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Antifungal Activity of Ethanol and Aqueous Extracts of *Euphorbia hirta* Linn against Clinical Isolates of Dermatophytes

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Abstract

Medicinal plants have evolved into essential preventive and therapeutic aids for various ailments. Dermatophytosis, a zoonotic disease caused by dermatophytes, is a significant public health problem, particularly in African countries. This study was conducted to determine the antifungal activity of ethanol and aqueous extracts of *Euphorbia hirta* against clinical isolates of dermatophytes. Fresh leaves, stems, flowers, and whole plants of *Euphorbia hirta* were collected and processed for further studies. Samples of clinical isolates and broken hair samples of dermatophytes were collected from the dermatology unit of Barau Dikko-Teaching Hospital, Kaduna. The samples were subjected to microscopic examination and culture identification using standard protocols. Standardization of test organisms and determination of the antifungal activities of ethanol and aqueous extracts of *Euphorbia hirta* against clinically isolated dermatophytes were performed using standard methods. The results revealed that the antifungal susceptibility of the ethanol extract of the whole plant was higher than that of the other extracts. Similar highest susceptibility patterns were observed with the ethanol extract of the whole plant against *Trichophyton* rubrum, *T. species*, and *Epidermophyton fluccosum*. Most *T. rubrum* isolates had higher MIC values for 100% aqueous extracts, ranging from 0.05-6 µg/mL. In contrast, 100% ethanol extracts were more effective in inhibiting *T. rubrum* at lower MIC values (0.04–1 µg/ml).

Keywords: Antifungal activity, *Euphorbia hirta,* Dermatophytes, Clinical isolates

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Introduction

Medicinal plants have evolved into essential preventive and therapeutic aids for various ailments. Dermatophytosis, a zoonotic disease caused by dermatophytes, is a significant public health problem, particularly in African countries (Diso *et al*., 2020). A high prevalence of fungal infections among school children in Nigeria has been reported (Ezomike *et al*., 2021). Dermatophytes, including species of *Trichophyton*, *Microsporum*, *Aspergillus*, *Epidermophyton*, and *Candida*, infect both sexes and all age groups (Alex *et al*., 2016). These infections are highly contagious, spreading through skin-to-skin contact, hair, skin, nails, and fomites, with recurrences common among school children due to environmental conditions (Alex *et al*., 2016; Diso *et al*., 2020).

The main dermatophyte genera, including *Microsporum*, *Epidermophyton*, and *Trichophyton*, invade the outer layers of the skin and keratin-rich appendages (Wisal and Salim, 2010). It has been reported that *Trichophyton* and *Microsporum* are the primary dermatophytes responsible for human infections (Wisal and Salim, 2010). These infections are more virulent when transmitted from animals to humans than when transmitted from human to human (Wisal and Salim, 2010). Andrews and Burns (2008) reported that *Trichophyton tonsurans* causes over 90% of infections, while *Microsporum* species account for 5%. The rise of multidrug-resistant pathogens has driven the exploration of the antimicrobial activities of medicinal plants. Plant extracts offer opportunities for novel drug development because of their chemical diversity (Alexandra *et al*., 2018). Nigeria is rich in medicinal plants used to treat pathogenic infections. Such plants, like *Euphorbia hirta*, are widely distributed across tropical continents (Alexandra *et al*., 2018; Al-Huqail *et al*., 2019). Meda *et al*. (2023) described Euphorbia (Euphorbiaceae) as the third largest genus of flowering plants with rich pharmacological properties, with *Euphorbia hirta* being particularly useful (Ernst *et al*., 2015). The use of *Euphorbia hirta* in African communities often relies on trial-and-error approaches without scientific validation. Reports on the antifungal effects of different parts of *E. hirta* from various locations sometimes produce inconsistent results (Rajeh *et al*. 2010). The widespread use of antibiotics for infectious diseases has detrimental effects on humans (Gupta *et al*., 2018). Misdiagnosis of dermatophyte infections often occurs, especially with the use of creams and steroid ointments, leading to ineffective treatment (Gupta *et al*., 2018). The rapid increase in antibiotic resistance has prompted investigations into novel organic molecules with antimicrobial properties from plants. These natural products offer minimal cost and are safer alternatives to synthetic drugs (Gupta *et al*., 2018). This study was conducted to determine the antifungal activity of ethanol and aqueous extracts of *Euphorbia hirta* against clinical isolates of dermatophytes.

Materials And Method

Plant Collection and Preparation

Fresh leaves, stems, flowers, and whole plants of *Euphorbia hirta* were collected from Centenary Park, opposite 44 Reference Army Hospital, Kaduna North, Kaduna, Nigeria. The entire plant was identified and authenticated by a Botanist, from the Department of Biological Sciences, Nigerian Defence Academy, with voucher number: (NDA/BIOH/2024/43). The collected plant parts were rinsed with tap water, air-dried under shade for 14 days, reduced to a coarse powder using a pestle and mortar, and further ground to a fine powder using a Kenwood electric blender. The powdered samples were stored in airtight bottles for further analysis.

Extraction of plant materials

Thirty grams (30 g) of each powdered sample (leaves, stem, flowers, and whole plant) was soaked in 200 ml of ethanol in a 1-liter conical flask covered with cotton wool, plugged, and wrapped with aluminum foil. The mixture was shaken vigorously and left to stand for 24 h in a shaking water bath maintained at 29°C. For aqueous plant extraction, 30 g of each powdered sample (leaves, stem, flowers, and whole plant) was soaked in 200 ml of distilled water in a 1-liter sterile conical flask. The mixtures were filtered using a muslin cloth and Whatman No. 1 filter paper. Each extract was placed in a water bath at 40°C and allowed to evaporate, leaving a pure extract (Sosa *et al*., 2016). The percentage yield of the crude extracts was determined.

Collection of Clinical Samples

Samples of clinical isolates and broken hair samples of dermatophytes were collected from the dermatology unit of Barau Dikko-Teaching Hospital, Kaduna, and were immediately transported in an ice bag to the Department of Microbiology, Kaduna State University for mycological screening. The samples were subjected to microscopic examination and culture identification using standard methods (Larone, 2011).

Preparation of Test Organisms

The fungal culture was maintained on Potato Dextrose Agar (PDA) mixed with chloramphenicol (0.45%) and cycloheximide (0.5%) and then incubated at 30°C for 7 days, subsequently stored in triplicates as stock fungal spore cultures at 4°C (Aberkane *et al*., 2002). The Fungal Stock Culture was maintained by inoculating and harvesting each isolate into six freshly prepared PDA and then incubating at 28°C for 5-7 days (Aberkane *et al*., 2002). Spore suspensions were prepared by harvesting PDA slants containing the fungal stock culture. The harvested fungal spores were washed with the harvesting medium. The

supernatants were discarded, and spore pellets were re-suspended in harvesting medium, standardized, and stored at 4°C until required for use within two weeks (Olowosulu *et al*., 2005).

Determination of Inhibition Zone Diameter (IZD)

Standardized spore suspensions of the test fungus were flooded onto PDA plates. Flamed corn borer was used to bore cups in the set PDA plates, and 0.1 ml of the graded concentration of the crude extracts were dispensed into each cup on the PDA plates using a micropipette, and allowed to stand for 60 min for thorough distribution of the antifungal agents into the agar before incubating at 30°C for 72-96 hours. The fungal inhibition zone diameter (mm) on PDA was determined using a caliper (Mbata and Nwajagu, 2006). The minimum inhibitory concentrations (MIC) of the crude extracts and reference antibiotics were determined using a tetrazolium microplate assay, slightly modified from the serial broth microdilution method, as previously described by Eloff (1998).

Data Analysis

Data were analyzed using Microsoft Excel and SPSS (version 25.0). All data are expressed as mean ± SD. Statistical significance was set at $P < 0.05$.

Results

Antifungal Susceptibility of the Extracts against Dermatophytes

The antifungal susceptibility test demonstrated that the ethanol extract of the whole plant was more effective against dermatophytes than the other extracts. The highest susceptibility patterns were observed with the ethanol extract of the whole plant against *Trichophyton* rubrum (20.0 mm), T. species (19.0 mm), and *Epidermophyton fluccosum* (16.0 mm) (Table 1).

These results were comparable to those of ketoconazole, with inhibition zones of 20.0, 19.5, and 16.0 mm, respectively. The zone of inhibition for the extracts against *Trichophyton rubrum* was 13.5 mm (stem), 12 mm (leaf), 10.5 mm (flower), and 20 mm (whole-plant). For *Trichophyton* species, the zones were 11.5, 10, 7, and 19 mm for the stem, leaf, flower, and whole plant, respectively. *Epidermophyton fluccosum* had inhibition zones of 11, 10, 7, and 15 mm. *Aspergillus niger* had zones of 10.5, 11, 6, and 17.5 mm, while Microsporum species showed zones of 11, 10, 7, and 12 mm for the stem, leaf, flower, and whole plant, respectively.

Similarly, the aqueous extracts of the stem, leaves, flower, and whole plant showed the following zones of inhibition for *Trichophyton rubrum*: 17.5 mm (stem), 14 mm (leaves), 13 mm (flower), and 17 mm (whole plant). For *Trichophyton* species, the zones were 14, 11, 12, and 15.5 mm for stem, leaf, flower, and whole plant, respectively. *Epidermophyton fluccosum* showed inhibition zones of 12, 14.5, 14.5, and 14 mm. *Aspergillus niger* had zones of 11, 13, 12, and 13 mm, while *Microsporum* species had zones of 10.5, 12.5, 6, and 12.5 mm for stem, leaf, flower, and whole plant, respectively (Table 1).

Table 1: Antifungal Activity of the Extracts against Dermatophytes

		Diameter of zone on Inhibition (mm)				
Solvent	Extract	*T. rubrum*	*E. fluccosum*	***A. niger***	***M. species***	***T.* species**
Ethanol	Stem	13.5	11.5	10.5	11.0	11.5
	Leaves	12.0	10.0	11.0	10.0	10.0
	Flower	10.5	7.0	6.0	7.0	7.0
	Whole plant	20.0	16.0	17.5	12.0	19.0
Aqueous	Stem	17.5	12.0	11.0	10.5	14.0
	Leaves	14.0	14.5	13.0	12.5	11.0
	Flower	13.0	14.5	12.0	6.0	12.0
	Whole plant	17.0	14.0	13.0	12.5	15.5
	DMSO	2.75	1.5	2.0	1.7	1.5
	Ketoconazole	20.0	16.0	19.0	18.5	19.5

Minimum Inhibitory Concentration (MIC) of the Extracts on Fungi Isolates

Table 2 presents the MIC ranges that inhibited 50% (MIC50) and 90% (MIC90) of the isolates for ethanol and aqueous *Euphorbia hirta* extracts.

Table 2: Minimum Inhibitory Concentration of Fungi Isolates for *E. hirta* Extracts and Control

Extract (100%)	Isolates	No of Isolates	MIC Range (µg/ml) 0.02	0.05	0.125	0.25
Aqueous Leaves	*Trichophyton* species	11	0	0	0	57
	Trichophyton rubrum	8	0	0	0	0
	Aspergillus niger	4	0	0	0	0
	Microsporum species	3	0	1	0	0
	Epidermophyton fluccosum	2	0	0	0	0
Ethanol Leaves	*Trichophyton* species	11	0	2	15	25
	Trichophyton rubrum	8	0	0	0	0
	Aspergillus niger	4	0	0	0	9
	Microsporum species	3	0	0	0	0
	Epidermophyton fluccosum	2	1	0	0	0
Aqueous Stem	*Trichophyton* species	11	0	2	15	25
	Trichophyton rubrum	8	0	12	28	45
	Aspergillus niger	4	0	1	0	2
	Microsporum species	3	0	0	1	0
	Epidermophyton fluccosum	2	1	0	0	0
Ethanol Stem	*Trichophyton* species	11	0	0	0	0
	Trichophyton rubrum	8	0	1	12	18
	Aspergillus niger	4	0	1	0	2
	Microsporum species	3	0	0	1	0
	Epidermophyton fluccosum	2	0	0	0	0
Aqueous Flower	*Trichophyton* species	11	0	0	0	0
	Trichophyton rubrum	8	0	0	0	55
	Aspergillus niger	4	0	0	0	0
	Microsporum species	3	0	1	0	0
	Epidermophyton fluccosum	2	0	0	0	2
Ethanol Flower	*Trichophyton* species	11	0	0	0	0
	Trichophyton rubrum	8	0	0	0	50
	Aspergillus niger	4	0	0	0	0
	Microsporum species	3	0	1	0	2
	Epidermophyton fluccosum	2	0	0	0	1
Aqueous whole plant	*Trichophyton* species	11	2	1	2	0
	Trichophyton rubrum	8	56	42	6	2
	Aspergillus niger	4	0	0	0	0
	Microsporum species	3	5	8	1	5
	Epidermophyton fluccosum	2	1	2	1	0
Ethanol whole plant	*Trichophyton* species	11	1	2	0	0
	Trichophyton rubrum	8	1	2	0	0
	Aspergillus niger	4	37	26	2	0
	Microsporum species	3	3	5	0	2
	Epidermophyton fluccosum	2	0	0	2	1
DMSO	*Trichophyton* species	11	0	0	0	0
	Trichophyton rubrum	8	1	0	0	0
	Aspergillus niger	4	0	0	0	1
	Microsporum species	3	0	2	3	5
	Epidermophyton fluccosum	2	0	1	2	1
Ketoconazole	*Trichophyton* species	11	0	0	0	0
	Trichophyton rubrum	8	0	1	0	0
	Aspergillus niger	4	1	0	1	1
	Microsporum species	3	0	0	0	3
	Epidermophyton fluccosum	2	0	1	0	0

DMSO (Dimethyl sulfoxide)

Minimum Fungicidal Concentration (MFC) of the Extracts

The results showed that most *T. rubrum* isolates had higher MFC values for 100% aqueous extracts, ranging from 0.05–6 µg/ml. In contrast, 100% ethanol extracts inhibited *T. rubrum* at lower MIC values (0.04–1 µg/ml). For *E. floccosum*, the MFC values for 100% aqueous extracts ranged from 0.125–0.5 µg/ml. For *T. species*, The 100% aqueous extracts inhibited growth at an MFC range of 0.05–4 µg/ml, while the 100% ethanol extracts inhibited growth at 0.02–6 µg/ml. The growth of *Aspergillus niger* was inhibited by 100% ethanol extracts at 0.05 to 2 µg/ml, while 100% aqueous extracts showed an MFC of 0.02 to 5 µg/ml. For *Microsporum* species, 100% aqueous extracts had MFC values ranging from 0.5 to 6 µg/ml, whereas 100% ethanol extracts had MFC values ranging from 0.05 to 3 µg/ml (Table 3).

Table 3: Minimum Fungicidal Concentration (MFC) (µg/ml) of the Extracts

Organism/Range	Aqueous Extract (100%)	Ethanol Extract (100%)	DMSO
T. rubrum	0.05 – 6.0	0.04 – 1.0	0.5 – 3.0
FMC_{50} (µg/ml)	1	0.5	1
MFC_{90} (µg/ml)	5	1	3
E. fluccosum	0.125 – 0.5	0.02 – 0.5	0.125 – 4
MFC_{50} (µg/ml)	0.25	0.03	2
MFC_{90} (µg/ml)	0.5	0.5	2
T. species	0.05 – 4	0.02 - 6	0.05 – 3
MFC_{50} (µg/ml)	1	1	1
MFC_{90} (µg/ml)	3	5	2
Aspergillus niger	0.02 – 5	0.05 - 2	0.05 – 4
MFC_{50} (µg/ml)	0.5	0.5	2
MFC_{90} (µg/ml)	3	1	3
Microsporum species	0.5 – 6	0.05 - 3	0.5 – 3
MFC_{50} (µg/ml)	1	0.5	2
MFC_{90} (µg/ml)	4	2	1

Key: MFC = Minimum Fungicidal Concentration; *T. rubrum, Trichophyton rubrum; E. fluccosum, Epidermophyton fluccosum; T.* species, *Trichophyton* species; DMSO, (dimethyl sulfoxide) = Negative control.

Discussion

Research indicates that *Euphorbia hirta* exhibits diverse properties, including morphological, phytochemical, pharmacological, pharmaceutical, therapeutic, and nutritional benefits (Kumar *et al*., 2010). This study was conducted to determine the antifungal activity of ethanol and aqueous extracts of *Euphorbia hirta* against clinical isolates of dermatophytes.

The ethanol extract of the whole plant exhibited the highest susceptibility against *Trichophyton rubrum*, *Trichophyton* species, and *Epidermophyton fluccosum*. This contradicts the report of Chukwuka *et al*. (2013), who found *Cladosporium* species to be the most sensitive to UV-C irradiation. This variation may be due to the study location and sample site. Vera *et al.* (2019) reported that, *E. hirta* leaf extracts were effective against *C. albicans.* Among the dermatophytes tested, *Trichophyton rubrum* was more sensitive to the ethanol extract of the whole plant than to the aqueous extract. This could be attributed to the extraction solvent, which is a significant factor affecting the chemical composition and biological activity of plant extracts. Previous studies have reported that crude ethanol extract exhibits more consistent antifungal activity than crude aqueous extract because most of the identified plant components that are active against fungi are aromatic or saturated organic compounds (Diso *et al*., 2020). Ethanol easily extracts the bioactive components of plants. This makes it an ideal solvent for initial extraction (Vera *et al.,* 2019).

Conclusion

Euphorbia hirta ethanol and aqueous extracts demonstrated effective antifungal activity against clinical dermatophytes, with the ethanol extract exhibiting higher activity. These extracts are potential antimicrobial candidates.

Reference

Aberkane A, Cuenca-Estrella M, Gommez- Lopez A et al (2002). Comparative evaluation of two different methods of inoculums preparation for antifungal susceptibility testing of filamentous fungi. *Journal of Antimicrobial Chemotherapy* 50: 19-22.

Alem A, Gebremedhin M, Gizachew A (2016). Prevalence and etiologic agents of dermatophytosis among primary school children in Harari Regional State, Ethiopia. *Journal of Mycology* 2016: 1- 5

Alexandra NW, Emberger-Klein A, Menrad K (2018). Why people use herbal medicine: insights from a focus group study in Germany. *BMC Complementary and Alternative Medicine* 18(92): 1-9

Al-Huqail AA, Said IB, Mohamed ZM, Salem HM et al (2019). Antifungal, antibacterial, and antioxidant activities of *Acacia saligna* (Labill.) H. L. Wendl. flower extract: HPLC Analysis of phenolic and flavonoid compounds. *Molecules* 24: 700. 2-14.

Andrews MD, Burns M (2008). Common Tinea infections in children. *American Family Physician* 77: 1415-20

Chukwuka KS, Iwuagwu MI, Uka UN (2013). Evaluation of nutritional components of *Caricapapaya L*. At different stages of ripening. *IOSR Journal of Pharmacy and Biological Science,* 6(4): 13-16.

Diso SU, Adam JS, Mu'azu L, Abdallah MS, Ali M (2020). Isolation and characterization of some Fungi associated with superficial Fungal infections. *ARC Journal of Dermatology* 5(1): 12-16

Eloff JN (1998). A sensitive and quick microplate method to determine the minimal inhibitory concentration of plant extracts for bacteria. *Planta Medica* 64: 711–713.

Ernst M, Grace OM, Saslis-Lagoudakis CH, Nilsson N et al (2015). Global medicinal uses of *Euphorbia L*. (Euphorbiaceae). *Journal of Ethnopharmacology* 176:90-101

Ezomike NE, Ikefuna AN, Onyekonwu CL, Ubesie AC et al (2021). Epidemiology and pattern of superficial fungal infections among primary school children in Enugu, South-East Nigeria. *Malawi Medical Journal*, 33(1): 21-27. doi: 10.4314/mmj. v33i1.4.

Gupta D, Kumar M et al (2018). An *in vitro* investigation of antimicrobial efficacy of *Euphorbia hirta* and *Murraya koenigii* against selected pathogenic microorganisms. *Asian Journal of Pharmacy and Clinical Research* 11(5): 359-363 DOI: http://dx.doi.org/10.22159/ajpcr.2018.v11i5.24578

Kumar S, Malhotra R, Kumar D (2010). *Euphorbia hirta*: Its chemistry, traditional and medicinal uses, and pharmacological activities. *Pharmacogny Review* 4(7):58-61. doi: 10.4103/0973-7847.65327. PMID: 22228942; PMCID: PMC3249903.

Larone DH (2011). Medically important fungi: A guide to identification, 5th ed. *American Society for Microbiology*. Washington, D.C.

Mbata T, Nwajagu C (2006). Dermatophytes and other fungi associated with hair-scalp of nursery and primary school children in Awka, Nigeria. *The Internet Journal of Microbiology* 3:2.

Meda RNT, Kam SE, Kagambega W, Zongo E et al (2023). A review on bioactive compounds isolated from *Euphorbia hirta* L. *American Journal of Plant Sciences* 14: 710-726

Olowosulu AK, Ibrahim YKE, Bhatia PG (2005). Studies on the antimicrobial properties of formulated creams and ointment containing *Raphia nitida* heartwood extract. *Journal of Pharmacy and Bioresources* 2(2): 124-130

Rajeh MA, Zuraini Z, Sasidharan S, Latha LY, Amutha S (2010). Assessment of Euphorbia *hirta* L. leaf, flower, stem and root extracts for their antibacterial and antifungal activity and brine shrimp lethality. *Molecules* doi: 10.3390/molecules15096008.

Sosa AA, Bagi SH, Hameed IH (2016). Analysis of bioactive chemical compounds of *Euphorbia lathyrus* using gas chromatography-mass spectrometry and Fourier transform infrared spectroscopy. *International Journal of Pharmacognosy and Phytochemical Research* 8(5): 109-126

Vera SBB. Car, LMP (2019). *In vitro* activity of Asthma plant (*Euphorbia hirta* Linnaeus) leaf extract against *Trichophyton mentagrophytes*, *Candida albicans* and *Malassezia pachydermatis. Philippine Journal of Veterinary and Animal Sciences 45*(2):126-131

Wisal AG, Salim MO (2010). Isolation and identification of dermatophytes from infected camels, *Sudan Journal of Veterinary Research* 25: 94–53

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