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Antifungal Activity of Different Extracts of *Andrographis paniculata* on Fungi Isolated from Barbing Salon Equipment

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ABSTRACT

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 *Andrographis paniculata* (king of bitters) 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 collected from Bori, Omoku and Port Harcourt Cities. Samples were cultured using dermatophyte test medium and sabouraud dextrose agar. Fungal isolates were identified using standard mycological techniques. Forty-four (44) fungi belonging to six genera isolated were *Aspergillus flavus*, *Aspergillus terrus*, *Fusarium solani*, *Mucor indicus*, *Rhizopus nigricans*, *Trichophyton rubrum* and *Penicillium italicum*. Fungi were subjected to ethanol, methanol and crude (aqueous) extract of *A. paniculata*. Results showed that the highest level of inhibitions for ethanol extract of *A. paniculata* in Bori were *Aspergillus terrus* (100%) and *Trichophyton rubrum* (100%), in Omoku, *Fusarium solani* (100%), *Aspergillus terrus* (100%) and *Trichophyton rubrum* (100%). In Port Harcourt, *Penicillium italicum* (100%), *Aspergillus flavus* (100%), *Fusarium solani* (100%), *Rhizopus nigricans* (100%) and *Trichophyton rubrum* (100%). For methanol extracts all fungal isolates from Bori were inhibited. All isolates of Omoku except *Penicillium italicum* (33.3%) and *Rhizopus nigricans* (33.3%) were inhibited. All isolates of Port Harcourt were inhibited except *Aspergillus terrus* (33.3%). Crude extracts showed minimal inhibitory activity with the highest being *Penicillium italicum* (33.3%) for Bori, *Penicillium italicum* (33.3%) and *Rhizopus nigricans* (33.3%) for Omoku and *Fusarium solani* (33.3%) for Port Harcourt. The activities of *Andrographis paniculata* extracts on the fungal isolates were concentration dependent especially as high concentrations of the extracts proved more potent than lower concentrations. The extracts have the potential to be used in treating mycoses that might arise as a result of using barbing equipment contaminated with pathogenic fungi.

Keywords: Barbing Salon Equipment, *Andrographis paniculata,* Antifungal Activity, Plant Extract, Inhibition, Mycoses.

Introduction

The number of barbing salons in Rivers State is quite high, and there seem to be a steady increase in the establishment of new salons. The advent of electric clippers for barbing operation replaced the traditional use of razor blade and other sharp objects following technological advancement (Mackenzie *et al*., 2005). The inappropriate disinfection or sterilization methods used in many barbing salons and the re-use of barbing equipment have heightened the concern regarding communicable diseases associated with the scalp (Mackenzie *et al*., 2005).

Fungal infections (mycoses) such as ringworm, dandruff and other impetigo-like lesions have been reported to be infections associated with barbering operations. Disinfection which is the removal or destruction of pathogenic microorganisms that may cause infection from surfaces such as the blade of a barbing clipper is usually carried out by the use of disinfectants (Boyce, 2000). The use of kerosene, diesel, ethanol, petrol and other cleaning agents for the sterilization of clippers, combs and brush is common practice among barbers in Nigeria (Kligman *et al*., 2011).

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Often referred to as "King of Bitters", *Andrographis paniculata* is an herbaceous plant, in the family Acanthaceae (Jarukamjorn and Nemoto, 2008). It is an annual plant and every part of the plant tastes very unpleasantly (Sabu, and Kuttan, 2006). *Andrographis* can be said to be "omnipresent" in its local territory. It develops in pine, evergreen and deciduous backwoods territories, along streets and in towns (Alpha Omega Labs, 2008). Having recognized it's therapeutic properties, it is additionally developed effectively, on the grounds that it develops in a wide range of soil (Alpha Omega Labs, 2008).

The antimicrobial properties of *A. paniculata* and its safe upgrading are what the plant is well known for (Khan, 2007). Research has demonstrated that *A. paniculata* has a shockingly wide scope of pharmacological impacts with astonishing value and include anticancer (Kumar *et al*., 2004), others are; anti-malarial, antifungal and anti-typhoid (Sabu, and Kuttan, 2006), antiviral (Wiart *et al.*, 2005), antibacterial (Mishra *et al*., 2009), antidiabetic (Zhang and Tan, 2000), cardiovascular, infertility and psychopharmacological movement (Mandal *et al.*, 2001). The plant has also shown promise for the easing of emotional impact of infections of the respiratory system (Coon and Ernst, 2004), for those with incessant weariness disorder and fibromyalgia (Khan, 2007). The proximate composition of various plants used for therapeutic purposes in various localities, is a thing of value for these species of plants. Its phytochemical constituents are key to understanding the impacts and pathway of therapeutic plants as a rule (Atangwho *et al*., 2009).

Traditional and complementary medicine (TCM) which can be defined as a group of diverse medical and healthcare systems, practices, or products that are designed to prevent, treat, or manage illnesses and preserve the mental and physical well-being of individuals (W.H.O., 2000.). TCM methods can be classified into 5 major categories of practice: whole medical systems, mind-body techniques, biologically based therapies, manipulative and body-based therapies, and energy therapies (Ben-Arye *et al*., 2008). Although the clinical efficacy of many TCM methods is controversial because of a lack of scientific evidence, TCM use is increasing significantly throughout the world (Ernst *et al*., 2004).

Andrographis paniculata has been emphasized as being a traditional remedy to many microbial diseases and it is based on this and other related studies, that it was selected and researched, to ascertain its potential effectiveness against fungi isolated from salon equipment.

Materials and Methods

Study Area

The study area is Rivers State in Southern Nigeria. Three (3) study locations; 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 $4^0$40'22''N $7^0$22'13''E. It is the traditional headquarters of the Ogoni people and the second largest city in Rivers State after Port Harcourt with an estimated population of about 250,000 people (Demographia, 2016). Bori serves as the commercial centre for the Ogoni, Andoni, Opobo and other ethnic nationalities. The Bori urban area has adjourning communities like Yeghe, Zaakpon, Bua Kaani, Kor, and Kpong (Hamilton, 2003).

Omoku is a town in the South-Western senatorial district in Rivers State, with an estimated population of about 200,000 people as at 2016 (Demographia, 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 $5^0$20'37''N $6^0$39'24''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 $4^0$49'27''N $7^0$2'1''E. As at 2016, the Port Harcourt urban area has an estimated population of 1,865,000 inhabitants in 2016 (Demographia, 2016).

Port Harcourt city is highly congested and is the only major city in River State. Port Harcourt is a major industrial centre as it accommodates many multinationals. Salons that have at least 10 haircuts per day were taken for the study.

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Sample Collection

A total of one hundred and eighty (180) samples were collected from the different salons by swabbing the surface of the cutting edge of the clipper, combs, and brush 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. The surface area of each equipment was calculated according to the method of Neusley *et al*. (2018) using the formula;

$CFU/cm^2 = A^xB/C$

Where,

CFU = Colony Forming Unit

A = CFU/ml of the suspension

B = sample surface area (of the equipment)

C = volume of diluents used for sample collection.

Isolation and Characterization of Fungal Isolates

Swab samples were dipped in 1ml 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 25^0C 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. The cut piece was transferred into the drop of lactophenol cotton blue on the slide using a sterile inoculating needle with which the piece of fungus was teased. The slide was then covered with a microscope coverslip and viewed under the ×10 and ×40 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 of *Andrographis paniculata*

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

Preparation and Extraction of Leaf Extracts of *Andrographis paniculata*

Batch-wise, 50g of the leaves of *Andrographis paniculata* (Balance used: Digital Scout Pro balance (Model SPU601) were washed with sterile distilled water, air dried and subsequently pulverized into powder using a sterile mortar (thoroughly washed with detergent, rinsed with water, and further rinsed with 95% alcohol).

The air dried leaf powder was sieved with a hand sieve and the sieved powdered forms 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 4^oC until required for use.

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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 organisms (spores) over the agar surface using the above swab. The seeded agar surface was allowed to dry for 5 to 10 minutes. The tip of a 16mm customized well cutter was sterilized by heating on Bunsen burner flame, allowed to cool, and used for well preparation after seeding the SDA plates with the test organisms.

Four (4) wells were prepared in each plate (as four replicates of the same test). 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 27^{o}C for 72 hours. 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%).

The susceptibility pattern (%) of Ethanolic extract, Methanolic extract, and aqueous extract of *Andrographis paniculata* on fungi isolated from barbing salon equipment in the locations are presented in Tables 1, 2, and 3 respectively.

Table 1: Mean Values of Total Fungal Population (×10^{2}CFU/cm^2) of Barbing Salon Equipment of Locations

Salon equipment	Fungal Population (×10^{2}CFU/cm^2) of Barbing Salon Equipment of Locations		
	Bori	Omoku	Port Harcourt
Clipper	34.34±5.44^a	49.00±23.96^a	45.66±13.56^a
Brush	66.95±13.88^b	93.26±11.58^b	78.88±32.22^a
Comb	40.09±5.39^b	53.02±13.11^a	74.61±23.69^a

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

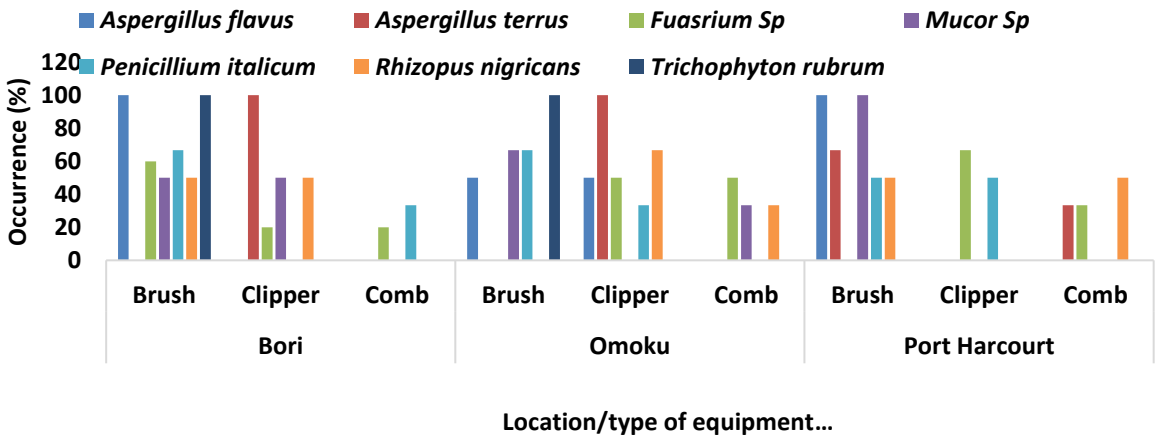

Fig 1: Percentage occurrence of different fungal isolates in salon equipment in the different locations

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Table 2: Susceptibility pattern (%) of Ethanolic extract on fungi isolated barbing equipment in the locations

Fungal Isolates	Susceptibility pattern of ethanolic extract on fungi in Barbing Locations					
	Bori		Omoku		Port Harcourt	
	Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)	Positive (%)
Penicillium italicum (n=8)	1(33.33)	2(66.67)	1(33.33)	2(66.67)	0(0.00)	2(100)
Aspergillus flavus (n=5)	1(50)	1(50)	1(50)	1(50)	0(0.00)	1(100)
Aspergillus terreus (n=5)	0(0.00)	1(100)	0(0.00)	1(100)	1(33.33)	2(66.67)
Fusarium spp (n=10)	1(20)	4(80)	0(0.00)	2(100)	0(0.00)	3(100)
Mucor spp (n=7)	1(50)	1(50)	1(33.33)	2(66.67)	1(50)	1(50)
Rhizopus nigricans (n=7)	1(50)	1(50)	1(33.33)	2(66.67)	0(0.00)	2(100)
Trichophyton rubrum (n=2)	0(0.00)	1(100)	0(0.00)	1(100)	0(0.00)	0(0.00)

Key: Negative = growth, Positive = No Growth

Table 3: Susceptibility pattern (%) of Methanolic extract on fungi isolated barbing equipment in the locations

Fungal Isolates	Susceptibility pattern of Methanolic extract on fungi in Barbing Locations					
	Bori		Omoku		Port Harcourt	
	Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)	Positive (%)
Penicillium italicum (n=8)	0(0.00)	3(100)	1(33.33)	2(66.67)	0(0.00)	2(100)
Aspergillus flavus (n=5)	0(0.00)	2(100)	0(0.00)	2(100)	0(0.00)	1(100)
Aspergillus terreus (n=5)	0(0.00)	1(100)	0(0.00)	1(100)	1(33.33)	2(66.67)
Fusarium spp (n=10)	0(0.00)	5(100)	0(0.00)	2(100)	0(0.00)	3(100)
Mucor spp (n=7)	0(0.00)	2(100)	0(0.00)	3(100)	0(0.00)	2(100)
Rhizopus nigricans (n=7)	0(0.00)	2(100)	1(33.33)	2(66.67)	0(0.00)	2(100)
Trichophyton rubrum (n=2)	0(0.00)	1(100)	0(0.00)	1(100)	0(0.00)	0(0.00)

Negative = growth, Positive = No Growth

Table 4: Susceptibility Pattern (%) of Aqueous extract on fungi isolated barbing equipment in the locations

Fungal Isolates	Susceptibility pattern of Aqueous extract on fungi in Barbing Locations					
	Bori		Omoku		Port Harcourt	
	Negative (%)	Positive (%)	Negative (%)	Positive (%)	Negative (%)	Positive (%)
Penicillium italicum (n=8)	2(66.67)	1(33.33)	2(66.67)	1(33.33)	2(100)	0(0.00)
Aspergillus flavus (n=5)	2(100)	0(0.00)	2(100)	0(0.00)	1(100)	0(0.00)
Aspergillus terreus (n=5)	1(100)	0(0.00)	1(100)	0(0.00)	3(100)	0(0.00)
Fusarium spp (n=10)	4(80)	1(20)	2(100)	0(0.00)	2(66.67)	1(33.33)
Mucor spp (n=7)	2(100)	0(0.00)	3(100)	0(0.00)	2(100)	0(0.00)
Rhizopus nigricans (n=7)	2(100)	0(0.00)	2(66.67)	1(33.33)	2(100)	0(0.00)
Trichophyton rubrum (n=2)	1(100)	0(0.00)	1(100)	0(0.00)	0(0.00)	0(0.00)

Key: Negative = growth, Positive = No Growth

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Discussion

This study has demonstrated the fungal population and fungal diversity in barbing salon equipment in Rivers State. The mean total heterotrophic fungal counts from salon equipment in Bori showed that brushes had the highest counts; the least heterotrophic fungal counts were recorded from clippers while combs had the second highest counts in the same location. Statistical analysis showed that there were statistical differences (except clipper) in the total heterotrophic fungal counts recorded amongst the salon equipment with counts recorded for brushes being significantly ($P<0.05$) higher than those recorded for clippers.

The heterotrophic fungal counts recorded for brushes in barbing salons located in both Omoku and Port Harcourt were higher than counts obtained for clippers and combs of the respective locations. The total heterotrophic fungal counts in this study are lower than values reported in a previous study (Mbajiuka *et al.*, 2014; Stanley *et al.*, 2019). This could be as a result of the sampling technique or the life style of residents in the study locations. The high fungal counts recorded in these other studies could also be attributed to overuse or inadequate treatment or washing of brushes and considering the large surface area of the brushes, microorganisms could be logged between the bristles of the brushes. Other factors that could have orchestrated high fungal load in the brushes amongst other salon equipment is the number of the salon equipment available per haircut and the type of treatment used for sterilization on the salon equipment.

Some of the fungal isolates from this study are potential pathogens known to cause disease. While other isolates are soil inhabiting fungi and mostly known to cause disease in plants (Chimbekujwo, 2000; Kanashiro *et al*., 2020) there have been reports of some diseases caused by fungal isolates from the present study.

Aspergillus flavus has been reported to be a leading cause of invasive aspergillosis, particularly in tropical regions. It has been implicated in various infections, including keratitis and otomycosis. It also produces aflatoxins, which are potent carcinogens that affect liver health (Sarvestani *et al*., 2022). *Aspergillus terreus*, reported to cause invasive pulmonary aspergillosis, spondylodiscitis, prosthetic joint infections, and cutaneous infections.

A fatal case of invasive pulmonary aspergillosis due to *A. terreus* in an immunocompetent COVID-19 patient was reported by Abolghasemi *et al*. (2021). *A. terreus* is also known for its resistance to amphotericin B, thereby, complicating treatment. It produces various secondary metabolites with potential antimicrobial and anticancer properties. A rare instance of spondylodiscitis caused by *A. terreus* following an abdominal stab wound has also been documented (Tagaki *et al*., 2019).

Mucor indicus is significant in causing necrotizing fasciitis, particularly in immunocompromised patients. A pediatric bone marrow transplant recipient developed necrotizing fasciitis caused by *M. indicus*, highlighting its potential as a human pathogen (Bloch *et al*., 2018).

Tinea capitis is one of the major diseases caused by dermatophyte species of genera *Trichophyton* (Emele and Oyeka, 2008). *Trichophyton* is one of the main causative organisms in most poor African countries. The other is *Microsporum* (Havlickova *et al*., 2008). Host preference and natural habitat are basis for determining the origin of the etiologic agent. Humans (anthropophilic dermatophytes), animals (zoophilic dermatophytes), or soil (geophilic dermatophytes) all serve as natural reservoir for dermatophytes (Adamski and Batura-Gabryel, 2007).

The antifungal activities of ethanolic and methanolic extracts of *A. paniculata,* demonstrated high success rate in the inhibition of the fungal isolates in this study. The antifungal activity of ethanolic extracts of *A. paniculata*, on *Penicillium italicum, Aspergillus flavus, Aspergillus terrus, Fusarium* sp., *Mucor* sp., *Rhizopus nigricans* and *Trichophyton rubrum* isolated from salons in Bori showed that the fungal isolates were 90% susceptible (inhibited) to ethanolic extracts of *A. paniculata* except *Trichophyton rubrum* which was only 50% susceptible.

The ethanolic extracts of *A. paniculata*, on fungal isolates from salons in Omoku showed *Penicillium, Aspergillus terreus, Mucor* and *Trichophyton rubrum* were 90% susceptible while *Aspergillus flavus* and *Rhizopus* sp were 50% susceptible to ethanolic extracts of *A. paniculata*. Findings also showed that the ethanolic extracts of *A. paniculata* had 100% antifungal activity on *Penicillium, Aspergillus flavus, Aspergillus terrus, Fusarium, Mucor, Rhizopus* and

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Trichophyton rubrum isolated from barbers' shop in Port Harcourt. The antifungal activity of *O. A. paniculata* is well documented. Abubakar *et al*. (2012) reported good inhibitory effects against *Trichophyton mentagrophytes, Trichophyton rubrum, Microsporum canis, Candida albicans, Candida krusei, Candida tropicalis,* and *Aspergillus niger* in a study of the antifungal activity of *A. paniculata* extracts and active principles against skin pathogenic fungal strains in vitro.

The existence of several secondary metabolites has been related to the antifungal activities of *A. paniculata*, and the concentrations of these secondary metabolites in substantial amounts are reported to impact the antimicrobial activities of *A. paniculata* (Athikomkulchal *et al.*, 2006; Abubakar *et al.*, 2012). Other investigations have shown that *A. paniculata's* antimicrobial action is due to the synergistic effects of a chemicals extracted from the plant (andrographolide), which exhibited more antimicrobial activity than the plant extracts (Mbaveng *et al.*, 2008; Sopa *et al*., 2008).

Unlike the antifungal properties of the ethanolic extracts, the methanolic extracts proved to possess better antifungal activity. This observed differences in the antifungal 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 (Tsado *et al.*, 2016; Tourabi *et al.*, 2023). Thus, the higher potency of antifungal function exhibited by the methanol extract suggests that the methanol extract possessed higher qualitative phytochemical composition than the ethanol extract.

The antifungal activity of aqueous extract of *A. paniculata* on the fungal isolates was poor as the extract showed minimal inhibitory activity against the fungal isolates. This buttresses the point that the amount of phytochemicals extracted by a solvent is dependent on the extracting solvent which implies that water is a less extracting solvent compared to ethanol and methanol (Tsado *et al.*, 2016; Tourabi *et al.*, 2023).

Presently, the use of medicinal plants alongside conventional medicine is of great significance in the Nigerian health care system, a type of health care referred to as "herbalism" (Amegbor, 2014). Due to the constant rise in sophistication across the world, it is essential to refer to herbal medical practice as alternative or complimentary medicine, so as to appeal to large populations of people regardless of their cultures and/or religions.

Conclusion

The susceptibility of the fungal isolates to plant extracts especially ethanolic and methanolic, shows potential for further studies on these plant extracts as this might provide the highly sought solution for some fungal diseases. This study indicates that ethanol and methanol are better extracting solvents than water. The occurrence of dermatophytes (*Trichophyton rubrum*) and other fungi was higher in brush than other equipment, indicating that more concentration is given to other equipment compared to brush.

Recommendations

It is recommended that, there is the need for public awareness on the mode of spread and simple preventive measures as to reduce the prevalence of dermatophytes in barbing salons. There is a need for regulation in establishing new salons as well as, train and retrain those already operating one. There is need for public health personnel to check antiseptic procedures used by the barbers and advise them on the correct procedures to use in order to reduce transmission of the infection. 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.

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