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GC-MS Analysis of Bioactive Components of Methanolic Stem Bark Extract of *Lannea acida* (Anacardiaceae)

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Abstract

The aim of the present study was to elucidate the bioactive compounds present in methanolic back extract of *Lannea acida* extracts using Gas chromatography–mass spectrometry (GC-MS) techniques The methanolic back extracts of *L. acida* was analyzed for GC-MS using a HP-5MS capillary column (30 m ×250 µm, i.d., 0.25 µm film thickness) in an Agilent 5975 series MDS gas chromatograph. The GC-MS mass spectra of the identified compounds were compared with those of the National Institute of Standards and Technology database library and Dr Duke library. The results shown the presence of Hexadecanoic acid (20.59%), Tetradecanoic acid (18.18%) and Octadecanoic acid (13.77%) were found as the major component in the methanolic bark extract and the eight minor components such as Dodecanoic acid (8.51%), Methoxyacetic acid,2-tetradecyl ester and Eicosanoic acid (7.62%) respectively, 7,10-Octad ecadienoic acid, methyl ester and trans-13-Octadecenoic acid, methyl ester (7.70%) respectively, Hexadecanoic acid, methyl ester (4.86%), Dibutyl phthalate (4.12%), Decanoic acid (2.16%) were found in methanolic extract extracts. The results of the present study generated by GC-MS spectrum revealed the presence of the bioactive compounds in the plant which offered a platform for the traditional usage of *L. acida* in treatment of several diseases.

Keywords

Lannea acida, Phytochemical and GC-MS

Introduction

Secondary metabolite are bioactive compounds of plant source which are regarded as secondary metabolites and they are synthesized naturally in all parts of the plants which include, bark, root, leaves, flower stem, fruits, seeds which contain active components [1]. The therapeutic value of a plant depends on some the chemical constituents that produce a definite physiological action on the human body. The most essential of these bioactive constituents of plants includes flavonoids, alkaloids, terpenoids, tannins, and steroids. [2]. Understanding of the chemical constituents of plants is necessary, not only for the discovery of therapeutic agents but also because such evidence may be of value in disclosing new sources of drug [3]. The determination of biological active compounds from plant material is mainly dependent on the type of solvent used in the extraction procedure [4]. Therefore, it becomes a necessity to use many type of solvents in screening plant materials for phytochemical constituent.

Lannea plants belong to the family Anacardiaceae and are used in traditional medicine in the management of infectious diseases. The bark decoction of *Lannea acida* is drunk for fever and malaria, whilst an infusion is taken for snake bite and a paste is applied on fractures, injuries and wounds [5]. It is also an excellent fruit tree for dry lands and commonly called, awere kogun in akoko area of ondo state, akogun in ondo town, “faruhi” in Fulani-Fulfulde (Nigeria), “fa`ar´u” in Hausa, “Mipadi” in Giziga, or “Timbiya” in Moundang in Cameroun and growth in Sub-Saharan Africa. Barks of *L. acida* are traditionally used in Nigeria to treat anal hemorrhoids, malaria, diarrhea, dysentery, malnutrition, and

debility and in Cameroon to treat dysmenorrhea, amenorrhea, and infertility, while the leaves treat rheumatism. Information provided by the traditional healer in Akoko side of Ondo state revealed that the bark aqueous or alcoholic extract is used to treat malaria, also Moutourwa (Far North Region of Cameroon) revealed that the maceration of *L. acida* stem bark in local alcoholic drink (palm wine) is used to treat diarrhea and gynecological complaints.

The Nigeria *L. acida* has been employed traditionally in the controlling of various ailments, notably infectious conditions affecting human immune system for many years. Aqueous decoctions or alcoholic extracts of *L. acida* have diverse folkloric claims of effectiveness for treatment of malaria, diabetes, hypertension, cardiovascular diseases, menopausal syndrome, infertility, rheumatism, agglutination and in conditions generally requiring modulation of the immune system [6]. It is equally used locally as an antimicrobial and antispasmodic agent. Some of its age-long ethno medicinal uses have not been strongly validated.

The genus *Lannea* belongs to the family Anacardiaceae and consists of about 40 species of shrubs or trees native to tropical Africa. For a decade now, there were a number of historic advances in analytical techniques, including GC-MS that has been used as a prevailing tools for identification and determination of components of plants [7]. The present study was carried out to validate the bioactive compounds present in the methanolic extract of *L. acida* with the aid of GC-MS techniques, which may provide an understanding in its use to determine the compounds that are responsible for the activities observed. as a traditional medicine.

Materials and Methods

Experimental Plant Material

The stem bark of *L. acida* was collected from Ugbe town from a location ($7^0$15′42.9″N $5^0$15′01.9″E) of Ondo state and was authenticated at the Department of Plant Science and Biotechnology, Adekunle Ajasin University, and a sample specimen deposited at the herbarium for future reference.

Extract Preparation

The stem back of *L. acida* was allowed to dry at room temperature. They were pulverized in mechanized laboratory grinder (Manesty, England) to fine powder. The dried back weighing 500g were soaked in 1L of absolute methanol. The mixture was thoroughly mixed and filtered after 48 hour using a Buchner vacuum filter. The filtered supernatant was evaporated to dryness with a Rotary evaporator.

Phytochemical Test

The methanolic extract was screened for the presence of some secondary metabolite such as saponin, tannin, alkaloids, terpenoid, steroid, Quinone, flavonoids and cardiac glycosides as directed by sofowora [8].

Extraction and Preparation of the Methanolic Back Extract of *L. acida*

The stem back of *L. acida* was allowed to dry at room temperature. They were pulverized in mechanized laboratory grinder (Manesty, England) to a fine powder. The dried stem back weighed 1.6 kg were soaked in 5.5 L of absolute methanol. The mixture was thoroughly mixed and filtered after 72 hours using a Buchner vacuum filter. The filtered supernatant was evaporated to dryness with a Rotary evaporator. The percentage yield of the extract was determined according to the expression provided by Banso and Adeyemo (2006).

$$\text{Percentage yield} = \frac{\text{Weight of extract}}{\text{Weight of ground plant material}} \text{ X } 100$$

GC-MS Analysis

The methanolic extracts obtained from the bark of *L. acida* was analyzed separately by GC-MS using a HP-5MS capillary column (30 m ×250 µm, i.d., 0.25 µm film thickness) in an Agilent 5975 series MDS gas chromatograph (Agilent Technologies, 7890A GC system) coupled to a water GCT Premier mass spectrometer (Waters Corporation, Milford, MA, USA). The carrier gas was helium with a constant flow rate of 3 mL/min. The oven temperature was initially kept at 100^{o}C for 3 min then ramped at 10 ^{o}C/min to 300 ^{oC}. The temperature was gradually increased from 8^{o}C/min to 350 ^{o}C and held isothermally for 10 min. An amount of 8.µl of the sample (100 ppm in chloroform) solutions was injected in the splitless mode. The relative% amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatograms was a Turbo mass spectra were obtained by EI at 70 eV over the scan range m/z 50-800. The compounds were identified by comparison of their mass spectra with those of the NIST 05 L mass spectral library having more than 62,000 patterns. The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. The name, molecular weight and structure of the components of the test materials were ascertained

Identification of Components

Identification was based on the molecular structure,

molecular mass and calculated fragments. Interpretation on mass spectrum GC MS was conducted using the database of National Institute Standard and Technology (NIST) having more than 62,000 patterns. The name, molecular weight and structure of the components of the test materials were ascertained. The relative percentage amount of each component was calculated by comparing its average peak area to the total area. The spectrum of unknown components was compared with the version, 2005, software, Turbo mass 5.2. This is done in order to determine whether this plant species contains any individual compound or group of compounds, which may substantiate its current commercial and traditional use as an herbal medicine. Further it helps to determine the most appropriate methods of extraction these compounds.

Results

Phytochemical Analysis

The phytochemical screening of the Lannea acida bark extract showed the presence of various chemical constituents (Table 1). Alkaloids, saponins, flavonoids, terpenoids, tannin, phenol, Quinone, Vitamin C and Vitamin A.

Table 1. Qualitative phytoconstituents of *Lannea acida* extracts.

Sample	Saponin %	Flavonoids %	Alkaloids %	Terpenoids mg/100g	Tannin mg/100g	Phenol mg/100g	Quinone	Vitamin A mg/100g	Vitamin C mg/100g
Qualitative Analysis	+++	+++	+++	++	+	+	+++	+++	+++

(+) = positive (less present}, (+++) More present

GC-MS Analysis

The brown colour methanolic extract of *L. acida* stem bark showed eleven peaks from the chromatogram. These peaks indicated the presence of eleven compounds (1-11) in the extract. The molecular formula, percentage constituents and molecular masses of the compounds are shown in

Table 2. Compound 1 was identified as Decane and has molecular formula of $C_{10}H_{22}$ (MW 142) with base peak area of 2.16%. Compound 2 was identified as Dodecane with molecular formula of $C_{12}H_{22}$ (MW 170) and base peak area of 8.51%. Compound 3 was named Tetradecanoic acid with molecular formula of $C_{14}H_{28}O_2$ (MW 228) and base peak area of 18.18%. Compound 4 was named Hexadecanoic acid with molecular formula of $C_{16}H_{34}O_2$ (MW 226) and base peak area of 20.59%. Compound 5 was named Octadecadienoic acid with molecular formula of $C_{18}H_{38}O_2$ (MW 254) and base peak area of 13.77%. Compound 6 was named Hexadecanoic acid, methyl ester with molecular formula of $C_{17}H_{34}O_2$ (MW 270) and base peak area of 4.86%. Compound 7 was named Dibutyl phthalate with molecular formula of $C_{16}H_{22}O_4$ (MW 278) and base peak area of 4.12%. The compound is a phenolic derivative compounds. Compound 8 was also a fatty acid known as Eicosane acid with the molecular formula $C_{20}H_{42}O2$ (MW 282) and has a base peak area of 7.62%. The compound 9 was identified as Methoxyacetic acid,2 tetradecyl ester with molecular formula of $C_{17}H_{34}O_3$ (MW 286) and base peak area of 7.62% which occurred as a result of the cleavage of a butyl group (C_4H_7) from the compound. Compound 10 was a fatty acid identified as 7,10-Octadecanoic acid, methyl ester with a molecular formula of $C_{19}H_{34}O_2$ (MW 294). It showed a base peak area of 4.70%. Compound 11 was also a fatty acid known as trans-13-Octadecanoic acid, methyl ester with the molecular formula $C_{19}H_{36}O_2$ (MW 296) and has a base peak area of 4.70%. GC-MS analysis of the volatile chemical compositions of methanolic stem bark extract of *L. acida* were highly complex containing saturated and unsaturated fatty acids The results of *L. acida* extracts, the GC-MS of *L. acida* plant shown in Table (2 and 3) and Figure (1).

Table 2. Components detected in methanolic bark extract of *Lannea acida* GC-MS.

No	RT	Name Of Compounds	M. Formula	MW	Peak Area%
1	4.895	Decanoic acid	$C_{10}H_{22}O_2$	142	2.16
2	7.830	Dodecanoic acid	$C_{12}H_{26}$	170	8.51
3	10.073	Tetradecanoic acid	$C_{14}H_{28}O_2$	228	18.18
4	11.864	Hexadecanoic acid	$C_{16}H_{32}O_2$	226	20.59
5	14.313	Octadecanoic acid	$C_{18}H_{36}O_2$	254	13.77
6	15.875	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	4.86
7	16.305	Dibutyl phthalate	$C_{16}H_{22}O_4$	278	4.12
8	16.705	Eicosanoic acid	$C_{20}H_{42}$	282	7.62
9	16.705	Methoxyacetic acid,2-tetradecyl ester	$C_{17}H_{34}O_3$	286	7.62
10	17.706	7,10-Octadecadienoic acid, methyl ester	$C_{19}H_{34}O_2$	294	4.70
11	17.706	trans-13-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	296	4.70

Table 3. Activity of phytochemicals identified in the methanolic bark extract of *Lannea acida.* By GC-MS.

S/No	Name of Compound	Nature of Compound	Biological Activity
1	Methoxyacetic acid,2-tetradecyl ester	Acetic acid compound	Antibacterial
2	7,10-Octadecadienoic acid, methyl ester	Linoleic acid ester	Anti-cancer
3	trans-13-Octadecenoic acid, methyl ester	Linoleic acid ester	Anti-inflammatory, antiandrogenic, cancer preventive, dermatitigenic, irritant, ant leukotriene—D4, hypocholesterolemic, 5-alpha reductase inhibitor, anemiagenic, insectifuge, flavor
4	Octadecanoic acid	Linoleic acid	Anti-inflammatory, Hypocholesterolemic Cancer preventive
5	Hexadecanoic acid	Fatty acid	Antioxidant, hypocholesterolemic
6	Hexadecanoic acid, methyl ester	Fatty acid-Palmitic acid methyl ester	Antioxidant, Hypocholesterolemic, Nematicide, Pesticide, Antiandrogenic flavor, Hemolytic,5-Alpha reductase inhibitor, Anti-inflammatory
	Dodecanoic acid	Fatty acid	Flavour
9	Tetradecanoic acid	Fatty acid	Antioxidant, hypercholesterolemia, cancer-preventive, cosmetic

Reference: Dr Duke and NIST Phytochemical and Ethnobotanical databases.

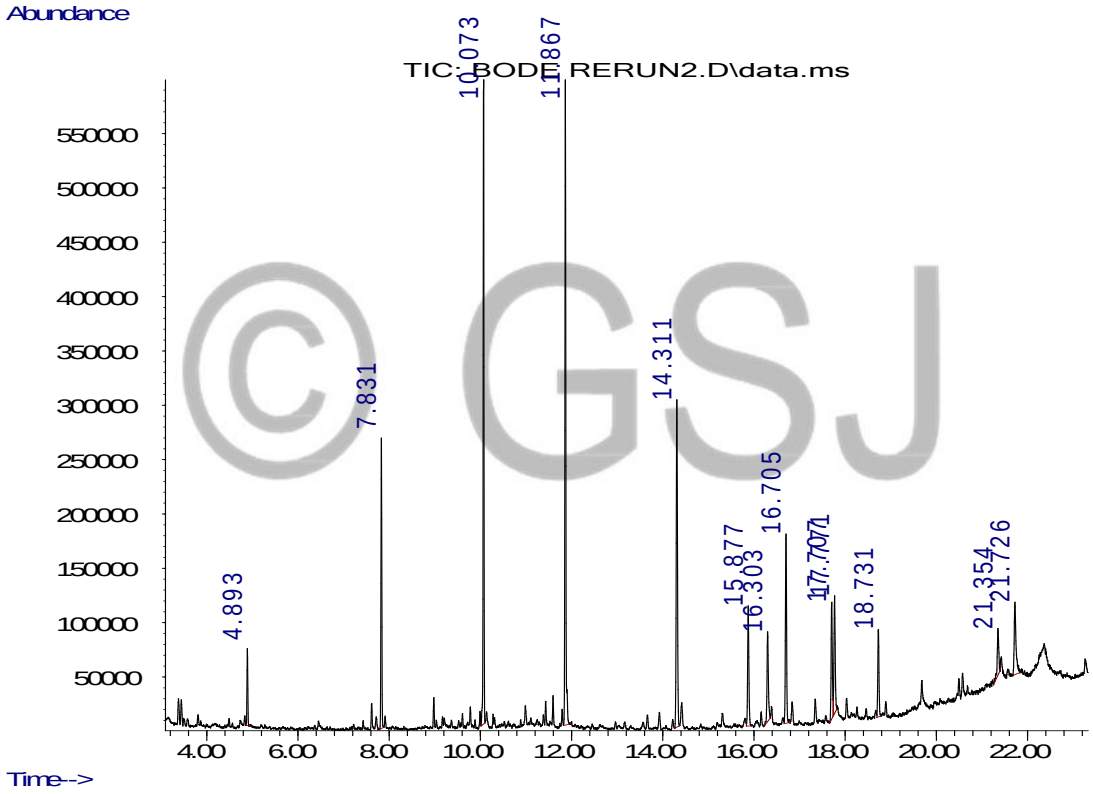

Figure 1. GC-MS analysis of Lannea acida methanolic back extract.

Discussion

The methanolic extract was screened for the presence of some secondary metabolite such as saponin, tannin, alkaloids, terpenoid, steroid, Quinone, flavonoids and cardiac glycosides as directed by sofowora [8]. In the present study, the investigation of phytochemical screening was done as directed by sofowora [8]. The result revealed that the methanolic extract of *L. acida* recorded the presence of alkaloid, flavonoid, phenol, tannins, saponins, and quinine. (Table.1).

Phytochemical constitutes such as flavonoids, tannins, and several aromatic compounds or secondary metabolites of plants serves as defense mechanism against predation by several microorganisms. The therapeutic properties of medicinal plants are probably due to the presence of several secondary metabolites such as alkaloid, flavonoid, tannins, phenolic compounds, Terpenoids, saponins and Quinone [12]. The presence of alkaloisds, saponin, flavonoids, phenolic compounds, tannins, and terpenoids are used in analgesic, antiplasmodic and bactericidal activities [13]. While Vitamine C is a potent antioxidant. Thus the preliminary screening test may be useful in the discovery of the bioactive values and consequently may lead to the drug design and development.

The studies on the active compound in Methanolic stem bark of *L. acida* extract by GC MS analysis vividly showed the presence of its compounds. The active compounds with their Retention Time (RT), Molecular Weight (MW), Molecular formula (MF) and Concentration (peaks areas%) as well as their biological activities are presented in (Table.2 & 3). The GC-MS chromatogram of the eleven peaks of the major compounds detected was shown in (Figure-1). The results revealed that Hexadecanoic acid (20.59%) Tetradecanoic acid (18.18%) and Octadecanoic acid (13.77%) were found as the one major component in the methanolic extract and the eight minor components such as Dodecanoic acid (8.51%), Methoxyacetic acid,2-tetradecyl ester and Eicosanoic acid (7.62%) respectively, trans-13-Octadecenoic acid, methyl ester and 7,10-Octadecadienoic acid, methyl ester (4.70%) respectively, Hexadecanoic acid, methyl ester (4.86%), Dibutyl phthalate (4.12%) and Decanoic acid (2.16%). The result of this study offer a platform of using *Lannea acida* and its active compounds as herbal medication for various diseases.

Conclusion

The presence of various bioactive compounds validates the use of *L. acida* plant for various ailments by traditional practitioners. However isolation of different phytochemical components used for biological action will positively give successful effects. It could be established that *L. acida* contains various bioactive compounds. So it is recommended as a plant of phytomedicinal importance. However, there is need for further studies to ascertain fully its bioactivity, toxicity profile, and its effect on the ecosystem.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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