

Phytochemical Screening, Antibacterial Activity and Fatty Acids from *Heliotropium Indicum*

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ABSTRACT

Heliotropium indicum are used in ethnomedicine for treating skin rashes, inflammation, tumors and various diseases of microbial origin. Previous work focused on phytochemical constituents, antibacterial activities and isolated compounds. The whole plant was air dried and pulverized (600 g). It was extracted with ethanol and concentrated to dryness in *vacuo* (20 g). Screening for phytochemical groups was performed using qualitative methods. The antibacterial activity was performed using agar diffusion and antifungal activity using ditch plate method. Compounds were characterized using Gas-chromatography Mass Spectrometry. The results of the phytochemical screening revealed the presence of phenols, saponins, terpenoids and cardiac glycosides. The results further showed the crude extract of the plant to be moderate to strong antibacterial activity with zones of inhibition ranging from 12.0 + 1.6 mm and 25 +1.7 mm. the crude extracts of *Heliotropium indicum* demonstrated antibacterial properties which confirmed the traditional use of this plant in treating skin rashes and boil. In addition, the presence of Palmitate and Linoleic acid in the plant as revealed in this study justified the use of this plant in treating hypertension.

Key words: *Heliotropium indicum*, Antibacterial Activity, Gas-Chromatography Mass Spectrometry, Phytochemical.

INTRODUCTION

Medicinal plants play an energetic role in the discovery of new therapeutic agents, thus growing interest in the use of pharmaceutical consumption.^{1,2}

The name “heliotrope” originates from the old idea that the inflorescence of these plants turned their rows of flowers to the sun. the meaning “helio” in greek is “sun” and “tropein” from where the word ‘tropium’ is derived means ‘to turn’.

Heliotropium indicum Linn. Belongs to family Boraginaceae (Figure 1). This family is well marked in their characteristics and not easily confused with any other. The genus *Heliotropium* comprises about 250 species. it is commonly known as Indian heliotrope. Leaves are 4-10 cm long and 2-5 cm wide. *H. Indicum* is used in ethnomedicine for giving protection to human from many diseases from ancient time. The different ethno pharmacological report suggest that *Heliotropium indicum* to heal skin infections, poisonous animal bites, stomach problems and nervous diseases. Many countries in Africa use it to treat tumors and inflammation. Traditionally, this plant is widely used against many pathological disorders including wound healing, antidote, bone fracture, febrifuge, cures eye infection, menstrual disorder, nerve disorder, kidney problem, and antiseptic purpose.³

Fatty acids, both saturated and unsaturated play a major role in metabolism, as a metabolic fuel, as a necessary component of all membranes and it is also used as a gene regulator. In addition, fatty acids have a number of industrial uses.³ Obtaining fatty acids from plant sources is more beneficial for one’s health. A well-planned plant-based diet fatty acids

rich food promotes heart health, ensuring the proper growth and development of your child and relieving pain. In this present study, focus shall be mainly on the phytochemical constituents, antimicrobial activity and fatty acids from *Heliotropium indicum*.⁴

MATERIALS AND METHODS

Plant material and general experimental methods

Fresh leaves of *Heliotropium Indicum* were collected in the month of June, 2021 from the neighbourhood of Ado-Ekiti Ekiti state, Nigeria. The plant was authenticated by the herbarium staff of the Botanical department at the Ekiti State University.

Sample preparation

Heliotropium indicum (950g) was harvested and dried for two weeks and separately ground into fine powder using an electric blender. 950 g of the leaves were air dried at room temperature for about four days. Thereafter, the dried leaves were pulverized



Figure 1: *Heliotropium indicum* Linn. Belongs to family Boraginaceae.

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and extracted using 95% ethanol at room temperature. The extracts were concentrated to dryness and the residue were obtained as a greenish black solid, after which the residues were transferred into a pre-weighed sample containers and were stored and later used for phytochemical screening and antioxidants activity.

Antimicrobial screening

The agar diffusion (cup plate) method was used for this examination. Molten and cooled agar 60 ml (45°C) were separately inoculated with the nutrient broth culture of the test organisms (0.6 ml) and mixed thoroughly. The inoculated medium was then carefully poured into sterile Petri dishes (24 cm Petri dish) and allowed to set. Thereafter, cups (9 mm diameter) were aseptically bored into the solid nutrient agar using a sterile corn borer. The test solutions 100 µL each were then introduced into each of the cups ensuring that no spillage occurred. Also, the same volume of the standard antimicrobial agent and the solvent were introduced into some of the cups to act as positive and negative controls respectively. The plates were left at room temperature. Antimicrobial screening 72 hours. The extract was then filtered using Whatman paper. The agar diffusion (cup plate) method was used for this examination. Molten and cooled agar 60 ml (45°C) were separately inoculated with the nutrient broth culture of the test organisms (0.6 ml) and mixed thoroughly. The inoculated medium was then carefully poured into sterile Petri dishes (24 cm Petri dish) and allowed to set. Thereafter, cups (9 mm diameter) were aseptically bored into the solid nutrient agar using a sterile cork borer. The test solutions 100 µL each were then introduced into each of the cups ensuring that no spillage occurred. Also, the same volume of the standard antimicrobial agent and the solvent were introduced into some of the cups to act as positive and negative controls respectively. The plates were left at room temperature for 2 hours to allow for diffusion into the medium and thereafter incubated face upwards at 37°C for 24 hours. Sample was tested in duplicate and the diameters of zone of inhibition were measured to the nearest millimeter using a transparent ruler.

GC-MS

The GC was carried out using Hewlett- Packard 6890 GC series equipped with FID and HP-5 capillary column (cross linked 5% diPh, 95% dimethylpolysiloxane, 30 m × 0.32 mm i.d.; 0.25 µm film thickness).

The column temperature was programmed at 50-210 at a rate of 3°C/min. The injector and detector temperatures were 220°C and 270°C, respectively. Samples (1 µL of the oil solutions in chloroform, 2 mg/mL) were injected by the split less technique into nitrogen carrier gas (0.8 mL/min).

Preliminary qualitative phytochemical analysis

Test for alkaloids: Alcoholic extract (10 ml) was stirred with 5 ml of 1 % HCl on a steam bath. Mayer's (1.35 gm mercuric chloride in 60 ml water + 5 gm potassium iodide in 10 ml water) and Wagner's reagents (1.27 g of iodine and 2 g of potassium iodide in 100 ml of water) were added, white and reddish brown color precipitate respectively, were taken as evidence for the presence of alkaloids.

Test for flavonoids: Crude extract was mixed with 2 ml of 2 % solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

Test for saponins: Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

Test for phenols and tannins: Crude extract was mixed with 2 ml of 2 % solution of FeCl₃. A blue –green or black coloration indicated the presence of phenols and tannins.

Test for glycosides (Salkowski's test): Crude extract was mixed with 2 ml of chloroform. The 2 ml of concentrated H₂SO₄ was added carefully and shaken gently. A reddish brown colour indicated the presence of steroidal ring that is glycine portion of glycoside

Keller-kilani test: Crude extract was mixed with 2 ml of glacial acetic acid containing 1-2 drops of 2 % solution of FeCl₃. The mixture was then poured into another test tube containing 2 ml of concentrated H₂SO₄. A brown ring at the interphase indicated the presence of cardiac glycosides.

Test for terpenoids: Alcoholic extract (2 ml) was dissolved in chloroform (2 ml) and evaporated to dryness. Concentrated sulphuric acid (2 ml) was then added and heated for about 2 min. A greyish colour was considered an indication for the presence of terpenoids.

RESULT

Tables 1 to 4.

Table 1: Phytochemical constituents of *Heliotropium indicum*.

Secondary metabolites	<i>Heliotropium indicum</i>
Alkaloids	+++
Cardiac glycosides	++
Flavonoids	++
Terpenoids	+++
Saponins	+++
Tannins	-
Phenols	++

Table 2: Antibacterial test on ethanolic extract of *Heliotropium indicum*.

Test Organisms	Ethanol Crude Extract of H.I (25mg/ml)	CnE	Streptomycin (1 mg/ml)
<i>B.cereus</i>	25.7 +1.7	NI	22.0 +1.2
<i>E.coli</i>	22.0+1.2	NI	25.0+1.6
<i>Paureginosa</i>	16.0+1.4	NI	20.0+1.2
<i>S.aureus</i>	12.0+1.6	NI	20.0+1.2

Mean+standard deviation of determinations

B.cereus: *Bacillus cereus*, *E.coli*: *Escherichia coli*, *Paureginosa*: *Pseudomonas aureginosa*, *S.aureus*: *Staphylococcus aureus*.

NI- No Inhibition, CnE- Control of solvent

Table 3: Antifungal test on ethanolic extract of *Heliotropium indicum*.

Test Organism	Ethanol crude Extract H.I (25 mg/ml)	CnE	Fluconazole (1 mg/ml)
<i>Aspergillus flavus</i>	0.0	NI	35.0 + 0.3
<i>Penicillium camemberti</i>	0.0	NI	40.0+0.6
<i>Candida Albican</i>	0.0	NI	30.0+0.2
<i>Fusarium species</i>	0.0	NI	42.0+0.6

CnE =Control, NI= No inhibition

Table 4: Compounds detected in the GC-MS screening of the oil extract of *Heliotropium indicum*.

Retention Time	Area in %	Compounds detected
14.874	10.66	Hexanoic acid
16.630	4.62	Linoleic acid ethylester
16.830	13.24	Phenol
14.874	13.83	Hexadecanoic acid
11.519	0.52	Tetradecanoic acid
5.309	0.71	Glutaric acid
16.696	3.73	Octadetrienoic acid

DISCUSSION

Heliotropium indicum ethanol extract was strongly active against *B. cereus* at the zone of inhibition 25.7 +1.7 mm more than streptomycin that serves as control drug. Its ethanol extract was strongly active against *E. coli* at the zone of inhibition 22.2 +1.2 mm but it's also moderately active against *P. aureginosa*.

It was not active against all other fungi mentioned in Table 2. This plant can be used to treat various infections caused by *B. cereus*, *S. aureus* and *P. aureginosa*. The potency of the extract was strong in the polar portion. Alkaloids, terpenoids and saponins were strongly present in the plant. Cardiac glycoside, phenols and flavonoids were moderately present in the plant but tannin was not present. Seven compounds are present in the *Heliotropium indicum* which are majorly the fatty acids. Application of Linoleic ethyl ester can be used as one of the drugs that can be used to treat fluke's infestation and measles infestation. Application of Glutaric acid can be used as one of the drugs that can be used for retentive memories in children.

CONCLUSION

The crude extract of *Heliotropium* showed strong antimicrobial activity for the first time. The crude extract of the plant revealed some secondary metabolites. The constituents of the plants revealed some fatty acids like linoleic acids, octadetrienoic acids and so on for the first time.

Further investigation could be carried out to isolate the active fatty acids that could be used in the pharmaceutical industry.

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