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GC-MS analysis of leaf extracts of *Terminalia macroptera* and *Dioclea reflexa*, two medicinal plants used for the treatment of respiratory tract disorders

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ABSTRACT

Objective: To analyze the phytochemicals that are present in two medicinal plants which are used for the treatment of respiratory tract infections by gas chromatography-mass spectrometer.
Methods: The plant leaves were extracted with *n*-hexane and methanol separately. Both extracts were analyzed for present phytochemicals using the method described by Harborne, 1985 while only methanol extracts were subjected to gas chromatography-mass spectrometer analysis.
Results: Phytochemical screening of the methanolic extracts of *Terminalia macroptera* (*T. macroptera*) revealed the presence of glycosides, tannins, flavonoids, saponins and steroids while that of *Dioclea reflexa* (*D. reflexa*) showed the presence of flavonoids, saponins and steroids. The *n*-hexane extracts were devoid of the screened phytochemicals. Twelve and twenty-five compounds were identified in the leaves of *T. macroptera* and *D. reflexa* respectively. These compounds were fatty acids, fatty acid esters, other esters, heterocyclics and phenolics. The most abundant compound in *T. macroptera* was benzenetriol (53.30%) while the predominant compounds in *D. reflexa* were dodecanoic acid, methyl ester (15.31%), 5, 5, 8a-trimethyl-3, 5, 6, 7, 8, 8a-hexahydro-2H-chromene (9.73%), 10-octadecenoic acid, methyl ester and 2-hexadecanoic acid, methyl ester (8.95%). Benzofuran, 2, 3-dihydro, 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol and hexadecanoic acid, methyl ester were common in both plant extracts. The antimicrobial properties of the leaves of these plants could be responsible for their use in the treatment of respiratory tract infections.
Conclusions: Some of the identified phytochemicals in the plant leaves are responsible for its use in the treatment of respiratory tract infections.

1. Introduction

The respiratory tract is very vulnerable to infection than other parts of the body. It is an infection of the nose, throat and lungs. The infection could either be caused by bacteria, virus or fungi. Some examples of respiratory tract infections include colds, sinusitis, *etc.* Ashworth *et al.,* 2005[1] reported that about a quarter of the population of England and Wales visit their doctors because of a respiratory tract infection each year. Most of treatments in these infections involve the use of antibiotics. According to National Institute for Health and Care Excellence clinical guidelines[2] 60% of all antibiotics prescribed in general practice were for respiratory tract infection treatment. In recent times there is a report of an increase in the resistance of disease causing pathogens to antibiotics. This has led to a renewed search for more potent drugs. Research is now focused on plants which are referred to as nature's pharmacy. Researchers have shown that plants possess chemical substances such as polyphenols, alkaloids, flavonoids, saponins, terpenoids, *etc*. which occur in small quantities that are beneficial to the healthcare of man[3-6]. The isolation, identification and characterization of these chemicals coupled with a series of biological tests and screening could lead to the development of new drugs. *Terminalia macroptera* (*T. macroptera*) and *Dioclea reflexa* (*D. reflexa*) are two medicinal

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plants that are commonly used for the treatment of respiratory tract infections in Western Nigeria. They are members of Combretaceae and Fabaceae families respectively and are found in Western and Central Africa. The aim of this research work is to identify the phytochemicals that are present in these two medicinal plants which are used for the treatment of respiratory tract infections.

2. Materials and methods

2.1. Sample collection and preparation

The plant leaves were collected from a herbalist in Omu Aran, Kwara State, Nigeria. The plant was identified by matching the local name with that written in the book 'vernacular names of Nigerian plants Yoruba' by Gbile and Soladoye, 2012[7]. The leaves were taken to the Department of Biological Sciences, Landmark University where they were air dried in the laboratory for two weeks and pulverized into fine powder. The powder was kept in sealed container until further use.

2.2. Extraction

A total of 200 g of the powdered leaves was extracted with *n*-hexane and methanol using a Soxhlet extractor. The extracts were concentrated by distillation and evaporation.

2.3. Phytochemical screening

The crude extracts were subjected to phytochemical screening using the method described by Harborne, 1985[8]. They were screened for the presence of tannins, saponins, steroids, flavonoids, alkaloids and glycosides.

2.4. GC-MS analysis

The methanolic extract was subjected to gas chromatography-mass spectrometer (GC-MS) analysis. The instrument used was Agilent 5975C inert MSD with triple axis detector (Agilent Technologies, Santa Clara, USA). Capillary column Agilent 19091S-433HP-5MS with dimensions 30 m × 250 μm × 0.25 μm was incorporated into the instrument. Helium gas was used as the carrier gas with flow rate of 1.5 mL/min. The injection temperature was set at 100–240 ˚C while the oven temperature was initially set at 100 ˚C and gradually increased to 300 ˚C at a rate of 5 ˚C/min. The mode employed was split mode with split ratio of 50:1. One microliter of sample was introduced into the column and the run time was 49 min. For the MS programme, the inlet line temperature was 200 ˚C, source temperature 250 ˚C, ionization energy 70 eV, mass scan (m/z) was from 50–600 amu. Solvent delay was for 5 min.

3. Results

Phytochemical screening and GC-MS analysis of *T. macroptera* and *D. reflexa* leaves were carried out. In this research work the phytochemical screening of the plant leaves extracts revealed the presence of glycosides, tannins, flavonoids, saponins, steroids and terpenoids in the methanolic leaf extract of *T. macroptera* while alkaloids were absent as shown in Table 1. For *D. reflexa* the identified phytochemicals were flavonoids, steroids and terpenoids as presented in Table 2. All the screened phytochemicals were absent in the *n*-hexane leaf extracts.

Table 1
Phytochemical screening of leaf extracts of *T. macroptera.*

Extracts	Glycosides	Tannins	Flavonoids	Saponins	Steroids	Alkaloids	Terpenoids
n-Hexane	-	-	-	-	-	-	-
Methanol	+	+++	+++	++	+	-	+

-: Absent; +: Present; ++: Moderately present; +++: Highly present.

Table 2
Phytochemical screening of leaf extracts of *D. reflexa.*

Extracts	Glycosides	Tannins	Flavonoids	Saponins	Steroids	Alkaloids	Terpenoids
n-Hexane	-	-	-	-	-	-	-
Methanol	-	-	++	-	+	-	++

-: Absent; +: Present; ++: Moderately present.

Table 3
GC-MS report for *T. macroptera.*

Peak number	Retention time	Molecular weight	Molecular formula	Name of compound
1	24.631	120	C_8H_8O	Benzofuran, 2, 3-dihydro
2	25.495	140	$C_7H_8O_3$	1, 2-Benzenediol, 3-methoxy
3	30.098	256	$C_{17}H_{20}O_2$	Benzene, 1, 1´-(1-methylethylidene) bis(4-methoxy)-
4	31.504	126	$C_6H_6O_3$	1, 2, 3-Benzenetriol
5	33.366	206	$C_{14}H_{22}O$	Phenol, 2, 4-bis(1, 1-dimethylethyl)-
6	39.320	338	$C_{22}H_{42}O_2$	Phytol acetate
7	39.666	296	$C_{20}H_{40}O$	3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol
8	39.996	270	$C_{17}H_{32}O_2$	Hexadecanoic acid, methyl ester
9	40.428	256	$C_{19}H_{32}O_2$	*n*-Hexadecanoic acid
10	41.111	292	$C_{18}H_{30}O_2$	9, 12, 15-Octadecatrienoic acid, methyl ester, (Z, Z, Z)-
11	41.504	278	$C_{18}H_{30}O_2$	9, 12, 15-Octadecatrienoic acid, (Z, Z, Z)-
12	41.591	623	$C_{39}H_{75}O_5$	Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester

The GC-MS analytical report for the methanolic extracts of both plant leaves were present in Tables 3 and 4. It revealed twelve and twenty-five phytocomponents in *T. macroptera and D. reflexa* respectively.

Biological properties of some of the identified phytocomponents were shown in Table 5. These biological properties were obtained from Dr. Jim Duke's Phytochemical and Ethnobotanical online database[9].

The total ion chromatogram for both plants methanolic leaf extracts were depicted in Figures 1 and 2.

4. Discussion

Oxidation reactions in the body are normal processes but it becomes an issue to tackle when free radicals such as reactive oxygen species, superoxides, peroxy, alkoxy, hydroxyl and nitric oxide radicals are generated and accumulated. These free radicals can attack lipids in cell membranes, DNA, enzymes, proteins and carbohydrates causing them to function improperly and resulting in diseases such as cancer, congestive dysfunction, *etc*.[9]. Synthetic drugs used for the treatment of these diseases have been reported to possess side effects and are very expensive. Therefore, there is the need to look for an alternative means of combating these ailments. Plants are referred to as nature's chemical store. Phytochemical analysis is a way of identifying the type, class or individual phytochemicals that are present in plant samples. The phytochemical screening of *T. macroptera and D. reflexa* showed the presence of some major phytocomponents such as flavonoids, tannins, saponins, terpenoids, steroids and glycosides in the plant leaves. Flavonoids and tannins are polyphenols that have been reported to possess antioxidant properties which enable them to combat free radicals due to their lower reduction potential than those of the free radicals[10,11]. Tannins also provide protection against microbial degradation of dietary proteins in the semen and have

Table 4
GC-MS report for *D. reflexa.*

Peak number	Retention time	Molecular weight	Molecular formula	Name of compound
1	9.187	117	$C_5H_{11}NO_2$	Glycine, N, N-dimethyl-, methyl ester
2	13.052	208	$C_{10}H_{12}SN_2O$	2-(1-Phenylethylamino)-2-thioxo-acetamide
3	14.380	120	C_9H_{12}	Benzene, 1, 2, 4-trimethyl
4	18.732	124	C_7H8O2	Phenol, 2-methoxy
5	21.120	191	$C_{11}H_{13}NO_2$	Butyl -3-(3-pyridyl) propanoate
6	24. 623	120	C_8H_8O	Benzofuran, 2, 3-dihydro
7	26.909	150	$C_9H_{10}O_2$	2-Methoxy-4-vinylphenol
8	28.150	182	$C_9H_{10}O_4$	Formic acid, 2, 6-dimethoxyphenyl ester
9	31.254	164	$C_{10}H_{12}O_2$	Trans-isoeugenol
10	31.708	206	$C_{10}H_{22}O_4$	Ethanol, 2-[2-(2-butoxyethoxy) ethoxy]-
11	33.311	214	$C_{13}H_{26}O_2$	Dodecanoic acid, methyl ester
12	35.126	190	$C_{13}H_{18}O$	3-(4-Isopropylphenyl)-2-methylpropionaldehyde
13	36.579	190	$C_{13}H_{18}O$	3-(4-Propylphenyl)-2-methylpropionaldehyde
14	37.184	208	$C_{13}H_{20}O_2$	2-Cycohexene-1-one, 4-(3-hydroxy-1-butenyl)-3, 5, 5-trimethyl-, [R-[R*, R*, (E)]]
15	38.158	242	$C_{15}H_{30}O_2$	Methyltetradecanoate
16	38.653	180	$C_{10}H_{12}O_3$	4-((1E)-3-hydroxy-1-propenyl)-2-methoxyphenol
17	38.967	281	$C_{19}H_{28}NO$	2-Aminomethyl-1-(2´-hydroxy-4´, 6´-dimethylphenyl)-5, 6, 7, 8-tetrahydronaphthalene
18	39.242	180	$C_{12}H_{20}O$	5, 5, 8a-trimethy-3,5, 6, 7, 8, 8a-hexahydro-2H-chromene
19	39.305	193	$C_{11}H_{15}NO_2$	Isonicotinic acid, pentyl ester
20	39.509	208	$C_{11}H_{12}O_4$	2-Propenoic acid, 3-(4-hydroxy-3-methoxyphenyl)-, methyl ester
21	39.658	278	$C_{20}H_{38}$	9-Eicosyne
22	39.996	270	$C_{17}H_{34}O_2$	Hexadecanoic acid, methyl ester
23	41.096	296	$C_{19}H_{36}O_2$	10-Octadecenoic acid, methyl ester
24	41.190	296	$C_{20}H_{40}O$	Phytol
25	41.237	298	$C_{19}H_{38}O_2$	Heptadecanoic acid, 16-methyl, methyl ester

Table 5
Biological properties of some of the identified compounds.

Compound	Type	Biological activity
n-Hexadecanoic acid	Palmitic acid	Antimicrobial, hypocholesterolemic, nematicide, pesticide, lubricant, hemolytic, 5-α-reductase inhibitor
n-Hexadecanoic acid methyl ester	Fatty acid ester	Antioxidant, hypocholesterolemic
9, 12, 15-Octadecatrienoic acid, methyl ester	Linolenic acid	Antiinflammatory, insectifuge, hypocholestrolemic, nematicide, hepatoprotective, antihistaminic, antieczemic, antiandrogenic, antiarthritic
3, 7, 11, 15-Tetramethyl-2-hexadecen-1-ol	Terpene alcohol	Antimicrobial
Phytol	Diterpene	Antimicrobial, diuretic, antioxidant, antiinflammatory
Octadecanoic acid methyl ester	Fatty acid ester	Antimicrobial

Source: Dukes. Phytochemical and Ethnobotanical Databases. Phytochemical and Ethnobotanical Databases. www.ars-grin.gov/cgi-bin/duke/ethnobot.pl 2013[9].

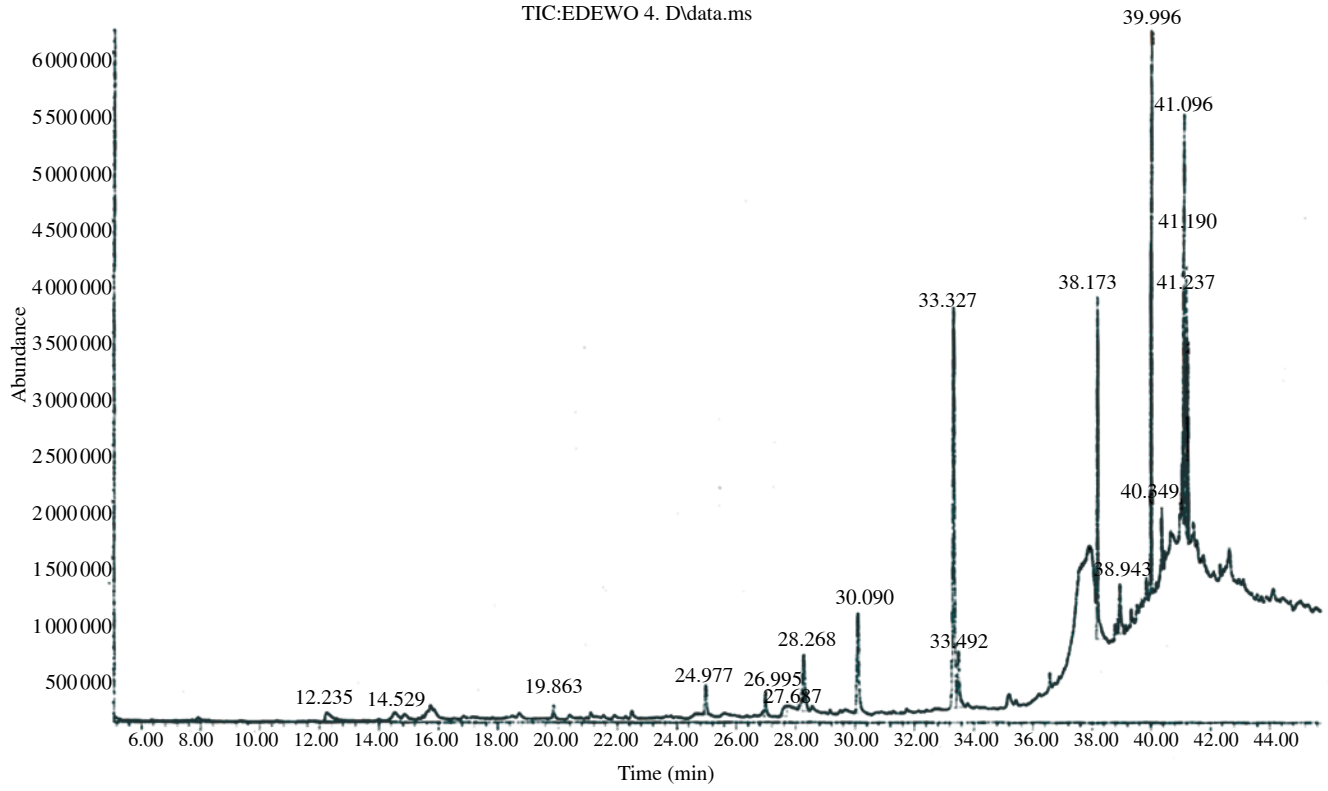

Figure 1. Total ion chromatogram for *D. reflexa.*

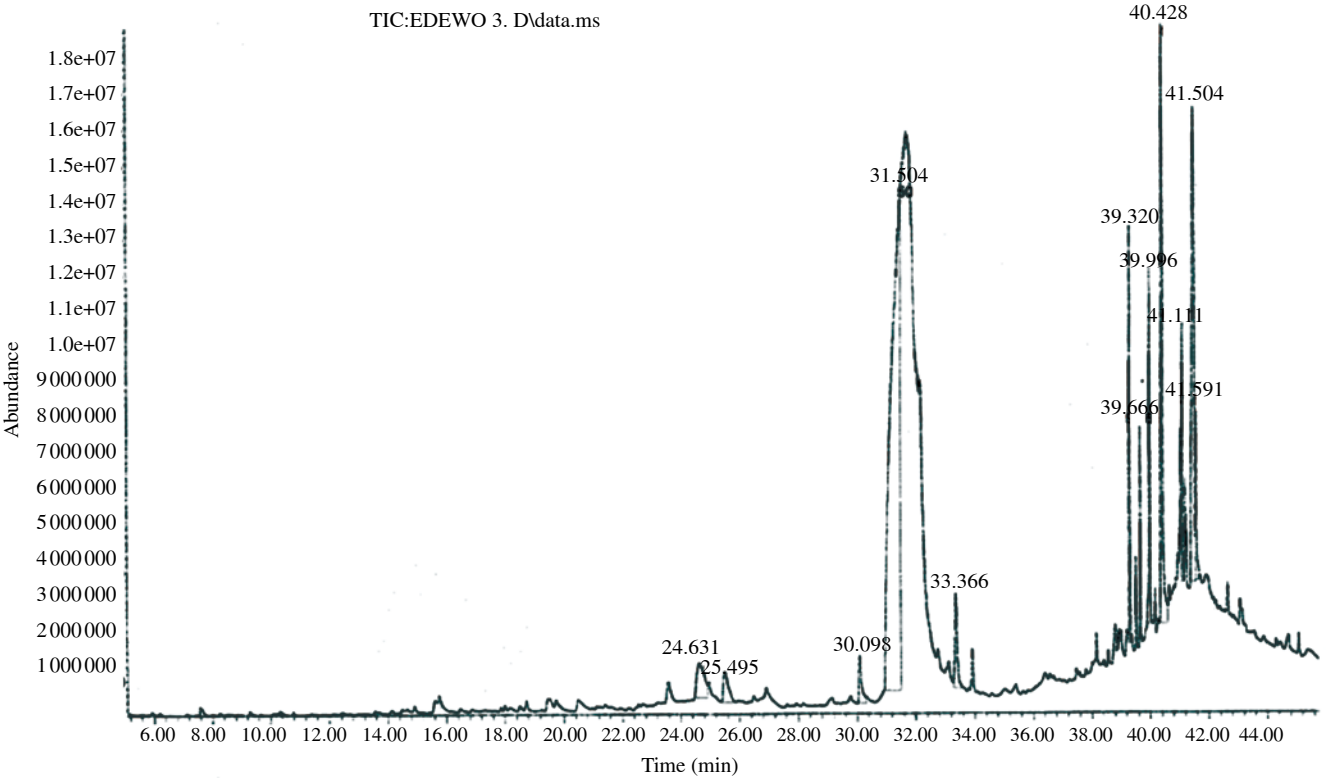

Figure 2. Total ion chromatogram for methanolic leaf extract from *T. macroptera.*

antidiarrhoeal and antihemorrhagic properties[12] while saponins have been reported to have the capacity to reduce cholesterol, exhibit hemolytic, antifungal, anti-inflammatory, molluscidal, and foaming properties[13]. Terpenoids have been reported to exhibit antiplasmodial, antineoplastic and anti viral activities[14]. Researchers have shown that steroids and steroidal alcohols possess cholesterol-lowering ability. When incorporated into the body it has the ability to compete with cholesterol for micelles which transport lipids and cholesterol into the intestinal mucosa thereby reducing the absorption of cholesterol into the gastrointestinal tract[15].

GC-MS is an important analytical tool used in the separation and identification of compounds in complex mixtures like plant extracts. The presence or absence of a particular component of a mixture can be ascertained by GC-MS analysis. The retention time obtained for that particular compound can be used for its identification. The size of the peak on the chromatogram is directly proportionally to the amount of the component in the mixture analyzed. The GC-MS analysis of the methanolic leaf extract of *T. macroptera* revealed the presence of twelve compounds (Table 4). These phytochemicals belong to a class of heterocyclics, phenolics, aromatic alcohols, fatty acids, fatty acid esters and other esters. The first compound to be identified was benzofuran, 2, 3-dihydro with retention time of 24.63 min while the last was octadecanoic acid, 2-hydroxy-1, 3-propanediyl ester (retention time 41.59 min). The most abundant compound is benzenetriol (53.30%) followed by 9, 12, 15-octadecatrienoic acid, (Z, Z, Z) (12.097%). For *D. reflexa* a total of twenty five compounds were identified (Table 5). These compounds are heterocyclics, phenolics, fatty acid esters, alcohols, alkaloids, flavonoids and carbonyls. The first compound to emerge was glycine, N, N-dimethyl, methyl ester (retention time 9.187 min), while the last compound was heptadecanoic acid, 16-methyl, methyl ester. The predominant compounds are dodecanoic acid, methyl ester (15.31%), 5, 5, 8a-trimethyl-3, 5, 6, 7, 8, 8a-hexahydro-2H-chromene (9.73%), 10-octadecenoic acid, methyl ester and hexadecanoic acid, methyl ester (8.95%). These compounds were identified based on their retention time, molecular formula and molecular weight. The mass spectrum of each unknown compound was compared with those in National Institute of Standards and Technology library version 2011 stored in the computer database of the equipment so as to obtain the name and molecular formula of the unknown compound. Some of the identified compounds have been reported to exhibit biological properties as presented in Table 5. Three compounds were identified as being common to both plant leaves. These are benzofuran, 2, 3-dihydro, 3, 7, 11, 15-tetramethyl-2-hexadecen-1-ol and hexadecanoic acid, methyl ester. These compounds might be responsible for the plant leaves activity against disease causing pathogens that attack the respiratory tract.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

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