IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Human Journals
Research Article
March 2015 Vol.:2, Issue:4

The Phytochemical Screening and Antimicrobial Activity of the Leaves of *Monodora myristica,* (Gaertn) Dunal, *Acanthus montanus* (Ness) T. Anders and *Alstonia bonnei* De Wild

IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals
ISSN 2349-7203
HUMAN

Osuagwu, G. G. E. and Onwuegbuchulam, N. P.

Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike Umuahia Abia State, Nigeria.

Submission: 2 March 2015
Accepted: 10 March 2015
Published: 25 March 2015

HUMAN JOURNALS

www.ijppr.humanjournals.com

Keywords: Antimicrobial activity, inhibition zone, phytochemicals, *Monodora myristica*, *Acanthus monantus*, *Alstonia boonei*

ABSTRACT

The phytochemical screening and the antimicrobial activity of the leaves of *Monodora myristica*,, *Acanthus montanus* and *Alstonia bonnei* were investigated to ascertain their pharmaceutical potential. The research showed that the leaves of these plants contained alkaloids, flavonoids, phenols, saponins, steroids and tannins. Quantitatively, the percentage phytochemical content of the leaves of the plants was as follows: alkaloids (3.85- 11.65), flavonoids (3.16- 5.20), phenols (0.38- 3.37), saponins (3.71- 11.24), steroids (1.05- 5.45) and tannins (0.14- 0.19). The ethanolic extracts of the leaves of the plants were tested against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, and *Klebsiella pneumoniae* by the agar diffusion method. The investigation reveals that the ethanolic extracts of the samples showed moderate to high activity against all tested pathogenic organisms. The diameter of the inhibition zone ranged from 5.50-27.00 mm. The minimum inhibitory concentration of the extract ranged from 62.50- 250 mg/ ml. The results obtained show that the leaves of the plants are good sources of phytochemicals and have antimicrobial activity against the pathogenic microorganisms, thus could be exploited as alternative antimicrobial drugs for the treatment of diseases caused by these pathogens.

INTRODUCTION

Plant use for the maintenance of good health is well documented (Burkill, 1995; Moerman, 1996; Edeoga and Eriata, 2001). Reports have shown that, base of many modern pharmaceuticals used today for the treatment of various aliments are plants and plant based products (Kamba and Hassan, 2010). Plants have been generally used worldwide for the treatment of diseases. About 80 % of the World's populations rely on plant based medicine for their health care (WHO, 2001). WHO (1996) report, observed that the majority of the population in the developing countries still depend on herbal medicine to meet their health need. The use of plants or their products to meet societal health need stems from the fact that indiscriminate use of commercial antimicrobial drugs commonly utilized in the treatment of infectious diseases has led to the development of multiple drug resistance (Gupta *et al*., 2008), the adverse effect on host associated with the use of convectional antibiotics (Gupta *et al*., 2008), the safety and cost effectiveness of the use of plants in traditional as well as in modern medicine (Koche *et al*., 2011) and high cost, adulteration and increasing toxic side effects of these synthetic drugs (Shariff, 2001). Thus the need to develop alternative antimicrobial drugs from medicinal plants for the treatment of infectious diseases has been advocated due to the fact that antimicrobials of plant origin have been found to have enormous potentials (Werner *et al*., 1999). Furthermore, the work of Perumalsamy and Ignacimuthu (2000), indicated that antimicrobials from plant from plant origin are effective in the treatment of infectious diseases and on the other hand, simultaneously mitigates many side effects that are linked with synthetic antimicrobials.

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 usefulness 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 (Bishnu *et al*., 2009; Edeoga *et al*.,2005).

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 (Edeoga and Eriata, 2001). They are also used as starting materials in the manufacture of steroidal drugs and carryout protective function in animals, thus

are used as medicine especially steroidal alkaloids (Maxwell *et al*., 1995; Stevens *et al.*, 1992). Isolated plant alkaloids and their synthetic derivatives are used as basic medicinal agent for their analgesic, antispasmodic and antibacterial effect (Ogukwe *et al*., 2004). Flavonoids are known to carryout antioxidant, protective effects and inhibit the initiation, promotion and progression of tumors (Kim *et al*., 1994; Okwu, 2004). Isoflavones, some kind of flavonoids are phytoestrogen which effectively modulate estrogen levels in human (Okwu and Omodamiro, 2005). A type of flavonoid anthocyanin helps in reducing the incidence of cardiovascular diseases, cancer, hyperlipidemias and other chronic diseases (de Pascual-Teresa and Sanchez-Ballesta, 2008). Phenolic compounds in plants are potentially toxic to the growth and development of pathogens (Singh and Sawhney, 1988). Research reports also show that phenolic compounds carry out potent antioxidant activity and wide range of pharmacological activities which include anti-cancer, antioxidant and platelet aggregation inhibition activity (Rein *et al*., 2000; Rice – Evans *et al*., 1996;). Saponins play essential roles in medicine. These include serving as expectorant and emulsifying agent (Edeoga *et al*., 2009) and having antifungal properties (Osuagwu *et al*., 2007). Tannins are reported to inhibit pathogenic fungi (Burkill, 1995). 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 (Haslam, 1996). Steroid containing compounds are of importance in pharmacy due to their role in sex hormones (Okwu, 2001). Steroids such as equine estrogen are implicated in the reduction of risks of coronary heart and neurodegenerative diseases in healthy and young postmenopausal women (Perrella *et al*., 2003). At low concentration tannins show antimicrobial, cytotoxic and astringent properties (Zhu *et al*., 1997; Ijeh *et al*., 2004).

The phytochemical screening of some plants has been carried out and they are found to be rich in alkaloids, phenols, flavonoids, saponins and tannins (Iniaghe *et al*., 2009; Ganjewala *et al*., 2009; Omoyeni and Aluko, 2010; Osuagwu and Eme, 2013; Osuagwu and Ihenwosu, 2014). The antimicrobial activities of plants have been reported (Arshad *et al*., 2010; Kamba and Hassan, 2010, Koche *et al*., 2011; Osuagwu and Akomas, 2013; Osuagwu and Nwoko, 2014). They are therefore used in the treatment of many diseases such as rheumatism, diarrhea, malaria, elephantiasis, cold, obesity, dysentery, high blood pressure, malnutrition, gonorrhea and others (Burkill, 1995; Edet *et al*., 2009; Akuodor *et al*, 2010).

Monodora myristica, is a tropical tree of Annonaceae family. Its seeds are widely sold as inexpensive nut Meg substitute (NNMDA, 2008). *M. myristica* tree can reach a height of 35 m and 2 m in diameter at breast height. The fruit is a berry of 20 cm in diameter and is smooth green and spherical and later become woody. It is attached to a long stalk which is up to 60 cm long. Inside the fruit, the numerous oblongoid, pale brown 1.5 cm long seeds are surrounded by a whitish fragment pulp. The seeds contain 5.9 % colourless essential oil (NNMDA, 2008). The plant parts have many medicinal values (Weiss, 2002; Akinwunmi and Oyedapo, 2013).

Acanthus montanus, is a thinly branched perennial with basal clusters of oblong to lance- shaped glossy, dark green leaves reaching up to 30 cm long. The leaves have silver marks and wavy margins (NNMDA, 2008). *A. montanus* is used in African traditional medicine for the treatment of urogenital infections urethral pains, endometritis, urinary disease, cystitis, aches, hypertension and dysfunctions (NNMDA, 2008; Igoli *et al*., 2005; Adeyemi *et al*., 2004).

Alstoniabonnei is a very large deciduous tropical forest tree belonging to the Apocynaceae family. It is native to tropical West Africa and it is also a tall forest tree which can reach 45 m in height and 3 m in girth. The leaves are borne in whorls at the nodes, oblanceolate shaped with the apex rounded to acuminate with lateral veins (Burkill, 1997). The fruits are pendulous, paired, slender follicles up to 16 cm long, containing seeds bearing a tuft of silky, brown floss at either end to allow dispersal by the wind. The latex is white and abundant (Burkill, 1997). The bark is used to treat malaria, typhoid fever, gonorrhea, asthma, dysentery, sores, ulcer, snake bites, rheumatic pains and toothache (NNMDA, 2008; Abbiw, 1990; Adetoye *et al*., 2012). The latex is applied to snake bite, skin complaints and swelling caused by filarial infections. The leaves are used to reduce Oedema and to treat sores and malaria (NNMDA, 2008; Kumar *et al*., 2011).

The objectives of the study is to ascertain the presence and quality of some basic phytochemicals in the leaves of *M. myristica*, *A. montanus* and *A. bonnei* to determine the antimicrobial activity of the leaves of these plants on selected human pathogenic microorganisms, in view of utilizing them as alternative source of antimicrobial drugs used in the treatment of diseases.

MATERIALS AND METHODS

Plant samples

The leaves of *Monodora myristica*, and *Alstonia bonnei* were collected from the premises of Michael Okpara University of Agriculture Umudike Umuahia Abia State Nigeria and that of *Acanthus montanus* were collected at the back of the New Industrial market AhiaekeIbeku Umuahia Abia State Nigeria. The leaves were identified by the taxonomic unit of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture Umudike Umuahia Abia State Nigeria.

Preparation of samples for analysis

The leaves of *M. myristica*, *A. montanus* and *A. bonnei* were air dried at room temperature for one week. The dried samples were ground using Thomas Wiley milling machine. The milled samples were stored in clean sample bottles, corked and stored at room temperature in the laboratory.

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 (1984).

An alcoholic extract was used and obtained by dispersing 2g of the powered 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 (1984).

2 ml of the aqueous extract was added into a test tube and a few drops of Bench Concentrated ammonia (NH_4) 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 (1984) methods.
The fat free sample was boiled with 50 ml of ether for 15 minutes. 5 ml of the extract was pipette into a 50 ml flask and 10 ml of distilled water added into it. 2 ml of ammonia hydroxide solution and 5 ml of concentrated amyl alcohol were also added. The mixture was allowed to react for 30 minutes for colour development.

Test for the presence of saponins

The presence of saponins in the samples was determined using Harborne (1984) 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 (1984).

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 (1984).

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^{o}C for 30 minutes, cooled in a desiccator 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.

$$\% \text{ Alkaloid} = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where

W_1 = weight of filter paper

W_2 = 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 (1984).

5 g of the powdered sample was placed into a conical flask and 50 ml of water and 2 ml 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. 10 ml 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^{o}C. It was cooled in a desiccator and weighed. The quantity of flavonoid was determined using the formula.

$$\% \text{ Flavonoid} = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where:

W_1= Weight of empty filter paper

W_2= Weight of paper + Flavonoid extract

Determination of phenols

The concentration of phenols in the leaves of the leaves of the plants was determined using the folin- cioCaltean colorimetric method described by Pearson (1976).

0.2 g of the powdered sample was added into a test tube and 10 ml of methanol was added to it and shaken thoroughly the mixture was left and to stand for 15 minutes before being filtered using Whatman No. 42 filter paper. 1 ml of the extract was placed in a test tube and 1 ml folin-cioCaltean 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,

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

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, 1984).

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^{o} 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^{o}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 pre-weighed evaporation dish. It was dried at 60^{o} C in the oven and reweighed after cooling in a desiccator. 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

$$\% \text{ Saponin} = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where

W_1 = weight of evaporating dish

W_2 =weight of dish + sample

Steroid determination

The steroid content of the leaves of the plants was determined using the method described by Harborne (1984).

5 g of the powdered sample was hydrolysed by boiling in 50 ml hydrochloric acid solution for about 30 minutes. It was filtered using Whatman filter paper No. 42, 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^{o}C for 5 minutes 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 No. 42 was used to filter the mixture properly. The dry extract was then

cooled in a desiccator 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

$$\% \text{ Steroid} = \frac{W_2 - W_1}{\text{Weight of sample}} \times \frac{100}{1}$$

Where,

W_1= weight of filter paper.

W_2 = 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).

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 water 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 *Monodora myristica,, Acanthus monantus* and *Alstonia bonnei* were prepared using the method of Ijeh *et al*.,(2005).

Fifty grams of the powdered sample were soaked in 200 ml of absolute ethanol and allowed to stand for 24 hours. They were filtered using Whatman No. 1 filter Paper. The filtrates were evaporated to dryness with rotary evaporator at 40^{o}C to thick residues. The residues were dissolved in deionized water to obtain the desired plant extracts for the antimicrobial tests.

Preparation of Innocular

The human pathogens; *Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae, Proteus mirabilis, Pseudomonas aeruginosa* and *Klebsiella pneumonia* 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^{o}C for 24 hours.

Antimicrobial activity test

The sensitivity of the test organism to the ethanolic extracts of the leaves of *Monodora myristica,, Achantus montanus* and *Alstonia bonnei* was carried out using the diffusion method described by Ebi and Ofoefule (1997).

20 ml of the molten nutrient agar was seeded with 0.2 ml 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 8.0 mm 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^{o}C for 24 hours. The zones of inhibition in millimetres were measured and recorded.

The test was carried out in the Laboratory of 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) was used to determined the minimum inhibitory concentration.

Six grams of nutrient agar were dissolved in 250 ml 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 5 mg/ml, 15 mg/ml, 20 mg/ml and 25 mg/ml were made from the original test samples. The Petri dishes were then placed in the incubator at 37^{o}C for 24 hours. The inhibition zones in millimetres were measured and recorded.

Statistical Analysis

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

RESULTS AND DISCUSSION

The result of the determination of the phytochemical composition and the antimicrobial activity of the leaves of *Monodora myristica, Acanthus montanus*, and *Alsonia bonnei* were summarized in Tables 1-4.

The phytochemical screening of the leaves of *M. myristica*, *A. montanus* and *A. bonnei* showed that the leaves of the plants contain alkaloids, flavonoids, phenols, saponins, steroids and tannins (Table 1). Research reports indicate the presence or these phytochemicals in other plants (Iniagbe *et al*., 2009; Omoyeni and Aluko, 2010; Osuagwu and Eme, 2013). The presence of these phytochemicals in the leaves of these plants confer them for their medicinal value (Arbonnier, 2004; NNMDA, 2008; Vadivu *et al* 2008). The pharmaceutical and therapeutic potentials of plants and their products are as a result of the presence of these phytochemicals in them (Edeoga *et al*., 2005; Bishnu*et al*., 2009). The phytochemical constituent in percentage of the leaves of the plants is summarized in Table 2. The percentage phytochemical content of the leaves of the plants is as follows: alkaloids (3.50- 11.66), flavonoids (3.56- 5.21), phenols (0.38- 3.37), saponins (3.71- 11.24), steroids (1.11- 5.45) and tannins (0.14- 0.18). The result shows that the leaves of these plants have appreciable amount of these phytochemicals, hence their medicinal

value. *A. bonnei*, had the highest amount of alkaloids (11.65 %) when compared with those of *M. myristica* (4.10 %) and the least was those of *A. montanus* (3.85 %). The saponins content of *A. bonnei* was also high (11.24 %), while that of *M. myristica* had the lowest amount of saponins (3.17 %). *A. montanus*, had the highest flavonoid content (5.21 %), while *A. bonnei* had the least flavonoid content (3.56 %). Tannin was the phytochemical that had the least concentration in the leaves of the plants. This observed variation in the concentration of the phytochemicals in the leaves of these plants has also been reported by other researchers (Ganjewala *et al*., 2009; Osuagwu and Eme, 2013).

The results of the antimicrobial activity of the ethanolic extracts of the leaves of *M. myristica*, *A. montanus* and *A. bonnei* on *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are summarized in Tables 3 and 4. The growth of all the pathogenic microorganisms used for the test was inhibited by the ethanolic extracts of the leaves of *M. myristica*, *A. montanus* and *A. bonnei* (Table 3). The inhibition zone ranged from 5.50- 27.5 mm. The leaf extracts of *A. montanus* had the highest inhibitory effect on all the pathogens used for the study, while the leaf extracts of *M. myristica* had the least inhibitory effect. *A. bonnie* had the highest inhibitory effect on *K. pneumoniae* (29 mm) and *E. coli* (27 mm). The ability of the extracts of the leaves of these plants to inhibit the growth of these pathogens confers medicinal value on them. The use of leaves of these plants in the treatment of diseases has been reported (Igoli *et al*., 2005; NNMDA, 2008; Adotey *et al*., 2012; Akinwunmi and Oyedapo, 2013). The antimicrobial activity of the leaves of other plants has been document (Arshad *et al*., 2010; Kamba and Hassan, 2010; Osuagwu and Nwoko, 2014; Osuagwu and Ihenwosu, 2014). The presence of bioactive substances (alkaloids, flavonoids, phenols, saponins, steroids, tannins and others) in the leaves of these plants confers on the extracts the ability to inhibit the growth of the test pathogenic microorganisms (Bishnu *et al*., 2009; Iniaghe *et al*., 2009; Omoyeni and Aluko, 2010). There is observed relationship between the concentration of the extracts and the rate of inhibition of the growth of the pathogens. There was a corresponding increase in the rate of inhibition of the growth of the pathogens as the concentration of the extracts increased. This trend was also reported by other researchers (Valarmathy *et al*., 2010; Subban *et al*., 2011). The minimum inhibitory concentration of the ethanolic extracts of the leaves of *M. myristica*, *A. montanus* and *A. bonnei* ranged from 62.50 to 250 mg/ ml (Table 4).

This investigation showed that the leaves of *M. myristica*, *A. montanus* and *A. bonnei* used in this research have high phytochemical content and have antimicrobial activity on the human pathogens used in the test. This shows that they are of high medicinal value. Thus could be exploited to be used in the formation of cheap alternative antimicrobial drugs which will be used to cure and control human infectious diseases.

Table 1: The Qualitative Analysis of the Phytochemicals in the leaves of *Monodora myristica,, Acanthus montanus and Alstonia bonnei*

Plant Species	Alkaloids	flavonoids	Phenols	Saponins	Steroids	Tannins
Monodora myristica,	+	+	+	+	+	+
Acanthus montanus	+	+	+	+	+	+
Alstonia bonnei	+	+	+	+	+	+

Key: + = Presence, - = Absence

Table 2: The percentage alkaloids, flavonoids, phenols, saponins, steroids and tannins constituents of the leaves of *M. myristica*, *A. montanus* and *A. bonnei*.

Plant Species	Alkaloids	Flavonoids	Phenols	Saponins	Steroids	Tannins
Monodoramyristica	4.10±0.08	3.781±0.21	3.146±0.05	3.712±0.17	3.210±0.70	0.184±0.07
Acanthusmontanus	3.85±0.01	5.210±0.04	3.370±0.10	5.030±0.42	5.450±1.400	1.80±0.14
Alstoniabonnei	11.655±0.07	3.561±0.21	0.382±0.04	11.240±1.15	1.106±0.07	0.138±0.05

Table 3: The antimicrobial activity of the ethanolic extracts of the leaves of *M. myristica*, *A. montanus* and *A. bonnei* on *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus pneumoniae*, *proteus mirabilis*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*

Pathogenic organisms	*M. myristica*	*A. montanus*	*A. bonnie*	Ciprofloxacin
Zone of Inhibition				
E. coli	14.00±1.41	27.50±2.71	27.00±2.41	48.50±3.50
S. aureus	10.50±0.71	21.00±1.40	22.00±1.85	37.00±2.41
S. pneumoniae	7.50±1.51	22.50±2.50	23.00±1.41	45.50±2.50
P. mirabilis	8.00±1.41	25.00±2.75	17.00±2.00	41.00±3.40
P. aeruginosa	5.50±0.80	19.00±1.45	17.50±2.12	31.00±2.20
K. pneumoniae	9.00±1.50	18.50±3.50	29.00±1.50	50.50±2.50

Table 4: The minimum inhibitory concentration (mg/ml) of the ethanolic extracts of the leaves of *M. myristica*, *A. montanus* and *A. bonnei* on *E. coli*, *S. aureus*, *S. pneumoniae*, *P. mirabilis*, *P. aeruginosa* and *K. Pneumoniae*.

Pathogenic organisms	***M. myristica***					***A. montanus***					***A. bonnei***				
MIC	**500**	**250**	**125**	**62.5**	**31.25**	**500**	**250**	**125**	**62.5**	**31.25**	**500**	**250**	**125**	**62.5**	**31.25**
E. coli	14	7.2	2.5	0.0	0.0	27.5	15.5	9.0	4.0	0.0	27.0	15.0	9.0	4.5	0.0
S. aureus	10.5	4.5	0.0	0.0	0.0	21.0	10.5	4.5	0.0	0.0	22.0	11.5	5.5	0.0	0.0
S. pneumonia	7.5	2.5	0.0	0.0	0.0	22.5	9.5	3.5	0.0	0.0	23.0	13.0	7.5	2.5	0.0
P. mirabilis	8.0	3.0	0.0	0.0	0.0	25.0	12.5	5.5	0.0	0.0	17.0	8.0	3.5	0.0	0.0
P. aeruginosa	5.5	2.0	0.0	0.0	0.0	19.0	8.5	3.0	0.0	0.0	17.5	7.5	4.5	0.0	0.0
K. pneumoniae	9.0	3.5	0.0	0.0	0.0	18.5	8.5	3.5	0.0	0.0	29.0	15.5	9.5	4.5	0.0

MIC = Minimum Inhibitory Concentration

REFERENCES

1. Abbiw, D. (1990). *Useful plants of Ghana: West African uses of wild and cultivated plants*. Kew, London, Intermediate Technology Publications, Royal Botanical Garden.
2. Adeyemi, O. O., Okpo, S. O. and Opaka, O. (2004). The analgesic effect of the methanolic extracts of *Acanthus montanus*. *J. EthnoPharmacol.90 (1):* 45- 48.
3. Adotey, J. P.K., Adukpo, G. E., Boahen, Y. O. and Armah, F. A. (2012). A review of the Ethnobotany and Pharmacological importance of *Alstoniabonnei* De Wild (Apocynaceae). *ISRN Pharmacology, 2012:* 1- 9.
4. Akinwunmi, K. F. and Oyedapo, O. O. (2013). Evaluation of antioxidant potentials of *Monodora myristica*, (Gaertn) dunel seeds. *African Journals of Food Science, 7 (9):* 317- 324.
5. Akuodor G. C.,Idris-Usman, M. S., Mbah, C. C.,Megiwar, U. A.,Akpan J. I.,Ugwu, T. C.,Okoroafor,D. O. and Osunkwo, U. A.2010.Studies of anti-ulcer,analgestic and antipyretic properties of ethanolic leaf extract of *Gongonemalatifolium*in rodents. *African Journal of Biotechnology,9(5)*:2316-2321.
6. Arshad, H., Shadma, H., Iffat, I. and Sarfara-Hussein.2010. Antibacteria activity of the leaves of *Cocciniaindica* (W and A) W of India. *Advances in Biological Research 4(5)*:241-248.
7. Baron,J. E. and Finegold, S. M.1990. *Methods for testing antimicrobial effectiveness*.In Bailey. Scotts Diagnostic Microbiology, Mossy C. V.(ed.)Missouri,pp 171-194.
8. Bishnu, J.U., Sunil, L. and AnuJa, S. 2009. Antibacterial properties of different medicinal plants; *Ocimum sanctum, Cinnamomumzeylanicum, Xanthoxylimarimatum and Origanum masorana*. *Kathmandu University Journal of Science, Engineering and Technology*.*5*:143-150.
9. Burkill, I. H.1995.The Useful Plants of West Tropical Africa (Vol. 3 Families J-L).*Royal Botanical Garden*,Kew, 605 pp.
10. Burkill, I. H.1997. *The useful plants of West Tropical Africa*. Vol. 4 families M – R. Royal Botanical Garden, Kew.605 p.
11. de – Pascual – Teresa, S. and Sanchez, Ballesta (2008). Anthocyanins: *From Plants to Health, 17(2)*: 281 – 289.
12. Ebi,G. C. and Ofoefule,S.I.1997.Investigation into the folkloric antimicrobial activities of *Landolphiaowerriance.Phytotherapeutic Research, 11*:147-151.
13. Edeoga,H. O. and Eriata,D. O.2001.Alkaloid,tannin and saponin contents of some Nigerian medicinal plants. *Journal of Medicinal and Aromatic Plant Science*, *23*:244-249.
14. Edeoga, H. O., Okwu, D. E. and Mbaebie, B. O. (2005). Phytochemical constituents of some Nigerian medicinal plants. *African Journal of Biotechnology, 4*: 685 – 688.
15. Edeoga, H.O., Osuagwu, G.G.E., Omosun, G., Mbabie, B.O. and A.S. Onwuka (2009). Chemical Characteristics and utility of some Rubiaceous medicinal plants. *Phytopharmacology and Therapeutic Values. 23*: 79-87.
16. Edet,E.E., Akpanabiatu,M. I., Eno,A. E., Umoh,I. B. and Itam,E. H. (2009). Effect of *Gongonemalatifolium*crude extract on some cardiac enzymes of alloxan-induced diabetic rats. *African Journal of Biochemistry,3(11)*:366-369.
17. Ganjewala, D. Sam, S. and Khan, K. H. (2009). Biochemical compositions and antibacterial activities of *Lantanacamara* plants with yellow, lavender, red and white flowers. *EurAsiaJournal of Biological Sciences*, *3 (10)*: 69-77
18. 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.
19. Harborne, J. B. (1984). *Phytochemical methods.* Second edition, Chapman and Hall Ltd., London. 288pp.
20. Haslam, E. (1996). Natural polyphenols (Vegetable tannins) as drugs possible mode of action. *Journal of Natural Products. 59*: 205 – 215.
21. Igoli, J.O., Ogaji, O. G., Tor- Ayiin, T. A., Igoli, N. P. (2005). Traditional medicine practiceamongst the Igede people of Nigeria. Part II. *Afri. J. Trad CAM 2(2):* 134- 152.
22. Ijeh, I. I., Njoku, O. U. and Ekenze, E. C. (2004). Medicinal evaluation of extracts of *Xylopiaaethipica* and *Ocimumgratissimum*. *J. of Med. Arm. Plant Sc. 26*: 41 – 49.

23. Ijeh, I. I., Omodamiro, O. D. and Nwanna, I. J. (2005). Antimicrobial effects of aqueous and ethanolic fractions of two spices, *Ocimumgratissimum* and *Xylopianetiopica*. *African Journal of Biotechnology, 4(9)*: 953 – 956.
24. Iniaghe, O. M., Malomo, C.O. and Adebayo, J. O. (2009). Proximate and phytochemical constituents of leaves of some *Acalypha* species. *Pakistan Journal of Nutrition, 8(3)*: 256 – 258.
25. Kamba,A. S. and Hassan,L.G. (2010).Phytochemical screening and antimicrobialactivities of *Euphorbiabalasamifera* leaves, stem and root against some pathogenic microorganisms. *African Journal of Pharmaceutical Sciences and Pharmacy,1(1)*:57-64.
26. Kim, S. Y., Kim, J. H., Kim, S. K., Ohandy, M. J. and Jung, M. Y. (1994). Antioxidant activities of selected oriental herb extracts. *J. Am. Oil Chem. Soc. 71*: 633 – 640.
27. Koche,D. K., Bhadange,D. G. AndKamble,K. D.2011.Antimicrobial activity of three medicinal plants. *Bioscience Discovery,2(1)*:69-71.
28. Kumar, P., Misra, C. S., James, J., Lipin- Dev, M. S., Arun, K. T. V. and Thanamani, V. (2011). Ethnobotanical and Pharmacological study of *Alstonia* (Apocynaceae) -*A Review. Journal of PharmaceuticalSciences and Research 3 (8):* 1394- 1403.
29. Maxwell, A., Seepersaud, M., Pingal, P., Mootoo, D. R. and Reynoids, W. F. (1995). 3-beta amino spirosolane steroidal alkaloids from *Solanumtriste. Journal of Natural Products,58*: 625 – 628.
30. Moerman, D. E. (1996). An analysis of the food plants of nature. *North America Journal of Ethnopharmacol. 52*: 1 – 22.
31. Nigeria Natural Medicine Development Agency (NNMDA) (2008). *Medicinal plants of Nigeria; South – East Nigeria*. Vol.1. Lisida consulting Lagos Nigeria.204pp.
32. Ogukwe, C.E., Oguzie, E.E., Unaegbu, C. and Okolue, B. N. (2004). Phytochemical screening of the leaves of *Sansevieratrifasciata J. Chem. Soc. Nigeria, 29(1)*: 8 – 10.
33. Okwu, D. E. (2001). Evaluation of the chemical composition of indigenous species and flavouring agents. *Global Journal of Pure and Applied Sciences, 7(3)*: 455 – 459.
34. Okwu, D. E. (2004). The phytochemicals and vitamins contents of indigenous spices of South Eastern, Nigeria. *J. Sust. Agric. Environ. 6:* 30 – 34.
35. Okwu, D. E. and Omodamiro, O. D. (2005). Effects of hexane extract and phytochemical content of *Xylopiaaethiopica* and *Ocimumgratissimum* on the uterus of guinea pig. *Bio. Research 3(2)*: 40 – 44.
36. Omoyeni, O. A. and Aluko, B. T. (2010). Qualitative determination of chemical and nutritional composition of *Cissuspetiolata* leaves. Electronic Journal of Environmental, Agricultural and Food Chemistry, *9 (2)*: 436 – 440.
37. Osuagwu, G.G.E. and Akomas C.B. (2013). Antimicrobial activity of three Species of Nigerian Pterocarpus (Jacq.). *International Journal of Medicinal and Aromatic Plants* 2(3): 178- 183.
38. Osuagwu G. G. E. andEme, C. F (2013). The Phytochemical Composition and Antimicrobial Activity of *DialiumGuineense, VitexDoniana* and *Dennettia Tripetala* Leaves. *Asian Journal of Natural and Applied Sciences*, 2(3):169 – 181.
39. Osuagwu G. G. E. and Ihenwosu, A. O. (2014). Phytochemical Composition and Antimicrobial Activity of the Leaves of *Alchorneacordifolia* (Schum and Thonn), *Sansevieraliberica* (GerandLabr) and *Uvariachamae* (P. Beauv). *American Journal of Phytomedicine and Clinical Therapeutics*, 2 (1): 1 – 12.
40. Osuagwu, G. G. E. and Nwoko, N (2014). The Phytochemical Screening and Antibacterial Activity of the Leaves of *CombretumPaniculatum*(Vent)*Solanium macrocarpon*(L) *and CatharanthusRoseus* (L) G. Don, *IOSRJournal of Pharmacy and Biological Sciences*. 9(1):58 – 65.
41. Osuagwu, G. G. E., Okwulehie, I. C. and Emenike, J. O. (2007). Phytochemical and Mineral content of the leaves of four Nigerian *Pterocarpus* species. *Int. J. Mol. Med. Adv. Sci. 3(1)*: 6 – 11.
42. Pearson, D. (1976). *Chemical Analysisss of Food.* (7th ed.) Churchill Livingstone, Edinburg. UK. 575p.
43. Perrella, J., Berco, M., Cecutt, 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.

44. Perulmalsamy,R. And Ignacimuthu, S. 2000. Antibacterial activity of some folklore medicinal plants used used by tribals in western charts of India. *JournalEthonopharmacology*, 69:68 71.
45. Rein, D., Paglieroni, J. Wun, T., Pearson, D. A., Schmhz, H. H.,Gossenlin, R. and Keen, C. L. (2000). Cocoa inhibits platelet activation and function. *Am. J. Clin. Nutr. 272*: 30 – 35
46. Rice-Evans, C. A., Miller, N. J. and Pogana, G. (1996). Structure-antioxidant activity relationships of flavonoids and phenolic acids: *Free Radical Biol. Med. 20*: 933 – 935.
47. Shariff, Z. U.(2001).*ModernHerbalTherapyforCommon* Ailments.Nature Pharmacy Series (Volume 1). Spectrum Books Limited,Ibadan,Nigeria.In Association with Safari Books(Export)Limited,United Kingdom.pp 9-84.
48. Singh, R. and Sawhney, S. K. (1988). Advances in Frontier Areas of Plant Biochemistry. Prentice Hall in India, New Delhi, pp 487.
49. Stevens, J. F., Hart, H. T., Hendriks, H. and Malingare, T. M. (1992). Alkaloids of some European and *Macaronesiansediodege* and *Semper vivodaee* (Crassulaceae). *Phytochemistry*,*31*: 159 – 163.
50. Subban, M., Annamalai,P. and Arumugame,C. T. (2011). Phytochemical screening and Antimicrobial activity of the leaves of *Memecylonumbellatum* burm. F. *JournalofApplied PharmaceuticalScience1(1)*:42-45.
51. Vadivu, R., Krithika, A., Biphab, C., Dedeopya, P., Shoeb, and Lakshmi, K. S. (2008). Evaluation of haptoprotective activity of the fruits of *Coccina grandis* Linn. *International Journal of Health Researc. 3:*163 – 168.
52. Valarmathy,K., Babu,P. S. and Abhilash,M. (2010).Antimicrobial activity of ethanolic extracts of varios plant leaves against selected microbial species. *ElectronicJournalofEnvironmental AgriculturalandFoodChemistry*, *9(8)*:1378-1382.
53. Werner,F., Okemo,P. and Ansorg,R. (1999). Antibacterial activity of East African Medicinal Plants. *JournalEthnopharmacology*,*60*:79-84
54. Weiss, E. A. (2002). *Spice Crops*. Oxon, CABI Publishing Company pp 102- 103.
55. World Health Organisation(WHO) (1996).Technical Series.Trace Element in Human Nutrition and Health:World Health Organization,Geneva pp. 199-205.
56. World Health Organisation(WHO) (2001). General guidelines for Methodologies on research and evaluation of traditional medicine.World Health Organization,Geneva *9(8)*:1378-1382
57. Zhu, M., Philipson, T. D., Greengrass, P. M., Bowmey, R. and Cal, T. (1997). Plant polyphenols: Biological active compounds of Non- selective binders to protein. *Phytochemistry*, 44: 441- 447.