IN VITRO ANTIBACTERIAL ACTIVITY OF THE EXTRACTS OF *PEPEROMIA PELLUCIDA* (L).

IdrisO.O[1].,Olatunji B.P.[1], MaduforP.[1],

[1]Department of Biological Sciences, AfeBabalola University Ado-Ekiti.

ABSTRACT

Background: *Peperomia pellucida* is an economic plant grown in West Africa. **Aim:** We investigated the phytochemical and antimicrobial activity of N-hexane, Ethyl acetate, and Ethanol extract of *Peperomia pellucida* whole plant that grows around Ado-Ekiti, Ekiti State, Nigeria. **Methods:** Preliminary screening was conducted on the powdered sample for the presence of secondary metabolites. 150g of the dried plant powdered sample was soaked with 750ml of solvents for 72 hours. The filtrates concentrated on water bath (40^0C) were tested against strains of some bacteria isolates including *Escherichia coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 34089, *Salmonella typhi* ATCC 22648, *Staphylococcus aureus* ATCC 25923 *and Pseudomonas aeruginosa*, using the agar well diffusion method. **Results:** Phytochemical screening of this plant showed the presence of antraquinone, tannins, flavonoids, alkaloids and glycosides. All extracts exhibited antimicrobial activities with the methanol extract exhibiting the least potency whilst the N-hexane extract exhibited the strongest potency with zone of inhibition 10-12mm at concentration of 25µg/ml. The MIC (200mg/ml) of the plant extracts was observed to be effective against the strains of organisms. **Conclusion:** The antimicrobial properties against the tested strains indicated the potential usefulness of *P. pellucida* in the treatment of various pathogenic diseases which in future can be developed as a potential antimicrobial agent.

Key words: *Peperomia pellucida*, Chemotherapeutic, Phytochemical

INTRODUCTION

Since prehistoric times, the treatment and cure of diseases has been one of the primary concerns of mankind of which through the aid of exceptional advancements in science and medicine, microorganisms called pathogens have been known to be the cause of diseases. Also, there is a worldwide increase in life threatening infections caused by these pathogenic microorganisms [1]. These microorganisms are becoming resistant to known antimicrobial agents [2, 3]. There is therefore an increased interest in the search for antimicrobial compounds. According to data of the Food and Agriculture Organization (FAO), more than 50,000 plant species are being used in the traditional folk medicine throughout the world [4]. This led to the pharmacological and chemical investigations of medicinal plants. Investigations have provided important advances in the therapeutic approach to several pathogens. *Peperomia pellucida*(L.), commonly known as shiny bush or silver bush belonging to family piperaceae is a common annual weed native to tropical North and South America, Africa and Asia. In Africa, it is found in Nigeria, Sierra Leone, Ghana and Democratic Republic of Congo (DRC). Regions where it can also be found include China, Brazil, Southern America and Asian countries [2,5,6,7,8]. Within the Yoruba speaking part of Nigeria, it is identified as "rinrin".The infusion added with milk is ethno-medicinally described to boost the immune system of sick people. The leaves of the plant are being used by the local people of Bangladesh in the treatment of excited mental disorder [1]. Pounded whole plant is used topically and as warm poultice for skin disorders such as boils, pustules, pimples and also used for headaches, rheumatic pains and impotence [9].

In Ayurvedic medicine, the plant is crushed and mixed with water to form a mixture, heated and administered orally to cure hemorrhage and also against kidney and prostate problems and against high blood pressure. Literatures revealed that the plant contain some secondary metabolites including, saponins, tannins, cardenolides, flavonoids, essential oils and carotol [10]. However, the full potential of the plant *Peperomia pellucida* is yet to be discovered, therefore it is imperative that this plant is thoroughly investigated.

This work focused on phytochemical property and antimicrobial activity of the whole plant of *Peperomia pellucida* that grows around Ado-Ekiti, Ekiti State, Nigeria.

MATERIALS AND METHODS

Collection and Identification of Plant Sample:

The whole plant of *P. pellucida* was handpicked from the vicinity of Afe Babalola University Ado-Ekiti in the month of May. Identified and authenticated at the herbarium unit of the Department of Botany, Ekiti State University, Ado-Ekiti, Ekiti State, Nigeria. The harvested plant materials were air dried for one month at room temperature (26^0C). The dried plant material was then ground to a fine powder using an electric blender and stored in sterile containers until use.

Collection of Microbial Isolates:

All bacterial strains were provided by National Institute of Pharmaceutical Research and Development (NIPRID) which included *Escherichia coli* ATCC 35218, *Klebsiella pneumonia* ATCC 34089, *Salmonella typhi* ATCC 22648, *Staphylococcus aureus*ATCC 25923 *and Pseudomonas aeruginosa*. The test organisms were maintained on nutrient agar slopes and kept in a refrigerator at 4°C.

Preparation of Plant Extract:

Three different solvents namely N-hexane, Ethyl acetate, Ethanol were chosen to be utilized for the sequential extraction. 150g of the dried plant powdered sample was soaked with 750ml of each of the solvents, mixed thoroughly and stored in air tight jars for 72 hours under strict observation. The extracts were filtered using Whatmann No.1 filter paper and the filtrate was then placed in a water bath at a temperature of 40°C with the lid of the jars left open until all the solvents successfully evaporated from the solution leaving behind a thick extract. The dried underlying crude extracts were kept in glass vials and stored in the refrigerator at 4°C until use.

Preliminary Phytochemical screening of *Peperomiapellucida* leaves

Phytochemical screening tests were carried out on *P.pellucida* for the following secondary plant metabolites: alkaloids, saponins, tannins, flavonoids, steroids, anthraquinones, and glycosides following the method described by Harborne, 1998 [11].

Test for flavonoids: 1 mL of NaOH was added to 3 mL of each extract. A yellow colouration indicates that flavonoids are present.

Test for steroids: 1 mL of concentrated sulphuric acid was added to 2 mL of each of the extracts. Observation of a red colouration indicates presence of steroids.

Test for saponins: 2 mL of distilled water was added to 2 mL of each extract and shaken vigorously. A persistent frothing indicates presence of saponins.

Test for tannins: 2 mL of 5% ferric chloride was added to 1 mL of each extract. Observation of a greenish precipitate indicates the presence of tannins.

Test for alkaloids: 1 mL of 1% HCl was added to 3 mL of each extract in a test tube. The mixture was heated for 20 mins, cooled and filtered. The filtrate was used for the following test: 2 mL of Meyer's reagent was added to 1 mL of the filtrate. Observation of a creamy precipitate indicates the presence of alkaloids.

Test for free AnthraquinoneAglycone: The powdered sample (0.5 g) was shaken with 5 ml of chloroform in a test tube for about 5 minutes, filtered, and the filterate was shaken with equal volume of 10% ammonia. Rose pink colouration in aqueous layer indicates the presence of free anthraqunoneaglycone.

Antimicrobial assay

The antimicrobial assay was done using the agar well diffusion method. An overnight culture of each organism was prepared by using small portion of the organism from the stock and inoculating each into 8ml sterile peptone water and incubated for 24hours at 37^{o}C. The various test bacteria were standardized using the 0.5 McFarland turbidity standards. From the overnight culture , 0.1ml of each organism was taken and put into the 9.9ml of sterile distilled water to get (1:100) of the dilution of the organism. An aliquot 0.1ml was taken from the dilution onto the surface of sterile plates of Mueller Hinton agar (MHA). A 6mm cork borer was used to make wells on the inoculated MHA agar. One milliliter of each crude extract was constituted with Dimethyl sulphoxide (DMSO) and introduced into designated wells. The DMSO served as the control and was introduced into a separate well as appropriate. These were left on the work bench for duration of 2 hours after which it was then incubated at 37^{o}C for 24hrs. The diameters of the zone of inhibition were measured in millimeters using a ruler [12]. The tests were conducted in duplicates. The minimum inhibitory concentration (MIC) was determined for each plant extract showing antimicrobial activity against the test isolates using broth micro dilution method [13]. The MIC values were taken from the lowest concentration of the extracts in the well of the tube that showed no turbidity after incubation. The turbidity of the wells in the subsequent tubes was interpreted as visible growth of microorganisms [14,15].

Antibiotic Susceptibility Test: The antimicrobial susceptibility test was done using the agar-disk diffusion method [16]. Fresh isolates were suspended in peptone water in comparison to 0.5 McFarland standards. Each of the isolates was inoculated onto the surface of a sterile Mueller

Hinton Agar plates using a sterile swab in order to ensure even distribution while streaking. The plates were allowed to dry for 15 minutes and antibiotic discs were placed on the surface of the agar plates using a sterile forceps. The plates were then inverted and incubated for 24 hours at 37°C. The antimicrobial disc include the Gram negative disc comprising of Ceftazidime 30µg, Cefuroxime 30µg, Cefixime 5µg, Augmentin 30µg, Ofloxacin5µg, Ciprofloxacin 5µg, Gentamicin10µg and Nitrofurantion 300µg which serves as positive control for Gram negative organisms and the Gram positive bacteria disc comprising of Erythromycin 5µg, Augmentin 30µg, Ofloxacin5µg, Gentamicin 10µg, Streptomycin 10µg, Cloxacillin5µg, Cefuroxime 30µg and Ceftazidime 30µg which serves as positive control for gram positive organisms. The antimicrobial activities were determined by the width of the zone of growth inhibition. The tests were conducted in duplicates [17,18].

RESULTS AND DISCUSION

Plants are an important source of potentially useful structures of development of the new chemotherapeutic agents. The first step towards this goal is the *in-vitro* antibacterial assay [19]. The importance of botanical, chemical and pharmacological evaluation of plant derived agents used in the treatment of human ailments has been increasingly recognized in the last decades [20]. The presence of these compounds in the plants has been attributed to most of their biological activities [21]. Many reports are available on the antiviral, antibacterial, antifungal, antihelmintic, antimolluscal and anti- inflammatory properties of the plants [22,23].

The phytochemical screening of this plant showed the presence of antraquinone, tannins, flavonoids, alkaloids and glycosides, while steroid was absent in all the solvent used in the extraction of *P. pellucida.* Also, saponin was absent in n-hexane and ethyl acetate extract and antraquinone was absent in methanol extract (**table 1**). The phytochemical contents of the leafy vegetables serve as supplements for food and also have the potential to improve the health status of its users through their antimicrobial properties. This study revealed the presence of a number of bioactive compounds which can be used as a lead compound for synthesizing drugs for various ailments. Alkaloids have been reported to be the most efficient therapeutically significant phytochemical [24]. Stray (1998) [25] reported that pure alkaloids and their derivatives are basic medicinal agents because of their analgesic, antispasmodic and bacterial properties. It has been reported that alkaloids can be used in the management of cold, fever and chronic catarrh [26].

Tannins are well known for their antioxidant and antimicrobial properties as well as for soothing relief, skin regeneration, as anti- inflammatory and diuresis [27]. Flavonoids are known for their antioxidant activity, and hence they help to protect the body against cancer and other degenerative disease such as Arthritis and Type II diabetes mellitus [28]. Glycosides, especially the cardiac glycosides act on the heart muscles and increase renal flow (dieresis). Herbal preparation containing cardiac glycosides is used for the treatment of congestive heart failure and cardiac arrhythmia. The presence of phytochemical compounds in this plant is responsible for the observed biological activity.

Out of the bacteria strains tested *Escherichia coli* showed resistance to all the antibiotics it was subjected to and also all the bacteria strains showed 100% resistance to augumentin (Table 2). The antimicrobial activities of the plant extracts tested *in vitro* against the five typed organisms *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Klebsiella pneumoniae and Salmonella typhi* using the agar diffusion method exhibited antibacterial activities with the methanol extract exhibiting the least potency whilst the N-hexane extract exhibited the strongest potency. The N-hexane and ethyl acetate extracts had zones of inhibition demonstrating susceptibility of the organisms between 10 to 12mm at concentration of 25µg/ml when compared **(table 3, 4 and 5).** Methanol extract shows antibacterial activities with zones of inhibition of 10mm at 200µg/ml **(table 5).** The minimum inhibitory concentration (MIC) is expressed in **table 6** where it was distinctly observed that at higher concentrations there was a stronger activity against microorganisms. The minimum inhibitory concentrations of the plant extracts were evaluated between the ranges of 25 – 200mg/ml.

The observed phytochemicals present in the plant could be responsible for its medicinal properties which affirm the use of this plant in the management of ailments in various localities especially gastro intestinal tract (GIT) infections as regards to the carefully selected and specified organisms of choice. The additive or synergistic action of these phytochemicals at target sites associated with physiological process may be responsible for the beneficial effects exerted by *Peperomia pellucida*. Further works need to be done in the future to correlate the specific compound with its biological property most importantly; the heart shape of the plant's leaf could be suspected cardio-specific in its activities. However, usage of such toxic chemical compounds at high doses should be properly monitored despite their medicinal benefits in the therapy of some ailments involving cell or tumour growth [29].

Table 1: Qualitative phytochemical properties of *Peperomia pellucida*

	NH	MEOH	EA
Alkaloids	+	+	-
Saponins	-	+	-
Tannins	+	+	+
Flavonoids	+	+	+
Steroids	-	-	-
Antraquinones	+	-	+
Glycosides	+	+	-

Key ~ NH: N- hexane extract, EA: ethyl acetate extract, MEOH: methanol extract, +: present, -: absent.

Table 2: Antibiotic Susceptibility Test

	Cefixime (5µg)	Nitrofuration (300µg)	Ciprofloxacin	Ceftazidime (30µg)	Cefuroxime (30µg)	Gentamicin (10µg)	Cefixime (5µg)	Augumentin (30µg)	Erythromycin	Cloxacillin (5µg)	Ofloxacin (5µg)
S.a	*	*	*	(R)0	(S)8	(S)27	(S)8	(R)0	(S)27	(R)0	(R)0
Sal	(S)18	(S)18	(S)13	(S)13	*	(S)18	(S)18	(R)0	*	*	(R)0
E. coli	(R)0	(R)0	(R)0	(R)0	(R)0	(R)0	(R)0	(R)0	*	*	*
Kleb	(S)16	(S)15	(S)15	(S)15	(R)0	(S)16	(S)16	(R)0	*	*	*
Ps.a	(R)0	(R)0	(S)16	(S)8	*	(S)16	(R)0	(R)0	*	*	(S)16

Score (R): resistant, (S): susceptible, *: not applicable, **S.a**: *Staphylococcus aureus,* **E. coli**: *Escherichia coli*,**Ps.a**: *Pseudomonas aeruginosa*, **Kleb**: *Klebsiellapneumonae*, **Sal**: *Salmonella typhi*.

Table3: Antibacterial activity of crude N-hexane extract of *P. pellucida.* Zone of inhibition (mm)

Organisms	N-hexane concentrations					
	25mg/ml	50mg/ml	100mg/ml	200mg/ml	-ve	+ve
Staphylococcus	10	12	14	16	-	38

***aureus*ATCC 25923**						
***Salmonella typhi* ATCC 22648**	12	14	16	18	-	36
***Escherichia coli* ATCC 35218**	10	12	14	16	-	36
***Klebsiella pneumonia* ATCC 34089**	10	12	14	16	-	34
***Pseudomonas aeruginosa*.**	12	14	16	18	-	36

N-hexane at various concentrations: 25mg/ml, 50mg/ml, 100mg/ml, 200mg/ml, -ve: negative control (methanol), +ve: positive control {Gentamicin at 10 mg/ml for bacteria}, -: no inhibition

Table 4: Antibacterial activity of crude methanol extract of *P. pellucida* Zone of inhibition (mm)

Organisms	**Methanol concentrations**					
	25mg/ml	**50mg/ml**	**100mg/ml**	**200mg/ml**	**-ve**	**+ve**
***Staphylococcus aureus* ATCC 25923**	-	-	-	10	-	38
***Salmonella typhi* ATCC 22648**	-	-	-	10	-	36
***Escherichia coli* ATCC 35218**	-	-	-	10	-	36
***Klebsiella pneumonia* ATCC 34089**	-	-	-	10	-	34
***Pseudomonas aeruginosa*.**	-	-	-	10	-	36

Methanol fraction at various concentrations: 25mg/ml, 50mg/ml, 100mg/ml, 200mg/ml, -ve: negative control (methanol), +ve: positive control (Gentamicin at 10 mg/ml for bacteria), -: no inhibition

Table 5: Antibacterial activity of crude ethyl acetate extract of *P. pellucida*Zone of inhibition (mm)

Organisms	**Ethylacetate concentrations**					
	25mg/ml	**50mg/ml**	**100mg/ml**	**200mg/ml**	**-ve**	**+ve**
***Staphylococcus aureus* ATCC 25923**	-	10	12	14	-	38

***Salmonella typhi* ATCC 22648**	-	10	12	14	-	36
***Escherichia coli* ATCC 35218**	-	-	-	10	-	36
***Klebsiella pneumonia* ATCC 34089**	10	12	14	16	-	34
***Pseudomonas aeruginosa*.**	-	-	10	14	-	36

Ethyl acetate fraction at various concentrations: 25mg/ml, 50mg/ml, 100mg/ml, 200mg/ml, -ve: negative control (methanol), +ve: positive control {Gentamicin at 10 mg/ml for bacteria}, -: no inhibition.

Table 6: Determination of the Minimum Inhibitory Concentration (MIC) of the Extracts.

Organism	**NH(200mg/ml)**	**MEOH(200mg/ml)**	**EA(200mg/ml)**
***Staphylococcus aureus*ATCC 25923**	25	62.5	27.5
***Escherichia coli* ATCC 35218**	27.5	31.25	31.25
***Klebsiella puemoniae* ATCC 34089**	31.5	62.5	100
***Salmonella typhi* ATCC 22648**	100	125	100
***Pseudomonas aeruginosa*.**	27.5	31.25	31.25

Key ~ NH: N- hexane extract, EA: ethyl acetate extract, MEOH: methanol extract

CONCLUSION

The potency of *P. pellucida* phytochemicals in the inhibition of bacteria is fast becoming a thing of interest in the fields of medicine, microbiology, biochemistry and life related sciences. The antibacterial properties of *P. pellucida* against the selected strains which included *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 35218, *Klebsiella puemoniae* ATCC 34089, *Salmonella typhi* ATCC 22648 and *Pseudomonas aeruginosa* has indicated the potential usefulness of *P. pellucida* in the treatment of various pathogenic diseases which in future can be developed as a potential antimicrobial agent with perhaps reduced toxicity and adverse effects when compared with synthetic chemotherapeutic agents and thus can be seen as potential source of useful antibacterial drugs. Further study is however recommended in order to isolate, identify, characterize and elucidate the structure of the plant's bioactive components.

REFRENCES

1. Khan A, Rahman M, Islam MS.Neuropharmacological effects of *Peperomiapellucida*leaves in mice. *DARU* 2008;16 (1) 35-40.
2. Ghani, A., *et al*.,. Medicinal plants of Bangladesh. Dhaka, Bangladesh: Asiatic Society of Bangladesh, 1998; 3.
3. De Smet, PAG. Herbal remedies, New England. Journal Medicines 2002;347: 2046-2056.
4. Schippmann U, Leaman DJ, Cunningham AB. (2002). Impact of Cultivation and Gathering of Medicinal Plants on Biodiversity: Global Trends and Issues, Biodiversity and the Ecosystem Approach in Agriculture, Forestry and Fisheries. Satellite Event on the Occasion of the 9th Regular Session of the Commission on Genetic Resources for Food and Agriculture, Inter-Departmental Working Group on Biological Diversity for Food and Agriculture, Rome, pp. 1–21.
5. Bayma JD, Arruda MS, Müller AH, ArrudaAC, Canto WC.A dimericArC_2 compound from *Peperomiapellucida*.Phytochemistry, 2000;55:779-782.
6. Santos PR, Moreira DL, Guimaraes EF, Kaplan MA. Essential oil analysis of 10 piperaceae speciesfrom the Brazilian Atlantic forest.Phytochemistry 2001;54:547-551.
7. Arrigoni-Blank de Fátima, M, Oliveira, RL, Mendes, SS. Seed germination, phenology, and antiedematogenic activity of *Peperomiapellucida* (L.) H. B. K. BMC. Pharmacol. 2002;2:12-

19.
8. Arrigoni-Blank de Fátima M, Dmitrieva, EG, Franzotti, EM, Antoniolli, AR, Andrade, MR, Marchioro, M. Anti- inflammatory and analgesic activity of *Peperomiapellucida* (L.) HBK (Piperaceae). J Ethnopharmacol, 2004;91(2-3):215-8.

9. Http://www.geocities.com/mmsi1902/herbal_aware.htm.

10. Khan MR, Omotoso AD.Antibacterial activity of Hygrophilastricta and *Peperomiapellucida'*.Fitoterapia2002; **73(3)**:251-254.

11. Harborne, J.B. "Phytochemical Methods" Harborne JB ed. Chapman & Hall, London.1998

12. Bauer AW, Kirby WWM, Sherris JC, Turk M. Antibiotic susceptibility testing by Standardized single disc method.Am JClinPathol.1966; 45:493-496.

13. Basri DF, Fan SH The potential of aqueous and acetone extracts of galls of Quercusinfectoria as antibacterialagents. India J. Pharmacol 2005; 37 (1):26-29.
14. Vollokova AD,Kostalova and Sochorova. Isoquinoline alkaloid from mahoniaaquifolium stem bark is active against Malseizia species. Journal Microbiology, 200146: 107-111.

15. Ogbulie JN, Ogueke CC, Okoli IO and Anyawu, BN. Antibacterial activities and toxicological potential of crude ethanolic extracts of Euphorbia hirta. African Journal of Biotechnology 2007;Vol. 6 **(13)**, 1544-1548.

16. CLSI, performance standards for Antimicrobial Disk Susceptibility Test; Approved Standard 7th edition, 2013. Volume 33.

17. DeeniYY, and Sadiq NM. Antimicrobial properties and phytochemical constituents of the leaves of African mistletoe (*Tapinanthusdodoneifolius*(DC) Danser) (Loranthaceae): an etnomedicinal plant of Hausa land, Northern Nigeria.Journal Ethnopharmacology.2002;83: 235-240.

18. Zaidan MR, Noor A, Badrul AR, Adlin A, Norazah A, Zakiah I (2005). In vitro screening of five local medicinal plants for antibacterial activity using disc diffusion method.Trop. Biomed. 22(2):165-790.

19. Tona LK, Kambu N, Ngimbi K., Cimanga. Antiamoebic and Phytochemical screening of some Congoleaea medicinal plants.Journal Enthopharmacol.2003;62:57-65.

20. Gbile ZO. Ethnobotany, Taxonomy and Conservation of Medicinal Plants. In: The State of Medicinal Plants Research in Nigeria, Sofowora, A. (Ed.). University of Ibadan Press, Ibadan, Nigeria.1986; pp13-29.

21. Seenivasan P, ManicklamJ andSavarimuthu I. In vitro antibacterial activity of some plant essential oils.Bmc Complement Altern. Med 2006; 6: 1-8

22. Samy RP and Ignacimuthu S. Antibacterial activity of some folklore medicinal plants used by tribals in western Ghats in India. Journal Enthopharmacology.2000; 69:pp 63-71.

23. Govindarajan R, Vijayakumar M, Singh CHV, Rao A, Shirwaikar AK, Rawat S. and Pushpangadan P. Antiulcer and antimicrobial activity of *Anogeissuslatifolia.* Journal Ethnapharmacology.2009; 106: 57-61.

24. Njoku, PC, and Akemefula, M.I. Phytochemical and nutrient evaluation of *Spondiasmombin* leaves. Pak. J. nutrition.2007; 6: 613-615.

25. Stray F.The Natural Guide to Medicinal herbs and plants. Tiger Book International, London. 1998.

26. Gill LS. Ethnomedicinal uses of plants in Nigeria.University of Benin Press, Benin City. 1992; 275p

27. Okwu DE. andOkwu, MR. Phytochemicals and vitamin content in indeginous spices of South Eastern Nigeria. Journal for Sustainanceof Agricultural Environment. 2004; 6: 140-147.

28. Lee, KG. andShibumoto, J.Journ. Agric. Food Chem.2002 50: 4947-4955.

29. Ganiyat KO, Patricia AO and Bamidele BO. Phytochemical, toxicity, antimicrobial and antioxidant screening of leaf extracts of *Peperomiapellucida*from Nigeria. Advances in Environmental Biology,2011;5(12): 3700-3709, ISSN 1995-0756.