plasma and urine glucose levels at the end of the study period. This could be a pointer to the fact that prolonged administration of high doses can potentiate deleterious health effects. This study recorded increased risk profile of PEVA at a dose level of 400 mg/kg which is associated with duration of administration. There should, therefore, be balance between the choice of therapeutic dose and duration of PEVA administration in order to maximize its possible health benefits.

CONCLUSION

In conclusion, the outcome of the study suggests that PEVA can be an herbal alternative in the treatment or management of subjects manifesting alterations in feeding pattern, glucose homeostasis and urine volume that is Cd-induced. Nevertheless, a high-risk profile at high doses is not unlikely.

To better understand the health benefits of dietary polyphenols, it is important to appreciate a proper classification of their considerable chemical complexity and diversity so that isolated forms can be extensively studied.

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Original Article

ScopeMed

Assessment of an ethanolic seed extract of *Picralima nitida* ([Stapf] Th. and H. Durand) on reproductive hormones and its safety for use

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ABSTRACT

Background: *Picralima nitida* seed extract (PNE) has aphrodisiac and contraceptive effect. **Aim:** To investigate the effect of PNE on reproductive hormones. **Materials and Methods:** The size and length of the combs of white leghorn day-old chicks treated with testosterone (0.5-1.5 mg/kg), cyproterone (3-30 mg/kg), or PNE (50-500 mg/kg) for 7 days, as well as cyproterone (10, and 30 mg/kg) on PNE-induced, and PNE (50-500 mg/kg) on testosterone-induced comb growth, were measured in the chick comb test. The effect of PNE the percentage change in an oviduct-chick weight ratio of Rhode Island Red layer day-old chicks treated with 17-β-estradiol (0.1-0.9 μg), PNE (30-300 mg/kg) or vehicle, for 6 days, was determined in the chick uterotrophic assay. Liver and kidney function was well lipid, and hematological profile tests were conducted to assess safety. **Results:** 7-day treatment with PNE and testosterone increased significantly ($P \leq 0.01$-0.001) while cyproterone significantly decreased ($P \leq 0.001$) comb growth dose-dependently. Qualitatively, testosterone and PNE treatment resulted in relatively brighter red combs. Cyproterone caused significant inhibition ($P \leq 0.001$) of both testosterone and PNE-induced comb growth. Co-administration of testosterone and PNE suppressed comb growth significantly ($P \leq 0.001$). Administration of 17-β estradiol and PNE increased ($P \leq 0.001$) oviduct-chick weight ratio dose-dependently. No significant changes were observed in assessing liver and kidney function, lipid profile, and hematological parameters. **Conclusion:** PNE exhibits both androgenic (partial testosterone agonist) and estrogenic activity. It has no detrimental effects on the blood, liver, and kidney tissue with prolonged use.

KEY WORDS: Androgenic effect, estrogenic activity, libido, partial agonist, testosterone

INTRODUCTION

Reproductive hormones are chemical substances that regulate the reproductive process which include sexual behavior, mating, gametogenesis, embryonic and fetal development, gestation, parturition, and even lactation [1]. Earlier work by author (in the process of being published) on the assessment an ethanolic seed extract of *Picralima nitida* ([Stapf] Th. and H. durand) on reproductive and developmental indices arrived at a conclusion that acute administration of *P. nitida* extract enhances sexual behaviors in both males (aphrodisiac effect) and females possible by affecting reproductive hormones. Its chronic administration in females, however, reduces the chances of fertility, i.e. caused contraception, but had no teratogenic or abortifacient effect during pregnancy. The study also established the fact that in males, enhanced libido associated with an acute intake of the extract diminishes significantly with prolonged usage, with a significant reduction sperm count as well. The study further indicated safety as far as acute usage of the preparation was concerned (no observed adverse effect levels was lower than 1000 mg/kg and lethal dose beyond 2000 mg/kg in mice).

Per the findings, females seeking contraception might find the anti-fertility effects beneficial, but it is a form of reproductive toxicity to others seeking fertility or conception. Males who have reduced sexual abilities, or those who want to further enhance

their sexual abilities who continue to take this preparation could develop low sperm count, and this could make them infertile. Caution is, therefore, needed during prolonged use of *P. nitida* preparations in the treatment of various ailments such as malaria, stomach problems, pneumonia, jaundice, measles, cough, typhoid fever, and gonorrhea [2]. Despite the widespread use of *P. nitida* traditionally, very limited data on its effects on reproductive hormones is available. What could be the fate of men and women using these for contraception as far as blood, liver and kidneys are concerned? These are some reasons why this study was carried out to assess the effects of the ethanolic seed extract *P. nitida* on reproductive hormones in both male and females to provide further explanation to earlier observations with regards to androgenic and estrogenic effects and to further ascertain its safety on prolonged usage.

MATERIAL AND METHODS

Duration of Study

This study was commenced in January 2013 and completed in March 2015 during which period experimental data were collected and analyzed.

Plant Materials

Fresh fruits of *P. nitida* were collected from the KNUST Botanical Gardens in January 2013. Its authenticity was confirmed by Dr. Kofi Annan, Department of Herbal Medicine, FPPS, KNUST, Ghana (Specimen voucher number: KNUST/HM1/2013/S054). The pods were cut open to collect the seed, which were then air dried.

Experimental Animals

In the investigation of androgenic and estrogenic effects of PNE, White leghorn and Rhode Island Red layer day-old chicks, purchased from Akati Farms, Kumasi Ghana were used. For safety assessment, Sprague-Dawley rats (180-220 g) were used. All animals were maintained in the Animal House of the Department of Pharmacology, KNUST, housed in stainless steel cages (34 × 47 × 18 cm) with soft wood shavings as bedding. The chicks were fed *ad libitum* with chick starter mash and the rats with normal pelleted rat chow all obtained from Agricare Ltd, Kumasi, Ghana. They were kept under normal conditions of humidity (60-75%) and temperature (25 ± 3°C). All animals were handled humanely throughout the experiment as recommended by the Declaration of Helsinki and the Guiding Principles in the Care and Use of Animals.

Preparation of *P. nitida* Seed Extract (PNE)

The dried seeds of *P. nitida* were milled into powder. A 2.25 kg quantity of the powder was sequentially extracted with 70% ethanol by cold maceration technique for the two consecutive 72-h periods. The extract obtained was concentrated using a rotary evaporator (Rotavapor R-215, BUCHI Labortechnik AG, Flawil, Switzerland) at 60°C to yield a syrupy mass which was subsequently dried at 40°C, in a hot air oven. The solid mass obtained (279.9 g: Percentage yield 12.44), labeled as PNE, was reconstituted in normal saline for dosing in this study.

Drugs and Chemicals

17-β estradiol (Fortress Diagnostics Limited, UK), testosterone propionate (Jinling Pharmaceutical, China), cyproterone acetate (Bayer, Germany) were used in this study.

Androgenic effect of *PNE*

Chick comb test

The method described by Dorfman (1969) [3] was used with slight modifications to study the androgenic effect of PNE. Day old white leghorn chicks, after 14 days acclimatization to the experimental laboratory conditions, were randomly assigned to 10 treatment groups ($n = 10$). The length and height of the combs of each of the chicks in the various groups were measured and recorded. Doses were administered as follows for 7 days. Group 1, the control group, was treated orally with distilled water (vehicle), Groups 2-4 were treated intramuscularly with 0.5, 1.0, and 1.5 mg/kg testosterone propionate, respectively, Groups 5-7: Received orally 3, 10, and 30 mg/kg cyproterone acetate, respectively, whiles Groups 8-10 received, orally, 50, 100, 500 mg/kg PNE, respectively. The length and height of the combs of each chick were measured and recorded 24 h after the last drug administration.

Effect of cyproterone on testosterone and PNE-induced comb growth

To estimate the effects of cyproterone on PNE, 20 white leghorn chicks were randomly divided into four groups ($n = 5$) and treated as follows; chicks in Group 1 were treated with only testosterone (0.6 mg/kg; i.m) while Group 2 received testosterone (0.6 mg/kg; i.m) and cyproterone (10 mg/kg; *p.o*). Group 3 was treated with PNE (30 mg/kg; *p.o*) and Group 4 both cyproterone (10 mg/kg; *p.o*) and PNE (30 mg/kg; p.o). Doses of testosterone and PNE used in this study were estimated from the ED_{50} values from the chick comb test. 24 h after the last administration, change in comb growth (length and height) were measured.

Effect of PNE on testosterone-induced comb growth

To evaluate the effects of PNE on testosterone, 20 single comb white leghorn chicks were grouped into four ($n = 5$) and treated as follows; chicks in Group 1 were administered testosterone (0.6 mg/kg; i.m). Group 2 was administered testosterone (0.6 mg/kg; i.m) and PNE (50 mg/kg; *p.o*). Group 3 was administered testosterone (0.6 mg/kg; i.m) and PNE (100 mg/kg; *p.o*) while Group 4 received testosterone (0.6 mg/kg; i.m) and PNE (500 mg/kg). 24 h after the last administration, change in comb growth (length and height) were measured.

Estrogenic Effect of PNE

Chick uterotrophic assay

This test is based on the principle that elevated levels of natural estrogens and phytoestrogens in female animals during the early stages of development, dose dependently, increases the uterine/body weight ratio [3-5]. Day old Rhode Island Red layer chicks were randomly assigned to seven groups ($n = 6$). Groups 1-3 were treated subcutaneously with 0.1, 0.3, or 0.9 μg of 17-β-estradiol twice daily. Groups 4-6 were treated orally with 30, 100, and 300 mg/kg of PNE, respectively. Group 7, the control group, was treated subcutaneously with 0.2 % v/v corn oil (vehicle) control. Dosing was done 12 h for 6 continuous days. During treatments, chicks were weighed every other day before feeding. On the 6th day of treatment, the chicks were weighed, euthanized with ether, dissected, and the oviduct was isolated; whiles carefully removing any attached connective tissue. The oviduct was immediately weighed. Weights were then normalized with the final body weight of the chick and expressed as a percentage using the formulas below.

$$\text{Percentage oviduct-chick weight ratio} = (\text{oviduct weight [g]} \div \text{chick weight [g]}) \times 100$$

The percentage change in weight was then plotted against the log of the concentration of the various treatments to obtain log concentration-response curves.

Safety Assessment

Four groups of male Sprague-Dawley rats ($n = 10$) were used with Group I receiving distilled water only whiles Groups II-IV received 30, 100, and 300 mg/kg of PNE, respectively, for 14 days. On the last day of administration, blood samples obtained from the jugular vein were collected into tubes containing gel and clot activator (Channel MED, China) and centrifuged at 3,000 × g for 5 min, to obtain plasma for liver and kidney function and the lipid profile tests using the Vital Scientific Flexor Junior Chemistry Analyzer. Blood samples were also collected into ethylenediaminetetraacetic acid tubes for hematological analyzes using the Sysmex KX 21NTM Automated Hematoanalyzer.

Ethical Considerations

This study was conducted at the Department of Pharmacology, KNUST in compliance with: OECD Principles of Good Laboratory Practices ENV/MC/CHEM (98)17, EEC Good Laboratory Practices (90/18/EEC) and FDA Good Laboratory Practice Standards (Part 58 of 21 CFR). All experiments were approved by The Committee on Animal Research, Publication and Ethics (CARPE) with ethics reference number FPPS/PCOL/0017/2012.

Data Analysis

GraphPad Prism for Windows Version 5 (GraphPad Software, San Diego, USA) was used for all statistical analysis. Data were analyzed using one-way Analysis of Variance followed by Newman-Keuls *post-hoc* test for comparison between control and treatment groups. $P \leq 0.05$ was taken to be statistically significant.

RESULTS

Chick Comb Test

7 days treatment with PNE (30-300 mg/kg) and testosterone (0.5-1.5 mg/kg) increased significantly ($P \leq 0.01$-0.001), while cyproterone acetate significantly decreased ($P \leq 0.001$) comb growth (size and length) in a dose-dependent manner; compared to the vehicle treated chicks [Figure 1]. The qualitative assessment revealed that chicks treated with testosterone (1.0, and 1.5 mg/kg) and all doses of PNE had relatively brighter red combs and wattle [Figure 2], and well-developed feathers. Using the comb length as the response, the ED_{50} of testosterone and PNE was estimated to be 0.6, and 27.37 mg/kg, respectively. The slope rate of growth was steep at low doses (50-100) mg/kg but very gentle at high doses (100-500) mg/kg. PNE exhibited partial agonist-like activity in the study [Figure 3]. Cyproterone acetate inhibited comb growth at all dose levels with the highest inhibition was observed at 30 mg/kg.

Effect of Cyproterone on Testosterone and PNE-Induced Comb Growth

Cyproterone caused a significant inhibition of both testosterone (72.84 ± 5.39%; $P \leq 0.001$) and PNE (81.57 ± 9.00%; $P \leq 0.001$) induced comb growth. The appearance of wattle was also inhibited until the last day of treatment [Figure 4].

Effect of PNE on Testosterone-Induced Comb Growth

Co-administration of testosterone and PNE suppressed comb growth significantly (70.05 ± 6.182%, $P \leq 0.001$). All treated chicks developed light pink combs and wattles [Figure 5].

Chick Oviduct Test

A 6-day continuous administration of 17-β estradiol and PNE increased dose-dependently ($P \leq 0.001$) the percentage oviduct-chick weight ratio [Figures 6 and 7]. The efficacy exhibited by both treatments were not significantly different, but 17-β estradiol was more potent at increasing oviduct weight compared to PNE as indicated in the estimated ED_{50}'s (Estradiol: 0.25 μg, PNE: 1.5 mg/kg) [Figure 8]. However, no significant differences in body weights were observed [Figure 9].

Safety Assessment

No significant changes were observed in blood biochemical and hematological parameters when compared to control. All hematological parameters were in normal ranges in the control and tested animals. Both red blood cells (RBC) and white blood cells (WBC) were increased, but insignificant compared to control. At a lower dose of 30 mg/kg, cholesterol and

triglyceride levels were slightly increased but levels decreased with higher doses. Aspartate transaminase (AST) and alanine transaminase (ALT) levels, although slightly increased were within the acceptable range. Blood urea and creatinine were also not significantly affected [Table 1].

DISCUSSION

P. nitida is used extensively across countries in Africa for the treatment of an appreciable number of ailments [6]. In view of the fact that among the population using the plant product is males and females of childbearing age, this study aimed at investigating the effect of the plant on reproductive hormones and also its safety for use. Earlier work by authors and also by other workers has demonstrated adverse effects of the PNE on male reproduction [7]; and its contraceptive capability in females on chronic usage. The effect of the PNE on male chick comb growth and female chick oviduct were assessed.

Comb growth in male chicks is highly androgen dependant. These chicks demonstrate an exaggerated response to induction or elevation of androgens (particularly testosterone) in their system by the expression of male secondary sex characteristics as revealed by changes in the comb, wattle, and ear lobe [8]. This makes the chick comb growth model ideal for screening androgens. Testosterone's androgenic effects have been attributed to its principal metabolite, 5α-dihydrotestosterone, known to have a fivefold affinity for the androgen receptor than

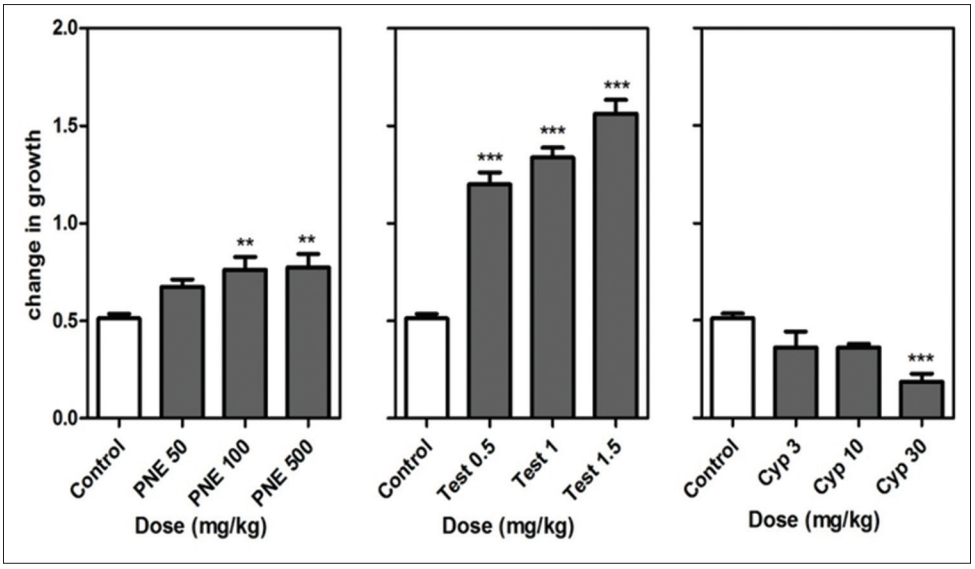

Figure 1: Effect of *Picralima nitida* seed extract [PNE] (50-500 mg/kg), Testosterone [Test] (0.5-1.5 mg/kg), and Cyproterone acetate [Cyp] (3-30 mg/kg) on chick comb growth. Data expressed as mean± standard error of mean, n=10. Significant different from control: $^{**}P \leq 0.01$; $^{***}P \leq 0.001$ (one way Analysis of Variance followed by Newman keuls post hoc test)

Table 1: Effect of *Picralima nitida* on some hematological and biochemical parameters in Sprague-Dawley rats

Parameters	Control	PNE 30 mg/kg	PNE 100 mg/kg	PNE 300 mg/kg
WBC ($\times 10^3$/uL)	4.36±0.62	5.68±0.93	6.9±0.70	6.58±0.54
RBC ($\times 10^6$/uL)	8.40±0.17	9.25±0.36	9.67±0.29	9.32±0.16
Hematocrit (%)	42.68±0.87	48.48±2.48	47.67±0.29	47.85±0.95
Hemoglobin (g/dL)	12.82±0.34	13.95±0.73	13.97±0.27	13.78±0.32
MCV (fl)	50.88±1.17	52.35±0.78	49.33±1.19	51.35±0.17
MCH (pg)	15.28±0.50	15.05±0.31	14.43±0.24	14.8±0.09
MCHC (g/dL)	30.02±0.50	28.78±0.40	29.3±0.40	28.78±0.18
Cholesterol (mmol/l)	2.87±0.13	4.03±0.62	2.54±0.16	2.77±0.15
Triglycerides (mmol/l)	1.53±0.05	2.05±0.51	0.93±0.12	1.20±0.16
AST (μ/L)	40.24±17.73	67.97±35.41	33.40±14.88	80.37±34.03*
ALT (μ/L)	4.42±2.33	5.65±1.69	4.9±2.77	5.93±1.15
ALP (μ/L)	132.5±7.11	143.9±6.54	138.5±9.08	141.8±9.11
Albumin (g/l)	43.52±1.03	38.44±0.84	41.29±0.78	40.37±0.82
Globulins (g/l)	31.40±0.94	33.00±2.80	29.40±1.70	32.65±1.59
Total protein (g/l)	74.87±1.02	71.83±1.92	71.17±0.85	72.77±2.05
Creatinine (μmol/l)	556.7±29.32	578.0±40.14	563.7±17.27	510.9±30.25
BUN (mmol/l)	17.50±1.40	15.93±0.85	18.30±0.76	15.67±1.12

Data expressed as mean±SEM, n=10. *$P \leq 0.05$ (one-way ANOVA using Dunnetts *post-hoc* test). WBC: White blood cells, RBC: Red blood cells, MCV: Mean corpuscular volume, MCH: Mean corpuscular hemoglobin, MCHC: Mean corpuscular hemoglobin concentration, AST: Aspartate transaminase, ALT: Alanine transaminase, ALP: Alkaline phosphates, BUN: Blood urea nitrogen, PNE: *Picralima nitida* seed extract, ANOVA: Analysis of variance, SEM: Standard error of mean

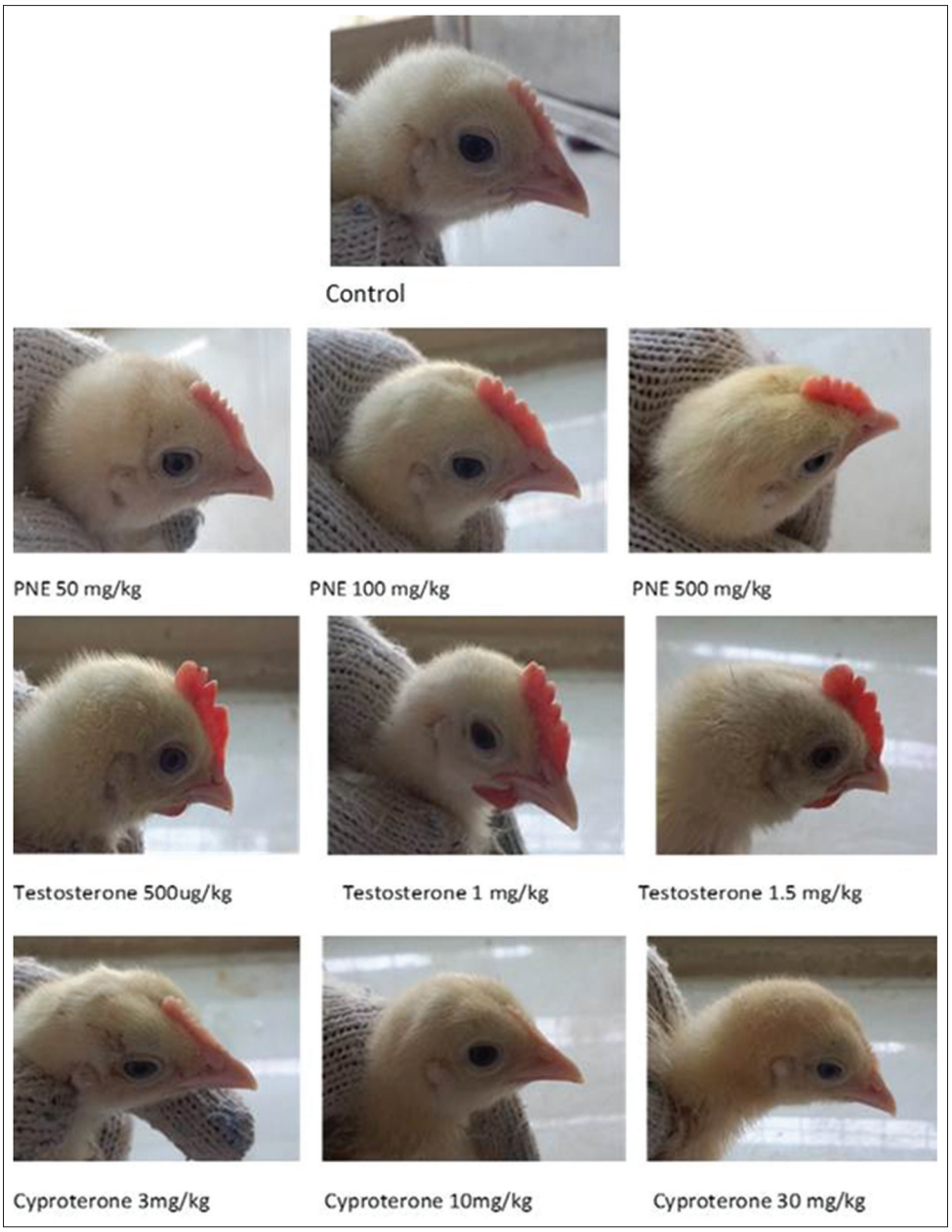

Figure 2: Representative images of chick combs after 7 days of treatment with *Picralima nitida* seed extract (PNE) (50-500 mg/kg), testosterone (0.5-1.5 mg/kg), and cyproterone acetate (3-30 mg/kg). Note the comb growth (and wattle) with associated with testosterone and PNE treatment; and the comb-growth inhibition with cyproterone treatment

testosterone in both mammals and Aves [8-10]. PNE activity was comparable to testosterone. Cyproterone's ability to block the androgenic activity of PNE indicates that PNE acts by directly or indirectly stimulating the avian androgen receptor.

The ability of PNE to stimulate comb growth may corroborate with our earlier observations of enhanced mating and mounting behaviors in treated rodents as well as its purported traditional usage as an aphrodisiac [6,11]. In general, there is an association between sexual behavior in male (and in females) and elevated serum testosterone levels [12]. Indeed, in conditions of diminished libido such as in hypogonadism, or menopause, there is enhanced sexual desire on testosterone administration [13]. In our previous study, we reported that PNE affects mating behaviors in rats. Subsequently in this study, we have demonstrated that PNE affects reproductive hormones principally testosterone and estradiol. Aphrodisiacs, according to Sandroni's (2001) [14], can be classified based on their ability to either increase libido sexual pleasure or potency. Because PNE alters the levels or activity of specific sex hormones, it can be said to be an aphrodisiac that increases libido.

Co-administration of testosterone and PNE, however, suppressed comb growth significantly suggesting that PNE could be a partial agonist on androgen receptors, characteristic of dose-response curves of testosterone and PNE obtained

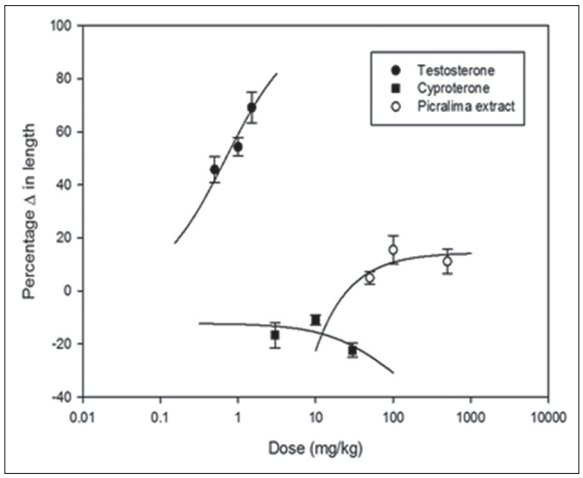

Figure 3: Log-dose-response of *Picralima nitida* seed extract (50-500 mg/kg), testosterone (0.5-1.5 mg/kg), and cyproterone acetate (3-30 mg/kg) on chick comb growth. Values plotted are mean ± standard error of mean, $n = 10$

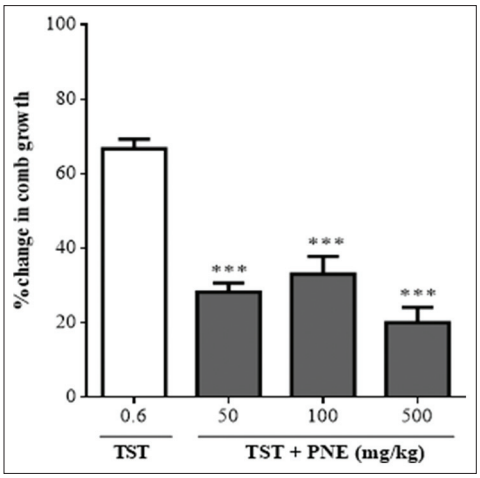

Figure 5: Effects of *Picralima nitida* seed extract [PNE] (50-500 mg/kg) on testosterone propionate [TST] (0.6 mg/kg)-induced comb growth. Values plotted are mean ± standard error of mean, n=5. *** $P \leq 0.001$ (one-way Analysis of Variance followed by Newman-Kuels post-hoc test)

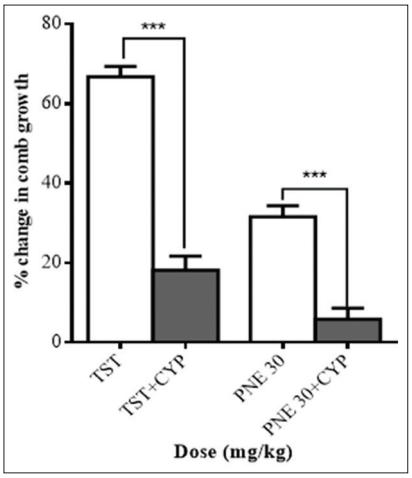

Figure 4: Effects of cyproterone [Cyp] (10 mg/kg) pre-treatment on testosterone propionate [TST] (0.6 mg/kg) and Picralima nitida seed extract [PNE] (30 mg/kg)-induced comb growth. Values plotted are mean ± standard error of mean, n=5. *** $P \leq 0.001$ (one-way Analysis of Variancefollowed by Newman-Kuels *post-hoc* test)

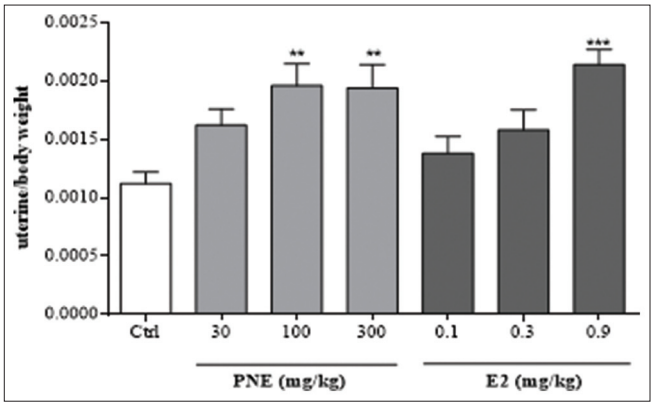

Figure 6: Effect of *Picralima nitida* seed extract [PNE] (30-300 mg/kg), and (b) 17- ß estradiol [E2] (0.1-0.9 µg) on chick uterine to body weight ratio. Values plotted are mean ± standard error of mean, n=5. Significant difference from control: ** $P < 0.01$; *** $P < 0.001$ (one-way Analysis of Variance followed by Newman-Keuls *post hoc* test)

[Figure 1]. The partial agonist activity observed in this study may explain why the subacute use of PNE led to a reversal of the acute aphrodisiac effects as well as alterations in male the reproductive parameters such as sperm count. Estrogens have been shown to block the activation of the androgen receptors by testosterone [15]. PNE demonstrates significant estrogenic activity. The partial agonist activity exhibited by PNE could be due to antagonism by estrogenic activity or the presence of estrogenic elements in PNE.

Androgens are known for their ability to stimulate erythropoeisis and anemia is associated with androgen deprivation [16]. In all the hematological assessments, PNE increased levels (although not statistically significant) of hemoglobin, hematocrit, mean corpuscular hemoglobin (MCH), MCH concentration, as well

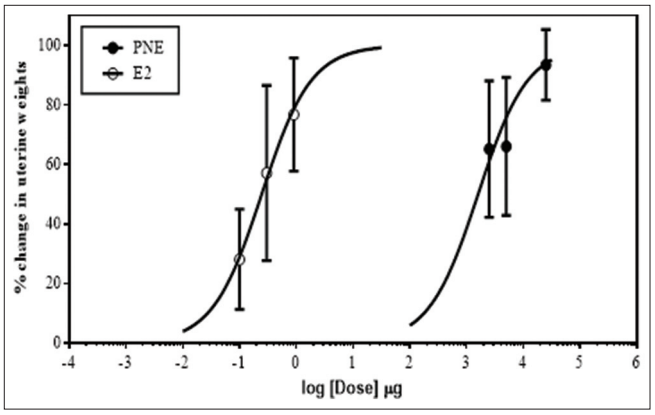

Figure 7: Dose-response curves of *Picralima nitida* seed extract [PNE] (30-300 mg/kg) and 17- ß oestradiol [E2] (0.1-0.9 9 *µg*) with respect to percentage change in uterine to body weight in the chick uterotrophic assay. Each point represents the mean ± standard error of mean, n=5

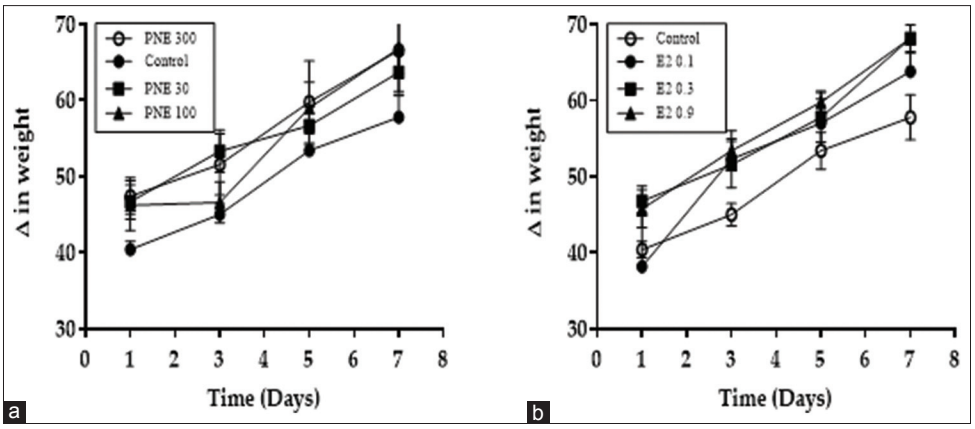

Figure 8: Time-course curve of the effects of (a) *Picralima nitida* seed extract [PNE] (30-300 mg/kg), and (b) 17- ß oestradiol [E2] (0.1-0.9 *µg*) on weight change. Values plotted are mean ± standard error of mean, *n*=6

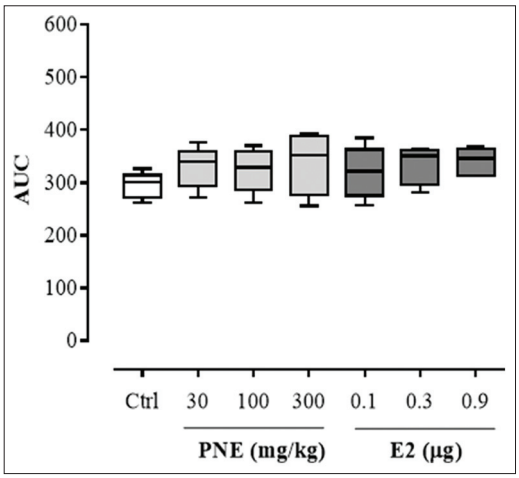

Figure 9: Effects of *Picralima nitida* seed extract [PNE], and 17- ß oestradiol [E2] on body weight changes in chick uterotrophic assay. Area under the curve (AUC) values plotted are mean ± standard error of mean, *n*=6. The lower and upper margins of the boxes represent 25th and 75th percentiles with the extended arms representing the 10th and 90th percentiles respectively. The median line is shown as the horizontal line within the box

as RBC. This could possibly account for the reddening of the combs.

In previous studies, PNE prolongs estrous phase in rats *in vivo* and possessed uterotonic effects *in vitro* [17]. Using the chick uterotrophic assay, we have demonstrated that PNE has uterotrophic effects which were comparable to estradiol. The chick uterotrophic assay is based on the principle that elevated levels of natural estrogens and phytoestrogens in female animals during the early stages of development, dose-dependently, increase the uterine/body weight ratio [3-5]. The uterus responds to estrogens in two-ways. An initial response would be an increase in weight due to water imbibition. This response is followed by a weight gain due to tissue growth. The results of these studies indicated that PNE either directly or indirectly enhance the activity of estradiol and or gonadotrophins, luteinizing (LH), and follicle-stimulating hormone.

Exhibiting androgenic and estrogenic activity for an extract is not unusual because of the presence of a myriad of components; whereas some components may contribute to the overall androgenic effect, others may have inhibitory effects. The phenolic components of the crude soy extract, for example, have been demonstrated to have an affinity for both estrogen and androgen receptors - although the components have a relatively lower affinity than the cognate agonist [18].

The seed extract of *P. nitida* has been shown to possess alkaloids that have opioid binding activity [19,20]. Opioids could modulate gonadotrophin, LH release and LH regulates testosterone and estrogen secretion by the gonads. Actions of opioids on the endocrine system are largely mediated through the Mu (μ) receptors [21,22]. Interestingly, more than five alkaloids isolated from PNE, have Mu receptor binding affinity [20]. Subsequently by influencing LH levels by PNE can possess estrogenic and androgenic effects. Indeed, chronic opioid administration tends to decrease serum testosterone and LH [23] which may explain why enhanced libido with acute administration dwindles with chronic use.

Safety assessment on PNE indicated its safety for use as far as its effect on blood; the liver and kidneys were concerned. Administration of herbal products has the propensity to cause significant changes in the structure, function, metabolic transformations, and concentration of biomolecules and enzymes. Such changes may lead to pathological and/or clinical effects [24]. Assessment of hematological parameters helps to determine the damaging effect of xenobiotics on blood. The non-significant increase in WBC number may probably be due to normal immune responses to foreign bodies. Furthermore, the insignificant changes in RBC count, hemoglobin and hematocrit suggest that PNE is unlikely to cause anemia. It could be that the extract has the potential to stimulate erythropoietin release in the kidney, which is the humoral regulator of red blood cell formation. This confirms previous studies on the hematological effects of *P. nitida* saponin extracts [25].

There were no significant changes in levels of cholesterol, triglycerides (TAG), low-density lipoprotein (LDL), and

high-density lipoprotein (HDL), to be attributed to the drug treatments. Cholesterol levels could decrease during the treatment period because there is a possibility of drugs to causes general damage, blockage of an enzyme system for steroidogenesis in the ovary and the capacity of the liver to store cholesterol due to general damage [26]. Elevation of cholesterol, TAG, and LDL, and a decrease in HDL would increase the risk of cardiovascular disorders [27-29].

In the liver function test, ALT, alkaline phosphates (ALP), and total proteins (albumin and globulins) did not change significantly, while AST was slightly elevated than those in the control group. ALT and AST are liver associated enzymes that are indirect measures of liver homeostasis [30]. Hepatocellular injury leading to the permeability of intracellular enzymes into the bloodstream is accompanied by elevated ALT and AST [31]. AST is also present in red cells, cardiac, and skeletal muscles, therefore, not specific to the liver [32,33]. Thus, the increment in AST observed cannot be attributed to hepatocellular damage as it is also associated with other tissues. Thus, PNE is not hepatotoxic at the dose levels used. Increased in serum ALP is associated with liver disease caused by intra or extra hepatic cholestatis and some destruction of the hepatic cell membrane, as well as extrahepatic and intra hepatic bile duct obstruction [34]. The kidneys were also not affected as control blood urea nitrogen (BUN), and creatinine levels did not change significantly with treatments. BUN and creatinine are used to evaluate kidney function; to help diagnose kidney disease, and to monitor acute or chronic kidney dysfunction or failure. Elevated of these in blood suggests impaired kidney function which could be acute or chronic kidney disease, damage, or failure [35].

CONCLUSION

The ethanolic seed extract of *P. nitida* exhibits both androgenic (partial testosterone agonist) and estrogenic activity. It has no detrimental effects on the blood, liver, and kidney tissue with prolonged use.

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Original Research

Comparative evaluation of the aphrodisiac efficacy of sildenafil and *Carpolobia lutea* root extract in male rabbits

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ABSTRACT

Aims: In spite of the folkloric use of the root of *Carpolobia lutea* as a sexual stimulant in man, there has been limited scientific proof of its efficacy. This study compares the efficacy of methanol extract of *C. lutea* root (MECLR) and sildenafil on the sexual activity of male rabbits. **Methods:** 20 adult male rabbits were grouped into four of five rabbits each. Groups 1-4 were treated orally for 28 days with 2 ml/kg 1% Tween-20 (vehicle), 40 mg/kg MECLR, 80 mg/kg MECLR, and 0.5 mg/kg sildenafil citrate (SC), respectively. Sexual activities of males from each group were assessed by cohabiting them with sexually receptive female at estrus on days 0, 1, 3, and 5 using digital camera mounted on mating arena. Serum testosterone and nitric oxide concentration of the corpora cavernosa homogenates were also determined. **Results:** MECLR caused a dose-dependent significant increase in mount frequency, intromission frequency and ejaculatory latency (EL) while it reduced mount latency, intromission latency and post EL (similar to SC) when compared with the control. MECLR also caused significant increase in nitric oxide concentration in corpora cavernosa but no change in serum testosterone concentration. **Conclusions:** Results suggest that MECLR enhances male sexual activity possibly by augmenting nitric oxide concentration. This study thus provides a novel scientific rationale for the use of *C. lutea* in the management of penile erectile dysfunction and impaired libido.

KEY WORDS: *Carpolobia lutea*, aphrodisiac, efficacy, male, rabbits

INTRODUCTION

Male sexual dysfunction, which mostly includes erectile dysfunction (ED) and premature ejaculation, is the most common problem that contributes to infertility, distress, relationship problems and low quality of life [1]. While sexual dysfunction rarely threatens physical health, it can bring a heavy psychological toll; depression, anxiety, and debilitating feelings of inadequacy. ED accounts for 45% of male sexual dysfunction in Nigeria [2]. ED is experienced some of the time by most men who have reached 45 years of age, and it is projected to affect 322 million men worldwide by 2025 [3]. ED is usually underestimated in many developing countries including Nigeria [4,5] probably because it is not a life-threatening condition and due to the associated stigma. Herbal preparations have enjoyed the patronage of most people in rural and urban areas of Nigeria [6]. In Africa, several plants have been used for many years to improve sexual stimulation and performance. Ang *et al*. [7] reported the use of *Aristolochia indica*, *Crocus sativus*, *Alpinia galanga*, and *Allium cepa* to improve sexual activities with varying degrees of success. *Carpolobia lutea*, (polygalaceae) is a small plant that often grows to 15 ft in height. Its juicy fruits are consumed by people of Southern Nigeria [8]. The plant is well distributed in West and also Central Africa [9]. It is popularly known in Southwest and South Eastern Nigeria particularly among the Eket tribe as a potent aphrodisiac. *C. lutea* is widely used by traditional herbal practitioners to treat male erectile disorders and facilitate delivery [9]. The root decoction is reportedly used as malarial remedy [10,11], anti-inflammatory/anti-arthritic [12,13], anthelmintic and anti-sterility agent [14,9].

Though ethno-botanical survey has revealed that the root decoction of *C. lutea* is used to enhance sexual activity [15],

very few experimental studies have been performed to ascertain these claims of efficacy. Orthodox treatments that could serve as options for ED are also expensive, not readily available and present unpleasant side-effects. As compared with orthodox drugs, *C. lutea* is cheap, readily available, and greatly consumed by local population. This study therefore investigated and compared the aphrodisiac efficacy of methanol extract of *C. lutea* root (MECLR) with sildenafil citrate (SC) in adult male rabbits.

METHODS

Plant Material

C. lutea plant was collected from Alade village in Akinyele Local government, Oyo State, Nigeria, in December 2013. The plant was identified, authenticated and assigned voucher number FHI 109755 at the Forestry Research Institute of Nigeria, Ibadan, Nigeria.

Preparation of Plant Extract

C. lutea root was washed, cut into pieces and oven dried at 40°C to a relatively constant dry weight. 4 kg of the oven dried, pulverized sample was soaked in 15 l of absolute methanol in a glass bowl at room temperature for 72 h. It was then filtered with Wattmann filter paper. The filtrate was concentrated in a rotary evaporator at 40°C [16] to yield the methanol extract (brown oily substance) which was stored in a refrigerator at −4°C.

Determination of LD_{50}

Nine male Wistar rats were equally grouped into 3.50 mg/kg of MECLR was administered to the first group and subsequently observed for signs of toxicity or mortality over a 24-h period. The dose was then repeated on the absence of mortality. Same procedures were then followed for the two remaining groups at fixed dose of 300 and 2000 mg/kg body weight (BW) respectively. LD_{50} was subsequently determined according to OECD 423 guideline [17].

Experimental Animals

20 adult male and female rabbits (6 months) weighing 1.5-2.0 kg sourced from the animal house of the Department of Veterinary Physiology University of Ibadan were used. Animal was allowed to acclimatize for 3 weeks before commencement of study. They were fed standard pelletized rodent feed and water ad libitum. Female rabbits used for the evaluation of sexual behavior were from the same strain with male and were prepared according to the method of Anders [18]. All the experimental procedures were done following guidelines of the University of Ibadan Animal Ethics Committee

Experimental Protocol

20 adult male rabbits (1.5-2.0 kg) were divided into four equal groups and treated daily (orally) for 28 days with 2 ml/kg 1% Tween-20 (control), 40 mg/kg MECLR), 80 mg/kg MECLR and 0.5 mg/kg SC. The male rabbits were cohabited with sexually receptive female at estrus, and sexual activities of the male were observed and recorded on days 0, 1, 3 and 5 using a digital camera mounted on the mating arena [19]. Animals were treated 1 h prior to mating on days 1, 3, and 5 [20].

Mating Behavior Test

Mating behavioral tests were carried out according to the methods of Anders [18] and as modified by Gauthaman *et al.*, [21] and Guohua *et al.*, [22]. Healthy males showing brisk sexual activity were paired with receptive females in estrus at ratio 1:1 in the mating arena. Estrus was artificially induced by sequential administration of estradiol benzoate (10 μg/100 g) orally and progesterone (0.5 mg/100 g BW) subcutaneously, 48 h and 4 h respectively prior to pairing [23]. Estrus was confirmed as described by the methods of Marcondes *et al.*, [24]. Pairing was also conducted 16.00 h each day in the same arena with same light intensity for 30 min. Recorded event frequencies and phases were later transcribed from the mounted digital camera. Mount frequency, mount latency, intromission frequency (IF), intromission latency (IL), ejaculatory latency (EL), and post EL (PEL) as indicators of male sexual behavior were analyzed following the adaptations of Gauthaman *et al.*, [21] and Guohua *et al.*, [22].

Blood Collection and Hormone Assay

Blood samples were collected on day 0 (basal), 5 and 28 from each animal through retro-orbital sinus with a 70 μl heparinized capillary tube into a plain serum bottle. Samples were centrifuged at 3000 revolutions per minute for 15 min to obtain serum. Serum testosterone concentration was thereafter determined using a double antibody enzyme-link immunosorbent assay kit (Rapidlab Testosterone kit, Italy).

Determination of Corpus Cavernosum Nitric Oxide

Male rabbits were sacrificed by cervical dislocation on day 28. Corpus cavernosum were then excised, weighed, and immediately homogenized in phosphate buffer (pH 7.4) and centrifuged. Nitric oxide concentration in each tissue homogenate was determined using Griess reaction method [25,26]. Samples were diluted fourfold with distilled water and deproteinized by adding $1/20^{th}$ volume of zinc sulfate (300 g/L) to give a final concentration of 15 g/L. After centrifugation at l000 g for 15 min (room temperature), 100 μL of supernatant was applied to a microtiter plate well, followed by 100 μL of Griess reagent (1 g/L sulfanilamide, 25 g/L phosphoric acid, and 0.1 g/L N-1-naphthylethylenediamine). After 10 min of color development at room temperature, the absorbances were measured on a microplate reader (Titertek Multiskan MCC/340; Flow Lab, McLean, VA) at a wavelength of 540 nm. Each sample was assayed in duplicate wells. Background values were obtained by treating samples as described but using 25 g/L phosphoric acid instead of complete Griess reagent.

Statistical Analysis

Data were analyzed using prism Graph pad version 5.0 and presented as mean ± standard error of the mean. Comparison between means was done using analysis of variance. Values were considered statistically significant at $P \leq 0.05$.

RESULTS

Effect of MECLR on Toxicity/Lethality and LD_{50}

No mortality was recorded when the starting dose of 50 and 300 mg/kg was repeated as well as the first administration of 2000 mg/kg. Only one animal died after repeating the dosage of 2000 mg/kg [Table 1]. 2500 mg/kg BW was obtained as the LD_{50} of MECLR in this study.

Effect of MECLR on Mount and IL

As shown in [Figures 1 and 2], there was significant, dose-dependent decrease in mount and IL in MECLR treated rabbits when compared to control. In addition, there was significant decrease when MECLR treated rabbits were compared to SC treated group.

Effect of MECLR on Mount and IF

There were significant, dose-dependent increases in mount and IF when MECLR treated rabbits were compared to control and SC groups [Figures 3 and 4].

Effect of MECLR on EL and PEL Period

As shown in [Figures 5 and 6], there were significant dose-dependent decreases in ejaculatory and PEL periods in MECLR treated groups as compared to control and SC treated groups.

Effect of MECLR on Serum Testosterone and Corpora Cavernosa Nitric Oxide Concentration

As shown in [Table 2] there were no significant differences in serum testosterone concentrations when MECLR treated groups were compared to control, and SC treated group. However, nitric oxide concentration increased significantly in MECLR (80 mg/kg), and SC treated groups as compared to control [Figure 7].

DISCUSSION

The background for classifying any medicinal plant as having the potential to stimulate and enhance sexual vigor was enunciated by Ratnasooriya and Dharmarsiri [27]. They opined that medicinal plant with a tendency to stimulate and enhance sexual behavior should produce a statistically significant increase in mount and IF and also reduce significantly mount and IL; since these indices are indicators of sexual arousability, motivation, and vigor. Results from this study indicated that MECLR at 40 and 80 mg/kg BW enhanced sexual activity comparable to SC.

Table 1: Effects of MECLR on toxicity/lethality and LD_{50} in male rabbits

Number of animals	Dosage (mg/kg BW)	Number of lethality
3	50	0
3	300	0
3	2000	1

MECLR: Methanol extract of *Carpolobia lutea* root, BW: Body weight

Table 2: Effect of MECLR on serum testosterone concentration (ng/mol)

Group	Day (0) (basal) (ng/mol)	Day 5 (ng/mol)	Day 28 (ng/mol)
Control	7.18±3.39	15.65±5.97	5.05±3.98
40 mg/kg BW	8.78±3.23	18.80±0.79	7.70±0.75
80 mg/kg BW	8.25±4.05	10.53±0.62	9.56±0.54
0.5 mg/kg BW SC	5.05±4.21	17.80±0.83	0.90±0.64

Data presented as mean±standard error of mean., n=5, MECLR: Methanol extract of *Carpolobia lutea* root, BW: Body weight, SC: Sildenafil citrate

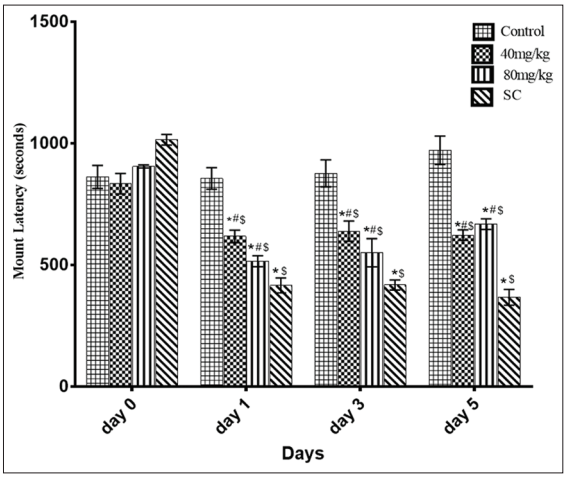

Figure 1: Effect of methanol extract of *Carpolobia lutea* root on mount latency. *$P \leq 0.05$ when compared with control group; #$P \leq 0.05$ when compared with sildenafil citrate group; \$$P \leq 0.05$ when compared with day 0 (Basal)

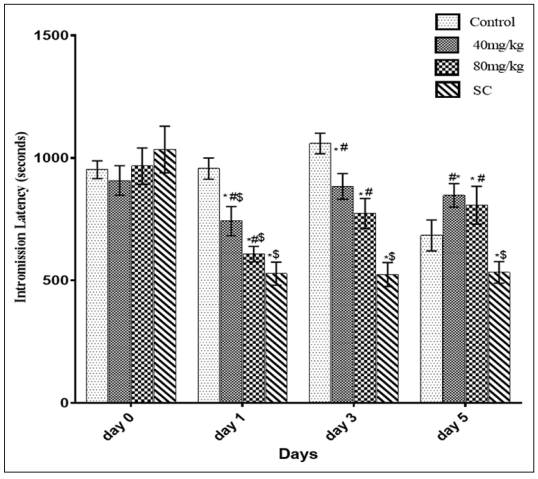

Figure 2: Effect of methanol extract of *Carpolobia lutea* root intromission latency. *$P \leq 0.05$ when compared with control group, #$P \leq 0.05$ when compared with sildenafil citrate group, \$$P \leq 0.05$ when compared with day 0 (Basal) $n = 5$

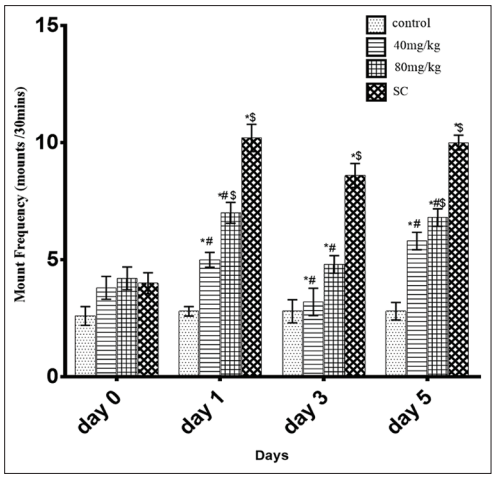

Figure 3: Effect of methanol extract of *Carpolobia lutea* root on mount frequency. *$P \leq 0.05$ when compared with control group, #$P \leq 0.05$ when compared with sildenafil citrate group, \$$P \leq 0.05$ when compared with day 0 (Basal). $n = 5$

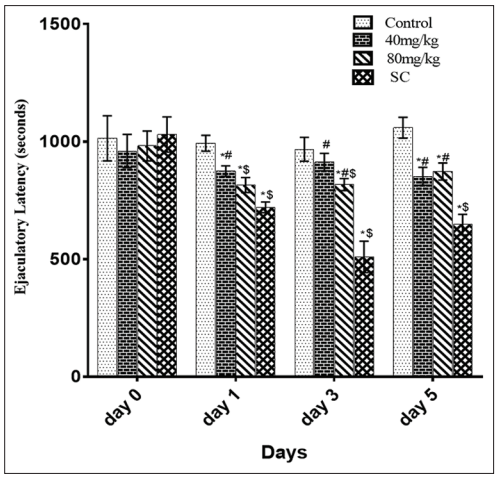

Figure 5: Effect of methanol extract of *Carpolobia lutea* root on ejaculatory latency. *$P \leq 0.05$ when compared with control group, #$P \leq 0.05$ when compared with sildenafil citrate group, \$$P \leq 0.05$ when compared with day 0 (Basal). $n = 5$

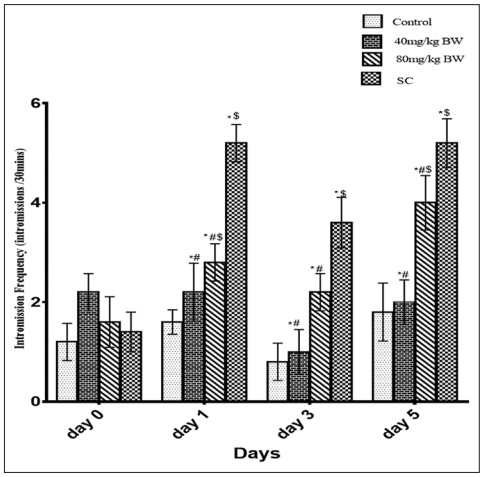

Figure 4: Effect of methanol extract of *Carpolobia lutea* root on intromission frequency. *$P \leq 0.05$ when compared with control group, #$P \leq 0.05$ when compared with sildenafil citrate group, \$$P \leq 0.05$ when compared with day 0 (basal). ***n*** = 5

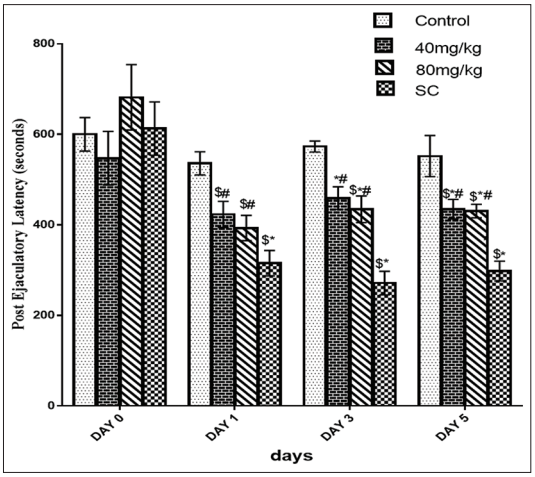

Figure 6: Effect of methanol extract of *Carpolobia lutea* root on post ejaculatory latency. *$P < 0.05$ when compared with control group, #$P < 0.05$ compared with sildenafil citrate group, \$$P \leq 0.05$ when compared with day 0 (basal). $n = 5$

This is evidenced by the statistically significant reduction in mount/intromission, and PEL; and the statistically significant increase in mount and IF of both MECLR and sildenafil-treated group as compared with control. Yakubu and Jimoh [28] have earlier reported on the capability of aqueous *C. lutea* root extract in enhancing mount/IF and EL after paroxetine-induced sexual impairment in male rats. The increase in sexual activity observed in the animals treated with MECLR could be due to phytochemicals present in the plant. *C. lutea* belongs to the polygalacaea families who are rich in saponins and flavonoids [28,29]. Medicinal plants such as *Tribulus terrestris* [21], *Anemopaegma arvense* [30], Arrabidaea *chica* [31] and Turnera diffusa [32] which also contain phytochemicals like saponins and flavonoids have been documented to enhance sexual activities. Flavonoids are widely distributed in flowering plants and therapeutic potential ascribed to them include antioxidant and hemodynamic activities. The antioxidant potential helps provide protection against cellular damage to erectile tissues that can cause ED as a result of stress [33] or leydig cells damage leading to decrease in testosterone and loss of libido [34]. Flavonoids ability to enhance hemodynamic flow benefits the activity of nitric oxide synthase that stimulates the production of nitric oxide. Nitric oxide then activates guanylylcyclase to produce cyclic GMP (cGMP) a potent vasodilator. Saponins are regarded as adaptogens or anti-stress agents. Although, their mechanism of action is still unclear, they have been reported to help improve non-specific resistance of the body after exposure to various stressing factors [35]. Saponins like flavonoids can help to enhance penile erection by preventing the damaging effect of stress on erectile tissues. In addition some saponins have also been reported to inhibit phosphodiesterase 5 [22] thereby potentiating activity of the potent vasodilator cGMP.

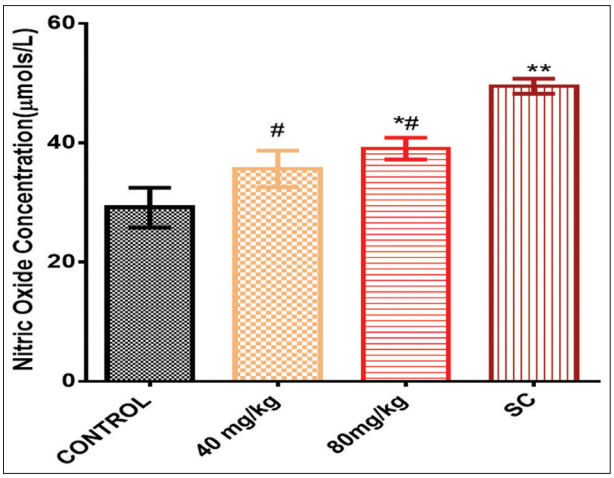

Figure 7: Effect of methanol extract of *Carpolobia lutea* root on nitric oxide concentration in corpus cavernosum. *$P \leq 0.05$; **$P \leq 0.001$ when compared with control. #$P \leq 0.05$ when compared with sildenafil citrate $n = 5$

Testosterone have always been assumed to play a major role in male erectile function as evidenced by the observation that men with marked decrease in testosterone concentration have a significant reduction in the frequency, amplitude and rigidity of erection [36,37]. However, the level of testosterone required to cause ED is debatable. Testosterone is a steroid hormone produced from cholesterol. In males, it is primarily synthesized in the Leydig cells of the testes under the influence of follicle stimulating hormone (FSH) and luteinizing hormone (LH). FSH regulates the number of Leydig cells in the testes while LH controls how much testosterone the Leydig cells produce by regulating the expression of 17-β-hydroxysteroid dehydrogenase (an enzyme that mediate the rate limiting-step of testosterone synthesis). Result from this study shows that there is no significant difference in serum testosterone concentration of male rabbits treated with MECLR despite its aphrodisiac potential. Similar observation was reported by Gonzales *et al*., [38] when the root extract of *Lepidium meyenii* produces no significant effect on serum reproductive hormones (LH and Testosterone) but with aphrodisiac and fertility-enhancing property. This observation may imply that the increase sexual activity in MECLR treated rabbits may not be mediated through the hormonal (testosterone) pathway.

There was significant increase in cavernosa nitric oxide (NO) concentration in 80 mg/kg MECLR treated rabbits comparable to sildenafil-treated groups. Nitric oxide (NO) synthesis is enhanced by NO synthase [39]. The ability of MECLR to enhance cavernosa concentration of NO may help explain its aphrodisiac potential. *Panax ginseng* used as a sexual stimulant has been reported to enhance nitric oxide synthesis in the corpora cavernosa [32]. Production of Nitric oxide is known to activate guanylyl cyclase to produce cGMP a potent vasodilator that acts by lowering intracellular calcium.

Comparatively, sildenafil exerted more potent action than MECLR in this study. This is quite understandable as MECLR used is still in its crude and unpurified form. It will be useful in future to compare the intracellular mechanisms of action of MECLR against sildenafil. It will also be appropriate to investigate the effect of MECLR on cavernosa NO concentration on injection of inhibitors of NO synthase. Furthermore, it will be interesting to know if MECLR like sildenafil can inhibit the breakdown of cGMP by serving as inhibitors of phosphodiesterase V (the prominent phosphodiesterase found in male cavernosa) without accompanying side effects associated with sildenafil.

CONCLUSION

The result from this study shows that MECLR enhances sexual activity in male rabbits by augmenting NO concentration in the corpus cavernosum. This provides a novel scientific basis for the folkloric use of this plant in stimulating and enhancing sexual activity.

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Original Research

ScopeMed

Studies on anti-hyperglycemic effect of *Euphorbia antiquorum* L. root in diabetic rats

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ABSTRACT

Background/Aim: To determine the anti-hyperglycemic effect of *Euphorbia antiquorum* L. root. **Materials and Methods:** The study evaluates the anti-hyperglycemic effect of *E. antiquorum* root in streptozotocin-nicotinamide-induced Type 2 diabetes mellitus and fructose-induced insulin resistance models. Alcohol and aqueous extracts of *E. antiquorum* root were administered at doses 200 and 400 mg/kg p.o. Serum levels of glucose, total cholesterol, triglycerides, glycosylated hemoglobin (GHb), and hepatic levels of malondialdehyde, glutathione, and glycogen were estimated. **Results:** Treatment with the alcohol and aqueous extracts of *E. antiquorum* roots resulted in significant ($P < 0.001$) lowering of serum blood glucose and GHb levels in both the models. Flavonoids, phenolic compounds, and glycosides were detected in the preliminary phytochemical screening. **Conclusion:** Root of *E. antiquorum* showed promising anti-hyperglycemic effect which may be due to the presence of important phytochemicals.

KEY WORDS: *Euphorbia antiquorum,* hyperglycemia, insulin resistance, oxidative stress

INTRODUCTION

Diabetes mellitus is a group of heterogeneous disorders associated with hyperglycemia and glucose intolerance due to insulin deficiency, impaired effectiveness of insulin action, or both. Herbal based anti-diabetic drugs are being developed which could replace some of the currently used oral hypoglycemic drugs to ensure better therapeutic outcome and acceptability [1].

Snuhee is an important drug in Ayurveda, for which *Euphorbia neriifolia* L., is the accepted botanical source and *Euphorbia antiquorum* Linn. (Euphorbiaceae) is used as substitute [2]. The leaf, stem, latex, and root of *Snuhee* are used in Ayurveda for the treatment of abdominal disorders, diabetes, edema, psychosis, leprosy, coryza, anemia, and rheumatoid arthritis [3-5]. *E. antiquorum* is used as a sex stimulant [6], laxative [7], and anti-fertility agent [8]; in rheumatism, toothache and nervine diseases [9]; in the treatment of inflammation, swellings on breast, and as a purgative [10]; for earache, dropsy, syphilis, and leprosy [11]. The plant is also used in veterinary practice [12,13]. In the Siddha system of medicine, *E. antiquorum* is known as *Sathura kalli* and is used in the treatment of skin diseases, urticaria, kapham, abdominal disorders, constipation, leucorrhea, and leprosy [3].

The phytoconstituents isolated from *E. antiquorum* are 3-0- angeloyligenol [14]; Eupha 7, 9 (11) 24-trien-3ß-ol ("antiquol C") and certain triterpenes from the latex [15]; terpenoids - friedelane-3ß, 30-dioldiacetate, 30-acetoxyfridelan-3ß-ol, and 3ß-acetoxy fridelan-30-ol from the stem [16]; ingenane type of diterpene esters were isolated from 5 *Euphorbia* species [17]; a diterpene antiquorin along with fridelane-3ß-ol and taraxerol was also isolated from *E. antiquorum* [18].

The stem of *E. antiquorum* has been subjected to extensive pharmacological evaluations including

anti-hyperglycemic [19]; anti-inflammatory and anti-arthritic [20]; antibacterial [21]; antitussive [22]; antibacterial and antifungal [23]; hepatoprotective and antioxidant [24] activities. Anti-hyperglycemic and aldose reductase inhibition activity studies have been reported on some isolated terpenoids [25]. In the present study, evaluation of anti-diabetic property of *E. antiquorum* root has been undertaken since no such studies are reported.

MATERIALS AND METHODS

Plant Material

Roots of *E. antiquorum* were collected from the forest surroundings of Tirunelveli, Tamil Nadu, India, during March 2011. The plant material was identified and authenticated by Dr. S. N. Yoganarasimhan, Plant Taxonomist, following various floras [26,27]. Voucher herbarium specimen (Sri Lalitha 045) along with a sample of the drug tested has been deposited at the herbarium and crude drug museum of Faculty of Pharmacy, M. S. Ramaiah University of Applied Sciences, Bengaluru, Karnataka, India.

Preparation of Extracts

Total alcohol extract was prepared by soxhlation with 95% v/v ethanol (yield 15.4% w/w). The total aqueous extract was prepared by maceration with chloroform water (0.25% v/v of chloroform in distilled water) (yield 13.8% w/w). The alcohol and aqueous extracts were suspended in 2% w/v acacia solution in distilled water for pharmacological studies.

Phytochemical Studies

The dried extracts were subjected to preliminary phytochemical screening to detect the presence of various phytochemical constituents and the extracts were further standardized by high-performance thin-layer chromatography (HPTLC) [28]. Camag HPTLC system equipped with Linomat V applicator, TLC scanner 3, Reprostar 3 with 12 bit CCD camera for photo documentation, controlled by WinCATS-4 software was used. All the solvents used were of HPLC grade obtained from Merck, India. All weighing were done on Precisa XB 12A digital balance. The extract concentration used was 5 mg/ml and pre-coated aluminum plates with silica 60 F_{254} (10 cm × 10 cm) as stationary phase was used. Ethyl acetate:pyridine:water:methanol (80:20:10:5) was used as the mobile phase. Developed plates were then scanned under the wavelengths 254 nm, 366 nm, and 425 nm using deuterium, mercury and tungsten lamps, respectively and photo documented using Camag Reprostar 3.

Pharmacological Studies

Animals

Albino rats (Wistar strain) of either sex 8-12 weeks old, weighing 170-250 g were used in acute toxicity and anti-diabetic studies. The animals were maintained as per Committee for the Purpose of Control and Supervision of Experiments on Animals guidelines and kept at 12 h dark/12 h light cycle. This study was approved by the Institutional Animal Ethics Committee of the institution (IEAC certificate no. MSRCP/M-40/2011).

Acute Toxicity

Acute toxicity studies were carried out following OECD guidelines 420 [29].

Fructose-Induced Insulin Resistance

Insulin resistance was induced in rats by chronic fructose feeding (40% fructose + 60% normal rat chow, 25 g/100 g b.w/day) for a period of 21 days. After 21 days fasting, serum glucose levels were checked and animals with moderate diabetes having serum glucose ≥180 mg/dl were further grouped into the positive control, standard and extract treated groups. Vehicle treated non-diabetic rats were assigned as the normal control group (Group I). Diabetic rats were divided into six groups of six animals each. Untreated diabetic rats served as the positive control group (Group II). Group III was administered standard anti-diabetic drug pioglitazone (10 mg/kg, b.w, p.o). Groups IV-VII were administered the alcohol and aqueous extracts at doses 200 and 400 mg/kg, respectively for 28 days. After 28 days animals were fasted overnight and on the 29th day, blood samples (<1 ml) were collected from the retro-orbital sinus under ether anesthesia. Serum was separated from the clotted blood by centrifugation at 12,000 rpm for 10 min and used for the estimations [30,31].

Streptozotocin (STZ)-Nicotinamide (NA) Induced Type 2 Diabetes Mellitus (NIDDM)

Diabetic mellitus (NIDDM) was induced by a single injection of freshly prepared solution of STZ (65 mg/kg b.w. intraperitoneal [i.p.]) in 0.1 mol/L cold citrate buffer (pH 4.5), 15 min after the administration of NA (230 mg/kg b.w. i.p). After 14 days, fasting serum glucose levels were checked for the development of diabetes. Animals with fasting serum glucose levels ≥180 mg/dl were further grouped into the positive control, standard and extract groups. Vehicle treated non-diabetic rats were assigned as the normal control group (Group I). Group II was the positive control, in which vehicle-treated diabetic rats were included. Group III was the standard group which was administered with glimepiride 0.5 mg/kg. Groups IV and V were administered the alcohol extract at dose 200 and 400 mg/kg, respectively, and Groups VI and VII were administered the aqueous extracts of *E. antiquorum* roots at doses 200 and 400 mg/kg respectively. Each group consisted of six animals. The treatment schedule was once daily for 28 days by oral administration. On the 29th day, blood (<1 ml) was withdrawn by retro-orbital puncturing under ether anesthesia. The animals were kept for overnight fasting prior to blood withdrawal [32-34].

Glucose [35], total cholesterol (TC) and triglycerides (TG) [36], glycosylated hemoglobin (GH_b) [37] were tested in serum for both models using commercial diagnostic kits.

Following blood withdrawal, the animals were sacrificed by an excess of anesthesia and liver was isolated. The liver was washed and used for preparation of homogenates - 10% w/v liver homogenate in 0.15 M potassium chloride buffer, used for the estimation of malondialdehyde (MDA) [38]; 10% w/v liver homogenate in 0.25% w/v sucrose in phosphate buffer (pH 7.4), used for the estimation of glutathione (GSH) [39]; 1% w/v liver homogenate in 5% trichloroacetic acid, used for the estimation of the liver glycogen [40].

Statistical Analysis

The data were expressed as mean ± SEM and tested with one-way analysis of variance followed by Tukey Kramer multiple comparison test.

RESULTS

Phytochemical Analysis

Preliminary phytochemical analysis revealed the presence of carbohydrates and glycosides; phenolic compounds and tannins; flavonoids.

HPTLC Studies

The alcohol extract at 254 nm revealed 6 phytoconstituents with no characteristic fluorescence [Figure 1]. At 366 nm, 3 phytoconstituents were revealed of which, one spot having R_f 0.59 exhibited blue fluorescence and another with R_f 0.66 exhibited light blue fluorescence. At 425 nm, 1 phytoconstituent having R_f 0.92 was revealed.

The aqueous extract revealed 18 phytoconstituents at 254 nm with no characteristic fluorescence [Figure 2]. At 366 nm, the aqueous extract revealed 10 spots and those with R_f values 0.64 and 0.75 were prominent. Spot with R_f 0.64 exhibited light blue fluorescence, whereas the one with R_f 0.75 exhibited dark blue fluorescence. The alcohol extract revealed 3 phytoconstituents with Rf values 0.03, 0.34, and 0.92 at 425 nm.

Acute Toxicity

Both the alcohol and aqueous extracts were found to be safe up to 2000 mg/kg.

Fructose-Induced Insulin Resistance

Administration of fructose for 21 days caused the development of hyperglycemia (≥180 mg/dl) in all the animals. The treatment with extracts of *E. antiquorum* roots 200 and 400 mg/kg resulted in significant ($P < 0.001$) lowering of serum blood glucose levels compared to the untreated diabetic control animals.

Serum of the diabetic control animals showed significantly ($P < 0.001$) increase in the TC levels. Serum TG levels were also high in the untreated diabetic animals. Treatment with extracts significantly reduced the elevated lipid levels.

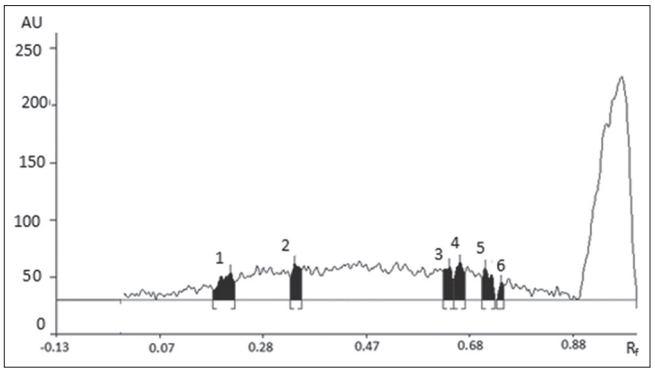

Figure 1: High-performance thin-layer chromatography fingerprint of alcohol extract of the root of *Euphorbia antiquorum* at 254 nm

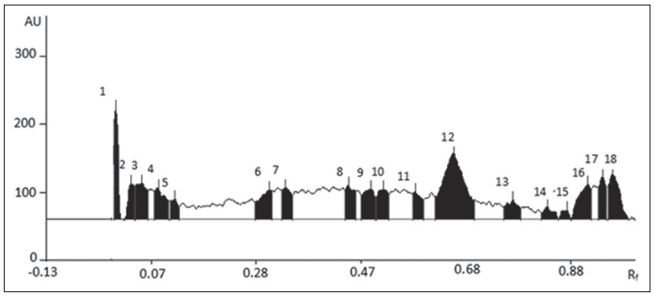

Figure 2: High-performance thin-layer chromatography fingerprint of aqueous extract of the root of *Euphorbia antiquorum* at 254 nm

Significant ($P < 0.001$) reduction in TC levels was observed in the groups treated with 400 mg/kg dose of both extracts. However, TG levels were significantly lowered with the dose of 200 mg/kg as well. GH_b levels were significantly ($P < 0.001$) lowered in the all test drug-treated groups when compared to control [Table 1].

Hepatic GSH levels decreased significantly ($P < 0.001$) in the positive control rats. This was significantly ($P < 0.05$ and $P < 0.001$) increased in animals treated with the higher dose of alcohol and aqueous extracts respectively. Liver glycogen levels decreased significantly ($P < 0.001$) in the positive control group. In groups treated with the alcohol extract, liver glycogen levels increased significantly ($P < 0.01$, $P < 0.001$ for 200 and 400 mg/kg, respectively). The aqueous extract at 400 mg/kg dose also showed significantly ($P < 0.001$) increase in hepatic glycogen levels. Hepatic MDA levels were significantly ($P < 0.001$) high in the diabetic control rats, indicating lipid peroxidation. However, treatment with extracts significantly ($P < 0.001$) reduced the extent of lipid peroxidation [Table 2].

STZ-NIDDM

Fasting serum glucose levels of positive control rats were significantly ($P < 0.001$) higher than the normal rats. The alcohol and aqueous extracts of *E. antiquorum* roots exhibited significant anti-hyperglycemic effects. There was a significant ($P < 0.001$) decrease in serum glucose levels with 200 and 400 mg/kg of alcohol and aqueous extracts. GH_b levels were significantly increased in the diabetic control animals and were

significantly ($P < 0.001$) lowered in the extract treated groups [Table 3].

Serum TC levels were significantly ($P < 0.001$) increased in the positive control group. There was a significant ($P < 0.001$) decrease in the cholesterol levels in animals treated with the higher dose of alcohol and aqueous extracts. The serum TG levels were also significantly ($P < 0.001$) high in the diabetic control group, and this was significantly ($P < 0.001$) controlled in the extract treated groups.

Hepatic GSH levels were significantly reduced ($P < 0.001$) in the positive control animals and significant ($P < 0.001$) increase was observed in both the extract treated groups. Administration of STZ and NA caused extensive lipid peroxidation which was evidenced by the significant ($P < 0.001$) increase in hepatic MDA levels in the diabetic control animals. Lipid peroxidation was also significantly ($P < 0.001$) lowered in the groups treated with the alcohol and aqueous extracts of *E. antiquorum* roots. A significant increase in liver glycogen levels was observed in the alcohol ($P < 0.01$, $P < 0.001$) and aqueous ($P < 0.001$) extract treated groups [Table 4].

DISCUSSION

Administration of both STZ and NA by i.p. injection induces experimental diabetes in rats. STZ (2-deoxy-2-({[methyl (nitroso) amino] carbonyl} amino)-β-D-glucopyranose) is a naturally occurring compound, produced by *Streptomyces achromogenes*, and it causes pancreatic β-cell damage. NA is administered partially to protect the insulin-secreting cells against STZ [41].

The anti-hyperglycemic activity of *E. antiquorum* extracts was compared with glimepiride, the second generation anti-hyperglycemic drug. Oral administration of *E. antiquorum* extracts and glimepiride to STZ-NA-induced diabetic rats decreased the serum glucose levels.

Increase TC and TG levels were observed in the untreated diabetic control rats. In diabetic rats treated with *E. antiquorum*

Table 1: Effect of *E. antiquorum* root extracts on serum parameters in fructose-induced insulin resistance

Treatment/groups	Glucose	TC	TG	GH_b
Normal control	101.14±2.54	41.95±1.69	70.69±1.67	12.40±0.27
Positive control	249.82±7.42[a]	77.26±8.01[a]	80.90±4.38[ns]	17.86±0.80[ns]
Standard (pioglitazone)	141.17±9.09***	32.05±2.16***	36.27±2.71***	4.54±0.37***
Alcohol extract 200 mg/kg	181.29±3.87***	69.74±2.90[ns]	75.22±2.36***	9.29±2.35***
Alcohol extract 400 mg/kg	154.68±6.46***	71.23±6.51***	67.79±3.92***	9.08±1.35***
Aqueous extract 200 mg/kg	190.54±4.56***	67.19±5.20[ns]	81.40±2.66***	8.34±0.77***
Aqueous extract 400 mg/kg	136.56±12.62***	41.62±4.56***	58.84±1.93***	7.45±1.35***

One-way analysis of variance. The values are expressed as mean±SEM; n=6 animals in each group. Tukey-Kramer multiple comparison test ***P<0.001, **P<0.01, *P<0.05 versus positive control; [a]P<0.001 versus normal control group. SEM: Standard error of mean, TC: Total cholesterol, TG: Triglycerides, GH_b: Glycosylated hemoglobin, *E. antiquorum: Euphorbia antiquorum*

Table 2: Effect of *E. antiquorum* root extracts on hepatic parameters in fructose-induced insulin resistance

Treatment/groups	Liver weight	MDA	GSH	Glycogen
Normal control	4.03±0.45	1.09±0.10	61±1.53	1079.32±73.26
Positive control	3.35±0.22[b]	2.15±0.12[a]	32.10±1.57[a]	606.46±19.97[a]
Standard (pioglitazone)	3.94±0.09[ns]	0.18±0.01***	69.75±1.95***	2062.55±59.62***
Alcohol extract 200 mg/kg	3.69±0.18*	1.19±0.17***	42.42±1.75[ns]	942.51±104.73**
Alcohol extract 400 mg/kg	3.78±0.12[ns]	0.45±0.03***	53.67±10.57*	1237.98±13.26***
Aqueous extract 200 mg/kg	3.35±0.14***	0.98±0.07***	46.25±0.97[ns]	836.17±36.79[ns]
Aqueous extract 400 mg/kg	3.54±0.09***	0.58±0.02***	65.22±1.20***	1072.92±16.16***

One-way analysis of variance. The values are expressed as mean±SEM; n=6 animals in each group. Tukey-Kramer multiple comparison test ***P<0.001, **P<0.01, *P<0.05 versus positive control; [a]P<0.001, [b]P<0.01 versus normal control group. SEM: Standard error of mean, MDA: Malondialdehyde, *E. antiquorum: Euphorbia antiquorum*, GSH: Glutathione

Table 3: Effect of *E. antiquorum* root extracts on serum parameters in STZ-NIDDM

Treatment/groups	Glucose	TC	TG	GH_b
Normal control	101.14±2.54	41.95±1.69	70.69±1.67	12.40±0.27
Positive control	221.5±20.89[a]	83.31±2.49[a]	101.45±3.35[a]	18.90±0.58[a]
Standard (glimepiride)	86.98±1.05***	40.94±2.28***	41.14±1.70***	4.94±0.42***
Alcohol extract 200 mg/kg	142.16±2.60***	78.15±2.0[ns]	65.11±2.84***	9.59±0.67***
Alcohol extract 400 mg/kg	107.70±2.16***	52.72±3.8***	68.69±2.70***	9.59±0.67***
Aqueous extract 200 mg/kg	160.36±3.82***	77.49±2.4 [ns]	74.10±2.30***	11.95±0.72***
Aqueous extract 400 mg/kg	151.51±3.56***	33.00±3.07***	83.75±2.30***	5.69±0.46***

One-way analysis of variance. The values are expressed as mean±SEM; n=6 animals in each group. Tukey-Kramer multiple comparison test ***P<0.001, **P<0.01, *P<0.05 versus positive control; [a]P<0.001 versus normal control group. SEM: Standard error of mean, TC: Total cholesterol, TG: Triglycerides, GH_b: Glycosylated hemoglobin, *E. antiquorum: Euphorbia antiquorum*

Table 4: Effect of *E. antiquorum* root extracts on hepatic parameters in STZ-NIDDM

Treatment/groups	Liver weight	MDA	GSH	Glycogen
Normal control	4.03±0.45	1.09±0.10	61±1.53	1079.32±73.26
Positive control	3.35±0.22[b]	2.16±0.09[a]	26.88±1.62[a]	517.96±94.03[a]
Standard (glimepiride)	4.11±0.06**	0.36±0.02***	73.39±0.40***	1550.82±26.88***
Alcohol extract 200 mg/kg	3.61±0.06[ns]	1.25±0.10***	42.86±0.30***	872.10±38.30**
Alcohol extract 400 mg/kg	3.85±0.03[ns]	0.71±0.02***	49.50±2.88***	985±56.21***
Aqueous extract 200 mg/kg	3.74±0.06[ns]	1.56±0.04***	44.74±0.43***	961.36±19.98***
Aqueous extract 400 mg/kg	3.85±0.06[ns]	0.77±0.04***	62.17±1.01***	1207.36±49.14***

One-way analysis of variance. The values are expressed as mean±SEM; $n=6$ animals in each group. Tukey-Kramer multiple comparison test ***$P<0.001$, **$P<0.01$, *$P<0.05$ versus positive control; [a]$P<0.001$, [b]$P<0.01$ versus normal control group. NIDDM: Nicotinamide-induced Type 2 diabetes mellitus, MDA: Malondialdehyde, *E. antiquorum: Euphorbia antiquorum*, GSH: Glutathione

extracts and glimepiride, the levels of TC and TG were significantly lowered as compared to the diabetic control. GH_b levels increase over long periods of time in diabetes. In the diabetic condition, an excess of glucose present in the blood reacts with hemoglobin to form GH_b. The rate of glycation or glycosylation is proportional to the concentration of glucose in the blood. In the current study, the untreated diabetic rats indicated the higher levels of GH_b in blood compared to the normal rats. Serum of animals treated with the *E. antiquorum* extracts and glimepiride showed a significant decrease in GH_b levels.

The liver plays an important role in buffering the post-prandial hyperglycemia and is involved in the synthesis of glycogen. Diabetes mellitus impairs the normal ability of the liver to synthesize glycogen. Glycogen depletion causes the mobilization of fat to meet the body's metabolic demands [42]. Hepatic glycogen levels were significantly ($P < 0.001$) lowered in the untreated diabetic control group and this abnormality was brought back to near normal levels in the extract treated groups.

The untreated diabetic animals in the present study registered low levels of GSH and high levels of MDA, suggesting its increased utilization to overcome the oxidative stress, while the significant elevation of GSH levels in the treated animals coincided with a significant decline in lipid peroxidation.

Fructose is an important dietary source of carbohydrates and is a simple sugar present in fruits and honey. Fructose induces insulin resistance by obesity-associated mechanisms. Hepatic triglyceride accumulation may result in protein kinase C activation and insulin resistance due to increased uptake of free fatty acids. The high-fructose diet was found to increase the serum levels of glucose, TG and TC, a phenomenon commonly associated with diabetes mellitus [43]. These are known to be high-risk factors in the development of cardiovascular disorders including hypertension. Results of this study showed that *E. antiquorum* root extracts possess lipid-lowering effects in fructose-induced insulin resistance.

Administration of alcohol and aqueous extracts reduced the MDA level in fructose-fed rats to levels similar to those of normal rats. This finding suggests that chronic oral treatment with higher doses of alcohol and aqueous root extracts of *E. antiquorum* prevent lipid peroxidation in the fructose-induced diabetic rats. The reduction in plasma MDA levels in normal rats treated with the extract provides further evidence that the extract possess anti-diabetic activity.

Flavonoids, phenolic compounds and glycosides were detected in preliminary phytochemical screening of the root extracts of *E. antiquorum*. Earlier evidence reveal the anti-diabetic potential of these phytoconstituents and the presence of these phytoconstituents in the extracts of *E. antiquorum* root could be responsible for their anti-diabetic activity [42,44].

The results of this study confirmed the anti-diabetic potential of *E. antiquorum* root and helps in substantiating the use of *E. antiquorum* as a potential drug in the treatment of diabetes. The study also substantiates the use of *E. antiquorum* as a substitute for *E. neriifolia* which is the accepted botanical source of the Ayurveda drug *Snuhee*.

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Original Research

Efficacy and phytochemical analysis of latex of *Calotropis procera* against selected dermatophytes

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ABSTRACT

Background: Since ancient time, increased interest has been witnessed in the use of an alternative herbal medicine for managing, and the treatment of fungal diseases worldwide. This may be connected to the cost and relative toxicities of the available antifungal drugs. It has been a known tradition practiced in the northern part of Nigeria that parents and teachers use the white latex of *Calotropis procera* to treat *Tinea capitis* in children attending the local religious school in the area. This study was conducted in 2009 to ascertain the above claim. **Materials and Methods:** Fresh latex of *C. procera* was screened for their antifungal activity against species of dermatophytes: *Trichophyton* spp., *Microsporum* spp. and *Epidermophyton* spp. using the agar incorporation method. **Results:** The result shows that the latex inhibits the *in vitro* growth of these pathogenic fungi to varying extents with *Trichophyton* spp. being the most susceptible ($P < 0.05$) and thus highly inhibited by the latex followed by the *Microsporum* spp. and *Epidermopyton* spp. was least inhibited. These inhibitions followed a dose-dependent trend as undiluted latex (100%) gave the highest inhibitory impacts ($P < 0.05$) when compared to serially diluted latex. The phytochemical analysis of the fresh latex indicated the presence of alkaloids, saponin, tannins, steroids, flavonoids, anthraquinone, and triterpenoids. **Conclusion:** The findings of this study confirmed the perceived usefulness of the latex in the treatment of *T. capitis* (ringworm) practiced in our society and therefore, its use topically in the treatment of dermatomycotic infection is encouraged.

KEY WORDS: Agar incorporation method, antifungal activity, dermatomycotic, latex

INTRODUCTION

Dermatophytes are a group of three fungal genera that can invade keratinized tissues and therefore able to cause superficial infections of the skin, nails and hair, thereby producing a disease referred to as ringworm in human and animals [1]. The infection is mostly cutaneous, i.e. restricted to the non-living cornified layer of the skin due to the innate inability of these groups of fungi to penetrate beyond the keratinized tissues or organs of the immunocompetent host [2].

The genus Calotropis (*Calotropis gigantea* and *Calotropis procera*) belongs to the family of Asclepiadaceae. These shrubs have been reported to exhibit a lot of medicinal properties which includes the antimicrobial, antimycotic, and anti-inflammatory effect [3].

C. procera is commonly called calotrope; other names are King's crown, kapok tree, Tumfafiya (in Hausa language), Bomubomu (Yoruba language). It is a spreading shrub with large grey-green leaves and large green inflated fruit similar in shape to a mango. A whitish sap (the latex) oozes out when the plant's stem is broken. The plant is native to tropical Africa and Asia. Studies from phytochemical analysis of *C. procera* suggest the presence of biologically active compounds such as Alkaloids, steroids, triterpenes; others include madaralbun, madarfluavil, caoutchouc, and calotropin [4].

The use of plant extracts for medicinal purposes is very widespread in the world, Nigeria inclusive. Many of these medicinal plants were being used against infectious disease causing agents, which are frequent nowadays, due to the emergence and increase in antimicrobial resistance and poor hygienic condition of our

environments. The increasing incidences of fungal infections coupled with the gradual rise in azole resistance and available antibiotics had highlighted the need to find more alternative antifungal agents from other sources [5]. Several plants have been shown to contain some significant amount of antifungal activity on a wide range of microorganisms [6]. The aqueous extract of the aerial part of *C. procera* is a prominent decoction used in Saudi Arabia for the treatment of varieties of diseases such as muscular spasm, joint pain, constipation, and fever [6]. Locally, the extract of *C. procera* is used topically for the treatment of ringworm [7]. In northern Nigeria, the latex, leaves, root, stem bark, and fresh follicles of *C. procera* were used in indigenous practice to treat topical fungal diseases, convulsion, asthma, cough and inflammation [8,9].

In Sokoto town (the area of the study), a study by Ameh and Okolo in 2004 [10] revealed the incidence of dermatomycosis among primary school pupils, and the study has attributed the observed incidence to the domestic animals as an important predisposing factor. It is evident from a survey (personal communication with the elderly people within the locality) that fresh latex of *C. procera* has been used from ancient time as a topical antimycotic treatment in the area.

This study was carried out to elucidate the antimycotic activity of the fresh latex of *C. procera* against selected representative species of the dermatophytes (the etiologic agent of dermatomycosis), and clarify the above claim by the local people.

MATERIALS AND METHODS

Plant Material

Fresh latex analysis of variance (the white liquid secretion) of *C. procera* was collected from the farmlands around Usmanu Danfodiyo University permanent site, Sokoto, Nigeria, in October 2009. The latex was collected into a sterile wide-necked screw-capped container by deliberately breaking the smooth stem and milky sap ooze out from the stem; this was repeated continuously until the required volume of the latex was tapped. The fresh latex was serially diluted 2-fold and 5-fold with sterile distilled water to give 50% and 20% of the original latex concentration respectively.

Phytochemical Analysis

The fresh latex of *C. procera* was sent to the Biochemistry laboratory of the department of Biochemistry, Faculty of Science, Usmanu Danfodiyo university, Sokoto for phytochemical analysis. The latex was analyzed for the presence of alkaloids, flavonoids, tannin, saponin, triterpenoids, anthraquinones, and glycosides compounds using the standard colorimetric procedures as described by Sofowora and Kennedy and Thorley [11,12].

The Fungal Species Used

Representative isolate each of *Trichophyton* spp., *Microsporum* spp. and *Epidermophyton* spp. were used in this study. The isolates were kindly provided by Mr. Abdulrahman Barau of the Mycology laboratory of the Biological science department of the Usmanu Danfodiyo University, Sokoto.

Antifungal Susceptibility Testing

Fresh latex of *C. procera* was examined for its antifungal properties against *Trichophyton* spp., *Microsporum* spp. *and Epidermophyton* spp. - the causative agents of dermatomycosis (ringworm). Four dilution groups were prepared for this study, this includes a negative control (sterile distilled water instead of latex), original, fresh latex (100%), 2-fold serially diluted latex (50%) and 5-fold serially diluted latex (20%) groups.

The antifungal assay of the latex was conducted using the agar incorporation method as described by Taudou and Dwivedi and Dubey [13,14]. Briefly, the aforementioned concentrations (sterile distilled water, 100%, 50% and 20% latex) were aseptically mixed in a ratio of 1:3 with sterile sabouraud dextrose agar (SDA) and poured in 150 mm × 30 mm petri dishes, allowed to solidify and seeded in duplicates with fungal isolates previously cultivated on SDA. The inocula was aseptically cut with a sterile 10 mm cork borer, seeded in the middle of the petri dishes and incubated at 28°C-30°C in the dark. The growth of the dermatophytes on each culture plate was measured linearly (growth diameter) by the use of transparent millimeter rule daily for 6 days.

Statistical Analysis

An SPSS 20® statistical software was used for statistical analysis. The data generated in the study were presented in the form of tables using frequency distribution. Average daily mycelial growth was analyzed using one-way (ANOVA) with Tukey's multiple comparisons testing to determine the significant differences between the control and experimental groups. All comparisons were considered to be significant at $P < 0.05$.

RESULTS

The latex of *C. procera* was found to inhibit the *in vitro* growth of the three dermatophytic fungi studied to varying extents. Tables 1-3 show a measure of a diameter of mycelial spread for each of the fungi tested. Statistical analysis of the result shows that *Trichophyton* spp. was the most susceptible, and thus highly inhibited by the latex followed by the *Microsporum* spp. and *Epidermophyton* spp. was the least inhibited. It is observed that

Table 1: Measure of mycelial spread of *Trichophyton* spp. grown on SDA incorporated with varying concentration of *C. procera* latex

Latex concentration	Average diametric mycelial spread (mm) over days					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
(Negative control)	5.6	32.6	55.6	81.0	86.6	89.6
Undiluted (100%)	6.7	7.5	14.0	21.4	21.1	39.7
2-fold dilution (50%)	6.8	7.4	15.2	20.2	30.7	45.8
5-fold dilution (20%)	5.6	10.2	12.3	25.7	35.7	51.6

C. procera: Calotropis procera, SDA: Sabouraud dextrose agar

100% (undiluted latex) of *C. procera* gave the highest inhibitory impact on the dermatophytes, whereas 20% latex recorded the lowest.

The diametric mycelial spread of the *Trichophyton* spp. ranges from 6.7 mm to 39.7 mm; the *Microsporum* spp. 7.3 mm to 65.1 mm and *Epidermophyton* spp., 8.3 mm to 72.7 mm. Statistical analysis shows that *Trichophyton* spp. was most susceptible, then *Microsporum* spp. and *Epidermophyton* spp. the least inhibited by the 100% latex ($P < 0.05$) [Table 1].

Similar trends of growth of the dermatophytes were recorded at the other concentration of 50, and 20% latex. However, the inhibition of their growth by the latex reduced with decreasing concentrations. All the three concentrations of the latex tested (i.e. 100, 50 and 20%) were significantly ($P < 0.05$) better than the control (standard). The result of this study indicated that *Trichophyton* spp. was the most sensitive, followed by *Microsporum* spp. while the *Epidrmophyton* spp. was the most resistant ones.

The result of the phytochemical analysis of the fresh latex of *C. procera* shows the presence of alkaloids, saponin, tannins, steroids, flavonoids, anthraquinone and triterpenoids [Table 4].

Table 2: Measure of mycelial spread of *Microsporum* spp. grown on SDA incorporated with varying concentration of *C. procera* latex

Latex concentration	Average diametric mycelial spread (mm) over days					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
(Negative control)	10.0	16.3	32.6	45.5	69.0	84.0
Undiluted (100%)	7.3	10.8	18.2	29.2	49.5	65.1
2-fold dilution (50%)	7.0	10.2	14.0	39.0	58.6	70.8
5-fold dilution (20%)	8.8	13.3	22.0	37.3	52.4	62.0

C. procera: Calotropis procera, SDA: Sabouraud dextrose agar

Table 3: Measure of mycelial spread of *Epidermophyton* spp. grown on SDA incorporated with varying concentration of *C. procera* latex

Latex concentration	Average diametric mycelial spread (mm) over days					
	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6
(Negative control)	15.0	30.0	46.5	72.0	84.0	88.5
Undiluted (100%)	8.3	20.7	38.3	52.7	61.2	72.7
2-fold dilution (50%)	11.6	18.1	32.8	46.0	56.5	56.5
5-fold dilution (20%)	12.3	16.8	28.8	45.2	51.0	68.7

C. procera: Calotropis procera, SDA: Sabouraud dextrose agar

Table 4: Results of the phytochemical analysis of the latex of *C. procera*

Phytochemicals	Amount
Alkaloids	+++
Saponins	++
Flavenoid	++
Tannins	+
Steroids	+++
Anthraquinone	+++
Triterpenoids	+++

Key: +: Trace amount, ++: Moderate amount, +++: Appreciable amount, *C. procera: Calotropis procera*

DISCUSSION

Various parts of *C. procera* has been reported to be used in many countries for the treatment of varieties of diseases, such as muscular spasm, joint pain, constipation, skin diseases and etc. [6]. The results of the present study indicated that the latex of *C. procera* has antifungal potentials against dermatophytes. This finding agreed with that of Kuta, 2008, who reported the same tradition of using the *C. procera* extracts in Gwari communities of Niger State, Nigeria, for the treatment of ringworm which stimulated his interest in evaluating the aqueous extracts of the plant and found it to display a significant inhibitory effect on the dermatophytes tested even at low concentration of the extracts.

The findings of this study are also in agreement with that of Halua and Vidyasagar 2012 who evaluated leaves extracts of two calotropis species (*C. gigantea* and *C. procera*) using three different solvents against dermatophytes and *Aspergillus flavus* with chloroform extract having the highest inhibition observed. Similarly, *C. procera* leaves extract was reported to have antifungal activity towards the tree dermatophytes genera: *Microsporum* spp., *Trichophyton* spp. and *Epidermophyton* spp. [15].

Furthermore, Iqbal *et al.* [3] reported the comparative efficacy of the chloroform and ethyl acetate *C. procera* leaf and latex extracts which proved active against some dermatophytes and other pathogenic fungi. However, the only dermatophytic fungi used in that study (*Microsporum boulardii*) was not inhibited by the extracts. This contrast with the present studies and could be explained by differences in the preparation of the plant products used and the methodology of the assay used to assess the efficacy of the plant latex.

Studies have reported several plant extracts to inhibit the growth of dermatophytes. Some of which include that of Alade and Irobi [16], who established that the ethanolic extract of *Acalypha wilkesian* had an antifungal effect on *Trichophyton mentagrophytes*, *Trichophyton rubrum*, *A. flavus* and *Candida albicans*. Verástegui *et al.* [17], showed that the alcoholic extract of *Agave lecheguilla* has an antifungal effect on *Microsporum gypseum*, *Candida albicans*, and *Candida neoformans*. Chevallier[18] described the use of extract of *Ulmus campestris* (Elm tree), *Melissa ofinicalis* (Balm tree), and *Juglans duclouxiana* (Walnut tree) against various dermatomycotic infections.

The result of the phytochemical analysis of the fresh latex of *C. procera* has indicated the presence of alkaloids, saponin, tannins, steroids, flavonoids, anthraquinone and triterpenoids. This result is similar to other reports on the leaves, stem and roots of *C. procera* in other studies [9,19,20]. Previous studies of phytochemical analysis of *C. procera* suggest the presence of biologically active compounds such as alkaloids, steroids, triterpenes; others include madaralbun, madarfluavil, caoutchouc, and calotropin, a very active poison of the digitalis type [21]. However, the chemical components responsible for the antifungal activity, and the mechanisms of action remain to be investigated. Though the

mechanism of action of the drug is not known, but antimycotics generally inhibit fungal growth by either disrupting fungal membrane permeability, inhibiting sterol synthesis, inhibiting the nucleic acid synthesis, or protein synthesis [22].

The results of the present study show that the plant latex is an effective antimycotic agent against dermatomycosis *in vitro*. This finding shows that there is an element of truth in the claim of traditional healers on the medicinal value of this plant as an antidermatophytic agent. Therefore, the use of the plant latex in treating dermatomycotic infections should be encouraged and the government shall pay more attention to our local medicinal plants and help in processing them, which will create more job opportunities and will bring about a reduction in the cost of conventional antifungal drugs.

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Review Article

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Potential antimalarials from African natural products: A reviw

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ABSTRACT

Malaria remains an overwhelming infectious disease with significant health challenges in African and other endemic countries globally. Resistance to antimalarial drugs has become one of the most momentous challenges to human health, and thus has necessitated the hunt for new and effective drugs. Consequently, few decades have witnessed a surfeit of research geared to validate the effectiveness of commonly used traditionally medicines against malaria fever. The present review work focuses on documenting natural products from African whose activity has been reported *in vivo* or *in vitro* against malaria parasite. Literature was collected using electronic search of published articles (Google Scholar, PubMed, Medline, Sciencedirect, and Science domain) that report on antiplasmodial activity of natural products from differernts Africa region. A total of 652 plant taxa from 146 families, 134 isolated antimalarial compounds from 39 plants species, 2 herbal formulations and 4 insect/products were found to be reported in literature from 1996 to 2015. Plants species from family Asteraceae (11.04%), Fababceae (8.128%), Euphorbiaceae (5.52%), Rubiaceas (5.52%), and Apocyanaceae (5.214%), have received more scientific validation than others. African natural products possess remarkable healing properties as revealed in the various citations as promising antimalarial agents. Some of these natural products from Africa demonstrate high, promising or low activities against *Plasmodium* parasite. This study also shows that natural products from Africa have a huge amount of novel antimalarial compounds that could serve as a leads for the development of new and effective antiplasmodial drugs. However, in a view of bridging the gap in knowledge, clinical validation of these natural products are of paramount importance.

KEY WORDS: Africa, insect, malarial, plants, plasmodial

INTRODUCTION

Malaria remains an overwhelming infectious disease with significant health challenges in African and other endemic countries globally. Over the last decade, prevalence of malaria has been increasing at an alarming rate, especially in third world countries. According to the rescent reports 3.3 billion peoples are at risk of contacting the infection of which 1.2 billion are at high risk. In 2013, an estimated 198 million cases of malaria with 755,000 deaths, 90% of which occur in Africa were documented [1]. According to Joy *et al*. [2], in Africa 3000 children die of malaria daily. Nigeria, the giant of Africa has been reported with the highest prevalence of malaria cases in African region, with all-year round transmission in the South, and more seasonal in the North [3]. About 60 million Nigerians, have malaria more than once in a year, with pregnant women and children (under 5 years) being more susceptible to the attack due to their low resistance and therefore constitute 92% of the prevalence [4].

The species of *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium Malariae*, and *Plasmodium falciparum* have been implicated in the etiology of the infection [5]. However, the control of these parasites using synthectic antimalarial drugs such as primaquine and chloroquine have been hindered by rapid parasite resistance to these drugs over the few decades [3]. The drug resistance developed by these parasites has therefore necessitated the hunt for more effectual antimalarial agents from natural products. In malaria endemic countries of the world, natural and traditional products (plants and insects/products) are commonly used arsenal to to combat malaria [6]. Therefore, there exists a brawny thought that if these natural products used by the traditional herbalists were not helpful, malaria would have shattered Africa long time ago [7]. Following an extensive survey of the literature, Willcox and Bodeker [8], documented over 160 families of plants with over 1200 species traditionally used for malaria treatment, some of which have been scientifically validated *in vitro* and/or *in vivo* for their claimed activity against the infection. Furthermore, conventional antimalarial drugs such as: Quinine and artemisinin were originated from plant extracts: *Cinchona calisaya* [9] and *Artemisia annua* [10], respectively. This has enthused many researchers especially in Africa to further intensify the search for antimalarial agents

from plant/insect compendium. Currently, the available review on anti-malarial agents focus only on Nigerian plants [11,12], alkaloids, and terpenoids only [5]. This paper has been presented to detail the efforts of African scientists toward finding more effective and cost efficient antimalarial agents from plants and insect (natural products). This will serve as an updated source for recent progress in the recognition of promising antimalaria agents. This paper will also motivate and served as point of reference for scientists, who are willing to work on the subject matter.

MATERIALS AND METHODS

Information for this study was obtained as described previously [11,13], using electronic search of published articles on Google Scholar, PubMed, Medline, Sciencedirect, Science domain. The search keywords include malaria, antimalarial, ethnobotany, African medicinal plants, natural product, antimalaria compounds, suppressive, curative, *in vitro*, *in vivo*, *P. falciparum*, and *P. berghei*. Informations documented on the natural products reviewed in here include the plant species, family, part of the plant used, extraction solvent, methods of antimalarial study (*in vivo/in vitro* or suppressive/curative), strain of the parasite tested, degree of activities and isolated compounds of African grown natural product from 1996 to 2015. Natural products whose level of antimalarial activities was not indicated by author, as well as that were reported outside African countries were completely excluded from this study.

RESULTS AND DISCUSSION

Figure 1 presented the regional distribution of African plants with antimalaria activities. A total of 652 plant species from 146 families and 4 insects/products were found. The activities of 558 plants were found to be reported *in vivo*, while 94 were reported *in vivo*. Plants species from family Asteraceae (11.04%), Fababceae (8.128%), Euphorbiaceae (5.52%), Rubiaceas (5.52%), Apocyanaceae (5.214%), Rutaceae (4.90%), Anonaceae (3.844%), Meliaceae (3.844%), Lamiaceae (3.52%), Combrataceae (2.76%), and Poaceae (2.60%) have received more scientific validation than others. About 36.80% of plants reviewed were grown in West Africa especially Nigeria, Bennin, 31.90% from South Africa, 13.03% from North Africa, 10.88% from Central Africa while 7.36% of the plants were grown from East Africa. The species, family, part use, extraction solvent, as well as inhibitory concentration 50% (IC_{50}) or minimal inhibitory concentration of *in vitro* assayed plant were presented in Tables 1-5. Datas on *in vivo* assayed plants were shown in Table 6. Phyto-chemistry studies of the anti-malarial plants led to the isolation of 134 specific antimalarial compounds from 39 plants species (Table 7).

For the purpose of this work and in accordance with WHO guidelines [14], antimalarial activity of plant extract reviewed in here was classified as highly active (IC_{50} < 5 μg/ml), promising activity (IC_{50} = 5-15 μg/ml), moderate activity (IC_{50} = 15-50 μg/ml), while extract with IC_{50} > 50 μg/ml were considered to be inactive. Furthermore, some authors presented their results

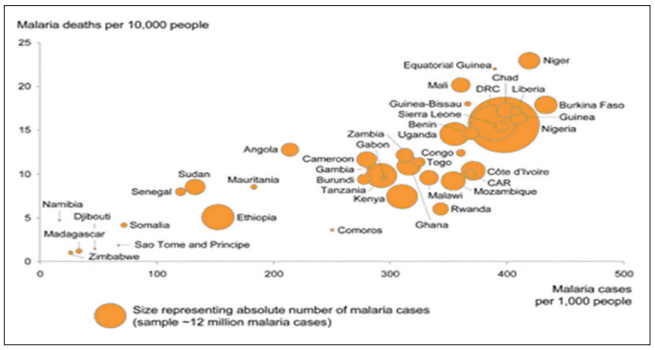

Figure 1: Malaria cases and death in Africa: Countries with negligible burden, such as Algeria, Botswana, Cape Verde, Egypt, Eritrea, Mayotte, Morroco, Swaziland, and South Africa, are not shown

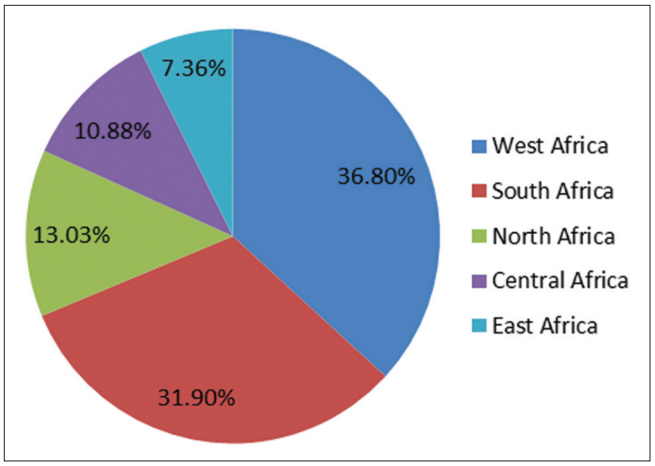

Figure 2: Regional distribution of African plant with potential antimalarial activities

in form of parasite inhibition at particular dose; however, degree of activities reported for such plants could not be classified.

Anti-malarial Activity of Plants from West Africa

Out of the total 170 plants species (53 family) found in West Africa, only 23 were highly active (IC_{50} < 5 μg/ml). The most outstanding activity were demonstrated by methanol stem barks extract of *Parkia biglobosa* (IC_{50} = 0.51 μg/ml) [15]. Ether leaf extract of the *Tithonia diversifola* (IC_{50} = 075 μg/ml) [16], aqueous (AQS) leaf extract of the *Nauclea latifolia* (IC_{50} = 0.60 μg/ml) [17], and *Guiera senegalensis* (IC_{50} = 0.79 μg/ml) [18]. The high antiplasmodial activities demonstrated by these plants render them a good candidate for the identification and isolation of anti-malarial compounds that could serve as a backbone for drug development [13]. A total of 27 plants species demonstrate promising activity (IC_{50} = 5-15 μg/ml), 55 plants species demonstrate moderate activity (IC_{50} = 15-50 μg/ml), while extract from remaining plant species were inactive (IC_{50} > 50 μg/ml).

It is generally known that the bioactive constituents of plant extracts varies with the solvent used in the extraction process [19,20]. These variations were observed in antimalarial activity of West Africa plant. For example dichloromethane

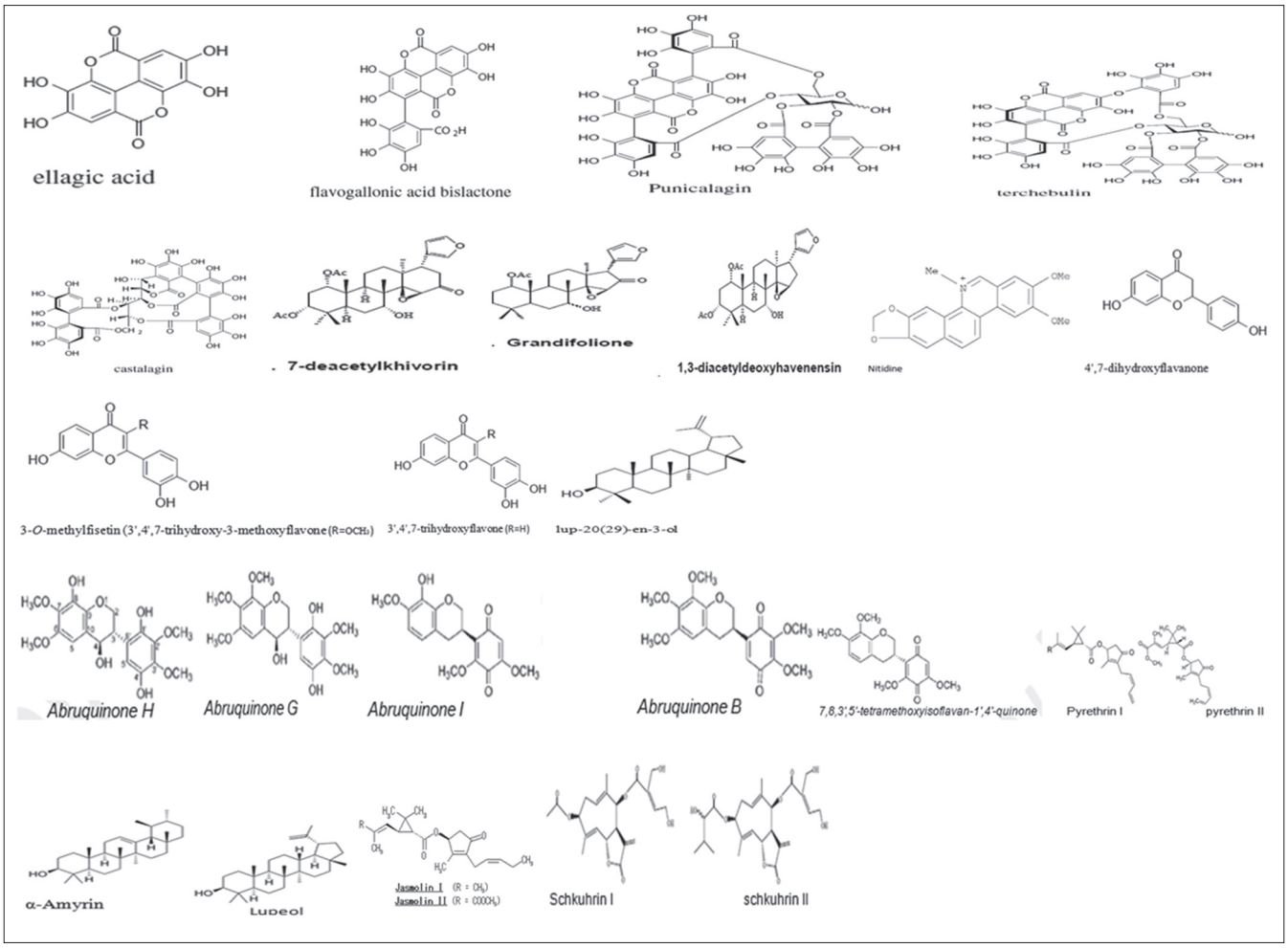

Figure 3: Structure of some antimalarial chemical compounds isolated from African plants

(DCM) extracts from leaf of *Celtis integrifolia* show promising activity (IC_{50} = 10.0) while the methanol and AQS extract were moderately active (IC_{50} = 30.2 and 38.4) against *Pfk1* [21]. DCM extract from aerial part of *Acanthospermum hispidum* show promising activity (IC_{50} = 7.5) while the methanol extract were completely inactive (IC_{50} = 55.6) against *P. f3D7* [22]. DCM extract from leaf of *Carica papaya* was highly active (IC_{50} = 2.6) while the aqueous extract was inactive [23]. The differences reported in antiplasmodial activities with variations in extraction solvent reflect the differences in the availability and concentration of bioactive agents in the extracts [13]. Although traditional healers commonly use water in preparing plants extract for medicinal application, it is surprising that most of the AQS plants extracts reported were either inactive poorly active. These poor activities could be explained by the fact that the AQS extracts were not prepared according to the traditional methods, which often involves boiling for several hours [24].

A total of 64 compounds from extracts of West African plants were reported for antiplasmodial activities. Alkaloids, flavonoids, quinines, terpenes, triterpenoids, polyphenols, and to a lesser extend sterols are the most common implicated phytochemicals in the extracts. Out of the 64 compounds isolated from West African plant 28 were highly active (IC_{50} < 5 μg/ml), 11 demonstrate promising activity (IC_{50} = 5-15 μg/ml), 4 shows moderate activity (IC_{50} = 15-50 μg/ml) while others were completely inactive *in vitro* against malaria parasites. The most interesting results were those of Simalikalactone D from leaf of *Quassia amara* [25], Samaderines B, X and Z from stem of *Quassia indica* [26], Picratidine and Picranitidine from seed of *Picralima nitida* [27], gedunin from leaf of *Azadiracta indica* [28], Fagaronine from roots of *Fagara zanthoxyloides* [29] and Ellagic acid from leaf of *Alchornea cordifolia* [30]. All these compounds excerpt high antiplasomodial activity with IC_{50} < 0.1 μg/ml.

Anti-malarial Activity of Plants from South Africa

Although literature survey revealed a very few researcher (working on antimalaria potency of indigenous plants) from South Africa, Quantitatively South African plants were the most *in vitro* investigated (198 plants from 59 families) plants from Africa. However, only 16 of the plant extracts from this region were highly active (IC_{50} < 5 μg/ml), 54 demonstrated promising activity (IC_{50} = 5-15 μg/ml), 39 demonstrate moderate activity (IC_{50} = 15-50 μg/ml), while others were inactive (IC_{50} > 50 μg/ml) *in vitro* against plasmodial parasite. Although AQS leave extract of *Vahlia capensis*, *Nicolasia*

Table 1: *In vitro* antimalarial activities of West African plants

Plant species	Family	Part use	Solvents	Parasite strain	IC_{50} (mg/ml)	Parasite inhibition	Country	References
Celtis integrifolia	Ulmaceae	Leaves	DCM/MeOH/ MeOH/AQS/AQS	*Pfk1*	10.0/30.2/ 20.7/38.4		B.Faso	Sanon *et al.* [21]
Opilia eltidifolia	Opiliaceae	Aerial part	AQS	*Pfk1*	83.176		Togo	Koffi *et al.* [31]
Abrus precatorius	Papillionaceae	Leaf	MeOH	*Pfk1*	53		Nigeria	Saganuwan *et al.* [32]
Acanthospermum hispidum	Asteraceae	Arial part and stem	DCM/MeOH/ AQS and eth	*P. f3D7 and Pf FcB1*	7.5/47.1/ 55.6 and 13.7		Benin/ I.Coast	Bero *et al.* [22]/ Zirihi *et al.* [33]
Adenia cissampeloides	Passifloraceae	Whole plant	EtOH	*pf3D7*	8.52		Ghana	Annan *et al.* [34]
Adenia rumicifolia	Passifloraceae	Leaf	MeOH	*pf K1*	>100		Ghana	Jonathan *et al.* [35]
Afzelia africana	Fabaceae	Leaf	MeOH	*3D7/K1*	31.55/39.72		Nigeria	Shuaibu *et al.* [36]
Albizia ferruginea	Fabaceae	Leaf	EtOH	*Pf FcB1*	>50		I.Coast	Zirihi *et al.* [33]
Alchornea cordifolia	Euphorbiaceae	Leaf	EtOH	*Pf FcB1*	>50		I.Coast	Zirihi *et al.* [33]
Alstonia boonei	Apocynaceae	Stem bark	EtOH	*Pf FcB1*	>50 ug/ 0.2 mg		I.Coast/ Nigeria	Zirihi *et al.* [33]/Bello *et al.* [37]
Alternanthera pungens	Amaranthaceae	Whole	EtOH	*Pf FcB1*	>50		I.Coast	Zirihi *et al.* [33]
Anacardium occidentale	Anacardiaceae	Back	AQS/Ethano	*Pf*		73% at 100/ 76 at 100	Nigeria	Sha'a *et al.* [38]
Anchomanes difformis	Araceae	Root	DCM/MeOH/ AQS	*P. f3D7*	>100/>100/ >100		Benin	Bero *et al.* [22]
Anogeissus leiocarpus	Combrataceae	Leaf	MeOH	*FcB1*	2.6		Nigeria	Okpako and Ajaiyeoba [39]
Anogeissus leiocarpus	Combretaceae	Stem bark	MeOH	*3D7/K1*	10.94/13.77		Nigeria	Shuaibu *et al.* [36]
Anthocleista djalonensis	Loganiaceae	Stem bark	EtOH	*FcB1*	>50		I.Coast	Zirihi *et al.* [33]
Anthocleista nobilis	Loganiaceae	Leaves	DCM/MeOH/ MeOH/AQS/AQS	*Pfk1*	1.8/2.5/ 12.5/13.1		Burkina Faso	Sanon *et al.* [21]
Anthonotha macrophylla	Caesalpiniaceae	Stem	EtOH	*Pf FcB1*	>50		I.Coast	Zirihi *et al.* [33]
Aspilia africana	Asteracae	Leaves	EtaC/AQS/ MeOH	*D10*	9.3/22.7/ 23.1		Nigeria	Waako *et al.* [40]
Azadirachta indica	Meliaceae	Leaves	Eth	*W2*	2.40		Nigeria	Benoit *et al.* [41]
Baillonella toxisperma	Sapotaceae	Barks	EtOH	*Pfk1*		99% at 9.6	Benin	Lagnika *et al.* [42]
Baillonella toxisperma	Sapotaceae	Barks	EtOH	*Pfk1*		99.2 at 9.6	Benin	Lagnika *et al.* [42]
Balanites aegyptiaca	Balanticeae	Leaves	MeOH	*pf*	24.56		Togo	Simplice *et al.* [43]
Bersama abyssinica	Melianthaceae	Leaf	EtOH	*Pf FcB1*	23.9		I.Coast	Zirihi *et al.* [33]
Bidens engleri	Asteraceae	Leaves	EtOH	*pf 3D7*	101		B.Faso	Traoré-Coulibaly *et al.* [44]
Boswellia dalzielii	Burceraceae	Leaves	EtOH/MeOH	*Pfk1/3D7*	14.59	62.2 at 9.6	Benin/ Nigeria	Lagnika *et al.* [42]/ Shuaibu *et al.* [36]
Byrsocarpus coccineus	Connaraceae	Arial part	DCM/MeOH/ AQS	*P. f3D7*	41.6/54.7/ >100		Benin	Bero *et al.* [22]
Caesalpinia bonduc	Caesalpiniaceae	Root	MeOH	>100	pf K1		Ghana	Jonathan *et al.* [35]
Carica papaya	Caricaceae	Leaves	Pet/DCM/ MeOH/AQS	*P. f*	16.4/2.6/ 10.8/>50		Nigeria	Melariri *et al.* [23]
Carpolobia lutea	Polygalaceae	Arial part	DCM/MeOH/ AQS	*P. f3D7*	19.4/85.4/ >100		Benin	Bero *et al.* [22]
Cassia alata	Caesalpiniaceae	Leaf	EtOH	*Pf FcB1*	>50		I.Coast	Zirihi *et al.* [33]
Cassia arereh Del	Fabaceae	Leaves	Chloroform	*K562S*	12.5		Sudan	Hager *et al.* [45]
Cassia occidentalis	Caesalpiniaceae	Leaf	EtOH	*Pf FcB1*	36.9		I.Coast	Zirihi *et al.* [33]
Cassia occidentalis	Fabaceae	Root	MeOH	*pf K1*	>100		Ghana	Jonathan *et al.* [35]
Cassia podocarpa	Ceasalpiniaceae	Leaves	EtOH	*pf 3D7*	22		B. Faso	Traoré-Coulibaly *et al.* [44]
Cassia sieberiana	Fabaceae	Leaf	MeOH	*3D7/K1*	>200		Nigeria	Shuaibu *et al.* [36]
Cassia singueana	Fabaceae	Leaf	Eth/chl/ethyl	*Pfk1*		82.1/96.4/ 85.7 at 500	Nigeria	Saidu *et al.* [47]
Cissus populnea	Amplidaceae	Leaf	MeOH	*Pfk1*	19.91		Nigeria	Shuaibu *et al.* [36]
Cissus quadrangulari	Vitaceae	Whole plant	DCM/MeOH	*Pfk1*	23.9/52.8		Mali	Bah *et al.* [48]
Citrus aurantifolia	Rutaceae	Leaf	MeOH	>100	pf K1		Ghana	Jonathan *et al.* [35]
Citrus limon	Rutaceae	Leaves	Pet/DCM/MeOH/ AQS	*P.fk1*	37.2/5.0/ >50/12.0>50		Nigeria	Melariri *et al.* [23]
Cleistopholis patens	Annonaceae	Leaf	MeOH	*pf K1*	8.7		Ghana	Jonathan *et al.* [35]
Cnestis ferruginia	Connaraceae	Root	MeOH	*pf K1*	>100		Ghana	Jonathan *et al.* [35]
Cocoa	-	Powder	MeOH	*Pfk1*	1.6103		Ghana	Amponsah *et al.* [49]
Cocos nucifera	Arecaceae	Seed	Hex	*W2*	10.6		Nigeria	Adebayo *et al.* [50]
Combretum collinum	Combretaceae	Bark	DCM/MeOH/ MeOH/AQS/AQS	*Pfk1*	3.6/4.5/ 7.4/6.8		B. Faso	Sanon *et al.* [21]
Combretum glutinosum	Combretaceae	Leaves	MeOH/hydroMeOH	*W2*	53/43.6		B. Faso	Ouattara *et al.* [51]

(Cond..)

Table 1: *(Continued...)*

Plant species	Family	Part use	Solvents	Parasite strain	IC_{50} (mg/ml)	Parasite inhibition	Country	References
Combretum molle Hyptis spicigera	Combretaceae	Leaves	Eth	*pf 3D7*	25		B. Faso	Traoré-Coulibaly *et al.* [44]
Combretum sericeum	Combretaceae	Leaves	AQS/eth/DCM	*pf 3D7*	68/>100/9		B. Faso	Traoré-Coulibaly *et al.* [44]
Commiphora kerstingii	Burseraceae	Leaf	Eth/chl/ethyl	*Pfk1*	64.3/75/82.1		Nigeria	Saidu *et al.* [47]
Crataeva religiosa	Capparidaceae	Leaf	MeOHhylene chloride/ Cyclohexane	*Pfk1*		88% at 9.6/ 87.7 at 9.6	Benin	Lagnika *et al.* [42]
Cucumis MeOHuliferus	Curcurbitaceae	Leaves	EtOH	*pf 3D7*	>100		B. Faso	Traoré-Coulibaly *et al.* [44]
Cymbopogon citratus	Poaceae	Leaves	Pet/DCM/MeOH/ AQS	*pf*	9.1/7.6/12.1/ 15.9/>50		Nigeria	Melariri *et al.* [23]
Cymbopogon citratus	Poaceae	Leaves	Essential oil	*P. f3D7*	47.92		Benin	Kpoviessi *et al.* [52]
Cymbopogon giganteus	Poaceae	Leaves	Essential oil	*P. f3D7*	11.22		Benin	Kpoviessi *et al.* [52]
Cymbopogon nardus	Poaceae	Leaves	Essential oil	*P. f3D7*	52.61		Benin	Kpoviessi *et al.* [52]
Cymbopogon schoenantus	Poaceae	Leaves	Essential oil	*P. f3D7*	43.15		Benin	Kpoviessi *et al.* [52]
Daniellia oliveri	Fabaceae	Leaves	MeOH	*3D7/K1*	23.14/32.97		Nigeria	Shuaibu *et al.* [36]
Desmodium velutinum	Fabaceae	Leaves	AQS/eth/DCM	*pf 3D7*	>100/35/9		B.Faso	Traoré-Coulibaly *et al.* [44]
Dialium guineense	Leguminosae	Arial part	DCM/MeOH/ AQS	*P. f3D7*	42.1/>100/ 65.5		Benin	Bero *et al.* [22]
Elaeis guineensis	Palmaceae	leaf	EtOH	*pf3D7*	1.195		Ghana	Annan *et al.* [34]
Entada africana	Fabaceae	Leaves	MeOH	*pf*	>100		Togo	Simplice *et al.* [43]
Erigeron floribundus	Asteracea	Stem and leaf	EtOH	*Pf FcB1*	36.9		I.Coast	Zirihi *et al.* [33]
Erythrina senegalensis	Fabaceae	-	MeOH	*3D7/K1*	199.0/153		Nigeria	Shuaibu *et al.* [36]
Erythrina senegalensis	Fabaceae	Leaf	Eth	*K1*	1.82		I.Coast	Kamanzi *et al.* [53]
Euphorbia hirta	Euphorbiaceae	Whole plant	Ethyl/MeOH	*Pfk1*	25.04/2.45		Nigeria	Oyindamola *et al.* [54]
Euphorbia hirta	Euphorbiaceae	Whole plant	EtOH	*Pf FcB1*	44.7		I.Coast	Zirihi *et al.* [33]
Fagara macrophylla	Rutaceae	Stem bark	EtOH	*Pf FcB1*	2.3		I.Coast	Zirihi *et al.* [33]
Fagara zanthoxyloides	Rutaceae	Leaf	AQS	*3D7*	4.90		Nigeria	Kassim *et al.* [29]
Ficus capensis	Moraceae	Leaf	EtOH	*Pf FcB1*	4.53		I.Coast	Zirihi *et al.* [33]
Ficus capraefolia	Moraceae	Leaves	DCM/MeOH/ MeOH/AQS/AQS	*Pfk1*	0.2/11.2/ 2.1/38.4		B.Faso	Sanon *et al.*
Ficus platyhylla	Moraceae	Leaf	MeOH	*3D7/K1*	15.28/13.77		Nigeria	Shuaibu *et al.* [36]
Ficus thonningii	Moraceae	Leaf	MeOH	*3D7/K1*	14.09/25.06		Nigeria	Shuaibu *et al.* [36]
Ficus thonningii	Moraceae	Leaf	MeOH/hex/eth	*Pf*	21.1/10.4/15.3		Nigeri	Falade *et al.*, 2014
Funtumia elastica	Apocynaceae	Stem bark	EtOH	*Pf FcB1*	3.3		I.Coast	Zirihi *et al.* [33]
Guiera senegalensis	Combrataceae	Leaf	AQS	*FcB1*	0.79		Nigeria	Ancolio *et al.* [18]
Harungana madagascariensis	Hypericaceae	Stem bark	MeOH/eth	*K1/P. y*	3.6/0.052		Nigeria	Ndjakou Lenta *et al.* [82]/ Iwalewa *et al.*, 2008
Heliotropium indicum	Boraginaceae	Arial part	DCM/MeOH/ AQS	*P. f3D7*	>100/>100/ >100		Benin	Bero *et al.* [22]
Hyptis spicigera	Lamiaceae	Leaves	OH	*pf 3D7*	Imactive		B.Faso	Traoré-Coulibaly *et al.* [44]
Irvingia gabonensis	Simaroubaceae	Stem bark	EtOH	*Pf FcB1*	2.16		I.Coast	Zirihi *et al.* [33]
Jatropha curcas	Euphorbiaceae	Leaf	Ethyl/MeOH	*Pfk1*	2.39/11.53		Nigeria	Oyindamola *et al.* [54]
Jatropha tanjorensis	Euphorbiaceae	Leaf	EtOH/AQS/ hydro etha	*Pfk1*	10.86/4.4/48.0		Nigeria	Omoregie and Sisodia (2012)
Keetia leucantha	Rubiaceae	Leaf/twig	DCM/MeOH/ AQS	*P. f3D7*	13.8/>100/ >100		Benin	Bero *et al.* [22]
Keetialeucantha	Rubiaceae	Twigs	DCM/AQS	*P. f3D7*	11.3/>100		Benin	Bero *et al.* [103]
Khaya grandifoliola	Maliaceae	Stem	MeOH–MeOHhylene chloride	*W2*	13.23		Nigeria	Bickii *et al.* (2000)
Khaya senegalensis	Maliaceae	Stem	MeOH	*3D7/K1*	28.12/15.46		Nigeria	Shuaibu *et al.* [36]
Khaya senegalensis	Meliaceae	Entire plant	Diethyl ether	*Pfk1*	-	98% at 9.6	Benin	Lagnika *et al.* [42]
Khaya senegalensis	Meliacea	Leaf	Eth/chl/ethyl	*Pfk1*	-	75/82/82 at 500	Nigeria	Saidu *et al.* [47]
Khaya senegalensis	Meliacea	Leaf	MeOH	*3D7*	>50		Nigeria	El Tahir *et al.* (1999)
Khaya senegalensis	Meliaceae	Entire plant	Cyclohexane	*Pfk1*	-	86.3 at 9.6	Benin	Lagnika *et al.* [42]
Lonchocarpus cyanescens	Fabaceae	Leaf	MeOH	*3D7/K1*	52.56/75.46		Nigeria	Shuaibu *et al.* [36]
Lophira alata	Ochnaceae	Leaf	MeOH/hex/eth	*Pfk1*	5.3/2.5/59.4		Nigeri	Falade *et al.*, 2014

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Table 1: *(Continued...)*

Plant species	Family	Part use	Solvents	Parasite strain	IC_{50} (mg/ml)	Parasite inhibition	Country	References
Lophira lanceolata	Ochnaceae	Bark	DCM/MeOH/ MeOH/AQS/AQS	*Pfk1*	5.5/9.8/ 14.7/4.7		B.Faso	Sanon *et al.* [21]
Mangifera indica	Anacardiaceae	Stem bark	EtOH	*Pf FcB1*	>50		I.Coast	Zirihi *et al.* [33]
Mareya micrantha	Euphorbiaceae	Stem	EtOH	*Pf FcB1*	27.6		I.Coast	Zirihi *et al.* [33]
Melanthera scandens	Asteracea	Whole	EtOH	*Pf FcB1*	>50		I.Coast	Zirihi *et al.* [33]
Microdesmis keayana	Pandaceae	Leaf	EtOH	*Pf FcB1*	>50		I.Coast	Zirihi *et al.* [33]
Microglossa pyrifolia	Asteracea	Stem and leaf	EtOH	*Pf FcB1*	33.1		Ivory Coast	Zirihi *et al.* [33]
Millettia zechiana	Fabaceae	Stem	EtOH	*Pf FcB1*	16.1		I.Coast	Zirihi *et al.* [33]
Mitragyana stipolosa	Rubiaceae	Leaf	MeOH	*3D7/K1*	>200		Nigeria	Shuaibu *et al.* [36]
Momordica balsamina	Cucurbitaceae	Leaf	MeOH	*3D7/K1*	199.0/250.55		Nigeria	Shuaibu *et al.* [36]
Momordica cissoides	Cucurbitaceae	Whole	MeOH	*pf K1*	>100		Ghana	Jonathan *et al.* [35]
Morinda lucida	Rubiaceae	Leaf	MeOH/MeOHcl/ pet ether	-	5.70/5.2/3.9		Nigeria	Cimanga *et al.* [55]
Morinda morindoides	Rubiaceae	Leaf	EtOH	11.6	Pf FcB1		I.Coast	Zirihi *et al.* [33]
Morinda morindoides	Cucurbitaceae	Root	MeOH	>100	pf K1		Ghana	Jonathan *et al.* [35]
Moringa oleifera	Moringaceae	Leaf	MeOH	*3D7/K1*	>200		Nigeria	Shuaibu *et al.* [36]
Nauclea latifolia	Rubiaceae		AQS	*(FcB1)*	0.60		Nigeria	Benoit-Vical *et al.* [17],
Nauclea latifolia	Rubiaceae	Bark	EtOH	*Pf FcB1*	8.9		I.Coast	Zirihi *et al.* [33]
Ocimum gratissimum	Labiatae	Leaf	Ethyl/MeOH	*Pfk1*	1.84/22.52		Nigeria	Oyindamola *et al.* [54]
Ocimum gratissimum	Lamiilaceae	Ariael/Leaf/ stem	Oil/eth	*Pfk1*	55/41/45		Benin	Kpoviessi *et al.* [52]
Oncoba spinosa	Flacourtiaceae	Seed	MeOH	*Pfk1*	>100		Ghana	Jonathan *et al.* [35]
Opilia celtidifolia	Opiliaceae	Leaves	DCM/MeOH/ MeOH/AQS/AQS	*Pfk1*	2.8/16.2/ 61.2/15.1		B.Faso	Sanon *et al.* [21]
Opilia celtidifolia	Opiliaceae	Aerial part	AQS	*Pfk1*	83.176		Togo	Koudouvo *et al.* [31]
Opilia celtidifolia	Opiliaceae	Roots	AQS/DCM	>100/<11	pf 3D7		Burkina Faso	Traoré-Coulibaly *et al.* [44]
Parinari curatellifolia	Chrysobalanceae	Leaves	MeOH	*pf*	>100		Togo	Simplice *et al.* [43]
Parkia biglobosa	Leguminosae	Stem barks	MeOH	*pf*	0.51		Nigeria	Modupe *et al.* [15]
Parquetina nigrescens	Asclepiadaceae	Leaf	EtOH	*Pf FcB1*	21.2		I.Coast	Zirihi *et al.* [33]
Pavetta corymbosa	Rubiaceae	Aerial part	MeOH/AQS	*Pf*	2.041/6.025		Togo	Koudouvo *et al.* [31]
Phyllanthus amarus	Euphorbiaceae	Leaf	Ethyl/MeOH	*Pfk1*	5.62/22.32		Nigeria	Oyindamola *et al.* [54]
Phyllanthus muellerianus	Euphorbiaceae	Leaf	EtOH	*Pf FcB1*	9.4		I.Coast	Zirihi *et al.* [33]
Physalis angulata	Olanaceae	Whole	EtOH	*Pf FcB1*	7.9		I.Coast	Zirihi *et al.* [33]
Picralima nitida	Apocynaceae	Roots, stem bark/fruit	MeOH	0.188/0.545/ 1.581	Pfk1		I.Coast	Francois *et al.* [56]
Piliostigma thonningii	Leguminosae	Leaf	Ethyl/MeOH	*Pfk1*	3.56/38.86		Nigeria	Oyindamola *et al.* [54]
Pleiocarpa mutica	Apocynaceae	Root	MeOH	*Pfk1*	16.7		Ghana	Jonathan *et al.* [35]
Prosopis africana	Leguminaceae	Leaf	MeOH	*3D7/K1*	14.97/15.2		Nigeria	Shuaibu *et al.* [36]
Psidium guajava	Myrtaceae	Leaves	Pet/DCM/MeOH/ AQS	*Pfk1*	15.5/6.0/21.6/ >50/>50		Nigeria	Melariri *et al.* [23]
Pupalia lappacea	Amaranthaceae	Arial part	DCM/MeOH/ AQS	*P. f3D7*	50.29/>100/ >100		Benin	Bero *et al.* [22]
Pycnanthus angolensis	Myristicaceae	Stem bark	EtOH	*Pf FcB1*	18.2		I.Coast	Zirihi *et al.* [33]
Quassia amara	Simaroubaceae	Leaf	AQS	*FcB1*	8.90		Nigeria	Bertani *et al.* [57]
Rauvolfia vomitoria	Apocynaceae	Root bark	EtOH	*Pf FcB1*	2.5		I.Coast	Zirihi *et al.* [33]
Rhigiocarya racemifera	Menispermaceae	Leaf	EtOH	*Pf FcB1*	>50		I.Coast	Zirihi *et al.* [33]
Rothmania longiflora	Rubiaceae	Stem	MeOH	*pf K1*	>100		Ghana	Jonathan *et al.* [35]
Rourea coccinea	Connaraceae	Arial part	DCM/MeOH	*Pf3D7*	41.6/54.7		Benin	Bero *et al.* [22]
Sansevieria liberica	Dracaenaceae	Arial part	DCM/MeOH/ AQS	*P. f3D7*	44.5/>100/ >100		Benin	Bero *et al.* [22]
Schrankia leptocarpa	Mimosaceae	Leaf/twig	DCM/MeOH/ AQS	*P. f3D7*	34.3/>100/ >100		Benin	Bero *et al.* [22]
Securidaca longipedunculata	Polygalaceae	Leaf	DCM	*P. f3D7*	6.9		Mali	Bah *et al.* [48]
Securinega virosa	Euphorbiaceae	Leaves	DCM/MeOH/ MeOH/AQS/AQS	*Pfk1*	P. f3D7		B.Faso	Sanon *et al.* [21]
Sida acuta	Malvaceae	Leaves	EtOH/H20	*FcM29*	3.90/0.92		Nigeria	Banzouzi *et al.* [58]
Solanum indicum	Olanaceae	Fruit	EtOH	*Pf FcB1*	41.3		I.Coast	Zirihi *et al.* [33]
Solanum nigrum	Olanaceae	Fruit	EtOH	*Pf FcB1*	>50		I.Coast	Zirihi *et al.* [33]
Striga hermonthica	Orobanchaceae	Whole pla	MeOH	*Pfk1*	274.8		Nigeria	Okpako and Ajaiyeoba, [39]
Strychnos spinosa	Loganiaceae	Leaf	DCM/MeOH/ AQS	*P. f3D7*	15.6/>100/ >100		Benin	Bero *et al.* [22]

(Cond..)

Table 1: *(Continued...)*

Plant species	Family	Part use	Solvents	Parasite strain	IC_{50} (mg/ml)	Parasite inhibition	Country	References
Strychnos spinosa	Loganiaceae	Stem bark	EtOH	21.8	Pf FcB1		I.Coast	Guede *et al.* [33]
Stylosanthes erecta	Fabaceae	Aerial parts	DCM/MeOH	*Pfk1*	21.9/23.3		Mali	Bah *et al.* [48]
Swartzia madagascariensis	Leguminosae	Roots bark	AQS, MeOH, hydroMeOH	*W2*	50.6/60.5/ 15.5		B.Faso	Ouattara *et al.* [51]
Tamarindus indica	Caesalpiniaceae	Fruit	AQS/MeOH	*Pfk1*	4.786/55.544		Togo	Koudouvo *et al.* [31]
Tapinanthus dodoneifolius	Euphorbiaceae	Leaves	DCM/MeOH/ MeOH/AQS/AQS	*Pfk1*	6.5/5.2/ 20.6/43.7		Burkina Faso	Sanon *et al.* [21]
Tapinanthus sessilifolius	Lorantheciae	Leaves	MeOH	*Pfk1*	200.5		Nigeria	Okpako and Ajaiyeoba [39]
Terminalia avicennioides	Combretaceae	Leaves	DCM/MeOH/ MeOH/AQS/AQS	*Pfk1*	1.6/1.9/ 5.4/2.6		B.Faso	Sanon *et al.* [21]
Terminalia avicennoides	Combretaceae	Stem bark	MeOH	*3D7/K1*	12.28/14.09		Nigeria	Shuaibu *et al.* [36]
Terminalia catappa	Combretaceae	Leaf	Ethyl/MeOH	*Pfk1*	3.05/7.42		Nigeria	Oyindamola *et al.* [54]
Terminalia ivorensis	Combretaceae	Stem bark	EtOH	*pf3D7*	6.949		Ghana	Annan *et al.* [34]
Tinospora bakis	Menispermaceae	Roots	AQS	*W2*	59.8		B.Faso	Ouattara *et al.* [51]
Tithonia diversifola	Asteraceae	Leaves	Ether	*FCA*	0.75		Nigeria	Goffin *et al.* [16]
Trema orientalis	Ulmaceae	Leaf	Ethyl/MeOH	*Pfk1*	1.99/6.79		Nigeria	Oyindamola *et al.* [54]
Trichilia emethanolica	Meliaceae	Leaf	DCM/MeOH/ AQS	*P. f3D7*	59.2>100 >100		Benin	Bero *et al.* [22]
Trichilia emthenolica	Meliaceae	Leaves	DCM/MeOH	*Pfk1*	11.9/47.6		Mali	Bah *et al.* [48]
Turreae heterophylla	Meliaceae	Root	MeOH	*pf K1*	>100		Ghana	Jonathan *et al.* [35]
Uvaria chamae	Annonaceae	Twig	MeOH	*Pfk1*	21.6		Ghana	Jonathan *et al.* [35]
Vernonia amygdalina	Asteraceae	Leaves	Pet/DCM/MeOH/ AQS	*Pfk1*	14.2/4.1/10.7/ >50/>50		Nigeria	Melariri *et al.* [23]
Vernonia colorata	Compositae	Stem	MeOH	*Pfk1*	>100		Ghana	Jonathan *et al.* [35]
Vitex doniana	Verbenaceae	Stem bark	Ethyl/MeOH	*Pfk1*	3.87/34.17		Nigeria	Oyindamola *et al.* [54]

DCM: Dichloromethane, MeOH: Methanol, EtOH: Ethanol, HO: Hydroxide, AQS: Aqueous, pf: *Plasmodium falciparum*, IC_{50}: Inhibitory concentration 50%

Table 2: *In vitro* antimalarial activities of South African plants

Plant species	Family	Part use	Solvents	Parasite	IC_{50} (μg/ml) P. inhibition	Country	References
Abrus precatorius	Fabaceae	Whole	DCM/MeOH (1:1)	*Pf NF*	3.99	S. Africa	Makoka *et al.* [62]
Acacia erioloba	Fabaceae	Root	DCM/MeOH (1:1)	*Pf NF*	10.7	S. Africa	Makoka *et al.* [62]
Acacia nilotica	Fabaceae	Twig	DCM/MeOH (1:1)	*P. f D10*	13	S. Africa	Clakson *et al.* [24]
Acacia tortilis	Fabaceae	Whole plant	DCM/MeOH (1:1)	*P. f D10*	4.8	S. Africa	Clakson *et al.* [24]
Achyranthes aspera	Amaranthaceae	Whole plant	DCM/MeOH (1:1)	*P. f D10*	9.9	S. Africa	Clakson *et al.* [24]
Agathosma apiculata	Rutaceae	Whole plant	DCM/MeOH (1:1)	*Pfk1*	0.209	S. Africa	Makoka *et al.* [63]
Agathosma puberula	Rutaceae	Root	DCM	*P. f D10*	33	S. Africa	Clakson *et al.* [24]
Agathosma puberula	Rutaceae	Roots	DCM	*Pfk1*	8.35	S. Africa	Makoka *et al.* [63]
Ageratum conyzoides	Asphodelaceae	Whole plant	DCM/MeOH (1:1)	*P. f D10*	27	S. Africa	Clakson *et al.* [24]
Albizia versicolor	Fabaceae	Root	DCM	*2 Pf-NF54*	2.12	S. Africa	Bapela *et al.* [64]
Alepidea amatymbica	Apiaceae	Whole	DCM/MeOH (1:1)	*P. f D10*	12.5	S. Africa	Clakson *et al.* [24]
Alepidea amatymbica	Apiaceae	whole plant	DCM/MeOH (1:1)	*Pfk1*	3.7	S. Africa	Makoka *et al.* [63]
Aloe ferox	Asphodelaceae	Whole plant	DCM/MeOH (1:1)	*P. f D10*	8	S. Africa	Clakson *et al.* [24]
Aloe maculata	Asphodelaceae	Whole plant	DCM/MeOH (1:1)	*P. f D10*	12.4	S. Africa	Clakson *et al.* [24]
Aloe marlothii	Asphodelaceae	Leaf	DCM	*P. f D10*	74	S. Africa	Clakson *et al.* [24]
Annona senegalensis	Annonaceae	leaf	DCM/MeOH (1:1)	*P. f D10*	35	S. Africa	Clakson *et al.* [24]
Anthocleista grandiflora	Gentianaceae	Leaves	DCM	*P. f D10*	>100	S. Africa	Clakson *et al.* [24]
Anthocleista grandiflora	Loganiaceae	Stem bark	DCM	*Pf-NF54*	8.69	S. Africa	Bapela *et al.* [64]
Artabotrys brachypetalus	Annonaceae	Leaf	DCM/MeOH (1:1)	*P. f D10*	>100	S. Africa	Clakson *et al.* [24]
Artabotrys monteiroae	Annonaceae	Twigs	DCM/MeOH (1:1)	*P. f D10*	8.7	S. Africa	Clakson *et al.* [24]
Artabotrys monteiroae	Annonaceae	leaves	DCM/MeOH (1:1)	*Pfk1*	8.79	S. Africa	Makoka *et al.* [63]
Artemisia afra	Asteraceae	Leaf	DCM	*P. f D10*	5	S. Africa	Clakson *et al.* [24]
Artemisia afra	Asteraceae	leaves	DCM/MeOH	*Pfk1*	6.22/13.3	S. Africa	Makoka *et al.* [63]
Asparagus virgatus	Asparagaceae	Whole plant	DCM/MeOH (1:1)	*P. f D10*	8	S. Africa	Clakson *et al.* [24]
Asystasia gangetica	Acanthaceae	Twigs	DCM/MeOH (1:1)	*P. f D10*	16	S. Africa	Clakson *et al.* [24]
Asystasia gangetica	acanthaceae	leaves	DCM/MeOH (1:1)	*4 Pfk1*	4.2	S. Africa	Makoka *et al.* [63]
Barringtonia racemosa	Lecythidaceae	Leaf	DCM/MeOH (1:1)	*P. f D10*	18	S. Africa	Clakson *et al.* [24]
Berula erecta	Apiaceae	Whole	DCM/MeOH (1:1)	*P. f D10*	6.6	S. Africa	Clakson *et al.* [24]
Bidens pilosa	Asteraceae	Leaf	DCM	*P. f D10*	8.5	S. Africa	Clakson *et al.* [24]
Bridelia micrantha	Euphorbiaceae	Twig	DCM/MeOH (1:1)	*P. f D10*	59.3	S. Africa	Clakson *et al.* [24]
Bridelia mollis	Phyllanthaceae	Roots	DCM	*Pf-NF54*	3.06	S. Africa	Bapela *et al.* [64]
Bruguiera gymnorhiza	Rhizophoraceae	Twigs	DCM/MeOH (1:1)/AQS	*P. f D10*	11.7/>100	S. Africa	Clakson *et al.* [24]
Burchellia bubaline	Rubiaceae	Twigs	DCM/MeOH (1:1)	*P. f D10*	18	S. Africa	Clakson *et al.* [24]

(Cond..)

Table 2: *(Continued...)*

Plant species	Family	Part use	Solvents	Parasite	IC_{50} (μg/ml)	P. inhibition	Country	References
Capparis tomentosa	Capparaceae	leaf	DCM	*P. f D10*	65		S. Africa	Clakson *et al.* [24]
Capparis tomentosa	Capparidaceae	Root	DCM	*Pf-NF54*	2.19		S. Africa	Bapela *et al.* [64]
Cardiospermum halicacabum	Sapindaceae	Whole plant	DCM/MeOH (1:1)	*P. f D10*	20		S. Africa	Clakson *et al.* [24]
Carissa edulis	Apocynaceae	Stem	DCM	*P. f D10*	33		S. Africa	Clakson *et al.* [24]
Catha eduli	Celastraceae	Seed	DCM/DCM/MeOH (1:1)	*P. f D10*	10/46		S. Africa	Clakson *et al.* [24]
Catha edulis	Celastraceae	Root	DCM	*Pfk1*	4.91		S. Africa	Makoka *et al.* [63]
Centella asiatica	Apiaceae	Leaf	DCM/MeOH (1:1)	*P. f D10*	8.3		S. Africa	Clakson *et al.* [24]
Cephalanthus natalensis	Rubiaceae	Leaf	DCM/MeOH (1:1)	*P. f D10*	24.3		S. Africa	Clakson *et al.* [24]
Clausena anisata	Rutaceae	Twig	DCM/MeOH (1:1)	*P. f D10*	88		S. Africa	Clakson *et al.* [24]
Clausena anisata	Rutaceae	Roots	DCM/MeOH (1:1)	*Pf NF*	3.61		S. Africa	Makoka *et al.* [62]
Clematis brachiata	Ranunculaceae	Leaves/stems/ flowers	DCM/MeOH (1:1)	*P. f D10*	20		S. Africa	Clakson *et al.* [24]
Clematis brachiata	Ranunculaceae	Roots	DCM	*Pf-NF54*	5.36		S. Africa	Bapela *et al.* [64]
Clerodendrum glabrum	Verbenaceae	twigs	DCM/MeOH (1:1)	*P. f D10*	19		S. Africa	Clakson *et al.* [24]
Clerodendrum glabrum	Verbenaceae	leaves	DCM	*Pf-NF54*	8.89		S. Africa	Bapela *et al.* [64]
Clutia hirsuta	Euphorbiaceae	Whole plant	DCM	*P. f D10*	15		S. Africa	Clakson *et al.* [24]
Clutia pulchella	Euphorbiaceae	Root	DCM/MeOH (1:1)	*Pf NF*	3.19		S. Africa	Makoka *et al.* [62]
Combretum zeyheri	Combretaceae	Twigs	DCM/MeOH (1:1)	*P. f D10*	15		S. Africa	Clakson *et al.* [24]
Conyza albida	Asteraceae	Whole plant	DCM/MeOH (1:1)	*P. f D10*	2		S. Africa	Clakson *et al.* [24]
Conyza albida	Asteraceae	whole plant	DCM/MeOH (1:1)	*Pfk1*	5.79		S. Africa	Makoka *et al.* [63]
Conyza podocephala	Asteraceae	Whole plant	DCM/MeOH (1:1)	*P. f D10*	6.8		S. Africa	Clakson *et al.* [24]
Conyza podocephala	Asteraceae	whole plant	DCM/MeOH (1:1)	*Pfk1*	5.45		S. Africa	Makoka *et al.* [63]
Conyza scabrida	Asteraceae	Flower	DCM/MeOH (1:1)	*P. f D10*	7.8		S. Africa	Clakson *et al.* [24]
Conyza scabrida	Asteraceae	Leaves	DCM/MeOH (1:1)	*Pfk1*	6.66		S. Africa	Makoka *et al.*[63]
Crinum macowanii	Amaryllidaceae	Bulbs	DCM/MeOH (1:1)	*P. f D10*	26		S. Africa	Clakson *et al.* [24]
Crotalaria burkeana	Fabaceae	Leafs	DCM	*P. f D10*	30		S. Africa	Clakson *et al.* [24]
Croton gratissimus	Euphorbiaceae	Leaf	DCM	*P. f D10*	3.5		S. Africa	Clakson *et al.* [24]
Croton menyhartii	Euphorbiaceae	Leaves	DCM/MeOH (1:1)	*Pfk1*	2.63		S. Africa	Makoka *et al.* [63]
Croton menyhartii Pax	Euphorbiaceae	Leaf	DCM/MeOH (1:1)	*P. f D10*	1.7		S. Africa	Clakson *et al.* [24]
Momordica balsamina	Cucurbitaceae	Whole plant	DCM/MeOH (1:1)	*P. f D10*	18		S. Africa	Clakson *et al.* [24]
Cussonia spicata	Araliaceae	Leaf	DCM	*P. f D10*	45		S. Africa	Clakson *et al.* [24]
Cussonia spicata	Araliaceae	Root	DCM	*Pf-NF54*	3.25		S. Africa	Bapela *et al.* [64]
Cymbopogon validu	Poaceae	Whole plant	MeOH/DCM (1:1)	*P. f D10*	5.8		S. Africa	Clakson *et al.* [24]
Cymbopogon validus	Poaceae	Leave	DCM/MeOH (1:1)	*Pfk1*	6.67		S. Africa	Makoka *et al.* [63]
Dicerocaryum eriocarpum	Pedaliaceae	Leaf	AQS	*Pfk1*		48% at 50	Namibia	Iwanette *et al.* [59]
Dichrostachys cinerea	Fabaceae	Roots	DCM	*Pf-NF54*	2.10		S. Africa	Bapela *et al.* [64].
Diosma sp.	Rutaceae	Root	DCM/MeOH (1:1)	*P. f D10*	55		S. Africa	Clakson *et al.* [24]
Diospyros mespiliformis	Ebenaceae	Roots	DCM	*Pf-NF54*	4.40		S. Africa	Bapela *et al.*
Diplorhynchus condylocarpon	Apocynaceae	Root	DCM	*P. f D10*	26.5		S. Africa	Clakson *et al.* [24]
Dodonaea viscose	Sapindaceae	Leaf	DCM/MeOH (1:1)	*P. f D10*	15.5		S. Africa	Clakson *et al.* [24]
Drypetes gerrardii	Meliaceae	Stem/leaves	DCM/MeOH (1:1)	*Pf NF*	0.50/21.60		S. Africa	Makoka *et al.* [62]
Ekebergia capensis	Maesaceae	Fruit	DCM/MeOH (1:1)	*P. f D10*	10		S. Africa	Clakson *et al.* [24]
Ekebergia capensis	Meliaceae	Fruit/twig	DCM/MeOH (1:1)	*Pfk1*	3.5/13.3		S. Africa	Makoka *et al.* [63]
Ekebergia capensis	Meliaceae	Roots	DCM/MeOH (1:1)	*Pf NF*	6.81		S. Africa	Makoka *et al.* [62]
Elephantorrhiza elephantina	Fabaceae	Root	DCM/MeOH (1:1)	*P. f D10*	28		S. Africa	Clakson *et al.* [24]
Euclea natalensis	Ebenaceae	Stem	DCM/MeOH (1:1)	*P. f D10*	5.3		S. Africa	Clakson *et al.* [24]
Euclea natalensis	Ebenaceae	roots	DCM/MeOH (1:1)	*7.59*	Pfk1		S. Africa	Makoka *et al.* [63]
Eucomis autumnalis	Hyacinthaceae	Bulbs	DCM	*P. f D10*	70		S. Africa	Clakson *et al.* [24]
Eucomis autumnalis	Asparagaceae	flowers/buds	DCM	*22.1*	Pfk1		S. Africa	Makoka *et al.* [63]
Euphorbia heterophylla	Euphorbiaceae	Whole plant	DCM/MeOH (1:1)	*P. f D10*	40		S. Africa	Clakson *et al.* [24]
Euphorbia tirucalli	Euphorbiaceae	Leaf	DCM	*P. f D10*	12		S. Africa	Clakson *et al.* [24]
Flacourtia indica	Flacourtiaceae	Root	DCM	*P. f D10*	86.5		S. Africa	Clakson *et al.* [24]
Flueggea virosa	Euphorbiaceae	Leaves/twigs	DCM/MeOH (1:1)	*P. f D10*	19		S. Africa	Clakson *et al.* [24]
Gloriosa superba	Colchicaceae	Whole plant	DCM/MeOH (1:1)	*P. f D10*	17		S. Africa	Clakson *et al.* [24]
Gnidia cuneata	Thymelaeaceae	Leaf	DCM/DCM/MeOH (1:1)	*P. f D10*	31.1/51		S. Africa	Clakson *et al.* [24]
Gnidia kraussiana	Thymelaeaceae	Tuber	DCM/MeOH (1:1)	*P. f D10*	16		S. Africa	Clakson *et al.* [24]
Gomphocarpus fruticosus	Apocynaceae	Fruit	DCM/MeOH (1:1)	*P. f D10*	26		S. Africa	Clakson *et al.* [24]
Helichrysum nudifolium	Asteraceae	Whole	DCM/MeOH (1:1)	*P. f D10*	6.8		S. Africa	Clakson *et al.* [24]
Helichrysum nudifolium	Asteraceae	whole plant	DCM/MeOH (1:1)	*9.36*	Pfk1		S. Africa	Makoka *et al.* [63]
Helichrysum pedunculatum	Asteraceae	Whole	DCM/MeOH (1:1)	*6.46*	Pf NF		S. Africa	Makoka *et al.* [62]

(Cond..)

Table 2: *(Continued...)*

Plant species	Family	Part use	Solvents	Parasite	IC_{50} (μg/ml) P. inhibition	Country	References
Hermannia depressa	Sterculiaceae	Whole plant	DCM/MeOH (1:1)	*P.fD10*	6.9	S. Africa	Clakson *et al.* [24]
Hippobromus pauciflorus	Sapindaceae	Leaf	DCM/MeOH (1:1)	*P.fD10*	34	S. Africa	Clakson *et al.* [24]
Hypericum aethiopicum	Hypericaceae	Leaves/flowers	DCM/MeOH (1:1)	*P.fD10*	14	S. Africa	Clakson *et al.* [24]
Hypericum aethiopicum	Hypericaceae	Leaves	DCM/MeOH (1:1)	*2.35*	Pfk1	S. Africa	Makoka *et al.* [63]
Hypoxis colchicifolia	Hypoxidaceae	Bulb	DCM: Ethyl acetat	*>100*	pf D10	S. Africa	Mthokozisi *et al.*, 2013
Hyptis pectinata	Lamiaceae	Leaves/stems/ fruit	DCM/MeOH (1:1)	*P.fD10*	17.5	S. Africa	Clakson *et al.* [24]
Justicia flava	Acanthaceae	Whole plant	DCM/MeOH (1:1)	*P.fD10*	31	S. Africa	Clakson *et al.* [24]
Kigelia africana	Bignoniaceae	Leaf	DCM	*P.fD10*	51	S. Africa	Clakson *et al.* [24]
Kirkia wilmsii	Kirkiaceae	Leaf	DCM/MeOH (1:1)	*P.fD10*	3.7	S. Africa	Clakson *et al.* [24]
Lannea discolor	Anacardiaceae	Fruit	DCM	*P.fD10*	25	S. Africa	Clakson *et al.* [24]
Leonotis leonurus	Lamiaceae	roots	DCM/MeOH (1:1)	*P.fD10*	15	S. Africa	Clakson *et al.* [24]
Leonotis leonurus	Lamiaceae	leaves	DCM/MeOH (1:1)	*2.9*	Pfk1	S. Africa	Makoka *et al.* [63]
Leonotis nepetifolia	Lamiaceae	Whole plant	DCM/MeOH (1:1)	*P.fD10*	15	S. Africa	Clakson *et al.* [24]
Leonotis ocymifolia	Lamiaceae	Leaf	DCM	*P.fD10*	17	S. Africa	Clakson *et al.* [24]
Leonotis ocymifolia	Lamiaceae	Leaves	DCM/DCM/MeOH (1:1)	*2.7/4.5*	Pfk1	S. Africa	Makoka *et al.* [63]
Leucas martinicensis	Lamiaceae	Whole plant	DCM/MeOH (1:1)	*P. f D10*	13.3	S. Africa	Clakson *et al.* [24]
Lippia javanica	Verbenaceae	Roots	DCM/DCM/MeOH (1:1)/ AQS	*P. f D10*	3.8/27/27	S. Africa	Clakson *et al.* [24]
Macrostylis squarrosa	Rutaceae	stem	DCM/MeOH (1:1)	*P.fD10*	10	S. Africa	Clakson *et al.* [24]
Maesa lanceolata	Maesaceae	Twigs	DCM/MeOH (1:1)	*P.fD10*	5.9	S. Africa	Clakson *et al.* [24]
Maytenus senegalensis	Celastraceae	Root	DCM	*P.fD10*	15.5	S. Africa	Clakson *et al.* [24]
Maytenus undata	Celastraceae	Leaf	DCM	*P.fD10*	>100	S. Africa	Clakson *et al.* [24]
Maytenus undata	Celastraceae	Roots	DCM	*Pfk1*	8.53	S. Africa	Makoka *et al.* [63]
Mimusops caffra	Sapotaceae	Leaf	DCM: Ethyl acetat	*pf D10*	2.14	S. Africa	Mthokozisi *et al.*, 2013
Mimusops obtusifolia	Sapotaceae	Bark	DCM: Ethyl acetat	*32.5*	pf D10	S. Africa	Mthokozisi *et al.*, 2013
Nicolasia costata	Asteraceae	Leaf	AQS	*Pfk1*	21.5% at 50	Namibia	Iwanette *et al.* [59]
Ocimum americanum	Lamiaceae	Whole plant	DCM/MeOH (1:1)	*P.fD10*	4.2	S. Africa	Clakson *et al.* [24]
Oedera genistifolia	Asteraceae	Whole	DCM/MeOH (1:1)	*Pf NF*	2.88	S. Africa	Makoka *et al.* [62]
Olea europaea	Olacaceae	Leaf	DCM/MeOH (1:1)	*P.fD10*	12	S. Africa	Clakson *et al.* [24]
Osteospermum imbricatum	Asteraceae	Stem	DCM/MeOH (1:1)	*P.fD10*	7.3	S. Africa	Clakson *et al.* [24]
Ozoroa sphaerocarpa	Anacardiaceae	Whole	DCM	*Pf NF*	12.9	S. Africa	Makoka *et al.* [62]
Pappea capensis	Sapindaceae	Root	DCM	*Pf NF*	10.10	S. Africa	Makoka *et al.* [62]
Pappea capensis	Sapindaceae	Root/leaves	DCM/MeOH (1:1)	*Pf NF*	5.33/9.67	S. Africa	Makoka *et al.* [62]
Pappea capensis	Sapindaceae	Twigs	DCM	*Pf-NF54*	5.47	S. Africa	Bapela *et al.* [64]
Parinari curatellifolia	Chrysobalanaceae	Leaves/flowers	DCM	*P.fD10*	17	S. Africa	Clakson *et al.* [24]
Parinari curatellifolia	Rosaceae	Stem bark	DCM	*Pf-NF54*	6.99	S. Africa	Bapela *et al.* [64]
Parkinsonia aculeata	Fabaceae	Twigs	DCM/MeOH (1:1)	*P.fD10*	9	S. Africa	Clakson *et al.* [24]
Pelargonium alchemilloides	Gentianaceae	Whole plant	DCM/MeOH (1:1)	*P.fD10*	15	S. Africa	Clakson *et al.* [24]
Pentzia globosa	Asteraceae	Leaf	DCM	*P.fD10*	12.5	S. Africa	Clakson *et al.* [24]
Pentzia globosa	Asteraceae	Roots/stem bark	DCM	*Pfk1*	4.27/6.04	S. Africa	Makoka *et al.* [63]
Piliostigma thonningii	Fabaceae	Leaf	DCM/MeOH (1:1)	*P.fD10*	32	S. Africa	Clakson *et al.* [24]
Pittosporum viridiflorum	Pittosporaceae	Whole plant	DCM/AQS	*P.fD10*	3/>100	S. Africa	Clakson *et al.* [24]
Plantaginaceae Plantago majo	Plantaginaceae	Whole plant	DCM/AQS	*P.fD10*	21.5/>100	S. Africa	Clakson *et al.* [24]
Plumbago zeylanica	Plumbaginaceae	Roots	DCM	*P.fD10*	43	S. Africa	Clakson *et al.* [24]
Plumbago zeylanica	Plumbaginaceae	Leaves	DCM/MeOH (1:1)	*12.4*	Pfk1	S. Africa	Makoka *et al.* [63]
Pollichia campestris	Illecebraceae	Twigs	DCM/MeOH (1:1)	*P. f D10*	6.8	S. Africa	Clakson *et al.* [24]
Pseudarthria hookeri	Fabaceae	Leaf	DCM/MeOH (1:1)	*P. f D10*	100	S. Africa	Clakson *et al.* [24]
Psiadia punctulata	Asteraceae	Twig	DCM	*P. f D10*	9	S. Africa	Clakson *et al.* [24]
Psoralea pinnata	Fabaceae	Leaves	DCM	*Pf NF*	8.46	S. Africa	Makoka *et al.* [62]
Ptaeroxylon obliquum	Ptaeroxylaceae	Root	DCM	*P. f D10*	19	S. Africa	Clakson *et al.* [24]
Ptaeroxylon obliquum	Rutaceae	Leaves	DCM	*Pfk1*	10.9	S. Africa	Makoka *et al.* [63]
Pterocarpus angolensis	Fabaceae	Stem	DCM	*P. f D10*	15	S. Africa	Clakson *et al.* [24]
Pyrenacantha grandiflora	Icacinaceae	Roots	DCM	*Pf-NF54*	5.82	S. Africa	Bapela *et al.* [64]
Ranunculus multifidus	Ranunculaceae	Whole plant	DCM/MeOH (1:1)	*P. f D10*	2.3	S. Africa	Clakson *et al.* [24]
Rapanea melanophloeos	Myrtaceae	Leaf	DCM/MeOH (1:1)	*P. f D10*	44	S. Africa	Clakson *et al.* [24]
Rauvolfia caffra	Apocynaceae	Fruit	DCM	*P. f D10*	88	S. Africa	Clakson *et al.* [24]
Rauvolfia caffra	Apocynaceae	Roots	DCM	*Pfk1*	8.44	S. Africa	Makoka *et al.* [63]
Rauvolfia caffra	Apocynaceae	Stem	DCM	*Pf-NF54*	2.13	S. Africa	Bapela *et al.* [64]

(Cond..)

Table 2: *(Continued...)*

Plant species	Family	Part use	Solvents	Parasite	IC_{50} (μg/ml) P. inhibition	Country	References
Rhizophora mucronata	Rhizophoraceae	Leaf	DCM/MeOH (1:1)	*P. f D10*	24	S. Africa	Clakson *et al.* [24]
Ricinus communis	Euphorbiaceae	Leaf	DCM/MeOH (1:1)	*P. f D10*	27.5	S. Africa	Clakson *et al.* [24]
Rumex crispus	Polygonaceae	Leaf	DCM	*P. f D10*	36.8	S. Africa	Clakson *et al.* [24]
Rumex sagittatus	Poaceae	Whole plant	MeOH/DCM (1:1)	*P. f D10*	18	S. Africa	Clakson *et al.* [24]
Rutaceae agathosma apiculata	Rutaceae	Whole plant	DCM/MeOH (1:1)	*P. f D10*	5.2	S. Africa	Clakson *et al.* [24]
Salvia repens	Lamiaceae	Whole plant	DCM/MeOH (1:1)	*P. f D10*	10.8	S. Africa	Clakson *et al.* [24]
Salvia repens	Lamiaceae	whole plant	DCM/MeOH (1:1)	*Pfk1*	7.61	S. Africa	Makoka *et al.* [63]
Scaevola plumieri	Goodeniaceae	Twig	DCM	*P. f D10*	11	S. Africa	Clakson *et al.* [24]
Schefflera umbellifera	Araliaceae	Leaf	DCM/DCM/MeOH (1:1)	*P. f D10*	3.7/19.5	S. Africa	Clakson *et al.* [24]
Schefflera umbellifera	Araliaceae	Roots	DCM/MeOH (1:1)	*Pfk1*	2.7	S. Africa	Makoka *et al.* [63]
Schkuhria pinnata	Asteraceae	Whole	DCM/MeOH (1:1)	*Pf NF*	2.19	S. Africa	Makoka *et al.* [62]
Senecio oxyriifolius	Asteraceae	Whole	DCM/MeOH (1:1)	*P. f D10*	13	S. Africa	Clakson *et al.* [24]
Senna didymobotrya	Fabaceae	Leaf	DCM/MeOH (1:1)	*P. f D10*	40	S. Africa	Clakson *et al.* [24]
Senna petersiana	Fabaceae	Leaf	DCM/MeOH (1:1)	*P. f D10*	>100	S. Africa	Clakson *et al.* [24]
Senna petersiana	Fabacea	Leaves	DCM	*Pf-NF54*	22.5	S. Africa	Bapela *et al.* [64]
Setaria megaphylla	Poaceae	Whole plant	MeOH/DCM (1:1)	*P. f D10*	4.5	S. Africa	Clakson *et al.* [24]
Setaria megaphylla	Poaceae	Whole plant	DCM/MeOH (1:1)	*Pfk1*	4.44	S. Africa	Makoka *et al.* [63]
Spilanthes mauritiana	Asteraceae	Stem	DCM	*P. f D10*	38	S. Africa	Clakson *et al.* [24]
Strychnos madagascariensis	Strychnaceae	Stem	DCM	*P. f D10*	70	S. Africa	Clakson *et al.* [24]
Strychnos potatorum	Strychnaceae	Leaf	DCM/DCM/MeOH (1:1)	*P. f D10*	60/>100	S. Africa	Clakson *et al.* [24]
Strychnos pungens	Strychnaceae	Leaf	DCM/DCM/MeOH (1:1)	*P. f D10*	12.6/80.4	S. Africa	Clakson *et al.* [24]
Syzigium cordatum	Myrtaceae	Twigs	DCM/MeOH (1:1)	*P. f D10*	14.7	S. Africa	Clakson *et al.* [24]
Syzygium cordatum	Myrtaceae	Leaves	DCM	*6.15*	Pf-NF54	S. Africa	Bapela *et al.* [64]
Tabernaemontana elegans	Apocynaceae	Stem bark	DCM/MeOH: AQS	*Pf-NF54*	0.33/0.83	S. Africa	Bapela *et al.* [64]
Tarchonanthus camphorates	Asteraceae	Whole	DCM/MeOH (1:1)	*P. f D10*	6	S. Africa	Clakson *et al.* [24]
Tarchonanthus camphorates	Asteraceae	Whole plant	DCM/MeOH (1:1)	*Pfk1*	6.23	S. Africa	Makoka *et al.* [63]
Tecomaria capensis	Bignoniaceae	Leaf	DCM/MeOH (1:1)	*P. f D10*	11.6	S. Africa	Clakson *et al.* [24]
Tetradenia riparia	Lamiaceae	Leaf	DCM	*P. f D10*	>100	S. Africa	Clakson *et al.* [24]
Tridax procumbens	Asteraceae	Whole	DCM/MeOH (1:1)	*P. f D10*	17	S. Africa	Clakson *et al.* [24]
Triumfetta welwitschii	Tiliaceae	Leaf	DCM/MeOH (1:1)	*P. f D10*	3.6	S. Africa	Clakson *et al.* [24]
Turraea floribunda	Meliaceae	Bark/Leaf/ Roots	DCM/MeOH (1:1)	*Pf NF*	4.52/12.7/ 5.56	S. Africa	Makoka *et al.* [62]
Vahlia capensis	Vahilaceae	Leaf	AQS	*Pfk1*	26.6% at 50	Namibia	Iwanette *et al.* [59]
Vangueria infausta	Rubiaceae	Fruit	DCM/MeOH (1:1)	*P.f D10*	23	S. Africa	Clakson *et al.* [24]
Vangueria infausta	Rubiaceae	Roots	DCM	*Pf-NF54*	1.84	S. Africa	Bapela *et al.* [64]
Vernonia colorata	Asteraceae	Twig	DCM/MeOH (1:1)	*P.f D10*	14.1	S. Africa	Clakson *et al.* [24]
Vernonia fastigiata	Asteraceae	Leaf	DCM	*P.f D10*	10	S. Africa	Clakson *et al.* [24]
Vernonia hirsuta	Asteraceae	Whole	DCM/MeOH (1:1)	*P. f D10*	14	S. Africa	Clakson *et al.* [24]
Vernonia hirsute	Asteraceae	Whole plant	DCM/MeOH (1:1)	*Pfk1*	10.2	S. Africa	Makoka *et al.* [63]
Vernonia mespilifolia	Asteraceae	Leaves	DCM/MeOH (1:1)	*Pf NF*	5.09	S. Africa	Makoka *et al.* [62]
Vernonia myriantha	Asteraceae	Leaf	AQS	*P. f D10*	>100	S. Africa	Clakson *et al.* [24]
Vernonia natalensis	Asteraceae	Whole plan	DCM	*P. f D10*	19.5	S. Africa	Clakson *et al.* [24]
Vernonia natalensis	Asteraceae	whole plant	DCM/MeOH (1:1)	*Pfk1*	8.53	S. Africa	Makoka *et al.* [63]
Vernonia oligocephala	Asteraceae	Roots	DCM	*P. f D10*	>100	S. Africa	Clakson *et al.* [24]
Vernonia oligocephala	Asteraceae	Leaves	DCM	*7.69*	Pfk1	S. Africa	Makoka *et al.* [63]
Ximenia americana	Olacaceae	Roots	DCM	*Pf-NF54*	28.2	S. Africa	Bapela *et al.* [64]
Ximenia caffra	Olacaceae	Leaf	DCM/MeOH (1:1)	*P. f D10*	55	S. Africa	Clakson *et al.* [24]
Xylopia parviflora	Annonaceae	Leaves	DCM	*Pf-NF54*	2.19	S. Africa	Bapela *et al.* [64]
Xysmalobium undulatum	Araliaceae	Whole plant	DCM/MeOH (1:1)	*P. f D10*	6	S. Africa	Clakson *et al.* [24]
Zehneria scabra	Cucurbitaceae	Whole plant	DCM/MeOH (1:1)	*P. f D10*	5.5	S. Africa	Clakson *et al.* [24]
Ziziphus mucronata	Rhamnaceae	Leaf	DCM	*P. f D10*	12	S. Africa	Clakson *et al.* [24]

DCM: Dichloromethane, MeOH: Methanol, EtOH: Ethanol, HO: Hydroxide, AQS: Aqueous, pf: *Plasmodium falciparum*, IC_{50}: Inhibitory concentration 50%

costata, and *Dicerocaryum eriocarpum* exhibit 48.0%, 26.6%, and 21.5 48% parasite inhibition at 50 μg/ml [59], their level of activities could not be ascertained. A total of 15 compounds were isolated from South African plant, 7 of which demonstrated high activities (IC_{50} > 5 μg/ml) while others show promising activities (IC_{50} 5-15 μg/ml). The most highly active compound is 13-epi-dioxiabiet-8(14)-en-18-ol isolated from leave extracts of *Hyptis suaveolens* (IC_{50} = 1.0 μg/ml) [60]. Despite the traditional use against malarial fever, most of the plants reviewed show no noticeable antiplasmodial activity, the traditional uses of this plant against malarial infection could only be linked to their antipyretics or immune modulatory effect to alleviate the

Table 3: *In vitro* antimalarial activities of North African plants

Plant	Family/common name	Part use	Solvents	IC_{50} (μg/ml)	P.strain	P.inhibion at conc	Country	References
Sisymbrium irio	Brassicaceae	Leaves	EtOH		*pf D6*	3 at 15.867	Egypt	Shimaa *et al.* [68]
Acacia nilotica	Fabaceae	Seed	MeOH	0.9/4.1	*3D7 and Dd2*		Sudan	El-Tahir *et al.* [68]
Acanthospermum hispidum	Asteraceae	Aerial shoots	MeOH	4.9	*pfDd2*		Sudan	El-Tahir *et al.* [70]
Aerva javanica	Amaranthaceae	Whole	Pet ether/ CLF (1:1)		*pf*	100 at 500	Sudan	Ahmed *et al.* [66]
Alhagi graecorum	Papilionaceae	Leaves	EtOH		*pf D6*	47 at 15.867	Egypt	Shimaa *et al.* [68]
Amaranthus lividus	Amaranthaceae	Leaves	EtOH		*pf D6*	46 at 15.867	Egypt	Shimaa *et al.* [68]
Ambrosia maritime	Asteraceae	Whole	Pet ether/ chlorof (1:1)		*pf*	94.112 at 500	Sudan	Ahmed *et al.* [66]
Anastatica hierochuntica	Cruciferae	Leaves	EtOH		*pf D6*	44 at 15.867	Egypt	Shimaa *et al.* [68]
Aristolochia bracteata	Aristolchiaceae	Stem	Chloroform/ AQS/MeOH	12/210/ 59	*Pf3D7*		Sudan	El-Tahir *et al.* [70]
Aristolochia bracteo-lata	Aristolochiaceae	Whole	Pet ether/ chlorof (1:1)		*pf*	100 at 500	Sudan	Ahmed *et al.* [66]
Artemisia Absinthium	Asteraceae	Leaves	EtOH		*pf D6*	52 at 15.867	Egypt	Shimaa *et al.* [68]
Aster squamatous	Compositae	Leaves	EtOH		*pf D6*	45 at 15.867	Egypt	Shimaa *et al.* [68]
Balanites aegyptiaca	Balantitiaceae	Stem	MeOH	55	*pfDd2*		Sudan	El-Tahir *et al.* [69]
Beta vulgaris	Chenopodiaceae	Leaves	EtOH		*pf D6*	32 at 15.867	Egypt	Shimaa *et al.* [68]
Camellia sinensis	Theaceae	Leaves	EtOH		*pf D6*	44 at 15.867	Egypt	Shimaa *et al.* [68]
Cartagena ipecacuanha	Rubiaceae	Root	EtOH		*pf D6*	70 at 15.867	Egypt	Shimaa *et al.* [68]
Cassia tora	Caesalpiniaceae	Aerial part	MeOH	5.2	*Pf3D7*		Sudan	El-Tahir *et al.* [69]
Chenopodium murale	Chenopodiaceae	Leaves	EtOH		*pf D6*	39 at 15.867	Egypt	Shimaa *et al.* [68]
Cichorium endivia	Asteraceae	Leaves	EtOH		*pf D6*	44 at 15.867	Egypt	Shimaa *et al.* [68]
Cichorium intybus	Asteraceae	Leaves	EtOH		*pf D6*	42 at 15.867	Egypt	Shimaa *et al.* [68]
Cinnamomum cassia	Lauraceae	Bark	EtOH		*pf D6*	44 at 15.867	Egypt	Shimaa *et al.* [68]
Citrullus colocynthis	Cucurbitaceae	Seed	Pet ether/ chlorof (1:1)		*pf*	97.96 at 500	Sudan	Ahmed *et al.* [66]
Citrus reticulate	Rutaceae	Leaves	EtOH		*pf D6*	33 at 15.867	Egypt	Shimaa *et al.* [68]
Conyza dioscoridis	Compositae	Leaves	EtOH		*pf D6*	38 at 15.867	Egypt	Shimaa *et al.* [68]
Corchorus olitorius	Tiliaceae	Leaves	EtOH		*pf D6*	37 at 15.867	Egypt	Shimaa *et al.* [68]
Croton zambesicus	Euphorbiaceae	Fruit	Pet ether/ chlorof (1:1)		*pf*	82.35 at 500	Sudan	Ahmed *et al.* [66]
Curcuma aromatic	Zingebracea	Rhizomes	EtOH		*pf D6*	52 at 15.867	Egypt	Shimaa *et al.* [68]
Cymbopogon proximus	Poaceae	Leaves	EtOH		*pf D6*	47 at 15.867	Egypt	Shimaa *et al.* [68]
Cymbopogon schoenanthus	Asteraceae	Aerial part	MeOH		*Pfk1*	100 at 8.00	Sudan	Intisar *et al.* [65]
Cyperus alopecuroides	Cyperaceae	Leaves	EtOH		*pf D6*	28 at 15.867	Egypt	Shimaa *et al.* [68]
Cyperus rotundus	Cyperacea	Leaves	EtOH		*pf D6*	44 at 15.867	Egypt	Shimaa *et al.* [68]
Daucus carota	Apiaceae	Leaves	EtOH		*pf D6*	41 at 15.867	Egypt	Shimaa *et al.* [68]
Desmostachia bipinnata	Poaceae	Leaves	EtOH		*pf D6*	44 at 15.8	Egypt	Shimaa *et al.* [68]
Emblica officinalis	Phyllanthaceae	Leaves	EtOH		*pf D6*	100 at 15.867	Egypt	Shimaa *et al.* [68]
Eruca sativa	Brassicaceae	Leaves	EtOH		*pf D6*	33 at 15.867	Egypt	Shimaa *et al.* [68]
Ficus carica	Moraceae	Leaves	EtOH		*pf D6*	36 at 15.867	Egypt	Shimaa *et al.* [68]
Gardenia jovis tonatis	Rubiaceae	Stem bark	MeOH	4.3/49	*3D7 and Dd2*		Sudan	El-Tahir *et al.* [70]
Gardenia lutea	Rubiacene	Fruit	Pet ether/ chlorof (1:1)		*pf*	97.67 at 500	Sudan	Ahmed *et al.* [66]
Gardenia lutea	Rubiaceae	Stem bark	MeOH	5.2/3.3	*3D7 and Dd2*		Sudan	El-Tahir *et al.* [70]
Glycyrrhiza glabra	Fabaceae	Roots and rhizomes	EtOH		*pf D6*	49 at 15.867	Egypt	Shimaa *et al.* [68]
Helianthus annus	Poaceae	Seed	MeOH		*Pfk1*	100 at 4.00	Sudan	Intisar *et al.* [65]
Hibiscus sabdariffa	Malvaceae	Flowers calyx and epi-calyx	EtOH		*pf D6*	34 at 15.867	Egypt	Shimaa *et al.* [68]
Hyphaene thebaica	Arecaceae	Fruits	EtOH		*pf D6*	35 at 15.867	Egypt	Shimaa *et al.* [68]
Lawsonia inermis	Lythraceae	Leaves	EtOH		*pf D6*	0 at 15.867	Egypt	Shimaa *et al.* [68]
Lupinus termis	Fabaceae	Seeds	EtOH		*pf D6*	0 at 15.867	Egypt	Shimaa *et al.* [68]
Malva parviflora	Malvacea	Leaves	EtOH		*pf D6*	50 at 15.867	Egypt	Shimaa *et al.* [68]
Mentha longifolia	Labiatae	Leaves	EtOH		*pf D6*	47 at 15.867	Egypt	Shimaa *et al.* [68]
Morus alba	Moraceae	Leaves	EtOH		*pf D6*	29 at 15.867	Egypt	Shimaa *et al.* [68]
Nigella sativa	Ranuneulaceae	Seed	Pet ether/ chlorof (1:1)		*pf*	100 at 500	Sudan	Ahmed *et al.* [66]
Opuntia ficus indica	Cactaceae	Leaves	EtOH		*pf D6*	52 at 15.867	Egypt	Shimaa *et al.* [68]
Origanum majorana	Lamiaceae	Leaves	EtOH		*pf D6*	41 at 15.867	Egypt	Shimaa *et al.* [68]
Peganum harmal	Nitrariaceae	Seed	EtOH		*pf D6*	70 at 15.867	Egypt	Shimaa *et al.* [68]
Phaseolus vulgaris	Papilionaceae	Leaves	EtOH		*pf D6*	41 at 15.867	Egypt	Shimaa *et al.* [68]
Phragmites communis	Poaceae	Leaves	EtOH		*pf D6*	0 at 15.867	Egypt	Shimaa *et al.* [68]

(Cond..)

Table 3: *(Continued...)*

Plant	Family/common name	Part use	Solvents	IC_{50} (μg/ml)	P.strain	P.inhibion at conc	Country	References
Pimpinella anisum	Umbelliferae	Fruits	EtOH		*pf D6*	44 at 15.867	Egypt	Shimaa *et al.* [68]
Polgonum glabrium	Polgonaceae	Leaf	EtOH	6.6	*P. fk1*		Sudan	Khadiga *et al.* [71]
Psidium guajava	Myrtaceae	Leaves	EtOH		*pf D6*	50 at 15.867	Egypt	Shimaa *et al.* [68]
Pulicaria crispa	Asteraceae	Whole	Pet ether/ chlorof (1:1)		*pf*	96.65 at 500	Sudan	Ahmed *et al.* [66]
Punica granatum	Lythraceae	Fruit	EtOH		*pf D6*	96 at 15.867	Egypt	Shimaa *et al.* [68]
Quercus infectoria	Fagaceae	Galls	EtOH		*pf D6*	100 at 15.867	Egypt	Shimaa *et al.* [68]
Ricinus communi	Euphorbiaceae	Leves	EtOH		*pf D6*	40 at 15.867	Egypt	Shimaa *et al.* [68]
Salix subserrata	Salicaceae	Leaves	EtOH		*pf D6*	0 at 15.867	Egypt	Shimaa *et al.* [68]
Senna alexandrina	Fabaceae	Fruit	MeOH		*Pfk1*	100 at 2.0	Sudan	Intisar *et al.* [65]
Sesamum indicum	Pedaliaceae	Leaves	EtOH		*pf D6*	0 at 15.867	Egypt	Shimaa *et al.* [68]
Sesbania sesban	Leguminosae	Leaves	EtOH		*pf D6*	0 at 15.867	Egypt	Shimaa *et al.* [68]
Solenostema argel	Ascepiadaceae	Leaf	Pet ether/ chlorof (1:1)		*pf*	98.82 at 500	Sudan	Ahmed *et al.* [66]
Solenostemma argel	Apocyanaceae	Leaves	EtOH		*pf D6*	43 at 15.867	Egypt	Shimaa *et al.* [68]
Sonchous cornatus	Asteraceae	Aerial shoots	MeOH	340	*pfDd2*		Sudan	El-Tahir *et al.* [70]
Spinacia oleracea	Chenopodiaceae	Leaves	EtOH		*pf D6*	2 at 15.867	Egypt	Shimaa *et al.* [68]
Tamarindus indica	Fabaceae	Fruit	EtOH		*pf D6*	0 at 15.867	Egypt	Shimaa *et al.* [68]
Tamarindus indica	Caesalpiniaceae	Fruit/stem bark	MeOH	>500/10	*Pf3D7*		Sudan	El-Tahir *et al.* [69]
Tamarix nilotica	Tamaricaceae	Leaves	EtOH		*pf D6*	8 at 15.867	Egypt	Shimaa *et al.* [68]
Thymus vulgaris	Lamiaceae	Leaves	EtOH		*pf D6*	0 at 15.867	Egypt	Shimaa *et al.* [68]
Tilia cordata	Tiliaceae	Leaves	EtOH		*pf D6*	3 at 15.867	Egypt	Shimaa *et al.* [68]
Tinospora bakis	Menispermaceae	Whole	Pet ether/ chlorof (1:1)		*pf*	92.94 at 500	Sudan	Ahmed *et al.* [66]
Trifolium alexandrinum	Leguminosae	Leaves	EtOH		*pf D6*	0 at 15.867	Egypt	Shimaa *et al.* [68]
Withania somnifera	Solanaceae	Leaves	EtOH		*pf D6*	15 at 15.867	Egypt	Shimaa *et al.* [68]
Zingiber officinale	Zingebracea	Rhizome	EtOH		*pf D6*	38 at 15.867	Egypt	Shimaa *et al.* [68]
Zizyphus spina-christi	Rhamnaccae	Leaves	EtOH		*pf D6*	0 at 15.867	Egypt	Shimaa *et al.* [68]

DCM: Dichloromethane, MeOH: Methanol, EtOH: Ethanol, HO: Hydroxide, AQS: Aqueous, pf: *Plasmodium falciparum*, IC_{50}: Inhibitory concentration 50%

Table 4: *In vitro* antimalarial activities of plants from East African

Plant	Family/common name	Part use	Solvents	IC_{50} (μg/ml)	Parasite	Country	References
Cassia abbreviata	Caesalpiniaceae	Roots	DCM/MeOH	40.0/>100	*3D7*	Mozambique	Ramalhete *et al.* [72]
Tabernaemontana elegans	Apocynaceae	Leaves	n-hex/DMC/et/MeOH	59/26.9/>100/>100	*3D7 P.f*	Mozambique	Ramalhete *et al.* [72]
cTrichilia eMeOHica	Meliaceae	Seed	n-hex/DMC/et/MeOH	>100	*3D7 P.f*	Mozambique	Ramalhete *et al.* [72]
AcAcaacia karroo	Fabaceae	Aerial parts	n-hex/DMC/et/MeOH	99/60/20.2/>100	*3D7 P.f*	Mozambique	Ramalhete *et al.* [72]
Acokanthera oppositifolia	Apocynaceae	Leaves	Hex/DCM/MeOH	>50/19.5/>50	*FcB1*	Kenya	Sylvain *et al.* [73]
Acokanthera schimperi	Apocynaceae	Stems/leaves	Hex/DCM/MeOH	31.5/9.8/>50	*FcB1*	Kenya	Sylvain *et al.* [73]
Alangium chinense	Alangiaceae	Aerial parts	Hex/DCM/MeOH	>50/6.15/2.8	*FcB1*	Kenya	Sylvain *et al.* [73]
Aristolochia elegans	Aristolochiaceae	seed	MeOH/DCM	>50	*3D7 P.f*	Rwanda	Muganga *et al.* [74]
Schefflera actinophylla	Araliaceae	Leaves	n-hex/DMC/et/MeOH	32.5/36.3/41.7/>100	*3D7 P.f*	Mozambique	Ramalhete *et al.* [72]
AAloe parvibracteata	Aloaceae	Leaf	n-hex/DMC/et/MeOH	>100	*3D7 P.f*	Mozambique	Ramalhete *et al.* [72]
Bridelia cathartica	Euphorbiaceae	Roots	n-hex/DMC/et/MeOH	99/>100/44	*3D7 P.f*	Mozambique	Ramalhete *et al.* [72]
Cadaba farinosa	Capparaceae	Aerial parts	Hex/DCM/MeOH	>50/6.2/>50	*FcB1*	Kenya	Sylvain *et al.* [73]
Carissa edulis	Apocynaceae	Stem bark	Eth/MeOHh	26.37/>50	*PfD6*	Kenya	Ayuko *et al.* [75]
Cassia abbreviate	Fabaceae	Stem bark	n-hex/DMC/et/MeOH	>100/40/>100/>100	*3D7 P.f*	Mozambique	Ramalhete *et al.* [72]
Cassia occidentalis	Fabaceae	Roots	n-hex/DMC/et/MeOH	19.3/59.9/31.9/88.2	*3D7 P.f*	Mozambique	Ramalhete *et al.* [72]
Conyza aegyptiaca	Asteraceae	Leaf	MeOH/DCM/AQS	22.7/36.8/>50	*3D7 P.f*	Rwanda	Muganga *et al.* [74]
Crossopteryx febrifuga	Rubiaceae	Aerial parts	n-hex/DMC/et/MeOH	0/44.4/0/>100	*3D7 P.f*	Mozambique	Ramalhete *et al.* [72]
Fuerstia africana	Lamiaceae	Leaf	MeOH/DCM	6.9/40.2	*3D7 P.f*	Rwanda	Muganga *et al.* [74]
Kigelia africana	Bignoniaceae	Leaves	Eth/MeOHh	13.5/25.7	*PfD6*	Kenya	Ayuko *et al.* [75]
Leonotis leonurus	Lamiaceae	Aerial parts	n-hex/DMC/et/MeOH	>100/45.4/38.4/>100	*3D7 P.f*	Mozambique	Ramalhete *et al.* [72]
Lippia javanica	Verbenaceae	Root	Eth/MeOHh	12.12/1.35	*PfD6*	Kenya	Ayuko *et al.* [75]
Markhamia lutea	Bignoniaceae	Leaf	MeOH/DCM	>50/29	*3D7 P.f*	Rwanda	Muganga *et al.* [74]
Maytenus heterophylla	Celastraceae	Root	Eth/MeOHh	18.9/13.07	*PfD6*	Kenya	Ayuko *et al.* [75]
Microglossa pyrifolia	Asteraceae	Leas	MeOH/DCM/AQS	4.2/1.5	*3D7 P.f*	Rwanda	Muganga *et al.* [74]
Microglossa pyrifolia	Asteracceae	Leaves	Eth/MeOHh	14.7/1.59	*PfD6*	Kenya	Ayuko *et al.* [75]
Mitragyna rubrostipulata	Rubiaceae	Stem	MeOH/DCM	>50/39.9	*3D7 P.f*	Rwanda	Muganga *et al.* [74]
Momordica balsamina	Cucurbitaceae	Aerial parts	n-hex/DMC/et/MeOH	>100/35.5/1.0/46.9	*3D7 P.f*	Mozambique	Ramalhete *et al.* [72]
Parkinsonia aculeata	Caesalpiniaceae	Aerial parts	n-hex/DMC/et/MeOH	24.5/26.3/36.4/54.9	*3D7 P.f*	Mozambique	Ramalhete *et al.* [72]
Periploca linearifolia	Asclepiadacae	Stem bark	Eth/MeOHh	25.7/1.6	*PfD6*	Kenya	Ayuko *et al.* [75]

(Cond..)

Table 4: *(Continued...)*

Plant	Family/common name	Part use	Solvents	IC_{50} (μg/ml)	Parasite	Country	References
Pittosporum tobira	Pittosporaceae	Aerial parts	n-hex/DMC/et/MeOH	34.4/44.6/4.8/>100	*3D7 P.f*	Mozambique	Ramalhete *et al.* [72]
Plumbago auriculata	Plumbaginaceae	Aerial parts	n-hex/DMC/et/MeOH	45.9/40.2/53.8/80.0	*3D7 P.f*	Mozambique	Ramalhete *et al.* [72]
Rumex abyssinicus	Polygonaceae	Root	MeOH/DCM/AQS	>50/4.3	*3D7 P.f*	Rwanda	Muganga *et al.* [74]
Rumex bequaertii	Polygonaceae	Root	MeOH/DCM/AQS	>50	*3D7 P.f*	Rwanda	Muganga *et al.* [74]
Schizozygia coffaeoides	Apocynaceae	Stems	Hex/DCM/MeOH	19.75/9.70/>50	*FcB1*	Kenya	Sylvain *et al.* [73]
Scolopia zeyheri	Flacourtiaceae	Aerial parts	Hex/DCM/MeOH	>50/7.5/>50	*FcB1*	Kenya	Sylvain *et al.* [73]
Senna didymobotrya	Fabaceae	Twig	n-hex/DMC/et/MeOH	57.6/92/>100/56	*3D7 P.f*	Mozambique	Ramalhete *et al.* [72]
Solanecio mannii	Asteraceae	Leaf	MeOH/DCM	21.6/18.2	*3D7 P.f*	Rwanda	Muganga *et al.* [74]
Strychnos henningnsii	Strychnaceae	root	Eth/MeOHh	25.0/1.07	*PfD6*	Kenya	Ayuko *et al.* [75]
Strychnos usambarensis	Strychnaceae	root	Eth/MeOHh	15.65/23.82	*PfD6*	Kenya	Ayuko *et al.* [75]
Terminalia mollis	Combretaceae	Leaf	MeOH/DCM	>50	*3D7 P.f*	Rwanda	Muganga *et al.* [74]
Tithonia diversifolia	Asteraceae	Fruit	MeOH/DCM	8.1/1.1	*3D7 P.f*	Rwanda	Muganga *et al.* [74]
Toddalia asiatica	Rutaceae	Roots	Hex/DCM/MeOH	10/5.75/39	*FcB1*	Kenya	Sylvain *et al.* [73]
Trimeria grandifolia	Flacourtiaceae	Leaf	MeOH/DCM	>50	*3D7 P.f*	Rwanda	Muganga *et al.* [74]
Zanthoxylum chalybeum	Rutaceae	Stem	MeOH/DCM	42.5/41.5	*3D7 P.f*	Rwanda	Muganga *et al.* [74]

DCM: Dichloromethane, MeOH: Methanol, EtOH: Ethanol, HO: Hydroxide, AQS: Aqueous, pf: *Plasmodium falciparum*, IC_{50}: Inhibitory concentration 50%

Table 5: Anti-malarial activity of plants from Central Africa

Plant	Family	Part use	Solvents	Parasite	IC_{50} (μg/ml)	P.inhibition	Country	References
Entandrophragma angolense	Meliaceae	Stem bark	Hex/MeOH	*pf W2*	33.4/26.2		Cameroon	Jean *et al.* [80]
Achromanes difformis	Araceae	Leaf	Chloroform/MeOH (1:1)	*pf F32*		114 at 10	Cameroon	Harikrishna *et al.* [81]
Albizia zygia	Mimosaceae	Stem	MeOH		1.10		Cameroon	Lenta *et al.* [82]
Alchornea cordifolia	Euphorbiaceae	Leaf	AQS	*Pfk1*	4.84		Congo	Muganza *et al.* [76]
Alchornea floribunda	Euphorbiaceae	Leas root	AQS	*Pfk1*	20.80		Congo	Muganza *et al.* [76]
Alstonia boone	Apocynaceae	Stem	AQS	*Pfk1*	>64		Congo	Muganza *et al.* [76]
Anisopappus chinensis	Asteraceae	Whole	MeOH/DCM/AQS	*pf3D7*	8.82/6.53/76.51		Congo	Lusakibanza *et al.* [77]
Anisopappus chinensis	Asteraceae	Whole plant	MeOH	*pf3D7*	8.82		Congo	Frédérich *et al.* [83]
Annona muricata	Annonaceae	Leaf	chloroform/MeOH (1:1)	*pf F32*		46.6 at 10	Cameroon	Harikrishna *et al.* [81]
Anonidium mannii	Annonaceae	Stem	AQS	*Pfk1*	>64		Congo	Muganza *et al.* [76]
Artocarpus communis	Moraceae	Leaf	MeOH	-	4.00		Cameroon	Boyom *et al.* [83]
Autranella congolensis	Sapotaceae	Stem	AQS	*Pfk1*	35.45		Congo	Muganza *et al.* [76]
Boscia angustifolia	Capparaceae	Leaf	DCM/MeOH		107.9/37.6		Mali	Bah *et al.* [48]
Calycobolus	Convolvulaceae	Stem	AQS	*Pfk1*	>64		Congo	Muganza *et al.* [76]
Caralluma tuberculata	Asclepiadaceae	Leaf	EtOH/P.eth/ISal		9.7/2.5/2.7		Congo	Tona *et al.* [46]
Cassia occidentalis	Caesalpiniaceae	Leaf	EtOH/P.eth/ISal		2.8/1.5/186		Congo	Tona *et al.* [46]
Cleome rutidosperma	Cleomaceae	Leaf	chloroform/MeOH (1:1)	*pf F32*		12.3 at 10	Cameroon	Harikrishna *et al.* [81]
Copaifera religiosa	Fabaceae	Back	DCM/MeOH	*pf FCB*	13.4/500.7		Gabon	Lekana-Douki *et al.* [84]
Cymbopogon citratus	Poaceae	Leaf	Chloroform/MeOH (1:1)	*pf F32*		42 at 10	Cameroon	Harikrishna *et al.* [81]
Dalhousiea africana	Leguminosae	Leaf	AQS	*Pfk1*	>64		Congo	Muganza *et al.* [76]
Drypetes gossweileri	Euphorbiaceae	Stem	AQS	*Pfk1*	>64		Congo	Muganza *et al.* [76]
Enantia chlorantha	Annonaceae	Stem	AQS	*Pfk1*	7.77		Congo	Muganza *et al.* [76]
Enantia chlorantha	Annonaceae	Stem/stem bark	MeOH	*Pfk1*	4.79/2.06		Cameroon	Boyom *et al.* [83]
Entandrophragma palustre	Meliaceae	Stem	MeOH/DCM/AQS	*pf3D7*	15.8/17.6/>100		Congo	Lusakibanza *et al.* [77]
Entandrophragma palustre	Meliaceae	Stem bark	MeOH	*pf3D7*	15.84		Congo	Frédérich *et al.* [83].
Euphorbia hirta	Dilleniaceae	Whole plant	EtOH/P.eth/ISal		2.4/1.2/2.6		Congo	Tona *et al.* [46]
Frostyrax lepidophyllus	Huaceae	Root	AQS	*Pfk1*	>64		Congo	Muganza *et al.* [76]
Garcinia kola	Clusiaceae	Stem bark	EtOH/P.eth/ISal		2.9/1.6/41.7		Congo	Tona *et al.* [46]
Garcinia punctata	Clusiaceae	Stem	AQS	*Pfk1*	36.56		Congo	Muganza *et al.* [76]
Harungana madagascariensis	Clusiaceae	Stem	AQS	*Pfk1*	9.64		Congo	Muganza *et al.* [76]
Isolona hexaloba	Annonaceae	Stem	AQS	*Pfk1*	15.28		Congo	Muganza *et al.* [76]
Jatropha curcas	Euphorbiaceae)	Root	AQS	*Pfk1*	>64		Congo	Muganza *et al.* [76]
Mammea africana	Clusiaceae	Stem	AQS	*Pfk1*	28.57		Congo	Muganza *et al.* [76]
Mangifera indicus	Anacardiaceae	Leaf	Chloroform/MeOH (1:1)	*pf F32*		46.1 at 10	Cameroon	Harikrishna *et al.* [81]
Manniophyton fulvum	Euphorbiaceae	Leaf/root	AQS	*Pfk1*	22.44/>64		Congo	Muganza *et al.* [76]
Massularia acuminata	Rubiaceae	Stem	AQS	*Pfk1*	>64		Congo	Muganza *et al.* [76]
Melia azedarach	Meliaceae	Leaves	MeOH/DCM	*pf3D7*	55.13/19.14		Congo	Lusakibanza *et al.* [77]
Melia azedarach	Meliaceae	Leaves	MeOH	*pf 3D7*	44.62		Congo	Frédérich *et al.* [83]

(Cond..)

Table 5: *(Continued...)*

Plant	Family	Part use	Solvents	Parasite	IC_{50} (μg/ml)	P.inhibition	Country	References
Mellotus appositofolius	Euphorbiaceae	Leaf	Chloroform/ MeOH (1:1)	*pf F32*		40 at 10	Cameroon	Harikrishna *et al.* [81]
Morinda morindoides	Rubiaceae	Leaf	EtOH/P.eth/ISal		94.2/1.8/15.3		Congo	Tona *et al.* [46]
Musanga cecropioides	Cecropiaceae	Stem	AQS	*Pfk1*	>64		Congo	Muganza *et al.* [76]
Napoleona vogelii	Lecythidaceae	Stem	AQS	*Pfk1*	>64		Congo	Muganza *et al.* [76]
Ocimum gratissimum	Lamiaceae	Leaf	AQS	*Pfk1*	7.25		Congo	Muganza *et al.* [76]
Penianthus longifolius	Menispermaceae	Root	AQS	*Pfk1*	27.10		Congo	Muganza *et al.* [76]
Physalis angulata	Solanaceae	Whole	MeOH/DCM/ AQS	*pf3D7*	1.27/1.96/ 23.10		Congo	Lusakibanza *et al.* [77]
Physalis angulata	Solanaceae	Whole plant	MeOH	*P.f3D7*	1.27		Congo	Frédérich *et al.* [77]
Picralima nitida	Apocynaceae	Stem	AQS	*Pfk1*	36.76		Congo	Muganza *et al.* [76]
Picralima nitida	Apocynaceae	Seed	Hex/MeOH	*pf W2*	129.6/10.9		Cameroon	Jean *et al.* [80]
Piper guineense	Piperaceae	Leaf/root/stem	AQS	*Pfk1*	>64		Congo	Muganza *et al.* [76]
Piper umbellatum	Piperaceae	Leaf	chloroform/ MeOH (1:1)	*pf F32*		36.2 at 10	Cameroon	Harikrishna *et al.* [81]
Piptadeniastrum africanum	Leguminosae	Stem	AQS	*Pfk1*	6.11		Congo	Muganza *et al.* [76]
Polyalthia oliveri	Annonaceae	Stem	MeOH	-	4.30		Cameroon	Boyom *et al.* [83]
Polyalthia suaveolens	Annonaceae	Leaf/root back/ stem	AQS	*Pfk1*	>64		Congo	Muganza *et al.* [76]
Pyrenacantha klaineana	Cacinaceae	Leaf	AQS	*Pfk1*	5.46		Congo	Muganza *et al.* [76]
Quassia africana	Simaroubaceae	Leaf/root back	AQS	*Pfk1*	0.46/1.27		Congo	Muganza *et al.* [76]
Schumanniophyton magnificum	Rubiaceae	Stem bark	Hex/MeOH	*78.1/ 28.7*	pf W2		Cameroon	Jean *et al.* [80]
Scorodophloeus zenkeri	Leguminosae	Root/stem back	AQS	*Pfk1*	>64		Congo	Muganza *et al.* [76]
Staudtia kamerunensis	Myristicaceae	Stem back	AQS	*Pfk1*	>64		Congo	Muganza *et al.* [76]
Strychnos icaja	Loganiaceae	Root	MeOH/DCM/ AQS	*pf3D7*	0.69/0.84		Congo	Lusakibanza *et al.* [77]
Strychnos icaja	Loganiaceae	Root bark	MeOH	*pf3D7*	0.69		Congo	Frédérich *et al.* [83]
Symphonia globulifera	Clusiaceae	Leaf	MeOH		4.1		Cameroon	Lenta *et al.* [82]
Tefracera pogge	Dilleniaceae	Leaf	EtOH/P.eth/ISal		36.9/1.7/21.8		Congo	Tona *et al.* [46]
Tetrapleura tetraptera	Leguminosae	Fruit/stem back	AQS	*Pfk1*	>64		Congo	Muganza *et al.* [76]
Tetrapleura tetraptera	Fabaceae	Back	MeOH	*pf FCB*	13.1		Gabon	Lekana-Douki *et al.* [84]
Thomandersia hensii	Acanthaceae	Leaf	AQS	*Pfk1*	41.12		Congo	Muganza *et al.* [76]
Thomandersia hensii	Acanthaceae	Stem bark	Hex/MeOH	*pfW2*	53.9/68.2		Cameroon	Jean *et al.* [80]
Triclisia dictyophylla	Menispermaceae	Leaf	Aqueous	*Pfk1*	5.13		Congo	Muganza *et al.* [76]

DCM: Dichloromethane, MeOH: Methanol, EtOH: Ethanol, HO: Hydroxide, AQS: Aqueous, ISal: Isoamyl alcohol, pf: *Plasmodium falciparum*, IC_{50}: Inhibitory concentration 50%

malarial symptoms rather than exerting direct antiplasmodial activity [61].

Anti-malarial Activity of Plants from North Africa

Appreciable amounts of plants were found in north Africa, out of the 79 plants found in this region only 4 plants had IC_{50} > 5 μg/ml (highly active), although IC_{50} not documented, 100% inhibition were documented for methanol extract from *Helianthus annus* seed at 4 μg/ml and methanol fruit extracts of senna alexandrina at 2 μg/ml [65], the activities demonstrated by this plants could be considered highly active if compared with the WHO guideline. However, Pet ether/chloroform extract from *Aerva javanica*, *Aristolochia bracteo-lata*, *Gardenia lutea*, *Citrullus colocynthis*, and *Nigella sativa* from Sudan show 100% parasite inhibition at 500 μg/ml [66]. Despite the significant parasite inhibition demonstrated by these plants, there activities could be classified under not active due to large dose of extract. A total of 23 compounds were isolated from north African plant, this extracts demonstrate interesting and varied antiplasmodial activities, however, the most noticeable activities is 3',4',7-trihydroxyflavone (IC_{50} = 0.078 μg/ml) from *Albizia zygia* against *Pfk1* [67].

Anti-malarial Activity of Plants from East Africa

Only 44 plants from East Africa were reviewed for *in vitro* activities against malarial parasite. 15 plants extracts reviewed from this region had IC_{50} > 5 μg/ml (highly active), 4 extracts had IC_{50} = 5-15 μg/ml (promising active), 32 extract had IC_{50} = 15-50 μg/ml (moderate active), while others were inactive (IC_{50} > 50 μg/ml).

Anti-malarial Activity of Plants from Central Africa

The majority of the plants grown in this region show very poor activities against *Plasmodium* parasite.

Out of 67 plants found to have been studied for anti-plasmodial (*in vitro*) activity in Central African, only 17 extracts from the plants demonstrate high antiplasmodial activity (IC_{50} value < 5 μg/ml). The most noticeable activities was demonstrated by AQS leaf extract of *Quassia africana* IC_{50} = 0.46 μg/ml [76] and methanol root back extract from *Strychnos icaj*a, IC_{50} = 0.69 μg/ml [77]. How ever 15 out of the 17 isolated compounds from central Africa were highly active (IC_{50} value < 5 μg/ml) against *Plasmodium*, the most

Table 6: Anti-malarial activity of isolated compounds from African plant

Compounds	Plant species	Family	Part used	Solvent	Pstrain	IC_{50} µg/ml	Country	References
1,2-didehydroancistrobertsonine D	*Ancistrocladus robertsoniorum*	Ancistrocladaceae	Leaves	MeOH	*PfK1/pf NF54*	1.4/5.0	Kenya	Bringmanna *et al.* [86]
1,3-deacetyldeoxyhavenensin	*Khaya anthotheca*	Meliaceae	Seed	Petroleum ether	*pf K1*		Uganda	Obbo *et al.* [87]
13_-*epi*-dioxiabiet-8 (14)-en-18-ol	*Hyptis suaveolens*	Lamiaceae	Leaf	Pet ether	*Pf*	0.1	South Africa	Chukwujekwu *et al.* [60]
1H-indole-5-carbaldehyde	*Monodora angolensis*	Annonaceae	Stem/root bark	-	*Pf K1*	>21	Tanzania	Nkunya *et al.* [88]
2,3,6-trihydroxy benzoic acid	*Sorindeia juglandifolia*	Anacardiaceae	Met	-	*Pf W2*	16.5	Cameroon	Raceline *et al.* [89]
2,3,6-trihydroxy methyl benzoate	*Sorindeia juglandifolia*	Anacardiaceae	Met	-	*Pf W2*	13	Cameroon	Raceline *et al.* [89]
2,6-dihydroxyfissinolide	*Khaya senegalensis*	Meliaceae	Back	-	*3D7*	0.12	Nigeria	Khalid *et al.* (1998)
3-(1,1-dimethyl-but-2-enyl)-5-(3-methyl-but-2-enyl)-1H-indole	*Isolona cauliflora*	Annonaceae	Stem/root bark	-	*Pf K1*	>21	Tanzania	Nkunya *et al.* [88]
3′,4′,7-trihydroxyflavone	*Albizia zygia*	Leguminosae	Back	DCM/MeOH.	*PfK1*	0.078	Sudan	Abdalla and Laatsch [67]
3-geranylindole	*Monodora angolensis*	Annonaceae	Stem/root bark	-	*Pf K1*	>21	Tanzania	Nkunya *et al.* [88]
3-O-methylfisetin (3′,4′, 7-trihydroxy-3-methoxyflavone	*Albizia zygia*	Leguminosae	Back	DCM/MeOH	*PfK1*	>0.078	Sudan	Abdalla and Laatsch, [67]
4′,7-dihydroxyflavanone	*Albizia zygia*	Leguminosae	Back	DCM/MeOH	*PfK1*	>0.078	Sudan	Abdalla and Laatsch, [67]
4-[3-(1,1-dimethyl-but-2-enyl)-1H-indol-5-yl]-but-3-en-2-one or caulidine B	*Isolona cauliflora*	Annonaceae	Stem/root bark	-	*Pf K1*	>21	Tanzania	Nkunya *et al.* [88]
4_-hydroxy-5,6,7,3_, 5_-pentamethoxyflavone	*Ageratum conyzoide*	Asteraceae	Aerial parts	MeOH	*Pfk1*	3.59	Sudan	Nour *et al.* [90]
5-(3-methyl-2-butenyl)-1H-indole and 5-(3-methylbuta-1, 3-dienyl)-1H-indole	*Isolona cauliflora*	Annonaceae	Stem/root bark	-	*Pf K1*	>21	Tanzania	Nkunya *et al.* [88]
5,6,7,3_,4_,5_-hexamethoxyflavone	*Ageratum conyzoide*	Asteraceae	Aerial parts	MeOH	*Pfk1*	2.99	Sudan	Nour *et al.* [90]
5,6,7,5_-tetramethoxy 3_, 4_-methylenedioxyflavone	*Ageratum conyzoide*	Asteraceae	Aerial parts	MeOH	*Pfk1*	4.26	Sudan	Nour *et al.* [90]
5,6,7,8,3_,4_, 5_-heptamethoxyflavone (5_-methoxynobiletine)	*Ageratum conyzoide*	Asteraceae	Aerial parts	MeOH	*Pfk1*	>5	Sudan	Nour *et al.* [90]
5,6,7,8,5_-pentamethoxy-3_, 4_-methylenedioxyflavone (eupalestin)	*Ageratum conyzoide*	Asteraceae	Aerial parts	MeOH	*Pfk1*	4.57	Sudan	Nour *et al.* [90]
6-(3-methyl-2-butenyl)-1H-indole-	*Monodora angolensis*	Annonaceae	Stem/root bark	-	*Pf K1*	>21	Tanzania	Nkunya *et al.* [88]
6-(3-methyl-but-2-enyl)-1, 3-dihydro-indol-2-one	*Monodora angolensis*	Annonaceae	Stem/root bark	-	*Pf K1*	21	Tanzania	Nkunya *et al.* [88]
6-(3methylbuta-1, 3-dienyl) 1H-indole	*Monodora angolensis*	Annonaceae	Stem/root bark	-	*Pf K1*	>21	Tanzania	Nkunya *et al.* [88]
6-(4-oxo-but-2-enyl)-1H-indole	*Monodora angolensis*	Annonaceae	Stem/root bark	-	*Pf K1*	>21	Tanzania	Nkunya *et al.* [88]
7,8,3′,5′-tetramethoxyisoflavan-1′,4′-quinone	*Abrus precatorius*	Fabaceae	Whole plant	DCM/MeOH (1:1)	*PfK1*	8.9	South Africa	Yoshie *et al.* [91]
7,9-dimethoxy-2, 3-methylenedioxybenzophenanthridine	*Zanthoxylum rubescens*	Rutaceae	Stem bark	MeOH: water (1:1)	*3D7/FCM29*	72.2/92.4	I.Coast	Penali *et al.* [92]
7-deacetylkhivorin	*Khaya anthotheca*	Meliaceae	Seed	Petroleum ether	*pf K1*	1.37	Uganda	Obbo *et al.* [87]
Abruquinone I	*Abrus precatorius*	Fabaceae	Whole plant	DCM/MeOH (1:1)	*PfK1*	20.4	South Africa	Yoshie *et al.* [91]
Abruquinone B	*Abrus precatorius*	Fabaceae	Whole plant	DCM/MeOH (1:1)	*PfK1*	4.1	South Africa	Yoshie *et al.* [91]
Abruquinone B	*Abrus precatorius*	Fabaceae	Aerial part	-	*Pfk1*	1.50	Nigeria	Limmatvapirat *et al.* [93]
Abruquinone G	*Abrus precatorius*	Fabaceae	Aerial part	-	*Pfk1*	1.50	Nigeria	Limmatvapirat *et al.* [93]
Abruquinone G	*Abrus precatorius*	Fabaceae	Whole plant	DCM/MeOH (1:1)	*PfK1*	>20	South Africa	Yoshie *et al.* [91]
Abruquinone H	*Abrus precatorius*	Fabaceae	Whole plant	DCM/MeOH (1:1)	*PfK1*	8.0	South Africa	Yoshie *et al.* [91]

(Cond..)

Table 6: *(Continued...)*

Compounds	Plant species	Family	Part used	Solvent	P.strain	IC_{50} µg/ml	Country	References
Akuammicine	*Picralima nitida*	Apocynaceae	Seed	EtOH	*D6*	0.45	Nigeria	Okokon *et al.* [27]
Akuammigine	*Picralima nitida*	Apocynaceae	Seed	EtOH	*W2*	0.530	Nigeria	Okokon *et al.* [27]
Akuammine	*Picralima nitida*	Apocynaceae	Seed	EtOH	*W2*	0.73	Nigeria	Okokon *et al.* [27]
Alstonine	*Picralima nitida*	Apocynaceae	Seed	EtOH	*D6*	0.95	Nigeria	Okokon *et al.* [27]
Ancistrobertsonine A	*Ancistrocladus robertsoniorum*	Ancistrocladaceae	Leaves	MeOH	*PfK1/pf NF54*	15.9/23.7	Kenya	Bringmanna *et al.* [86]
Ancistrobertsonines B	*Ancistrocladus robertsoniorum*	Ancistrocladaceae	Leaves	MeOH	*PfK1/pf NF54*	9/>23.0	Kenya	Bringmanna *et al.* [86]
Ancistrobertsonines C	*Ancistrocladus robertsoniorum*	Ancistrocladaceae	Leaves	MeOH	*PfK1/pf NF54*	4.5/10.1	Kenya	Bringmanna *et al.* [86]
Ancistrobertsonines D	*Ancistrocladus robertsoniorum*	Ancistrocladaceae	Leaves	MeOH	*0/4.8*	0/4.8	Kenya	Bringmanna *et al.* [86]
Ancistrobrevine B	*Ancistrocladus robertsoniorum*	Ancistrocladaceae	Leaves	MeOH	*PfK1/pf NF54*	20/4.7	Kenya	Bringmanna *et al.* [86]
Annonidine F	*Monodora angolensis*	Annonaceae	Stem/root bark	-	*Pfk1*	21	Tanzania	Nkunya *et al.* [88]
Arborinine	*Teclea trichocarpa*	Rutaceae	Leaves	MeOH	*Pfk1*	1.61	Kenya	Mwangi *et al.* [94]
Bis[6-(5,6-dihydrochelerythrinyl)] ether	*Zanthoxylum rubescens*	Rutaceae	Stem bark	MeOH: AQS (1:1)	*3D7/FCM29*	15.3/14.9	I.Coast	Penali *et al.* [92]
Cajachalcone	*Cajanus cajan*	Fabaceae	Leaves	Ethyl acetate	*Pfk1*	2.0	Nigeria	Ajaiyeoba *et al.* [95]
Cassiarin A	*Cassia siamea*	Fabaceae	Leaves	Ammonium acetate	*Pfk1*	0.020	Nigeria	Oshimi *et al.* [96]
Castalagin	*Terminalia avicennoides and Anogeissus leiocarpus*	Combretaceae	Stem bark	Met	*3D7/K1*	10.57/9.63	Nigeria	Shuaibu *et al.* [36]
Chelerythine	*Zanthoxylum chalybeum*	Rutaceae	Root bark	EtOH	*Pf 3D7*	1.35	Rwanda	Muganga *et al.* [74]
Chrysopentamin	*Strychnos usambarensis*	Loganiaceae	Leaves	EtOAc	*Pf 3D7*	579	Rwanda	Frédérich *et al.* [97]
Cinerin II	*Chrysanthemum cinerariifolium*	Asteraceae	Flower	n-hexane	*PfK1*	5.8	South Africa	Yoshie *et al.* [91]
Cryptolepine	*Cryptolepis sanguinolenta*	Periplocaceae	Root	Aqueous	*Pfk1*	0.44	Nigeria	Augustine *et al.* [98]
Cryptolepine	*Sida acuta*	Malvaceae	Leaf	Met	*Pfk1*	0.114	Nigeria	Frederich *et al.* [99]
Cryptolepine	*Cryptolepis sanguinolenta*	Periplocaceae	Leaves	EtOH	*Pfk1*	0.23	Guinea-Bissau	Paulo *et al.* [100]
Cryptolepinoate	*Cryptolepis sanguinolenta*	Periplocaceae	Roots	EtOH	*Pfk1*	<23	Guinea-Bissau	Paulo *et al.* [100]
Cryptolepinoic acid	*Cryptolepis sanguinolenta*	Periplocaceae	Roots	EtOH	*Pfk1*	<23	Guinea-Bissau	Paulo *et al.* [100]
Cynaropicrin	*Vernonia mespilifolia*	Asteraceae	Leaves	DCM/MeOH	*Pf NF*	1.56	South Africa	Makoka *et al.* [62]
Dioncopeltine A	*Triphyophyllum peltatum*	*Dioncophyllaceae*	Stem bark	DCM-NH3	-	0 (*in vivo*) 50 mg/kg	I.Coast	Francois *et al.* [101]
Dioncopeltine A	*Triphyophyllum peltatum*	*Dioncophyllaceae*	Stem bark	DCM-NH3	-	49 (*in vivo*) 50 mg/kg	I.Coast	Francois *et al.* [101]
Dioncophylline B	*Triphyophyllum peltatum*	*Dioncophyllaceae*	Stem bark	DCM-NH3	-	47 (*in vivo*) 50 mg/kg	I.Coast	Francois *et al.* [101]
Dioncophylline C	*Triphyophyllum peltatum*	*Dioncophyllaceae*	Stem bark	DCM-NH3	-	100(*in vivo*) 50 mg/kg	I.Coast	Francois *et al.* [101]
Eburnamine	*Pleiocarpa mutica*	Apocynaceae	Root	MeOH	*Pfk1*	163.3	Ghana	Jonathan *et al.* [35]
Ellagic acid	*Alchornea cordifolia*	Euphorbiaceae	Leaf	Eth	*FcM29*	0.08	Nigeria	Banzouzi *et al.* [30]
Ellagic acid	*Terminalia avicennoides and Anogeissus leiocarpus*	Combretaceae	Stem bark	Met	*3D7/K1*	12.14/11.2	Nigeria	Shuaibu *et al.* [36]
Emodin	*Cassia siamea*	Fabaceae	Stem bark	Ethylacetate	*Pfk1*	5.00	Nigeria	Ajaiyeoba *et al.* [102]
Fagaronine	*Fagara zanthoxyloides*	Rutaceae	Root	Aqeou	*3D7*	0.018	Nigeria	Kassim *et al.* [29],
Feruraldehyde	*Keetia leucantha*	Rubiaceae	Twigs	DCM	*P.f 3D7*	>100	Benin	Bero *et al.* [103]
Flavogallonic acid	*Terminalia avicennoides and Anogeissus leiocarpus*	Combretaceae	Stem bark	Met	*3D7/K1*	8.889/ 8.35	Nigeria	Shuaibu *et al.* [36]
Fr2	*Terminalia avicennoides and Anogeissus leiocarpus*	Combretaceae	Stem bark	Met	*3D7/K1*	9.98/ 21.83	Nigeria	Shuaibu *et al.* [36]
Gedunin	*Azadiracta indica*	Meliaceae	Leaf	Met	*W2*	0.02	Nigeria	Saxena *et al.* [28]
Gedunin	*Khaya grandifoliola*	Meliaceae	Stem back	Met	*W2*	1.25	Nigeria	Agbedahunsi *et al.* [104]
Grandifolione	*Khaya anthotheca*	Meliaceae	Seed	Petroleum ether	*pf K1*	0.73	Uganda	Obbo *et al.* [87]

(Cond..)

Table 6: *(Continued...)*

Compounds	Plant species	Family	Part used	Solvent	P.strain	IC_{50} µg/ml	Country	References
Guieranone A	*Guiera senegalensis*	Combrataceae	Root	DCM	*PfW2*	1.29	B.Faso	Julien *et al.* [105]
Harmalan	*Guiera senegalensis*	Combrataceae	Root	DCM	*PfW2*	22.43	B.Faso	Julien *et al.* [105]
Harman	*Guiera senegalensis*	Combrataceae	Root	DCM	*PfW2*	3.29	B. Faso	Julien *et al.* [105]
Hydroxybenzaldehyde	*Keetia leucantha*	Rubiaceae	Twigs	DCM	*P.f*	>100	Benin	Bero *et al.* [103]
Hydroxycryptolepine	*Cryptolepissanguinolenta*	Periplocaceae	Leaves	Chlorophorm	*Pf K1*	<23	Guinea-Bissau	Paulo *et al.* [100]
Isostrychnopentamine	*Strychnos usambarensis*	Loganiaceae	Leaves	EtOAc	*pf FCA 20*	120	Rwanda	Frédérich etal., 2004
Jasmolin I	*Chrysanthemum cinerariifolium*	Asteraceae	Flower	n-hexane	*PfK1*	5.3	South Africa	Yoshie *et al.* [91]
Jasmolin II	*Chrysanthemum cinerariifolium*	Asteraceae	Flower	n-hex	*PfK1*	5.0	South Africa	Yoshie *et al.* [91]
Jatrorrhizine	*Penianthus longifolius*	Menispermaceae	Stem bark	-	*PfK1*	0.35	Cameroon	Bilda *et al.* [79]
Kopsinine	*Pleiocarpa mutica*	Apocynaceae	Root	MeOH	*PfK1*	>200	Ghana	Jonathan *et al.* [35]
Lemairamide	*Zanthoxylum rubescens*	Rutaceae	Stem bark	MeOH: water (1:1)	*3D7/FCM29*	89.7/101.1	I.Coast	Penali *et al.* [92]
Liriodenine	*Glossocalyx brevipes*	Siparunaceae	Leaf	-	*Pf D6*	2.37	Cameroon	Mbah *et al.* [106]
Lup-20 (29)-en-3-ol	*Albizia zygia*	Leguminosae	Back	DCM/MeOH	*PfK1*	>0.078	Sudan	Abdalla and Laatsch, [67]
Lupeol	*Cassia siamea*	Fabaceae	Leaf	Ethylacetate	*Pfk1*	5.00	Nigeria	Ajaiyeoba *et al.* [102]
Melicopicine	*Teclea trichocarpa*	Rutaceae	Leaves	MeOH	*Pfk1*	12.45	Kenya	Mwangi *et al.* [94]
Methyl canadine	*Zanthoxylum chalybeum*	Rutaceae	Root bark	EtOH	*Pf 3D7*	2.01	Rwanda	Muganga *et al.* [74]
Nitidine	*Zanthoxylum chalybeum*	Rutaceae	Root bark	EtOH	*Pf 3D7*	0.17	Rwanda	Muganga *et al.* [74]
N-nornitidine	*Zanthoxylum rubescens*	Rutaceae	Stem bark	MeOH: water (1:1)	*3D7/FCM29*	Inactive	I.Coast	Penali *et al.* [92]
Normelicopicine	*Teclea trichocarpa*	Rutaceae	Leaves	MeOH	*Pfk1*	4.45	Kenya	Mwangi *et al.* [94]
Oleanolic acid	*Keetia leucantha*	Rubiaceae	Twigs	DCM	*P.f 3D7*		Benin	Bero *et al.* [103]
palmitine	*Penianthus longifolius*	Menispermaceae	Stem bark	-	*Pfk1*	0.23	Cameroon	Bilda *et al.* [79]
Picraline	*Picralima nitida*	Apocynaceae	Seed	EtOH	*W2*	0.66	Nigeria	Okokon *et al.* [27]
Picranitidine	*Picralima nitida*	Apocynaceae	Seed	EtOH	*W2*	0.038	Nigeria	Okokon *et al.* [27]
Picratidine	*Picralima nitida*	Apocynaceae	Seed	EtOH	*D6*	0.017	Nigeria	Okokon *et al.* [27]
Pleiocarpamine	*Pleiocarpa mutica*	Apocynaceae	Root	MeOH	*pf K1*	17.6	Ghana	Jonathan *et al.* [35]
Pleiocarpine	*Uvaria chamae*	Apocynaceae	Root	MeOH	*pf K1*	>200	Ghana	Jonathan *et al.* [35]
Pleiomutinine	*Pleiocarpa mutica*	Apocynaceae	Root	MeOH	*pf K1*	5.2	Ghana	Jonathan *et al.* [35]
Punicalagin	*Terminalia avicennoides and Anogeissus leiocarpus*	Combretaceae	Stem bark	Met	*3D7/K1*	9.42/8.779	Nigeria	Shuaibu *et al.* [36]
Pyrethrin I	*Chrysanthemum cinerariifolium*	Asteraceae	Flower	n-hexane	*PfK1*		South Africa	Yoshie *et al.* [91]
Pyrethrin II	*Chrysanthemum cinerariifolium*	Asteraceae	Flower	n-hexane	*PfK1*	4.0	South Africa	Yoshie *et al.* [91]
Quindoline	*Cryptolepis sanguinolenta*	Periplocaceae	Leaves	EtOH	*Pf K1*	<23	Guinea-Bissau	Paulo *et al.* [100]
Samaderines B	*Quassia indica*	Simaroubaceae	Stem	-	*PFK1*	0.071	Nigeria	Kitagawa *et al.* [26]
Samaderines E	*Quassia indica*	Simaroubaceae	Stem	-	*PFK1*	0.210	Nigeria	Kitagawa *et al.* [26]
Samaderines X	*Quassia indica*	Simaroubaceae	Stem	-	*PFK1*	0.015	Nigeria	Kitagawa *et al.* [26]
Samaderines Z	*Quassia indica*	Simaroubaceae	Stem	-	*PFK1*	0.071	Nigeria	Kitagawa *et al.* [26]
Schkuhrin I	*Schkuhria pinnata*	Asteraceae	Whole	DCM/MeOH	*Pf NF-54*	2.05	South Africa	Makoka *et al.* [62]
Schkuhrin II	*Schkuhria pinnata*	Asteraceae	Whole	DCM/MeOH	*Pf NF*	1.67	South Africa	Makoka *et al.* [62]
Scopoletin	*Keetia leucantha*	Rubiaceae	Twigs	DCM	*P.f 3D7*	>100	Benin	Bero *et al.* [103]
Simalikalactone D	*Quassia amara*	Simaroubaceae	Stem		*FcB1*	0.010	Nigeria	Bertani *et al.* [25]
Skimmianine	*Teclea trichocarpa*	Rutaceae	Leaves	MeOH	*Pfk1*	5.60	Kenya	Mwangi *et al.* [94]
Stigmasterol	*Keetia leucantha*	Rubiaceae	Twigs	DCM	*P.f 3D7*	>100	Benin	Bero *et al.* [103]
Strychnopentamine	*Strychnos usambarensis*	Loganiaceae	Leaves	EtOAc	*117*	pf FCA 20	Rwanda	Frédérich *et al.* [97]
Tagitinin C	*Tithonia diversifola*	Asteraceae	Leaves	-	*FCA*	0.330	Nigeria	Goffin *et al.* (2002)
TCA1	*Cassia alata*	Caesalpiniaceae	Leaves	DCM	*P.f*	0.94	Congo	Kayembe *et al.* [78]
TCA2	*Cassia alata*	Caesalpiniaceae	Leaves	DCM	*P.f*	0.23	Congo	Kayembe *et al.* [78]

(Cond..)

Table 6: *(Continued...)*

Compounds	Plant species	Family	Part used	Solvent	P.strain	IC_{50} µg/ml	Country	References
TCA3	*Cassia alata*	Caesalpiniaceae	Leaves	DCM	*P.f*	0.44	Congo	Kayembe *et al.* [78]
TCA4	*Cassia alata*	Caesalpiniaceae	Leaves	DCM	*P.f*	0.52	Congo	Kayembe *et al.* [78]
Terchebulin	*Terminalia avicennoides and Anogeissus leiocarpus*	Combretaceae	Stem bark	Met	*3D7/K1*	8.89/8.49	Nigeria	Shuaibu *et al.* [36]
Tetrahydroharman	*Guiera senegalensis*	Combrataceae	Root	DCM	*PfW2*	8.56	Burkina Faso	Julien *et al.* [105]
TOG1	*Ocimum gratissimum*	Lamiilaceae	Leaves	DCM	*P.f*	0.32	Congo	Kayembe *et al.* [78]
TOG2	*Ocimum gratissimum*	Lamiilaceae	Leaves	DCM	*P.f*	0.27	Congo	Kayembe *et al.* [78]
TOG3	*Ocimum gratissimum*	Lamiilaceae	Leaves	DCM	*P.f*	1.41	Congo	Kayembe *et al.* [78]
TOG4	*Ocimum gratissimum*	Lamiilaceae	Leaves	DCM	*P.f*	3.96	Congo	Kayembe *et al.* [78]
TOG5	*Ocimum gratissimum*	Lamiilaceae	Leaves	DCM	*P.f*	0.44	Congo	Kayembe *et al.* [78]
TOG6	*Ocimum gratissimum*	Lamiilaceae	Leaves	DCM	*P.f*	0.65	Congo	Kayembe *et al.* [78]
TOG7	*Ocimum gratissimum*	Lamiilaceae	Leaves	DCM	*P.f*	0.52	Congo	Kayembe *et al.* [78]
Urosilic acid	*Morinda lucida*	Rubiaceae	Leaves	-	*Pf*	3.1	Nigeria	Cimanga *et al.* [55]
Ursolic acid	*Keetia leucantha*	Rubiaceae	Twigs	DCM	*P.f 3D7*	14.8	Benin	Bero *et al.* [103]
Vanillin	*Keetia leucantha*	Rubiaceae	Twigs	DCM	*P.f 3D7*	>100	Benin	Bero *et al.* [103]
Zanthomamide	*Zanthoxylum rubescens*	Rutaceae	Stem bark	MeOH: water (1:1)	*3D7/FCM29*	133.8/149.9	I.Coast	Penali *et al.* [92]
α-Amyrin	*Teclea trichocarpa*	Rutaceae	Leaves	MeOH	*Pfk1*	0.96	Kenya	Mwangi *et al.* [94]
β–Sitosterol	*Teclea trichocarpa*	Rutaceae	Leaves	MeOH	*Pfk1*	8.20	Kenya	Mwangi *et al.* [94]

DCM: Dichloromethane, MeOH: Methanol, EtOH: Ethanol, HO: Hydroxide, AQS: Aqueous, ISal: Isoamyl alcohol, pf: *Plasmodium falciparum*, IC_{50}: Inhibitory concentration 50%

Table 7: *In vivo* antiplasmodial activities of African plants

Plants	Family	Part	Dose (mg/kg)	Solvent	Model	% Inhibition	Survival days	Parasite	Country	References
Pyrenacantha staudtii	Icacinaceae	Leaf	100/200/500	Aqueous	Sup	61/63.4/58.0		p.b	Nigeria	Olorunniyi and Morenikeji [107]
Morinda lucida	Rubiaceae	Root	400	Met	Cur	-	29	p.b	Nigeria	Umar *et al.* [108]
Phytolacca dodecandra	Phytolacaceae	Leaves	100/200/400	Meth	Cur	18/50/55		p.b	Ethiopia	Adinew [109]
Olea europaea	Oleaceae	Leaf	40/80/120	Etha		30/55/80	-	p.b	Nigeria	Akanbi [110]
Acacia auriculiformis	Fabaceae	Leaf	350/700/1050	Eth	Sup	69/72/76	15/18/20	p.b	Nigeria	Okokon *et al.* [111]
Acacia nilota	Leguminoseae	Roots	300	Hex/EtlC/MeOH	Cur	71/50/66	-		Nigeria	Jigam *et al.* [112]
Adansonia digitata	Malvaceae	Stem bark	100	AQS/org	Sup	60.47/32.90	20	p.b	Kenya	Musila *et al.* [113]
Ageratum conyzoides	Asteraceae	Leaf	100/200/400	AQS	Sup	70.49/82.20/89.87		p.b	Nigeria	Victoria *et al.* [114]
Ageratum conyzoides	Asteraceae	Leaves	400	AQS/hex/chlo	Sup	89.87/61.74/52.61		p.b	Nigeria	Victoria *et al.* [114]
Alstonia boonei	Apocynaceae	Root back	200	Met	Sup/prop/cur	62.2/58.8/66.4	-	p.b	Nigeria	Onwusonye and Uwakwe [115]
Amaranthus spinosus	Amaranthaceae	Stem	200	Water	Sup	789.36	-	p.b	Burkina Faso	Hilou *et al.* [116]
Anthocleista grandiflora	Gentianaceae	Stem bark	300/500/700	Met	Supprsive	14/32/68		p.b	Nigeria	Odeghe *et al.* [116]
Anthocleista vogelii	Loganiaceae	Stem	100/200/400	Eth	Sup	48.5/78.5/86.6%		p.b	Nigeria	Lebari *et al.* [117]
Artocarpus altilis	Moraceae	Stem bark	ED50	-	Pro/sup/cur	ED50214.2/ 227.2/310.2	-	p.b	Nigeria	Adebajo *et al.* [118]
Aspilia africana	Asteraceae	Leaf	100/200/ 400 mg/kg	Eth	Suppressie	79.42/84.28/92.23	22/25/28 days	p.b	Nigeria	Christian *et al.* [119]
Aspilia africana	Asteraceae	Leaf	100/200/400	Et	Sup	22/25/28	-	p.b	Nigeria	Akuodor *et al.* [121]

(Cond..)

Table 7: *(Continued...)*

Plants	Family	Part	Dose (mg/kg)	Solvent	Model	% Inhibition	Survival days	Parasite	Country	References
Azadiracta indica	Meliaceae	Leaf/back	800	AQS	Sup	79.6/68.2	-	*Py*	Nigeria	Isah *et al.* [122]
Bombax buonopozense	Bombacaceae	Leaf	200/400/600	Met	Sup/cur	65/78/86	25/28/29	p.b	Nigeria	Akuodor *et al.* [120]
Bridelia ferruginea	Euphorbiaceae	Bark	400	Methanol	Pro/sup/cura	-	26/16/27	p.b	Nigeria	Kolawole and Adesoye, [123]
Byrsocarpus coccineus	Connaraceae	Leaf	100, 200 and 400	Met	Cur/sup	81/88/92	20/22/26	p.b	Nigeria	Joseph *et al.* [124]
Calpurnia aurea	Fabaceae	Leaf	60	Hydromethanol	Sup, cur and pro	51.15, 47.77 and 36.8%	9.6/10/8	p.b	Ethiopia	Mebrahtu *et al.* [125]
Canthium glaucum	Rubiaceae	Root	100 mg/kg/day	Aq/org	Sup	31.98/43.76	20	p.b	Kenya	Musila *et al.* [113]
Cassia singueana	Fabaceae	Root	50/100/150/200	MeOH		48.22/66.51/79.06/80.45			Nigeria	Adzu *et al.* [126]
Cassia singueana	Fabaceae	Roots	50/100/200	MeOH	Sup	48/66/79	-	p.b	Nigeria	Adzu *et al.* [126]
Cassia singueana	Fabaceae	Bark	200/400/800	EtOH	Sup	37/72/90		p.b	Nigeria	Lydia *et al.* [127]
Catha edulis	Celasteraceae	Leaf	1000	MeOH	Supp	13.7		p.b	Ethiopia	Tsige *et al.* [128]
Chrozophora senegalensis	Euphorbiaceae	Whole	75	EtOH	Sup	51.80%	-	p.b	Nigeria	Jigam *et al.* [129]
Chrysophyllum albidum	Sapotaceae	Bark	1000/1500	MeOH	Sup	74.20/62.90%		p.b	Nigeria	Adewoye *et al.* [127]
Cissampelos mucronata	Menispermaceae	Leaf	200	EtOH	Sup/cur/pro	68.4/60.0/73.7%	-	p.b	Nigeria	Katsayal and Obamiro, [128]
Clerodendrum violaceum	Verbenaceae	Leaf	13	EtOH	Supp	100 after 21 days		p.b	Nigeria	Balogun *et al.* [129]
Crateva adansonii	Capparaceae	Leaves	200/400/600	MeOH	Cur	0.00/37.71/40.41			Nigeria	Tsado *et al.* [130]
Croton macrostachyus	Euphorbiaceae	Leaf	200/400	MeOH	Sup	39/69	6/7 days	p.b	Ethiopi	Laychiluh *et al.* [131]
Croton zambesicus	Clusiaceae	Root	81/57/57	EtOH/n-Hex/DCM	Sup	86.18/57.88/75.39	-	p.b	Nigeria	Okokon and Nwafor, [132]
Cryptolepis sanguinolenta	Apocyanaceae	Leaf	36	AQS	Sup	25	-	p.b	Nigeria	Augustine *et al.* [98]
Cymbopogon citratus	Poaceae	Leaf	200/400/800	EtOH	Sup/cur/pro	82,84,99/66,74,83/43,56,70	-	p.b	Nigeria	Uraku *et al.* [133]
Cymbopogon citrutus	Poaceae	Bark	200/400/800	EtOH	Sup	50/77/100	-	p.b	Nigeria	Lydia *et al.* [127]
Dicliptera verticillata	Acanthaceae	Leaf	290/580/870	Ethanol	Sup	59.14/70.67/83.66	--	p.b	Nigeria	Ettebong *et al.* [134]
Dodonaea angustifolia	Sapindaceae	Seed	600	AQS/met	Cur	62.02/86.21%	11.3/11.25	-	Ethiopia	Andualem, [135]
Dodonaea angustifolia	Sapindaceae	Seed	100 mg/kg	AQS/butanol	Sup	35.79/48.6		p.b	Ethiopia	Berhan *et al.* [136]
Eleucine indica	Poaceae	Leave	600	Etha	Sup/pro	64.67/56.34	27	p.b	Nigeria	Ettebong *et al.* [137]
Enantia chlorantha	Annonaceae	Stem bark	-	-	Sup	317.9	-	p.b	Nigeria	Adebajo *et al.* [118]
Entada abyssinica	Fabaceae	Leaves	600	AQS/met		39.2/66.4	11.45/11.65		Ethiopia	Andualem, [135]
Faidherbia albida	Fabaceae	Stem bark	100/200/400	Eth	Sup	24/72/89	-	p.b	Nigeria	Sulaiman *et al.* [137]
Ficus platyphylla	Moraceae	Stem bark	300	Ethanol	Sup	43.50	28	p.b	Nigeria	Isma'il *et al.* [138]
Flacourtia indica	Flacourtiaceae	Leaves	100	AQS/org	Sup	0.2/87	7/8.6	pf ANKA	Kenya	Caroline *et al.* [139]
Hoslundia opposita	Lamiaceae	Roots	100	AQS/org	Sup	90/41	9.2/9.6	pf ANKA	Kenya	Caroline *et al.* [139]
Hyptis spicigera	Lamiaceae	Leaf	200/400/800	Eth	Sup/cur/pro	55,83 and 94/82, 96,96/53, 57, 70		p.b	Nigeria	Uraku *et al.* [133]
Hyptis suaveolens	Lamiaceae	Leaf	10/25/50	Eth	Sup/cur	22.39 and 6.06/33.69 and 10.22/42.76 and 18.03		p.b	Nigeria	Dawet *et al.* [140]
Languas galanga	Zingiberaceae	Rhizome	400	Methanol	Sup/cur/pro	65/67/52		p.b	Nigeria	Abdulelah *et al.* [141]
Launaea cornuta	Asteraceae	Leaf	100 mg/kg/day	AQS/org	Sup	38.13/31.04	0	p.b	Kenya	Musila *et al.* [113]
Lecaniodiscus cupanioides	Sapindaceae	Root	50/150/250	AQS	Sup	70/10/20%	17/19/17 days	p.b	Nigeria	Nafiu *et al.* [142]
Lippia multiflora	Verbanaceae	Leaf	200/400	Meta	Cur	13.35% and 50.94		p.b	Nigeria	Jigam *et al.* [143]
Momordica balsamina	Cucurbitaceae	Leaf	200/600	Hexane/ethylacetat/met	Sup	13.78 and 9.41/28.08 and 27.29/45.21 and 53.07		p.b	Nigeria	Jigam *et al.* [144]
Morinda lucida	Rubiaceae	Leaves	200	Eth/meth/pet chl	Sup	63/68/72	-	p.b	Nigeria	Cimanga *et al.* [55]
Morinda lucida	Rubiaceae	Root	100/200/400	Met	Sup/cur	56/59/67	18/20/23	p.b	Nigeria	Umar *et al.* [145]
Morinda morindiodes	Rubiaceae	Leaf/stem/root	100	Met/AQS	Sup	-	21.5 and 17.5/16.5/19.5 and 21.5	p.b	Nigeria	Soniran *et al.* [146]

(Cond..)

Table 7: *(Continued...)*

Plants	Family	Part	Dose (mg/kg)	Solvent	Model	% Inhibition	Survival days	Parasite	Country	References
Moringa oleifera	Moringaceae	Seed	50/100/ 200 ml/kg	n-hexane/ ethanolic		61%/70%/97% (n-hexane) and 61%/65%/100%(eth)		p.b	Nigeria	Olasehinde *et al.* [147]
Murraya koenigii	Rutaceae	Leaf	ED50	-	Pro/sup/cur	ED50195.6/287.1/252.4	-	p.b	Nigeria	Adebajo *et al.* [118]
Nauclea latifolia	Rubiaceae	Root	ED50	-	Pro/sup/cur	ED50189.4/279.3/174.5	-	p.b	Nigeria	Adebajo *et al.* [118]
Ocimum basilicum	Lamiaceae	Leaf	200/400/800	Eth	Sup/cur/pro	50,58,76/61,69,82/34,45,60		p.b	Nigeria	Uraku *et al.* [133]
Ocimum gratissimum	Lamiaceae	Leaves	100	AQS/org	Sup	17/88	8.2/9.6	pf ANKA	Kenya	Caroline *et al.* [139]
Olea europaea	Oleaceae	Root	40/80/120	Eth	Cur	33.4%, 59.0% and 79.1%	-	p.b	Nigeria	Osheke *et al.* [148]
Otostegiaintegrefolia	Lamiaceae	Leaf	800	Met/aqueous/ chloroform	Sup	56.77/44.45/39.16	-	p.b	Ethiopia	Solomon, [149]
Paullinia pinnata	Sapindaceae	Leaves	12.5/25/50	Ethanol	Cur	53/69/69	-	p.b	Nigeria	Maje *et al.* [150]
Pedilanthus tithymaloides	Euphorbiaceae	Latex	25/5/10% w/v		Cur	36.29/69.35/79.03	-	p.b	Nigeria	Adzu *et al.* [151]
Phyllanthus fraternus	Euphorbiaceae	Leaf	50/100/200	Aqueous	Cur	77.23%, 85.15% and 86.39%	-	p.b	Nigeria	Matur *et al.* [152]
Pseudocedrala kotschyi	Meliaceae	Leaf	100-400	Etha	Sup	71/90/91	-	p.b	Nigeria	Akuodor *et al.* [153]
Pseudocedrela kotschyi	Meliaceae	Leaf	200	Eth/teeth/aqueous	Supp	39.43/26.99/28.36	-	p.b	Nigeria	Dawet and Yakubu, [154]
Quassia amara	Simaroubaceae	Leaves	200	Hex	Sup	0.05 p.density	-	p.b	Nigeria	Ajaiyeoba *et al.* [155]
Quassia undulata	Simaroubaceae	Leaves	200	Hex	Sup	0.16 p.density	-	p.b	Nigeria	Ajaiyeoba *et al.* [155]
Salacia senegalensis	Celestraceae	Leaves	1000/1200/ 1400	Meth	Sup/pro/cur	66.47 and 80.33/66.57 and 75.41/64.90 and 82.72	-	p.b	Nigeria	Adumanya *et al.* [156]
Smilax krausiana	Smilaceae	Root	72	Ethano	Sup/pro	62.68/51.6	-	p.b	Nigeria	Jude *et al.* [156]
Solanum incanum	Solanaceae	Leaves	100	Aqe/org	Sup	14/31	8.8/8	pf ANKA	Kenya	Caroline *et al.* [139]
Sphenocentrum jollyanum	Menispermaceae	Root/leaf	200 mg/kg	Met		74.4/54.1		p.b	South Africa	Olorunnisola and Afolayan, [157]
Spilanthes uliginosa	Compositae	Leaf	200/400/800	EtOH	Sup/cur/pro	50,58 and 76/59, 70, 80/ 32, 47, 55		p.b	Nigeria	Uraku *et al.* [133]
Stachytarphets cayennensia	Verbenaceae	Leaf	90/80/170	EtOH	Sup	64/77/78		p.b	Nigeria	Okokon *et al.* [158]
Striga hermonthica	Orobanchaceae	Whole	400	Met	Sup	68.5	-	p.b	Nigeria	Okpako and Ajaiyeoba, [39]
Tapinanthus sessilifolius	Lorantheciae	Leaf	400	Met	Sup	51.3	-	p.b	Nigeria	Okpako and Ajaiyeoba, [39]
Tetrapleura tetraptera	Fabaceae	Fruit	900	EtOH	Sup	76.37	-	p.b	Nigeria	Okokon *et al.* [159]
Trichilia emetica	Meliaceae	Leaves	300	Hexane/methanol	Sup	79.19/95.83	-	p.b	Nigeria	Sulaiman *et al.* [137]
Trichilia emetica	Meliaceae	Leaf	300	Hex/MeOH	Sup	79.19/95.83		p.b	Nigeria	Ijeoma *et al.* [160]
Uvariopsis congolana	Amaranthaceae	Stem/leaf		MeOH		4.47/4.57		p.b	B.Faso	Hilou *et al.* [116]
Vernonia amygdalina	Asteraceae	Leaf	200/400/600	Met	Sup	17.15/35/58.24	-	p.b	Nigeria	Madaki [161]
Zanthoxylum chalybeum		Stem bark	100	Aq/org	Sup	44.93/27.56	20	p.b	Kenya	Musila *et al.* [113]

DCM: Dichloromethane, MeOH: Methanol, EtOH: Ethanol, HO: Hydroxide, AQS: Aqueous, ISal: Isoamyl alcohol, pf: *Plasmodium falciparum*

noticeable compounds were TCA1 to TCA4 isolated from DCM leaf extract of *Cassia alata* and TOG1 to TOG7 isolated from DCM extracts of *Ocimum gratissimum* from Congo [78], as well as palmitine from stem bark extract of *Penianthus longifolius* from Cameroon [79], all these compounds excerpt there *in vitro* antimalarial activities with $IC_{50} < 1$ μg/ml.

African Plants with Ameliorative Effects on Plasmodial-Induced Pathological Changes

Histopathology

Methanol bark extract of *Chrysophyllum albidum* (750-1500 mg/kg/day) exhibited significant antiplasmodial effects both. The extract also ameliorated the liver pathological symptoms of enlarged liver, hepatocellular necrosis, aggregations of periportal mononuclear cell, and Kup-ffer cell hyperplasia that were severe in the untreated mice [129].

Histological study of kidney and pancreas of *P. berghei* infected rat treated with *Mormodiaca charantia* (100 mg/kg) revealed and mild atropy of the glomeruli and mild degeneration of the islet of langerhan as oppose to severe degeneration observed in untreated controls [131]. *Aframomum sceptrum* leaf extract (350 mg/kg) shows moderately brought central vein, hepatic cell with preserved cytoplasm and prominent nucleus as oppose to severe effect expressed by the parasitized untreated mice [163]. Histological study on *P. berghei* parasitized rats treated with methanol extract from leaves of *Acalypha wilkesiana* reveals that the extract may excerpt meso hepatoprotective effect during malarial infection as there were no observable cellular defects on the liver histo-structure as observed in there untreated control [164].

Liver photomicrograph study of *Plasmodium berghei* infectedmice treated with ethanol extract from stem bark of *Ficus platyphylla at* 300 mg/kg shows the clearance of K¨upffer's cells-laden malaria pigment and normal lobular architecture as opposed to the dilated hepatic sinusoids congested with hypertrophied, K¨upffer's cells-laden malaria pigment and parasitized red blood cells that were observed in untrated mice. The extracts also produced chemosuppression of 43.50% and increase the life span of the mice (28 days) [141].

Biochemical parameters

Methanol bark extract of *Chrysophyllum albidum* has been reported to prevented hyperproteinemia due to hyperglobulinaemi in *P. berghei* parasitized mice (Adewoye *et al.*, 2010). According to Ketema *et al.* [128], administration of at 300 mg/kg to *P. berghei* infected rats significantly elevated the activites of serum aspartate aminotransferase (AST), alanine transaminase (ALT) and decrease albumin level compare to the controls. There reports could be translated that administration of that following malarial infection could increase the risk of jaundice or jeopardized the integrity of renal and liver functions.

Recently, Akanbi, [109], investigated AST, ALT, and ALP activities in heart and liver of *P. berghei* parasitized mice treated with *Anogeissus leiocarpus* methanol extract at 100 and 200 mg/kg. There results revealed that the extract at 200 mg/kg was not able to prevent the parasite induced alteration in the organs (heart and liver) ALP, ALT, and AST activities. However, the activities reported at 100 mg/kg were comparable with the normal control mice. These findings could be explained by our earlier discussion, that natural products excert dose dependent effect, the extract A. *leiocarpus* at 100 mg significantly prevented *P. berghei* induced organs damage, this could be an interaction between the infective condition and the constituents of the extract. A. *sceptrum* extract (250 and 350 mg/kg) when administered to *P. berghei* infected mice prevent parasite induced liver damage by preventing the elevations of liver and serum ALP, AST, and ALT, than in parasitized mice. The extract was able to preserve the ALT activity to a comparable level with the normal rat [166]. Methanol leaf extract of A. *wilkesiana* significantlyameliorated parasite-induced oxidative stress as revealed by significant reduction in liver malondialdehyde and reversed effects on reduced superoxide dismutase, glutathione-P (GSH-P), reduced-GSH and catalase as reported in the parasitized untreated rats [164].

Hematology

Balogun *et al.*, 2012 evaluated the effectiveness of *M. charantia* (100 mg/kg) in ameliorating biochemical and histological alteration in malarial and diabetic co-infected rats, and reported that the extracts improved the packed cell volume (PCV), hemoglobin (Hb), and red blood cell (RBC) of the mice comparable with the chloroquine treated mice. According to Balogun *et al.* [129], ethanol leaf extracts from *Clerodendrum violaceum* at 13 mg/kg for 14 days significantly improved the *P. berghei* induced alteration in RBC, PCV, Hb, white blood cell (WBC), and platelet count of infected mice. Methanol leaf extract from Nigerian *Abrus precatorius* at 25-100mg/kg also improve weight gain, RBC, Hb, MCV, and MCH of *P. berghei* infected mice [32]. Methanol extract from *Catha edulis* obtained from Ethiopia, when administered to *P. berghei* infected mice at dose of 300 mg/kg reduced the levels of hematological parameters including platelets count, WBCs and Hb levels [128]. Ethanol extract from leaves of *H. suaveolens* had a dose dependent effect on *P. berghei* in infected mice with chemosuppresion of 10.22% and 33.69% at 25 mg/kg and 42.7% and 18.03% at 50 mg/kg against established and early infection respectively. The extract was however unable to prevent parasite induced anemic condition as indicated by significant reduction in RBC, HB, and PCV of the treated mice [140]. Crude extract from *Croton macrostachyus* prevented weight loss but produce no ameliorative affect on hematocrite of *P. berghei* infected mice [172].

Antiplasmodial Activity of Insect/Products

While more than 95% of African scientist who works on validating the therapeutic claims of natural product against infectious and protozoan disease focused on plants very few documentation [167,168,173, 178], exist on validation of other natural products like insect against malarial disease.

Musca domestica

Adult houseflies (*M. domestica)* are known as carriers of disease, supprisingly in the study of Shittu *et al.* [167], methanol extract from fourth instar stage (maggot) of the fly was able to suppressed *P. berghei* replication, improved mice life span (34 days) and ameliorated parasite induced anemia when evaluated for it antimalarial activities at 600 mg/kg against *P. berghei* parasitized mice. Maggot of housefly has also been reported to be effective against other protozoan disease [18]. This is not supprising as several literatures have documented the therapeutic effects of house fly maggot. Clinically, live maggots has been used to aid wound healing back then in 19th Century (Maggot Debridement Therapy), traditionally it has been reported to be used as antibacterial, antiviral, anti-osteomyelitis, anti-decubial necrosis, antitumor, anti-immunosuppressive agents and also for curing malnutritional stagnation [169-172].

Honey bee

Shittu and Eyihuri [173], evaluated the antiplasmodial effect of bee sting, from their reports *P. berghei* paeasitisized mice were treated with intradermal bee sting. According to their results bee stings produce 56.6% chemosupression and prolong the lifespan to 20 and 15 days for early and established infection, respectively. The hematological studies show that the level of packed cell, the bee sting also improved the PCV, HB, RBC compared to untreated control, the bee sting however was reported to increase the WBC of the mice. Their study justify the traditional believe that mild honey bee attacked could be useful against malarial fever, however, the bee sting induced elevation of WBC reported by Shittu and Eyihuri [170], point out immunostimulatory effect of the constituent release from the bee sting.

Honey and propolis

Although honey from *Apis florea* and *Apis andreniformis*, were reported to excert no significant activity at 10 ug/l when tested against *pfk1* parasitized mice ethanol extract of propolis from the same species exhibit significant activities with IC_{50} value of 4.48 g/ml [174]. Olayemi [178], also administered bee propolis to *P. berghei* parasitize rat at dose of 600 mg/kg and reported that the extract significantly inhibited the parasite replication and improve the PCV of the mice.

Herbal Formulations

UDU

Duru *et al.* [173], studied the effect of "udu," an herbal preparation commonly use to treat malaria by Isiala Mbano people of Imo State, Nigeria on visceral organ, lipid profiles, and weight of rats. There results revealed that the herbal preparation produces no significant effect on organs. However, blood lipid profile parameters were altered in test rats compared with the reference value [176].

Saabmal

Antimalarial herbal formulation called SAABMAL was investigated at 200 and 400 mg/kg against *P. berghei* infected mice in a four days suppressive test. The formulation was able to suppress the (29.39-100%) parasite replication in a dose-dependent fashion. The formulation was also more effective than chloroquine in prolonging the survival time of mice [177].

CONCLUSION AND FUTURE PROSPECTS

This study has documented the list of African natural products with potential antimalaial activities. Some of these natural products demonstrated, high, promising, or low activities against *Plasmodium* species. Some of the plant ameliorated the parasite induced pathological changes while few others did not. The study also shows that natural products from Africa have a considerably huge amount of novel antimalarial compounds that could serve as a lead for the development of new and effective antiplasmodial drugs. It is hoped that pertinent scientist stakeholders will look further into some of these compounds for detailed authentication and subsequent commercialization. However, despite incessant comprehensive and mechanism-orientated assessments of Africa natural products, there is still inadequate information concerning procedures to be adopted for quality control, authentication and standardization of crude plant products. Furthermore, in a view of bridging the gap in knowledge, clinical validation of some of these natural products is of paramount importance.

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Review Article

Antiviral properties of caffeic acid phenethyl ester and its potential application

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ABSTRACT

Caffeic acid phenethyl ester (CAPE) is found in a variety of plants and well-known the active ingredient of the honeybee propolis. CAPE showed anti-inflammatory, anticarcinogenic, antimitogenic, antiviral, and immunomodulatory properties in several studies. The beneficial effects of CAPE on different health issues attracted scientists to make more studies on CAPE. Specifically, the anti-viral effects of CAPE and its molecular mechanisms may reveal the important properties of virus-induced diseases. CAPE and its targets may have important roles to design new therapeutics and understand the molecular mechanisms of virus-related diseases. In this mini-review, we summarize the antiviral effects of CAPE under the light of medical and chemical literature.

KEY WORDS: Antiviral effect, caffeic acid phenethyl ester, caffeic acid phenethyl ester-like compounds

INTRODUCTION

Caffeic acid phenethyl ester (CAPE) has been used all over the World, especially in Asian and other geographical areas as a traditional medicine since ancient times. It is an active phenolic component of propolis (Figure 1) of honeybee hives and possesses a plethora of important biological activities. CAPE is thought to be responsible for various well-known effects of propolis, including antibacterial, antioxidant, anti-inflammatory, immunomodulatory, and anticancer activities [1-3]. It is a well-documented inhibitor of nuclear factor kappa B (NF-κB), which may be an action mechanism for CAPE-mediated anti-inflammatory and antineoplastic effects [4,5]. Classically, CAPE reduces prostaglandins and leukotriene synthesis, acting as a potent anti-inflammatory agent. CAPE down-regulates inflammation by blocking NF-κB and influences some mediators including adhesion molecules, cytokines, and inducible nitric oxide synthase [5,6]. Additionally, CAPE used as an antioxidant and anti-inflammatory agent in a number of studies about human diseases. Its beneficial effects have been reported in the treatment of cancer, diabetes, kidney, liver, and neurological diseases [4,7-9]. On the other hand, recent findings provide new insights into the molecular mechanisms involved in the antiviral effect and activities of this natural compound.

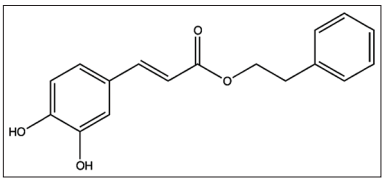

Figure 1: Chemical structure of caffeic acid phenethyl ester

Therefore, the aim of this mini-review article is to highlight the antiviral properties of CAPE, focusing on the mechanisms of action.

GENERAL CHARACTERISTICS OF CAPE

The commercial form of CAPE is a white powder, which is soluble in ethanol, dimethyl sulfoxide, and ethyl acetate (50 mg ml^{-1}). Its empirical formula is $C_{17}H_{16}O_4$ and has 284.3 g mol^{-1} molecular weight. It can be either extracted from propolis by using extraction methods or be synthesized by several methods such as response surface methodology from caffeic acid and phenethyl alcohols [10]. The molar conversion ratio was found to be 96% [11] and 91.2% [12]. According to the current literature, it is asserted that CAPE has no significant toxic effects or minimum toxicity. The pharmacokinetics of CAPE has been characterized; the body clearance values were ranged from 42.1 to 172 ml min^{-1} kg^{-1} decreasing with the higher dose of CAPE. The calculated volume distributions were ranged between 1,555 and 5,209 ml kg^{-1}, which decreases with dose. The estimated elimination half-life was ranged from 21.2 to 26.7 min showing independence from the dose. From this point of view, it can be suggested that CAPE is distributed extensively into the tissues, eliminated very rapidly from the tissue, and has a high volume of distribution and short elimination half-life [13]. The *in vitro* stability of CAPE in different biological samples was investigated. CAPE is hydrolyzed to caffeic acid after 6 h within rat plasma *in vitro* and is hydrolyzed to caffeic acid as the major metabolite *in vivo* [14].

TARGETS IN ANTIVIRAL THERAPY AND ANTIVIRAL EFFECTS OF CAPE

Although fewer drugs were licensed for the treatment of viral infections up to now, the current antiviral drugs repertoire has been increasing. Antiviral drugs are generally divided into four classes; (i) drugs that inhibit uncoating of viral RNA (amantadine, rimantadine, and gamma globulins); (ii) drugs that inhibit viral nucleic acid synthesis (DNA polymerase inhibitors; entecavir, acyclovir, idoxouridine, vidarabine, etc.); (iii) drugs that inhibit late protein synthesis and processing (protease inhibitors); and (iv) immunomodulators (interferons). There are various strategies for antiviral drug development including inhibition of virus adsorption, virus-cell fusion, viral DNA or RNA synthesis (viral DNA polymerase, reverse transcriptase), IMP dehydrogenase, S-adenosylhomocysteine hydrolase, and inhibition of viral enzymes such as protease and neuraminidase [15]. At the earliest, Sud'ina *et al.* suggested various activities and molecular targets of CAPE including antiviral effect inhibiting HIV-1 integrase [1]. Therefore, CAPE is believed to have a potential for anti-HIV therapy. At the same time frame, Fesen *et al.* reported that the integration step is efficiently inhibited by CAPE than the initial cleavage step by HIV-1 integrase (Figure 2) [16]. According to their results, CAPE was the only compound that inhibited the integration step to a substantially greater degree than the initial cleavage step of the enzyme. It was confirmed by another study that CAPE had been found to inhibit the activity of HIV-1 [17]. The mechanism of this inhibition is attributed to the unique molecular structure of CAPE which inhibited the reaction involved by NF-κB [2], and interrupted the method of the treatment of multiple growing points in the life cycle of HIV [18]. CAPE was demonstrated to inhibit the integration step relative to the cleavage step of integration reaction selectively. CAPE was unable to bind DNA significantly [15]. Moreover, it is reported that the effect of CAPE derivatives on hepatitis C virus (HCV) proliferation has been investigated to develop more effective anti-HCV compounds [16]. As it was mentioned before, CAPE inhibits the enzyme activity of some endogenous and viral proteins as well as a transcription of NF-κB. CAPE also suppresses HCV replication enhanced by morphine mediated NF-κB activation [19]. However, the molecular mechanisms of this action have not been fully understood. Shen *et al.* examined chemical structure and antiviral activity suggested that the length of the n-alkyl side chain and catechol moiety is responsible for the anti-HCV activity of CAPE [20]. Their study revealed that CAPE and its analog possess a significant inhibitory effect on HCV replication. HCV NS3, which is a viral protease, was decreased at the protein level by treatment with CAPE in a dose-dependent manner, corresponding to the viral replication. In addition, CAPE and its esters, in a concentration range of 1.0 to 109.6 mM, have also been tested in an HCV replicon cell line of genotype 1b and found effective against replication of HCV. These studies suggest that CAPE and CAPE-like esters are promising therapeutic reagents for HCV treatment [21]. On the other hand, HTLV-1 is an etiologic agent for aggressive, the lethal malignancy of CD4 T-lymphocytes called adult T-cell leukemia and some other clinical disorders[22]. The viral tax protein has been accepted as a key factor in HTLV-1 pathogenicity. Shvarzbeyn and Huleihel found that CAPE strongly prevented both tax binding to inhibitor of κBα and its induced degradation by Tax, whereas it did not interfere in the nuclear transport of tax or NF-κB proteins (Figure 2) [22].

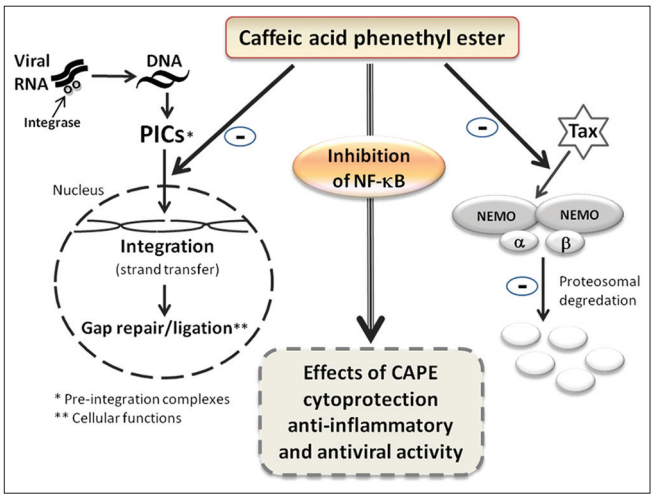

Figure 2: Featured two hypothetical opinions about the antiviral effects of caffeic acid phenethyl ester (CAPE). Integration step is efficiently inhibited by CAPE than the initial cleavage step by HIV-1 integrase (on the left), and CAPE strongly prevents both Tax binding to IκBα and its induced degradation by Tax (on the right)

ANTIVIRAL AND INHIBITORY EFFECTS OF CAPE DERIVATIVES AND CAPE-LIKE COMPOUNDS

CAPE and four CAPE-like compounds (methyl caffeate; ethyl 3-(3,4-dihydroxyphenyl)acrylate; phenethyl dimethyl caffeate, and phenethyl 3-(4-bromophenyl)acrylic) synthesized from commercial caffeic acid were investigated for their anti-HIV replication *in vitro* and immune modulation effects *in vivo* [23]. In these studies, CAPE and other derivatives significantly inhibited HIV replication although the mechanism was unknown. The different effects of treatment on HIV replication and cytokine modulation are guided that the compounds had a virological and immunological response by different mechanisms. Among them, CAPE can selectively inhibit virus-transformed and oncogene-transformed rodent cells and human tumor cells. Two decades ago, it was found that the integrase is essential for viral replication and a possible target for antiviral agents [24,25]. However, most of these compounds possess little or no activities in tissue cultures and have no selectivity in their action mechanisms. These results indicate that CAPE-like compounds do not selectivity eliminate the activation of HIV integrases or the compounds inhibiting HIV integrase do not enter the cells [23]. In a study, 30 different compounds have been tested as HIV integrase inhibitors based on the structural lead provided by CAPE [26]. All of them were designed to test specific properties of the parent CAPE structure, which might be important for activity. The examined properties that have a potential to inhibit integrase were side chain length and composition, rigs substitution, and phenyl ring conformational orientation. Dinucleotide cleavage and strand transfer, which were two sequential steps in the measured combined effects, were found to be lower in the analogs than those of CAPE. Additionally, in literature, there are other studies on other viral agents including influenza and adenovirus [27]. Kishimoto *et al*. [28] reported that CAPE at 8.8 μM inhibited the growth of Type A and B influenza virus by 95% and 92%, respectively. In the other study, treatment of the cells with an anti-IL-6 receptor antibody and CAPE reduced the detached cell number, viral titers, and improved cell viability after infection with the pandemic influenza virüs [29].

CONCLUSION

Modern medicines currently available for antiviral treatment are very expensive and sometimes ineffective; therefore, the alternative agents from natural sources need to be extensively investigated. CAPE seems to be one of such promising agents for antiviral treatment because of accumulating *in vivo* and *in vitro* data. In this regard, clinical trials are needed to test the availability of CAPE alone and in combination with existing regimens.

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Review Article

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Situational analysis and future directions of AYUSH: An assessment through 5-year plans of India

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ABSTRACT

AYUSH is an acronym for Ayurveda, Yoga and Naturopathy, Unani, Siddha, and Homeopathy. These are the six indigenous systems of medicine practiced in India. A department called Department of Indian System of medicine was created in March 1995 and renamed to AYUSH in November 2003 with a focus to provide increased attention for the development of these systems. Very recently, in 2014, a separate ministry was created under the union Government of India, which is headed by a minister of state. Planning regarding these systems of medicine was a part of 5-year planning process since 1951. Since then many developments have happened in this sector albeit the system was struggling with a great degree of uncertainty at the time of 1st 5-year plan. A progressive path of development could be observed since the first to the 12th 5-year plan. It was up to the 7th plan the growth was little sluggish and from 8th plan onward the growth took its pace and several innovative development processes could be observed thereafter. The system is gradually progressing ahead with a vision to be a globally accepted system, as envisaged in 11th 5-year plan. Currently, AYUSH system is a part of mainstream health system implemented under National Rural Health Mission (NRHM). NRHM came into play in 2005 but implemented at ground level in 2006 and introduced the scheme of "Mainstreaming of AYUSH and revitalization of local health traditions" to strengthen public health services. This scheme is currently in operation in its second phase, since 1st April 2012, with the 12th 5-year plan. The scheme was primarily brought in to operation with three important objectives; choice of treatment system to the patients, strengthen facility functionally and strengthen the implementation of national health programmes, however, in some places it seems to be a forced medical pluralism owing to a top-down approach by the union government without considerable involvement of the concerned community. In this study, the 5-year planning documents have been reviewed, from the 1st plan to 12th plan, to enable reflection and throw some light into the future directions of AYUSH system.

KEY WORDS: Ancient medical manuscripts, Indian systems of medicine, Indian systems of medicine informatics, mainstreaming of Ayurveda, Yoga and Naturopathy, Unani, Sidha and Homeopathy, medical tourism

INTRODUCTION

AYUSH is an abbreviation for Ayurveda, Yoga and Naturopathy, Unani, Siddha, and Homeopathy. These are the six Indian systems of medicine (ISM) prevalent and practiced in India and in few neighboring Asian countries. A department called Department of ISM was created in March 1995 [1,2]and renamed to AYUSH in November 2003 [3] with an objective to provide augmented attention for the expansion of these systems. However history unearths that AYUSH system is filled with many ups and downs since its traditional form of ISM to its present form of AYUSH. Western medicine dominated the ISM during the period of British rule in spite of the strength and public faith on these systems of medicine. It was in the year 1835, these systems faced the nadir when Lord Macaulay settled the controversy over whether government should support indigenous or western learning. He ordered that western knowledge should be exclusively fostered in all areas governed by East India Company. Subsequently, eastern medical wisdom was actively discouraged, and the western medicine was recognized as the only legitimate system of medicine to be followed [2]. The current situation is obviously not the same as it was during the British rule. The latest developments in the sector of AYUSH are many, such as mainstreaming of AYUSH and revitalization of local health traditions, inception of many national level institutions such as All India Institute of Ayurveda and above all creation of a separate ministry under the union Government of India [4,5]. Similarly, many programs have been initiated by the Central Government and are implemented at a different level. Moreover, India is a land known for pluralistic system of healthcare with the firm presence of allopathic system of medicine.

Post-independence Indian economy has been based on the concept of planning. This has been carried out on the basis of

a long-term planning process known as 5-year planning. The 5-year plans in India are developed, executed, and monitored by the planning commission [6]. The planning commission is chaired by the prime minister of India and a nominated deputy chairman who enjoys the rank of a cabinet minister. The 1st 5-year plan was launched in 1951 and two subsequent 5-year plans were formulated until 1965. 5-year planning could not take place during 1966-1969 owing to Indo-Pakistani War and other humanitarian crisis such as drought, devaluation of the currency, a general rise in prices and erosion of resources. During this period, three successive annual plans were made and subsequently the 4th 5-year plans was launched in 1969. Since the 1st 5-year plan, health sector has been an integral part of planning process. Health sector planning is one among the 13 sectors identified by Government of India for planning until the 12th 5-year plan. AYUSH sector forms the part of health sector planning [6]. Since the 1st 5-year plan, the sector started appearing under the sector of health planning.

At the time of 1st 5-year plan, a great deal of uncertainty was prevailing regarding the position and future course of development of indigenous system of medicine. Planning pertaining to indigenous system of medicine took place from its nascent stage owing to its very raw status during that period. However, it was felt that the controversy with regard to the truths and merits of any particular system of medicine can only be settled on the benchmark of research. Scientifically designed investigations will, in the course of time, decide the value and validity of different techniques and those which can justify their existence will necessarily become the part of the integrated system of medicine [7].

There are a number of areas where strategies and recommendations are available in 5-year plan documents, but this brief review focused broadly on situational analysis and future directions of AYUSH. In this review, the acronyms ISM and homeopathy (ISM and H) and AYUSH have been used interchangeably owing to their reference during different 5-year plans.

SITUATIONAL ANALYSIS

Current Situation

The ministry of AYUSH, Government of India, released a detailed status of AYUSH system as on 1st April 2014. Few important statistics is represented below in a tabular form [Table 1] for quick reference as per the need of this article [1].

AYUSH Situation during Each 5-Year Plan

While reviewing the 5-year plans, a progressive path of development could be observed. It was up to the 7th plan the growth was little sluggish and from the 8th plan onward the growth took its pace and several innovative development processes could be observed thereafter. Important policy prescriptions, strategies and events in each 5-year plan from 1st 5-year plan to 12th 5-year plan are described in Table 2 following a brief analysis from the 7th plan to 11th plan. Delineation regarding 1st plan to the 6th plan is only tabulated in Table 2.

At the beginning of the 7th plan there were 4.5 lakhs practitioners of indigenous medicine serving in rural areas of different states in India. They were working in far-flung rural areas where they were enjoying acceptance and privilege of doing so [8]. After a similar phase in the 8th 5-year plan, the system was in a position which could be measured on the basis of its strengths and weaknesses at the beginning of the 9th 5-year plan. The strengths during that period could be measured by the number of AYUSH practitioners serving in remote rural areas/urban slums which accounted more than 6 lakhs of such practitioners. Similarly, the weaknesses were also of great concern. It included lack of qualified teachers along with poor quality training standards in training institutes. There were lack of essential staff, infrastructure, and diagnostic facilities in secondary and tertiary care institutions. Potential of AYUSH drugs and therapeutic modalities were not fully exploited, and the existing AYUSH practitioners were not fully utilized to improve access to health care [9].

It was after the 9th 5-year plan a detailed mention of different segments of AYUSH was found in the planning documents. The principal approaches in the 9th 5-year plan were to improve the quality of primary, secondary and tertiary care in AYUSH. It included investment in human resource development for AYUSH to bring marked improvement in the quality of services rendered by these practitioners. In addition, it focused on preservation, promotion and cultivation of medicinal plants and herbs and completion of the pharmacopoeia for all systems of AYUSH. It focused on drawing up a list of essential drugs belonging to these systems and encouraged good manufacturing practices (GMP) to ensure quality control of drugs. Most importantly 9th 5-year plan promoted research and development a therapeutic trial of especially on new drug formulation, therapeutic trial of potential drugs through strengthening of the central research councils and coordination with other research agencies. Special emphasis was laid on encouraging research

Table 1: Current status of AYUSH in India as on 1st April 2014

Components	Ayurveda	Yoga and naturopathy	Unani	Sidha	Homeopathy	Total
Number of practitioners	399400	1764	47683	8173	279518	736538
AYUSH hospitals	2838	42 (7+35)	257	265	213	3615
Bed strength	43170	1107 (85+1022)	3379	2305	6834	56805
Dispensaries	15153	214 (138+76)	1289	845	7199	24700
UG colleges	260	18	41	8	186	513
PG colleges	100	-	9	3	39	151

Source: Ministry of AYUSH, Government of India, UG: Under-graduate, PG: Post-graduate

Table 2: Events/policy prescriptions during each 5-year plan

5-year plans	Duration	Important events/strategies/policy prescriptions	References
1st	1951-1956	ISM and H was a part of health and family welfare planning process	[7]
2nd	1956-1961	Budgetary allocation was increased from 37.5 lakhs to 1 crore in the center and 5.5 crore in states	[26]
3rd	1961-1966	A 4 years diploma course in Ayurveda was introduced with the blend of both traditional and modern medicine	[27]
4th	1969-1974	Budgetary allocation still increased to 15.83 crores	[28]
5th	1974-1979	Reiteration of the 4th 5 year plan strategies. Central councils were formed, CCIM in 1970 and CCH in 1973	[29]
6th	1980-1985	Coordinated efforts for the management of communicable and non-communicable diseases with the help of ISM&H drugs were proposed	[30]
7th	1985-1990	Proposals were made to utilize ISM&H practitioners in family welfare, MCH and UIP programs as they serve in far-flung rural areas with a great degree of acceptance	[8]
8th	1992-1997	Integration of ISM&H with modern medicine in health care services was envisioned	[9]
9th	1998-2002	Creation of para-professionals in ISM&H was proposed. Mainstreaming of AYUSH and revitalization of local health traditions was proposed	[10]
10th	2002-2007	Inclusion of ISM&H in all levels of heath care, accreditation system of ISM&H education, zero base budgeting was introduced	[11]
11th	2007-2012	Strengthening professional education, strategic research programs, promotion of best clinical practice, technology up gradation in industry, setting internationally acceptable pharmacopoeial standards, conserving medicinal flora, fauna and metals, Utilization of AYUSH workforces in national health programs were important strategies	[31]
12th	2012-2017	Availability of AYUSH services in 100% of districts through NABH accredited hospitals, Improving quality of education and training and developing Centers of excellence in government and private sectors, promoting quality research to validate the efficacy and safety of AYUSH remedies, ensuring availability and conservation of medicinal plants, accelerating pharmacopeial work, ensuring availability of quality drugs, positioning AYUSH national institutes as leaders in SAARC region, propagation of AYUSH for global acceptance as systems of medicine are the ongoing 12th 5-year plan strategies	[32]

CCIM: Central Council of Indian Medicine, ISM and H: Indian systems of medicine and homeopathy, MCH: Maternal and Child Health, NABH: National Accreditation Board for Hospitals and Healthcare providers, R & D: Research and Development, SAARC: South Asia Association for Regional Cooperation, UG: Under-graduate, UIP: Universal Immunization Programme, AYUSH: Ayurveda, Yoga and Naturopathy, Unani, Sidha and Homeopathy, CCH: Central Council of Homeopathy

aimed at improving AYUSH inputs in national health programs during 9th 5-year plan [10].

During the 10th 5-year plan, it was felt that despite all the efforts the AYUSH systems have not realized their full potential because the existing AYUSH systems at all such as primary, secondary and tertiary level health care institutions lack essential staff, infrastructure, facilities and drugs. At the same time, the potential of AYUSH drugs and therapeutic modalities has not been fully exploited. There was a lack of quality control and GMP resulting in the use of spurious and substandard drugs. The quality of AYUSH practitioners has been below par; many AYUSH colleges lack essential facilities, qualified teachers and hospitals for practical training. There was also no system for continued medical education (CME) for periodic updating of knowledge and skills during the 10th plan period. It was also found that the AYUSH practitioners were not involved in national disease control programs or family welfare programs. Medicinal plants have been over-exploited and as a result, the cost of AYUSH drugs has increased and spurious products were getting into the market [11].

The vision statement of 11th 5-year plan was very appealing as the same mentions about mainstreaming of AYUSH by designing strategic intervention for wider utilization of AYUSH both domestically and internationally. National Rural Health Mission (NRHM) came into play in 2005 but implemented at ground level in 2006 and introduced the concept of "Mainstreaming of AYUSH and Revitalization of Local Health Traditions" to strengthen public health services [4,12,13]. Under the broader umbrella of mainstreaming of AYUSH and revitalization of local health traditions AYUSH facilities have been co-located with 331 (44.3%) District Hospitals (DH), 1885 (36.3%) Community Health Centers (CHC), and 8461 (34.6%) Primary Health Centers (PHC) by 31st April 2014. Similarly, 2.61, 0.46 and 0.1 million of rural population were being served per DH, CHC, and PHC, respectively, in the country in 2014. Contractual appointment of 10933 AYUSH Doctors and 5419 AYUSH Paramedical staff has been recorded by this time. Uttar Pradesh ranked top (1829 appointments) in the contractual appointment of AYUSH Doctors followed by states of Bihar and Odisha that accounts for 1384 and 1316 appointments, respectively. Likewise, 5419 contractual appointments of AYUSH Paramedical Staffs were recorded by 31st March 2014. A maximum of 1584 paramedical staffs were appointed in the state of Andhra Pradesh, followed by Uttar Pradesh, Madhya Pradesh, Tamil Nadu, Uttarakhand and Rajasthan that appointed 733, 526, 475 413 and 401 paramedical staffs respectively. Arunachal Pradesh, Delhi, Goa, Madhya Pradesh, West Bengal and Daman Diu are the only five States and one Union Territory (UT) where there are no contractual appointments of AYUSH Doctor. There were 11 States and UTs where no AYUSH Paramedical staffs were appointed on a contractual basis as on 31st March 2014 that includes Arunachal Pradesh, Assam, Bihar, Delhi, Gujarat, Himachal Pradesh, Mizoram, Nagaland, West Bengal, D and N Haveli and Daman and Diu [14]. The Figures 1 and 2 shows the percentage of contractual appointment of AYUSH doctors and AYUSH paramedical staffs respectively by 31st March 2014 in different Indian states [14].

Albeit the AYUSH doctors have contributed to the equitable distribution of health workforces in rural areas [15] but the system has many implementation problems as per various

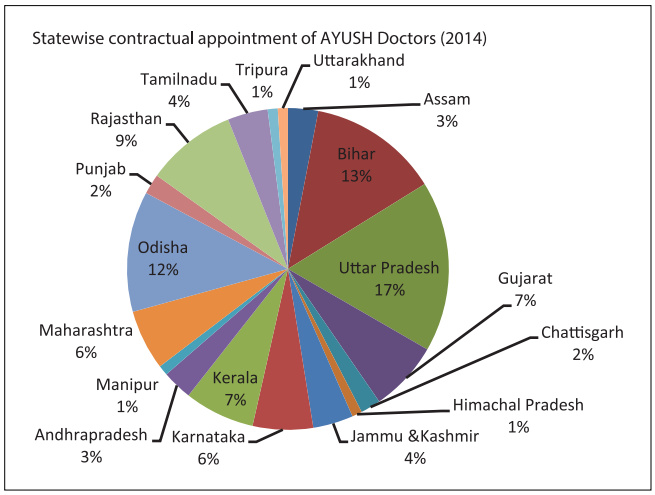

Figure 1: Percentage of contractual appointment of Ayurveda, Yoga and Naturopathy, Unani, Sidha and Homeopathy doctors by 31st March 2014

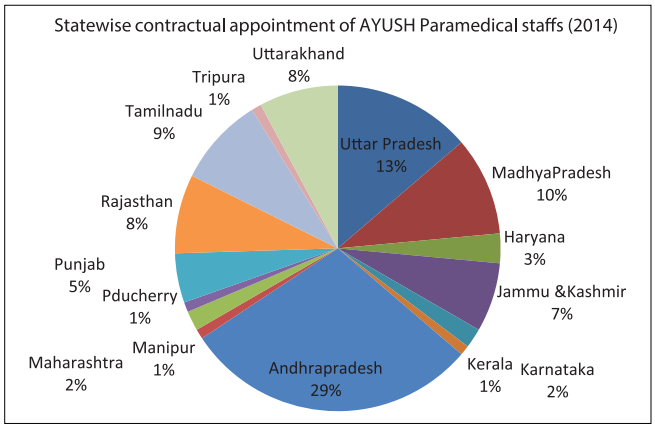

Figure 2: Percentage of contractual appointment of Ayurveda, Yoga and Naturopathy, Unani, Sidha and Homeopathy paramedical staffs by 31st March 2014

studies [16]. At the same time, some of the studies reveal that mainstreaming of AYUSH in some places is perceived as forced medical pluralism as the implementation is a top-down approach without due consideration of local needs [17]. Furthermore, the appointment of AYUSH doctors throughout India is mainly contractual which seriously impacts the motivation level and thereby affects the service delivery. The Planning Commission had recommended provision of contractual recruitment, training, and involvement of human resources for Program Implementation Plans [18]. Studies reveal that security and salary are the two important motivating factors for AYUSH doctors working in CHCs and PHCs [19-21]. Akin to modern medical doctors the AYUSH doctors do not have facilities for CME, which is utmost important for efficient service delivery [22]. There is again the lack of standard clinical guidelines for the AYUSH system which needs to be developed for uniform service delivery. In some places, the placement of AYUSH clinics and OPDs are so located that the signage for AYUSH services is not prominently visible. This spatial marginalization prevails against the operational guidelines for the provision of a separate physician consultation room and a distinct space for storing medicines in the co-located PHCs and CHCs [23]. The deployment of AYUSH doctors for IEC activities rather than specialized services contribute to significant subordination and de- professionalization in the overall health care service delivery [16,24]. Logistics and supply chain management are also a matter of concern as the same affects uninterrupted service delivery.Such delays in supply and erratic replenishment of AYUSH medicines have also been observed in one of the Indian states (Andhra Pradesh) [21]. In addition, studies from Delhi show lack of a robust logistic mechanism with improper indentation, supply and storage of medicines [16]. Moreover, suggestions have been made for integration of modern doctors with AYUSH doctors for utmost patient care [25].

FUTURE DIRECTIONS

Given the present scenario and penetration of AYUSH system in mainstream health care system in India, following are some of the areas in this domain which can be developed, strengthened and augmented for better prospects of this system.

Medical Tourism

India is believed to be the rapidly growing medical tourism destination globally. Low-cost medical care has attracted people from far across the globe. Apart from corporate hospitals attracting patients, India is also known for its rich heritage coupled with its own system of medicine such as Ayurveda and Yoga. These two systems of therapies are very much popular in some of the Indian states especially in Kerala and some of the north Indian states such as Himachal Pradesh, Uttaranchal, etc. Spas, Therapy centers and Beauty salons have attracted a lot of foreign tourists to India. Furthermore, certain therapies are becoming enormously popular and tourists/visitors come to India for such therapies like *Panchkarma* and Yoga. Medical tourism not only popularizes India systems of medicine but also offers better scope for foreign exchange earnings. Little has been done to create a chain of *Panchkarma* Centers and establish centers of excellence for yoga therapy, meditation and teaching. This area can be explored for propagating the Indian medical heritage and improving economy[33].

Ancient Medical Manuscripts

At the present day, a complete catalog of Indian manuscripts is hard to find. These manuscripts are found scattered in oriental libraries and private custody in India and elsewhere in the world. Furthermore, these manuscripts are found in maimed condition with the families of traditional *Vaidyas* and nondescript libraries. Given the condition, urgent remedial measures are required to prevent the irreversible loss of this ancient medical wisdom. Their retrieval is important to preserve these ancient medical doctrines which would provide a wealth of knowledge and thrust to research and clinical application [2]. Sporadically, attempts have been made by few organizations to treasure this medical bequest. The initiatives by the Institute of Trans-Disciplinary

Health Sciences and Technology (Previously known as Institute of Ayurveda and Integrative Medicine), Bengaluru, India with its Center for ISM informatics and Theoretical Foundations are laudable. It was started in 1995 to give increased focus for the modernization of ISM to bring enhanced access for a variety of research purposes. A number of CDs have been prepared by this organization on the medicinal plants on various ISM including Ayurveda, Sidha, Unani, and Homeopathy. The center for development of advanced computing, Pune, India which is a premier research and development (R and D) organization under Government of India is also contributing to this field of knowledge. A software, AyuSoft, has been prepared by this organization on various functionalities of Ayurveda [34].

Research in ISM

ISM is the ethnic legacy deeply buried in the cultural belief of Indian population. Some of the treatment procedures, therapies and drugs of ISM have unbroken traditions of acceptance and practice and have been practiced over centuries. Hence, it is not always desirable to validate these practices on modern scientific parameters. The need for fundamental, clinical and therapeutic research in ISM can hardly be over emphasized. Users demand the evidence of safety and efficacy of these systems of medicine owing to the present day focus on evidence-based medicine. For the last 40 years, research councils have been conducting research, yet a lot remains to be done. The major problem with the research in the realm of ISM is that it is not up-to-date which needs to keep pace with time [2]. Very often irrational use of herbal drugs has been reported which is an issue of concern that needs to be monitored through governmental efforts, research and development and quality control measures in the realm of ISM drugs [35]. Moreover, the present day approach of evidence-based medical care requires research and development to receive wider acceptance among users.

Research Publication and Access to Information

Research publications in the realm of AYUSH are very poor. Although these days mushrooming of scientific journals are found in the market, but very few meet the required scientific rigor. At present a list of 3 PUBMED indexed journals of Ayurveda, 38 non PUBMED indexed journals, 4 Hindi Ayurveda Journals, 26 Journals of Complementary and Alternative Medicine and 11 magazines of Ayurveda have been documented [34,36]. Except Ayurveda scientific publications in other systems of Indian medicine is negligible. Dedicated journals pertinent to specific system of Indian medicine is hard to find. Homeopathy has one dedicated journal, Indian Journal of Research in Homeopathy, published as an official publication of Central Council of Research in Homeopathy [37]. Scholars of other systems of Indian medicine depend on relevant journals for publication of their scientific work. The total number of scientific publications in different systems of AYUSH until date is 21076 which include 14664, 1396, 2104, 640, and 2272 publications of publications of Ayurveda, Yoga and Naturopathy, Sidha and Homoeopathy respectively [38]. The standard of most of these journals is of great concern. Many of the journals of the Indian medicine are identified as predatory journals by Jeffrey Beall from the University of Colorado [39]. Given the situation, there is an urgent need to create awareness among the scholars of ISM about poor quality journals, training programs on research methods, and scientific drafting skills to the researchers of Indian medicine [40].

Veterinary Medicine

ISM are not reflected merely in the treatment of human beings. Other important dimensions like veterinary medicine are also addressed in detail through these systems [25]. Description regarding the management of various animal pathologies is found in the classical treatises of Ayurveda. This represents a whole new spectrum of knowledge and opportunity. Although sporadic developments are seen in this realm and few pharmaceutical companies are producing formulations for animal diseases but is very little to be counted [2].

ISM Informatics

The present era is aptly called as digital era as computer has immensely influenced human life. The realm of health care is no exception in this case. The field of medical or health informatics is growing very rapidly. However, progress in the field of ISM informatics is not advancing at par with the medical/health informatics. Although ISM informatics is a part of the broader umbrella of health informatics, but the developments specific to ISM informatics is very negligible. Sporadic developments are happening elsewhere in the realm of ISM informatics but is not at the desired pace. Akin to health informatics, ISM informatics is a specialized field which is a judicious mix of the principles of India systems of medicine and information technology. ISM informatics would be a paradigm shift to bring automated applications in the field of clinical medicine, biomedical research or information storage and retrieval. The urgent need for the development of ISM informatics is also accrued to the wider acceptance of these systems of medicine owing to their safe and efficacious therapeutics on many of the human diseases. Another glaring picture is that websites are burgeoning imparting information, education and communication in matters related to ISM. However, the authenticity of these sources is skeptical which needs to be monitored with governmental effort. In addition, there are several novice areas which could be explored and worked out for better access, operation and above all for better utility of I [35]. Given the current growth of Indian system of medicine, Table 3 lists out few areas where future research in ISM informatics can be planned.

CONCLUSION

After independence, when the process of long-term planning was started 5-year plan took its birth and in that all the development and technology sectors started appearing. Since then health and family welfare planning became imperative as a social sector planning. Health has always been given due importance in the planning process owing to its very complex

Table 3: Future research areas of ISM informatics

ISM information storage and retrieval	Electronic medical records
Clinical laboratory information system	Electronic prescriptions for patients
Decision support system for ISM physicians	Health education and information through computers
Hospital information system	Telemedicine
Nursing information system	Computer-assisted ISM drug discovery and development
Dietetic and nutrition information system	Computer-mediated instruction in medicine
	Research databases in ISM system [35]

ISM: Indian Systems of Medicine

nature of affiliation with other sectors of development known as the social determinants of health. ISM, or AYUSH in its present form, became a part of health, and family welfare planning since then. In the entire planning process, the ISM and H have faced lot of criticism and appraisal owing to its various characteristic features. At the very outset, the system struggled with great degree of uncertainty (as described in 1st 5-year plan) and progressed ahead with a vision to be a globally accepted system (as envisaged in 11th 5-year plan). Healthy and positive acceptance of this system requires great degree of determination at Governmental level both at the center and the state. Mainstreaming of AYUSH is currently in operation which opened a window for these systems to become a part of mainstream health care system. Under NRHM AYUSH doctors have contributed to the equitable distribution of health workforce in rural India however the system has many implementation problems as per various studies. It was primarily brought in to operation with three important objectives; choice of the treatment system to the patients, strengthen facility functionally and strengthen the implementation of national health programs, however, in some places it seems to be a forced medical pluralism owing to a top-down approach by the union government. With the new government at the center in India, the planning commission was dissolved and a new organization was set up in 2014, National Institution for Transforming India-Ayog. NITI-Ayog. It stands for National Institution for transforming India-Ayog which replaces planning commission and serves as the Government of India's policy think tank. This organization would function in similar fashion like the planning commission.

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Letter to the Editor

Inhibition of plasminogen activator inhibitor-1 release from human endothelial cells by *Angelica keiskei* Koidzumi (Ashitaba) chalcones is structure-dependent

Dear Editor,

The endothelium is a monolayer of cells that lines the inner surface of vessels and plays a crucial role in maintaining hemostatic balance [1]. All blood vessels, from the largest arteries and veins to the smallest venules are lined with endothelial cells, which prevent thrombosis via anticoagulant and antiplatelet mechanisms. Endothelial cells are also involved in hemostatic pathways triggered by vascular injury and limited clot formation in areas where hemostasis is needed. Various conditions such as diabetes, metabolic syndrome, hypertension, and smoking can cause endothelial cell dysfunction [2] that can lead not only to atherosclerosis but also a predisposition toward thrombosis and stroke [1].

Endothelial cells are considered to contribute under various conditions to elevated levels of plasma plasminogen activator inhibitor-1 (PAI-1), which is the major physiological inhibitor of tissue type plasminogen activator (tPA) and urokinase PA (uPA) *in vivo* and it plays a crucial role in the regulation of fibrinolysis [3]. High plasma PAI-1 levels disrupt the fibrinolytic system, resulting in a prothrombotic state that is associated with the development of thrombotic disorders such as stroke, cardiovascular diseases, and deep vein thrombosis [4,5]. Maintaining physiological plasma levels of PAI-1 might represent a promising intervention for these diseases. However, drugs that can suppress PAI-1 release remain unknown and insight into the regulation of PAI-1 release is also inadequate. *Angelica keiskei* Koidzumi (Ashitaba) is a perennial plant that grows mainly along the pacific coast of Japan. Chalcones isolated from Ashitaba have various biological properties [6,7]. The major constituents of the chalcone fraction are xanthoangelol A (XA) (~50%) and 4-hydroxyderricin (4-HD) (~50%). Trace amounts of other chalcones such as xanthoangelols B (XB), D (XD), E (XE), and F (XF) have the basic structure of XA or 4-HD with a slightly modified side chain [Figure 1]. A previous study of mice has shown that Ashitaba exudate inhibits lipopolysaccharide (LPS) induced increases in plasma PAI-1 and that XA inhibits this increase in plasma whereas 4-HD does not [8]. The inhibition activity seemed to be due to the inhibition of nuclear factor kappa B activation. However, the molecular structure of chalcones that are essential for the suppression of PAI-1 production remained undetermined. Here, the effects of 4-HD XA, XB, XD, XE, and XF on tumor necrosis factorsα (TNFα) stimulated PAI-1 production in endothelial cell-like EA. hy926 cells were analyzed and the structure that is critical for such activity is discussed.

EA. hy926 cells (ATCC, Manassas, VA, USA) were seeded in Dulbecco's Modified Eagle's Medium (DMEM) (Sigma-Aldrich, St. Louis MO, USA) at a density of 2.0×10^4 cells per gelatin-coated well in 96-well plates at 37°C in a humidified atmosphere of 95% air and 5% CO_2. Chalcones in dimethyl sulphoxide (DMSO) were added to DMEM containing 1% fetal bovine serum and the cells were incubated for 3 h followed by a 24-h incubation with TNFa (10 ng/mL). Thereafter, PAI-1 concentrations were measured using total PAI-1 ELISA kits (Molecular Innovations, Southfield, MI, USA). Cytotoxic effects of test compounds on EA.hy926 cells were detected using cell counting kit-8 (Dojindo, Kumamoto, Japan). All values are expressed as means ± standard error of the mean. Differences between the two groups were analyzed using Mann–Whitney U tests and $P < 0.05$ was considered to represent significance.

Xanthoangelol (5 and 10 μm) dose-dependently suppressed the TNFα-induced PAI-1 increase in the culture medium. The inhibition rates of PAI-1 were 38.7% ± 9.7% ($n = 3$) and 75.4% ± 1.5% ($n = 3$), respectively. Xanthoangelol was significantly cytotoxic at 25 μm, but not at either 5 or 10 μm. Cell viability in the presence of 5 μm and 10 μm XA was respectively 120.0% ± 11.8% and 120.7% ± 13.5% compared with the control value ($n = 3$), whereas that in the presence of 25 μm XA was only 0.9% ± 0.1% of the control value ($n = 3$). We then assessed the effects of 10 μm XA, XB, XD, XE, XF, and 4-HD on the PAI-1 concentration in the medium [Figure 2]. The inhibition rate of XA was about 77.1%, whereas XB that has a long side chain with a hydroxyl group bound to the A ring was not inhibitory. None of XD, XE, and 4-HD that has a short side chain in the A ring exerted an inhibitory effect. None of XB, XD, XE, and 4-HD were noticeably cytotoxic at a concentration of 10 μm, whereas XF was extremely cytotoxic, which prevented evaluation of its inhibition rate. Isoliquiritigenin, a chalcone obtained from liquorice root, does not have a long side chain bound to the A ring. This chalcone did not inhibit PAI-1 activity

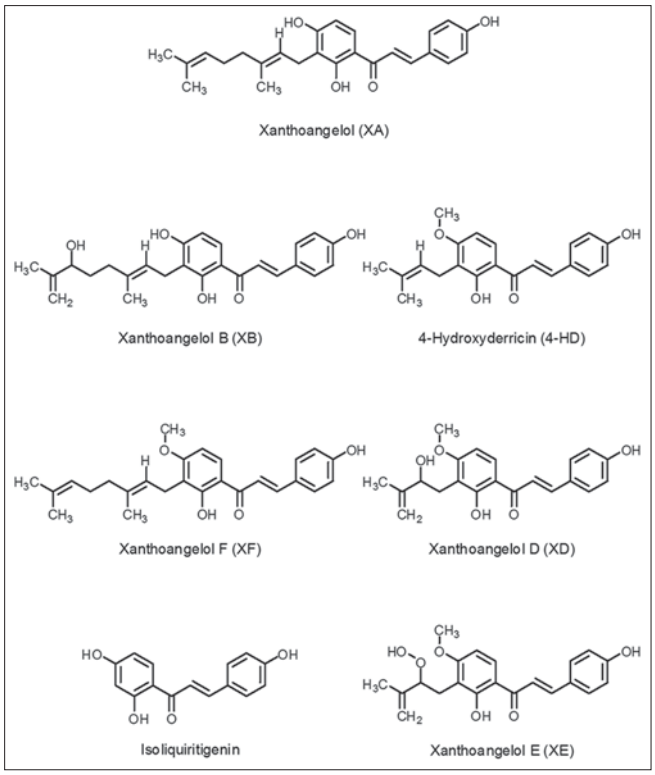

Figure 1: Chemical structure of Ashitaba chalcones and isoliquiritigenin

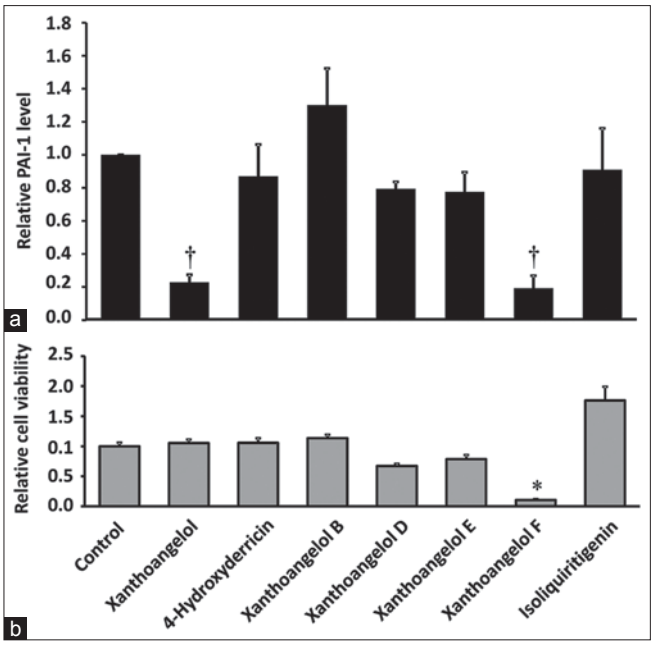

Figure 2: Effect of Ashitaba chalcones on tumor necrosis factorsα (TNFα)-induced plasminogen activator inhibitor-1 (PAI-1) increases in medium of EA.hy926 cells. Cells were incubated with 10 μm chalcones in DMSO for three hours followed by TNFα (10 ng/mL) for 24 h. Thereafter, PAI-1 concentrations were measured using total human PAI-1 ELISA. Longitudinal axis shows relative PAI-1 levels in the medium. Concentration of PAI-1 in medium without TNFα stimulation is expressed as one unit (a). Chalcones cytotoxicity was assessed by cell counting (b). The absorbance of control cells is expressed as one unit. Data are expressed as means ± SEM ($n = 7$ for xanthoangelol A and 4-hydroxyderricin, $n = 3$ for xanthoangelol F, $n = 4$ for others). $^{*}P < 0.05$; $^{\dagger}P < 0.01$ versus control

and was not cytotoxic. These findings suggested that having a long hydrocarbon chain with moderate hydrophobicity on the A ring is critical for expressing chalcone activity. Orally- or intraperitoneally administered Ashitaba exudate suppresses LPS induces PAI-1 increases in mouse plasma [8]. Therefore, the present results suggested that the inhibition of PAI-1 production in mouse plasma by Ashitaba exudate administered orally or intraperitoneally was mainly due to the effect of XA. The present study also found that the main Ashitaba chalcone XA inhibited TNFα-induced PAI-1 mRNA increases in EA.hy926 cells (data not shown). This indicated that the inhibitory mechanism of the blunted increase of PAI-1 antigen in the medium of EA.hy926 cells proceeds via the inhibition of PAI-1 mRNA expression.

The present results indicated that XA, the main constituent of chalcone, inhibits PAI-1 release from endothelial cells induced by inflammation. High plasma PAI-1 levels disrupt the fibrinolytic system, which results in a prothrombotic state that is associated with the development of thrombotic disorders. Therefore, we considered that maintaining the physiological levels of PAI-1by XA prevents thrombus formation and avoids thrombotic disorders. The clinical co-administration of XA with tPA might reduce required doses of tPA and prevent adverse reactions.

However, we investigated the effects of chalcones only on endothelial cells *in vitro*. Many types of cells interact and regulate endothelial cell functions. The effect of co-culture endothelial cells with other cells is very important for objectively assessing the actions of agents. Indeed, co-culture of endothelial cells with other cells modulates PAI-1 production from endothelial cells [9,10]. Astrocytes that comprise one type of glia cells co-cultured with human brain capillary endothelial cells enhance PAI-1 expression in a blood-brain barrier model [11,12]. Even more complex effects from other cells should modulate endothelial cell function *in vivo* and change PAI-1 production from endothelial cells. Therefore, careful consideration is needed to assess the effect of chalcones on PAI-1 production in endothelial cells *in vitro*.

CONCLUSIONS

The main xanthoangelol subtype in Ashitaba was the most potent inhibitor of a TNFα stimulated PAI-1 increase in the culture medium of human EA.hy926 endothelial cells. The side hydrocarbon chain played an important role in this process and small modifications to the hydrocarbon chain or the addition of a small functional group to the A ring of XA influenced the inhibitory activity. The present findings indicated that Ashitaba could serve as an antithrombotic agent and that more novel antithrombotic agents could be developed to suppress PAI-1. Further investigation into the antithrombotic action of Ashitaba is warranted.

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