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Antimicrobial and Phytoconstituent Analysis of the Leaf Extracts of Annual Swordfern (*Nephrolepis undulata* Afzel. Ex Sw.)

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ABSTRACT *Nephrolepis undulata* (sword fern) is a medicinal plant with its different parts used traditionally for treatment of various ailments including stomach ache and cough. This study was aimed at evaluating the antimicrobial activities of the leaf extracts of *N. undulata* against selected microorganisms (*K. pneumoniae, E. coli, S. aureus, B. subtilis, C. albicans, M. audiounii*) using the agar diffusion method. The presence of bioactive phytochemicals was also investigated using standard procedures and GC-MS assay. The results of the inhibition zone diameter (IZD) ranged from7.8±0.3 to 28.0±0.0 mm, 8.5±0.0 to 25.5±0.5 mm and 9.5±1.5 to 24.5±0.5 mm for the methanol, petroleum ether and chloroform extracts respectively. The minimum inhibitory concentration (MIC) showed that the methanol extract had better antimicrobial activity followed by the petroleum ether and chloroform extracts with the MIC values ranging from 0.9 to 6 mg/ml, 0.9 to 8 mg/ml and 4 to 10 mg/ml for the methanol, petroleum ether and chloroform extracts respectively. The IZD and MIC results further revealed the Gram-positive organisms to be more susceptible than the Gram-negative organisms whereas the fungus *C. albicans* showed intermediate susceptibility and *M. audiounii* was the least susceptible. Qualitative phytochemical screening results revealed the presence of some secondary metabolites; carbohydrates, reducing sugars, saponins, steroids, tannins, flavonoids and alkaloids in the pulverized leaf of *N. undulate* while GC-MS analysis revealed the presence of vast array of phytochemicals such as 2-Nitrobutan-1-ol, Glycerin, Isosorbide dinitrate, Cyclohexanmine, 5-butyldihydro-2(3H)thiophenone, 4H-pyran-4-one, Isosorbidedinitrate, 2-Furanone, Benzaldehyde, 2-Nitro-1-buten-3-ol, 5-Hydroxymethylfurfural, 1-Decanol, 6-Acetyl-beta-d-mannose, 3-n-Butylthiolane, Pentadecane, 3,4-Anhydro-d-galactosan, Hydrocoumarin, Heneicosane, N6-phenyl acetic acid, Glutamine, Coumarin, Heneicosane, 9,9-Dimethoxybicyclo, 3-O-methyl glucose, n-Hexadecanoic acid and [1, 1'-Bicyclopropyl]-2-octanoic acid. These positive finding validates the ethnomedicinal application of the plant in management of various ailments. It further demonstrates the potential of using the plant as raw material in the pharmaceutical industries for future drug discovery and development.

Keywords: Antimicrobial, Phytochemical, GC-MS, *Nephrolepis undulata*, extracts

Introduction

Plant-derived medicines have been a part of traditional health care in most parts of the world (Ekunsanmi, 2005). There is an increasing interest in plants as sources in treatment of diseases (Ekunsanmi, 2005; Dowe *et al*., 2016). The increase in interest has continued to emerge in herbal medicine owing to increasing microbial resistance to orthodox antibiotics, problems of adverse side effects and the ready availability of natural herbs (Okigbo and Mmeka, 2006). *Nephrolepis undulata* is a fern native to tropical Africa and Madagascar (Oloyede *et al*., 2014). It is however distributed worldwide especially in northern Australia, southern America and Asia (Patil and Dongare, 2014). It belongs to the family Nephrolepidiaceae. The tufts have finely toothed sword-shaped fronds which rise from short, erect, hairy leaf stems. It grows to 2-3 feet tall in its terrestrial habitat and sometimes as epiphytes on palm trees (Patil and Dongare, 2014). It has many common names including annual sword fern, helecho and ladder fern. In India, young leaves are cooked as vegetables (Perumalsamy *et al.*, 1999). Decoctions of fresh fronds have been used to treat fever (Oloyede *et al*., 2014). It is also used to treat cough and skin diseases in India (Perumalsamy *et al.*, 1999). The rhizomes are used for rheumatism, chest congestion and anorexia; The leaves are used in the treatment of wounds, stomache ache, jaundice and pregnancy booster (Kalembe *et al*., 2014; Patil and Dongare, 2014).

Members of the genus *Nephrolepis* has gained wide application to herbal medicine practitioners owing to their negligible toxicity and the presence of a wide array of bioactive phytochemicals (Garcia *et al*., 2012; Savoia, 2012). Studies revels that other species of *Nephrolepis* such as *N. cordifolia* and *N. biserrata* are highly nutritious containing ascorbic acid, carbohydrate, protein and some mineral elements with very low level of oxalate and cyanide (Oloyede *et al*., 2014). Oloyede *et al*. (2014) and Kalembe *et al*. (2014) also reported the presence of cardiac glycosides *N. abrupt*, *N. brownie* and *N. davalliae* in addition to flavonoids, alkaloids, tannins, saponins and phenols. The presence of saponins in these ferns contribute immensely to their antimicrobial activities. Saponins are secondary plant metabolites with potent antifungal, antibacterial, anti-inflamatory and phytoprotectant properties (Bairy *et al*., 2002). This means that the primary function of phytochemicals and secondary metabolites are to protect the plants against microbial attack and to aid their survival or adaptation (Garcia *et al*., 2012; Savoia, 2012). However, when these plants are consumed by herbivores or for health reasons, their supposedly phytoprotectant phytochemicals and nutrients are transferred and utilized for similar purpose in the consumer (Okwu, 2004; Dowe *et al*., 2016). Pure isolated alkaloids and their synthetic derivatives are important basic medicinal agents for their analgesic effect and bactericidal properties (Durling, *et al*., 2009). Flavonoids carry out various biological functions and studies have recognized flavonoids for their antioxidant, anti-inflammatory properties (Okwu, 2004; Kalembe *et al*., 2014). At low concentration, tannin can inhibit the growth of microorganisms and act as antifungal agent at higher concentration (Adekunle and Ikumapayi, 2006). Recently, coumarins such as scopoletin and chalcones have been isolated as antitubercular constituents of the plant *Fatoua pilosa* (Garcia *et al*., 2012). In addition, phytoalexins, which are hydroxylated derivatives of coumarins, which are produced in plants in response to microbial

infections, have been found to exert marked antibacterial and antifungal activity (Garcia *et al*., 2012). Researchers also found that capsaicin compound of plant extract was an important microbial inhibitory component among tested fractions and significantly reduced the expression of genes of *Vibrio cholerae* (Yamasaki *et al*., 2011).

More than twelve thousand antimicrobial compounds from plants or plant secondary metabolites have been identified and isolated (Firas *et al*., 2008; Habamu *et al*., 2010). Comparative studies have shown that some plant extracts are nearly more active than conventional or orthodox antibiotics (Ekunsami, 2005; Firas *et al*., 2008). This is particularly so for most microorganism resistant to conventional antibiotics while being susceptible to various plant extracts. For instance, Ekunsanmi, (2005) reported antibiotic resistant strains of *S. aureus*, *K. pneumonia* and *E. coli* which were susceptible to garlic extract. In related studies, aqueous and methanol extract of *Massularia acuminata, Moringa oleifera, Chromonella odorata, Mangifera indica, Nephrolepis cordifolia, Casia alata*, etc were reported to be active against clinical bacterial and fungal isolates which were resistant to some existing antibiotics (Kareem *et al*., 2012; Kalembe *et al*., 2014; Dowe *et al*., 2016). Therefore, the aim of this study is to evaluate the methanol, chloroform and petroleum ether extracts of *N. undulata* leaf for antimicrobial activity and the presence of bioactive plant metabolites.

Materials and Methods

Materials

Materials used include Sohxlet apparatus, table-sized autoclave, mettler weighing balance and glass wares of pyrex, England. All solvents were of the Analar grade, obtained from JHD, Guandgua Chemical Ltd., China. Microbiological media were obtained from Biotech., India and include Mueller Hinton agar, Nutrient broth, Sabouraud Dextrose agar and Sabouraud Dextrose broth.

Collection and Identification of Plant

The plant was collected from Ewu Monastery, Esan Central Edo State and Identified by Uwumarongie Henry of Pharmacognosy department, University of Benin who had previously authenticated the plant at the Forestry Research Institute of Nigeria (FRIN), in Ibadan, Oyo state where a voucher specimen was made with assigned herbarium number: FHI110381.

Preparation of the plant extracts

The leaves of the plants were prepared and extracted using Soxhlet apparatus. In the process, the leaves of the plant were collected and washed using distilled water. It was then air dried for two weeks before milling into fine powder using a mechanical milling machine. Approximately 200 g of the powdered plant was seperately extracted with 2000 ml of methanol, chloroform and petroleum ether using the Soxhlet apparatus. The extract was then concentrated to dryness at 60^{o}C using a thermostatical-

ly controlled water bath. The yields were weighed, percentage yield calculated to be 13.11%, 9.07% and 7.13% corresponding to the methanol, chloroform and petroleum ether extract respectively. The extracts were introduced into sterilized sample bottles and stored in the refrigerator at 4^{o}C prior to analyses.

Phytochemical screening

Qualitative screening of the phytochemical components of the pulverized plant was carried out using the method outlined by Harborne (1998) to detect the presence of alkaloids, saponins, tannins, flavonoids, anthracene derivatives, cyanogenic glycosides, carbohydrates, reducing sugars and steroids.

GC-MS Analysis

The GC-MS analysis was carried out according to the method described by Isahq *et al*. (2015). In the process, the carrier gas used was Helium at a flow rate of 1.2 ml/ min. The inlet temperature was maintained at 230^{o}C. The oven temperature was programmed initially at 50^{o}C for 5 minutes. Then programmed to increase to 300^{o}C at a rate of 10^{o}C ending with 25 minutes with a total run time of 45 minutes. The source temperature was maintained at 230^{o}C and the MS Quad at 150^{o}C. The ionization mode used was electron ionization mode at 70eV. Total Ion Count (TIC) was used to evaluate for compound identification and quantification. The Spectrum of the separated compound was compared with the database of the spectrum of known compound saved in the NIST02 Reference Spectra Library. Data analysis and peak area measurement was carried out using Agilent Chemstation Software.

Antimicrobial assay of the extracts

Overnight broth cultures of the selected pure isolates *Staphylococcus aureus*, *Klebsiella pneumoniae, Escherichia coli*, *Bacillus subtilis*, *Candida albicans* and *Microsporum audouinii*) were adjusted to 0.5 McFarland turbidity standard and further diluted (1:100 using normal saline solution) to yield microbial suspension of approximately 10^{6}cfu/mL; Antimicrobial sensitivity of the extracts were performed using the modified agar well diffusion method (Firas *et al*., 2008; Habamu *et al.,* 2010). Wells of 7 mm in diameter were made into uniformly streaked Mueller Hinton agar/Sabouraud dextrose agar plates. Each well was filled with 0.1 ml of the extract at varying concentrations. The same quantity of Tween-80 (10%) served as negative control while 1µg/ml of Ciprofloxacin and 10µg/ml of Ketoconazole were used as positive controls for bacteria and fungi respectively. All plates were incubated in an upright position. However, bacteria plates were incubated overnight at 37^{o}C and fungal plates were incubated at room temperature (25^{o}C) for 72 hr. The absence or presence of growth was observed on the plates and the diameter of clear zone was measured in mm and recorded. The experiments were done in duplicates and the mean zones of inhibitions calculated.

Determination of Minimum Inhibitory Concentration's (MIC's) of the extracts

The MIC's of the extracts that showed activity against the organisms were determined by the agar dilution method (NCCLS, 2003; Lalitha *et al*., 2004). From the extract stock concentration of 100 mg/mL, lower concentrations were prepared by incorporation into the molten Mueller Hinton agar at different volumes to obtain a range of concentrations of between 0.1-10 mg/ml. Then a loop-full volume of one in hundred dilution (1:100) of 0.5MacFarland turbidity standard of microbial suspensions obtained from overnight broth were spotted on the surface of the agar plates at marked segment of the various plate concentrations of the test extracts and plates were incubated at 37^{o}C for 24 hours. The same procedure was repeated using Sabouraud agar but incubation was at room temperature (25±2^{o}C) for 48-72 hours after which the lowest concentration at which there was no observable bacterial/ fungal growth was recorded as the MICs.

Statistical Analysis

Statistical analysis was done using SPSS software version 16 (SPSS Inc. Chicago) and results presented in tables as mean ± standard error of mean. Paired t-Test and one-way analysis of variance (ANOVA) was used to compare data for level of significance.

Results

The qualitative phytochemical analysis of the pulverized plant powder detected the presence of carbohydrates, reducing sugars, saponins, steroids, tannins, flavonoids and alkaloids (Table 1).

Table 1: Qualitative phytochemical constituent of the pulverized plant

CONSTITUENTS	STATUS
Reducing sugars	+
Carbohydrates	+
Saponins	+
Anthracene derivatives	-
Cyanogenetic glycosides	-
Steroids	+
Tannins	+
Flavonoids	+
Alkaloids	+

Key: + = present, - = absent

Results of the antimicrobial activity of the methanol, petroleum ether and chloroform extract as represented by the inhibitory zone diameters (IZDs) are presented in Table 2, 3 and 4 respectively. The results of the inhibition zone diameter (IZD) ranged from7.8±0.3 to 28.0±0.0 mm, 8.5±0.0 to 25.5±0.5 mm and 9.5±1.5 to 24.5±0.5 mm for the methanol, petroleum ether and chloroform extracts respectively. The IZDs were concentration dependent and varied among the organisms and the various extracts with the methanol extract (Table 2) showing the highest range of IZDs (7.8±0.3-28.0±0.0 mm), the least set of IZDs (< 25 mm even at its highest concentration of 50 mg/ml) was shown by the chloroform extract (Table 4) with a complete loss of activity at 1 mg/ml of the extract.

The results of minimum inhibitory concentration (MIC) are shown in Table 5. The methanol extract recorded the least set of MICs on the average, followed by the petroleum ether extract and the chloroform extract which showed the highest MIC against *M. audouinii*.

Table 2: Antimicrobial activities of the methanol extract

Organisms	Zones of Inhibition (mean ± S.E.M mm)						
	Concentrations (mg/ml)	CIP	KET	Tween-80			
	1	10	30	50	1µg/ml	10µg/ml	(10%)
K. pneumoniae	−	14.0 ±1.0	17.0 ±1.0	23.5± 0.5	32.8 ±0.2	−	−
E. coli	8.0±0.5	14.7 ±0.3	18.5 ±0.5	23.8± 0.2	31.7 ±0.3	−	−
S. aureus	11.0±0.5	16.5 ±0.5	23.0 ±1.0	26.5± 0.5	33.0 ±2.0	−	−
B. subtilis	12.3±0.8	16.3 ±0.7	24.0 ±1.0	28.0± 0.0	33.5 ±0.5	−	−
C. albcans	7.8±0.3	15.3 ±1.3	19.5 ±0.5	24.0± 1.0	−	31.5 ±1.5	−
M.audouinii	−	14.0 ±0.0	19.0 ±1.0	23.5± 0.5	−	30.5 ±0.5	−

Key: S.E.M = Standard Error of Mean, − = No activity, CIP = ciprofloxacin, KET = Ketoconazole

Table 3: Antimicrobial activities of the petroleum ether extract

Organisms	Zones of Inhibition (mean ± S.E.M mm)						
	Concentrations (mg/ml)	CIP	KET	Tween-80			
	1	10	30	50	1µg/ml	10µg/ml	(10 %)
K.pneumonia	−	12.7 ±3.0	17.0 ±1.0	22.5 ±0.5	32.5 ±2.5	−	−
E. coli	−	14.0 ±1.0	18.5 ±2.5	23.7 ±0.3	33.5 ±1.5	−	−
S. aureus	8.5±0.0	16.3 ±0.7	19.0 ±3.0	24.5 ±0.5	34.0 ±1.0	−	−
B.subtilis	10.5±2.0	17.8 ±0.2	21.5 ±2.5	24.5 ±1.5	34.0 ±2.0	−	−
C.albicans	−	13.2 ±1.3	18.0 ±1.0	22.5 ±1.5	−	30.5 ±0.5	−
M.audouinii	−	11.2 ±1.3	17.0 ±1.0	22.5 ±0.5	−	30.0 ±1.0	−

Key: S.E.M = Standard Error of Mean, − = No activity, CIP = ciprofloxacin, KET = Ketoconazole

Table 4: Antimicrobial activities of the chloroform extract

Organisms	Zones of Inhibition (mean ± S.E.M mm)						
	Concentrations (mg/ml)	CIP	KET	Tween-80			
	1	10	30	50	1µg/ml	10µg/ml	(10 %)
K. pneumoniae	−	11.8 ±0.2	16.0 ±1.0	21.0 ±0.0	32.0 ±1.0	−	−
E. coli	−	13.5 ±0.5	16.5 ±0.5	22.7 ±0.3	33.5 ±0.5	−	−
S. aureus	−	14.0 ±1.0	18.5 ±2.5	23.0 ±1.0	31.0 ±2.0	−	−
B. subtilis	−	14.8 ±0.2	20.7 ±0.3	24.5 ±0.5	33.5 ±0.5	−	−
C. albicans	−	12.7 ±0.3	17.0 ±1.0	23.0 ±0.0	−	33.0 ±2.0	−
M. audouinii	−	9.5± 1.5	15.0 ±2.0	21.5 ±0.5	−	32.5 ±1.5	−

Key: S.E.M = Standard Error of Mean, − = No activity, CIP = ciprofloxacin, KET = Ketoconazole

Table 5: MICs of the extracts against the test organisms

Organisms	Methanol Extract	Petroleum ether extract	Chloroform Extracts
	MICs (mg/ ml)		
K. pneumoniae	4	6	8
E. coli	0.9	4	6
S. aureus	0.7	0.9	4
B. subtilis	0.5	0.7	4
C. albicans	2	4	8
M. audiounii	6	8	10

The most active methanol extract was subjected to GC-MS analysis and the results of the chromatogram shows the presence of vast array of chemical groups represented by the various peaks (detected compounds) are shown in figure 1.

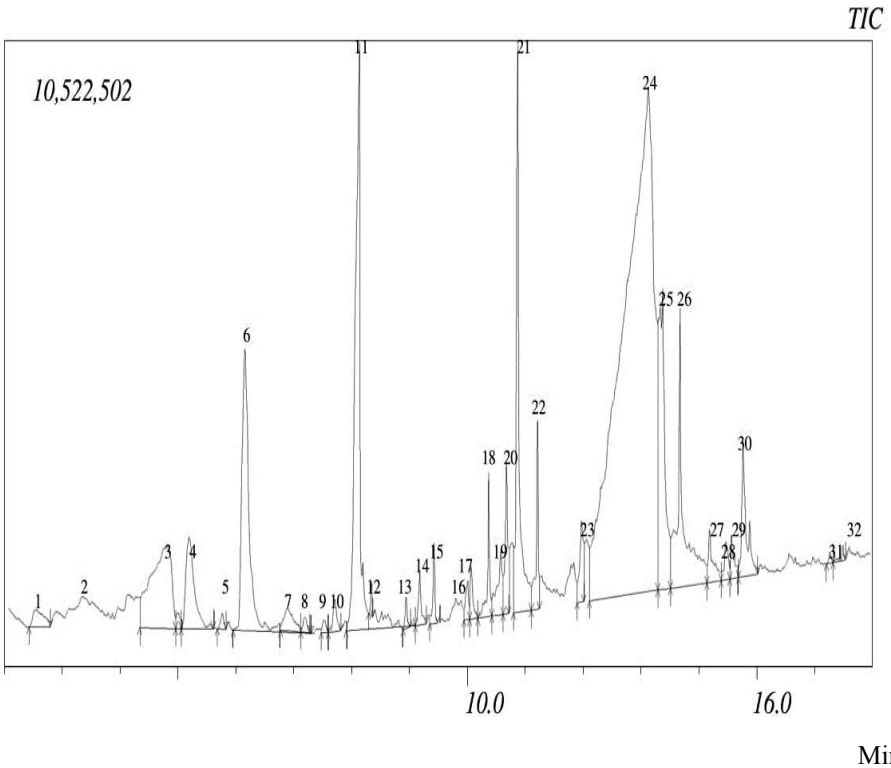

Figure 1: GC-MS sample chromatogram of the methanol extract of *N. undulata*

Peak TIC

Peak#	R Time	I Time	F Tim	Area	Area%	Heig	Height	A/	Mar	Name
1	2.483	2.358	2.725	413341	0.61	29939	0.44	13.8		1-Butanol, 2-nitro-
2	4.759	4.283	4.908	356590	5.26	14121	2.07	25.2	V	Glycerin
3	4.945	4.908	5.008	123798	0.18	25781	0.38	4.8	V	Isosorbide Dinitrate
4	5.142	5.008	5.567	161725	2.39	15748	2.30	10.1	V	Cyclohexanamine, N-3-
5	5.719	5.633	5.783	103855	0.15	26689	0.39	3.8		5-Butyldihydro-
6	6.116	5.908	7.267	419171	6.19	48385	7.08	8.4	S	4H-Pyran-4-one, 2,3-dihydro-
7	6.851	6.733	7.092	451286	0.67	38670	0.57	11.6	TV	Isosorbide Dinitrate
8	7.161	7.092	7.250	125040	0.18	26496	0.39	4.7	TV	2-Furanone, 3,4-
9	7.493	7.442	7.567	87990	0.13	22222	0.32	3.9	V	Benzaldehyde, 4-benzyloxy-2-
10	7.678	7.567	7.783	260833	0.38	55883	0.82	4.6	V	2-Nitro-1-buten-3-ol
11	8.106	7.875	9.083	651290	9.61	100898	14.75	6.2	SV	5-Hydroxymethylfurfural
12	8.314	8.267	8.342	93809	0.14	56245	0.82	1.6	T	1-Decanol, 2-hexyl-
13	8.919	8.867	8.983	135171	0.20	54094	0.79	2.5	T	6-Acetyl- beta -d-mannose
14	9.159	9.083	9.267	323274	0.48	93606	1.37	3.4		3-n-Butylthiolane
15	9.406	9.333	9.467	290564	0.43	13736	2.01	21.1	V	Pentadecane
16	9.987	9.925	10.025	242921	0.36	66172	0.97	3.6	V	3,4-Anhydro-d-galactosan
17	10.051	10.025	10.167	264119	0.39	91220	1.33	2.9	V	Hydrocoumarin
18	10.357	10.167	10.408	616198	0.91	24849	3.63	24.8		Heneicosane
19	10.566	10.408	10.608	701619	1.04	11149	1.63	62.9	V	Adenosine, N6-phenylacetic
20	10.666	10.608	10.700	808678	1.19	26235	3.84	30.8	V	Glutamine
21	10.857	10.783	11.092	333559	4.92	97637	14.28	34.2	V	Coumarin
22	11.205	11.092	11.242	874770	1.29	32456	4.75	27.0	V	Heneicosane
23	11.971	11.892	12.008	598828	0.88	13910	2.03	43.1	V	9,9-
24	13.138	12.108	13.300	3237552	47.77	86248	12.61	37.5	V	3-O-Methyl-d-glucose
25	13.347	13.300	13.517	381504	5.63	48338	7.07	7.8	V	3-O-Methyl-d-glucose
26	13.686	13.517	14.150	327850	4.84	42540	6.22	7.7	V	n-Hexadecanoic acid
27	14.203	14.150	14.400	502339	0.74	82566	1.21	6.0	V	[1,1'-Bicyclopropyl]-2-
28	14.488	14.400	14.550	326795	0.48	64635	0.95	5.0	V	9-Octadecenoic acid, (Z)-
29	14.586	14.550	14.700	298418	0.44	70774	1.03	4.2	V	9,9-
30	14.785	14.700	15.033	128641	1.90	22769	3.33	5.6	V	Oleic Acid
31	16.297	16.233	16.350	55875	0.08	18071	0.26	3.0		[1,1'-Bicyclopropyl]-2-
32	16.517	16.350	16.567	90496	0.13	25016	0.37	3.6	V	[1,1'-Bicyclopropyl]-2-

Discussion

Results of the qualitative phytochemical screening (Table 1) reveals the presence of carbohydrate and reducing sugars in the leaves, several other studies have detected same in the leaves of other plants especially as the seat of photosynthetic activities (Nobmann *et al*., 2009; Okwu, 2004; Josephs and Dowe 2016). The antimicrobial properties of tannins, alkaloids, flavonoids and saponins has been established and reported (Okwu, 2004; Adekunle and Ikumapayi, 2006; Kalembe *et al*., 2014). For instance, saponins has bitter taste, foaming property and serve as mild detergent that solubilizes cell permeability barriers and consequent lysing of bacterial and fungal cells (Okwu, 2004; Dowe *et al*., 2016).

The antimicrobial activities of plant extracts and other agents are determined by the presence of growth inhibitory zones diameter (IZD) on seeded agar plate (Cheesbrough, 2006). The IZDs shown by the different extracts were concentration dependent (Table 2, 3 and 4). Extracts were considered active at zone of inhibition of >7 mm; a benchmark established due to the diameter of the cork borer used to make the agar wells. In a related study in which cork borer diameter of 9 mm was used by Ndukwe *et al*. (2005) and Usman *et al*. (2005), they considered activity of their test extract at IZD of > 9 mm. A stronger antimicrobial agent will create a larger IZD because a lower concentration of the agent is enough to inhibit the growth of microbial cells (Cheesbrough, 2006; Dowe *et al*., 2016). The study showed that the methanol, petroleum ether and chloroform extract of *N. undulata* and the positive control (Ciprofloxacin and Ketoconazole) were active against the test microorgan-

isms when compared with the negative control (10% Tween-80) which showed no activity. However, even at a higher extracts concentration of 50 mg/ml, the positive controls were observed to show higher inhibition zone diameters which may be attributed to the crude nature of the extracts as opposed to ciprofloxacin and ketoconazole (positive controls) which already are in their purified and compounded form. This result is in agreement with those reported by Dowe *et al*. (2016) in which the methanol extract of *M. acuminata* showed higher IZDs than the chloroform and petroleum ether extract. In contrast, Habbu *et al*. (2010) reported higher IZDs for the chloroform extract of *Spondias mombi* relative to the n-Butanol and petroleum ether extracts.

The MICs are quantitative indices used to measure the effectiveness of antimicrobial agents against microorganisms and are of importance in fixing benchmark for dosing concentration of antimicrobial agents (Bairy *et al*., 2002; Firas *et al*., 2008; Vinothkumar *et al*., 2012). The lower the MIC value the more potent the antimicrobial agent, conversely, the higher the MIC value, the less potent the antimicrobial agent. *M. audiounii* followed by the bacterium *K. pneumoniae* were least susceptible to the inhibitory effect of the plant extracts whereas *B. subtilis* was most susceptible. The MIC results shows the gram-positive organisms to be more susceptible to the inhibitory effect of the plant extracts compared to the gram-negative organisms whereas *C. albicans, E. coli,* and *S. aureus* showed intermediate susceptibility relative to the two extremes. In a related study, Oshomo and Idu (2012), reported lower MICs for *B. subtilis S. mutans*, *S. aureus* and *C. albicans* compared to other gram negative dental caries isolates. Some organisms are generally more susceptible than others depending largely on their structural framework; some such as gram negative bacteria are often more resistance than their gram positive counterpart due to an outer phospholipid membrane with structural lipopolysaccharides components that reduce the cell wall penetration ability of antimicrobial compounds (Chessebrough, 2006; Rahman *et al.,* 2011).

Studies have been carried out to discover useful antibacterial and antifungal compounds from plants (Perumalsamy *et al.,* 1999; Singh *et al.,* 2012). In line with this, the GC-MS analysis of the most active methanol extract of *N. undulata* (Fig. 1) reveals a large array of compound beginning with the first peak of 1-Butanol followed by 2-nitro group which is synonymous with 2-Nitro-1-butanol (IUPAC name: 2-Nitrobutan-1-ol). This compound was found by Soheila *et al*. (2016) to inhibit quorum sensing process among microbial cells. The next peak shows the compound Glycerin, followed by Isosorbide dinitrate, Cyclohexamine, 5-butyldihydro-2(3H) thiophenone, 4H-pyran-4-one, Isosorbide dinitrate, 2-Furanone, Benzaldehyde, 2-Nitro-1-buten-3-ol, 5-Hydroxymethylfurfural which registered the highest peak and highly abundant in the extract. According to Durling *et al*. (2009), 5-Hydroxymethylfurfural (HMF), a heat-induced food toxicant present in a vast number of plants, has been suggested to be genotoxic after being bioactivated by the sulfotransferase SULT1A1. It has been shown to possess DNA damaging effect either activated or otherwise in microbial cells. The next peak that followed was peak 12 which represents 1-Decanol, next was 6-Acetyl-beta-d-mannose, followed by 3-n-Butylthiolane (peak 14), Pentadecane (peak15), 3,4-Anhydro-d-galactosan (peak 16), Hydrocoumarin (peak 17), Heneicosane (peak18), N6-phenyl acetic acid (peak 19), Glutamine (peak 20), Coumarin (peak 21) otherwise known as benzopyrone

also registered a high peak; other compounds represented by peak 22 to 32 include Heneicosane, 9,9-Dimethoxybicyclo, 3-O-methyl glucose, n-Hexadecanoic acid and [1, 1'-Bicyclopropyl]-2- octanoic acid. According to Soheila *et al*. (2016), coumarin or benzopyrone represented by peak 21 is a compound with pleasant smell reported to have fungicidal and bactericidal activity and it acts by coagulating cell cytoplasm. Suppoting results established the presence of coumarins such as scopoletin and chalcones isolated as antitubercular constituents of the plant *Fatoua pilosa* (Garcia *et al*., 2012).

Conclusion

This study has demonstrated significant concentration dependent activity of the extracts against the test organisms as opposed to the negative control (10% Tween-80). This means that the Tween-80 to water combination (10:90) used in diluting out the varying concentration of extracts did not enhance or reduce the activity of the extracts and standard antimicrobial agents used. The methanol extract was more active, followed by the petroleum ether extract and the chloroform extract which was least active. However, the difference in activity between the petroleum ether extract and chloroform extract was not statistically significant but the difference in activity between either of the petroleum ether or the chloroform extract and the methanol extract was statistically significant (*$P < 0.05$). There is the presence of several bioactive plant compounds or metabolites which correlates with the antimicrobial properties of the plant. The findings from this study supports the continuous ethnomedicinal uses of the plant as well as its potential applicability in the Pharmaceutical industries.

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