Journal of Microbiology Research 2012, 2(4): 108-113
DOI: 10.5923/j.microbiology.20120204.08

Effects of *Ocimum Gratissimum* Leaves on Common Dermatophytes and Causative Agent of *Pityriasis Versicolor* in Rivers State, Nigeria

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Abstract This antifungal study on *Ocimum gratissimum* is rare in Nigeria and hence this study seeks to justify the ethobotanical uses of the plant. The antifungal activity of *Ocimum gratissimum* used by traditional medicine practitioners 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 different 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, Nigeria. The results of the study revealed the significant inhibitory effect of *Ocimum gratissimum* at five different concentrations of 250mg/ml, 200mg/ml, 150mg/ml, 100mg/ml and 50mg/ml used. The diameter zones of inhibition exhibited by the extracts against the test fungal species ranged between 12.50 and 20mm. The minimum inhibitory concentrations (MICs) of the ethanol extract of *O. gratissimum* was 50.01, 52.40, 63.06 and 63.09 mg/ml for *Malassezia furfur*, *Microsporum*, *Trichophyton* and *Epidermophyton,* respectively. Assessment of the various MICs showed that *Ocimum gratissimum* has great potential for use as an antidermatophytic agent. The study showed that the extracts from the leaves of *Ocimum gratissimum* had pronounced antifungal activities on all the fungi tested. The study has shown that the leaves extracts of *O. gratissimum* are quite promising and have strongly indicated the antifungal activity spectra of leaves extract of the plant. In conclusion, the results from this study indicated potentials of leaves extract of *Ocimum gratissimum* as a source of antifungal compounds.

Keywords Antifungal activity, Antidermatophytic Agent, Ethanol Extract, Mics, *Ocimum Gratissimum*, *Malassezia furfur*, *Microsporum*, *Trichophyton* and *Epidermophyton*

1. Introduction

Traditional medicine is a popular form therapy in developing countries and its use is widely documented in various literatures[1-2]. Due to the huge potential of plant herbs in the discovery of novel therapeutic molecules, various national governments have placed traditional medicine under the supervision of health ministries[3]. In many rural areas of Nigeria the use of herbal plants as treatments for various bacterial infections still thrives. Some of these plants have been analysed and shown to be effective against well known pathogens such as *Staphylococcus aureus* and *Salmonella typhimurium*[4-5].

Ocimum gratissimum L. is a shrub belonging to the family Lamiaceae. It is commonly known as Scent leaf or Clove basil and is found in many tropical countries. Africa and Asia are however, the two continents where most variants of the plant exists[3,6-7]. *O. gratissimum* is found in the tropical and warm temperature regions such as India and Nigeria[8-9]. Some of the vernacular names in Nigeria include: (Ncho-anwu, Ahuji) Igbo, (Efinrin,) Yoruba, (Aramogbo) Edo and (Daidoya) Hausa[9-11]. *O. gratissimum* has been described to have other species in the flora of tropical West Africa. These include: *Ocimum viride* Linn, *Ocimum suave* Linn, *Ocimum basilicum* Linn and *Ocimum canum* Sims[9,11].

Mshana et al.[12] reported their numerous medical uses. The *Ocimum* oil has been described to be active against several species of bacteria and fungi. These include *Listeria monocytogenes, Shigella, Salmonella* and *Proteus,* for fungi *Trichophyton rubrum, Trichophyton mentagrophytes, Cryptococcus neoformans, Penicillum islandi cum*, and *Candida albicans[*4,9,13-16]. From recent findings, *O. gratissimum* has proved to be useful in the medication for people living with Human Immunodeficiency Virus (HIV), and Acquired Immuno Deficiency Syndrome virus AIDs[9,17]. In Congo, *O. gratissimum* decoction is used for

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Published online at http://journal.sapub.org/microbiology

gonorrheal infection, vaginal douches for metritis and vaginitis and used in treatment of mental illness[9,18].

Antibacterial action of ethanolic extracts against a range of pathogenic bacteria such as *Escherichia coli*, *Streptococcus viridians*, *Klebisiella pneumonia*, *Pseudomonas aeruginosa, Listeria monocytogenes and Proteus vulgaris* has been documented[2,11]. The Ocimum oil is also active against several species of bacteria (*Staphylococcus aureus, Listeria monocytogenes, Escherichia coil*, Shigella, Salmonella and Proteus) and fungi (*Trichophyton rubrum, T. Mentagrophytes, Cryptococcus neoformans, Penicillium islandi cum,* and *Candida albicans*[4,11,13-16,19-21].

Though a lot of literature on the antibacterial action of the plant is available, little information is known about its antifungal action. Antifungal study on *Ocimum gratissimum* 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 *Ocimum gratissimum* on various dermatophytes including *Malassezia furfur*

2. Materials and Method

2.1. Plant Collection and Identification

Fresh leaves of *Ocimum gratissimum* were collected form 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. Preparation and Extraction of Crude Extract

The leaves of *Ocimum gratissimum* were separated manually. The materials were cleaned with sterile distilled water; air dried and finely ground using a grinder mill. Twenty grams of the fine powder from *Ocimum gratissimum* leaves were placed in 250 ml of solvent (95% ethanol), placed in a conical flask and refluxed at 50℃ for 60 min[22]. The extracts were filtered through Whatman filter paper No. 1 and ethanol extracts were evaporated to dryness using a hot air oven at a much reduced temperature (40℃). The residues obtained were dissolved in 1% dimethyl sulphoxide (DMSO). The weight of the extract was determined and stored below ambient temperature.

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. Specimens 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

Table 1. Antimicrobial Activity of Ethanolic Extracts of *Ocimum gratissimum* on the Fungal Isolates

Concentration of Extract (mg/ml)	Zones of Inhibition (mm)			
	Epidermophyton	Trichophyton	Microsporum	Malassezia furfur
250	22.40	20.20	18.50	21.50
200	18.50	18.70	17.20	20.50
150	18.10	16.80	16.40	20.20
100	17.50	15.50	15.10	18.50
50	15.90	13.00	12.50	16.20

The ethanolic extract of the *Ocimum gratissimum* leaves was applied on the fungal isolates *Epidermophyton, Trichophyton, Malassezia furfur, Microsporum* using agar diffusion as described by Matasyoh *et al.*[6] and Nwaeze and Eze[3] in order to determine their antifungal activity on these isolates. The fungal isolates were allowed to grow on a Sabouraud dextrose agar (SDA) (Oxoid) at 25℃ until they sporulated. The fungal spores were harvested after sporulation by pouring a mixture of sterile glycerol and distilled water to the surface of the plate and later scraped the spores with a sterile glass rod. The harvested fungal spores and bacterial isolates were standardized to an OD600nm of 0.1 before use. One hundred microliter of the standardized fungal spore suspension was evenly spread on the SDA (Oxoid) using a glass spreader. Wells were then bored into the agar media using a sterile 6 mm cork borer and the wells filled with the solution of the extract 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 h to allow for proper diffusion of the extract into the media. Plates were incubated at 25℃ for 96 h and later observed for zones of inhibition. The effect of the extract on fungal isolates was not compared with amphothericin B and miconazole at a concentration of 1 mg/ml.

2.7. Minimum Inhibitory Concentration (MIC)

The MIC estimation of the crude extract of *Ocimum gratissimum* was determined using the methods of Sahm and Washington[23] and Matasyoh *et al.*[6]. Two-fold dilutions of the crude extract was prepared and 2 ml aliquots of different concentrations of the solution were added to 18 ml of pre-sterilized molten SDA for fungi at 40℃ to give final concentration regimes of 0.050 and 10 mg/ml. The medium was then poured into sterile Petri dishes and allowed to set. The surface of the medium was allowed to dry under laminar flow before streaking with 18 h old fungal cultures. The plates were later incubated at 25℃ for up to 72 h for fungi, after which they were examined for the presence or absence of growth. The MIC was taken as the lowest concentration that prevented the growth of the test fungus.

3. Results

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

At the end of the incubation, the plates (Petri dishes) were collected and the zones of inhibition that developed were measured. The average of the zones of inhibition for each extract was calculated. Results are shown in Table 1. The zones of inhibition exhibited by the extracts against the test fungal species ranged between 12.50 and 20mm. In general, zones of inhibition decreased with decrease in the concentration of the extract (Table 1).

3.2. The Minimum Inhibitory Concentration of the Ethanol Extracts

Table 2. Minimum Inhibitory Concentrations of the Ethanolic Extract of *Ocimum gratissimum*

Isolates	Minimum Inhibitory Concentrations (mg/ml) values
Epidermophyton	63.09
Trichophyton	63.06
Microsporum	52.40
Malassezia furfur	50.01

Table 2 shows the minimum inhibitory concentrations of the ethanolic extracts of *Ocimum gratissimum* (mg/ml). The highest MIC value of 63.09mg/ml was obtained for *Ocimum gratissimum* against *Epidermophyton* and least MIC value of 50.01mg/ml was obtained against *Malassezia furfur* (Table 2). Results of the minimum inhibitory concentration (MIC) are summarized in Table 2. Higher concentrations of the *O. gratissimum* extract were needed to inhibit *Epidermophyton* when compared to *Malassezia furfur.* The MIC of the ethanol extract of *O. gratissimum* was 50.01, 52.40, 63.06 and 63.09 mg/ml for *Malassezia furfur*, *Microsporum*, *Trichophyton* and *Epidermophyton,* respectively (Table 2).

4. Discussion

The aim of this study is to investigate the antifungal activity of the leaves of *Ocimum gratissimum* against *Pityriasis versicolor* and other dermatophytoses. The antifungal activity of the plant extract was tested on selected clinical fungal isolates. The upsurge in the prevalence of side effects of many synthetic antimicrobial agents and incidence of multidrug resistant bacteria has spurred scientists on the research for plant based antimicrobial of therapeutic potentials. *Ocimum gratissimum* present such potential of high medicinal value[9]. In this study, the ethanol extracts of *O. gratissimum* showed high antifungal activity against the isolates of *Malassezia furfur*, *Microsporum*, *Trichophyton* and *Epidermophyton* at 50.01, 52.40, 63.06 and 63.09 mg/ml concentrations respectively. The ethanol extracts of the leaves of *O. gratissimum* contain substances with antifungal properties. This agrees with the works of Olowokudejo and Pereira-Sheteolu[24], Sofowora[25] and Ilori et al.[26]. The extracts were active against the fungi of medical importance. The volatile oils of *O. gratissimum* and *O. bacilicum* were established to produce inhibitory effect against the oral microbial flora tested in a study by Ahonkhai et al.[2]. Braga et al.[27] suggested that the fractions of *O. gratissimum* L. could be used as a source of natural products derived from this plant, as it has the ability to change antibacterial activity, providing a new weapon against the problem of bacterial resistance to antibiotics.

It has also been demonstrated that the eugenol isolated

from O. *gratissimum* presented antimcrobial activities [15,20-21,28-30], insecticidal activities[20,21,31-33], anti-helmintic activities[34], nematicidal activities[35] or which have fungistatic properties[20,21,36]. Medicinally, they are used in aromatherapy, as insect repellents, larvicidal and insecticidal agents[2,37-39]. Extracts of *O. gratissimum* has also been reported to have hypotensive effects, strong insect repellent effects and also showed significant antimicrobial effects against both fungi and bacteria[2,39-41], while others like *Cymbopogon citratus, Citrus sinensis* and *Citrus maxima* have been reported to possess remarkable antimicrobial effects against some bacteria and fungi [2,41-42].

Several studies in Nigeria and outside Nigeria has reported similar findings on the inhibitory effect and antifungal activities of *Ocimum gratissimum* against fungi and other organisms of medical importance. Ethanol extracts of *Ocimum gratissimum* showed more inhibitory effect compared to the aqueous extracts in a study by Nwinyi et al.[9]. Hexane and methanol extracts of *Ocimum basilicum* also inhibited three isolates out of 23 strains of *Candida albicans* in a study by Adigzel et al.[43]. Nwosu and Okafor[14] reported the antifungal activities of extracts of *O. gratissimum* collected from southeastern Nigeria against seven pathogenic fungi including *Trichophyton rubrum* and *T. mentagrophytes.* Nwosu and Okafor[14] also suggested the possible use of certain plant extracts in the treatment of subcutaneous phycomycosis in humans and animals. Other reports have shown smooth muscle contracting lipidsoluble principles[44] and antimutagenic activity[45] in organic solvent extracts of leaves of *O. gratissimum.* Ilori et al.[26] have reported the antidiarrhoeal activities of leaf extracts of *O. gratissimum* investigated by disc diffusion and tube dilution methods. Hydro-distilled volatile oils from the leaves of *Ocimum gratissimum* L. (Lamiaceae) showed active antimicrobial activity against a pathogenic fungus, *Candida albicans* in a study by Matasyoh *et al.*[46]. There was also a marked antifungal activity against *Candida albicans[*46].

In this study, extraction from the leaves of *Ocimum gratissimum* was done using ethanol. Ethanol is believed to be a suitable solvent for the extraction process[47], any of the other solvents could result in a greater yield of extract. Adebolu and Oladimeji[5] reported the use of cold water and steam distillation as methods of extraction. Extraction of flavenoids from the plant using methanol was reported by Ighodaro *et al.*[48]. In a recent study by Koche et al.[11], ethanolic extracts of *Ocimum gratissimum* at different concentrations inhibited the growth of *E. coli* and *L monocytogenes*.

The diameter zones of inhibition exhibited by the extracts against the test fungal species ranged between 12.50 and 20mm. This present study showed that *Ocimum gratissimum* had significant inhibitory effect at high concentrations (250mg/ml and 200mg/ml) using the agar diffusion method. *Ocimum gratissimum* gave a wide zone of inhibition of 22.40mm for *Epidermophyton* at 250mg/ml. For *Malassezia furfur*, *Trichophyton* and *Microsporum,* the zones of inhibition recorded at 250mg/ml were 21.50mm, 20.20mm and 18.50mm. These organisms showed varying degrees of susceptibility at the various concentrations. The diameter zones of inhibition reported in this study is higher compared to the zones of inhibition exhibited by the same extracts (2 + 0.01 – 10 + 0.10 mm) on *C. albicans* in a study by Matasyoh *et al.*[46]. In a study by Koche et al.[11], the concentration of 250mg/ml inhibited the isolate with highest diameter zone of inhibition ranging from 22mm to 25mm. Also, Mbata and Saikia[21] showed that *O. gratissimum* leaves ethanolic extracts at different concentrations inhibited the growth of *Listeria monocytogenes*. This is similar to our observation except for the fact that fungal isolates was used in this present study.

In this study, the minimum inhibitory concentrations (MICs) determined by the agar diffusion method, which are the least concentrations of the plant extract that inhibited fungal growth was between 50.01 and 63.09 mg/ml. The MIC of the ethanol extract of *O. gratissimum* was 50.01, 52.40, 63.06 and 63.09 mg/ml for *Malassezia furfur*, *Microsporum*, *Trichophyton* and *Epidermophyton,* respectively. It showed that a higher concentration of the *O. gratissimum* extract was needed to inhibit *Epidermophyton* when compared to *Malassezia furfur.* This is comparable to what was obtained elsewhere by some authors. Matasyoh et al.[20] reported that the MIC for the fungus *C. albicans* was 50 mg/ml. In a study by Nweze and Eze[3], the MICs value that inhibited the growth of *C. albicans* (ATCC 90028) were also 50 mg/ml. In the study by Matasyoh *et al.*[46], the MIC was between 10.00 and 2.50 mg/ml, this differs from the MIC value obtained in our study. According to Koche et al.[11], the extracts inhibited the growth of the bacterial isolate in a concentration dependent manner with MICs of 9.25mg/ml.

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 *O. gratissimum*. In a study by Lima et al.[49], *O. gratissimum* was found to be the most active in inhibiting 80% of the dermatophyte strains tested and producing zones greater than 10 mm diameter. Similarly, Nwosu and Okafor[14] reported that the extracts of *O. gratissimum* inhibited the growth of *Trichophyton rubrum* and *T. mentagrophytes*. In addition, Mbata and Saikia[20] showed that *O. gratissimum* oils have properties that can inhibit the growth of *psychrophils* and heat resistant organisms and suggested there were need for the use of this plant and its derivatives for the primary purpose of flavouring foods and antimicrobial activities. These established a good support to the use of this plant in herbal medicine and as base for the development of new drugs and phytomedicine[21]. The study by Mann[7] showed that *O. gratissimum* extractants are potential sources of antimicrobial and preservative agents. In a study by Nweze and Eze[3], the extract of *O. gratissimum* showed good but varying *in vitro* activities against all the isolates tested including typed fungal strain of *Candida albicans*

(ATCC 90028).

5. Conclusions

The study showed that the extracts from the leaves of *Ocimum gratissimum* had pronounced antifungal activities on all the fungi tested. The preliminary screenings of *O. gratissimum* results are quite promising and have strongly indicated the antifungal activity spectra of leaves extract of the plant. As the findings of study compared favourably with previous studies on the antimicrobial activity of *Ocimum gratissimum* against fungal infections, the plant holds great promise for use as both an antibacterial and antifungal agent. Further studies should be carried out to unravel the identity of the active ingredients as well as its medicinal properties. Other methods of extraction should be tried to determine the best method for optimal yield of the medicinal ingredients. *In-vivo* testing using laboratory animals should also be carried out.

ACKNOWLEDGMENTS

We are particularly thankful to the herbarium Unit of the University of Port Harcourt, Rivers State, Nigeria and Miss. Okoyomo EP for her assistance in the collection of fresh leaves of *Ficus exasperata* vahl Oredo Local Government Area, Benin City, Edo State, Nigeria.

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