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Gas Chromatography–Mass Spectrometry (GC-MS) Analysis of Bioactive Components of Ethyl acetate Root Extract of *Guiera senegalensis* J.F. Gmel

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ABSTRACT

Guiera senegalensis J.F. Gmel is used in West African Ethnomedicine for treating diarrhoea, dysentery, malaria, cough and microbial infections. The methanol and ethyl acetate root extracts of *G. senegalensis* have been shown to be effective against diarrhoea and also have antibacterial activity. The plant was therefore investigated for its bioactive components. The ethyl acetate root extract was investigated using Gas Chromatography-Mass Spectrometry (GC-MS) analysis. Nine components were identified, n-Hexadecanoic acid (46.6%) as the major component followed by 9-Hexadecenoic acid (20.93%), methyl ester (7.75%), 7-Octadecenoic acid- methyl ester, 1, 2-benzene dicarboxylic acid – diisoctyl ester having (6.97%) respectively; 2–pentanone - 4–hydroxy–4–methyl acid diethyl phthalate (2.32%), Decane–6–ethyl–2–methyl and nonane, 3–7–dimethyl with (1.55%) compositions respectively.

INTRODUCTION

In view of the fact that man lays a great premium on his life, he therefore tries to maintain a good state of health at all cost. Medicinal plants are some of the tools which he uses to maintain this state of good health. The use of medicinal plants is as old as the existence of man, who has tried various methods and materials to cure himself from disease, using the so many available plants growing around him. Although the use of plants by man as a way of treatment of diseases has evolved by trial and error, but the conclusion today is that over hundreds and thousands of years, man has at last amassed several medicinal plants with economic applications. Plants have played very important roles, all over the world since creation. They are used as medicines, food, shelter, clothing, cosmetics, flavours and species (Gamaniel 2000; Cordell, 2006).The current World Health Organization (WHO) report indicates that over 85% of the population in sub-Saharan Africa, including Nigeria depends on herbal traditional medicine for their health care needs (Odugbemi, 2008). Recent statistics have shown that 75-90% of the rural population in the rest of the world still rely on herbal medicine for their main healthcare (Odugbemi, 2008). Nigeria, like China, India and many other countries had a long practice of medicine. Varieties of medicinal plants occurring in the Northern parts of Nigeria and their uses have been studied and documented as a contribution to the promotion and development of traditional medicine in view of the interest of the WHO and Scientific Technical Research Commission (STRC) of the African Union (AU). The plant *Guiera senegalensis* J.F. Gmel is a member of the family Combretacea (Hutchinson and Dalziel 1954). It is a small shrub with green leaves. The plant is widely distributed in Nigeria, Senegal, Gambia, Mali Niger and Burkina Faso (Touzeau, 1973). The root concoction is used to cure diarrhea, dysentery and microbial infections. The methanol and ethyl acetate root extracts of *G. senegalensis* have been proven to have both antidiarrhoeal and antimicrobial activities (Shettima *et al.*, 2012abc). Taking into consideration the medicinal importance of this plant, the ethyl acetate root extract of *G. senegalensis* was analyzed using GC-MS. This work will help to identify the bioactive components. GC-MS is the best technique to identify bioactive constituents of long chain hydrocarbons, alcohols, acids, ester, alkaloids, steroids, amino and nitro compound etc (Muthulaksmi *et al*., 2012).

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MATERIALS AND METHODS

Sample Collection and Identification

The plant material (root) of *G. senegalensis* was collected in Jere Local Government Area of Borno State, Nigeria. It was identified and authenticated by a plant taxonomist from the Department of Biological Sciences, University of Maiduguri. A voucher specimen with number BCH GR 00I was deposited at the herbarium of the Biochemistry Department, University of Maiduguri, Nigeria. Fresh root of *G. senegalensis* was dried in the open air and ground to powder form and kept in cellophane bag at 4°C before extraction.

PLANT EXTRACTION

Heat Treatment

The root of *G.senegalensis* was washed to remove particles and dust. The washed root was heated at 80°C for 10 minutes and 60° C for 30 minutes. (Joslyn, 1970)

Maceration Method

About 2000 g of the weighed, powdered dried root (sample) was partitioned using ethyl acetate. The sample was placed in a stopped container with the solvent for a period of at least three days with frequent agitation until the soluble matter has dissolved. The mixture then was strained. The marc (the damp solid matter) was pressed, and the combined liquids were clarified by filteration (Harborne, 1988). The crude extract obtained was concentrated to dryness at 40°C – 45°C using a water bath. The concentrate (extract) was weighed, labelled and kept for further use.

Gas Chromatography – Mass Spectrometry (GC-MS)

Analysis of Ethyl acetate Root Extract of G. senegalensis

A shimadzu Qp – 2010 plus GC-MS was used. The GC-MS was equipped with a split injector and an ion – trap mass spectrometer detector together with a fused – silica capillary column having a thickness of 1.00µm, dimensions of 20m x 0.22mm and temperature limits of 60°C to 325°C. The column temperature was programmed between 60°C and 250°C at a rate of 3.0ml/min. The temperature of the injector and detector were at 250°C and 200°C respectively. Helium gas was used as a carrier gas at a flow rate of 46.3 cm/sec.

Identification of Components

Interpretation of mass spectrum of GC-MS was done using the computer-aided matching of unknown spectra with spectra of known compounds from the Library of spectra from the National Institute of Standards, Washington, USA having more than 62,000 patterns.

The fragmentation patterns of the identified compounds were then examined for consistency with known data from literature (Williams and Howe, 1972). In addition, the hit quality (which indicates how closely matched the compound is with the Library data) was used to further verify the identity of the compounds in the sample. The name, molecular weight and the structure of the components of the test materials were ascertained, the relative percentage composition of each component was calculated by comparing its average peak area to the total area. Software adopted to handle mass spectra and chromatogram was a Turbomass.

RESULTS AND DISCUSSION

GC-MS Analysis

The components present in the ethyl acetate root extract of *G. senegalensis* were identified by GC-MS. The chromatogram is shown in (Fig. 1). The active principles with their retention time (RT) molecular formula, molecular weight (MW) and percentage composition in the ethyl acetate root extract of *G. senegalensis* is presented in (Table 1). Nine components were identified in the ethyl acetate root extract. The compounds were n-Hexadecanoic acid (46.6%) as the major component followed by 9-Hexadecenoic acid (20.93%), hexadecanoic acid, methyl ester (7.75%), 7–octadecenoic acid–methyl ester, 1, 2–benzenedicarboxylic acid diisooctyl ester having (6.97%) respectively, 2 pentanone, 4-hydroxy–4–methyl and diety phthalate (2.32%), Decane 6–ethyl–2–methyl–and nonane 3–7– dimethyl having (1.55%) respectively. Figures 2 and 3a to3i show the mass spectrum and structures of the identified components. Major phytocompounds obtained and their biological activities have been tabulated in (Table 2).

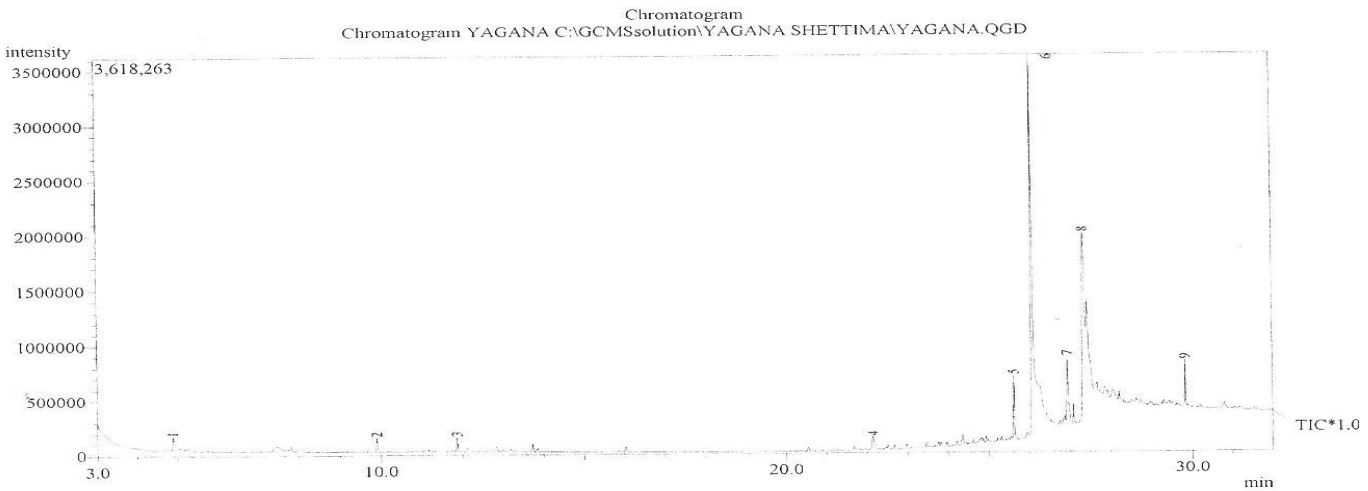

Fig.1**:** GC-MS Chromatogram of Ethyl acetate Root Extract of *Guiera senegalensis.*

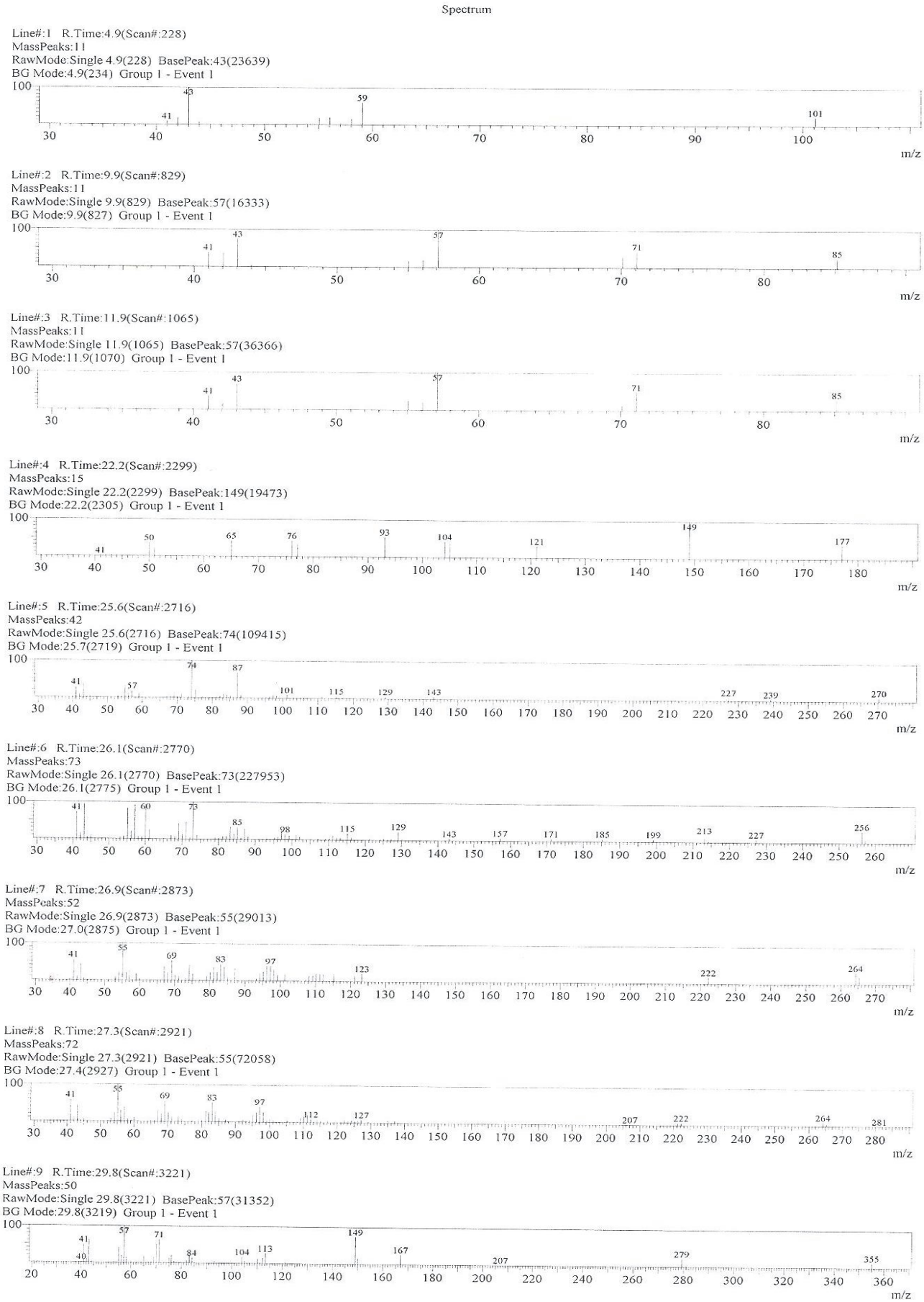

Fig. 2: Spectrum of the Nine Compounds Detected in the Ethyl acetate Root Extract of *Guiera senegalensis*.

Tabl.e 1: Components Detected in Ethyl acetate Root Extract of *G. senegalensis.*

Peak	RT	Name of the compound	Mol. Formula	MW	Peak height	Peak (%)
1	4.80	2- pentanone4 – hydroxyl-4 – methyl	$C_6H_{12}O_2$	116.3	0.3	2.32
2	9.90	Decane,6-ethyl-methyl	$C_{13}H_{28}$	184	0.2	1.55
3	11.86	Nonane 3, 7 – dimethyl – 2, 6 – octadienyl – 3 – hydroxyl benzoate	$C_{11}H_{24}$	156	0.2	1.55
4	22.15	Diethyl phthalate	$C_{12}H_{14}O_4$	222	0.3	2.32
5	25.62	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	270	1.0	7.75
6	26.07	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256	6.4	49.6
7	26.93	7-octadecenoic acid methyl ester	$C_{19}H_{36}O_2$	296	0.9	6.97
8	27.33	9-Hexadecenoic acid	$C_{16}H_{30}O_2$	254	2.7	20.93
9	29.83	1, 2-benzenedicarboxylic acid, diisooctyl ester	$C_{24}H_{38}O_4$	390	0.9	6.97
		Total		12.9cm		99.98

Key:
RT = Retention time
MW = molecular weight

Table. 2: Biological Activities of Phytocomponents Identified in the Ethyl acetate Root Extract of *G. senegalensis*

S/N	Name of the compound	Nature of compound	Activity
1	2-pentanone, 4-hydroxy-4-	Ketone	Antimicrobial
2	Decane,6-ethyl-2-methyl	Organic acid	Industrial uses such as solvents for lacquers, paints, inks, flavouring acid
3	Nonane, 3, 7-dimethyl-2, 6 – octadienyl – 3 – hydroxyl benzoate	Ester	Antitumoral antimicrobial antioxidative
4	Diethyl phthalate	Organic acid	Antimicrobial plasticizer in a wide variety of consumer goods
5.	Hexadecanoic acid, methyl ester	Organic acid	Antimicrobial
6	n-Hexadecanoic acid	Organic acid	Antimicrobial
7	7-octadecenoic acid, methyl ester	Organic acid	Antibacterial
8	9-Hexadecenoic acid	Organic acid	Pesticide and antibiotic
9	1, 2-benzenedicarboxylic acid, diisooctyl ester	Organic acid	Antimicrobial

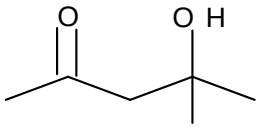

Fig. 3a: Structure of 2-pentanone, 4-hydroxy-4-methyl

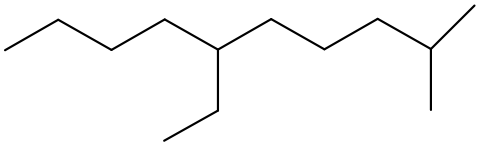

Fig. 3b: Structure of Decane, 6-ethyl-2-methyl

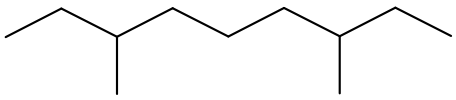

Fig. 3c: Structure of Nonane, 3,7-dimethyl-

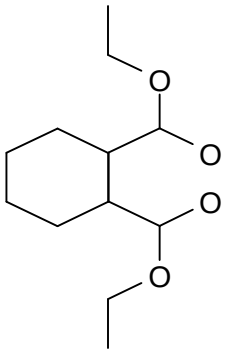

Fig. 3d: Structure of Diethyl phthalate (1, 2-Benzenedicarboxylic acid,2-ethoxy ethyl ester).

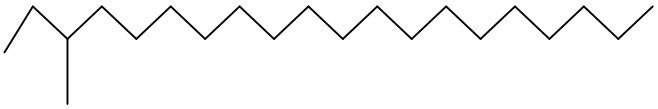

Fig. 3e: Structure of Hexadecanoic acid, methyl ester

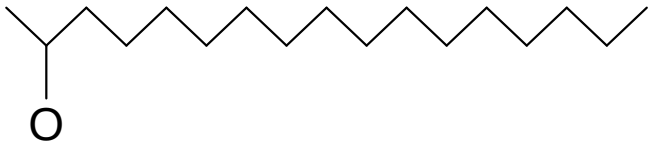

Fig. 3f: Structure of n-Hexadecanoic acid

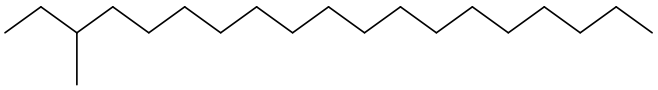

Fig. 3g: Structure of 7-Octadecenoic acid, methyl ester

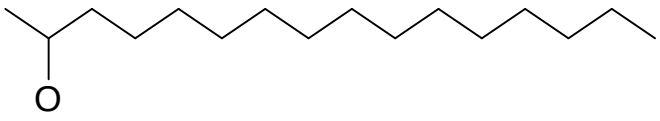

Fig. 3h: Structure of 9-Hexadecenoic acid

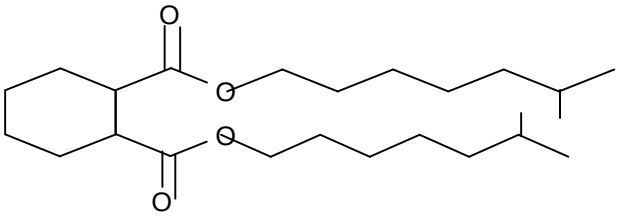

Fig. 3i: Structure of 1, 2-Benzenedicarboxylic acid, diisooctyl ester

CONCLUSION

In the present study nine compounds from the ethyl acetate root extract of *Guiera senegalensis* were identified by Gas-chromatography–Mass spectrometry (GC-MS) analysis. The biological activities of each of the identified phytocomponents range from antimicrobial, antioxidant and antitumoral activities. The nature of the identified compounds are mostly organic acids. The research findings have shown that the root of *Guiera senegalensis* is extensively rich in secondary metabolites. The plant root has a high potential for a vast number of bioactive compounds which justified its use for various ailments by traditional practitioners.

These findings have provided scientific basis to the ethnomedical usage of the plant. However, isolation of the individual phytochemical constituents, subjecting it to biological activity and toxicity profile will give fruitful results.

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