

**ANTIFUNGAL EFFECTS OF *Azadirachta indica* AND *Ocimum gratissimum* ON DERMATOPHYTES****¹Grillo Adebayo Joseph, ²Ukhureigbe Oluchi Miriam, ³Egwari Louis Osayenum, ¹Olasupo Nurudeen Ayoade and ⁴Adeniyi A. Bolanle**¹Department of Microbiology, Faculty of Science, Lagos State University, Lagos State, Nigeria.²Department of Microbiology, Faculty of Science, Federal University, Oye-Ekiti, Ekiti State, Nigeria.³Department of Microbiology, College of Pure and Applied Sciences, Landmark University, Nigeria.⁴Department of Pharmaceutical Microbiology, Faculty of Pharmacy, University of Ibadan, Oyo State, Nigeria.*Corresponding Author's Email: ukhureigbemiriam1983@gmail.com**ABSTRACT**

Dermatophytes pose a global burden with a worldwide prevalence of 20 - 25%. This study was aimed at the evaluation of antifungal effects of leaf extracts of *Azadirachta indica* and *Ocimum gratissimum* on dermatophytes. Forty nine skin scrapings were obtained from patients at the Skin Disease Clinic, Lagos University Teaching Hospital Annex, Yaba. Dermatophytes were isolated on Sabouraud dextrose agar and identified by microscopic and biochemical methods. The aqueous and ethanolic extractions of the plant leaves was done by maceration and Soxhlet method respectively. The antifungal activity was evaluated using the broth dilution method. *Trichophyton mentagrophytes* had the highest percentage occurrence of 43.24, followed by *T. equinum* (27.03), and the lowest was 5.41 for *Microsporum gypseum*. The aqueous extracts of both plants completely inhibited all the dermatophytes at 250mg/ml. The minimum inhibitory concentration (MIC) of *A. indica* was 125mg/ml against all the dermatophytes. However, for *O. gratissimum*, the MIC was 63mg/ml against *T. equinum*, *T. verrucosum* and 125 mg/ml against *T. mentagrophytes*. For ethanol extract of *A. indica*, the highest percentage reduction in radial growth (%RRG) was 94.84 at 50 mg/ml against *T. equinum* and the lowest %RRG was 0.33 at 5 mg/ml against *E. floccosum*. For ethanol extract of *O. gratissimum*, the highest %RRG was 95.71 at 50 mg/ml against *T. verrucosum* and the lowest was 23.6 at 5 mg/ml against *M. gypseum* even as *E. floccosum* was not inhibited at that concentration. The results indicated the effectiveness of studied plant extracts in controlling the growth of dermatophytes.

Keywords: *Azadirachta indica*, Minimum inhibitory concentration (MIC), *Ocimum gratissimum*, Percentage reduction in radial growth (%RRG)

INTRODUCTION

Dermatophytes are parasitic fungi that grow on the epidermis of the skin, hair and nails. They include the fungi genera *Epidermophyton*, *Microsporum* and *Trichophyton*. Dermatophytes are cosmopolitan and encompass more than 50 species (Segal & Elad, 2021). These agents are transmitted by direct contact with infected part of the body or indirect contact with fomites such as clothing, shoes, nails, clippers, nail fibres, showers, lockers, room floor, bed sheets and carpets (Moskaluk & VandeWoude, 2022). They colonize the keratinized layer of the skin and cause a range of infections such as Athlete's foot (*Tinea pedis*), ringworm of the scalp (*Tinea capitis*), ringworm of the nails (*Tinea unguium*), and ringworm of the skin (*Tinea corporis*). Risk factors that enhance transmission include hot weather, immunocompromised condition and poor ventilation. Various antifungals have been proposed to be effective against different fungal agents. However, the world is currently faced with increasing threats of antifungal drug resistant microorganisms as well as increasing adverse effects of synthetic drugs (Geddes-McAlister & Shapiro, 2019). Therefore, efforts towards the development of new antimicrobials that lack resistant trait and possess minimal adverse effects is increasing (Miethke *et al.*, 2021). In recent times, the search for natural alternative (local herbs) that will inhibit microbial agents of infections has been attracting much attention among biological and medical scientists (Abubakar & Sandamu, 2026). The high cost of synthetic chemical drugs in developing countries, like Nigeria and the fact that a lot of people, especially in the developing countries of Africa, Asia and the Caribbean still believe in the

efficacy of herbs have also led to the upsurge of scientific interests in medicinal plants.

Azadirachta indica (family Meliaceae) commonly known as "Dongoyaro" in Nigeria, is a tree plant, planted for shade and medicinal purposes in the West, East and Central Africa (Akinnibosun *et al.*, 2009). The leaves and bark are separately, and sometimes together, used for treatment of fever through oral administration, inhalation or hydrotherapy. *Ocimum gratissimum* (family; Labiate) also known as "Efinrin" in Yoruba land and "Ncha anwu" in Igbo land both in Nigeria, grows wild in Western and Eastern parts Nigeria and it is sometimes cultivated as garden plant. The leaves and stem of *Ocimum gratissimum* (*O. gratissimum*) are, separately, used as mouth wash, the cold water extract of the leaf is used for treatment of stomach discomfort, whereas fresh leaves are used topically for curing skin diseases (Priyanka *et al.*, 2018). Leaves of *Azadirachta indica* (*A. indica*) contains quercetin, sitosterol phlebotanins, hydroxymethyl anthraquinone and phenol; the bark contains nimbin, nimbidin, Nimboesterol, tannin and margisine whereas the seeds contain diacetylnimbin, azadiractin and meliantriol. Over the past two decades, the incidence of dermatophytosis has increased, and the population at risk has increased dramatically, with prevalence rate of about 29% being reported in Nigeria. Overcrowded environment, male gender and poor hygiene increases susceptibility to this infection. The country suffer massive financial loss through health expenses on this infection that could easily be prevented. In Nigeria, only a few studies are available on antifungal effects of *Azadirachta indica* and *Ocimum gratissimum* on dermatophytes.

Therefore, this study is aimed at determining the in vitro activity of leaf extract of *A. indica* and *O. gratissimum* against test dermatophytes. The establishment of the effectiveness of local plants against dermatophytes may lead to the development of plant based antifungal drugs which will reduce the cost of treatment of dermatophytosis and also ensure safer natural treatment regimen.

MATERIALS AND METHODS

Inclusion Criteria

All consenting patients suspected to be infected by dermatophytes that were present in the skin clinic at the period of study, and whose parents consented, were included for the study. Patients with severe and mild clinical symptoms were recruited for the study.

Non consenting patients and those that were suspected not to be infected by dermatophytes and those whose parents did not give consent to their children's participation in the study were excluded from the study (Bhosale *et al.*, 2022).

Sample Collection

Forty nine samples of skin scrapings were aseptically collected from patients at the dermatology unit of the Lagos University Teaching Hospital (LUTH) Annex, Yaba, Lagos, in a sterile envelope (Egwuatu *et al.*, 2019).

Direct Examination of Samples

Samples were evaluated for hyphae and arthrospores by placing 3 drops of 20% potassium hydroxide on the slide containing the sample (Hardy diagnostic, USA) and analyzing the set up after five minutes using X10, x20 and x40 objective of microscope (Chandler, 2022).

Isolation of Dermatophytes

Each scraping was aseptically placed into sabouraud dextrose agar (32.5g/500ml) containing chloramphenicol (3.025gramme/500ml) and cycloheximide (0.25gramme/500ml), in order to inhibit the growth of bacteria and saprobic fungi respectively (Bhosale *et al.*, 2022).

The plates were incubated at 28°C for one to two weeks and examined at three days interval for fungal growth. The first step taken to characterize the dermatophytes was based on macroscopic examination of the pure culture. The macroscopic examination of dermatophytes was characterized by duration of growth, surface morphology and change in colony pigmentation on both front and reverse side of the plate.

Indirect Microscopic Examination

The isolates were examined microscopically for morphological features (formation of macro conidia, microconidia, and hyphae) of fungi using lactophenol cotton blue mounts of the pure culture (Egwuatu *et al.*, 2019).

Biochemical Identification of Isolates

Isolates were further assayed for urea and vitamin utilization in urea medium and rice grain medium respectively. They were also evaluated for tolerance for Sodium chloride, hydrolysis of casein, and in vitro hair perforation (Egwuatu *et al.*, 2019).

Plant Sample Collection

Fresh leaves of *A. indica* and *O. gratissimum* were collected from botanical garden of the University of Lagos (Latitude 6° 30' 59.99' N and Longitude 3° 23' 5.99' E) in Lagos State, Nigeria. The identification of the plants was performed at the

Department of Botany and Microbiology, University of Lagos. The leaves were washed with distilled water, dried at 600pm at 40°C for 24hr in the hot air oven and eventually ground to pass through 2-5 mm mesh (Mbata *et al.*, 2009).

Preparation of Aqueous Extract of Plants

Aqueous extracts of the plants were prepared by soaking 100g of the ground leaves of each plant in 200ml distilled water in sterile beaker for 2 days. The extracts were sieved using muslin cloth into each beaker for each plant species (Kator & Hosea, 2018).

Preparation of Ethanol Extract of Plants

Ethanol extracts were prepared from the finely ground plant leaves using Soxhlet extraction apparatus. One hundred and twenty-one (121g) of the powdered leaf sample of each plant was subjected to extraction with 2 litres of 95% ethanol at about 70°C for 10h (Okigbo *et al.*, 2010).

Both aqueous and ethanol extracts were concentrated in a rotary evaporator at 40°C, after which the concentrated extracts were weighed and stored in the refrigerator at 8°C before use. The aqueous and ethanol extract of *A. indica* yielded 21.2g and 25.0g respectively, while the aqueous and ethanol extract of *O. gratissimum* produced 23.0g and 28.0g respectively.

Preparation of Different Concentrations of Ethanol Extract

Four dilutions of each of the ethanol extracts were prepared as follows: 0.5g of ethanol extract was added to 10ml distilled water in a test tube, producing 50mg/ml concentration of extract. Two millilitres of this 50mg/ml solution was diluted to 5ml by adding 3ml sterile water. This gave 20mg/ml concentration of extract. From the 20mg/ml extract solution, 2.5ml was obtained and diluted to 5ml by adding 2.5ml sterile distilled water. This gave 10mg/ml of the extract. 2.5ml of the 10mg/ml was diluted to 5mls by addition of 2.5ml sterile distilled water to produce 5mg/ml ethanol extract solution.

Preparation of Different Concentrations of Aqueous Extract

Doubling dilutions of 0.5g/ml (i.e. 500mg/ml) of each aqueous extract was constituted out to obtain ½ (250mg/ml), ¼ (125mg/ml), ⅛ (63mg/ml), 1/16 (31 mg/ml), 1/32 (16mg/ml) dilutions.

Inocula Preparation and Standardization

The isolates were inoculated into Sabouraud dextrose Agar (SDA) and incubated at 37°C for 24h. Suspensions of the isolated dermatophytes which were prepared in normal saline, were made by scraping the culture surface with the tip of a sterile loop and dissolving it in 1ml of 0.9% normal saline until the suspension's turbidity was the same as that of 0.5 Macfarlan standard (Rubinchik & Pasetka, 2010). The turbidities of the suspensions formed were adjusted by using normal saline until it matched the turbidity of 0.5 McFarland standard (final inoculums equivalent to a bacterial count of approximately 1.5×10^8 cfu/ml).

Plant Extracts Sensitivity Assay

One (1) ml of each dilution of extract was aseptically placed into the sterile petri-dishes according to the labeling, while other plates were left without extract to serve as control. Prepared SDA was poured into petri-dishes and allowed to solidify after gentle swirling of plates to ensure even mixing of the medium and plant extract. Each plate in each group was inoculated and streaked with the dermatophyte suspension

already adjusted to 0.5 McFarland standard, and the set up incubated at 25° C for 14 days.

Interpretation of Susceptibility Assay

The diameter of zone of inhibition which shows the degree of susceptibility or resistance of the test organisms) was measured in milliliter and recorded and then interpreted as resistant, intermediate or susceptible according to Clinical Laboratory Standard Institute (CLSI) guideline (CLSI, 2016) (Sánchez-Bautista et al., 2018).

RESULTS AND DISCUSSION

Prevalence of Dermatophytes

The results in Table 1 show that a total of 37 isolates of dermatophytes were obtained from the skin scrapping samples and *Trichophyton mentagrophytes* had the highest percentage occurrence of 43.24, followed by *T. equinum* (27.03), *T. verrucosum* (13.51). The lowest % occurrence was 5.41 recorded for *Microsporum gypseum*.

Table 1: Prevalence of Dermatophytes Isolated in the Study

Dermatophyte	Number of Isolates	Percentage Occurrence
<i>Trichophyton equinum</i>	10	27.03
<i>Trichophyton mentagrophytes</i>	16	43.24
<i>Microsporum gypseum</i>	2	5.41
<i>Epidermophyton floccosum</i>	4	10.81
<i>Trichophyton verrucosum</i>	5	13.51

n= number of each species of isolate obtained; N= total number of isolates obtained

The Minimum Inhibitory Concentrations of Aqueous Extracts of *Azadirachta indica* and *Ocimum gratissimum*

Tables 2 and 3 show that the aqueous leaf extracts of both plants completely inhibited the growth of all the dermatophytes and *Candida albicans* at 250mg/ml. The

minimum inhibitory concentration (MIC) of *A. indica* was 125 mg/ml against all the dermatophytes but, against *C. albicans* it was 250 mg/ml. However, for *O. gratissimum*, the MIC was 63mg/ml against *T. equinum*, and *T. verrucosum* but against *T. mentagrophytes* it was 125 mg/ml.

Table 2: Minimum Inhibitory Concentration of Crude Water Extract of *Azadirachta indica* against Dermatophytes and *Candida albicans*

Isolated Dermatophytes	Doubling Dilutions						Control
	Neat	1/2(250mg/ml)	1/4(125mg/ml)	1/8(63mg/ml)	1/16(31mg/ml)	1/32(16mg/ml)	
<i>Trichophyton equinum</i>	-	-	-	+	++	+	+++
<i>Trichophyton mentagrophytes</i>	-	-	-	+	++	+	+++
<i>Candida albicans</i>	-	-	+	+	++	++	+++
<i>Microsporum gypseum</i>	-	-	-	+	+	++	+++
<i>Epidermophyton floccosum</i>	-	-	-	++	++	++	+++
<i>Trichophyton verrucosum</i>	-	-	-	+	++	++	+++

Neat =

Broth medium with extract (-) = no growth

Without microorganism (+) = little growth

Control =

Broth medium with microorganism (++) = Average growth

Without extract (+++) = Normal growth

Table 3: Minimum Inhibitory Concentration of Crude Water Extract of *Ocimum gratissimum* against Dermatophytes and *Candida albicans*

Isolated Dermatophytes	Doubling Dilutions						Control
	Neat	1/2(250mg/ml)	1/4(125mg/ml)	1/8(63mg/ml)	1/16(31mg/ml)	1/32(16mg/ml)	
<i>Trichophyton equinum</i>	-	-	-	-	+	+	+++
<i>Trichophyton mentagrophytes</i>	-	-	-	+	+	+	+++
<i>Candida albicans</i>	-	-	+	+	+	++	+++
<i>Microsporum gypseum</i>	-	-	+	++	++	++	+++
<i>Epidermophyton floccosum</i>	-	-	+	++	++	+++	+++
<i>Trichophyton verrucosum</i>	-	-	-	-	+		+++

Neat=

Broth medium with extract

(-) = no growth

Without microorganism (+) = little growth
 Control=
 Broth medium with microorganism (++) = Average growth
 Without extract (+++) = Normal growth

The Determination of the Effect of Ethanol Extracts of the Plants at different Concentrations on the Growth Diameter of Isolated Dermatophytes

The results in Tables 4 and 5 show that for ethanol extract of *A. indica*, the highest percentage reduction in radial growth (%RRG) was 94.84 at 50 mg/ml against *T. equinum*, followed by 90.07 at 20 mg/ml against *T. equinum*, and the lowest was

0.33 at 5 mg/ml against *E. floccosum*. For ethanol extract of *O. gratissimum*, the highest %RRG was 95.71 at 50 mg/ml against *T. verrucosum*, followed by 95.51 at 50 mg/ml against *T. mentagrophytes* and the lowest was 23.6 at 5 mg/ml against *M. gypseum* even as *E. floccosum* was not inhibited at that concentration.

Table 4: Effect of Ethanol Extract of *Azadirachta indica* at different Concentrations on the Growth Diameter of Isolated Dermatophytes

Isolated dermatophytes	Growth Diameter (cm)									
	Control	50mg/ml		20mg/ml		10mg/ml		5mg/ml		
		Mean growth	%reduction	Mean Growth	%reduction	Mean growth	%reduction	Mean growth	% reduction	
<i>Trichophyton equinum</i>	2.52	0.13	94.84	0.25	90.07	0.5	79.37	0.93	63.10	
<i>Trichophyton mentagrophytes</i>	3.56	0.51	85.67	1.15	67.70	1.72	51.69	1.91	46.35	
<i>Candida albicans</i>	3.63	0.75	79.34	0.82	77.41	1.12	69.15	1.42	60.88	
<i>Microsporium gypseum</i>	3.94	1.52	61.42	2.25	42.89	2.53	35.79	3.86	2.03	
<i>Epidermophyton floccosum</i>	6.03	3.19	47.10	3.85	36.15	4.21	30.18	6.01	0.33	
<i>Trichophyton verrucosum</i>	4.66	1.12	75.97	1.80	61.37	2.05	56.01	2.50	46.35	

*= Mean of three replicates 14 days after inoculation +0.05 (SE)

Table 5: Effect of Ethanol Extract of *Ocimum gratissimum* at different Concentrations on the Growth Diameter of Isolated Dermatophytes

Isolated Dermatophytes	Growth Diameter (cm)									
	Control	50mg/ml		20mg/ml		10mg/ml		5mg/ml		
		Mean Growth	%Reduction	Mean Growth	%Reduction	Mean Growth	%Reduction	Mean Growth	% Reduction	
<i>Trichophyton equinum</i>	2.52	0.12	95.24	0.25	90.07	0.52	79.37	0.93	63.10	
<i>Trichophyton mentagrophytes</i>	3.56	0.16	95.51	1.10	69.10	1.32	62.92	1.72	51.69	
<i>Candida albicans</i>	3.63	0.21	94.22	0.50	86.23	1.12	69.15	1.51	58.40	
<i>Microsporium gypseum</i>	3.94	0.50	84.31	1.12	71.57	2.35	40.36	3.01	23.60	
<i>Epidermophyton floccosum</i>	6.03	3.25	46.10	3.85	36.15	4.10	30.01	6.03	0.00	
<i>Trichophyton verrucosum</i>	4.66	0.20	95.71	0.51	89.06	1.24	73.39	1.74	62.66	

* = Mean of three replicates 14 days after inoculation +0.05 (SE)

The Comparison of Potency of Ethanol Leaf Extracts of *Ocimum gratissimum* and *Azadirachta indica* against each Dermatophyte

The results in Figures 1 – 6 show higher percentage reduction in growth diameter for *O. gratissimum* than *A. indica* for all the dermatophytes. The results at 50 mg/ml are as follows;

Figure 1, *O. gratissimum* had 95.2% reduction in growth diameter compared to 94.84% for *A. indica* against *T. equinum*.

Figure 2, *O. gratissimum* (95.51%) compared to *A. indica* (85.67%) against *T. mentagrophytes*.

Figure 3, *O. gratissimum* (94.22%) compared to *A. indica* (79.34%) against *Candida albicans*.

Figure 4, *O. gratissimum* (84.31%) compared to *A. indica* (61.42%) against *Candida albicans*.

Figure 5, *O. gratissimum* (46.1%) compared to *A. indica* (47.1%) against *Candida albicans*.

Figure 6, *O. gratissimum* (95.71%) compared to *A. indica* (75.97%) against *Candida albicans*.

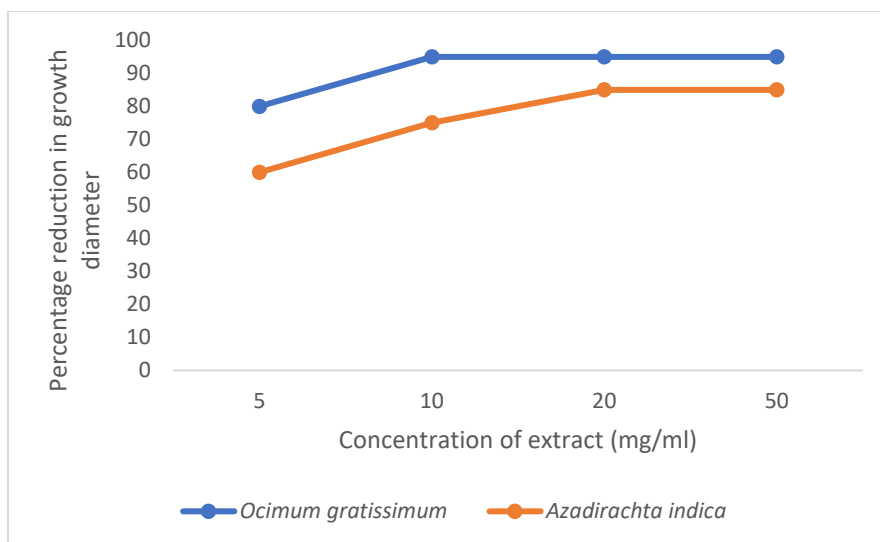


Figure 1: Comparative Potency of Ethanol Leaf Extracts of *Ocimum gratissimum* and *Azadirachta indica* against *Trichophyton equinum*

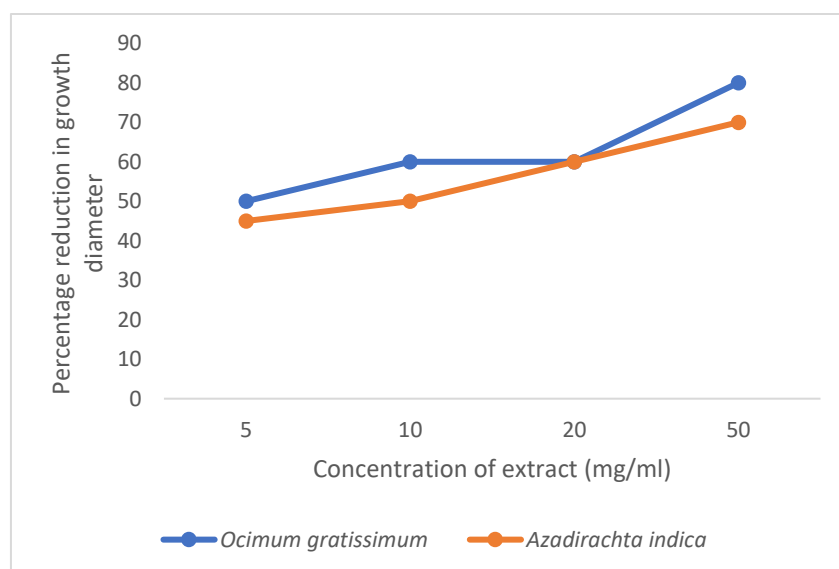


Figure 2: Comparative Potency of Ethanol Leaf Extracts of *Ocimum gratissimum* and *Azadirachta indica* against *Trichophyton mentagrophytes*

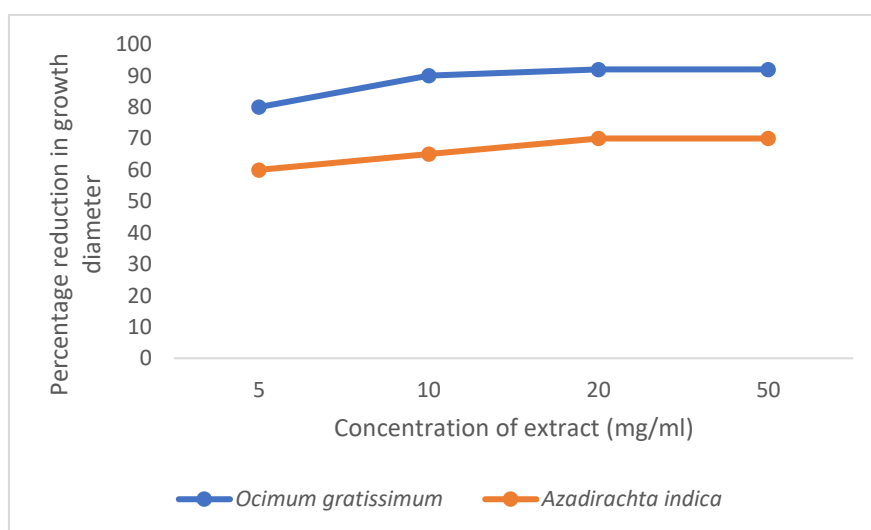


Figure 3: Comparative Potency of Ethanol Leaf Extracts of *Ocimum gratissimum* and *Azadirachta indica* against *Candida albicans*

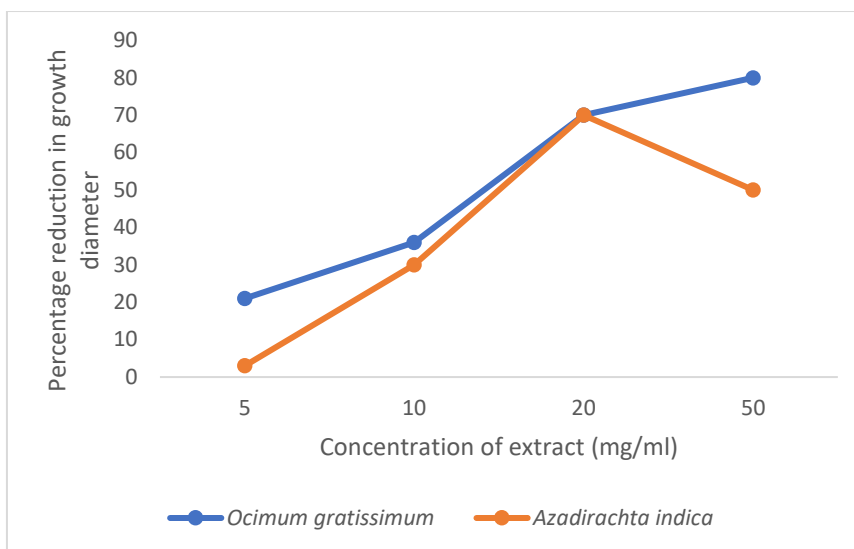


Figure 4: Comparative Potency of Ethanol Leaf Extracts of *Ocimum gratissimum* and *Azadirachta indica* against *Microsporium gypseum*

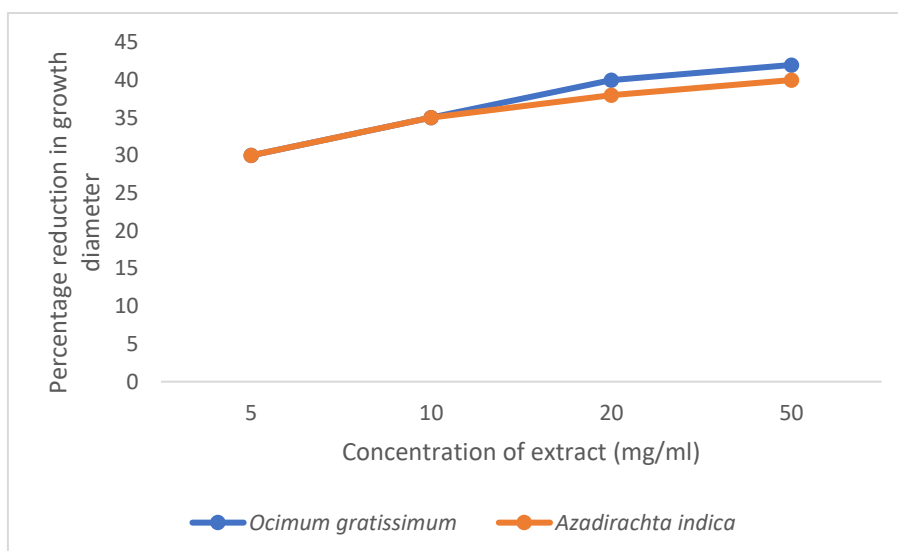


Figure 5: Comparative Potency of Ethanol Leaf Extracts of *Ocimum gratissimum* and *Azadirachta indica* against *Epidermophyton floccosum*

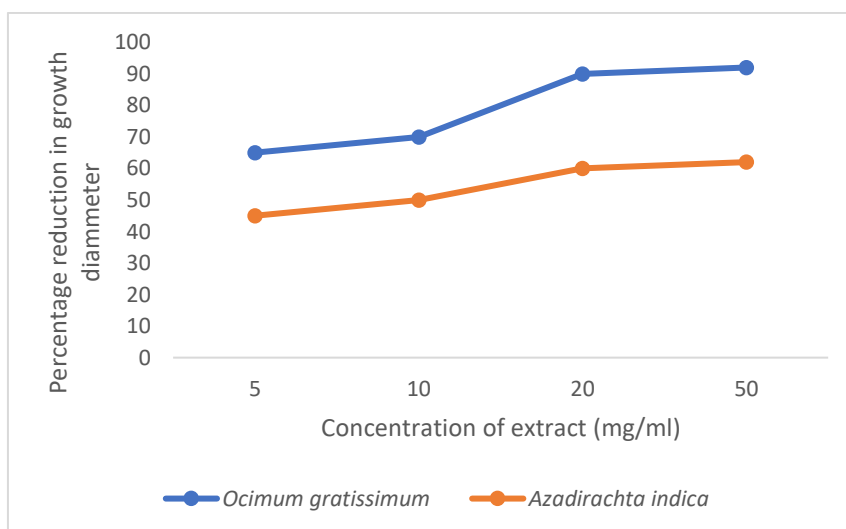


Figure 6: Comparative Potency of Ethanol Leaf Extracts of *Ocimum gratissimum* and *Azadirachta indica* against *Trichophyton verrucosum*

Discussion

In this present study, five dermatophytes spp. consisting of *Trichophyton equinum*, *T. mentagrophytes*-highest, *Microsporum gypseum*-lowest, *Epidermophyton floccosum* and *T. verrucosum* were identified. In agreement with this study, Lavanya (2013) identified *T. equinum* and *E. floccosum* among fungi isolated from skin scraping. *Epidermophyton floccosum* was the only geophilic specie and the second least number of dermatophyte isolated, as a result of sporadic nature of the disease caused by this specie; however, this was different from a study where *E. floccosum* was the most common dermatophyte isolated (Ansari et al., 2019). The low incidence of *Trichophyton verrucosum* obtained in this study, could be associated with low rates of interaction of patients with cattle which served as reservoir for the dermatophytes. Although in a particular study, *T. verrucosum* recorded the highest number of dermatophytes isolated, suggesting that prevailing pathogens vary among different regions and change with time in accordance with the existing living and hygiene conditions (Nweze & Eke, 2018).

Plants are becoming an important source of potentially useful structures for the developments of new chemotherapeutic agents (Mahesh & Satish, 2008). Medicinal plants have attracted the attention of several biological communities (Penecillia & Magno, 2011). The results of antifungal test of extracts of both plants against isolated dermatophytes, indicated that both aqueous and ethanol extracts of the leaves of *A. indica* and *O. gratissimum* possess antifungal activity against some dermatophytes; these plant materials therefore could be endowed with components that are either polar and non-polar which enhance their solubility in water and ethanol respectively. Moreover, they generally produce secondary metabolites which constitute an important source of antimicrobial agents and pharmaceutical drugs (Varaprasad et al., 2009).

However, aqueous extract of *O. gratissimum* and *A. Indica* were more potent against *T. equinum*, *T. mentagrophytes*, and *T. verrucosum* than *M. gypseum* and *E. floccosum*, while that of *A. indica* was more potent against *T. equinum*, *T. mentagrophytes*, *M. gypseum*, and *T. verrucosum* than *E. floccosum*. This is in agreement with Kaur & Gill, (2023) that reported higher activity of *A. indica* against *T. mentagrophytes*, *T. rubrum*, and *T. verrucosum* than against *E. floccosum*. However, Mbakwem-Aniebo et al (2012) reported lower MIC of *O. gratissimum* against *Microsporum* sp. than against *Trichophyton* sp. and *Epidermophyton* sp. The lower susceptibility of *E. floccosum* to the studied plant extracts in the present study and which is supported by many previous reports may be due to the peculiar physiology, metabolism, and adaptation strategies of the organism (Izah, 2018).

Extracts of *O. gratissimum*, has antifungal effect on *T. tonsurans* and *T. mentagrophytes*. This is in agreement with studies by, Iwuagwu et al. (2018) that reported antifungal properties of *O. gratissimum* against pathogenic fungi of rice, and Rodrigues et al. (2019) on the antifungal activities of *A. indica* in submerged cultures of aflatoxigenic *A. parasiticus*. Antifungal activity was more pronounced at higher concentrations (250mg/ml and 125ml/ml) than at lower concentrations (63mg/ml, 31mg/ml and 16mg/ml) for the aqueous extracts of both plants. Interestingly, the aqueous extract of *O. gratissimum* was comparatively more active than that of *A. indica*; and as such recorded MIC of 31 mg/ml against *T. equinum* and 63mg/ml against *T. verrucosum*. The minimum inhibitory concentration (MIC) of *A. indica* against *T. equinum*, *T. mentagrophytes*, *M. gypseum* and *T. verrucosum* was 125mg/ml. The aqueous extracts of both

plants inhibited all the dermatophytes at 250mg/ml. It was observed that, the aqueous extracts of both plants showed loss of activity after 2 fold double dilution, with exception of *O. gratissimum* against *T. equinum*. This is important in deciding dosage and mode of administration. For topical use, the crushing and rubbing of the plant leaves on infected skin without dilution may be found effective on treatment of infections by dermatophytes, and therefore can be incorporated into wound dressing ointments or local antiseptics.

The results based on percentage reduction in radial growth (RRG) shows that, the ethanol extracts of *O. gratissimum* and *A. indica* at the concentrations used, considerably and effectively reduced the radial growth of the dermatophytes except *E. floccosum* at 5mg/ml when there seemed to be no inhibition at all. The results of the antifungal sensitivity test showed that the antimicrobial potential of the ethanol extracts on *T. mentagrophytes* and *T. rubrum* was higher than that of distilled water (Adejumo & Bamidele, 2009). This is in agreement with a particular study, in which the results of the antifungal sensitivity test showed that the antimicrobial potential of ethanol extract on dermatophytes; *T. mentagrophytes* and *T. rubrum* was higher than those of distilled water. In this study, the better in-vitro activity of *O. gratissimum* over *A. indica* against the dermatophytes may be attributed to differences in their phytochemical constituents. Similarly, interesting results were also observed on the study by Koba et al., (2009) showing inhibitory effects of *O. gratissimum* where it elicited antifungal activities against various dermatophytes, imperfect filamentous fungi and pathogenic yeasts. This is in disagreement with another study which depicted methanol extract of *A. indica* (Neem) to have a higher inhibitory property than *O. gratissimum* against fungal isolates probably as a result of high abundance of tannin, Glycosides and phenolics in the neem plant (Aguguo et al., 2020)

In this study, the exhibition of least sensitivity of *E. floccosum* to the ethanol leaf extracts of *O. gratissimum* is a reflection of similar response to the aqueous extract of the plants. At 5mg/ml of *O. gratissimum*, there was no reduction in radial growth of *E. floccosum*. Compared with *O. gratissimum*, *A. indica* had better performance against *E. floccosum*. However, at higher concentrations of 50 mg/ml and 20 mg/ml, the ethanol leaf extracts of both plants recorded effectively high % RRG to all the dermatophytes including *E. floccosum*. This is important in considering dosage, such that higher concentrations of plant extracts may be required for effective treatment of infections, by *E. floccosum* and *M. gypseum* than those required for the other dermatophytes.

Both aqueous and ethanol extracts of the two plants have been demonstrated to be potent against the isolated dermatophytes, indicating effective diffusibility of the bioactive principles in both solvents. The high inhibitory activity in these extracts could be due to the concentration of the antimicrobial constituents of the solvent. The inhibitory effects of plants on microorganisms may be therefore, due to the presence of certain phytochemicals such as flavonoids, alkaloids, phenols and saponins (Babayi et al., 2004). The low activity of plants at a particular concentration to some test organisms may also be due to the higher volatility of the oil, leading to the escape or evaporation of the oil. Moreover, absence of in-vitro activity does not warrant disapproval of the ethno botanical utilizations as this may suggest extracts acting in an indirect way where active ingredient exist as a precursor requiring activation in-vivo.

CONCLUSION

Trichophyton mentagrophytes was the most prevalent among the dermatophyte isolates. The leaf extracts of *O. gratissimum* and *A. indica* were both effective in inhibiting the growth of the dermatophytes, therefore, justifying the ethno-medicinal uses of the plants. However, *O. gratissimum* was more effective, than *A. indica*, against each dermatophyte. The outcome of this study thus, indicated the potential of the two plants as veritable sources of lead compounds for antifungal drugs production. However, further studies are required to determine the specific bioactive compounds and mechanisms of action responsible for the antifungal properties of the two plants. New milestone in antifungal therapy can be achieved by the development of natural products from these medicinal plants that address unfulfilled therapeutic needs against fungal infections.

REFERENCES

- Abubakar, N., & Sandamu, H. S. (2026). Antibacterial activity of *Allium sativum* (garlic) against *Neisseria gonorrhoeae* and *Treponema pallidum*. *FUDMA Journal of Sciences*, 10(1): 253 – 258.
- Adejumo T.O., & Bamidele, B. S. (2009). Control of dermatophyte-causing agents (*Trichophyton mentagrophytes* and *Trichophyton rubrum*) using six medicinal plants. *Journal of Medicinal Plants Research*, 3(11): 906 - 913.
- Aguguo, K., Umedum, C. U., Nzeagwu, M. O., Opara, N. K., Onyema, C. T., Okorie, C. C., Umejiego, I. B., Korie, M. C., Onwubuche, B. C., & Uzoho, I. J. (2020). Antifungal activity of two plant extracts against fungi isolated from poultry droppings in Owerri, Imo State, Nigeria. *Nigerian Journal of Microbiology*, 34(1): 5070 – 5079.
- Akinnibosun, H. A., Rotimi J., & Aigbogun, O. C. (2009). "Larvicidal activity of leaf extracts of neem- *Azadirachta indica* A. Juss. (Family: Meliaceae) against mosquito larvae of *Aedes aegypti* Linn. (Diptera Culicidae). *Plant Archives*, 9(1): 509 - 513.
- Ansari, S., Ahmadi, B., Norouzi, M., Ansari, Z., Afsarian, M. H., Lotfali, E., & Rezaei-Matehkolaei, A. (2019). *Epidermophyton floccosum*: nucleotide sequence analysis and antifungal susceptibility testing of 40 clinical isolates. *Journal of Medical Microbiology*, 68(11): 1655 - 1663.
- Babayi, H., Kolo, I., Okogun, J., & Ijah, U. J. J. (2004). The antimicrobial activities of methanolic extracts of *Eucalyptus camaldulensis* and *Terminalia catappa* against some pathogenic microorganisms. *Biokemistri*, 16(2): 106 – 111.
- Bhosale, N. K., Prabha, R., Munuswamy, R., Pramodhini, S., & Easow, J. M. (2022). A comparative study on the phenotypic versus molecular identification of clinical dermatophytes. *Journal of Pure and Applied Microbiology*, 16(2): 1138 - 1146.
- Chandler, D. (2022). Direct microscopy in the dermatology clinic: enhancing the management of skin infections and infestations. *Clinical Experimental Dermatology*, 47(6): 1023 – 1029.
- Egwuatu, T. O., Ukhureigbe, O. M., Ojo, S. K. S., Ogeneh, B. O., Osanyinlusi, S. A., & Ajayi, O. E. (2019). Risk factors associated with *Tinea capitis* among pupils in Lagos, Ekiti and Ogun States, Nigeria. *Nigerian Journal of Microbiology*, 33(1): 4380 – 4386.
- Geddes-McAlister, J., & Shapiro, R. S. (2019). New pathogens, new tricks: emerging, drug-resistant fungal pathogens and future prospects for antifungal therapeutics. *Annals of the New York Academy of Sciences*, 1435(1):57 - 78.
- Iwuagwu, C. C., Onejeme, F. C., Ononuju, C. C., Umechuruba, C. I., & Nwogbaga, A. C. (2018). Effects of plant extracts and synthetic fungicides on the radial growth of *Phoma oryzae* on rice (*Oryza sativa* L) in some rice growing areas of South Eastern Nigeria. *Journal of Plant Pathology and Microbiology*, 9(12): 468 - 473.
- Izah, S. C. (2018). Some determinant factors of antimicrobial susceptibility pattern of plant extracts. *Research and Review Insights*, 2(3): 1 – 4.
- Kator, L., & Hosea, Z. Y. (2018). In vitro potential of aqueous extracts of plant leaves to inhibit pathogenic fungi. *Asian Journal of Research in Biochemistry*, 2(3):1 - 18.
- Kaur, S., & Gill, P. K. (2023). In-vitro anti-dermatophytic activity of different medicinal plant extracts. *International Journal of Indigenous Herbs and Drugs*, 8(5): 27 - 32.
- Koba, K., Poutouli, P. W., Raynaud, C., & Sanda, K. (2009). Antifungal activity of the essential oils from *Ocimum gratissimum* L. grown in Togo. *Journal of Scientific Research*, 1(1): 164 – 171.
- Lavanya, V. (2013). Isolation and identification of fungi causing dermatophytosis in a tertiary care hospital in bagalkot: Doctoral dissertation, Rajiv Gandhi University of Health Sciences (India). ProQuest Dissertations Publishing, 30559268.
- Mahesh, B., & Satish S. (2008). Antimicrobial activity of some important medicinal plants against plant and human pathogens. *World Journal of Agricultural Sciences*, 4 (5): 839 – 843.
- Mbakwem-Aniebo, C., Onianwa, O., & Okonko, I. O. (2012). Effects of *Ocimum gratissimum* leaves on common dermatophytes and causative agent of *Pityriasis Versicolor* in Rivers State, Nigeria. *Journal of Microbiology Research*, 2(4): 108 – 113.
- Mbata, T. I., Duru, M. C., & Onwumelu, H. A. (2009). Antibacterial activity of crude seed extracts of *Buchholzia coriacea* E. on some pathogenic bacteria. *Journal of Developmental Biology and Tissue Engineering*, 1(1): 001 - 005.
- Miethke, M., Pieroni, M., Weber, T., Brönstrup, M., Hammann, P., Halby, L., Arimondo, P. B., Glaser, P., Aigle, B., Bode, H. B., & Moreira, R. (2021). Towards the sustainable discovery and development of new antibiotics. *Nature Reviews Chemistry*, 5(10): 726 - 749.
- Moskaluk, A. E., & VandeWoude, S. (2022). Current topics in dermatophyte classification and clinical diagnosis. *Pathogens*, 11(9): 957 – 963.

- Nweze, E. I., & Eke, I. E. (2018). Dermatophytes and dermatophytosis in the eastern and southern parts of Africa. *Medical Mycology*, 56(1): 13 - 28.
- Okigbo, R. N., Okeke, J. J., & Madu, N. C. (2010). "Larvicidal effects of *Azadirachta indica*, *Ocimum gratissimum* and *Hyptis suaveolens* against mosquito larvae. *Journal of Agricultural Technology*, 6(4): 703-719: 703-719.
- Penecilla, G. L., & Magno, C. P. (2011). Antibacterial activity of extracts of twelve common medicinal plants in Philipines. *Journal of Medicinal Plants Research*, 5 (16): 3975 - 3981.
- Priyanka, C., Shivika, S., & Vikas, S. (2018). *Ocimum gratissimum*: A review on ethnomedicinal properties, phytochemical constituents, and pharmacological profile. In: Kumar, N. (ed) *Biotechnological approaches for medicinal and aromatic plants*, (pp.251-270). Springer Nature.
- Rodrigues, M. P., Astoreca, A. L., Oliveira, Á. A. D., Salvato, L. A., Biscoto, G. L., Keller, L. A. M., Rosa, C. A. D. R., Cavaglieri, L. R., Azevedo, M. I. D., and Keller, K. M. (2019). In vitro activity of neem (*Azadirachta indica*) oil on growth and ochratoxin A production by *Aspergillus carbonarius* isolates. *Toxins*, 11(10):579 - 591.
- Rubinchik, E., & Pasetka, C. (2010). Ex vivo skin infection model. In: Giuliani, A., Rinaldi, A. (eds) *Antimicrobial Peptides: Methods in Molecular Biology*, vol. 618 (pp.359-369). Humana Press, Totowa, NJ..
- Sánchez-Bautista, A., Coy, J., García-Shimizu, P., & Rodríguez, J. C. (2018). From CLSI to EUCAST guidelines in the interpretation of antimicrobial susceptibility: What is the effect in our setting? *Enfermedades infecciosas microbiologia clinica* (English ed.), 36(4): 229 - 232.
- Segal E., & Elad, D. (2021). Human and zoonotic dermatophytoses: epidemiological aspects. *Frontiers in Microbiology*, 6(12): 713 - 532.
- Varaprasad, B., Rajesh, K. C., Varahalarao, V., & Prasanth, K. K. (2009). Medicinal plant as alternative biocontrol agents in the control of seed borne pathogen *Macrophomina phaseolina*. *Varaprasad et al., 2009 of Pharmacy Research*, 2(6): 1045 – 1048.

