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ARTICLE

Nauclea latifolia Stem Extracts: Antimicrobial Activity and Gas Chromatography-Mass Spectrometry Analysis

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Abstract: *Nauclea latifolia* Sm. Rubiaceae is a native Southeast Nigerian tree known for its medicinal characteristics and is commonly found in various regions of Africa. *N. latifolia* is known to possess broad-spectrum medicinal bioactivities. In this assay the pulverized stem was extracted using methanol and chloroform. The extracts were analyzed using Gas Chromatography-Mass Spectrometry (GC-MS). The *in vitro* antimicrobial analysis was performed based on Broth dilution method using arrays of fungi and bacteria. A total of 10 and 12 compounds were isolated from the methanol and chloroform extracts, respectively, including docosanoic acid, arachidic acid, stearic acid, palmitic acid, oleic acid, and squalene. The methanolic extract showed activity against *S. typhi* with MBC/MIC ratio of 1.0 while the chloroform extract showed activity against *S. mutans* also with MBC/MIC ratio of 1.0. All other MBC/MIC ratios ranged from 2–8 while those of the fungi was from 1.0 to 4.0 for both extracts. Our results were comparable to those of the standard drugs. Therefore, *N. latifolia* stem extracts contained phytochemicals of relevance in ethnomedicine.

Keywords: *Nauclea latifolia*; GCMS; Bactericidal; Squalene; Ethnomedicine.

Introduction

Medicinal therapeutics of plant origin are important due to the presence of medicinal phytochemicals in plant extracts. Phytochemicals are plant-based compounds that give plants their physical characteristics and medicinal applications[1], some are employed in defensive mechanisms[2]. *Nauclea latifolia* Sm. (Family: Rubiaceae, Local name: Uvuru inu) is a medicinal plant quite abundant in Southeast Nigeria and several regions of Africa. It is an evergreen spreading shrub with multiple stems - bears flowers and possesses a large red ball-like fruit with projecting stamens[3-6]. The roots and stems are employed by Nigerian locals for the treatment of different ailments[3-8]. It contains numerous pharmaceutical phytochemicals such as alkaloids, terpenoids, phenols, steroids, and saponins[9-12]. *N. latifolia* has been reported to possess nutritional benefits[13]. World Health Organization (WHO) hypothesize that 80% of the world population depends on herbal remedies as a primary source of medicine. Millions of ethnic Africans rely on these herbal remedies for pharmaceuticals and chemotherapeutics[14].

Nigerian communities make use of different parts of *N. latifolia* for the treatment of diabetes, jaundice, malaria, fever, dysentery, diarrhea and hypertension. Its antibacterial, antiplasmodial and antidiabetic activities have been revealed by various biological screening research[6,12]. *N. latifolia* is reported to contain plant-based secondary metabolites such as: triterpenes, indole alkaloids, saponins, and steroids[6,12]. *N. latifolia* root decoction is a herbal remedy prepared using different solvents and has long been used traditionally for treating and managing different diseases and health conditions. Its medicinal uses vary from one traditional setting

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to another, including: fever, septic mouth, pain, dental caries, dysentery, malaria, diarrhea, and various diseases of the central nervous system such as epilepsy, Alzheimer's and sclerosis etc.[3]. The minimal side effect of medicinal plants favor their preference among local natives in Nigeria. Therefore, it is scientifically important to explore medicinal plants for their therapeutic benefits.

Phytochemicals in *N. latifolia* ethanolic leaf extract exhibit synergy in bioactivity[1]. These phytochemicals prevent diseases and generally promotes health status of individuals. Plant secondary metabolites are free radical scavengers, inhibits carcinogen-activating enzymes and increases the synthesis and activity of carcinogen detoxifying enzymes[1]. Various compounds have been isolated from *N. latifolia* such as caffeic acid, quercetin, isoquercitrin, chlorogenic acid, 3,5-*O*-dicaffeoylquinic acid, nauclefolinine, nauclefine, and naucletine etc.[12].

The GC-MS technique is an analytical protocol employed in the identification of stable or volatile low molecular-weight compounds. It is a fast technique, reproducible, specific, and easy to use. It is widely favored in the identification of trace amounts of metabolites. Also, it is less expensive, readily available, and mostly employed in metabolite elucidations, evaluations and quantifications.

The novelty of this research stems from the variation in phytochemical composition of plants due to geographical location necessitated by factors such as type of soil, light intensity, precipitation, humidity etc. hence, we report for the first time the phytochemicals isolated from *N. latifolia* sourced from an agrarian community that is reliant on ethnomedicine. Therefore, the objectives of this study were to determine the secondary metabolites found in *N. latifolia* stem that are responsible for its pharmaceutical application in ethnomedicine and to correlate its medicinal benefits to the presence of phytochemicals identified using GCMS. Finally, the study aim to determine its antimicrobial activity using the broth dilution method.

Materials and methods

Chemicals

All chemicals used were analytical grade and HPLC standard. Muller Hilton broth (MHB) (Becton Dickson, USA). Clotrimazole and Neomycin were purchased from Sigma-Aldrich, Germany

Plant collection

The research was undertaken partly in the laboratories of the Department of Industrial Chemistry, Ebonyi State University, Abakaliki and the National Research Institute for Chemical Technology (NARICT), Zaria under the supervision of the Department of Pure and Industrial Chemistry, Nnamdi Azikiwe University, Awka. The plant stem (Figure 1) was collected in November, 2021. The stems were collected from some of their natural habitats in Nnodo located at Ebonyi Local Government Area, Ebonyi State. The Plant was identified and authenticated as *N. latifolia* by a taxonomist (Prof. S.C. Eze) at the Department of Applied Biology Ebonyi State University, Abakaliki and was labeled as ESU/FCAB/0034. The stems were washed thoroughly with tap water to remove sand and dust particles, and then rewashed using distilled water. The stems were dried and cut into smaller pieces followed by sun drying for 72 hours then, pulverized in a local mortar into a fine powder and stored in a glass jar.

Figure 1. *Nauclea latifolia* plant

Preparation of Plant Extracts

Constituents of the pulverized stems were extracted by soaking 100 g in methanol and chloroform separately for 72 hours. The solutions were filtered through a Whatman filter paper No. 42. The filtrate was evaporated to 1/5 of its initial volume under vacuum using a rotary

evaporator (Ohaus, USA) and re-filtered. Extracts were dried and kept in sterile glass vials and placed in the refrigerator until further use.

Gas chromatography-mass spectroscopy analysis

The GCMS spectra of the extracts were obtained using the GC-MS-QP2010 PLUS Series at NARICT to determine the structure of phytochemicals present in the extracts (Supplementary files)

Bacterial and fungi strain

The clinical isolates of bacteria and fungi were obtained from the University of Nigeria Teaching Hospital. All strains of bacteria and fungi were isolated from clinical specimens of hospitalized patients identified based on the Center for Disease Control and Safety Network (CDC/NHSN) criteria Mogana et al.[15]. For the experiment three different microbial culture (NCTC – National Collection of Type Cultures) reference and ten clinical isolates were used

Reference Strains: *Escherichia coli* (NCTC 10418), *Staphylococcus aureus* (NCTC 6571), *Proteus vulgaris* (NCTC 4175)

Clinical isolates: *Bacillus sp., Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumonia, Staphylococcus albus, Enterobacter aerogenes,* and *Streptococcus mutans* while the fungi were *Aspergillus flavus, Aspergillus niger, Candida albicans*

Method for Antimicrobial analysis

Minimum Inhibitory Concentration (MIC) The MIC was based on the microtiter broth dilution method published by Mogana et al.[15] in accordance with the guideline of the Clinical and Laboratory Standards Institute (CLSI). Correspondingly, in 2.0 mL microcentrifuge tubes (Eppendorf), extracts stock solutions were made via dissolution of dry plant extracts in methanol (MeOH) to a final concentration of 128 mg/mL. The stock solution was serially diluted from 64 mg/mL to 0.25 mg/mL in 96-well microplates with MHB and 100 µL of this suspension was included in each well. Each strain was also subjected to sterility and growth control tests. For bacteria, incubation of microtiter plates was done at 37°C for 24 hours while for fungi it was for 48 hours at same incubation temperature. To monitor microbial growth, 40 µL solution of 0.4 mg/mL *p*-iodonitrotetrazolium was included in each well after incubation at 37°C. Subsequently, microplate incubation was carried out at 37°C for 30 minutes and 24 hours for bacteria and fungi respectively, with MIC values measured visually. The MIC was determined by taking the least concentration of each extract that didn't show observable growth. The MIC value was chosen as the concentration that entirely stopped bacterial/fungal growth i.e. the first clear well. The experiments were repeated to validate activity. The sensitivity of microorganisms were evaluated via positive control studies. For bacterial strains, Neomycin was used at an initial concentration of 0.10 mg/mL in sterile water, and for fungus strains, Clotrimazole at 0.10mg/mL initial concentration in MeOH was used i.e. 1.00 mg/mL was made in MeOH, and subsequent dilution was done in sterile water to 0.10mg/mL. These experiments final concentrations was from 25.00–0.19 µg/mL. MeOH was used as negative control.

Minimum bactericidal concentration (MBC) The MBC was estimated using the microtiter broth dilution method published by Mogana et al.[15]. After 24 hours incubation at 37°C the lowest extract concentration responsible for the death of 99.9% of the bacterial inocula was considered as the MBC. From the well gotten from the MIC experiment, 10 µL were taken followed by two wells above the MIC well's values and spread on MHA plates. The colonies were incubated at 37°C and enumerated after 18–24 hours. Sample concentration values producing < 10 colonies was taken as the MBC. Each experiment was done in triplicate.

Results and Discussion

Results of GCMS of *N. latifolia* revealed the presence of 10 and 12 peaks for the methanol and chloroform extracts (Figure 2), respectively. Constituents of each peak were reported in Table 1 (methanol extract) and Table 2 (chloroform extract) (supplementary file). To identify the pharmacological relevance of identified compounds, each compound was matched with known pharmacological references.

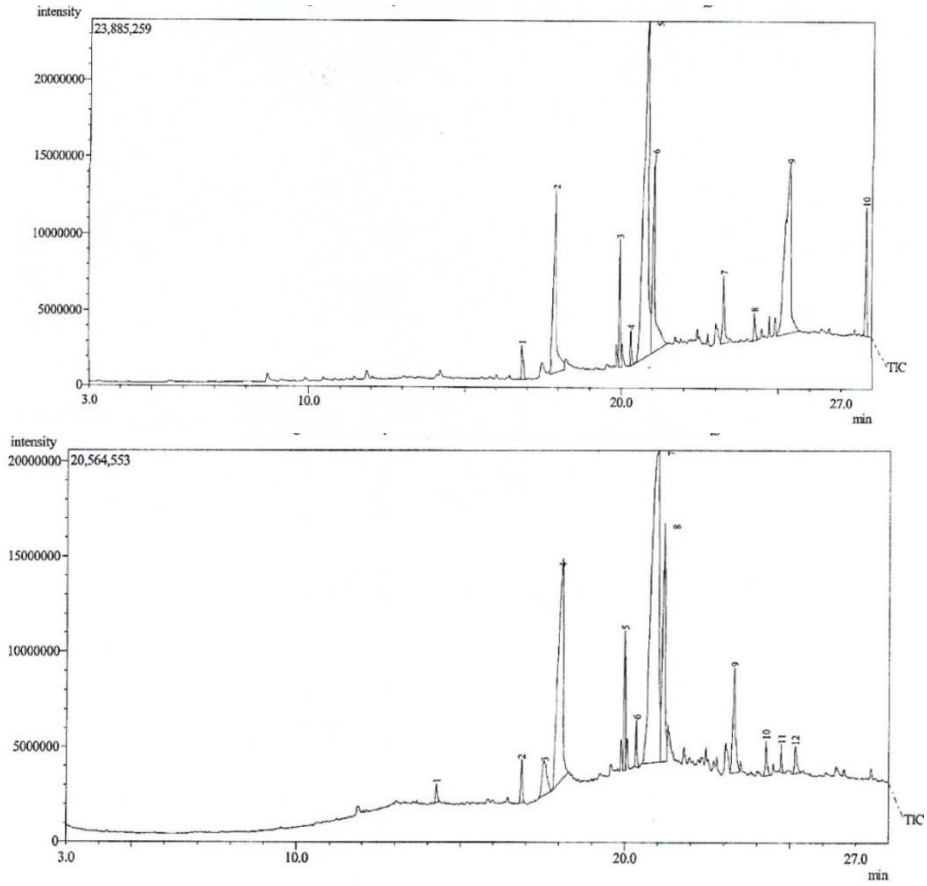

Figure 2. GCMS spectra of methanol (A) and chloroform (B) stem extracts of *N. latifolia*

Table 1. Compounds identified in the stem methanol extract of *N. latifolia* using GC-MS analysis

Peak Number	Retention Index	Peak Area (%)	Compound	Pharmacology	Literature
1	1814	1.29	Methyl 14-methyl pentadecanoate	Antibacterial, antioxidant	[16-18]
2	1968	14.01	Palmitic acid	Antibacterial	[19]
3	2085	3.67	Methyl (13E)-13-octadecenoate	Antisickling	[20]
4	2077	0.95	Stearic acid methyl ester	Antifungal, antioxidant	[21]
5	2175	37.07	Oleic acid	Suppresses triacylglycerol absorption and prevents obesity, antioxidant, mosquitocidal, toxicity	[22-24]
6	2167	11.65	Stearic acid	Anticardiovascular and anticancer	[25]
7	1968	2.98	Arachidic acid	Antioxidant	[26]
8	2007	0.98	9-octadecenal	Antimicrobial	[27]
9	2545	23.22	Cholesterol methyl ether	Antiatherogenic	[28]
10	2917	4.19	Squalene	Enhance antimicrobial activity, antiobesity/antidiabetic, anticancer, skin protective agent	[29-31]

Table 1 displayed Ten (10) compounds that were isolated and identified from the methanol extracts of *N. latifolia*. Methyl 14-methyl pentadecanoate was one of the compounds identified from *Sarcophyton flexuosum* that showed potential DPPH scavenging

properties[18]. A study on the antimicrobial and antioxidant activities in the methanol and chloroform extracts of six important medicinal plants sourced from North Iran also, identified methyl 14-methyl pentadecanoate as a potential antioxidant phytochemical[17]. Similarly, Khalil *et al*.[16] showed the antibacterial activity of methyl 14-methyl pentadecanoate against neonate sepsis induced by *K. pneumonia.* The inhibitory activity of *N. latifolia* against *K. pneumonia* was reported in Table 3.

Free fatty acids such as palmitic, oleic, myristic and stearic acids are known to possess antibacterial activities including methicillin-resistant Staphylococcus aureus (MRSA) activity and their activities are relatives to the length of their chains[19]. Palmitic acid was found to be active against *S. aerus*, *E. coli*, *Streptococcus*, *Pneumococci*, *Bacillus* sp. variants used in the study[19] which supports the antibacterial activity of *N. latifolia*. Dietary stearic acid has been found to regulate mitochondria *in vivo* and thus can decrease cardiovascular and cancer risk in humans[25].

Chikezie *et al*.[20] identified methyl (13E)-13-octadecenoate as a constituent of *Anarcardium occidentale, Psidium guajava* and *Terminalia catappa* that altered the membrane stability of sickle erythrocyte, hence the presence of methyl (13E)-13-octadecenoate in *N. latifolia* stem suggested antisickling characteristics which justify the use of *N. latifolia* in the management of sickle cell disease. Pinto *et al*.[21] showed the antifungal and antioxidant activity of stearic acid methyl ester suggesting the use of *N. latifolia* as antifungal agent and in the management of oxidative stress-related conditions such as Alzheimer, sickle cell, cancer, parkinson disease etc.

Table 2. Compounds identified in the stem chloroform extract of *N. latifolia* using GC-MS analysis

Peak Number	Retention Index	Peak Area (%)	Compound	Pharmacology	Literature
1	1769	0.62	Myristic acid	Enhance antimicrobial activity	[32]
2	1878	1.38	Methyl hexadecanoate	Anti-inflammatory, antioxidant, anti-apoptotic	[33, 34]
3	1976	3.23	9-Hexadecenoic acid	Anti-inflammatory, anticancer	[35]
4	1968	22.15	Palmitic acid	Antibacterial	[19]
5	2085	3.76	Methyl petroselinate	Antioxidant, α-glucose inhibitor	[36]
6	2077	1.20	Methyl octadecenoate	Co-nutritional	[37]
7	2175	47.49	Oleic acid	Suppresses triacylglycerol absorption, antiobesity, antioxidant, mosquitocidal, toxicity	[22-24]
8	2167	11.83	Stearic acid	Anticardiovascular, anticancer	[25]
9	2366	5.30	Arachidic acid	Antioxidant	[26]
10	2007	1.16	9-octadecenal	Antimicrobial	[27]
11	2475	0.65	Methyl behenate	Antihypoglycemic	[38]
12	2564	1.24	Docosanoic acid	Suppresses triacylglycerol absorption, antiobesity	[24]

Table 2 indicated the presence of twelve compounds in the chloroform extract of *N. latifolia* (Fig. 1). Methyl behenate was characterized in the methanolic extract of *Ocium basilium* showing hyperglycemic effect in rats[38] which may explain the use of *N. latifolia* in controlling high blood sugar. Squalene enhances the activity of astaxanthin towards *in vivo* oxidative stress and control obesity, and diabetes. It's a cancer chemoprotective and skin protective agent[29-31]. Methyl petroselinate was among compounds of *Cydonia oblonga* fruit that showed antioxidant and α-glucosidase inhibitory

activity[36] supporting earlier claims on the use of *N. latifolia* in diabetes treatment.

Oleic acid and arachidic acid have been found to show radical scavenging activity i.e. antioxidant potentials[22, 26] while, 9-hexedecenoic acid, a trans fatty acid, suppresses TNF-α-induced inflammatory gene expression in endothelial and hepatocellular carcinoma[35] thus, extracts of *N. latifolia* may recover liver cancer.

Similarly, enhanced antimicrobial activity of nonadecanoic acid has been shown by Ding et al.[32] and Oluwasina et al.[27] who reported that 9-octadecenal was among phyto-constituents that showed antimicrobial potential in toothpaste formulated from extracts of *Syzygium aromaticum*, *Dennettia tripetala* and *Jatropha curcas* latex. These studies can explain the use of *N. latifolia* stem as chewing stick due to its activity against oral pathogens. The atherogenic effect of cholesterol methyl ether has been studied[28] thus, *N. latifolia* methanol stem extracts may mediate atherosclerosis.

Eicosapentaenoic and docosahexaenoic acids have been profiled for their nutritional benefits, and methyl octadecenoate was identified as a co-nutritional in fish oil nutritional capsules[37]. Yakubu et al.[39] identified various compound entirely different from those reported herein however, oleic acid was found to be common to both their work and those reported herein. In particular, myristic and Palmitoleic acids isolated from *N. latifolia* stem showed appreciable *in vitro* antimicrobial activity[10] supporting the observations herein.

Methyl hexadecanoate showed putative anti-inflammatory, antioxidant, and anti-apoptotic roles against isoproterenol-induced myocardial injury in rats[33] and modulates cyclophosphamide-induced cardiotoxicity[34] thus supporting the anti-apoptotic activity of *N. latifolia* stem extracts.

Therefore, the presence of these phytochemicals in both the methanol and chloroform extracts of *N. latifolia* reveals its richness of pharmaceuticals of medicinal relevance.

Table 3. Minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), minimum fungicidal concentration (MFC), MBC/MIC, and MFC/MIC ratios of methanol and chloroform stem extracts of *Nauclea latifolia* (mg/mL), negative control and control drugs against test bacteria and fungi organisms

Bacteria/Fungi	Plant extracts and control drugs					
	Parameters determined	Methanol	Methanol extract	Chloroform extract	Neomycin	Clotrimazole
E. coli NCTC 10418	MBC (mg/mL)	-	4.0 ± 0.01	4.0 ± 0.00	1.0 ± 0.00	NA
	MIC (mg/mL)	-	2.0 ± 0.00	0.5 ± 0.00	0.5 ± 0.00	NA
	MBC/MIC	-	2.0 ± 0.00 (+)	8.0 ± 0.00 (-)	2.0 ± 0.00 (+)	NA
S. aureus NCTC 6571	MBC (mg/mL)	-	8.0 ± 0.00	8.0 ± 0.02	2.0 ± 0.00	NA
	MIC (mg/mL)	-	2.0 ± 0.00	4.0 ± 0.00	0.5 ± 0.01	NA
	MBC/MIC	-	4.0 ± 0.00 (+)	2.0 ± 0.00 (+)	4.0 ± 0.00 (+)	NA
Proteus vulgaris (NCTC 4175)	MBC (mg/mL)	-	16.0 ± 0.00	8.0 ± 0.03	1.0 ± 0.00	NA
	MIC (mg/mL)	-	8.0 ± 0.00	2.0 ± 0.01	0.25 ± 0.00	NA
	MBC/MIC	-	2.0 ± 0.00 (+)	4.0 ± 0.00 (+)	4.0 ± 0.00 (+)	NA
Bacillus Sp. LCI	MBC (mg/mL)	-	4.0 ± 0.00	16.0 ± 0.00	2.0 ± 0.00	NA
	MIC (mg/mL)	-	2.0 ± 0.00	4.0 ± 0.00	1.0 ± 0.00	NA
	MBC/MIC	-	2.0 ± 0.00 (+)	4.0 ± 0.00 (+)	2.0 ± 0.00 (+)	NA
Salmonella typhi LCI	MBC (mg/mL)	-	4.0 ± 0.00	4.0 ± 0.00	2.0 ± 0.00	NA
	MIC (mg/mL)	-	4.0 ± 0.00	2.0 ± 0.00	1.0 ± 0.00	NA
	MBC/MIC	-	1.0 ± 0.00 (+)	2.0 ± 0.00 (+)	2.0 ± 0.00 (+)	NA

Bacteria/Fungi	Parameters determined	Plant extracts and control drugs				
		Methanol	Methanol extract	Chloroform extract	Neomycin	Clotrimazole
Pseudomonas aeruginosa LCI	MBC (mg/mL)	-	4.0 ± 0.01	16.0 ± 0.00	1.0 ± 0.02	NA
	MIC (mg/mL)	-	1.0 ± 0.00	8.0 ± 0.01	1.0 ± 0.01	NA
	MBC/MIC	-	4.0 ± 0.00 (+)	2.0±0.00 (+)	1.0 ± 0.00 (+)	NA
Klebsiella pneumonia LCI	MBC (mg/mL)	-	2.0 ± 0.00	4.0 ± 0.00	1.0 ± 0.00	NA
	MIC (mg/mL)	-	0.5 ± 0.00	2.0±0.00	0.5 ± 0.00	NA
	MBC/MIC	-	4.0 ± 0.00 (+)	2.0 ± 0.00 (+)	2.0 ± 0.00 (+)	NA
Staphylococcus albus LCI	MBC (mg/mL)	-	16.0 ± 0.01	32.0 ± 0.01	1.0 ± 0.00	NA
	MIC (mg/mL)	-	4.0 ± 0.00	16.0 ± 0.00	0.25 ± 0.03	NA
	MBC/MIC	-	4.0 ± 0.00 (+)	2.0 ± 0.00 (+)	4.0 ± 0.00 (+)	NA
Enterobacter aerogenes LCI	MBC (mg/mL)	-	32.0 ± 0.00	32.0 ± 0.00	4.0 ± 0.00	NA
	MIC (mg/mL)	-	8.0 ± 0.00	4.0 ± 0.00	2.0 ± 0.00	NA
	MBC/MIC	-	4.0 ± 0.00 (+)	8.0 ± 0.00 (-)	2.0 ± 0.00 (+)	NA
Streptococcus mutans LCI	MBC (mg/mL)	-	4.0 ± 0.00	2.0 ± 0.00	1.0 ± 0.00	NA
	MIC (mg/mL)	-	0.5 ± 0.00	2.0 ± 0.00	0.5 ± 0.01	NA
	MBC/MIC	-	8.0 ± 0.00 (-)	1.0 ± 0.00 (+)	2.0 ± 0.00 (+)	NA
Aspergillus flavus LCI	MFC (mg/mL)	-	2.0 ± 0.01	4.0 ± 0.01	NA	0.5 ± 0.00
	MIC (mg/mL)	-	0.5 ± 0.00	2.0 ± 0.00	NA	0.25 ± 0.00
	MFC/MIC	-	4.0 ± 0.00 (+)	2.0 ± 0.00 (+)	NA	2.0 ± 0.00 (+)
Aspergillus niger LCI	MFC (mg/mL)	-	2.0 ± 0.00	4.0 ± 0.00	NA	1.0 ± 0.00
	MIC (mg/mL)	-	2.0 ± 0.00	1.0 ± 0.00	NA	1.0 ± 0.01
	MFC/MIC	-	1.0 ± 0.00 (+)	4.0 ± 0.00 (+)	NA	1.0 ± 0.00 (+)
Candida albicans LCI	MFC (mg/mL)	-	4.0 ± 0.00	8.0 ± 0.00	NA	2.0 ± 0.00
	MIC (mg/mL)	-	2.0 ± 0.01	4.0 ± 0.01	NA	1.0 ± 0.00
	MFC/MIC	-	2.0 ± 0.00 (+)	4.0 ± 0.00 (+)	NA	2.0 ± 0.00 (+)

NA Not applicable, - no activity, *(-)* Bacteriostatic/fungistatic, *(+)* Bactericidal/fungicidal

The results shown in Table 3 suggest that both stem methanol and chloroform crude extracts of *N. latifolia* were bacteriostatic and fungistatic and, the results of the MIC, MBC and MFC were comparable to those of the standard drug (Neomycin and Chlotrimazole) although the standard drug showed better activity. It was also observed that the methanol extract showed better *in vitro* antibacterial and antifungal activity. Studies have shown that potent antibiotics have low MIC values however, as bacteria become less susceptible to an antibiotic the MIC values increases[15,40]. Therefore, Table 3 revealed that the methanol and chloroform extracts of *N. latifolia* are potential antibacterial and antifungal agent and should be explored further.

Furthermore, the results of the MIC and MBC of *N. latifolia* stem methanol and chloroform extracts showed that the MIC values ranged from 0.5mg/mL to 32.0 mg/mL. In comparison, the MBC values ranged from 2.0 to 32.0 mg/mL for both *N. latifolia* stem methanol and chloroform extracts. Also, the MFC was 2.0 to 4.0 mg/mL. The MFC values (Table 3) *N. latifolia* stem methanol extract showed better antifungal and

antibacterial activities compared to the chloroform extract.

The MIC and MBC results show that *N. latifolia* methanol and chloroform extracts can serve as a broad-spectrum antimicrobial agent even at a very low concentration thus confirming the use of *N. latifolia* stem extracts by ethno medicine experts for the treatment of ailments caused by pathogens.

Conclusion

The outcomes of this research showed that *N. latifolia* is rich in bioactive phytochemicals that are effective against various microorganisms and have pharmacological relevance. Furthermore, the phytochemical constituents and pharmaceutical properties of *N. latifolia* stem extract is solvent dependent. Future research should investigate the individualistic pharmacological importance of identified phytochemicals of *N. latifolia* and their randomized synergistic activity as combinatorial therapeutic in the treatment of different ailments and medical conditions. Also, it is necessary to validate the presence of fatty acids by methylating the samples and subsequently comparing them with the original extracts because many fatty acids do not volatilize under the GCMS experimental conditions. Also, future research should explore in silico, *in vivo* and clinical trials to gain insight into further therapeutic benefits of *N. latifolia* stem extracts. It is necessary to consider other spectroscopic tools to determine with certainty the structure of compounds in the extracts of *N. latifolia* stem i.e. the limitations of this study is in the isolation and spectra characterization of specific compounds that are responsible for each specific therapeutic application of *N. latifolia* stem extract.

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Authors' contribution

K.I.I.: Investigation, Data curation, Supervision, Conceptualization, Writing – original draft, Writing – review and editing, Formal analysis, Methodology, Visualization, Software; O.O.: Writing – editing; J.A.: Writing – editing.

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