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PRELIMINARY PHYTOCHEMICAL SCREENING AND ANTIBACTERIA/ANTIFUNGAL ACTIVITIES OF LEAF EXTRACTS OF EUPHOBIA HIRTA

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ABSTRACT
The phytochemical Screening, Antibacterial and Antifungal activities of the leaf extracts of Euphorbia hirta , was evaluated, using ethanol and hot water as a solvent to determine the active components. Maceration method was adopted in extracting the active components. This revealed the presence of saponins, alkaloids, flavonoids, phenol, tannins, and steroids. Antibacterial screening of clinical isolates of Staphylococcus aureus and E. coli, using disk diffusion method, indicated a high sensitivity for S. aureus and a low sensitivity for E. coli. Ethanol extracts were more active than hot water extracts. Inhibition zones for Staphylococcus aureus and Escherichia coli at 100mg/ml and 50mg/ml was 10mm, 7mm, 7mm, and 5mm respectively. For hot water extracts. No inhibition zone was observed at 50mm/ml for both microorganisms. Both had 5mm at 100mm/ml. Screening of clinical isolates of Mucor species, Candida albican,and Aspergillus species using agar well diffusion method showed high sensitivity for Mucor species, and Candida albican. Aspergillus species had low sensitivity. Inhibition at 100mg/ml, 50mg/ml/25mg/m was15mm,12mm,10mm,/12mm,5mm,10mm,/11mm,6mm.-ethanol extract. For hot water at 100mg/ml, 50mg/ml, inhibition zone was 13mm, 10mm, 5mm,/11mm,5mm 6mm.Aspergillus sp.and Candida aureus, had no inhibition at 25mm/ml/50mm/ml. This result suggests that Euphorbia hirta can be used in traditional medicine as remedy for infections caused by the test microorganisms as well as in the treatment of such ailments.
Key words: Phytochemical, sensitivity, inhibition zone, antibacterial, antifungal, extraction.

INTRODUCTION

In recent years, multiple drug resistance in human pathogenic micro-organisms, has developed, due to indiscriminate use of commercial antimicrobial drugs, commonly used in the treatment of infections. This situation, coupled with the undesirable side effects of certain antibiotics and the emergence of previously uncommon infections, is of serious medical concern, (Ahmed, et al; 1998). Many infectious diseases, have been known to be treated with herbal remedies, throughout the history of mankind. Thus the continuous and urgent need to discover, new antimicrobial compounds with diverse chemical structures and novel mechanisms of activities, for new and re-emerging infections/infectious diseases (Begum, et al; 2003).In modern medicine, plants play very important roles as raw materials, for some important drugs. The herbs provide starting materials, for the synthesis of conventional drugs. Medicinal plants have curative actions, due to the presence of complex chemical constituents. (Farnsworth, 1994). According to world health organization (WHO), more than 80% of the world's population rely on traditional medicines for their primary health care needs. Euphorbia hirta known in the Philippines as Tawa-Tawa, or Gatas Gatas. It is known in Nigeria by the Yorubas as 'Akun Esano, Iroko, or Iju, . "Kadanya" ' or Nonkuruyaa in Hausa, Itasin Uloko in Edo. Igbos-Owerri people call it Ba-ala. In Mbaise precisely, in Imo State, Igbos and indigenes call it Ogbu na Izu (that which kills within one week or eight market days).It is commonly used in treating eczema (Ugwa) in Igbo land. It is also included, in the herbal combinations used in cooking for women who newly put to birth- i.e. (Aju Mbaise).The plant specie, is an annual prostrate weed of the family Euphorbiaceae. Euphorbia is the largest genus in this family, with

about 600 species. It is characterized by the presence of white milky latex, which is more or less toxic. Lattices of Euphorbia, ingens, Euphorbia, mey, Euphorbia tiruscalli and Euphorbia, trianglaris, are possible sources of rubber. This milky juice exuded by Euphorbia, when broken is more or less poisonous and is used as an ingredient in arrow poison (Holodzie, et al; 2005). Euphorbia hirta is a slender-stemmed annual hairy plant with many branches from base to top, spreading up to 40cm in height. The leaves are greenish in colour. The stem is rounded, solid, hairly with abundant milk sap. The flowers are unisexual and found in axillary cymes at each leaf node. They lack petals and are generally on stalk. The fruit is a capsule, with three valves and produces tiny oblong four-sided red seeds. It has a white or brown tap root. (Williamson, 2002). The pharmacological importance of the indigenous plant herbs and fibres over the years, have been overlooked by the scientific world. However, photochemical investigations on these plants, their components, have revealed that many of these plants, posses anti-malarial, antiviral, and antifungal, antibiotic purposes.

ETHNOPHARMACOLOGY

Euphorbia hirta is used in the treatment of gastro- intestinal disorders (diarrhea, dysentery, intestinal parasitoids etc), Bronchial and respiratory diseases (asthma, bronchitis, hay fever etc), (Singh and Sinha 1996).Aqueous extracts also inhibit aflatoxin contanuavation, in rice, wheat and grains. Again aqueous extracts, exhibits auxiolytic, analygestic sap used in the treatment eyelid styles. Leaf poultrice is used on swellings (Williamson 2002). Roots of E. hirta, are used for snake bites (Williamson 2002). The Palypheralic extracts have amoebic (Tona et al; 2000) and antispasmodic activity (Gnecco, et al; 1996), Alcoholic extracts of whole plants, show hypoglycemic activity in rats (sood, et al; 2005). Again it has a sedative effect on the genital urinary tract (Kirtotar, et al; 2003).

MATERIALS AND METHODS

Leaves of Euphorbia hirta were collected from the surroundings of Federal University of Technology, Owerri. Clinical isolates of staphylococcus aureus, E. Colis, Mucor species, Aspergillus species and Candida albican, were also used. S. aureus was isolated from the skin, E. coli.¸and C. albican from urine, Mucor species and Aspergillus species from fruit justice kept open over night. The bacterial, was cultural on a nutrient and Mac Conkey agar, while the fungi was cultured on SDA.(Sabourand Dextrose Agar). Fresh leaves properly washed and rinsed in sterile water, where oven dried at 40^{o}C for two days. The dry leaves were pulverized using mechanical grinder machine and stored in air tight containers. Extraction of plant materials, was done with ethanol and hot water. For hot water, 10g of sample was soaked in 200 mls of hot water in an air tight container, which was then mixed vigorously by shaking, allowed to stand for two days with periodic shaking, every 24hrs. Extracts were then filtered off with sterile paper (Whitman no 1). For ethanol plant extract, soxhlet extraction method was used. Filtrate was evaporated to dryness at 40^{o}C in a vacuum and stored at 50^{o}C in a refrigerator for use. Freshly prepared ground samples were chemically tested for the presence of chemical constituents using a standard procedure (Trease and Evans, 1983). Both aqueous and ethanolic extracts were screened for their photochemical basis using, the standard method of Harbone, (1973).Extraction methods employed include: Maceration, Percolation, Digestion, Infusion, Decoction.

RESULTS

PHYTOCHEMICAL SCREENING OF PLANT EXTRACT

Table 1: RESULT ON THE PHYTOCHEMICAL SCREENING

Botanical Name	Common Name	Steroids	Saponin	Tannins	Flavonoid	Alkaloid	Phenol
Euphorbia Hirta	Asthma weed	+	+	+	+	+	+

KEY: (+) POSITIVE (-) NEGETIVE

This test, revealed the presence of Tannis, Saponins, Alkaloids, Flavonoids, Steroids, Phenol, in Euphorbia hirta.

COLONY MCIROSCOPY AND BIOCHEMICAL CHARACTERISITCS OF BACTERIAL ISOLATES USED IN THE WORK

Table 2: IDETIFICATION OF BACTERIA USED IN THE RESEAERCH WORK

Colony Morphology	Gram reaction	Shape	Catalase	Motility	Coagulase	Oxidase	Probable organism
Creamy Circular Colonies on Nutrient Agar, pink on MacConkey Agar	-ve	Short Rods	+ve	+ve	-ve	+ve	Escherichia Coli
White tiny Colonies on Nutrient agar	+ve	Coci	+ve	-ve	+ve	-ve	Staphyloco - ccus aurerus

ANTIMICROBIAL SENSITIVITY TESTING OF HOT WATER AND ETHANOL EXTARCTS OF THE PLANT EUPHORBIA HIRTA

Table 3: Diameters of Inhibition of Bacteria Growth by Leaf Extracts of Euphorbia Hirta

SOLVENT	CONCENTRATION	ORGANISMS	
		Staphylococcus	Escherichia coli
ETHANOL	100mg 50mg	10mm 5mm	12mm 10mm
HOT WATER	100mg 50mg	5mm -	10mm 5mm

The zone of inhibition was recorded, by checking the diameter of the clearance area, from one end across the disc to the other end in millimeter. For Staphylococcus aureus at 100mg ,the inhibition zone was 10mm and 7mm. At 100mg for hot water extract it was 5mm and no clear zone at 50mg. Escherichia coli, at 100mg, the inhibition zone was 7mm and 5mm with 50mg and for water extract with 100mg the inhibition zone was 5mm and no clear zone with 50mg. Staphylococcus aureus had higher zones of inhibition than that of the Escherichia coli.

TABLE 4: DIAMETERS OF INHIBITION ZONE RECORDED ON THE FUNGI USING ETHANOL AND HOT WATER EXTRACT

SOLVENT	CONCENTRATION	ORGANISMS		
		Aspergillus species	**Candida albican**	**Mucor** species
Ethanol	100mg 50mg 25mg	10mm 5mm -	12mm 10mm 6mm	15mm 12mm 11mm
Hot water	100mg 50mg 25mg	5mm - -	10mm 5mm -	13mm 11mm 6mm

From the result, Mucor species has the highest zone of inhibition of 15mm with 100mg of ethanol, 12mm with 50mg and 11mm with 25mg of ethanol extract, for hot water extract it has 13mm with 100mg, 11mm with 50mg and 6mm with 25mg of hot water extract. This is followed by Candida albican which has, inhibition zone of 12mm with 100mg of ethanol extract, 10mm with 50mg and 6mm with 25mg of ethanol extract and 13mm with 100mg of hot water extract, 11mm with 50mg. No clear zone was seen with 25mg. The least zone of inhibition is observed in Aspergillus species

Table 5a: CONTROL WITH COMMERCIAL DISK FOR BACTERIA

Micro organisms	Erythromycin	Gentamycin	Streptomycin	Tetracycline	Chloramphenicol
Staphylococcus aureus	Nil	12mm	10mm	8mm	13mm
Escherichia coli	Nil	Nil	11mm	Nil	Nil

Erythromycin showed no inhibition for both Staphylococcus and Escherichia coli. 12mm inhibition was recorded on Staphylococcus aureus using Gentamycin. Streptomycin had 10mm, Tetracycline 8mm and Chloramphenicol 13mm. Using Gentamycin on Escherichia coli, no inhibition was observed. Streptomycin had 11mm. inhibition. Tetracycline and Chloramphenicol no inhibition.

Table 5b: ANTIBIOTIC USED AS CONTROL FOR FUNGI

Micro organisms	Erythromycin	Gentamycin	Streptomycin
organism	Econazole	Clotrimoxazol	Fluconazol
Mucor spp	12mm	Nil	9mm
Aspergillus spp	6mm	15mm	6mm
Candida albican	8mm	10mm	20mm

The highest zone of inhibition was observed in Mucor species when Econazole was used against the three organisms. Aspergillus species showed the highest inhibition when Clotrimoxazole was used against the three organism, Candida albican showed the highest inhibition when Fluconazole was used against the three organisms.

DISCUSSION

The results of this study revealed that the crude leaf extracts of Euphorbia hirta, contains Saponins, Alkaloids, Flavonoids, Steroids, Phenol, and Tannins. Hostettman et al., [1995] reported the presence of these substances in Euphorbia hirta. The inhibition effects of the leaf extracts on the test organisms may be due to the presence of these phytochemical components. Although the leaf extract showed limited activity against the bacteria, Escherichia coli and the fungi, Aspergillus species, marked activity was exhibited against the bacteria Staphylococcus aureus and the fungi, Mucor species. The low activity recorded in the bacteria Escherichia coli and in the fungi, Aspergillis species using ethanol and hot water extracts, corresponds to the work of [Abubakar, 2009]. This is in line with the works of Kela and kujefi [1995] who reported that antibiotics are not the only antimicrobial agents. The results of the antibacterial screening of the plants extract showed that ethanol extract of 100mg/ml concentration on staphylococcus aureus had inhibition zone of 10mm while Escherichia coli showed 7mm. At 50mg/ml, it had 7mm for Staphylococcus aureus and 5mm for Escherichia coli. The aqueous extract of 100mg/ml for staphylococcus aureus showed inhibition zones of 5mm and 5mm for Escherichia coli. At 50mg/ml concentration there was no inhibition. The results of the Antifungal screening of the plants extracts showed that the ethanol extract at 100mg/ml, Aspergillus species had inhibition zone of 10mm. Candida albican had 12mm and Mucor species had 15mm. at 50mg/ml Aspergillus species had 5mm, Candida albican had 10mm and Mucor species had 12mm. at 25mg/ml, Aspergillus species had no inhibition zone, Candida aibican had 6mm and Mucor species showed 11mm inhibition zone. At 25mg/ml, Aspergillus spp and Candida albican had no inhibition .Mucor spp had showed inhibition zone of 6mm.

CONCLUSION

The leaf extract of Euphorbia hirta used in the study possesses antibacterial and antifungal properties and the constituents of the plant extract can be useful in the chemotherapy of some microbial infections. The results of phytochemical screening reveals the presence of steroids, tannins, saponins, flavonoids, alkaloids and phenol in Euphorbia hirta since activities of the ethanol and hot water plant extracts were comparable to that of the standard antibiotics. It is therefore inferred, that the degree of activity of a plants extract is a collective function of the type of the extraction solvent used, organisms involved and the specific concentration of the bioactive agent present. Again, inhibitory effect of ethanol extracts were more than that of hot water extracts.

RECOMMENDATION

It is therefore recommend that more development in the extraction methods should be carried out in order to further investigate the active compound. A combined microbiological and biochemical screening as well as an extensive phytochemcial study of wide array of available plants species and their extracts for bioactive agents is essential in order to isolate the particular chemical compounds and actual active ingredients that are responsible for observed antimicrobial effects on particular organisms by the plant under study.

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