Preliminary Investigation into the Anti-Microbial Properties of Methanolic and Aqueous Extracts of the Leaves of *Euphorbia hirta* Linn (Family: Euphorbiaceae)

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Abstract

The methanolic and water extracts of the leaves of Euphorbia hirta (Linn) were screened for antimicrobial activities. The antimicrobial properties were investigated using the sensitivity test and minimum inhibitory concentration (MIC). The results obtained showed that Euphorbia hirta (Linn) has antimicrobial properties on Staphylococcus aureus, Pseudomonas aeruginosa, Candida albicans, and Aspergillus niger. The extracts showed greater activity against the bacteria organisms than the fungal organisms.

Key words: Antimicrobial activity, Methanol extract, Water extract, *Euphorbia hirta*

Introduction

Traditional medicine can be defined as the total combination of knowledge and practice, whether explicable or not, used in diagnosing, prevention or eliminating physical, mental and social disorders. This may rely exclusively on past experience or observation handed down from generation to generation while bearing in mind the original concept of nature which includes the material world, the sociological environment whether living or dead and the metaphysical forces of the universe (Sofowora, 1986; Berdy, 1982). Disease treatment and preventing in Nigeria were for many years handled solely by traditional healers (herbalists) who inherited the act from their fore-fathers. The traditional medical practitioners make use of largely plant parts (Alake and Irobi, 1992; Ebi and Ofoefule, 1997). One of such plants is *Euphorbia hirta* (Linn). This plant is known and used by many natives in the treatment of candida infections especially in the infants. The aims and objectives of this research therefore, are to study in details, the antibacterial and antifungal properties of the plant *Euphorbia hirta* (Linn) so as to validate or otherwise the claim of the herbalists, and also to improve the existing usage.

Materials and Methods

The following materials were used as procured: methanol (Riedel de Haen, Hanover, Germany), nutrient agar (Oxoid, England), and Sabouraud Dextrose Agar (Lab' M, England).

Collection, identification (authentication) and preparation of plant parts: The aerial parts of the plant *Euphorbia hirta* (Linn) were collected from Akanu Ibiam Stadium, University of Nigeria, Nsukka. It was identified and classified by Mr. J. N.C. Ekekwe, a plant taxonomist with Botany Department, University of Nigeria, Nsukka. The leaves were separated from the rest of the plant and sun-dried for 2 days. With the aid of a milling machine, the dried leaves were reduced to fine powder and stored in airtight containers until used.

Methanolic extraction: One hundred grams of the dried leaves of *Euphorbia hirta* (Linn) was weighed and soaked in methanol and the mixture was then allowed to stay for 18 h, a process known as cold maceration. The mixture was passed through filter paper and the greenish coloured filtrate collected into a clean pre-weighed large-surface area, evaporating dish and evaporated on a boiling water bath. The weight of the residual extract was obtained and recorded.

Aqueous extraction: Another 100 g of the dried leaves of *Euphorbia hirta* (Linn) was weighed and soaked in water and the mixture was then allowed to stay for 18 h. The mixture was then passed through filter paper and a yellowish-orange coloured filtrate collected into a clean pre weighed large-surface area dish. The weight of the residual extract was obtained and recorded after evaporation on a water bath.

Stock solution: A 500 mg quantity of the extract was weighed and dissolved in 5 ml of the solvent (dimethylsulphoxide or water), to give a concentration of 100 mg/ml, which was then used as the stock solution.

Using the 100 mg/ml a two-fold serial dilution method was carried out to obtain the following concentrations: 100, 50, 25, 12.5, 6.25 (mg/ml).

Determination of minimum inhibition concentration (MIC): Dilution of the extract was made using 500 mg of the dried leave extract of *Euphorbia hirta* dissolved in 5 ml of the solvents (DMSO or water) to give 100 mg/ml. This was serially diluted (two-fold) to obtain five different

concentrations and these used to determine the MIC for both the fungi and bacteria. Two drops of the diluted, extracted material of *Euphorbia hirta* (Linn) was introduced into holes in an agar plate seeded with either *Candida albicans*, *Aspergillus niger*, *Staphylococcus aureus*, or *Pseudomonas aeruginosa* to determine the activity of the extract. After overnight incubation (24 h) at 37 °C for bacteria and room temperature (25 °C) for fungi, the zones of inhibition were recorded. A plot of the log concentration against inhibition zone diameters was used to determine the MIC of the agent. Water or dimethylsulphoxide was used as the control.

Results and Discussion

From the results in Tables 1 and 2, the methanolic and water extracts of the powdered leaves of *Euphorbia hirta* (Linn) have some antimicrobial activities against *Staph. aureus*, *Ps. aeruginosa*, *Candida albicans* and *Aspergillus niger*. This wide spectrum makes the materials a very good antimicrobial agent.

Table 1: Results of preliminary investigation of the antimicrobial effects of the extracts (methanol extract)

Organisms	Activity growth	Zone of inhibition (mm)
Staphylococcus aureus	+	23
Pseudomonas aeuginosa	+	22
Candida albicans	+	15
Aspergillus niger	±	12

Key: - No growth inhibition, + Growth inhibition, ± Partial growth inhibition (Bacteriostatic effect). Both the dimethylsulphoxide and water showed no activity.

Table 2: Results of preliminary investigation of the antimicrobial effects of the extracts (water extract)

Organisms	Activity growth	Zone of inhibition (mm)
Staphylococcus aureus	+	22
Pseudomonas aeruginosa	+	17
Candida albicans	±	12
Aspergillus niger	-	-

Key: No growth inhibition, + Growth inhibition, ± Partial growth inhibition (Bacteriostatic effect). Both the dimethylsulphoxide and water showed no activity.

When water, dimethylsulphoxide and methanol were used, no activity was noted. The MIC of the methanolic extract against *Staphylococcus aureus* is 2.0 mg/ml, *Pseudomonas aeruginosa* is 3.2 mg/ml, *Aspergillus niger* is 5.0 mg/ml, and *Candida albicans* is 2.5 mg/ml as shown in Table 3. For the water extract, the MIC against *Staphylococcus aureus* is 2.5 mg/ml and *Ps. aeruginosa* is 2.0 mg/ml. The MIC were extrapolated from the plots in Fig. 1. The activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* was consistently more pronounced in the entire test than against the test fungi. Though the concentration of the extract that showed antimicrobial activity against the organisms is high when compared to those standard antibiotics (from literature values), the result of this work gives credence to the ethnomedical use of the plant in tonsillitis and candida infections in children.

Table 3: MIC of the methanolic and aqueous extracts against the test microorganisms

Name of organism	Minimum inhibitory concentration (mg/ml)	
	Methanol Extract	Aqueous Extract
Staphylococcus aureus	2.0	2.5
Pseudomonas aeruginosa	3.2	2.0
Candida albicans	2.5	-
Aspergillus niger	5.7	-

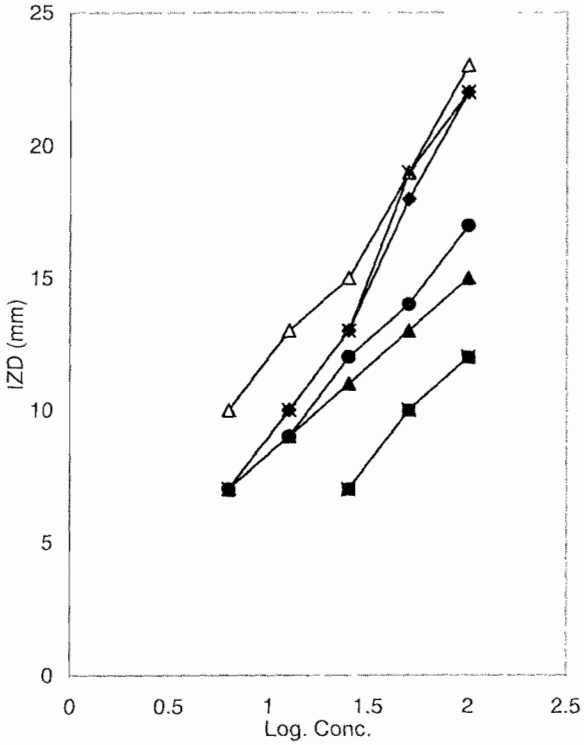

Fig. 1: Plot of inhibition zone diameter against the log concentration of the extracts against various test organisms: *Staph. aureus* Δ methanol extract; Ж aqueous extract; *Pseudomonas aeruginosa* ♦ methanol extract; ▲ aqueous extract; *Candida albicans* • methanol extract; *Aspergillus niger* ■ methanol extract.

Medicinal plants offer a great variety of chemical substances that can be harnessed for use in the treatment of human and animal ailments. All that is required is thorough characterization and where necessary purification. Most modern antibiotics are from fungi and many compounds have been isolated from lichens (Adikwu and Esimone, 1998; Esimone et al, 1999; Esimone and Adikwu, 2002). The tropical forest is endowed with a variety of organisms, both plants and animals that can yield a wide variety of biologically active compounds that can be harnessed for the treatment of various ailments plaguing man.

More work is recommended on the characterization and isolation of the active ingredient. This may result in a compound with better therapeutic efficacy.

References

Adikwu, M U. and Esimone, C O. (1998). Susceptibility of tetracycline and ampicillin resistant strains of *Staphylococcus aureus* to bioactive column fractions the lichen *Ramalina farinacea.* (L.). Ach. J. Pharm. Pharmacol. 50(Supplement), 38.

Alade, P. I. and Irobi, O. N. (1992), Antimicrobial activities of crude leaf Extract of *Acalyphya wilkesiana.* J. Ethnopharmacol., 39: 171-174

Berdy, J. (1982) Handbook of Antibiotics from Higher Forms of Life: Higher Plants. Boca Raton: CRC Press.

Ebi, G. C. and Ofoefule, S. I. (1997), Investigation into the folkloric antimicrobial activities of *Landolphia owarrience*, Phytotherapy Research 11: 145 – 151.

Esimone, C O. and Adikwu, M U. (1999). Antimicrobial activity and cytotoxicity of *Ramalina farinacea.* Fitoterapia, 70, 428-431.

Esimone, C O and Adikwu, M U (2002). Susceptibility of some clinical isolates of *Staphylococcus areus* to bioactive column fractions from the lichen *Ramalina farinacea* (L) Ach. Phytotherapy Research, 16, 494-496.

Sofowora, A. (1986). The State of Medicinal Plants Research in Nigeria: Proceedings of a Workshop. Ife pp. 53 – 58.