FULL LENGTH RESERCH ARTICLE

ANTIBACTERIAL ACTIVITY OF AQUEOUS AND ETHANOLIC LEAF EXTRACTS OF *Peperomia pellucida* (L.) H. B. & K. (PIPERACEAE) ON THREE GRAM-NEGATIVE BACTERIA ISOLATES.

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ABSTRACT

The antibacterial activity of aqueous and ethanolic leaf extract of *Peperomia pellucida* was investigated on *Escherichia coli, Proteus mirabilis* and *Pseudomonas aeruginosa* using Agar-well diffusion method. Results showed that *E. coli* displayed the highest susceptibility in water extract (17.4mm–21.2mm) followed by *P. mirabilis* (12.4mm–15mm) and least in *P. aeruginosa* (10.2mm–12.24mm). Conversely, the ethanolic extract showed the highest inhibition in *P. aeruginosa* (13.4mm–19.6mm) followed by *Proteus mirabilis* (10.2mm–18.2mm) and the least was in *Escherichia coli* (0.0mm–12.2mm). The results revealed that ethanol is better than water as solvent for extraction of *P. pellucida* for it to show its highest inhibitory activity on *Proteus mirabilis* and *P. aeruginosa* while water is the best solvent for extraction of *P. pellucida* for it to show its highest inhibitory activity on *E. coli*.

Keywords: Antibacterial activity, *Peperomia pellucida*, *Escherichia coli, Proteus mirabilis*, *Pseudomonas aeruginosa*, leaf extracts.

INTRODUCTION

The continuous evolution of bacteria resistant to currently available antibiotics has necessitated the search for novel and more effective antibacterial compounds (Akinnibosun *et al.*, 2008). Bacterial resistance to almost all available antimicrobials has been recorded, making antibiotics resistance a global concern. Ethnopharmacologists, botanists, microbiologists and natural-product chemists are working to discover phytochemicals and leads, which could be developed for treatment of infectious diseases (Kavitha & Padma, 2008). Efforts in this regard have focused on plants because of their use historically and the fact that a good portion of the world's population, particularly in developing countries, rely on plants for the treatment of infectious and non infectious diseases (Martinez *et al.*,1996; Jadeja *et al.*, 2005; Aibinu, 2006).

Despite the millions of chemical structures currently available for screening for therapeutic value, natural products particularly of plants origin remain a most important source of new drugs (Odugbemi, 2006). Among the benefits derived from using medicine obtained from plants are their relative safety compared to synthetic alternative (Iwu, *et al*., 1999, Idu *et al*., 2007).

This study was carried out to determine the antibacterial activity of the crude extract of *Peperomia pellucida* against three vegetative Gram-negative bacteria namely *Escherichia coli, Pseudomonas aeuriginosa* and *Proteus mirabilis.*

Materials and Methods

Plant material: *Peperomia pellucida* (L) H.B.& K. is a weed of damp, moist places, common in West Africa belonging to the family piperaceae. It is one of the most widely distributed families of flowering plant that consists of more than 12 genera comprising about 1,400 species distributed throughout tropical and subtropical region in both hemispheres (Sengupta & Ray, 1987; Tchoumbougnang *et al*., 2009). The genus *Peperomia* represents nearly half of the piperaceae (Wagner *et al.,* 1999). The plant grows erect and succulent. The stem is fleshy, round, delicate and glabrous. The leaves are alternate on stalks about 1-2 cm long. The blades are broadly-ovate, heart-shaped at the base, entire at the margins, rather thin, about 1.8-2.5cm long and 1.2-1.8cm wide and smooth on both surfaces. The inflorescence consists of slender spikes, 1-6cm long arising at stem terminals as well as leaf axils. The flowers are numerous, scattered on the spikes, without petals, but with only two stamens and an ovary. The fruits are minute, 1–seeded berries (Akobundu & Agyakwa, 1998). The plant have fibrous root system (Wagner *et al.,* 1999).The plant is a weed of damp moist places, common in West Africa. It was introduced form tropical America (Akobundu & Agyakwa, 1998). It develops during rainy period and thrives in loose humid soil under the shade of trees.

Collection of plant sample: The plant was collected from University of Benin, Ugbowo Campus, Benin City, Edo State, Nigeria and identified with appropriate literature (Akobundu & Agyakwa, 1998).

Processing of plant materials: After collection and identification, the plant samples were washed with distilled water and dried in open air under a shade in order to prevent the ultra violet rays from inactivating the chemical substances present in the plant. The dried

leaves were pulverized using a mortar and pestle to a fine powder and stored in an air tight glass jar.

Preparation of aqueous extracts: Five grammes of the dried pulverized leaf powder was soaked in 50 ml of distilled water for 24 hrs at the end of which the mixture was centrifuged at 3000 rpm. The supernatant was serially diluted with sterile distilled water to get four different dilutions as follows: 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} which were the concentration at which antibacterial activity was carried out.

Preparation of ethanolic extracts: Five grammes of the dried pulverized leaf powder was soaked in 50 mls of alcohol (95% Ethanol) for 24 hrs at the end of which the mixture was centrifuged at 3000 rpm. The supernatant was serially diluted to get four different dilutions as follows: 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} which were the concentrations at which antibacterial activity was carried out.

Research bacteria: Three bacteria isolates namely *Pseudomonas aeruginosa, Escherichia coli* and *Proteus mirabilis* were used. *Pseudomonas aeruginosa* is the most important genus in the Pseudomonadaceae family and contains straight or slightly curved Gram-negative rods, 0.5-1.0 µm by 1.5-5.0µm in length that are motile with one or several polar flagella and lack prothecea or sheaths. These chemoheterophs are aerobic and carry out respiratory metabolism with oxygen (O_2) (and sometimes nitrate) as the electron acceptor. The genus is an exceptionally heterogeneous taxon composed of 700 more species. Many can be placed in one of five rRNA homology groups (Prescott *et al*., 2005). *Escherichia coli* and *Proteus mirabilis* had been previously described (Akinnibosun *et al*., 2008)

Cultures of *P. aeruginosa, E. coli* and *P. mirabilis* were collected from urine, boil and wounds of infected patients from the Department of Medical Microbiology, University of Benin Teaching Hospital (UBTH), Benin City and identified using cultural, morphological and biochemical tests (Akinnibosun *et al*., 2008). The bacteria isolates were maintained on nutrient agar slants at 4 ^{0}C. Prior to the test, the innocula of the test organisms were aseptically transferred from the stock culture to separate Petri-dishes containing sterile broth and incubated at 37 ^{0}C for 18 hrs.

Determination of Antimicrobial Activity: Agar-well diffusion method (Stoke, 1975) was used to measure the zone of inhibition. Six Petri-dishes were poured with already sterilized nutrient agar to the level of obtaining a standard well and allowed to set. The organisms, dissolved in nutrient broth were poured into set petri-dishes and uniform distribution was ensured. Sterile cork borer of 10 mm diameter was used to punch holes in the agar. Each of the holes (4 in number) in each petri-dish were filled with 0.3µl of the serially diluted extracts and kept in an incubator for 24 hrs at 37 ^{0}C for the organisms to grow. In this assay the degree of sensitivity was expressed as a measure of the diameter of the inhibition of growth in millimetres.

RESULTS

The antimicrobial properties (as shown by zones of inhibition) of the different concentrations of dried leaves extracts of the plant on test isolates are shown in Table 1 while the cultural, morphological and biochemical tests results are shown in Tables 2, 3 and 4 respectively.

TABLE 1. ZONES OF INHIBITION OF LEAF EXTRACTS ON DIFFERENT BACTERIA ISOLATES.

Organism	Extract Concentration	Zones of Inhibition(mm)	
		Water Extract	Ethanol Extract
E. coli	10^{-1}	21.2	12.0
	10^{-2}	19.6	0.0
	10^{-3}	17.8	0.0
	10^{-4}	16.4	0.0
P. Mirabilis	10^{-1}	15.0	18.2
	10^{-2}	13.8	15.0
	10^{-3}	12.0	12.6
	10^{-4}	12.4	10.2
Pseudomonas aeruginosa	10^{-1}	12.24	19.6
	10^{-2}	11.8	17.4
	10^{-3}	11.4	14.2
	10^{-4}	10.2	13.4

TABLE 2. CULTURAL CHARACTERISTICS OF TEST BACTERIA

Organisms	Cultural characteristics
E. coli	Smooth like droplets and are cream coloured on nutrient agar
P. mirabilis	Swarming nature on nutrient agar and characteristic fishy dour.
Pseudomonas aeruginosa	Smooth round colonies with fluorescent greenish colour on nutrient agar.

TABLE 3. MORPHOLOGICAL CHARACTERISTICS OF TEST BACTERIA

Organisms	Gram Reaction	Form	Arrangement
E. coli	Negative	Short rod	Single
P. mirabilis	Negative	Pleomorphic rod	Cluster and tetrads
Pseudomonas aeruginosa	Negative	Straight rod	Single

TABLE 4. BIOCHEMICAL CHARACTERISTICS OF TEST BACTERIA

Organisms	Coagulase	Motility	Oxidase	Catalase	Methyl red	Sugar fermentative
E. coli	-	-	-	+	+	+
P. mirabilis	-	+	-	-	-	+
Pseudomonas aeruginosa	-	+	+	+	+	-

Key: - = Negative, + = Positive

DISCUSSION

The water and ethanolic crude extracts of the leaves were found to be effective against all the test organism at the different extract concentrations except that ethanol extract has no effect on *E. coli* at lower concentrations of 10^{-2} -10^{-4}. The antimicrobial activity of the different extracts actually increase with increase in concentration, this is in agreement with the findings of Dabai & Muhammad (2008) who observed an increase in antibacterial activity of leaf and bark extracts of *Bantrinia thonningi*, *Angeiossus schimperi* and *Cassia occidentalis* on *Staphylococcus auerus*, *Salmonella typhi* and *E. coli* when the extracts concentrations increases from 30mg/ml^{-1} to 120mg/ml^{-1}. The results obtained in this study on the anti-microbial efficacy of the dried leaf extracts of *Peperomia pellucida* on *P. mirabilis* and *P. aeruginosa* showed the highest inhibitory activity using ethanol as the medium of extraction similar result was obtained by Ammara *et al*., (2009). They opined that the stronger extraction capacity of ethanol could have produced greater active constituents responsible for anti-microbial activity. This suggests that the active components of this plant may be highly polar compound, and the active principle dissolved completely in the alcohol after soaking the leaves for 24 hrs. This showed ethanol to be better than water as solvent for extraction of *P. pellucida* when used on some pathogenic bacteria.

The recent emergence of bacterial infections and resistant strains has stimulated the investigation of plants used in traditional medicine as sources of anti-infective agent (NNIS, 2004).The use of medicinal plants in the world and especially in Nigeria, contributes significantly to primary health care delivery (Gill, 1992). All the test organisms were susceptible to the water extract though in varying degree. The inhibitory concentrations were different and varied according to different organisms, concentrations and extraction medium. This is in consonance with the report of Ammara *et al*., (2009). *E. coli* in water extract showed the highest susceptibility (17.4-21.2mm), followed by *P. mirabilis* (12.4-15mm) while the least was *P. aeruginosa* (10.2-12.24mm).

P. aeruginosa showed the highest susceptibility in ethanolic extract (13.4-19.6mm) followed by *P. mirabilis* (10.2-18.2mm) and the least in ethanolic extract was *E. coli* (0.0-12.2mm). Rauha *et al.,* (2000) reported that alcoholic extract of *Starchytarpheta jamaicensis* showed antimicrobial activity on *E. coli*, *P. aeruginosa*, *Candida albicans*, and *P. mirabilis*.

The test organisms were susceptible to the crude extracts, indicating that the crude extracts is not cell bound and that the active principle had a broad antimicrobial spectrum (Bankole, 1992). Of all the organisms tested, *P. aeruginosa* was the most susceptible in ethanolic extract while *E. coli* showed the highest susceptibility in water extract. This indicates that in case of *P. aeruginosa* and *Proteus mirabilis* infections, the ethanolic extracts of *Peperomia pellucida* if employed will produced a positive effect, while using alcoholic extract on *E. coli* might not be very effective as little or no inhibition was produced at the different concentrations used.

The results of this investigation support the claims by local practitioners of ethnomedicine in the therapeutic efficacies of this herb. The antimicrobial action of the medicinal herb used in this study has shown that the plants extracts is a potential source of antimicrobial agent against *E. coli, P. mirabilis* and *P. aeruginosa* and could be used in the management of nosocomial infection.

REFERENCES

Aibinu, I. (2006). Medicinal plants as antimicrobials. *In*: T. Odugbemi (Ed.). *Outlines and pictures of medicinal plants from Nigeria.* University of Lagos Press, Akoka, Yaba, Lagos State, Nigeria.

Akinnibosun, F. I., Akinnibosun, H. A., Ibeh, I. N. & Osaghae, F. (2008). Antibacterial activity of *Phyllanthus amarus* Schum. and Thonn. on five vegetative organisms. *Plant Archives* 8(2):563-568.

Akobundu, I. O. & Agyakwa C. W. (1998). *A Handbook of West African Weeds* (2nd edn.). International Institute of Tropical Agriculture, Ibadan, Oyo State, Nigeria.

Ammara, H., Salma, R., Farah, D. & Shahid, M. (2009). Anti microbial activity of some plant extracts having hepatoprotective effects. *Journal of Medicinal Plant Research* 3(1):20-23.

Bankole, A. A. (1992). The antimicrobial Activity of the crude seed extracts of three plants used in Nigerian ethnomedicine. University of Benin, Benin City.

Dabai, Y. U. & Muhammad, S. (2008). Antibacterial activity of some Nigeria medicinal plants. *Science World Journal* 3(2):43-44.

Gill, L. S. (1992). *Ethnomedical uses of Plants in Nigeria*. University of Benin Press, Nigeria.

Idu, M., Omogbai, E. K. I., Aghimine, G. E. Amaechina, F., Timothy, O. & Omonigho, S. E. (2007). Preliminary phytochemistry, antimicrobial properties and acute toxicity of *Stachytarpheta jamaicensis* (L.) Vahl. leaves, *Trends in Medical Research* 2:193-198.

Iwu, M. W., Duncan, A. R. & Okon, C. O. (1999). New Antimicrobial of plant origin. In: *Perspective on New crops and New uses.* Janick, J. (Ed.), Alexandra Press, VA.

Jadeja, D., J. Parekh & Chanda, S. (2005). Efficiency of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity. *Turkish Journal of Biology* 29:203-210.

Kavitha, D. & Padma, P. R. (2008). Biotherapeutic potential of flower and bark extracts of *Couroupita guianensis* Aubl. *Plant Archives* 8(2): 569-771.

Martinez, M.J., Betamcourt, J., Alonso-Gonzalez, N. & Jauregai, A. (1996). Screening of some Cuban medicinal plants for antimicrobial activity. *Journal of Ethnopharmacology* 52:171-174.

National Nosocomial Infections Surveillance (NNIS) (2004). System report, Data summary from January 1992 through June 2004, *Infections Control* 3: 470-485.

Odugbemi, T. (2006). *Outlines and Pictures of Medicinal Plants from Nigeria*. University of Lagos Press, Akoka, Yaba, Lagos State, Nigeria.

Prescott, L. M., Harley, J. P. & Klein, O. A. (2005). *Microbiology* 6th edition. Mc-Graw Hills, New York.

Rauha, J., Remes, P., Heinonea, W., Hopia, A., Kyjala, T., Pithlaja, K., Vaonela, H. & Vaorela, P. (2000). Antimicrobial effects of finished plants extracts containing flavonoids and other phenolic compounds. *International Journal of Food Microbiology* 56:3-12.

Sengupta, S. & Ray, A. B. (1987). The chemistry of *Piper* species: a Review. *Fitoterapia* 58: 157-166.

Stoke, E. J. (1975). *Clinical Bacteriology*. 4th edn. Edward Arnold Publisher.

Tchoumbougnang, F., Jazet, D. P. M., Sameza, L. M., Fombotioh N., Wouatsa, N. A. V., Amvam, Z. P. H. & Menut, C. (2009). Comparative essential oils composition and insecticidal effect of different tissues of *Piper capense* L. *Piper guineense* Schum. et Thonn., *Piper nigrum* L. and *Piper umbellatum* L. grown in Cameroun. *African Journal of Biotechnology* 8(3):424-431.

Wagner, W. L., Herbst, D. R. and Sohmeg S. H. (1999). *Manual of the flowering plants of Hawaii.* Revised edition. Bernice P. Bishop Museum special Publication University of Hawaii Press, Honolulu.

World Health Organization (W.H.O.). (1979). Resolution on traditional medicine programme W.H.O. Document NO. EB 63R4. W.H.O. Geneva.