



PHYTOCHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE LEAVES OF *ALCHORNEA CORDIFOLIA* (SCHUM AND THONN), *SANSEVIERA LIBERICA* (GERARD LABR) AND *UVARIA CHAMAE* (P. BEAUV)

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ABSTRACT

The phytochemical content and antimicrobial activity of the leaves of *Alchornea cordifolia*, *Sansevieria liberica* and *Uvaria chamae* were investigated. The ethanolic extracts of the leaves of the plants were tested against some human pathogens (*Escherichia coli*, *Salmonella typhi*, *Shigella flexineri*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Candida albicans*). The antimicrobial activity of the plants extracts was carried out using the Agar well diffusion method. The phytochemical screening showed that the leaves of the all plant species had flavonoid, tannins and steroids. Alkaloid and phenol occurred in *A. cordifolia* and *U. chamae*, while saponins occurred in *S. liberica* and *U. chamae*. Averagely, the percentage phytochemical constituent of the leaves of the plants are as follows; Alkaloid (8.77-10.15), flavonoid (5.33 -6.67), phenols (0.75 -1.12), saponin (1.41 -5.96), steroid (0.6 -3.33) and tannin (0.001 -0.067). The result obtained indicated that the leaf extracts inhibited all the test pathogens. The inhibition zone ranged from 7.04 to 25.57 mm which was higher than that of the commercial antibiotics Penicillin (5.25 to 12.25mm) used as control. The extracts of *A. cordifolia* had the highest activity on *E. coli* and least activity on *S. aureus*, while *S. liberica* had the highest activity on *S. aureus* and least on *S. typhi*. On the other hand, the leaves of *U. chamae* had the highest activity on *E. coli* and least on *C. albicans*. Concentration affected the ability of the extracts to inhibit the growth of the pathogens. The higher the concentration of the extracts, the higher the rate of inhibition of the pathogens. The minimum inhibitory concentration (MIC) ranged from 3.11 to 18.04 mg/ml). The results obtained indicate that the leaves of these plants are good sources of phytochemical and have antimicrobial activity against the pathogens tested, thus could be exploited as alternative antimicrobial drugs for treatment of diseases caused by these pathogens.

INTRODUCTION

The use of plants in the maintenance of good

health is well reported [1,2]. It has also been reported that the bases of many modern pharmaceuticals used today for the treatment of various ailments are plants and plant based products [3]. Plants have been generally utilized for the treatment of diseases worldwide. About 80% of the world populations depend on plants based medicine for their health care [4]. WHO (1996), [5] also observed that the majority of the population in the developing countries still

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rely on herbal medicines to meet their health need. The use of plants and plant based products to meet societal health need is due to the fact that the indiscriminate use of commercial antimicrobial drugs in treatment of infectious diseases has led to the development of multiple drug resistance [6] and the adverse effects on host, associated with the use of conventional antibiotics. Many of these indigenous plants contain bioactive compounds that exhibit physiological activities against bacteria and other microorganisms and are also used as precursors for the synthesis of useful drugs. Thus the use fullness of these plant products in medicine is due to the presence of bioactive substances such as alkaloids, tannins, flavonoids, phenolic compounds, steroids, resins and other secondary metabolites which they contain and are capable of producing definite physiological action in the body [7,8]. Phytochemicals are known to carry out important medicinal roles in the body. Alkaloids are known to have a powerful effect on animal physiology. They play some metabolic role and control development in living system [2]. They are also used as starting materials in the manufacture of steroidal drugs and carry out protective function in animals, thus are used as medicine especially steroidal alkaloids [9,10]. Isolated pure plant alkaloids and their synthetic derivatives are used as basic medicinal agent for their analgesic, antispasmodic and bactericidal effect [11]. Flavonoids are known to carry out antioxidant, protective effects and inhibit the initiation, promotion and progression of tumors [12,13]. Isoflavones, some kind of flavonoids are phytoestrogen which effectively modulate estrogen levels in human [14]. A type of flavonoid anthocyanin helps in reducing the incidence of cardiovascular diseases, cancer, hyperlipidemias and other chronic diseases [15]. Phenolic compounds in plants are potentially toxic to the growth and development of pathogens [16]. Research reports also show that phenolic compounds carry out potent antioxidant activity and wide range of pharmacologic activities which include anti- cancer, antioxidant and platelet aggregation inhibition activity [17,18]. Saponins play essential roles in medicine. These include serving as expectorant and emulsifying agent [19] and having antifungal properties [20]. Tannins are reported to inhibit pathogenic fungi [1]. They are also associated with many human physiological activities such as stimulation of phagocytic cells and host mediated tumor activity and a wide range of infective actions [21]. Steroid containing compounds are of importance in pharmacy due to their role in sex hormones [22]. Steroids such as equine estrogen are implicated in the reduction of risks of coronary heart and neurodegenerative diseases in healthy and young postmenopausal women [23]. At low concentration tannins show antimicrobial, cytotoxic and astringent properties [24,25].

The phytochemical screening of some plants has been carried out and they are found to be rich in alkaloids, phenols, flavonoid, saponin and tannins [20,26-28]. The

antimicrobial activities of plants have been reported [29,30,3]. They are therefore used in the treatment of many diseases such as rheumatism, diarrhea, malaria, elephantiasis, cold, obesity, dysentery, high blood pressure, malnutrition, gonorrhoea and others [1,31,32].

Alchmea cordifolia belongs to the Euphorbiaceae family. It is widely spread in secondary forest in Nigeria and Democratic Republic of Congo. The leaves of *A. cordifolia* are used in the treatment of gastrointestinal and liver disorder, elephantiasis, sickle cell disease and epilepsy [33]. They are also used as anti-inflammatory Laxative, and analgesic and for treatment of eye infection.

Sansevieria liberica belongs to Agavaceae family. It is a perennial rhizomatous plant with variable leaves. The leaves of *S. liberica* are used for the treatment of gonorrhoea, pile, asthma and eczema [34].

Uvaria chamae belongs to Annonaceae family. The leaves of *U. chamae* are used in the treatment of wounds, lesions, ophthalmia, yellow fever and jaundice [33].

The objectives of this investigation is to ascertain the presence and quantity of some phytochemicals in the leaves of *A. cordifolia*, *S. liberica* and *U. chamae* and to determine the antimicrobial activity of the leaves of these plants, in view of their use as alternative sources of antimicrobial drugs used in the treatment of diseases.

MATERIALS AND METHODS

Plant samples

The leaves of *Alchmea cordifolia* were collected from the forest strip of the Forestry Department, College of Natural and Environmental Management, Michael Okpara University of Agriculture, Umudike Umuahia, Abia State. Those of *Sansevieria liberica* were collected from a residential compound in National Root Crop Research Institute, Umudike, Umuahia, while the leaves of *U. chamae* were collected from the forest strip of the Forestry Research Institute, Okwuta, Isieke Umuahia. The plants were identified by Mr. N. Ibe of the Forestry Department, College of Natural and Environmental Management, Michael Okpara University of Agriculture Umudike, Umuahia Abia State, Nigeria. The leaves of *U. chamae* and *A. cordifolia* were air dried for one week, while those of *S. liberica* were oven dried for at 60°C for 48 hours using Genab model mimo 175/F/ OG oven. The leaves were ground to powder using Wood land electric grinding machine. Powdered samples were stored in the Plant Science and Biotechnology laboratory to be used for analysis.

Determination of the phytochemical content of the plant samples

Both qualitative and quantitative tests were carried out on the samples to determine the presence and the amount of the phytochemicals in the powdered



samples.

Qualitative analysis of the plant samples

Test for presence of alkaloids.

The presence of alkaloids in each sample was investigated using the method described by Harborne (1973) [35].

An alcoholic extract was used and obtained by dispersing 2g of the powdered sample in 10 ml of ethanol. The mixture was through shaken before filtering using Whatman No (40) filter paper. 2 ml of the filtrate was added into a test tube and 3 drops of pirovic acid was mixed with it. The formation of light green colouration indicates presence of alkaloid.

Test for the presence of flavonoid

The determination of presence of flavonoid in the sample was carried out using the acid alkaline test described by Harborne (1973) [35].

2ml of the aqueous extract was added into a test – tube and a few drops of Bench Concentrated ammonia (NH₄) were also added. The formation of a yellow colouration shows presence of flavonoid. Confirmatory test was carried out by adding few drops of concentrated hydrochloric (HCL) into the yellow solution which turned colourless.

Test for the presence of phenols.

The presence of phenols in the sample was carried out using the Harborne (1973) [35] methods.

The fat free sample was boiled with 50ml of ether for 15 minutes. 5ml of the extract was pipette into a 50ml flask and 10ml of distilled water added into it. 2ml of ammonia hydroxide solution and 5ml of concentrated amyl alcohol were also added. The mixture was allowed to react for 30 minutes for colour development.

Test for the presence of saponin

The presence of saponins in the samples was determined using Harborne (1973) [35] method.

Two tests were involved in the investigation, the froth test and emulsion test.

In the froth test, 2 ml of the aqueous extract was mixed with 5 ml of distilled water in a test tube. The mixture was shaken vigorously. A stable froth on standing indicates the presence of saponins.

In the emulsion test, 3 drops of groundnut oil, was added to the aqueous extract mixed with 5 ml of distilled water and shaken well. Formation of emulsion indicates the presence of saponins.

Test for the presence of tannin

The presence of tannins in the samples was determined using the method described by Harborne (1973) [35].

2 ml of the aqueous extract filtrate and 3 ml distilled water was put into a test tube. A few drops of 0.1% ferric chloride was added to the mixture. The formation of a very dark precipitate indicated presence of tannin.

Quantitative determination of the phytochemical constituents of the plant samples

Alkaloid determination

The determination of the concentration of alkaloid in the leaves of the plants was carried out using the alkaline precipitation gravimetric method described by Harborne (1973) [35]. 5 g of the powdered sample was soaked in 20 ml of 10% ethanolic acetic acid. The mixture was stood for four (4) hours at room temperature. Thereafter, the mixture was filtered through Whatman filter paper (No 42). The filtrate was concentrated by evaporation over a steam bath to ¼ of its original volume. To precipitate the alkaloid, concentrated ammonia solution was added in drops to the extract until it was in excess. The resulting alkaloid precipitate was recovered by filtration using previously weighed filter paper. After filtration, the precipitate was washed with 9% ammonia solution and dried in the oven at 60°C for 30 minutes, cooled in a dessicator and reweighed. The process was repeated two more times and the average was taken. The weight of alkaloid was determined by the differences and expressed as a percentage of weight of sample analyzed as shown below.

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{\text{Weight of sample}} \times 100$$

Where:-

W₁ = weight of filter paper

W₂ = weight of filter paper + alkaloid precipitate

Flavonoid determination

The flavonoid content of the leaves of the plant was determined by the gravimetric method as was described by Harborne (1973) [35].

5g of the powdered sample was placed into a conical flask and 50ml of water and 2ml HCl solution was added. The solution was allowed to boil for 30 minutes. The boiled mixture was allowed to cool before it was filtered through Whatman filter paper (No 42). 10ml of ethyl acetate extract which contained flavonoid was recovered, while the aqueous layer was discarded. A pre weighed Whatman filter paper was used to filter second (ethyl-acetate layer), the residue was then placed in an oven to dry at 60°C. It was cooled in a dessicator and weighed. The quantity of flavonoid was determined using the formular.

$$\% \text{ Flavonoid} = \frac{W_2 - W_1}{\text{Weight of Sample}} \times 100$$



Where:-

W₁= Weight of empty filter paper

W₂= Weight of paper + Flavonoid extract

Determination of phenols

The concentration of phenols in the leaves of the plants was determined using the folin-cio Caltean colorimetric method described by Pearson (1976) [36].

0.2 g of the powdered sample was added into a test tube and 10ml of methanol was added to it and shaken thoroughly the mixture was left and to stand for 15 minutes before being filtered using Whatman (No42) filter paper. 1 ml of the extract was placed in a test-tube and 1 ml folin-cio Caltean reagent in 5ml of distilled water was added and color was allowed to develop for about 1 to 2 hours at room temperature. The absorbance of the developed colour was measured at 760 nm wave. The process was repeated two more times and an averaged taken. The phenol content was calculated thus.

$$\% \text{ Phenol} = 100 / w \times AU / AS \times C / 100 \times VF / VA \times D$$

D

Where,

W= weight of sample analyzed

AU= Absorbance of test sample

AS= Absorbance of standard solution

C= concentration of standard in mg/ml

UF= total filtrate volume

VA= Volume of filtrate analyzed

D= Dilution factor were applicable

Determination of saponins

The saponin content of the sample was determined by double extraction gravimetric method (Harborne, 1973).

5 g of the powered sample was mixed with 50 ml of 20% aqueous ethanol solution in a flask. The mixture was heated with periodic agitation in water bath for 90 minutes at 55° C; it was then filtered through what man filter paper (No 42). The residue was extracted with 50 ml of 20% ethanol and both extracts were poured together and the combined extract was reduced to about 40 ml at 90°C and transferred to a separating funnel where 40 ml of diethyl ether was added and shaken vigorously. Separation was by partition during which the ether layer was discarded and the aqueous layer reserved. Re extraction by partitioning was done repeatedly until the aqueous layer become clear in color. The saponins were extracted, with 60 ml of normal butanol. The combined extracts were washed with 5% aqueous sodium chloride (NaCl) solution and evaporated to dryness in a preweighed evaporation dish. It was dried at 60° C in the oven and reweighed after cooling in a dessicator. The process was repeated two more times to get an average. Saponin content was determined by difference and calculated as a percentage of the original sample thus

$$\% \text{ Saponin} = \frac{W_2 - W_1}{\text{Weight of Sample}} \times 100$$

Where

W₁ = weight of evaporating dish

W₂ = weight of dish + sample

Steroid determination

The steroid content of the leaves of the plants was determined using the method described by Harborne (1973) [35].

5g of the powdered sample was hydrolysed by boiling in 50 ml hydrochloric acid solution for about 30minutes. It was filtered using Whatman filter paper (N042), the filtrate was transferred to a separating funnel. Equal volume of ethyl acetate was added to it, mixed well and allowed separate into two layers. The ethyl acetate layer (extract) recovered, while the aqueous layer was discarded. The extract was dried at 100°C for 5minutes in a steam bath. It was then heated with concentrated amyl alcohol to extract the steroid. The mixture becomes turbid and a reweighed Whatman filter paper (N042) was used to filter the mixture properly. The dry extract was then cooled in a dessicator and reweighed. The process was repeated two more times and an average was obtained.

The concentration of steroid was determined and expressed as a percentage thus

$$\% \text{ Steroid} = \frac{W_2 - W_1}{\text{Weight of Sample}} \times 100$$

Where,

W₁= weight of filter paper.

W₂ = weight of filter paper + steroid

Tannin determination

The tannin content of the leaves of the plants was determined using the Folin Dennis spectrophotometric method described by Pearson (1976) [36].

2 g of the powered sample was mixed with 50 ml of distilled water and shaken for 30 minutes in the shaker. The mixture was filtered and the filtrate used for the experiment. 5 ml of the filtrate was measured into 50 ml volume flask and diluted with 3 ml of distilled water. Similarly 5 ml of standard tanuric acid solution and 5 ml of distilled was added separately. 1 ml of Folin- Dennis reagent was added to each of the flask followed by 2.5 ml of saturated sodium carbonate solution. The content of each flask was made up to mark and incubated for 90 minutes at room temperature. The absorbance of the developed colour was measured at 760.nm wave length with the reagent blank at zero. The process was repeated two more times to get an average. The tannin content was calculated as shown below

$$\% \text{ tannin} = 100/W \times AY / AS \times C / 100 \times VF / VA \times D$$

Where,



W= weight of sample analysed
 AY=Absorbance of the standard solution
 C= Concentration of standard in mg /ml.
 VA= volume of filtrate analysed
 D= Dilution factor where applicable

Determination of antimicrobial activity

Preparation of plant extracts.

The ethanolic extracts of the leaves of *Alchornea cordifolia*, *Sansevieria liberica* and *Uvaria chamae* were prepared using the method of Ijeh et al. (2005) [37].

Fifty grams of the powdered sample were soaked in 200ml of absolute ethanol and allowed to stand for 24 hours. They were filtered using Whatman No1 Filter Paper. The filtrates were evaporated to dryness with rotary evaporator at 40°C to thick residues. The residues were dissolved in deionised water to obtain the desired plant extracts for the antimicrobial tests.

Preparation of Innocular

The human pathogens; *Escherichia coli*, *Staphylococcus aureus*; *Shigella flexneri*; *Klebsiella pneumoniae*; *Salmonella typhi* and *Candida albicans* used in the research were obtained from the stock culture of the Microbiology Laboratory, Federal Medical Centre, Umuahia, Abia State, Nigeria. Viability test of each isolate was carried out by resuscitating the organism in buffered peptone broth and thereafter sub-cultured into nutrient agar medium and incubated at 37°C for 24 hours.

Antimicrobial activity test

The sensitivity of the test organism to the ethanolic extracts of the leaves of *A. cordifolia*, *S. liberica* and *U. chamae* was carried out using the diffusion method described by Ebi and Ofoefule (1997) [38].

20ml of the molten nutrient agar was seeded with 0.2ml of broth culture of the test organisms in sterile Petri dishes. The Petri dishes were rotated slowly to ensure a uniform distribution of the organisms. They were left to solidify and dish cups of 8.0mm diameter were made in the agar using a sterile Pasteur pipette. The Petri-dishes were allowed to stand for about 30 minutes at room temperature to allow for the proper diffusion of the extracts to take place. The plates were then incubated at 37°C for 24 hours. The zones of inhibition in millimetres were measured and recorded.

The test was carried out in the Laboratory of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria.

Minimum Inhibitory Concentration (MIC) Test

The agar dilution method described by Baron and Finegold (1990) [39] was used to determine the minimum inhibitory concentration.

Six grams of nutrient agar were dissolved in 250ml of distilled water in a conical flask. After sterilization, the nutrient agar was poured into sterilized Petri dishes to solidify. The microorganisms were introduced into the wells using swap sticks. Extracts of 5mg/ml, 15mg/ml, 20mg/ml and 25mg/ml were made from the original test samples. The petri dishes were then placed in the incubator at 37°C for 24 hours. The inhibition zones in millimetres were measured and recorded.

Preparation of Antibiotics Stock Solution

500mg of Penicillin was dissolved in 5ml of distilled water for the antimicrobial assay. 12g of nutrient agar was dissolved in 250ml of distilled water in a conical flask. The nutrient agar was poured into sterilized Petri dishes after sterilization. After solidification, wells were made using a sterilized cork borer and microorganisms were introduced. The dissolved antibiotics solution was poured into the wells using a dropping pipette after which the Petri dishes were incubated for 24 hours at 37°C. The inhibitory zones in millimetre were measured and recorded.

Statistical Analysis

The tests were carried out in triplicate; data obtained were analysed using mean and standard deviation.

RESULT AND DISCUSSION

The results of the evaluation of the presence of phytochemicals in the leaves of *Alchornea cordifolia*, *Sansevieria liberica* and *Uvaria chamae* were summarized in table 1.

The leaves of all the plant species had flavonoids, steroids and tannins. Alkaloids and phenols were present in the leaves of *A. cordifolia* and *U. chamae* and were absent in *i. liberica*. Saponins occurred in the leaves of *S. liberica* and *U. chamae* but not in the leaves of *A. cordifolia*. The presence of these phytochemicals has conferred to the leaves of these plants their medicinal value [33,34]. These phytochemicals are known to have antimicrobial activity [40].

The quantitative estimation of the phytochemical composition of the leaves of the three plant species is summarized in table 2.

The alkaloid content of the leaves of the plants ranged from 8.77 to 10.15%. *U. chamae* had more alkaloid when compared to that of *A. cordifolia*. Alkaloids are known to exhibit marked physiological activity when administered to animals. Pure isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agents for analgesic, antispasmodic and bactericidal effects [41].

The concentration of flavonoids in the leaves of the plant species ranged from 5.33 to 6.67%. The leaves of *U. chamae* had the highest amount of flavonoid, while



those of *A. cordifolia* had the least flavonoids. The presence of flavonoids in the leaves indicates their medicinal value. Flavonoids are antioxidants and free radical scavengers which prevent oxidative cell damage, have strong anticancer activity and protect the cells against all stages of carcinogenesis. Flavonoids in intestinal tract lower the risk of heart disease [22].

The leaves of *A. cordifolia* had the highest percentage of phenols (1.12%), while the leaves of *U. chamae* had 0.75% of phenols. The leaves of *S. liberica* had no phenol. The presence of phenolic compounds in the leaves of *A. cordifolia* and *U. chamae* indicates that they may act as antimicrobial agents. Phenols and phenolic compounds are extensively used in disinfection and remain the standard with which other bactericides are compared [20].

The concentration of tannins in the three plant species is quite low. It ranged from 0.001% to 0.067%, which is quite low. Tannins have been reported to possess astringent properties, hasten the healing of wound and inflamed mucous membranes [13,42]. The results of the antimicrobial activity of the leaves of *A. cordifolia*, *S. liberica* and *U. chamae* are summarized in tables 3 and 4.

The ethanolic extracts of the leaves of the three plant species used for the test displayed antimicrobial activity in varying degrees against the test pathogens (table 3). The inhibition zone ranged from 7.04 to 25.57 mm and

these values were higher than those of the commercial antibiotics Penicillin (5.25 to 12.25 mm) used as control (table 3). The antimicrobial activity of the leaves of other plants has also been documented. The ability of the extracts to inhibit the growth of the microorganisms might be as a result of the presence of bioactive substances (alkaloids, flavonoids, phenols, saponin, steroid, tannin) in their leaves. The leaf extracts of *A. cordifolia* showed the highest antimicrobial activity with inhibition zone (9.77 – 25.57 mm), while those of *U. chamae* had the least antimicrobial activity (7.04 – 19.00mm). This observed trend might be related to the concentration of these bioactive constituents in them. *A. cordifolia* had more inhibitory effect on *E. coli* and least on *S. aureus*, while *S. liberica* had the highest inhibitory effect on *S. aureus* and least on *S. typhi*. On the other hand, *U. chamae* had the highest inhibitory effect on *E.coli* and least on *C. albicans*.

Generally, the concentration of the extracts affected the rate of inhibition of growth of pathogens. As the concentration of the leaf extracts increased, the rate of the inhibition of the growth of the pathogens increased. This trend has also been observed by other researchers [43,44]. The minimum inhibitory concentration of the ethanolic extracts of the leaves of the three plant species ranged from 3.11 to 23.90 mg/ml (table 4).

Table 1. The qualitative analysis of the phytochemicals in the leaves of *Alchornea cordifolia*, *Sansevieria liberica* and *Uvaria chamae*

Plant species	Alkaloid	Flavonoid	Phenol	Saponin	Steroid	Tannin
<i>Alchornea cordifolia</i>	+	+	+	–	+	+
<i>Sansevieria liberica</i>	–	+	–	+	+	+
<i>Uvaria chamae</i>	+	+	+	+	+	+

Key + = presence - = absence

Table 2. The percentage alkaloid, flavonoid, phenol, saponin, steroid and tannin contents of the leaves of *A. cordifolia*, *S. liberica* and *U. chamae*

Plant species	Alkaloid	Flavonoid	Phenol	Saponin	Steroid	Tannin
<i>A. cordifolia</i>	8.77 + 0.03	5.33 + 0.15	1.12 + 0.02	0.00 + 0.00	0.99 + 0.02	0.67 + 0.03
<i>S. liberica</i>	0.00 + 0.00	6.50 + 0.10	0.00 + 0.00	1.41 + 0.08	3.33 + 1.53	0.001 + 0.00
<i>U. chamae</i>	10.15 + 0.20	6.67 + 0.15	0.75 + 0.02	5.96 + 0.40	0.65 + 0.25	0.05 + 0.002

Table 3. The antimicrobial activity of the ethanolic extracts of the leaves of *A. cordifolia*, *S. liberica*, and *U. chamae* on *Escherichia coli*, *Salmonella typhi*, *Shigella flexneri*, *Staphylococcus aureus*, *Klebsiella pneumonia* and *Candida albicans*.

Pathogenic organisms	Zone of Inhibition (mm)			
	<i>A. cordifolia</i>	<i>S. liberica</i>	<i>U. chamae</i>	Penicillin stock solution
<i>E. coli</i>	25.57 + 2.07	16.91 + 0.90	19.00 + 1.25	7.25 + 2.17
<i>S. typhi</i>	14.66 + 0.57	10.76 + 0.79	10.18 + 1.36	6.75 + 3.27
<i>S. flexneri</i>	21.07 + 1.06	13.56 + 0.72	11.06 + 7.10	10.25 + 2.95
<i>S. aureus</i>	9.77 + 0.39	17.78 + 0.46	15.03 + 1.05	12.25 + 3.96
<i>K. pneumonia</i>	17.39 + 1.38	14.51 + 1.01	17.03 + 1.17	8.75 + 3.96
<i>C. albicans</i>	24.42 + 0.92	14.52 + 0.05	7.04 + 2.36	5.25 + 1.79



Table 4. Minimum inhibitory concentration (mg/ml) of the ethanolic extracts of the leaves of *A. cordifolia*, *S. liberica* and *U. chamae* on *E. coli*, *S. typhi*, *S. flexneri*, *S. aureus*, *K. pneumonia* and *C. albicans*

Pathogenic organism	<i>A. cordifolia</i>				<i>S. liberica</i>				<i>U. chamae</i>			
	MIC	5	15	20	25	5	15	20	25	5	15	20
<i>E. coli</i>	13.0	17.8	18.0	23.9	7.0	12.0	12.3	14.2	15.1	16.0	16.3	18.0
<i>S. typhi</i>	5.0	8.1	9.1	11.2	3.3	5.0	6.9	9.9	4.0	6.5	7.0	8.0
<i>S. flexneri</i>	5.3	10.1	10.4	18.0	5.3	7.3	8.9	10.3	3.1	6.9	7.3	9.1
<i>S. aureus</i>	3.2	4.3	4.8	5.3	8.1	10.0	11.6	10.4	10.4	11.3	12.3	12.9
<i>K. pneumonia</i>	10.7	12.0	12.3	15.5	6.1	7.3	9.3	12.2	11.2	13.0	13.1	15.1
<i>C. albicans</i>	16.1	18.0	18.0	21.1	10.1	11.9	11.1	12.1	5.1	5.8	6.4	6.1

MIC = Minimum Inhibitory Concentration (mg/ml)

CONCLUSION

This investigation has revealed that the leaves of the three plant species studied have high phytochemical content and have antimicrobial activity on the test human pathogens used in this research. This is an indication that

they are of high medicinal value. Thus they could be exploited to be used in the formation of alternative antimicrobial drugs which will be used to cure and control human diseases.

REFERENCES

- Burkill IH. (1995). The Useful Plants of West Tropical Africa (Vol 3 Families J-L). *Royal Botanical Garden, Kew*, 605.
- Edeoga HO and Eriata DO. (2001). Alkaloid, tannin and saponin contents of some Nigerian medicinal plants. *Journal of Medicinal and Aromatic Plant Science*, 23, 244-249.
- Kamba AS and Hassan LG. (2010). Phytochemical screening and antimicrobial activities of *Euphorbia balsamifera* leaves, stem and root against some pathogenic microorganisms. *African Journal of Pharmaceutical Sciences and Pharmacy*, 1(1), 57-64.
- World Health Organisation (WHO) (2001). General guidelines for Methodologies on research and evaluation of traditional medicine. World Health Organization, Geneva 9(8), 1378-1382.
- World Health Organisation(WHO) (1996). Technical Series Trace Element in Human Nutrition and Health, World Health Organization, Geneva pp 199-205.
- Gupta C, Amar P, Ramesh G, Uriya C and Kumari, A. (2008). Antimicrobial activity of some herbal oils against common food-borne pathogens. *African Journal of Microbial Research*, 2, 258-261.
- Bishnu JU, Sunil L and Anu Ja S. (2009). Antibacterial properties of different medicinal plants, *Ocimum sanctum*, *Cinnamomum zeylanicum*, *Xanthoxylum arimatum* and *Origanum masorana* Kathmandu University. *Journal of Science, Engineering and Technology*, 5, 143-150.
- Edeoga HO, Okwu DE and Mbaebie BO. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology*, 4, 685 – 688.
- Maxwell A, Seepersaud M, Pingal P, Mootoo DR and Reynolds WF. (1995). 3-beta amino spirostane steroidal alkaloids from *Solanum triste*. *Journal of Natural Products*, 58, 625 – 628.
- Stevens JF, Hart HT, Hendriks H and Malingare TM. (1992). Alkaloids of some European and Macaronesian sedidege and *Semper vivodaee* (Crassulaceae). *Phytochemistry*, 31, 159 – 163.
- Ogukwe CE, Oguzie EE, Unaegbu C and Okolue BN. (2004). Phytochemical screening of the leaves of *Sansevieria trifasciata*. *J Chem Soc Nigeria*, 29(1), 8 – 10.
- Kim SY, Kim JH, Kim SK, Ohandy MJ and Jung MY. (1994). Antioxidant activities of selected oriental herb extracts. *J Am Oil Chem Soc*, 71, 633 – 640.
- Okwu DE. (2004). The phytochemicals and vitamins contents of indigenous spices of South Eastern Nigeria. *J Sust Agric Environ*, 6, 30–34.
- Okwu DE and Omodamiro OD. (2005). Effects of hexane extract and phytochemical content of *Xylopiya aethiopica* and *Ocimum gratissimum* on the uterus of guinea pig. *Bio Research*, 3(2), 40 – 44.
- De Pascual Teresa S and Sanchez Ballesta. (2008). Anthocyanins. *From Plants to Health*, 17(2), 281 – 289.
- Singh, R and Sawhney, S K. (1988). Advances in Frontier Areas of Plant Biochemistry, Prentice Hall in India, New Delhi, pp 487.
- Rein D, Paglieroni J Wun T, Pearson DA, Schmhzh HH, Gossenlin R and Keen CL. (2000). Cocoa inhibits platelet activation and function. *Am J Clin Nutr*, 272, 30 – 35.
- Rice-Evans CA, Miller NJ and Pogana G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol Med*, 20, 933 – 935.



19. Edeoga HO, Osuagwu GGE, Omosun G, Mbabie BO and Onwuka AS. (2009). Chemical Characteristics and utility of some Rubiaceae medicinal plants. *Phytopharmacology and Therapeutic Values*, 23, 79-87.
20. Osuagwu GGE, Okwulehie IC and Emenike JO. (2007). Phytochemical and Mineral content of the leaves of four Nigerian Pterocarpus species. *Int J Mol Med Adv Sci*, 3(1), 6– 11.
21. Haslam E. (1996). Natural polyphenols (Vegetable tannins) as drugs possible mode of action. *Journal of Natural Products*, 59, 205 – 215.
22. Okwu DE. (2005). Phytochemical, vitamins and mineral contents of two Nigerian medicinal plants. *Int J Mol Med Adv Sci*, 1, 378–381.
23. Perrella J, Berco M, Cecutti A and Bhavnani B. (2003). Potential role of the interaction between equine estrogen low density lipoprotein (LDL) and high density lipoprotein (HDL) in the prevention of coronary heart and neurodegenerative disease in postmenopausal women. *Lipid in Health Disease*, 2, 4.
24. Zhu M, Philipson TD, Greengrass PM, Bowmey R and Cal T. (1997). Plant polyphenols, Biological active compounds of Non- selective binders to protein. *Phytochemistry*, 44, 441- 447.
25. Ijeh II, Njoku OU and Ekenze, EC. (2004). Medicinal evaluation of extracts of *Xylopi aethipica* and *Ocimum gratissimum*. *J of Med Arm Plant Sc*, 26, 41 – 49.
26. Iniaghe OM, Malomo CO and Adebayo JO. (2009). Proximate and phytochemical constituents of leaves of some *Acalypha* species. *Pakistan Journal of Nutrition*, 8(3), 256 – 258
27. Ganjewala D, Sam S and Khan KH. (2009). Biochemical compositions and antibacterial activities of *Lantana camara* plants with yellow, lavender, red and white flowers. *EurAsia Journal of Biological Sciences*, 3 (10), 69-77.
28. Omoyeni OA and Aluko BT. (2010). Qualitative determination of chemical and nutritional composition of *Cissus petiolata* leaves. *Electronic Journal of Environmental, Agricultural and Food Chemistry*, 9 (2), 436 – 440.
29. Arshad H, Shadma H, Iffat I and Sarfara-Hussein. (2010). Antibacteria activity of the leaves of *Coccinia indica* (W and A)W of India. *Advances in Biological Research*, 4(5), 241-248
30. Koche DK, Bhadange DG And Kamble KD. (2011). Antimicrobial activity of three medicinal plants. *Bioscience Discovery*, 2(1), 69-71
31. Edet EE, Akpanabiatu MI, Eno AE, Umoh IB and Itam EH. (2009). Effect of *Gongonema latifolium* crude extract on some cardiac enzymes of alloxan-induced diabetic rats. *African Journal of Biochemistry*, 3(11), 366-369.
32. Akuodor GC, Idris-Usman MS, Mbah CC, Megiwar UA, Akpan JI, Ugwu TC, Okoroafor DO and Osunkwo UA. (2010). Studies of anti-ulcer, analgesic and antipyretic properties of ethanolic leaf extract of *Gongonema latifolium* in rodents. *African Journal of Biotechnology*, 9(5), 2316-2321.
33. Arbonnier M. (2004). Trees, shrubs and lianas of West African dry zones CIRAD MARGRAF publishers, GMBH AJ Wageningen, The Netherland, 573.
34. Nigeria Natural Medicine Development Agency (NNMDA). (2008). Medicinal plants of Nigeria, South – East Nigeria Vol1 Lisida consulting Lagos Nigeria, 204.
35. Harborne JB. (1973). Phytochemical methods Chapman and Hall Ltd, London, 278.
36. Pearson, D (1976) Chemical Analysis of Food (7th ed) Churchill Livingstone, Edinburg UK 575.
37. Ijeh II, Omodamiro OD and Nwanna IJ. (2005). Antimicrobial effects of aqueous and ethanolic fractions of two spices, *Ocimum gratissimum* and *Xylopi aethipica*. *African Journal of Biotechnology*, 4(9), 953 – 956
38. Ebi GC and Ofoefule SI. (1997). Investigation into the folkloric antimicrobial activities of *Landolphia owerriance*. *Phytotherapeutic Research*, 11, 147-151
39. Baron JE and Finegold SM. (1990). Methods for testing antimicrobial effectiveness In Bailey Scotts Diagnostic Microbiology, Mossy CV(ed) *Missouri*, 171-194.
40. Ebanu RU Essien A and Ekpa OD. (1995). Nutritional and Potential Medicinal Values of the Leaves of *Lasianthera Africana* (Beauv). *Global Journal of Pure and Applied Science*, 1, 1-7.
41. Stray F. (1998). The National guide to medicinal herbs and plants Tiger books International London, 12- 16.
42. Kozioc MJ and Marcia MJ. (1998). Chemical composition, nutritional evaluation and economic prospects of *Spondias purpurea* (Anarcadiaceae). *Economic Botany*, 52, 373 – 380.
43. Subban M, Annamalai P and Arumugame CT. (2011). Phytochemical screening and Antimicrobial activity of the leaves of *Memecylon umbellatum burm F*. *Journal of Applied Pharmaceutical Science*, 1(1),42-45
44. Valarmathy K, Babu PS and Abhilash M. (2010). Antimicrobial activity of ethanolic extracts of various plant leaves against selected microbial species. *Electronic Journal of Environmental Agricultural and Food Chemistry*, 9(8), 1378-1382

