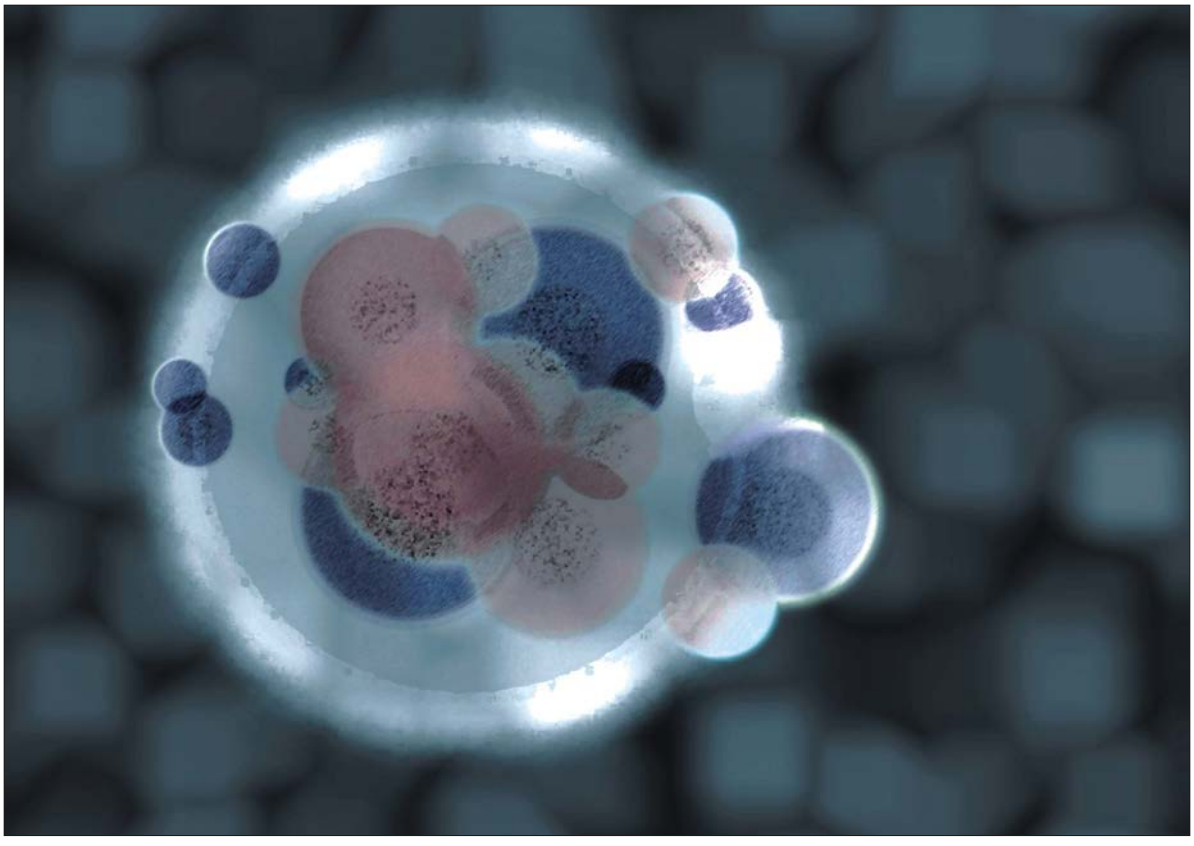

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Preliminary Phytochemistry and Antimicrobial Screening of Methanol Extract of *Baissea axillaris* Hau. Leaf

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Abstract: This study was aimed at determining the phytochemistry and antimicrobial properties of the methanolic extract of *Baissea axillaris* leaf. Phytochemical analyses of *B. axillaris* leaf using procedures described by Trease and Evans, revealed the presence of saponins, phenolic compounds, eugenol oil, glycosides and tannins. Methanolic extract of the leaf showed antimicrobial activities against some organisms. The extract was most active against *Staphylococcus aureus* yielding the highest zone of inhibition (15 mm) as well as the least minimum inhibition concentration of 125 μg mL^{-1}. It was least effective against *Escherichia coli* as minimum inhibition concentration was above 500 μg mL^{-1}.

Key words: Phytochemistry, antimicrobial, methanolic extract, *Baissea axillaris*, leaf

INTRODUCTION

Plants have been a major source of medicine and the presence of plant secondary metabolites have been implicated for most plants therapeutic activities (Ogunleye and Ibitoye, 2003; Aibinu, 2006). Phytomedicines derived from plants have shown great promise in the treatment of intractable infectious diseases (Idu *et al*., 2007a).

Reports on the antibiotic properties of various plants are clearly documented (Pessini *et al*., 2003; Oyedeji *et al*., 2005; De Campos *et al*., 2005; Kalyoncu *et al*., 2006; Avato *et al*., 2006; Rojas *et al*., 2006).

Ethnomedicinally, the Urhobos (in the Niger Delta region of Nigeria) administer decoction of *Baissea axillaris* Hau. orally for the treatment of hypertension (Ayinde and Amaechina, 2005).

B. axillaris belongs to the family Apocynaceae. It is a climbing perennial shrub. The leaves are 2.5-4 cm by, 1.2-2.7 cm broad, glabrous on both surfaces, arranged in opposite pairs with short stipules. It exudes milky latex when plucked or cut. The apical and young stems are tender, becoming more ligneous and tough towards the base and with age.

The plant is often found interwoven with edges, other woody supports (dead or alive) and fences (especially mesh-like iron fences) where it forms a thick evergreen mass of twisted stems and leaves above the ground. It thrives better in water-rich soil and ample supply of sunlight.

This study is aimed at determining the phytochemistry and antimicrobial properties of the methanolic extract of *B. axillaris* leaf against some disease causing microorganisms.

MATERIALS AND METHODS

The study was conducted in 2006. Sample of *B. axillaris* was harvested in the month of March around Ugbowo axis, Benin City, Nigeria. The leaves were plucked off with bare fingers, cleaned off debris and dried in a Gallenkhamp laboratory oven set at 40°C for 18-24 h. The dried, brittle leaves were pulverized in an electric mill.

Methanol was used to extract 3.5 kg of the powered leaves by soxhlet extraction. A rotary evaporator was used to concentrate the extract into a viscous paste (Somchit *et al*., 2003). This was preserved in a properly covered bottle container and kept in a refrigerator till used for experiments.

Tests for alkaloids, saponins, tannins and anthraquinones were according to Trease and Evans (1996).

For antimicrobial screening, strains of some microorganisms including *Klebsiella arogenes*, *Staphylococcus aureus*, *Bacillus subtilis*, *Esherichia coli* and *Pseudomonas aureginosa* were obtained from the Department of Pharmaceutical Microbiology, University of Benin Teaching Hospital, Benin City, Nigeria. A standard resistant strain of *E. coli* ($J62k_{12}$) served as control.

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Gutter method was used to determine preliminary activities of the extract on the microorganisms. Sterilized nutrient agar was poured into three Petri dishes, covered and allowed to set. The medium was partitioned into six segments by using a marker pen to draw parallel lines on the bottom of plates. With the aid of a sterilized loop, each organism including the control was streaked across a segment on the agar surface. A sterilized spatula was then used to cut out a continuous gutter in the agar across the streaks.

Highly concentrated solution of the extract was poured into the gutter. This was repeated for all the plates. The Petri dishes were covered and transferred to an incubator set at 37°C and left for 24 h, a sufficient period for the organisms to grow. Clearance of streak growth from the gutter margin indicated inhibitory activity of extract.

Punch hole method (Stokes, 1975) was used to determine the zone of inhibition. Five Petri dishes were poured with already sterilized nutrient agar to the level of obtaining a standard well, covered and allowed to set. The organisms were dissolved in nutrient broths and each poured over the entire surface of a set agar plate, ensuring even distribution. Sterile cork borer, 10 mm in diameter was used to punch three adequately spaced holes in the media. For each plate, the holes were filled with a few drops of the extract. The Petri dishes were covered and kept in an incubator set at 37°C for 24 h to allow the organisms grow.

The average inhibition diameters were measured using a meter rule to measure two points and taking the average. The values were compared with the activity of Gentamycin (as standard) and water (as control).

To determine the Minimum Inhibition Concentration (MIC) 9 agar plates were prepared and three each were flooded with the same organism. Seven holes were punched in each plate and filled with 0.2 mL volume containing 500 μg mL^{-1} of the extract in different dilution. Double dilution of the extract was carried out. Double strength nutrient broth of 5 mL was pipetted into seven universal bottles. They were labelled A, B, C, D, E, F and G. Using sterile pipette, 5 mL of the extract was pipetted into each of the bottles and properly mixed. Only nutrient broth was put in another bottle labelled H to serve as control. The plates were incubated at 37°C for 24 h. The order of concentrations in each of the bottles is as follows: A-500, B-250, C-125, D-62.5, E-31.25, F-15.6 and G-7.8 μg mL^{-1}.

RESULTS

Table 1 revealed the presence of secondary metabolites including saponins, phenolic compounds, eugenol oil and glycosides but no trace of alkaloids, flavonoids and anthraquinones.

Table 2 showed the extract had inhibitory activity against *S. aureus*, *B. subtillis* and *E. coli* but inactive against *K. arogenes*, *P. aeruginosa* and $J62K_{12}$.

The highest zone of inhibition was recorded against *S. aureus* and lowest for *E. coli* (Table 3), while the MIC was 125 and 500 μg mL^{-1} for *S. aureus* and *B. subtilis* respectively (Table 4).

Saponins have been reported as active antifungal agents, while tannins prevent the development of microrganisms by precipitating microbial protein and making nutritional proteins unavailable to them (Sodipo *et al*., 1991). Zakaria *et al*. (2006) reported on the *in vitro* antibacterial properties of *Corchorus olitorius*. Also, Idu *et al*. (2007b) reported that the stem of *Stachytarpheta jamaicensis* contained saponins, tannins and flavonoids and the alcoholic extract demonstrated antimicrobial activities.

In the present study, the presence of similar phytochemicals, including phenolic compounds, eugenol

Table 1: Phytochemical analyses of *B. axillaris* leaf

Secondary metabolites	Observation
Alkaloids	-
Saponins	+
Phenolic compounds	+
Eugenol oil	+
Glycosides	+
Flavonoids	-
Anthraquinones	-
Tannins	+

++ = Present; - = Absent

Table 2: Antimicrobial activity of methanol extract of *B. axillaris* leaf using gutter method

Organisms	Inhibitory activity
Klebsiella arogenes	-
Staphylococus aureus	+
Bacillus subtilis	+
Escherichia coli	+
Pseudomonas aeruginosa	-
$J62k_{12}$	-

+ = Inhibited; - = Not inhibited

Table 3: Zone of inhibition (diameter in mm) due to activity of methanol extract of *B. axillaris* leaf

Organisms	H_2O	Extract	Gentamycin
Staphylococus aureus	-	15.00	5
Bacillus subtilis	-	10.00	5
Escherichia coli	-	9.00	5

Table 4: Minimum Inhibition Concentration (MIC) of methanol extract of *B. axillaris* leaf

	Extract concentration (μg mL^{-1})						
Organisms	500	250	125	62.5	31.25	15.6	7.8
S. aureus	+	+	+	-	-	-	-
B. subtilis	+	-	-	-	-	-	-
E. coli	-	-	-	-	-	-	-

+ = Inhibited; - = Not inhibited

oil and glycosides could be responsible for the antimicrobial property of the methanolic extract of *B. axillaris* leaf. It was most effective against *S. aureus*, yielding the highest zone of inhibition (15 mm) and lowest MIC (125 μg mL^{-1}) compared with the lower zone of inhibition (10 mm) and a higher MIC (500 μg mL^{-1}) obtained for *B. subtilis* (Table 3, 4). It was least effective against *E. coli*.

CONCLUSION

The use of plants as antimicrobials has been long established though the effectiveness varies. *B. axillaris* possesses promising antimicrobial properties, especially against *S. aureus*, *B. subtilis* and *E. coli* which are major pathogens for human infections, varying from food poisoning or minor skin infections to severe life threatening infections.

However, further research is required to establish the *in vivo* activities as well as the therapeutic index of this plant in respect to the management and possible cure of infectious diseases.

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