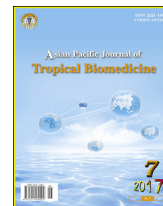




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Chemical constituents, *in vitro* antimicrobial and cytotoxic potentials of the extracts from *Macaranga barteri* Mull-Arg



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ABSTRACT

Objectives: To investigate antimicrobial and cytotoxic potentials as well as chemical constituents of extracts from *Macaranga barteri* (*M. barteri*).

Methods: Antimicrobial activity was carried out using micro-dilution, cell culture and GC–MS methods were employed to determine the cytotoxicity and chemical constituents of the extracts respectively.

Results: Marked activity was observed in methanol (ME) fraction [MIC₅₀: (0.0977–6.2500) mg/mL] compared to hexane and ethyl acetate fractions. *Aeromonas hydrophila* (environmental strain) and *Shigella sonnei* (ATCC 29930) were the most susceptible pathogens to ME and ciprofloxacin (CI) at MIC₅₀ value of 0.0977 and < 0.0195 mg/mL respectively. *Cryptococcus neoformans* (ATCC 66031) was susceptible to ME at 0.1953 mg/mL compared to fluconazole at 10.0000 µg/mL. Decreased viability of the Vero cells was observed at the concentrations of 0.1–1.0 mg/mL. The lethal dose (LC₅₀) of hexane, ethyl acetate and methanol fractions were recorded at (0.30 ± 0.07), (0.52 ± 0.05) and (0.22 ± 0.04) mg/mL, respectively. Some of the compounds identified from ME were caryophyllene (25.21%), neophytadiene (11.90%), α-humulene (7.67%), phytol (4.40%), ethyl ester hexadecanoic acid (4.04%) and nerolidol (2.83%) which were known to have various antimicrobial activities.

Conclusions: Methanol fraction of *M. barteri* is a potent and safe antimicrobial and antifungal alternative which can be useful in the search for new antimicrobial drugs. The study also confirmed the orthodox usage of *M. barteri* in combating infectious diseases.

1. Introduction

The emerging resistance to antibiotics by pathogenic organism which is on increase in Africa has led to the restriction of their efficacy in combating microbial infections. Communicable diseases in tropical countries are major source of illness and death, resulting in almost half of deaths in the populace [1]. *Staphylococcus aureus* (*S. aureus*), a Gram-positive and facultative anaerobic bacterium which invade their host via the mucous membrane is an opportunistic pathogen associated with wound infection and food poisoning. *Pseudomonas aeruginosa* is the pathogenic organism linked with infections related to

urinary tract while *Plesiomonas shigelloides* contaminations have been associated with gastroenteritis [2,3]. Fungi, particularly *Candida* sp. and *Cryptococcus* sp. were implicated as the key pathogens responsible in the patients that are critically ill. *Candida albicans* (*C. albicans*) infection is one of the opportunistic fungal infections when cluster of differentiation 4 (CD4) count is between 200 and 500/mm³ as an initial sign of immunodeficiency. These test organisms have been reported to have the capacity to build up resistance genes to antibiotics [4]. However, phytomedicine has displayed great potential in combating infectious diseases in Africa. In fact, medicinal plant materials serve as models for most Western drugs, and they are the richest bio-resource for food supplements, traditional and modern medicines, with herbal medications displaying promising results in the treatment of diseases including those of microbial lineage [5].

Macaranga barteri (*M. barteri*) Mull-Arg (Euphorbiaceae) is a plant that develops up to 20 m in height, and it is commonly

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found in different parts of West Africa (Sierra Leon, Liberia, Ivory Coast, Ghana and Equatorial Guinea) with significant presence in Nigeria [6]. It is locally called ‘aarasa’ and ‘owariwa’ among the Yoruba tribe from Nigeria while it is called ‘opam-kokoo’ by the Akan tribe from Ghana. The bark and leaf, either powdered or in decoction, are traditionally used in Nigeria as a vermifuge [7]. The plant is used as a worm expellant and medicine to reduce fever in the Democratic Republic of Congo [6]. Its mixture with other *Macaranga* spp. is used to relieve cough and bronchitis [6]. While its leaves are active in the management of gonorrhoea in Sierra Leone, it is routinely used in Ivory Coast as an aperient and anti-anaemic tonic [6]. The pharmacological significance of the leaf extract of *M. barteri* as antioxidant and antimicrobial agent have also been reported [7,8]. *M. barteri* is rich in phenolic and these have been identified as its major constituents implicated for its anti-inflammatory activity [9].

Although, attempts have been previously made to demonstrate the antimicrobial potential of *M. barteri* [8], however, the reports were not exhaustive against a wide collections (clinical, environmental and reference strains) of fungi, Gram-positive and Gram-negative bacteria. This has necessitated a more detailed and comprehensive work which is the goal of this study. Hence, this study assesses the antimicrobial potentials of different leaf extracts of *M. barteri*. It also elucidates the chemical constituents of the plant, probably responsible for the displayed activity. The cytotoxicity of the extracts was also examined to ascertain its safety in the management of microbial infections.

2. Materials and methods

2.1. Bacteria and fungi strains

The bacteria and fungi strains used were gotten from the collections of microbial stock in the Department of Plant Sciences, University of the Free State, (UFS) South Africa. The Gram-positive bacteria included *Bacillus pumilis* ATCC 14884, *S. aureus* OK2b, *Streptococcus faecalis*, *S. aureus* OK2a, *Listeria* and *S. aureus* ATCC 6538. Gram-negative bacteria were *Pseudomonas aeruginosa*, *Plesiomonas shigelloides*, *Aeromonas hydrophila* (*A. hydrophila*), *Shigella sonnei* (*S. sonnei*) ATCC 29930, *Salmonella typhi*, *Salmonella typhimurium*, *Escherichia coli* ATCC 8739, *Proteus vulgaris* CSIR0030, *Proteus vulgaris* ATCC 6830, *Enterobacter faecalis* KZN, *Acinetobacter calcoaceticus anitratus* CSIR, *Shigella flexneri* KZN and *Klebsiella pneumoniae* ATCC 13047. The fungi included *C. albicans*, *Trichophyton mucoides* and *Cryptococcus neoformans* (*C. neoformans*). Sub-culturing of bacteria and fungi cultures were done twice to ensure purity. The fungi and bacteria were inoculated in Sabouraud dextrose and nutrient broths respectively. The incubation was achieved at 37 and 27 °C for bacteria and fungi respectively between (24–72) h. The turbidity of the cultures was adjusted to 0.5McFarlands equivalent using sterile saline solution.

2.2. Cell culture

Normal African green monkey kidney epithelial (VERO) cell lines were obtained at the Department of Paraclinical Science cell line laboratories, University of Pretoria, South Africa. The cells were cultured in Dulbecco's Modified Eagle's Medium

(DMEM) complemented with 10% foetal bovine serum (FBS), 100 units/mL penicillin and 100 µg/mL streptomycin at 37 °C under a humidified atmosphere containing 5% CO₂.

2.3. Sample collection and authentication

M. barteri was selected for the study based on ethnobotanical information gathered from south-western part of Nigeria. Fresh leaves of *M. barteri* were collected in June 2015 along Eruwa-ido Road, Ibadan, Nigeria. Authentication of the plant was done by Mr. Soyewo of the Herbarium of Forestry Research Institute of Nigeria (FRIN). Voucher specimen FHI 110189 was dropped at the herbarium of the Institute.

2.4. Preparation of extract and fractions

The fresh leaves were collected and dried under shade for 2 weeks, the dried samples was then grounded to fine powder. The pulverised plant material (500 g) was subjected to cold extraction by percolation for a week using 2.0 L of 99% ethanol. The extract was filtered and concentrated to dryness using rotary evaporator at 35 °C to yield 226.34 g ethanol crude extract (45.27% w/w of dry plant material). The crude ethanol extract (226.34 g) was then macerated using a CoorTM porcelain mortar and pestle (Aldrich and sigma, Germany) with hexane (3 × 100 mL), ethyl acetate (3 × 100 mL) and methanol (3 × 100 mL) successively, to ensure the fractionation of the non polar, relatively polar and polar constituents respectively. Each of the resulting fractions was separately concentrated to dryness using rotary evaporator at 35 °C to give hexane (19.58 g) ethyl acetate (45.46 g) and methanol (63.95 g) fractions. The respective fraction was weighed and kept inside labelled sample bottle and stored in the refrigerator.

2.5. Antimicrobial assay

Micro-dilution technique was used to evaluate the MIC using 96-well plates according to the method of Bussmann *et al.* [10] and Sabiu and Ashafa [11] with modification. Different concentrations (0.0244–12.5000) mg/mL of the fractions were prepared using twofold serial dilutions in Mueller-Hinton broth (Merck, South Africa) and sabouraud dextrose broth (Lab M, UK) respectively for bacterial and fungi strains. Ciprofloxacin (Geltec, Bangalore, India) and fluconazole (Austell Laboratory, South Africa) were used as positive controls (0.0195–10.0000) µg/mL and 5% solvent of extraction as negative control. Each strain (50 µL) deposited in the wells was adjusted to be equal to 0.5 McFarland standards and incubated at 37 °C (fungi, 27 °C) for (24–72) h. Exactly, 40 µL 0.2 mg/mL of colourless tetrazolium salt (P-iodonitrotetrazolium) solution was pipetted into each well and further incubated for 30 min. The assay was performed in duplicate and concentration at which 50% (IC₅₀) of microbial growth was inhibited was determined.

2.6. Cytotoxicity assay

Cytotoxicity of the each fraction from *M. barteri* was tested against African Green Monkey kidney (VERO) cells using slightly modified procedure [12,13]. The cells were cultured in DMEM (Sigma, SA), added with L-glutamine (2 mM), NaHCO₃ (16.5 mM), 0.1% gentamicin (Virbac) and 5% foetal

calf serum (Sigma). Confluent monolayer culture suspensions of the cells at a density of 2×10^3 cells per well were seeded into 96-well tissue culture microtitre plates, followed by incubation for 24 h at 37 °C using 5% CO₂ incubator. The MEM was removed from the cells, after which fractions at different concentrations, doxorubicin and negative controls were added before incubating for 5 d. Inverted microscope was used to check for cytopathic effect of the fractions on the cells. Thirty µL (30 µL) of 5 mg/mL MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) in phosphate-buffered saline (PBS) was added to each well prior to incubation for another 4 h at 37 °C to quantify the cell proliferation and viability. The medium was cautiously taken out of wells without interfering with the MTT concentrate and washed twice with PBS. The liquid was removed from the cells and 50 µL of dimethyl sulphoxide (DMSO) was placed in each well to dissolve the crystallized MTT formazan. The absorbance of the reduced MTT was taken on microtitre plate reader (Versamax USA) at 570 nm. The results were calculated as a percentage growth of the control cells. The values at which 50% of the cells were inhibited (LC₅₀) were calculated.

2.7. Gas chromatography–mass spectrometric (GC–MS) analysis of *M. barteri* methanol fraction

The GC–MS analysis of the chemical constituents from methanol fraction of *M. barteri* was carried out on Agilent Technologies 6890 Series gas chromatograph together with an Agilent 5973 Mass Selective detector which is operated by

Agilent Chemstation software. The column was an agilent eHP-5MS capillary column (30.00 m × 0.25 mm internal diameter, 0.25 µm film thickness). Ultra-pure helium was used as the carrier gas at flow rate and linear velocity of 0.57 mL/min and a 27.5 cm/s, respectively. The injector temperature was fixed at 250 °C. The initial oven temperature (50.0 °C), was set to rise to 250 °C at the rate of 15 °C/min and hold time of 4 min at each increment. Injections of 1 µL were made in the splitless mode with a split ratio of 20:1. The mass spectrometer was run in the electron ionization mode at 70 eV and electron multiplier voltage at 1859 V. Other MS operating factors were set appropriately. Identification of the components was accomplished by matching the mass spectral data, retention times and fragmentation pattern of the unidentified components of the sample analysed with those from Wiley libraries and National Institute of Standards and Technology (NIST).

3. Results

3.1. Antibacterial and antifungal activity

The results of antibacterial and antifungal activity of *M. barteri* fractions are given in Table 1 and Table 2, respectively. The hexane (HE), ethyl acetate (EAE) and methanol (ME) fractions exhibited both bacterial and fungal growth inhibition potential between the range of 0.0244 to 12.5000 mg/mL. Marked activity was detected with ME at half maxima minimum inhibitory concentration (MIC₅₀) of 0.0977 to 6.2500 mg/mL followed by EAE (0.3906 to

Table 1

MIC range of the bacteria at 50%.

Strains of bacteria	Gram+/-	Reference number	Source	HE*	EAE	ME	CI
<i>Staphylococcus aureus</i>	+	ATCC 6538	Human lesion	6.2500	3.1250	1.5625	0.1560
<i>Staphylococcus aureus</i>	+	OK2a	wound sepsis	0	6.2500	6.2500	0.1560
<i>Staphylococcus aureus</i>	+	OK2b	wound sepsis	6.2500	6.2500	0.7813	0.1560
<i>Bacillus pumilus</i>	+	ATCC 14884	–	0	0	3.1250	2.5000
<i>Streptococcus faecalis</i>	+	–	ES*	3.1250	0.7813	0.3906	0.0390
<i>Listeria</i> sp.	+	–	ES	0	6.2500	3.1250	1.2500
<i>Pseudomonas aeruginosa</i>	–	–	ES	6.2500	0	1.5625	0.3130
<i>Plesiomonas shigelloides</i>	–	–	ES	0	0	6.2500	0.6250
<i>Aeromonas hydrophila</i>	–	–	ES	0.7813	0.3906	0.0977	<0.0195
<i>Shigella sonnei</i>	–	ATCC 29930	–	0.7813	0.3906	0.0977	<0.0195
<i>Salmonella typhi</i>	–	–	ES	12.5000	6.2500	3.1250	0.2500
<i>Salmonella typhimurium</i>	–	–	ES	0	6.2500	6.2500	0.0780
<i>Escherichia coli</i>	–	ATCC 8739	Faeces	12.5000	6.2500	1.5625	0.3130
<i>Proteus vulgaris</i>	–	CSIR0030	–	0	12.5000	3.1250	5.0000
<i>Proteus vulgaris</i>	–	ATCC 6380	–	12.5000	6.2500	3.1250	1.2500
<i>Enterobacter faecalis</i>	–	KZN	–	12.5000	6.2500	3.1250	5.0000
<i>Acinetobacter calcaoceticus anitratus</i>	–	CSIR	–	0	0	0	5.0000
<i>Shigella flexneri</i>	–	KZN	–	0	0	0	5.0000
<i>Klebsiella pneumoniae</i>	–	ATCC 13047	–	12.5000	6.2500	3.1250	5.0000
<i>Klebsiella pneumoniae</i>	–	–	ES	0	6.2500	3.1250	5.0000

*HE, Hexane extract; EAE, Ethyl acetate extract; ME, Methanol extract; CI, Ciprofloxacin; ES, Environmental strain; MIC₅₀ (mg/mL).

Table 2

MIC range of the fungi at 50%.

Strains of fungi	Reference number	Source	HE*	EAE	ME	Flu
<i>Cryptococcus neoformans</i>	ATCC 66031	–	0.7813	0.3906	0.1953	10.0000
<i>Trichophyton mucoides</i>	ATCC 201382	–	3.1250	3.1250	0	>10.0000
<i>Candida albicans</i>	ATCC 2091	–	6.2500	0	6.2500	0.6250

*HE, Hexane extract; EAE, Ethyl acetate extract; ME, Methanol extract; Flu, Fluconazole; MIC₅₀ (mg/mL).

6.2500 mg/mL) and HE (0.7813 to 12.5000 mg/mL). *A. hydrophila* (ES) and *S. sonnei* (ATCC 29930) were the most susceptible pathogens tested with the extracts and antibiotics (positive control) with ME and ciprofloxacin (CI) having MIC₅₀ value of 0.0977 and <0.0195 mg/mL respectively. *C. neoformans* (ATCC 66031) was susceptible to ME, EAE and HE at respective MIC₅₀ value of 0.1953, 0.3906 and 0.7813 mg/mL compared to fluconazole (10.0000 µg/mL). Meanwhile fluconazole (Flu) was more active against *C. albicans* (ATCC 2091) at 0.625 µg/mL MIC₅₀ value. *Acinetobacter calcoaceticus anitratus* (CSIR) and *Shigella flexneri* (KZN) were resistant to all the tested fractions (HE, EAE and

ME). Inhibitory efficacy of leaf ME was more distinct while that of EAE was moderate.

3.2. Cytotoxicity

The hexane, ethyl acetate and methanol fractions from *M. barteri* exhibited dose dependent effects on VERO cell lines viability. Cytotoxicity was not induced by the fractions at the lowest concentration (0.05 mg/mL). However, there was decrease in the viability of the cells by the fractions at higher concentrations (0.1–1.0 mg/mL). The LC₅₀ (mean ± SD) of the hexane, ethyl acetate and methanol fractions from *M. barteri*

Table 3

Compounds identified from the methanol fraction of *M. barteri*.

S/N	Constituents	Retention time (Min)	Area %	Molecular formula	Molecular weight (g/mol)
1	Benzamide*	8.41	0.29	C ₃₃ H ₂₈ NO ₈	636
2	2,2-Bis(4-nitrobenzyl)-1-phenylbutane-1,3-dione	8.47	0.19	C ₂₄ H ₂₀ N ₂ O ₆	432
3	2-Acetophenone	8.56	0.31	C ₁₁ H ₁₂ O ₂	176
4	2,2-Bis(4-nitrobenzyl)-1-phenylbutane-1,3-dione	8.74	0.21	C ₂₄ H ₂₀ N ₂ O ₆	432
5	1,2,3-Trimethylbenzene	9.04	2.94	C ₉ H ₁₂	120
6	1,2-Diacetoxyp propane	9.48	0.76	C ₇ H ₁₂ O ₄	160
7	3-Hydroxy-1-phenyl-4-hexen-1-one	9.55	0.33	C ₁₂ H ₁₄ O ₂	190
8	Methyl vinylcarbinol	10.83	0.20	C ₄ H ₈ O	72
9	2-Isobutyl-3-methylfuran	11.77	0.18	C ₉ H ₁₂ O	136
10	Acetyltetrahydrofuran	12.27	0.06	C ₆ H ₁₀ O ₂	114
11	Isopropenyl dodecanoate	12.44	0.24	C ₁₅ H ₂₈ O ₂	240
12	2-hydroxy-2-methyl Propanoic acid	12.47	0.16	C ₅ H ₁₀ O ₃	118
13	Undecane*	15.31	1.62	C ₁₁ H ₂₄	156
14	Caryophyllene*	15.80	25.21	C ₁₅ H ₂₄	204
15	aromadendrene	16.04	0.73	C ₁₅ H ₂₄	204
16	α-Humulene	16.27	7.67	C ₁₅ H ₂₄	204
17	3-Hydroxy-4(3H)-quinolinone	16.46	0.07	C ₉ H ₇ NO ₂	161
18	Caryophylla-2(12),6-dien-5-one	16.70	0.92	C ₁₅ H ₂₂ O	218
19	Germacrene B	16.77	1.93	C ₁₅ H ₂₄	204
20	Germacrene D	16.99	0.64	C ₁₅ H ₂₄	204
21	Nerolidol*	17.43	2.83	C ₁₅ H ₂₆ O	222
22	Spathulenol	17.80	1.45	C ₁₅ H ₂₄ O	220
23	Tetradecane*	17.84	2.31	C ₁₄ H ₃₀	198
24	β-Elementene	17.90	0.41	C ₁₅ H ₂₄	204
25	N-Acetylbenzamide	18.22	0.06	C ₉ H ₉ NO ₂	163
26	α-Linalool-2-penten-4-one	18.49	2.51	C ₁₀ H ₁₈ O	154
27	Caryophyllene oxide	18.94	1.48	C ₁₅ H ₂₄ O	220
28	N-Phenylacrylamide	19.03	0.09	C ₉ H ₉ NO	147
29	2,12-Epoxycaryophyll-5-ene	19.63	1.12	C ₁₅ H ₂₄ O	220
30	4-Methyl-1,3-oxathiolane	19.75	0.24	C ₄ H ₈ OS	104
31	Methylethynylcarbinol	20.04	0.14	C ₄ H ₆ O	70
32	n-Octadecane	20.11	1.55	C ₁₈ H ₃₈	254
33	Neophytadiene*	20.51	11.90	C ₂₀ H ₃₈	278
34	Citronellyl propionate	20.76	2.41	C ₁₃ H ₂₄ O ₂	212
35	trans-Phytol*	20.96	4.40	C ₂₀ H ₄₀ O	296
36	4-Methylpentanoic acid	21.42	0.07	C ₆ H ₁₂ O ₂	116
37	n-Hexadecanoic acid*	21.75	2.10	C ₁₆ H ₃₂ O ₂	256
38	2-Hexyl-2-decenal	22.02	0.07	C ₁₆ H ₃₀ O	238
39	Ethyl ester hexadecanoic acid*	22.09	4.04	C ₁₈ H ₃₆ O ₂	284
40	n-Docosane	22.16	0.64	C ₂₂ H ₄₆	310
41	Citronellyl butyrate	23.29	2.63	C ₁₄ H ₂₆ O ₂	226
42	Cyclodecene	23.45	0.85	C ₁₀ H ₁₈	138
43	2,5,8-Heptadecatrien-1-ol	23.51	1.66	C ₁₇ H ₃₀ O	250
44	Dodecanedialdehyde	23.75	0.48	C ₁₂ H ₂₂ O ₂	198
45	9,12,15-Octadecatrien-1-ol	23.82	3.71	C ₁₈ H ₃₂ O	264
46	Ethyl Hept-6-enoate	24.08	0.21	C ₉ H ₁₆ O ₂	156
47	2-Keto-butryic-acid	24.15	0.10	C ₄ H ₆ O ₃	102
48	Pentadecanal	24.31	5.22	C ₁₅ H ₃₀ O	226
49	1-Aza-4-thiapentane	27.81	0.14	C ₃ H ₉ NS	91
50	2-Ethoxycarbonyloxy-1-phenyl-2-nonene	29.92	0.53	C ₁₈ H ₂₆ O ₃	290
		Total	100.00		

*Compounds reported to have antimicrobial potential.

against Vero cells were (0.30 ± 0.07) , (0.52 ± 0.05) and (0.22 ± 0.04) mg/mL, respectively.

3.3. Analysis of the GC–MS chromatogram of methanol fraction from *M. barteri*

Fifty compounds were identified from the GC–MS analysis of the methanol fraction of *M. barteri*, representing 100% of the total constituents (Table 3). The major constituents were caryophyllene (25.21%), neophytadiene (11.90%), α -humulene (7.67%) and pentadecanal (5.22%). Compounds present in a significant quantity include phytol (4.40%), ethyl ester hexadecanoic acid (4.04%), 9,12,15-Octadecatrien-1-ol (3.71%), nerolidol (2.83%), Citronellyl butyrate (2.63%), α -Linalool-2-penten-4-one (2.51%), Citronellyl propionate (2.41%), Tetradecane (2.31%), *n*-Hexadecanoic acid (2.10%), 2,5,8-heptadecatrien-1-ol (1.68%), Undecane (1.66%), Germacrene B (1.93%), *n*-Octadecane (1.55%), Caryophyllene oxide (1.48%), Spathulenol (1.45%) and 2,12-epoxycaryop-hyll-5-ene (1.12%). Other compounds were present in a traceable amount (< 1%).

4. Discussion

Natural products especially from plant source have been in use for centuries in traditional medicine for variety of purposes and some have demonstrated their antimicrobial activity against selected pathogens. The rise in multi-drugs resistant strains of human pathogenic organisms spurs the need to explore new antimicrobial agents from relatively affordable and readily available local alternative sources. In numerous instances, phytochemicals have proven to be more active than synthetic chemical compounds [14]. This is as a result of complex combination of constituents which facilitates their interaction with several molecular targets. Meanwhile isolation of pure, pharmacologically active constituents still remains an essential process in new drug formulation [14].

Activity observed in this study gave a clear indication of the importance and potentials of medicinal plants. Marked antibacterial activity of the extracts (HE, EAE and ME) was observed against *A. hydrophila* and *S. sonnei*. *A. hydrophila* is implicated in gastroenteritis, which occurs mostly in young children and immuno-compromised individuals. *A. hydrophila* is also associated with cellulitis, myonecrosis, eczema and necrotizing fasciitis [15,16]. Similarly, *S. sonnei* mostly affects the vulnerable ones such as, infants and toddlers, the elderly, travellers, and individuals with acquired immune deficiency syndrome (AIDS) with increasing multidrug resistance rates [17]. *C. neoformans* which was also noted to be susceptible to the fractions in this study is an opportunistic fungus that causes infections mostly in the lungs. It can also result in meningitis and encephalitis as a secondary infection for AIDS patients [18]. The hexane, ethyl acetate and methanol fractions from *M. barteri* were non-toxic against Vero cells, with respective LC₅₀ value of (0.30 ± 0.07) , (0.52 ± 0.05) and (0.22 ± 0.04) mg/mL. Plant extracts are considered toxic when the LC₅₀ is ≤ 20 μ g/mL [19]. This implies the safety in the usage of the extract of *M. barteri*.

Marked activity observed in methanol fraction compared to others in the current study was in conformity with the earlier report by Natarajan *et al.* [20]. Methanolic extracts of many plants have been stated to exhibit promising antimicrobial

activity compared to other solvent extractants. The reason being that, methanol, a polar solvent, extracts variety of important phytochemicals, such as phenols, tannins, proanthocyanidins, alkaloids, garlic acid, flavonols and fatty acids, which harbour antimicrobial potentials [21,20] that is in line with the current findings. The GC–MS analysis of the methanol fraction of *M. barteri* was carried out because of the marked activity observed compared to other fractions. Some of identified compounds from methanol fraction of *M. barteri* as reflected by its GC–MS analysis were previously reported to have antimicrobial activities. Caryophyllene (25.21%) and neophytadiene (11.90%) which are the two major constituents of the methanol fraction were shown to have antimicrobial [22,23] and antibacterial [24] activities. Other compounds with reported antimicrobial activities, present in the methanol fraction of *M. barteri* has revealed by its GC–MS analysis are benzamide, undecane, tetradecane, phytol, hexadecanoic acid and ethyl ester hexadecanoic acid [25–27]. The findings of Chan *et al.* [28] indicate the antimicrobial and antifungi capacities of nerolidol which was one of the significant constituents (2.83%) of *M. barteri* methanol fraction. The presence of these identified bioactive chemical constituents of diverse pharmacological potentials in methanol fraction of *M. barteri* might be accountable for its antimicrobial potential as evidently shown in this study.

The present study showed that *M. barteri* demonstrated promising antibacterial and antifungi activities. The usage is also considered relatively safe and non-toxic within the tested doses. These findings validate the traditional usage of the plant in combating of microbial related infections.

Conflict of interest statement

We declare that there is no conflict of interest.

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