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Evaluation of Chemical Compositions and *in vitro* Antimicrobial Activity of Extracts from *Dennettia tripetala* Leaves

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Abstract

Dennettia tripetala plant is commonly used in the south eastern Nigeria as appetite stimulant, cough suppressant and to stop excess saliva. This work evaluated the chemical compositions and *in vitro* microbial activity of leave extracts of the plant. The result of present study revealed the presence of alkaloid (26.14 ± 0.6%), flavonoid (21.0 ± 2.0%), saponnins (3.77 ± 0.7%), phenols (0.33 ± 0.29%) and tannins (0.68 ± 0.17%) in the leaves. The vitamin, proximate and mineral compositions showed appreciable amount of the parameters determined. Ascorbic acid had the highest content (20.0 ± 4.1), followed by thiamin (2.01 ±0.08%), while niacin was the least (0.43 ± 0.03%). The *in vitro* antibacterial activity results showed that both the water and ethanol extracts exhibited activity against *Escherichia coli*, *Proteus mirabilis*, *Klebsiella pneumonia* and with no activity observed on the strains of *Pseudomonas aeruginosa*. Eleven fatty acids were identified from the methanol extract subjected to GC/MS analysis. Palmitic acid had the highest composition of 26.17% while caprylic acid had the least (0.48%). The result of the chloroform extract on GC/MS analysis showed seventeen peaks indicating the presence of seventeen (17) compounds. Compound 7, an alcohol with molecular formula $C_{20}H_{40}O$ (m/z 296), for which the base peak occurred at m/z 43 (C_3H_7) and identified as (2E)-3,7,11,15-tetramethyl-2-hexadecen-1-ol had the highest percentage composition of 19.93%; while compound 3 had the least percentage composition of 1.53% and was identified as menth-1,3,8-triene with molecular formula $C_{10}H_{14}$ (m/z 134). The research findings justify some of the traditional uses of the plant leaves.

Keywords

Bacteria, Bioactive, *Dennettia tripetala*, Fatty Acids, Leaves

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1. Introduction

Plants constitute very important part of human diet in the form of spices, herbs, vegetables, fruits, etc. Condiments and spices are products of plants which are mostly used for seasoning and flavouring thereby enhancing the taste of foods, beverages and drugs. The chemical compounds present in food like spices, condiments, herbs, etc have been revealed by researchers to have protective effects evidenced by decrease in the risk of certain diseases and health problems (Salah *et al.*, 1995, Del-Rio *et al.*, 1997 and Okwu and Ekeke, 2003). It is used as anti-inflammatory and antinociceptive (Oyemitan *et al.,* 2008).

The important elements and/or compounds present in food as nutrients have nutritional, medicinal, refreshing properties etc. They could also be used as condiments and flavouring agents (Gordon and Weny, 1992; Stray, 1998; Okwu and Emenike, 2006). Moreover, plants serve as one of the sources of fatty acids and essential oils. Most naturally occurring fatty acids have a chain of even numbers of carbon from 4 to 28 (Bruice, 2007).

Dennettia tripetala (annonaaceae) is a plant found mostly in

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tropical rainforest region and sometimes in the Savannah area. It is commonly known as pepper fruit. It has alternate simple and estipulate leaves which are often fragmented and usually with smooth margins. The young leaves have spicy taste and are sometimes used to prepare 'pepper soup'. The leaves and seeds have been found to be useful in the treatment of cough, fever and are also used in enhancing appetite, clear throats, check excess saliva, relieve coated tongues and stop nausea. The leaves and fruits are used with other herbs for the treatment of cough, infantile convulsion, vomiting, worm infestation and typhoid (Ejechi and Akpomedaye, 2005; Ukeh *et al.*, 2012). The essential oil has been reported to inhibit the growth of tomato-rot fungi (Ejechi *et al*., 1999). Onyechi *et al.* (2013) have made use of the fruits in the formulation of herbal tablets by direct compression. Taking the medicinal role of *D. tripetala* into consideration, this research is therefore aimed at evaluating the chemical compositions and antibacterial activity of the leaf extract against some selected bacterial strains and characterising the bioactive compounds using GC/MS analysis for the possible use of the plant's leaves in the development of new drug for the treatment and prevention of infections.

2. Materials and Methods

The *D. tripetala* leaves used for this research were gotten from Umuekeogo Ogbor Ovuru in Abo Mbaise Local Government Area of Imo State, Nigeria. The green leaves were detached from the stem, air-dried and ground into powdered form. The powder was then stored in an air tight container until required for analysis.

2.1. Phytochemical Analysis

The determination of alkaloid, saponin and phenol was done according to the method of Harborne (1973) as described by Obadoni and Ochuko (2001). Tannin determination was done by the method of Van Burden and Robinson (1981) and flavonoid determination was carried out according to Boham and Kocipal (1974) method [cited in Okoronkwo *et al*., 2011].

2.2. Proximate Analysis

The moisture, ash, crude lipid, crude fibre and protein content determinations were done by the recommended methods of Association of Official Analytical Chemists (AOAC, 1999).

The energy value was estimated as described by Asibey-Berko and Tayle (1999) in KCal/100g by multiplying the percentage crude protein, crude lipids (fats) and carbohydrate by recommended factors 2.44, 8.37 and 3.57 respectively as used in vegetable analysis,

$$FE = (\%CP \times 2.44) + (\%Lipids \times 8.37) + (\%CHO \times 3.57)$$

where, FE = Food Energy (g/cal); CP = Crude Protein; CHO = Carbohydrate

2.3. Vitamin Analysis

The determination of vitamin A was done by the method of Davie (1976), thiamine (B_1), riboflavin (B_2) and niacin (B_3) were estimated by spectrophotometric method; while vitamin C (ascorbic acid) was calculated titrimetrically by the method of Barakat *et al*. (1973) [cited in Okoronkwo *et al*., 2011]

2.4. Mineral Determination

2 g of the plant sample was weighed and subjected to dry ashing in a well cleaned crucible at 550^{o}C in a muffle furnace. The resultant ash was dissolved in 5 ml of HNO_3/HCl/H_2O (1:2:3) and heated gently on a hot plate until brown fumes disappeared. The mineral solution in the crucible was transferred into a 100 ml volumetric flask by filtration through a Whatman No. 42 filter paper and the volume was made up to mark with deionized water. The solution was used for elemental analysis by atomic absorption spectrophotometer (Shahidi *et al.*, 1999).

Phosphorus content of the sample digest was determined colorimetrically by the method of Naphapetian and Bassiri (1975).

2.5. Antibacterial Activity Test

The ethanol and aqueous extracts of the plant samples were prepared by cold percolation method. 20 g of each of the pulverized samples was soaked in different 200 ml of ethanol and 200 ml of water respectively for 48 hrs for optimum extraction with intermittent shaking to get a concentration of 10%, after which they were filtered with Whatman paper No. 1 into vials. The filtrate of each extract was dried until a constant dry weight of each extract was obtained. The extracts were stored at 4^{o}C for further use.

Nutrient agar was used as medium in the antibacterial assay. The cultures were diluted with fresh nutrient agar to achieve optical density corresponding to 2.0 x 10^6 colony forming units (CFU/ml) for bacteria. The ethanol and water extracts of the samples were screened against a total of five bacterial strains. The test bacteria organisms were *Staphylococcus aureus, Proteus mirabilis, Klebsiella pneumonia*, *Pseudomonas aeruginosa* and *Escherichia coli,* which were collected from the stock culture of the Microbiology Laboratory of the Federal Medical Centre Umuahia, Abia State, Nigeria. The screening activity was performed by filter paper disc method (Valsaraji, *et al.*, 1997).

The antibacterial assay was done by preparing the plates which were inoculated in the screening test 0.1g of dried

ethanol and water extract respectively dissolved in 0.9 ml of ethanol and water to get concentrations of 0.1 g/ml. Standard solution containing 0.1 g/ml ciprofloxacin was also prepared. About 0.2 ml plant extract, ethanol, water and standard ciprofloxacin were loaded on separate 4 mm sterile disc with ethanol and water respectively served as controls for ethanol and water extracts.

Standard ciprofloxacin disc was used to compare the efficacies of the antibacterial activities of the extracts. A loaded disc was placed on the surface of inoculated medium and the substance allowed to diffuse for 5 minutes and plates were incubated for 24 hours at 37^{o}C. At the end of incubation, inhibition zones formed around the discs were measured with transparent ruler in millimetre. The experiment was performed in triplicate and the mean value was recorded.

2.6. Extraction of Leaves of *D. tripetala* for Fatty Acid Characterisation

The extraction of the crude sample from the leaves of the plant sample was done using methanol. 15 g of ground leaves was put into conical flask to which 150 ml of methanol was added and then allowed to stand for 24 hours. It was then filtered and filtrate was then allowed to evaporate to dryness. The extract was subjected to Gas Chromatography/Mass spectrometer (Hewlett Packard 6890 series, England) adapted only for the determination of fatty acid composition.

2.7. Extraction of Leaves for Other Bioactive Components for GC/MS Characterisation

The extraction of the crude sample from the leaves of the plant sample was done by cold percolation using chloroform. 15 g of ground *Dennettia tripetala* leaves was put into conical flask to which 150 ml of chloroform was added and left to stand for 24 hrs. It was then filtered and the residue was re-soaked for another 24 hrs with fresh 150 ml of chloroform and again filtered for total extraction of the plant material. The filtrate was then allowed to evaporate to dryness under room temperature. The extract was then subjected to Gas Chromatography/Mass Spectrometer (GC/MS) analysis.

The compounds present were identified by matching the peaks with computer Wiley MS libraries and confirmed by comparing mass spectra of the peaks and those from literatures.

3. Results and Discussion

3.1. Phytochemical Analysis

The result of the phytochemical composition of the *Dennettia tripetala* leaves (Table 1) showed that alkaloids had the highest content by composition with a value of 26.14%, followed by flavonoid with a value of 21.0%, while phenol had the least with a value of 0.33%.

Table 1. Phytochemical Composition of *Dennettia tripetala* leaves.

Phytochemicals	Composition (%)
Alkaloid	26.14 ± 1.0
Phenol	0.33 ± 0.29
Saponin	3.77 ± 0.7
Tannin	0.68 ± 0.17
Flavonoid	21.0 ± 3.5

Each data is the mean of three replicates ± standard deviation

The high content of alkaloid found in this part of the plant indicates its potential use as basic medicinal agent. Most plants used in curing diseases especially malaria have been reported to contain alkaloid (Okwu and Okwu, 2004; Stray, 1998). It could further be used as disinfectant due to the presence of phenols since phenols are used as cleaning agents (Urguiaga and Lagton, 2000). Saponins have useful biological activities which include antimicrobial and anti-inflamatory effects (Okwu, 2001; Sodipo *et al*., 2000). Tannins are used to hasten wound healing due to its antiseptic nature. It also helps to provide resistance against parasites to human bodies (Tiger, 1998). Flavonoids are water soluble antioxidants. They stop free radicals effect, prevent oxidative damage of the cell, have strong anticancer activities and protect the body against all stages of carcinogen (Wang, 2000; Birt *et al*., 2001).

3.2. Vitamin and Proximate Composition of Leaves

The results of the vitamins and proximate compositions are shown in Table 2. It showed that this part of the plant is a good source of some quality food nutrients. It is not only sufficiently rich in calories but also contains other essential nutrients such as proteins, vitamins and minerals.

Table 2. Vitamin and Proximate Composition of *D. tripetala* leaves.

Parameter	Composition
Thiamine (vitamin B_1) (mg/100g)	2.01 ± 0.08
Riboflavin (vitamin B_2) (mg/100g)	1.86 ± 0.02
Niacin (vitamin B_3) (mg/100g)	0.43 ± 0.03
Ascorbic acid ((vitamin C) (mg/100g)	20.0 ± 4.1
Crude fibre (%)	8.7 ± 1.2
Moisture (%)	9.3 ± 0.7
Ash (%)	6.2 ± 0.3
Fat (Lipid) (%)	18.2 ± 0.7
Protein(%)	0.7 ± 0.2
Carbohydrate (%)	56.9 ± 1.76
Energy value (g/cal)	393 ± 9.3

Each data is the mean of three replicates ± the standard deviation

Plant leaves are good source of vitamins. The ascorbic acid had the highest content of 20.0±4.1%, followed by thiamine

with a value of 2.01 ±0.08% and the least, niacin with a value of 0.43±0.03%. Ascorbic acid has many functions which include prevention of cell damage and healing of wounds etc; and leafy vegetables are among its major sources. The presences of other vitamins such as riboflavin, thiamine and niacin which are always in trace amount are essential for the body metabolism.

The percentage compositions of the protein, carbohydrate and fat contents were 0.7±0.2, 56.9±1.76 and 18.2±0.7 respectively. The presence of crude fibre in the plant leaves indicates that it might act as an index in the intestine by absorbing toxins and carrying out harmful substances (Roger, 2002 and Akobundu, 1999). The carbohydrate content acts as a mild sweetener and also adds to the bulk of the diet (Roger, 2002).

3.3. Mineral Composition of Leaves

Result of the mineral compositions indicated that calcium had the highest content, followed by zinc (Table 3). Cobolt was not detected in the plant sample. The high content of calcium in the plant leaves is necessary for blood coagulation and formation of bone cells. The presence of zinc could mean that the leaves can play a valuable role in the management of diabetes. Calcium and phosphorus are associated with each other for growth and maintenance of bones, teeth and muscles (Turan *et al*., 2003 and Dosunmu, 1997).

Table 3. Mineral composition of *D. tripetala* leaves.

Mineral Elements	Composition (mg/100g)
Calcium (Ca)	1760.0
Cadmium (Cd)	0.01
Cobalt (Co)	Not Detected
Copper (Cu)	3.85
Iron (Fe)	10.41
Potassium (K)	10.40
Magnesium (Mg)	25.02
Sodium (Na)	0.75
Lead (Pb)	0.01
Zinc	18.36
Phosphorus (P)	3.85

Iron is a component of haemoglobin and its concentration (mg/100g) was 10.41. It also helps in oxygen transportation. The concentration of sodium was low and this might be an advantage due to direct relationship of sodium intake with hypertension in humans (Dahl, 1974). Phosphorus and magnesium in the plant leaves are required by the body for healthy living (Okwu, 2005).

3.4. Antibacterial Activities

The result of the inhibitory activities of the ethanol and water extracts of the leaves relative to that of the standard is shown in Table 4.

The results of the *in vitro* antibacterial activity against some selected bacteria showed that the tested bacterial strains were sensitive to the extracts. The growth inhibition zones and efficacies of extracts relative to standard antibacterial agents showed that the water extracts of the sample was active (14 mm) than ethanol extract (10 mm) against *Escherichia coli*.

Table 4. Antibacterial zone of inhibition (mm) of *D tripetela* leaves extracts.

Bacterial strains	Standard Inhibition	Ethanol	Water
Escherichia coli	20.0	10.0	14.0
Staphylococcus aureus	21.0	-	6.0
Proteus mirabilis	19.8	10.0	8.0
Pseudomonas aeruginosa	17.0	-	-
klebsiella pneumonia	21.0	12.5	7.0

Both ethanol and water extracts of the sample showed activity of 10.0 mm and 8.0 mm respectively on *Proteus mirabilis* and 12.5 mm and 7.0 mm respectively on *klebsiella pneumonia*. Furthermore, only the water extract exhibited activity against the *Staphylococcus aureus* strains and there was no activity observed on the strains of *Pseudomonas aeruginosa*.

The observed inhibitory role of the extracts explain the reasons for the use of plant leaves in traditional medicine as cough suppressant, clearing of the throat, checking excess saliva, relieving coated tongue and stopping nausea (Ejechi *et al*., 1999). Also, the phytochemicals present are capable of inhibiting the growth of microorganisms. Plant extracts have been used medicinally to inhibit microbial activities and extracts of the different parts of most plants are active against some bacteria (Obomanu *et al.*, 2005) and there are evidences that increasing number of people across the globe depend on traditional herbal remedies for health care (Saeed *et al*., 2004).

3.5. Fatty Acid Characterisation

The chromatogram of GC/MS characterisation of fatty acid composition of methanol extract of *Dennettia tripetala* leaves is shown in Fig.1 and Table 5 and from the result, the following fatty acids were identified: caprylic, capric, lauric, myristic, myristol, palmitic, palmitoleic, stearic, oleic, linoeic and linolenic acids. Palmitic acid had the highest concentration with a value of 26.1711% at the retention time of 8.247 mins while caprylic acid had least the percentage concentration with a value of 0.484% at the retention time of 1.76 mins.

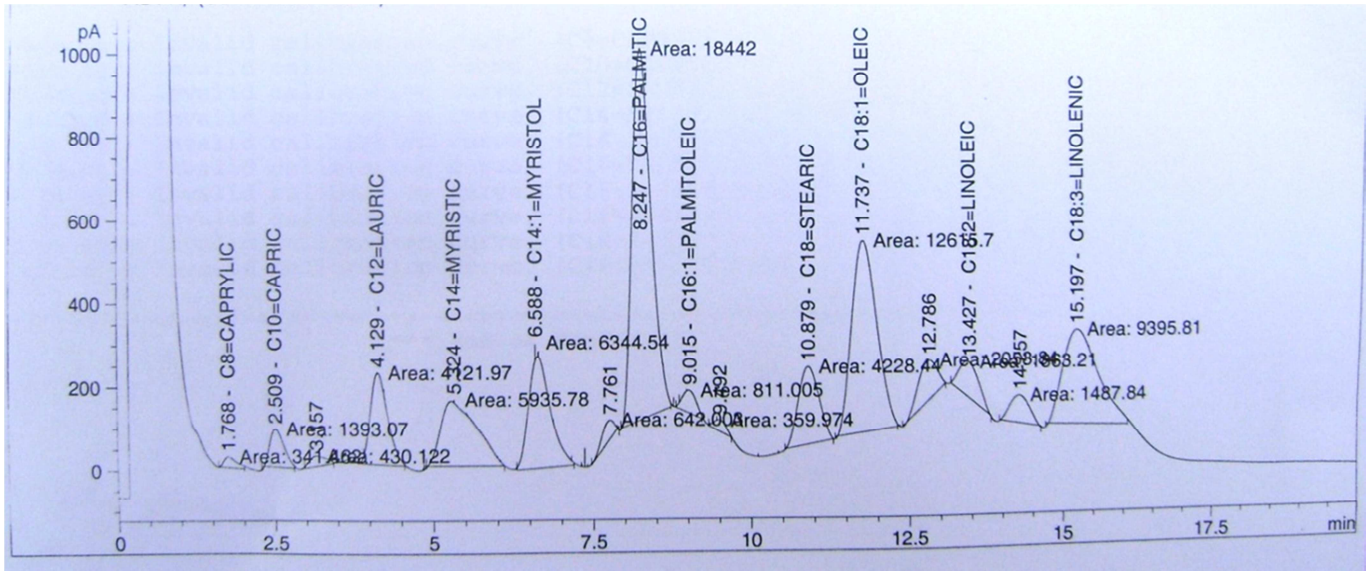


Fig. 1. Chromatogram of GC/MS characterisation of methanol extract of *Dennettia tripetala* leaves.

Table 5. Fatty Acid Composition of *Dennettia tripetala* leaves.

S.No.	Trival Name	IUPAC Name	Retention Time (mins)	Area (%)	Molecular weight (g/mol)	Molecular formula
1	Caprylic	Octanoic acid	1.768	0.4845	144.21	$C_8H_{16}O_2$
2	Capric	Decanoic acid	2.509	1.9769	172.26	$C_{10}H_{20}O_2$
3	Lauric	Dodecanoic acid	4.129	5.8495	200.32	$C_{12}H_{24}O_2$
4	Myristic	Tetradecanoic acid	5.324	8.4235	228.37	$C_{14}H_{28}O_2$
5	Myristoleic	Tetradec-9-enoic acid	6.588	9.0035	226.36	$C_{14}H_{26}O_2$
6	Palmitic	Hexadecanoic acid	8.247	26.1711	256.42	$C_{16}H_{32}O_2$
7	Palmitoleic	Hexadec-9-enoic acid	9.015	1.1509	254.41	$C_{16}H_{30}O_2$
8	Stearic	Octadecanoic acid	10.879	6.0006	284.48	$C_{18}H_{36}O_2$
9	Oleic	Cis-9- Octadecenoic acid	11.737	17.9030	282.46	$C_{18}H_{34}O_2$
10	Linoleic	Cis, cis-9,12- Octadecadienoic acid	13.427	2.6441	280.45	$C_{18}H_{32}O_2$
11	Linolenic	Cis, cis-9,12,15- Octadecatrienoic acid	15.197	13.3336	278.43	$C_{18}H_{30}O_2$

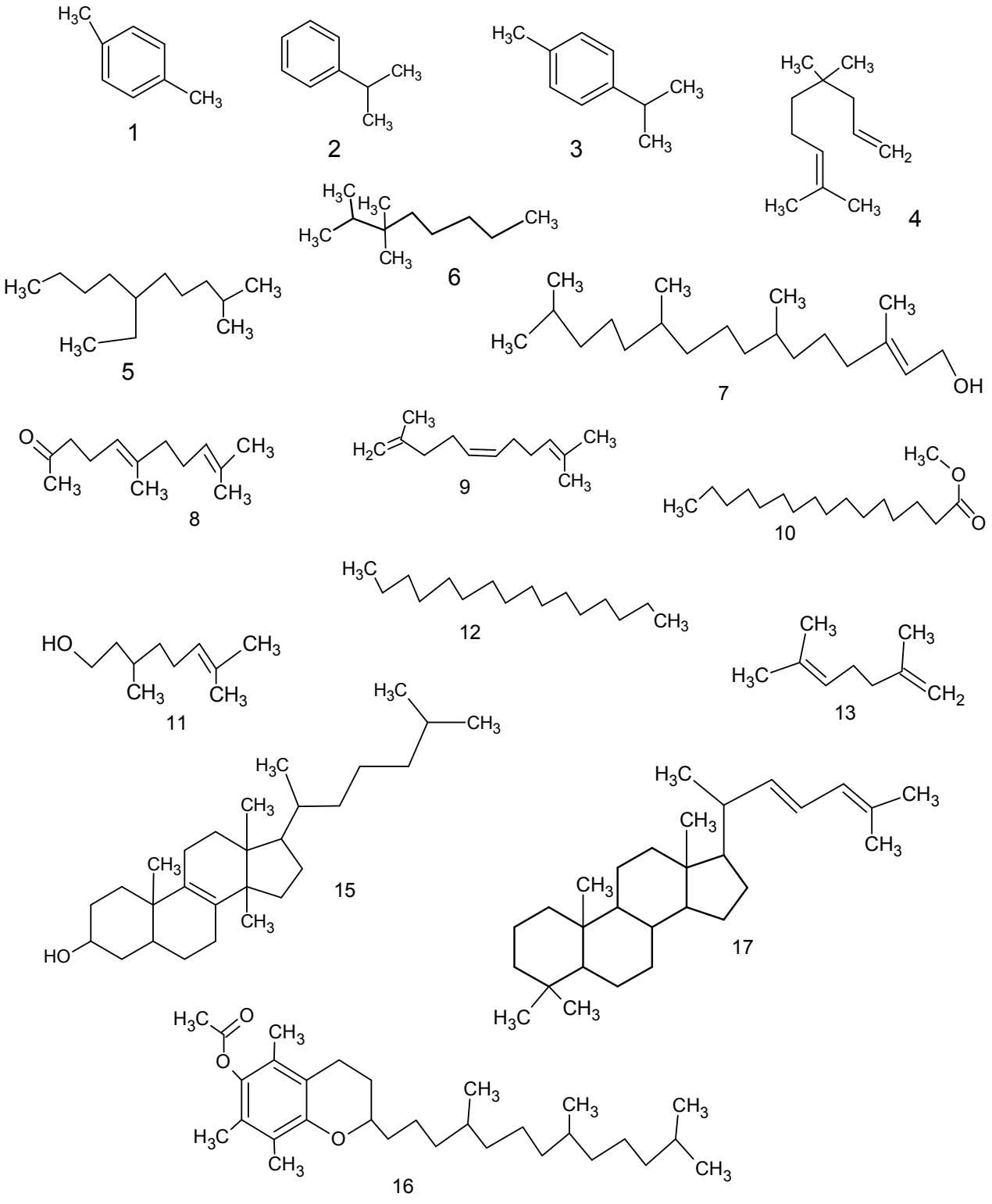
Caprylic acid (octanoic acid) is used in the treatment of some bacterial infections. Capric acid, a saturated fatty acid is used in organic synthesis and industrially in the manufacture of pharmaceuticals. Lauric acid is used in the manufacture of flavourings, cocoa butter, margarine, soaps, shampoos and other surface active agents (Nakatsuji *et. al*., 2009). Myristic acid is used in cosmetics and medicinal preparations. Palmitic acid increases the risk of developing cardiovascular diseases. Palmitoleic acid helps to reduce weight gain which in turn combats obesity (Nestel *et al*., 1994). Oleic acid is used as an insect pheromone and linoleic acid is one of the essential fatty acids that human and animals ingest for good health (Cunnane and Anderson, 1997). It is used in the treatment of dermatitis and also in the making of soap emulsifiers and quick drying oil. Linoleic acids have anti-inflammatory, acne reductive and moisture retentive properties when applied on the skin (Beare-Rogers *et* al., 2001).

3.6. Identified Bioactive Compounds from the Chloroform Extract

The result of chloroform extracts of the sample on GC/MS analysis showed seventeen peaks indicating the presence of seventeen (17) compounds in the extract (Table 6) and their structures shown in scheme 1.

The first three compounds of the chloroform extract (compounds 1 – 3) were hydrocarbons and their percentage composition were 7.67, 4.60 and 1.53% which were identified as 1,4-dimethyl benzene, isopropyl benzene and menth-1,3,8-triene with molecular formula C_8H_{10} (m/z 106), C_9H_{12} (m/z 120) and $C_{10}H_{14}$ (m/z 134) respectively. The various base peaks of the three compounds were observed at m/z 90, 105 and 119 respectively. The fourth compound is an alcohol. Its constituent in the extract was 21.5% with a molecular formula $C_{11}H_{20}O$ (m/z 168) and base peak of m/z 43. Compound 5 and 6 were also hydrocarbon with

molecular formula $C_{13}H_{28}$ (m/z 184) and $C_{11}H_{24}$ (m/z 156) respectively. Compound 5 was identified as 6-ethyl, 2-methyl decane that had a base peak at m/z 57 which resulted due to cleavage at C_4H_9 (m/z 57). Other prominent peaks occurred at m/z 43 (C_3H_7), m/z 71 (C_5H_{11}) and m/z 84 (C_6H_{13}). These similar cleavages were observed for compound 6 which was identified as 2,3,3-trimethyl octane. Compound 7 is an alcohol with molecular formula $C_{20}H_{40}O$ (m/z 296). This is the compound with the highest percentage composition (19.93%). Its base peak occurred at m/z 43 (C_3H_7) and was identified as (2E)-3,7,11,15-tetramethyl -2-hexadecen-1-ol.



Scheme 1. Structures of identified compound from the chloroform extract of the leave.

Table 6. Identified Bioactive compounds from the chloroform extract of *D. tripetala* leaves shown by GC/MS analysis.

S.No.	Retention Time (mins)	% Composition	Molecular weight	Molecular formula	IUPAC Name
1	4.2	7.67	106	C_8H_{10}	1,4 dimethyl benzene
2	6.4	4.60	120	C_9H_{12}	[1-methyl ethyl] benzene
3	8.3	1.53	134	$C_{10}H_{14}$	p-menthan-1,3,8-triene
4	14.8	2.15	168	$C_{11}H_{20}O$	4,8-dimethyl, -1,7-nondien-4-ol
5	18.5	2.45	184	$C_{13}H_{28}$	6-ethyl-2-methyl decane
6	23.2	4.60	156	$C_{11}H_{24}$	2,3,3-trimethyl octane
7	23.9	19.93	296	$C_{20}H_{40}O$	(2E)-3,7,11,15-tetramethyl -2-hexadecen-1-ol
8	24.9	11.04	194	$C_{13}H_{22}O$	(SE)-6-,10-dimethyl-5,9-undecadien-2-one
9	26.1	1.97	192	$C_{14}H_{24}$	(SZ)-2,6,10-trimethyl-1.5,9-undecatriene
10	27.9	1.97	268	$C_{17}H_{32}O_2$	7-Hexadecenoic acid
11	28.1	7.67	159	$C_{10}H_{20}O$	3,7-dimethyl-6-octen-1-ol
12	29.5	4.60	226	$C_{16}H_{34}$	n-hexadecane
13	32.4	3.07	124	$C_{10}H_{14}$	2,6-dimethyl-1,5-heptadiene
14	33.3	3.99	-	-	-
15	38.1	3.37	400	$C_{28}H_{48}O$	Cholest-8-en-3-ol
16	40.9	3.68	472	$C_{31}H_{52}O_3$	Vitamin E acetate
17	43.7	3.68	412	$C_{29}H_{48}O$	4,4-dimethyl-cholesta-22,24-dien-5-ol

Compound 8 is a ketone with molecular formula $C_{13}H_{20}O$ (m/z 194) and the base peak at m/z 43 (C_3H_7). This compound constituted 11.04% which is the second highest composition. It was identified as (SE)-6-,10-dimethyl-5,9-undecadien-2-one. Compound 9 is a hydrocarbon that comprised of 1.97% with molecular formula $C_{14}H_{24}$ (m/z 194) and base peak at m/z 69 and was identified as (SZ)-2,6,10-trimethyl-1.5,9-undecatriene. Compound 10, a fatty acid had approximately percentage composition of 1.97% similar to compound 9 but with molecular formula of $C_{17}H_{32}O_2$ (m/z 268). Its base peak occurred at m/z 55. Compound 11 was third to the largest composition of all the compounds with a value of 7.67%. This compound is an alcohol with molecular formula $C_{10}H_{20}O$ (m/z 159); its base peak occurred at m/z 71 and was identified as 3,7-dimethyl-6-octen-1-ol. Compound 12 is a normal straight chain hydrocarbon which was identified as n-hexadecane with molecular formula $C_{16}H_{34}$ (m/z 226) and base peak at m/z 57. The percentage composition of this compound in the extract was 4.60%. Compound 13 is a branched chain alkene with a yield of 3.07% and a molecular formula of $C_{10}H_{14}$ (m/z 124). Compound 14 had a percentage composition of 3.99% and its base peak occurred at m/z 57, but the compound was not fully identified due to lack of authentic library spectra of the corresponding compound because identification of compounds were carried out by searching commercial library databases. Compound 15 had a steroid nucleus with molecular formula $C_{28}H_{48}O$ (m/z 400) and base peak at m/z 43. This compound was identified as cholest-8-en-3-ol. Compound 16 was identified as vitamin E acetate with percentage composition of 3.68%. The base peak occurred at m/z 430 with molecular formula of $C_{31}H_{52}O_3$ (m/z 472). Compound 17 was identified as a steroid (4,4-dimethyl-cholesta-22,24-dien-5-ol) with molecular formula of $C_{19}H_{48}O_3$ (m/z 412) and percentage composition of 3.68%. The presence of alcohol based compounds identified among other bioactive components in the leaves of *Dennettia tripetela* justifies the reason for its use as an antimicrobial agent.

4. Conclusion

D. tripetala leaves contained phytochemicals, fatty acids and other bioactive compounds that have their important uses. Also, the extracts were found to have inhibitory activities against some of the selected bacteria strains. This justifies some of the reasons for the traditional applications of the part of the plant in the treatment of some ailments.

References

[1] Akobundu, E. N. T. (1999). Healthy Food in Human Nutrition. *J. of Sustainable Agric. and Environ.* 1: 1 – 7.

[2] Asibey-Berko E, and Tayle F. A. K. (1999). Proximate Analysis of some underutilized Ghanaian Vegetables. *Ghana J. Sci.* 39: 91-92.

[3] Beare-Roger, J. Dieffebacher A. And Holm, J. V. (2001). Lexicon of lipid nutrition IUPAC Publisher pp 685 -744.

[4] Birt D. F., Hendrich S. and Wang W. (2001). Dietary agents in cancer prevention: Flavonoids and isoflavonoids. *Pharmacol Ther*; 90:157–177.

[5] Bruice P. Y. (2007). Organic Chemistry 5th ed. Pearson Education International USA

[6] Cunnane, S and Anderson, M. (1997). Pure linoleate deficiency in the ratinfluence on growth. Lipid resource publishers, pp 2216-2223

[7] Dahl, L. K. (1974). Salt and Hypertension. *American J. of Clinical Nutrition* , 25: 231 – 238.

[8] Davie B. H. (1976). Analytical Methods: Carotenoids. In*: Chemistry and Biochemistry of Plants Pigments (Vol. 4)*. Godwin TW (ed). Academic Press, London, pp. 125-127.

[9] Del-Rio, A., Obdulio, B. G., Castilo, J., Marin, F. R. and Ortuno, A. (1997). Uses and Properties of Citrus Flavonoids. *J. of Agric.l and Food Chem.* 45: 4505 – 4515.

[10] Dosunmu M. I. (1997). Chemical composition of the fruit of *Tetrapleura tetreptra* and the physico-chemical Properties of its oil. *Global J. Pure Appl. Sci.* 3: 61-67.

[11] Ejechi, B. O., and Akpomedaye D. E. (2005). Activity of essential oil and phenolic acid extracts of pepper fruit (*Dennetia tripetala* G. Baker) against some food - borne microorganisms. Afr. J. Biotech. 4: 258 – 261.

[12] Ejechi, B. O.; Nwafor, O. E. and Okoko, F. J. (1999). Growth inhibition of tomato-rot fungi by phenolic acids and essential Oil extracts of pepper fruit (*Dennetia tripetela*). *Food Res.* 32:395 399.

[13] Gordon, M. H. And Weny, X. C. (1992). Antioxidant Properties of Exracts from Tanshen (Salvia Mictiorrhiza Bunge). *J. Food Chem* 44:119 – 122.

[14] Nakatsuji, T., Kao, M. C. And Huangi, C. M. (2009). Antimicrobial properties of Lauric Acid against Propionibacterium Acnes. *J. of Investigative Dermatology*, Ching. pp 2480 – 2488.

[15] Nephaepetiian, A and Bassiri, A. (1975). *Changes in Concentration and interrelationships of Phylate, P, Mg, Cu, Zn, in Wheat during Maturation. J. Agric. Food Chem. 23:* 1179 – 1182.

[16] Nestel, P., Clifon, P. and Noakes, M. (1994). Effects of increasing dieting palmitoleic acid, palmitic acid and oleic acid on plasma lipid of hypercholesterolemic men. *J. of lipid research*, 35: 656-662.

[17] Obadoni, B. O. and Ochuko, P. O. (2001). "*Phytochemical Studies and Comparative Efficacy of the Crude Extracts of some Homostatic Plants in Edo and Delta States of Nigeria*". *Global J. Pure Appl. Sci., 8b:* 203 208.

[18] Obomanu, F. G.; Fekarurhobo, G. K. and Howard, I. C. (2005). Antimicrobial activity of extracts of leaves of *Lepidagathis alopecuroides* (VAHL). *J. of Chem. Society of Nig.*, 30:33-35

[19] Okoronkwo, N. E.; Mbachu, A. K and Nnaukwu, N. C. (2011). Chemical Compositions and GC/MS Characterisation of Fatty Acids Content of *Dennettia Tripetala* Leaves. *Proceedings of the Seventh International Conf. on Sustainable Dev.*. 7: (10) 18 – 24

[20] Okwu, D. E. and Emenike, I. N. (*2006*). Evaluation of the phytonutrients and vitamin content of citrus fruits. *Int*. *J*. Mol. *Med*. Adv. *Sci*. 2: *1- 6*.

[21] Okwu, D. E, (2001). Improving the Nutritive Value of Cassava Tapioca Meal with Loc al Spices. *J. of Neutrac., Functional and Med. Food.* 3:43 – 51.

[22] Okwu, D. E., Morah, F. N. I. and Anam, E. M. (2005). Isolation and characterization of phenanthrenic alkaloid uvariopsine from *Dennettia tripetala* fruits. *J. Med. Aromatic Plant Sci.,* 27: 496 – 498.

[23] Okwu, D. E. (2005). Phytochemicals, vitamins and mineral contents of two Nigerian Medicinal Plants. *Inter. J. of Mol. and Adv. Sci.. 1:375 – 381.*

[24] Okwu, D. E. and Ekeke, O. (2003). Phytochemical screening and mineral composition of Chewing Sticks in South Eastern Nigeria. *Global J. of Pure and applied Sci.* 9:235 – 238.

[25] Okwu, D. E. and Okwu, M. E. (2004). Chemical composition of *Spondias mombimlinn* Plant Parts. *J. of Sustainable Agric. and Environ.*. 6: 140 – 147.

[26] Onyechi J. O., Chime S. A., Onyishi I. V. and Eneiga A. (2013). Formulation of *Dennettia tripetala* Tablets by Direct Compression: Standardization and Quality Control. *Int. J. Pharm. Sci. Rev. Res*., 22(2), 1: 1 – 4.

[27] 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. *Afr. J. Trad. Comp. Alt. Med*. 5(4): 355 – 362.

[28] Roger, G. D. P. (2002). *Encyclopedia of Medicinal Plants*. Education and Health Library Editorialsafeliz S. L. Spalm 1: 153 – 154, 265.

[29] Saeed, M., Arshad, M., Ahmad, E. and Ishaque (2004) Ethnophytotherapies for the treatment of various diseases by the local people of selected areas of NWFP (Pakistan) *Pakistan J.l of Biol. Sci.* 7 (7): 1104 – 1108.

[30] Salah, N., Miller, N. J., Paganga, G., Tiburg, L., Bolwel, G. P., Rice, E. and Evans, C. (1995. Polyphenolic Flavonols as Scavanger of Aqueous Phase Radicals as Chain-breaking Antioxidants. *.Arch Biochem. Bioph* 2:339 – 346.

[31] Shahidi F, Chavan U. D., Bal A. K., Mckenzie D. B. (1999). Chemical Composition of Beach Pea (*Lathyrus Maritimus* L.) Plant parts. *Food Chem*. 64: 39-44.

[32] Sodipo, O. A., Akiniyi, J. A., and Ogunbamoru, J. V. (2000). Studies on certain Characteristics Extracts of Bark of Pansinystalia mucruceras. (K. Schemp). Pierre Exbeille. *Global J. of Pure and Applied Sci.* 6: 83 – 87.

[33] Stace, C. A. (1980). *Plant Taxanomy and Biosystematics.* Edward Arnold Publication Ltd., London. p. 279.

[34] Stray, F. (1998). *The Natural Guide to Medicinal Herbs and Plants.* Tiger Books International, London. pp. 12 – 16.

[35] Tiger, L. (1998). *The Natural Guide to the M edicinal Herbs and Plants (1st edition)*. Tiger Books Plc. Twitchenhanze, UK. pp.12 – 15.

[36] Turan M., Kordali S., Zengin H., Dursun A. and Sezen Y. (2003). Macro and Micro- Mineral content of some wild edible leaves consumed in Eastern Anatolia. *Acta Agric. Scand., Sect. B, Plant Soil Sci.* 53: 129-137.

[37] Ukeh D. A., Oku E. E., Udo I. A., Nta A. I. and Ukeh J. A. (2012). Insecticidal effect of fruit extracts from Xylopia aethiopica and *Dennettia tripetala* (annonaceae) against Sitophilus oryzae (coleoptera: curculionidae). *Chi. J. Agric. Res*. 72(2): 195 - 200.

[38] Urquiaga, I. and Leighton F. (2000). Plants Polyphenol Antioxidants and Oxidative Stress. *Bio. Res.* 33: 105 – 159.

[39] Valsaraji, R., Pushpangadan, P., Smith, U. W., Adsergen A. and Nyman, U. (1997). "*Antimicrobial Screening of Selected Medicinal Plants from India*". *J. of Ethnopharm. . Vol. 58. pp. 75 – 83.*

[40] Van Burden, T. P. and Robinson W. C. (1981). "*Formation of Complexes between Protein and Tannin Acid*". *J. of Agricultural Food Chemistry*. USA. pp. 77 – 99.

[41] Wang H. K. (2000). The therapeutic potential of flavonoids. *Expert Opin Invest Drugs* 9:2103–2119.