

PHYTOCHEMICAL SCREENING AND ANTIMICROBIAL ACTIVITIES OF CRUDE METHANOLIC EXTRACT OF *PTELEOPSIS HABEENSIS* (AUBREV EX KEAY) STEM BARK AGAINST DRUG RESISTANT BACTERIA AND FUNGI

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ABSTRACT- Herbal medicines have been in use since ancient times for many age related diseases for which no modern medicine or only palliative therapy is available. The screening of plants extracts has been of great interest to scientists for the discovery of new drugs effective in the treatment of several diseases. The leaves, fruits and stem barks of *Pteleopsis habeensis* were collected from Yankari Game Reserve Bauchi, Nigeria in June, 2012. The plant was authenticated at the Department of Biological sciences, Ahmadu Bello University, Zaria, Nigeria. Phytochemical screening of crude aqueous stem bark extract of *Pteleopsis habeensis* revealed the presence of steroid, triterpenes, cardiac glycoside, saponins, tannins, alkaloids and flavonoids. Crude extract exhibited antimicrobial activities against drug resistant *Escherichia coli* and Methicillin resistant *Staphylococcus aureus* using agar well diffusion and broth dilution methods. The MIC of the extract against the test organism was 3.125 and its MBC was 1.562mg/ml. However, the extract had no activity against drug resistant *Candida albicans*. This study has therefore showed that *Pteleopsis habeensis* aqueous stem bark extract has only antimicrobial activity and hence a potential source of a candidate drug for the treatment of infection(s) associated with drug resistant bacteria.

(2012), Stated that despite the increase in the production of synthetic drugs, natural plant drug materials are still economically significant in the world and large quantities are harvested. Plants among other source have remained a veritable source of bioactive compounds with medicinal values and have been the most explored and exploited for their bioactive medicinal components

In the recent years, alternative medicine, which involves the use of plants, is attracting attention globally. Countries in Africa, Asia, and Latin America use traditional medicine to help meet some of their primary healthcare needs. In spite of vast improved health and longevity in the United State and Europe, millions of their people are turning back to traditional herbal medicine in order to prevent or treat many illness WHO (2006) in Sule *et al.* (2011) and Sofowora (2008). In Africa today, up to 80% of the population uses traditional medicine in primary health care, World health organisation (WHO, 2006). Many African plants are used in traditional medicine as antimicrobial agents but only few have been documented.

I. INTRODUCTION

The history of the usage of plant as a source of medication is as old as man's history on earth. The earlier recorded plants in medicine dates back to about 3000 BC and this was oil obtained from the plant *Hydrocarpus gaertn*, use for the treatment of leprosy. Even before then other plants like opium poppy (*Papaver somniferum* L.) and castor seed oil (*Ricinus communis* L.) were being use in Egypt. Earliest Egyptian use of herbs /plants in traditional medicine can be found in Eber Papyrus written around 1500 BC, as well as Chinese pharmacopeia written around 1122 BC. The Indian Ayurveda that was described around 1,200 BC with list of 127 plants is still in use in over 1,400 dispensaries in India. The records of later civilization of Greece, Rome, Arabia, Europe, Africa Australia and America showed their use of plants as medicine. In Nigeria our ancestors use plants since time immemorial for the treatment of many common ailments right from conception, delivery to adult hood (Owunobi, 1989).

The alarming rate at which traditional medicine is now patronized by all segments of the society the rich, the poor, educated and the uneducated- clearly signifies one thing, "the realization that traditional medicine which has long been taken for granted and rejected for decades has a crucial role to play in making affordable health care delivery system available to the entire populace. Their constituents that have always served as "lead compounds" or templates for the rational development of drugs are of more specific efficacies and fewer side effects than synthesized drugs (Owunobi, 1989; Yakubu and Musa 2012).

The acceptance of traditional medicine as an alternative form of health care has led researchers to further investigate medicinal plant. Thus scientist focus has now tilted towards more research on medicinal plants. According to Sule *et al* (2011) large body of evidence has accumulated to demonstrate the promising potential of medicinal plants used in various traditional, complementary and alternate systems of treatment of human diseases. Recently some higher plant products have attracted the attention of microbiologists to search for some

Most of these plants recognized by these ancient societies as having medicinal properties are still in use. Soladoye *et al.*

phytochemicals for their exploitation as antimicrobials, such plant products would be biodegradable and safe to human health (Kumar *et al.* 2008; Wang *et al.*, 2010) in (Sule *et al.*, 2011).

Pteleopsis habeensis (Aubrev ex Keay), which belongs to the family Combretaceae, is known as Lalen giwa in Hausa language in northern part of Nigeria of Sub Saharan Africa. *Pteleopsis* is a small genus of about 10 species, occurring in 3 localities, all in tropical Africa. The area of distribution of *Pteleopsis habeensis* is restricted to only a few regions: the Bandiagara escarpments in Mali (with the plant population possibly extending into Burkina Faso), the Akosombo and Bui regions in Ghana, and the Yankari Game Reserve and its immediate surroundings in Nigeria. It is possibly also present in Benin.(Oyen, 2010).

In Mali *Pteleopsis habeensis* forms low thickets with *Combretum micranthum* G.Don. In the Yankari Game Reserve in Nigeria it covers several square kilometers, and forms pure stands of coppice-like woodland of numerous thin stems, uniform in height and girth, forming a closed canopy 8–15 m high.

The plant; *Pteleopsis habeensis* occurs on sandstone, in general on gravelly slopes along watercourses. The habitat, although associated with stream beds, is very dry, as the soils are sandy and well-drained, and the species is never found in or near the actual streambed (Hawthorne, 1998).

II. MATERIALS AND METHODS

A. Collection and Identification of plant material

The leaves, fruits and stem barks of *Pteleopsis habeensis* were collected from Yankari game reserve Bauchi, in the month of June 2012. The plant was authenticated in herbarium of the Department of Biological Sciences, Ahmadu Bello University, Samaru, Zaria, Nigeria as *Pteleopsis habeensis*.

B. Stem bark Processing and Extraction Procedure

The stem bark of *Pteleopsis habeensis* were washed with deionized water and sun-dried for 14 days after which they were oven-dried for 15 minutes at 40 °C to ensure proper drying. The plant materials; stem bark, was pulverized into powder using pestle and mortar,

One hundred grams (100g) of the pulverized stem bark, was weighed into three 500 ml conical flasks containing 250 ml of 70% Methanol. The content was stirred gently using stirring rod. The flask was properly corked and labelled. The flask containing the solvent and the plant part was kept on a clean table in the laboratory for 72 hours for the extraction to take place. After 72 hours of extraction, the contents of both flask was sieved into a different sterile 250 ml conical flasks using Watman No.1 filter paper fitted into a glass funnel. The flask was properly labelled

while the supernatant was discarded. The flask with containing the *Pteleopsis* stem bark extract was transferred into a water bath adjusted to 40 °C and left for an hour to evaporate the solvent and concentrate the extract into paste.

C. Phytochemical analysis

The phytochemical analysis of the *Pteleopsis habeensis* stem bark extract was carried out using the standard method described by (Trease and Evans, 1978). Tests for alkaloids, tannins, flavonoids, saponins, anthraquinones and cardiac glycosides were carried out using the standard procedures.

D. Antimicrobial screening

1. Test Microorganisms

Two bacterial and one fungal species were employed as test organisms. These are;

Escherichia coli, *Methicillin resistant Staphylococcus aureus* and *Candida albicans*, which were obtained as fresh pure cultures from the department of microbiology of Ahmadu Bello University, Zaria , Nigeria.

2. Antibacterial Assay

Antibacterial activity of the crude methanolic extract of *Pteleopsis habeensis* stem bark was carried out using the agar well diffusion method as described by Cheesebrough (2002). Varying concentrations of the extract were prepared and tested for antibacterial activity using nutrient agar .Fifteen (15) millilitres of a Molten Nutrient Agar was separately poured to 12 Petri dishes and allowed to jelled. Six of the plates containing the nutrient agar were each separately seeded with one loopful of equivalent to macfland standard three of *Escherichia coli*. The remaining six nutrient agar plates were each seeded with one loopful of equivalent to macfland standard three of *Methicillin resistant Staphylococcus aureus*. Holes were bored in each of the plates to introduce the various concentrations of the extracts. The plates were incubated at 37°C for 24 hours. The zones of inhibitions were measured using transparent metre rule. The Minimum Inhibitory Concentrations (MICs) and minimum bactericidal (MBC) were recorded after 24 hours.

3. Antifungal Assay

Similarly the Antifungal activity of the stem bark extracts was carried out using the agar well diffusion method as described above using sabouroid dextrose agar. The plates were incubated at room temperature for 48 hours and inhibition of growth was noted. The minimum inhibitory concentrations (MICs) and minimum fungicidal concentrations (MBC) were recorded after 7 days.

Constituents	Tests	inference
Cardiac glycoside	Kelle Kilkani	+
Saponins	Froth forming test	+
Steroid & Triterpenes	Lieberman	+
Buchard Flavonoids	NaOH	+
Tannins	Fe Cl ₃	+
Alkaloids Dragendoff/Wagner		+

KEY: + = Present,

Table 1: Phytochemical constituents of Leaves Extracts of *Pteleopsis habeensis*

Table 2: Antimicrobial Activities of the Crude Methanolic Stem bark Extract of *Pteleopsis habeensis*

Concentration (mg/ml)	Zone of Inhibition in (mm)		
	<i>C. albicans</i>	<i>E. Coli</i>	<i>S. aureus</i>
100	-ve	25	24
50	-ve	22	22
25	-ve	20	20
12.5	-ve	18	18

Key: -ve means no activity.

Table 3: Minimum Inhibitory Concentration (MIC) of Crude Methanolic Extract of *Pteleopsis habeensis* Stem bark in mg/ml

Text organisms	Minimum Inhibitory Concentration mg/ml								
	100	50	25	12.5	6.25	3.125	1.562	0.781	
<i>E. Coli</i>	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	
<i>S. aureus</i>	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	

Key: -ve = no growth, +ve = growth

Table 4: Minimum Bactericidal Concentration (MBC) of Crude Methanolic Extract of *Pteleopsis habeensis* Stem bark in mg/ml

Test organisms	Minimum Bactericidal Concentration (MBC) mg/ml								
	100	50	25	12.5	6.25	3.125	1.562	0.781	
<i>E. Coli</i>	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	
<i>S. aureus</i>	-ve	-ve	-ve	-ve	-ve	-ve	+ve	+ve	

Key: -ve = no growth, +ve = growth

III. RESULT

Phytochemical screening of the crude methanolic extract of *Pteleopsis habeensis* leaves Table 1, showed the presence of Cardiac glycosides, alkaloids, flavonoids, tannins, saponins, and steroid and triterpenes.

The result of the antimicrobial screening Table 2, showed that *Pteleopsis habeensis* crude Methanolic stem bark extract has antibacterial activities against gram positive and gram negative bacteria, but it showed no antifungal effect against the tested fungus; *C.albicans*. *Escherichia coli* were observed to be slightly more sensitive to the crude extract, with zone of inhibition of 25 mm as compared to the 24 mm observed with *S. aureus*. The minimum inhibitory concentrations Table 3 against the *Escherichia coli* and *Methicillin resistant Staphylococcus aureus* were similar 1.562g/ml. It was also observed that the two bacteria responded similarly to the crude methanolic extract of *Pteleopsis habeensis* stem bark, each exhibited an MBC of 3.125mg/ml in table 4.

IV. DISCUSSION

The results presented in Tables 2 showed that crude stem bark methanolic extract of *Pteleopsis habeensis* was found to inhibit the growth of only the bacteria excluding the fungus. *Escherichia coli*, showed the highest sensitivity with zone of inhibition 25 mm. *Escherichia coli* and *MRSA* showed the same MIC 1.562mg/ml and MBC 3.125mg/ml this suggests that the two organisms may be responding similarly to the extract. Higher plants have been shown to be potential source for new antimicrobial agents. The screening of plant extracts has been of great interest to scientists for the discovery of new drugs effective in the treatment of several diseases Alim *et al.*, (2009) in (Sule 2011).

Tannins are known to have biological activities. Parekh and Chanda (2007) in Sule *et al.* (2011), reported that tannins reacts with proteins to provide the typical tanning effect which is important for the treatment of inflamed or ulcerated tissues. Herbs that have tannins as their main components are astringent in nature and are used for treating intestinal disorders such as diarrhoea and dysentery. Therefore the presence of Tannins observed in *Pteleopsis habeensis*, leaves suggests that the plant could be used for treating intestinal disorder, inflammatory conditions as well as ulcers.

Saponins were reported to act as act as antifungal secondary metabolite (Onwuliri and Wonang, 2004, 2005) The presence of saponins observed in *Pteleopsis habeensis* extracts suggest that the plant can be use as an anti-inflammatory. Saponins were also reported as surface active agents who interfere with or alter the permeability of the cell wall, and this facilitates the entry of toxic materials or leakages of vital constituents from the cell Onwuliri and Wonang, (2004, 2005) in Sule *et al* 2011. Just *et al.* (1998), revealed the inhibitory effect of saponins on inflamed cells. The presence of Saponins observed in the present study in the aqueous and methanol extracts of the leaves and stems bark of *Pteleopsis habeensis*, suggests that the plant could be potential source of antimicrobial and anti inflammatory drugs.

Flavonoids, another secondary metabolite detected in *Pteleopsis habeensis* extracts, are believed to exhibit a wide range of biological activities like antibacterial (Rahman *et al*, 2003). Flavonoids were phenolic in nature and acts as

cytoplasmic poisons, which also have been reported to inhibit the activity of enzymes (Dathak and Iwu, 1991).

The presence of alkaloids observed in the leaves of *Pteleopsis habeensis* indicates that the leaf could be use as an important therapeutic agent. This is supported by the report of **Bethwell and Kisangau (2006), which showed that alkaloids** interact with macromolecular targets; receptors and enzymes; resulting in venom-inactivation, analgesic and anti-inflammatory action.

V. CONCLUSION AND RECOMMENDATION

The results obtained from this study shows that the crude methanolic stem bark extracts of *Pteleopsis habeensis* has good therapeutic and antimicrobial activities. It is therefore suggested that further studies be carried out to determine the bioactive component of *Pteleopsis habeensis* stem bark. This will enable the pharmaceutical industry to make mass production so as to ease the global challenge of antimicrobial drug resistant. It could be interesting to also further investigate and establish the safety of this plant for it's possible application in the treatment of infections associated with drug resistant bacteria.

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