Bayero Journal of Pure and Applied Sciences, 3(2): 65 - 68
Received: September, 2010
Accepted: October, 2010
ISSN 2006 - 6996

ANTIBACTERIAL PROPERTIES AND PRELIMINARY PHYTOCHEMICAL ANALYSIS OF METHANOLIC EXTRACT OF MISTLETOE (*Tapinanthus bangwensis*)

***Ekhaise, F. O[1]., Ofoezie, V. G.[2] and Enobakhare, D. A[3].**
[1,2]Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City and [3]Department of Crop Sciences, Faculty of Agricultural Sciences, University of Benin, Benin City. [1]Email:fredfldyn@yahoo.com or
*Correspondence author: aiyevbosa.ekhaise@gmail.com

ABSTRACT
Tapinanthus bangwensis (Mistletoe) is a parasitic plant grown on citrus plant and a variety of other plants, used by most communities in Nigeria for the treatment and management of ailments such as diabetics, blood pressure, asthma, epilepsy, cancer of the ovary and breast and acquired immunodeficiency syndrome (AIDS). The study was aimed at investigating the antibacterial properties and the phytochemical constituents of the leaves extract. Crude extract of Tapinanthus bangwensis leaves were screened for its antibacterial and phytochemical properties on Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis and Klebsiella pneumonia. The results showed that the extract at varying concentrations has antibacterial activity on the test organisms. The extract inhibited the growth of the bacterial isolates in a concentration dependent manner with MICs (minimum inhibitory concentration) ranging from 10 – 50mg/ml. Partial purification (Thin Layer Chromatography) of the crude extract revealed five (5) components. Characteristics of these components were viewed by Infrared and UV/VIS spectrophotometer showing the presence of alkaloid, saponin, tannin, steroid and flavonoid. The observed antibacterial activity was believed to be due to the presence of the phytochemical constituents.

Keywords: Herbalism, Loranthanceae, Phytochemicals, Test organisms

INTRODUCTION

It is widely believed that traditional medicine sometimes called herbalism is the most ancient method of curing diseases (Evans, 2005). It has been known that plants are the first and only true medicines ever used by man. However, in Nigeria, until recently, the practices of the use of herbs has been kept by secrecy and shrouded in dreaded magical incantations, rituals and sacrifices. It is now very clear that the potency of the plants and its parts does not depend on such exhibition.

The use of plants for medical purposes is an important part of the culture and tradition in Africa. Thus, about 80% of the population depends directly on the traditional medicine for the primary health care (Ekhaise and Okoruwa, 2001). Today, there has been an increasing incidence of multiple resistances in human pathogenic microorganisms in recent years, largely due to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases (Eloff, 1998). The number of resistant strains of microbial pathogens is growing since penicillin resistance and mutli-resistance pneumococci caused a major problem in South Africa in 1977 (Eloff, 1998; Marchese and Shito, 2001). The development of resistance of antimicrobial agent has forced scientists to search for antibacterial substance from alternative sources such as medicinal plants. This has also made medicinal plants to receive much attention as an alternative therapy as against synthetic drugs. Traditionally, extracts of mistletoe (*Tapinanthus bangwensis*) have been used against a variety of diseases such as disorders in female reproductive system, cancer, arthritis, rheumatism, epithelial tumors, hypertension, asthma, nervousness and epilepsy (Evans, 2005). Mistletoe species have been used in folk medicine by cultures on almost every continent at some point in history.

Mistletoe (*Tapinanthus bangwensis*) belongs to the family Loranthanceae. It is a well known evergreen parasitic plant, which grows on deciduous trees in ball-like bush (Kay, 1986; Evans, 2005). It is an excellent medicinal plant. The evergreen, leathery leaves are of a yellow-green colour. The berries are whitish, somewhat opaque and sticky; birds have the habit of distributing the sticky seeds by sharpening their beaks on branches or passing the undigested seeds in droppings. Mistletoe can grow on either edible or non-edible trees, while only those that grow on edible plants are used for medicinal purposes (Evans, 2005). The growth of Mistletoe on different kinds of plants, are of disease curring specificity, for example, mistletoe grown on Guava, Kolanuts and Citrus are specific for curing diseases like cancer, hypertension, nervousness and insomnia, while those grown on cocoa is best used for curing diabetes. This study was aimed at investigating the antibacterial and preliminary phytochemical analysis of the methanolic extract of *Tapinanthus bangwensis*.

MATERIALS AND METHODS

Source of Mistletoe

The mistletoe bunches were plucked from branches of citrus plant (Tangerine) in citrus farm located at Faculty of Social Sciences, University of Benin, Benin City. The plant was identified as *Tapinanthus bangwensis* and confirmed by Dr. E. Aigbokhian of the Department of Botany, Faculty of Life Sciences, University of Benin, Benin City. The leaves were washed thoroughly with sterile water and spread and dried on clean trays for experimentation.

Sources of Test Organisms

The microorganisms used for this research were clinical isolates of Gram positive and Gram-negative bacteria. These include *Staphylococcus aureus, Escherichia coli, Proteus mirabilis, Klebsiella pneumonia* and *Pseudomonas aeruginosa* obtained in stock cultures from the Department of Microbiology, University of Benin Teaching Hospital (UBTH), Benin City, Edo State, Nigeria. The purity and identification of each of the bacterial isolates were confirmed using the standard procedures of biochemical and physiological examinations (Buchanan and Gibbons, 1974; Gerhardt *et al*., 1994).

Preparation of Extract

The properly air-dried mistletoe leaves were grinded into powder using a sterilized Thomas-Wiley Milling Machine. The aqueous extract of the sample was prepared by maceration procedure. Two hundred grams (200g) of the powder was soaked in a glass jar containing 500ml of methanol supplied by Fluka (Buchs, Switerzerland), which was used as the extracting solvent.

The suspension was vigorously shaken for even dissolution and the extraction was done with variation in time, for 2, 4, 6, 10 and 12hours. The filtrate was concentrated to get the crude extract and this was kept at 4^0C for further experimentations (Sofowora, 1993).

Partial purification of the crude extract

Partial purification of the crude extract was achieved through thin layer chromatography (TLC), infrared spectrophotometric analysis and the ultraviolet spectrum analysis. The one dimensional thin layer chromatography method was used to separate the crude extract into five components. Plate measuring 20 x 20cm was prepared using the silical gel as the coating material with the aid of a spreader to a uniform thickness of 0.2cm. The developing solvents used were methanol and chloroform (1:1). The chromatogram and the spots were viewed under UV and infrared spectrophotometer. The phytochemical components were screened for, using the method of Soforowa (1993).

Test for antibacterial property of *Tapinanthus bangwensis*

Susceptibility tests of the crude extract of *T. bangwensis* were carried out using the modified agar diffusion techniques of Garrod, *et al.,* (1981) and Irobi (1992).

Determination of Minimum Inhibiting Concentration

The techniques described by Irobi *et al.,* (1993), Rusell and Fur (1977) was used for this experiment. The extracts were incorporated into nutrient broth in test tubes at varying concentrations. Control experiment of tubes containing the growth medium and each of the test bacterium, excluding the extract was also set. The experiments were incubated at 37^0C/24hr and were performed in triplicate for each test isolate. The lowest concentration of extract that did not allowed growth within the incubation period was taken to be the minimum inhibitory concentration.

RESULTS

Table 1 summarizes the quantitative determination of the antibacterial properties of the methanol extracts of *Tapinanthus bangwensis* (mistletoe) on the test organism. The plant extract showed antibacterial activity against all the test organisms. Extraction of the crude antibacterial active principle was maximum at extraction time of 2hr, 4hr and 6hr but most effective at the 2hr extraction time. The minimum inhibitory concentrations measured were 10mg/ml, 25mg/ml, 25mg/ml and 50mg/ml for *P. aeruginosa, Proteus mirabilis, Klebsiella pneumonia, Staphylococcus aureus and Escherichia coli* respectively.

Partial purification of the crude extract by TLC showed five components with RF – values: 4.20, 4.40, 4.60, 5.20 and 5.30 (Table 2) respectively. The phytochemical screening revealed the presence of alkaloid, tannin, saponin, steroid and flavonoid (Table 3).

Table 1: Effect of extraction periods on the inhibitory action of the methanolic crude extract of *T. bangwensis*.

	Extraction period (hr) diameter zones of inhibition (mm)					
Test Organism	**2hr**	**4hr**	**6hr**	**10hr**	**12hr**	**24hr**
S. aureus	2.0	1.5	1.0	1.0	1.0	0.5
P. mirabilis	1.5	1.0	0.5	1.0	1.0	0.5
K. pneumonia	3.0	1.0	1.0	0.5	0.5	1.0
E. coli	2.0	2.0	0.5	0.5	0.5	0.5
P. aeruginosa	6.0	0.5	0.5	1.0	0.5	0.5

Table 2: Purified spots of the five components showing the retention factor (R_f –factor)

Spot	R_f-factor
C_1	4.20
C_2	4.40
C_3	4.60
C_4	5.20
C_5	5.30

Table 3: Phytochemical Constituent of *Tapinanthus bangwensis*

Phytochemical Constituent	*Tapinanthus bangwensis*
Alkaloid	+
Tannin	+
Saponin	+
Steroid	+
Flavonoid	+

Key: + = Positive, - = Negative

DISCUSSION

The results obtained from this study showed that the methanolic extract of *Tapinanthus bangwensis* exhibited antibacterial properties, which inhibited the growth of the test organisms to varying degree. Thus the crude extract posses some active ingredients that can inhibit the growth of microorganisms responsible for some common hospital infections.

The findings agreed with the work of Ekhaise and Okoruwa, 2001; Aluyi *et al.*, 2003, and Esimone, *et al.,* 1998, which report that various extract of plant exhibit antibacterial properties against the growth of some common clinical isolates.

The presence of antibacterial properties in mistletoe is of great importance in healthcare delivery system, since it could be used as an alternative to orthodox antibiotics, in the treatment of infections due to the microorganisms tested, especially as they frequently develop resistance to known antibiotics (Singleton, 1990) and will reduce the cost of obtaining health care. The observed antibacterial effects corroborate its traditional uses.

Preliminary phytochemical screening revealed the presence of alkaloids, tannins, saponin, steroid and flavonoid. These are believed to be responsible for the observed antibacterial effects of plant extracts (Nwze*, et al.,* 2004), for the treatment of several infections in Africa, indigenous medicinal plants are often the only means (Fennell *et al*., 2004). This highlights the continuous interest in laboratory screening of medicinal plants, not only to determine the scientific rationale for their usage, but also to discover new active ingredients.

CONCLUSION

African medicinal plants have been screened for their *invitro* antibacterial activities. It could be inferred that the extract tested had pronounced inhibitory effect against all test organisms. The test gave validity to the traditional use as a natural antibacterial. It is therefore, recommended that, the public and private organizations should encourage researches towards the use of indigenous herbal medicine in the fight against common infections in our society.

REFERENCES

Aluyi, H.S.A., Ekhaise, F.O. and Irhuegbae, B.O. (2003). Antimicrobial properties of *Palisorta hirsuta* (Thumb) K. Schum. *Nigerian Journal of Applied Science* 21:96-100.

Buchanan R. E. and Gibbons, E.N. (1974). Bergey's Manual of Determinative Bacteriology. 8th edn. Williams and Wilken. Co., Baltimore. 1300pp.

Ekhaise, F.O. and Okoruwa, P. (2001). Antibacterial activity of Aloe vera (Aloe Garbadensis) extract on *Staphylococcus aureus*. *Tropical Journal of Environmental Science and Health* **4:** 28-37.

Eloff, J.N (1998). Which extract should be used for the screening and isolation of antibacterial components from plants? *Ethopharmacology* **60:** 1-8.

Esimone, C.O., Adikwu, M.U. and Okonta, J.M. (1998). Preliminary antimicrobial screening of the ethanolic extract from the lichen *Usnea subflovidans.* (L). *Journal of Pharmaceutical Research and Development* 3(2): 99-101.

Evans, J. (2005). Mistletoe: Good for more than free kisses. *Journal of American Botanical Council.* 68: 50 – 59.

Fennell, C. W., Lindsey, E. E., McGraw, L. J., Sprag, S. G., Staffort, G. I., Elgorashi, E. E., Grace, O. M., and Van Staden, J. (2004). Assessing African Medicinal Plants for efficacy and safety: Pharmacological screening and toxicity. *Journal of Ethnopharmacology.* 94: 205 – 217.

Garrod, L.P., Lambert, H.P. and Grady, O.A. (1981). *Antibiotics and Chemotherapy*. Church Hill. Livingstone Press. London. 385pp.

Gerhardt, P., Murray, R.G.E., Wood, W.A. and Krieg, N.R. (1994). *Methods for General and Molecular Bacteriology.* ASM Press, Washington DC. 791pp.

Irobi, O.N. (1992). Activities of *Chromolaena odorata* (compositae) leaf extract against *Pseudomonas auruginosa* and *Streptococcus faecalis. Journal of Ethnopharmacology* 37:81 – 83

Irobi, O. N., Daramola, S. O., Tasie, S. B., Oyekke, S. B. and Tsado, M. L. (1993). Bacterial properties of crude extracts of *Mitracarpus villos I* (SW) D. C (Synonym *Mitracarpus scaber* Zucc.) Rubiaceae. *Nigerian Journal of Microbiology* 9:9 – 12.

Kay, M.A. (1986). *Healing with plants in the American and Mexican West.* 2nd edn. University of Arizona Press. Texas. 178pp.

Marchese, A. and Shito, G.C. (2001). Resistance patterns lower respiratory tract pathogens in Europe. *International Journal of Antimicrobial Agents* **16:** 25-29.

Nwze, E.I., Okafor, J.I. and Njoku, O. (2004). Antimicrobial activities of methanolic extracts of *Trema guineensis*. (Schumm and Thorn) and *Morinda lucida* Benth used in Nigerian Herbal Medicinal Practices. *Journal of Biological Research and Biotechnology* 2(1): 39-46.

Russell, A.D. and Fur, J.R. (1977). Antibacterial activity of a new chloroxylenol preparation containing ethylenediamine tetraacetic acid. *Journal of Applied Bacteriology* 43:253-260.

Singleton, P. (1999). Bacteria in Biology, Biotechnology and Medicine. 4th edn. John Wiley and Sons Ltd. New York. 337pp.

Sofowora, A. (1993). *Medicinal plants and traditional medicine in Africa*. Spectrum Books Ltd., Ibadan, Nigeria, 289pp.