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ORIGINAL RESEARCH

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May fermented Baltic Sea herring help in conditions of gut disorders, such as gastric catarrh and heartburn?

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ABSTRACT

Background: It has been suggested that disruption of the gut microbiota can be significant with respect to pathological intestinal conditions, such as irritable bowel syndrome (IBS), gastric catarrh (GC), and heartburn (HB). Through history, an essential part of the colonization of the human gut took place by ingestion of food preserved by fermentation. The natural replenishment of microbes via food and beverage is today low because food is "sterilized" through boiling, broiling, and pasteurization. Modulating the gut microbiota with fermented food products may hence be considered as a strategy to treat such conditions. Fermented Baltic Sea herring (FBSH) is an example of a *Lactobacillus*-fermented food product, which was tested in the present study.

Methods: A 30-day open study was performed in 42 volunteers with IBS, GC, or HB. Volunteers were recruited by advertisements in daily newspapers. The volunteers were provided with gelatin capsules for the study, each containing approximately 100 mg freeze dried FBSH. They were also provided with forms that contained columns and rows for every test day where the volunteers were ask to fill in number of capsules taken, and to report possible improvements according to a 0–10 scale, where 10 stands for full recovery.

Results: The most reported common disorder symptom was IBS and 7 of 14 of these volunteers reported recovery, with a mean recovery of 4.4. All of the 9 volunteers reported recovery from GC, with a mean recovery of 8.4. Five of 6 volunteers reported recovery from HB, with a mean recovery of 6.8.

Conclusion: Although the present study is a small open study, the overall results are exciting and merits further studies in volunteers, ideally in a double-blind placebo-controlled manner.

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Butyric acid; fermented Baltic Sea herring; gastric catarrh; gut microbiota; heartburn; irritable bowel syndrome; lactobacillus fermented; open study; probiotics; volunteers

Introduction

Controlled clinical trials have identified probiotics that favorably prevent or improve the symptoms of various gut disorders including inflammatory bowel disease, irritable bowel syndrome (IBS), and infectious and antibiotic-associated diarrhea [1]. Most of these products are supplemented milk products. The list of probiotic microorganisms is long and includes various species of *Lactobacillus*, *Bifidobacterium*, *Streptococcus*, *Saccharomyces boulardii*, and *E. coli* [1]. The used bacteria are mainly chosen because milk is an ideal medium for them to grow in and give tasty products. The World Health Organization defines probiotics as "live microorganisms which when administered in adequate amounts confer a health benefit on the host." However, diet *per se* is extremely important in shaping the human gut microbiota, one of the most densely populated microbial ecosystems in nature [2].

The natural replenishment of microbes via food and beverage is today low because food is "sterilized" through boiling, broiling, and pasteurization.

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Moreover, the technique to preserve food by fermentation is essentially forgotten among modern people.

Fermented Baltic Sea herring (FBSH) is an example of a *Lactobacillus*-fermented food product. The oldest archeological finding of fish fermentation is more than 9,000 years old [3]. FBSH or *surströmming* in Swedish (combination of *sour* and the local name of herring, *strömming*, in the Baltic Sea) seems to date back to at least the fourteenth century [4]. The fermentation is a result of interplay between the bacteria and gut enzymes from the Baltic Sea herring, according to the Swedish National Food Agency. The fermentation results in pungent smelling acids are formed in the fish such as propionic acid, butyric acid, and acetic acid [4]. Hydrogen sulfide is also produced. The osmotic pressure of the brine rises above the level where bacteria responsible for rotting can thrive and prevents decomposition of fish proteins. This condition enables *Haloanaerobium* bacteria to prosper and decompose the fish glycogen into organic acids, making it further acidic. The Swedish National Food Agency conducted trials in the 1970s by adding known food pathogens such as *Staphylococcus aureus*, *Bacillus cereus*, and *Clostridium perfringens* to FBSH, but none of these microorganisms could be shown to grow in FBSH, thus indicating that there is an efficient barrier towards growth of unwanted bacteria in FBSH [4]. For more details the reader is referred to [4].

There are to the best of our knowledge no scientific studies showing beneficial effects of FBSH. In fact, a search at PubMed using the search term "FBSH" results in 0 hits, whereas "probiotics" results in almost 15,000 hits. Nevertheless, one of us (JE, better known as Skogsjan) got the idea that FBSH may cure certain stomach problems, such as heartburn (HB) and gastritis, many years ago. JE observed that these problems seemed to improve after consumption of FBSH. JE hence initiated more systematic studies and found further support for his idea. However, JE also realized that treatment with FBSH due to its pungent smell was associated with invincible problems for most individuals to consume it. Except for people living in certain parts of Sweden, there are relatively few that consume FBSH. He hence developed a method enabling FBSH to be freeze dried and encapsulated (EpicAid®), as described in a US patent (US 6,572,883 B1). As an early part of the developmental process, the present study in volunteers was conducted in 2004. During that time the company Rebiotica AB was formed and the production of EpicAid was scaled up and prepared for marketing. A manufacturing plant was established in Sandviken Municipality, Sweden. However, at that time the Baltic Sea herring contained higher levels of dioxins and polychlorinated biphenyls (PCBs) than the permitted levels for fish in the European Union (EU). Although Sweden was granted exceptions from these rules when it came to marketing of Baltic Sea herring as foodstuff, a local supervisory authority in Sandviken (Miljö och Hälsoskyddsnämnden) surprisingly decided to stop Rebiotica from marketing EpicAid, i.e., the freeze-dried form of FBSH. Rebiotica AB complained to the Swedish Responsible Authority, the National Food Agency, but the Agency maintained the local decision. This decision halted any further development of the product, and the results from the above-mentioned study were not published at that time. However, today selected Baltic Sea herring is available that contains less dioxins and PCBs, well within the EU's rules, and it is in this context results from the study is now published. JOGK came into the project soon after the in-life phase of the present study and drafted the raw data, which now has been compiled into an article.

Materials and Methods

An open 30-day study was performed during 2004 in volunteers with IBS, gastric catarrh (GC), HB, and other related but undefined stomach problems. Forty-two volunteers (22 females and 20 males) were recruited by advertisements in the daily newspapers "Gefle Dagblad" and "Dagens Industri." Prior to the start of the study, each volunteer filled out a form with her/his name, address, telephone number, birth date, disease symptoms, and possible use of medicines; only medicines relevant to stomach problems are given in the Results section. There is no information in the raw data whether or not the symptoms have been confirmed by a physician. Because symptoms from GC and HB are rather clear, lack of this information is probably of minor importance. On the other side as discussed later on, diagnosis of IBS is highly problematic even for physicians. The volunteers were provided with capsules for the study, each containing approximately 100 mg freeze dried FBSH. FBSH, manufactured at Oscars Surströmming, Söråker, Sweden were used. The FBSH was divided into small pieces and freeze-dried by means of a conventional freeze-drying equipment. After freeze-drying, the herring was

grinded, and subsequently, encapsulated in gelatine capsules, essentially impermeable to the scent emitted from the powder, as described in the US patent 6,572,883 B1. The volunteers were provided with forms that in addition to volunteers' ID (name plus birth date) contained columns and rows for every test day where the volunteers were ask to fill in the number of capsules taken in the morning and in the evening (they were recommended to take 1–6 capsules twice daily (2 × 1–6) based on the necessity), and to report possible improvements according to a 0–10 scale, where 0 stands for no change and 10 for complete recovery from the disease symptoms. Thirty-one volunteers (20 females and 11 males) fulfilled the study and returned the forms. Thirty of them were eligible for inclusion in the study and one was excluded because of protocol violation. Statistical difference where appropriate was tested by a two-tailed *t*-test.

Results

Thirty-one patients were recruited to participate in the study, 20 females (22–63 years old) and 11 males (31–64 years old) (Table 1). Some protocol violations occurred but these were relatively minor and mainly of the character "data missing" regarding medications. The most common disorder symptom (alone or in combination with other symptoms) was IBS. Seven of 14 volunteers with IBS reported recovery from the symptoms and the mean (± S.E.M.) recovery was 4.4 ± 1.1, corresponding to 44%. Nine of 9 volunteers reported recovery from GC (alone or in combination with other symptoms) with a mean ± S.E.M. recovery of 8.4 ± 0.6 (84%). Five of 6 volunteers reported recovery from HB (gastroesophageal reflux; alone or in combination with other symptoms) with a mean (± S.E.M.) recovery of 6.8 ± 1.5 (68%). The results are summarized in Figure 1. The difference between the IBS and the GC group was statistically significant ($p = 0.0124$; two-tailed *t*-test).

Six out of 9 non-responders had IBS symptoms the other four had HB, undefined stomach problems, and hiatus hernia, respectively. The underlying mechanisms behind IBS are heterogeneous and available treatment unsatisfactory [5]. Although changes in microbiota may play an important role in IBS many other factors seem to be of importance. This heterogeneity may explain the lower numbers of responders in the volunteers with IBS than in volunteers with GC and HB.

Discussion

To the best of our knowledge, this is the first study investigating the effects of FBSH on GC, HB, and IBS. Although the present feasibility study is an open non-placebo controlled study, the results are interesting. The beneficial effect on GC and HB is astonishing and we do not believe that this effect is a pure placebo effect. The significantly lower efficacy in the IBS group compared to the GC group may in fact serve as a "built-in" control, with respect to placebo effects.

Our relation to microorganisms is ancient, and the interplay between the microorganisms and the host was an important part of the evolution of multicellular eukaryotes. In terms of cell number, adult humans are more prokaryotic than eukaryotic with 90% of our cells estimated to be of microbial origin, and only 10% of human origin [6–9]. It has been estimated that our gut contains in the range of 1,000 bacterial species and 100-fold more genes than are found in the human genome. This community is commonly referred to as our hidden metabolic "organ" due to its immense impact on human wellbeing, including host metabolism, physiology, nutrition, and immune function, and protection of the colonized host against invasion by alien microbes [1,9,10]. It is now generally accepted that the "central genome dogma," i.e., a causal chain going from DNA to RNA to proteins and downstream to biological functions, should be replaced by the "fluid genome dogma," that is, complex feed-forward and feed-back cycles that interconnect organism and environment by epigenomic programing and reprograming throughout life and at all levels [8]. The epigenomic programing is the net sum of interactions derived from our own metabolism and microbiota as well as external factors such as diet, pharmaceuticals, environmental compounds, and so on. Foods and gut microbiota are the two most important environmental factors in epigenomic programing, and are most pronounced in pregnancy and early in life.

Colonization of the gut starts already during normal delivery, where the infant is exposed for large amounts of maternal microbes [1,9,11]. During the following colonization and up to an age of 2 years, more than 1,000 species will be established in the gut. Through history, an essential part of the colonization of the human gut took place by ingestion of food preserved by fermentation. There are good reasons to anticipate that food earlier contained

Table 1. Baseline characteristics, FBSH daily dose (number of capsules), and outcome (recovery) in each volunteer.

Volunteer	Gender (F/M)	Age (years)	Symptoms	Medications	Daily dose (capsules)	Recovery[a] (0–10)
#1	M	49	HB[b]	No medications	2 × 2	10
#2	F	22	GC[c]	Data missing	2 × 2	8
#3	F	39	IBS[d]	No medications	2 × 4	0
#4[e]	F	63	HB	Omeprazol[f]	2 × 2	0
#5	M	31	HB + Anxious stomach	No medications	2 × 2	6
#6	F	50	IBS	No medication	2 × 2 (w. 1) 2 × 4 (w. 2–4)	7
#7	M	64	GC	Ranitidine[g]	2 × 4	7
#8[h]	F	47	HB + GC	Lansoprazol[f]	2 × 4	10
#9	F	24	IBS	No medications	2 × 4	0
#10[j]	F	53	IBS	No medications	2 × 3 (w. 1–2) 2 × 4 (w. 3)	0
#11[h]	F	49	IBS	No medications	2 × 3	6
#12	F	60	Undefined stomach pr.	Data missing	2 × 2–3	10
#13	F	58	IBS + HB	No medications	2 × 2	7
#14	F	35	IBS + HB + GC	No medications (prev. omeprazol[f])	2 × 2 (w. 1) 2 × 4 (w. 2–4)	8
#15	F	54	GC + ulcerous colitis	Combizym (digestion enzymes mixture)	2 × 2	10
#16[e]	M	50	IBS	No medication (normally ranitidine[g])	2 × 2	0
#17	F	39	IBS	No medications	2 × 2	7
#18	M	59	GC	Esomeprazol[f]	2 × 2 (w. 1) 2 × 3 (w. 1) 2 × 4 (w. 2–4)	5
#19[j]	M	32	GC	No medications (prev. omeprazol[f])	2 × 2 (w. 1) 2 × 4 (w. 1–4)	9
#20	F	31	IBS	No medications	2 × 6 (w. 1–2) 2 × 3 (w. 3–4)	10
#21	M	43	GC + peptic ulcer disease	Lansoprazol[f] (prev ranitidine[g])	2 × 4	9
#22	F	49	GC	No medications	2 × 2 (w. 1) 2 × 1 (w. 2–4)	10
#23	F	52	IBS	Dimetikon and Lact Bac	2 × 2 (w. 1) 2 + 3 (w. 2) 2 × 3 (w. 3) 2 × 4 (w. 4)	8
#24	F	23	IBS	No medications	2 × 1 (w. 1) 2 × 2 (w. 2–4)	0
#25	F	46	IBS	No medications	2 × 1 (w. 1) 2 × 2 (w. 2–3) 2 × 3 (w. 4)	0
#26[k]	M	61	IBS	No medications	2 × 2	–
#27	F	46	Undefined stomach pr.	Data missing	2 × 2–4	0
#28	M	47	IBS	No medications	2 × 1	9
#29	M	47	Hiatus hernia[l]	Lansoprazol[f] (prev. omeprazol)	2 × 2 2 × 4	0
#30	M	35	Undefined stomach pr.	Data missing	Data missing	6
#31	F	31	Undefined stomach pr.	Data missing	2 × 1	7

[a]At the end of the 30-day study.
[b]HeartBurn (gastroesophageal reflux).
[c]Gastric Catarrh (gastritis).
[d]Irritable Bowel Syndrome.
[e]Interrupted the study after 2 weeks.
[f]H^+/K^+-ATPase proton pump inhibitor.
[g]H_2-receptor antagonist.
[h]Went through two 30-day studies and this result is from the second study.
[i]Interrupted the study after 3 weeks.
[j]Interrupted in the middle of week 3; the given recovery (9) is from that time point.
[k]Filled in the scheme incorrect with respect to recovery; used "X" instead of a figure between 0–10.
[l]Protrusion of a part of the stomach through the diaphragm at the esophageal opening.

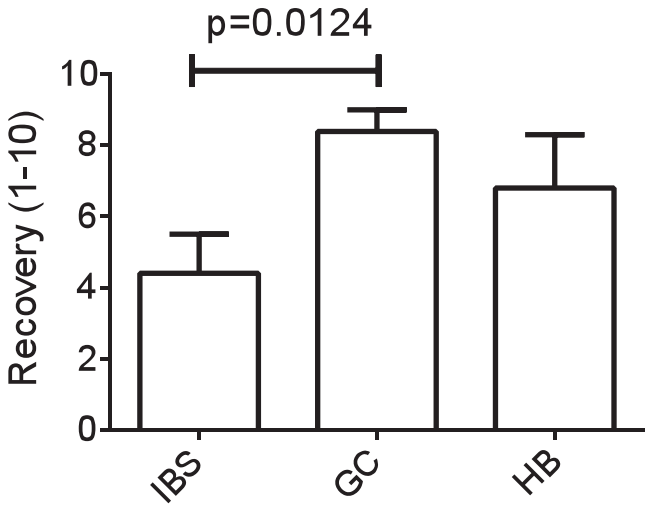

Figure 1. Recovery from IBS, GC, and HB. Statistical difference was tested by a two-tailed *t*-test.

more microbes than today, both to the total number and number of species. After finding that certain microbes were linked to serious diseases, an indiscriminate chase was started, using antibiotics, desinfectants, food preservatives, and so on. However, the underlying concept that "germ-free" humans should stay healthy showed to be completely wrong. We have instead been confronted with antibiotic resistance and depletion of the complex gut microflora.

Probiotics exert antimicrobial effects against hostile microbes by release of antimicrobial molecules and by taking up space [1,9]. The important benefits of probiotics come from their ability to metabolize complex carbohydrates and produce lactic acid and short chain fatty acids, such as butyric acid. Butyric acid reduces bacterial translocation, improves the organization of tight junctions, and stimulates the synthesis of mucin, a glycoprotein maintaining the integrity of the intestinal epithelium. Although no scientific studies have been conducted on FBSH in this particular perspective and probably not in any other perspectives either, interestingly, lactic acid and butyric acid are essential ingredients of FBSH.

The *Helicobacter pylori* bacterium is present in individuals with chronic gastritis and gastric ulcers. This bacterium is also linked to the development of duodenal ulcers and stomach cancer. *H. pylori* is present in about 50% of the world population but only causes problems in 10%–15%. It is spiral-shaped with polar flagella that live near the surface of the human gastric mucosa. It has evolved intricate mechanisms to avoid the bactericidal acid in the gastric lumen [12]. This interaction sometimes results in severe gastric pathology. *H. pylori* infection is the strongest known risk factor for the development of gastroduodenal ulcers, with infection being present in 60%–80% of gastric and 95% of duodenal ulcers. Many researchers, including Sheu et al. [13], have demonstrated that products containing certain *Lactobacillus* species can reduce *H. pylori* densities in humans. Antimicrobial actions of FBSH against *H. pylori*, in particular those related to butyric acid and other short chain fatty acids, may play an important role in its efficacy against GC and HB. Interestingly, Wang et al. [14] noted that there was a clear segregation between the microbiota of colorectal cancer patients and healthy volunteers, with respect to a decrease in the abundance of butyric acid producers.

IBS is one of the most common gastroenterological diagnoses, experienced by around 11% of the population [5]. Symptoms consist of abdominal pain associated with erratic bowel habit and variable changes in stool form and frequency, suggesting considerable heterogeneity in underlying mechanisms. Despite IBS' high prevalence, these mechanisms are poorly understood and treatment is unsatisfactory. This may explain the apparently much lower efficacy of FBSH in volunteers with IBS in comparison to those with GC or HB.

Limitations in study design

The open design of the study does not take into account the placebo effects that could be rather pronounced in a study like the present. The lack of information whether the gut symptoms have been confirmed by a physician is of course another limitation.

Conclusion

The results of the present study, in particularly those related to GC and HB, are exciting and merits further studies in volunteers, ideally in a double-blind placebo-controlled manner. In addition characterization of the active FBSH ingredients and their mechanisms of action are needed.

Conflict of Interest

The authors declare no conflict of interest.

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Ethnomedicinal survey and documentation of healing river sources among the Yoruba People (Ijesha land), Nigeria

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ABSTRACT

Background/Aim: Ethnomedicinal practices in developing countries have been widely utilized has the major source of healing human illnesses and diseases. The Yoruba people of Ijesha land, Osun State, Nigeria, have many medicinal plants and river sources in meeting their health needs. The study documented some medicinal plants and river sources used as ethnomedicine among the Ijesha people.
Methods: The ethnomedicinal survey focused on five major markets in Ijesha land which include Atakumosa, Sabo, Owena-Ijesha, Ibokun, Ijebu-Ijesa, and Kajola-Ijesha. One hundred and fifty key respondents were interviewed using a cross-sectional and purposive sampling method in gathering and collecting information on plants ethnomedicinal uses and different healing rivers sources. The healing river sources, usages, and some practices were documented in this study.
Results: The findings identified 57 medicinal plants belonging to 37 families including Fabaceae, Euphorbiaceae, Solanaceae, Malvaceae, Asteraceae, and Leguminosae. Among the medicinal plants, *Azadirachta indica*, *Cymbopogon citrates*, *Vernonia amygdalina*, and *Zingiber officinale* had the highest fidelity level of 100%. The local names, parts used, ethnomedicinal uses, and five healing rivers sources were documented alongside with their uses, risky practices at foresight (that could make the hygienic/safe status questionable).
Conclusion: The study provided baseline information on the use of medicinal plants and documentation of some healing river sources since both sustain health and cure illness among the people.

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Natural healing; rivers; ethnobotanical; Ijesha land; Yoruba; fidelity level; traditional medicine

Background

Over the years, ethnomedicinal practices, especially in developing countries, have contributed in meeting various health demands of the populace [1–3]. These traditional practitioners include herbalist, bone setter, spiritual therapist, circumciser, traditional birth attendant, psychotherapist, music therapist, aroma therapist, water therapist, homoeopathist, etc [4–7]. They are illustrious and well known among communities as a competent health service provider. In the southwestern Nigeria, they are known locally as destiny seers (Aworawo), bead seers (Olopele), oracle consultant (Oni-Ifa), sand tray seers (Oni-Iyanrin), insanity healers (Awo-were), and local Islamic healers who often participated in healing process and their practices include charms, amulets, incantations, and spiritual births [7]. The churches (Aladura) also belong to the traditional healing branch [7–10]. These churches are very strong in healing and other spiritual traditions such as the use of healing water, special soaps, bathing arrangements, and the use of holy water [9,11–12], and their household are referred to as *Ile oloogun*, *Ile elegbo igi*, *Alagbo Adahunse*, or *Ile Alaadura* [13]. Ethnobotanical studies and documentation guides identification, selection, and development of potential drug candidates from medicinal plants [14]. Several studies

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have documented different ethnomedicinal surveys in the southwestern Nigeria [15] in the management of *diabetes mellitus* [16–18]; malaria [14,19]; treatment of ulcer [20]; and used as phytocosmetics [21]. Also, World Health Organization reported that about 75% of African countries including Nigerians depend on medicinal plants for primary health care [12]. Borokini and Omotayo [22] reported the need to document and recover the ancient traditional use of plants from the older and rapidly declining generation so as to maintain and preserve medicinal plants and to also form a basis for scientific research into the relevant phytochemical principles in anticipation of medicines that could be used by populace, especially primary health care. These utilizations vary from different cultures, families, and individual experienced people [23]. Healing water is both practical and symbolical in traditional healing [24,25]. Water is an essential constituent, and it plays a special role in all ethnomedicinal uses. This suggests the basis for the Yoruba beliefs that consider water to be a vital and sacred origin of life [26,27]. In Christianity religion, water healing is generally recognized and used in curing all kinds of illness, diseases, and even opening the womb of barren women [28]. It is noticeable that a cleric (Pastors, Alfas, Evangelists, Prophets, Prophetess, and Uztaazs) uses natural therapy practices such as the use of coconut and its oil, local black soap (Ose-dudu), red soap (Ose-Ajase), kernel oil (Adin), fry palm oil (Epo-Ojere), spiritual births in rivers, use of birds or fowls (pure white or black), salts, and other natural prescribed materials. Studies have also reported different ethnomedicinal practices among different localities and its advantages and disadvantages have been highlighted [29–33]. Rinne also examined the role of healing water among the traditional Yoruba healers in southwest Nigeria [7]. The objectives of the present study aimed to document the medicinal plant knowledge and some healing river sources used as natural remedies among the Yoruba people of Ijesha land, Nigeria, since their utilization and practices are directed toward treatment and cure.

Materials and Methods

The study area

The ethnomedicinal survey was carried out among the Yoruba people of Ijesha land southwestern Nigeria, and the town covers a total area of about 73.6 km^2 with the population of about 300,000 people. The town lies along the forest region in the heart of the Yoruba with a clear boundary from the Ekitis to their east and at the intersection of roads from Ile-Ife, Oshogbo, Ado Ekiti, and Akure [34]. African traditional religion has been the earliest religion practice among the Ijeshas, but the most popular one is Ogun (god of Iron) [34]. Ijesha land presently covers six local government areas in Nigeria, which includes Ilesha West, Ilesha East, Atakumosa West, Atakumosa East, Oriade, and Obokun (local government areas within Osun State (Fig. 1)). They enjoy trading kolanut, cloth weaving, cocoa farming, blacksmithing, and other export services which make other Yoruba descendants refer to them as *Osomalo—Magba Owo-Mi-Loni*—meaning I will not sit until I have collected my money, which displays an inflexible strength of debt collection. Some prominent tourist sites include Olumirin waterfall, popularly known as Erin-Ijesha waterfall that is located in Erinmo-Ijesha land and the palace of the King, Adimula of Ijesha land usually celebrate the annual "Iwude" Ijesha festival.

Ethnobotanical survey

The survey focused on five markets in Ijesha land, including Atakumosa, Sabo, Owena Ijesha, Ibokun, Ijebu-Ijesa, and Kajola-Ijesha markets (Fig. 1). The respondents were selected based on a cross-sectional and purposive survey method in gathering and collecting information from the respondents on ethnomedicinal uses of plant and different healing rivers sources in Ijesha land. Some traditional practitioners including the cleric (Alfa and pastors) were also interviewed during the study. However, the healing river sources information was carried out mainly in Ilesha town.

Data collection

One hundred and fifty key guided interviews were carried out among local herb sellers, hunters, herbalists, and elderly people in the market and their towns. The interviews were done in their native language (Yoruba language) while the information gathered was sorted and the local names given were interpreted to their respective scientific names by Mallam Namadi Sanusi in the Department of Biological Sciences, Ahmadu Bello University Zaria, Kaduna State, Nigeria.

Ethical consent

The purpose of the study was explained to the respondents (traditional herb sellers, traditional medical practitioners, and herbalists), and informed consent was obtained from each of the respondents.

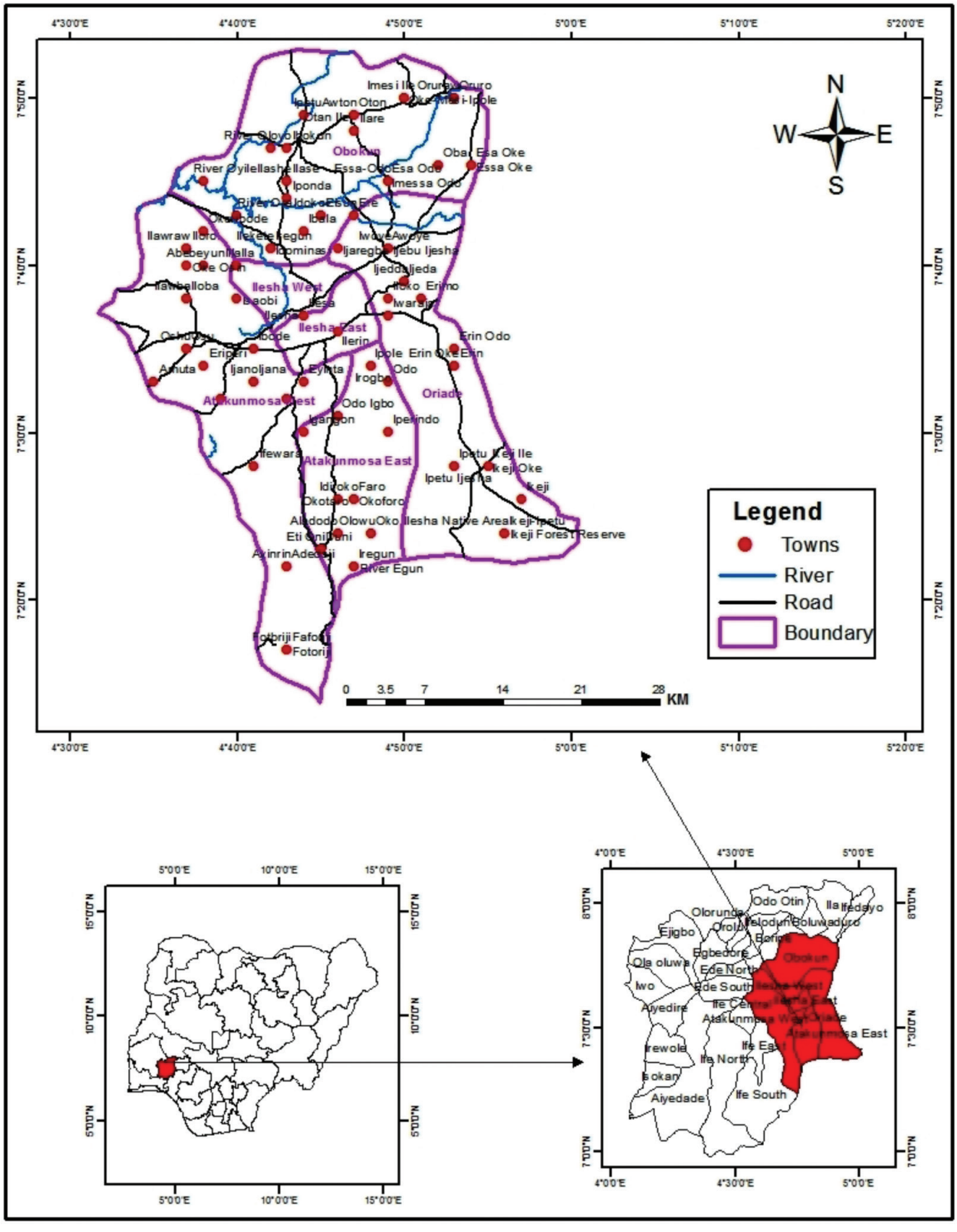

Figure 1. Geographical location of Ijesha land, showing rivers, roads, towns, and boundary in Osun State, Nigeria. Source: (Authors).

Table 1. Demographic details of the respondents (n = 150).

Variable	Categories	Number of respondent (n = 150)	Percentage of respondent (%)
Gender	Male	95	63.33
	Female	55	36.67
Age	21–30 years	13	8.67
	31–40 years	29	19.33
	41–50 years	50	33.33
	51–60 years	39	26.00
	61 years and above	19	12.67
Training level	No formal education	85	56.67
	Primary six	40	26.67
	Secondary school	16	10.67
	Nigeria Certificate in Education (NCE)/technical/vocational	5	3.33
	Diploma/degree	4	2.67
Respondents livelihood	Farmer/market people	85	56.67
	Herb seller	40	26.67
	Herbalist/priest (Alfa or pastors), priests	20	13.33
	Civil servants/retiree	5	3.33

Data analysis

The family, botanical name, common name, local name, Voucher Number, morphological parts used, and the ethnomedicinal uses of the identified plants are presented in a tabular form. The diversity of the uses of medicinal plants was evaluated by calculating the fidelity level (FL). The FL of the plants was analyzed by adopting the method in [35]

$$\text{FL}\ (\%) = \frac{Np \times 100}{N}$$

where Np represents the number of respondent that reported a use of a plant species to treat a particular ailment, and N represents the total number of respondents in the study area.

Results

Demographic/personal information of respondents

A total of 150 respondents were interviewed. The respondents were mainly Farmer/market people (56.67%), Herb sellers (26.67%) Herbalist/priest (Alfa or pastors) (13.33%), and Civil servants/retiree (3.33%) as presented in Table 1.

Medicinal plants used among the Ijesha land, Osun State, Nigeria

During this survey, a total of 57 medicinal plants species belonging to 37 families were recorded. The surveyed plants are arranged alphabetically using their scientific name, common name, voucher number, their families, ethnomedicinal uses, plant parts used, and their vernacular names in Yoruba, Nigeria language. The FL of the plants was recorded with the lowest and highest values being 45.33% and 100%, respectively (Table 2). The Fabaceae family has the highest number of plant species (five species), followed by Euphorbiaceae, Malvaceae, and Solanaceae with four species each, and then Asteraceae and Leguminosae with three species.

Discussion

The demographic details of the respondents (Table 1) showed (63.33%) males and (36.67%) females. Adebo and Alfred reported that men were experts in herbal medicine and widely known in treating some deadly diseases at affordable prices. The womenfolk were not heard but it was discovered that in almost all local markets in Nigeria, women engaged in the sales of herbal plants and medicine and also in the treatment of some diseases [36,37]. The highest age respondents (33.33%) were between 41 and 50 years old while there was low participation (8.67%) between the ages of 21 and 30 years old. These aged groups (41–50 years old) of the society were observed to be more knowledgeable about the traditional medicinal uses than younger generation. Zerabruk and Yirga [38] confirmed that a traditional healer is knowledge of secret professional that should be known by elderly ones and for male practitioners. The reason of less traditional medicinal knowledge among the younger generation could be due to urbanization and assimilation of alien culture [39]. Also, (56.67%) of the respondents had no formal

Table 2. List of medicinal plants and their ethnomedicinal uses used among the Ijesha land, Osun State, Nigeria.

Scientific name	Common name	Voucher number	Family name	Yoruba name (Nigeria)	Part used	Ethnomedicinal uses	FL (%)
Abelmoschus esculentus Moench	Okra, lady's finger	ABS1032	Malvaceae	*Ila*	Fruit, seeds	Reduce stomach flatulence, fevers, dysentery, catarrhal infections, emollient, and tonic	60.00
Abrus precatorius L.	Crab's eye	ABS1036	Fabaceae	*Oju-ologbo*; *Omisinminsin*	Root, leaf, seeds	Colds, cough, convulsion, rheumatism, contraceptive, antimicrobials, aphrodisiac, and antidote for poison	73.33
Ageratum conyzoides	White weed	ABS1057	Asteraceae	*Imi-Esu*	Aerial part	Stomach pain, antidiarhoea, antimicrobial, the juice sap could be drop on wound, bruises and as an insect repellent	80.00
Albizia lebbeck L. (Benth)	Silk flower	ABS1039	Fabaceae	*Igbagbo*	Seeds, leaf stem-bark	Astringent, mouthwash, river-blindness, and gonorrhoea	56.67
Alchornea laxiflora (Benth.) Pax & K. Hoffm	Lowveld bed-string	ABS1037	Euphorbiaceae	*Pepe*	Stem, roots, leaf	Chewing sticks, venereal diseases, emmenagogue, ring worm, (leaves traditional wraps for cola nuts). Relives pain	60.67
Amaranthus viridis L.	Green amaranth	ABS1043	Amaranthaceae	*Tete-abalaye*	Leaf, roots	Anthelmintic, dysentery, gonorrhoea, and stop blindness	89.33
Azadirachta indica A. Juss.	Neem	ABS1003	Meliaceae	*Dongoyaro*	Leaf stem	Use to treat fever and the stem could be used to wash mouth	100.00
Bryophyllum pinnatum	Miracle leaf	ABS1056	Crassulaceae	*Ewe abomoda*	Leaf	Use to cure hypertension, to stop haemorrhage, wound, diabetes, syphilis, weak erection, infertility, anti-microbial, and many other diseases.	92.67
Cajanus cajan (L.) Millsp.	Pigeon pea	ABS1047	Fabaceae	*Otili*	Leaf, seeds	Cure smallpox, chicken pox, measles antimicrobial, and chewing stick	59.33
Cannabis sativa L.	Indian Hemp	ABS1051	Cannabaceae	*Igbo*	Leaf stem-twigs.	Used in mental retardation treatment, depression, migraine, diarrhoea, sores, whooping cough, and head lice including antifungal	84.67
Carica papaya L.	Pawpaw	ABS1002	Caricaceae	*Ibepe*, *Gbegbere*	Leaf	Use as one of the plant to treat fever, and malaria symptoms.	97.33
Chromolaena odorata (L.) R. M. King & H. Rob		ABS1005	Asteraceae	*Ewe Akintola*	Leaf	The juice is usually dropped on wound; Antimicrobial	65.33
Citrus aurantifolia (Christm) Swingle	Bitter orange/lime orange	ABS1052	Rutaceae	*Osan-wewe*	Leaf, stem, root, fruit	Fever, cough, jaundice, antimicrobials abdominal pain and internal wound, hypertensive recipe and used in recipe for spiritual healing and preparation	94.00
Cocos nucifera L.	Coconut palm	ABS1050	Arecaceae	*Agbon*	Bark, root, nuts	Treatment of respiratory disorder, the oil (Adin) also used in treatment of skin and antiseptic, hair loss, diuretic, anthelmintic, laxative	85.33

Continued

Scientific name	Common name	Voucher number	Family name	Yoruba name (Nigeria)	Part used	Ethnomedicinal uses	FL (%)
Colocasia esculenta L. Schott	Cocoyam	ABS1018	Araceae	*Koko*	leaf	Use in management of hypertension, to chase ant	71.33
Corchorus olitorus L.	Mallow jute	ABS1055	Malvaceae	*Ewedu*, *Oyoyo*	Leaf	Fever, worms, diarrhoea, anthelmintic, and asthma	97.33
Costus afer L.	Ginger lily	ABS1054	Costaceae	*Ireke-omode*	Stem, roots, fruit, juice	Coughs, diabetes, rheumatic swellings, and anti-venom.	78.00
Croton zambesicus Muell. Arg.	Bushveld	ABS1046	Euphorbiaceae	*Ajekobale*	Leaf twigs	Prevent piles, gonorrhoea, arthritis, diarrhoea, cure impotence and also used among spiritual healers to stop witches and coming around your house after burning.	73.33
Cymbopogon citrates (DC.) Stapf	Lemon grass	ABS1049	Poaceae	*Ewe tea*	Leaf, roots	Used in the treatment of malaria, cough, stomach ache, stimulant, cold, and to cure ringworm	100.00
Dioscorea dumetorum (Kunth)Pax	African bitter yam	ABS1045	Diocoreaceae	*Esuru*	Leaf, tuber	Could stop abdominal pain and ache, to ease labor during child delivery, vomiting, insanity and fever.	56.67
Elaeis guineensis Jacq.	Africa palm oil	ABS1053	Arecaceae	*Igi-ope*	Palm oil	Malaria, addition to insanity treatment asthma, measles and skin rashes	85.33
Entada africana Guill. & Perr.	Entada	ABS1041	Fabaceae	*Ayunre-banana*	Bark	Astringent, antimicrobials, abortifacient and malaria.	94.00
Erythroxylum coca Lam.	Coca	ABS1031	Erythroxylaceae		Leaf	Local anaesthesia, sedative.	97.33
Ficus exasperate Roxb.	Sand paper leaf	ABS1006	Moraceae	*Ewe epin*	Leaf	Used to treat high blood pressure, stomach crumps, antimicrobial and fibroid.	66.67
Garcinia kola Heckel.	Bitter kola	ABS1042	Clusiaceae	*Orogbo*	Seeds, root, stem-bark, fruits	Antimicrobials, dysentery, swollen, cough, fever, toothache and respiratory disorders, headache and cancer	92.67
Hibiscus sabdariffa L.	Roselle plant	ABS1040	Malvaceae	*Sobo*	Flower	Diuretic, coughs, dressing wounds, beverage	52.00
Ipomoea batatas L. (Lam.)	Sweet potato	ABS1044	Convolvulaceae	*Anamo*	Leaf, tuber	Boils, wounds, nasal congestion, asthma, purgative, antimicrobials.	97.33
Jathropha multifidi L.	Coral plant/physic nut	ABS1015	Euphorbiaceae	*Ogege*	Juice sap. leaf/stem	Use to wash tongue thick white sputum or (Efuu) tuberculosis, indigestion, to relieve internal cough but in a little quantity.	86.67
Kigelia africana (Lam.) Benth.	Sausage tree	ABS0148	Bignoniaceae	*Pandoro*	Root, stem bark, fruits, Leaf	Cardiac arrest, malaria, dysentery, rheumatism, gonorrhoea' Haemorrhage and cough.	73.33
Lawsonia inermis L.	Henna tree	ABS1038	Lythraceae	*Laali*	Leaf	To boost sperm production jaundice, gonorrhoea, leucorrhoea, menstrual disorder, skin diseases and malaria.	78.67
Leucaena leucocephala Lam.	Tamarind, jumpy bean	ABS1035	Fabaceae		Leaf	Antimicrobial and blood tonic.	87.33

Continued

Scientific name	Common name	Voucher number	Family name	Yoruba name (Nigeria)	Part used	Ethnomedicinal uses	FL (%)
Ludwigia abbysinica A. Rich.	Water primrose	ABS1034	Onagraceae	*Ako ewuro odo*	Whole plant	Purgative, pain, swollen, fevers, anti-helminthic, cough and goat weed.	66.67
Mimosa pudica L.	Sensitive plant	ABS1033	Leguminosae	*Patanmo*	Leaf	Guinea worms piles, kidney disease and boils	59.33
Mirabilis jalapa L.	Four o' clock plant.	ABS1030	Nyctaginaceae	*Tanaposo*	Root Leaf	Wounds healing and purgative purposes	97.33
Momordica charantia L.	African cucumber, bitter melon	ABS1001	Cucurbitaceae	*Ejerin were*	Leaf and stem	Stomach pain, Indigestion Diabetes, piles, jaundice, sore, antimicrobials and Ease of ejaculation for men	73.33
Morinda lucida Benth.	Brimstone-tree	ABS1029	Rubiaceae	*Oruwo*	Leaf, stem bark, root	Malaria, diabetes, heart diseases, purgative, emetic, diuretic, jaundice, flatulence, Anti-cancer.	87.33
Nicotiana tabbacum L.	Tobacco	ABS1028	Solanaceae	*Taaba*	Seed, leaf	Sniff in the nose to cure catarrh, migraine and nasal congestion	96.67
Ocimum basilicum L.	Sweet and hairy basil	ABS1027	Lamiaceae	*Efinrin*	Aerial part	Gonorrhoea, catarrhal conditions, cough, constipation, dysentery, ringworm, carminative, stimulant, and hypertension	84.67
Parkia biglobosa Benth.	locust bean	ABS1026	Leguminosae	*Igi-iru*	Leaf, bark, seeds, fruit pulp	It helps and protect the eyes from cataract and blindness gonorrhoea, aid wound healing, malaria and also increase the blood level	92.00
Parquetina nigrescens (Afzel.) Bullock	African parquetina	ABS1013	Asclepiadaceae	*Ewe Ogbo*	Leaf	Squeeze the leaf and prepare with water to increase blood in the body. Abortificient; It cures constipation It also boosts the memory of children that have low retentive memory. Cure diabetes and use to heal insanity.	49.33
Piper guineense Schumach	Climbing black pepper	ABS1025	Piperaceae	*Atare*	Dried seed	Additional ingredient in many herbal and spiritual cures. These include rheumatism, antipyretic, insecticides stomach ache, mental illness, antimicrobials and also use to chase away evil spirit.	65.33
Senna alata (L.) Roxb.	Candle bush	ABS1024	Caesalpiniaceae	*Asunwon oyinbo*	Leaf, seeds, stem, bark	Skin rashes, dysentery, ringworm, eczema, bronchitis, and stomach ache.	79.33
Sida acuta Burm. F.	Wireweed	ABS1023	Malvaceae	*Osokotu*	Leaf, root	Malaria, antipyretic, and boils	96.67
Solanum bicolor Willd. Ex Roem. & Schult	Guinea corn.	ABS1020	Solanaceae	*Oka baba*; *Oka-pupa*	Leaf, whole plant, grains	Help diabetic patients, nursing mothers, diuresis and as meal supplements	59.33
Solanum melongena L.	Egg plant	ABS1022	Solanaceae	*Igba*, *igba-ijesu.*	Fruits	Fertility and hormonal balance, Diuretic, and purgative	45.33
Solanum nigrum L.	Black-nightshade	ABS1021	Solanaceae	*Efo odun*	Aerial part	Malaria, gonorrhoea, inflammatory swellings, skin diseases, ringworms, boils, hypertensions	75.33
Talinum triangulare (Jacq.) Wild.	Water leaf	ABS1012	Portulaceae	*Gbure*	Leaf	Increase blood level and clean urinary tract	89.33

Continued

Scientific name	Common name	Voucher number	Family name	Yoruba name (Nigeria)	Part used	Ethnomedicinal uses	FL (%)
Tectona grandis L.f.	Teak tree	ABS1011	Lamiaceae	*Igi tikii*	Fruit, seeds, bark	Head ache, skin rashes ,antimicrobial, astringent, and chewing sticks	64.67
Telfairia occidentalis Hook.f.	Fluted pumpkin	ABS1010	Cucurbitaceae	*Efo Egusi*	Aerial part & leaf	Increases blood level and cure many intestinal disorders.	83.33
Terminalia catappa L.	Church fruit	ABS1004	Combretaceae	*Furutu*	Leaf	Used to treat cough; as cardiac tonic and increase diuresis	54.67
Tetracarpidium conophorum Hutch. & Dalziel	African walnut	ABS1007	Euphorbiaceae	*Awusa*; *Asala*	Bark, seed and leaf	Used as recipes for treatment of epilepsy, the seed is aphrodisiac and used to treat malaria, asthma and gonorrhoea	97.33
Tetrapleuratetraptera Schumach. and Thonn	Prekese	ABS1019	Leguminosae	*Aridan*	Leaf fruit	Used to treat pain, convulsion (Giri), diabetes, antimicrobial and mosquito repellent.	50.67
Thaumatococcus danielli Benth	Miracle berry	ABS1009	Marantaceae	*Ewe eran*	Leaf and fruit	It cures diabetes and mainly used as natural meal wrapper especially moi-moi, pounded yam which usually add more sweetening to the food	45.33
Theobroma cacao L.	Cocoa	ABS1008	Sterculiaceae	*Koko*	Seed and leaf	Stimulant and aid wound healing especially internal bleeding	65.33
Vernonia amygdalina Delile.	Bitter leaf	ABS1017	Asteraceae	*Ewuro*	Leaf, stem	Aid digestion, antidiabetic, cure stomach ache, chewing stick and also reduce pile and antimicrobial	100.00
Vitellaria paradoxa C. F Gaertn	Shea butter	ABS1014	Sapotaceae	*Ori*	Seed, oil	Use in relieving wound and pain. In formulation with palm oil as (Ero)	86.67
Zingiber officinale Roscoe.	Ginger	ABS1016	Zingiberaceae	*Ata-ile*	Rhizomes	Cure cough, cold, piles, used in spiritual recipes, fever, chicken pox, malaria and for herb soup to cure catarrh	100.00

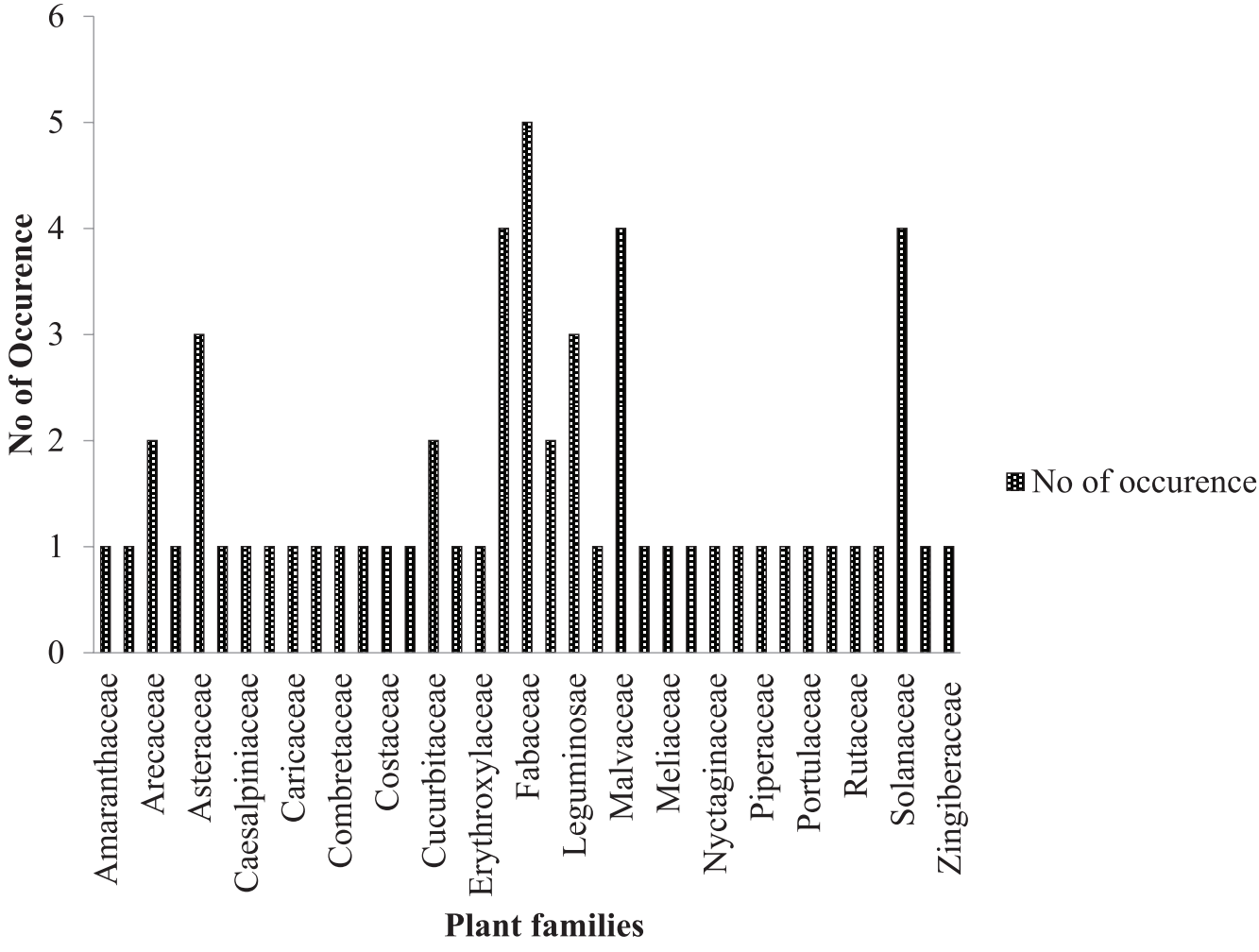

Figure 2. Distribution of plant families in the study area.

education and (26.67%) only attained primary education. The major livelihood (56.67%) was farming and market selling, and there was low participation of civil servants/retiree (3.33%) during the study.

Furthermore, 57 names of plant species were documented alongside with their ethnomedicinal uses (Table 2). Some of the plants documented in the survey have also been reported in different ethnobotanical surveys [11,36,40–43] for similar ethnomedicinal uses. Their Yoruba names and parts of plants (leaves, stem bark, fruit, roots, and flowers) used would guide future studies since local names play a vital role in the ethnobotanical study of a particular tribe or a region [44–45]. The ethnomedicinal uses of the medicinal plants including antimicrobial, aphrodisiac activity, anti-diabetes, and potential sources of curing cough, fever, malaria, hypertension, boils, skin rashes, infertility and hormonal imbalances, rheumatism, ease of sperm ejaculation and among others have been documented. These information could serve as a data base toward scientific exploitation, tool for knowledge sharing, and documenting cultural heritage for sustainable development in the country as suggested by [46]. There was a significant occurrence of Fabaceae, Leguminosae, Malvaceae, Solanaceae, Asteraceae, and Curcubitaceae in the taxonomical families during the study (Fig. 2). The above botanical families have previously been reported to be the most used medicinal plant families of Nigeria [16,17,19,21,47,48]. Scientific studies on these plant families could provide insights into their rich phytoconstituents and understandings of the pharmacological actions of their active compounds [49]. The majority of the plants had high FL, and the highest FL of 100% was recorded for four plant species of which two species, namely, *Azadirachta indica* and *Cymbopogon citrates* were used in the treatment of malaria. *Vernonia amygdalina* is used in the treatment of diabetes and *Zingiber officinale* is used in the treatment of cold. These plants are widely used in many ethnobotanical practices around the

Table 3. Distribution and name of some healing rivers in Ijesha land.

River sources name	Location	Uses and practices	Risky practices at foresight (that could make the hygienic/safe status questionable)
Omi-Ayo	Oke—oye Ilesa	For drinking and bathing under instruction of Prophet	Sources polluted. It is contaminated with soaps, scrubbers and other diabolic materials
Omi- ipade meta	Along Osun state college of Education Ilesa-Ibodi	Specialized water for drinking and bathing sick people and other spiritual purposes	Clean, safe for drinking and managed by someone in charge
Omi abele known as Omi-Oko	In Ilaje around Isokun Road, Ilesa	Specialized water for drinking and other spiritual purposes. In the past days, the king's men fetched the water early in the morning for the king to drink.	Safe for drinking but stagnant.
Omi Erinmo	Erin Ijesha (Waterfall)	Specialized water for drinking and other spiritual purposes	Clean, safe for drinking and other spiritual purposes. Shoes are not allowed around the river
Omi Baba Erinle	Imo, Ilesa	Specialized water for healing and spiritual purposes	Clean and safe. Shoes and bathing are not allowed around the areas.

world with sufficient scientific validations of their ethnomedicinal use [50].

Finally, five healing river sources in Ijesha land (Table 3) documented their ethno uses, practices, and possible cautions on their hygienic status. Qualitative responses from respondents were quoted on their experiences and psychological impacts of bathing in rivers. The following quotations summarize their experiences:

> *I was told by a Prophet from Lagos state, Nigeria to bath in Omi-Ayo in Ilesha for 21 days. I was scared because of different people I saw bathing. I saw some people bathing with red soap, cloth with blood, and some were using prepared concoction to bath in the same water (Omi-Ayo from Ilesa, Nigeria).*

> *In fact, I saw a mentally sick person that was brought in a white garment clothes who they were praying that insanity in the person should enter the river and also delivering the woman from the power of witchcraft (Omi-Ayo from Ilesha, Nigeria*).

> *I saw a man pouring water in an empty basket and I was shocked when the man started cursing the basket and using the basket to bath openly in the midst of people (Omi-Ayo from Ilesa, Nigeria).*

> *I was told by Man of God from Ekiti state to come and fetch water from where three rivers points meet (Omi-Ipade meta). He told me to fetch the water for drinking and bathing and that I should keep it and use it (Omi-Ipade meta from Ilesa, Nigeria).*

There is a possible risk associated with bathing in rivers including bacterial, viral, and other diseases could be contracted by man through exposure to sewage polluted bathing water or beach sand [51]. This could have effect on their health being and might pose further threat on life in anticipation for cure. These unsafe practices of healing river waters bathing or drinking will have a harmful effect on human including skin infections and other opportunistic diseases.

Conclusion

The study provided baseline information on the use of medicinal plants for the treatment of malaria, gonorrhoea, abdominal pain, treat cough, diabetes, fever, and hypertension. The healing river sources identified some risk practices at foresight that could make river sources utilization and safe status questionable. This ethnomedicinal survey would guide further exploitation into phytomedicine and natural remedy research toward drug development.

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Conflict of Interest

The authors declared no conflict of interest.

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An ethnoveterinary study on plants used in the treatment of dermatological diseases in Central Anatolia, Turkey

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ABSTRACT

Aim: The aims of the present study are to determine the significant plant species utilized in ethnoveterinary medicine of Central Anatolia region (Turkey), identify methods used for different veterinary preparations, and to compare the plants used in the treatment of different animal dermatological diseases in other regions of Turkey and different parts of the world.

Methods: Interviews were conducted with 173 individuals in total by means of a semi-structured questionnaire, between 2009 and 2013, for the purpose of recording traditional veterinary remedies and practices employed in animal health care. In order to evaluate the reliability and richness of the knowledge of medicinal plants in the area, quantitative indices, such as "informant consensus factor (FIC)," "use value (UV)," "relative frequency citation," and "fidelity level," were used for the data analysis.

Results: The findings of this study have revealed about 26 species, including herbs, trees, and green algae belonging to 22 botanical families utilized in the treatment of veterinary dermatological diseases by breeders in Central Anatolia. In the present study, the highest FIC score (0.90) was identified for cracked nipples. It was determined that Pine tar and *Cydonia oblonga* were used for the above-mentioned purpose. The second highest FIC value (0.87) was identified for ringworm. A number of medicinal plants were very popular and utilized intensively in the present research area. In accordance with the calculation performed on the basis of the UV, it was determined that *Pinus nigra* (0.43) and *Allium sativum* (0.28) had the highest UVs.

Conclusion: The current study has emphasized the ethnoveterinary knowledge of plants recently in use and their new usage in the Central Anatolia region of Turkey.

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Ethnoveterinary medicine; medicinal plants; dermatology; Turkey

Introduction

Ethnobotanical studies investigate plants used in the folk tradition of different regions and countries, as well as plants used in ethnoveterinary practices [1]. Ethnoveterinary medicine (EVM), which is the scientific term used for traditional animal health care, contains the knowledge, skills, practices, methods, and beliefs about animal health care present among the members of a certain community [2]. Many farmers use a variety of ethnoveterinary knowledge for the purpose of maintaining the health of their domestic animals, and they have utilized it in order to prevent and treat livestock ailments [3].

No scientific studies have been conducted on the ethnoveterinary medicinal plants in Central Anatolia. Thus, the current study was carried out for the purpose of recording the indigenous knowledge about the usage, management, and conservation status of ethnoveterinary medicinal plants. Furthermore, in the present study, it was aimed to record the plants utilized in the treatment of animal dermatological diseases and emphasize their preparation, processing, and administration in the present research area,

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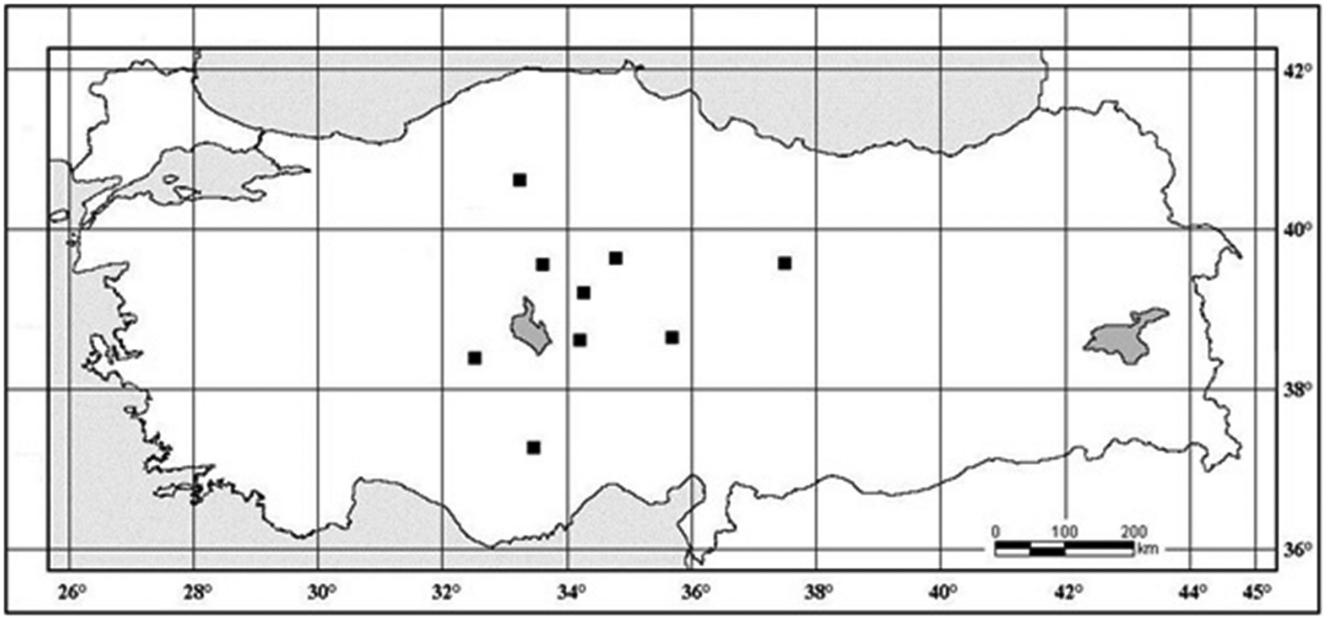

Figure 1. Map showing the regions studied in the Central Anatolia, Turkey.

and to compare to the plant species used in other regions of Turkey and in other countries. Moreover, the results acquired in the present study may be used for further scientific studies.

Materials and Methods

Study area

Central Anatolia represents one of the seven geographical regions of Turkey. The area in question is situated in the central region of Turkey (Fig. 1). The area of Central Anatolia is 151,000 km^2, and it makes up 21% of Turkey's territory. The study area falls within the latitudes 32°31'N and 37°02'N and longitudes 37°11'E and 40°36'E [4].

There is a rich flora in the area due to the region's climatic characteristics, geological structure, and location [5]. Temperatures range from −25°C/−13F to 40°C/104F, with the rainfall of only 413 mm/15 inches per year. Forests including *Pinus nigra* J. F. Arnold, *Quercus pubescens* Willd., and *Juniperus oxycedrus* L. are mainly situated in the higher sections of the mountains, such as the Akdağlar, which contain a considerable number of these trees utilized for acquiring tar [6].

Although some ethnobotanical information [7,8] is available for the Central Anatolia region of Turkey, several studies investigating plants utilized in EVM have been encountered in the literature [9–11]. There are a number of studies on the EVM for the other regions of Turkey [12–17].

The Ph.D. fieldwork of the first author [18], which was carried out between 2009 and 2010 in the province of Middle Red River of the Central Anatolia region (Çankırı, Kırıkkale, Kırşehir, Kayseri, and Yozgat), mostly contains data on EVM. The second fieldwork followed the previous study in the eastern part of the Central Anatolia region, Upper Red River province (Sivas) [19]. Our research group conducted the last study in Konya province of the Central Anatolia region (Aksaray, Karaman, and Konya) [20].

Interviews with local people

In order to collect EVM information, 2015 individuals from nine cities (Aksaray, Çankırı, Karaman, Kayseri, Kırıkkale, Kırşehir, Konya, Sivas, and Yozgat) in Central Anatolia were interviewed by means of semi-structured and structured questionnaires. Interviews were conducted by taking notes and performing the audio or video recordings of the interviewees when it was possible. In total, 173 people comprising almost 9% of the total informants gave information about plants used in the treatment of animal dermatological diseases. Mainly elderly individuals involved in the breeding and maintenance of livestock, such as farmers and shepherds, or who were working in agriculture provided information on EVM. All the individuals interviewed were males aged 50 years on an average, who still have the richest knowledge of traditional domestic medicine.

The information included plant species and family, vernacular name, the parts of the plant utilized, methods of preparation (i.e., infusion, poultice, powder, and latex) and administration, popular use, use value (UV), bioactive compounds, recorded literature uses, and locations.

Plant materials

Field studies were performed during a 4-year period (2009–2013). In the above-mentioned period, 26 plant taxa, which are used in the treatment of veterinary dermatological diseases, were recorded. The standard text entitled "Flora of Turkey and the East Aegean Islands" [21,22] was used for the identification of the scientific names of plant species. The comparison of the plants with herbarium accessions was performed at Selçuk University (Konya). A specialist (Tugay O., botanist) from the Biological Department of Selçuk University assisted us in order to ensure proper identification.

Data analysis

The informant consensus factor (FIC) was used to demonstrate the homogeneity of the information. The FIC was calculated using the formula mentioned as follows: FIC = (Nur – N_t)/(Nur – 1), where Nur is the number of use citations in each category and N_t is the number of the species utilized [23]. The FIC gets a low value (close to 0) in case the plants are selected randomly or in case that there is no exchange of information about the usage of plants among informants. The FIC gets a high value (close to 1) in case of a well-defined selection criterion in the community and/or in case the informants share the information [24].

The UV, which represents a quantitative method showing the relative importance of species that are known on a local scale, was also determined by using the formula below: UV = U/N, where UV is the use value of a species, U is the number of citations per species, and N is the number of informants [23].

The fidelity level (FL) is beneficial for recognizing the plants that are mostly preferred by respondents in order to cure particular ailments. The main purpose of the FL is to calculate the importance of plant species for a specific objective [25]. The FL value was estimated by means of the following formula: FL = $N_p/N \times 100$, where N_p refers to the number of respondents who reported the utilization of medicinal plants for a specific main ailment and N refers to the total number of respondents who indicated the same plant for any ailment [26]. The closer the FL value is to 1, the higher is the number of respondents who have utilized the plant species in question for a specific usage. A high FL value means that a specific plant species is frequently used by the respondents in the research area for the treatment of a specific ailment category [27].

The frequency citation (FC) was acquired by means of the formula below: FC = (number of times when a specific species was mentioned/total number of times when all species were mentioned) × 100. The above-mentioned formula is employed for a better relative expression of citations. The following formula: relative frequency citation (RFC) = FC/N ($0 < RFC < 1$) was used to calculate the RFC. In order to acquire the index in question, the number of respondents indicating a useful species FC or frequency of citation is divided by the total number of respondents in the questionnaire (N) without considering the categories utilized [27]. In the present study, the FL, RFC, and FC were determined for the plant cited most frequently.

Results

The findings of the present study have indicated 26 species, including herbs, trees, and green algae belonging to 22 botanical families utilized by breeders in Central Anatolia to treat veterinary dermatological diseases (Table 1). Rosaceae, Fabaceae, and Amaryllidaceae were the most common representatives of these families used to treat eight dermatological disease categories with the percentages of 13.6, 9.09, and 9.09, respectively (Fig. 2). There was one species in the other families, as indicated in Figure 2.

Traditional ethnoveterinary plants were identified for the treatment of dermatological diseases of domestic animals. The most treated dermatological diseases encountered were categorized into eight groups. In this study, it was determined that 16 (31.4%) plant species were used for treating open skin wounds, 9 (17.6%) plant species were used for treating mange, 7 (13.7%) plant species were used for treating ringworm, 5 (9.8%) plant species were used for treating papillomatosis and sunstroke-sunburn, 4 (7.8%) plant species were used for treating interdigital dermatitis, 3 (5.9%) plant species were used for treating dermatitis madidans, and 2 (3.9%) plant species were used for treating cracked nipples (Fig. 3).

The parts of plants utilized most frequently are leaves (27.6%), fruit (17.2%), wood, latex and seed (10.34%), and bulb (6.93%). Gum, resin, bark,

Table 1. List of the plants used in the EVM of Central Anatolia region, and scientific literature review.

Family	Plant species/ voucher number	Vernacular name	Part(s) used	Preparation/ administration[a]	Popular use[b] (therapeutic effect)	UV[c]	Bioactive compounds/ recorded literature uses (pharmacological activity)	Locations[c]
Amaranthaceae	*Beta vulgaris* L. *var. altissima* Döll O. Tugay 1865 26.909	Şeker pancarı	Root	Powdering/E	OSW	0.01	Terpenoids/anti-inflammatory [28,29]	Y
Amaryllidaceae	*Allium cepa* L. O. Tugay 3234 26.916	Soğan	Bulb	Poultice/E	OSW ID	0.07	Essential oils, flavonoids/anti-inflammatory, antimicrobial [30]	A, Kar, Ko
Amaryllidaceae	*Allium sativum* L.*	Sarımsak	Bulb	Crushing (mixed yogurt) /I Pounding (mixed salt or lemon juice, vinegar)/E	SS, R, M ID	0.28	Allicin, ajoene/antiseptic, antibacterial [31], antiparasitic [32–34]	Ç, Kar, Ko, S, Y
Anacardiaceae	*Rhus coriaria* L. O. Tugay 2952 26.910	Sumak	Fruit	Infusion/E	OSW R	0.20	Tannin, Phenols (myricetin)/ Antibacterial [35] antioxidant [36]	A, Kar, Ko, S
Brassicaceae	*Brassica oleracea* L. O. Tugay 1444 26.911	Lahana	Leaves	Poultice/E	SS	0.01	Wound healing [37]	Ç
Cladophoraceae	*Cladophora glomerata* L. Not founded	Yosun	Leaves	Topical application/E	OSW	0.05	Carotenoids, phenols/ antihemorrhagic, antibacterial [38]	A
Convolvulaceae	*Convolvulus arvensis* L. O. Tugay 1727 26.912	Çoban döşeği otu	Leaves	Poultice/E	OSW	0.02	Not reference	Ko
Cupressaceae	*Juniperus oxycedrus* L. O. Tugay 1827 26.913	Katran ardıcı	Wood (juniper tar) Branch	Distilled tar (mixed butter)/E Smoke/E	M, OSW, P	0.05	Phenols (giacol, ethyl, creosol)/ Antiseptic, antiparasitic, antipruritic, anti-inflammatory [39], antifungal [40]	A, Kar, Ko, S, Y
Euphorbiaceae	*Euphorbia macroclada* Boiss. O. Tugay 1544 26914	Sütleğen otu	Latex	Topical application/E	OSW P	0.05	Polyphenolics, terpenoids/ antibacterial, antifungal [41,42]	Kar, Ko, S
Fabaceae	*Astragalus* L. AK. 1050	Geven	Spina	Punksiyon/E	P	0.01	Not reported	A
Fabaceae	*Ceratonia siliqua* L.*	Keçiboynuzu	Latex	Topical application/E	P	0.01	Tannins, polyphenols/ antimicrobial, antiproliferative [43,44]	Kar, Ko
Fagaceae	*Quercus pubescens* Willd. O. Tugay 2244 26915	Tüylü meşe	Bark Wood (ash)	Powdering/E Burning to ashes/E	OSW M	0.02	Flavonoids, tannins/antiseptic [45], antiparasitic [46], antifungal [47]	Kar, Kay, Ko, Y
Linaceae	*Linum usitatissimum* L. O. Tugay 5600 26.929	Keten	Seed (linseed oil)	Topical application/E	SS, R, M	0.09	Phenols (linoleic, linolenic, oleic acids)/wound healing [48]	Kır, Kırş, Ko, S, Y
Lythraceae	*Lawsonia inermis* L.*	Kına	Leaves	Powdering/E	R	0.06	Tannin, lawson/antifungal [49]	Ko, S, Y
Moraceae	*Ficus carica* L.*	İncir	Latex	Topical application/E	P	0.01	Proteolytic enzymes/Keratolytic, proteolytic [44,50]	Kar, Ko

Continued

Family	Plant species/ voucher number	Vernacular name	Part(s) used	Preparation/ administration[a]	Popular use[b] (therapeutic effect)	UV[c]	Bioactive compounds/ recorded literature uses (pharmacological activity)	Locations[c]
Oleaceae	*Olea europaea* L.*	Zeytin	Fruit (olive oil)	Topical application/E	SS M	0.14	Phenols/antimicrobial [51,52]	Kar, Kırş, Ko, S
Pedaliaceae	*Sesamum indicum* L.*	Susam	Seed (tahini)	Topical application/E	R	0.08	Sesamin, sesaminol, sesamolin/ antioxidant [53], antifungal [54]	A, Kar, Ko
Pinaceae	*Pinus nigra* J.F. Arnold O. Tugay 2219 26.921	Karaçam	Gum Pine resin Wood (tar)	Direct application/E Powdering/E Distilled tar/E	DM, OSW, ID SS, OSW, CN, ID R	0.43	Essential oils/antimicrobial [55], vulnerary [56], antiparasitic [57]	A, Ç, Kar, Kay, Kır, Ko, S, Y
Plantaginaceae	*Plantago lanceolata* L. O. Tugay 1493 26.922	Damarlıca	Leaves	Poultice/E	OSW	0.03	Flavonoids, polysaccharides/ antiseptic [58], antihemorrhagic, antihelmintic [57]	Kır, Kay
Rosaceae	*Cydonia oblonga* Mill. O. Tugay 3510 26.923	Ayva	Seed	Crushing/E	CN	0.02	Pectin, tannin/wound healing [59], antioxidant [60]	A, Kar, Ko
Rosaceae	*Malus pumila* Mill. O. Tugay 3194 26.924	Elma	Fruit (vinegar)	Topical application/E	DM, OSW, P, M	0.08	Acetic acid/antiseptic [31,51,52]	A, Ç, Kar, Kay, Ko, Y
Rosaceae	*Prunus persica* (L.) Batsch.*	Şeftali	Leaves	Infusion/E	OSW	0.10	Polyphenols/antimicrobial, antioxidant [61]	A, Kar, Ko
Rutaceae	*Citrus limon* (L.) Burm. f. O. Tugay 10.273 26.926	Limon	Fruit	Topical application/E	OSW, M	0.02	Citric acid/antiseptic [62]	Ko
Scrophulariaceae	*Verbascum cheiranthifolium* Boiss. O. Tugay 3114 26.927	Sığırkuyruğu-kurtkulağı-bozkulak	Leaves	Poultice/E	OSW	0.03	Flavonoids, iridoids, saponins, polysaccharides/Wound healing [63]	Ç, Ko, S, Y
Solanaceae	*Nicotiana tabacum* L.*	Tütün	Leaves	Infusion/E	M	0.01	Alkaloid (nicotine)/antiparasitic [64]	Ko
Vitaceae	*Vitis vinifera* L. O. Tugay 3518 26.928	Üzüm	Fruit (molasses)	Topical application/E	R	0.08	Phenolic acids/antifungal [65]	A, Kar, Kır, Ko

[a]Administration: E = external; I = internal.
[b]Popular use: DI = dermatitis madidans; OSW = open skin wounds; SS = sunstroke and sunburn; CN = cracked nipples; P = papillomatosis; R = ringworm; ID = interdigital dermatitis; M = mange.
[c]Locations: A = Aksaray; Ç = Çankırı; Kar = Karaman; Kay = Kayseri; Kır = Kırıkkale; Kırş = Kırşehir; Ko = Konya; S = Sivas; Y = Yozgat.
*The voucher number was not given to these plants because they were purchased from the markets.

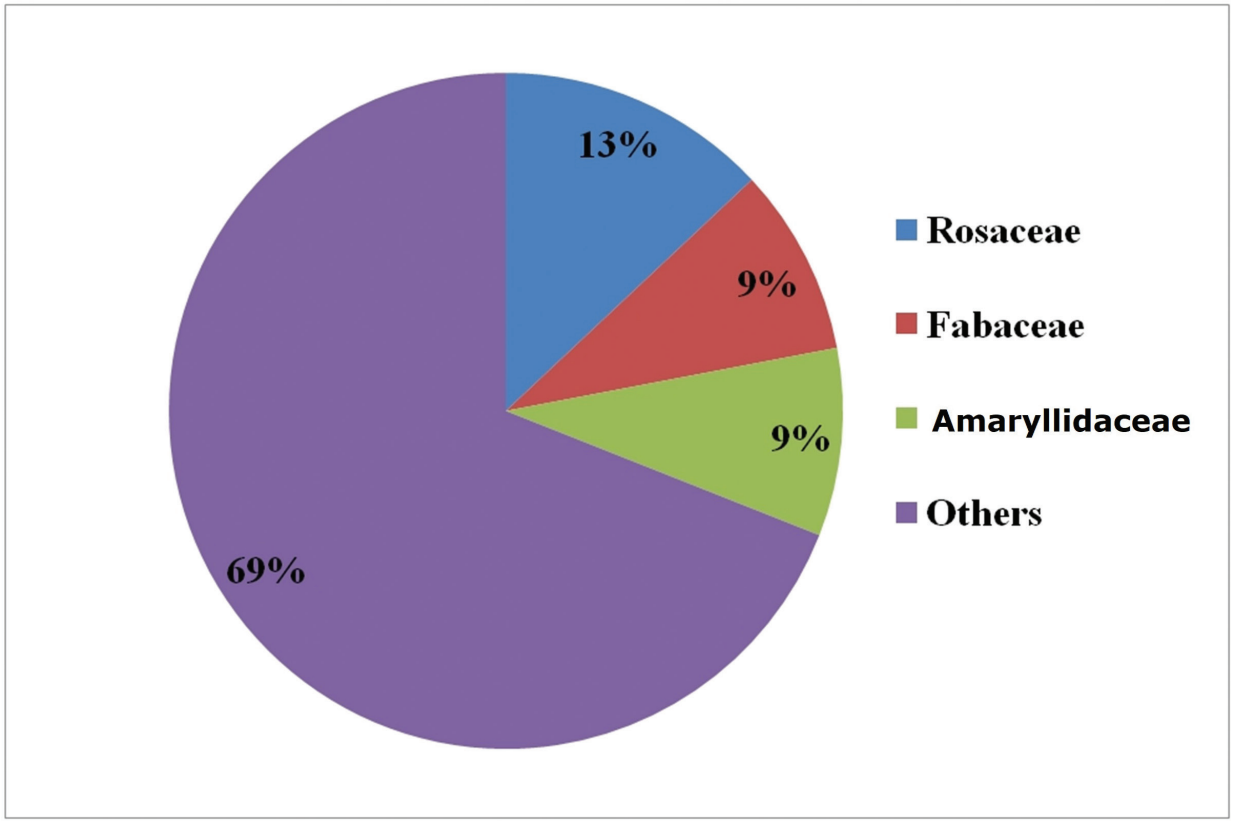

Figure 2. Relative frequency of plant species by family utilized for veterinary purposes in Central Anatolia.

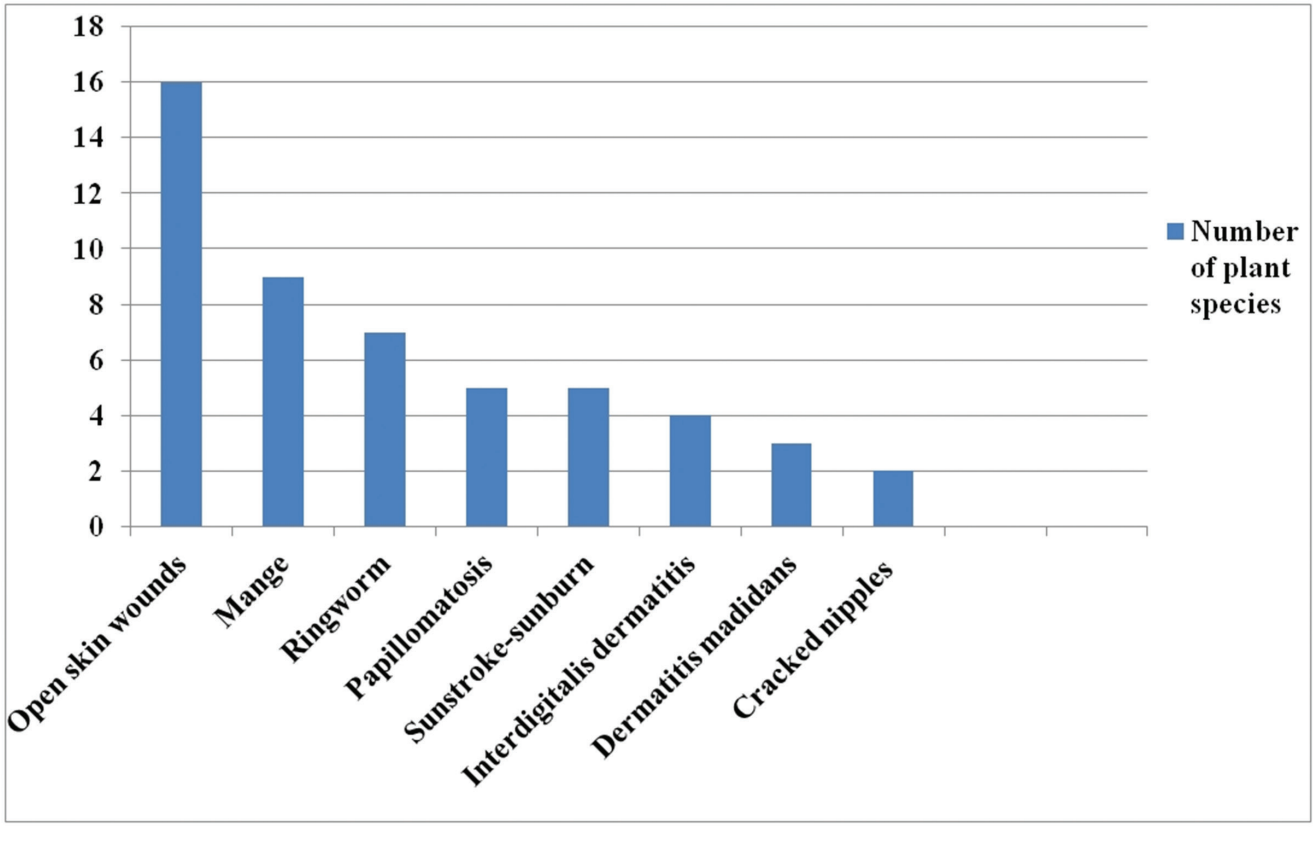

Figure 3. A number of reported plants used for the treatment of different veterinary dermatological diseases.

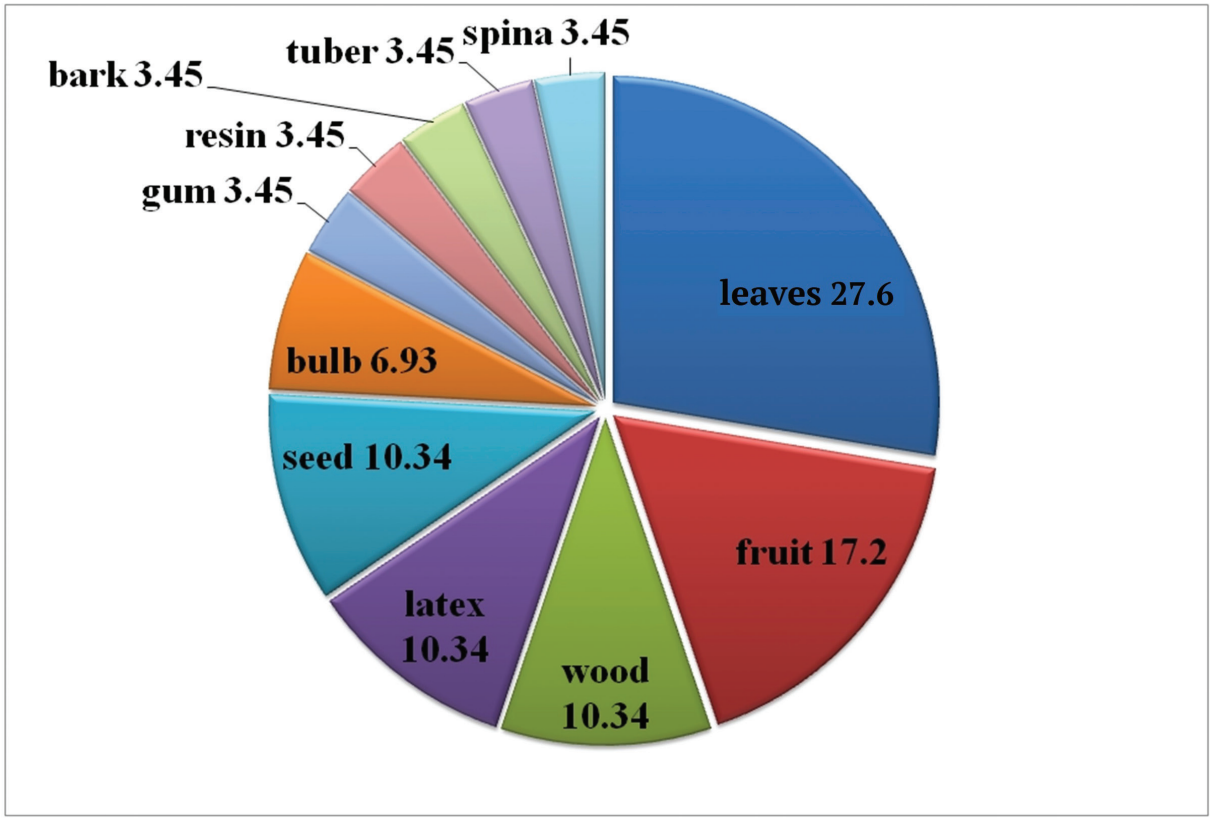

Figure 4. Fractions of plant part used in EVM of Central.

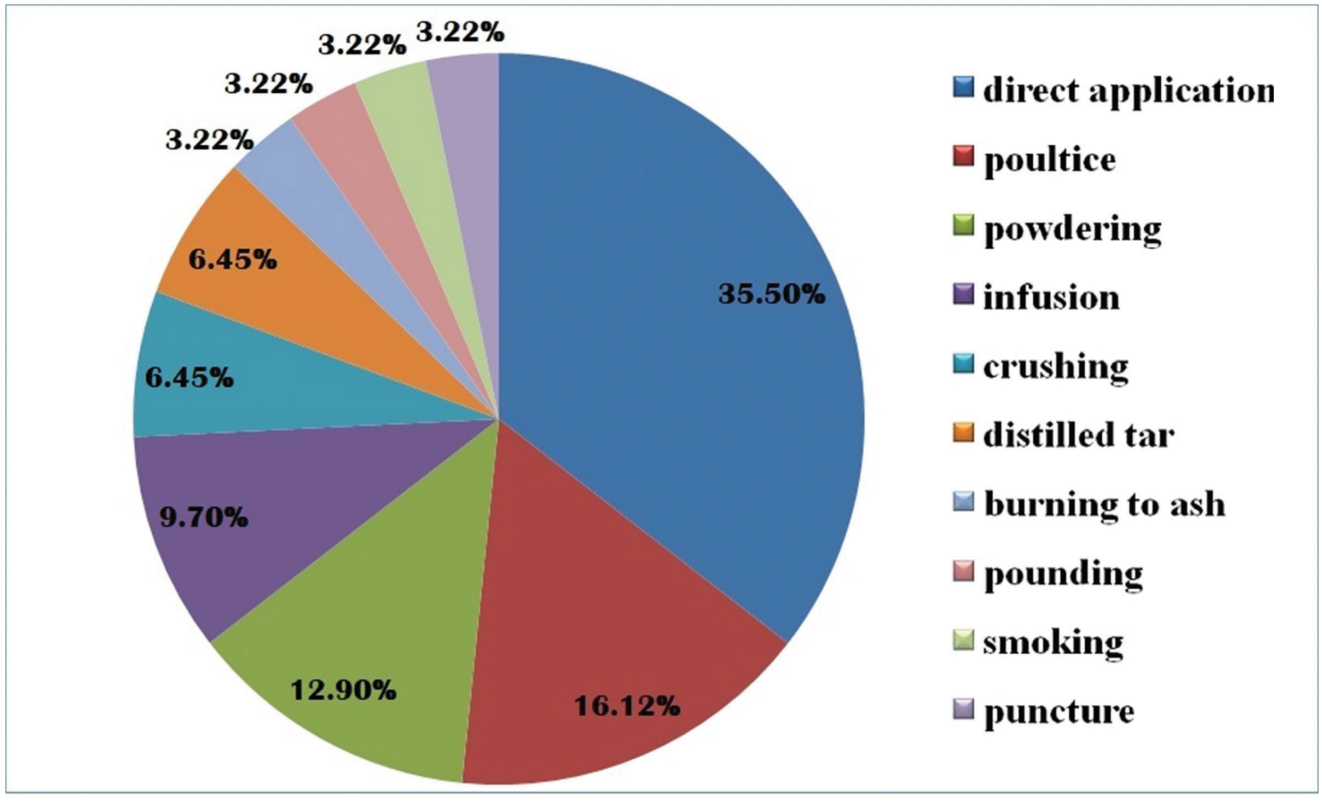

Figure 5. Methods of preparation and administration used in the EVM of Central Anatolia..

Table 2. Categories of animal dermatological diseases treated in Central Anatolia region, with associated FIC.

No	Dermatological diseases	Use citations	All use citations (%)	FIC
1	Dermatitis madidans	7	3.1	0.66
2	Open skin wounds	73	32.3	0.86
3	Sunstroke and sunburn	23	10.2	0.81
4	Cracked nipples	12	5.3	0.90
5	Papillomatosis	7	3.1	0.17
6	Ringworm	48	21.2	0.87
7	Interdigital dermatitis	14	6.2	0.85
8	Mange	42	18.6	0.80

Table 3. List of most frequently cited plants.

Plant species	No. of interviews in which it was cited	FC (%)	RFC
Pinus nigra	53	74.64	0.43
Allium sativum	48	67.60	0.39
Rhus coriaria	43	60.56	0.35
Olea europaea	25	35.21	0.20
Prunus persica	18	25.35	0.14
Linum usitatissimum	17	23.94	0.13
Malus pumila	16	22.53	0.13
Vitis vinifera	14	19.71	0.11
Sesamum indicum	13	18.30	0.10
Allium cepa	12	16.90	0.09
Beta vulgaris	11	15.49	0.08

tuber, and Spina were used in a low proportion (3.45% each one) (Fig. 4). Moreover, in this study, products of plant origin, for example, olive oil, vinegar, tahini, linseed oil, and molasses were utilized alone or in combination with other substances for the preparation of remedies. We noted that preparations are administered in two ways, being internal administration and external administration, in order to treat dermatological diseases. The drugs are usually applied externally rather than internally on the area affected. External use was more common when compared to internal use (96% *versus* 4%) (Table 1).

It was identified that local people made medicinal preparations from plants for treating purposes by employing simple methods. The most popular methods of application are making a direct application (35.5%), poultice (16.1%), powdering (12.9%), and infusion (9.7%). The preparation of plants by crushing and distilled tar (6.45%) was reported on only two occasions, while burning to ash, pounding, smoking, and puncture (3.22%) were the least used preparation methods (Fig. 5).

The FIC was determined for all disease categories, and it varied between 0.17 and 0.90. Table 2 shows disease categories with relatively higher FIC values: the highest FIC score (0.90) was identified for cracked nipples. It was determined that Pine tar and *Cydonia oblonga* Mill. were used for the above-mentioned purpose. The second highest FIC value (0.87) was identified for ringworm, which was followed by open skin wounds with the FIC of 0.86, and interdigital dermatitis with the FIC value of 0.85. The sunstroke and sunburn were ranked to be the fifth ailment with the FIC value of 0.81. Mange had the FIC value of 0.80. Dermatitis madidans was ranked as the seventh with the FIC value of 0.66. Papillomatosis was determined to have the lowest FIC value of 0.17. Upon examining this study, it is observed that the FIC values are high, i.e., the FIC value is close to 1. The higher FIC values are determined for the medicinal plants that are considered to have an effect on the treatment of a particular disease. No study, in which the calculation of the FIC values has been performed, has been carried out by people from our region.

A number of medicinal plants were very popular and utilized intensively in the present research area. In accordance with the calculation performed on the basis of the UV, it was determined that *Pinus nigra* (0.43), *Allium sativum* L. (0.28), *Rhus coriaria* L. (0.20), *Olea europaea* L. (0.14), and *Prunus persica* (L.) Batsch. (0.10) had the highest UVs (Table 1). The high UV of plant species indicates that the plants in question represent the most recommended, utilized, and known by the respondents, which means the importance of these plants.

It was identified in the present study that the plants cited most frequently had minimum 10 or more citations (Table 3). The RFC was determined for the plant cited most frequently, and it ranged from 0.08 to 0.43 (Table 3). *Pinus nigra* (0.43), *Allium sativum* (0.39), and *Rhus coriaria* (0.35) were determined to be the plant species that had the highest RFC.

The FL was found for the plants cited most frequently (26 plants), and the plant species with the FL between 50 and 100 (three plants) were regarded as important and significant (Table 4). Two plants (*Pinus nigra* and *Malus pumila* Mill.) had the highest FL of 100%, and they were used to treat dermatitis madidans. *Euphorbia macroclada* Boiss. had the FL value of 57%, and it was followed by *Olea europaea* with the FL value of 47%. The high fidelity value of medicinal plants proved the fact

Table 4. FL values for the most cited plants.

Dermatological diseases	Plant species	N_p	N	FL (%)
Dermatitis madidans	*Pinus nigra*	7	7	100.0
	Malus pumila	7	7	100.0
Open skin wounds	*Prunus persica*	18	73	24.65
Sunstroke and sunburn	*Linum usitatissimum*	6	23	26.08
	Olea europaea	6	23	26.08
Cracked nipples	*Pinus nigra*	8	12	66.66
	Cydonia oblonga	4	12	33.33
Papillomatosis	*Euphorbia macroclada*	4	7	57.14
Ringworm	*Allium sativum*	19	48	39.58
Inderdigital dermatitis	*Pinus nigra*	12	14	85.71
Mange	*Olea europaea*	20	42	47.61

that the plants in question were preferred more by respondents when compared to other plants in the same category, and it also confirmed their frequent usage by the respondents.

The collection of the resources is mostly performed from their natural habitat due to the fact that most of them are autochthonous plants in the region (presented in Table 1 with the voucher numbers). The collection of such species as *Plantago lanceolata* L., *Convolvulus arvensis* L., *Verbascum cheiranthifolium* Boiss., *Rhus coriaria*, and *Euphorbia macroclada* that are found in natural habitat. Such plant species as *Beta vulgaris* L., *Allium cepa* L., and *Malus pumila* are grown, while *Allium sativum*, *Olea europaea*, *Prunus persica*, *Ceratonia siliqua* L., *Ficus carica* L., *Sesamum indicum* L., *Lawsonia inermis* L., and *Nicotiana tabacum* L. are obtained from local markets.

Discussion

Despite the fact that the present study does not focus on bioactive compounds, it contains effective compounds and phytochemical references [28–65] on a number of the plants that are listed in the current paper (as shown in Table 1), in particular those with higher consensus of use or with a greater number of veterinary uses.

On the other hand, plant species, which previously had been shown to have very good wound healing, antimicrobial, antiparasitic, and antifungal properties in laboratory studies were mentioned by the interviewees. For example, *Pinus nigra* (tar, resin, and gum) exhibited strong antimicrobial [55], wound healing [56], and antiparasitic [57] activity properties. *Allium sativum* was found to have antibacterial [31] and antiparasitic [32–34] effects. *Rhus coriaria* was found to have potent antibacterial [35] and antioxidant properties [36]. *Olea europaea* was determined to have antimicrobial effects [51,52]. *Prunus persica* extracts were analyzed in recent phytopharmacological studies and antimicrobial, antioxidant properties were found [61]. *Cladophora glomerata* L. was found to be the endemic plant used for a hemostatic effect [38] in Central Anatolia, Turkey. The traditional usage of the plants in the research area may be confirmed by the above-mentioned effects.

When comparing the findings of the present study with other ethnoveterinary studies in other areas of Turkey, *Pinus nigra* is also found to be the most relevant plant [14,15]. Pinus tar is used on animals as a treatment for mange, tick, and to cure wounds inflicted by wolves in Afyonkarahisar province, Central Western Turkey [66]. For widespread species, similar uses were found in different regions of Turkey. For example, *Allium cepa* for open skin wounds [14,15], *Ficus carica* (latex) for papillomatosis [12,14] and mange [13], *Allium sativum*, *Nicotiana tabacum*, and *Quercus pubescens* (ash) for mange are used in the EVM of the Lower Euphrates Basin [14]. Olive oil is used for ringworm, open skin diseases [13], and mange [12]; tar is used for dermatitis madidans and mange in the Aegean region [13] as well as open skin diseases in Antalya province [16].

Ethnoveterinary practices are discussed according to the reports of similar procedures in other countries. Tobacco (*Nicotiana tabacum*) is used in Central Anatolia in preparations against mange (Table 1) as it is in Israel [67]. Tobacco is usually used as a folk remedy in different regions of Africa, America, and Europa, especially as a parasiticide [68–70]. It is used to cure mange, wounds, and eyes infections in Sardinia (Italy) [1].

The use of garlic (*Allium sativum*) as an antiseptic, antifungal, and antiparasitic agent is reported here (Table 1). The above-mentioned findings are parallel to the results of the study conducted by Martínez and Luján [69], who recorded garlic as a remedy for wounds and injuries in Argentina. The garlic is most commonly known to have an antiparasitic effect [71,72], but it is reported in EVM in Italy also as a gastrointestinal agent [70]. Moreover, it is used in Ethiopia to treat evil eye, hepatitis, and blackleg [2].

An olive oil is used to treat mange in Central Anatolia, Turkey (Table 1). A similar report was

also given by Piluzza et al. [1] from the island of Sardinia. In Spain, the olive oil is employed as a detoxifying agent internally [73], and as a vulnerary, antiseptic, and cicatrizing agent [74], and to cure mastitis [75], and to treat eye infections in Israel [67] as in Greece [76].

Infection of wolf bite and open skin wounds in cows and sheep are prevented with the poultice of *Allium cepa* in Central Anatolia (Table 1). *Allium cepa* is used orally once a day to treat worms in the Indian EVM [72]. In Spain, it is used to facilitate delivery [73]. Furthermore, the bulb of *Allium cepa* is used as an anti-inflammatory agent in the Iberian Peninsula [74]. Papillomatosis is treated with *Juniperus oxycedrus*, *Malus pumila*, *Euphorbia macroclada*, the latex of *Ficus carica*, and *Ceratonia siliqua* in Central Anatolia (Table 1). Furthermore, the warty area is eliminated with the thorns of field *Astragalus* L. Similar use is described for it in Israel [67].

The usage of fig tree (*Ficus carica*) as an antifungal agent reported here (Table 1) was described before in Italy for the treatment of warts in humans [77]. Furthermore, it is used against scabies in Israel [67] and insect bites in West Bank of Palestine [78]. Also, it is utilized as an antitussive agent in Galicia, Spain [71].

For healing purposes against sunburn, the fresh leaves of *Brassica oleracea* L. are wrapped on the cow skin in Central Anatolia. The juice of the young leaves of *Brassica oleracea* is used against gastric ulcer [71]. The decoction of the seeds or the leaves was considered to act as a vermifuge agent in Italy [77].

According to the finding of the present study, the poultice of *Plantago lanceolata* cures skin wounds (Table 1). *Plantago* spp. has also been frequently reported in the European EVM as a vulnerary drug [70]. *Plantago lanceolata* leaves are used as an antiseptic post-labor drug in cows [74]. It is used for the treatment of diarrhea in British Columbia, Canada [79], and in the Iberian Peninsula [74].

The leaves of *Lawsonia inermis* are used to treat the ringworm of cattle in Central Anatolia (Table 1), but according to Upadhyay et al. [24], the leaves of the plant in question are given to cattle for the purpose of curing body heat in India. *Lawsonia inermis* has also been used for the treatment of trauma and ulcers in West Bank of Palestine [78]. On the national and international scale, we have noted the antifungal effect of *Lawsonia inermis* leaves as a new usage identified for the first time in Turkey. *Pinus nigra* (tar, resin, and gum) and *Juniperus oxicedrus* (juniper tar) are very common plants in Turkey. These plants were employed in the study area as an antiseptic, a parasiticide, and for wound healing remedies (Table 1) in domestic animals. Pine tar is employed for the purpose of healing wounds and keeping flies out [79]. *Juniperus oxicedrus* is used as the most promising agent against ectoparasites in the European EVM [70]. It is used in Italy as an antiseptic, a parasiticide and a purgative (Spain) remedy in animals [73,80].

The seeds of *Linum usitatissimum* L. (linseed oil) were used to treat mange and ringworm, to heal sunburn, or to prevent sunstroke in the study area (Table 1). The use of linseed oil against mange and ringworm was not cited in other countries, even though it is used against otitis in humans in Italy [81]. Furthermore, it was indicated for the first time that *Cladophora glomerata* is used as a homeostatic agent in comparison with the results of various international studies.

This survey indicated that the seed of *Sesamum indicum* (tahini) was used to treat ringworm (Table 1), although it is employed for the retention of the placenta in the Indian EVM [72]. Moreover, sesame tahini and carob (*Ceratonia siliqua*) syrup mixed and rubbed on affected skin areas are used for wound healing in EVM in Israel [67].

While the crushed seeds of *Cydonia oblonga* are used to heal cracked nipples in Central Anatolia (Table 1), *Cydonia oblonga* leaves are used to treat diarrhea in EVM in Turgutlu/Manisa [82] and ethnomedicine in Balıkesir/Turkey [83]. The bark of *Quercus pubescens* has been employed for the same purpose in the Iberian Peninsula [74].

Prunus persica has been used in Pakistan as a parasiticide remedy in ruminants [84], *Vitis vinifera* L. (grape syrup) has been used to cure belly-ache in Israel [67], and these are not cited as wound healing elsewhere. *Verbascum cheiranthifolium* has been used for wound healing in the study area (Table 1). Different species of genus Verbascum have traditionally been used in the treatment of diseases of domestic animals and human in Spain, Italy, and Ethiopia [3,73,74,80]. However, among them, only the use of *Verbascum sinaiticum* as a treatment of wound healing is similar to our records in Central Anatolia.

It was indicated in the literature that many plant species are utilized in human medicine and veterinary medicine. However, for two species, *Astragalus* L. (Spina) and *Convolvulus arvensis*, bibliographic references on their usage in veterinary and human medicine have not been found in the scientific

studies. Furthermore, the high coincidence was identified between the plant species employed in human and veterinary medicine since humans utilize particular plants to treat both themselves and their domestic animals, which play a significant role in their daily life. Many plant species reported (22) are medicinal plants used in human medicine.

According to Pirbalouti et al. [85], about one-third of all traditional medicinal plants are employed for curing skin disorders and wounds. The comparison of our results with other published sources from the distinct regions of Turkey and all over the world has indicated that new usages for therapeutic purposes have been revealed in this study and same or similar plants are often utilized for curing dermatological diseases.

Conclusion

This survey demonstrates that EVM is still widespread in certain societies, and especially in rural areas. Thus, there should be prevention for the destruction of EVM in terms of keeping the cultural tradition, as well as conserving the data on beneficial plant species.

We believe that the present study will stimulate further ethnoveterinary research, particularly in the other regions of Turkey, and clinical studies on treating livestock by using promising plants, for the purpose of re-establishing veterinary phytotherapy as an integral element of sustainable treating of the health issues of livestock reared under organic and conventional conditions.

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Conflict of Interest

The authors declare no conflict of interest.

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In vitro-scientific evaluation on anti-*Candida albicans* activity, antioxidant properties, and phytochemical constituents with the identification of antifungal active fraction from traditional medicinal plant *Couroupita guianensis* Aubl. Flower

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ABSTRACT

Background: *Couroupita guianensis* Aubl. has many therapeutic uses in the practice of traditional medicine.
Objective: The current research was conducted to evaluate the anti-*Candida albicans* activity, antioxidant properties, and phytochemical constituents with the identification of antifungal active fraction from *C. guianensis* flower.
Methods: Anticandidal, antioxidant activities, and determination of total phenolic contents (TPCs) of *C. guianensis* flower extract were carried out. Identification of antifungal active fraction was done by using solvent partitioning technique.
Results: The extract inhibited *C. albicans* with a minimum inhibitory concentration value of 12.5 mg/ml. Time-kill assay suggested that *C. guianensis* flower extract had completely inhibited *C. albicans* growth and also exhibited prolonged antiyeast activity. The alterations in morphology and complete collapse of the yeast cells after 36 hours of exposure to the extract were observed through microscopic observations. Ethyl acetate fraction was considered as an active fraction on the basis of zone of inhibition by solvent partitioning technique. Phytochemical analysis of the extract showed the presence of major classes of phytochemicals alkaloids, phenolic compounds such as flavonoids, tannins, steroids, glycosides, and saponin. The extract exhibits antioxidant activity with an Inhibitory Concentration (IC_{50}) value of 93.2 ± 0.011 µg/ml in the 2,2-diphenyl-1-picrylhydrazyl assay and 46.48 ± 0.13 µg/ml in the Hydrogen peroxide scavenging activity (HPSA) assay with TPCs of 32.2 ± 0.22 mg of gallic acid equivalents/100 g of extract.
Conclusions: The extract of *C. guianensis* flower with good anticandidal and antioxidant activities could be an effective agent to treat the *Candida albicans* infection.

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Antiyeast; *C. albicans*; *C. guianensis* flower; antioxidant; free radical

Introduction

The new-fangled drug resistance to human pathogenic fungus is repetitively being reported from all over the world [1]. Nonetheless, this situation is a threat in developing as well as developed countries [2]. Resistance to the antifungal drug has taken to undesirable implication for mortality, morbidity, and healthcare in the community. In Malaysia, HIV/AIDS cases have been reported since 1986 by the Ministry of Health [3]. Since then, the number of patient with HIV positive has been increasing. In such condition, where patients' immune system

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is compromised, infections that are opportunistic such as cryptococcosis, penicilliosis, and candidiasis are common [4]. Indirectly, fungal infection holds a critical problem to health and major root for mortality worldwide. Despite the increase in the spectrum of antifungal agents, the antifungal regimen has fallen far behind bacterial chemotherapy [5]. For instance, Amphotericin B is a macrocyclic type compound and used as "gold standard" for being less toxic. It was made available in the early 60's and prescribed up until now. Also, griseofulvin, terbinafine, and itraconazole are considered as the drug of choice for fungal infections. Unfortunately, these drugs have been withdrawn from the market as it has been replaced by new antifungal drugs [6].

Regardless of dedication to the development of new therapeutic strategies, there are only a limited number of available drugs to fight against fungal infections. Indeed, only four molecular classes that target three distinct fungal metabolic pathways are currently used in clinical practice to treat essentially fungal infections: fluoropyrimidine analogs, polyenes, azoles, and echinocandins. Therefore, a search for novel antifungal drugs selectively acting on new targets with fewer or no side effects is extremely necessary. Against this backdrop, researchers are forced to identify and explore a non-chemical, non-classical approach which is plant-based therapeutic agents that in fact are cheaper, safer, and effective antifungal drugs through systematic research are blatant [7]. Medicinal plants rich in natural sources have been used to treat mankind for various diseases since antiquity. The utilization of crude extracts of plant parts and phytochemicals for treating diseases is as old as the human species. Generally, plants produce secondary metabolite which exhibits antibacterial, antifungal, and insecticidal with minimal environmental impact and not toxic to human cells in contrast to the synthetic antifungal agent. This urged the evaluation of medicinal plants as a source of potential antifungal agent based on their usage as therapeutic agents such as *Couroupita guianensis* Aubl. flower extract.

Couroupita guianensis tree is native to the tropical north-eastern South America, especially the Amazon rainforest. The flowers are pinkish red with a yellowish tinge on the outside, heavenly scented. These are 3" to 5" waxy flowers growing directly on the bark of the trunk [8]. *Couroupita guianensis* has been referred by traditional healers to possess multifarious role, given the fact that all the parts can be utilized for medicinal application. Traditionally, the soft mass of the fruits is rubbed on the infected skin as antiseptics and to ease a toothache [9]. The juices of leaves are used to cure odontalgia, skin ailments, and shamans. Besides the leaves is also used to treat protozoal infections, stomach ache, and enteral gas formation, as antithrombotic, vitalize hair and has vasodilator properties [10,11]. Flowers are solely employed to cure scorpion poison, cold, intestinal gas formation, and stomach ache. The infusion of leaves and flower units are used for cold, abdomen ache, and pain associated with the inflammatory process [10].Due to the emergence of antifungal drug resistance, lack of curative effect, high cost, and toxicity, a new prototype antifungal agent with antioxidant properties is needed to address this situation [12]. Hence, the present investigation was conducted to demonstrate the anti-*Candida albicans* activity, antioxidant properties, and phytochemical constituents of traditional medicinal plant *C. guianensis.*

Materials and Methods

Plant collection and extract preparation

The flowers of *C. guianensis* were collected from Universiti Sains Malaysia and authenticated at the Herbarium of the School of Biological Sciences, Universiti Sains Malaysia, Pulau Pinang, Malaysia, where a sample was deposited (Voucher specimen: USM/HERBARIUM/11577). The flower sample was rinsed thoroughly —two to three times with running tap water, chopped, and shade dried at room temperature for 2 weeks and then homogenized to fine powder using a conventional blender for ease of extraction of active compounds. A sample of 100 g of plant powder was soaked in 500 ml (1:5) of 80% methanol at retention time (RT) (23°C ± 2°C) for 7 days. The filtrate from each extraction was concentrated under vacuum on a rotary evaporator (Buchi, Switzerland) at 40°C and the concentrated extract was finally poured into Petri dishes and brought to dryness at 40°C in an oven. The resultant extract paste is stored at RT in dark.

Preliminary phytochemical screening

Phytochemical assays were carried out on 80% methanol on the *C. guianensis* flower extract using standard procedures to determine the presence of flavonoids, saponins, steroids tannins, and anthraquinone glycoside [13–15]. One mg/ml of flower extract stock solution was prepared. The test was based on the visual observation of a change in color or formation of precipitate after the addition of specific reagents.

Test for tannins

Three milliliter of *C. guianensis* flower extract was treated with —two to three drops of 1% lead acetate and observed for yellow precipitate formation.

Test for saponins

To 6 ml of water was added 2 ml of *C. guianensis* flower extract and shaken vigorously. Formation of foam layer up to 1 cm showed the presence of saponins.

Test for steroids

One milliliter of *C. guianensis* flower extract was dissolved in 10 ml chloroform. An equal volume of concentrated sulphuric acid was added from the wall of test tube. The upper layer turns into red and the sulphuric acid layer shows a yellow with green fluorescence which indicates the presence of steroids.

Test for flavonoids

In 1 ml of *C. guianensis* flower extract, a drop of diluted sodium hydroxide was too added. Formation of yellow color appeared which will turn colorless upon addition of few drops of dilute acid which indicates the presence of flavonoids.

Test for alkaloids

To 2–3 ml of *C. guianensis* flower extract, Mayer's reagent was added. Formation of yellow precipitate indicated the presence of alkaloids.

Test for terpenoids

To 0.2 g of the *C. guianensis* flower extract, 2 ml of chloroform ($CHCl_3$) and concentrated H_2SO_4 (3 ml) were carefully added to form a layer. A reddish-brown coloration of the interface was formed to indicate positive results for the presence of terpenoids.

Test for anthraquinone glycosides (Borntrager's test)

A small amount of *C. guianensis* flower extract was hydrolyzed with hydrochloric acid for few hours in a water bath. The mixture was then treated with chloroform. An equal volume of diluted ammonia solution was added. Pink color formation indicates the presence of glycosides.

Determination of total phenolic content

Preparation of standard

The total phenolic content (TPC) in *C. guianensis* flower extract was determined according to the method of Folin–Ciocalteu [16] using gallic acid as the standard. Six different concentrations of gallic acid in methanol were prepared (10, 20, 40, 80, 120, and 200 µg/ml) in triplicates from a stock solution of 1 mg/ml. In a 20 ml test tube, 1 ml of gallic acid of each concentration was added and to that 5 ml of folin reagent (10%) was added to each tube. The blue colored mixture was shaken well and left in dark at room temperature for 30 minutes. It is important to keep the solutions in dark as the reagents are very sensitive and will react with light. After 90 minutes, the absorbance of the solutions in each tube was measured at 725 nm on a spectrophotometer (Thermo Scientific Multiskan Spectrum plate reader, Lithuania). A graph of absorbance against concentration was plotted as the standard. The procedure was repeated with 1 mg/ml of plant sample.

Determination of total phenolic content in C. guianensis flower extract

Stock solution of *C. guianensis* flower extract was prepared in 1 mg/ml. Then, triplicates of the extract were prepared with 200 µl from stock solution with various concentrations (10, 20, 40, 80, 120, and 200 µg/ml). The absorbance of each concentration of the extract was recorded. The TPC of extracts was expressed as mg gallic acid equivalents (GAE) per gram of sample. The TPC in all samples was calculated using the formula:

$C = cV/m$

C = TPC mg GAE/g

C = concentration of gallic acid obtained from calibration curve in mg/ml

V = volume of extract in ml

Candida albicans

Yeast isolate used in this study was *C. albicans* obtained from the Microbiology Department of Universiti Sains Malaysia Hospital, Kelantan. This yeast strain was isolated from a patient. The yeast strain was stored in 50% glycerol stock at −80°C to maintain their long-term viability. For all the experiments, the yeast strain was subcultured for single colonies on Sabouraud Dextrose Agar (SDA) and incubated at 37°C for 18 hours in an incubator (Loading Modell 100-800, Memmert, Schwabach, Germany).

Candida albicans *inoculum preparation*

Inoculum size is very important and has to be standardized at a certain value to obtain reliable, reproducible, and significant results. Therefore, inoculum size was standardized throughout this

study. A loop (25 µl) of yeast was aseptically obtained from a pure single colony from SDA and was suspended in 10 ml of Sabouraud dextrose broth (HiMedia, Mumbai, India). Sufficient inoculums were added until the turbidity was equivalent to 0.5 McFarland (10^6 CFU/ml) standard (bio-Meriuex, Marcy Petoile, France).

Disk diffusion method

Anticandidal activity was determined by a modification of the disk diffusion method by Harris and Coote [17]. Paper disk (Advantec 90 mm, Toyo Roshi Kaisha, Ltd., Japan) with a diameter of 6 mm was sterilized by autoclaving at 121°C for 15 minutes and kept at room temperature until used. A 100 µl of mid-exponential phase yeast with the turbidity of 0.5 McFarland standard was spread onto SDA and left to dry for 1 hour at room temperature. Then, the sterile disk was placed on the surface of the plates. Sterile paper disks were impregnated with 20 µl of *C. guianensis* flower extract (100 mg/ml). An 80% methanol (v/v) was used as a negative control. Miconazole nitrate (30 µg/ml) (Duchefa Biochemie, Netherlands) was used as a positive control. The plates were incubated in an incubator (Memmert) for 18 hours at 37°C. The test was conducted in triplicate. Anticandidal activity was determined by measuring the diameter of inhibition zone around the disk.

Minimal inhibitory concentration

Minimal inhibitory concentration (MIC) was determined based on the 2-fold broth dilution method. The *C. guianensis* flower extract (2,000.00 mg) was dissolved in 80% methanol (10 ml) to reconstitute an extract solution of 200.00 mg/ml as stock. Subsequently, a serial dilution technique was carried out with 2.5 ml of the stock solution being transferred to a test tube containing 2.5 ml nutrient broth medium to give a concentration of 100.00 mg/ml. Next, 2.5 ml of solution from the first test tube was transferred into another second test tube containing nutrient broth medium that gave rise to a concentration of 50 mg/ml and similarly the technique was continued until a final concentration of 0.098 mg/ml was achieved. An inoculum size of 0.5 ml yeast with the turbidity of 0.5 McFarland standard was added to each test tube by maintaining the final concentration of the extract in each test tube. After 48 hours of incubation at 37°C, the tubes were examined for yeast growth. Growth was observed in those tubes where the concentration of the extract was below the inhibitory level where the broth medium turned into turbid or looks cloudy. The MIC value of the extract was taken as the lowest concentration that showed no growth or non-turbid in the test tube [18].

Minimum fungicidal concentration

To determine the minimum fungicidal concentration (MFC) value, all the tubes used in the MIC study which did not show any visible growth of the yeast after the incubation period were diluted (1:4) in fresh Potato Dextrose Broth (PDB) before subcultured on the surface of the freshly prepared Potato Dextrose Agar (PDA) plates and incubated at 37°C for 48 hours. The MFC was recorded as the lowest concentration of the extract that did not permit any visible fungus colony growth on the appropriate agar plate after the period of incubation [19].

Time-kill study

The time killing study of *C. guianensis* flower extract against *C. albicans* was conducted with 1/2, 1, and 2 times MIC over time whereby a growth profile curve was plotted [20]. A 16-hour culture was harvested by centrifugation, washed twice with phosphate saline, and re-suspended in phosphate saline. The suspension was adjusted using the McFarland standard and was then further diluted in phosphate saline to achieve an approximation of 10^7 colonies forming unit (CFU/ml). *Couroupita guianensis* flower extract was added to aliquots of 25 ml PDB in 50 ml Erlenmeyer flask and was placed in a water bath at 37°C with amounts corresponding to the concentration of 1/2, 1, and 2 times of MIC value (12.5 mg/ml) upon the addition of the inoculums. Free medium without extract was used as a control. Next, 100 µl of *C. albicans* inoculum was added to all Erlenmeyer flasks. After the addition of the inoculums, 1 ml portion was removed from Erlenmeyer flask and the growth of *C. albicans* was monitored using this portion by measuring the optical density by using UV/spectrophotometer at 540 nm (UV-9100, Ruili Co, China). The growth of *C. albicans* was measured at every 4 hours throughout 48 hours by the above method. After that, a graph was plotted to determine the effect of *C. guianensis* flower extract on the growth profile of *C. albicans*.

Morphological changes of C. albicans after treatment with C. guianensis flower extract

The morphological change of *C. albicans* treated with *C. guianensis* flower extract was observed with a scanning electron microscope (SEM).

Preparation of the antifungal agent from plant extract

MIC value (12.5 mg/ml) was used as the concentration of treatments for *C. guianensis*. The fungal sample was harvested for electron microscopic observation. For treatment purpose, the *C. albicans* was inoculated in 10 ml PDB and then incubated at 25°C for 2 days. The final density of the fungal suspension was adjusted with phosphate saline to achieve approximately 1×10^8 CFU/ml, inoculated on PDA plate and incubated at 37°C for 6 hours. Two ml of *C. guianensis* flower extract at the concentration of MIC was then dropped on the inoculated agar and was further incubated for 48 hours at the same incubation temperature. A 50% methanol treated culture was taken as a control. A small block of *C. albicans* containing agar was cut and withdrawn from the inoculated plates at 0, 12, 24, 36, and 48 hours intervals of extract treatment, after which the plates are sealed with parafilm and stored at 4°C before being processed for the SEM (FESEM LEO Supra 50VP, Carl Zeiss, and Germany) viewing [21].

Preparation of the sample for SEM viewing

A segment between 5 and 10 mm was obtained from the treated plate for SEM examination. A double-stick adhesive tab was used to place the specimen on a planchette. The subsequent process is carried out in a fume hood. The planchette was secure in a Petri dish and a vial containing 2% osmium tetroxide was placed in a deserted area of the plate. Latterly, the plate was parafilmed, and vapor fixation of the sample proceeded for 1 hour. After 1 hour, the planchette was subjected to slushy nitrogen (−210°C) and shifted to "peltier-cooled" stage of freeze dryer (Emitech K750) for 10 hours. The freeze dried sample is then coated with 5–10 nm gold prior to SEM viewing under following conditions: *L* = SE1, Working Distance (WD) = 21 mm, and Extra High Tension (EHT) = 10.0 kV to study the effect of the extract on *C. albicans* cell [21].

Solvent partitioning (Liquid–liquid extraction)

The *C. guianensis* flower extract was dissolved in 90% methanol before further partitioned in hexane: methanol: water (100:90:10 v/v/v) and yield hexane fraction. Subsequently, the aqueous layer formed was further partitioned into ethyl acetate (100 ml) to yield ethyl acetate fraction. Consequently, the aqueous layer formed was further partitioned with butanol (100 ml) and yielded butanol fractionation. The remaining aqueous layer was collected as water fraction. The entire fraction was evaporated to dryness in the rotary evaporator. Each fraction was tested for their antifungal activity using the disk diffusion assay. The most active fractions were further analyzed using gas chromatography/mass spectrometry (GC/MS).

Gas chromatography/mass spectrometry

The GC/MS analysis of the ethyl acetate fraction from *C. guianensis* flower extract was performed using an Agilent 6890N series II gas chromatograph interfaced with an Agilent 5973 series quadrupole mass spectrometer (Palo Alto, CA) and equipped with an autosampler, Agilent 7673A. Helium gas (99.999%) was used as a carrier gas with a constant flow rate of ±1 ml/minute. Mass transfer line and injector temperature were set at 220°C and 290°C, respectively. The temperature set for oven was from 50°C to 150°C at 3°C/minute, then held isothermal for 10 minutes and finally raised to 250°C at 10°C/minute. The ethyl acetate crude fractions were diluted with methanol solvent into 10 mg/ml. The diluted samples (1 µl) were injected in the split mode with split ratio 120:1. The delay time was 2 minutes and the total running time was 120 minutes. The relative percentage of the chemical ingredients in ethyl acetate fractions from *C. guianensis* flower extract was expressed as percentage by peak area normalization. The relative percentage amount of each component was deliberated by comparing its average peak area to the total area. Software used to handle mass spectra and chromatograms was a GC-MS solution version 2.53.

***Antioxidant assays*2,2-diphenyl-1-picrylhydrazyl free radical scavenging assay**

The *in vitro* determination of antioxidant activity was done according to the method described by Hatano et al. [22]. Five mg/ml stock solution of *C. guianensis* flower extract was prepared, and distributed into six different concentrations (10, 20, 40, 80, 120, and 200 µg/ml) in triplicates, by adding up the volume in each tube to 300 µl with distilled water. A control tube was prepared with 300 µl of distilled water. 2,2-diphenyl-1-picrylhydrazyl (DPPH) (0.1 mM) solution was prepared by dissolving 3.9 mg of DPPH in 100 ml methanol and stirred overnight at 4°C. To each 0.5 ml extract solution, 2.5 ml of 76 0.1 mM DPPH solution was added, which was prepared freshly. This sample was vigorously shaken using vortex machine and left in dark for 60 minutes at room temperature. It is important to note that DPPH is reactive with light and may affect the readings of absorbance. The reduction of the DPPH radical was determined by measuring the absorption at 517 nm using a spectrophotometer.

The radical scavenging activity (RSA) was calculated as a percentage of DPPH discoloration using the equation:

$$\%RSA = [(A\ control - A\ sample)/A\ control] \times 100\%$$

Where A control is the absorbance of the solution without the extract and A sample is the absorbance of the solution containing extract with different concentration. Ascorbic acid was used as standard and a triplicate was performed.

Hydrogen peroxide assay

Hydrogen peroxide scavenging activity of the *C. guianensis* flower extract was determined by using the method described by Jayaprakash et al. [23]. A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (pH 7.4). Various concentrations of flower extract (10–200 µg/ml) in methanol (1 ml) were added to 2 ml of hydrogen peroxide solution. The absorbance of H_2O_2 was determined after 10 minutes, measured at 230 nm against a blank solution that contained extracts in phosphate buffer without H_2O_2. The experiment was carried out in triplicate and the percentage of H_2O_2 scavenging of the flower extract was calculated using the equation:

$$\%scavenged\ (H_2O_2) = [(Abs\ control - Abs\ sample)/Abs\ control] \times 100$$

Statistical analysis

Experimental results are expressed as means ± standard deviation. All measurements were replicated three times. The data were analyzed by an analysis of variance and *t*-test. *P* values less than 5% were considered statistically significant ($p < 0.05$). The IC_{50} values were calculated from the linear regression analysis.

Results

Preliminary phytochemical screening

The phytochemical analyses of *C. guianenesis* flower extract by the qualitative method are presented in Table 1. The presence or absence of the phytochemical was indicated with a positive and negative sign. The intensity of the present compounds was designated as "+," "++," and "+++." The results showed that the *C. guianenesis* flower extract contains a wide range of phytoconstituents including alkaloids, flavonoids, saponins, steroids, tannins, anthraquinone glycoside, and terpenoids. The *C. guianensis* flower extract was rich in tannins, flavonoids, and terpenoids and was indicated with "+++" sign. Moderate amount of alkaloids and saponins were found to be the constituent of *C. guianensis* flower extract which was represented with "++" sign in Table 1. As for anthraquinone glycoside and steroids, insignificant amount was present and which was represented with "+" sign.

Table 1. Phytochemical screening of *Couroupita guianensis* flower extract.

Chemical constituents	Results
Alkaloids	++
Flavonoids	+++
Saponins	++
Steroids	+
Tannins	+++
Anthraquinone glycoside	+
Terpenoids	+++

(+): weak positive test (if the reagent has slight color opacity).
(++): positive test (if the reagent produces observable color intensity).
(+++): test strongly positive (if the reagent produces heavy color intensity).

Total phenolic content

The TPC in the *C. guaianensis* flower extract was measured using the Folin–Ciocalteu's reagent and the result was expressed in terms of gallic acid equivalent. Gallic acid was used as a standard and the calibration curve was prepared with the range of concentration from 10 to 200 µg/l. The standard curve equation obtained was $y = 0.0052x$, $R^2 = 0.9993$ (Fig. 1). The absorbance value obtained for *C. guianensis* flower extract at the concentration of 100 µg/ml was substituted in the standard curve equation. The TPC of *C. guianensis* flower extract was found to be 32.2 ± 0.22 mg of GAE/100 g of extract.

Anticandidal activity

Couroupita guianensis flower extract showed a good inhibitory activity against *C. albicans* at 100 mg/ml. Therefore, the MIC value was determined in this study with a maximum concentration of 100 mg/ml.

Minimum inhibitory concentration and Minimum fungicidal concentration

The MIC value for *C. guianensis* flower extract was depicted in Figure 2. The MIC values confirmed the existence of inhibitory effects on *C. albicans* tested in the study, with MIC value of 12.5 mg/ml for the *C. guianensis* flower extract. There is no visible growth of *C. albicans* observed in test tube with the *C. guianensis* flower extract with the concentration of 100.0, 500.0, 25.0, and 12.5 mg/ml and therefore, the MIC value was determined as 12.5 mg/ml concentration indicating the lowest concentration which inhibits the growth of *C. albicans*.

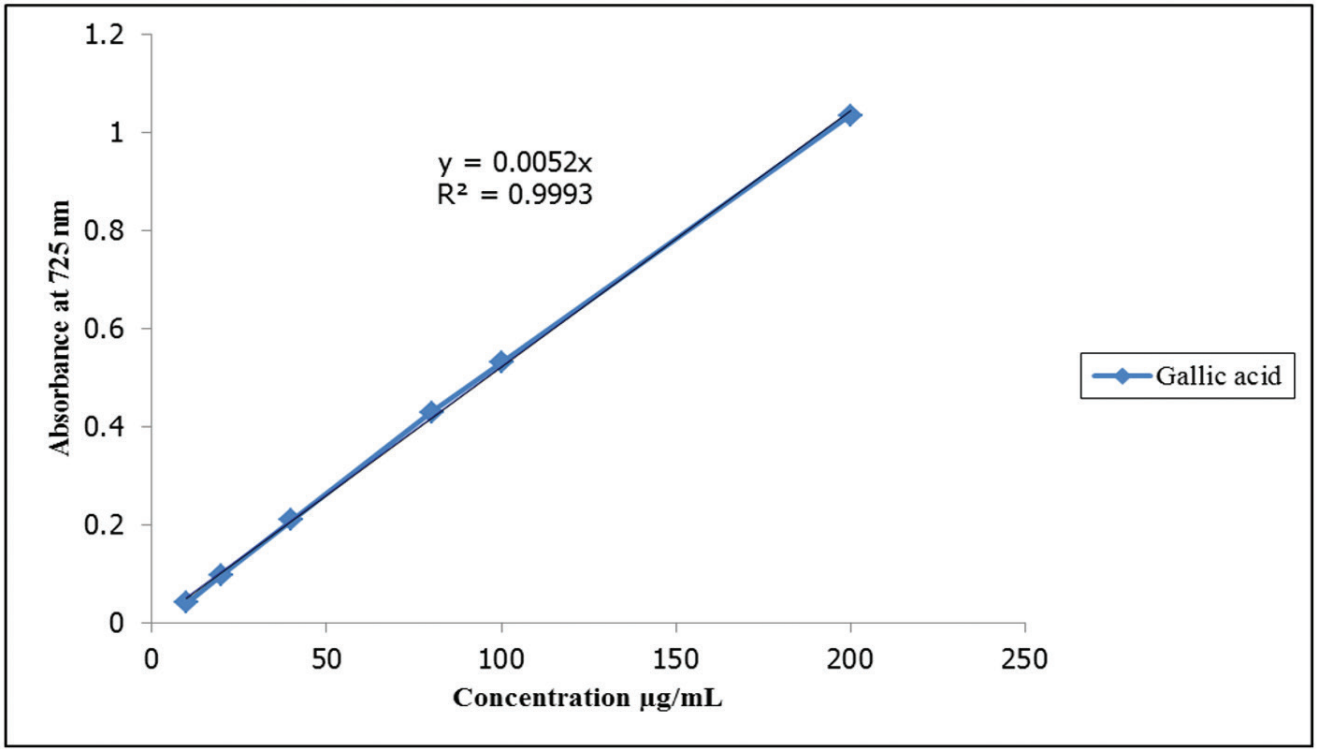

Figure 1. Standard calibration curve (gallic acid) for the quantification of total phenolic content in *Couroupita guianensis* flower extract.

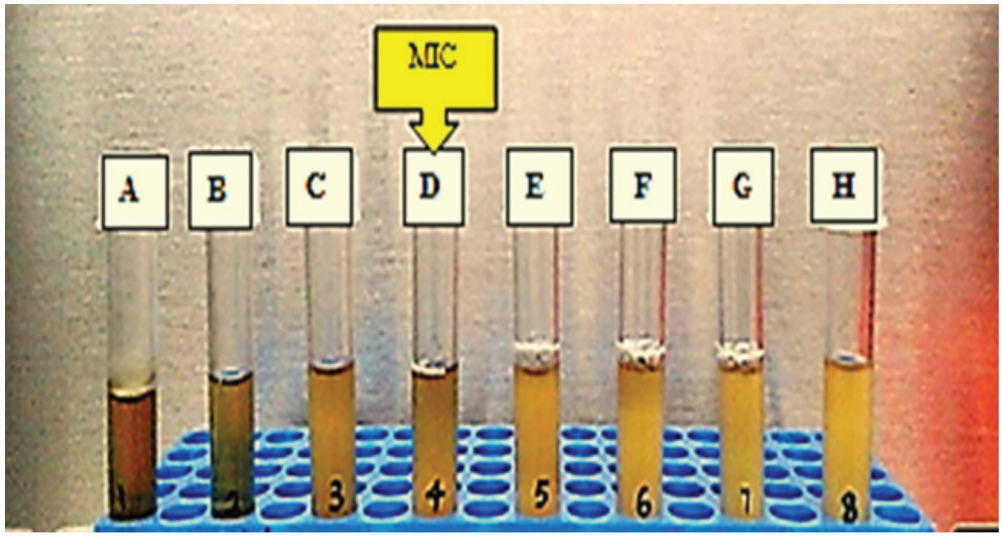

Figure 2. Minimum Inhibitory Concentrations (MIC) in mg/ml of the *Couroupita guianensis* flower extract against *Candida albicans* by broth dilution method. (A) 100 mg/mL, (B) 50.0 mg/mL, (C) 25.0 mg/mL, (D) 12.5 mg/mL, (E) 6.25 mg/mL, (F) 3.125 mg/mL, (G) 1.56 mg/mL, (H) 0.78 mg/mL.

The minimal fungicidal concentration

The minimal fungicidal effect of the *C. guianensis* flower extract was determined by pipetting out 0.1 ml yeast culture from the mixture obtained in the determination of MIC tubes (100, 50, 25, and 12.5 mg/ml) which did not show any growth and subcultured on to PDA agar and incubated at 37°C for 48 hours. The concentration of which there was no single colony was determined and recorded as 25 mg/ml of *C. guianensis* flower extract. It was noted that the *C. guianensis* flower extract MFC value (25 mg/ml) was 2-fold higher than MIC value (12.5 mg/ml).

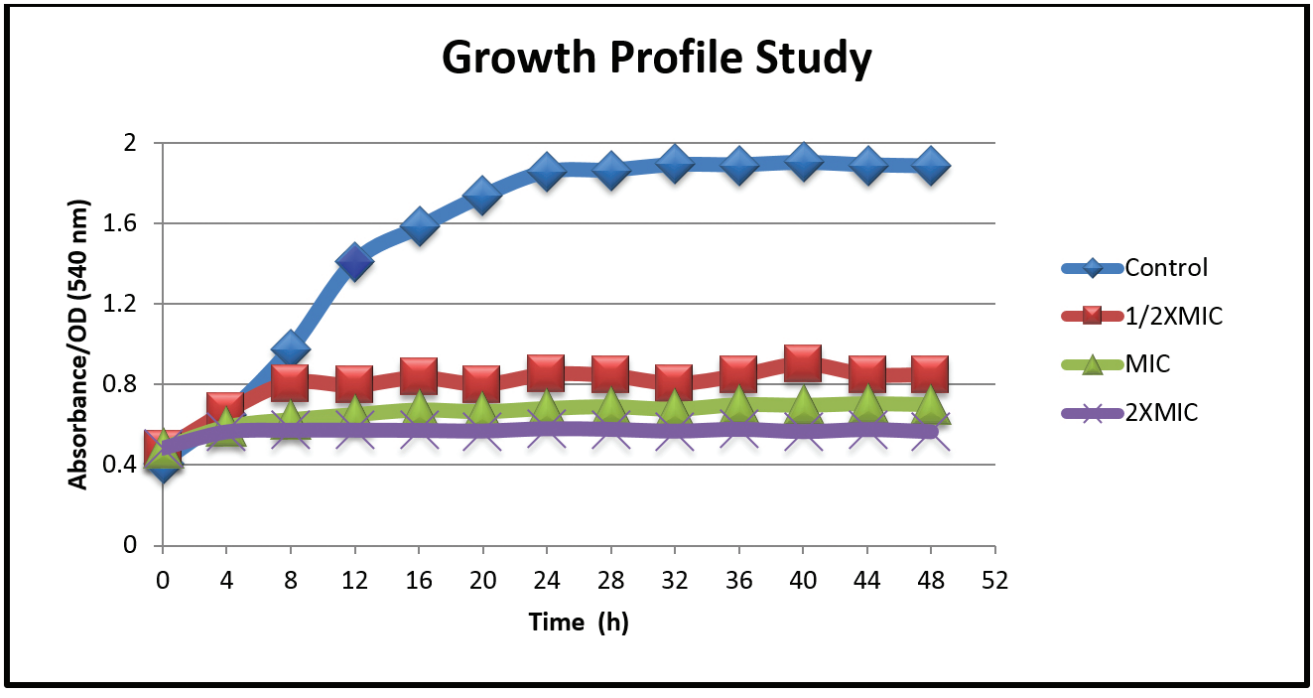

Figure 3. Growth profile for *Candida albicans* in Potato Dextrose Broth (PDB) with 0 (control), ½ MIC (6.25 mg/mL), MIC (12.5 mg/mL), and 2MIC (25.0 mg/mL) concentration of *Couroupita guianensis* flower extract.

Time-kill study

The growth profile study of *C. albicans* treated with 1/2 MIC (6.25 mg/ml), MIC (12.5 mg/ml), 2 MIC (25.0 mg/ml) concentration of *C. guianensis* flower extract, and untreated control group are shown in Figure 3. The growth profile of *C. albicans* in the presence of various MIC concentrations of *C. guianensis* flower extract was studied to evaluate the ability of the extract to eradicate *C. albicans* growth *in vitro*. In the case of 1- and 2-fold MIC concentrations, the *C. guianensis* flower extract inhibited the yeast growth within 4 hours and subsequent regrowth was not seen. However, subsequent regrowth was seen in *C. albicans* treated with 1/2 MIC concentrations of *C. guianensis* flower extract. The flower extract of *C. guianensis* exhibited a concentration and time-dependent killing profile. This observation confirmed the fungicidal effect of the *C. guianensis* flower extract on *C. albicans* at the concentration with MIC value.

Scanning electron microscope

The morphological features of photomicrographs by the untreated and *C. guianensis* flower extract treated *C. albicans* at various incubations time were shown in Figure 4. Untreated or control cells of *C. albicans* (Fig. 4A) show many regular spherical or oval in shape cells with smooth cell wall and some cells undergoing budding stage. After 12 hours of exposure to the *C. guianensis* flower extract as shown in Figure 4B; the formation of viscous material and a small number of cells presented with cavitation was witnessed. Figure 4C displays 24 hours treated cell with rough and wrinkled bodies, cells appear to be elongated and tend to form a clustered group of cells. After 36 hours of exposure (Fig. 4D), shrunken and sign of cell ruptures begins to be visible. A complete disruption of *C. albicans* cell wall with a rough, irregular, excessive shrinkage surface morphology, and vesicular formation were observed at 48 hours (Fig. 4E).

Solvent partitioning (Liquid–liquid extraction)

The crude *C. guianensis* flower extract was partitioned with various solvent systems, namely, hexane, ethyl acetate, and butanol and yielded hexane, ethyl acetate, butanol, and the aqueous fractions which were evaporated and weighed. The anticandidal activity of all the four fractions of *C. guianensis* was performed at the concentration of 50 mg/ml and the zone of inhibition was compared among all four fractions to determine the best active fraction for anticandidal activity as shown in Figure 5. Each fraction tested against *C. albicans* exhibited different diameter for the zone of inhibition against with the ethyl acetate fraction that showed a higher anticandidal activity.

GC–MS analysis

Interpretation of mass spectrum GC–MS for the ethyl acetate fraction of *C. guianensis* flower extract

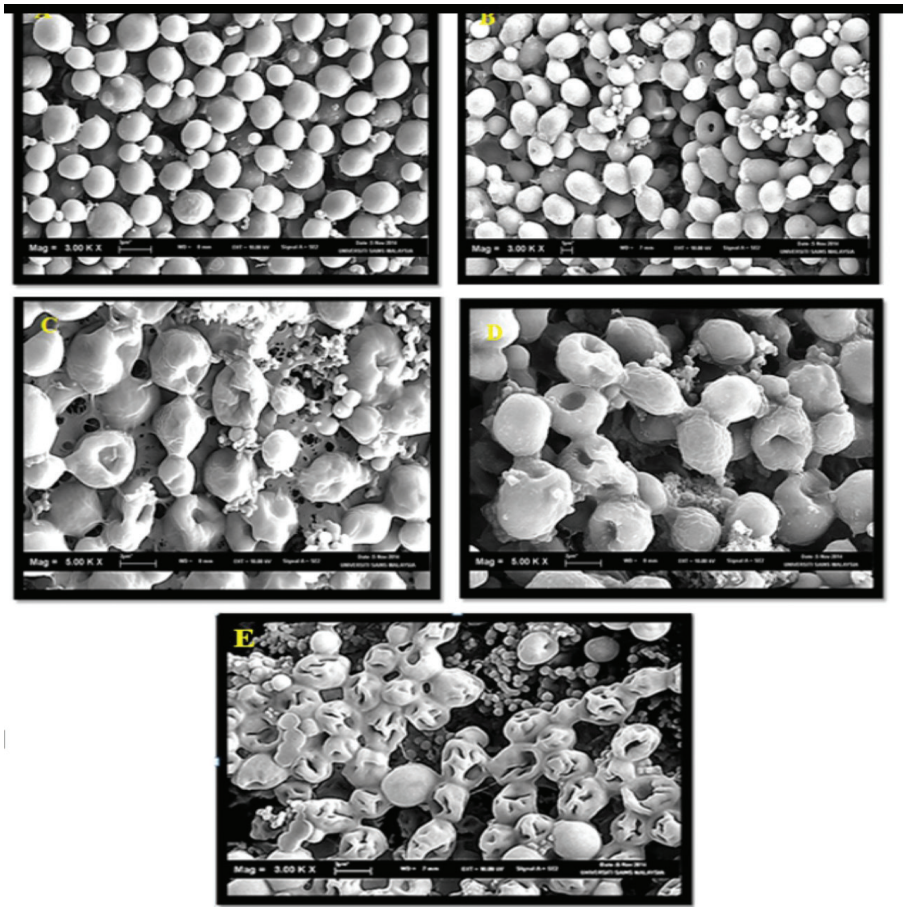

Figure 4. Scanning Electron Micrograph of the untreated and *Couroupita guianensis* flower extract treated cells of *Candida albicans*. A, Control cells of *C. albicans*. B, 12 h; C, 24 h; D, 36 h and E, 36 h of *C. albicans* cells treated with 12.5 mg/mL of *C. guianensis* flower extra.

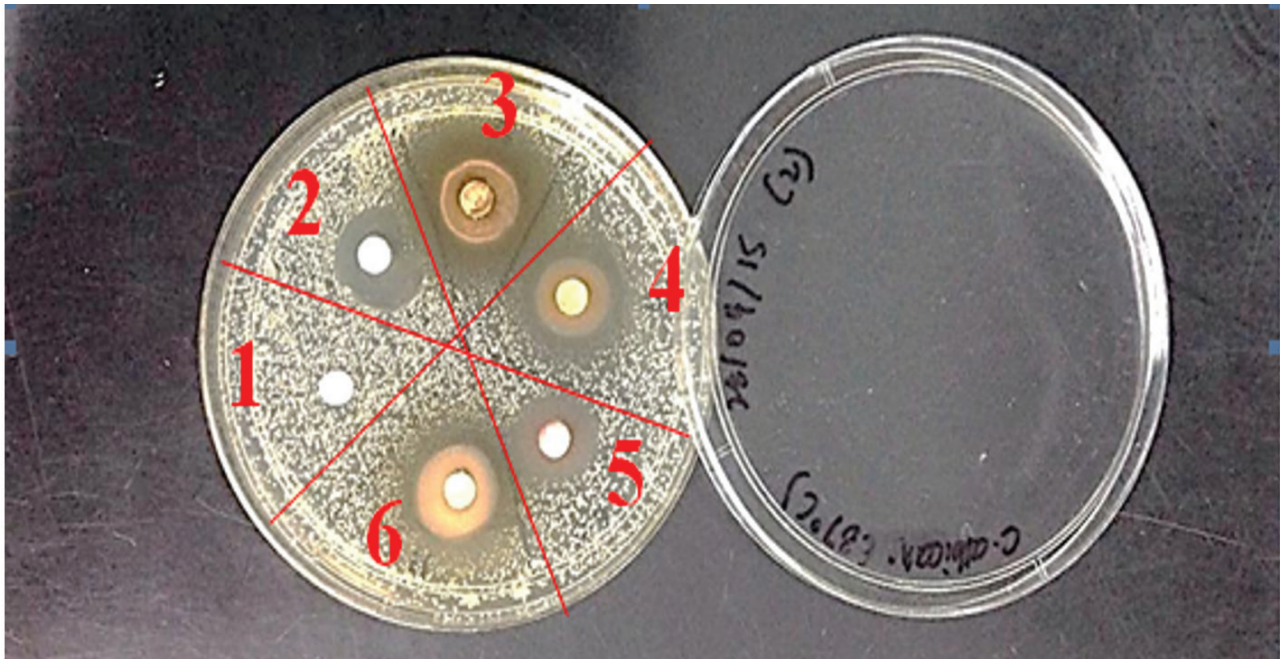

Figure 5. Antifungal activity of partition fractions of *Couroupita guianensis* flower against *Candida albicans* by disc diffusion method; (1) negative control (methanol), (2) positive control (miconazol nitrate (30 µg/mL)), (3) ethyl acetate fraction, (4) butanol fraction, (5) water fraction, (6) hexane fraction. Each fraction was tested at 50 mg/mL.

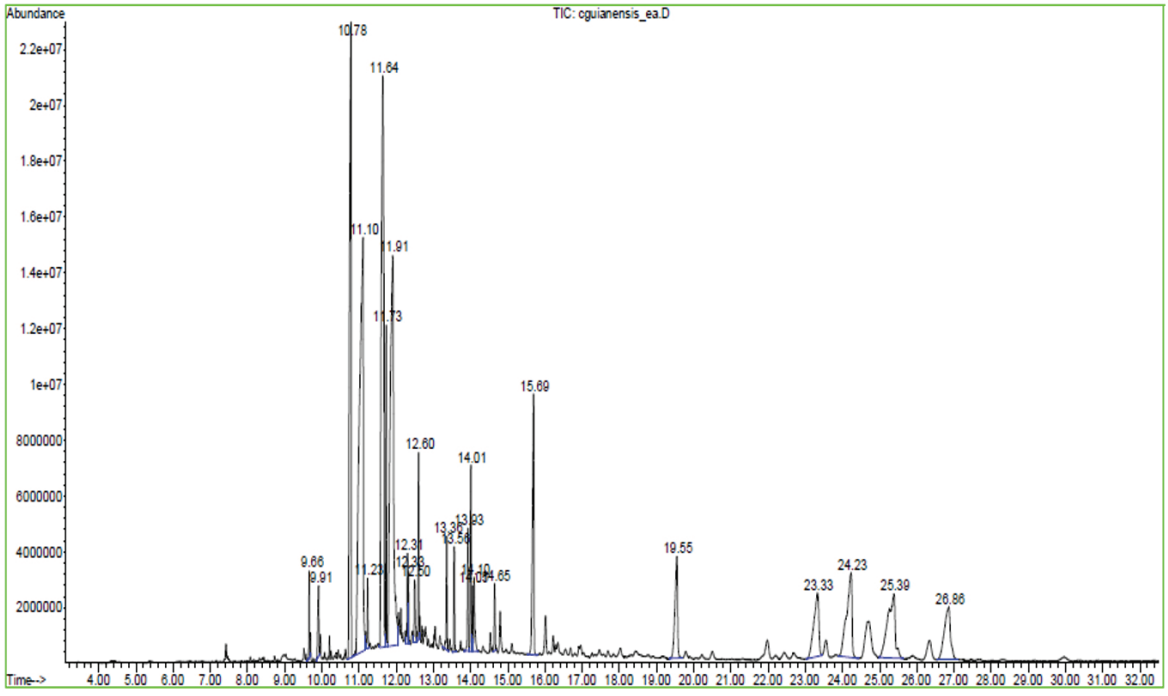

Figure 6. Gas chromatogram obtained for ethyl acetate fraction of *Couroupita guianensis* flower extract.

was conducted using the database of National Institute Standard and Technique (NIST). The spectrum of the unidentified component was compared with the spectrum of the known components stored in the NIST library. Qualitative analyses of ethyl acetate fractions of *C. guianensis* flower by using GC–MS showed the presence of 25 compounds in ethyl acetate fraction of *C. guianensis* flower extract. Figure 6 shows the gas chromatogram of ethyl acetate fraction of *C. guianensis* flower extract. The active principle, an area of the peak in concentration (%), and RT are presented in Table 2.

The identified compounds are: methyl tetradecanoate, tetradecanoic acid, pentadecanoic acid, 14-methyl-, n-hexadecanoic acid, heptadecanoic acid, 9,12-octadecadienoic acid (Z,Z)-, octadecanoic acid, 9,12-octadecadienoic acid (Z,Z)-, 9H-carbazole, 2-methyl-, benzamide, 2,3,4,5-tetrafluoro-N-(3-methylthio-1,2,4- triazol-5-yl)-, 11-eicosenoic acid, eicosanoic acid, 2,5-diphenyltetrazole, docosanoic acid, 1-propene, 3-(2-cyclopentenyl)-2-methyl-1,1-diphenyl-, (2,3-diphenylcyclopropyl)methyl phenyl sulfoxide, trans-, 1-propene, 3-(2-cyclopentenyl)-2-m ethyl-1,1-diphenyl-, methadone N-oxide, borinic acid, 2,6,10,14,18,22-tetracosahexaene,2,6,10,15,19,23-hexamethyl-, (all-E)-, vitamin E, stigmasta-7,16-dien-3-ol, (3.beta.,5. alpha.)-, beta-amyrin, alpha-amyrin, 9,19-cyclolanost-25-en-3-ol, 24-methyl-, and (3.beta.,24S)-.

Free radical scavenging ability on 2,2-diphenyl-2picrylhydrazyl

The DPPH radical scavenging ability of the *C. guianensis* flower extract was recorded in terms of % inhibition as shown in Table 3 with the gallic acid as standard reference. The inhibition rate shows the capacity of the *C. guianensis* flower extract to reduce the absorption of the DPPH free radicals. The result obtained for DPPH free RSA of *C. guianensis* flower extract was in a concentration dependent manner by which the activity or the inhibition percentage gradually increased with concentration. The IC_{50} value was calculated from linear regression analysis and the value obtained for *C. guianenesis* extract was 93.2 ± 0.011 µg/ml, and for the standard gallic acid was 32.31 ± 0.08 µg/ml. The results of this study indicate *C. guianensis* flower extract has a noticeable scavenging effect on DPPH radicals.

Hydrogen peroxide radical scavenging activity

The scavenging ability of *C. guianensis* flower extract on hydrogen peroxide is shown in Figure 7 by comparing with the gallic acid as standard. The *C. guianensis* flower extracts were capable of scavenging hydrogen peroxide in a concentration dependent manner. A linear regression curve was used to calculate IC_{50} values. The IC_{50} for *C. guianensis* flower extract for scavenging of hydrogen peroxide was 46.48 ± 0.13 µg/ml compared ($p < 0.05$) to standard

Table 2. Total ionic chromatogram of ethyl acetate fraction of *C. guianensis* flower extract with RT and peak area.

Peak	$^{a}R_t$	Area (%)	Compound
1	9.66	0.50	Methyl tetradecanoate
2	9.91	0.79	Tetradecanoic acid
3	10.78	11.95	Pentadecanoic acid, 14-methyl-, me 100727 005129-60-2 99 thyl ester
4	11.10	17.11	n-Hexadecanoic acid
5	11.24	0.50	Heptadecanoic acid
6	11.64	15.89	9,12-Octadecadienoic acid (Z,Z)-
7	11.73	2.42	Octadecanoic acid
8	11.91	15.53	9,12-Octadecadienoic acid (Z,Z)-
9	12.33	0.64	9H-Carbazole, 2-methyl-
10	12.33	0.42	Benzamide, 2,3,4,5-tetrafluoro-N-(3-methylthio-1,2,4-triazol-5-yl)-
11	12.50	0.81	11-eicosenoic acid
12	12.60	1.33	Eicosanoic acid
13	13.36	1.00	2,5-diphenyltetrazole
14	13.56	0.88	Docosanoic acid
15	13.93	1.19	1-propene, 3-(2-cyclopentenyl)-2-m ethyl-1,1-diphenyl-
16	14.01	1.88	(2,3-diphenylcyclopropyl)methyl ph enyl sulfoxide, trans-
17	14.05	0.54	1-propene, 3-(2-cyclopentenyl)-2-methyl-1,1-diphenyl-
18	14.10	0.92	Methadone N-oxide
19	14.65	0.72	Borinic acid
20	15.69	4.26	2,6,10,14,18,22-tetracosahexaene,23-hexamethyl-, (all-E)-
21	19.55	2.93	Vitamin E
22	23.33	3.55	Stigmasta-7,16-dien-3-ol, (3.beta.,5.alpha.)-
23	24.23	5.11	Beta.-amyrin
24	25.38	5.49	Alpha.-amyrin
25	26.86	3.63	9,19-cyclolanost-25-en-3-ol, 24-methyl-, (3.beta.,24S)-

gallic acid which was 33.12 ± 0.03 µg/ml The regression correlation ($R2$) was measured for *C. guianensis* flower extract and the standard gallic acid and $R2$ was found to be 0.9895 and 0.9336, respectively. It can be inferred from the findings that *C. guianensis* flower extract possesses the ability to inhibit oxidation by virtue of the presence of phenolic compounds.

Discussion

Phytochemical analysis

Phytochemical screening of *C. guianensis* flower extract revealed the presence of various bioactive compounds such as alkaloids, flavonoids, saponins, steroids, tannins, anthraquinone glycoside, and terpenoids which have been linked to antifungal activity [24]. It is therefore possible that these compounds may be responsible for the excellent antifungal properties, which was exhibited by *C. guianensis* flower extract. The phytochemical screening results showed that the tannin, flavonoids, terpenoids, moderate amount of saponins, steroids, and anthraquinone were present in the *C. guianensis* flower extract and may be responsible for the observed good anticandidal and antioxidant activities of flower extract [25–37]. Flavonoids have the ability to quench and efficiently mop up the damaging radical species [30]. The damaging effect caused by the free radicals in cells is repaired, healed, and protected by the flavonoids because they are strong antioxidant [31]. Hence, flavonoids in the *C. guianensis* flower extract may be responsible for the observed antioxidant activity in this study. The mechanism of terpenoids action as an antimicrobial agent is not fully understood but is contemplated to involve in membrane disruption of the pathogens [34]. Therefore, the disruption of *C. albicans* membrane *C. guianensis* flower extract may be associated with the existing of terpenoids in the extract. Furthermore, previous reports mentioned that saponins containing plants exhibit antifungal activity against human pathogenic yeast *C. albicans*, *Candida glabrata*, and *Candida tropicalis* [35]. Saponins appear to act by

Table 3. Evaluation of DPPH free radical scavenging activity of *Couroupita guianensis* flower extract.

Concentration (µg/ml)	*Couroupita guianensis*	Standard (gallic acid)
10	10.69953 ± 0.11*	12.46 ± 0.03
20	26.47887 ± 0.09*	24.19 ± 0.02
40	30.61033 ± 0.08*	52.64 ± 0.01
80	41.97183 ± 0.09*	97.21 ± 0.02
100	51.07981 ± 0.13*	98.24 ± 0.03
200	71.06103 ± 0.09*	98.67 ± 0.04

Results are expressed as mean ± SEM; *statistically significant compared to standard gallic acid ($P < 0.05$).

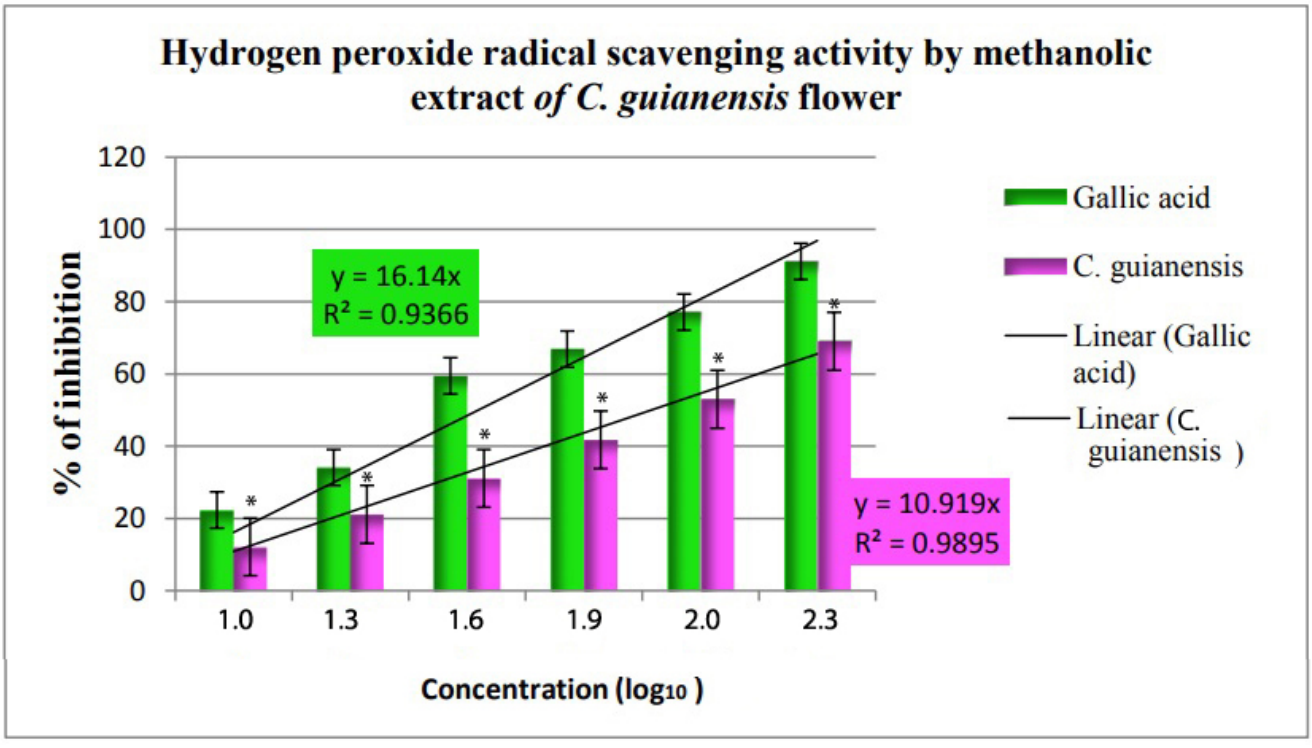

Figure 7. Scavenging effect of *Couroupita guianensis* flower extract on hydrogen peroxide compared to Gallic acid. Each value expressed as mean ± S.E.M. *statistically significant compared to standard Gallic acid ($P < 0.05$).

disrupting the membrane integrity of the cells of fungal [36]. Steroid or sterol and anthraguinone are the derivatives of terpenoids and flavonoids, respectively. These compounds indirectly attribute to the exertion of antifungal properties of *C. guainensis* flower extract as flavonoids and terpenoids possess good antifungal properties [37].

Total phenolic content

Phenols are important plant phytochemical because of their action as primary antioxidants and scavenging capability on free radicals due to the presence of hydroxyl groups. They play an important role in counteracting the free radicals, quenching singlet's and triplets oxygen [38]. Phenolic compound has a unique chemical structure responsible for free RSA. The mechanism of action of phenolic compound relies on the accepting or donating *electron*(s) to eliminate the *unpaired* condition of the free radicals. The antioxidant capacity (AOC) of a compound relies greatly on the number and location of hydroxyl groups [39]. According to Koleva et al. [40], antioxidant activity increases with TPC and there is a linear correlation between the phenolic content and antioxidant activity. In this study, the phenolic content in *C. guianensis* flower was moderately high (32.2 mg GAE/100 g of extract) and was determined to correlate between the content of phenolic compound in a plant with the antioxidant activity [41]. Most medicinal plant with potential pharmacological effect has TPC ranged from 30 to 200 mg GAE/100 g of extract [13]. The method engaged in this study to evaluate the content of phenolic is roughly proportional to the number of phenolic hydroxyl groups in a given extract, but for reducing or scavenging capacity, it is enhanced when two phenolic hydroxyl groups are oriented ortho or para. Moreover, it was reported that different phenolic compounds have different responses in this analysis [42].

Anticandidal activity

The results expressed from the disk diffusion method are qualitative data where the obtained results gave an initial idea of the anticandidal activity of *C. guianensis* flower extract. The highest concentration of 100 mg/ml was applied for the screening of the anticandidal activity in this study since the crude extract of *C. guianensis* flower was used. *C. guianensis* flower extract exhibited a favorable anti-yeast activity against *C. albicans* with a MIC and MFC value of 12.5 and 25.0 mg/ml, respectively. The MIC value is the lowest concentration that completely inhibits any visible fungal growth [43]. Meanwhile, the MFC is a determinant for inhibited growth (static) or no-growth (cidal) after incubation. The observed anticandidal activity may be attributed

to the rich plant content of active compounds which is an important source of microbicides. The MFC value (25 mg/ml) obtained in this study was 2-fold higher than the MIC value (12.5 mg/ml). This finding suggests that the *C. guianensis* flower extract was fungistatic at lower concentration and fungicidal at higher concentration. Moreover, the crude plant extract, which has fewer or no side effects with MIC values between 2.5 and 15 mg/ml, has good potential to be the candidate extract to obtain new antifungal compounds [44,45]. Therefore, the findings from this study suggest that the *C. guianensis* flower extract may be a potential lead extract for the isolation of novel anticandidal compound(s).

Growth profile study

The presence of the active component in the *C. guianensis* flower extract may act synergistically to produce good antifungal effect as observed in the growth profile study [46]. Moreover, the observed antifungal activity may also be credited with the high percentage of phenols group in the *C. guianensis* flower extract. Various reports in the literature are in agreement that the antifungal activity of a particular plant is mainly attributed to the phytochemicals such as tannins, alkaloids, terpenoids, flavonoids, and saponins [47]. The time-killing study revealed prolonged anticandidal activity when *C. albicans* was exposed to *C. guianensis* flower extract at 0.5 MIC, MIC, and 2 MIC for 48 hours. The findings of this study also clearly indicated the potential of *C. guianensis* flower extract to be developed as a therapeutic agent against *C. albicans* infection. To verify this hypothesis, *C. albicans* cells (untreated and extract treated) were observed through SEM techniques.

Scanning electron microscope

Microscopy was employed to obtain detailed information about the *in situ* ultrastructural changes of *C. albicans* caused by *C. guianensis* flower extract. The most important structure that enhances the pathogenicity of *C. albicans* is the cell wall. Adhesion of *C. albicans* to the host cell is the prerequisite for colonization and an essential step in establishing an infection. Therefore the succession of anticandidal activity of a potential medicinal plant extract is by acting on the few layers of the cell wall and penetration of the cell wall. The interaction of the bioactive compounds in the plant with the fungal cell aids the breakage of the cell wall [48]. Hence, the extracellular morphological changes of flower extract treated with *C. albicans* were observed by using SEM. The untreated cells were elongated and showed few daughter cells budding out of the parent cells. The microscopic examination of *C. albicans* using SEM showed that the cells treated with *C. guianensis* flower extract decreased in size, appeared irregular in shape with cell wall modifications, and clear depressions on the cell surface with holes. Interestingly, the exposure of *C. albicans* cells to *C. guianensis* flower extract increased the disruption of the cell wall and cell membrane structures.

About 90% of the *C. albicans* cell wall is carbohydrate [48]. There are three basic constituents that make up the cell walls polysaccharide. First is the polymers of glucose containing β-1,3 and β-1,6 linkages, second is the unbranched polymers of N-acetyl-D-glucosamine containing β-1,4 bonds (chitin), and the third one is polymers of manose protein and 1%–7% of lipids. It can be postulated that the *C. guianensis* flower extract could possibly be acting upon one or more of the cell wall constituent which results in detaching or breaking the cell wall structure and encounter the *C. albicans* infection which warrants further study. These SEM micrographs study confirmed the evidence of anticandidal potential of *C. guianensis* flower extract.

Solvent partitioning (liquid–liquid extraction) and GC/MS analysis for antifungal active fraction identification

Bioassay-guided fractionation is widely used in the isolation and identification of the bioactive compound from plant extracts [49]. Hence in this study, this method was employed to identify antifungal active fraction from *C. guianensis* flower extract. Fractionation of compounds from the crude form of medicinal plant is important in the search of bioactive principle(s) from organic fractions. This method is based on the differential solubility of compounds in the crude extract between two different solvents employed. The chloroform was initially used as solvent in the fractionation process, but due to the reported toxicity it led to discontinuing of its use and in favor, ethyl acetate was employed in this study [50]. The bioassay-guided (antifungal activity) fractionation revealed that ethyl acetate showed a higher zone of inhibition compared to butanol. To reveal the presence of the bioactive component in the antifungal ethyl acetate fraction of *C. guianensis* flower was further analyzed with GC/MS. The Benzamide, 2,3,4,5-tetraflouro-N(-3-methylthio-1,2,4-triazol-5-yl), 9,12-Octodecadienoic acid (Z,Z), Octadecadienoic acid, Pentadecanoin acid, 14-methyl-, Squalene, and Tetradecanoic acid were identified as possible antifungal agents in

C. guianensis flower extract active fraction. Therefore, it is possible that these active components in *C. guianensis* flower extract was mainly responsible for the observed anticandidal effects in this study especially against the *C. albicans* that might warrant further detail studies.

Antioxidant studies

One of the most common sources of free radicals in the human biological system is generated during infection [51] of pathogenic microorganisms. Therefore, in this study, the antioxidant activity of the *C. guianensis* flower extract was studied besides the anticandidal activity, to evaluate the ability of the flower extract to counteract the adverse effects of free radicals after the post-infections which lead to various life-threatening health problems [52,53]. Since the possible toxicity of the synthetic antioxidants such as butylated hydroxytoluene and butylated hydroxyanisole have been increasingly reported, the attention of seeking for a new source of antioxidant has been directed to natural antioxidant [54]. Consequently, in this study, the antioxidant activity of the *C. guianensis* flower extract was evaluated as a natural antioxidant. Plants such as *C. guianensis* have almost boundless ability to synthesize secondary metabolites that have been reported to possess remarkable antioxidant activities. Hence, in the current study, the phytochemical screening of *C. guianensis* flower extract is also done to determine whether the antioxidant phytochemicals are responsible for the observed anticandidal and AOC of the *C. guianensis* flower extract.

DPPH assay and hydrogen peroxide assay

AOC assays have been developed on the basis of the chemical reaction mechanisms involved. In general, single electron transfer is used to measure an antioxidant's reducing capacity, and the hydrogen atom transfer is for quantifying the hydrogen atom donating capacity. In this study, the antioxidant activity of *C. guanensis* flower extract was evaluated by two different methods based on these two mechanisms [55]. Single electron transfer-based assays quantify the capability of a compound (antioxidant) to donate an electron to reduce radically. Single electron transfer-based assays take after the redox titration in classical chemical analysis and can be defined by the following electron-transfer (redox) reaction:

$$\text{Free radicals} + e\ (\text{antioxidant}) \longrightarrow \text{reduced free radicals} + \text{oxidized antioxidant}$$

The most common single electron transfer is DPPH assay [55]. Assays based on hydrogen atom transfer measures the ability of an antioxidant to scavenge free-radicals by donating hydrogen atom. In most hydrogen atom transfer based methods, the free radicals will remove the antioxidants, which becomes radical itself. One of the important hydrogen atom transfer assays is hydrogen peroxide assay [56].

DPPH is a stable free radical and their mechanism of action is limited to extracellular compartment. The donation of electrons by antioxidant to the DPPH radicals made a resultant change from purple to yellow in the solution. As DPPH receive one electron in the presence of antioxidant or free radical scavenger, the absorption reduces and results in the decolorization. This reaction is stoichiometric with the respect to a number of electrons gained [57]. Hydrogen peroxide itself is not mostly reactive with biologically important molecules but is an intracellular precursor for formation of hydroxyl radicals which are poisonous to the cell. Hydrogen peroxide can inactivate a number of enzymes directly since it can cross the cell membrane rapidly. Once hydrogen peroxide enters the living cell, it is converted into free radical called hydroxyl radicals (• OH), reacts with biomolecules, causes tissue damage and cell death [53]. In both scavenging assays carried out in this study, the results showed a strong ability of *C. guianensis* flower extract to scavenge the DPPH and hydrogen peroxide free radicals with IC_{50} values of 93.2 ± 0.011 μg/ml and 46.48 ± 0.13 μg/ml. According to Kumaran and Karunakaran [58], a standard antioxidant such as quercetin on DPPH assay and hydrogen peroxide gives a value between 10 and 35 μg/ml.

The result of this study supports the claim that there is no correlation between the TPC and total antioxidant potential. As reported, phenolic compound attributes to great scavenging assets due to the presence of special active group known as a hydroxyl group (OH) [59]. The Folin–Ciocalteu phenol reagent used to quantify the TPC present in an extract is specific only to polyphenols containing hydroxyl as their active group, leaving behind amino substitute phenol (NH_2). This statement may explain the low correlation between total polyphenol contents and the antioxidant activity of *C. guianensis* flower extract. It is suggested that this plant may have both amino substitute phenol (NH_2) and hydroxyl substitute phenol (OH) by which a major factor for excellent scavenging activity. The phenolic compounds in the *C. guianensis* flower extract are powerful chain breaking antioxidants

because of their scavenging ability associated their active groups [60]. It is proven that the antioxidant activity is not directly dependent on absolute measurement of the phenolic content as mention in Folin–Ciocalteu, which only measures the presence of hydroxyl group but it also dependent on different structure types of phenolic compound and that has a role in antioxidant capacities [61].

Conclusion

The present study clearly demonstrated that *C. guianensis* flower extract exhibited good anticandidal and antioxidant activities. Ethyl acetate fraction from *C. guianensis* flower extract was the most effective agents for anticandidal activity. The anticandidal and antioxidant activities in *C. guianensis* flower extract may contributed by the presence of various phytochemical in the extract which was support by phytochemical analysis in this study. These findings provide promising baseline information for the potential use of *C. guianensis* flower in the treatment of oxidative damage and infections associated with the studied microorganisms.

Conflict of Interest

The authors declare no conflict of interest.

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