Phytochemical and Antimicrobial analysis of the crude extract, petroleum ether and chloroform fractions of Euphorbia heterophylla Linn Whole Plant

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ABSTRACT

The whole plant of *Euphorbia heterophylla* Linn family Euphorbiaceae, was screened for secondary metabolites and evaluated for its antimicrobial activities using standard procedures. The antibacterial activities of the methanol extract, pet ether, chloroform and methanol: water fractions were tested on gram negative and gram positive bacteria (*Staphylococcus albus*, *Staphylococcus aureus*, *Proteus mirabilis*, *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumonae*) while the antifungal activity of the extract and fractions were tested on *Fusarium oxysporum, Aspergillus flavus*, *Aspergillus niger* and *Candida albicans*. The phytochemical screening showed the presence of alkaloids, tannins, cardiac glycosides and saponins in the whole plant of *E. heterophylla*. The active constituents present in the whole plant of *E. heterophylla* against the test organisms are of varying polarities with the crude extract showing significant activity against *Staph albus*, *Proteus mirabilis*, *E. coli*, *Salmonella typhi* and *Kleb. pneumonae* at all the doses tested and no effect on *Staph. aureus, Fusarium oxysporum, Aspergillus flavus and Candida albicans.*

Key word: *Euphorbia heterophylla* Linn, antimicrobial, pet. ether, chloroform fractions

INTRODUCTION

Infectious diseases are main causes of morbidity and mortality in man, especially in developing countries.[1] The emergence of multidrug resistant organisms to known antibiotics leading to several deaths worldwide has led to the interest of scientists in exploring plants, herbal products and traditional medicine for the identification of safe and effective remedies to ailments of microbial origin.[2]

Euphorbia heterophylla Linn belongs to the family Euphorbiaceae, a family represented by the trees, shrubs, herbs and characterized by presence of white milky latex which is more or less toxic.[3] It consists of about 300 genera and about 7,000 species.[4] The genus *Euphorbia* is one of the largest genera in the Euphorbiaceae family with about 1,600 species[5] which has been subjected to numerous chemical studies.[6] *E. heterophylla* Linn (syn. *E. geniculata*) commonly known as spurge weed is an erect, annual weed growing to about 3 ft high and locally abundant.

In East Africa, *E. heterophylla* is used for the treatment of gonorrhea and to accelerate wound healing. It is also used as a purgative,[7] a lactogenic agent,[8] as a cure for migraine and warts[9,10] while, the latex of the plant is used as fish poison, insecticide and poisons.[11] Previous biological studies have reported the antibacterial activity of the leaf of *E. heterophylla*,[9,12] its anti-inflammatory activity[13] as well as the wound healing potentials.[14]

Several compounds such as friedelin, β-sitosterol, myricyl alcohol, ellagic acid were isolated from the stem of *E. heterophylla*. Alanine, cysteine, serine, aspartic acid, methionine, proline, glutamic acid, stigmasterol, lupeol, beta-amyrin, stigmasterol glucoside, benzoic acid, 4-hydroxylbenzoic acid and quercetin were isolated from the leaves.[10,15] In addition, the roots have been reported to possess diterpenes.[16]

This study attempts to investigate the antibacterial and antifungal activities of the ethanol crude extract, pet ether and chloroform fractions of the whole plant of *E. heterophylla*.

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MATERIALS AND METHODS

Plant collection and authentication

Whole plant of *E. heterophylla* was collected at Sagamu, Ogun State, Nigeria and authenticated at the Forestry Research Institute of Nigeria FRIN with voucher number F.H.I. 100463 were a voucher specimen was deposited.

Plant preparation and extraction

The air dried and powdered whole plant of *E. heterophylla* was refluxed with ethanol for eight hours. The crude extract was evaporated to dryness to give the ethanol extract. The dried crude ethanol extract was reconstituted in methanol-water (1:3) and partitioned successively against pet ether and chloroform.

Phytochemical Screening.

The air dried powdered plant was screened for the presence of secondary metabolites by using standard procedures.[17]

Microorganisms

Laboratory culture of gram positive and gram negative bacteria (*Staphylococcus aureus*, *Staphyloccocus albus*, *Proteus mirabilis*, *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumonae)* were obtained from the microbiology Department of the Faculty of Basic Medical Sciences, Olabisi Onabanjo University while the fungi were obtained from the laboratory stock of the Department of Pharmaceutical Microbiology, University of Ibadan.

Preparation of bacterial cultures

Each organism was taken from prepared agar slope and inoculated into each of the six 5 mls of nutrient broth. The broth culture was incubated at 37°C for 24 hours. 0.1 ml of 1 in 100 dilution of the overnight culture was added to 20 mls of the melted and cooled nutrient agar at 45°C. The bottles were rolled between the palms to mix and poured into labelled sterile Petri dishes. The seeded Petri dishes were allowed to sit and dried in an incubator for twenty minutes by means of a sterile cork borer (diameter 0.7 cm).

The concentration of the crude extract, pet ether and chloroform fractions (90 mg/ml; 45 mg/ml and 22.5 mg/ml). The antibiotic discs used were Ampicillin, Gentamycin, Nitrofurantoin, Tetracycline and Colistin.

The plates were left at room temperature for six hours to allow the plant extracts diffuse into the agar medium and then incubated at 37°C for twenty-four hours.

Preparation of fungal cultures

Fungal organisms except *Candida albicans* were inoculated in malt extract broth. These were incubated at room temperature for four days and 0.5 ml of each was introduced into 30 mls saline to make 1 in 60 dilutions. 0.3 ml of each dilution was spread over the surface of the Petri dishes containing the Tryptone soya agar. Tryptone soya agar was prepared by dissolving 1 g of Tryptone Soya agar in 250 mls of water and boiled, the agar was then dispensed in 15 mls into universal bottles and autoclaved at 121°C. Cork borer (diameter 0.7 cm) was used to bore wells. 90 mg/ml; 45 mg/ml and 22.5 mg/ml concentrations of *E. heterophylla* whole plant extract were used and Tioconazole (5 µg/ml) was used as control. The plates were left at room temperature for six hours to allow the plant extract diffuse into the agar medium and then incubated at 37°C for ninety-six hours.

RESULTS

Results and Discussion

Previous phytochemical analysis have reported the absence of alkaloids,[13,15] Tannins,[15] saponins[18] and cardiac glycosides in the leaves of *E. heterophylla*[1,15] however, this study reports the presence of alkaloids, tannins which conformed with some previous reports,[18,19] cardiac glycosides[18] and saponins in the whole plant of *E. heterophylla.*

Table 1: Phytochemical screening analysis of *E. heterophylla* whole plant

Alkaloids	Cardiac glycoside	Saponin glycosides	Anthraquinone	Cyanogenetic glycosides	Tannins
+	+	+	–	–	+

– = Absent, + = Present.

Table 2: Antimicrobial screening of the crude extract of *E. heterophylla* whole plant

Dose	ZONE OF INHIBITION (cm)					
	E. coli	*Staph. aureus*	*Proteus mirabilis*	*Salmonella typhi*	*Klebsiella pneumoniae*	*Staph albus*
90 mg/ml	1.8	0.0	1.1	1.5	1.4	1.8
45 mg/ml	1.4	0.0	1.0	1.0	1.3	1.7
22.5 mg/ml	0.0	0.0	0.6	0.4	1.0	1.4

Table 3: Antimicrobial screening of the pet ether fraction of *E. heterophylla* whole plant

Dose	ZONE OF INHIBITION (cm)					
	E. coli	*Staph aureus*	*Proteus mirabilis*	*Salmonella typhi*	*Klebsiella pneumoniae*	*Staph albus*
90mg/ml	1.1	0.0	0.0	1.1	1.3	1.5
45 mg/ml	0.8	0.0	0.0	0.9	1.3	1.3
22.5 mg/ml	0.0	0.0	0.0	0.7	1.2	1.1

Table 4: Antimicrobial screening of the chloroform fraction of *E. heterophylla* whole plant

Dose	ZONE OF INHIBITION (cm)					
	E. coli	*Staph. aureus*	*Proteus mirabilis*	*Salmonella typhi*	*Klebsiella pneumoniae*	*Staph. albus*
90 mg/ml	0.0	0.0	0.0	1.5	0.0	0.8
45 mg/ml	0.0	0.0	0.0	0.8	0.0	0.0
22.5 mg/ml	0.0	0.0	0.0	0.6	0.0	0.0

Table 5: Antimicrobial screening of the MeOH – H_2O of *E. heterophylla* whole plant

Dose	ZONE OF INHIBITION (cm)					
	E coli	*Staph aureus*	*Proteus mirabilis*	*Salmonella typhi*	*Klebsiella pneumoniae*	*Staph. albus*
90 mg/ml	1.4	0.0	0.0	0.0	0.0	1.4
45 mg/ml	0.0	0.0	0.0	0.0	0.0	1.3
22.5 mg/ml	0.0	0.0	0.0	0.0	0.0	0.0

Gentamycin(10 mcg/ml) = 2.1 cm (Staph albus); Ampicillin (10mcg)= 1.1 cm (Proteus mirabilis); Tetracycline (10 mcg) = 0.6 cm( ); Nitrofurantoin (200 mcg) = 1.4 cm (Kleb pneumonia); Nalidixic (30 mcg) = 2.0 cm (E. coli); Chloramphenicol (10 mcg) = 0.4cm (Salmonella typhi)
(Diameter of cork borer = 0.7 cm).

Table 6: Antifungal screening of the MeOH – H_2O of *E. heterophylla*

Dose	ZONE OF INHIBITION (cm)			
	Fusarium oxysporium	*Aspergillus niger*	*Aspergillus flavus*	*Candida albicans*
90 mg/ml	0.0	2.4	0.0	0.0
45 mg/ml	0.0	0.0	0.0	0.0
22.5 mg/ml	0.0	0.0	0.0	0.0

Tioconazole (5 µg/ml) *A. flavus*; *A. niger*, *Candida albicans* = 2.0 cm.
(Diameter of cork borer = 0.7 cm).

The presence of saponin and cardiac glycosides in *E. heterophylla* showed that the diverse groups of compounds in the plant are related biosynthetically. The crude extract of the whole plant of E. *heterophylla* showed significant antibacterial activity against *Staph albus*, *Proteus mirabilis*, *E. coli*, *Salmonella typhi* and *Kleb. pneumonae* at all the doses tested and had no effect on *Staph. aureus*. The biological activity observed in the crude extract was however retained in the pet ether fraction for *E. coli, Salmonella typhi, Klebsiella pneumonia* and *Staph. aureus* while the chloroform fraction had significant activity against Salmonella *typhi which* showed the active constituents are of varying polarities.

There was no activity observed in the crude extract, pet ether and chloroform fractions of *E. heterophylla against Staph. aureus* while the activity observed for *Proteus mirabilis* in the crude extract was lost in both the pet. ether and chloroform fractions.

Against *Klebsiella pneumonae*, the crude extract activity was replicated in the pet ether fraction suggesting that the active constituents are non-polar while for *Staph albus*; the activity of *E. heterophylla* was more in the non-polar fraction and in the mother liquor again showing that the active constituents are of varying polarities.

The extract of *E. heterophylla* had comparable activity as Nitrofurantoin on *Klebsiella pneumonae* while it had a slightly lower activity compared to Nalidix acid and Gentamycin on *E. coli* and *Staph. albus*. The activity of *E. heterophylla* was comparable to that of Ampicillin on *Proteus mirabilis* and that of Colistin on *Salmonella typhi*.

E. heterophylla exhibited antifungal activity against *A. niger* and no activity on *Fusarium oxysporum*, *A. flavus* and *Candida albicans*. The presence of saponins and alkaloids has been reported to be responsible for various pharmacological properties with alkaloids exerting toxic effects against cells of foreign organisms.[20,21] The broad spectrum antimicrobial activity exhibited by the ethanol extract, pet. ether and chloroform fractions of *E. heterophylla* could therefore be

attributed to the presence of the alkaloid, cardiac glycosides, tannins, saponins in the whole plant of *E. heterophylla*. Further study is required to identify the active constituents responsible for these antibacterial and antifungal activities.

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