**Antifungal Activity of Different Extracts of *Ocimum gratissimum* on Fungi Isolated from Salon Equipment in Rivers State, Nigeria**

**Abstract**

This research was aimed to evaluate the antifungal activity of *Ocimum gratissimum* on fungal isolates from salon equipment in Rivers State, Nigeria. Most barbing equipment are contaminated by fungi due to unproven methods of cleaning and sterilization. This research was carried out to investigate the antifungal potential of *Ocimum gratissimum* on fungi isolated from salon equipment in Rivers State, Nigeria. A total of one hundred and eighty (180) swab samples from clipper, brush and combs were subjected to standard mycological procedures such as culturing using dermatophyte test medium and sabouraud dextrose agar and identified using macroscopic and microscopic method. Total number of identified fungal isolates (44), belonging to six genera from the study locations werse, *Aspergillus flavus*, *Aspergillus terrus*, *Fusarium solani*, *Mucor indicus*, *Rhizopus nigricans*, *Trichophyton rubrum* and *Penicillium italicum* and subjected to ethanol, methanol and crude (aqueous) extract of *Ocimum gratissimum*. Results showed that ethanol extracts of *Ocimum gratissimum* completely inhibited the growth of *Trichophyton rubrum* (100%), *Mucor indicus* (100%) and *Rhizopus nigricans* (100%). Methanol extracts showed complete inhibition (100%) of all fungal isolates except *Penicillium italicum* (66.67%, Bori), while the results of the crude extract of *Ocimum gratissimum* showed that only *Aspergillus terrus*, *Fusarium solani*, *Mucor indicus* and *Rhizopus nigricans* were inhibited at 33.33% respectively. The activity of the extracts on the fungal isolates was concentration dependent especially as high concentrations of the extracts proved more potent than those with low concentrations. The extracts hold a potential in treating diseases that might arise as a result of using barbing equipment contaminated with pathogenic fungi.

**Keywords: *Ocimum gratissimum*, antifungal activity, salon equipment, macroscopic and microscopic**

**INTRODUCTION**

“Recent observations show an increase in the establishment of new barbing salons in Rivers State and the personnel in these salons are mostly individuals with negligible or no knowledge on the control of infectious diseases. The likelihood of salon equipment acting as vehicles for acquisition of contagious diseases after visiting the salon is backed up by research” (Kondo *et al*., 2006). “There are approximately 300,000 hairs on the scalp of most humans growing at the rate of approximately half an inch per month” (Elewski, 2000). “Therefore, there would be at least a monthly visit to the barbing salon for a haircut. Electric clippers came into existence and replaced the use of razor blade and other sharp objects in response to technological advancement” (Mackenzie *et al*., 2005). “The re-use of barbing equipment and the disinfection or sterilization methods used in many barbing salons have elevated the interest on communicable diseases associated with the scalp” (Mackenzie *et al*., 2005).

“Some common agents used by salon operators in Nigeria for sterilization include kerosene, diesel, ethanol and fuel. This has become a common practice among practitioners in Nigeria” (Kligman *et al*., 2011). “Ringworm and dandruff which are fungal infections are backed by research to be associated with barbing operations. Opportunistic pathogens are usually present in non-living cornified layers of the skin and its appendages” (Kligman *et al*., 2011).

“Host resistance and the inoculum size are major determinants in the establishment of a microbial infection during barbing. The immunologic status of the host however, seems to play a vital role on the severity of the infection” (Mackenzie *et al*., 2005).

“Ocimum gratissimum L. commonly known as clove basil belonging to family Lamiaceae is an important aromatic and medicinal plant existing wild or cultivated in various tropical and subtropical parts of the globe. The plant possesses two unique features firstly it contains essential oil with diversity in chemical composition and water stress tolerance capacity. Plant oil is considered the Mother Nature’s chemical factory” (Alexander, 2016). “A unique composition of alcohols, aldehydes, ketones, ethers, esters, lactones, oxides, peroxides, tannins and flavonoids” (Matasyoh *et al*., 2008). “The studies related to the efficacy of essential oil of Ocimum gratissimum against gram-negative, gram-positive bacteria, fungi, and its regular consumption as spice without affecting the activities of conventional antibiotics makes it a strong antimicrobial herbal product throughout the tropics” (Ahonkhai *et al*., 2009).

“The plant extract has potentiality in suppressing the growth of Basidiobolus haptosporus, B. ranarum, causing subcutaneous infection in children below ten years, along with Trichophyton rubrum and T. mentagrophytes that cause skin, feet and fingernail infections in human” (Lemos *et al*., 2005). This research was aimed at evaluating the antifungal activity of *Ocimum gratissimum* on fungal isolates from salon equipment in Rivers State, Nigeria.

**Materials and Methods**

**Study Area**

The study area was Rivers State in Southern Nigeria. Three (3) study locations viz; Bori, Omoku and Port Harcourt were chosen for the purpose of this research. These locations represent the three (3) senatorial zones in Rivers State. Bori is a city in Khana Local Government Area, Rivers State with coordinates of 4040’22’’N 7022’13’’E. It is the traditional headquarters of the Ogoni people and the second largest city in Rivers State after Port Harcourt. Bori serves as the commercial centre for the Ogoni, Andoni, Opobo and other ethnic nationalities (Hamilton, 2003).

Omoku is a town in the South-Western senatorial district in Rivers State, with a population of about 200,000 people (Demogrophia, 2016). It is located in the Southern part of the state, having boundary with Delta state and Imo state. It is the headquarters of Ogba/Egbema/Ndoni Local Government Area with coordinates of 5020’37’’N 6039’24’’E.

Port Harcourt is the capital and largest city of Rivers State. It is a major city in the Rivers East senatorial district with coordinates of 4049’27’’N 702’1’’E. As at 2016, the Port Harcourt urban area has an estimated population of 1,865,000 inhabitants (Demogrophia, 2016). The city is highly congested and is the only major city in Rivers State.

**Sample Collection**

One hundred and eighty **s**amples were collected from the different salons by swabbing the surface of the cutting edge of the clippers, combs, and brushes using sterile moist swab stick (Michael *et al*., 2016). Samples were collected twice a month for two months. Samples were then transferred into sterile tubes containing 1mililitre of sterile distilled water to avoid drying and transported to the laboratory in ice pack containers. The name, source, and location were noted on the swab sticks and brought to the laboratory under sterile/aseptic conditions for microbiological analysis. Salons that have at least 10 haircuts per day were taken for the study. The surface area of each equipment was calculated according to the method of Neusley *et al*. (2018) using the formula;

Cfu/cm2 = AxB/C

Where, A= cfu/ml of the suspension

B= sample surface area

C= volume of diluents used for sample collection

**Isolation and Characterization of Fungal Isolates**

Swab samples were dipped in 10ml of sterile normal saline and subsequently diluted into test tubes containing sterilized 9ml of normal saline to make 10-1 to 10-4 dilutions. Aliquots (0.1ml) of the dilutions were inoculated using sterile pipette onto Saboraud Dextrose Agar, and onto Dermatophyte Test Medium (DTM) for the isolation of dermatophytes (Elis *et al*., 2007). Plates were inoculated in duplicates and incubated at 25°C for 2 to 5 days (Elis *et al*., 2007). After incubation, pure cultures of fungal isolates were obtained by aseptically inoculating representative colonies of different morphological types on the culture plates onto freshly prepared Saboraud Dextrose Agar and Dermatophyte Test Medium plates and incubated at 250C for 2 to 5 days. The isolates were identified based on macroscopic characteristics (growth characteristics, pigment formation, texture) as well as microscopic morphology (formation of macroconidia and microconidia or other typical elements). The microscopical identification was done by lactophenol cotton blue mounts. In this method, a drop of lactophenol cotton blue was placed on a grease-free slide and the aerial mycelium of the investigated fungal isolates was cut and transferred into the drop of lactophenol cotton blue on the slide using a sterile inoculating needle. The slide was covered with a microscope coverslip and viewed under the x10 and x40 magnification lens of the compound microscope. Characterization of fungal isolates was drawn from matching results with those reported by **Kidd** *et al.* (2023) and Elis *et al.* (2007).

**Collection and Identification of Leaves**

The leaves of *Andrographis paniculata* used in this study were obtained from a nursery in the Rivers State University, and identified by professionals from Plant Science and Biotechnology Department, Rivers State University. The leaves were immediately taken to the laboratory.

**Preparation of Leaves**

The leaves were washed with sterile distilled water, air dried and subsequently ground into powder using a pre-cleaned mortar (thoroughly washed with detergent and rinsed with 95% alcohol), and were kept wrapped in filter paper cartridges according to the method of Okigbo and Ajalie (2005). The sieved powdered form of the leaves was then soaked in 500ml of ethanol, methanol, and water for 48 hours.

Batch-wise, 50g of the leaves (Balance used: Digital Scout Pro balance (Model SPU601) were pulverized using a sterile (washed thoroughly with detergent and rinsed with 95% alcohol) mortar to obtain a powdery sample. The air dried leaf powder was sieved with a hand sieve and the sieved powdered form of the leaves were then soaked in 500ml of ethanol, methanol and water for 48 hours following the method of Okigbo & Ajalie (2005). The crude extracts (filtrate) were filtered using Whatman no 1 filter paper. The supernatant was discarded and residue was put in 100 ml beaker and later transferred to an evaporator where the aqueous solvent was evaporated at low temperature to obtain constant weight of powder (Flores, *et al.,* 2009)*.* The standard extract powder (concentrate) which was obtained in the process was stored in a refrigerator at 4oC until required for use.

**Antifungal Susceptibility Test**

Agar well diffusion and susceptibility testing was carried out by employing the method of Magaldi *et al*. (2004). Sterile cotton swab were taken and dipped in 48 hours old culture of each test organism. The entire surface of Sabouraud Dextrose Agar (Lab M Limited, UK) was seeded, first horizontally and vertically to ensure even distribution of organism over the agar surface using the above swab. The seeded agar surface was allowed to dry for 5 to 10 minutes. The concentrate of the extracts were diluted into four concentrations **(**0.25g/ml to 0.016µg/ml) using the two-fold dilution method, the tip of a 16mm customized well cutter was sterilized by heating on Bunsen burner flame and used for well preparation after seeding the SDA plates with the test organisms. Four (4) wells were prepared in different plates. As soon as the wells were prepared, 0.1ml of reformulated plant extract (using the initial solvent of extraction) was poured in each well using sterile micro-tip following the method of Magaldi *et al*. (2004).

All Sabouraud agar plates were incubated at 27oC for 72 hours. After incubation, the results of sensitivity tests were used as basis for estimating activity levels of the extracts. The reporting was done to indicate the presence or absence of fungal growth. (**Kaur *et al*., 2014).**

**Results**

Total number of fungal isolates (44), belonging to six genera and their percentage occurrence from the study locations were, *Aspergillus flavus* 5(11.36%) *Aspergillus terrus* 5(11.36%)*, Fusarium solani* 10(22.72%)*, Mucor indicus* 7(15.91%)*, Rhizopus nigricans* 7(15.91%)*, Trichophyton rubrum* 2(4.54%) and *Penicillium italicum* 8(18.2%).

**Table 1: Mean Distribution of Fungal Population from Salon Equipment in Bori, Omoku and Port Harcourt** (x102cfu/cm2)

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Salon equipment |  | Bori | Omoku | Port Harcourt |
| **Clipper** |  | 34.34±5.44a | 49.00±23.96a | 45.66±13.56a |
| **Brush** |  | 66.95±13.88b | 93.26±11.58b | 78.88±32.22a |
| **Comb** |  | 40.09±5.39b | 53.02±13.11a | 74.61±23.69a |

 \*Means with same superscript across the column shows no significant difference (*p*≥0.05)

THF- Total Heterotrophic Fungi

**Fig 1: Percentage Occurrence of Different Fungal Isolates In Salon Equipment From The Different Locations**

**Table 2: Percentage Susceptibility Pattern of Ethanolic Extract on Fungal Isolates from Bori, Omoku and Port Harcourt**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Organisms | Bori | Omoku | Port Harcourt |  |
| Negative (%) | Positive (%) | Negative (%) | Positive (%) | Negative (%) | Positive (%) |  |  |
| *Penicillium italicum* (n=8) | 1(33.33) | 2(66.67) | 0 | 3(100) | 0 | 2(100) |  |  |
| *Aspergillus flavus* (n=5) | 1(50) | 1(50) | 0 | 2(100) | 0 | 1(100) |  |  |
| *Aspergillus terreus* (n=5) | 0 | 1(100) | 0 | 1(100) | 1(33.33) | 2(66.67) |  |  |
| *Fusarium* spp (n=10) | 1(20) | 4(80) | 0 | 2(100) | 1(33.33) | 2(66.67) |  |  |
| *Mucor* spp (n=7) | 0 | 2(100) | 0 | 3(100) | 0 | 2(100) |  |  |
| *Rhizopus nigricans* (n=7) | 0 | 2(100) | 0 | 3(100) | 0 | 2(100) |  |  |
| *Trichophyton rubrum* (n=2) | 0 | 1(100) | 0 | 1(100) | 0(0.00) | 0(0.00) |  |  |

Negative = growth, Positive = No Growth

**Table 3: Percentage Susceptibility Pattern of Methanolic Extract on Fungal Isolates from Bori, Omoku and Port Harcourt**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Organisms | Bori | Omoku | Port Harcourt |  |
| Negative (%) | Positive (%) | Negative (%) | Positive (%) | Negative (%) | Positive (%) |  |  |
| *Penicillium italicum* (n=8) | 1(33.33) | 2(66.67) | 0 | 3(100) | 0 | 2(100) |  |  |
| *Aspergillus flavus* (n=5) | 0 | 2(100) | 0 | 2(100) | 0 | 1(100) |  |  |
| *Aspergillus terreus* (n=5) | 0 | 1(100) | 0 | 1(100) | 0 | 3(100) |  |  |
| *Fusarium* spp (n=10) | 0 | 5(100) | 0 | 2(100) | 0 | 3(100) |  |  |
| *Mucor* spp (n=7) | 0 | 2(100) | 0 | 3(100) | 0 | 2(100) |  |  |
| *Rhizopus nigricans* (n=7) | 0 | 2(100) | 0 | 3(100) | 0 | 2(100) |  |  |
| *Trichophyton rubrum* (n=2) | 0 | 1(100) | 0 | 1(100) | 0(0.00) | 0(0.00) |  |  |

Negative = growth, Positive = No Growth

**Table 4: Percentage Susceptibility Pattern of Crude Extract on Fungal Isolates from Bori, Omoku and Port Harcourt**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Organisms | Bori | Omoku | Port Harcourt |  |
| Negative (%) | Positive (%) | Negative (%) | Positive (%) | Negative (%) | Positive (%) |  |  |
| *Penicillium italicum* (n=8) | 3(100) | 0 | 3(100) | 0 | 2(100) | 0 |  |  |
| *Aspergillus flavus* (n=5) | 2(100) | 0 | 2(100) | 0 | 1(100) | 0 |  |  |
| *Aspergillus terreus* (n=5) | 1(100) | 0 | 1(100) | 0 | 2(66.67) | 1(33.33) |  |  |
| *Fusarium* spp (n=10) | 4(80) | 1(20) | 2(100) | 0 | 2(66.67) | 1(33.33) |  |  |
| *Mucor* spp (n=7) | 2(100) | 0 | 2(66.67) | 1(33.33) | 2(100) | 0 |  |  |
| *Rhizopus nigricans* (n=7) | 2(100) | 0 | 2(66.67) | 1(33.33) | 2(100) | 0 |  |  |
| *Trichophyton rubrum* (n=2) | 1(100) | 0 | 1(100) | 0 | 0(0.00) | 0(0.00) |  |  |

Negative = growth, Positive = No Growth

**Discussion**

The mean distribution of fungal population from salon equipment in Bori, Omoku and Port Harcourt, showed that brushes had the highest mean population when compared to other equipment from same locations. However, apart from Port Harcourt, the mean population showed significant difference between brushes and other equipment. This suggests that attention from the salon operators might be more on clippers and combs compared to brushes.

The antifungal activity of methanolic and ethanolic extracts of *O. gratissimum* demonstrated high success rate in the inhibition of the fungal isolates in the different locations in this present study.The antifungal activity of ethanolic extracts of *O. gratissimum* on fungal isolates from salons in Bori, Omoku and Port Harcourt, show that *Mucor spp, Rhizopus nigricans* and *Trichophyton rubrum* had 100% positive results (no growth). The other isolates showed varying percentages of negative (20-50%) and positive results (50-80%). For methanolic extract, only *Penicillium italicum* isolate from Bori, had a negative result which indicated growth. All other fungal isolates from the three locations had 100 % positive results (no growth).

However, despite the susceptibility of these isolates to other extracts of *O. gratissimum,* the crude (aqueous) extract of same plantshowed lower antifungal activity and couldn’t completely inhibit their proliferation. The crude extract antifungal activity of *O. gratissimum* showed 100% negative results (growth) for all fungal isolates from Bori, except *Fusarium* sp. (80% negative). The results were similar for Omoku isolates except for *Mucor* and *Rhizopus* sp. (66.67% negative result respectively). The same pattern of results followed the fungal isolates from Port Harcourt, with only *Aspergillus terrus* and *Fusarium* sp. showing less negative results (66.67% respectively).

The inhibitory activities of the extract have been attributed to the presence of Hexadecanoic acid (37.21%), oleic acid (25.38%), and octadecanoic acid (16.19%) in the extracts of *O. gratissimum.* (Uchegbu *et al*.,2019). The extracts of *O. gratissimum* has previously been discovered to have other antifungal properties. The suppression of development of *Trychophyton rubrum* and *T. mentagrophytes* was confirmed by Lima *et al.* (1993) and Nwosu and Okafor (1995). Thymol, a component of *O. gratissimum* essential oil, was shown to be extremely potent against *T. rubrum, T. mentagrophytes, Candida neoformans, Candida albicans,* and *Malassezia pachydermatis* by Chand *et al.* (2002). The major components identified were n-Hexadecanoic acid (37.21%), oleic acid (25.38%), and octadecanoic acid (16.19%). These compounds are believed to contribute significantly to the plant's antifungal properties. The essential oil demonstrated varying degrees of antifungal activity against pathogens such as *Aspergi*

Unlike the antifungal properties of the ethanolic extracts of all three plants, the methanolic extracts proved to possess better antifungal activity. This observed differences in the antimicrobial effect of same plant showing varied response could be attributed to the affinity or level of phytochemicals extracted by the extracting solvent. This agreed with previous studies that solvents which includes n-hexane, ethyl acetate, methanol and water used for extraction possess different affinities for phytochemical compounds (Ibrahim *et al.,* 2015; Tsado *et al.,* 2016). Thus, the higher potency of antimicrobial function exhibited by the methanol extract suggests that the methanol extract possessed higher qualitative phytochemical composition than the ethanol and crude extracts.

**Conclusion**

The findings from this study emphasizes the presence of diverse fungal species on salon equipment, including *Aspergillus flavus*, *Aspergillus terreus, Fusarium solani, Mucor indicus, Rhizopus nigricans*, *Trichophyton rubrum*, and *Penicillium italicum*, across the different study locations. The fungal contamination was highest in brushes, followed by combs and clippers, which indicates that improper cleaning techniques and frequent use, contribute to microbial load.

The antifungal activity of *O. gratissimum* extracts demonstrated significant efficacy in inhibiting fungal isolates. The methanolic extract exhibited the highest antifungal potency, showing complete inhibition of most fungal isolates. Ethanolic extracts also demonstrated strong inhibitory effects, whereas crude extracts showed lower antifungal activity. The observed differences in antifungal potency among extracts could be attributed to the solubility and affinity of bioactive compounds in different solvents.

These findings suggest that *O. gratissimum* extracts, particularly methanolic extracts, could serve as a potential alternative antifungal agent for controlling fungal contamination in salon environments.

**Recommendations**

1. The methanolic extract of *O. gratissimum* demonstrated significant antifungal activity and could be incorporated into antifungal sprays or disinfectants for salon equipment, depending on the outcome of further research.
2. Pharmaceutical companies should partner more with research students and channel more energy towards plant remedies for various diseases including dermatophyte infection as plant extracts have shown great potential.
3. There is a need for regulation in establishing new salons as well as, train and retrain those already operating one. Regulatory bodies and health agencies should enforce routine inspections and microbiological assessments of salon equipment to prevent fungal infections and ensure public safety.
4. The need for public sensitization on the mode of spread, simple preventive measures and proper sterilization methods to reduce the prevalence of dermatophytes in barbing salons is recommended.

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