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Research Article

GAS CHROMATOGRAPHY-MASS SPECTROMETRIC ANALYSIS OF METHANOLIC LEAF EXTRACTS OF *LANNEA KERSTINGII* AND *NAUCLEA DIDERRICHII*, TWO MEDICINAL PLANTS USED FOR THE TREATMENT OF GASTROINTESTINAL TRACT INFECTIONS

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ABSTRACT

Objective: The leaves of *Lannea kerstingii* and *Nauclea diderrichii* are traditionally used for the treatment of gastrointestinal infections. The aim of this work is to determine the phytochemicals that are present in the leaves.

Methods: Phytochemical screening was carried out to determine the type of phyto-components present while a gas chromatography-mass spectrometry (GC-MS) analysis was to separate and identify the different phytochemicals that are present in the leaves.

Results: The phytochemical screening showed the presence of tannins, flavonoids, saponins, and steroids in the methanolic extracts of both plants while only steroids were present in the n-hexane extract of *N. diderrichii*. Alkaloids, anthraquinone glycosides, and terpenoids were absent in all extracts. The GC-MS revealed 16 compounds in the methanolic extract of *L. kerstingii* with dodecanoic acid, methyl ester as the predominant compound (24.276%) and 26 compounds in *N. diderrichii* with phenol, 2,4-bis(1,1-dimethylethyl) - (17.253%) as the most abundant. Seven compounds were identified to be common in the two plants methanol extracts. Some of the identified compounds possess biological activities.

Conclusion: Some of the identified compounds could be responsible for the biological activity of the plants leaves, especially its effect on gastrointestinal pathogens.

Keywords: *Lannea kerstingii*, *Nauclea diderrichii*, Gastrointestinal infection, Gas chromatography-mass spectrometri.

INTRODUCTION

Gastrointestinal infections affect the stomach and the small intestine. These type of infections are the most widely encountered in primary health care. Although they may not always be severe, they can be quite serious and contagious in particular locations. According to the WHO, 2008 [1], gastrointestinal infections are responsible for about 3 million deaths globally each year. Moreover, effective vaccines are not available and treatments with available drugs are unsatisfactory. There is also the issue of resistance of the infection causing pathogens to the available antibiotics [2]. Therefore, there is the need to look for an alternative means of the treatment for these infections.

Medicinal plants have been used by different civilizations for the treatment of several health related matters. A traditional health practitioners either use a single plant part, whole plant, or a combination of plant parts. Research has shown that these medicinal plants contain phytochemicals such as flavonoids, alkaloids, and saponins, which occur in small quantities to be responsible for the biological and pharmacological activities of these plants [3-6]. *Lannea kerstingii* and *Nauclea diderrichii* are medicinal plants that are used primarily for the treatment of diarrhea and other stomach related ailments. They belong to the family of plants known as Anacardiaceae and Rubiaceae, respectively. This research work was undertaken to determine the phytochemicals that are present in the plants leaves using a gas chromatography-mass spectrometry (GC-MS) as our analytical tool.

METHODS

Sample collection and preparation

The plant samples were collected from the traditional herbal practitioner in Omu-Aran, Kwara State, and Nigeria. The plants were identified using the book "Vernacular names of Nigerian plants Yorub" written by Gbile and Soladoye [7]. The plants leaves were air dried, pulverized into fine powder and stored in clean storage bottles. All chemicals used were purchased from Sigma-Aldrich. The solvents were distilled before use.

Extraction

About 200 g of the pulverized leaves was extracted with n-hexane and methanol using a soxhlet extractor. The extracts were concentrated by distilling off the solvent and dried using a rotary evaporator.

Phytochemical analysis

The extracts were subjected to phytochemical analysis using the method described by Harborne, 1993 [8].

GC-MS analysis

The methanolic extracts were subjected to GC-MS analysis. Model 7890A, Agilent Technologies interfaced with a mass selector detector model 5975°C was used. The electron ionization was kept at 70 eV with an ion source temperature at 250°. Helium gas was used as the carrier gas while HP-5MS (30 mm × 0.25 mm × 0.320 µm) was used as the stationary phase. The oven temperature was kept at 80°C held for 4 minutes and ramped to 270° at the rate of 3.5°C/minutes holding for 6 minutes. 1 µl was injected into the column at 300°C. The split mode was employed with a split ratio of 50:1.

RESULTS

The phytochemical screening of the leaf extracts of the plants is given in Tables 1 and 2 while the GC-MS reports are presented in Tables 3 and 4. The total ion chromatograms are shown in Figs. 1 and 2 while the structures of the identified compounds are presented in Figs. 3 and 4.

DISCUSSION

The evaluation of the biological properties and identification of the chemical components of the leaf extracts of two medicinal plants

Table 1: Phytochemical screening of leaf extracts of *L. kerstingii*

Extracts	Anthra quinone glycosides	Tannins	Flavonoids	Saponins	Steroids	Alkaloids	Terpenoids
n-hexane	–	–	–	–	–	–	–
Methanol	–	++	+	+	+	–	–

–: Absent, +: Present, ++: Highly present, *L. kerstingii*: *Lannea kerstingii*

Table 2: Phytochemical screening of leaf extracts *N. dedirrichii*

Extracts	Anthra quinone glycosides	Tannins	Flavonoids	Saponins	Steroids	Alkaloids	terpenoids
n-hexane	–	–	–	–	+	–	–
Methanol	–	+	++	+	+	–	–

–: Absent, +: Present, ++: Highly present, *N. dedirrichii*: *Nauclea dedirrichii*

Table 3: GC-MS report on the methanolic leaf extract of *L. kerstingii*

Serial number	Retention time	Name of compound	Molecular weight	Molecular formula
1	12.235	6-oxa-bicyclo[3.1.0] hexan-3-one	98	$C_5H_6O_2$
2	14.529	Silane, [3-(2,3-epoxypropoxy) propyl] ethoxydimethyl	218	$C_{10}H_{22}SiO_3$
3	19.863	Octanoic acid, methyl ester	158	$C_9H_{18}O_2$
4	24.977	Phosphonic acid, methyl-, bis (trimethylsilyl) ester	240	$C_7H_{21}PO_3Si_2$
5	26.995	Decanoic acid, methyl ester	186	$C_{11}H_{22}O_2$
6	27.687	Hydroquinone	110	$C_6H_6O_2$
7	28.268	Eugenol	164	$C_{10}H_{12}O_2$
8	30.090	1H-pyrrole, 3-ethyl-5-[(4-ethyl-3, 5-dimethyl-2H-pyrrol-2-ylidene) methyl]-2, 4-dimethyl-	256	$C_{16}H_{21}N_2$
9	33.327	Dodecanoic acid, methyl ester	214	$C_{13}H_{26}O_2$
10	33.492	Phenol, 2-methoxy-4-(2-propenyl)-, acetate	206	$C_{12}H_{14}O_3$
11	38.173	Methyltetradecanoate	242	$C_{15}H_{30}O_2$
12	38.943	1-propene-1, 2, 3-tricarboxylic acid, tributyl ester	342	$C_{18}H_{30}O_6$
13	39.996	Hexadecanoic acid, methyl ester	270	$C_{17}H_{34}O_2$
14	40.349	n-hexadecanoic acid	256	$C_{16}H_{32}O_2$
15	41.096	16-octadecenoic acid, methyl ester	296	$C_{19}H_{36}O_2$
16	41.190	9-octadecamide	281	$C_{19}H_{23}NO$
16	41.237	Heptadecanoic acid, 16-methyl, methyl ester	298	$C_{19}H_{38}O_2$

GC-MS: Gas chromatography-mass spectrometry, *L. kerstingii*: *Lannea kerstingii*

Table 4: GC-MS report on methanolic leaf extract of *N. diderrichii*

Serial number	Retention time	Name	Molecular weight	Molecular formula
1	8.347	Pilocarpine	208	$C_{11}H_{18}N_2O_2$
2	8.433	13-heptadecyn-1-ol	252	$C_{17}H_{32}O$
3	10.350	4-cyclopentene-1,3-dione	96	$C_5H_4O_2$
4	10.782	Benzyloxy (butyl) dimethylsilane	222	$C_{13}H_{22}SiO$
5	11.764	3-azabutyl-1-ol, 4-cyclopropyl-3,3-dimethyl	144	$C_8H_{18}ON$
6	12.408	6-oxa-bicyclo[3.1.0]hexa-3-one	98	$C_5H_6O_2$
7	14.262	2-propanol, 1,1,1-trichloro-2-methyl	142	$C_4H_7Cl_2O$
8	14.608	Silane, [3-(2,3-epoxypropoxy) propyl] ethoxymethyl	218	$C_{10}H_{23}SiO_3$
9	14.898	1, 2-cyclohexane dione	112	$C_6H_8O_2$
10	15.621	Phenol	94	C_6H_6O
11	15.747	2-oxabicyclo[3.2.0]hepta-3, 6-diene	94	C_6H_6O
12	21.222	4H-pyran-4-one, 2,3-dihydro-3,5-dihydroxy-6-methyl	144	$C_6H_8O_4$
13	21.921	1,1-dimethyl-2-methyl-4-penten-2-ol	160	$C_9H_{17}O$
14	24.686	Catechol	110	$C_6H_6O_2$
15	24.961	Heptadecane	240	$C_{17}H_{36}$
16	27.726	Hydroquinone	110	$C_6H_6O_2$
17	30.075	1H-pyrrole, 3-ethyl-5-[(4-ethyl-3,5-dimethyl-2H-pyrrol-2-ylidene) methyl]-2,4-dimethyl	256	$C_{14}H_{24}O_4$
18	33.319	Phenol, 2,4-bis (1,1-dimethylethyl)-	206	$C_{14}H_{22}O$
19	35.126	2H-1-benzopyran, 6,7-dimethoxy-2,2-dimethyl	220	$C_{13}H_{16}O_3$
20	38.166	Methyltetradecanoate	242	$C_{15}H_{30}O_2$
21	39.847	9-octadecenamide, (Z)-	281	$C_{18}H_{38}NO$
22	39.996	Hexadecanoic acid, methyl ester	270	$C_{17}H_{34}O_2$
23	40.357	Hexadecanoic acid	256	$C_{16}H_{32}O_2$
24	41.009	Pentacosanoic acid, methyl ester	396	$C_{26}H_{52}O_2$
25	41.096	16-octadecenoic acid, methyl ester	296	$C_{19}H_{36}O_2$
26	41.190	Isophytol	296	$C_{20}H_{40}O$

GC-MS: Gas chromatography-mass spectrometry, *N. diderrichi*: *Nauclea diderrichi*

Table 5: Biological properties of some of the identified compounds

Serial number	Compounds	Biological properties
1	Heaxadecanoic acid, methyl ester	Antimicrobial
2	Octadecanoic acid, methyl ester	Antimicrobial
3	Dodecanoic acid, methyl ester	Antieczemeic
4	4H-pyran-4-one, 2, 3-dihydro-3, 5-dihydroxy-6-methyl	Antimicrobial, antifouling
5	n-hexadecanoic acid	Hypocholesterolemic, nematicide, pesticide, lubricant, hemolytic, 5-α-reductase inhibitor, Antimicrobial,

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

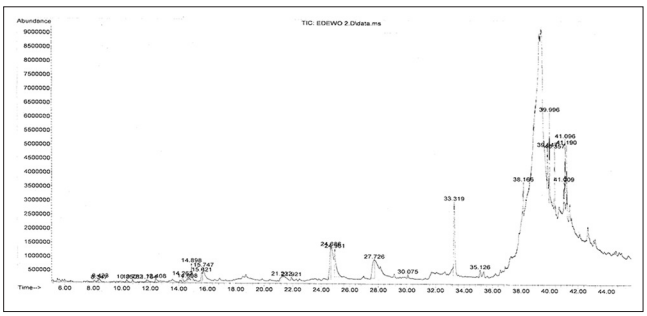

Fig. 1: Total ion chromatogram for *Nauclea diderrichi*

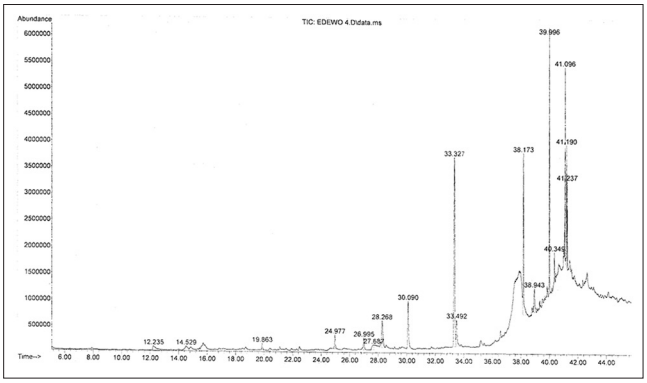

Fig. 2: Total ion chromatogram for *Lannea kerstingii*

(*L. kerstingii* and *N. diderrichii*) used for the treatment of gastrointestinal disorders was carried out. The phytochemical screening of the *L. kerstingii* revealed the presence of tannins, flavonoids, saponins, and steroids while alkaloids and anthraquinone glycosides were absent in the methanol extract. All the screened phytochemicals were absent in the n-hexane extracts as shown in Table 1. For *N. diderrichii,* tannins, flavonoids, saponins, and steroids were present in the methanol extract as shown in Table 2. The leaves of both plants possess similar phytochemicals. The intensity of tannins was stronger in *L. kerstingii* while the intensity of flavonoids was stronger in *N. diderrichii*. The GC-MS analysis of *L. kerstingii* revealed the presence of 16 compounds which are made up of fatty acids, fatty acid esters, other esters, terpenoids, ketones, and quinone. The first compound to emerge was 6-oxa-bicyclo [3.1.0] hexan-3-one with a percent total of 1.779, while the last compound to emerge was heptadecanoic acid, 16-methyl, methyl ester with a percent total of 4.135. The most abundant compound in the methanol extract was dodecanoic acid, methyl ester with a percent total of 24.276 while the least was Silane, [3-(2,3-epoxyproxoy)propyl] ethoxydimethyl with a percent total of 0.812. For *N. Dedirrichii,* a total of 26 compounds were separated and identified. The first compound to emerge was pilocarpine (0.525%) and it was also the least abundant while the last was heptadecane (3.744%). The most abundant compound was phenol, 2, 4-bis(1,1-dimethylethyl)-(17.253%). The compounds separated by the GC were identified based on their retention time, peak area and molecular formula. The interpretation of the mass spectrum was conducted using the NIST 2011 database stored in the computer

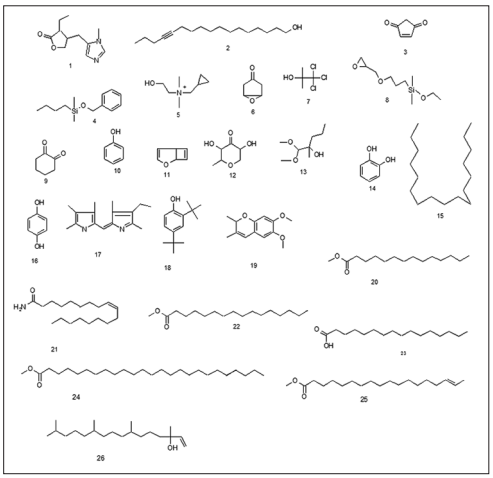

Fig. 3: Structures of compounds identified in the methanolic extract of *Nauclea diderrichii*

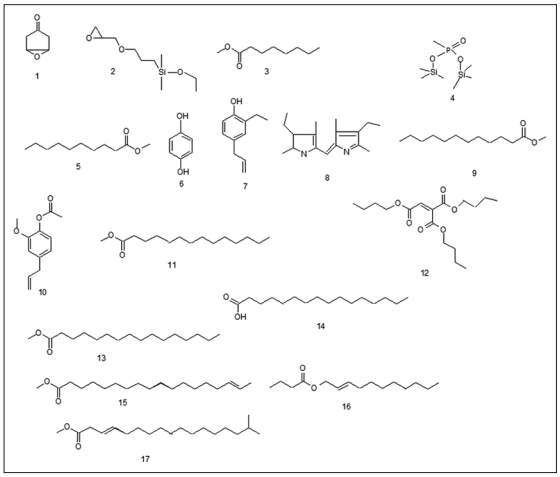

Fig. 4: Structures of compounds identified in the methanolic extract of *Lannea kerstingii*

component of the GC-MS equipment. The name, molecular weight, and structure of the unknown compounds were ascertained by comparing them with those stored in the NIST library version (2011). The relative abundance of each compound was also determined as shown in Tables 3 and 4. The total ion chromatograms are presented in Figs. 1 and 2 while the structures of the identified compounds are as shown in Figs. 3 and 4. Eight compounds were identified to be common in the two plants leaves methanol extracts. The identified compounds are 6-oxa-bicyclo [3.1.0], hexa-3-one, Silane, [3-(2,3-epoxypropoxy)propyl] ethoxymethyl, catechol, methyltetradecanoate, hexadecanoic acid, methyl ester, 16-octadecenoic acid, methyl ester, and 9-octadecanamide. These compounds could be responsible for their biological and pharmacological properties. A some of the identified compounds have proven biological properties as shown in Table 5 [9]. Compounds such

as heptadecane, pentacosane have been identified in the rhizomes of *Nervilia aragoana* [10], hydroquinone, n-hexadecanoic acid and hexadecanoic acid methyl ester in the leaves of *Cassia italic* and *Wrightia tinctoria* [11, 12]. The isolation, characterization, pharmacological and in depth biological studies of the identified compounds may lead to novel drugs that can be effective against gastrointestinal disorders.

CONCLUSION

The presence of the identified phyto-components could be responsible for the medicinal properties of the plants leaves. The eight compounds that are common to both plant leaves could be the bioactive compounds that make the plants leaves active against gastrointestinal infections.

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