PRELIMINARY PHYTOCHEMICAL ANALYSIS AND ANTIBACTERIAL ACTIVITY OF *Pergularia tomentosa*

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Abstract

Fresh samples of leaves and stems of *Pergularia tomentosa* were investigated for phytochemical and antibacterial properties. The samples were dried in air and ground to powder. Three solvents methanol, n-hexane and ether were used to extract the samples. The samples were screened for alkaloids, tannins, saponins and glycosides. Antibacterial screening was done using the Disc diffusion method. The test bacteria used are *Bacillus cereus*, *Escherichia coli* , *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Results of the phytochemical analysis indicated that the methanol extracts of the plants' leaves and stem contain alkaloids, saponins, tannins and glycosides. The methanol extracts of the leaves (MEL) showed antibacterial activity affecting *E. coli*(14mm) and *P. aeruginosa* (10mm);while the ether extract (EEL) showed activity on *B. cereus* (4mm) and *S. aureus* (2mm). In case of the stem, only the methanolic extracts (MES) showed an inhibition of 6mm against *P. aeruginosa*. The implication is that this plant contains some active antibacterial components extractible by methanol and ether. Therefore there is need for further screening using various solvents and bacteria.

Keywords: *Pergularia tomentosa*, Phytochemical, Sensitivity, Bacteria

Introduction

There has been increased attention on plants research recently all over the world because of their potential in various traditional systems. Dahanukar *et al.*(2000) have reported tht n-ore than 13,000 plants have been studied within a period of five (5) years(1994-98).Plants and plants' products have been used for years in several ways for the treatment of diseases such as bacterial diseases, physiological disorders and mental problems. Some of the bacterial diseases include gonorrhoea, syphilis, dysentery, pneumonia and tuberculosis. Several drugs used today especially antibacterial agents originated from the purification of plants extracts and isolation of the active ingredients (Sofowora, 1985; Rates, 2001).

The plant *Pergularia tomentosa* is of the *Asclepiadaceae* family. The synonyms for this plant include *Daemia extensa* R.B, *Asclepias daemia forsk*, *Daemia tomentosa*, *Pergularia daemia* (forsk) and *Pergularia extensa*. It is known as 'fatakko' in the Hausa language of Northern Nigeria.P. tomentosa is found inthe tropics and subtropics, growing to heights of up to one metre They are milkweeds and cardiac glycosides are known to be present in almost all species of this genus (Walters and Elvin, 1977). The leaves have anthelmintic properties and are used in Nigeria against guinea worm. Hussein *et al.*(1999) have reported the molluscicidal activity of methomyl,a cardiac glycoside and methiocarb isolated from *P. tomentosa* against land snails. The plant is also used in traditional medicine for the treatment of many diseases caused by bacteria.

Sofowora(1985) reported that the leaves of the plant have been used to cure diarrhoea. The juice or latex is applied to boils and abscesses for fast treatment. Their availability and efficacy in such treatment provides a good basis for examining their pharmacological potential. It is in this regard that this preliminary investigation of the phytochemical properties and antibacterial activities of *Pergularia tomentosa* on some bacterial species-*Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Bacillus cereus* is carried out.

Materials and Methods

Fresh samples of *Pergularia tomentosa* leaves and stem were obtained within the main campus of the Usmanu Danfodiyo University Sokoto and duly authenticated by the Botany Unit, Department of Biological Sciences of the University. The leaves were separated from the stem, and each of them was separately air dried, ground, sieved and kept at ambient temperature.

Extraction

0.5kg each of powdered stem and leaves were separately extracted with n-hexane, diethyl ether and methanol using soxhlet apparatus for four hours each. The extracts were concentrated using rotary evaporator at a temperature of 50°C and then evaporated to dryness in a hot air oven at the same temperature. The methanol extract of the leaves was labelled MEL while that of the stem as MES. Similarly, the n-hexane and ether extracts of the leaves and stem were labelled HEL, HES, EEL and EES respectively.

The dried extracts were kept in a refrigerator for subsequent use.

Phytochemical and Chromatographic(TlC) Studies

The methanol extracts of both leaves (MEL) and stems (MES) were used in the phytochemical screening to test for the presence of alkaloids, tannins, phlobatannins, saponins, anthraquinones and glycosides using the methods described by Sofowora (1985).

Chromatographic (TLC) studies of n-hexane extracts was carried out using benzene- ethyl acetate (3:1) and n-butanol-acetic acid-water (4:1:5) solvent mixtures. The separated components were viewed under UV light at wavelengths 254nm and 366nm and the respective R_f values were recorded.

Antibacterial Assessn.ent

a) Bacteria species

The bacteria tested include *E. coli*, *S. aureus* and *P. aeruginosa* which were clinical specimens; and B. cereus which was a non-clinical specimen were obtained as pure cultures from the microbiology unit of Usmanu Danfodiyo University, Sokoto. The choice of these bacteria species was made so as to have a wide group of bacteria, both gram-positive and gram- negative.

b) Media preparation

Nutrient agar was prepared according to the manufacturer's instructions and the streaking done as described by (Ogundana, 1989).

c) Sensitivity Test

The antibacterial sensitivity test carried out was the disc diffusion method as described by Bauer *et al.* (1966). A uniform concentration of 50mg/cm^3 was used throughout so that the relative efficacy of the extracts could be measured. A positive test was indicated by a zone of inhibition of the bacterial growth around the discs.

Results

The results of the phytochemical screening of the leaves and the stem extracts are summarized in Table 1.The components found were alkaloids, tannins, saponins and glycosides. Anthraquinones and phlobatannins were not found in both the leaves and the stem extracts.

Table 1 : Results of the phytochemical screenings on methanol extracts

Component	Alkaloids	Tannins	Saponins	Anthraquinones	Pholobatannins	Glycosides
MEL	+	+	+	-	-	+
MES	+	+	+	-	-	+

Key: + = present
- = absent

The results of the chromatographic analysis of the n-hexane extract using the two solvent systems are shown in Table 2. A component with R_F value of 0.95 is common to both the leaves and the stem extracts in the two solvent systems. In addition, the number of spots was more in the benzene/ethyl acetate system than that of butanol/acetic acid/water.

Table 2: R_f Values of the chromatographic analyses on hexane extract

Fraction	Solvent System	
	n-butanol-acetic acid -water	Benzene-ethylactate(3:1)
HEL	0.95	0.08,0.65,0.73, 0.82 and 1.08
HES	0.95, 0.58	0.06,0.73,0.79 and 0.87

The antibacterial activities for the leaves extracts against four bacteria tested in various solvents are presented in Table 3.The zone of inhibition in mm against each bacterium was recorded. The highest antibacterial activity was noticed against *E.coli* (14mm), which was sensitive to the leaves extract in methanol (MEL).

Table 3: Antibacterial activity of leaf extracts in various solvents

Leaf Extract	Zone of Inhibition (mm)			
	B.cereus	*E.coli*	*P. aerugenosa*	*S. aureus*
MEL	0	14	10	0
EEL	4	0	0	2
HEL	0	0	0	0

The least activity (2mm) was noticed against S. aureus in ether extracts (EEL).For the stem extracts, the antibacterial activities in various solvents are presented in Table 4. Only P. *aeruginosa* was sensitive to the stem extract in methanol whereas all the other bacteria were insensitive.

Table 4: Antibacterial activity of stem extracts in various solvents

Stem Extract	Zone of Inhibition (mm)			
	B.cereus	*E.coli*	*P. aerugenosa*	*S. aureus*
MES	0	0	6	0
EES	0	0	0	0
HES	0	0	0	0

Discussion

Alkaloids were detected in both the methanolic extracts of the leaves and the stem. Alkaloids are used as analgesics, stimulants, anaesthetic, hallucinogens and antibacterials. Tannins are present in both MEL and MES if these tannins can be isolated from the crude extracts and purified, they can be used in tanning leather, dyeing fabric, making ink and in various medical applications. Tannins have received much attention in recent times as it was suggested that the consumption of tannin-containing beverages could cure or prevent a variety of ills(Cowan,1999). Glycosides are non-reducing substances that on hydrolysis by reagents or enzymes yield one or more reducing sugars(Balbaa *et al.*, 1976). Glycosides are found to be present in both MEL and MES.

Their presence gives more support to the use of the plant for heart disorders and bacterial diseases which is also in agreement with Gurdeep(1976). Examples of some antibacterial agents derived from glycosides include streptomycin, neomycin, kanamycin and gentamycin(Trease and Evans, 1989; Dangoggo *et al.*, 2001). Glycosides found in *P. tomentosa* are strong toxicants against land snails(Hussain *et al.*, 1994) and if isolated may lead to the synthesis of many highly effective molluscicides. Saponins are found to be present in all the methanolic extracts examined. The saponins have applications in foaming fire extinguisher, emulsifying, insecticides, etc.

The organs in which particular plant constituents reside differ widely. Some constituents are accumulated exclusively in some particular organs. In some cases a particular constituent may be found in different organs such as leaves, stem bark, roots, seeds, etc. Yet, there are other constituents that are found all over the body organs of a plant. Therefore, the appearance of identical R_f values in the chromatographic analyses on both HEL and HES in BAW solvent is indicative that the two parts contain the substance represented by the R_f value 0.95 likewise in the benzene–ether chromatogram there appears identical R_f value of 0.73 for both for HEL and HES. This R_f value may be representing the same compound as in BAW and the difference in R_f values of 0.95 in BAW and 0.73 in benzene-ether may be accounted for by the difference in the developing solvent. However, this compound is not likely to be responsible for any antibacterial activity since neither HEL nor HES extract shows any such activity. Yet, the information is important as it can be used to eliminate the active constituent from non-active ones.

The inhibition zone in mm against each bacterium was recorded. The highest antibacterial activity was noticed against *E.coli* (14mm), which was sensitive to the leaves extract in methanol (MEL). The least activity (2mm) was noticed against S. aureus in ether extracts (EEL). Generally, *B. cereus* and S. aureus were not sensitive to methanolic extracts. Similarly, *E.coli* and *P. aeruginosa* were not affected by the ether extracts. In all no bacteria was sensitive to n-hexane extracts and it can therefore be said that the methanol extract (MEL) is the best solvent for the extraction of the component with antibacterial property from this plant.

The antibacterial activities of the stem extracts in various solvents as given in Table 4, indicated that only P. aeruginosa was sensitive to the stem extract in methanol whereas all the other bacteria were insensitive. Comparing the two results i.e. leaves and stem in various solvents, the leaves extracts were found to be more potent than the stem extracts. Also, there is more antibacterial activity with the MEL extracts than either the HEL or EEL. Therefore, it could be said that the active ingredient of the drug is most soluble in the polar solvents. This agrees with the findings of Sofowora[2].This probably explains the use of the plant in treating diarrhoea and wounds.

Conclusion

The phytochemical studies of *P. tomentosa* revealed the presence of alkaloids, glycosides, tannins, saponins which when isolated and purified can be used for various therapeutic, domestic and industrial purposes. Presence of tannins provides more evidence for the use of the plant in tanning leather.

The antibacterial effect of the extract on the four bacterial species (*Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) also gives support to its potential use of the plant as an antibacterial agent. The plant is of economic importance and more research needs be done on it to isolate and investigate the active ingredient, minimum inhibitory concentration, mechanism of action, toxicity study and also to widen the choice of bacterial species.

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