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A preliminary study on the antimicrobial activities and gas chromatography-mass spectrometry (GC-MS) analysis of the ethyl acetate extract of *Dennettia tripetala* G. Baker seeds

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Abstract

The study investigated the antimicrobial properties and the chemical composition of ethyl acetate extract of *Dennettia tripetala* (pepper fruit) seeds. Crude extract obtained by maceration of pulverized seeds in ethyl acetate was evaluated for antimicrobial activity against *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Candida albicans* and *Aspergillus niger* using standard agar-well diffusion method. GC-MS method was used to determine the chemical constituents of the extract. The extract was oily, yellowish-brown with a yield of 1.66 % and had activity against most of the test microorganisms, with inhibition zone diameters ranging between 10 to 25 mm. About 41 chemical constituents were present in the extract with formic acid methyl esters and fatty acids accounting for 57.23 and 18.49 % respectively. Ethyl acetate extract of *Dennettia tripetala* seeds possessed antimicrobial activity against bacteria but not fungi. The observed activity may be due to the presence of formic and fatty acid esters in the seed. The study further established a scientific proof for the traditional use of *Dennettia tripetala* seed extracts in treating microbial infections.

Keywords: **Antimicrobial activity, *Dennettia tripetala*, ethyl acetate extract, GC-MS**

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INTRODUCTION

The need to explore natural supply of novel antimicrobial agents has increased in the past three decades, due in part, to the growing resistance of microorganisms to the few existing synthetic antimicrobial agents. *Dennettia tripetala* G. Baker (Annonaceae) also known as pepper fruit is an indigenous spicy medicinal plant found within the tropical rain forest region and occasionally in Savana areas of Nigeria (Okwu and Morah, 2004). It is locally called *Ako* (Edo), *Opipi* (Idoma), *Nakarika* (Ibibio), *Mmimi* (Igbo), *Ata Igbere* (Yoruba). Extract from the seed (whole, powder, or decoction) is used as an effective insecticide in grain protection against weevils in particular (Akinbuluma *et al*., 2015). Its seeds are used (in various forms) to protect maize grains in storage and may be consumed singly or taken with kola nut, garden egg or palm wine particularly during the entertainment of a guest

(Okigbo, 1980). As masticators and stimulants, *Dennettia tripetala* seeds are applied to diets of pregnant and postpartum women to aid uterine contraction (Okwu and Morah, 2004). The seeds are also used in some parts of Nigeria as spice in flavouring and seasoning foods, such as meat, vegetable, soup and sausage (Ikpi and Uku, 2008). Important nutritive substances of *Dennettia tripetala* fruit/seeds are minerals, vitamins, and fibre whereas the major phytochemicals are thiamine riboflavin, niacin and alkaloids (Okwu *et al*., 2005).

Essential oil from its leaves has been reported to possess significant antibacterial, anti-nociceptive and anti-inflammatory activities (Oyemitan *et al*. 2008; Okoh *et al*., 2016). Extracts of the root and leaves are commonly used by the local herbalist in folk medicine in combination with other medicinal plants to treat various ailments including fever, infantile convulsion, typhoid, worm infestation, vomiting and stomach upset. It has also been reported that the essential oil and phenolic acid extract of *Dennettia tripetala* can inhibit the growth of food-borne micro-organisms (Iseghohi, 2015). Another study found that the leaves of *Dennettia tripetala* were effective in inhibiting the growth of the rot-causing fungus *Sclerotium rolfsii* in cocoyam both *in vitro* and *in vivo* (Nwachukwu and Osuji, 2008).

Dennettia tripetala seeds are putative candidates for the discovery of pharmacologically active plant derived components, as different parts of the plant have been used ethno-medicinally in the treatment of various infections and preservation of food in Nigeria. This study evaluated the antimicrobial activity of ethyl acetate extract of pepper fruit seed (*Dennettia tripetala*), with the aim of justifying its use in ethnomedicine and also to elucidate the chemical component responsible for the observed activity using GC-MS.

MATERIALS AND METHODS

Materials

Ethyl acetate and dimethyl sulphoxide (DMSO) (JHD Science-Tech Co., China), ciprofloxacin powder (Sigma Aldrich, Taufkirchen, Germany), ketoconazole (Biochemika, Mombai, India) were used in the study. Ripe fruits of *Dennettia tripetala* were purchased from a local market in Benin City in the months of April and May. The fleshy parts of the fruit were removed, and the seeds washed and sun-dried to a constant weight for three (3) days. The dried seeds were stored in a desiccator under silica until use.

Methods

Preparation of crude extracts

About 500 g of dried *Dennettia tripetala* seeds was pulverized and the powder macerated with 2.5 litres of ethyl acetate for 72 hours with stirring at intervals. Extract mixture was filtered with Whatman filter paper No 1 and the resulting filtrate concentrated with a rotary evaporator at 40 °C. The extract obtained was stored in a refrigerator at 4 °C in air-tight bottles for further studies.

Preparation of test microorganisms

Prior to use, test microorganisms were sub-cultured from stock into sterile nutrient agar plates for bacteria and Sabouraud dextrose agar for fungi and incubated overnight at 37 °C for bacteria (*Bacillus subtilis*, *Escherichia coli*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*) and at 35°C for 48 hours for fungi (*Aspergillus niger* and *Candida albicans*). After incubation, identical colonies from the overnight plates were suspended in sterile broth for 12 hours at room temperature and adjusted to 0.5 McFarland standard to give an inoculum size of approximately 10^8 CFU/mL. The adjusted inocula were diluted 1:100 to give inoculum size of approximately 10^6 CFU/mL (CLSI, 2015).

Preparation of antibiotic stock solutions

Stock solutions of ciprofloxacin and fluconazole with final concentrations of 0.5 and 2.0 mg/mL, respectively were prepared in 10 % dimethyl sulphoxide (DMSO) solution. The stock solution of the extract was also prepared in 10 % DMSO solution by dissolving 1.0 g of extract in 1.0 mL of solvent to give a final concentration of 1000 mg/mL.

Test for antimicrobial activity of the extract

Antimicrobial susceptibility test was carried out using agar well diffusion method with some modifications (Jorgensen and Turnidge, 2007). Sterile Mueller Hinton agar was prepared and poured aseptically into different petri dishes, each containing 30 mL and allowed to set. The petri dishes were dried in a hot air oven for about 10 minutes at 40 °C. The dried plates were incubated overnight to rule out contamination and then streaked with the test bacteria using a sterile swab stick. A sterile cork borer (10 mm) was used to bore four (4) wells in each agar plate. The agar disks were

removed, and two drops of molten agar was introduced into each of the well to seal the base of the well. Two of the wells were filled with 200 µL (equivalent to 200 mg) of the extract per well while the other two wells were filled with 200 µL (equivalent to 0.1 mg or 100 µg) of ciprofloxacin per well. Inoculated plates were all incubated at 37 °C for 18 - 24 hours. The procedure was repeated using Sabouraud agar, the test fungi and fluconazole. Here, while two wells were filled each with 200 µL of the extract, the other two wells were each filled with 200 µL (equivalent to 0.2 mg or 200 µg) of fluconazole. The inoculated plates were incubated at 35 °C for 48 hours. Negative control (10 % DMSO solution) and positive control (viability test for used organisms) were carried out for each set of experiment. Inhibition zone diameters (IZDs) were measured in millimetres (mm) as an index of the killing or inhibitory action of the test agent against a given microorganism.

Determination of minimum inhibitory concentration (MIC)

Agar dilution method of Afolayan and Meyer (1997) was used in this study for the determination of minimum inhibitory concentration (MIC) of the extract. A two-fold serial dilution of the test extract was prepared to give concentrations of 12.5, 25.0, 50.0, 100 and 200 mg/mL. A two-fold serial dilution was also done for the stock solution of ciprofloxacin/fluconazole to give concentrations of 0.02, 0.04, 0.08, 0.16, 0.32 and 0.40 µg/mL. Nutrient agar and Sabouraud dextrose agar were separately prepared according to the manufacturer's instruction. Calculated volumes ranging from 0.125 - 4.0 mL of the extract and 0.4 - 12.8 µL of ciprofloxacin/fluconazole with nutrient agar or Sabouraud dextrose agar was poured into 10 mL glass petri dish and rocked gently to mix properly giving a concentration range of 12.5 - 200 mg/mL of the extract and 0.02 - 0.40 µg/mL of ciprofloxacin/fluconazole in the agar plates. Test bacteria or fungi inoculum was streaked with the aid of a sterile wire loop on each plate. Inoculated plates were incubated at 37 °C for 18 - 24 hours for bacteria and at 35 °C for 48 hours for the fungi. After incubation, the plates were visually examined for growth in the inoculated spots. The lowest concentration of the extract/standard that inhibited growth was considered as the MIC.

Determination of minimum bactericidal and fungicidal concentrations (MBC and MFC)

The MBC and MFC were determined from the MIC plates by sub-culturing into agar plates that did not contain any extract. The plates were then incubated at 37 °C for 18 - 24 hours for bacteria and at 35 °C for 48 hours for the fungi. After incubation, the plates were visually examined for growths and the lowest concentration of the extract that showed no visible growth was considered as the MBC/MFC in the bacterial and fungal plates respectively.

Gas chromatography - mass spectrometry analysis

GC-MS analysis of the extract was done using GCMS-QP2010 SE (Shimadzu, Japan) with capillary column specification: DB-5MS (5.0 % phenylmethyl siloxane as stationary phase), 0.25 x 30 mm (internal diameter) and 1.0 µm (film thickness) in an Agilent 6890N gas chromatograph and Agilent Technologies 5973 Network Mass Selective Detector. The carrier gas was helium with a constant flow rate of 3.22 mL/min. The inlet temperature was maintained at 250 °C. The oven temperature was initially kept at 60 °C for 3.4 min then ramped at 12 °C/min to 240 °C. The temperature was gradually increased from 60 °C/min to 290 °C and held isothermally for 2 min. An amount of 1.0 µL of the sample solutions was injected in the split mode with split ratio 15:1. Mass spectra were obtained by electron ionization at 70 eV over the scan range m/z 1428. The compounds were identified by comparison of their mass spectra with those of NIST 05 L mass spectra library. The spectra match factor limit was set as 700 and any components with match factor less than 700 were not considered.

Statistical analysis

Data were reported as mean from triplicate determination for inhibition zone diameters (IZD) ± standard error. Data analysis and peak area measurement of raw GC-MS data were carried out using Agilent Chemstation and Pherobase software.

RESULTS

The ethyl acetate extract of *Dennettia tripetala* seed was observed to be an oily liquid, yellowish-brown in color with a low yield of 1.66 %. Results from the antimicrobial activity of the extract shown in Table 1 revealed that six (6) of the seven (7) test microorganisms were susceptible to the inhibitory effect of the ethyl acetate extract of *Dennettia tripetala* seeds. The extract showed no inhibitory action against *Aspergillus niger*. Table 2 outlines the minimum inhibitory concentrations (MICs) and the

minimum bactericidal concentrations (MBCs) of the ethyl acetate test extract and standard drugs against susceptible microorganisms. An MIC of 25.0 mg/mL was observed for *Staphylococcus aureus* while *Escherichia coli*, *Klebsiella aerogenes, Bacillus subtilis* and *Pseudomonas aeruginosa* had MICs of 12.5 mg/mL respectively. *Candida albicans* exhibited an MIC > 200 mg/mL. The MBC results of the test extract for *Staphylococcus aureus* (50.0 mg/mL) was observed to be twice its MIC value (25 mg/mL) while that of *Pseudomonas aeruginosa* (50.0 mg/mL) was four times its MIC value (12.5 mg/mL). *Escherichia coli, Klebsiella aerogenes* and *Bacillus subtilis* exhibited similar MBCs of 12.5 mg/mL respectively as their MICs. The standard test drug, ciprofloxacin had MIC and MBC values of 0.02 µg/mL for all test bacteria while fluconazole displayed an MIC value of 0.32 µg/mL for *Candida albicans*.

Table 1: Inhibition zone diameters of the *Dennettia tripetala* seeds extract

Test microorganisms	Ethyl acetate extract (mm)	Ciprofloxacin (mm)	Fluconazole (mm)
Staphylococcus aureus	15.0 ± 0.12	34.0 ± 0.24	NA
Escherichia coli	25.0 ± 0.11	32.0 ± 0.16	NA
Klebsiella spp	20.0 ± 0.14	25.0 ± 0.18	NA
Bacillus subtilis	15.0 ± 0.13	22.0 ± 0.10	NA
Pseudomonas aeruginosa	25.0 ± 0.02	32.0 ± 0.11	NA
Candida albicans	10.0 ± 0.04	NA	23.0 ± 0.22
Aspergillus niger	0	NA	34.0 ± 0.12

Key: NA - (Not applicable), mm - (Millimeter), Values **±** Standard error

Table 2: Minimum inhibitory concentrations/minimum bactericidal concentrations of the test extract and standard drugs against susceptible microorganisms

Test microorganisms	Ethyl acetate extract (mg/mL)		Ciprofloxacin (µg/mL)		Fluconazole (µg/mL)	
	MIC	MBC	MIC	MBC	MIC	MBC
S. aureus	25.0 ± 0.011	50.0 ± 0.025	0.02 ± 0.001	0.02 ± 0.001	NA	NA
E. coli	12.5 ± 0.011	12.5 ± 0.013	0.02 ± 0.002	0.02 ± 0.002	NA	NA
Klebsiella spp	12.5 ± 0.021	12.5 ± 0.014	0.02 ± 0.001	0.02 ± 0.001	NA	NA
B. subtilis	12.5 ± 0.022	12.5 ± 0.011	0.02 ± 0.003	0.02 ± 0.003	NA	NA
P. aeruginosa	12.5 ± 0.013	50.0 ± 0.012	0.02 ± 0.001	0.02 ± 0.001	NA	NA
C. albicans	> 200	> 200	0.00	0.00	0.32 ± 0.011	NA

Key: NA (Not applicable), Values **±** Standard error

GC-MS chromatogram (Figure 1) showed several peaks with their corresponding retention time and on comparison of this mass spectral with NIST02 reference spectral library, 41 phytocompounds were identified (Table 3). The predominant compound identified with known antimicrobial properties was formic acid, (2-(methylphenyl) methyl ester) with a peak

percentage area of 57.23. Other components present with their peak percentage areas include the following; 9,12-octadecadienoic acid (6.98), nerolidyl acetate (4.89), n-hexanoic acid (3.81), cis-9-hexadecenal (4.50), octadecanoic acid (1.08), naphthalene methanol (1.56), 1,6-octadien-3-ol (1.25), humulene (1.34), caryophyllene (1.67) and vitamin E (0.67).

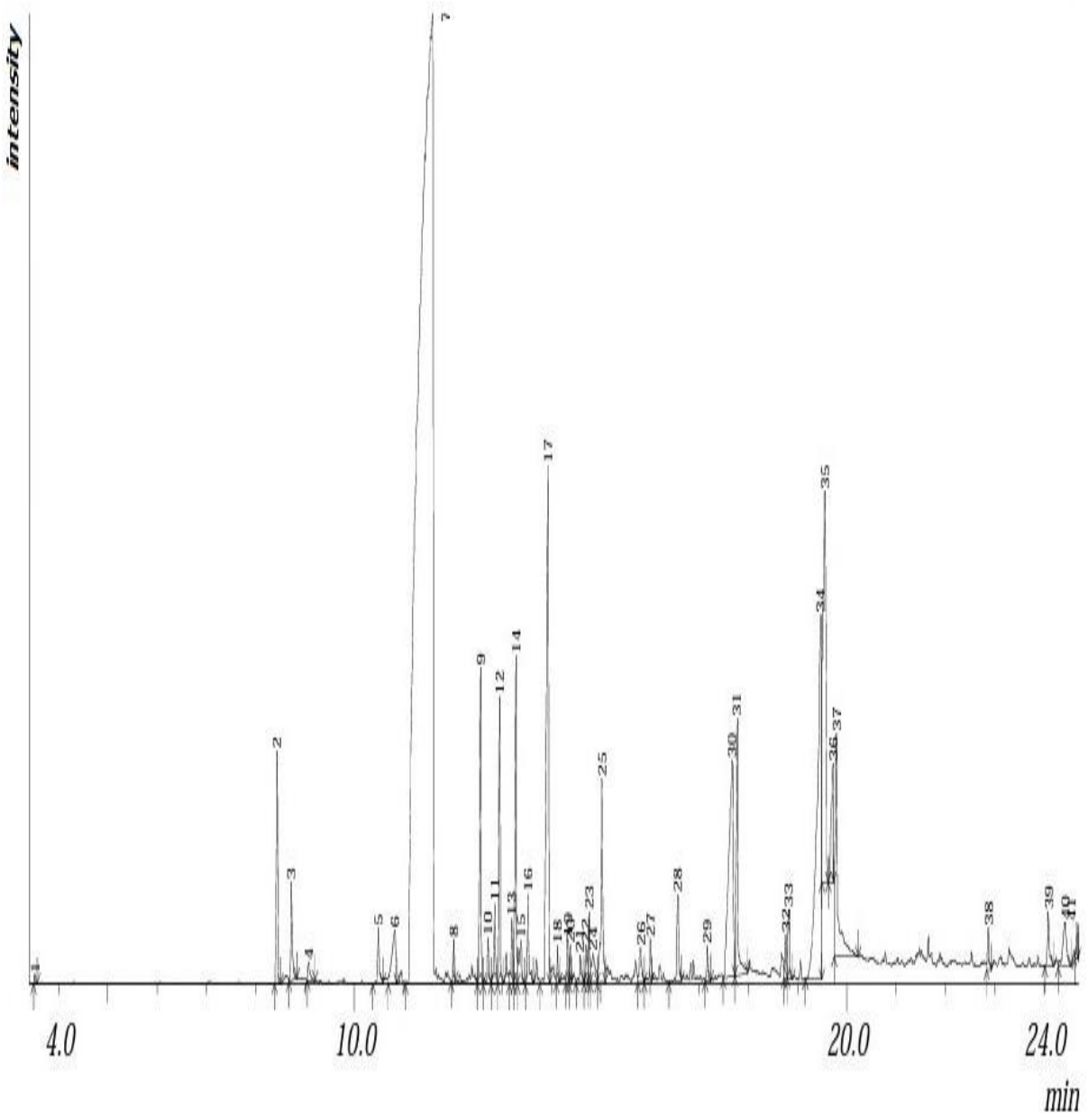

Figure 1: GC-MS chromatogram of the ethyl acetate extract of *Dennettia tripetala* seed

Table 3: Chemical components identified by the GC-MS analysis of the ethyl acetate extract of *Dennettia tripetala* seed

Peak no.	Ret. time	Ret. index	Peak area	Area (%)	Mol. wt.	SI (%)	Chemical compound
1	3.510	686	145663	0.03	102	96	Propanoic acid
2	8.439	1082	7001926	1.25	154	97	1,6-Octadien-3-ol
3	8.725	1136	3226675	0.57	122	96	Phenylethyl alcohol
4	9.074	1138	800321	0.14	117	89	Benzyl nitrile
5	10.491	1259	2243724	0.40	164	93	Acetic acid
6	10.826	1100	4509698	0.80	146	79	Ketone
7	11.589	1271	321318185	57.23	150	92	Formic acid
8	12.017	1221	1029146	0.18	204	89	Copaene
9	12.559	1494	9377899	1.67	204	97	Caryophyllene
10	12.718	1440	1439812	0.26	204	92	(E)-beta-Famesene
11	12.850	1410	2716630	0.48	164	95	Trans-Isoeugenol
12	12.948	1579	7505048	1.34	204	95	Hunulene
13	13.198	1216	1775880	0.32	204	95	beta-Copaene
14	13.280	1458	9278524	1.65	204	96	alpha-Famesene
15	13.383	1555	2737145	0.49	206	88	Phenols
16	13.530	1469	3111189	0.55	204	92	Naphthalene
17	13.938	1754	27480493	4.89	264	92	Nerolidyl acetate
18	14.124	2017	950122	0.17	280	92	9-Eicosene
19	14.327	1507	1158076	0.21	220	93	Caryophyllene oxide
20	14.383	1614	946466	0.17	222	93	5-Azulene methanol
21	14.591	1592	1306940	0.23	220	85	12-Oxabicyclopentane
22	14.708	1598	1449917	0.26	222	79	alpha-Acorenol
23	14.764	1626	2249908	0.40	222	95	gama-Eudesmol
24	14.844	1580	2462246	0.44	222	83	1-Naphthalenol
25	15.029	1593	8769873	1.56	222	90	2-Naphthalene methanol
26	15.808	2366	1875504	0.33	312	79	Eicosanoic acid
27	16.012	1854	1543692	0.27	242	86	1-Hexadecanol
28	16.569	1598	3925564	0.70	222	81	2-Naphthalene methanol
29	17.166	1878	917397	0.16	270	92	Hexadecanoic acid
30	17.676	1968	21402416	3.81	256	96	n-Hexadecanoic acid
31	17.772	1978	10252506	1.83	284	93	Hexadecanoic acid
32	18.754	2266	1435791	0.26	334	89	Cyclopropaneoctanoic acid
33	18.813	2085	2313935	0.41	296	89	9-Octadecenoic acid
34	19.470	2183	39205921	6.98	280	90	9,12-Octadecadienoic acid
35	19.554	1808	25243413	4.50	238	87	Cis-9-Hexadecenal
36	19.714	2167	6040055	1.08	284	88	Octadecanoic acid
37	19.782	2177	13569428	2.42	312	89	Octadecanoic acid ethyl ester
38	22.868	2704	1429954	0.25	390	89	Bis(2-ethylhexyl) phthalate
39	24.086	2719	2374997	0.42	376	92	Oxalic acid
40	24.426	3149	3752739	0.67	430	78	Vitamin E
41	24.662	1835	1127741	0.20	254	82	Hexadecanal

Ret. = Retention, Mol. wt. = Molecular weight, SI = Similarity index

DISCUSSION

In this study the percentage yield of the ethyl acetate extracts was 1.6569 %, indicating that the solvent used had low extracting properties. The maceration method used in the extraction may also account for the low yield of the extract. A previous work has shown that methods such as Soxhlet and steam distillation extractions gives higher extractive yields from plant seeds (Carvalho *et al*., 2012). Polarity is also an important factor to consider as polar solvents tend to give a higher yield than non-polar solvents (Zhang *et al.,* 2018).

Susceptibility of a test microorganism to an antimicrobial agent is evident by the presence and sizes of growth inhibitory zones on seeded agar plate. This zone is measured as an index of the killing or inhibitory action of the extract against the test microorganism. In this screening test, the inhibition zone diameters (IZDs) ranged between 10 - 25 mm, making the ethyl acetate extract of *Dennettia tripetala* fairly active against *Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Bacillus subtilis, Klebsiella spp and Candida albicans* but not against *Aspergillus niger*. Extracts are considered to be active at inhibition zone diameters > 10 mm (EUCAST, 2017). It was observed that the inhibition zone diameters displayed by the seed extract were smaller compared to those exhibited by ciprofloxacin and fluconazole against test bacteria and fungus respectively. This may be attributable to the crude nature of the extract as compared to ciprofloxacin and fluconazole which are already in their pure forms. Previous researchers have found that the essential oil of *Dennettia tripetala* can inhibit the growth of food-borne microorganism such as *Staphylococcus aureus, Salmonella spp, E. coli* and a host of others (Okoh *et al*., 2016).

The GCMS analysis of the extract identified about 41 compounds with the most abundant (over 50 %) being formic acid, a fatty acid ester. Though other fatty acids such as octadecadienoic, n-hexanoic and octadecanoic acids were present in the extract, but their levels were not significant. Results from previous works show an abundance of fatty acids from the methanol, ethanol and benzene extracts of the leaves and whole fruit of *Dennettia tripetala* (Okoronkwo *et al*., 2015; Larayetan *et al*., 2018). The whole fruit methanol extract analysis revealed the presence of eighteen (18) compounds with the major constituent being oleic acid, palmitic acid, stearic acid, estragole and phytol while the methanol, ethanol and benzene extracts of the leaves showed sixteen (16), fifteen (15) and five (5) compounds, respectively with the presence of myristic, palmitic, palmitoleic, oleic and linolenic acids in varying amounts common to all the extracts. Therefore, the high number and different constituents found in the ethyl acetate extract of the seed of *Dennettia tripetala* may be attributable to the presence of different constituents in different parts of the plant and the type of solvent and method used in the extraction process (Zhang *et al*., 2018; Nguyen *et al*., 2019). Furthermore, the antimicrobial activity of the extract may be traced to the fatty acid components in the extract identified by the GCMS analysis. The eight (8) fatty acid methyl esters which accounted for 18.49 % of the total constituents of the extract have been known to exhibit antibacterial action (Chandrasekran *et al*., 2011). Previous works have shown that fatty acids possess antibacterial properties as bioactive fraction of hexadecanoic, octadecanoic and oleic acids possess antibacterial activity against a variety of bacteria (Okoh *et al*., 2016; Larayetan *et al*., 2018). This may be due to the ability of fatty acids to intercalate into the bacterial cell membrane causing increased fluidity, permeability changes and consequently the unstable bacterial cell lyses. Release of lethal secondary degradation products of fatty acid peroxidation like hydrogen peroxide and reactive oxygen species may also be responsible for the observed antibacterial action (Braide *et al.,* 2012). Additionally, auto oxidation of unsaturated fatty acids creates short chain aldehydes which have antibacterial properties on their own (Hassan *et al*., 2011).

Vitamin E is a family of lipophilic antioxidants in cellular membrane that scavenge reactive oxygen species. In aerobic microorganisms, oxygen is the terminal electron acceptor in the generation of energy, thus, the non-availability of this oxygen as a result of the antioxidant activity of vitamin E inhibits the generation of energy and consequently reduces metabolic activity of the bacterial cell leading to bacteriostasis. Since growth inhibition cannot continue indefinitely, the growth inhibited bacterium eventually dies (bactericidal action). Thus, presences of this free radical scavenger in our extract may in part account for the observed inhibitory action. Nerolidyl compounds are naturally occurring sesquiterpene alcohol found in the essential oils of many plants and flowers and they are used as flavoring agents and in perfumery. They are also known for their various biological activities

including antioxidant, antifungal, anticancer and antimicrobial activity (Chan *et al*., 2016). The presence of this compound in the extract may be responsible for the distinct odor of the pepper fruit seed and also confer some antimicrobial property.

Formic acid which accounted for 57.23 % of the chemical constituent of our extract has been reported to show high antibacterial activity against enteric bacteria. This is in line with our findings where larger inhibition zones (20 - 25 mm) were observed against all the Gram negatives. This is due to the ability of this short chain molecule to readily diffuse through the protein channels embedded in the lipid bilayer of the Gram-negative bacteria (Stanojevic-Nikolic *et al*., 2016). Accordingly, our extract showed higher antibacterial activity against *E. coli, Klebsiella spps* and *Pseudomonas aeruginosa* with MIC of 12.5 mg/mL.

This extract was active against *Candida albicans*. Previous reports have demonstrated that low concentrations of formic acid do induce apoptosis-like programmed cell death in *Candida* with minimum lethal effect on mammalian cells (Lastauskienė *et al*., 2014). This extract was however not active against *Aspergillus niger*. This fungus has increased resistance to many antifungal agents through mechanisms of resistance such as increased drug efflux, alteration in the drug target site and development of compensatory pathways (Berkow and Lockhart, 2017).

CONCLUSION

The observed antibacterial activity of these seeds has established a scientific basis for its traditional uses in treatment of infections and consequently laid down a platform in the search for a lead molecule that could be a potential antibacterial and antifungal agent of natural origin. Further study is however required to isolate the active principle from the seeds for the development of a new antimicrobial agent.

Conflict of Interest

The authors have no conflict of interest to declare

REFERENCES

Afolayan, A.J. and Meyer, J.M. (1997). The antimicrobial activity of 3,5,7-trihydroxyflavone isolated from the shoot of *Helichrysum aureonitens*. *Journal of Ethnopharmacology*. **57**: 177-181.

Akinbuluma, M.D., Adepetun, M.T. and Yeye, E.O. (2015). Insecticidal effect of ethanol extracts of *Capsicum frutescens* and *Dennettia tripetala* against *Sitophilus zeamais* Motschulsky on stored maize. *International Journal of Research in Agriculture and Forestry*. **2**: 1-6.

Berkow, E.L. and Lockhart, S.R. (2017). Fluconazole resistance in *Candida* species: A current perspective. *Infection and Drug Resistance*. **10**: 237-245.

Braide, W., Odiong, J. and Oranusi, S. (2012). Phytochemical and antibacterial properties of the seeds of watermelon (*Citrullus lanatus*). *Prime Journal of Microbiology Research*. **2**: 99-104.

Carvalho, R.H.R., Galvão, E.L., Barros, J.Â.C., Conceição, M.M. and Sousa, E.M.B.D. (2012). Extraction, fatty acid profile and antioxidant activity of sesame extract (*Sesamum indicum* L.). *Brazilian Journal of Chemical Engineering*. **29**: 409-420.

Chan, W.K., Tan, L.T., Chan, K.G., Lee, L.H. and Goh, B.H. (2016). Nerolidol: A sesquiterpene alcohol with multi-faceted pharmacological and biological activities. *Molecules*. 21: 529.

Chandrasekaran, M., Senthilkumar, A. and Venkatesalu, V. (2011). Antibacterial and antifungal efficacy of fatty acid methyl esters from the leaves of *Sesuvium portulacastrum* L. *European Review for Medical and Pharmacological Sciences.* **15**: 775-780.

Clinical and Laboratory Standards Institute (2015). Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard - Tenth Edition. CLSI document M07-A10. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania, USA.

European Committee on Antimicrobial Susceptibility Testing Breakpoint Table for Interpretation of MIC and Zone of Inhibition Diameter. Version 7.0, 2017.

Hassan, L.A., Hasnah, M.S., Sakina, M.A., Waleed, S.K. and Abdelwahab, S.I. (2011). *In vitro* antimicrobial activities of

chloroformic, hexane and ethanolic extracts *of Citrullus lanatus var. citroides* (wild melon). *Journal of Medicinal Plant Research*. **5**(8): 1338-1344.

Ikpi, D. and Nku, C. (2008). Effect of ethanolic extract of *Dennettia tripetala* fruit on haematological parameters in albino Wistar rats. *Nigerian Journal of Physiological Sciences*. **23**: 13-17.

Iseghohi, S.O. (2015). A review of the uses and medicinal properties of *Dennettia tripetala* (pepperfruit). *Medical Sciences* (Basel, Switzerland). **3**(4): 104-111.

Jorgensen, J.H. and Turnidge, J.D. (2007). In: Manual of Clinical Microbiology (Murray, P.R., Baron, E.J., Jorgensen, J.H., Landry, M.L. and Pfaller, M.A., Eds.), 9th edn. ASM Press, Washington D.C., pp. 1152-1172.

Larayetan, R., Osanekwu, S. and Sokwo, M. (2018). Phytochemical components of methanolic fruit extract of *Dennettia tripetala*. *Journal of Functional Materials and Biomolecules*. **2**(2): 48-52.

Lastauskienė, E., Zinkevičienė, A., Girkontaitė, I., Kaunietis, A. and Kvedarienė, V. (2014). Formic acid and acetic acid induce a programmed cell death in pathogenic *Candida* species. *Current Microbiology*. **69**(3): 303-310.

Nguyen, D.H., Anh Ta, N.T., Bui, A.V., Do, T.H. and Nguyen, H.C. (2019). Evaluation of the use of different solvents for phytochemical constituents, antioxidants and *in vitro* anti-Inflammatory activities of *Severinia buxifolia*. *Journal of Food Quality*. Article ID 8178294.

Nwachukwu, E.O. and Osuji, J.O. (2008). Evaluation of plant extracts for antifungal activity against *Sclerotium rolfsii* causing cocoyam cormel rot in storage. *Research Journal of Agriculture and Biological Sciences*. **4**: 784-787.

Okigbo, B.N. (1980). *Plants and Food in Igbo Culture*. Ahiajoku Lecture, Published by Cultural Division, Ministry of Information, Culture, Youth and Sports, Owerri Imo State, Nigeria.

Okoh, S.O., Iweriegbor, B.C., Okoh, O.O. Nwodo, U.U. and Okoh, A.I. (2016). Bactericidal and antioxidant properties of essential oils from the fruits *Dennettia tripetala* G. Baker. *BMC Complementary and Alternative Medicine*. **16**: 486.

Okoronkwo, N.E., Ajomiwe, M.O. and Ike-Amadi, C.A. (2015). Comparative study of fatty acid composition of *Dennettia tripetala* leaves extracted with different solvents. *Chemistry Journal*. **1**(2): 35-40.

Okwu, D.E. and Morah, F.N.I. (2004). Mineral and nutritive value of *Dennettia tripetala* fruits. *Fruits* **59**: 437-442.

Okwu, D.E., Morah, F.N.I. and Anam, E.M. (2005). Isolation and characterization of phenanthrenic alkaloid uvariopsine from *Dennettia tripetala* fruits. *Journal of Medicinal and Aromatic Plants.* **27**: 496-498.

Oyemitan, I.A., Iwalewa, E.O., Akanmu, M.A. and Olugbade, T.A. (2008). Antinociceptive and anti-inflammatory effects of essential oil of *Dennettia tripetala* G. Baker (Annonaceae) in rodents. African Journal of Traditional, *Complementary and Alternative Medicines*. **5**: 355-360.

Stanojevic-Nikolic, S., Dimic, G., Mojovic, L., Pejin, J., Djukic-Vukovic, A. and Kocic-Tanackov, S. (2016). Antimicrobial activity of lactic acid against pathogen and spoilage microorganisms. *Journal of Food Processing and Preservation*. **40**: 990-998.

Zhang, Q.W., Lin, L.G. and Ye, W.C. (2018). Techniques for extraction and isolation of natural products: A comprehensive review**.** *Chinese Medicine*. **3**:20