



IJPPR

INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals

ISSN 2349-7203




Human Journals

Research Article


March 2015 Vol.:2, Issue:4

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The Antimicrobial Activity of the Leaves of Some Wild Cucurbitaceae Species from South-East Nigeria



IJPPR
INTERNATIONAL JOURNAL OF PHARMACY & PHARMACEUTICAL RESEARCH
An official Publication of Human Journals



ISSN 2349-7203

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Submission: 26 February 2015
Accepted: 4 March 2015
Published: 25 March 2015

Keywords: Antimicrobial activity, inhibition zone, minimum inhibition concentration, Cucurbitaceae species

ABSTRACT

The antimicrobial activity of the leaves of some wild Cucurbitaceae species from South-East Nigeria on some human pathogenic microorganisms was investigated. The leaves of *Momordica charantia*, *Luffa cylindrical* and *Trichosanthes cucumerina* were tested against *Salmonella typhi*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus faecalis*. Both ethanolic and aqueous extracts of the leaves of the plants were used for the study. The leaves of all the three plant species used for the test inhibited the growth of the microorganisms except *E. coli*. The inhibition zone ranged from 6.00±0.0 to 9.50±0.7 mm. Generally, the ethanolic extracts had more inhibitory effect when compared with those of the aqueous extracts. *T. cucumerina* leaf extracts tend to have more inhibitory effect on the growth of the pathogens when compared with the leaves of *L. cylindrical* and *M. charantia*. The aqueous extracts of *L. cylindrical* had no inhibitory effect on any of the pathogenic microorganisms. The concentration of the extracts affected their ability to inhibit the growth of microorganisms. The higher the concentration, the greater the rate of inhibition of pathogens. The minimum inhibitory concentration of the extracts ranged from 2.00 – 5.50 mg/ml. The results from this investigation show that the leaves of these plants have the ability to inhibit the growth of these microorganisms and can be exploited to be used as alternative antimicrobial drugs for the treatment of diseases caused by these pathogens.



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INTRODUCTION

The use of plants as remedies for treatment of diseases can be traced back to the pre-historic times. A wide range of medicinal plant parts such as roots, stems, flowers, fruits, twigs, exudates and modified plant organs has been used for extraction of raw drugs (Ankita, 2012). People in the rural areas of the world depend largely on medicinal herbs for the treatment of several ailments because; they constitute indispensable components of traditional medicine practice due to low cost, easy access and ancestral experience (Muthumani *et al.*, 2010; Sangh *et al.*, 2012). The use of plants and plant based products to meet societal health needs stems from the fact that indiscriminate use of commercial antimicrobial drugs commonly utilized in the treatment of infectious disease has led to the development of drug resistance (Gupta *et al.*, 2006; Osuagwu and Akomas, 2013), the adverse effect on hosts associated with the use of conventional 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 effect of these synthetic drugs (Shariff, 2001). These effects have made the use of alternative antimicrobial drugs from medicinal plants highly sought for because antimicrobials of plant origin have been found to have enormous therapeutic potentials (Werner *et al.*, 1999). Perumalsamy and Ignacimuthu (2000) also reported that antimicrobials from plant origin are effective in the treatment of infectious disease and on the other hand simultaneously mitigated many of the side effects that are linked with synthetic antimicrobials.

The medicinal value of these plants lies in some chemical substances that produce definite physiological action on the human body (Edeoga *et al.*, 2005). These chemical substances are bioactive non-nutrient plant compounds that have protective on disease preventive properties (Mallikharjuna *et al.*, 2007). 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). The phytochemical screening of the leaves of *M. charantia*, *L. cylindrical* and *T. cucumerina* has been carried out and found to contain alkaloids, flavonoids, phenols, and tannins (Edeoga *et al.*, 2010). The leaves of plants are reported to have high nutritive value (Osuagwu and Edeoga, 2014).

The antimicrobial activity of plants and plant products have been documented (Arshad *et al.*, 2010; Kariba and Hassan, 2010; Koche *et al.*, 2011; Osuagwu and Akomas, 2013; Osuagwu and Eme, 2013; Osuagwu and Ihenwosu, 2014). These plants therefore are used in the treatment of many diseases such as rheumatism, diarrhoea, malaria, elephantiasis, cold, obesity, dysentery, high blood pressure, malnutrition, gonorrhoea and others (Arbonnier, 2004; NNMDA, 2009; Akuodor *et al.*, 2010).

Momordica charantia is an annual climbing herb vine growing up to 3 – 4 m in length. Stems are slender, 5 angled and longitudinally furrowed. Tendril is simple or branched, leaves are lobed and have foetid odour. Flowers are monoecious, axillary and solitary. Fruits are pendulous, green and becoming orange or yellow when mature. The size and shape vary, but it is often pear shaped or oblong and tapering. Its length is 10 – 12 cm and 5 – 8 cm in diameter (Tindall, 1983; Edeoga *et al.*, 2010). *M. charantia* has laxative emetic and emmenagogue properties (Akobundu and Agyakwa, 1998). It is also been documented to be used in the treatments of arthritis, cold, hypertension, fever, eczema, herpes, influenza, diabetes, intestinal worm (Abascal and Yarmell, 2005; Schutes, 1990).

Luffa cylindrical is an annual climbing vine with several lobed leaves. The leaves give off a rank odour when crushed and are covered with short stiff hairs. Flowers are monoecious. The male flowers occur in clusters, while the female flowers are solitary. Fruits are smooth and cucumber shaped. The exterior is green and sometimes mottled with longitudinal lines (Akobundu and Agyakwa, 1998; Edeoga *et al.*, 2010). *L. cylindrical* exhibits emetic and laxative properties. It also relieves asthma and intestinal worms (Chakravarthy, 1990; Nagao, *et al.*, 1991).

Trichosanthes cucumerina is a climbing annual herb. Leaves are hairy, dentate 10 – 25 cm in length and 15 cm in diameter. They emit foetid odour when damaged. Flowers are monoecious, axillary and white male flowers occur in long racemes with panicles up to 30 cm in length. The female flowers are solitary. Fruits are cylindrical with waxy surface, slender and tapering 40 – 120 cm in length and 4 – 10 cm in diameter (Tindall, 1983). Trichosanthin from *T. cucumerina* is reported to have the ability to inhibit the replication of human immune deficiency virus (HIV), infected lymphocyte and phagocytes, making it a potential therapeutic agent for AIDS (McGrath *et al.*, 1989). It can also act as a natural antibiotics, expectorant and laxative (Nget *et al.*, 1991).

Furthermore, it is used to treat and cure constipation, jaundice, diabetes, cold, bronchitis, cough and asthma (Ng *et al.*, 1991; Chopra *et al.*, 1986).

This study aimed at determining the antimicrobial activity of the leaves of the three wild Cucurbitaceae species from South-Eastern Nigeria in view of their utilization as alternative source of antimicrobial drugs in the control and treatment of infectious diseases.

MATERIALS AND METHODS

Plant samples

The leaves of *Momordica charantia* were obtained in a household garden in Amawom Oboro, Ikwuano Local Government Area, Abia State, Nigeria, while the leaves of *Luffa cylindrical* were obtained from the forest strip of Forestry Department, College of Natural Resources and Environmental Management, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria. The leaves of *Trichosanthes cucumerina* were obtained from a refuse dumps site at Ihim Ibere, Ikwuano Local Government Area, Abia State, Nigeria. The plant samples were identified by the Taxonomic Unit of the Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Umuahia, Abia State, Nigeria.

The leaves of the plants were sun-dried for 5 days and later dried in Selecta Model 150 – 900 L ovens at 45 °C for 24 hours. The leaves were then ground using Thomas Willey machine into fine powder and stored in screw cap bottles for use.

Preparation of plant extracts

Preparation of ethanolic plant extract

The ethanolic extracts of the leaves of *M. charantia*, *L. cylindrical* and *T. cucumerina* were prepared using the methods 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 °C to thick residues. The residues were dissolved in deionised water to obtain the desired plant extracts for antimicrobial tests.

Preparation of aqueous plant extracts

The aqueous extracts of the leaves of *M. charantia*, *L. cylindrical* and *T. cucumerina* were prepared using the method of Ijeh *et al.*, (2005).

Fifty grams of the powdered sample were soaked in 200 ml of water 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 °C to thick residues. The residues were dissolved in deionised water to obtain the desired plant extracts for antimicrobial tests.

Preparation of Innocular

The pathogenic micro-organisms, *Enterococcus faecalis*, *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Pseudomonas aeruginosa* used in the study 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 organisms in buffered peptone broth and thereafter sub-cultured into agar medium and incubated at 37 °C for 24 hours.

Antimicrobial activity test

The sensitivity of the test organisms to the ethanolic and aqueous extracts of the leaves of *M. charantia*, *L. cylindrical* and *T. cucumerina* 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 °C for 24 hours. The zones of inhibition in millimetre 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) was used to determine 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 50 mg/ml, 100 mg/ml and 200 mg/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

500 mg of Gentamycin was dissolved in 5 ml of distilled water for the antimicrobial assay. 12 g of nutrient agar was dissolved in 250 ml 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 means and standard deviation.

RESULTS AND DISCUSSION

The results of the antimicrobial activity of the ethanolic and aqueous extracts of the leaves of *Momordica charantia*, *Luffa cylindrical* and *Trichosanthes cucumerina* are summarized in Tables 1 – 4.

The ethanolic extracts of the leaves of all the three plants used in the study inhibited the growth of all the test organisms except *Escherichia coli*. The inhibition zone ranged from 6.00 – 9.50 mm (Table 1). The ethanolic extracts of *L. cylindrical* had the highest inhibition of the growth of *E. faecalis* (9.50 mm), followed by the extracts of *M. charantia* (8.00 mm), while those of *T.*

cucumerina had the least inhibitory effect on *E. faecalis* (7.50 mm). The growth of *P. aeruginosa* was only inhibited by the ethanolic leaf extracts of *T. cucumerina* and not by the leaf extracts of the other two plants indicating that *T. cucumerina* had compounds which inhibited the growth of the pathogen but were absent in the other plants.

The use of plants in treatment of some diseases stems from the fact that they have the ability to inhibit these pathogens. The use of leaves in these plants in the treatment of disease has been documented (Akobundu and Agyakwa, 1998; Kage *et al.*, 2009; Edeoga *et al.*, 2010).

The antimicrobial activity of the leaves of other plants has been reported (Arshad *et al.*, 2010; Kamba and Hassan, 2010; Osuagwu and Eme, 2013, Osuagwu and Ihenwosu, 2014). The inhibition of the growth of these human pathogens by the leaf extracts might be as a result of presence of bioactive substances (alkaloids, flavonoids, phenols, saponin, steroids and others) in their leaves (Bishnu *et al.*, 2009; Osuagwu and Ihenwosu, 2014). The study of Edeoga *et al.*, (2010) revealed the presence of these bioactive substances in the leaves of these plants.

There were varying effects of the aqueous extracts of the leaves of the plants in the growth of the test microorganisms. The aqueous extracts of *M. charantia* inhibited the growth of *Staphylococcus aureus* and *Salmonella typhi* and had no effect on the other pathogenic organisms. The aqueous leaf extracts of *T. cucumerina* inhibited only the growth of *S. aureus* and *P. aeruginosa*. On the other hand, the aqueous leaf extracts of *L. cylindrical* did not affect the growth of any of the pathogens.

These observed greater effects by the ethanolic leaf extracts may suggest that most of the bioactive substances were more soluble in ethanol than water. There is an observed relationship between the concentration of the extracts and the rate of inhibition of the pathogens. There was corresponding increase in the rate of inhibition of the growth of the pathogens as the concentration of the extracts increased. This trend was also observed by other researchers (Subban *et al.*, 2011; Osuagwu and Eme, 2013; Osuagwu and Nwoko, 2014). The minimum inhibitory concentration of the ethanolic extracts of the leaves of the three plants ranged from 2.00 – 5.5 mm mg/ml.

This investigation reveals that the ethanolic leaf extracts of *M. charantia*, *L. cylindrical* and *T. cucumerina* have antimicrobial activity on the test human pathogenic microorganisms used in

this research except *E. coli* and that the aqueous leaf extracts affected only three of the human pathogens (*S. aureus*, *P. aeruginosa* and *S. typhi*). This shows that the leaves of these plants are of medicinal value. Thus could be exploited to be used in the formations of cheap alternative antimicrobial drugs which will be used to control and cure human infectious disease caused by these pathogens.

Table 1: The antimicrobial activity of the ethanolic extracts of the leaves of *Momordica charantia*, *Luffa cylindrical* and *Trichosanthes cucumerina* on *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*.

Pathogenic organisms	<i>M. charantia</i>	<i>L. cylindrical</i>	<i>T. cucumerina</i>	Gentamycin stock solution
<i>E. coli</i>	0.00	0.00	0.00	21.50±2.12
<i>E. faecalis</i>	8.00±0.20	9.50±0.71	8.00±0.10	19.50±0.71
<i>S. aureus</i>	7.50±0.71	6.00±0.90	7.50±0.55	37.00±1.41
<i>P. aeruginosa</i>	0.00	0.00	6.00±0.50	20.50±0.71
<i>S. typhi</i>	6.00±1.00	5.50±0.20	6.00±0.75	18.50±1.75

Table 2: The antimicrobial activity of the aqueous extracts of the leaves of *Momordica charantia*, *Luffa cylindrical* and *Trichosanthes cucumerina* on *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*.

Pathogenic organisms	<i>M. charantia</i>	<i>L. cylindrical</i>	<i>T. cucumerina</i>	Gentamycin stock solution
<i>E. coli</i>	0.00	0.00	0.00	21.50±2.12
<i>E. faecalis</i>	0.00	0.00	0.00	19.50±0.71
<i>S. aureus</i>	7.50±0.40	0.00	6.00±0.20	37.00±1.41
<i>P. aeruginosa</i>	0.00	0.00	6.00±0.45	20.50±0.71
<i>S. typhi</i>	6.00±1.00	0.00	0.00	18.50±1.75

Table 3: The minimum inhibitory concentration (MIC) of the ethanolic extracts of the leaves of *Momordica charantia*, *Luffa cylindrical* and *Trichosanthes cucumerina* on *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*.

Pathogenic organisms	<i>M. charantia</i>			<i>L. cylindrical</i>			<i>T. cucumerina</i>		
	50	100	200	50	100	200	50	100	200
<i>E. coli</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>E. faecalis</i>	4.5	5.0	5.5	0.0	5.0	6.5	4.5	5.0	5.0
<i>S. aureus</i>	3.5	4.0	5.0	3.0	3.2	4.0	0.0	3.5	5.5
<i>P. aeruginosa</i>	9.0	0.0	0.0	0.0	0.0	0.0	3.0	4.0	5.0
<i>S. typhi</i>	3.0	4.0	4.8	0.0	3.0	3.5	2.0	3.0	3.5

Table 4: The minimum inhibitory concentration (MIC) of the aqueous extracts of the leaves of *Momordica charantia*, *Luffa cylindrical* and *Trichosanthes cucumerina* on *Escherichia coli*, *Enterococcus faecalis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*.

Pathogenic organisms	<i>M. charantia</i>			<i>L. cylindrical</i>			<i>T. cucumerina</i>		
	50	100	200	50	100	200	50	100	200
<i>E. coli</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>E. faecalis</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
<i>S. aureus</i>	0.0	4.0	5.0	0.0	0.0	0.0	3.0	3.5	3.8
<i>P. aeruginosa</i>	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.0	3.5
<i>S. typhi</i>	2.0	3.0	3.8	0.0	0.0	0.0	0.0	0.0	0.0

REFERENCES

1. Abascal, K. and Yarmell, E. (2005). Using bitter melon to treat diabetes. *Altern. Coplen. Ther.*, 11 (4): 179-184.
2. Akobundu, I. O. and Agyakwa, C. W. (1998). A handbook of West African weeds. Second edition, revised and expanded. International Institute of Tropical Agriculture. Ibadan, Nigeria. Pp 244- 247.
3. 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, analgesic and antipyretic properties of ethanolic leaf extract of *Gongonema latifolium* in rodents. *African Journal of Biotechnology*, 9(5):2316-2321.
4. Ankita, S. Parminder, K. and Gupta, R. (2012). Phytochemical screening and antimicrobial assay of various seed extracts of Cucurbitaceae family. *International Journal of Applied Biology and Pharmaceutical Technology*, 3 (3): 401- 409.
5. Arbonnier, M. (2004). Trees, shrubs and lianas of West African dry zones. CIRAD MARGRAF publishers, GMBH. AJ. Wageningen, The Netherland. 573 pp.
6. Arshad, H., Shadma, H., Iffat, I. and Sarfara-Hussein. 2010. Antibacterial activity of the leaves of *Coccinia indica* (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*, *Cinnamomum zeylanicum*, *Xanthoxylum arimatum* and *Origanum masorana*. *Kathmandu University Journal of Science, Engineering and Technology*. 5:143-150.
9. Chakravarty, H. L. (1990). Cucurbits of India and their role in the development of vegetable crops, P 325-334. In : D. M. Bates, R.W. Robinson and C. Jeffrey (eds.). *Biology and Utilization of the Cucurbitaceae*. Cornell University press, Ithaca New York.
10. Chopra, R. N., Nayar, S. L. and Chopra I. C. (1986). Glossary of India medicinal plants. Council of Scientific and Industrial Research, New Delhi.
11. Ebi, G. C. and Ofoefule, S. I. 1997. Investigation into the folkloric antimicrobial activities of *Landolphia owerrance*. *Phytotherapeutic Research*, 11:147-151.
12. 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.
13. Edeoga, H. O., Osuagwu, G. G. E., Omosun, G. Mbaebie, B. O. and Osuagwu, A. N. (2010). Pharmaceutical and therapeutic potential of some wild Cucurbitaceae species from South- East Nigeria. *Recent*
14. Edet, E. E., Akpanabiatu, M. I., Eno, A. E., Umoh, I. B. and Itam, E. H. (2009). Effect of *Gongonema latifolium* crude extract on some cardiac enzymes of alloxan-induced diabetic rats. *African Journal of Biochemistry*, 3(11):366-369.
15. 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.
16. Ijeh, I. I., Omodamiro, O. D. and Nwana, I. E. 2005. Antimicrobial effects of aqueous and ethanolic fractions of two species, *Ocimum gratissimum* and *Xylopiiaethiopica*. *African Journal of Biotechnology*, 4(9):053-956.
17. Kage, D. N., Malashetty, V. B., Seetharam, Y. N. Suresh, P. and Path, S. B. (2009). Effects of ethanol extract of whole plant of *Trichosanthes cucumerina* var *cucumerina* L on Gonadotropins ovarian follicular kinetics and oestrous cycle for screening of antifertility in albino rats. *Int. J. Morphol.* 27 (1): 173- 182.
18. Kamba, A. S. and Hassan, L. G. 2010. Phytochemical screening and antimicrobial activities of *Euphorbia balasamifera* leaves, stem and root against some pathogenic microorganisms. *African Journal of Pharmaceutical Sciences and Pharmacy*, 1(1):57-64.
19. Koche, D. K., Bhadange, D. G. and Kamble, K. D. 2011. Antimicrobial activity of three medicinal plants. *Bioscience Discovery*, 2(1):69-7

20. Mallikharjuna, P. B., Rajama, L. N., Seetharam and Sharanabasappa, G. K. (2007). Phytochemical studies of *Strychnos potatorum* L. A medical Plant. E-Journal of Plant Chemistry, 4(4): 510- 518.
21. McGrath, M. S., Hwang, K. M., Caldwell, S. E., Gaston, I., Luk, K. C. Wu, P., Ng, V. L. Crowe, S., Daniels, J., Marsh, J., Deinhart, T., Lekas, P. V., Vennari, J., Yeung, H. W. and Lifson, J. D. (1989). GLQ223: an inhibitor of human immunodeficiency virus replication in acutely and chronically infected cells of lymphocyte and mononuclear phagocyte lineage. Proc. Natl. Acad. Sci. (USA) 86: 2844- 2848.
22. Muthumani, P., Meera, R., Subin, M., Matthew, J., Devi, O., Kameswari, B. and Priya, B. E. (2010). The Phytochemical screening, anti-inflammatory, bronchodilator and antimicrobial activities of the seeds of *Luffa cylindrical*. Research Journal of Pharmaceutical, Biological and Chemical Sciences. 1 (4): 11- 22.
23. Nagao, T., Tanaka, R., Iwase, Y., Hanazono, H. and Okabe, H. (1991). Studies on the constituents of *Luffa acutangula* Roxb. I. Structures of acutosides A-G, oleanane-type triterpene saponins isolated from the herb. Chem. Pharm. Bul. 39: 599- 606.
24. Ng. T. B., Feng, Z., Li, W. W. and Yeung, H. W. (1991). Improved isolation and further characterization of beta-trichosanthin, a ribosome-inactivating and abortifacient protein from tubers of *Trichosanthes cucumeroides* (Cucurbitaceae). Int. J. Biochem., 23: 561- 567.
25. Nigeria Natural Medicine Development Agency (NNMDA) (2008). Medicinal plants of Nigeria; South – East Nigeria. Vol.1. Lisida consulting Lagos Nigeria.204pp.
26. Osuagwu, A. N. and Edeoga, H. O. (2014). Nutritional properties of the leaf, seed and pericarp of fruit of four Cucurbitaceae species from South- East Nigeria. IOSR Journal of Agriculture and Veterinary Sciences, 7 (9) iv: 41- 44
27. 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.
28. Osuagwu G. G. E. and Eme, C. F (2013). The Phytochemical Composition and Antimicrobial Activity of *Dialium Guineense*, *Vitex Doniana* and *Dennettia Tripetala* Leaves. Asian Journal of Natural and Applied Sciences, 2(3):169 – 181.
29. Osuagwu G. G. E. and Ihenwosu, A. O. (2014). Phytochemical Composition and Antimicrobial Activity of the Leaves of *Alchornea cordifolia* (Schum and Thonn), *Sansevieria liberica* (Gerard Labr) and *Uvaria chamae* (P. Beauv). American Journal of Phytomedicine and Clinical Therapeutics, 2 (1): 1 – 12.
30. Osuagwu, G. G. E. and Nwoko, N (2014). The Phytochemical Screening and Antibacterial Activity of the Leaves of *Combretum Paniculatum* (Vent) *Solanium macrocarpon* (L) and *Catharanthus Roseus* (L) G. Don. IOSR Journal of Pharmacy and Biological Sciences. 9(1):58 – 65.
31. Perulmalsamy,R. And Ignacimuthu,S. 2000. Antibacterial activity of some folklore medicinal plants used by tribals in western charts of India. JournalEthnopharmacology,69:68-71.
32. Sangh, P., Kumar, A., Sharma, K. and Jha, K. K. (2012). *Luffa cylindrical*: An important medicinal plant. J. Nat. Prod. Plant Resour., 2 (1): 127- 134.
33. Schultes, R.E. (1990). Biodynamics cucurbits in the New World tropics, p. 307- 317.In: Bate D. M., Robinson, R. W. and Jeffrey, C. (eds). Biology and utilization of the Cucurbitaceae. Cornell University Press, Ithaca, New York.
34. 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.
35. Subban,M., Annamalai,P. and Arumugame,C. T. 2011. Phytochemical screening and Antimicrobial activity of the leaves of *Memecylonumbellatum burm. F.* JournalofAppliedPharmaceuticalScience1(1):42-45.
36. Tindall, H. D. (1983). Vegetables in the Tropics. AVI., Westport, CT.
37. Werner,F., Okemo,P. and Ansorg,R. 1999. Antibacterial activity of East African Medicinal Plants. JournalEthnopharmacology,60:79-84.