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Bioactivity of leaf and bark extractives of *Prosopis africana* (Guill., Perrott. and Rich.) Taub. against some multidrug-resistant microbes

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The aqueous, methanolic, ethanolic and n-Hexane leaf and bark of *P. africana* extracts were tested for antimicrobial activity against five clinical pathogens: *Acinetobacter baumanii, Escherichia coli* 25922, *Escherichia coli* ESBL, Methicillin-Resistant *Staphylococcus aureus,* and *Candida albicans* ELI. Antimicrobial sensitivity test was carried out using the agar well diffusion method at a stock and varying concentrations of 500, 1000 and 1500 mg/mL. Results indicated that n-hexane leaf and bark extracts could not inhibit the organisms. Aqueous leaf extracts inhibited *A. baumanii*, MRSA, *E. coli* ESBL and *E. coli* 25922, while aqueous bark extract inhibited *A. baumanii*, MRSA, *E. coli* 25922, *C. albicans* ELI. Methanolic leaf extracts inhibited *A. baumanii,* MRSA, *E. coli* ESBL, while Methanolic bark extracts inhibited *A. baumanii*, MRSA, *E. coli* ESBL, *E. coli* 25922, *C. albicans* ELI. Ethanolic bark extracts inhibited *A. baumanii,* MRSA, *E. coli* ESBL, *E. coli* 25922, *C. albicans* ELI. The Minimum Inhibitory Concentration of the extracts for leaf and bark ranged from 1500 mg/mL – 23.43 mg/mL. Minimum Bactericidal Concentration was observed in aqueous leaf extracts against *E. coli* 25922 at 1500 concentration. Antibiotics susceptibility test indicated multidrug resistance by the test organisms with only Ofloxacin, Gentamycin, Ceftazidime and Nitrofurantoin eliciting inhibitory activity against *A. baumanii*, *E. coli* ESBL and *E. coli* 25922, respectively. Preliminary phytochemical screening revealed that *P. africana* leaf and bark extractives contained beneficial phytochemicals responsible for their high bioactivity against the selected clinical isolates.

Keywords: Gram-negative and positive, MBC, MFC, MIC, Phytochemical, Susceptibility test

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Introduction

The search for plants with antibiotic or therapeutic potential has been a continued process as the trends of multidrug resistance among emerging and re-emerging bacterial and fungal pathogens to the available modern drugs or antibiotics increases[1,2]. Several findings have proven the potential of medicinal plants in traditional medicine in treating several human diseases. Today, natural products derived from plants are being continuously tested for new drugs with new modes of pharmacological action to develop novel drugs. However, antibiotics were discovered to provide the source for the therapy of microbial infections. However, excessive use of antibiotics has become a major factor for the emergence and spread of multidrug-resistant strains[3]. Infectious diseases caused by these multidrug-resistant strains remain the leading cause of death. Thus, people are focusing on alternative novel antimicrobial agents to combat such pathogens[4,5].

Phytochemicals are biologically active compounds derived from plant physiological processes and confer colour, flavour, smell, texture, and several biological properties, including antimicrobial properties to the plants[6]. Phytochemicals possess disease-curative potentials by inhibiting the growth of pathogenic microorganisms[7,8]. C. albicans cause 70% of all human Candida infections. *C. albicans* is a fungus usually present on the skin and in mucous membranes such as the vagina, mouth, or rectum. MRSA (methicillin-resistant *Staphylococcus aureus*) has been known to cause various diseases ranging from skin and soft tissue infections, bone and joint infections, pneumonia, bacteremia, and endocarditis[9]. *E. coli* is associated with ear infections[10]. *Acinetobacter baumannii* causes fatal meningitis[11] and pneumonia[12]. These pathogenic micro-organisms are resistant to many widely used antibiotics, and the acquisition of resistance to many other antibiotic classes results in multidrug-resistant (MDR) strains. Ethnobotanical surveys carried out around the world have reported several plants used in the treatment of infectious diseases, including *Prosopis africana*[13,14].

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Prosopis africana, also known as African mesquite, belongs to the Fabaceae family and the sub-family Mimosoideae. It is native to the North, Central and West Africa. According to Yarkwan[15], Different parts of the tree have been successfully used traditionally for years for the treatment of diseases such as bronchitis, dermatitis, gonorrhoea, dysentery, malaria, rheumatism, sore throat, fevers, stomach cramps, skin diseases, headache, toothache, and as a dressing for wounds or cuts. Apart from the medicinal uses, the freshly budding leaves, shoots and pods are used as fodder[15]. The wood has a high thermal value of about 1720 J/kg and produces excellent charcoal and firewood. The bark and root, rich in tannin, are useful as a dyestuff, and the pod ashes are a source of potash for making local soap used to treat skin disease and fevers, and as eye washes[15]. The rich protein seeds are a very important condiment in soups and other dishes, and it is used as human and animal feed (Yarkwan)[15]. *P. africana* has also been reported to possess several medicinal uses. Almost all its parts are reportedly used in the traditional treatment of various diseases. The stem bark is used as a remedy for dysentery, gonorrhea, bronchitis, and skin diseases[16].

According to Abdullahi and Kabir[16], the twigs, leaves, bark, and secondary roots are used to treat typhoid fever, dental decay, malaria, and stomach cramps in many parts of Nigeria[17]. The root poultice is also used to manage bronchitis, dermatitis, tooth decay, dysentery, malaria, stomach cramps, dental caries, sore throat, toothache, tooth decay, and as a dressing for wounds[16]. Pharmacological studies have shown *P. africana* root extract to exhibit strong antimicrobial activity against *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia* with MIC values of 20, 50 and 50 mg/mL, respectively[18]. *P. africana* has also been reported to inhibit the growth of *Salmonella typii*, *Streptococcus pyogenes*, Methicillin Resistant *Staphylococcus aureus* (MRSA), *K. pneumonia*, *P. aeruginosa* and *Candida albicans*, at MICs ranging from 12.5 to 50 mg/mL[17,18]. It has also been reported to show a significant inhibitory activity on the growth of *S. typii*, *S. pyogenes*, MRSA, *K. pneumonia*, *P. aeruginosa*, and *C. albicans* at MICs ranging from 12.5 to 50 mg/mL[17,18].

The phytochemical profile and the bio-activities of *P. africana* against various pathogenic microbes and cancer cells have been reported. Adetutu[19], reported *P. africana* to contain lipid, phytochemical and essential oils, which were able to protect the liver against *B. bergei*-induced damages. Elmezughi *et al.*[20] isolated a compound with a structural formula of $C_{16}H_{14}O_6$ from *P. africana*, which showed activity against *Mycobacterium aurum* with a MIC of 413.63 µM as compared to the standard drug (ethambutol at 29.36 µM). The compound showed MIC pf 827.81 µM against *S. aureus* compared to gentamicin as a standard at 0.31 µM. They also tested the cytotoxicity of the compound against ZR75 (Breast cancer cell). It showed 40% cell viability against the ZR75 cell line with low toxicity against human cell lines[20]. Likewise, the anti-Plasmodium activity of *P. africana* was investigated by Adetutu[19]. The aqueous bark extracts showed significant anti-malaria activities at 200 mg/kg body weight with a 90.02% chemo-suppressive effect compared to other plant extracts and the standard chloroquine (61.70%). All these studies have suggested a promising future for the extracts of *P. africana* being used as phytotherapy in managing the diversity of human diseases. In this study, we evaluated the antimicrobial activities of different extracts of *P. africana* against selected Gram-positive and Gram-negative bacteria and pathogenic fungi. The aim is to evaluate its biological and pharmacological properties and ascertain the toxicity attached to their use in folkloric and curative medicine.

Materials and Methods

Collection, identification and preparation of plant materials

Fresh leaf and stem bark of disease-free *P. africana* were collected in December 2017 from the University of Ilorin natural plantation and were authenticated at the Herbarium of the Department of Plant Biology, University of Ilorin, Nigeria. A voucher specimen was deposited in the departmental herbarium with the UILH/001/472 authentication number. The plant materials were prepared via washing, shade drying and pulverized using a pestle and wooden mortar. The powdered plant material was sieved with fine mesh and kept in plastic bags before extraction.

Extraction process and phytochemical analysis

One hundred grams of each powdered plant material was percolated in 500 mL of methanol, ethanol, distilled water and n-hexane, placed on a rotatory shaker for 48 hours at 150 rpm and filtered using muslin cloth. The filtrates were concentrated at 45°C using a water bath and then subjected to GC-MS analysis to reveal the potent phytochemical content of the plant materials.

Test organisms

The test organisms include *Acinetobacter baumannii, Candida albicans* ELI, Methicillin-resistant *Staphylococcus aureus* (MRSA), *Escherichia coli* ESBL and *Escherichia coli* 25922 were obtained from the medical microbiology laboratory, University of Ilorin teaching hospital, Ilorin, Kwara State.

Preparation and standardization of bacterial inoculum

Bacterial inoculum was standardized using McFarland's standards. It was carried out by picking test organisms growing as pure culture in McCartney bottles and transferring them into 10 mL of distilled water in test tubes. Growing media was prepared according to the manufacturer's specifications. The media were sterilized at 121°C for 15 min using an autoclave.

Antimicrobial Susceptibility Test (AST)

The antimicrobial screening was carried out using the agar well diffusion method as described by Lino and Deogracious[21] with slight modifications. The test bacteria were first cultivated in nutrient broth at 37°C for 18 h. Each of the cultures was then adjusted to 0.5 McFarland turbidity standards and inoculated (0.2 mL each) onto Muller Hinton agar (MHA, Oxoid) plates (diameter: 15 cm). A sterile cork borer was used to bore six wells (6 mm diameter). The plates were allowed to stand for 30 min to allow the extract to diffuse into the agar, then incubated at 37°C overnight. Antibacterial activity was determined by observing, measuring and recording the zones of inhibition (ZOI) to the nearest millimetres (mm) using a meter rule[22].

Two types of antibiotic disc (Abtek antibiotic disc) were used depending on the Gram staining property of the test organism under observation, i.e. Gram-positive and Gram-negative. The positive disc was pressed down on the media containing Methicillin-resistance *Staphylococcus aureus*, and the negative discs for *A. baumanii, E. coli* 25922, *and E. coli* ESBL. The plates were slightly opened, and the appropriate antibiotic disc was placed inside using sterile forceps. The plates were incubated upright at 37°C for 18 h. After incubation, the inhibition zones around each antibiotic disc were measured to the nearest millimetre (mm) using a metric rule. The antibiotics used and their corresponding concentrations are as follows: Gram-negative: Ceftazidime (Caz) 30 µg, Cefuroxime (Crx) 30 µg, Gentamicin (Gen) 10 µg, Ceftriaxone (Ctr) 30 µg, Erythromycin (Ery) 5 µg, Cloxacilin (Cxc) 5 µg, Ofloxacin (Ofl) 5 µg, Augmentin (Aug) 30 µg. Gram-positive: Amoxicillin (Amx) 25 µg, Cotrimoxazole (Cot) 25 µg, Nitrofurantoin (Nit) 300 µg, Gentamicin (Gen) 10 µg, Nalidix acid (Nal) 30 µg, Ofloxacin (Ofl) 30 µg, Augmentin (Aug) 30 µg, Tetracycline (Tet) 30 µg. For fungi isolates: E-test strip MIC, Voruocenazole (1 µg), Nystatin (100 units), Fuconazole (25 µg), Micafungin.

Minimum Inhibitory Concentration (MIC) of plant extracts and test organisms

MIC of the extracts were determined for each test organism in triplicates at 400, 200, 100, 50, and 25 mg/mL. The sub-cultured test organisms were standardized according to Cheesebrough[23]. One mL of nutrient broth was added, and then a loopful of the test organism previously diluted to 0.5 McFarland turbidity standard was introduced to the test tubes. All test tubes were incubated at 37°C for 24 h[22]. The highest dilution of the extracts that inhibit the growth but do not kill the organism was defined as MIC. The active plant extracts, which showed an inhibition zone in some test plates from the disc diffusion method, were further tested to determine MIC values by the broth macro dilution method described by NCCLS[24].

Determination of Minimum Bactericidal Concentration (MBC)

The MBC was determined by the method of Semnani *et al.*[25]. All the tubes that showed no microbial growth (no turbidity) after 24 h of incubation were subcultured onto the surfaces of freshly prepared Mueller-Hinton agar and incubated at 37°C for another 24 h. The concentration at which no growth was observed in the broth inoculation and on the plate is the concentration at which the microbial cells are lysed or killed. Hence, MBC (for bacteria) and Minimum Fungal Concentration (MFC)[22]. The plates from the MIC assay showing growth on inoculated plates but not in the broth after incubation were indicated to have a bacteriostatic/fungistatic effect, i.e. at that concentration, the organism is inhibited from growing when it has no access to fresh nutrients.

Statistical analysis

To show the differences in the zone of inhibition of the extracts against the selected clinical organisms, an analysis of variance (ANOVA) was performed. Significant differences between means were determined by Duncan's multiple range tests using Statistical Package for the Social Sciences.

Results and Discussion

Antimicrobial sensitivity test

The results of the sensitivity tests of *P. africana* leaf and bark extracts against *A. baumanii*, *E. coli* 25922*, E. coli* ESBL, MRSA and *C. albicans* ELI inoculated on nutrient agar was measured by checking for clear zones on the plates after incubation (zones of inhibition).

The ZOI of the test organisms on aqueous, ethanolic, methanolic and N-hexane leaf and bark extracts of *P. africana* was observed using 500 mg/mL stock concentration. The susceptibility test result revealed that the aqueous bark extract was observed to inhibit the growth of all the test organisms except *E. coli* ESBL. The aqueous leaf extract inhibited the growth of all organisms except *C. albicans* ELI. The methanolic bark extract inhibited the growth of all test organisms. The methanolic leaf extract inhibited the growth of three (3) test organisms: *A. baumanii*, *E. coli* ESBL and MRSA. The ethanolic bark extract inhibited the growth of all test organisms. The ethanolic leaf extract had no ZOI against all the test organisms. No inhibition was observed for the n-hexane bark and leaf extracts against all the test organisms (Table 1).

Effects of varying concentrations of the extracts on the test organisms

The effects of varying concentrations of the aqueous, methanolic, ethanolic and n-hexane extracts were assayed against the test organisms. This was achieved by diluting the stock concentration to obtain the desired concentration. The desired concentrations were 1000 and 1500 mg/mL. There were significant variations in the activity of the *P. africana* extracts, which was judged by the ZOI exerted by each extract. The activities of the extracts were similar for ethanolic and aqueous bark extracts with ZOI of 17.3 and 17 mm, respectively, against *E. coli* 25922. At the same time, there were significant differences between aqueous leaf and ethanolic bark extracts at 500 mg/mL. ZOI was highest (14.3 mm) for *E. coli* ESBL and *A. baumanii* (19.7 mm) when inhibited by aqueous leaf extract at 500 mg/mL while MRSA and *C. albicans* were most sensitive to ethanolic bark extract at 500 mg/mL with ZOI of 15.7 and 18.3 mm. However, n-hexane extracts could not inhibit any organisms' growth at any of the concentrations. At 1000 mg/mL, *A. baumanii* was most sensitive to aqueous leaf extract (ZOI of 18.7 mm), followed by *E. coli* 25922 (16.3 mm) to aqueous bark extract. ZOI was similar for methanolic leaf and bark extract against *A. baumanii* and *E. coli* ESBL. At 1500 mg/mL, only aqueous leaf and bark extract inhibited the growth of *A. baumanii* with ZOI of 12.7 and 14.0 mm, respectively. *C. albicans* was selectively sensitive to aqueous bark (14.7 mm), methanolic bark (13.7 mm) and ethanolic bark (18.3 mm) at 500 mg/mL. At 1000 mg/mL, only aqueous bark had inhibitory effects on *C. albicans* (ZOI = 14.0 mm), while none of the extracts had inhibitory effects at 1500 mg/mL

Generally, no inhibitory activity was observed in the n-hexane bark, leaf extract, and ethanolic leaf extract. In the aqueous, methanolic and ethanolic extracts, inhibitory effects were observed at higher concentrations but diminished as the concentration reduced (Table 2).

Minimum Inhibitory Concentration of the crude extracts

The minimum inhibitory concentration of the aqueous (bark and leaf), methanolic (bark and leaf), and ethanolic (bark) extracts were determined by observing the turbidity of the varying concentrations of the extracts in the test tube after incubation. The minimum inhibitory concentration of the extracts was observed as 1500, 750, 375, 187.5, 93.75, 46.875, and 23.43 mg/mL for the organisms, respectively (Table 3-7).

Table 1 — Zones of inhibition (mm) for the stock concentration of 500 mg/mL of the bark and leaf extracts of *P. africana* against the selected pathogenic organisms

Test organism	Crude extracts							
	Aqueous		Methanolic		Ethanolic		n-Hexane	
	Leaf	Bark	Leaf	Bark	Leaf	Bark	Leaf	Bark
A. baumanni	19.7	14	13.3	13.7	N	17.7	N	N
E. coli 25922	15.7	17.3	N	14.3	N	17	N	N
E.coli ESBL	14.3	N	13.7	13.3	N	14	N	N
MRSA	17.3	14.7	13.3	12	N	15.7	N	N
C. albicans ELI	N	14.7	N	13.7	N	18.3	N	N

Key: N = No zone of inhibition: MRSA = *Methicillin-resistant Staphylococcus aureus*

Table 2 — Zones of inhibition (mm) for varying concentrations of *P. africana* leaf and bark crude extracts on the selected pathogenic micro-organisms

Conc. (mg/mL)	Crude extracts		Test organisms				
			E. coli 25922	*E. coli ESBL*	*A. baumanii*	MRSA	*C. albicans ELI*
500	Aqueous	Leaf	15.7[b]	14.3[a]	19.7[a]	17.3[a]	N
		Bark	17.3[a]	N	14[c]	14.7[c]	14.7[b]
	Methanolic	Leaf	N	13.7[b]	13.3[d]	13.3[d]	N
		Bark	14.3[c]	13.3[a]	13.7[d]	12	13.7[c]
	Ethanolic	Leaf	N	N	N	N	N
		Bark	17[a]	14[a]	17.7[b]	15.7[b]	18.3[a]
	N-hexane	Leaf	N	N	N	N	N
		Bark	N	N	N	N	N
1000	Aqueous	Leaf	N	N	18.7[a]	N	N
		Bark	16.3[a]	N	14.3[b]	N	14[a]
	Methanolic	Leaf	N	12.3[a]	12.7[c]	N	N
		Bark	12.7[b]	12.6[a]	11.3[c]	13	N
	Ethanolic	Leaf	N	N	N	N	N
		Bark	N	N	10[d]	N	N
	N-hexane	Leaf	N	N	N	N	N
		Bark	N	N	N	N	N
1500	Aqueous	Leaf	N	N	12.7[b]	N	N
		Bark	N	N	14[a]	N	N
	Methanolic	Leaf	N	N	N	N	N
		Bark	N	N	N	N	N
	Ethanolic	Leaf	N	N	N	N	N
		Bark	N	N	N	N	N
	N-hexane	Leaf	N	N	N	N	N
		Bark	N	N	N	N	N

Key: N = No zone of inhibition

Table 3 — Minimum Inhibitory Concentrations of the aqueous leaf extract

Organisms	Varying concentrations of aqueous leaf extract (mg/mL)							
	1500	750	375	187.5	93.75	46.875	23.43	MIC Conc. (mg/mL)
A. baumanii	-	-	+	+	+	+	+	750
MRSA	-	-	-	+	+	+	+	375
E. coli 25922	-	-	+	+	+	+	+	750
E. coli ESBL	-	-	-	+	+	+	+	375

Key for MIC: (-) No Growth (+) Growth

Table 4 — Minimum Inhibitory Concentration of the aqueous bark extract

Organisms	Varying concentrations of aqueous bark extract (mg/mL)							
	1500	750	375	187.5	93.75	46.875	23.43	MIC Conc. (mg/mL)
A. baumanii	+	+	+	+	+	+	+	Nil
MRSA	+	+	+	+	+	+	+	Nil
E. coli 25922	+	+	+	+	+	+	+	Nil
C. albicans ELI	+	+	+	+	+	+	+	Nil

Key for MIC: (-) No Growth (+)Growth

Table 5 — Minimum Inhibitory Concentration of the methanolic leaf extract

Organisms	Varying concentrations of methanolic leaf extract (mg/mL)							
	1500	750	375	187.5	93.75	46.875	23.43	MIC (mg/mL)
A. baumanii	-	-	-	+	+	+	+	375
MRSA	-	-	-	+	+	+	+	375
E. coli ESBL	-	-	+	+	+	+	+	750

Key for MIC: (-) No Growth (+) Growth

Table 6 — Minimum Inhibitory Concentration of the methanolic bark extract

Organisms	Varying concentrations of methanolic bark extract (mg/mL)							
	1500	750	375	187.5	93.75	46.875	23.43	MIC (mg/mL)
A. baumanii	-	-	+	+	+	+	+	750
MRSA	-	+	+	+	+	+	+	1500
E. coli ESBL	-	-	-	+	+	+	+	375
E. coli 25922	-	-	+	+	+	+	+	750
C. albicans ELI	-	-	+	+	+	+	+	750

Key for MIC: (-) No Growth; (+) Growth

Table 7 — Minimum Inhibitory Concentration of the ethanolic bark extract

Organisms	Varying concentrations of ethanolic bark extract (mg/mL)							
	1500	750	375	187.5	93.75	46.875	23.43	MIC (mg/mL)
A. baumanii	-	-	-	+	+	+	+	375
MRSA	-	-	-	+	+	+	+	375
E. coli ESBL	-	-	-	+	+	+	+	375
E. coli 25922	-	-	-	+	+	+	+	375
C. albicans ELI	-	-	-	+	+	+	+	375

Key for MIC: (-) No Growth (+) Growth

Table 8 — Minimum bactericidal and fungicidal concentration of the aqueous and methanolic leaf extract

	Organisms	Varying concentrations			
		1500	750	375	MBC/MFC Conc. (mg/mL)
Aqueous leaf extract	*A. baumanii*	+	+	+	Nil
	MRSA	+	+	+	Nil
	E. coli 25922	-	+	+	1500
	E. coli ESBL	+	+	+	Nil
Methanolic leaf extract	*A. baumanii*	+	+	+	Nil
	MRSA	+	+	+	Nil
	E. coli ESBL	+	+	+	Nil

Key: (+) growth (-) no growth

Minimum bactericidal and fungicidal concentration of the crude extracts

This is the least concentration capable of inhibiting and killing the inoculum. It is the least concentration, showing no observable growth in the test tube and the plate. Samples from the test tubes used in the MIC assays, which did not show observable signs of turbidity (growth) after incubation, were streaked out on solidified NA/PDA plates using a sterile cotton swab and incubated at 37°C for 24 h. The lowest concentration that shows no growth on plates after incubation indicated a bactericidal/fungicidal effect and was taken as the MBC (for bacteria) and as the MFC (for fungi)[18].

There was a minimum bactericidal concentration for aqueous leaf extracts on *E. coli* 25922 at 1500 mg/mL (Table 8). However, no minimum bactericidal and fungicidal concentration was observed for the remaining extracts on all the test organisms (Tables 8 and 9).

Standard antibiotics susceptibility test

The antimicrobial activity of standard antibiotics was assayed on the test organisms. An appropriate antibiotic disc was used for the corresponding test organisms. Tables 10-12 revealed the inhibitory activity observed on all the standard antibiotics.

For MRSA, only 30 µg of Ofloxacin was able to inhibit the growth of MRSA with ZOI of 33.5 mm. In contrast, other antibiotics could not (Table 10). 30 µg Ceftazidime, 10 µg Gentamicin and 5 µg Ofloxacin inhibited *E. coli* 25922 with ZOI of 12.0, 23.0, and 27.7 mm. This showed that Ofloxacin was more potent. *A. baumanii* was inhibited by 10 µg Gentamicin, 10 and 5 µg Ofloxacin, while only Nitrofurantoin at a dose of 300 µg was able to inhibit *E. coli* ESBL (Table 11). This result shows that only a few antibiotics could suppress the growth of these micro-organisms. However, in the case of *C. albicans*, none of the selected antifungals could inhibit its growth (Table 12), which showed that it is multidrug resistant.

Table 9 — Minimum bactericidal and fungicidal concentration of the aqueous, methanolic and ethanolic bark extract

Extract type		Varying concentrations			
	Organisms	1500	750	375	MBC/MFC Conc. (mg/mL)
Aqueous bark	*A. baumanii*	+	+	+	Nil
	MRSA	+	+	+	Nil
	E. coli 25922	+	+	+	Nil
	C. albicans ELI	+	+	+	Nil
Methanolic bark	*A. baumanii*	+	+	+	Nil
	MRSA	+	+	+	Nil
	E. coli ESBL	+	+	+	Nil
	E. coli 25922	+	+	+	Nil
	C. albicans ELI	+	+	+	Nil
Ethanolic bark	*A. baumanii*	+	+	+	Nil
	MRSA	+	+	+	Nil
	E. coli ESBL	+	+	+	Nil
	E. coli 25922	+	+	+	Nil
	C. albicans ELI	+	+	+	Nil

Key: (+) growth (-) no growth

Table 10 — Antimicrobial activity of standard Gram-positive antibiotics disc on the test organisms

Standard antimicrobial agent (µg)	Zones of Inhibition (mm)
	MRSA
Amoxicillin 25	0.0
Cotrimoxazole 25	0.0
Nitrofurantoin 300	0.0
Gentamicin 10	0.0
Nalidix acid 30	0.0
Ofloxacin 30	33.5
Augumentin 30	0.0
Tetracycline 30	0.0

Table 11 — Antimicrobial activity of standard Gram-negative antibiotics disc on the test organisms

Standard antimicrobial agent (µg)	Diameter of zones of inhibition (mm)		
	E. coli 25922	*E. coli* ESBL	*A. baumanii*
Ceftazidime 30	12.0	0.0	0.0
Cefuroxime 30	0.0	0.0	0.0
Gentamicin 10	17.0	0.0	14.3
Nitrofurantoin 300	23.0	22.0	0.0
Erythromycin 15	0.0	0.0	0.0
Cloxacilin 5	0.0	0.0	0.0
Ofloxacin 5	27.7	0.0	22.3
Augumentin 30	0.0	0.0	0.0

Quantitative and qualitative analysis of the aqueous leaf and bark extracts using GC/MC techniques

From the result of the antimicrobial testing, MIC, MBC and MFC. The aqueous leaf and bark extracts had greater inhibitory activities than other extracts. These two extracts were taken for GC-MS screening. The Preliminary bio-active components carried out on aqueous extracts of the leaf and bark using GC/MS techniques are presented in Fig. 1 and 2, Tables 13 and 14.

Table 12 — Antimicrobial activity of standard antibiotic disc on the selected pathogenic fungal organisms

Standard antimicrobial agent	Diameter of zones of inhibition (mm)
	Candida albicans ELI
E-test strip MIC	0.0
Voruocenazole (1 µg)	0.0
Nystatin (100 units)	0.0
Fuconazole (25 µg)	0.0
Micafungin	0.0

The aqueous leaf and bark extracts contained active bio-active components responsible for their inhibitory activities against the selected test organisms.

Discussion

P. africana has been used in most rural communities to treat a handful of infectious diseases due to its apparent medicinal properties that stand it out from other herbal plants. Various studies have shown that plants are rich in phenolic substances that are active against several micro-organisms. Bioactive substances have been reported to confer resistance to plants against bacteria, fungi and pests. This, therefore, explains the demonstration of antimicrobial activities of *P. africana* in this study[26,23]. The antibacterial and antifungal activity of the plant extracts on the test organisms justifies the active ingredients observed in herbal extracts in preparing crude drugs from medicinal plant materials. The inhibitory activities exhibited by the extracts tend to agree with the report of El-Mahmood *et al.*[27], all of whom linked the antimicrobial properties of the plant

Fig. 1 — Phytochemical profile of *P. africana* bark aqueous extract as revealed by GC-MS.

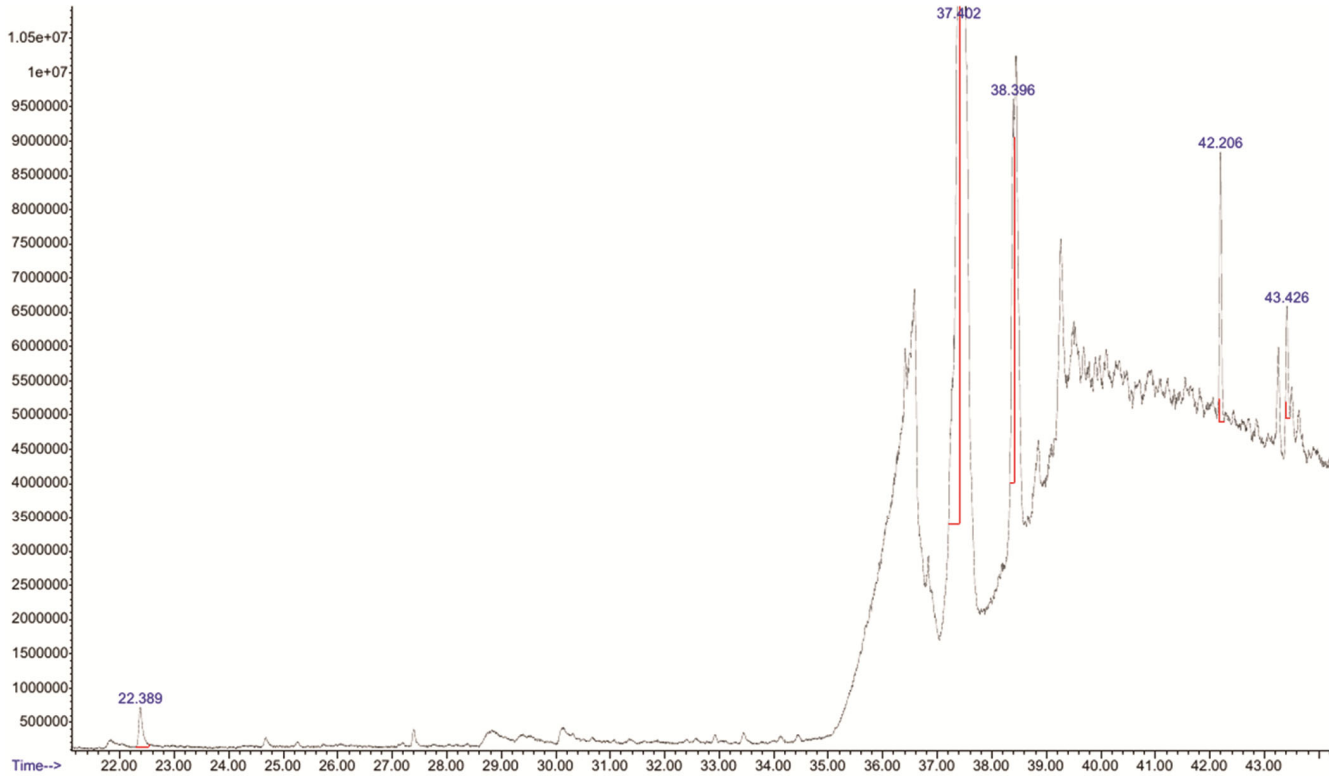

Fig. 2 — Phytochemical profile of *P. africana* leaf aqueous extract as revealed by GC-MS.

Table 13 — Major bio-active components of aqueous bark extract of *P. africana*

Bark water extract components	Area %	RT	MF	MW (g/mol)
Cyclotetrasiloxane, octamethyl-	8.85	13.19		
Benzoic acid, 5-methyl-2-trimethyl silyloxy-, trimethylsilyl ester	8.85	13.19	$C_{12}H_{18}O_2Si$	222.36
Cyclohexasiloxane, dodecamethyl-	10.04	19.26	$C_{12}H_{36}O_6Si_6$	444.92
Cyclohexasiloxane, dodecamethyl-	9.84	25.26	$C_{12}H_{36}O_6Si_6$	444.92
2H-1, 4 -Benzodiazepin -2- one, 7 - chloro - 1, 3 - dihydro - 5 -phenyl-1-(trimethylsilyl)-	9.84	25.26	-	
Pentasiloxane, dodecamethyl-	14.02	30.69	$C_{12}H_{36}O_4Si_5$	384.840
Octasiloxane, 1, 1, 3, 3, 5, 5, 7, 7, 9, 9, 11, 11, 13, 13, 15, 15 - hexadecamethyl-	14.02	30.69	-	
Cycloheptasiloxane, tetradecamethyl-	14.02	30.69	$C_{14}H_{42}O_7Si_7$	519.08
Cyclooctasiloxane, hexadecamethyl-	6.33	35.69	$C_{16}H_{48}O_8Si_8$	593.23
Benzoic acid, 2, 4–bis [(trimethylsilyl) oxyl] -, trimethylsilyl ester	6.33	35.69	-	
Silane,[(4-(1,2-bis((trimethylsilyl)oxy) ethyl)-1,2-phenylene]bis(oxy) bis [trimethyl-	6.33	35.69	-	
1 - Benzyl - 1, 2, 3 - triazole	28.14	36.43	$C_9H_9N_3$	159.19
N - Benzyl - 1H - benzimidazole	28.14	36.43	$C_{14}H_{12}N_2$	208.26
3 -Benzyl -5-chloro- 1, 2, 3-triazole 1 - oxide	28.14	36.43	$C_9H_8ClN_3O$	209.63
Benzimidazol-5-amine, 1-(4- ethoxyphenyl)-	22.77	37.25	$C_{15}H_{15}N_3O$	253.30
Benz (a) anthracene - 7 - carbonitrile	22.77	37.25	$C_{19}H_{11}N$	253.30
Phthalic acid, 3, 5-dimethylphenl 4- methoxyphenyl ester	22.77	37.25	$C_{23}H_{20}O_5$	376.40

RT = Retention time; MW = Molecular weight (g/mol); MF = Molecular formula

Table 14 — Major bio-active components of aqueous leaf extract of *P. africana*

Leaf water extract components	Area %	RT	MF	MW (g/mol)
2 (1H)- Quinolinone, hydrazine	3.70	22.39	$C_9H_9N_3$	159.19
3-Methyl - 2 - butanone, 4-t-butyldime thylsilyloxy	3.70	22.39		
Benzene, 1, 3-hexadienyl-	60.76	30.40	$C_{12}H_{14}$	158.40
2-chloro - 1 - (2-methyl - allyl) -1, 2 ,3 ,4 - tetrahydro-naphthalene	60.76	30.40	-	-
(2 ,3-Diphenylcyclopropyl) methyl phenyl sulfoxide, trans-	60.76	30.40	$C_{22}H_{20}OS$	332.46
1 - butyne, 4 - chloro - 3 methyl -3 – phenyl	17.89	38.40	$C_{11}H_{11}Cl$	178.66
Hexa - 2, 4 – dienylbenzene	17.89	38.40	$C_{12}H_{14}$	158.24
Benzene, 1, 3 - hexadienyl -	17.89	38.40	$C_{12}H_{14}$	158.40
.alpha. – Benzylsuccinic acid	12.39	42.21	-	-
(2, 3 -Diphenylcyclopropyl) methyl phenyl sulfoxide, trans -	12.39	42.21	$C_{22}H_{20}OS$	332.46
Benzenemethanamine, N- (phenylmethylene) -	12.39		-	-
(2, 3 - Diphenylcyclopropyl) methyl phenyl sulfoxide, trans -	5.25	43.43	$C_{22}H_{20}OS$	332.46
1 - propene, 3 -(2 -cyclopentenyl) - 2 - methyl - 1, 1 - diphenyl-	5.25	43.43	-	-
1H – Indole, 5 - methyl - 2 – phenyl-	5.25	43.43	-	-

RT = Retention time; MW = Molecular weight (g/mol); MF = Molecular formula

to the presence of the bio-active secondary metabolites (Table 1). Aqueous (bark and leaf), methanolic (bark and leaf) ethanolic (bark) extracts of *P. africana* showed inhibitory activities against *A. baumanii*, *E. coli* 25922, *E. coli* ESBL, MRSA and *C. albicans* at stock concentrations of 500, 1000, 1500 mg/mL but the ethanolic (leaf) and n-hexane (bark and leaf) extracts of *P. africana* showed no inhibitory activity against the test organisms. Therefore, n-hexane (bark and leaf) and ethanolic (leaf) had no significant inhibitory effect on all the test organism isolates, and this indicated that the active components necessary to confer significant inhibitory effects on the pathogens did not dissolve in considerable amounts as the extracts did not confer inhibitory effect at a stock concentration of 500, 1000, and 1500 mg/mL. Different solvents have various degrees of solubility for different phyto-constituents[28]. This is a significant discovery as it means that other solvents for extraction should be focused on, for the bioactive compounds present in the plant materials necessary to inhibit the selected pathogens (Table 2).

The activity of the plant extracts against Gram-positive and Gram-negative bacteria and clinical fungal

isolates indicates the presence of broad-spectrum antibiotic compounds in the plant. The activity of the extracts also varied between solvents, with the methanolic (bark) and ethanolic (bark) extracts demonstrating the highest activity against all the test organisms. Aqueous (bark and leaf) extract also showed the highest activity except for *C. albicans*, resistant to water leaf extract, and *E. coli* ESBL, resistant to water bark extract. It has been reported that different phytochemicals have different degrees of solubility in different types of solvents depending on their polarity. In traditional settings, water is the widely used solvent used to prepare these concoctions[29]. The higher activity demonstrated by these organic solvents (methanolic and ethanolic bark) in this work indicates that less bioactive components were extracted when water was used as a solvent. The variations may reflect the difference in the solvents, which equally define the phytochemicals released. This suggests that water solvent is better for dissolving bio-active compounds in *P. africana.* Standard antibiotics were also used to test their antimicrobial activity against the test organisms. Standard antibiotic discs for Gram-negative and positive were used to test for the antimicrobial activity of the various antibiotics against the corresponding organisms.

The antimicrobial activity of gram-positive antibiotic discs was tested against MRSA. Inhibition was only observed in Ofloxacin with a zone of inhibition of 33.5 mm (Table 10). For the Gram-negative organisms, the highest inhibitory activity was observed in Ceftazidime, Gentamicin, Nitrofurantoin and Ofloxacin against *E. coli* 25922 with diameters of 12.0, 17.0, 23.0, and 27.0 mm, respectively. Inhibitory activity was also observed in Nitrofurantoin against *E. coli* ESBL with a diameter of 22.0 mm. The highest inhibitory activity was observed in Gentamicin (Gen) 10 µg and Ofloxacin 5 µg against *A. baumanii* with diameters of ZOI as 22.3 and 14.3 mm, respectively. No inhibitory activity was observed in all the antibiotics against *C. albicans*, compared with aqueous, methanolic and ethanolic extracts, which had antimicrobial activity on the organism (*C. albicans)* (Table 12). This indicates that phyto-constituents present in the plant extracts are more potent than the standard antibiotics to exert antimicrobial effects on the organisms. This finding is similar to the finding of Adetutu[19] where aqueous bark extracts of *P. africana* showed significant anti-malaria activities at 200 mg/kg body weight with 90.02% chemo-suppressive effect compared to other plant extracts and the standard chloroquine (61.70%). The antimicrobial activity of Ofloxacin observed against the Gram-positive and Gram-negative bacteria indicates it is a broad-spectrum antibiotic. Standard antibiotics are refined and purified products. At the same time, extracts of herbal medicines are mixtures of various plant constituents, some of which can interfere with antimicrobial activity and are subjected to degradation and decomposition on storage[29].

The active plant extracts that showed inhibition zones in some test plates from the disc diffusion method were further tested to determine MIC values by the broth macro dilution method described by NCCLS[24]. The MIC of the leaf and bark extracts ranged from 1500 – 23.43 mg/mL for aqueous (bark and leaf), methanolic (bark and leaf) and ethanolic bark extracts. The effects of the extracts correlate with the reports that micro-organisms vary widely in their degree of susceptibility to antimicrobial agents[29]. High MIC values are an indication of high activity, while low MIC values are an indication of low activity. In this study, the water (leaf), methanol (bark and leaf) and ethanol (bark) had higher MIC values for the entire test organism at 1500, 750, and 375 mg/mL, respectively, thus indicating higher susceptibility to the efficacy of the extract. Lower MIC values of 187.5, 93. 75, 46.875, and 23.43 mg/mL were obtained for the entire organisms, thus indicating lower activity of the extracts against the organisms. (Table 3, 5, 6, and 7). No inhibitory activity was observed in the water (bark) extracts against *A. baumanii*, *E. coli* 25922, MRSA, and *C. albicans* ELI*,* and it was speculated that the organism was resistant to the phytochemicals present in the water (bark) extracts.

Preliminary bio-active components on aqueous leaf and bark using GC/MS techniques showed that Aqueous leaf extracts contained diverse phytochemicals of different concentrations (Tables 13 and 14). The aqueous bark extracts had the highest concentration of 1-Benzyl-1,2,3-triazole, 3-Benzyl-5-chloro-1,2,3-triazole 1-oxide and N-Benzyl-1H-benzimidazole (28.14%) while hexadecamethyl-Cyclooctasiloxane, trimethylsilyl ester Benzoic acid, 2,4-bis[(trimethylsilyl)oxy] and Silane, [(4-(1,2-bis((trimethylsilyl)oxy) ethyl)-1,2-phenylene]bis(oxy) bis [trimethyl- had the lowest concentration of 6.33% (Table 13 and 14). This is similar to the findings of Adetutu[19] to contain lipid, phytochemical and

essential oils, which were able to protect the liver against *B. bergei*-induced damages. Elmezughi *et al.*[20] isolated a compound with a structural formula of $C_{16}H_{14}O_6$ from *P. africana*, which showed activity against *Mycobacterium aurum* with a MIC of 413.63 µM as compared to the standard drug (ethambutol at 29.36 µM). The compound showed MIC pf 827.81 µM against *S. aureus* compared to gentamicin as a standard at 0.31 µM. The researchers then attributed the activity of the compound to the lipophilicity of the compound, which helped in their penetration of the mycobacterial cell wall. They also tested the cytotoxicity of the compound against ZR75 (Breast cancer cell). It showed 40% cell viability against the ZR75 cell line with low toxicity against human cell lines[20].

Generally, the present study has shown that the aqueous (bark and leaf), methanolic (bark and leaf), and ethanolic (bark) extracts were only able to inhibit the growth of the organisms but did not exert a killing effect on the test organisms and this suggests that the extracts were bacteriostatic. Aqueous leaf extract inhibits the growth of the organisms and still exerts a killing effect (bactericidal) on *E. coli* 25922 at 1500 mg/mL concentration. However, the study still suggests a promising future for the extracts being used as phytotherapy against multidrug-resistant organisms with reference to their wide range of activity.

Conclusion

The findings of this study showed that the aqueous (bark and leaf), methanolic (bark and leaf), and ethanolic (bark) extracts of *P. africana* have great potential as a phyto-drug and as an inhibitory agent against the activities of the selected pathogenic micro-organisms. The results have substantiated the acceptance of the folkloric use of this plant leaf and bark in treating microbial infections. The phytochemical constituents of *P. africana* should be extensively studied to actualize the most active antimicrobial ingredients and their mechanism of action. This helps explain possible reasons for its narrow spectrum of activity, as observed in this study. Further studies should be carried out to obtain biological and pharmacological evaluations of these extracts to ascertain the toxicity attached to their use in folkloric and curative medicine.

Conflict of interest

The authors have no conflicts of interest to declare.

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