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ORIGINAL ARTICLE

Studies on Extracts of Three Medicinal Plants of South-Western Nigeria: *Hoslundia opposita, Lantana camara* and *Cymbopogon citratus*

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ABSTRACT

Assessment of antibacterial activity and phytochemical screening of extracts of three medicinal plants from South-Western Nigeria was done using *Streptococcus mutans, Klebsiella pneumonia, Pseudomonas aeruginosa, E .coli, and Staphylococcus aureus* isolated from patients admitted to Teaching Hospital Ado-Ekiti. The aqueous and methanol extracts had zones of inhibition suggesting susceptibilities of the organisms (1.0 to 11.0mm at concentration of 10µg/mL). Ethyl acetate extract showed antibacterial activities with zones of inhibition that varied from 1.0 to 5.0mm at 20µg/mL. The extracts of *Lantana camara* and *Hoslundia opposita* were also potent inhibitors of bacteria growth, showing the least MIC of 2.0µl/mL on most bacterial strains tested. The compounds tested in the plants samples include: tannins, phlobatanins, saponins, flavonoids, terpenoids and cardiac glycosides. The concentration of the following mineral elements were also determined: sodium, potassium, calcium, zinc, iron, manganese, lead and phosphorus while the percentage composition of ash, moisture, protein, fat, fibre carbohydrates and sugars were determined in the proximate analysis. This study shows that the plant samples contain a considerable but varying amount of each of these compounds. The plants samples contain all the minerals except lead. These findings have important implications in traditional medicine and these implications are discussed.

Key words: *Hoslundia opposite, Lantana camara, Cymbopogon citrates,* antibacterial activity, phytochemicals.

Introduction

For centuries, plant-based medications have been man's prime therapeutic weapons and are still in the frontline for treating a large number of diseases (Ameyaw *et al*., 2005). Traditional medicine is defined by the World Health Organization as the sum total of knowledge or practices whether explicable or inexplicable, used in diagnosing, preventing or eliminating a physical, mental or social disease which may rely exclusively on past experience or observations handed down from generation to generation, verbally or in writing. It also comprises therapeutic practices that have been in existence often for hundreds of years before the development of modern scientific medicine and are still in use today without any documented evidence of adverse effects (Elujoba *et al*., 2005).

It is estimated that there are 250,000 species of plants on earth (Borris, 1996). A relatively small percentage (1 to 10%) of these is used as foods by both human and other animal species. It is possible that even more are used for medicinal purposes. (Moerman, 1996).In Africa, drugs used mainly for curative purpose are prepared from plants, leaves, and herbs that are known to have medicinal values before the advent of Europeans medicine. Prescription of dosages and knowledge of active ingredient in the medicinal plants were

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however not known by the herbalist not to talk of the investigation of its effects on human body. Toxic or otherwise large amounts of raw materials are usually used in the preparation, which results in wastage and generally in overdosing of the patients (Sofowora, 1992).

The World Health Organization (WHO) has since urged developing countries of the world to utilize the resources of traditional medicine for achieving the goals of primary Health care. This injunction stems round the various advantages of traditional medicine namely, low cost, affordability, accessibility and acceptability and perhaps low toxicity, the practitioners are ready sources of medical manpower. It is also expected that in recognizing them and hence utilizing the advantages, the various disadvantages of the practice can also be resolved. These include lack of adequate scientific proof, imprecise diagnosis and dosage, unstandardized medicines and ocultic practices (Elujoba *et al*., 2005).

Much work has been done on medicinal plants in relation to their antimicrobial activities. The specific drug contained in medicinal plants are known as active principles, divided chemically into a number of group which include the glycosides, alkaloids, volatile oils, resins and steroids. Quite a number of these chemical compounds of plant origin have been shown to posses antimicrobial activities (Corthout, 1993). The aerial parts of the medicinal plant part of *Hoslundia opposite* is known to treat gonorrhea, cystitis, hookworm, cough, fever, colds, wounds and bilharziaz (Chhabra *et al*.,1984).

Many tropical plants have been confirmed to contain compounds like alkaloid, glycosides, saponins, tannins, waxes, volatile oils, carbohydrates, quinine etc. These compounds have been confirmed to be responsible for most of the biological activities of these plants. Those plants in which the phytochemistry has been determined include: *Asparagus africana, Boerhavia diffusa, Corrbretum racement, Emilia cocinea, Phylianthus discoidus, Grewia capinifolia, Lamea welwitsell* etc. (Chaisa-Wadi *et al.*, 2003).

The Nigerian plants selected for this study are: *Hoslundia opposita, Lantana camara* and *Cymbopogon citrates* with the objectives of determining the antibacterial activities, phytochemical and mineral element composition of leaf extract of the three plants.

Materials and methods

Medicinal plants

Fresh leaves of *Hoslundia opposita, Lantana camara* and *Cymbopogon citrates* were collected at different locations in Ado-Ekiti and Ikere Ekiti, Ekiti State, Nigeria. The plants were identified and authenticated at the herbarium unit of the Department of Plant Science University of Ado-Ekiti, Nigeria and voucher specimen deposited.

Extraction procedures

Extracts of the plants were prepared according to the method of Olukoya *et al.*,(1995). Seventy gram of each dried, grinded leaves were soaked separately in 200mL of sterile distilled water, methanol and ethyl acetate for seven days at 30 – 32^{0}C. The extracts were filtered through a Millipore filter (0.25µm). The resulting filtrate was concentrated under reduced pressure at 50^{0}C and then transferred into a well labeled sterile bottle.

Test organisms

The test organisms which included *klebsiella pneumonia*, *Staphylococcus aureus*, *Streptococcus mutans*, *Escherichia coli* and *Pseudomonas aeruginosa* were clinical strains obtained from the stock culture of the microbiology laboratory, teaching hospital, Ado-Ekiti, Ekiti state, Nigeria.

Antimicrobial assay

The antimicrobial assay was done using the agar diffusion method. The various test bacteria were standardized using the 0.5 McFarland turbidity standards. These standardized strains were inoculated onto the surface of sterile plates of Diagnostic sensitivity test agar (DST). Cork borer (6mm) was used to make wells on the inoculated DST agar. One milliliter of each concentration of extracts was introduced into designated wells. These were allowed to be absorbed into the agar, and then incubated at 37^{0}C for 24h. The antimicrobial activities were determined by the width of the zone of growth inhibition (Bauer, 1996).

Minimum inhibitory concentrations

One hundred mL of Mueller-Hinton broth was diluted with extract in each well of a microtiter plate. 100µl of stock solution of aqueous, methanol and ethyl acetate (400mg/mL) was added respectively and subsequently two fold serially diluted with Mueller – Hinton broth. The inoculum suspension (20µl) of each bacterial strain was then added in each well containing the extract dilution and the Mueller- Hinton broth. The final concentration of the extracts was measured respectively.

Phytochemical screening of extracts

The phytochemical screening of the extracts was done on the aqueous extracts and the powdered specimens using standard procedure as described by Edoga *et al.,* 2005 and Sofowora, 1992 . The following qualitative tests were carried out as follows:

Test for tannins

About 0.5 gram of dried powdered samples was boiled in 20mL of water and filtered. A few drops of 0.1% ferric chloride was added and observed for brownish green or blue black colouration.

Test for phlobatannins

The aqueous extract of each sample was boiled with 1% aqueous hydrochloric acid. Observation of deposits of a red precipitate was taken to indicate the presence of phlobatannins.

Test for saponins

Two grams of each powdered sample was boiled in a water bath and filtered. The boiled samples were one milliliter of filtrate was mixed vigorously to form a stable persistent froth. A formation of emulsion was observed after mixing the froth with about 3 drops of olive oil to indicate the presence of saponins.

Test for flavonoids

The method described by Sofowora (1992) was used to determine the presence of flaxonoids. Five milliliters of diluted ammonia solution were added to a portion of the aqueous filtrate of each plant extract followed by the addition of concentrated H_2SO_4. A yellow coloration which disappeared after some minutes indicates the presence of flavonoids.

Test for steroids

Two milliliters of acetic anhydride was added to 0.5g ethanol extract of each sample with 2mL of H_2SO_4. A colour change of violet to green is indicative of the presence of steroids.

Test for terpenoids

The Salkowski test was used to test for the presence of terpenoids. Five milliliter of each aqueous extract was mixed with 2mL of chloroform and 3mL of concentrate H_2SO_4. A reddish brown colouration of the interface was used to indicate the presence of terpenoids.

Test for cardiac glycosides

Five milliliter of each aqueous extract was treated with 2mL of glacial ascetic acid which contains one drop of ferric chloride solution. One milliliter of H_2SO_4 was later added. The formation of a brown ring indicates the presence of cardenoids.

Quantitative Determinations

To determine the quantity of each of the constituents, 2g of each powdered sample was first defatted with 100mL of diethyl ether for 2h using a soxhlet apparatus.

Determination of total phenol

This was determined by spectrophotometric methods. Each fat-free sample of the plants was boiled with 50mL of ether for 15min. 5mL of the extract is added to 10mL of distilled water. Two milliliter of ammonium hydroxide solution and 5mL concentrated amyl alcohol were also added. The mixture was left for 30mins after which it was observed for colour development. The optical density was read at a wavelength of 505nm.

In summary, the concentrations of the following chemical constituents were determined using standard procedures as previously described: total phenols, alkaloids, tannins, saponins, flavonoid, moisture content, ash content, mineral, fat, crude protein, fiber and soluble carbohydrates.

Results

The results on the investigation of antimicrobial activities of aqueous, methanol and ethyl acetate extracts of leaves of *Hoslundia opposita, Lantana camara* and *Cymbopogon citrates* are presented in Tables 1-3. Methanol and aqueous extracts of *Hoslundia opposite* showed appreciable antibacterial activities at concentration of 20µg/mL. This was observed on both Gram positive and negative bacteria. The zones of inhibition ranged from 6.5 to 11.00mm. It was however observed that at concentration of 10µg/mL of the aqueous and methanol extracts, all the test organisms showed mild susceptibility. Activity of ethyl acetate extract was far lower when compared to the other extracts (Table 1).

The antibacterial effect of *Lantana camara* was represented in Table 2. At concentration of 20µg/mL, all the bacteria were more sensitive to the methanol and aqueous extract than that of ethyl acetate when compared. Activities of *Lantana camara* against Gram positive and Gram negative bacteria showed an indication that the extracts of the plants are of broad spectrum. The zone of inhibition ranges from 6.00 to 10.00mm.

The antibacterial effect of *Cymbopogon citratus* was represented in Table 2. At 20µg/mL, methanol and aqueous extract of *C. citrates* inhibited *Klebsiella Pneumoniae, Streptococcus mutans* and *E. coli* while *Staphylococcus aureus* and *Pseudomonas aeruginosa* were resistant to the extracts. The zone of inhibition ranged from 4.00 to 10.00mm.

The qualitative phytochemical tests showed that *H. opposita* contained alkaloids, tannin, saponin and flavonoid while only tannin and steroid were found in *C. citratus*. Table 4 below shows the result of the qualitative analysis of the phytochemical components of the plants. The percentage composition of tannin was highest in *H. opposta* (6.83%) while that of oxalate was least (0.09%) In *L. camara*, the percentage composition of saponin was the highest (6.93%) and *C. citratus* contains extremely low percentage composition of the phytochemicals, alkaloids, flavonoid and saponin were not detected. The details are shown in table 5. The proximate analysis of the powdered samples revealed that each sample contain considerable amount of nutrients. The percentage content of proteins in the samples was highest while all the samples contain considerably low quantity of moisture and fat. The details of the mineral content analysis shows that the plants sample contain extremely high amount of phosphorus, potassium, calcium, sodium and magnesium. The composition of copper and manganese was the minimum. The proximate analysis of the three plants (Table 6) revealed that the three plants have appreciable amount of ash, moisture content, protein, fat, fibre, carbohydrate and minimal yields for sugar (between 6.0mg/100g or 0.01%). The mineral analysis showed the plant samples contain the following elements: sodium, potassium, calcium, magnesium, zinc, iron, copper, manganese and phosphorus. Lead was not present in all the plant sampled investigated. The result of the mineral content determination is shown in table 7.

Table 1: Antibacterial effects of *Hoslundia opposite* extracts. (zone of inhibition in mm)

Test organisms	AQUEOUS		METHANOL		ETHYL ACETATE	
	10µg/mL	20µg/mL	10µg/mL	20µg/mL	10µg/mL	20µg/mL
Klebsiella spp	6.0	10.0	7.0	11.0	2.0	4.0
S. aureus	4.0	7.0	5.0	9.0	1.0	3.5
Strep. Mutans	5.0	8.0	4.0	6.5	2.0	4.5
E. coli	5.0	7.5	6.0	9.5	3.0	4.50
Ps. aeruginosa	4.0	6.5	5.0	7.0	1.0	3.0

Table 2: Antibacterial effects of *Lnatana camara* extracts. (zone of inhibition in mm)

Test organisms	AQUEOUS		METHANOL		ETHYL ACETATE	
	10µg/mL	20µg/mL	10µg/mL	20µg/mL	10µg/mL	20µg/mL
Klebsiella spp	4.5	8.0	6.0	9.0	1.5	3.0
S. aureus	4.0	7.5	6.0	8.0	2.0	4.0
Strep. Mutans	6.0	8.5	6.5	8.0	2.0	4.5
E. coli	6.0	10.0	7.0	10.0	3.0	5.0
Ps. aeruginosa	2.0	6.0	4.0	7.0	-	-

Table 3: Antibacterial effects of *Cymbopogon citrates* extracts. (zone of inhibition in mm)

Test organisms	AQUEOUS		METHANOL		ETHYL ACETATE	
	10µg/mL	20µg/mL	10µg/mL	20µg/mL	10µg/mL	20µg/mL
Klebsiella spp	4.5	8.0	6.0	9.0	1.5	3.0
S. aureus	4.0	7.5	6.0	8.0	2.0	4.0
Strep. Mutans	6.0	8.5	6.5	8.0	2.0	4.5
E. coli	6.0	10.0	7.0	10.0	3.0	5.0
Ps. aeruginosa	2.0	6.0	4.0	7.0	-	-

Table 4: Qualitative analysis of the phytochemical of the medicinal plants. (Hoslundia opposita, Lantana camara and Cymbopogon citratus).

Plant	Alkaloid	Tannin	Saponin	Steroid	Phlobatanin	Terpenoid	Flavonoid	Cardiac glycoside
Hoslundia opposita	+	+	-	-	-	-	+	+
Lantana camara	+	+	+	-	-	-	+	-
Cymbopogon Citrates	-	+	-	+	-	-	-	-

Table 5: Quantitative phytochemical analysis of plant extract.

Plant	Alkaloid %	Phenols %	Tannin %	Flavonoid %	Saponion %	Phytin phosphorus %	Phytate %	Oxalate %
Hoslundia opposita	1.27	2.92	6.83	1.36	5.80	0.39	0.70	0.09
Lantana camara	2.34	2.38	5.68	1.29	6.93	0.22	0.71	0.08
Cymbopogon citratus	ND	1.29	2.67	ND	ND	0.30	1.08	0.12

Key: + ----- Present; - ---- Absent; ND---- Not detected

Table 6: Proximate analysis of the plants

Plant	% Ash	% Moisture content (MC)	% Protein content (CP)	% Fat	% Fibre	% Carbohydrate CHO	Mg/100g Sugar
Hoslundia opposita	12.01	3.13	17.58	6.42	10.16	50.71	6.12
Lantana camera	11.06	2.84	17.62	4.24	12.22	52.03	5.82
Cymbopogoncitratus	13.57	3.28	8.81	2.14	31.58	40.68	6.53

Table 7: Determination of the mineral contents of the plants (ppm).

	Na	K	Ca	Mg	Zn	Fe	Cu	Mn	Pb	P
Hoslundia opposita	648.90	917.34	1073.79	719.39	522.40	59.87	0.97	2.90	ND	1187.72
Lantana camara	770.90	1017.99	1160.31	785.73	526.78	89.94	1.98	5.93	ND	973.51
Cymbopogoncitratus	785.88	1082.00	851.37	729.08	681.47	89.22	0.95	4.75	ND	231.54

Discussion

The aqueous and methanol extracts of *Hoslundia opposita, Lantana camara* and *Cymbopogon citrates* when used against some pathogenic organisms such as *Klebsiella pneumoniae, Staphylococcus aureus*, *Streptococcus mutans*, *Pseudomonas aeruginosa* and *Escherichia coli* showed marked antibacterial activity. This observation against both Gram-positive and Gram – negative organisms indicated broad spectrum of activity since all organisms were sensitive to the extracts. However, the methanol extract proved to be more effective, probably the best extracting agent for these plants. The poor anti bacterial activity observed with extract of ethyl acetate implicated this agent as not good for extraction of active components of these plants.

The phytochemical screening and quantitative estimation of the percentage crude yield of chemical constituents of the plants showed that the leaves are rich in alkaloids, flavonoids, tannin, saponins and cardiac glycosides. There are previous reports that these compounds have also be found in leaves of other plant e.g. *Ocimum sanctum and Centella asiastice* etc. The presence of these compounds in the plants has been attributed to most of their biological activities (Seenivasan *et al,* 2006).

Several studies have been carried out to determine the chemical composition of these plants, some of which have revealed that these plants, particularly *H. opposita* and *L. camara* contain essential oils, whose chemical constituents are fully characterized. Other studies are underway to determine the usefulness and the implication of the presence of the compounds in the oils present in the plants (Tonzibo *et al.,* 2006; Randrianalijiana *et al.,* 2005). The chemical composition of the leaves of these plants was determined in this study and this, in our opinion, makes this study novel and innovative.

A study by Gills (1992) revealed that *C. citratus* contains alkaloids which is a stark contrast to the observation in this study. In our view, the best explanation for this observation could be that plants and their natural constituents are subject to variations due to differences in their locations and climatic condition of where they thrive. It was observed in this study that *C. citratus* contain steroids. Steroids are important compounds of interest in pharmacy due to their various uses in inducing the production of sex hormone in

humans and animals. The detection of major compounds in the leaves has important implications as these compounds have been found to confer important biological activities including antibacterial, antiviral, antidiabetic and antihepatotoxic on the plants (Iwu, 1993; Corthout, 1993). These biological activities could be explored in African traditional medicine.

All the plants studied can be seen to be potential source of useful antibacterial drugs. Further studies are however recommended on these plants in other to isolate, identify, characterize and elucidate the structure of the bioactive components.

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