

Antifungal Activity of *Alchornea cordifolia* and *Ficus exasperata* Leaves Against *Trichophyton mentagrophyte* and *Trichophyton verrucosum*

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: The use of plant based natural products as alternative remedy for mycosis has gained global prominence, hence the need to explore the antifungal potentials of readily available herbs.

Aim: To compare the antifungal activity of the ethanol leaf extracts of *Alchornea cordifolia* and *Ficus exasperata* against *Trichophyton mentagrophyte* and *Trichophyton verrucosum* with that of conventional griseofulvin.

Method: Plant samples were extracted with ethanol via maceration. Phytochemical screening was carried out using standard techniques. The ethanol extract of the fresh and dried leaves of *Ficus exasperata* and *Alchornea cordifolia* were compared with griseofulvin (0.03mg/ml) for activity against *Trichophyton mentagrophyte* and *Trichophyton verrucosum* using agar well diffusion method at varying concentrations.

Results: Both crude drugs contains flavonoids, saponins, tannins, and cardiac glycosides. *Ficus exasperata* extracts also contains phlobatannin and terpene. The extracts exhibited a dose dependent fungal inhibition. The highest concentration (150mg/ml) of *A.cordifolia* and *F.exasperata* extracts exerted the highest zones of inhibition (16.7mm, 19.3mm, and 19.00mm, 19.7mm respectively) against *T.mentagrophyte*. The zones of inhibition of the test drugs against *T.verrucosum* were 20mm, 19.3mm, and 21.3mm, 24.7mm respectively. The least mean zone of inhibition was observed at 25mg/ml. Minimum inhibitory concentration (MIC) of 50mg/ml was obtained for *Alchornea cordifolia* fresh and dried leaves and *Ficus exasperata* dried leaves while minimum inhibitory concentration of 25mg/ml was obtained for the *Ficus exasperata* (fresh) leaves.

Conclusion: These results clearly elucidates the potentials of fresh and dry extracts of *A.cordifolia* and *F.exasperata* as a good source of antifungal compounds.

Keywords: *Ficus exasperata*, *Alchornea cordifolia*, Antifungal, Inhibitory, Phytochemical, Extract

INTRODUCTION

Over the years, plants have continually formed the basis for the development of sophisticated traditional medicine system which provides mankind with new healthcare remedies. Although some of the therapeutic properties attributed to plants have proven to be erroneous, medicinal plant therapy is based on the empirical findings of hundreds and probably thousands of years of use (Rios and Recio, 2005). Plants have been utilized as medicines for thousands of years (Samuelsson, 2004), these medicines usually occur in various herbal dosage forms such as tinctures, alcoholic beverages, tablets, capsules, ointment, tea, poultices, powders, creams etc (Kumadoh and Ofori-Kwakye, 2017). The antimicrobial and antitoxin properties of some plants, herbs and their components have been documented since the late 19th century (Saadabi, 2006). Today, more than half of over-the-counter drugs and prescription drugs are derived from herbs. According to the World Health Organization (WHO), about 80% of the world population is taking interest in indigenous medicinal plant remedies (Pirzada *et al.*, 2009). A recent study has reported the effectiveness of the fresh juice from the stem pith of *Alchornea cordifolia* in the management of respiratory, wound, urinary tract and gastrointestinal tract infections since an invitro study of this plant part revealed a high potency against *Klebsiella pneumoniae*, *Kocuria varians*, *Streptococcus pyogenes*, and *Salmonella enterica* (Owhe-ureghe and Akpo, 2016). Earlier scientific investigations has validated the traditional use of the fresh pith and leaves of *Alchornea cordifolia* in the management of dental carries and toothache caused by oral bacteria isolates such as *S. mutans*, *S. aureus* etc (Akpo and Owhe-ureghe, 2013). Apart from the previously mentioned scientifically demonstrated therapeutic use of *Alchornea cordifolia* extracts, the bark extract of *Alchornea cordifolia* has recently been discovered as a potential remedy for infections caused by *Staphylococcus aureus*, and *Bacillus subtilis* (Akpo *et al.*, 2020)

Recently, there has been a surge in the incidence of fungal related infections. This is due to the growth of immunocompromised populations such as organ transplant recipients, cancer and HIV/AIDS patients. Other likely factors have been linked to rampant cases of antibiotics resistance and toxicity associated with prolonged usage of several orthodox antifungal medications which has in turn led to an extended and more intensive search for newer natural remedies that can combat these opportunistic fungal infections (Giordani *et al.*, 2001). Some of the natural agents utilized by traditional healers in management of superficial dermatophytoses are *Ficus exasperata* and

Alchornea cordifolia. *Ficus exasperata* is also known as sandpaper tree (“Ewe ipin” in Yoruba) (Adebayo and Ishola, 2009), it is an evergreen terrestrial agrotropical shrub or tree. *Alchornea cordifolia*, on the other hand is commonly called “Christmas bush” or “dovewood”, known as Ubobo and Ewwa in Urhobo and Isoko languages of Delta State respectively (Akpo and Owhe-ureghe, 2013). It is a perennial shrub with simple alternate broadly ovate leaves, brown woody stems and green hanging fruits widely distributed in Nigeria (Ngaha *et al.*, 2016). Dermatophytes are pathogenic fungi having high affinity for keratinized tissues like nails, skin, or hair causing superficial dermatophytoses in both human and animals (Coelho *et al.*, 2008). Typical examples of this class of fungi are *Trichophyton mentagrophyte*, *Trichophyton verrucosum*. Apart from the genus trichophyton, other genera such as *Epidermophyton* and *Microsporum* are also implicated in dermatophytoses (Weitzman and Summerbell, 1995). Specifically, it is noteworthy to state that *Trichophyton mentagrophyte* and *Trichophyton verrucosum* re causative organisms of *Tinea pedis*, *Tinea capitis*, *Tinea barbae* etc.

Fungal infections are frequently persistent and necessitates long term, costly treatment with antifungal medications. The majority of people either explore alternative treatment approaches or leave the health problem untreated since they may not be able to afford the expense of pharmaceuticals. Among the prevalent diseases that are disregarded because they are thought to be non-life threatening are dermatophytoses. Proper medical attention should be given to dermatophytoses because if they worsen and become persistent, they could impair the skin’s defense against future skin infections and exacerbate existing issues (Rouzad *et al.*, 2018)

Generally, pharmacological options for treatment of superficial mycoses, particularly infections caused by dermatophytes include the azoles, allylamines, and griseofulvin (Hector, 2005), but recently, the use of some natural plant products has emerged to inhibit the causative organisms. Recent studies have proven the effectiveness of ethylacetate extract of *Sennaalata* against *Trichophyton mentagrophyte* and *Trichophyton verrucosum* while also demonstrating the zero activity of both hot water and ethyl-acetate extracts of *Alchornea cordifolia* against the aforementioned dermatophytes (Akpo *et al.*, 2022). Considering the controversy between the efficacy of medicinal plants and antifungal drugs, this research is thus undertaken to identify dermatophytes isolated from the head and foot of infected patients, compare the antifungal activity of the ethanol leaf extracts of two medicinal plants (*Alchornea cordifolia* and *Ficus*

exasperata) against both agent of dermatophytoses with that of conventional griseofulvin, and determination of the minimum inhibitory

METHODOLOGY

Materials and Methods

Beakers, conical flasks, measuring cylinders pipettes, spatulas, spirit lamps, cotton wool, aluminium foils, sabourand dextrose agar and Mueller Hinton agar (Titan biotech limited, Rajasthan India), Chloramphenicol (Keng pong pharmaceutical factory, China), Griseofulvin (Greenfield Pharmaceutical, China), Ethanol (BDH Chemicals Limited, Poole, England) and Sterile distilled water was obtained from pharmaceutical chemistry laboratory of Delta state university, Abraka, Nigeria. All reagents used for this study were analytical grade reagents.

Plant Collection and Preparation

Fresh leaves of *Ficus exasperata* Vahl (Moraceae) and *Alchornea cordifolia* (Euphorbiaceae) were collected during rainy season (March, 2022) from different locations in the main campus of Delta state university, Abraka, Nigeria. Leaves of both plants were identified and authenticated at the herbarium of the Botany Department. *A. cordifolia* (Euphorbiaceae) had Voucher Number DELSUH 128 and *F. exasperata* Vahl (Moraceae), DELSUH 211. The leaves of each plant were rinsed in distilled water and then air-dried for seven days in the laboratory.

The extraction of the plant extracts was carried out according to Mbakwem *et al.* (2012) with little modifications the air-dried leaves of *Ficus exasperata* and *Alchornea cordifolia* were pulverized into fine powders with a mechanical blender. Also fresh leaves of each plant were pounded. Fifty grams of fine powder of each of the dried leaves were dissolved in 200ml of 70% ethanol in conical flasks. The flasks were allowed to stand for 72 hours. The resultant mixtures in both flasks were filtered using a Whatmann filter paper, and the filtrates were evaporated to a paste like form using a rotary evaporator. The paste-like ethanol extracts were stored in labeled sterile bottles at refrigeration temperature of 4°C before assay

Phytochemical screening of the ethanol extracts

The ethanol extract of each plant was subjected to phytochemical analysis to detect the presence or absence of secondary metabolites using standard

concentration of these plant extracts against the dermatophytes.

qualitative procedures according to Nwankwo *et al.* (2021)

Test for Alkaloids

Mayer's test

2ml of each extract was treated with Mayer's test reagent (1.36 g of mercuric chloride and 5gm of potassium iodide in 100 ml of water) and equal volume of hydrochloric acid. The formation of cream colored precipitate confirmed the presence of alkaloid.

Wagner's test

Few drops of Wagner's reagent (1.27 g of iodine and 2 g of potassium iodide in 100 ml of water) and hydrochloric acid was added to 2 ml of both plant extract. The immediate appearance of reddish brown precipitate confirmed the presence of alkaloid.

Dragendorff's test

To 2ml of each extract, 2ml of hydrochloric acid and Dragendorff's reagent was added. The formation of an orange red precipitate indicated the presence of alkaloid.

Test for Anthraquinones

Borntrager's test

5mg of each powdered plant extract was boiled with 10% ferric chloride solution and 1ml concentrated hydrochloric acid. The extract was then filtered and cooled. Each of the filtrate was shaken with diethyl ether. 1ml of dilute ammonia solution was incorporated into each test-tube containing the ether extract. The formation of pink or deep red color in the aqueous layer confirmed anthraquinone presence.

Test for Flavonoids

NaOH test

2ml of each crude extract was measured into different test tubes, then 2 ml of aqueous sodium hydroxide and 2ml of hydrochloric acid was added to the test-tubes. The formation of yellow orange color indicated flavonoid presence.

Sulfuric acid test

2ml of each extract was treated with equal volume of concentrated sulfuric acid and observed for the appearance of orange color.

Test for Phenols

Ferric chloride test

A small portion of each extract was treated with 2ml of 5% ferric chloride and observed. The presence of deep blue or black color indicated a positive result

Test for Sterols

Liebermann-Burchard test

To 1ml of each extract, few drops of chloroform was added, then drops of acetic anhydride and sulfuric acid were added and observed for the formation of dark pink or red color.

Test for Terpenoids

Liebermann Burchard test

To 1ml of each extract, chloroform, acetic anhydride and drops of sulfuric acid was added. This was observed for the formation of dark green color.

Test for Saponin

Froth test

2 ml of distilled water was added to 2 ml of each extract. The mixture of the extract and the water was vigorously agitated and observed for the formation of persistent foam.

Emulsion test

1ml of olive oil was added to 2mls of each extract and shaken vigorously. Formation of two liquid layers that are immiscible (emulsion), indicated the presence of saponin.

Haemolysis test

2 ml of plant extract dissolved in 1% normal saline was added to 2 ml v/v of blood in normal saline and mixed properly. The mixture was centrifuged and the red supernatant was compared with that of the control tube containing 2 ml of 10% blood in normal saline diluted with 2 ml of normal saline.

Test for Quinones

1ml of extract was measured into a test tube and then 1ml of concentrated hydrochloric acid was added to the test tubes containing each extracts. The formation of yellow color precipitate confirmed presence of quinones.

Test for Tannins

3ml of each extract was treated with equal volume of acetic acid solutions and observed for the formation of red color solution.

Test for Cardiac Glycosides

To 2ml of extract, 2ml dilute HCl and 2 ml sodium nitropruside in pyridine and sodium hydroxide solution were added. Formation of pink to blood red color indicates the presence of cardiac glycosides.

Sample Collection

Samples of infected hairs and feet were collected from ten school age children within Abraka metropolis after seeking the consent of their parents/guardian. These aforementioned children had notable clinical manifestations of dermatophytoses. The sites of infection were first cleaned with methylated spirit. Specimen from the scalp was collected using a blunt scalpel to scrap the lesion from the scalp. Scales from the feet were collected by scraping using blunt scalpel from the edge of the lesion. All the samples were collected on a clean piece of paper and the papers were folded to enclose the specimen, they were labeled and transferred to Pharmaceutical Microbiology Laboratory of the Faculty of Pharmacy, Delta State University, Abraka for analysis of associated dermatophytes.

Identification of Dermatophytes

The hair and toe scrapings were examined microscopically to examine the macroconidia and microconidia characters as well as presence of hyphae and athroconidia. Samples were treated with 10% Potassium hydroxide and covered with cover slides. Microscopy was carried out under low power and subdued light. Infected hair and toe scrapings were seen encased in regular sheath of arthrospores that doubled their normal thickness; lactophenol cotton blue stain was used to improve visualization.

The process of inoculation was carried out in accordance with Shinkafi and Manga, (2011). Saboraud Dextrose agar was prepared, sterilized and used for isolation. After sterilization, the media was

allowed to cool to 45^oc and chloramphenicol 500mg/l was introduced into the medium to inhibit the growth of bacteria and the sterile medium was poured into the sterile petri dishes and allowed to solidify. The collected specimen were placed on the surface of the media. The culture media was incubated at room temperature for seven days. After isolation, the culture was transferred to freshly prepared sabourand dextrose agar media to obtain pure cultures. Pure cultures were also maintained in sabourand dextrose agar slants at 4^oc. The test dermatophytes were identified by their cultural morphology and microscopic characteristics as described by Hartmann and Rohde (1980). Pure culture samples were isolated and treated with 10% potassium hydroxide solution by flooding on slides. Lactophenol cotton blue was added to the slides to improve visualization. The slides were covered with cover slides and viewed under the microscope. Microscopy was carried out under low power and subdued light. A dermatophyte identification chart by Hardy diagnostics (2013) was used to identify the dermatophytes. The dermatophytes identified were *Trichophyton mentagrophyte* and *Trichophyton verrucosum*.

Invitro Antifungal Assay

Agar well diffusion method

Invitro antifungal activity was performed in triplicates using the agar well diffusion method as described by Cheesbrough (2006) with modifications. An antifungal drug (griseofulvin) was used as standard drug. The fungal isolates were allowed to grow on Sabourand dextrose agar (SDA) at room temperature until they are sporulated. A broth culture was prepared using peptone water and the fungi isolates were inoculated into the test tubes. The organisms were allowed to grow in the broth culture for 48 hours. After 48 hours, the fungi organisms was evenly spread on, solidified Mueller Hinton agar previously supplemented with 2% glucose concentration and 5µg/ml methylene blue using a sterile swab stick. The ethanol extracts of the leaves were prepared using serial dilution method. 5ml of different concentrations

of the solution were prepared to give concentration regimes of 150, 100, 50, 25 and 12.5mg/0.1ml. Wells were then bored into the agar media using a sterile 8mm cork-borer and the wells filled with 0.1ml of the test samples taking care not to allow spillage of the solution to the surface of the agar medium. The plates were allowed to stand on the laboratory bench for 1 hour to allow for proper diffusion of the extract into the media. The plates were incubated at room temperature for 72 hours and later observed for zones of inhibition. The effect of the extract on fungal isolates was compared with griseofulvin at a concentration of 0.03mg/ml.

Minimum inhibitory concentration

The estimation of MIC of the crude extracts was carried out using serial dilution method as described by Akinpelu and Kolawole (2004). Serial agar dilutions of the ethanolic extracts of the plants were prepared and 2ml aliquots of different concentrations of the solution were mixed with 18ml of pre-sterilized molten Mueller Hinton agar at 45^oC to give final concentration regimes of 150, 100, 50, 25, 12.5, 6.25 and 3,125mg/ml. The medium was poured into sterile petri dishes and then allowed to set. A mycelia disc, 5mm in diameter, cut from the periphery of the 7 days old cultures, was aseptically inoculated onto the medium and incubated at room temperature in the laboratory for 7 days after which they were examined for the presence or absence of growth. The minimum inhibitory concentration (MIC) was taken as the lowest concentration that prevented the growth of the test microorganism.

Statistical analysis

Results were reported as mean ± standard deviation (SD). Statistical analysis was carried out using one-way analysis of variance (ANOVA). Statistical significance was considered at P<0.05.

RESULTS

Phytochemical Analysis

Investigations on the phytochemical screening of the ethanolic extract of both the fresh and dry leaves of *Ficus exasperata vahl* and *Alchornea cordifolia Schumach and Thonn* (Table 1), indicated the presence of saponins, cardiac glycosides, tannins and flavonoids in all four extracts. Phlobatannins and terpenes were absent in *Alchornea cordifolia* but

present in *Ficus exasperata* while anthraquinones were absent in *Ficus exasperata* but present in *Alchornea cordifolia*. Alkaloids were absent in all four extracts. The phytochemical analysis of the ethanol extract of fresh and dry leaves of *Alchornea cordifolia* and *Ficus exasperata* are shown in Table 1.

Table 1: Phytochemical analysis of the ethanolic extract of fresh and dry leaves of *Alchornea cordifolia schumach&thonn* and *Ficus exasperata vahl*.

Phytochemical Component	<i>Alchornea Cordifolia</i> (dry)	<i>Alchornea Cordifolia</i> (fresh)	<i>Ficus exasperata</i> (dry)	<i>Ficus exasperata</i> (fresh)
Alkaloid	-	-	-	-
Tannin	+	+	+	+
Saponin	+	+	+	+
Cardiac glycoside	+	+	+	+
Anthraquinone	+	+	-	-
Phlobatannin	-	-	+	+
Flavonoid	+	+	+	+
Terpene	-	-	+	+

Key: + = Present; - = Absent

Evaluation of the Antifungal Activity of the Different Plant Extracts

The highest mean zone of inhibition was obtained for *Ficus exasperata* (fresh and dry) at 150mg/ml with mean zone of 19.67 ± 0.58 and 19.00 ± 1.00 mm respectively against *Trichophyton mentagrophyte*. The result however showed a significant difference when compared with griseofulvin (control) at $P < 0.05$ (Table 2). A mean zone of 24.67 ± 0.58 mm and 21.33 ± 0.58 mm was obtained for *Ficus exasperata* (fresh and dry) at 150mg/ml against *Trichophyton verrucosum* respectively (table 3) but the result showed a significant difference at $P < 0.05$. At 100mg/ml, *Ficus exasperata* (fresh and dry) exhibited antifungal properties against *Trichophyton mentagrophyte* with mean zone of 17.33 ± 0.58 mm and 16.00 ± 1.00 mm respectively (table 2) while a mean zone of 21.33 ± 0.58 mm and 14.67 ± 0.58 mm respectively was obtained against *Trichophyton verrucosum* (table 3). However, the result showed a significant difference ($P < 0.05$). Also, at 50mg/ml, *Ficus exasperata* (fresh and dry) showed antifungal activities with a zone of 8.67 ± 0.58 mm and 10.67 ± 0.58 mm against *Trichophyton mentagrophyte* respectively (table 2) while a mean zone of 16.67 ± 1.5 mm and 8.67 ± 0.58 mm respectively was obtained against *Trichophyton verrucosum* (Table 3). The result also showed significant difference from the control drug (griseofulvin) at $P < 0.05$. The least zone of inhibition was exhibited by *Ficus exasperata* (fresh) at 25mg/ml with a mean zone of 11 ± 1.00 mm against *Trichophyton verrucosum* (table 3) which was significantly different from the control drug (griseofulvin) at $P < 0.05$. At 150mg/ml, *Alchornea cordifolia* (fresh and dry) with a mean zone of 19.33 ± 1.15 mm and 16.67 ± 0.58 mm respectively showed the highest mean

zone of inhibition against *Trichophyton mentagrophytes* (Table 2) while the highest mean zone of inhibition was obtained against *Trichophyton verrucosum* with a mean zone of 20 ± 1.00 mm (fresh) and 19.33 ± 0.58 mm (dry) (table 3) which was significantly different from the control drug (griseofulvin) at $P < 0.05$. At 100mg/ml, there was significant difference between *Alchornea cordifolia* (fresh and dry) when compared with the control (griseofulvin). The mean zone of 14.67 ± 1.53 mm (fresh) and 12.67 ± 0.58 mm (dry) was obtained against *Trichophyton mentagrophyte* (Table 2) while a mean zone of 16.33 ± 0.58 (fresh) and 14.67 ± 1.15 (dry) was obtained for *Trichophyton verrucosum* (table 3). At 50mg/ml, *Alchornea cordifolia* (fresh and dried) showed the least antifungal activity against both *Trichophyton mentagrophyte* and *Trichophyton verrucosum* with a mean zone of inhibition of 8.33 ± 0.00 , and 10.33 ± 0.58 , 9.33 ± 1.15 respectively (Tables 2 and 3). However, at 25 and 12.5mg/ml, *Ficus exasperata* (dry) and *Alchornea cordifolia* (fresh and dry) showed no antifungal activity against both tested dermatophytes (Tables 2 and 3) but at 25mg/ml *Ficus exasperata* (fresh) exhibited antifungal potentials with a mean zone of 11 ± 1.00 mm (Table 3). Griseofulvin (control), at 0.03mg/ml, with a mean score of 29.67 ± 0.58 mm against *Trichophyton mentagrophyte* showed significant difference when compared with the ethanolic leaf extracts of *Alchornea cordifolia* and *Ficus exasperata* at all concentrations used (150, 100, 50, 25 and 12.5mg/ml). However, a mean score of 25 ± 1.00 mm was obtained against *Trichophyton verrucosum* and the result showed significant difference when compared with the ethanol leaf extract of *Ficus exasperata* (fresh) at 150mg/ml.

Table 2: Invitro antifungal assay of ethanol extracts of *Ficus exasperata vahl* and *Alchornea cordifolia Schumach&Thonn* (fresh and dry) on *Trichophyton mentagrophyte*

Conc. of extracts (mg/ml)	Zone of inhibition (mm)	Zone of inhibition (mm)	Zone of inhibition (mm)	Zone of inhibition (mm)
	<i>Alchornea cordifolia</i> (dry)	<i>Alchornea cordifolia</i> (fresh)	<i>Ficus exasperata</i> (dry)	<i>Ficus exasperata</i> (fresh)
150	16.7±0.58 ^b	19.3±1.15 ^b	19.00±1.00 ^b	19.7±0.58 ^b
100	12.7±0.58 ^b	14.7±1.53 ^b	16±1.00 ^b	17.3±0.58 ^b
50	8.0±0.00 ^b	8.3±0.58 ^b	10.7±0.58 ^b	8.7±0.58 ^b
25	0.00	0.00	0.00	0.00
12.5	0.00	0.00	0.00	0.00
Griseofulvin 0.03mg/ml	29.7±0.58 ^a			

Values represented by same letters are not significantly different based on ANOVA at $P < 0.05$

Table 3: Invitro antifungal assay of ethanol extracts of *Ficus exasperata vahl* and *Alchornea cordifolia Schumach and Thonn* (fresh and dry) on *Trichophyton Verrucosum*

Conc. of extract (mg/ml)	Zone of inhibition (mm)	Zone of inhibition (mm)	Zone of inhibition (mm)	Zone of inhibition (mm)
	<i>Alchornea cordifolia</i> (dry)	<i>Alchornea cordifolia</i> (fresh)	<i>Ficus exasperata</i> (dry)	<i>Ficus exasperata</i> (fresh)
150	20±1.00	19.3±0.58	21.3±0.58	24.7±0.58
100	14.7±1.15	16.3±0.58	14.7±0.58	21.3±0.58
50	9.3±1.15	10.3±0.58	8.67±0.58	16.7±1.53
25	0.00	0.00	0.00	11±1.00
12.5	0.00	0.00	0.00	0.00
Griseofulvin 0.03mg/ml	25±1.00			

Values represented by same letters are not significantly different based on ANOVA at $P < 0.05$

Table 4 shows the minimum inhibitory concentrations of the ethanolic leaf extracts of *Ficus exasperata vahl* and *Alchornea cordifolia Schumach&Thonn*. The highest MIC value of 50mg/ml was obtained for *Ficus exasperata vahl* and *Alchornea cordifolia Schumach&Thonn*. The highest MIC value of

50mg/ml was obtained for *Ficus exasperata* (dry) and *Alchornea cordifolia* (fresh and dry) against *Trichophyton mentagrophyte* and *Trichophyton verrucosum* and least MIC value of 25mg/ml was obtained for *Ficus exasperata* (fresh) against *Trichophyton verrucosum*.

Table 4: The minimum inhibitory concentration (MIC) of the ethanolic extracts of *Ficus exasperata vahl* and *Alchornea cordifolia schumach & thonn* on *Trichophyton mentagrophyte* and *Trichophyton verrucosum*

Plant extract	Minimum Inhibitory Concentration (mg/ml)	
	<i>Trichophyton mentagrophyte</i>	<i>Trichophyton verrucosum</i>
<i>Ficus exasperata</i> (dry)	50	50
<i>Ficus exasperata</i> (fresh)	50	25
<i>Alchornea cordifolia</i> (dry)	50	50
<i>Alchornea cordifolia</i> (fresh)	50	50

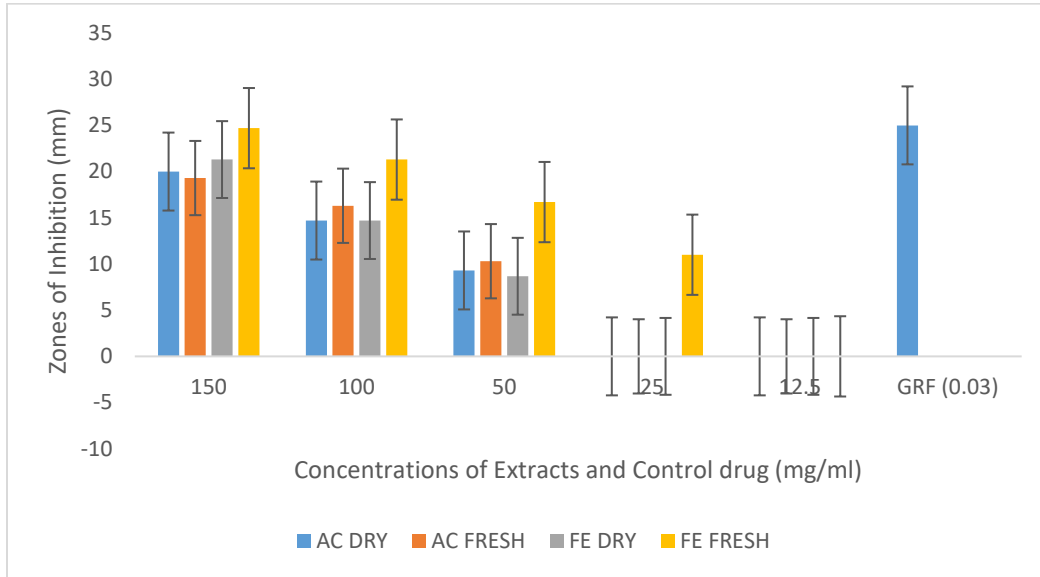


Fig. 1: *In-vitro* antifungal assay of ethanol extracts of *Ficus exasperata vahl* and *Alchornea cordifolia Schumach&Thonn* (fresh and dry) on *Trichophyton mentagrophyte*
 AC: *Alchornea cordifolia* , FE: *Ficus exasperata* GRF: Griseofulvin

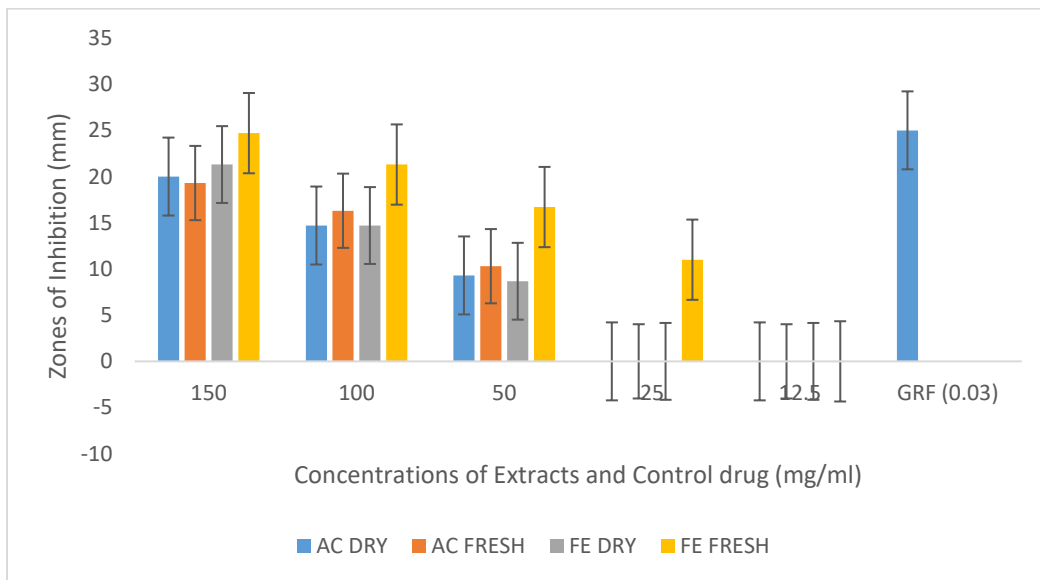


Fig. 2: *In-vitro* antifungal assay of ethanol extracts of *Ficus exasperata vahl* and *Alchornea cordifolia Schumach and Thonn* (fresh and dry) on *Trichophyton Verrucosum*
 AC: *Alchornea cordifolia* , FE: *Ficus exasperata* GRF: Griseofulvin

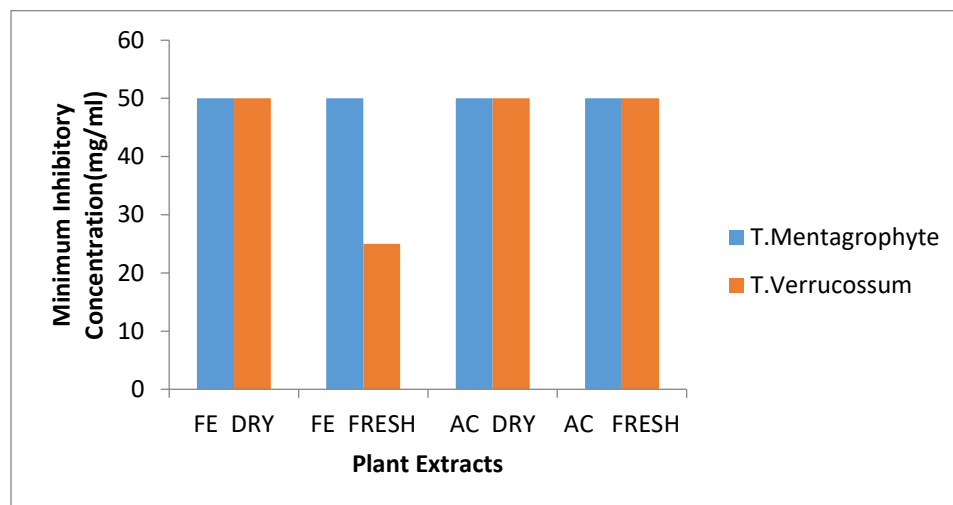


Fig. 3: The minimum inhibitory concentration (MIC) of the ethanolic extracts of *Ficus exasperata vahl* and *Alchornea cordifolia schumach&thonn* on *Trichophyton mentagrophyte* and *Trichophyton verrucosum*
AC: *Alchornea cordifolia* FE: *Ficus exasperata* GRF: Griseofulvin

DISCUSSION

The need to develop new antifungal agents stems from notable cases of resistance attributed to most microorganisms. This study explored the antifungal potentials of the dried and fresh ethanol extracts of *Alchornea cordifolia* and *Ficus exasperata* leaves. The medicinal value of these plants is mainly dependent on the presence of phytochemicals that elicits definite physiological action on the human body (Edeoga *et al.*, 2005).

Results obtained from the phytochemical screening of both fresh and dry leaf extracts of *Alchornea cordifolia* revealed the presence of tannins, cardiac glycosides, flavonoids and saponins. This result agrees with the findings of Agbor *et al.* (2004) and Palombo, (2006) who reported the presence of tannins and flavonoids in the leaf extract of *Alchornea cordifolia*. The presence of these secondary metabolites in plant extracts is an indication that the plant is of pharmacological importance (Adebayo and Ishola, 2009). Several plants which are rich in tannins are known to possess antifungal activities against a wide range of microorganisms (Doss *et al.*, 2009). The result of this study indicates that the ethanolic extracts of *Ficus exasperata vahl* and *Alchornea cordifolia* were similar in their antifungal potentials against *Trichophyton mentagrophyte* and *Trichophyton verrucosum*. Increase in the concentrations of the ethanolic leaf extracts gave rise to corresponding surge in mean zone of inhibition. The highest antifungal activity was observed at 150mg/ml for *Ficus exasperata vahl* (fresh and dry) against *Trichophyton mentagrophyte* and *Trichophyton verrucosum* respectively. This can also be seen in the case of a recent study in which ethanol leaf extract of *Ficus*

exasperata had significant inhibitory effect at higher concentrations of 200 and 250mg/ml against *malassezia furfur*, *Microsporum*, *Trichophyton* and *Epidermophyton*. (Mbakwem *et al.*, 2012) Also both ethanolic leaf extracts showed no antifungal effect at low concentrations of 25 and 12.5mg/ml except *Ficus exasperata* (fresh) which had the least mean zone of inhibition of 11.00mm against *Trichophyton verrucosum* at 25mg/ml. This is contrary to a study whose results showed all clinically tested dermatophytes were not inhibited at higher concentrations of crude extracts of *Senna alata linn* (Sule *et al.*, 2011). From the investigation, it was observed that *Trichophyton verrucosum* was more susceptible to both crude drugs as against *Trichophyton mentagrophyte*. This has been clearly elucidated by the mean zones of inhibition as presented in Tables 2 and 3. The findings of this present study clearly contradicts a recent report which revealed that the ethylacetate and hot water extracts of the stem piths of *Alchornea cordifolia* had no effect on the test dermatophytes but antifungal activity was clearly exhibited by ethylacetate extract of senna alata stem piths (Akpo *et al.*, 2022). Comparatively the ethanol extract of *Alchornea cordifolia* has been scientifically proven to possess moderate antifungal activity against the test dematophytes while the hot water and ethylacetate extracts exhibited zero activity against same dermatophytes in previous studies. This buttresses the ability of ethanol to extract better yields of phytoconstituents from crude drugs as well as limitations of water and ethylacetate in extracting good yields of phytoconstituents.

Furthermore, griseofulvin exhibited a higher zone of inhibition against the tested dermatophytes which was significantly different from both test drugs at $P < 0.05$. This may be attributed to the fact that conventional antibiotics are usually prepared from synthetic materials by means of reproducible manufacturing techniques and procedures, while herbal medicinal products prepared from plant and animal origins are subject to contamination and deterioration most of the time (El-mahmood and Ameh, 2007). The minimum inhibitory concentration (MIC) of 50mg/ml was

obtained for *Ficus exasperata* (dry) and *Alchornea cordifolia* (fresh and dry) against both test dermatophytes while *Ficus exasperata* (fresh) was 50mg/ml and 25mg/ml against *Trichophyton mentagrophyte* and *Trichophyton verrucosum* respectively. This is supported by findings of a recent study where the minimum inhibitory concentration (MIC) of the ethanol extract of *Ficus exasperata* (dry) was found to be 44.67mg/ml against *Trichophyton species* (Mbakwem *et al.*, 2012).

CONCLUSION

Conclusively, the activity of the ethanol extracts of *Ficus exasperata* and *Alchornea cordifolia* has been attributed to the presence of tannins, flavonoids, saponins and glycosides. The ethanolic leaf extracts were active against *Trichophyton mentagrophyte* and *Trichophyton verrucosum*. Therefore, the ability of the extracts to inhibit the growth of the tested fungal species is an indication of the broad-spectrum antimicrobial potential of *Ficus exasperata* vahl and

Alchornea cordifolia schumach&thonn, which makes them potent sources of antifungal drugs. In a nutshell, the findings of this study elucidates the potentials of fresh and dry extracts of *A.cordifolia* and *F.exasperata* as a good source of antifungal compounds, thereby supporting the use of these plants in the management of *Trichophyton mentagrophyte* and *Trichophyton verrucosum* induced fungal diseases.

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