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Antimicrobial Activities of *Jatropha curcas* and *Myristica fragrans* Seeds Extracts Against Pathogenic Isolates from Barber Clippers in Shomolu Local Council Development Area, Lagos State

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Abstract: There are several communicable diseases of the scalp that are of concern in barbering and this is because of the re-use of barbing clippers without appropriate disinfection or sterilization. Barber's clippers have been identified as a possible vehicle for pathogen transmission. This study investigated the comparison of antimicrobial potential and minimum inhibitory concentrations (MICs) of aqueous and ethanol extracts of Physic nut (*Jatropha curcas)* and nutmeg (*Myristica fragrans*) seeds against isolated microorganisms viz: *Staphylococcus aureus*, *Pseudomonas aeruginosa, Micrococcus, Proteus spp., Lactobacillus spp., Escherichia coli*, *Streptococcus pyogenes, Aspergillus niger*, *Aspergillus flavus, Penicillium fellutanum,* and *Mucor spp*. from the surface of the cutting edge of the barber's clipper from various barbing salons in Shomolu Local Council Development Area of Lagos State using agar well diffusion technique and the Clinical and Laboratory Standard Institute guidelines respectively. Aqueous and ethanol extracts of *J. curcas* and *M. frangrans* showed antimicrobial activity against almost all tested isolates. MICs of aqueous extracts of both seeds were between 12.5 and 50 mg/ml of extract in all susceptible isolates, while MICs of ethanol extracts was between 12.5 and 100 mg/ml. The ethanol extract of *J. curcas* had the highest antimicrobial activity of all the extracts, indicating it is the most potent antimicrobial for barber's clipper disinfection.

Keywords: Antimicrobial, *Jatropha curcas*, *Myristica fragrans*, Barber, Clippers, Shomolu LCDA

1. Introduction

There has been an increase in the establishment of barbing industry since early twenty first century and the majority of which is controlled by people with little or no knowledge on infection controlled practices. Although the barbing industry is known for its aesthetic activity, research however shows the possibility of it making its patrons feel sick by acquisition of contagious diseases [1]. All individuals have approximately 300,000 hairs on their scalp with a growth rate of approximately half an inch per month. Therefore, they are expected to visit a barber's shop at least once a month for a haircut. The use of barbing clipper for barbering operation is a substitute for the traditional use of razor blade and other sharp objects following advancement in science, technology and civilization [2]. There are several communicable diseases of the scalp that are of concern in barbering and this is because of the re-use of barbing clippers without appropriate disinfection or sterilization.

Some barbers are known for using Kerosene, diesel, fuel and other organic cleaning agents for the sterilization. A number of infections such as ringworm, dandruff and other impetigo-like lesion caused by bacteria have been reported to be infections associated with barbering operations. Causative organisms are usually present in non-living cornified layers of the skin and its appendages [3].

The person at risk may be the next client on whom the contaminated instrument is used. Organisms that can cause potentially serious infections may be transmitted where appropriate precautions are not taken. Thus there is need to regularly clean and disinfect barbers' clipper with preferably, relatively affordable, environmentally friendly, acceptable and equally efficient antimicrobials in order to reduce if not eliminate pathogenic microbes that could harbor barbers clippers.

Plants and their essential oils have been associated with antimicrobial activity since prehistoric times [4, 5]. Plants have been endowed with innate ability to synthesize aromatic substances such as phenols and their derivatives [6], these secondary metabolites have therapeutic tendencies. Due to the potential health benefit of plants of medicinal [7], medicinal plant extracts in recent times, have been developed and proposed for use in food as natural antimicrobials [8]. Some of their metabolites have been successfully used in the treatment and prevention of infectious diseases, cancer or in stimulating the immune system. The medicinal value attributed to plants is a function of the bioactive phytochemical constituents that produce definite physiological action on the human body [9]. The most important of these plants bioactive chemical constituents are flavoids, alkaloids, tannins and phenolic compounds [10, 11]. The biological activities reported to have been associated with plants and plant derived compounds can be categorized according to the disease area which include antimicrobial activity, anticancer activity, anti-inflammatory activity, nervous system activation/suppression, cardiovascular/ metabolic, immune modulating activity. The development of microbial antibiotic resistance has necessitated the global search for new antimicrobials of preferably plant origin [12-14]. Plant extracts, and pure compounds isolated from natural sources have formed the bedrock of modern chemotherapy [15]. Various botanicals have been reported to have antimicrobial activity [16-21]. Worldwide, the number of plants with medicinal properties is quite vast [17].

In a bid to source for relatively cheap and effective natural disinfectants for cleaning clippers of barbers, this study is aim at investigating the *in vitro* antimicrobial potentials of *J. curcas* and *M. fragrans* seeds exudate against potential pathogens isolated from clippers of barbers with a view to also determining the most suitable solvent for extraction of the antimicrobial active ingredient.

2. Materials and Methods

2.1. Microorganisms

The microorganisms used were isolated from clippers of barbers in Shomolu Local Council Development Area in Lagos State. Series of sub culturing was done until pure cultures were obtained and then preserved at 4°C.

2.2. Equipment

Autoclave (Charles Hearson & Co.Ltd England), Weighing balance (Genlab England), Oven N30C (Genlab England), Calibrated micro pipette (US Associates, India), Microscope (Olympus Optical Co.Ltd Japan), Incubator (Charles Hearson & Co. Ltd England).

2.3. Media

Nutrient Agar (Oxoid Ltd, England), Potato Dextrose Agar (Oxoid Ltd, England), MacConkey Agar (Oxoid Ltd, England).

2.4. Chemicals

Ethanol, Ciprofloxacin, Nystatin (Valeant Pharmaceuticals International, USA). All chemicals are of analytical grade.

2.5. Collection, Preparation of Seeds and Extraction

Seeds of Physic nut (*Jatropha curcas)* and nutmeg (*Myristica fragrans*) were purchased from Mile 12 market in Lagos State, Nigeria. Their identities were authenticated by an authority at the Department of Botany, University of Lagos, Akoka, Lagos State.

Extract preparation was done according to the method described by Leonard *et al.,* [22]. The seeds were collected and ground dry. The dried powder were put in ziplock bags and then moved to the laboratory. At the laboratory, the powder were weighed in 100 g each and put in beakers. Then the ethanol extracts done using 300 ml ethanol while the aqueous extracts were done using 300 ml water. They were allowed to soak for 72 h, afterwards, each extract was sieved through a muslin cloth and centrifuged at 5000 x g for 15min. The supernatant was collected and the solvent was evaporated to make the final volume one- third of the original volume and stored at 4°C in air tight reagent bottles for further studies.

2.6. Sampling of Clippers of Barbers

Barbing clippers were sampled randomly using swab sticks from various barbing salons in Shomolu Local Council Development Area, Lagos State. Six different areas (Anthony village, Onipanu, Bariga, Shomolu, Ilupeju and Obanikoro) were selected due to the high population density.

2.7. Isolation and Identification of Test Isolates

Microbial samples were collected by rotating a moist swab over the inner surface of the clipper as described by Gholamereza *et al*., [23]. Microbial samples were collected by rotating a moist swab over the surface of the cutting edge of the clipper. Swabs were then soaked in nutrient broth and incubated for 24 h. After 24 h, the broth were brought out and plated out on Nutrient Agar (NA), MacConkey Agar (MCA) and Potato Dextrose Agar (PDA) respectively. They were then incubated at 37°C for 24 h and at 28°C for 72 h respectively. After incubation, distinct colonies were picked and repeatedly sub cultured to obtain pure cultures on nutrient agar, incubated at 37°C for 24 h.

Bacterial cultures isolated were identified conventionally and based on a combination of cultural and morphological characteristics. A series of biochemical tests including catalase, coagulase, indole production, citrate utilization,

oxidase test, methyl red test and vogues proskaeur test. After the tests, isolates were characterized using Bergey's manual of determinative microbiology while fungi were identified on the basis of cultural and morphological characteristics as described by Samson *et al.,* [24].

2.8. Determination of Antimicrobial Activity

The Agar well diffusion method described by Pelczar *et al.,* [25] was used to determine the antimicrobial activity of the plant seeds extract. Nutrient Agar (NA) plates were seeded with 0.1 ml of 24 h cultures of the test bacteria. A 6 mm diameter well was made on the agar using a cork-borer. After which, a 0.3 ml volume of the plant extract was added to the well. The reference drugs for bacteria and fungi were ciprofloxacin and Nystatin respectively in the same volume as those of sample extracts served as positive control while each solvent served as negative control. The cultures were allowed to stand for 1 h for the pre-diffusion of the extract to occur. Plates were incubated for 24 h at 37°C (bacteria) and 72 h at 28°C (fungi) and the zones of inhibition were taken using a vernier calliper as an indication of antimicrobial activity.

2.9. Determination of Minimum Inhibitory Concentration

The minimum inhibitory concentration is the smallest amount (concentration) of a substance which will inhibit the growth of a particular microorganism. This is determined according to the National Committee for Clinical Laboratory Standard (NCCL) guidelines by double dilution method as reported by Leonard *et al.,* [22]. Sterile nutrient broth (4 ml) was added in five test-tubes and 4 ml extract was added. Doubling dilution was done to have extract concentrations (mg/ml) of 100, 50, 25, 12.5 and 6.25. Afterwards, 0.1 ml 0.5 Macfarland's standard of the test organism in normal saline (0.85% NaCl, w/v) were inoculated into the test tubes and incubated at 37°C for 24 h. Potato dextrose broth was used for fungal isolates and incubated at 28°C for 72 h. Controls were done by dispensing 4 ml broth and 4 ml of the extracts without test organism into the test tubes.

3. Results and Discussion

3.1. Characterization of Isolated Microorganisms

Bacteria isolated and identified in this study using biochemical test revealed viz: *Staphylococcus aureus* (Figure 1), *Pseudomonas aeruginosa, Micrococcus spp., Proteus spp., Lactobacillus spp., Escherichia coli* and *Streptococcus pyogenes* (Table 1.) while the fungal isolates were identified based on cultural and morphological characteristics as *Aspergillus niger, A. flavus, Penicillium fellutanum,* and *Mucor* species respectively (details not shown).

3.2. Antimicrobial Activity of Aqueous and Ethanolic Extracts from J. Curcas and M. Frangrans Seeds Against Test Microbes

The antimicrobial activity of the ethanol extracts of *J. curcas* and *M. frangrans* against pathogenic microorganisms isolated from cutting edge of the barber's clipper are presented in Figures 2 and 3. Ethanol extract of the seeds exhibited higher antimicrobial activity against all the test organisms than the aqueous. However, the reference drug had higher antimicrobial activity than the ethanol extracts of the two plant seeds against all test organisms. Ethanol extract of *J. curcas* had higher antimicrobial activity against *S. aureus* (15 mm), *A. niger* (13.5 mm) and *P. fellatum* (9.5 mm) than the ethanol extract of *M. frangrans* which had higher antimicrobial activity on the other two test organisms (*E. coli* and *S. pyogenes*) than that of *J. curcas* activity against test isolates at concentrations up to 50 mg/ml and above with the exception of *P. fellutanum* and *A. niger* with MIC at 100 mg/ml. The aqueous extract of *J. curcas* seed had higher antimicrobial activity against *A. niger* (14.5 mm), *S. aureus* (14 mm) and *Lactobacillus spp*. (13 mm) amongst other test isolates. The highest antimicrobial activity (13.5 mm) of aqueous extract of *M. frangrans* was recorded against *A. niger* as shown.

The known pathogenic microorganisms isolated from cutting edge of the barber's clipper are similar to that reported by Adamu *et al*., [26]. This results which also corroborate the findings of Roth and Jenner [27] highlights the potential risk associated with sharing of commercial used sharp instruments.

The antimicrobial activity and diameters of zones of inhibition ranges observed by the various botanicals used in this study are in agreement with and comparable to that of earlier reports [18, 20, 27-31]. The aqueous extract of *M. frangrans* exhibited antimicrobial activity against known pathogenic test isolates. The antimicrobial activity of *M. frangrans* is attributable to the bioactive presence compounds [33] while the antimicrobial activity of *J. curcas* plant parts according to [18, 33-38] has been attributed to the medicinal constituents and presence of certain phytochemicals which include saponins, tannins, alkaloids and glycosides [20, 33]. However, Arekemase [18] reported that the latex of *J. curcas* inhibited the growth of *Candida albicans* at higher concentration of the latex, while the aqueous extract of *J. curcas* roots failed to inhibit the growth of *Candida albicans* at concentrations tested. He further reported that the aqueous root extract of *J. curcas* exhibited antimicrobial activity against a number of microbes including, *Neiserria gonorrhea*, *Escherichia coli*, *S. aureus*, *Pseudomonas aeruginosa* and *Aspergillus flavus.* Similarly, Igbinosa *et al*. [34] reported the antimicrobial activity of stem bark aqueous extract of *J. curcas* against a wide range of bacterial isolates excluding *Klebsiella pneumonia*. In another study by Narayani *et al*. [31] no antimicrobial activity against *E. coli*, *Proteus* sp., *S. aureus* and *P. aeruginosa* was observed from aqueous leaf extract of *J. curcas.* However, Oloyede *et al*. [36] reported that aqueous extracts of *J. curcas* (up to a concentration of 500 mg/ml) showed antimicrobial activity against *K. pneumonia*, *E. coli* and *P. aeruginosa.* The disparities in the different reports may be attributable to differences in extract preparation and concentrations, and as

well as strain differences. Microbial antibiotics sensitivity patterns have been reported to be strain-dependent within a given species [39]. For example, certain strains of *Staphylococcus aureus* are resistant to methilicin (Methicillin-resistant *Stapylococcus. aureus*, MRSA), whereas some are not. The chloroform extracts of both plants have been reported to exhibit antibacterial and antifungal activities [32, 35, 40]. During the screening of *J. curcas* root and latex for phytochemicals which are responsible for antimicrobial activity, Arekemase [18] observed that high levels of phytochemicals were detected in the ethanol extracts than in aqueous and hexane extracts.

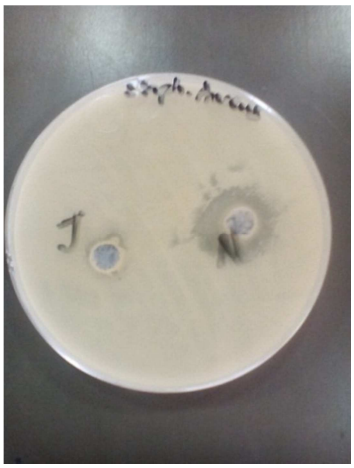

Figure 1. *Staphylococcus aureus showing zone of inhibition on agar medium incorporated with M. frangrans.*

Legend: J- Test organism without zone of inhibition; N- Test organism showing zone of inhibition

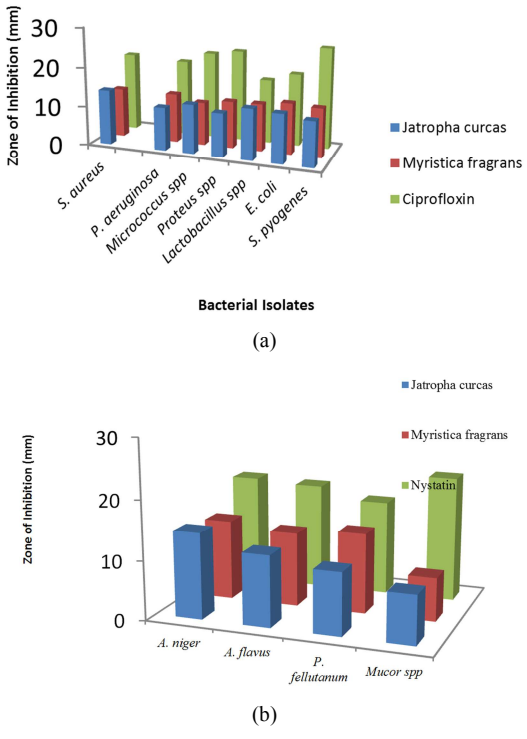

Figure 2. *Antimicrobial activity of aqueous extract of seed materials on test microorganisms (a) bacterial isolates (b) fungi isolates.*

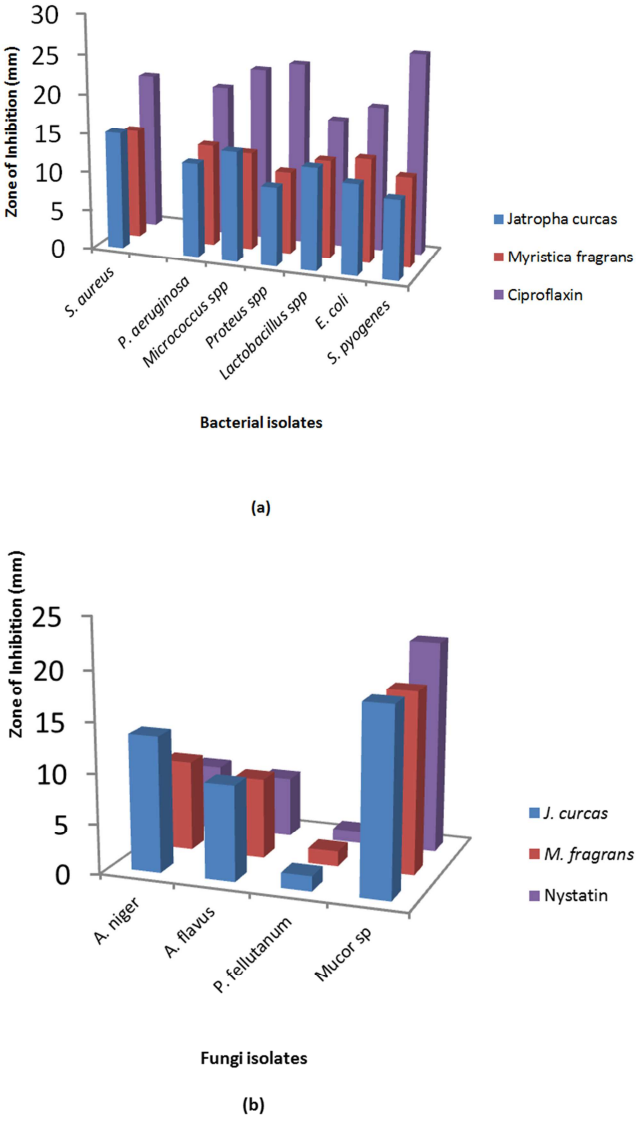

Figure 3. *Antimicrobial activity of ethanol extract of seed material on test microorganisms.*

3.3. Determination of Minimum Inhibitory Concentration (MIC) Using J. curcas and Myristica fragrans Extracts

The Minimum inhibitory concentration (MIC) of the both seed extracts against the test isolates are shown in Tables 2 and 3. The MIC of ethanol and aqueous extracts of *M. fragrans* against *S. aureus* were 25 mg/ml or less. The MIC of the ethanol extracts of *M. fragrans* against *S. pyogenes* and *E. coli* was 50 mg/ml or less, while that against the fungal species; *A. niger*, *P. fellutanum* and *Mucor* sp. was 100 mg/ml or less. Aqueous extracts of *M. fragrans* had a MIC of 50 mg/ml or less against almost all test isolates, with the exception of *S. aureus* and *A. niger,* where it found to be 25 mg/ml or less. The MIC of ethanol and aqueous extract of *J. curcas* against *S. aureus* and *A. niger* was 25 mg/ml or less, that of *S. pyogenes* was 50 mg/ml or less. The highest MIC value for ethanol extract of *J. curcas* was against *P. fellutanum and Mucor* sp., with a value of 100 mg/ml or less. The highest MIC value for the aqueous extract of *J. curcas*

was 50mg/ml, and against *S. pyogenes*, *E. coli, P. fellutanum* and *Mucor* sp.

The Minimum inhibitory concentrations (MICs) of solvent extracts against the test isolates in this study are comparable to other reports [18, 40] However, lower MICs values (0.5-1.0 mg/ml) were reported by Kawo *et al*. [41] for ethanol extracts of *C. procera* leaf against *E. coli* and *S. aureus.* The reason for this slight discrepancy may be attributable to a possible difference in the characteristics of bacterial strains used and differences in plant species used. The same reasons may explain the lower MICs values reported by Igbinosa *et al*. [33] for stem bark extracts of *J. curcas*. The MIC of ethanolic extracts of *M. frangrans* and *J. curcas* were the lowest of all the solvents extract, implying that ethanol extracts were the most potent (at lower concentration) and that ethanol was the best extracting solvent. The lower the MIC of a botanical against pathogens, the more desirable it is.

This study has substantiated earlier reports of the presence of and possible transmission of microorganisms with health significance through sharing of public clippers. The antimicrobial potentials of solvent extracts of *Myristica frangrans* and *Jatropha curcas* seeds for the disinfection of barber's clipper have also been demonstrated. The ethanol extract of *Myristica frangrans* had the highest antimicrobial activity against test pathogenic isolates.

Table 1. *Biochemical, Cultural and Morphological Characterizations of the bacterial isolates.*

CHARACTERISTICS	**Descriptions of Isolates**				
CULTURAL					
Margin	Smooth	Smooth	Smooth	Smooth	smooth
Colour	White	Yellowish-white	Green	White	White
Shape	Small and Irregular	Round	Round	Irregular	Irregular
MORPHOLOGICAL					
Cell Type	Cocci	Cocci	Rods	Rods	Rods
Cell Arrangement	Single	Clusters	Clusters	Clusters	Clusters
GRAM REACTION	+	+	-	-	+
SUGAR FERMENTATION TEST					
Lactose	-	+	-	-	
Maltose	+	+	+	-	
Sucrose	-	+	+	-	
Glucose	+	+	+	+	
BIOCHEMICAL TEST					
Coagulase	-	+	+	_	+
Indole	+	+	-	-	-
Catalase	+	+	+	+	-
Citrate	-	-	+	+	+
Oxidase	+	-	+	-	+
Probable Microorganism	*Micrococcus sp.*	*Staphylococcus auerus*	*Pseodomonas aerupinosa*	*Proteus spp*	*Lactobacillus spp*

Table 1. *Continued.*

CHARACTERISTICS	**Descriptions of Isolates**	
CULTURAL		
Margin	Smooth	Smooth
Colour	Black	Milkish-white
Shape	Smooth and Regular	Cocci in row
MORPHOLOGICAL		
Cell Type	Rods	Cocci
Cell Arrangement	Single	Row-form
GRAM REACTION	-	
SUGAR FERMENTATION TEST		
Lactose	+	
Maltose	+	
Sucrose	-	
Glucose	+	
BIOCHEMICAL TEST		
Coagulase	_	+
Indole	+	-
Catalase	+	-
Citrate	-	N/A
Oxidase	-	+
Probable Microorganism	*Escherichia coli*	*Streptococcus pyogenes*

Table 2. *Minimum inhibitory concentrations of J. curcas extracts on test bacterial and fungal isolates.*

Test Organism	Concentration of seed extract (mg/ml)*									
	Ethanol					Water				
	6.25	12.5	25	50	100	6.25	12.5	25	50	100
S. aureus	+	+	+	-	-	+	+	+	-	-
P. aerupinosa	+	+	-	-	-	+	+	-	-	-
Micrococcus sp.	+	+	+	-	-	+	+	+	-	-
Proteus sp.	+	+	+	-	-	+	+	+	-	-
Lactobacillus sp.	+	+	+	-	-	+	+	+	-	-
E. coli	+	+	+	-	-	+	+	+	-	-
S. pyogenes	+	+	+	-	-	+	+	+	-	-
A. niger	+	+	+	+	-	+	+	+	-	-
A. flavus	+	+	+	_	-	+	+	+	-	-
P. fellutanum	+	+	+	+	-	+	+	+	-	-
Mucor sp.	+	+	+	-	-	+	+	+	-	-

+, presence of growth (no inhibition); -, no growth (presence of inhibition)
*Triplicate determination

Table 3. *Minimum inhibitory concentrations of M. fragrans extracts on test bacterial and fungal isolates.*

Test Organism	Concentration of seed extract (mg/ml)*									
	Ethanol					Water				
	6.25	12.5	25	50	100	6.25	12.5	25	50	100
S. aureus	+	+	-	-	-	+	+	-	-	-
P. aerupinosa	+	+	+	+	-	+	+	+	-	-
Micrococcus sp.	+	+	+	+	-	+	+	+	-	-
Proteus sp.	+	+	+	+	-	+	+	+	-	-
Lactobacillus sp.	+	+	+	+	-	+	+	+	-	-
E. coli	+	+	+	-	-	+	+	+	-	-
S. pyogenes	+	+	+	-	-	+	+	+	-	-
A. niger	+	+	+	+	-	+	+	-	-	-
A. flavus	+	+	+	+	-	+	+	+	-	-
P. fellutanum	+	+	+	-	-	+	+	-	-	-
Mucor spp	+	+	+	+	-	+	+	+	-	-

+, presence of growth (no inhibition); -, no growth (presence of inhibition)
*Triplicate determination

4. Conclusion

The results of the present study have shown that the aqueous and ethanol extracts of *J. curcas* and *M. frangrans* seeds were found to possess antibacterial and antifungal activities. The extracts of these seeds inhibit the growth of the isolated bacteria and fungi. It could therefore be inferred that these seeds contain bioactive constituents which can effectively inhibit the growth of some microorganisms. This lends credence to the traditional use of this plant as a medicinal plant and also, could be natural disinfectants for cleaning clippers of barbers.

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