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ANTIBACTERIAL POTENTIAL OF *TAPINANTHUS BANGWENSIS (*AFRICA MISTLETOE*)* ETHANOLIC EXTRACTS ON SELECTED CLINICAL ISOLATES

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ABSTRACT

Antibiotics resistance has been recognized as an emerging problem worldwide both in human and veterinary medicine. *Tapinanthus bangwensis* is a well-known evergreen parasitic plant and an excellent medicinal plant belonging to the family Loranthanceae used as a remedy for several human and animal ailments that include stomach ache, diarrhea, dysentery, wound, arthritis, epilepsy and cancer traditionally. This study aimed was to assess the antibacterial potential of *T. bangwensis* on pathogenic bacteria and also study the qualitative and quantitative phytochemicals inherent in the plant. Ethanolic extracts of *T. bangwensis* leaf and stem was challenged with clinical isolates of *Staphylococcus aureus, Escherichia coli* and *Klebsiella Pneumoniae.* Antibacterial activity was investigated using agar well diffusion method while qualitative and quantitative phytochemical screening were carried out using Gas and mass spectroscopy method. Antibacterial effect of *T. bangwensis* leaf and stem showed various inhibitory effect against the microbial isolates. The leaf extract exhibited a higher zones of inhibition against *Escherichia coli* (18.70 ± 0.6mm) at a concentration of 1000 mg/ml while a zone of inhibition of 16.30±0.6mm was observed on *E. coli* using stem extract. *Klebsiella Pneumoniae* and *Staphylococcus aureus* had a zone of inhibition of 15.70±0.5 mm using stem extract respectively at 1000 mg/ml. The MIC was 250mg/ml for the crude extracts against the clinical isolates. The Minimum Bactericidal Concentration (MBC) recorded for the leaf extract was observed to be 1000mg/ml for *Staphylococcus aureus* and 500mg/ml for *Escherichia coli* and *Klebsiella Pneumoniae* whereas MBC for the stem extract recorded 500mg/ml for *Staphylococcus aureus* and 1000mg/ml for *Escherichia coli* and *Klebsiella Pneumoniae***.** The result obtained from the phytochemical screening revealed the presence of tannin, steroids, phenolics, flavonoids, anthocyanin, coumarin, terpenoids, glycoside, and alkaloid among others. Steroids and flavonoids was predominant in the leaf with the value of 309.68 ±0.94 mg/kg and 274.92 ± 1.09 mg/kg respectively while Glycoside had the least (12.67±0.01 mg/kg). In the stem 109.02±1.89 mg/kg recorded as the highest value with the lowest of 0.22±0.01 mg/kg for Saponin. These results could suggest the promising chemopreventive use of *T. bangwensis* and some of its active principles in the treatment of infections.
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1. INTRODUCTION

Since prehistoric times, humans have used natural products, such as plants, animals, microorganisms, and marine organisms in medicines to alleviate and treat diseases. According to fossil records, the use of plants as medicines by humans may be traced back at least 60,000 years (Shi et al., 2010). Most of the population of developing countries regularly depends on traditional medicine. The traditional health care practice of indigenous people pertaining to human health is termed , "alternative medicine," "complementary medicine or "Ethno medicine" which includes products processed or derived from living organisms, including plants, animals, insects, microorganisms, and marine organisms (Liu et al., 2011).

Traditional medicine has been used in wound treatment, bone healing, poisonous bite, and neurological disorders (Vedavathy et al., 2003). Medicinal plants have been used for centuries as remedies for human diseases and have provided new sources of chemical compounds with biological activity as antimicrobial agents (Das et al., 2010). The biologically active components of plant extracts and essential oils are used in most of the pharmaceutical industries because of their antimicrobial, antifungal, and antiviral properties. The World Health Organization (WHO) considers phytotherapy in its health programmes and continues to encourage the

integration of herbal cure with the orthodox medicine. Infectious diseases caused by bacteria, fungi, and viruses are still in increase and they are still the major threat to public health (Akinpelu et al., 2015).

Use of indigenous drugs of natural origin forms a major part of such therapies; more than 1500 herbals are sold as dietary supplements or ethnic traditional medicines (WHO, 2003). World Health Organization (WHO) has pointed it out that medicinal plants could be the best source to obtain a variety of drugs (Gislene et al., 2000). Therefore, there has been a global resurgence in the use of herbal preparations in disease management in all continents of the World and most developing countries are now integrating traditional herbal medicine into their health care systems (El-Mahmood et al., 2007).

T. bangwensis grow on a wide range of host trees, and it may reduce their growth and eventually they can kill the trees with heavy infestation (Plate 1).

Plate 1: *Tapinanthus bangwensis* Magnification (×40)

The evergreen, leathery leaves are of a yellow-green color. 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. *T. bangwensis* can grow on either edible or non-edible trees, while only those that grow on edible plants are used for medicinal purposes (Evans, 2005; Tizhe et al., 2016). The growth of *T. bangwensis* on different kinds of plants, are of disease curing specificity, for example, *T. bangwensis* 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. In Nigeria, *T. bangwensis* is used as a remedy for several human and animal ailments that include stomach ache, diarrhea, dysentery, wound and cancer. Ruminants and local fowls do relish it without any reported digestive disorder (Egbewande et al., 2011). Traditionally, extracts of mistletoe (*T. 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)

Despite the abundance of this plants in our environment here in Nigeria, little or no attention has been paid to the investigation of their medicinal potentials as compared to higher plants. This study aimed was to assess the antibacterial potential of *T. bangwensis* on pathogenic bacteria and also study the qualitative and quantitative phytochemicals inherent in the plant.

2. MATERIALS AND METHODS

Collection of plants

T. bangwensis plant was obtained in large quantity from kolanut tree (host plant) at different locations in Omu-Aran town kwara State, Nigeria using sharp penknife in March 2019. The plant was identified and authenticated at the Herbarium unit of the department of Plant Biology, Faculty of Life Sciences University of Ilorin.

Extraction and Preparation of *T. bangwensis* Extracts of Leaf and Stem

The leaf and stem of the plant were washed with distilled water and dried at room temperature in the Microbiology Laboratory, Kwara State University, Malete, until the it became crispy and of constant weight. The dried plant was pulverized using sterile Veronica standing tall Genius Mixer grinder, millennium quality electric blender (India) and kept in an air tight container prior extraction. Exactly 100g of each powdered sample was soaked in 1000ml of 70 percent ethanol for four days on the laboratory bench with regular agitation in order to homogenize. After extraction the extracts was evaporated using rotary evaporator until the extracts reached a solid form. From the solid extracts suitable concentrations were made using Dimethyl sulfo-oxide (DMSO) for further analysis.

Test organisms

Clinical isolates of *Escherichia coli, Klebsiella Pneumoniae,* and *Staphylococcus aureus* were procured from University of Ilorin teaching hospital, Kwara State, Nigeria. The isolates were confirmed using standard biochemical tests (Cheesbrough, 2006). The isolates were maintained on freshly prepared nutrient agar slants and kept in a refrigerator at 4ºC until required.

Standardization of inoculum

A loopfull growths from bacterial isolates were inoculated into nutrient broth incubated at 37 °C for 18 hours. The bacterial suspensions were diluted with

normal saline. The turbidity was adjusted and compare with standard tube (McFarland number 0.5) to yield a uniform suspension containing 1.5×10^8 CFU / ml. (CLSI, 2009).

Antibacterial Assay of *T. bangwensis*

The modified agar ditch diffusion method of Bamidele et al. (2013) was used. 0.2 ml of the standardized microbial suspension of the test organisms were mixed with 20 ml of molten Mueller Hinton agar at 40°C. The seeded agar was poured aseptically into sterile Petri dishes with a depth of about 6 mm and allowed to solidify. The solidified agar was punched with a 6 mm diameter sterile cork borer to create six wells on the agar. The wells were filled with 0.1 ml of the concentrations of the leaf extracts prepared by dissolving 0.3g of each crude extracts of *T. bangwensis* in 3ml of DMSO (dimethylsulfoxide) in different vials bottles to make a stock solution of 1000, 500, 250, 125 and 65.5 mg/ml. DMSO (dimethylsulfoxide) was used to fill one of the wells which served as the solvent control. Tests were carried out in duplicates and plates were incubated at 37°C for 24 h. The antibacterial activity was evaluated by measuring the inhibition zone diameter in millimeter (mm).

Minimal Inhibitory Concentration (MIC) of Extract

The Minimum Inhibitory Concentration (MIC) of the extracts was determined according to the methods described by Awe and Amobi (2015). Different concentration of the extract ranging between 65.5mg/ml and 1000mg/ml were prepared and standardized inocula of the test organisms added. Control cultures without extract were set up, both control and experimental tubes were incubated at 37^0C for 24hrs respectively. The MIC was reported as the lowest concentration extract that showed no visible growth.

Minimum Bactericidal Concentration (MBC)

Sterile Mueller-Hinton agar plates were separately inoculated with bacterial inocula from each of the test tubes that showed no evidence of growth. The plates were further incubated at 37 ^{o}C for 24 hours. The lowest concentration of the extract that showed no growth was noted and recorded as the minimum bactericidal concentration (Orji et al., 2013).

Reagents

Chemicals used were of analytical grade and were products of Randox Laboratory Limited UK.

Phytochemical screening of ethanolic extract of *T. bangwensis* **Leaf and Stem**

Qualitative phytochemical analysis of the extract was carried out using the method described by Odebiyi and Sofowora (1993) for the detection of saponins, tannins, phenolics, alkaloids, steroids, triterpenes, phlobatannins, glycosides and flavonoids. Phytosterols presence was tested for by method of Finar (1990) while for terpenoids the method of kokate (1999) was adopted.

Gas chromatography-mass spectroscopy

The quantitative screening were determined using the Gas Chromatograph (GC) and Mass spectroscopy (MS) as described by Raaman (2006) and Prabu et al. (2013). Chromatography-mass spectroscopy (GC-MS). Extract and control samples were centrifuged at 8,944 _ g for 10 min and then extracted with ethyl acetate in a separating funnel, dried in a rotary evaporator and re-dissolved in high analytical grade methanol for GC-MS analyses.

Statistical Analysis

Results of the Statistical analysis were expressed as mean and Standard deviation of triplicates and were statistically analyzed using ANOVA of SPSS statistical package of version 16.0 and levels of significance were evaluated using Duncan's Multiple range Test (DMRT) at $p<0.05$

3. RESULTS

The antibacterial prosperities of the ethanolic extract of *T. bangwensis* leaf and stem were evaluated by well diffusion assay against different microbial isolates. Table 1 shows the result of the zone of inhibition of the ethanolic extract of *T. bangwensis*. These results showed that this extracts give a good inhibition effect against Gram-positive and against Gram-negative bacteria with inhibition zone ranging from 12.7±0.6 - 18.7±0.6 mm. There is no significant *E. coli* is more susceptible to extracts than *S. aureus* and *K. pneumoniae* at the same concentration.The MIC (minimum inhibitory concentration) of the ethanolic leaf and stem extracts is shown in Table 2. Both leaf and stem extract of *T. bangwensis* inhibited all the test organisms at 125.0 mg/ml. The Minimum Bactericidal Concentration is presented in table 3 where *Escherichia coli* and *Klebsiella Pneumoniae* were found to be killed at a concentration of 500 mg/ml of leaf extract except for *Staphylococcus aureus* with 1000 mg/ml while in the stem extracts *Escherichia coli* and *Klebsiella Pneumoniae* were found to be killed at a concentration of 1000 mg/ml and *Staphylococcus aureus* at 500 mg/ml.

Table 4 shows both qualitative and quantitative phytochemical constituents determined to ascertain the biologically active compound present in the plant which might have conferred the antibacterial activity. The phytochemical screening revealed that leaf of *T. bangwensis* contains a very high amount of steroid (309.68±0.94 mg/kg) and flavonoids (274.92±1.09 mg/kg) , while in stem, steroids had the highest amount (109.02±1.89 mg/kg) with less of flavonoid (69.67±1.63 mg/kg)

Table 1: Zones of inhibitions of leaf and stem extract of *T. bangwensis* against selected clinical isolates.

Organisms/ Concentration of extract (mg/ml)	Zone of inhibition of extract (mm)										
	Leaf					Stem					Control
	65.5	125	250	500	1000	65.5	125	250	500	1000	DMSO
Klebsiella Pneumoniae	0.0	0.0	15.7±0.6^{b}	16.7±0.6^{b}	17.7±0.3^{a}	0.0	0.0	12.3±0.6^{a}	13.7±0.6^{a}	15.7±0.5^{a}	0.0
Staphylococcus aureus	0.0	0.0	14.7±0.6^{b}	15.7±0.6^{a}	17.3±0.6^{a}	0.0	0.0	12.0±0.6^{a}	13.4±0.6^{a}	15.7±0.5^{a}	0.0
Escherichia coli	0.0	0.0	12.7±0.6^{a}	14.7±0.6^{a}	18.7±0.6^{a}	0.0	0.0	12.7±0.6^{a}	14.3±0.6^{a}	16.3±0.6^{a}	0.0

Values are means of three replicate. Standard error of means of zones of inhibition. Values within the column having different superscripts are significantly different at P< 0.05

Table 2: Minimum Inhibitory Concentration of *T. bangwensis* on selected clinical isolates.

Organisms/ Concentration of extract (mg/ml)	Concentration of the extracts (mg/ml)									
	Leaf					Stem				
	65.5	125	250	500	1000	65.5	125	250	500	1000
Klebsiella Pneumoniae	+	+	-	-	-	+	+	-	-	-
Staphylococcus aureus	+	+	-	-	-	+	+	-	-	-
Escherichia coli	+	+	-	-	-	+	+	-	-	-

Key: - indicates No turbidity + indicates turbidity

Table 3: Minimum Bactericidal Concentration of *T. bangwensis* on selected clinical isolates

Organisms	Concentration of the extracts (mg/ml)	
	Leaf	Stem
Klebsiella Pneumoniae	500	1000
Staphylococcus aureus	1000	500
Escherichia coli	500	1000

Table 4: Qualitative and Quantitative Phytochemical Screening of Leaf and Stem Extract of *T. bangwensis*

S/N	Phytochemicals	Qualitative Screening		Quantitative Screening	
		Leaf	Stem	Leaf (mg/kg)	Stem (mg/kg)
1	Saponin	-	+	-	0.22±0.01^{a}
2	Tannin	+	-	56.01±0.02^{d}	-
3	Steroids	+++	++	309.68±0.94^{g}	109.02±1.89^{f}
4	Phenolics	+	-	34.94±0.18 ^{b}	-
5	Flavonoids	++	+	274.92±1.09^{f}	69.67±1.63^{e}
6	Anthocyanin	+	-	42.98±0.44^{c}	-
7	Coumarin	+	-	64.76±0.09 ^{e}	-
8	Triperpene	-	+	-	45.52±0.71^{d}
9	Phlobatanin	-	-	-	-
10	Terpenoids	+	+	13.64±1.10^{a}	11.11±0.17^{c}
11	Glycoside	+	+	12.67±0.01^{a}	2.60±0.02^{b}
12	Alkaloids	+	+	41.73±0.09^{c}	41.91±0.06^{d}

Key Key: - = Absent; + = slightly present; ++ = Moderately present; +++ = Strongly present
Values represented in the table are mean of triplicate readings and standard error of means of quantitative screening. Values within the column having different superscripts are significantly different at P< 0.05

4. DISCUSSION

Several works have been done to evaluate the antimicrobial and phytochemical compositions activities of different parts of diverse plants, with the aim of using these plants for the treatment of microbial infection as possible alternatives to synthetic drugs to which many infectious microorganisms have developed resistance (Ighodaro, 2012). This study showed the antibacterial potential of the ethanolic leaf and stem extracts of *T. bangwensis.* The results of the study

corroborate several investi-gations that established its use in traditional medicine. The leaf and stem extract inhibited the growth of *Staphylococcus aureus, Escherichia coli* and *Klebsiella Pneumoniae* which are causative agents of infectious diseases such as travellers diarrhea, vomiting, haemorrhagic colitis and gastro-intestinal tract infection.

The large zone of inhibition exhibited by the extracts against *Staphylococcus aureus, Escherichia coli* and *Klebsiella Pneumoniae* justifies their use by traditional practitioners in the treatment of sores, boils, and open wounds. This was in line with the study carried out by Orji et al. (2013) on the antimicrobial susceptibility of the leaves of African mistleloe (*Loranthus micranthus) at* various concentrations on *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Candida albicans, Aspergillus* species, *Penicillum* species. According to Aboaba et al. (2006) the antibacterial properties of plants are desirable tools in the control of undesirable microorganisms, especially in the treatment of infections. Therefore, the use of *T. bangwensis* by the herbal healers for treatment of gastro-intestinal disorder, diarrhea, dysentery, wound, cancer infections is in the right direction. Studies on the Minimum Inhibitory Concentration of the extracts showed inhibitory effects on the test organisms at the highest concentration. The extracts did not inhibit the bacterial isolates at lower concentrations. However, a concentration higher than 500 mg/ml for the ethanolic extracts of both leaf and stem is needed to have a bactericidal effect on the test organisms. No statistical difference was found among the organisms at different concentrations of stem extract studied at $p< 0.05$ while statistical difference occurs at different concentration of leaf extract except 1000mg/ml.

The ability of this plant to be capable of inhibiting the activity of various bacteria may be associated with the presence of the bioactive principle or the secondary metabolites including steroids, phenolics, flavonoids, tannin, anthocyanin, coumarin, terpenoids, glycoside, and alkaloid compounds. These compounds are known to be biologically active and therefore aid the antibacterial activities of *T. bangwensis*.

Flavonoids as anti-oxidants are beneficial to human health as reported by Jouad et al. (2001).The reasonable amount of Steroids, phenols, Tannin, Anthocyanin and Coumarin present in *T. bangwensis* is quite interesting. Steroids have been reported by Prashant *et al.* (2012) to have antidiarrheal effect while phenolic compounds possess important pharmacological values, some having anti-inflammatory properties (Nwauzoma et al., 2013). Tannins found in the leaf extract has been reported to have antioxidant, antibacterial and anticancer properties while the presence of cumarins in the leaf extract might have complemented the antibacterial potency of the leaf extract against the selected clinical isolate (Dharamanda, 2003).

Hanx et al. (2007) reported that anthocyanins possess antiplatelet aggregation and anti-inflammatory properties and coumarin a precursor of synthetic anticoagulant drug warfarin possesses a variety of biological activities including antibacterial, anti-inflammatory, antidiabetic, antioxidant and enzyme inhibitory activities (Poumale et al., 2013). These phytochemical compounds could be acting either alone or collectively in synergy to produce antimicrobial activity. The fact that the ethanolic extract of *T. bangwensis* and its fractions showed activity against most of the test organisms is a major breakthrough in appreciating the medicinal potential of the plant especially in the management of clinic community acquired and nosocomial associated infections.

5. CONCLUSION

Our findings show that leaf and stem of *T. bangwensis* is very rich chemical substances which offer great potential for food and pharmaceutical companies. Its preservation is therefore very important for further studies on its medicinal and other benefits.

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