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Effects of *Ficus Exasperata* Vahl on Common Dermatophytes and Causative Agent of *Pityriasis Versicolor* in Rivers State, Nigeria

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Abstract Antifungal study on *F. exasperate* is rare in Nigeria and hence this study seeks to justify the ethobotanical uses of the plant. The antifungal activity of *Ficus exasperata* vahl against the three major Dermatophytes – *Trichophyton, Microsporum, Epidermophyton* together with *Malassezia furfur* (the cause of *Pityriasis versicolor* (Eczema)), were studied by well-in-agar diffusion technique using 250mg/ml, 200mg/ml, 150mg/ml, 100mg/ml and 50mg/ml concentrations of ethanolic extracts. Isolates from the scalp, skin, toes and feet of forty individuals (mainly children) were obtained in four locations namely; Aluu, Choba, Rumuosi and Emohua areas of Rivers State. The results of *in vitro* antimicrobial screening of ethanol extracts from the plant displayed a wide range of activity on *Trichophyton, Microsporum, Epidermophyton* together with *Malassezia furfur*. The leaf ethanol extract of *Ficus exasperata* vahl inhibited the growth of all four fungal isolates at the five different concentrations (250mg/ml, 200mg/ml, 150mg/ml, 100mg/ml and 50mg/ml) to varying degrees. The diameter zones of inhibition exhibited by the extracts against the test fungal species ranged between 13.00 and 22.45mm. The minimum inhibitory concentrations (MICs) of the ethanol extract of *Ficus exasperata vahl* were 25.12, 39.81, 44.64 and 44.67 mg/ml for *Malassezia furfur*, *Microsporum*, *Trichophyton* and *Epidermophyton,* respectively. The results revealed the significant inhibitory effect of *Ficus exasperata vahl* on the fungal isolates. *F. exasperate* extracts has shown to be a promising potential of new antifungal drug especially as a potent vehicle in antifungal drug design. The zones of inhibition exhibited by the extract on the fungi suggest that it could be effective in the treatment of infections associated with these fungi isolates. The appreciable levels of inhibition recorded for *Ficus exasperata vahl* on the isolates indicates that this plant could be potent sources of novel antifungal drugs. The results from this study indicated potentials of leaves extract as a source of antifungal compounds. Further studies should be carried out to unravel the identity of the active ingredients.

Keywords Antifungal Agents, Dermatophytes, *Ficus Exasperata Vahl*, Mics, Zones of Inhibition

1. Introduction

Ficus species (Moraceae) are plants commonly known globally as the "fig plant". There are about 800 species occurring chiefly in Indomalaya and Polynesia[1]. Over 45 different species are found in Nigeria[2], and are primarily located in the rainforest, Savannah and besides rivers and streams. Ficus exasperata is a medicinal plant referred to as "sand paper plant" ("Ewe ipin" in Yoruba language of Western Nigeria). The leaf extract from F. exasperata is used for the treatment of various diseases. Different parts of the plant are used for treating eye-sores, ring worm, stomach pains and treating leprosy[3-5]. The leaf extract from F. exasperata reported to have diverse uses such as treating hypertensive patients[6], heamostative opthalmia, coughs, heamorrhoid[1] and epilepsy, high blood pressure, rheumatism, arthritis, intestinal pains, and colics, bleeding, ulcer and wounds[7-14]. Other uses of *Ficus exasperata* are stabilization of vegetable oils and food supplement[1].

In Nigeria, the young leaves of *F. exaperata* are prescribed as a common anti-ulcer remedy[1]. Various pharmacological actions such as anti-ulcer, anti-diabetic, lipid lowering and antifungal activities have been described for *F. Exasperate*[1, 15]. The essential oils of this genus are much studied. Other industrial uses of sand paper leaves are for polishing woods[16], stabilization of vegetable oils, suppression of foaming, supplement as food stock and antimicrobial[1]. The activities of leaf extract of *F. Exasperata* against some pathogenic organisms have been investigated[1,6]. Antifungal activities for *F. exasperata* have also been reported[15, 17] but is not extensive.

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Extraction is the key step for the recovery and isolation of bioactive phytochemicals from plant materials, earlier than component analysis. The analysis and extraction of plant compounds play a vital role in the development, upgrading and quality control of natural products formulations. Currently, there is growing public concern on the possible risks of the use of synthetic fungicides on food commodities to human health and environmental pollution[18]. Therefore, alternative option the use of bio-control agent or biofungicides can protect the environmental pollution as well as human health.

Antifungal study on *F. exasperate* is rare in Nigeria and hence this study seeks to justify the ethobotanical uses of the plant. Thus, this study was carried out to examine the antifungal activities of *F. exasperate* on common dermatophytes and the causative agent of *Pityriasis versicolor* thus contributing to the list of plants used in the treatment of infections.

2. Materials and Method

2.1. Plant Collection and Identification

Fresh leaves of *Ficus exasperata* vahl were collected from Oredo Local Government Area, Benin City, Edo State, Nigeria. They were identified at the herbarium of the University of Port Harcourt, Rivers State, Nigeria.

2.2. Extract Preparation

The plant leaves were air dried for one week and then ground into fine powders with a mechanical grinder. Twenty grams of the fine powder was dissolved in 250ml of ethanol (95%) placed in conical flasks. The flasks were then covered and shaken every 30 minutes for 6 hours and allowed to stand for 48hours afterwards. Empty conical flasks were also weighed. The solutions obtained from each of the mixtures were then filtered with Whatman filter paper #1 and placed in the empty conical flasks. The filtrate was evaporated to dryness using a hot air oven at a much reduced temperature. The weight of the extract was determined after which the latter were stored below ambient temperature. The crude extract was diluted with 30% dimethyl sulphoxide (DMSO) obtained from the mixture of 30ml dimethyl sulphoxide with 70ml distilled water in a 250ml conical flask. The mixture was shaken gently and covered afterwards. Two hundred and fifty grams per ml (250mg/ml) concentration of crude extract was obtained by dissolving 1g of extract in 4ml of 30% DMSO; 200mg/ml concentration of crude extract was obtained by dissolving 1g of extract in 4ml of 30% DMSO; 150mg/ml concentration of crude extract was prepared by dissolving 1.2g of extract in 8ml of 30% DMSO; 100mg/ml and 50mg/ml concentration of crude extract was prepared by diluting the 200mg/ml concentration of crude extract.

2.3. Specimen Collection

The specimens were collected from different parts of the body of various individuals of different age groups, mainly children. Forty (40) individuals were sampled. This procedure was carried out using forty (40) new surgical blades for each individual. Specimen were collected by scraping affected spots into clean sheets of paper which were then transferred into sterile containers that had been properly labelled with respect to each individual's data, these were brought to the laboratory for inoculation.

2.4. Clinical Appearance of Specimen

The lesions on the body of the individuals had various appearances. These ranged from the formation of dense to flat mass of skin which could be black or reddish in colour. Others were a mixture of black and red lesions. The shapes of the lesions were also variable. Some were circinate, these were also dry, irregular, and scaly with thin marginated epidermis, not healing at the centre. Some other lesions were diffused having broken hairs which were grey to white in appearance.

2.5. Specimen Inoculation

The Sabouraud Dextrose Agar (SDA) was prepared and poured into forty (40) sterile Petri dishes and allowed to solidify. The media was then inoculated with each of the specimen after which the culture was incubated at room temperature for growth to occur. Subculturing into fresh SDA agar was carried out after about four days of incubation. The plates were then incubated at room temperature for about four days. This was followed by macroscopic and microscopic examination.

2.6. Evaluation of Antifungal Activity

In-vitro antifungal activity was performed using the agar well diffusion assay as describe by Hufford *et al*.[19] with some modifications. The organism was grown in Sabouraud-dextrose broth (SDB) for 72 h at 25°C. The cells were harvested by centrifugation (2000 rpm, 3 min.). The cells were then washed and suspended in sterile 0.9 % saline to give a final concentration of 10^6 cfu/ mL. Disposable petri-dishes containing Sabouraud-dextrose agar were streaked with the suspension (10^6 cfu/mL) of four (4) different specie of fungi namely *Trichophyton*, *Microsporum*, *Epidermophyton* and *Malassezia furfur* (the cause of *Pityriasis versicolor*) using sterile cotton swabs. Cylindrical plugs were removed from agar plates by means of a sterile cork borer to produce wells with a diameter of 10 mm. To the well was added 100 mL of test solution. The activity was recorded as the width in mm of the inhibition following incubation of the plates at 25°C for 72 h. This process was repeated using the Potato Dextrose Agar (PDA) medium. The plates were allowed to stand for one hour for pre-diffusion of the extracts to occur.

2.7. Minimum Inhibitory Concentration (MIC) Determination

The method used to determine the MIC was the twofold

serial broth dilution assay in yeast nitrogen broth (Difco). The extract was initially tested using a concentration of 100 mg/mL in the first tube. The test compound was added to sterile Sabouraud-Dextrose broth as a solution in dimethyl sulphoxide (DMSO). The inoculum for the MIC determination was prepared as describe by Clark et al.[20]. With the help of calibrated sterile wire loop, 10mL of the 10^6 cfu/mL suspension of four (4) different specie of fungi namely *Trichophyton*, *Microsporum*, *Epidermophyton* and *Malassezia furfur* (the cause of *Pityriasis versicolor*) was used as inoculum for each tube. The MIC value was taken as the lowest concentration of compound that inhibited the growth of the test organisms after 24 and 48 h of inoculation at 25°C.

3. Results

3.1. Evaluation of the Antifungal Activity of the Different Plant Extracts

The ethanolic extract of *Ficus exasperata vahl* was applied on the fungal isolates *Epidermophyton, Trichophyton, Malassezia furfur, Microsporum* using agar diffusion in order to determine their antifungal activity on these isolates. At the end of the incubation, the plates (Petri dishes) were collected and the diameter zones of inhibition that developed were measured. The average diameter of the zones of inhibition for the extract was calculated. It ranged from 13.00mm to 22.45mm. Results are shown in Table 1. In general, diameter zones of inhibition decreased with decrease in the concentration of the extract[21-22].

Table 1. Antimicrobial Activity of Ethanolic Extracts of *Ficus exasperata vahl* on the Fungal Isolates

Concentration of Extract (mg/ml)	Zones of Inhibition (mm)			
	Epidermophyton	*Trichophyton*	*Microsporum*	*Malassezia furfur*
250	18.00	21.50	22.45	19.10
200	17.20	20.10	21.24	18.50
150	16.42	16.50	20.50	16.40
100	16.01	18.10	19.20	15.20
50	15.30	16.44	17.50	13.00

3.2. Minimum Inhibitory Concentrations of the Ethanolic Extracts of *Ficus exasperata vahl* (mg/ml)

Table 2 shows the minimum inhibitory concentrations of the ethanolic extracts of *Ficus exasperata vahl* (mg/ml). The highest MIC value of 44.67mg/ml was obtained for *Ficus exasperata vahl* against *Epidermophyton* and least MIC value of 25.12mg/ml was obtained against *Malassezia furfur* (Table 2). Generally, the MICs of the ethanol extract of *Ficus exasperata vahl* were 25.12, 39.81, 44.64 and 44.67 mg/ml for *Malassezia furfur*, *Microsporum*, *Trichophyton* and *Epidermophyton,* respectively (Table 2).

Table 2. Minimum Inhibitory Concentrations (MICs) of the Ethanolic Extracts of *Ficus exasperata vahl* Used (mg/ml)

Fungal isolates	Minimum Inhibitory Concentrations (mg/ml)
Epidermophyton	44.67
Trichophyton	44.64
Microsporum	39.81
Malassezia furfur	25.12

4. Discussion

The quest for plants with medicinal properties continues to receive attention as they are known for a range of biological activities which range from antibiotics to antitumor[23]. The aim of this study was to investigate the antifungal activities of *Ficus exasperata vahl* against *Pityriasis versicolor* and other dermatophytoses. The antifungal activity of the plant extracts were tested on selected clinical fungal isolates (*Malassezia furfur*, *Microsporum*, *Trichophyton* and *Epidermophyton*). *Malassezia furfur* is responsible for *Pityriasis versicolor*, a superficial infection of the skin[24]. *Epidermophyton, Microsporum, Trichophyton* is responsible for a number of other dermatophytic infections.

In this study, the ethanolic extract of *Ficus exasperata vahl* inhibited the growth of these fungal isolates: *Epidermophyton, Trichophyton, Malassezia furfur, Microsporum* at different concentration. The ethanolic extract of *Ficus exasperata vahl* exhibited a significant zone of inhibition against the tested strain of the fungal isolates. The minimum inhibition concentration (MIC) of the ethanolic extract was found to be 25.12, 39.81, 44.64 and 44.67 mg/ml for *Malassezia furfur*, *Microsporum*, *Trichophyton* and *Epidermophyton,* respectively. Also, our results showed that the plant extract had significant inhibitory effect at high concentrations of 250mg/ml and 200mg/ml. The extract gave a diameter zone of inhibition (22.45mm) against *Microsporum* at a concentration of 250mg/ml. At 200mg/ml, the same extract had a diameter zone inhibition diameter of 21.24mm against *Microsporum*. For *Epidermophyton,* the extract from *Ficus exasperata* vahl had a diameter zone inhibition of 18.00mm and 17.20mm at concentrations of 250mg/ml and 200mg/ml respectively. For *Trichophyton* and *Malassezia furfur,* the zones of inhibition were 21.50mm and 19.10mm respectively at 250mg/ml. *Ficus exasperata* vahl inhibited all four fungal isolates at the five different concentrations to varying degrees.

The activity of this extracts against *S. typhi, E. coli* and *V. cholerae* which are the potential causative agents of abdominal ailment in previous study by some authors, reported that young leaves of *F. exasperata* are used as remedy for some abdominal ailments[15, 25]. The decoction of the leaf has been established to exhibit significant reduction in intestinal motility in addition to its anti-ulcer activity with no sign of toxicity[26]. The stem bark and leaf is used traditionally in treatment of typhoid fever and various stomach related problems[1].

The current drug of choice for disseminated

dermatophytoses and *Pityriasis versicolor* is amphotericin B with MIC value of 0.39 mg/ml. Oladosu *et al.[*17] reported that the growth of the yeast, *C. abicans* was inhibited at low MIC (1.1µg/ml). Our results showed that the MIC values required to inhibit the growth of *Epidermophyton, Microsporum, Trichophyton and Malassezia furfur* were significantly higher (44.67mg/ml, 39.81mg/ml, 44.64mg/ml and 25.12mg/ml respectively). Adebayo *et al.[*25] also showed that *F. exasperata* leaf, stem bark and root contained active substances with the highest inhibitory activities against some human pathogens. The stem bark extract had MICs of 50 mg/ml on *P. aeruginosa,* 1.0 mg/ml on *S. typhi* and 75mg/ml on *S. aureus*.

The activities of leaf extract of *F. exasperata* against some pathogenic organisms have been extensively investigated[1, 6]. *F. exasperata* has been reported as one of the common medicinal plants used in Africa[27]. In many scientific literatures, plant extracts have been reported to have poor antibacterial and antifungal activity[28-30]. However, this study has shown that *F. exasperata* has good antifungal activity against common dermatophytes and *Pityriasis versicolor*. This is also supported by the findings of Oladosu *et al.*[17] where *F. exasperata* was shown to be effective against *Candida albicans.* The study by Taiwo et al.[31] showed that *F. exasperata* have antioxidant properties and may contribute to the treatment of oxidative damage-related diseases for which it is used in folk medicine. It has also been reported that the leaves of *F. exasperata* are used traditionally in the treatment of ringworm, a fungal infection[4].

Phytochemicals can be derived from any part of the plant like bark, leaves, roots, fruits, seeds, fruit rind, flowers, stem, whole plant, etc. and antimicrobial activity of different parts of the plant is reported in literature[32]. However, leaf is generally the preferred part for therapeutic purpose[23, 33]. This may be because leaves contain more number of secondary metabolites which may be responsible for its antimicrobial activity[23]. They can inhibit the growth of microbes in many ways such as by inhibiting protein synthesis, interfering with nucleic acid synthesis, breaking the peptide bonds, acting as chelating agents, inhibiting metabolic pathway, interfering with cell wall synthesis or by preventing utilization of available nutrients by the microorganisms. Some compounds also cause lyses of microbial cells[23].

In the study by Odunbaku et al.[1], the plant extract displayed antimicrobial activity and therefore justifies its ethnobotanical uses for the treatment of ophthalmic, coughs, colic and hemorrhoids. *F. exasperata* could be taken alongside some synthetic drugs, during the treatment of diseases caused by *E. coli* and *S. albus*[1, 34-35].

In this study, the plant extract inhibited the growth of the fungi *Epidermophyton, Microsporum, Trichophyton and Malassezia furfur* to a high degree. The zones of inhibition exhibited by the extract on the fungi suggest that it could be effective in the treatment of infections associated with these microorganisms. One of the limitation of this study is that the antifungal activities of the plant extracts were not tested against the antifungal activity of any known antifungal drug for comparison. However, the present study has lent support to the findings of previous stuides on the antifungal activities of *F. exasperata*.

5. Conclusions

The MIC results obtained showed that the plant extract was very active against the human pathogens tested for in this study. The finding in this study has justified the use of this plant in ethnomedicinal treatment of fungal diseases which are caused by some of these organisms used in this study. *F. exasperate* extracts could be a promising potential of new antifungal drug especially as a potent vehicle in antifungal drug design. The results obtained in this study also revealed the significant inhibitory effect of the ethanolic extract of *Ficus exasperata* vahl on the fungal isolates; *Trichophyton, Microsporum, Epidermophyton* and *Malassezia furfur* that causes *Pityriasis versicolor*. This indicates that the plant possesses medicinal activity that can inhibit the growth of this group of microorganisms. The appreciable levels of inhibition recorded for *Ficus exasperata vahl* on the isolates indicates that this plant could be potent sources of novel antifungal drugs. Further studies should be carried out to unravel the identity of the active ingredients as well as its medicinal properties.

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