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

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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 (Elloff, 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 (Elloff, 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 Loranthaceae. 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, Switzerland), 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 12 hours. The filtrate was concentrated to get the crude extract and this was kept at 4°C 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 0.2 cm. 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 Sofowora (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), Russel 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°C/24 hr 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 10 mg/ml, 25 mg/ml, 25 mg/ml and 50 mg/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*.

<table>
<thead>
<tr>
<th>Test Organism</th>
<th>Extraction period (hr)</th>
<th>diameter zones of inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>2hr 4hr 6hr 10hr 12hr 24hr</td>
<td></td>
</tr>
<tr>
<td>P. mirabilis</td>
<td>1.5 1.0 0.5 1.0 1.0 0.5</td>
<td></td>
</tr>
<tr>
<td>K. pneumonia</td>
<td>3.0 1.0 0.5 0.5 0.5 0.5</td>
<td></td>
</tr>
<tr>
<td>E. coli</td>
<td>2.0 2.0 0.5 0.5 0.5 0.5</td>
<td></td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>6.0 0.5 0.5 1.0 0.5 0.5</td>
<td></td>
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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.

REFERENCES


