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Research Articles

Synergistic Potential of the Leaves of Aspillia Africana (Compositae) and Psidium guajava (Myrtaceae) Against Some Selected Bacterial and Fungal Isolates

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Abstract

Background: The plethora of secondary metabolites inherent in plants that could be synthesized and formulated into useful drugs for the treatment of diseases is not fully understood.

Objectives: This research was carried out to investigate the antimicrobial potential of the leaves of Aspillia africana and Psidium guajava plants on some selected bacterial and fungal isolates.

Methods: Fresh and matured leaves of Aspillia africana and Psidium guajava were collected at the Federal College of Agriculture, Akure and the Botanical Garden, Obafemi Awolowo University (OAU), Ile-Ife, Nigeria. The plants were identified and voucher specimen deposited at IFE Herbarium. The pure isolates of the bacteria and fungi were obtained from Medical Microbiology unit, Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. The bacteria isolates were maintained on nutrient agar and the fungal isolates on Sabouraud Dextrose Agar (SDA). Extraction of the plant materials using methanol, ethanol and aqueous fractions were carried out using standard protocols. The isolates were inoculated on a nutrient broth. The Minimum Inhibitory Concentration (MIC) of the extracts was determined by broth dilution method while minimal bactericidal and fungicidal concentrations respectively were determined following established protocols.

Results and conclusion: The results showed that Bacillus cereus, Cornybacterium pyogenes, Klebsiella pneumonia and Escherichia coli showed higher degree of resistance to the plant extracts. The fungal isolates; Candida albicans, Tryptophyton rubrum, Penicillium expansium, and Aspergillus flavus were resistant to Psidium guajava extract as no significant biological activity was observed. All the extracts from the plants produced considerable antimicrobial activities with Streptococcus faecalis and Staphyloccocus. aureus. whereas, these organisms were resistant to ofloxacin, sparfloxacin, chloramphenicol, augmentin, ciprofloxacin and septrin. This study concludes that ethanolic extracts (100mg/ml) of the leaf of Aspillia africana is more potent than the methanolic and aqueous extracts of Psidium guajava. This study demonstrated that the therapeutic properties of Aspillia africana and Psidium guajava are better enhanced with their synergistic potential to the tested microorganisms.

Keywords: Aspillia Africana, Bactericidal, Psidium guajava, Synergistic, therapeutic
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INTRODUCTION

Crude extracts of medicinal plants notwithstanding stand out as veritable sources of potential resistance agents and the African biosphere promises to be a potential source of such compounds owing to its rich plant species diversity[1.] According to World Health Organization[2], a larger percentage of the world's population is based mainly on traditional medicine and a significant part of the therapy involves the use of plant materials or their bioactive components. There are many

findings on the therapeutic potentials of plant extracts against bacteria[3], hence, plants are still being recognized as the pivot of modern medicine for the prophylaxis of infectious diseases[4]. Therefore, the contributions of medicinally and pharmaceutically important plants to health care cannot be over-emphasized. There is a growing demand to discourage the usage of drugs with high clinical toxicity. However, the usual practice of oral administration of crude extracts poses threat to human health as the extracts may contain some toxic components[5]. These ambivalent challenges had necessitated the search for antibiotics in institutions, industries and the academic community [6]. Plants have major advantage over synthetic drugs because it's cost effective, readily available, cheaper alternative and pharmacological importance[7]. Several studies have been reported on the antibacterial activities of local plants such as *Euphorbia hirta*, *Kigelia Africana*, *Hibiscus sabdarifa* and *Mucuna pruriens*[8].

Aspillia africana (Compositae) is a tropical shrub, semi-woody herb from a perennial root widely grown in Nigeria where it is commonly known as *Yunrinyun* by the Yorubas, *Orangila* by Igbos and *Tozalin* by Hausas. The plant is about 2m high, polymorphic and ubiquitous on western land of Savannah. Its formulations had been used in the treatment of various bacterial infections such as gastro-intestinal disorders, corneal opacity and insect bites. The plant is widely used as haemostatic agent (spontaneous arrest of bleeding from damaged blood vessels). The fresh leaves are effective in wound healing, cuts and sores[9].

Table 1. Antimicrobial effect of methanolic (M), Ethanolic (E) and Aqueous (A) extracts of *Aspillia africana* and *Psidium guajava* on some selected bacterial and fungal isolates

	Aspillia africana (100mg/ml)			*Psidium guajava* (100mg/ml)		
Test bacteria	A	E	M	A	E	M
Providencia stuartii	-	12	4	8	12	4
Bacillus cereus	-	-	-	-	-	-
Staphylococcus aureus	15	16	3	7	12	15
Corynebacterium pyogenes	-	-	-	-	-	-
Streptococcus faecalis	4	8	-	10	15	4
Klebsiella oxytoca	-	-	-	-	-	-
Klebsiella pneumonia	-	-	-	-	-	-
Escherichia coli	-	-	-	-	-	-
Pseudomonas fluorescens	8	10	-	-	-	-
Serratia rubidae	-	-	-	4	7	-
Proteus mirabilis	-	-	-	-	-	-
Salmonella pullorum	-	-	-	-	-	-
Test fungi						
Trychophyton tonsurans	1	2	-	-	-	-
Candida albicans	-	-	1	-	-	-
Trychophyton rubrum	-	-	-	-	-	-
Penicillium expansium	-	-	-	-	-	-
Alternaria sp.	-	-	-	-	-	-
Fusarium sp.	3	5	2	-	-	-
Aspergillus niger	-	-	-	-	-	-
Aspergillus fumigates	-	-	-	-	-	-
Aspergillus flavus	-	-	-	-	-	-
Penicillium camenberti	-	-	-	-	-	-

CLSI. Performance standards for antimicrobial susceptibility testing. Clinical and laboratory standards institute, 29th ed, 2019.

Psidium guajava L. (Mytaceae) is one of such plants in folk medicine that had been extensively used for the treatment of various disease conditions like fever, dysentery, vomiting and inflamed gums[10]. The extract from the leaves were found to display analgesic and anti-inflammatory potntial[11], antimicrobial and antioxidant activities[12]. The alarming increase of resistance to antibiotics by microorganisms cannot be overemphasized. This had necessitated scientist to search for alternative strategies of mitigating this challenge. The objectives of the study are therefore to investigate the antimicrobial potential of the leaves of *Aspillia africana* and *Psidium guajava* plants on some selected bacterial and fungal isolates; and to investigate the synergistic antimicrobial effects of the extracts on the test organisms.

Materials and methods

Preparation and authentication of plant samples

A vey fresh and matured leaves of *Aspillia africana* and *Psidium guajava* were collected at the Federal College of Agriculture, Akure and Botanical Garden, Obafemi Awolowo University (OAU), Ile-Ife, Nigeria. The specimens were identified at IFE Herbarium,

Department of Botany, Obafemi Awolowo University, Ile-Ife and voucher deposited. Each plant sample was separately washed and rinsed several times in distilled water. The samples were dried to constant weight in an oven and ground into powdery form using laboratory blender. The powder (100g) obtained were extracted with 500ml of sterile distilled water, 500ml ethanol and 500ml methanol by soaking in water for 5days and then filtered. The filtrates were later concentrated at 45ºC using a motorized rotary evaporator (Resona England). The dried samples were kept in sterile bottles for future use.

Table 2. Synergistic antibacterial and antifungal effects of the plant extracts on the tested microorganisms

	Aspillia africana+ Psidium guajava		
	A	E	M
	(100mg/ml)		
Test bacteria			
Providencia stuartii	6	18	15
Bacillus cereus	22	4	3
Staphylococcus aureus	20	21	7
Corynebacterium pyogenes	1	1	2
Streptococcus faecalis	11	12	6
Klebsiella oxytoca	4	8	2
Klebsiella pneumonia	-	-	-
Escherichia coli	-	-	-
Pseudomonas fluorescens	11	1	6
Serratia rubidae	6	6	1.2
Proteus mirabilis	-	-	-
Salmonella pullorum	-	-	-
Test fungi			
Trychophyton tonsurans	-	-	-
Candida albicans	-	-	-
Trychophyton rubrum	-	-	-
Penicillium expansium	-	-	-
Alternaria sp.	-	-	-
Fusarium sp.	2	4	2
Aspergillus niger	-	-	-
Aspergillus fumigatus	-	-	-
Aspergillus flavus	-	-	-
Penicillium carmenberti	-	-	-

KEY: A – Aqueous extracts, E – Ethanolic extracts, M – Methanolic extract

CLSI. Performance standards for antimicrobial susceptibility testing. Clinical and laboratory standards institute, 29th ed, 2019.

Preparation of test organism

The pure isolates of the bacteria and fungi used in this research were obtained from Medical Microbiology unit, Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria. The bacteria isolates were maintained on nutrient broth and the fungal isolates on SDA. The isolates were constantly sub cultured at fourteen days interval. Before use, the isolates were inoculated into separate test tube that was plugged with adsorbent containing 10ml Mueller- Hinton broth which were incubated for 24h at 37ºC. The test organisms include bacteria namely, *Providencia stuartii, Bacillus cereus, Staphylococcus aureus, Corynebacterium Pyogenes, Streptococcus faecalis, Klebsiella oxytoca, Klebsiella pneumoniae, Escherichia coli, Pseudomonas flourescens, Serratia rubidae, Proteus mirabilis, Salmonella pullorum;*and fungi namely, *Trychophyton tonsurans, Candidia albicans, Trychophyton rubrum, Penicillium expansium, Alternaria* sp, *Fusarium* sp, *Aspergillus niger, Aspergillus fumigatus, Aspergillus flavus,* and *Penicillium camenberti*.

Preparation and Extraction of Plant Materials

Freshly harvested sampled leaves of *A. africana* and *P. guajava* were thoroughly washed with distilled and rinsed accordingly. Twenty gram each of the leaves was weighed on an electric weighing balance for methanol, ethanol and aqueous extraction. The leaves (20g) were later grounded with mortar and pestle and then transferred into labeled petridishes. Incubation was carried out at 37ºC for 24h. About 90ml each of the solvent were added to the grounded leaves for extraction, vigorously shaken for 30 minutes and allowed to settle.

Sensitivity Test: Antibacterial and Antifungal assay was carried out following established protocols.

Preparation of the medium

40g of Mueller-Hinton agar powder were carefully weighed into a clean conical Flask and 100 ml of sterilized distilled water was dispensed into the conical flask to achieve homogeneity using magnetic stirrer before autoclaving at 121°C for 15 min. The plates were allowed to cool for 2h before a cork borer (No 4) was flamed and used to bore wells (6mm). The plant extracts were filled into the wells and labelled appropriately.

Inoculation of medium

Inoculation of the medium was achieved using spread plate method. The fungal isolates selected were allowed to colonize the Sabouraud Dextrose Agar (SDA) at 25ºC until after sporulation. The fungal spores were harvested by pouring a mixture of sterile glycerol and distilled water (3:100 i.e 3 % glycerol) to the surface of the plate and later the spores were scraped . The harvested fungal spores were standardized before use. The plates were allowed to cool on the laboratory bench for one hour to allow for homogenous diffusion of the extract into the media. Plates were incubated at 25ºC for 96 hours and later observed for zones of inhibition.

Minimal inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of the extracts was determined following established protocols. About100mg/ml of the extracts were prepared and introduced into the test tubes containing 8ml of sterile Mueller-Hinton broth, 1ml of the different extract concentration and 1ml broth culture. The mixture was homogenized and then incubated at 37ºC for 24h. The

estimation of minimal inhibitory concentration of the plant extracts on the fungi was determined using established protocols. Plant extracts was prepared and 2ml of different concentrations of the solution were added to Sabouraud Dextrose Agar (SDA) at 40ºC to give the appropriate concentration. The medium was then poured to sterile Petri-dishes and allowed to cool. The extracts were then streaked with fungal spores The plates were incubated at 25ºC for 5days and were examined for the presence or absence of fungal growth. The MIC was taking as the lowest concentration that inhibited the growth of the test fungi.

Minimal bactericidal and fungicidal concentration (MBC and MFC)

Minimal bactericidal concentration (MBC) was determined by plating 1ml of the MIC positive tubes on nutrient agar to ascertain the bactericidal effect of the extracts. Samples were taken from plates with no significant growth in the MIC culture and sub-cultured on freshly prepared Mueller-Hinton agar plates for determination of MBC and SDA plates for MFC and later incubated at 25ºC for 5days.

Synergistic antimicrobial effects of the plant extracts against tested bacterial and fungal isolates:

The synergistic antimicrobial effects of the various combinations of the plant extracts for the test microorganisms were determined using the method employed by[14]. To demonstrate synergism, equal quantity of plant combinations were weighed and dissolved in the appropriate quantity of the dilution to give the 100mg/ml concentrations which was used for the antimicrobial test[13]. Antimicrobial susceptibility testing will be compared with clinical and laboratory standard institute (CLSI, 2019)[15].

Results

Antimicrobial effect of methanolic (M), Ethanolic (E) and Aqueous (A) extracts of *Aspillia africana* and *Psidium guajava* on some selected bacterial and fungal isolates

The zones of inhibition of the extracts ranged from 1-15mm for bacteria while fungal isolates ranged between 1 and 7mm respectively. The highest and lowest zones of inhibition of 15mm and 3mm respectively were obtained in *Staphyloccocus aureus* under the aqueous and methanolic fractions. *Bacillus cereus, Cornybacterium pyogenes, Klebsiella pneumonia* and *Escherichia coli* showed high degree of resistance to the plant extracts. The fungal isolates; *Candida albicans, Tryptophyton rubrum, Penicillium expansium,* and *Aspergillus flavus* were resistant to *Psidium guajava* extract as no significant activity was observed. There were no significant effect of the plant extracts against tested fungi isolates (Table 1).

Synergistic antibacterial and antifungal effects of the plant extracts on the tested microorganisms.

The fungal isolates were resistant to the plant extracts as no significant effect was observed. However, the highest zone of inhibition of 21mm was observed in *S. aureus* under the ethanolic fraction while the lowest (1.2mm) was noticeable in *Serratia rubidae* under the methanolic extract (Table 2). *Escherichia coli* and *Klebsiella pneumonia* were both resistant to the synergistic effect of the plant extracts.

Susceptibility of the test organisms to reference drugs

The susceptibility of the test organisms to antibiotics and antifungal drug is presented in Table 3. Some of the micoorganisms which were sensitive to the plant extracts were found to be resistant to some of the antibiotics used. All the plant extracts produced considerable antimicrobial activities with *Streptococcus faecalis and S. aureus,* whereas, these organisms were resistant to ofloxacin, sparfloxacin, chloramphenicol, augmentin, ciprofloxacin and septrin. (Table 3). However, the antifungal drug (Ketoconazole) was more effective on the test fungi than the plant extracts.

Minimum inhibitory concentrations (MICs) of the plant extracts against the tested micoorganims

The MIC ranged from 25 to 250µg/ml. The lowest MIC (25µg/ml) was recorded against *P. stuartii* with ethanolic extract of *P. guajava.* Also, the aqueous and ethanolic extracts of *P. guajava* and had a MIC of 25µg/ml on *S. aureus*. The highest MIC (250µg/ml) was recorded with the aqueous extract of *A. africana* against *P. fluorescence*, and methanolic extracts of *P. guajava* on *S. faecalis* (Table 4). The MIC of the plant extracts against selected fungi ranged from 150-300µg/ml. Aqueous and ethanolic extracts of *A. africana* recorded MIC of 250 µg/ml on *T. tonsurans* and *Fusarium* sp. respectively. The highest MIC (300 µg/ml) was obtained with methanolic extract of *A. africana* on *Fusarium* sp (Table 4).

Minimum bactericidal concentrations (MBCs) of the plant extracts against the tested organisms

The MBC ranged from 50-400µg/ml. The lowest MBC (50µg/ml) was obtained with ethanolic and aqueous extracts of *P. guajava* against *S. aureus.* The highest MBC (400µg/ml) was obtained with the aqueous and ethanolic extracts of *A. africana*, and methanolic extract of *P. guajava on S. faecalis* respectively (Table 5).

Discussion

Nigeria is repleted with reservoir of medicinal plants. The economic burden and health implications of antimicrobial resistance are quite alarming. The unquantifiable extent of the antimicrobial potency of plant extracts orchestrated by characterization of abundant secondary metabolites inherent in these plants necessitated research on traditional medicine. Findings from this study revealed that aqueous and ethanolic extracts of the leaf of *Aspillia africana* is more potent than the extracts of *Psidium guajava. A. Africana* posess antibacterial activity against *Staphylococcus aureus, Streptococcus feacalis* and *Pseudomonas fluorescens*. [16]reported earlier that the ethanolic extract of the leaf of *A. africana* posess antibacterial potentials against these microorganisms. However, *Psidium guajava* exhibited no antimicrobial activity against the tested fungal isolates. Among the bacterial isolates *Stapylococcus aureus* were the most sensitive to the plant extracts while

Table 3. Susceptibility of the test organisms to reference antibiotics and antifungal drugs

Test Bacteria	Diameter of Zones of Inhibition (µg)															
Gram negative	**OFL (5)**	**SP (10)**	**CHL (30)**	**AUG (30)**	**CPX (5)**	**SXP (5)**	**AMX (25)**	**GEN (10)**	**PEF (5)**	**STR (10)**	**APX (10)**	**Z ()**	**CT (30)**	**ERY (10)**	**N ()**	**CM ()**
Escherichia coli	15 (R)	17 (R)	14 (R)	13 (R)	18 (S)	13 (R)	13 (R)	16 (S)	17 (S)	14 (R)	-	-	-	-	-	-
Klebsiella pneumoniae	0 (R)	0 (R)	15 (S)	20 (S)	0 (R)	12 (R)	16 (R)	12 (R)	23 (S)	0 (R)	-	-	-	-	-	-
Klebsiella oxytoca	0 (R)	16 (R)	20 (S)	16 (R)	18 (S)	22 (S)	16 (R)	14 (R)	20 (S)	18 (S)	-	-	-	-	-	-
Pseudomonas fluorescens	0 (R)	13 (R)	20 (S)	12 (R)	18 (S)	14 (R)	20 (R)	16 (S)	12 (R)	20 (S)	-	-	-	-	-	-
Serratia rubidae	4 (R)	4 (R)	5 (R)	0 (R)	12 (R)	0 (R)	0 (R)	8 (R)	16 (S)	12 (R)	-	-	-	-	-	-
Proteus mirabilis	8 (R)	6 (R)	2 (R)	0 (R)	16 (R)	20 (S)	4 (R)	22 (S)	16 (S)	2 (R)	-	-	-	-	-	-
Salmonella pullorum	19 (S)	0 (R)	2 (R)	0 (R)	0 (R)	16 (S)	0 (R)	21 (S)	22 (S)	0 (R)	-	-	-	-	-	-
Providential stuartii	4 (R)	4 (R)	5 (R)	0 (R)	12 (R)	0 (R)	0 (R)	8 (R)	16 (S)	12 (R)	-	-	-	-	-	-
Bacillus cereus	-	-	-	-	-	-	0 (R)	24 (S)	26 (S)	20 (S)	0 (R)	24 (S)	0 (R)	4 (R)	8 (R)	10 (R)
Staphlococcus aureus	-	-	-	-	-	-	0 (R)	22 (S)	18 (S)	0 (R)	0 (R)	0 (R)	0 (R)	0 (R)	19 (S)	0 (R)
Corynebacterium pyogenes	-	-	-	-	-	-	12 (R)	3 (R)	21 (S)	0 (R)	19 (S)	12 (R)	4 (R)	10 (R)	19 (S)	0 (R)
Streptococcus faecalis	-	-	-	-	-	-	12 (R)	17 (S)	7 (R)	0 (R)	8 (R)	10 (R)	10 (R)	15 (S)	6 (R)	0 (R)

Test Fungi	**Concentrations of Ketofung (mg/ml)**		
	05	**10**	**20**
Trychophyton tonsurans	18	34	37
Candida albicans	18	25	33
Penicillium expansium	16	22	28
Alternaria sp.	11	13	17
Fusarium sp.	21	26	32
Aspergillus niger	16	18	24
Aspergillus fumigates	18	25	30
Aspergillus flavus	20	22	28
Penicillum camenberti	14	18	22
Trychophyton rubrum	17	22	27

OFL= ofloxacin (5µg) , SP= Sparfloxacin (5µg) , CHL= Chloramphenicol (30µg) , AUG= Augmentin (30µg), CPX= Ciprofloxacin (5µg), SXP= Septrin (25µg), AMX= Amoxicillin (25µg), GEN= Gentamycin (10µg), PEF= Pefloxacin (5µg), STR= Streptomycin (10µg), APX= Ampiclox (10µg), **Z= Zinnacef (µg)**, CT= Ceftriazone (30µg), ERY= Erythromycin (10µg), **N= Nitrofurantoin (µg), CM= Clarithomycin (µg)**

Corynebacterium pyogens and Escherichia coli were the most resistant. The prolific synergistic sensitivity of the test isolates to aqueous and ethanolic extracts of both plants is at variance with the findings of [17], who reported that ethanol as the best solvent for the extraction of bioactive components from plants. This study showed that *A. africana extract* was active on both Gram-negative (*P. fluorescence and P. stuartii*) and Gram-positive (*S. aureus and S. faecalis*) bacterial at 100mg/ml. Similar findings were reported by[18] on the evaluation of aqueous extracts of the leaves of *A. Africana* in Sierra Leone which exhibited antibacterial activities on both Gram-positive and Gram-negative organisms at concentrations which ranged from 0.1-0.5g/ml. However, the present study contradicts the findings of[17] who reported aqueous extract of *A. africana* to be active on *E. coli and Salmonella* sp at concentrations ranging from 0.1-0.5g/ml. In the present study, the extract of *Ps.* guajava exhibited inhibitory property on *S. aureus*, *S. faecalis* and *P. Stuartii* thus conforms to earlier reports by[19-20] and[21] who reported the antibacterial activity of *Ps. guajava* leaf. [20]also reported the efficacy of the plant extract in the treatment of diarrhoea, dysentery and wounds. Most of the microorganisms tested in this study have been implicated in various gastrointestinal infections such as watery diarrhoea, nausea, vomiting, abdominal pain, pediatric diarrhoea, typical gastroenteritis, necrotizing enterocolitis and/or dysentery (*E. coli*, *Klebsiella* sp. and *Salmonella* sp) and wound infection (*Staphylococcus* sp., *Streptococcus* sp., *Proteus* sp. and *Pseudomonas* sp). The synergistic antimicrobial effect of the various plants extracts showed that the zones of inhibition increased considerably compared to when extracts were used singly. The wider zones of inhibition recorded may be due to resultant effects of the bioactive compounds in the extracts. [21]reported in their studies on *in vitro* synergistic activities of some herbs on *S. typhi*, that antimicrobial activity of some plants extracts increase when used synergistically. However, *E. coli, P. mirabilis, P. expansium, Alternaria* spp, *A. fumigatus, A. flavus* and *P. carmenberti* all displayed resistance to all the combined extracts tested. This may be due to the possibility that the active ingredient of one particular plant may not be complementary to another, thereby resulting in neutralization or denaturalisation of the plants components. The results obtained from the minimum inhibitory concentration (MIC) of the various plants extracts showed that the plant extracts are very potent against the pathogens at the MIC of 25µg/ml. The high MIC obtained on the test fungi confirmed their low susceptibility to virtually all the extracts tested. The MICs of the fungal isolates ranged between 150 and 300µg/ml. The low susceptibility of these fungi to these extracts may likely be attributed to the fact that majority of fungi interact with plants in the field and would have naturally developed resistance to the active antimicrobial ingredients in these plants.

CONCLUSION

This study concludes that ethanolic extracts (100mg/ml) of the leaf of *Aspillia africana* is more potent than the methanolic and aqueous extracts of *Psidium guajava*. The plant extracts showed greater antimicrobial activity on the bacterial isolates than on the fungal isolates suggesting a broader spectrum of activity with ethanolic extract on the gram positive and the gram negative bacteria. The therapeutic properties of *Aspillia africana* and *Psidium guajava* is better enhanced with their synergistic potential to the tested microorganisms.

Table 4. Minimum inhibitory concentrations (MICs) of the plant extracts against the tested microorganisms

	Aspillia africana			*Psidium guajava*	
Test Bacteria	A	E	M	A	E
Providencia stuartii	-	50	M	A	E
Bacillus cereus	-	-	50	50	25
Staphylococcus aureus	50	50	100	-	-
Streptococcus faecalis	200	200	100	25	25
Klebsiella oxytoca	-	-	-	100	100
Klebsiella pneumonia	-	-	100	-	-
Escherichia coli	-	-	200	-	-
Pseudomonas fluorescence	250	200	-	-	-
Salmonella pullorum	-	-	250	-	-
Corynebacterium pyogenes	-	-	-	-	-
Serratia rubidae	-	-	-	-	-
Test Fungi			-	150	150
Trychophyton tonsurans	250	250			
Candida albicans	250	-	-	-	-
Trychophyton rubrum	-	-	-	-	-
Fusarium H.	250	250	-	-	-
Aspergillus niger	-	-	250	-	-

KEY: A – Aqueous extracts, E – Ethanolic extracts, M – Methanolic extract

CLSI. Performance standards for antimicrobial susceptibility testing. Clinical and laboratory standards institute, 29th ed, 2019.

Table 5. Minimum bactericidal concentrations (MBCs) of the plant extracts against tested microorganisms

	Aspillia africana			*Psdium guajava*	
Test Bacteria	A	E	M	A	E
Providencia stuartii	-	150	150	100	50
Bacillus cereus	-	-	300	-	-
Staphylococcus aureus	150	150	300	50	50
Streptococcus faecalis	400	400	-	250	200
Klebsiella oxytoca	-	-	200	-	-
Klebsiella pneumonia	-	-	400	-	-
Escherichia coli	-	-	-	-	-
Pseudomonas fluorescence	400	300	400	-	-
Salmonella pullorum	-	-	-	-	-
Corynebacterium pyogenes	-	-	-	-	-
Serratia rubidae	-	-	-	200	250

RECOMMENDATION

A further study on the proper formulation into drugs of the plant extracts for human consumption is highly

recommended. The toxicological studies of the extracts on experimental animals should be determined in order to ascertain its safety threshold on humans.

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