

Full Length Research Paper

Phytochemical and antimicrobial screening of methanol extract of *Heliotropium indicum* leaf

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Methanol extract of the leaf of *Heliotropium indicum* was evaluated for its antimicrobial activity against five bacterial isolates comprising of four Gram-negative namely: *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella* spp. and *Proteus mirabilis*; and one Gram positive, *Staphylococcus aureus* at 6.25, 12.5, 25, 50, 100 and 200 mg/ml including phytochemical analysis. While both *S. aureus* and *Klebsiella* spp. were inhibited at 50, 100 and 200 mg/ml with minimum inhibitory concentration (MIC) of 3 mg/ml; *P. aeruginosa* and *P. mirabilis* with MIC of 10 mg/ml were inhibited at 100 mg/ml and 200 mg/ml. *E. coli* with MIC of 20 mg/ml was inhibited only at 200 mg/ml concentration of the extract. Phytochemical analysis revealed the presence of plant metabolites as alkaloids, saponin and tannins. While activity-directed assay is advocated, the plant promises to be of tremendous assistance in the treatment of infections with which bacterial isolates used for the study are associated.

Key words: Phytochemical, antimicrobial, *Heliotropium indicum* alkaloids, saponin, tannin.

INTRODUCTION

The importance of medicinal plants in the prevention and treatment of diseases cannot be underscored. This can be achieved by medicinal plants serving as sources of "lead compounds" in the synthesis of potent antimicrobial agents and/or use directly as decoction or concoction locally.

The importance is further enhanced due to the fact that microorganisms have developed resistance to all known antimicrobial agents already in use thereby making search for new ones inevitable.

While some plants have been screened for this purpose, many are yet to be screened. One of the plants yet to be screened for its antimicrobial activity is *Heliotropium indicum*, also known as Indian heliotrope.

The plant is an annual, erect, branched hirsute plant about 15 to 50 cm high. The leaves are always opposite or alternate, ovate to oblong-ovate, somewhat hairy, acute or acuminate, base decurrent along the petiole and about 3 to 8 cm long. The flowers are calyx green and

about 3.5 mm in diameter. The fruits are dry 2 to 4 lobed of 2 or 4 nearly free, more or less united nutlets, 4 to 5 mm long. It is a common weed in waste places and settled areas, flowering the whole year round as shown in Figure 1.

In West Africa, the plant is reputed for use in vomiting, amenorrhea, high blood pressure, gumboils, clean up ulcers and for eye infections.

In Sierra Leone, the decoction of leaves is used for washing new born babies while the leaf powder is used for dermatitis, eczema, and impetigo in Senegal. Leaf decoction is used for thrush and poultices used for herpes and rheumatism in Indonesia and for wound healing in Thailand. The plant serves as remedy for sore throat and lung diseases in Taiwan.

In Nigeria, the plant is used for fevers and ulcers. In South-Western part of Nigeria, the plant is used for the treatment of inflammation especially inflamed joints. The use for this purpose has to do with the "doctrine of signature" as it is called 'ogbe akuko', that is 'cock's comb' which normally appears red in color like inflamed area. However, several activities have been linked to the plant scientifically. These include gastroprotective

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Figure 1. Photograph of *Heliotropium indicum* growing in its natural habitat.

activity (Adelaja et al., 2008), wound healing activity (Reddy et al., 2002), antitumour activity (Kugelman et al., 1976), anti-inflammatory activity (Srinivas et al., 2000), antituberculosis effect (Machinan et al., 2005), anti-proliferative effect (Moongkarndi et al., 2004), as well as immunostimulant effect (Ashoka et al., 2009).

Workers who have reported antimicrobial activity of the plant linked it with gastroprotective activity with no report on the antimicrobial activity per se.

This work aimed at screening the plant, *Heliotropium indicum*, for its physiochemical and antimicrobial profiles.

MATERIALS AND METHODS

Plants collection and identification

The whole plant of *H. indicum* L. (*Boraginaceae*) was collected in Sabo, Sagamu, Ogun State, Nigeria. The plant was identified and authenticated by Mr. Oshiyemi of the Forestry Research Institute of Nigeria, Ibadan, Oyo State, Nigeria. The authenticated plant has the Voucher number FHI 107093.

Sample preparation and extraction

The fresh leaves were sun-dried for about 2 weeks and separately ground into fine powder using a mechanical grinder. The method of Dupont et al (2006) was adopted for extraction with little modification. Briefly, 20 g portions of the powdered plant part was separately weighed and soaked in 100 ml methanol at ambient temperature for 72 h under regular shaking condition. The extract was then filtered using Whatman filter paper No 1. The filtrates

were evaporated to dryness using evaporating dish at 35°C and percentage yield calculated.

Phytochemical screening

The following phytochemical tests were carried out on the extracts. Test for alkaloids was performed using Wagner and Dragendoff reagents (Sofowora, 1994). 0.5 g of the extract was added to 5 ml of 1% aqueous hydrochloric acid on a steam bath. This was filtered and 1 ml portion treated with a few drops of Draggendoff reagent and another 1 ml portion similarly treated with Wagner's reagent. The formation of precipitates was an indication of the presence of alkaloids.

The blood haemolysis test was used to test for Saponins (Sofowora, 1994). The test for anthraquinones was done by shaking 0.5 g of the extract with 5 ml chloroform for 5 min. The mixture was filtered and the filtrate shaken with equal volume of 10% ammonia solution. A pink, violet or red color in the ammoniacal layer (lower layer) indicated the presence of free anthraquinones. Test for tannins was done using Ferric chloride test. A deep green coloration showed the presence of tannins (Trease and Evans, 1989). The Keller-Kiliani test was used to test for the presence of cardenolides. 0.5 g of the extract was dissolved in 2 ml of glacial acetic acid containing one drop of Ferric chloride solution. This was then underplayed with 1 ml of concentrated H₂SO₄. A brown ring obtained at the interphase indicated the presence of a deoxy sugar typical of cardenolides. About 1 g of the extract was dissolved in 5 ml of 2% potassium hydroxide and then filtered. Formation of precipitate on addition of 10% hydrochloric acid to the filtrate confirms the presence of flavonoids.

Antimicrobial screening

The pure cultures of the microbial strains were purchased from the Medical Pathology and Microbiology Department of the University College Hospital, Ibadan and included: *Escherichia coli*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella spp* and *Proteus spp*.

All were collected on slants in McCartney bottles containing Mueller-Hinton agar, re-characterized to confirm their identity through some conventional biochemical tests such as indole, catalase, citrate utilization, as well as Gram stain, to mention but a few and appropriately stored until needed.

A known weight of each extract was reconstituted in sterile distilled water to give the desired concentration of extract in milligram (mg). The bacterial suspensions were cultured in peptone water for 24 h. 0.2 ml of 10⁶ cfu/ml was used as inoculum size for all organisms. Each inoculum was mixed with 20 ml of Mueller-Hinton agar in Petri dishes. Wells (6 mm in diameter) were punched in the agar medium using sterile glass cork borer before being filled with 0.1 ml of plant extracts. On each plate, gentamycin was used as positive control while methanol was used as negative control. The plates were incubated for 24 h at 37°C and the diameters of inhibition zones were measured. Each experiment was done in duplicates.

Minimum inhibitory concentration (MIC) assay

This was determined by agar dilution method. Varying concentrations of the extract were prepared by serial dilution and allowed to set in plates. Each plate containing different concentration was streaked with test organism and incubated at 37°C for 24 h. The minimum concentration inhibiting the growth of test organism was recorded as the MIC.

Table 1. Phytochemical components of methanol leaf extract of *Heliotropium indicum*.

Test	Result
Secondary metabolites	
Alkaloid tests	
Wagner	Positive
Mayer	Positive
Picric acid	Positive
Anthracene derivative	
Test for free anthraquinone	Negative
Test for combined anthraquinone	Negative
Test for those that are resistance to mild hydrolysis	Negative
Test for saponin	Positive
Test for tannins	Positive

Table 2. Antibacterial activity of the methanol extract of *H. indicum*.

Organism	Mean diameter of zone of inhibition (mm)						
	Plant extract (mg/ml)					Control drug(mg/ml)	
	6.25	12.5	25	50	100	200	10
<i>Staphylococcus aureus</i>	-	-	-	7	8	14	14
<i>Pseudomonas aeruginosa</i>	-	-	-	-	6	11	7
<i>Proteus mirabilis</i>	-	-	-	-	8	12	22.5
<i>Escherichia coli</i>	-	-	-	-	-	12	22.5
<i>Klebsiella</i> spp.	-	-	-	6	9	14	27

Diameter of cork borer = 6 mm.

RESULTS

Extraction yield

$$\begin{aligned} \text{Percentage Yield} &= \frac{\text{Quantity of dried extract(g)}}{\text{Quantity of powdered sample(g)}} \times 100 \\ &= \frac{1.44}{20} \times 100 \\ &= 7.2\%^{w/w} \end{aligned}$$

DISCUSSION

The result reveals the percentage yield to be 7.2%w/w while phytochemical analysis reveals that the plant possess alkaloids, tannin and saponins as shown in Table 1. Antimicrobial screening showed that the plant extract was effective against all the organisms tested although at different concentrations as shown in Table 2.

While *S. aureus* and *Klebsiella* spp. responded positively by having zones of inhibition to three of five concentrations tested, that is, 50, 100 and 200 mg/ml; *P. aeruginosa* and *P. mirabilis* responded to two of five concentrations, that is, 100 and 200 mg/ml with *E. coli* showing susceptibility to only one concentration, that is, 200 mg/ml.

This observation can be attributed to the presence of metabolites like alkaloids, saponins and tannins whose antibacterial activity has been previously documented (Tschesche, 1970). Also, the stronger extraction capacity of methanol could have produced greater number of active constituents responsible for antibacterial activity.

The Minimum Inhibitory Concentration ranges between 3 and 20 mg/ml with MIC of *S. aureus* and *Klebsiella* at 3 mg/ml; *P. aeruginosa* and *P. mirabilis* at 10 mg/ml and *E. coli* at 20 mg/ml. This is comparable to the activity of the standard used at concentration of 10 mg/ml, which has activity on all the bacterial isolates used for the study.

However, the corollary to the finding is that the plant will serve a useful purpose in the treatment of infections to which these organisms are associated. Nonetheless, activity-directed assay is necessary on this plant with a

view to isolating and characterizing the active metabolite responsible for the observed activity.

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