In Vitro Antimicrobial Potential And Phytochemical Screening Of Ethanolic Leaf Extracts Of Euphorbia Hirta

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Citation

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Abstract

Recent attention in plant-derived extracts or compounds has risen in response to the spread of antimicrobial resistance and negative side effects associated with synthetic antimicrobials. *Euphorbia hirta* is a member of Euphorbiaceae family. This work was carried out to assess the antimicrobial activities and phytochemical constituents of leaf extracts of *Euphorbia hirta* against some microorganisms (*Staphylococcus aureus, Escherichia coli, Proteus spp, Candida albicans, Aspergillus flavus*, and *Fusarium* species). The antibacterial activity was done using the agar well diffusion technique. Concentrations of 50mg/ml, 100mg/ml, 200mg/ml and 400mg/ml were made. Ornidazole and Dimethyl Sulfoxide (DMSO) were used as positive control and negative control respectively. Antifungal activity was carried out using the agar dilution technique and miconazole and dimethyl sulfoxide (DSMO) were respectively used as positive and negative controls. The result showed that 400mg/ml of the extract consistently showed the highest zone of inhibitions with the highest antibacterial effect on Staphylococcus aureus. The extract also exhibited antifungal activities of varying degrees against the molds. *Aspergillus spp* and *Fusarium spp* showed no growth in agar containing 400mg/ml and 200mg/ml. Qualitative phytochemical studies exhibited the presence of saponins, flavonoids, terpenoids, alkaloids, and glycosides. The findings show that *E. hirta* extracts have promising antibacterial potential.

INTRODUCTION

Given the increasing number of antimicrobials available for various purposes, the search for new drugs is a never-ending labor because target microorganisms frequently develop new genetic variants that are resistant to currently available antimicrobial, limiting the effective shelf life of any antibiotic [1]. Recent interest in plant-derived preparations or compounds has risen in response to the unusual spread of antimicrobial resistance and negative effects associated with synthetic antimicrobial drugs [1, 2]. Because the natural compounds are often considered to be safer than synthetic ones, the world's attention is increasingly focusing on natural sources for production of antimicrobial medications [1]. More than 80% of the world's population relies on medications produced from these medicinal herbs for primary health care, according to the World Health Organization (WHO). The usage of medicinal plants as a source of disease alleviation may be dated back to the beginning of recorded history. These phytomedicines are both effective and eco-friendly [4, 5]. Phytomedicines are becoming more popular, and their use is becoming more widespread. Phytochemicals are a diverse group of bioactive compounds produced by plants. They are classified as secondary metabolites since the plants that produce these phytochemicals may not have for need the compounds [6]. Secondary metabolites are produced in all parts of the plant, including the bark, leaves, stem, root, flower, fruits, and seeds [7]. The number of phytochemical substances varies greatly between species and even between plants, depending on age and environmental and climatic factors [8]. In the current era of emerging infectious diseases and antimicrobial resistance, new infection-fighting strategies are urgently required to tackle microbial infections [9]. *Euphorbia hirta* is a member of the Euphorbiaceae family and belongs to the genus Euphorbia [10]. It's a short annual herb that is wild in tropical areas. It can reach a maximum height of 40 cm. *E. hirta* is a common herb among traditional herbal medicine practitioners. It's also known as a pill-bearing purge and asthma herb. The plant's stem is thin and reddish, with yellowish bristles covering it, especially in the immature parts of *E. hirta* [5]. The search for a more natural, alternative source of disease therapies has become a global

priority. The cost of therapy with synthetic medications, as well as the hazards associated with their continued use, necessitates the employment of a different treatment strategy. Currently, phytotherapeutic applications of our native plants are a welcome development. *E. hirta* is a tropical plant that can be found in both urban and rural areas, and if its antimicrobial properties are confirmed, it will mean a more wholesome, easily accessible phytotherapeutic alternative to more conventional pharmaceuticals antimicrobials, thereby contributing to the improvement of the healthcare system in our environment.

MATERIALS AND METHODS

PLANT COLLECTION AND TAXONOMY

Fresh leaves of *Euphorbia hirta* were obtained from bushes in Enugu State, Nigeria. The plant was properly identified and authenticated by the Department of Plant Science and Biotechnology, University of Nigeria, Nsukka.

EXTRACTION OF PLANT MATERIAL

Euphorbia hirta leaves were washed and laid out to air dry. Using an electric blender, the dried leaves were powdered to a fine consistency. The sample was loaded into the thimble and placed into the main chamber of the soxhlet extractor. Ethanol was added to the round bottom flask and placed onto the heating mantle. The soxhlet extractor was then attached above the round bottom flask. The solvent was refluxed for hours. To obtain the crude, the extracts were concentrated under decreased pressure using a rotator evaporator. The result was a dark green semi-solid mass. The extract was kept at 4°C in the refrigerator until it was needed.

TEST ORGANISMS

The test organisms used were six different isolates including *Staphylococcus aureus, Escherichia coli, Proteus spp.*, for bacteria, *Candida albicans, Aspergillus flavus, Fusarium spp*, for the fungi. The bacterial isolates including *Candida albicans* were sourced from clinical samples at the Microbiology Department of the University of Nigeria Teaching Hospital, Ituku Ozalla Enugu while molds were obtained from Research Laboratory in Enugu. The isolated organisms were properly identified by their colonial morphology, Gram-reactions, microscopic appearance, and specific biochemical reactions.

ASSAYS FOR ANTIBACTERIAL ACTIVITY

The antibacterial susceptibility of plant extracts on selected gram-positive and gram-negative bacteria was determined using an agar well diffusion experiment. The test organisms were inoculated onto Muller Hinton medium using a standardized bacterial suspension. On each plate, a sterile cork borer with a diameter of 5mm was used to make wells, with two of the wells put aside as controls. With the help of plastic pipettes, about 50µl of different concentrations (400mg/ml, 200mg/ml, 100mg/ml, and 50mg/ml) were put into the wells. As a positive control, the antibiotic Ornidazole was utilized. Each well was labeled correctly. The inoculated Petri dishes were left for the extract to diffuse before placing them in the incubator at 37°C for 24 hours for the growth of test organisms after which zones of inhibition were observed. A ruler was used to measure and record the diameter of the inhibitory zones. The tests were done in triplicate.

ASSAYS FOR ANTIFUNGAL ACTIVITY

The Agar dilution method was used for the determination of the antifungal activity of *Euphorbia hirta* ethanolic and hexane extract. The same 1000mg/ml of extract was made by dissolving 10g of extract in 10ml of DMSO. Dilution of the plant extract and the Sabouraud dextrose agar at 45°C containing chloramphenicol was made to get 10mls of agar for different concentrations, 400mg/ml (4.0ml of plant extract + 6.0ml of Sabouraud agar), 200mg/ml (2.0ml of plant extract 8ml of Sabouraud agar), 100mg/ml (1.0ml of plant extract + 9ml of Sabouraud agar) and 50mg/ml (0.5ml of plant extract + 9.5ml of Sabouraud agar respectively for ethanol and hexane. The plates were allowed to be set and dried in the incubator and a 4mm size of agar blocks from the 3-day old culture of molds (*Aspergillus flavus, Fusarium species*) was seeded on the agar. Miconazole was used as positive control and DMSO was used as a negative control. They were all incubated at room temperature and monitored daily for growth. The growth extension of the molds was measured using a meter rule for three consecutive days and the measurements were compared with that of the positive and negative control.

Antifungal activity of the extract for *Candida albicans* was done the same way the antibacterial activity was done. It was incubated at 37°C for 24 hours and the antifungal activity was determined by measuring the diameter of the inhibitory zone generated using a meter rule. The experiment was also, repeated three times, with the findings taken as the mean of the readings for the molds, and the percentage inhibition of

diameter growth was determined as follows:

% inhibition = C-T ÷C×100

Where C= diameter growth of negative control

T= diameter of the test.

PHYTOCHEMICAL ANALYSIS (QUALITATIVE)

Preliminary phytochemical analysis of the homogenous fine powder of Euphorbia hirta was done following standard procedures [11, 12, 13]. Qualitative analysis was carried out to identify the phytochemical compounds in the plant. Twenty grams of the sample was soaked in 100mls of ethanol for not less than 24hours. The extract was decanted and heated for 3minutes to concentrate it. The presence of alkaloids, saponin, flavonoids, steroids, phenols, glycosides, and tannin were checked for.

Tests for alkaloids

One gram (1g) of the extract was immersed in 5 ml of 10% ammonia solution and extracted with 15 ml of chloroform. The chloroform was vaporized to dryness, and remaining part was dissolved in 15 ml dilute sulphuric acid. A part of the solution was used to test for the general alkaloid, while the rest was used for specific assays.

When some drops of Mayer's reagent were introduced into the test tube containing the acidic solution, the appearance of an opalescence or yellowish precipitate demonstrated the presence of alkaloids.

In the second test tube, 2 ml of acidic solution were neutralized with a 10% ammonia solution. Dragendorff's reagent was introduced, and turbidity or precipitate was detected, indicating that alkaloids were present.

Test for Saponins

Frothing and emulsion are the two parameters that make up this test

In the frothing test, the crude extract was diluted in a test tube with 5mls of distilled water. The mixture was shaken vigorously for 5 minutes to observe for froth.

For the emulsion test, 3 ml of extract were added into a test tube, along with 5 drops of olive oil, and the mixture was agitated vigorously and checked for emulsion.

Test for Flavonoids

In a test tube, 5 ml of crude extract was mixed with 0.1g of metallic zinc, and 8ml of concentrated sulphuric acid was added to the mixture. Red coloration in the mixture indicated the presence of flavonoids.

Test for Steroids

A 0.5g extract was dissolved in 10ml anhydrous chloroform and then filtered. For the tests that followed, the solution was split into two equal parts. In the first part of the solution, one ml of acetic anhydride was introduced and then I ml of concentrated sulphuric acid was added down the side of the test tube to produce a layer underneath. The appearance of green coloration in the test tube demonstrated that steroids were present.

Salkowski's test

The second part of the solution was then mixed gently with concentrated sulphuric acid to produce a lower layer of acid. Terpenoids are demonstrated by the presence of a reddish-brown tint at the interface.

Test for Tannin

In a test tube, two milliliters (2 ml) of the extract were added and gently heated for two minutes before being allowed to cool. Three drops of ferric chloride solution were added. A green precipitate indicates the presence of tannin.

Glycoside Test

Two milliliters (2ml) of crude extract was added to 5 ml of equal volumes of Fehling's solutions A and each. The mixtures are then boiled in a water bath. A brick-red precipitate at the bottom of demonstrated the presence of sugars/glycosides.

STATISTICAL ANALYSIS

Graphpad prism version 7.0 (Graphpad, San Diego, CA, USA) was used for statistical analysis. Ordinary one-way Analysis of variance (ANOVA) and student t-test were used for comparison of mean differences between and among groups respectively at 95% confidence interval. P-value ≤ 0.05 is considered statistically significant.

RESULTS

The result on table 1 shows the comparisons of the ZIDs of the ethanol extract and control drug against the test organisms. From the table, 400mg/ml of the extract consistently showed the highest ZIDs on all the tested

microorganisms with the highest antimicrobial effect on *Staphylococcus aureus* with the least on *Proteus mirabilis*. The 50 mg/ml concentration showed the lowest ZID. Ordinary one-way ANOVA showed a statistically significant difference ($p<0.0001$) in the mean ZIDs of individual test organisms with respect to different extract concentrations and the control antibiotic (Ornidazole). Similarly there were significant differences ($p<0.05$) in the antimicrobial activity of different concentrations of the extract when compared across the different microbial isolates.

Table 1

Comparing the antimicrobial activities of Ethanol Extracts on the Test Organisms

	Mean zone of inhibition (mm) ± SEM				
Extract Conc. (mg/ml)	*Staph aureus*	*Escherichia coli*	*Proteus spp*	*Candida albicans*	P-value
400mg/ml	15.04 ± 0.28	13.11 ± 0.08	12.24 ± 0.10	10.14 ± 0.07	0.0431
200mg/ml	13.08 ± 0.30	12.27 ± 0.11	10.67 ± 0.15	9.27 ± 0.12	0.0278
100mg/ml	11.53 ± 0.10	10.57 ± 0.06	8.62 ± 0.18	8.38 ± 0.20	0.0336
50mg/ml	8.42 ± 0.51	8.25 ± 0.17	7.68 ± 0.08	7.50 ± 0.03	0.0643
Positive Control	19.37 ± 0.39	17.58 ± 0.15	25.15 ± 1.12	26.27 ± 0.04	<0.0001
P-value	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	

The result in table 2 shows the percentage inhibition diameter of growth (PIDG) for *Aspergillus spp* and *Fusarium spp* on agar plates containing different concentrations of the ethanol extract. From the table, *Aspergillus spp* and *Fusarium spp* showed lower PIDG in culture media containing 100mg/ml and 50mg/ml of extract concentration when compared with the 200mg/ml a, 400mg/ml, and the positive control (miconazole) plate. The table shows *Aspergillus spp* and *Fusarium spp* had the highest percentage inhibition diameter of growth (PIDG) of 100% at concentrations of 200mg/ml and 400mg/ml of the ethanol extracts. There was also no growth seen in culture plates containing the positive control (miconazole).

Table 2

Percentage inhibitory effects of ethanol extract on Aspergillus spp, Fusarium spp and control drug

	Percentage Inhibition Growth	
Extract Conc. (mg/ml	***Aspergillus spp***	***Fusarium spp***
400mg/ml	100%	100%
200mg/ml	100%	100%
100mg/ml	88.40%	82.20%
50mg/ml	83.20%	68.60%
Negative Control	0%	0%
Positive Control(Miconazole)	100%	100%

Table 3 shows the phytochemical study results demonstrating that *Euphoria hirta* possesses alkaloids, saponins, glycosides, flavonoids, and terpenoids.

Table 3

Preliminary Phytochemical screening of ethanol leaves Extract of Euphorbia hirta

Phytochemical constituents	Results
Alkaloids	+
Saponins	+
Tannins	-
Glycosides	+
Flavonoids	+
Terpenoids	+

+= Presence of the phytochemical while -= absence of the phytochemical

DISCUSSION

Natural plants have sparked curiosity as alternatives to synthetic antimicrobials over the years. Herbs used in traditional medicine have a high level of antimicrobial activity. Herbal products are increasingly being used as food supplements and in modern medicine [14]. The genus Euphorbia is a member of the family Euphorbiaceae, comprising about 2000 species occupying a multitude of habitats. The presence of latex is a unique characteristic of the family. The plant is routinely used by traditional healers to combat various ailments like amoebiasis, diarrhea, peptic ulcers, heartburn, vomiting, afflictions of the skin and mucous membranes and respiratory disorders, cough and colds [15, 16]. Antibacterial and antifungal activities of the ethanol leaf extracts of *E. hirta* have been evaluated in the present research work. The in vitro study of antimicrobial of plant extracts might be a first step toward the production of novel medicines. The antibacterial and antifungal properties of *E. hirta* against a range of microorganisms were assessed in this study (*Staphylococcus aureus, Escherichia coli, Proteus spp*) and fungi (*Aspergillus flavus, Fusarium spp*, and *Candida albicans*), the results obtained are consistent with the reports available. *E. hirta*'s efficacy on bacterial isolates has been studied by several researchers, with promising results [5, 9]. our work reported a smaller zone of inhibition when compared to some of the work. This could be due to the number of phytochemical compounds present, as well as the strain of organisms, which, according to some studies, varies greatly from species to species, and even from plant to plant, depending on age and environmental and climatic conditions [17]. The extract was also found to be most effective on gram-positive bacteria *Staphylococcus aureus*. This agrees with the work of some researchers that reported that plant extracts are more potent on Gram-positive organisms than gram-negative [18]. The large inhibitions

zones are shown by the extract against *S. aureus* justify the plant used in the treatment of sores, boils, and open wounds. The smaller zones of inhibitions shown by Gram-negative bacteria could be due to the presence of a thick phospholipid and lipopolysaccharide outer membrane layer that shields them from environmental influences and renders them highly resistant especially to synthetic antimicrobials [1, 19]. This study is at variance with the work of Nazeer [9] who reported a greater zonal inhibitory diameter on gram-negative organisms, *Escherichia coli* (22 ±1.04), *Pseudomonas aeruginosa* 20.17±1, *Klebsiella pneumoniae* (19), and *Staphylococcus aureus* (17.81± 3.61).

Antifungal activities of ethanolic extracts of *E. hirta* done were compared with miconazole nitrate. Miconazole is an antifungal drug from the azole family that is used to treat fungal infections. They show antifungal activity by inhibiting the enzyme lanosterol 1, 4-demethylase, which is required for the conversion of lanosterol to ergosterol, the most common sterol in fungal cell membranes and necessary for maintaining cell integrity, viability, function, and normal growth [20]. Results showed that the extracts displayed antifungal activity against *A. flavus*, *Fusarium spp*, and *Candida albicans*. The herb showed resistance at lower concentrations on the isolates, but total inhibition at a higher concentration. *E. hirta* has been shown to have a biochemically related compound with inhibitory effects similar to miconazole, as well as antifungal activity that varies with extract concentrations [20]. Our result agrees with the work of Nazeer [9] but is at variance with the study of Ahmad [5] who reported no activity on fungi. Karanga and Ndam also reported the antifungal activity of *E. hirta* on *Fusarium spp*. [20, 21.]

The screening of medicinal plants for antibacterial activity has become necessary in the search for remedies to the global increasing antibiotic resistance. Active efflux plays a crucial role in the development of both acquired and innate bacterial resistance; according to studies into the mechanisms of bacterial resistance. As a result, overcoming efflux has been seen as a reasonable alternative to avoiding the problem. Some plants have since been found to have bacterial efflux pump inhibitors [22]. When MDR inhibitors are combined with antibiotics in vitro, the activity of some drugs is greatly boosted, even against antibiotic-resistant bacteria strains. Plants produce a wide range of chemicals that have been shown to have therapeutic properties as antimicrobials and resistance modifiers. The African biosphere, which has the highest biodiversity of plant species, has the potential to be a source of therapeutically beneficial chemicals, particularly when combined with antimicrobial therapy [22]. Euphoria hirta can serve as one of these resistance modifiers. Secondary metabolites including alkaloids, saponins, glycosides, flavonoids, and terpenoids were detected in *E. hirta*. Alkaloids are organic heterocyclic nitrogen compounds that generate basic water-soluble salts. They have a variety of pharmacological properties. They generally have analgesic properties. They can intercalate with DNA, causing impaired cell division and death. This feature is thought to be the mechanism of alkaloid activity [23]. Photosynthesizing cells contain flavonoids. The interaction of flavonoids with microbial cell membranes is responsible for their antibacterial effect against several bacterial and fungal pathogens [24, 25]. Membrane permeability and rupture are caused by interactions with membrane proteins present on the bacterial cell wall [25]. Terpenoids have also been shown to have antimicrobial properties. Terpenoids' antibacterial activity is not well understood, however, it is thought to be due to the breakdown of the microbial membranes [26].

CONCLUSION

Our work has supported other works that *Euphorbia hirta* plant (Asthma weed) has both antifungal and antibacterial activities which increases with the increase in concentration. *E. hirta* is an herb that is often used by traditional healers to cure a variety of ailments. *E. hirta* extracts have promising antimicrobial potentials, nevertheless, the ornidazole, which was the control drug for bacteria had a larger inhibition zone on test organisms.

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