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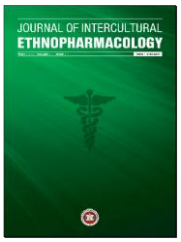

Original Research

Phytochemical and antibacterial activity of the extracts of *Fagara zanthoxyloides* on selected cariogenic and enteric bacterial isolates

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Keywords: *Fagara zanthoxyloides*; antibacterial; bacteria; chewing stick; extract.

Abstract

Background: *Fagara zanthoxyloides* belongs to the family Rutaceae. The stem and the root of the plant are both used as chewing stick in Nigeria particularly among the Yoruba ethnic group in the South-Western part of the country. This study determined the antimicrobial activity of the extracts from *F. zanthoxyloides* on selected cariogenic and enteric bacterial isolates.
Methods: Crude extracts were obtained by cold extraction method of the powdered stem in methanol-water mixture (MW) in ratio 3:2 and phosphate buffer saline (PBS). Filtrates obtained were concentrated in a rotary evaporator and lyophilized. Antimicrobial activity of the extracts, at a concentration of 25 mg/ml was tested against four bacterial isolates using agar well diffusion method. Phytochemical analysis of the plant extract for the presence of tannins, saponins, alkaloids and flavanoids was based on chemical examination.
Results and discussions: Extracts from methanol-water mixture showed some antimicrobial activity against *Lactobacillus brevis* (NCIMB 4617), *L. plantarum* (NCDO 1752) and *Escherichia coli* and *Proteus vulgaris.* The minimum inhibitory concentration (MIC) of extracts ranged between 1.57 and 12.5 mg/ml except in *E. coli* with value greater than 12.5.mg/ml. The phytochemical screening indicated that the extract tested positive for tannin, saponin, flavonoids and alkaloids. Results showed that *F. zanthoxyloides* would be valuable in the treatment of microbial diseases particularly those of the oral cavity. The considerable antibacterial activities exhibited by the extract of the plant thus justify the use as a teeth cleansing agent in the local setting. The problem of antibiotics resistance to synthetic drugs is also expected to be mitigated with the use of natural drugs.

INTRODUCTION

In many African homes, cleaning of teeth in the morning is by using the root or slim stem of certain plants until they acquire brush-like ends. The fibrous end is then used to brush the teeth thoroughly.In Nigeria and other parts of West Africa like Senegal, chewing sticks which are usually pencil-sized sticks of about 6 inches or 15mm long are used frequently during the day. The use of traditional agents in oral hygiene cuts across Africa: Sudan North Africa [1], East Africa [2], Southern Africa [3,4] (and Asia as well [5,6]. Thus the use of chewing stick is not peculiar to Africans. Almas [7] reported usage throughout the Greek, Roman, Jewish and Islamic Empires. They are used by many cultures since antiquity [8].

It is a common practice to clean the teeth first with one of these chewing sticks prior to the use of the recognized brand of toothpaste and a brush. The chewing sticks are believed to impart varying taste,

sensations and factors such as preferred taste or flavour [9]. These factors as well as therapeutic values may be put into consideration in choosing a particular kind of chewing stick. Freshly cut specimens are usually desirable because they are more easily chewed into a brush [9].

The usefulness of chewing sticks in oral hygiene maintenance has been considered comparatively effective as tooth brush [4,10]. Previous investigations have also demonstrated the antiplaque and antimicrobial action of extracts of some chewing sticks against general oral flora of the mouth, oral bacteria such as *Streptococcus mutans, Bacteroides gingivitis* and oral anaerobes commonly implicated in dental caries and orodental infections [11,12]. Chewing sticks therefore can safeguard against dental problems, which is probably the reason why dental caries (decay) is not rampant in certain part of Nigeria where the use of chewing sticks is frequent [13]. The use of chewing sticks has hence been encouraged by the World Health Organization [14, 15].

Chewing sticks in Nigeria are produced from the root, bark or stem of 24 different tree species [13], mostly tree and shrubs with only two climbers. One of these sources is a glaborous shrub or tree of up to 12m in height, *Fagara zanthoxyloides* also known as *Zanthoxylum zanthoxyloides.* It belongs to the family Rutaceae and is known by various local names in Nigeria (table 1). This plant is found in the savannah, dry forest and coastal areas of Nigeria and mostly in the southern part of the country [13].Chewing sticks are obtained by cutting either the stems or the roots into short slim pieces. A tingling peppery taste and numbness is provided by these two plant parts used as chewing sticks [13].

Table 1. Local names of Fagara zanthoxyloides by various Nigerian tribes

Nigerian Tribe	Local Name
Yoruba	Ata
Hausa	Fasakuwa
Fulani	Fasakorihi
Bini	Ughoghon
Itsekiri	Atako
Urhobo	Ujo
Ijaw	Korokumo

Source: Isawumi, 1978.

*F. zanthoxyloides*is known to be useful for various other purposes. It has been reported to possess antiplasmodial activity [16, 17]. Other ailment for which the root-bark extract is used includes: elephantiasis, toothache, sexual impotence, gonorrhoea, malaria, dysmenorrhoea and abdominal pain [18, 19]. In addition, studies have reported the anti-sickling [20, 21] and anticancer activity of extracts of *F. zanthoxyloides* [22].

Some of the interests in chewing sticks and their extracts have focused on their effects on microorganisms that are involved in oral infections [23, 24]. This comes as a result of the increasing prevalence of drug-resistant pathogens which has emerged as one of the most serious threats to the successful treatment of microbial diseases. Today, multiple drug resistant (MDR) microorganisms constitute a major public health problem [25]. This is a foremost reason why research for new antimicrobial agents especially those derived from natural herbs needs to be intensified. Besides, the call to regulate antibiotics use in order to prevent the selection for antibiotics resistant organism has been acknowledged. Hence, it is pertinent to uncover new grounds for the discovery of non-antibiotic substances with proven efficacy which, can be used in the treatment of microbial infections, including those that are encountered in dental practices. In view of the above, this study has therefore been designed to screen the extract of a selected brand of Nigerian chewing stick *F. zanthoxyloides* for its phytochemical constituents and assess the antibacterial activities of the extracts on selected typed oral micro-flora culture and enteric bacteria. The selection of the bacteria was based on their roles in oral hygiene and potential for causing dental diseases [26].

MATERIALS AND METHODS

Plant Collection and Preparation

The stems of *Fagara zanthoxyloides*used in the study were obtained commercially. The plant was identified in the Herbarium unit of Department of Botany, ObafemiAwolowo University (O.A.U), Ile-Ife, Nigeria. The stems were washed with clean water and air-dried until constant weight was obtained. Extracts were prepared by mincing the stem together with the bark into small pieces and grinding it with a laboratory mill into coarse powder. About 100g of the ground samples was measured into 2 conical flasks, one portion of the measured sample was covered with a litre of freshly prepared phosphate buffer saline (PBS) at pH 7.2 to serve as control and the other was covered with a litre of methanol-sterile distilled water mixture at a ratio 3:2. The mixtures were agitated at intervals for 4 days, after which the extracts were filtered into sterile flask. The extracts were concentrated in a rotary evaporator and lyophilized to recover the residue as sticky pastes which were further dried in a desiccator.

Antimicrobial Sensitivity Testing of Plant Extracts

The extracts were screened for antimicrobial activity using the agar well diffusion method. Each bacterial

inoculum (18-24 h old) was adjusted to turbidity of 0.5 McFarland standards. The inoculum was plated by pour-plate method using diagnostic sensitivity test agar and allowed to set. The required number of wells 5 mm to the edge of the plate was bored into the medium and labeled accordingly. Each well was then filled up with measured amount of extract at concentration of 25 mg/ml. Plates were incubated at 37 ^{o}C for 48 h. The relative susceptibility of the organisms to the extract as indicated by zone of inhibition around the wells were examined and recorded in millimeters (mm).

Minimum Inhibitory Concentration of Plant Extracts on Bacterial Isolates

The minimum inhibitory concentration of the extract was carried out using the method of Akinpelu et al. [27]. Two ml of extract at varying concentrations of 12.5, 6.26, 3.13, 1.57, 0.79 and 0.39 mg/ ml was mixed thoroughly with 18 ml of molten nutrient agar, poured into sterile Petri dishes and allowed to set. Plates with surface dried agar were then streaked with overnight both cultures of isolates. Lowest concentration at which there was no growth was recorded as the minimum inhibitory concentration.

Phytochemical Analysis of the Extract

Phytochemical screening was done following methods previously described [28, 29].

Test for Tannins: 5g of extract was stirred into 10 ml of distilled water and filtered. A few drops of ferric chloride reagent were then added to the filtrate. Presence of tannin is indicated by a blue-black, green or blue-green precipitate.

Test for Saponins: One gram of the extract was mixed with 5ml of distilled water. A few drops of Fehling's solution were added to the extract. Presence of green colour indicates the presence of saponins.

Test for Flavonoids: 0.25g of the extract was dissolved in 5ml or ethanol. 0.56g of Potassium Hydroxide (KOH) was added to 20ml ethanol in a beaker; about 0.5N of ethanolic-Potassium hydroxide was added to 1 ml of the filtrate. Presence of yellow colour indicates the presence of flavonoids.

Test for Alkaloids: About 0.5g of the extract was stirred into 5ml of 1% aqueous hydrochloric acid on a steam bath, 1ml of the filtrate was treated with a few drops of Mayer's reagent, another 1ml portion was treated with Dragendorff's reagent and the third 1ml portion was treated with picric acid solution. The production of turbidity or precipitation with any of these reagents was taken as preliminary evidence for the presence of alkaloids.

RESULTS

The antimicrobial activities of the extract of *F. zanthoxyloides* stem on selected cariogenic and enteric bacterial isolates at 25 mg/ml were as shown in table 2 with zones of inhibition range of between 11 mm and 16 mm. The minimum inhibitory concentrations of extracts against the selected bacterial isolates were represented by figure 1. This ranged between 1.57 and 12.5 mg/mℓ. The results of the phytochemical screening based on chemical examination of the plant extracts indicated that the extracts tested positive for tannin, saponin, flavonoids and alkaloids.

DISCUSSION

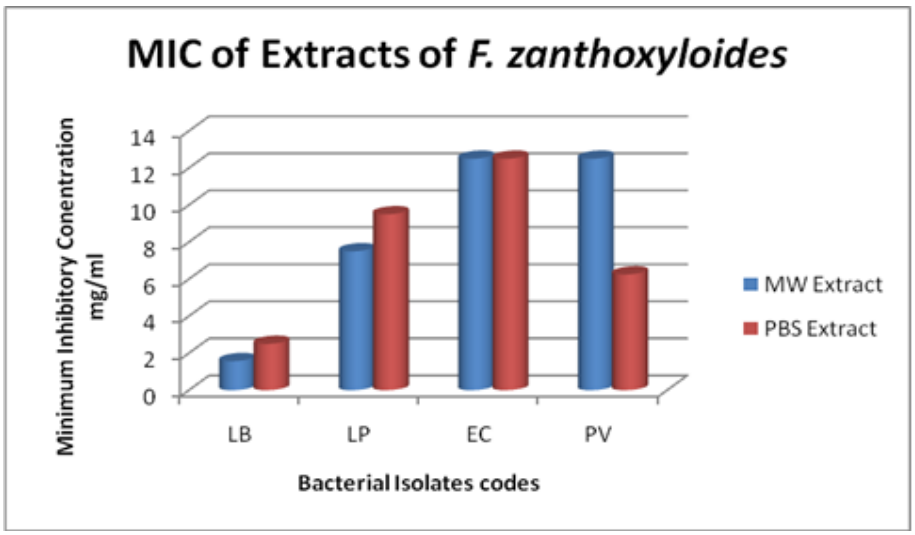
Both the two types of extracts, methanol-water (M-W) and phosphate buffer saline (PBS) showed some antibacterial activities. The minimum inhibitory concentration (MIC) of the extracts ranged between 1.57 and 12.5 mg/mℓ in all isolates except for *E. coli* where MIC value of the M-W extract was greater than 12.5 mg mℓ. The M-W extract inhibited significantly *L. brevis* NCIMB 4617while the PBS extract had considerable activity against *P. vulgaris.* According to Fabrey*et al.* [30], both types of crude extract of medicinal plants having MIC values below 8 mg/mℓ have been reported as being effective for antimicrobial therapy, hence the extracts can serve as effective antimicrobial against infections with *L.* brevis and *L. plantarum.* However, only the PBS extract offer a promising activity against *P. vulgaris* this is comparable to a previous study water extract of *F. Zanthoxyloides* showed activities against bacteria significant to periodontal disease [31].

The results of the phytochemical analysis of the plant extract revealed the presence of tannin, saponin, flavonoids and alkaloids. These compounds are known to be biologically active and have been shown to possess antimicrobial activity [32, 33]. The presence of alkaloids in this plant is in accordance to the report of Elujoba*et al.* [34] which described the main active ingredients in *F. zanthoxyloides* as alkaloids: berberine, fagaronine, chelerythrine, canthin-6-one and benzoic acid derivatives. Fagaronine has been shown to inhibit the growth and cell differentiation of human erythroleukemia K562 cells and L1210 murine leukemia cells [35]. The methanol extract of *F. zanthoxyloides* and fagaronine have been shown to be relatively safe [16, 36]. However, Takanishi*et al.* [37] reported that fagaronine showed toxicity only at a high pharmacological dose of 75 mg/kg of body weight in the comparison of the in vivo activities of fagaronine, nitidine, and other alkaloid derivatives against transplantable murine tumor P-388 cells.

Table 2. Zone of antimicrobial inhibition of extract

Bacteria	Zone of Inhibition MW extract	Zone of inhibition PBS extract
Lactobacillus brevis(CIMB 4617)	12 mm	0
Lactobacillus plantarum(NCDO 1752)	15 mm	15 mm
Escherichia coli (LIO)	16 mm	13 mm
Proteus vulgaris (LIO)	0	11 mm

Key:
NCIMB – National collection of Industrial, Marine-food bacteria
NCDO – National collection of diary organisms
LIO – Locally isolated organism
MW – Methanol-water mixture (ratio 3:2)
PBS – Phosphate buffer saline
0 – Resistant



Key:
LB - Lactobacillus brevis (NCIMB 4617)
LP - Lactobacillus plantarum NCDO 1752
EC - Escherichia coli (LIO)
PV - Proteus vulgaris (LIO)
MW – Methanol-water (ratio 3:2) extract (mg/ml)
PBS – Phosphate buffer saline extract (mg/ml)

Figure 1. Minimum Inhibitory Concentration (MIC) of extract of F. zanthoxyloides on selected bacteria isolates

CONCLUSION

The extracts from *F. zanthoxyloides* stem were found to possess antimicrobial activities. The two types of extracts inhibited the growth of at least three out of the four bacteria tested. The stem contains tannin, saponin, flavonoids and alkaloid which have been found to possess antimicrobial activities thus supporting the usefulness of this plant as local chewing stick. The result suggests the presence of antimicrobial ingredients in *F. zanthoxyloides* which is used as a chewing stick - a means for teeth cleansing in Nigeria. This thus makes the plant a viable candidate for antiplaque agent in the local setting.

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Original Research

Effect of *Momordica charantia* (bitter melon) on serum glucose level and various protein parameters in acetaminophen intoxicated rabbits

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Abstract

Aim: Liver function tests, including total plasma proteins, albumin, bilirubin and glucose were analyzed to find out the hepatocurative and hepatoprotective effects of *Momordica charantia*.

Method: The study was divided into two categories. In first category, the livers of rabbits were intoxicated with acetaminophen, and then *Momordica* fruit extract was given to observe its hepatocurative effects.

Results: The results indicated significant changes in concentrations of the parameters in acetaminophen-challenged rabbits. In the second category, treatment was started by giving *Momordica* fruit extract dose orally for 10 days and 15 days to two groups of rabbits, respectively. Then, livers of rabbits were damaged with acetaminophen and hepatoprotective effects of *Momordica* were observed.

Conclusion: The results showed that the animals treated with *Momordica* fruit extract experienced less liver damage due to acetaminophen intoxication, indicating that *Momordica* has hepatoprotective properties.

INTRODUCTION

Nutrigenomics has emerged as an important facet in current biology because of lesser tissue toxicity and off target effects. Various lines of evidence suggest utilization of plants with reference to their medicinal properties since the dawn of mankind. *Momordica charantia* or bitter melon has been used extensively in folk medicine as a remedy for diabetes [1, 2]. In the past decades, research has been focused on scientific evaluation of traditional drugs of plant origin. *Momordica charantia* is one such plant that has been frequently used as medicine [3]. *Momordica* has also been reported to show a wide range of biological activities including antioxidant, antiviral, antimicrobial, antiulcerogenic, anticacerous and antihepatotoxic activities [4, 5, 6, 7]. It has been found that this plant possesses effective components in preventing HIV [6]. The studies have also shown its efficacy in various cancers such as lymphoid leukemia, breast cancer, skin tumor, prostate cancer, squamous carcinoma of tongue and larynx [7].

Semiz & Sen found that *Momordica charantia* fruit extract was found to be containing free radical scavenging activity, which can exert a protective action against patho-physiological alterations caused by the presence of superoxide and hydroxide free radicals [8].

The liver diseases are constantly posing a challenge for human beings in the world at large. As liver is a major detoxifying and drug metabolizing organ of the body, therefore liver regulates many important metabolic functions. Hepatic injury is associated with distortion of these metabolic functions [9]. The diverse functional activity of the liver lends itself to the use of a number of different testing procedures. Multiple parameters are representatives of proper liver function. Of the substances released by damaged hepatic cells, enzymes and metabolites appearing in the blood are most useful

as indicators of possible liver injury. Also, the excretion of chemicals removed from the circulation into the bile is used to detect and assess severity of the damage [10]. The signs and symptoms of drug-induced hepatotoxicities are similar to those of natural disease [11]. Acetaminophen (paracetamol) is an analgesic and antipyretic drug that, in large overdose, can cause liver necrosis in man and laboratory animals [12, 13].

This project was carried out to evaluate the pharmacological properties of *M. charantia* in respect to hepatic diseases, and also to search for cheaper and effective therapeutic agents to mask the symptoms and ultimately cure the liver diseases.

MATERIALS AND METHODS

Plant Extract

The freshly obtained fruits of *M. charantia* were washed and air-dried. The whole fruits along with seeds were macerated in electric mixer then soaked in water and stirred vigoursely and left overnight. The mixture then filtered properly through sieve and the filtrate thus obtained was dried at reduced temperature.

Animals

Male rabbits (*Oryctolagus cunniculus*) weighing 1-2 kg were used for the study, purchased from veterinary center, Lahore. The animals were housed in the University Animal House to acclimatize in standard conditions and fed with standard diet and water.

Experimental Design

The study was divided in two categories.

Hepatocurative Studies

Five healthy rabbits were selected for the treatment. First blood sampling was done for control reading on day 0, normal values of liver function tests (LFTs) were recorded. To induce liver damage, acetaminophen was given for consecutive three days, the standard dose was 1500 mg/kg body weight, recommended by Kamran [14]. Blood was analyzed for LFTs on Day 3. Then from day 4 onwards, *Momordica charantia* fruit extract was given (5ml/ kg body weight), for 15 days on daily basis. Blood sampling was done at intervals, like on day 6, day 11, day 16 and day 20.

Hepatoprotective studies

This category was further divided into two phases,

i) Hepatoprotective (P 10) studies

This group consisted of five animals. Sampling of blood was done on day 0 for control reading. The treatment was started with the administration of *Momordica charantia* fruit extract daily (5 ml/kg body weight) from day 1 to day 10. After 10 days, the livers of rabbits were challenged with Acetaminophen (1500 mg/kg body weight) for three consectives days (11^{th} to 13^{th} day). Blood sampling was done on 13^{th} and16th day, and the changes in the concentrations of total plasma protein, albumin, bilirubin and blood glucose were observed.

ii) Hepatoprotective (P 15) studies

The group comprised five animals. For control reading, blood sampling on day 0 was done. In this category, *Momordica charantia* fruit extract daily (5 ml/ kg body weight) was given for 15 days from day 1 to day 15. Afterwards, the livers of rabbits were intoxicated with acetaminophen (1500 mg/kg body weight) for three consecutive days (16^{th} to 18^{th} day). Blood sampling was done on 18^{th} and 21^{st} day, and the changes in the concentrations of total plasma protein, albumin, bilirubin and blood glucose were observed.

Blood Sampling

The blood was collected from marginal ear vein of rabbit with 23/25 guage needle in the morning (8 am-9am). It was centrifuged at 3500 rpm for 5 minutes to obtain the serum.

Liver Function Tests (LFTs)

The liver function tests, including, total plasma proteins, albumin, bilirubin and glucose were studied.

Chemicals

Acetaminophen was used as a hepatotoxic drug. LFT test kits for total plasma proteins, albumin and glucose by Audit diagnostics, Ireland and for bilirubin by Biocon Diagnostik, Germany, were used for serological analysis.

Statistical Analysis

The data was analyzed by using ANOVA (One-way Analysis of Variance). The probability less than 0.05 ($P< 0.05$) was considered as significant represented by asterisks in tables.

RESULTS

Hepatocurative studies

Total plasma proteins

Blood sampling was done on 0 day for control reading. The concentration of total plasma proteins in untreated (control) animal was 79.1 $\pm$ 0.79 g/l. After 3 days acetaminophen administration there was significant decrease in serum total protein levels (39.7 $\pm$ 0.76 g/l) indicating liver damage. Total protein concentrations were found to be significantly increasing after treatment with *M. charantia* extract . The values were, 57.2 $\pm$ 0.81, 62.7 $\pm$ 1.01, 66.2 $\pm$ 0.96, 73.2 $\pm$ 0.64 g/l for 6^{th}, 11^{th}, 16^{th}, and 20^{th} day readings, respectively (Table 1).

Table 1. Variations among the concentrations of total plasma proteins, albumin, bilirubin and glucose during the hepatocurative treatment.

Treatments	Total Plasma Proteins (g/l)	Albumin (mg/dl)	Bilirubin (mg/dl)	Glucose (mg/dl)
Control (Day 0)	79.1 ± 0.79	8.8± 0.79	1.13 ±0.22	138.17 ± 0.9
Acetaminophen (Day 3)	39.7 ± 0.76*	5.03 ± 0.98*	4.08 ± 0.54*	97.9 ± 0.322*
Momordica extract (Day 6)	57.2 ± 0.81*	6.35 ± 0.81*	3.73 ± 0.25*	92.21 ± 0.34*
Momordica extract (Day 11)	62.7 ± 1.01*	6.9 ± 0.02*	3.11 ± 0.09*	90.34 ± 0.4*
Momordica extract (Day 16)	66.2 ± 0.96*	7.4 ± 0.1*	1.92 ± 0.02*	90.05 ± 0.67*
Momordica extract (Day 20)	73.2 ± 0.64*	7.75 ± 0.24*	1.69 ± 0.24*	84.76 ± 0.33*

Albumin

During the hepatocurative treatment, blood sampling was done on 0 day, for control reading. The concentration of albumin in untreated (normal) animal was 8.8±0.91mg/dl. After three days of acetaminophen intoxication there was significant fall in serum albumin levels i.e. 5.03 ± 0.98* mg/dl indicating liver damage. Then afterwards, animals were treated with *Momordica* extract, albumin concentrations were found to be significantly increasing with that treatment. The values were, 6.35 ± 0.81*, 6.9± 0.02*, 7.4± 0.01*, 7.75± 0.24*mg/dl at 6^{th}, 11^{th}, 16^{th}, and 20^{th} day readings respectively (Table 1).

Bilirubin

Blood sampling was done on 0 day, for control reading. The concentration of bilirubin in untreated (normal) animal was 1.13± 0.22 mg/dl. After that, acetaminophen was administered for three days and there was significant elevation in total bilirubin levels i.e. 4.08 ± 0.54 mg/dl, showing liver damage. Then afterwards, animals were treated with *Momordica* extract, the bilirubin concentrations were found to be significantly decreasing with that treatment. The values were, 3.73 ± 0.25, 3.11 ± 0.09, 1.92 ± 0.02, and 1.69± 0.24 mg/dl for 6^{th}, 11^{th}, 16^{th}, and 20^{th} day readings respectively, indicated in Table 1.

Glucose

The concentration of blood glucose in untreated (normal) animal was 138.17 ± 0.9 mg/dl. After that, acetaminophen was administered and there was significant decrease in blood glucose levels i.e. 97.9 ± 0.322* mg/dl, after 3 days, showing liver damage. Then afterwards, animals were treated with *Momordica* extract, blood glucose concentrations were found to be significantly decreasing with that treatment. The values are, 92.21 ± 0.34*, 90.34 ± 0.4*, 90.35 ± 0.67*, 84.76 ± 0.33* mg/dl for 6^{th}, 11^{th}, 16^{th}, and 20^{th} day readings respectively. (Table 1)

Hepatoprotective (P 10) studies

Total plasma proteins

In hepatoprotective (P_{10}) treatment the control (0 day) total plasma protein reading was 73.8 ± 1.3 g/l. After treatment with *M. charantia* extract (at day 10), total plasma protein reading was increased non-significantly (72.8 ± 1.1 g/l). There was a significant decrease in total plasma protein values i.e 54.8 ± 1.65 and 51.5 ± 0.57 g/l in 13^{th} day and 16^{th} day sampling, respectively (Table 2).

Table 2. Variations among the concentrations of total plasma proteins, albumin, bilirubin and glucose during the hepatoprotective (P 10) treatment.

Treatments	Total Plasma Proteins (g/l)	Albumin (mg/dl)	Bilirubin (mg/dl)	Glucose (mg/dl)
Control (Day 0)	73.8 ± 1.3	8.3 ± 0.61	1.05± 1.8	136.62 ± 1.05
Momordica extract (Day 10)	72.8 ± 1.1	8.09± 0.09	1. 08 ± 0.28	115.91 ± 0.96*
Acetaminophen (Day 13)	54.8 ± 1.65*	6.24 ± 0.88*	3.03 ± 0.43*	109.46 ± 0.44*
Acetaminophen (Day 16)	51.5 ± 0.57*	5.37 ± 1.2*	4.12 ± 0.85*	110.99 ± 0.46*

Albumin

The control (0 day) albumin reading was 8.3± 0.61 mg/dl. After treatment with *M. charantia* extract (at day 10), albumin reading was increased non-significantly (8.09 ± 0.09 mg/dl). After that, the animals were challenged with acetaminophen. There was a fall observed in albumin value i.e. 6.24 ± 0.88* and 5.37±1.2* mg/dl in 13th day and 16th day sampling, respectively (Table 2).

Bilirubin

In hepatoprotective (P_{10}) treatment the control (0 day) bilirubin reading was 1.05±1.8 mg/dl. Then during the treatment *Momordica* extract was administered into the animals. The value of bilirubin concentration was found to be 1.08±0.28 mg/dl. After that, the animals were challenged with acetaminophen. A rise was observed in bilirubin value i.e. 3.03±0.43* and 4.12±0.85* mg/dl in 13th day and 16th day sampling (Table 2).

Glucose

The control reading for blood glucose was 136.62±1.05 U/l. Then *Momordica* extract was given to the animals and the value of blood glucose concentration was found to be 115.91±0.96* mg/dl. After that, the animals were challenged with acetaminophen. There was a fall observed in blood glucose value i.e. 109.46±0.44* and 110.99±0.46* mg/dl in 13th day and 16th day sampling, after the liver damage, but the values were high as compared to the hepatocurative liver damage, showing that there is some protective properties in the extract (Table 2).

Hepatoprotective (P 15) studies

Total plasma proteins

In hepatoprotective (P_{15}) treatment the control (0 day) total plasma protein reading was 75.6 ± 1.09 g/l. After treatment with *M. charantia* extract (at day 15), total plasma protein reading was 75.3 ± 1.13 g/l).After that, the animals were challenged with acetaminophen. There was a fall observed in total plasma protein levels in serum i.e. 62.60 ± 0.75 and 54.9 ± 0.89 g/l in 18th day and 21st day sampling, after the liver damage, but the values were high as compared to the hepatocurative liver damage, showing that there are some protective properties in the extract. (Table 3).

Albumin

Similarly in hepatoprotective (P_{15}) treatment The control (0 day) albumin reading was 8.72±0.23 mg/dl. Then during the treatment *Momordica* extract was administered into the animals. The value of albumin concentration was found to be 8.6±1.12* mg/dl. After that, the animals were challenged with acetaminophen. There was a decrease in albumin value i.e. 7.4 ± 0.95* and 6.18 ± 0.56* mg/dl in 13th day and 16th day sampling, after the liver damage (Table 3).

Bilirubin

The control (0 day) bilirubin reading was 1.01±1.22 . mg/dl and then *Momordica* extract was administered into the animals at of 5ml/ kg body wt. The value of bilirubin concentration was found to be 0.98±1.65* mg/dl. Afterwards, the animals were challenged with acetaminophen. There was a rise observed in bilirubin value i.e. 2.31±0.45* and 3.67±0.87* mg/dl in 13th day and 16th day sampling, after the liver damage, as indicated in Table 3.

Glucose

The control (0 day) blood glucose reading was 136.04 ± 0.12 mg/dl. Then during the treatment *Momordica* extract was administered into the animals. The value of glucose concentration was found to be 104.22 ± 0.85* mg/dl. After that, the animals were challenged with acetaminophen. There was a increasing trend observed in blood glucose value i.e. 107.54 ± 1.3*and 111.86 ± 0.55* mg/dl in 13th day and 16th day sampling (Table 3).

Table 3. Variations among the concentrations of total plasma proteins, albumin, bilirubin and glucose during the hepatoprotective (P 15) treatment.

Treatments	Total Plasma Proteins (g/l)	Albumin (mg/dl)	Bilirubin (mg/dl)	Glucose (mg/dl)
Control (Day 0)	75.6 ± 1.09	8.72 ± 0.23	1.01± 1.22	136.04 ± 0.12
Momordica extract (Day 15)	75.3 ± 1.13	8.6 ± 1.12	0.98 ± 1.65*	104.22 ± 0.85*
Acetaminophen (Day 18)	62.6 ± 0.75*	7.4 ± 0.95*	2.31 ± 0.45*	107.54 ± 1.3*
Acetaminophen (Day 21)	54.9 ± 0.89*	6.18 ± 0.56*	3.67 ± 0.87*	111.86 ± 0.55*

DISCUSSION

The effect of *Momordica* fruit extract was experimentally evaluated during the current study. The parameters included Liver function tests like, total plasma proteins, albumin, bilirubin and Glucose concentrations in blood. For the induction of experimental hepatotoxicity, acetaminophen (paracetamol) was used. When therapeutic doses of acetaminophen are given, this metabolite is quickly metabolized to a non-toxic derivative by glutathione [15] and excreted in the urine as conjugates of cysteine and mercaptopuric acid. However overdose of paracetamole causes the damage to the liver [13]. When acetaminophen is taken in large doses or has been used long-term, the glucuronic acid or sulphate pathways become saturated and an increased amount of acetaminophen is metabolized by the cytochrome P-450 system to form the toxic metabolites. Glutathione conjugation increases but the amount of glutathione available is limited. Once the supply of glutathione becomes depleted, N-acetyl p-benzoquinoneimine binds covalently and irreversibly to hepatic cellular protein macromolecules and causes cell damage and death of hepatocytes [16, 17,18].

In this study we found that *M. charantia* brings the altered lavels of total plasma proteins, albumin and bilirubin of acetaminophen intoxicated mice to their normal levels. Our work correlates to Dandagi and coworkers who explore the hepaotoprotective activity of various extracts of *Ferula asafetida, M. charantia* and *Nardosta jatamansi* against experimental hepatotoxicity and the results demonstrated that the extracts of *Momordica charantia* Linn. have significant hepatoprotective activity [19]. The hepatoprotective role of *Momordica* extract in our findings seems to be due to enhanced antioxidant enzymes these enzymes have the capability to engulf reactive oxygen species that can damage the liver [20]. In another study by Chaudhri *et. al*, also found hepatoprotective activity of hydro-alcoholic extract of *M. charantia* leaves by estimation of SGOT, SGPT, ALP and total Bilirubin [20]. Similar results have been reported for some other ethnobotanical fruits and herbs [21, 22, 23].

In conclusion, the results of the present study indicate that the *M. charantia* fruit extract have both hepatoprotective (protect liver from injuries) and hepatocurative (Cures the injured liver) properties. Further studies are needed to see if a higher dose and different routes of administration of *M. charantia* have a hepatoprotective effect.

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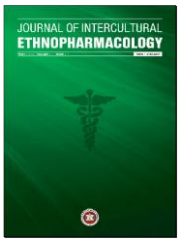

Original Research

Preliminary phytochemical analysis and cytotoxic potential of *Cucumis trigonus* Roxb

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Abstract

Aim: The present study involves in the investigation of various extracts of Cucumis trigonus Roxb. for its phytochemical constituents and also the cytotoxic potential of the Cucumis trigonus Roxb. ethanolic fruit extract.

Methods: The phytochemical screening with methanol, ethanol, petroleum ether, chloroform and aqueous extracts of Cucumis trigonus Roxb. was done by modern method of Peach and Tracey (1955) Cytotoxicity is estimated by staining technique using Trypan blue after the addition of drug, the dead cells are stained blue with Trypan blue. Confirmation studies were done by additional metabolic intervention experiments like (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide) MTT assays.

Results: Qualitative phytochemical analysis of the plant extracts were done and it was found that the presence of phytochemicals were maximum in the ethanolic extract when compared to other extracts. So the ethanolic extract of C. trigonus fruit was used for further investigations. In the cytotoxic study the ethanolic fruit extract concentration at 1.25 mg / ml was found to be the effective dose because at this concentration, it exhibited 50% cytotoxicity against Hep2 cells.

Conclusion: These results conclude that the ethanolic fruit extract of Cucumis trigonus possess a good phytochemical strength and also an effective cytotoxic potential.

INTRODUCTION

The uses of herbs to treat diseases are almost universal among non-industrialized societies. The World Health Organization (WHO) estimates that 80% of the world's population presently uses herbal medicine for some aspect of primary health care [1]. Herbalism is a traditional medicinal or folk medicinal practice based on the use of plants and plant extracts. Herbal medicines are popular remedies for diseases used by a vast majority of the world's population [2].

Plants are utilized as therapeutic agents since time immemorial in both organized and unorganized form. The healing properties of many herbal medicines have been recognized in many ancient cultures [3]. Plants provide a variety of resources that contribute to the fundamental needs of food, clothing and shelter. Among plants of economic importance, medicinal and aromatic plants have played a vital role in alleviating human sufferings [4]. The scope of herbal medicine is sometimes extended to include fungal and bee products, as well as minerals, shells and certain animal parts [5].

Numerous molecules have come out of Ayurvedic experiential base, examples include rauwolfia alkaloids for hypertension, holarrhena alkaloids in amoebiasis, baccosides in mental retention, picrosides in hepatic protection, phyllanthins as antivirals, many other

steroidal lactones and glycosides as immunomodulators [6]. Herbal drugs which are claimed to be safe are equally effective in comparison to allopathic drugs provide some answer to chronic diseases. However, these herbal drugs are marketed with innumerable pharmacological activities which are not mentioned in the text of various traditional systems of medicine [7].

Cucumis trigonus Roxburghii of family Curcurbitaceae is a perennial scabrid monoecious tendrillar herb with slender angled stem, leaves deep palmately five lobed, hispid on the nerves beneath and rounded at the apex. Male flowers are small and are found in clusters where as female flowers are solitary. Fruits are ellipsoid or sub-global, yellow or yellow with green stripes, seeds are white and ellipsoid. Cucumis trigonus is distributed throughout India, Srilanka, Afghanistan, Persia and Northern Australia. Roots, fruits and seeds are the medicinal parts of the plant. Roots are purgative and liver tonic. Fruits are used for stomachic, ascites, anemia and constipation and acts as a diuretic. Seeds have a unsaturated lipids as major constituents and acts as a coolant and astringent [8].

The present study is to analyze the phytochemical constituents and the cytotoxic activity of the Cucumis trigonus fruits against Hep2 cells with the help of MTT assay.

MATERIALS AND METHODS

Collection of the plant material

Cucumis trigonus Roxb. fruits were collected from Kovanur foot hills of Coimbatore district, Tamil Nadu, India during the month of July to August,2009. The plant was identified and authenticated by taxonomist Dr. K. Arumugasamy, Assistant Professor, Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India. Voucher specimen was deposited herbarium centre, Department of Botany, Kongunadu Arts and Science College, Coimbatore.

Qualitative phytochemical analysis of the fruit extract

The phytochemical screening with methanol, ethanol, petroleum ether, chloroform and aqueous extracts of Cucumis trigonus Roxb. was done by modern method [9] to identify the presence of alkaloids, flavonoids, tannins, saponins, triterpenes and glycosides.

Alkaloids

a.Dragendroff's test: (Kraut-potassium bismuth iodide) 8.0g of Bi (NO3)3. 5H2O was dissolved in 20ml of HNO3 and 2.72g of potassium iodide in 50ml of water. These were mixed and allowed to stand when KNO3 crystallizes out. The supernatant was decanted off and made upto 100ml with distilled water. The alkaloids were regenerated from the precipitate by treating with Na2CO3 followed by extraction of the liberated base with ether.

To 0.5ml of alcoholic, petroleum ether, chloroform and aqueous solution of plant sample added 2.0ml of HCl. To this acidic medium, 1.0ml of reagent was added. An orange red precipitate was produced immediately which indicates the presence of alkaloids.

b. Meyar's reagent (potassium iodide)

1.3g of mercuric chloride was dissolved in 60ml distilled water and 5.0g of potassium iodide in 10ml of water. The two solutions were mixed and diluted to 100ml with distilled water.

To 1.0ml of alcoholic, petroleum ether, chloroform and aqueous solution of samples few drops of reagent was added. Formation of white or pale yellow precipitate showed the presence of alkaloids.

Flavanoids

In the test tubes containing 0.5ml of alcoholic, petroleum ether, chloroform and aqueous solution of the plant, 5-10 drops of dilute HCl and small piece of zinc or magnesium were added and the solution was boiled for few min. In the presence of flavonoids, reddish pink or dirty brown color was produced.

Tannins

Ferric chloride test

To 1-2ml of alcoholic, petroleum ether, chloroform and aqueous solution of the plant, few drops of 5% aqueous Fecl3 solution was added. A bluish black color, which disappears on addition of a few ml of dilute H2SO4 was followed by the formation of yellowish brown precipitate.

Saponins

In a test tube containing about 5.0ml of alcoholic, petroleum ether, chloroform and aqueous solution of the plant, a drop of sodium bicarbonate solution was added. The mixture was shaken vigorously and kept for 3min. A honey comb like froth was formed and it showed the presence of saponins.

Steroids

Libermann-Burchard's test

To 1.0ml of alcoholic, petroleum ether, chloroform and aqueous solution of the plant, 1.0ml of conc. H2SO4 was added followed by the addition of 2.0ml of acetic anhydride solution. A greenish color developed and turned blue indicates the presence of steroids.

Terpenoids

Salkwski reaction

5.0ml of the alcoholic, petroleum ether, chloroform and aqueous solution of the plant was mixed in 2.0ml of chloroform and concentrated H2SO4 (3.0ml) was carefully added to form a layer. A reddish brown coloration in the inter phase formed to show positive results for the presence of terpenoids.

Resins

To 2.0ml of alcoholic, petroleum ether, chloroform and aqueous solution the plant sample, 5-10ml of acetic anhydride was dissolved by gentle heating, cooled and then 0.5ml of H2SO4 was added. A bright purple color rapidly changing in to violet was produced, indicating the presence of resins.

Glycosides

A small amount of alcoholic, petroleum ether, chloroform and aqueous solution of the plant sample was dissolved in 1.0ml of water and then aqueous sodium hydroxide solution was added. Formation of a yellow color indicates the presence of glycosides.

Phenols

a. Ferric chloride test

To 1.0ml of alcoholic, petroleum ether, chloroform and aqueous solution of the plant sample 2.0ml of distilled water followed by few drops of 10% aqueous Fecl3 solution were added. Formation of blue or green color indicates the presence of phenols.

b. Lead acetate test

1.0ml of alcoholic, petroleum ether, chloroform and aqueous solution of the plant sample was diluted to 5.0ml with distilled water and to this few drops of 1% aqueous solution of lead acetate was added. A yellow precipitate was formed to indicate the presence of phenols.

Cytotoxic Assay

Minimal essential media preparation:

Media is defined as a complex source of nutritional supplementation vital for the growth proliferation and maintenance of cells in vitro. The MEM vial was rinsed in the millipore distilled water, mixed well, closed and sterilized at 15lbs pressure at 121ºc for 15min.Add ingredients depending on the concentration of fetal calf serum (2% or 10%) and was mixed well and then shaken. Take care to avoid spills, pass CO2 using sterile pipette, shake the bottle, check pH and adjust to 7.2 to 7.4. The MEM bottles are kept for 2 days at 37ºc and checked for sterility, pH drop and floating particles. They are then transferred to the refrigerator.

Preparation of ingredients:

a. Penicillin and Streptomycin: (Concentration 100 IU of Penicillin and 100 µg of Streptomycin)

Dissolved both antibiotics in sterile millipore distilled water, so as to give a final concentration of 100 IU of penicillin and 100µg of streptomycin/ml. Mix well and distribute in 1ml aliquots. Stored at - 20º C.

b. Amphotericin B (Fungizone);20µg/ml

Dissolved in sterile millipore distilled water so as to give a final concentration of 20µg/ml and distribute in 1ml aliquots in vials. Store at - 20ºC.

c. L-glutamine(3%)

Weighed 3.0g of l-glutamine accurately and dissolved in 100ml sterile millipore distilled water and mixed well. Filtered through millipore membrane filter 0.22µ and distributed in 5ml aliquots in vials. Store at - 20ºC.

d. 7.5% Sodiumbicarbonate

Weighed requisite quantity of sodium bicarbonate (to give 7.5% solution) accurately and dissolve in 100ml of sterile millipore distilled water. Filtered through what mann filter paper No.4, distributed into bottles and sterilized at 121ºC, 15lbs pressure for 15min. Cooled and stored at - 4ºc.

e. Foetal calf serum

Brought FCS to room temperature. Inactivated at 56ºC in water bath for 30min. and cooled at room temperature. If floating particles were seen filtered through Seitz filter. Distributed in 100ml, 50ml, and 20ml quantities in sterile bottles and Stored at - 20ºC.

f. Trypsin, PBS, versene, glucose solution: (TPVG)

i. 2% Trypsin

Weighed 2.0g of trypsin accurately; dissolved in 100 ml sterile millipore distilled water with magnetic stirrer for 30min. Filtered through membrane filter. Stored at - 20ºC.

ii. 0.2%EDTA (versene)

Weigh 200mg of EDTA accurately. Dissolved in 100 ml of sterile millipore distilled water. Autoclaved at 15lbs/15min.

iii. 10% Glucose

Weighed 1.0g of glucose accurately. Dissolved in 100 ml of sterile millipore distilled water and filtered through What Mann filter paper and autoclaved at 15lbs / 15 min.

iv. TPVG: 100ml

For the preparation of 100ml of TPVG solution, 840 ml of PBS solution is mixed with 50 ml of 0.2% trypsin, 100 ml of 0.2% EDTA, 5 ml of 10% glucose and 5 ml

of penicillin and streptomycin and mix all the ingredients and adjusted the pH to 7.4 with 0.1 N HCl or 0.1 N NaOH. Distributed in 100 ml aliquots. Stored at - 20ºc.

MAINTENANCE OF CELL LINE

Thawing was done by bringing the medium and TPVG to room temperature. The tissue culture bottles were observed for growth, cell degeneration, pH and turbidity by seeing in an inverted microscope. Wipe the mouth of the bottle with cotton soaked in spirit to remove the adhering particles. Discard the growth medium in a discarding jar and keep distance between the jar and the flask. Then add 4 - 5 ml of MEM without FCS and gently rinsed with tilting. The dead cells and excess FCS are washed out and then discard the medium. TPVG was added over the cells and incubate at 37ºC for 5 min. for disaggregation. The cells become individual and it became a suspension. Add 5ml of 10% MEM with FCS by using serological pipette, if any clumps were present then repeat the process. After passaging, split the cells into 1:2, 1:3 ratio for cytotoxicity studies for plating method

MTT Assay

MTT assay is called as (3-(4, 5-dimethyl thiazol-2yl)-2, 5-diphenyl tetrazolium bromide was first proposed by Mossman in 1982. After incubation, remove the medium from the wells carefully for MTT assay. In each well wash with MEM (w/o) FCS for 2 - 3 times. Added 200µl of MTT (5mg/ml). Incubate for 6-7hr. in 5% CO2 incubator for cytotoxicity. After incubation added 1.0ml of DMSO in each well and mixed by pipette and left for 45sec. If any viable cells present formazan crystals after adding solubilizing reagent (DMSO) it shows the purple color formation. The suspension was transferred in to the cuvette of spectrophotometer and the O.D values are read at 595nm by taking DMSO as a blank.

Cell viability (%) = Mean OD / Control OD x 100

RESULTS

Qualitative phytochemical analysis of the fruit extract

The qualitative analysis which produces a "fingerprint" chromatogram obtained under standard conditions is very useful for quality control of phytochemicals. The phytochemical screening with aqueous, ethanol, methanol petroleum ether and chloroform extracts of Cucumis trigonus fruit extract showed to possess secondary metabolites which are clearly depicted in table 1.

Table1. Qualitative analysis of phytochemicals in the fruit extracts of *C.trigonus* Roxb.

S.NO	Chemical constituents	Water	Ethanol	Methanol	Pet. ether	Chloroform
1	Aklaloids	+	++	+	+	+
2	Flavonoids	-	+	-	-	-
3	Tannins	-	+	+	++	+
4	Saponins	+	++	++	-	-
5	Steroids	-	++	-	++	++
6	Terpenoids	++	++	-	-	++
7	Resins	-	+	-	+	-
8	Glycosides	+	+	-	+	-
9	Phenolic constituents	-	++	-	-	-

Cytotoxic assay

The ethanolic fruit extract was prepared from shade dried fruits of Cucumis trigonus to evaluate the cytotoxic efficacy using Hep2 cell line. The cytotoxic effect of varying concentrations of ethanolic fruit extract of Cucumis trigonus at the range of 10 mg / ml to 0.156 mg / ml on Hep2 cell lines was represented in Table 2 and Figure 1.

Table2. Cytotoxic effect of *C. trigonus* fruit extract on Hep2 cells

S. No.	Concentration (mg / ml)	Absorbance	Percentage of cell viability
1	10	0,02	4,08
2	5	0,15	30,61
3	2,5	0,21	42,85
4	1,25	0,27	55,1
5	0.625	0,35	71,42
6	0,3125	0,41	83,67
7	0.156	0,47	95,91
8	Cell control	0,49	100

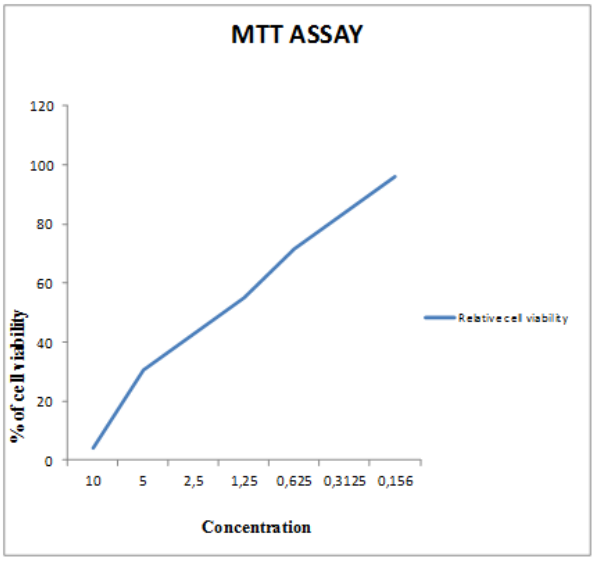

Fig.1. MTT assay of *C. trigonus* fruit extract

The above results indicate that the cytotoxic effect of the ethanolic extract of Cucumis trigonus against Hep2 cells is dose dependant. At low concentrations the extract was found to be less toxic towards the Hep2 cells whereas at higher concentrations the toxicity was increased. Our results are similar to that of [10]. The concentration at 1.25 mg / ml was found to be effective dose because at this concentration, it exhibited 50% cytotoxicity against Hep2 cells.

The cytotoxicity study of C. trigonus Roxb. fruit extract on Hep2 cells is given in the Figure 2, which depicts the percentage of dead cells at different concentrations of the fruit extract. This clearly indicates that on increasing the concentration of the fruit extract the dead cells increase which clearly depicts the percentage of cell viability.

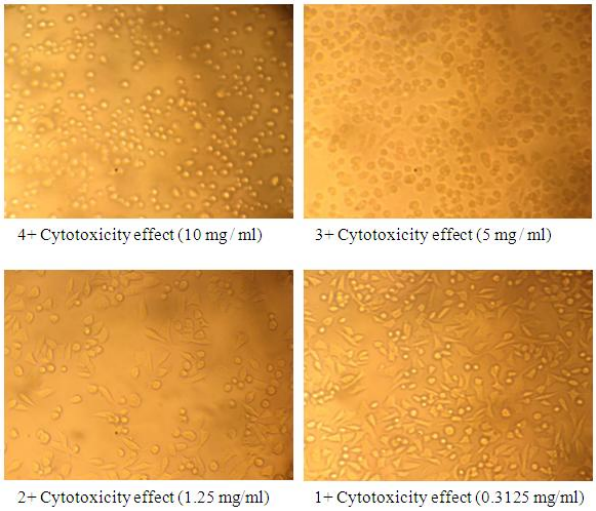

Fig. 2. Cytotoxicity effect of the fruit extract of *Cucumis trigonus* Roxb.

The cytotoxicity effect of ethanolic fruit extract of C. trigonus Roxb. might be due to the alkaloid and glycoside components of the fruit possessing anticancer activity. Figure 1 represents the cytotoxicity effect of Cucumis trigonus R. fruit extract.

DISCUSSION

Qualitative phytochemical analysis of the plant extract was done and it was found that the presence of phytochemicals were maximum in the ethanolic extract when compared to other extracts. The phytochemical study with ethanolic fruit extract of selected medicinal plant showed the presence of alkaloids, flavanoids, tannins, saponins, steroids, terpenoids, resins, glycosides and phenolic constituents. So the ethanolic extract of C. trigonus fruit was used for further investigations.

The cytotoxic effect of the ethanolic extract of Cucumis trigonus against Hep2 cells is dose dependant. At low concentrations the extract was found to be less toxic towards the Hep2 cells whereas at higher concentrations the toxicity was increased. The

concentration at 1.25 mg / ml was found to be effective dose because at this concentration, it exhibited 50% cytotoxicity against Hep2 cells. The cytotoxicity effect of ethanolic fruit extract of C. trigonus Roxb. might be due to the alkaloid and glycoside components of the fruit possessing anticancer activity.

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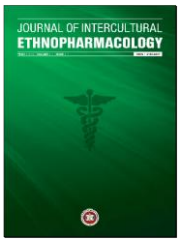

Original Research

Study of antinociceptive and anti-inflammatory activities of certain Iranian medicinal plants

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Keywords:
Antinociceptive, Hot-Plate, Carrageenan-Induced edema, Anti inflammatory, Fenugreek

Abstract

Aim: Four medicinal plants of Tri*gonella foenum-graecum, Zhumeria majdae, Achillea wilhelsii* and *Viola tricolor* are traditionally used in Iran as analgesic and for treatment of inflammatory disorders. At the present atudy, the antinociceptive and anti-inflammatory effects of these plants have been studied.
Methods: The antinociceptive and anti-inflammatory activity of methanol extracts of tested plants were evaluated using hot-plate and carrageenan-induced edema methods respectively. The plant extracts were studied by i.p administration at three doses of 100, 200 and 400mg/kg.
Results: In the hot-plate test, the extracts of *T. foeunm-graecum* (100 mg/kg) and *Z. majdae* (200 and 400m g/kg) significantly increased the tolerance to pain in female albino mice in comparison to control. The administration of *T. foenum-graecum* at doses of 100 and 200mg/kg and *V. tricolor* (400mg/kg) significantly reduced the paw edema in male rat which measured in all the times of observation after carrageenan administration in comparison to control and reference (Ibuprofen, 400mg/kg).
Conclusions*:* The present work comparatively demonstrated considerable antinociceptive and anti inflammatory effect of all of the tested plants especially *T. foeunm-graecum.* The results here confirm traditional uses of *T. foeunm-graecum* both as analgesic or anti inflammatory agents.

INTRODUCTION

In Iranian traditional medicine, many of the plants have been suggested as anti-inflammatory and to treat bruise, pain and rheumatism [1, 2]. Regarding wide range of undesired side effects of available anti-inflammatory and analgesic drugs, many studies are being directed to find compounds with lesser side effects. The present study was conducted to verify and compare the antinociceptive and anti-inflammatory activity of methanol extracts of four most common used medicinal plants.

Fenugreek (*Trigonella foenum graecum* L.; Fabaceae) is a plant whose seeds and leaves have several pharmacological effects such as hypoglycemia, hypocholestrolemia, antioxidant and appetite stimulation [3-6]. This plant under the name of "Shanbalileh", in Iranian traditional medicine has been used as hypoglycemic and antirheumatism [2].

Mohrkhosh (*Zhumeria majdae* Resh. f.& Wendelbo : Lamiaceae) has a limited geographical range in bandar-abbas in Hormozgan province in the southeastern of Iran [7]. Plant leaves have been used for many years as

treatment of stomachaches, antiseptic, carminative especially in infants and for treatment of painful menstruation [8]. Antibacterial and antioxidant activity of the essential oil of *Z.majdae* has been previously reported [9].

Achillea, a genus of Asteraceae family, has long been used in traditional medicine. Top flowering of the plant is used as antiflatulence, spasmolytic, antinociceptive and diuretic. Aerial parts of *A. wilhelmsii* K.Koch contains volatile oils, flavonoids, terpenoids, alkaloids, saponins and sesquiterpenlactones [10]. We have reported immune-stimulating effects of this plant [11].

Viola tricolor L., Violaceae commonly known as violet is used in folk medicine for various purposes such as anti inflammatory agent especially in common cold, it is externally used for mild seborrheic complains and various skin conditions such as eczema [1]. Modern pharmacological studies have demonstrated that violet has antimicrobial and cytotoxic activity and is useful for treatment of mild-to-moderate atopic dermatitis [12]. Chemical studies on *V. tricolor* have shown the presence of flavonoids, anthocyanins, coumarins, tannins, saponins, carotenoids and phenolic acids [13].

MATERIALS AND METHODS

Plant material

The dried seeds of the fenugreek were prepared from the local market and the other plants were gathered in June-2006 from Kerman and scientifically approved by Dr. Mirtajaldini, Department of Botany, Bahonar University, Kerman, Iran. A voucher specimen of the gathered plants was deposited in the herbarium of Faculty of Pharmacy, Kerman University of Medical Sciences, Kerman, Iran. Information of the tested plants has given in the Table 1.

Preparation of plant extract

500g of dried plant was extracted with methanol using percolation method [14]. The obtained extracts were dried under vacuum to give viscose mass. The yield of the extraction is given in Table 1. The extracts were suspended in 0.1% DMSO/normal saline (v/v) for the *in vivo* tests.**Drugs and reagents**

Ibuprofen tablet (Iran, Hakim Pharmaceutical Co. Ltd., Iran); Dexamethasone ampule (Sina-Daru Pharmaceutical Co. Ltd., Iran); Carboxymethylcellulose-sodium (CMC-Na, Iran); Carrageenan (type I, Sigma Chemical Co., St. Louis, MO, U.S.A.) and Hot plate (LE-7406, PANLAB, Spain) were used in the present study.

Animals

Female albino mice weighing 20–25 g and male rats weighing 250-300 g, were employed for antinociceptive and anti inflammatory tests respectively. The animals were obtained from the Neuroscience Research Center, Kerman University of Medical Sciences. They were housed in a room temperature 22 ± 2 °C at 12-h light:12-h dark cycle and had free access to food and water except during the time of experiments. Groups of 6 animals each were used in all tests. Animals were acclimatized to the laboratory for at least 1 h before testing and were used for once experiment only. This study complied with current ethical regulations on animal research (National Research Council of USA, 1996) and related rules of our school and all animals used in the experiment received humane care (NO, EC/KNRC/85-2).

Table 1. Extraction results and additional information of 4 tested plants used for studying their antinociceptive and anti inflammatory activities

Scientific name	Family	Common/folk name	Part used	Voucher No.	Yield of extraction
Trigonella foenum-graecum L.	Fabaceae	Fenugreek/ Shanbalileh	Seeds	-	23.5%
Zhumeria majdae Resh. f.& Wendelbo	Lamiaceae	- / Mohrkhosh	Aerial parts	KF1155	19.2
Achillea wilhelmsii C.Koch	Asteraceae	Santalin yarrow/ Boomadaran	Aerial parts	KF1387	21.4
Viola tricolor L.	Violaceae	Violet/ Banafsheh	flowers	KF1382	19.9

Hot plate latency assay in mice

In the hot-plate test, animals were habituated twice to the hot-plate in advance. The temperature of a metal surface was set at 55 ± 0.5 °C. The time that elapsed until the occurrence of either a hind paw licking or a jump off the surface was recorded as the hot-plate latency. After the determination of baseline response latencies, hot-plate latencies were determined at 30, 60 and 90 min after i.p administration of test drugs in comparison to control. A latency period of 30 s was defined as complete analgesia as cut off time to prevent damage to mice. Mice with baseline latencies of <5 s or >30 s were eliminated from the study (15, 16).

Carrageenan-induced rat paw edema

Edema was induced by injecting 0.1 ml of 0.5% carrageenan subcutaneously into the sub-plantar region of the left hind paw of Wistar rats. Equal volume of solvent was injected to right hind paw (16). Different doses of the extracts (100, 200 and 400 mg/kg) were administered ***i.p***. to left hind paw. The reference group received Ibuprofen (400 mg/kg, *i.p.*) and the control group received an equal volume of solvent. All drugs were administrated just after injection of carrageenan (T=0). The volume of hind paws were measured with a plethysmometer at 0 h (just after carrageenan injection), 1, 2, 3 and 4 h. Difference of two paws volume was considered as carrageenan-induced edema.

Statistical Analysis

Results are expressed as mean ± S.E.M. Differences between the control and treated groups were tested for significance using a one-way analysis of variance (ANOVA), followed by Tukey 's *t*-test.

RESULTS

The yield of extraction and the other information of the plants are given in Table 1. The results of hot plate test showed that the extract of *T. foenum graecum* (dose of 100mg/kg) in all the test times showed significant antinociceptive effect compared to control ($p<0.001$). The extract of *Z. majdae* has shown the most antinociceptive effect at dose of 200mg/kg (30 and 90 min) and 400mg/kg (30, 60 and 90 min), in comparison to control ($p<0.05$) (Table 2) . The results obtained from carrageenan-induced paw edema show the mean volume difference between right and left foot paws before and 4h. after *i.p* administration of normal saline and carrageenan respectively (Figure 1). As shown in this figure the index of carrageenan induced edema is completely different from the ones for normal saline. Methanol extract of *T. foenum graecum* seeds at the dose of 100 and 200mg/kg has inhibited paw edema at 1, 2 and 4h after carrageenan administration. As shown in Figure 2, these extracts notably inhibited paw edema in rat when given *i.p* at 4 h after carrageenan injection. At the dose of 400mg/kg, methanol extract of *V. tricolor* has shown the most inhibition of paw edema at 1, 2 and 4h after carrageenan administration. As shown in Figure 2, this extract notably inhibited paw edema in rat when given *i.p* at 4 h after carrageenan injection. None of these three active extracts showed significant activity in comparison to ibuprofen ($p>0.05$).

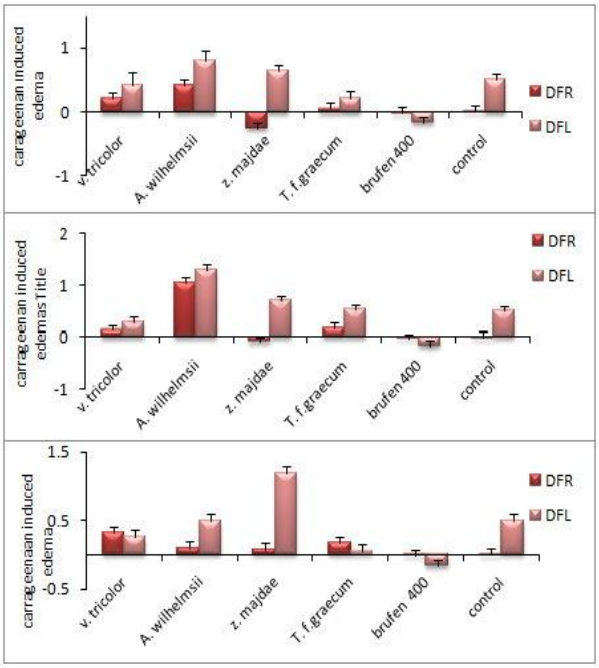

Fig 1. Effect of methanol extracts of 4 tested plants on carrageenan-induced paw edema in rat. DFR: Differences of volume of right foot paw before and 4h. after normal saline injection. DFL: Differences of volume of left foot paw before and 4h. after carrageenan injection (a): Dose 100mg/kg, (b) Dose of 200mg/kg, (c) Dose of 400mg/kg of each plant extract.

DISCUSSION

Pain and inflammation are induced in various clinical disorders like arthritis, cancer and vascular disease which disaster the patient. Some of medicinal plants have been traditionally used either for pain relief or as anti inflammatory agents. Our results of the antinociception in hot-plate test show that among the tested plants, the highest efficacy was exhibited by the methanol extracts of *T. foenum-graecum* (100mg/kg) and *Z. majdae* (200 and 400mg/kg) which significantly raised the pain threshold in comparison to control. The antinociception effect has occurred in different experimental times for each of the plants that would be due to the various analgesic metabolites of the plants which reached to maximum in different times [17]. *T. foenum-graecum* at the dose of 100mg/kg showed the

Table 2. Anti nociceptive effect of tested plant extract in hot-plate test at different doses (100, 200 and 400 mg/kg). Hot-plate latencies were determined at 0, 30, 60 and 90 min after *i.p* administration of test drugs to animal in comparison to control (n=6).

Treatment	Dose (mg/kg)	Latency time (sec) in definite intervals			
		0 min	30 min	60 min	90 min
T. foenum-graecum	100	9.3 ± 0.2*	6.2 ± 0.2*	8.5 ± 0.2*	7.3 ± 0.2*
	200	6.1 ± 0.2	4.7 ± 0.1	4.1 ± 0.1	5.1 ± 0.1
	400	5.9 ± 0.1	4.7 ± 0.2	6.2 ± 0.2	4.5 ± 0.1
A. wilhelmsii	100	5.5 ± 0.1	5.7 ± 0.07	5.8 ± 0.1	5.2 ± 0.1
	200	3.7 ± 0.04	3.9 ± 0.02	3.6 ± 0.07	3.8 ± 0.08
	400	5.0 ± 0.1	3.9 ± 0.07	4.3 ± 0.1	2.8 ± 0.07
V. tricolor	100	5.8± 0.1	4.3± 0.05	4.6± 0.07	4.6± 0.04
	200	6 ± 0.1	5.7 ± 0.1	5.1± 0.08	4.6 ± 0.1
	400	5 ± 0.1	3.6 ± 0.05	3.6 ± 0.04	3.8 ± 0.06
Control	-	5.5± 0.1	4.1 ± 0.08	3.7 ± 0.06	4.5 ± 0.08

*$p < 0.001$, vs. the control group.

most latency in licking and jumping of mice paw (Table 2). The hot-plate method is known as a test for detecting of opioids as well as the other CNS depressants which can respond to thermal stimuli. It is suggested that the extracts which have exhibited anti nociception effect in this method, might act through central mechanisms [18]. A report shows that in the naloxone pre-treatment animals, the antinociception of *T. foenum-graecum* seeds has been partially antagonized, it seems that some of endogenous opioids are involved in antinociception effect of the plant seeds [19]. The seeds of *T. foenum-graecum* contain saponins, alkaloids, flavonoids and salicylate [18, 20, 21], which may be responsible of the antinociception effect of the plant. Our findings also show that the methanol extract of *Z. majdae* has increased significantly the tolerance to pain at the dose of 400mg/kg. This effect may derive from the contribution of active components composing the plant such as linalool. The essential oil of this plant contains linalool and camphor as main compounds (53 and 26% respectively)[9]. Linalool produces antinociception in two experimental models of pain [22]. As Hosseinzadeh *et al.,* reports, the aqueous extract of the plant has shown antinociceptive and anti inflammatory activity at the doses of 1g and 800mg/k respectively [23] .Our findings show that the antinociceptive effect of methanol extract was more potent than that effect observed with aqueous extract of the plant which may be due to the presence of some monoterpenes like linalool in the methanol extract that can potentiate the antinociception of the extract. Linalool is a monoterpene which is sparingly soluble in aqueous solvent. The methanol extract of *A. wilhelmsii* failed to prolong latency time compared with controls in mice hot plate test. Carrageenan-induced paw edema in rat has known as a sensitive method for studying of non steroidal anti-inflammatory agents and show a biphasic event which is attributed to the different mediators. At the first (about 2 h after carrageenan injection), hyperemia mainly induces because of the release of histamine and serotonin, whereas prostaglandins and bradykinin potentiate the second phase of edema by mobilization of leukocytes [24]. Our results showed that among the tested plants, the extract of *T. foenum-graecum* at the doses of 100 and 200mg/kg and *V. tricolor* at the dose of 400mg/kg significantly reduced the paw edema throughout the entire period of observation in comparison to control ($p<0.05$) without significant difference with Ibuprofen (Figure 2). Presence of saponins and flavonoids as the major compounds in *T. foenum-graecum* and *V. tricolor* [13, 20] can approximately explain the anti inflammatory activity of these plants. A number of flavonoids, including quercetin, are able to inhibit both the cyclooxygenase and lipoxygenase pathways at relatively high concentrations. Flavonoids can also inhibit the nitric oxide synthase [25]. Thakur and et al, have reported no significant anti inflammatory activity of the aqueous extract of *T. foenum-graecum* seeds [26], whereas in another report the ethanol extract of the plant obtained in suxhelet method has exerted significant anti inflammatory effect. The latter report shows that the ethanol extract of *T. foenum-graecum*

seeds increases the peritoneal exudates as well as macrophage cell counts which indicates that this plant probably acts via activation of macrophages (18). Gastroprotective effect reported for the seeds of this plant is a valuable factor regarding the gastrointestinal disturbance caused by non steroidal anti inflammatory (NSAIDs) [27].

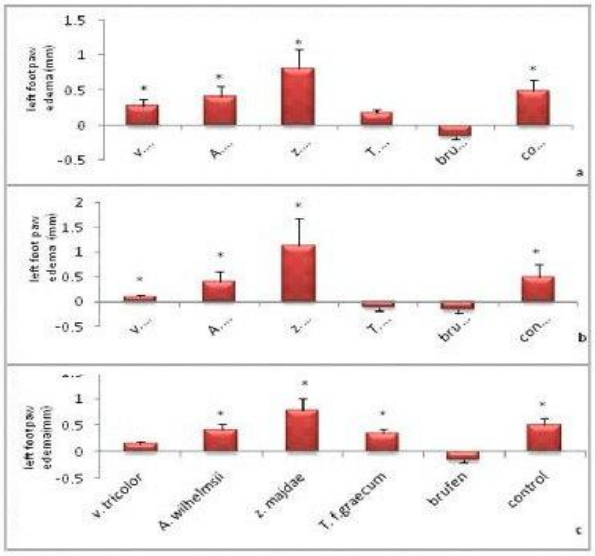

Fig 2. The DFL-DFR show the carrageenan-induced edema in the left foot paw (a): Dose 100mg/kg, (b) Dose of 200mg/kg, (c) Dose of 400mg/kg of each plant extract. *T. foenum graecum* seeds at the dose of 100 and 200mg/kg and *V. tricolor* At the dose of 400mg/kg have shown the most anti inflammatory activity at 1, 2 and 4h after carrageenan administration. Results are expressed as mean ± S.E.M. Differences between the control and treated groups were tested for significance using a one-way analysis of variance (ANOVA).* Significant differences with Ibuprofen group (p<0.05).

The pharmacology of *V. tricolor* has been studies lesser than the other plants. The flowers of this plant are a rich source of many secondary metabolites with anti inflammatory effect such as saponins, flavonoids, salicylic acid, carboxylic phenolic acids (caffeic acid, coumaric acids) and mucilages and have been used in a wide variety of skin disease such as eczema, seborrhea, impetigo and acne [28]. Therefore, the results of this study not only provided partial experimental evidence for the therapeutic efficacy of *V. tricolor* in the treatment of skin inflammatory disease, but also would be beneficial to the future studies and exploitation of this plant. In conclusion, our results in this study showed notably antinociceptive and anti inflammatory activity of all tested plant extracts (except *A. wilhelmsii*), which parallels the traditional use of these plants as analgesic and anti-inflammatory medicine. Our findings justify that only the seeds of *T. foenum-graecum* seeds possess significant antinociception and anti inflammatory effects which may be of potential benefit regarding its gastroprotective effect. This preliminary study needs more pharmacological and toxicological experiments before using of this plant as an official herbal drug for the clinical use. Further works for isolation and identification of the active components of the plant are carrying out.

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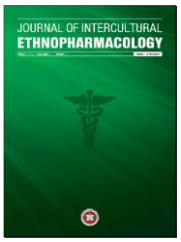

Original Research

Anti-inflammatory activity of *Russelia equisetiformis* Schlecht & Cham: identification of its active constituent

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Keywords:
Russelia equisetiformis, Scrophulariacae, lupeol isolation, Anti-inflammatory activity

Abstract

Aim: The present research work was carried out to isolate and identify the chemical constituent responsible for the antinflammatory activity of *Russelia equisetiformis.*

Method: Isolation was carried out using column chromatography. The structure of the isolated compound was identified and established using the available spectroscopic techniques including high-resolution electron mass spectrum. The anti-inflammatory activity was evaluated using egg albumin-induced paw edema, formaldehyde–induced arthritis and cotton pellet granuloma in-vivo tests. Prednisolone was used as a standard drug.

Results: Lupeol was isolated as colorless crystals (mp 213-215OC). Lupeol at the doses of 10, 20, 40 mg/kg produced significant ($P<0.05$) and dose-dependent inhibition of egg albumin-induced edema and edematous response to arthritis. In chronic model of granuloma pouch in rat, lupeol (20 and 40 mg/kg), significantly ($P<0.05$) and also dose-dependently reduce the granuloma weight.

Conclusion: These findings suggest that lupeol isolated from extract of *Russelia equisetiformis* possesses anti-inflammatory activity in acute and certain aspects of chronic inflammation.

INTRODUCTION

Russelia equisetiformis (Schlect & Cham) belongs to the family Scrophulariaceae [1]. It is a native of Tropical America, commonly known as firecracker, coral and fountain plant and may be cultivated easily, freely naturalized in sandy clearings, long streets, roadsides and grow accidentally in quite a number of places by the roadsides [2, 3]. It is an evergreen shrub with tiny dark green leaves, scale-like in structure, growing on thin rush-like stems. The branches are arched, bearing 1-2 inch long tubular flowers. The plant blooms abundantly round the year, if given optimum conditions [4]. Medicinally, the plant is used for the treatment of diabetes and leukemia in Southwestern, Nigeria [5]. Personal communication with the tribes in this part of Nigeria revealed that, the whole plant is used for the treatment of pain and inflammation.

The methanol extract of the whole plant is reported to have both analgesic and anti-inflammatory activities [6]. Phytochemically, the plant is reported to contain triterpenes of lupane type [7]. The aim of this study is to isolate compound responsible for the antiinflammatory activity of *Russelia equisetiformis.* Therefore, the major compound of n-hexane extract of *R. equisetiformis* was isolated and identified as lupeol and was studied for anti-inflammatory activity.

MATERIALS AND METHODS

The experimental protocols and procedures used in this study were approved by the Ethical committee, University of Ibadan, Ibadan, Nigeria and conform to the guideline of the care and use of animals in research and teaching (NIH publications no 85-93, revised 1985).

Plant material

The plant sample was collected in the month of October, 2010 from Bodija in the South West of Nigeria. The plant was identified in the herbarium of the Forest Research Institute; Ibadan, Nigeria, where voucher specimen was deposited with voucher's umber 106998. The plant sample was air-dried at room temperature, and reduced to powdery form using electric blending machine.

Extraction and isolation of lupeol

A 400 g of powdered whole plant material of *R. equisetiformis*was extracted with 100%methanol in the cold for 72 h; it was filtered and concentrated to dryness using rotary evaporator. The crude methanol extract was re-dissolved in water and partitioned successively with four organic solvents to obtain n-hexane, dichloromethane, ethylacetate and n-butanol fractions. These fractions were screened for antiinflammatory activity using the method described in the section on antiinflammatory tests. The n-hexane was found to be the most active fraction. 10g of hexane fraction packed in a column (92x6cm) with silica gel 60 (0.2-0.5mm) and was bulked using 100% hexane and subsequently hexane: ethylacetate at ratio 90:10, 80:20 and 70:30. Fractions obtained were into 3 different subfractions. The fraction (2.23g) showing TLC profile of lupeol was purified in a column (36x1.5cm) using silica gel 60(0.040-0.06mm) eluted with 50% hexane: ethylacetate in step 85:15, 75:25 and 65:35. TLC was sprayed with 1 % vanillinsulphuric acid reagent and heated to 110o C for 5 min to confirm the presence of lupeol. The structure of compound was established using the available spectroscopic techniques including high-resolution electron mass pectrum 1H NMR (δ [CDCl3 300MHz, 298K], 13C NMR. (δ[CDCl3 Hz, 298K]. Rf 0.32(hexane: Ethyl acetate 9:1 *v/v*). The isolated compound obtained was freshly prepared as a fine homogenized suspension in Tween-80 (2% *v/v*).

Animals

Wistar rats of either sex weighing about 120-160 g, divided into groups of six animals each, were used for this study. The animals were housed in a well ventilated pre- clinical animal house, College of Medicine, University of Ibadan, and were acclimatized in the laboratory for two weeks before experimentation, and fed with standard diet (Ladokun Feeds Nigeria Ltd) and water *ad libitum*.

Evaluation of Anti-inflammatory activity

Egg albumin-induced paw edema in rats

The rat paw edema method as described by [8] was used. Acute inflammation was measured in terms of change in volume of the rat hind paw [9] induced by sub-plantar injection of egg albumin [10 11] Animals of six per group received 10, 20 or 40 mg/kg of lupeol administered intraperitoneally. Thirty minutes later, edema was induced with 0.1 ml of fresh undiluted egg albumin injected into the sub plantar region of the right hind paw of the rats. The volume of distilled water displaced by the treated paw was measured before and after 3 and 24 h induction of edema. Control groups received either equivalent volume of vehicle ((2% *v/v*) or prednisolone (5 mg/kg). Inflammation was assessed as the difference between the zero time volume of the treated paw (*Vo*) and the volume at various times (*Vt*) after the administration of the phlogistic agent. Percentage inhibition of edema was calculated using [12 13] relation.

Inhibition of edema (%) = 100 x (1- $\frac{ax}{by}$)

Where a = mean paw volume of treated rats at various time after egg albumin injection; x = mean paw volume of treated rats before egg albumin injection; b = mean paw volume of control rats at various time after egg albumin injection; y = mean paw volume of control before albumin injection

Arthritis induced by formaldehyde in rats

The formaldehyde-induced arthritis method of [14] was used. On day 1, adult rats of six per group received 10, 20 or 40 mg/kg of lupeol administered i.p. Thirty minutes later; arthritis was induced by sub plantar injection of 0.1 ml of 2.5% formaldehyde solution and repeated on day 3. Arthritis was assessed by measuring the volume of distilled displaced by the paw before induction of arthritis and after induction once every day for 10 days. Control animals received either i.p. Prednisolone (5 mg/kg) or equivalent volume of vehicle (2%, *v/v* Tween 80). The edematous response was quantified as the area under the curve (AUC) of the time-course of the arthritic event. The AUC was calculated using the trapezoidal rule. The level of inhibition of arthritis was calculated using the relation

[1-$\frac{(\text{AUCt})}{\text{AUCc}}$] x100

Where AUCt = AUC of the control group; AUCt = AUC of the treated group.

Cotton-pellet granuloma test in rats

The effect of lupeol isolated from the plant' s extract on chronic inflammation was evaluated using cotton-pellet

granuloma test in rats [15]. On day 1, rats received 10, 20 or 40 mg/kg i.p. of lupeol. Control animals received either Prednisolone (5 mg/kg) or equivalent of vehicle (2% *v/v* Tween 80). Thirty minutes later autoclaved cotton pellets 50±1 were implanted on the back of the rats under diethyl ether anesthesia. Drugs were administered daily for 7 days. On day 8, animals were killed by overdose of ether. The pellets were dissected out, dried in an oven at 60^{o} Cand weighed to determine the level inhibition of granuloma. The level of inhibition of granuloma tissue development was calculated using the relation:

$$\frac{Tc - Tt_X}{Tc} 100$$

Data analysis

Data were expressed as mean ± SEM. Statistical analyses was performed by one-way, ANOVA followed by Dunnett' s test. P values < 0.05 were considered significant

RESULTS

Characteristically, lupeol showed a violet spot on TLC when sprayed with 1 % vanillin-sulphuric acid reagent and heated at 110^{o} C for 5 min. Lupeol was isolated as colorless crystals (mp 213-215^{O}C). The spectral data was found in accordance with the literature data of lupeol [16 17].The ^{13}C NMR spectra showed 28-carbon atoms giving a characteristic of pentacyclic saturated system and only one unsaturated carbon absorption at δ 150.9 ppm for alkenes. The hydroxyl carbon was found at δ 78.9. The compound was identified by comparison of its NMR spectrum with literature for Lupeol, a pentacyclic compound already isolated from other plants (Fig. 1)

^{1}H NMR ($CDCl_3$): δ 0.70 (3H, s); 0.76 (6H, s); 0.80 (3H, s); 0.92 (3H, s); 1.00 (6H, s);

^{13}C-NMR ($CDCl_3$): δ 40.0 – 48.2(C); 55.3 (CH, t); 50.4 (CH, t); 25.1 (CH_2, d); 150.9 (CH_2, s); 15.4 – 20.9 (CH_3, s); and 78.9 (ROH, t).

Lupeol produced dose-dependent inhibition of egg albumin-induced paw edema at10, 20 and 40 mg/kg after 24 h as compared to prednisolone (5 mg/kg) (Table 1). Investigation of the effect of lupeol on the proliferative phase of inflammation revealed that lupeol isolated from the hexane fraction demonstrated significant (*P>0.05*) inhibition of global edematous response to formaldehyde-induced arthritis in a dose-dependent manner, with maximum inhibitory effect of 56 %, greater than a standard anti-inflammatory steroid drug, prednisolone (50.8 %) (Table2). However, in the cotton pellet granuloma test, lupeol was not much effective as standard anti-inflammatory steroid drug (prednisolone) in inhibiting the growth of granuloma tissue (Table 3).

Table 1. Effect of lupeol isolated from R. equisetiformis on egg albumin-induced edema

Treatment	Dose mg/kg Or mg/kg	Edema volume (ml)		% inhibition	
		3 h	24 h	3 h	24 h
Control (2%Tween80	10	0.36± 0.01	0.31±0.01	-	-
Lupeol	10	0.34±0.01	0.24±0.01*	5	23
	20	0.32±0.01	0.21±0.01*	11	33
	40	0.28±0.01*	0.18±0.01*	23	44
Prednisolone	5	0.25±0.01*	0.15±.0.01*	31	55

*P< 0.05 vs. Control. Values of edema are mean ± S.E.M (n =6)

Table 2. Effect of lupeol isolated from R. equisetiformis on formaldehyde-induced arthritis in rats.

Treatment	Dose mg/kg Or mg/kg	Edema volume (ml)	% inhibition
Control (2%Tween80	10	4.59±0.06	-
Lupeol	10	2.52± 0.03*	45.0*
	20	2.18±0.03*	52.5*
	40	2.01±0.03*	56.0*
Prednisolone	5	2.33±0.03*	50.8*

*P< 0.05 vs. Control. Values of edema are mean ± S.E.M (n =6)

Table 3. Effect of lupeol isolated from R. equisetiformis on cotton pellet granuloma

Treatment	Dose mg/kg or mg/kg	Cotton pellet granuloma	
		Weight ofgranuloma (mg)	% inhibition
Control (2%Tween80)	10	186.67±1.21	-
Lupeol	10	171.34±1.24	8.0
	20	145.52±2.02*	22*
	40	128.15±2.24*	31*
Prednisolone	5	92.3±1.65*	50.6*

*P< 0.05 vs. Control. Values of granuloma weight shown are mean ± S.E.M (n =6)

DISCUSSION

Anti-inflammatory drugs, presently available for the treatment of various inflammatory disorders have one or more adverse or side-effects [18].The presence of anti-inflammatory activity in triterpenes seems interesting, since they possess hydro-aromatic ring system, which is more or less similar to that of steroidal antiiflammatory drugs, devoid of side-effects [19]. The inhibition of lupeol of the acute phase of edema caused by egg albumin, suggests that lupeol may suppress both the early and later phases of the acute inflammatory response. The lupeol may have inhibited the release or actions of the various chemical mediators such as easily histamine, 5HT, kinins and prostanoids known to mediate acute inflammation induced by phlogistic agents such as egg albumin.[20 21, 22, 23, 24, 25]. The disparity in the activity in the two chronic inflammatory models (formaldehyde-induced arthritis and cotton pellet granuloma), suggests that lupeol may predominantly affect certain aspects of inflammatory response associated with formaldehyde arthritis, while exerting little or no effect on the process involved in granulomatous inflammation. Granuloma of chronic inflammation comprises an accumulation of modified macrophages arranged in small clusters or nodular collections or surrounded by a cuff of lymphocytes [26] and is a consequence of cell-mediated immunity [27]. The inability of lupeol to inhibit granulomatous inflammation may be due to its inability to inhibit the accumulation of macrophages and lymphocytes in chronic inflammation and therefore has no effect on cellular mediated immune responses. However, these macrophages and lymphocytes at the inflammation or injury site are known to secrete peptide growth factors (PGF), which partly mediate the process of healing and repair [28]. It implies therefore, that lupeol may promote tissue repair by mediating the pro-healing actions of PGF, inhibit the expression of tissue necrotic factor (TNF), which is known to exacerbate the later stage of formaldehyde-induced arthritis [14]. Necrotic tissue on its own can perpetuate inflammation through several mechanisms such as the release of mediators from dead or dying passenger leukocytes.[29] Thus, it is possible that lupeol isolated from *R. equisetiformis* may modulate the arthritis event through mediators' release inhibition by preventing formaldehyde-induced tissue necrosis as well as tissue destruction seen in arthritis. However, it may have little inhibitory effect on cellular response associated with chronic inflammation. Also in arthritis, there is increase in the level of lipid peroxide, superoxide dimutase (SOD), glutathione peroxidase (GPx) and catalase (CAT).[30]. Lupeol and its esters have been reported to reduce the level of the above mentioned enzyme involved in lipid peroxidation in arthritic-induced animals [31]. Therefore, it is not unlikely, that lupeol isolated from *R. equisetiformis* extract demonstrated its anti-arthritic action, by reducing the alterations induced in arthritic animals in the levels of lipid peroxide, SOD, GPx and CAT. In summary, the major compound isolated from the hexane extract of *R. equisetiformis* possesses anti-inflammatory activity and may be the reason for its traditional use in inflammatory disease conditions. This study confirms that, the compound responsible for the anti-inflammatory activity is lupeol.

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Original Research

Preliminary phytochemical screening and evaluation of antibacterial activity of *Dichrocepala integrifolia* (L.f) O.kuntze

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Antibacterial activity, phytochemical screening, disc diffusion, broth dilution, Dichrocepala integrifolia

Abstract
Background: More than 80% of all common disease in Ethiopia is of infectious origin. Traditional medicine plays a great role in combating this disease. Dichrocepala integrifolia (L.f) O.kuntze is one of the traditional remedies used for treatment of wound infection.
Objective: To screen the major phytochemical constituents and evaluate antibacterial activity tests of leaf extracts of Dichrocephala integrifolia (L.f.) O. kuntze.
Methods: experimental study involving gradient extraction, disc diffusion and broth dilution were conducted. Phytochemical screenings were also done to assess the presence of major secondary metabolites.
Result: Chloroform extracts were the most effective where as ethanol extract were the least effective. S. typhi and pseudomonas aeruginosa were found to be the most sensitive and resistant microorganisms to all extract used at all test concentration, respectively. Accordingly from all test strains, S.typhi was inhibited by the minimum concentration of chloroform extract (MIC=125mg/ml).
Conclusion and Recommendation: In general the antimicrobial activity of the plant observed here support the tradition therapeutic claim of the society. Further study should be conducted in further evaluating its antimicrobial effectiveness and also purification of the active chemical constituents that could be responsible for its biological activity.

INTRODUCTION

Starting from the ancient time, medicinal plants have been used to prevent and treat various health problems. Plants are still an independent source of medication in the contemporary health care delivery system. Their role is twofold in the development of medicines and served as a natural blue print for the development of new drugs [1-3].

About 80% of the population in Ethiopia relies on herbal medicines. The use of herbs for remedies represents not only part of the struggle of the people to meet their essential drug needs but also it is integral component of the cultural beliefs and attitudes. More than 95% traditional preparations in the country are of plant origin. Anti microbial and wound healing plant products are among remedies that are commonly available in the markets [4-6].

In general these medicinal preparations have paramount importance in treatment of infectious diseases in Ethiopia where it accounts 80% of all common diseases. Though the currently available anti infective drugs are critically important in reducing the global burden of infectious disease, emergency of resistant strains limits their usefulness. So there is considerable incentive to discover new antimicrobial agents from plants [5-6].

Different researches have been conducted in different

countries worldwide in search for new antibacterial agents from traditionally used herbs. The screening of plant extracts and plant products for antimicrobial activity has shown that plants represent a potential source of novel antibiotic prototypes [7-10].

Though numerous medicinal plants are available in Ethiopia to be considered as potential sources of medicines, scientific studies supporting the traditional claims are lacking. *Dichrocephala integrifolia (L.f.) kuntze* is one of the commonly used traditional herbs used for treatments of wound infection and other ailments in Ethiopia [8].

Only limited studies are available worldwide regarding the antimicrobial activities of *Dichrocephala integrifolia (L.f.) kuntze* and its families. Ones study done on the same plant family indicated anti-inflammatory and anti-swelling activity of *Dichrocephala integrifolia (L.f.) kuntze*. The plant is also known to promote circulation for irregular menses and sprains. Another study done *Saussurea lappa coustus* of the same family of the plant again showed antimicrobial activity of its methanol extract against *S.auerus, E. coli* and *S. typhi* [11-13].

Despite of lacking scientific proof in assuring the antimicrobial activity of *Dicrocephala integrifolia (L.f) O.kuntze***,** it is commonly used by the societies for treatment of various ailments. So, the purpose of this study was to screen the major phytochemical constituents and evaluate antimicrobial activity of the leaf extracts to support the traditional therapeutic claim and to provide base line information for the scientific communities to carry on further study.

MATERIALS AND METHODS

Study design and period:

Laboratory based Experimental study design was conducted in pharmacy and biology laboratory of Jimma University from May, 15 to Aug 20, 2011.

Plant material

The fresh leaves of *Dicrocephala integrifolia (L.f) O.kuntze* were identified and collected in January 2011at '*Seto Semero'* and '*Mendera Kochi'* kebele of Jimma town and its botanical identity was confirmed by Dr. M. Remeesh department of Biology, Collage of natural science, Jimma University.

Preparation of Extract

The collected leaf of *Dichrocephala integrifolia (composite)* was dried, powdered and weighed. The gradient solvent extraction of powdered plant extract (70 mg) using soxhlet apparatus were undertaken using petroleum ether, ethyl acetate, chloroform and ethanol (80%), in an increasing order of polarity. After the plant parts were successively and exhaustively extracted with the solvents, each fraction was dried, weighed and screened for antimicrobial activity.

Microorganisms

Escherichia coli (ATCC 33849), *salmonella typhi* (ATCC 33458), *staphylococcus aureus* (ATCC 25923), *pseudomonas aerugenosa* (ATCC 27853), which are commonly known to cause wound infection were used to screen the antibacterial activity of the plant extracts.

Antibacterial screening

Disc diffusion methods were employed for antimicrobial screening of the extracts. The standard strains were reactivated and adjusted for the turbidity of the inoculums suspension using 0.5 McFarland's technique.

The dried surface of a Mueller-Hinton agar plate (MHA) (prepared by using 20ml of MHA on 150mm diameter Petri dish) was inoculated by streaking the swab over the entire sterile agar surface. This procedure was repeated by rotating the plate approximately 60° each time to ensure an even distribution of inoculums. Five different concentrations (200, 100, 50, 25, 12.5mg/ml) were prepared by dissolving the dried extract in the respective solvents used during gradient extraction. The sterile paper discs (what man filter paper 6mm in diameter) were impregnated with 20µl of the above extract. The discs loaded with the extract was placed on Muller-Hinton agar plates, which were previously inoculated with test strains and incubated at 37^{o}C for 16-18 hours. After incubation, inhibition zones were recorded as the diameter of the growth free zones around the disc using sliding vernier caliper in millimeters. Negative controls were prepared in the same way but using 20µl of pure solvent on sterile discs. Ciprofloxacin (5µg/disc) were served as positive control for *S.aureus, P.aeruginosa* and *E.coli* [14].

Determination of Minimum Inhibitory Concentration (MIC)

The Minimum Inhibitory Concentration (MIC) of the extracts was determined for each of the test organisms in test tubes. For these test, 0.5 ml of varying concentrations of the extracts (25, 50, 75, 100, 125, 150, 175, 200,225 and 250 mg/ml) were taken in the test tubes and nutrient broth (2 ml) were added and then a loop full of the test organism. Two control tubes, antibiotic control (tube containing extract and growth media without inoculums) and organism control (tube containing the growth medium, saline and the inoculums), were maintained for each test batch. The lowest concentration of the extract that produced no visible bacterial growth (no turbidity) when compared

with the control tubes were taken as MIC [15].

Phytochemical screening

The concentrated residue from the gradient solvent extracts of the plant material was used to detect the secondary plant metabolites including alkaloids, glycoside, flavanoids, phytosterols, saponins, tannins, carotenoids and anthraquinones based on the methodology described by Asfaw Debella [13].

RESULTS

The percentage yield of each fraction of *Dichrocephala integrifolia* is summarized in [table 1]. The maximum yield was obtained from the petroleum ether extracts (12.86%w/w) followed by chloroform extracts (10.14%w/w).

As it is clearly indicated in the table 2 below, preliminary phytochemical analysis showed alkaloids, saponnins, carotinoid and tannins detected in petroleum extract while phytosterols and saponins detected in ethanol extract. Alkaloids were detected in petroleum ether, ethyl acetate and chloroform fractions.

Regarding the antibacterial activity, all crude extract except ethanol exhibited an inhibitory effect against *S.aureus, E.coli and S. typhi* in a dose dependent manner as it is clearly shown in table 3. The chloroform extract found to be effective against all test strains used in the study except *pseudomonas aeruginosa*. On other hand the ethanol extract is only effective only against *E.coli and S. typhi* at high dose. *S. typhi* were the most sensitive but *P.aeruginosa* was the most resistant test strains to any of the plant extract examined at all test concentration.

The MIC values were also determined using broth dilution method for the most active extract, for Petroleum ether and chloroform extract, as demonstrated in table 4. Chloroform extract showed the lowest MIC value against *S. typhi* (125mg/ml) followed by petroleum ether extract against *S. typhi* (200mg/ml).

Table 1. Percentage yield of *Dichrocephala integrifolia* leaf gradient solvent extracts.

Plant species	Plant part used	Percentage (w/w) yield of the organic fractions			
		Petroleum ether	Ethyl acetate	Chloroform	Ethanol (80%)
Dichrocephala integrifolia	Leaf (70gm)	12.86	7.14	10.14	3.34

Table 2. Results of the phytochemical screening of the extracts of *Dichrocephala integrifolia*.

Major phytochemical tested.	fractions analyzed			
	Petroleum ether	Ethyl acetate	chloroform	Ethanol (80%)
Alkaloids	+	+	+	-
Glycosids	-	-	-	-
Phytosterols	-	+	+	+
Flavanoids	-	-	-	-
Saponins	+	+	-	+
Carotenoids	+	-	-	-
Anthraquinones	-	-	-	-
Tannins	+	-	+	-

*+ detected, - Not detected

Table 3. Antibacterial activity of leaf extract of *Dichrocephala integrifolia*

Fraction analyzed	Concentration (µg/disc)	Zone of inhibition (mm)			
		E.coli	*S.aureus*	*P.aeruginoa*	*S.typhi*
Petroleum ether	250	-	-	-	6
	1000	7	-	-	11
	2000	10	5	-	13
	3000	11	9	-	15
	4000	12	10	-	15
Ethyl acetate	250	-	-	-	4
	1000	5	-	-	5
	2000	6	3	-	8
	3000	7	5	-	8
	4000	8	6	-	9
Chloroform	250	6	4	-	9
	1000	11	8	-	13
	2000	13	9	-	15
	3000	15	9	-	20
	4000	16	11	-	22
Ethanol (80%)	250	-	-	-	-
	1000	-	-	-	-
	2000	-	-	-	5
	3000	5	-	-	7
	4000	6	-	-	8
Ciprofloxaciline	5	31	24	27	30

Table 4. Minimum inhibitory concentration of petroleum ether and chloroform extract of *Dichrocephala integrifolia*

Fraction analyzed	Minimum inhibitory concentration (mg/ml)		
	E.coli	*S.aureus*	*S.typhi*
Petroleum ether	225	275	200
Chloroform	200	250	125

DISCUSSION

The limitation of this study was, the antibacterial activities of the phytochemical constituents of studied plants were not investigated because of laboratory facility constraints.

In this study the highest percentage yield was obtained from Petroleum ether extract (12.86%). The maximum yield from petroleum ether might be important for further commercial production if the plant found to be antimicrobially effective.

In antimicrobial activity screening, it is the chloroform extracts of *dichrocephala integrifolia* that exhibited maximum bacterial growth inhibition. It is found to be very active against most microorganisms used similar to the study done in India. Another study done on similar species of *dichrocephala integrifolia* also indicate that petroleum extract exhibit the highest activity on most test microorganism which still support the present study since both are non polar portion of the plants that are antimicrobial active.

The alcoholic (ethanol) extract of this extract exhibit least activity than other plant extract used in the study. It was only active against *E.coli and S. typhi* which is still in general agreement with the methanol extract of *Saussurea lappa coustusc* of the same family with

dichrocephala integrifolia (composite) which had an activity against only E.coli but not *S. typhi* [13]. The activity difference of these two plants regarding *S. typhi* might be explained by test strain to strain differences and physicochemical difference of the plant.

From this study the lowest MIC value of chloroform extract 125 mg/ml was demonstrated against *S. typhi*, while the MIC values ranging between 200-275 mg/ml were demonstrated against the rest of the test bacteria. MIC value of chloroform extract is found be different from MIC value of *Holeptela integrifolia*, (50 mg/ml) [13]. This difference could be due to a number of factors such as time of collection of the plant material and climatic condition difference which might in turn affect the amount of active constituents in the plants.

The antimicrobial activity of *Dichrocephala integerifoliea leaf* extracts observed in this study might be due to the alkaloid, phytosterols, tannins, flavonoids and carotenoids detected to be present in the plant. But, their antibacterial activity remain to be proofed

In general the antimicrobial activities of the plant extract is found to be low compared to the standard antibiotics (ciprofloxacin) used in these study. But it is hoped that they might produce comparable effect after further purification and analysis of the active constituents.

In conclusion, the plant extract displayed an activity against *E. coli*, *S. aureus* and *S. typhi*, which could support the traditional claim of the society. So based on the findings, the authors recommended further study to be conducted concerning the chemical compositions and the structure elucidation of the active component of the plant.

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Original Research

Antimicrobial and phytochemical evaluation of the leaf, stem bark and root extracts of *Cyathula prostrata (L)* Blume against some human pathogens

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Cyathula prostrata, aqueous extract, ethanol extract, antimicrobial activity

Abstract
The antimicrobial activities of aqueous (cold and hot) and ethanolic extracts of leaf stem bark and root of *Cyathula prostrata* were investigated against some human clinical isolates of *Staphylococcus aureus, Streptococcus mutans, Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Salmonella typhi,* and *Candida albicans* using the Agar well diffusion method at extract concentration of 25mg/ml. Ciprofloxacin(5µg/ml) and Fluconazole (20µg/ml) drugs was used as positive reference standards to determine the sensitivity of the strains. Results obtained showed that all the test isolates were inhibited by various fractions of the leaf, root and stem bark extracts. The antimicrobial activities of the different plant parts were not significantly different ($P<0.05$), though the greatest activities were observed with the ethanolic fractions (14.0-25.5 mm), followed by the hot water (12.0-24.2 mm) and cold extracts (13.0-18.5 mm). An inhibition range of 24.0-25.5mm and 28.5mm were observed from ciprofloxacin and fluconazole drugs respectively. The percentage susceptibility of the most sensitive bacterial isolate (*E. coli*,) was 95.9% while the least (*K. pneumoniae*) had 40.0% sensitivity. *Candida albicans* had a percentage susceptibility of 57.5%. The minimum inhibitory concentration (MIC) ranged between 400 and 800µg/ml. The observed phytochemical compounds were saponins, tannins, flavonoids, alkaloids, cardiac glycosides and steroids. This study has justified the applications of *Cyathula prostrata* in the traditional herbal medicines and therefore holds a promise as a potential source of novel broad spectrum drug for treating infectious diseases.

INTRODUCTION

Over the years, plants and their extracts have been applied as herbal remedies for diverse human ailments. Presently, plant is still being utilized by numerous developing countries as sources of therapeutic agents because they believe medicinal plants are readily available, accessible, affordable, potent, and with relatively lower incidences of adverse reactions compared to modern conventional drugs [1]. Base on the growing knowledge of potency of traditional medicinal plants and coupled with fact that numerous infections agents are becoming resistant to synthetic drugs, researchers all over the world have intensified the screening of these acclaimed medicinal plants in order to provide a documented scientific backing and ultimately recommend them as novel sources of future antimicrobial agents. Therefore, the continuous screening of these acclaimed medicinal plants by scientists cannot be overemphasis.

Currently, several researchers have reported that numerous tropical plant possess antimicrobial properties against pathogenic micro-organisms. In their report, [2] demonstrated the activity of *Terminalia avicennoides* against *Vibrio cholerae* and *Salmonella typhi*. In the same vein, [3] reported the antimicrobial

activity of the leaf extract of *Anacardium occoccidentale* and *Gossypium hirsutum* against *Escherichia coli, Shigella dysentriae, Salmonella typhimurium, Staphylococcus aureus* and *Pseudomonas aeruginosa*. The leaf extract of *Kalanchoe pinnate* displayed antimicrobial activity against *Stsaphylococcus aureus, Escherichia coli, Bacillus sutilis, Proteus vulgaris, Pseudomonas aeruginosa* and *Candida albicans [*4]. In their studies, [5] reported that the root, stem bark and root extracts of *Parkia Clappertoniana* demonstrated significant antimicrobial activities against commonly implicated human clinical pathogens. Also, the stem bark extracts of *Vitellaria paradoxa* and *Caesalpinia pulcherrima* were reported possess significant antibacterial activities against some enteric pathogens like *Escherichia coli, Salmonella typhi, Klebseilla pneumoniae, Proteus mirabilis, and Shigella dysentriae* [6, 7]. Furthermore, the seed extracts of *African* nutmeg (*Monodora myristica*) were currently documented to possess broad spectrum antibacterial properties against some human pathogens [8]. With these promising results, there is still the need to search for more plants of medicinal value so as to complement the available arsenal of drugs and ultimately, increase the array of choices required for effective chemotherapy.

Cyathula prostrata (L) Blume (Amaratheceae) is an annual, branched herb/shrub reaching up to 1m with stem trails on the ground and bears leaves which are rhomboid-oblong and adhesive fruits [9]. Traditionally, various preparations of the leaves, stems and roots of this plant are used to treat a range of illnesses including articular rheumatism, cough, skin diseases, scabies, craw-craw, snake bites, bruises, liver problem, dysentery, diarrhoea, nausea, cholera vomiting blood, and many others in Nigeria and other African countries [9-11]. Among the Kurichayas tribe of Kannur District, a tea spoon of the dried powdered root is boiled in water and taken thrice daily as cure for fever [12]. When mixed with other plants (*Synedrella nodiflora* and *Aframomum melegueta*), and clay, it is used to treat heart trouble and bronchial infections while the fruit has been claimed to prevent miscarriages [9]. Scientically, the methanolic extract of *Cyathula prostrata* has been documented to be relatively non toxic in albino mice [11]. Also, it was recently documented that the methanolic extract of this plant possesses anti-inflammatory and analgesic properties, justifying its application in the traditional management of ailments associated with pains among others [13].

Despite the arrays of traditional applications to which the leaf, stem and root of *Cyathula prostrata* are subjected to, available literature revealed that there is paucity of information on the scientific elucidation of these plants as remedy for the acclaimed related ailments.

This study was therefore undertaken to evaluate the phytochemical properties and antimicrobial activities of aqueous (cold and hot water extract) and ethanolic extracts of this plant against *Staphylococcus aureus, Streptococcus mutans, Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Salmonella typhi* and *Candida albicans* which are routinely implicated in gastro-intestinal infections, oral diseases, wounds and other skin diseases in order to establish a scientific evidence for their traditional usage in Africa.

MATERIALS AND METHODS

Collection and Preparation of Plant material

Fresh plants of *Cyathula prostrata* were collected from farmland around the Amai Campus of Novena University, Delta State, Nigeria and were identified at the Department of Biological Sciences of the university courtesy of Prof. J. M. O. Eze (Botany unit). The leaves, stem, and roots were then washed thoroughly, separated and air-dried to crispiness on the laboratory workbench (prevailing room temperature of 30 ± 2^0C) for two weeks. The dried materials were reduced to coarse form using a pestle and mortar and further pulverized to very fine particles with an electric blender (Super Search Model 2815). The powdered leaves stem and root samples obtained were stored separately in polyethylene bags until needed for analysis.

Sterilization of materials

All glassware used in this research were washed with detergent, rinsed with distilled water, air dried and sterilized on a hot air oven at 121^0C for 2 hours. Each of the materials was wrapped with aluminium foil before sterilization. Distilled water and all prepared media were sterilized in the autoclave at 121^0C for 15 minutes. Cork borers and glass rods were sterilized by dipping into 70% alcohol prior to flaming in a Bunsen burner. The working bench was swabbed with 75% alcohol before and after each experiment.

Source of test micro-organisms

The pure clinical isolates of *Staphylococcus aureus, Streptococcus mutans, Escherichia coli, Bacillus cereus, Pseudomonas aeruginosa, Klebsiella pneumoniae, Proteus mirabilis, Salmonella typhi,* and *Candida albicans* were collected from Lahor Research Diagnostic and Environmental Consortium (LRDEC) in Benin City, Nigeria. The cultures were maintain at 35°C on nutrient agar (bacteria) and sabouraud dextrose agar (fungi) and used for the study

Standardization of test organisms

Prior to antimicrobial sensitivity test, 0.2 ml of

overnight culture of each organism was dispensed into 20 ml of sterile Mueller Hinton Broth (Hi-Media, India) and then incubated about 16-24h to standardize the cultures to approximately 10^6cfu/ml [14].

Extraction of Plant Material

Cold water Extraction: 200g of each powdered sample was soaked in 500ml of sterile distilled water, agitated manually, and allowed to extract for 48hours, before each extract was filtered using Whatmann No 1 Filter paper. The filtrates were evaporated in a water bath at 50^{o}Cto dryness. The yields were 13.5%w/w, 13.9%w/w, and 14.0%w/w the leaf, stem and root extracts respectively. The extracts were stored at 4^{o}C until needed.

Hot water Extraction: 200g of each weighed plant materials was soaked in 500ml of hot water boiled for 30minutes into a conical flask for 48 hours. Each extract was filtered using filter paper and evaporated to dryness using water bath at 50^{o}C. The yields were 14.5%w/w, 13.8%w/w, and 14.0%w/w the leaf, stem and root extracts respectively. The extracts were stored at 4^{o}Cuntil needed.

Ethanol extraction: 200g of the plant samples were soaked in 500ml of absolute ethanol for 48h at room temperature with occasional stirring. The content was filtered and evaporated to dryness in a water bath at 50^{o}C. The yields were 14.5%w/w, 15.4%w/w, and 14.0%w/w the leaf, stem and root extracts respectively. The extracts were collected and stored in the refrigerator at 4^{o}C until required for assay.

Sterility Test of the Plant Extracts

Each of the above extracts ethanolic and aqueous (cold and hot) extract) were tested for growth or contaminants. This was carried out by inoculating 1ml of each of them on sterile Mueller Hinton Agar and incubated at 37^{o}C for 24 hours. The plates were observed for growth. No growth in the extracts after incubation indicated that they were sterile. The different extracts were then assessed for antimicrobial activity.

Antimicrobial susceptibility testing

The agar well diffusion method of [14] with slight modification, was adopted for this assay. Mueller Hinton Broth (Hi-Media, India) was prepared as specified by the manufacturer, autoclaved and poured ascetically into sterile Petri dishes and allowed to gel. Then a loopful of the standardized bacterial cell suspension (10^6cfu/ml) was streaked evenly on each gelled agar plate. The leaf, stem bark and root extracts were reconstituted in 20% DimethylSulfoxide (DMSO) to obtain the working concentrations of 25mg/ml. 200µl of each extract was inoculated into three wells (6 mm Diameters) earlier bored with a sterile cork borer in each plate. The negative control was 200µl of 20% DMSO, while the positive control were 5µg/ml of ciprofloxacin (Ranbaxy Pharmaceuticals India) and 20µg/ml of fluconazole (Greenlife, pharmaceuticals India). The plates were allowed to stand for 30minutes on the work bench for pre-diffusion of the extracts to proceed before the growth of the organism commenced. The plates were incubated at 37°C for 24 h. The whole experiment was carried out in triplicate and the antibacterial activity of the extracts were determined after incubation period by measurement of mean diameter zones of inhibition produced by the extracts against the test organisms and results were recorded in millimeters(mm) using a transparent ruler.

Determination of the minimum inhibitory concentration (MIC)

The plant extracts that demonstrated significant antibacterial activity by the agar well diffusion method were subjected to MIC assay using the broth dilution method of [15]. One ml of 24 h culture of test organisms (10^7 CFU/ml) adjusted to McFarland turbidity standard were incubated in serial dilution of 100, 200, 400, 600, 800 and 1000 µg/ml of plant extracts in physiological saline at 37°C for 24 h. The concentration at which the lowest dilution with no detectable bacterial growth was considered as minimum inhibitory concentration (MIC).

Phytochemical screening of Extracts

The different extracts of *Cyathula prostsrata* were tested for the presence of phytochemicals such as steroids, saponins, alkaloids, flavonoids, terpenoids, cardiac glycosides, and tannins using the standard procedures described by [16].

Test for alkaloids. 0.5g of the sample was accurately weighed and defatted with 5% ethyl ether for 15mins. The defatted sample was extracted for 20mins with 5.0ml of aqueous HCl on a steam bath. The resulting mixture was centrifuged for 10mins at 3000rpm to remove filtrate (Supernatant). 1.0ml of the filtrate was treated with a few drops of Mayer's reagent and a second 1.0ml portion was treated similarly with Dragendorff's reagent. Turbidity or precipitation with either of these reagents was taken as evidence for the presence of alkaloids

Test for saponins: The ability of saponins to produce frothing in aqueous solution was used as screening test for the sample. 0.5g of dried extract was shaken with water in a test tube, frothing which persist on warming was taken as evidence for the presence of saponins.

Test for tannins: 5.0g of dried extract was stirred with 10.0ml of distilled water. This was filtered and ferric chloride reagent was added to the filtrate. A blue-black precipitate was taken as evidence for the presence of

tannins

Test for cardiac glycosides: 0.5g of dried extract was dissolved in 2.0ml of glacial acetic acid containing one drop of ferric chloride solution. This was then under laid with 1.0ml of concentrated H_2SO_4. A brown ring obtained at the interface indicated the presence of a cardenolides.

Test for flavonoids: 1.0ml of 10% lead acetate was added to 1.0ml of the extract contained in a test-tube. A formation of a yellow precipitate was taken as positive for flavonoids.

Test for steroids: 0.5g of the dried extract was extracted with 2.5ml of chloroform in a test tube and 1ml of concentrated sulphuric acid added to form a lower layer. A reddish-brown interface indicated the presence of steroids.

Test for Terpenoids: 0.5ml 0f the chloroform extract of the dried extracts was evaporated to dryness on a water bath and heated with 3ml of concentrated sulphuric acid for 10minutes on a water bath. A grey colour indicated the presence of terpenoids.

Statistical treatment of the results

The results were expressed as means ± standard error (SE). Significance of differences compared to the control groups was determined using students t-test.

RESULT

The antimicrobial activity test of *Cyathula prostsrata* showed that all the test isolates were susceptible to the organic and aqueous extracts at 25mg/ml concentration used (Table 1, 2, and 3). Generally, the root extract demonstrated the most inhibitory activity followed by the leaf extract and then the stem bark being the least, though their activities were not significantly different ($P<0.05$). The zones diameter of inhibition observed ranged between 14.0 – 25.5 mm for ethanolic extracts, 12.0 – 24.2 mm for cold water extracts and 13.0 – 18.5 mm for hot water extracts as against an inhibition range of 24.0-26.5mm and 28.5mm observed from ciprofloxacin and fluconazole drugs respectively. With the ethanolic extracts, at concentration of 25mg/ml, the most susceptible organisms (inhibition diameter>19.5mm) were *E. coli, S. aureus, P. aureuginosa, and S. typhi,* while the least susceptible isolates (inhibition diameter<14.5mm) were *K. pneumoniae* and *B. cereus* (Table 1). With the hot aqueous extracts, at concentration of 25mg/ml, only *E. coli,* and *P. aureuginosa* were the most sensitive (inhibition diameter>19.5mm) isolates, while the least susceptible isolates (inhibition diameter<14.5mm) were *P. mirabilis, E. coli,* and *P. aureuginosa* (Table 2). The result in Table 3 however shows that non of the cold aqueous extracts at 25mg/ml exhibited antimicrobial inhibition greater than 19.5mm, but had several inhibition zones that were less than 14.5mm against *S. mutans, C. albicans, P. mirabilis, E. coli* and *P. aeruginosa.* When compared with the reference standard drugs (Ciprofloxacin -5µg/ml and Fluconazole- 20µg/ml) used, it was observed that the percentage susceptibility of the most sensitive bacterial isolate (*E. coli,*) was 95.9% while the least (*K. pneumoniae*) had 40.0% sensitivity. While *C. albicans* had a percentage susceptibility of 57.5% (Table 1, 2, and 3).

Table 1. Diameter of Zones of inhibition of the test bacterial species to the ethanolic extracts and controls

Test Isolate	Leaf (25mg/ml)	Stem bark (25mg/ml)	Root (25mg/ml)	CIP (5µg/ml)	FLU (20µg/ml)	DMSO 20%
S.aureus	22.4±0.01	22.5±0.11	24.0±0.01	26.0	ND	-
S.mutans	15.0±0.00	15.1±0.01	15.5±0.05	25.0	ND	-
E.coli	25.0±0.01	24.5±0.04	25.5±0.03	26.5	ND	-
B.cereus	14.4±0.11	14.2±0.33	14.0±0.25	26.0	ND	-
P.aeruginosa	24.2±0.01	22.5±0.11	22.0±0.00	24.5	ND	-
K.pneumoniae	14.3±0.01	14.0±0.15	14.4±0.02	25.0	ND	-
P.mirabilis	15.5±0.11	15.0±0.12	16.0±0.00	24.0	ND	-
S.typhi	20.5±0.04	21.1±0.21	22.0±0.00	26.0	ND	-
C.albicans	16.4±0.11	15.2±0.14	16.0±0.24	ND	28.5	-

** Results are means of three replicate diameter zones of inhibition values (mm) ± standard deviations (SD), CIP= Ciprofloxacin; FLU= Fluconazole; 20% DMSO=Dimethylsulfoxide; -=No Inhibition.*

Table 2. Diameter of zones of inhibition of the test bacterial species to the hot water extracts and controls

Test Isolate	Leaf (25mg/ml)	Stem bark (25mg/ml)	Root (25mg/ml)	CIP (5µg/ml)	FLU (20µg/ml)	DMSO 20%
S.aureus	18.2±0.01	19.0±0.21	19.0±0.51	26.0	ND	-
S.mutans	14.5±0.00	15.0±0.02	14.5±0.12	25.0	ND	-
E.coli	24.0±0.03	23.5±0.04	24.2±0.05	26.5	ND	-
B.cereus	13.1±0.11	13.5±0.03	13.5±0.15	26.0	ND	-
P.aeruginosa	20.2±0.31	19.6±0.01	20.0±0.10	24.5	ND	-
K.pneumoniae	13.4±0.00	13.0±0.10	13.2±0.32	25.0	ND	-
P.mirabilis	14.0±0.21	14.2±0.22	13.0±0.10	24.0	ND	-
S.typhi	19.2±0.03	18.1±0.22	19.9±0.10	26.0	ND	-
C.albicans	15.1±0.21	14.5±0.24	14.5±0.04	ND	28.5	-

** Results are means of three replicate diameter zones of inhibition values (mm) ± standard deviations (SD), CIP= Ciprofloxacin; FLU= Fluconazole; 20% DMSO=Dimethylsulfoxide; -=No Inhibition.*

Table 3. Diameter of zones of inhibition of the test bacterial species to the cold water extracts and controls

TestIsolate	Leaf (25mg/ml)	Stembark (25mg/ml)	Root (25mg/ml)	CIP (5µg/ml)	FLU (20µg/ml)	DMSO 20%
S.aureus	14.5±0.02	14.8±0.01	15.0±0.11	26.0	ND	-
S.mutans	12.8±0.50	13.0±0.01	13.5±0.02	25.0	ND	-
E.coli	18.4±0.13	18.0±0.14	18.2±0.02	'26.5	ND	-
B.cereus	12.5±0.01	12.2±0.05	12.7±0.35	26.0	ND	-
P.aeruginosa	18.0±0.01	16.6±0.41	18.5±0.20	'24.5	ND	-
K.pneumoniae	12.3±0.10	12.0±0.00	12.5±0.22	25.0	ND	-
P.mirabilis	13.1±0.11	13.5±0.22	13.5±0.50	24.0	ND	-
S.typhi	16.4±0.12	15.1±0.24	16.0±0.30	26.0	ND	-
C.albicans	13.1±0.01	13.0±0.04	13.0±0.02	ND	'28.5	-

** Results are means of three replicate diameter zones of inhibition values (mm) ± standard deviations (SD), CIP= Ciprofloxacin; FLU= Fluconazole; 20% DMSO=Dimethylsulfoxide; -=No Inhibition.*

The result of the minimum inhibitory concentrations presented in Table 4, shows that the values of the different extracts ranged between 400 and 800µg/ml. With the ethanolic extracts, the MIC ranged from 400µg/ml on *S. aureus, E. coli, C. albicans* and *P aeruginosa*, to 600µ on *S. mutans*, and *S. typhi*, to 600-800µg/ml on *K. pneumonia, B. cereus and P. mirabilis*. The hot aqueous extracts however, had MIC ranged between 600µg/ml for *S aureus, E. coli, S. typhi, P. aeruginosa* and *C. albicans* and 800µg/ml for, *S. mutans, K. pneumonia, B. cereus and P. mirabilis*, while the cold water extract had MIC value of 600µg/ml on *S aureus* and *E. coli*, and 800µg/ml on *S. typhi, P. aeruginosa, C. albicans, S. mutans, K. pneumonia, B. cereus and P. mirabilis*(Table 4).

The result of the phytochemical analysis of *Cyathula prostsrata* presented in Table 5 shows that only alkaloids was detected in the ethanol extracts of leaf and stem bark, while terpenoids, tannins, flavonoids, saponins, glycosides and steroids were found in the leaf, stem bark and root extracts.

Table 4. Minimal Inhibitory Concentration (MICμg/ml) of Ethanol, and aqueous leaf, stem and root extracts against test isolate

Test Isolate	Ethanol Extract			Hot Aqueous Extract			Cold Aqueous Extract		
	Leaf	Stem	Root	Leaf	Stem	Root	Leaf	Stem	Root
S. aureus	400	400	400	600	600	600	600	800	600
S. mutans	600	600	600	600	600	600	800	800	800
B. cereus	600	800	600	800	800	800	800	800	800
E. coli	400	400	400	600	600	600	600	800	600
P. mirabilis	600	800	600	800	800	800	800	800	800
S. typhi	600	600	600	600	600	600	800	800	800
K. pneumonaiae	600	800	600	800	800	800	800	800	800
P. aeruginosa.	400	400	400	600	600	600	800	800	800
C. albicans	400	400	400	600	600	600	800	800	800

Table 5. Qualitative analysis of the phytochemicals in Leaf, Stem bark, Root extracts of Cyathula prostsrata

Phytochemicals	Leaf		Stem Bark		Root	
	AqE	ETE	AqE	ETE	AqE	ETE
Terpenoids	+	+	+	+	+	+
Tannins	+	+	+	+	+	+
Flavonoids	+	+	+	+	+	+
Saponins	+	+	+	+	+	+
Cardiacglycoside	+	+	+	+	+	+
Steroids	+	+	+	+	+	+
Alkaloids	-	+	-	+	-	-

Key: AqE = Aqueous extract ETE = Ethanol extract, + = Deteected, - = Not detected

DISCUSSION

Medicinal plants play a central role not only as traditional medicines but also as commercial commodities meeting the demand of distant markets. To compete with the growing market, there is need to expeditiously utilize and scientifically validate more medicinally useful plants. Because of the appearance of drug resistance to antimicrobial agents, more effort is being made to find alternative antimicrobial components. It had been suggested that natural products are a preferable option to synthetic ones. Literature indicates that medicinal plants are the backbone of traditional medicine [17], and the antimicrobial activity of plant extract is due to different chemical agent in the extract with antimicrobial compounds [18]. Many studies have been undertaken with the aim of determining the different antimicrobial and phytochemical constituents of medicinal plants and using them for the treatment of both topical and systemic microbial infections as possible alternatives to chemical synthetic drugs to which many infectious microorganisms have become resistant [7,19,20].

In this study the leaf, stem bark, and root extracts of *C. prostsrata* were found to inhibit all the test bacteria and fungus, indicating that this plant possesses significant in vitro antimicrobial properties. The slightly greater activities, though not significant ($P<0.05$), exhibited by the ethanol fractions of the various plant parts shows that this solvent dissolved a greater percentage of the actual bioactive ingredient of this plant than the hot and cold aqueous counterparts. This justifies the preference of local gin "ogogoro" as extraction solvent by herbal physician in the preparation of crude drugs from medicinal plant materials. Local gin obtained from fermented palm wine distillation is known to contain a high concentration of alcohol. When these solvents are used as herbal extractants, it may be possible that bioactive substances that are less soluble in water would then be dissolved by the solvent [21]. This observation is in consonance with the works of [22] and [23] who reported that the ethanol extract of *Piliostgma reticulatum* and *Aspila africana* generally

displayed the highest activities, followed by the hot aqueous and cold aqueous extract against *S. aureus, S. faecalis, P. aeruginosa, K. pneumonia, E. coli, S. dysentriae,* and *S. typhimurium.* But the result of this study is however in disagreement with the work of [5], where the hot water extracts of root, stem bark and leaf of *Parkia clappertoniana* were more active than their ethanol and cold water extracts against *E. coli* ATCC 11775, *P. aeruginosa* ATCC10145, *S. aureus* ATCC 12600. The relatively high antimicrobial activities of hot aqueous extract also indicate that significant amount of the bio-active components were extracted at elevated temperature. This finding is in line with the report of [24], that hot aqueous extraction expressed greater amount of the inherent bioactive chemicals thereby making them more available for antimicrobial activities over the cold water counterpart. This observation also suggests that the secondary bio-compounds of these plant extracts are to some extent stable at relatively high temperature, thereby justifying the efficacy of the whole plant extract even when boiled in water and utilized as herbal remedy.

The slightly greater antimicrobial activities recorded by root and leaf extract over the stem bark extracts in this study, suggest that more of the bioactive ingredients are lodged in these parts. Many practitioners may have observed these in the past that they almost always recommend the use of leaf and root extracts over that of stem bark of a medicinal plant for native medicine. This finding is in agreement with the observation of [5]

The significant antibacterial activities recorded by the ethanol and hot water extract of leaf, stem and root extract of this plant against *E. coli, S. aureus, P. aureuginosa, and S. typhi,* are worth-noting especially now that they are multiple-drug resistant species of these bacteria commonly implicated in several cases of human diseases such as gastro-intestinal, urinary tract and wound infections in Nigeria and other African countries [25-27]. The significant antibacterial activity of this plant extract against *E. coli* was however in disagreement with the observations of [28], who documented the antibacterial activity of *C. prostrata* and some other traditional Medicinal Plants (*Leucas aspera, Murraya koengigii, Oxalis corniculata, Alternanthera sessilis, Pagostemon benghalensis, Hydrocotyl rotendifolia, Cyathula prostrata, Piper peepuloides, Potentilla mooniana*) of North East India on *Escherichia coli.* They noted that the aqueous leaf extracts of *C. prostrata, P. benghalensis, H. rotendifolia* and *P. mooniana* could not inhibit the growth of *E. coli.* The failure of some extracts to exert antibacterial effect on test organisms in not enough to to conclude lack of antimicrobial property because the potency of extracts depends on the solvent and method used to obtain the extract, the age of plant when harvested and the amount of the active constituent, which can vary in quality and quantity from season to season [29, 30] (Rios and Recio,

2005). In addition, the greater. Thus, the variations of this finding with the previous report could be attributed to some of the aforementioned reasons.

The inhibition of *S. mutans, P. mirabilis, B. cereus,* and *K. pneumonia* equally suggests that this plant possesses broad spectrum antimicrobial properties and could be used in the treatment of dental caries, food poisoning, wound infections, and urinary tract infections(UTIs) of which these pathogens are commonly implicated [25,31]. Inhibition of *C. albicans* by this plant extracts also suggest that it possesses antifungal properties, and can thus be tried as antifungal agent for the treatment of refractory candidiasis (oral) that has been a major global challenge with HIV/AIDS patients [32]. This observed antifungal property justifies their applications as cure for craw-craw, scabies, ringworm and other skin diseases in Nigeria and several African countries [10-12]. This result is however not in agreement with the findings of [33], who reported that the aqueous extracts of some medicinal plants including *C. prostrata* could not reduced the mycelia growth of *Fusarium moniliforme,* crop spoilage mould. In this study significant inhibitory activity was observed from the ethanolic extracts than the aqueous extracts. Thus, the slight variation could be attributed to extracting solvent used as well as resistant nature of spores of moulds. This result further suggests more investigations on antifungal properties of this plant on a wider range of fungi.

The relatively low MIC values recorded by the extracts against the test isolates confirm the high activity of the extract at low concentrations. In their studies on the toxicity of the methanolic leaf extract of this plant (*C. prostrata*), [11] documented that the extract could be administered at a dose range of 100 mg/kg/BW without any side effects in mice. High activity of antimicrobial agent at low concentration, in relation to the standard reference drug is very essential for chemotherapeutic purposes because of their toxicity to the patient's system. This research was conducted on crude extract; it is believed that if the extract is further purified, stronger inhibitory results will be achieved.

The significant antimicrobial properties of the leaf, stem bark and root extracts of *Cyathula prostrate* could be attributed to the presence of the bioactive compounds detected in this study. Earlier researchers had demonstrated their antimicrobial activities [34-40]. Flavanoids are phenolics structure containing one carbonyl group complexes with extra cellular and soluble protein and with bacterial cell wall [35], thus exhibits antibacterial activity through these complexes [41]. Tannins on the other hand have been found to

form irreversible complexes with proline-rich proteins [19] resulting in the inhibition of the cell protein synthesis. Plants that have tannins as their main component are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery [42]. This could be the basis for the antimicrobial use of such plants in the treatment of diarrhoea and dysentery [10-12]. Saponins, however, are a special class of glycosides which possesses antifungal activities [43]. The significant activities of the extracts against *C albicans* in this study might be attributed to the action of this bioactive ingredient. Terpenoids have been demonstrated to be active against bacteria, fungi, viruses and protozoa [37, 44], which has enabled food scientists to use terpenoids present in essential oils of plants to control *Listeria monocytogenes [*36]. The mechanism of action of terpenes is by lipophilic membrane disruption. Indeed, [35] found that increasing the hydrophilicity of kaurene diterpenoids by addition of a methyl group drastically reduces their antimicrobial activity. The presence of cardiac glycosides and steroids have been documented to inhibit the many bacteria and found to possess antioxidant potentials [40].

In conclusion, this study has demonstrated that the leaf, stem bark and root extract of *Cyathula prostrata* possess bioactive ingredient with in vitro antibacterial and antifungal activities against some human pathogens, thereby justifying the application of their extracts as traditional herbal medicine. It is worth-noting that *in vitro* finding is not always dependable because plants which are effective *in vitro* might not work when used *in vivo* and some plants which showed little or no effect *in vitro* study might also be effective when evaluated in animals due to various factors that affect or favor the release of active ingredients in animal bodies. Therefore, it is recommended that further identification of the active constituents is needed to exploit them in evaluating efficacy and safety in vivo against the test pathogens.

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Review Article

Pharmaceutical market in Serbia

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Abstract

Marketing concept formed around the focus on the consumers, their needs, wants and demands, evolves in the case of pharmaceutical into a care of the complex interest of constituents generating demand on this market – pres scribers whose role is to select therapies, pharmacists who dispense drugs within a specialized distribution channel to the final consumer -patient, alongside the payers – the state and or insurance companies refund a part of or total costs of the pharmaceutical product. A special challenge that the subject raises is the existence of controversy generated from two sources. Marketing controversy stems from criticism leveled at the effectiveness and efficiency of marketing activities and the debatable ethical code of conduct

INTRODUCTION

Ever since the beginnings of history, medicine and pharmacy intertwine in man's effort to overcome the biological limitations of the human organism. Both have accompanied humanity on the way from magic and divine to rational and science based practices. The formation of pharmaceutical industry in the second half of 19th century marked the beginning of standardization of pharmaceutical products and regulation of the industry, and subsequently the sale and application of medicines. 150 years later, the industry is one of the most vital global industries, functioning on a specific market. According to pharmaceutical industry in the 20th century was marked by individuality, primary focus on science and the process of scientific learning, while the ethics and morals of the industry derived from medicine itself [1, 2]. The sum of human knowledge in the area of medicine and pharmacy have determined pharmaceutical industry over the past few decades, and provided a theoretical basis for predicting continued innovation which will ultimately transform the perception of the process of prevention, diagnosis and treatment. Today, discussing pharmaceutical industry implies several burning issues:

- Blockbuster drugs – contemporary pharmaceutical industry relies on the sale of a relatively small number of extremely successful drugs, whose annual sales, according to one of the criteria, exceed a billion US dollars. Blockbuster drugs are originator products, branded, and patent-protected drugs, the outcome of a long and costly R&D process. From the aspect of meeting humanity's medical needs, it is an objective fact that these drugs were developed to 'resolve' the morbidity statistics of highly developed countries - the chronic, high-incidence diseases of the modern man. The development of new blockbusters and their survival are challenged due to reduced efficiency of pharmaceutical companies R&D process, generic substitution, and the trend of personalizing therapies through bio pharmacy [3, 4].
- Generic drugs are the outcome of the limited period

of patent protection of the original pharmaceutical products, whose active ingredient becomes 'common good' upon expiry of patent protection. Apart from proven bioequivalence, generics guarantee the availability of high-quality drugs to the widest population, as competition in the production leads to a rapid fall in prices. Generic drugs do not bring about new quality or more effective therapy for an existing health problem, apart from wide availability of the drug. With the growing availability of healthcare, pressures on payers result in preference for cheaper generic products. The essence of the dilemma related to the choice of (and/or preference for) the originator or generic products rests in the question whether the pharmaceutical industry will maintain the levels of investment in R&D that will generate advances in therapy, which will mean better and more effective fulfillment of humanity's medical needs [5].

- Lifestyle drugs are a separate group of drugs intended for conditions that give rise to a philosophical/moral dilemma whether they can be regarded as pathological conditions requiring medical and/or pharmaceutical therapy. These are 'disorders' such as sexual dysfunction, hair loss, obesity, signs of skin aging *etc*. This group of drugs may be regarded as the 'latest fashion' on this market, and terms this group of drugs as 'Vanity Drugs'. The culture of the Western civilization actively contributes to the growth in demand for this category of pharmaceutical products, turning this trend into a new quest for the 'fountain of youth' [6, 7].

- Biotechnology is the outcome of qualitative advances of medicine/pharmacy towards predictive and preventive medicine. The biotechnology concept is based on the use of biological systems, organisms and their derivatives. The most promising bio technological discoveries are those in the field of recombinant DNY, with the potential of tailoring a man's/individual's genetic inheritance in such a way as to enable 'bypassing' biological limitations and irregularities. Biotechnology is related to the concept of individualized medicine/therapy tailored according to individual circumstances. In view of the still modest results, expectations from biotechnology are practically unlimited [8].

Marketing is both a scientific (theoretical) and practical discipline. The abundance of marketing theory in academic circles is beyond dispute. There is a developed set of patterns, a glossary of the discipline, and a considerable body of knowledge. Once this knowledge leaves the premises of academia and enters the reality of economic life, a justifiable question is posed - to what extent is a set generalized assumptions (and similar solutions) applicable in real life. This varies greatly from one industry to another. The application of marketing in pharmaceutical industry surpasses the framework of its application in the fast-moving consumer goods industry, where the discipline has reached its heights [9, 10].

STRATEGIC POSITION ANALYSIS

Qualitative research on an appropriate sample on the territory of Serbia, which included ten leading companies creating the offer of pharmaceuticals on this market, has shown that marketing practices function in authentically specific conditions. First of all, marketing function exceeds the framework of marketing profession and is taken over by pharmaceutical experts, who are, by their vocation and knowledge, closer to the nature, properties and application of the product that marketers would be. Such a form of solution objectively entails certain limitations from the aspect of knowledge of marketing principles, models and tools, which is reflected on their application in daily activities. Another significant determinant is based on the fact that this market functions within a strict regulatory framework, facing the application of marketing with a whole range of limitations [11]. The extensive body of available literature in the field of pharmaceutical marketing, supplemented by primary research, confirms the hypothesis that designing appropriate marketing strategies requires appreciation of specific conditions which distinctively define the pharmaceutical marked as a separate - specialized market**.** However, designing and implementing an appropriate marketing strategy requires an organizational culture supporting (and reflecting) marketing business philosophy. The nature of the purpose of products and specific de fragmentation of the decision on the choice of products into several constituents that generate the demand for these products results in a different view of marketing. The objective level of development of pharmaceutical industry in the Republic of Serbia and other systemic limitations of a relatively underdeveloped economy, as well as the modest size of the market, do not leave sufficient space for viewing the complex logic and practice of pharmaceutical marketing in one of the most controversial contemporary industries. The very size of the US pharmaceutical market and the strength of global pharmaceutical companies competing primarily on this (in many respects) 'archetype market', but also on other markets worldwide, reveal all the controversies related to the industry and its use of marketing. Is marketing a value free, negative or positive concept? It depends on the point of view. Marketing concept starts from the consumer [11, 12]. The goal of marketing is to use a careful analysis of consumers' needs (and wants) to create a value proposition that will be able to fully meet their

expectations (better than the competitors). On this task, marketing uses sophisticated methods for researching the market, consumer behavior and competition, seeking to disperse the care of consumer satisfaction throughout the whole organization through a process of internal marketing. Modern marketing is value-driven, where the consumers and other constituents of the environment are regarded as partners, and marketing itself is focused on creating and maintaining long-term relationships with the target environment, surpassing a relationship based on a simple transaction. An organization's profits (and survival) result from superior fulfillment of consumers' needs. In the marketers' words, marketing has a clear value framework. It would be naive to believe that it is practically impossible to forget and/or deliberately distort this marketing logic in order to achieve opportune interests. In contemporary marketing, content holds sway over form, as only the ideas adding consumer or stakeholder value reflect a long-term orientation of marketing on creating value added [11-13].

Analysis of economic indicators of business performance

Interviews with professionals involved in marketing in the pharmaceutical industry have revealed that this market shows a strong orientation of marketing on the content, information and knowledge in the function on higher quality of decisions made by prescribes, in a joint mission of providing patients with the best possible and/or available therapy. Consider the concept of evidence based medicine (EBM) conscientious, explicit and judicious use of current best evidence in making decisions about the care of individual patients [14]. Where does this evidence come from? They are the output of scientific research process, clinical trials, advances in and development of the medical profession. However, according to pharmaceutical industry has inserted itself into every aspect of medical practice from medical education to basic research and clinical care.' Modern society has opened space for the pharmaceutical industry to legitimately claim the right to such impact, removing from society:

- a part (or all) of the care of continued education of physicians and pharmacists;
- the need to disseminate information in medicine/pharmacy via formal channels; and
- to conduct clinical trials proving the effectiveness and safety of a drug at the expense of society [15, 16].

In all this, the assumption on which the ethical/moral aspect of this concept rests is that commercial interest will not overpower the medical/altruistic. If the marketing function, marketing organizational unit and marketing activities were removed, would the problem disappear as well? Or are we trapped in a 'tangle of moral compromise' where each of the parties gives something in exchange for something, but none of them is entirely satisfied? The crucial question is not whether pharmaceutical industry needs marketing. The root of the issue of relation between medicine/pharmacy and the way that they fulfill the needs of individuals/society is much deeper. The problem is the marketing. The Marketing is distorting information that we, as patients, read and understand. There really isn't any place for marketing in medicine. The problem is in drugs also, as at the present level of development of science and technology, with the current amount of human knowledge, drugs do not provide the ideal that we strive for. Another indicative fact is that new adverse effects are still being found for verified drugs used for many years (even decades). A part of this complex jigsaw puzzle, man's struggle against biological transience, is also the fact that man's appetites are growing, with expectations bordering on what is currently called regarded as science fiction. Despite all the disappointments, human expectations from medicine and pharmacy will keep growing. 'Surely, the scientists would argue, where the clinical differentiation is significant enough, marketing is superfluous.' Objectively, commentary is correct, however, even the best therapy will not yield satisfactory results from the aspect of the complex multitude of stakeholders if patients do not seek diagnosis and medical therapy (or at least not on time), if the informative function of marketing activities is lacking, or a set of additional services that may raise the patient compliance levels. Marketing is not a substitute for a product's therapeutic value, but may make an impact so as to realize this therapeutic value. According to developing new drugs is one thing; making them successful in the marketplace is another.' [16, 17].

THE AIM OF THE RESEARCH

Taking into consideration the nature of the initial hypothesis as the premise or assumption aimed at serving as a tool for organizing the available body of knowledge and the guiding principle of the research, it can be assumed that the pharmaceutical market is ruled by specific conditions that shape marketing mix instruments in a qualitatively new way (different than that of consumer goods). Pharmaceutical companies form their marketing strategies and resulting marketing activities with due regard to specific properties, which means that the analysis and consideration of all specific conditions present on the pharmaceutical product market are vital preconditions for designing appropriate marketing strategies (Research Hypothesis).The aim of the research is to view the extent to which

consideration of elaborated specific properties affects the strategic (and subsequently tactical) marketing decision-making of pharmaceutical companies in the Republic of Serbia. The nature of the issue, and also the comparatively small number of pharmaceutical companies that organize production on the territory of Serbia, have prevented generalization based on large numbers, thus determining the research as qualitative in nature [18].

THE SUBJECTS AND OBJECTIVES OF RESEARCH (RESEARCH QUESTIONS)

The course of research so far, based on the analysis of secondary data sources and the available literature, has provided only partial replies to research questions: The analysis of the state of and tendencies in pharmaceutical industry has provided the basic picture of contemporary pharmaceutical industry. A brief historic overview from roots to present date has enabled the understanding of the industry's logic and possible routes of development. The analytic approach to determining the players in the micro and macro marketing environment has produced insights into the pharmaceutical industry and market in all its complexity, numerous stakeholders and, frequently, conflicting interests.

The insights into the environment have enabled the detection and profiling of players forming the offer of pharmaceutical products and constituents of demand, primarily manifested through three key groups of stakeholders – consumers/patients, prescribes and health insurance payers, including the segment of expenditure on pharmacological therapies. The central section of the thesis deals with the analysis of marketing mix instruments, in an effort to elaborate on some of the key specific properties of marketing activities on the pharmaceutical market, distinguishing them from the marketing of consumer goods. The dilemmas, place and role of the marketing function and marketing strategies in the corporate strategy open the opportunities for primary research, which is to confirm or disprove the thesis that designing marketing strategy in pharmaceutical industry implies an analysis and consideration of a much broader spectrum of specific environmental factors. In addition to providing a cross section of the state of marketing practices on the pharmaceutical market of Serbia, the combined output of secondary and primary research will result in relevant conclusions that may be regarded as original contribution to the body of knowledge on marketing on this marketing. A research tool was designed for the purpose of gathering primary data [18, 19].

THE RESEARCH TOOL

The research tool selected for this thesis was a structured interview, comprising three sets of questions related to the following areas:

- definition of the target market;
- value proposition (marketing mix); and
- the evaluation of the place and role of marketing in pharmaceutical industry.

The motivation for such a setup of research structure has two determinants. The first is related to the fact that strategy is a comparatively permanent orientation of a company, determining both its market positioning and the indications of the intended routes of development. On the other hand, reluctance of economic subjects in Serbia to contribute to research is proverbial, where evading questions at the operative level is regarded as safeguarding the integrity of the company and facilitates approach to respondents. Furthermore, only by insights into the attitudes and logics of thinking on marketing strategy and the place of marketing on the pharmaceutical market facilitates identifying the patterns that characterize this market in the given conditions, which would not be possible at the level of analysis of operative marketing activities. In addition to a brief introduction defining the purpose of research and clarifying the initial hypothesis, each research subject is divided into a certain number of issues representing its more detailed determination. The preliminary version of the guide was tested for comprehensibility and content in a panel discussion with three persons whose work is related to the pharmaceutical market, and suggestions were used for adjusting the wording, in view of the fact that persons responsible for marketing activities in pharmaceutical companies are predominantly physicians and/or pharmacists. The research tool was sent for inspection to potential respondents in electronic form prior to the interview. The surveying unit was comprised of persons directly responsible for the companies' marketing activities, with an influence on the design of marketing strategies. The interviews lasted for 60 to 75 minutes. Five interviews were recorded in digital audio format, whereas the other half of the respondents preferred not to have the interviews recorded this way [16, 17].

Changes in the marketing practices in pharmaceutical industry

The most prominent issues raised under this subjects point partly to the wish of the participants on the pharmaceutical market in Serbia to see better regulated relationship and business conditions, and partly represent a view of the evolution in the marketing practices and the industry itself [19-21].

- All respondents who participated in the research believe that the most significant change that they expect is regulation of the relationships on the pharmaceutical market in Serbia. Basically, this process is about harmonizing the country's healthcare system, pharmacy network, pharmaceutical industry and appropriate legislation. The prevailing opinion is that the process of changes will be inevitable in view of the European integration process [2].

- Reformed healthcare systems, sources of healthcare finance (basic and additional healthcare insurance), equalization of private and national institutions both in healthcare and in pharmaceutical system, and better control of drug sale are some of the key issues to determine future conditions on the pharmaceutical market in Serbia. From the point of view of the level of service provided to final users, regulating the system should also rationalize the routes through which drugs reach consumers, eliminating intermediaries incapable of meeting required standards from the market competition. In the respondents' opinion, rationalized number of market participants will result in higher quality of service, better control of sale and preservation of the pharmacological properties of products in the chain from production sites to the place where they are used. Unregulated system has also resulted in numerous 'grey' routes by which drugs reach the patients. Although at the rear end of information revolution, consumers do not need Internet pharmacies to obtain prescription drugs without any obstacles. Ethical drugs can be purchased without prescriptions or any limitations in numerous pharmacies, but objectively, drugs are often available on open-air market stalls or through classified ads in local papers. A considerable number of drugs in the grey zone are of dubious origin and pharmacological properties, without package inserts and potentially harmful to any final consumer/patient. The unlimited availability of ethical drugs in the 'grey' zone also gives rise to forms of self-treatment that are dangerous for the patients' health, as they make diagnoses themselves or following advice of persons from immediate social environment, and chose therapies which will, in the best case, not produce either beneficial or harmful effects [2, 3].

- The marketing practices on the pharmaceutical products in Serbia must be of higher quality. The respondents' opinion is that quality should be sought through reducing pressure on prescribes, where the industry's activities aimed at them should be more educational and less commercial by nature. Commercial pressures on prescribes include various form of gaining loyalty, room gifts, to financial stimulations, covering costs of seminar attendance, visits to fairs. Numerous commercial incentives are not contrary to legislative solutions, but there is a profound doubt in the ethicality of such actions. One of the respondents expressed the opinion that pharmaceutical marketing is about to face 'going back to the roots'. Explicitly, it refers to investing in partnerships and trust between pharmaceutical industry, prescribes and patients that characterized the early stages of application of marketing within the healthcare system [3-8].

Respondents in the survey highlight prevention as a trend which will, in future, gain importance, in accordance with WHO's recommendations that each individual is responsible for his/her health. Prevention is achieved by raising he population's health culture and influence towards changing 'unhealthy lifestyles' [9].

- The development of biotechnology and genetic therapies will make the industry highly sophisticated. In some respondents' opinion, man's ability to impact on the 'avoidance' of serious health problems through gene modifications will not mean the end of classical pharmacy and 'common' drugs. Minor health disorders, especially acute, will still be treated with verified therapies of low unit price, which basically guarantees a future for generic producers [10].

- Numerous mergers and acquisitions have marked the development of the industry in the previous period. One of the respondents in the research has expressed an opinion that this trend will end in the forthcoming period. Some of the systems have become too large and it is obstructing communication between various functional areas, as well as different geographical centers. The decomposition of gigantic systems is also heralded by the practice of large pharmaceutical companies, to seek efficiency of R&D by encouraging mutual competition between their own research centers.

- New markets – the most significant participants in the research are becoming aware of the limited potential of the domestic market and turning to new market, with a primary focus on Russia and the Commonwealth of Independent States, Asian and African countries, but, to an extent, also to adjacent markets of the EU or countries gravitating to it.

- Due to their broad product range and assortment, wholesalers have a specific insight into the sales of various categories of drugs. It is very interesting to identify trends, which cannot be regarded as exclusively characteristic of Serbian market, such as a rise in the sale of lifestyle drugs. According to one of the respondents, three key trends leading to a proliferation of products in the sphere of OTC preparations or auxiliary medicinal devices, are based on:

- the desire for extended (or permanent) youth among

the female population ('hyaluronic acid is the absolute hit'), but the trend is slowly extending to include men as well;

- the desire of male population to preserve potency; and

- desire to maintain ideal body proportion (weight loss products) [11].

The emergence of small domestic producers offering products from these groups reveals another fact, that it is a profitable industry, as the consumers are willing to allocate considerable amounts of money for these products. It is not uncommon that a consumer unwilling to pay the cost of an ethical drug against a 'mundane' disease does not complain of the household budget restraints when buying the 'youth potion'. There is a consensus among respondents that the future pharmaceutical market in this region will be better regulated, and that marketing is expected to make a qualitative move from 'commercial to educational'. Primary objectives mentioned by the respondents include true therapeutic quality of drugs, higher quality of communication with prescribing physicians and pharmacists, and availability of drugs to patients [8].

IMPLEMENTATION OF RESEARCH RESULTS IN THE PRACTICE OFPHARMACEUTICAL COMPANIES IN SERBIA

One of the conclusions that impose themselves is that the circumstances of the functioning of the pharmaceutical market in Serbia, in view of the presence of a large number of generic producers, and relatively modest size of the market, have resulted in the existence of very similar views of and considerations on marketing pharmaceutical products. A key difference is obvious at the operative level, as only the difference in available resources enables the full utilization of marketing activities, as well as the tactical tools for stimulating various participants generating the demand for pharmaceutical products. Only in one case, analyzing the company's SWOT matrix, the respondent pointed out that the lack of marketing strategy can be regarded as the company's internal weakness. Objectively, it is not about the total lack of defined market strategy, in view of the fact that the company is one of the leaders in the industry. The respondent formulated his view as insufficiently defined marketing strategy in the segment where it represents a unique competitive advantage of his company in relation to others. Thinking about marketing strategy is pragmatic, as his opinion is that this unique competitive advantage must be enforced in the form of measurable tender criteria, as 'nice and charming' cannot be evaluated numerically when submitting offers for supplying large healthcare centers that the company services. Such an opinion can support the conclusion that the marketing activities in pharmaceutical companies on the territory of Serbia are primarily operative. Marketing's advisory role in strategic decision-making at the company level is correct, but the interviews have lead to an impression that there is space for a more active inclusion of marketing in generating strategic decisions at the company level. Focus on operative, daily duties may also be the result of the complex and difficult conditions in which the industry functions in Serbia, balancing between imposed limitations, its own orientation to generating profit, and de facto present social and ethical factor. Summing up the way of viewing the target market and segmentation criteria, it is clear that production logic is present, where the target market is viewed through the prism of the product's characteristics and purpose, and the group of prescribes using the given products. The interviews have not identified a more complex way of categorizing prescribes according to specific behavioral properties or attitudes. Value proposition on the domestic market, unified in the synergetic effect of the product, pricing, distribution and promotion, enhanced with a set of services and user support, is perceived as the same as among competitors. Criteria used for describing the uniqueness of one's own offer and advantage over the competitors are almost identical with all respondents [3-8].

CONCLUSION

'Marketing is a serious business.' As a rule, respondents agree that it is marketing that sells drugs. Despite recognizing and acknowledging the controversial nature of relationship between pharmaceutical industry and marketing, all respondents agree that marketing has its place, adding that, regardless of their products' clinical superiority, there is a need to convey information from the industry to physicians. The importance of marketing is additionally highlighted by the fact that a large number of producers with identical and/or similar offer are competing on a limited marketing, and targeting the same consumer groups [22]. Marketing activities become one of the dominant tools for encouraging physicians to prescribe a certain producer's drugs. The dominance of the operative level of marketing is prominent in several respondents' comment that marketing activities have no sense until the drug has reached wholesale and subsequently pharmacies, i.e. until the physicians start prescribing it. The logic of marketing, which is characteristic not only of the pharmaceutical market in Serbia, is predominantly based on promotional activities. Nevertheless, it must be pointed out that respondents prefer the opinion that this is in fact

informing prescribes and other target groups, and regard the

word ‘promotion’ as too ‘blunt’ for the type of activities that they conduct. For the ‘average’ consumer – the patient – oversized interest in pharmaceutical industry and the multitude of, often divergent, opinions on the benefits and/or dangers of drugs is a serious problem. Between the extremes that a drug may at the same be a means of returning an individual’s life back to normal, but also a way of permanently disrupting the functions of the organism, and even threaten life, one may very easily find oneself in an endless succession of delights and disappointments. However, the very nature of the product and the circumstances in which the individual uses them mean that humanity will never be able to distance the selves from the industry. As long as it is an industry rather than something else. The discourse on the mutual relationship between marketing and pharmacy can only be concluded by a lengthy debate on ethics. Is the aim of clinical trials to prove the effectiveness and safety of a drug, or to meet criteria that will enable the drug’s market launch? Is the purpose of information from pharmaceutical companies to raise levels of awareness of risks, symptoms and diagnostics of a disease, or is the primary motive to boost sales and/or market share? Is the risk factor a disease to be treated pharmacologically or not? Do physicians give balanced advice on alternative methods of treatment and a change of lifestyle in the patient’s best interest, or is it in their interest only to prescribe pharmacological therapy? There are other products that may be harmful to consumers if they are not produced and/or used appropriately, and other products that not everyone can afford, but there are few products that imply so many emotions, and so much pondering what is fair and what is not - from the human organism, nature, social environment. In the end, different cultures/societies have found different ways of rationalizing this controversy. Pharmaceutical industry has done a lot to objectively extend an individual’s lifespan, but people still have a finite number of years at their disposal. The complementary advances in medicine have enabled man to live longer and think less about biological limitations, but there is no supreme, ultimate and final result, approaching the mythical ideal, of which each individual has the conception of what it should (or must) look like. Does such a position have a price that affects the pricing of a pharmaceutical product as well? It certainly does. But everything has a price. Marketing is the connection between R&D and the production of a pharmaceutical, and the target audience in its broadest sense [3-8].

If nothing else, marketing is what makes the pill taste less bitter.

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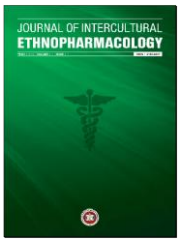

Review Article

Herbal antibacterials: a review

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Keywords:
Antibacterial plants, herbs, plant extract

Abbreviation
MIC: Minimum Inhibitory Concentration;
MBC: Minimum Bactericidal Concentration;
MFC: Minimum Fungicidal Concentration

Abstract

Plants are rich source of antibacterial agents because they produce wide array of bioactive molecules, most of which probably evolved as chemical defense against predation or infection. A major part of the total population in developing countries still uses traditional folk medicine obtained from plant resources With an estimation of WHO that as many as 80% of world population living in rural areas rely on herbal traditional medicines as their primary health care, the study on properties and uses of medicinal plants are getting growing interests. In recent years this interest to evaluate plants possessing antibacterial activity for various diseases is growing. Different solvent extracts (aqueous, alcohol and ethanol) of leaves, flower and seed of various plants selected based on an ethnobotanical survey from India were subjected to *in vitro* antibacterial activity assay against Gram-positive and Gram-negative bacteria employing different diffusion method. Based on local use of common diseases and Ethnobotanical knowledge, an attempt has been made to assess the antibacterial properties of selected medicinal plants viz. *Argemone mexicana* (Shialkanta), *Aster lanceolatus* (White panicle), *Capparis thonningii* and *Capparis tomentosa* (Woolly caper bush), *Cardiospermum halicacabum* (Balloonvine), *Cassia alata* (Herpetic alata), *Centaurea sclerolepis*, *Cinnamomum zeylanicum* (Cinnamon), *Curcuma longa* (Turmeric), *Cymbopogon nervatus*, *Ficus religiosa* (Peepal), *Indigofera aspalathoides* (Ajara), *Marrubium vulgare* (Horehound), *Medicago Spp.*(Medick, Burclover), *Morus alba* (Mulberry), *Ocimum sanctum* (Tulsi), *Origanum marjorana* (Marjoram), *Oxalis corniculata* (Amli), *Piper nigrum* (Kala mirch), *Plectranthus amboinicus* (Indian borage, Patharchur), *Plumeria acutifolia* (Kachuchi), *Salvadora persica* (Piludi), *Salvia repens* and *Syzygium aromaticum* (Clove) for potential antibacterial activity against some important bacterial strains, namely *Bacillus subtilis*, *Bacillus cereus*, *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas spp*, *Proteus spp*, *Salmonella Typhi*, *Escherichia coli*, *Shigella dysentriae*, *Klebsiella pneumoniae*. The plant extracts were more active against Gram-positive bacteria than against Gram-negative bacteria.

INTRODUCTION

India is well known for Ayurveda, which is one of important traditional medicine practiced. Herbs are widely exploited in the traditional medicine and their curative potentials are well documented [3]. Medicinal plants are relied upon by 80% of the world's population, and in India the use of plants as therapeutic agents remains an important component of the traditional medicinal system. Medicinal plants are a source of great economic value all over the world. Nature has bestowed on us a very rich botanical wealth and a large number of diverse types of plants grow in different parts of the country. Emergence of pathogenic microorganisms that are resistant/multi-resistant to major class of antibiotics has increased in recent years due to indiscriminate use of synthetic antimicrobial drugs [4]. In addition, high cost and adverse side effects are commonly associated with popular synthetic antibiotics, such as hypersensitivity, allergic reactions, and immunosupression and are major burning global

issues in treating infectious diseases [5]. This situation forced scientists to search for new antimicrobial substances. In the present scenario, there is an urgent and continuous need of exploration and development of cheaper, effective new plant based drugs with better bioactive potential and least side effects. Hence, recent attention has been paid to biologically active extracts and compounds from plant species used in herbal medicines [6].

Antimicrobials of plant origin have enormous therapeutic potential and have been used since time immemorial. They have been proved effective in the treatment of infectious diseases simultaneously mitigating many of the side effects which are often associated with synthetic antibiotics [7]. Many infectious diseases have been known to be treated with herbal remedies based on ethnobotanical knowledge. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for new drug leads because of the unmatched availability of chemical diversity. Therefore, researchers are increasingly turning their attention to folk medicine, looking for new leads to develop better drugs against microbial infections. Thus, it is anticipated that phytochemicals with adequate antibacterial efficacy will be used for the treatment of bacterial infections.

***Argemone mexicana* (Shialkanta)**

The sensitivity of two Gram-positive (*Staphylococcus aureus* and *Bacillus subtilis*) and two Gram-negative (*Escherichia coli* and *Pseudomonas aeruginosa*) pathogenic multi-drug resistant bacteria was tested against the crude extracts (cold aqueous, hot aqueous, and methanol extracts) of leaves and seeds of *Argemone mexicana* L. *(Papaveraceae)* by agar well diffusion method. The methanol extracts of *A. mexicana* (leaves and seeds) showed maximum antibacterial activity against *P. aeruginosa*, followed by *E. coli*, *B. sublitis*, and *S. aureus.* On the contrary, aqueous extracts (cold and hot) of *A. mexicana* seeds showed maximum activity against *B. subtilis*, followed by *P. aeruginosa*, *E. coli*, and *S. aureus*. Again, cold aqueous extract of *A. mexicana* leaves showed highest efficacy against *P. aeruginosa* followed by *B. subtilis*, *E. coli*, and *S. aureus* where as in case of hot aqueous extract of *A. mexicana* leaves, maximum sensitivity was shown against *E. coli*, followed by *B. subtilis*, *P. aeruginosa* and *S. aureus* [8].

***Aster lanceolatus* (White panicle)**

The activity of the ethanolic extract of the flowers has been demonstrated against *Streptococcus pyogenes* in diffusion in gel and the activity of the ethanol extract of the stems and leaves against *Salmonella typhimurium* and *Streptococcus pyogenes* in minimal inhibitory concentration [9].

***Capparis thonningii* and *Capparis tomentosa* (Woolly caper bush)**

The leaf methanol extract of *C. thonningii* and ethanol extract of *C. tomentosa* aerial parts were investigated *in vitro* antimicrobial activity using the agar disc diffusion technique. The extract of *C. tomentosa* displayed overwhelming concentration dependent antimicrobial properties, inhibiting the growth of *Staphylococcus aureus* and *Bacillus cereus*, far above that of ampicillin used in the study at a concentration of 1.0 g/ml. The methanol extract of *C. thonningii* also displayed a concentration related antibacterial activity, inhibiting the growth of *S. aureus* comparable to ampicillin at 1.0

g/ml. The extract was least active against to *Escherichia coli* with a mild activity at 1.0 g/ml [10].

***Cardiospermum halicacabum* (Balloonvine)**

The antibacterial properties of leaf and its callus extracts were screened against ten human pathogenic bacteria by cup diffusion method. Powdered leaf and leaf derived callus material was subjected to extraction of aqueous and with different organic solvents viz., petroleum ether, chloroform, methanol and ethanol using soxhlet apparatus. Among all the solvents tested, significant inhibitory activity was observed in ethanol extract of both leaf and leaf derived callus followed by methanol. It also observed that the activity was more pronounced on Gram-positive bacteria than Gram-negative bacteria. Pathogenic bacteria only *Bacillus subtilis* and *Bacillus cereus* belongs to Gram-positive bacteria showed susceptible for leaf aqueous extract when compared to *Staphylococcus aureus*. In Gram-positive bacteria *Bacillus subtilis* showed maximum inhibition. Whereas Gram-negative bacteria showed least susceptible for leaf aqueous extract [11].

***Cassia alata* (Herpetic alata)**

Crude ethanol and water extract of leaves from *Cassia alata* were tested *in vitro* against bacteria (*Staphylococcus aureus* and *Escherichia coli*) and *in vivo* to evaluate the effect of both extracts in liver cells of mice. Antibacterial activity of *Cassia alata* extracts on *Staphylococcus aureus* was detected. The water extract exhibited higher antibacterial activity than the ethanol extract from leaves (inhibition zone of 11-14 mm and 9-11 mm respectively). Escherichia coli showed resistant to both extract. Results were compared to commercial antibiotics, chloramphenicol, penicillin and aerofloxaxine, which had 19 mm, 29 mm and 22 mm respectively [12].

Centaurea sclerolepis

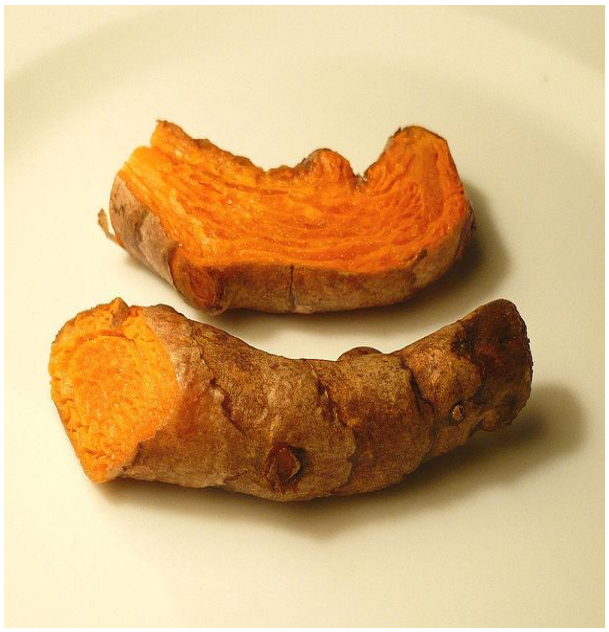
The antibacterial and general toxicity with brine shrimp lethality bioassay of the arctiin from the seeds of *C. sclerolepis* has been studied. According to the obtained MIC values, it is clear that arctiin's antibacterial activity against bacteria is lower than the positive control chloramphenicol. Whereas arctiin has activity between the ranges of 62.5-250 µg/ml against both Gram-positive (*S. aureus, M. luteus, B. cereus*) and Gram-negative (*E. coli, P.aeroginosa*) bacteria, the positive control chloramphenicol was more effective within the ranges of 31.25-125 µg/ml [13].

***Cinnamomum zeylanicum* (Cinnamon)**

The antimicrobial activity of cinnamon essential oil against *Paenibacillus larvae* was analyzed by means of a combination of *in vitro* techniques, such as the tube dilution method and bioautography, a method

employed to localize antibacterial activity on a chromatogram. Cinnamaldehyde and eugenol proved to have antibacterial effects against *P. larvae.* MIC and MBC for *C. zeylanicum* essential oil were between 25-100 µg/ml and 125-250 µg/ml, respectively, for all strains. Essential oil showed inhibitory capacity against strains of *P. larvae* [14].

***Curcuma longa* (Turmeric)**



Curcuma longa rhizome extracts were evaluated for antibacterial activity against pathogenic strains of Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*) bacteria. The use of essential oil from turmeric as a potential antiseptic in prevention and treatment of antibacterial infections has been suggested [15].

Cymbopogon nervatus

Antibacterial activity of essential oil of dried inflorescence of *Cymbopogon nervatus* was investigated. The essential oil remarkably inhibited the growth of tested bacteria except for *Salmonella typhi*. The maximum activity was against *Shigella dysenteriae* and *Klebsiella pneumonia* [16].

***Ficus religiosa* (Peepal)**

The antimicrobial activity of ethanolic extracts of *F. religiosa* (leaves) was examined using the agar well diffusion method. The test was performed against four bacteria: *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 6538), *Escherichia coli* (ATCC 11229) and *Pseudomonas aeruginosa (*ATCC 9027). The results showed that 25mg/ml of the extract was active against all bacterial strains. The antibacterial activity of different extracts from the bark of *F. religiosa* was tested against diarrhoeal enterotoxigenic *Escherichia coli* using disc diffusion method. The antibacterial activities of extract were compared with standard antibiotics. The sensitivity of the organisms

measured in terms of zone of inhibition ranged from 8.00 to 14.00 mm at 4mg/ml of different extract. The results revealed that methanol extract exhibits good activity compared to chloroform and aqueous extract. Petroleum ether and hexane extract did not show any activity [17].

***Indigofera aspalathoides* (Ajara)**

The alcohol extract and fractions were tested for evaluation of the MIC to *Mycobacterium tuberculosis* H37Rv strain with microplate technique using alamar blue. The antimycobacterial activity of alcohol extract and fractions were compared with rifampicin. The benzene, ethyl acetate fractions and extract showed inhibition 61%, 27% and 48% respectively at 100 µg/ml of MIC. The n-butanol and diethyl ether fractions were found to be less active 6 and 9 µg/ml [18].

***Marrubium vulgare* (Horehound)**

The antibacterial activity of the methanolic extract of *Marrubium vulgare* whole plant was tested by disc diffusion method. Zones of Inhibition produced by methanolic extract in a dose of 50, 100, 200, 400 and 600 mg/ml against selected strains was measured and compared with those of standard discs of antibiotic ciprofloxacin (10 µg/ml). The study revealed that methanolic extract of the crude drug was very much effective against *B. subtilis, S. epidermidis* and *S. aureus* (Gram-positive bacteria) and moderately effective against *P. vulgaris* and *E. coli* while ineffective in case of *P. aeruginosa* (Gram-negative bacteria).Thus on the basis of the results it is inferred that the methanolic extract of *M. vulgare* whole plant had *in vitro* antibacterial [19].

***Medicago Spp.* (Medick, Burclover)**

The antimicrobial activity of saponins from *Medicago sativa*, *M. arborea* and *M. arabica* against a selection of medically important yeasts, Gram-positive and Gram-negative bacteria was investigated. The antimicrobial activity was especially high against Gram-positive bacteria (*Bacillus cereus, B. subtilis, Staphylococcus aureus* and *Enterococcus faecalis*) with *M. arabica* being the species showing a broader spectrum of action [20].

***Morus alba* (Mulberry)**

Antibacterial activity of Kuwanon G (active antibacterial constituent) was investigated by the MIC test and the viable cell count method. MIC of Kuwanon G against *Streptococcus mutans* causing dental caries was determined to be 8 µg/ml. The bactericidal test

showed that Kuwanon G completely inactivated *S. mutans* at the concentration 20 µg/ml in 1 min. Kuwanon G also significantly inhibited the growth of other cariogenic bacteria such as *Streptococcus sobrinus* and *Streptococcus sanguis* and *Porpyromonas gingivalis* causing periodontitis. Transmission electron microscopy (TEM) of Kuwanon G treated cells demonstrated remarkable morphological damage of the cell wall and condensation of the cytoplasm [21].

***Ocimum sanctum* (Tulsi)**

The antibacterial activity of ethanol extracts was determined by agar well diffusion method. The plant extracts were more active against Gram-positive bacteria than against Gram-negative bacteria among all the pathogens, all Gram-positive bacteria were inhibited by all four plant extract. All Gram-negative bacteria *i.e. Pseudomonas* spp, *Proteus* spp, *Escherichia coli*, *Shigella dysenteriae*, *Klebsiella pneumonia* and *Salmonella typhi* were showed zone of inhibition against extract of *Ocimum sanctum* [22].

***Origanum marjorana* (Marjoram)**

In vitro microbicidal activity of the methanol extract of *Origanum marjorana* L. was tested against six bacteria (*Bacillus subtilis, B. megaterium*, *Escherichia coli*, *Proteus vulgaris*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*). The methanol extract of *O. marjorana* can be used as an effective herbal protectant against different pathogenic bacteria [23].

***Oxalis corniculata* (Amli)**

The inhibitory activity was highly significant in the aqueous extracts of *Oxalis corniculata*. Most of the plant extracts showed significant antibacterial activity than bacitracin. MIC of aqueous extract of twelve plants varied between 4-50 µl. Results indicate the potential of these plants for further work on isolation and characterization of the active principle responsible for antibacterial activity and its exploitation as whereas *Oxalis Acacia nilotica* varied between 9-35.5 mm. Whereas *corniculata* was effective against all the tested bacteria in case of *Lawsonia inermis* it varied between 9 to except *Shigella sonnei* and *Proteus mirabilis* [24].

***Piper nigrum* (Kala mirch)**

Effectiveness of organic extracts of *Piper nigrum* fruit against pathogenic strains of *Escherichia coli* (MTCC 723), *Staphylococcus aureus* (MTSS 96), S*treptococcus pyogenes* (MTSCC 442), *Proteus mirabilis* (MTCC 1429) by tube dilution method. The study revealed that 70% alcoholic hot extract had higher antibacterial activity as compared to chloroform hot and petroleum ether cold extracts [25].

***Plectranthus amboinicus* (Indian borage, Patharchur)**

The aqueous extract was found to be antibacterial and it was studied against various Gram-positive and Gram-negative bacterial strains by using MIC, agar well diffusion method to find zone of inhibition. The MIC results of aqueous extract of *Plectranthus amboinicus* indicated that *Proteus vulgaris*, *Bacillus subtilis* and *Staphylococcus aureus* were least susceptible among the organisms tested and *Escherichia* coli, *Klebsiella pneumonia and Pseudomonas aeruginosa* are not shown any inhibition to aqueous extract of *Plectranthus amboinicus* [26].

***Plumeria acutifolia* (Kachuchi)**

The *in vitro* antimicrobial activity of *P. acutifolia* stem bark ethanol extract against the microorganisms employed was assessed qualitatively and quantitatively by the presence or absence of inhibition zones, zone diameters, MIC and MBC or MFC values. The data indicated that Gram-positive *E. faecalis* was the most sensitive strain tested to the ethanol extract of *P. acutifolia* stem bark with the greatest inhibition zone of 21 mm. The *B. subtilis* and *S. aureus* were also found to be more sensitive with inhibition zones of 26 and 26 mm, respectively. The ethanol extract of *P. acutifolia* stem bark also showed excellent activity against tested Gram-negative bacteria. *E. coli* was the most sensitive organism among Gram-negative bacteria with the inhibition zone of 33 mm more than that of standard gentamycin positive control. *K. pneumoniae*, *P. aeruginosa* and *S. typhimurium* also exhibited significant sensitivities to the tested ethanol crude extract with the inhibition zones of 18, 16 and 20 mm respectively. The extract did not show any toxic symptoms against the tested mice [27].

***Salvadora persica* (Piludi)**

The aqueous extract of *S. persica* was active against all oral pathogens and *Streptococcus* species were the most sensitive; the highest inhibitory activity was seen against *S. faecalis* (zone of inhibition: 22.3 mm) using the extract concentration of 200 mg/ml, while the weakest activity was demonstrated against *P. aeruginosa*. On the other hand, the methanol extract of *S. persica* stems showed less inhibitory activity against the tested bacteria than did the aqueous extract. *L. acidophilus* and *P. aeruginosa* resisted all methanol extract concentrations, while *S. faecalis* was the most susceptible bacteria (zone of inhibition: 17.7 mm) to the highest extract concentration. The aqueous extract exhibited better antifungal results than the methanol extract and the strongest activity was observed. According to both antimicrobial assays the aqueous extract inhibited all isolated microorganisms, especially the *Streptococcus* species, and was more efficient than the methanol extract, which was resisted by *L. acidophilus* and *P. aeruginosa*. The strongest antibacterial activity was observed using the aqueous extract against *S. faecalis* (zone of inhibition: 22.3 mm; MIC: 0.781 mg/ml) [28].

Salvia repens

The antibacterial activity of the aerial parts of *Salvia repens* has shown that the acetone extract inhibited the growth of *Bacillus cereus*, *Streptococcus pyrogens* and *Escherichia coli* bacteria tested at MIC of 0.5 mg/ml. The methanol extracts effectively inhibited the growth of both *Staphylococcus epidermidus* and *Micrococcus kristinae* at minimum concentration of 0.5 mg/ml. At 0.1 mg/ml the methanol extract inhibited the following *B.cereus*, *S. pyrogens* and *E. coli* bacteria whose inhibition concentration was below 0. 5 mg/l. The activity of the water extracts of the plants against Gram-negative and Gram-positive bacteria has shown inhibition at 1.0 and 2.5 mg/l, respectively [29].

***Syzygium aromaticum* (Clove)**

The antibacterial property of *S. aromaticum* was studied. Compare to ethanolic extract, methanolic extract was showing best result against Gram-positive culture *Staphylococcus aureus* (MTCC 2940) and two Gram-negative cultures *Pseudomonas aeruginosa* (MTCC 2453) and *E. coli* (MTCC 739). The MIC value was determined by using broth dilution methods. Methanolic extract of clove was subjected to get the MIC against test organisms and it was found to be 2.31 mg/ml for *E. coli*, 0.385 mg/ml for *Stapylococcus aureus* and 0.01 mg/ml for *Pseudomonas aeruginosa*. The addition of metal ions (Zn^{++}, Cu^{++}, Pb^{++}, Ca^{++}, Mg^{++}, Fe^{++}) along with methanolic extract of clove samples gave positive results against test organisms. The metal ions increased antibacterial properties of clove samples but after optimization at various concentrations it could not increase the antibacterial activity of samples compare to 10%, 20% and 30% [30].

DISCUSSION

Plant essential oils and extracts have been used for many thousands of years, in food preservation, pharmaceuticals, alternative medicine and natural therapies. It is necessary to investigate those plants scientifically which have been used in traditional medicine to improve the quality of healthcare. Plant extracts are potential sources of novel antimicrobial compounds especially against bacterial pathogens. *In vitro* studies showed that the plant extracts inhibited bacterial growth but their effectiveness varied [31]. The antimicrobial activity of many plant extracts has been reviewed.

In general, the cell walls of Gram-negative organisms are more complex than Gram-positive organisms because of high lipid content and act as a diffusional barrier and make less susceptible to the antibacterial

agents than the Gram-positive organisms [32]. In spite of this permeability difference, however, aqueous extract, ethanol extract, methanol extract have still exerted some degree of inhibition against Gram-negative organisms as well.

The present antibacterial review of the plant extracts demonstrates that folk medicine can be as effective as modern medicine to combat pathogenic microorganisms. The millenarian use of these plants in folk medicine suggests that they represent an economic and safe alternative to treat infectious diseases.

CONCLUSION

Many medicinal plants have been found effective in the cure of bacterial diseases. Due to increasing antibiotic resistance in microorganisms and side effects of synthetic antibiotics medicinal plants are now gaining popularity in the treatment of bacterial infections. The use of traditional medicines and medicinal plants in most developing countries as therapeutic agents for the maintenance of good health has been widely observed. Furthermore, an increasing reliance on the use of medicinal plants in the industrialized societies has been traced to the extraction and development of drugs and chemotherapeutics from these plants as well as from traditionally used herbal remedies. Medicinal plants are considered as clinically effective and safer alternatives to the synthetic antibiotics. According to World Health Organization, medicinal plants would be the best source to obtain a variety of drugs. Extensive research in the area of isolation and characterization of the active principles of these plants are required so that better, safer and cost effective drugs for treating bacterial infections can be developed.

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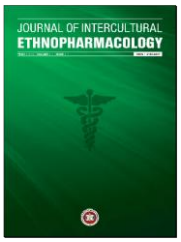

Review Article

Ethno-dentistry: popular medicinal plants used for dental diseases in India

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Abstract
There is a long and venerable history of the use of plants to improve dental health and promeote oral hygiene. Plant contain phytochemicals such as alkanoids, tannins, essential oils and flavanoids which have pronounced defensive and curative activity. India is a vast country with people from different cultures and communities. There are many species of medicinal plants belonging to various families which are being used, traditionally, to control and cure a variety of dental problems by the Indian population. The proper documentation of traditional knowledge may be helpful to promote further research in dental science.

INTRODUCTION

Oral diseases continue to be a major health problem worldwide [1]. Dental caries and periodontal diseases are among the most important global oral health problems, although conditions such as oral and pharyngeal cancers and oral tissue lesions are also significant health concerns [2]. Despite general advances in the overall health status of the people living in industrialized countries, including oral and dental health, the prevalence of dental caries in school aged children is up to 90% and the majority of adults are also affected [1]. Oral health is integral to general well-being and relates to the quality of life that extends beyond the functions of the craniofacial complex. The link between oral diseases and the activities of microbial species that form part of the microbiota of the oral cavity is well established [3].Over 750 species of bacteria inhabit the oral cavity (50% of which are yet to be identified) and a number of these are implicated in oral diseases [3]. The global need for alternative prevention and treatment options and products for oral diseases that are safe, effective and economical comes from the rise in disease incidence (particularly in developing countries), increased resistance by pathogenic bacteria to currently used antibiotics and chemotherapeutics, opportunistic infections in immunocompromized individuals and financial considerations in developing countries [4, 5].

The term 'Medicinal Plant' is not a taxonomic term, but based on the utility of the plants. Any plant used in any system of medicine can be categorized as a medicinal plant. In spite of the tremendous progress in the development of medical science, plants continue to be an important source of drugs in many countries around the world. During past two decades reliability and usage of herbal product has become of increasing importance, due to the side effects and complications of many chemical and synthetic medicines. About 25 % of drugs are derived from plants and many other are formed from prototype compounds isolated from plant species [6]. Kanwar et al. [7] reported that about two million traditional health practitioners use over 7500 medicinal plant species.

Table 1. List of useful plant parts and their active constituents

Plant	Generic Name	Useful parts	Active constituents	Properties
Aloe	Emblica officinalis	Fruit	Vitamin C	Anti-inflammatory
Amla	Aloe barbadensis	Leaves	Anthraquinone	Antioxidant
Babool	Acacia arabica	Bark	Tannins	Astringent
Blackberry	Rubus fructicosus	Leaves,root	Tannins	Astringent
Bloodroot	Sanguinaria canadensis	Root	Alkanoids	Astringent
Blueberry	Vaccinium myrtillus	Ripe berries	Anthocyanosides	Antioxidant
Caraway	Caram carvi	Dried ripe fruit	Volatile oil	Anti-inflammatory
Chamomile	Matricaria chamomilla	Dried flowers	Volatile oils, biflavanoids	Anti-inflammatory
Clove	Syzgium aromaticum	Flower buds	Volatile oil, Tannins	Antiseptic, analgesic
Eucalyptus	Eucalyptus globosus	Leaves	Volatile oil	Anti-inflammatory
Green tea	Camellia sinensis	Leaves	Polyphenols	Antibacterial
Horsetail	Equisetum arvense	Stem	Silicic acid and silicates	Antibacterial
Liquorice	Glycyrrhiza glabra	Root	Glycyrrhizin, flavanoids	Anti-inflammatory, antioxidant
Miswak	Salvadora persica	Bark, leaves	Tannins, volatile oils	Anti-inflammatory
Moringa	Moringa oleifera	Leaves,stem, roots	Carotenoids, vitamin C	Anti-inflammatory, Astringent
Mulberry	Morus alba	Fruits	Anthocyanosides	Antioxidant
Myrrh	Commiphora molmol	Stem	Resin, gums, volatile oil	Antibacterial, astringent, analgesic, anticancer
Neem	Azadirachta indica	Leaves	Terpenoids	Antioxidant, anti-inflammatory, antibacterial
Peppermint	Menthe piperita	Leaves	Volatile oils	Analgesic, counterirritant
Propolis		Resin itself	Flavonoids	Antioxidant, antibacterial, antibacterial,
Raspberry	Rubus idaeus	Leaves	Tannins	Anti-inflammatory, Astringent
Rhatany	Krameria triandra	Root-bark	Tannic acid	Astringent
Rose	Rosa canina	Hips,leaves,flower	Tannins,vitaminC	Astringent,Antibacterial
Sage	Salvia officinalis	Leaves	Essential oil	Antioxidant
Stinging nettle	Urtica dioica	Root, leaves	Polysaccharides, lectins	Anti-inflammatory
Tormentil	Potentilla erecta	Dried roots	Tannins	Anti-inflammatory
Tulsi	Ocimum sanctum	Leaves	Ursolic acid, apigenin, luteolin	Anti-inflammatory
Turmeric	Curcuma longa	Dried roots	Tannins	Analgesic, anti-inflammatory
White oak	Qurecus alba	Bark	Tannins	Astringent
Cranberry	Vaccinium macrocarpon	Fruits	Flavanoids, triterpinoids	Anti-oxidant
Echinacea	Echinacea purpurea	Root	Alkylamides	Immune system stimulant

A lot of research has been carried out on the utilization of medicinal plants in the treatment of a variety of ailments [7-12], more especially during last 2-3 decades, as a result the commercial use and exploitation of these herbal medicines has increased markedly as pointed out in the detailed review by Joshi [13]. However there are only a few reports on theutility of medicinal plants in the treatment of specific disease. For example Sadangi et, al. [14] have reported 10 species of medicinal plants used in the treatment of ear and mouth diseases by the tribal people of Kalahandi district, Jadhav [15] has documented 15 species of medicinal plants used in different types of fever, while Kadel and Jain [16] reported that 34 plant species are being used for the treatment of snakebite in Madhya Pradesh and Chhatisgarh states.

Teeth are very hard but sensitive organs which are implanted in the jaw bones. They not only help in the biting and grinding of food but also aid speech. Any malfunctioning of the teeth or a disease of the gums

disturbs the process of digestion. Lack of oral hygiene, an excess of fleshy food and sweets harm our teeth by causing pyorrhoea, toothache, bleeding gums and dental caries. The use of medicinal plants to treat dental problems has been discussed from time to time by many researchers, viz. the use of Argemone maxicana, Azadirachta indica and Ocimum basilicum in dental health care has been reported by Singh and Dhakre [11] while the use of Hedychium spicatum and Zanthoxylum aromatum has been reported by Arya and Prakash [12]. Acharya et,al. [17] have reported 26 herbal medicines used to treat dental diseases. In addition, Kanwar et,al. [7] have reported the use of Achyranthes aspera, Aegle marmelos and Vitex negundo in dental care by the locals of Kangra district and Tomar [18] has reported the use of six species of plants by the local people of Meerut district (India) to treat dental caries. Sharma and Joshi [9] have reported the use of 30 species of medicinal plants in Almora district, with 5 of these plant species being used by the local people for dental health care.

Various plants used for the treatment of dental disease by Indian population

Many plants are being studied for their potential as phytonutrients or phytotherapy (Jiang et al,)[19]. The literature suggests several plants and plant parts which have anti-inflammatory, antioxidants, antibacterial, astringent and other useful properties. These properties can be made use of in the treatment of dental diseases. Compared to plant derived drugs that often consist of one single natural compound in combination with other chemicals, herbs or phytotherapy materials often contain multiples bioactive components with multiple targets during intakes and therapy. Various plants along with their useful parts and active constituents have been listed in Table 1.

Common uses of various medicinal plants

Among the reported plants, leaves were the dominant parts in oral care uses (25.44%), followed by root (20.17%), seed/nut/fruit (18.42%), bark (14.03%), young stem/stem/rachis (12.28%), whole plant (9.65%) and gum/ latex (8.77%). Among the utilization, most of the plants were used to relieve from toothache (29.82%) followed by, used as dentifrice/ toothbrush (25.43%), mouthwash/gargle (16.66%), against common dental diseases (14.03%), mouth related stomatitis/ulcer/gingivitis (12.28%) and gum bleeding/disorders (10.53%). The mode of utilization of these plants is either in the form of gargle of decoction of plant part(s), powder of dried material or toothbrush.

CONCLUSION

Healthy teeth are fundamental for the proper functioning of the human body. Proper and regular hygiene is required to prevent dental problems. In allopathy, the treatment of dental problems is expensive and cannot be afforded by poor people. So, these types of herbal medicines, which are almost free, are a great help. However these medicines are limited to rural areas and, so, it is necessary to carry out research into these medicines and make available to every part of the country. It was also observed that elderly people have more knowledge about these traditional herbal therapies than younger people. The main reason for this is the superstitious nature of local people. They do not reveal the methods used to prepare "magic" herbal medicines to anyone, even their family members, because they think that if they do, the effective medicinal qualities of the herbs will be lost. As a consequence, with the death of the elderly knowledgeable persons in these remote rural areas, this traditional knowledge could be lost forever.

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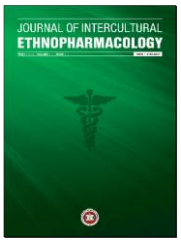

Case Report

Incidental radiological finding of charm needles in the hip region: a potential surgical precaution

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Abstract

We report an unusual incidental radiographic finding of this 71 year old Malay lady who suffered a closed neck of femur fracture due a fall at home which had undergone total hip replacement at our establishment .This is one of the only papers showing incidental occurrence of susuk or charm needles in hip region in orthopaedic field.

A 71 year old Malay woman was admitted with pain in the left hip. She suffered a closed neck of femur fracture, due to a fall at home one month prior to the admission, and was bed ridden. The woman had a history of diabetes mellitus type 2, bronchial asthma, left hemi paresis secondary to stroke and right breast carcinoma treated with mastectomy 24 years ago. Despite these co-morbidities, she was able to ambulate with bearable pain and limping before she became bed-ridden a week prior to admission due to localized unbearable pain aggravated by movement and not responding to analgesics. Past history was non contributory.

Clinical examination revealed an obese woman with normal vital signs. The lungs were clear. Examination of the right breast revealed a previous mastectomy scar with intact skin without any sign of local recurrent axillary lymphadenopathy. The diffusely swollen left hip was tender on palpation, held in partial flexion position with its motion restricted by pain. The left lower limb had no obvious foot drop. Radiograph of the pelvis (figure 1) revealed a Garden-IV fracture neck of the left femur with periacetabular osteopenia. Multiple thin radiolucent metal foreign bodies were noted in both hip regions. These spear-like thin-shaped solid metal foreign bodies appear to be strategically placed over the pubis and right hip region. Beside the presence of bilateral femoral artery calcification, there was no other significant soft tissue abnormality. Laboratory investigations, including full blood count, renal profile and liver function tests, were within normal ranges. Chest radiographs revealed no significant abnormality. A diagnosis of neglected Garden-IV fracture neck of left femur was made with the presence of multiple susuk (charm needles) in the hip region.

The patient was optimized with subcutaneous short-acting insulin, fractionated heparin and thrombo-embolic stockings and deep breathing exercises prior to a planned total hip arthroplasty. Skin traction of the affected limb was applied to alleviate pain. A cemented

total hip replacement via a standard Southern-Moore approach was done approximately a week later. Patient consented for total hip replacement (THR) but refused consent for removal of susuk needles. No attempt was made to remove foreign bodies in the tissue around the left hip. Both the acetabular cup and femoral stem were fixed in acceptable positions (Figure 2).

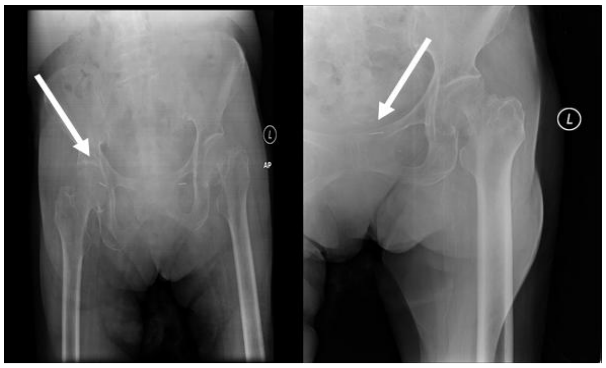

Fig. 1. Pre operative radiograph showing a Garden Type IV fracture neck of the left femur with periacetabular osteopenia. Multiple thin radiolucent metal foreign bodies noted bilaterally in the hip region.

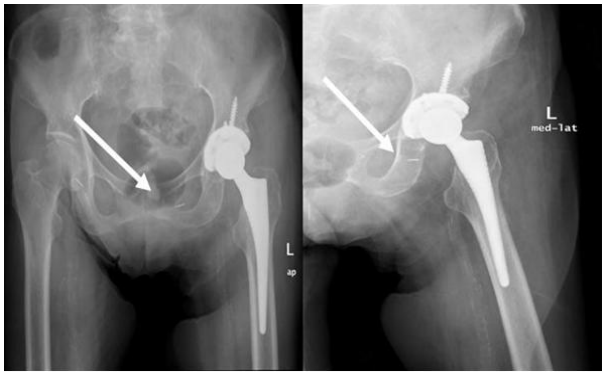

Fig. 2. Post-operative radiograph showing an cemented Total hip replacement and two foreign bodies below the left hip and pubic tubercle

Post-operatively, the patient was given epidural cocktail analgesia for two days. She was able to ambulate with a walker by day three post-operatively. Patient was then taught appropriate physiotherapy regimes, and allowed to be discharge on post-operative day four. On follow-up at two months post-THR, she was delighted to be able to ambulate with a walking frame, and had a painless good range of hip motion. Radiographs show the acetabulum cup and femoral stem in situ and in good alignment, with two foreign bodies being noted. The two month post procedure follow up revealed a delighted patient with good range of motion of hip and ambulating with walking frame.

DISCUSSION

Susuk or charm needles are needles made of gold or other precious metals that are inserted into the soft tissues of the body to act as talisman. The practice of inserting susuk follows a cultural superstitious belief that it either enhances beauty and youth, or is used as treatment of headaches and joint aches, or is used for protection against injury and accidents. This mystic practice is found among some South-East Asian people, especially Muslim females. Most susuk wearers are secretive about their hidden talismans. The practice of susuk wearing and its relevance to dentistry has been reported in south-east Asia [1-3]. In addition to oro-facial region, susuk have also been reported in occipital, thorax, chest wall regions [4,5]. The threat of foreign bodies in modern day surgical practice is evident. Migration of these substances causing vascular and nerve injuries in the extremities have been reported. These foreign bodies also cause increase risk of infection whether immediately upon insertion or at a later date [5]. Most foreign body infections require removal of the device before cure is possible. With globalization, discovery of charm needles on radiographs may become more frequent.

In conclusion, a thorough history and careful imaging are essential to a correct diagnosis. It is important to be aware of this practice among some Asian populations, to avoid misdiagnosis and mismanagement of these patients.

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