Fatty acids analysis of petroleum ether crude extracts from three parts of Sterculia setigera Del.

Nahla M. M. Taha[1] and Barakat M. Mudawi[2]

1. Department of chemistry, Faculty of Medicine, University of Science and Technology, P.O Box 30 Omdurman, Sudan
2. Department of chemistry, Faculty of Science, University of Khartoum, P.O Box 321 Khartoum, Sudan

Abstract

The main objective of this study was to identify and measure the fatty acid constituents of seeds, roots and stem bark of Sterculia setigera plant using gas chromatography-mass spectrometry (GC-MS) method. The main fatty acids in petroleum ether seed extract are: linoleic acid(25.53%), palmitic acid(18.59%), oleic acid(17.65%), sterculic acid (12.1%), malvalic acid(9.35%) and stearic acid(7.96%).Whereas palmitic acid (32.33%), oleic acid(24.95%), stearic acid(21.34%) are the dominant fatty acids of the stem bark petroleum ether extract. This extract also contains non-fatty acid compounds squalene(4.17%) and phytol(0.87%). Ethyl ester derivatives of fatty acids were the most dominant constituents in root. The petroleum ether extract of the root contains palmatic acid, ethyl ester(28.43%,), 17-methyl-stearic acid,methyl ester(12.06%), oleic acid(10.71%) , elaidic acid,ethyl ester (5.89%). In addition to fatty acid derivatives, the root extract contains the triterpene alpha.-Amyrin(11.87%) and the sterol stigmast-7-en-3-ol,(3,beta.,5.alpha.,24S)-(6.78%).

Keywords: Sterculia setigera, fatty acids, gas chromatography/mass spectroscopy

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Introduction

Oils extracted from plant sources have a rich history of use by local people as a source of food, energy, medicine and for cosmetic applications. It has been used in the production of lubricants, soaps and personal care products, as well as in the topical treatment of various conditions. Due to toxic effects of synthetic oils, there is a growing trend to replace them and revert to the use of natural oils in the cosmetic and pharmaceutical industries.

Sterculia setigera Del., English name karaya gum tree, belongs to the family Sterculiaceae. It is a deciduous savanna tree up to 15m high. Bark grey purple, slash red with paler streaks, exuding white gum and watery sap. In Sudan, it grows wildly in the Blue Nile area, Kassala, Northern Darfur, southern Kordofan and Red Sea hills. Traditionally, the stem bark has been used for treatment of jaundice, bilharzia, asthma, bronchitis, dysentery and wounds. Leaves used as pain killers and for treating malaria. The seeds can be eaten and contain edible oil. Seeds and stem bark are used in treatment of dermatosis.

Materials and Methods

Sampling of Plant materials:

Seeds, stem bark and roots of Sterculia setigera, Del. were collected from November to December from Umabdalla village which is located in Southern Kordofan state, western of Sudan. The plant identified and collected by the help of Dr. Abass Hassan Ali manager of national seed research centre in El Obied, North Kordofan, and his team. Then it was authenticated by the Plant Taxonomist Dr. Haidar Abd Algadir, Medicinal and Aromatic plant research Institute (MAPRI), national research centre, Khartoum, Sudan. Voucher specimens were deposited at the herbarium of the institute. Collected plant parts were cleaned, chopped into pieces, air dried under shade for 4 weeks, and then powdered using a clean electric blender then carefully stored.

Extraction of plat materials:

A quantity of 100 g sample from pulverized seeds, stem bark and roots of Sterculia setigera was introduced separately into a thimble while 500mL of petroleum ether (40 – 60°C) were added to

a pre-weighed round bottom flask. Both thimble and round bottom flask were attached to the extraction unit. The sample was extracted with boiling petroleum ether for 6 hours. Solvent was evaporated under reduced pressure using rotary evaporator apparatus. The residue was dried in an oven to a constant weight and the yield percentage was calculated as follows:

$$\frac{\text{Weight of flask and extract (g) -Weight of empty flask(g)}}{\text{Weight of sample(g)}} \times 100$$

The seed produced 30.877g of brownish yellow oil. Root produced 1.81g brown oily substance and stem bark 2.44g Whitish green solid.

In order to study the fatty acids content of lipid of the parts of the plant by GC/MS each extract was converted to the simplest convenient volatile derivative, methyl esters.

Preparation of fatty acids methyl esters*:*

7ml of alcoholic sodium hydroxide prepared by dissolving 2 g sodium hydroxide in100ml methanol were added to 2mL of the petroleum ether extract. Then 7ml of alcoholic H_2SO_4(1%) were added. The mixture was shaken by vortex for 3 minutes and left overnight. 2mL of supersaturated NaCl and 2ml normal hexane were then added. After the mixture shaken for three minutes, hexane layer was collected. 5µL from hexane layer diluted with 5ml diethyl ether and dried over anhydrous Na_2SO_4. Finally it was filtered through syringe filter 0.45 µm and filtrate from each sample was transferred directly to the GC-MS vial and1.00 µL injected into the GC-MS.

The gas chromatography-mass spectrometry analysis (GC-MS):

Fatty acids esters present in Sterculia setigera extracts were identified and measured using a Shimadzu QP 2010 system GC-MS(Japan) equipped with capillary column(30m length, 0.25mm i.d, 0.25µm film thickness). Helium was used as the carrier gas at 1ml/min, and initial temperature set 50℃, temperature ramp 15℃/min to 250℃ and spectra were obtained over m/z: 100-800.

Results and Discussion

The oil content of seed was found 30.877% which nearly is the same as that obtained by Practice Bazongo et al. which was(30.7%) but higher than that reported by Elkhaleifa (25.25%). The fatty acid composition of the petroleum ether extracts of seed, stem bark and root of Sterculia setigera were determined by GC coupled with mass spectrometer. The fatty acid composition of seed oil is shown in Table 1 and Figure 1. Unlike root and stem, the oil of seed contains unsaturated fatty acids more than saturated. The seed oil also contains cyclopropenoid acids (21.73%) whereas these acids were not detected in root or stem bark pertoleum ether extracts. The most abundant fatty acids in seed oil were linoleic acid (25.53%) followed by palmitic acid(18.59%) and oleic acid (17.65%) stearic acid(7.96%). The fatty acids percentages of seed oil are different from those previously reported. Practice Bazongo et al. reported that the most dominant fatty acids were palmitic acid(25.8%), sterculic acid(20.0%), linoleic acid(19.8%) and cyclopropenoid acids(31.6%)while the dominant fatty acids reported by Miralles J. were linoleic acid(30%), oleic acid(21%), palmitic acid(20%) and cyclopropenoid fatty acids(17.1%).

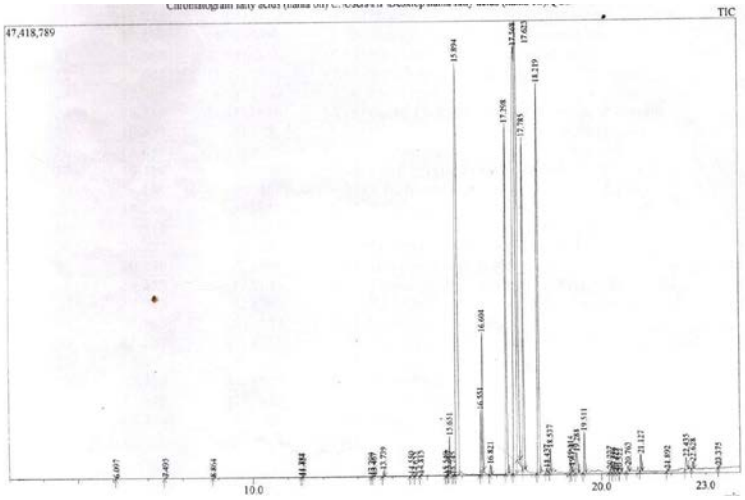

Fig.1: Gas Chromatogram of the Petroleum ether extract of Sterculia setigera seed.

Table 1: Result of GC-MS analysis of petroleum ether extract of Sterculia setigera seed

R_t in min.	Compound name	Chemical form.	Area %
7.495	Nonanoic acid, methyl ester	$C_{10}H_{20}O_2$	0.02
11.388	Butylated Hydroxytoluene	$C_{15}H_{24}O$	0.01
13.390	9,12,15- Octadecatrienoic acid, methyl ester,(Z,Z,Z)-(linolenic acid, methyl ester 18:3)	$C_{19}H_{32}O_2$	0.01
13.467	Cis-9-Tetradecenoic acid, methyl ester, Methyl myristoleate	$C_{15}H_{28}O_2$	0.01
13.739	Tetradecanoic acid, methyl ester(myristic acid, methyl ester)	$C_{15}H_{30}O_2$	0.11
14.550	5-Octadecenoic acid, methyl ether	$C_{19}H_{36}O_2$	0.03
14.655	4-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	0.01
14.815	Pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	0.03
15.549	7,10-Hexadecadienoic acid, methyl ester	$C_{17}H_{30}O_2$	0.01
15.651	9-Hexadecenoic acid, methyl ester, (Z)-(palmitoleic acid,methyl ester)	$C_{17}H_{32}O_2$	0.73
15.745	11-docosenoic acid, methyl ester,(11Z)-	$C_{23}H_{44}O_2$	0.01
15.894	Hexadecanoic acid, methyl ester(palmitic acid, methyl ester)	$C_{17}H_{34}O_2$	18.59
16.551	Methyl 9,12-heptadecadienoate(linolelaidic acid, methyl ester)	$C_{18}H_{32}O_2$	1.26
16.604	Cis-10-Heptadecenoic acid, methyl ester	$C_{18}H_{34}O_2$	2.58
16.821	Heptadecanoic acid, methyl ester(margaric acid,methyl ester)	$C_{18}H_{36}O_2$	0.2
17.298	Methyl 2-octylcyclopropene-1-heptanoate(malvalic acid)	$C_{19}H_{34}O_2$	9.35
17.568	9,12-Octadecadienoic acid(Z,Z)-, methyl ester (linoleic acid, methyl ester)	$C_{19}H_{34}O_2$	25.53
17.623	9-Octadecenoic acid(Z)-, methyl ester(Oleic acid, methyl ester)	$C_{19}H_{36}O_2$	17.65
17.785	Octadecanoic acid,methyl ester(stearic acid, methyl ester)	$C_{19}H_{38}O_2$	7.96
18.219	Methyl 2-octylcyclopropene-1-octanoate (methyl sterculate)	$C_{20}H_{36}O_2$	12.10
18.437	Cis-10-Nonadecenoic acid, methyl ester	$C_{20}H_{38}O_2$	0.09

18.537	10-Nonadecenoic acid, methyl ester, (E)	$C_{20}H_{38}O_2$	0.45
19.114	Cyclopropaneoctanoic acid, 2-[[2-[(2-ethylcyclopropyl)methyl] cyclopropyl]methyl]-,methyl ester	$C_{22}H_{38}O_2$	0.28
19.151	9,12-Octadecadienoyl chloride, (Z,Z)	$C_{18}H_{31}ClO$	0.11
19.288	9-Octadecenoic acid, 1,2,3-propanetriyl ester,(E,E,E)	$C_{57}H_{104}O_6$	0.72
19.511	18-methylnonadecanoic acid, methyl ester	$C_{21}H_{42}O_2$	0.82
20.237	11-Octadecenoic acid, methyl ester	$C_{19}H_{36}O_2$	0.06
20.336	Heneicosanoic acid, methyl ester	$C_{22}H_{44}O_2$	0.04
20.522	9,12-Octadecadienoic acid,ethyl ester	$C_{20}H_{36}O_2$	0.02
20.763	9-Octadecenal, (Z)	$C_{18}H_{34}O$	0.21
21.127	20-methyl heneicosanoic acid, methyl ester.	$C_{23}H_{46}O_2$	0.33
21.892	Tricosanoic acid, methyl ester	$C_{24}H_{48}O_2$	0.04
22.435	9-octadecenoyl chloride, (Z)-Oleoyl chloride	$C_{18}H_{33}ClO$	0.39
22.628	Tetracosanoic acid,methyl ester	$C_{25}H_{50}O_2$	0.16
23.375	Squalene	$C_{30}H_{50}$	0.05
	Total		99.97
	SA		28.30
	USA		49.17
	CPA		21.73
	*Other compounds		0.77

Key: R_t =Retention time, Chemical form. = Chemical formula, SA = saturated fatty acids (esters), USA= unsaturated fatty acids(esters), CPA = cyclopropenoid fatty acids(methyl esters). *Other compounds = aldehyde, triterpene,acid chlorides and a derivative of phenol.

Regarding stem bark petroleum ether extract 14 components were identified. They were shown in Table 2 and Figure 2. The major fatty acids(expressed as methyl esters) are palmitic acid (32.33%), Oleic acid (24.95%), stearic acid (21.34%). There are considerable amounts of 3,3-Dimethylnonadecane(9.34%), squalene(4.17%), arachidic acid(2.01%), linoleic acid(2.10%) and phytol(0.87%). Saturated fatty acids were the most dominant in stem bark.

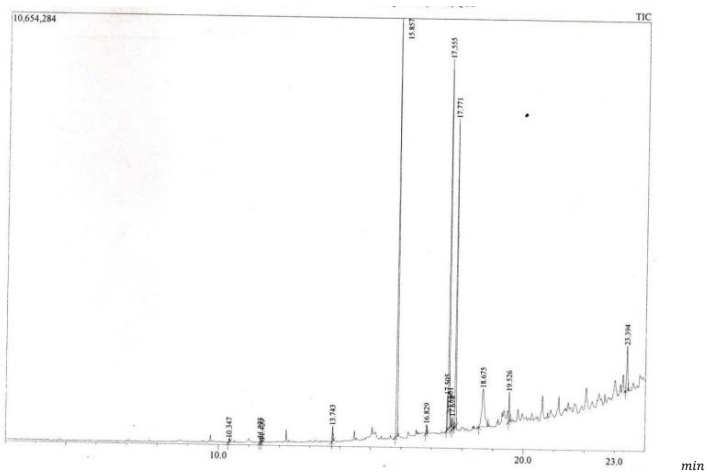

Fig.2: Gas Chromatogram of the Petroleum ether extract of Sterculia setigera stem bark

Table 2: Result of GC-MS analysis of petroleum ether extract of Sterculia setigera stem bark

R_t in min.	Compound name	Chemical form.	Area %
10.347	Nonanoic acid, 9-oxo-, methyl ester	$C_{10}H_{18}O_3$	0.23
11.373	Phenol,2,4-bis(1,1- dimethylethyl)-	$C_{14}H_{22}O$	0.19
11.420	Dodecanoic acid, methyl ester	$C_{13}H_{26}O_2$	0.08
13.743	Tetradecanoic acid, methyl ester(myristic acid, methyl ester)	$C_{15}H_{30}O_2$	0.88
15.857	Hexadecanoic acid, methyl ester (palmitic acid,methyl ester)	$C_{17}H_{34}O_2$	32.33
16.829	Heptadecanoic acid, methyl ester	$C_{18}H_{36}O_2$	0.55
17.505	9,12-Octadecadienoic acid (Z,Z)-, methyl ester	$C_{19}H_{34}O_2$	2.10
17.555	9-Octadecenoic acid(Z) , methyl ester (Oleic acid, methyl ester)	$C_{19}H_{36}O_2$	24.95
17.601	9-Octadecenoic acid, methyl ester, (E)- (Elaidic acid,methyl ester)	$C_{19}H_{36}O_2$	0.95
17.678	Phytol	$C_{20}H_{40}O$	0.87
17.771	Octadecanoic acid, methyl ester (stearic acid,methyl ester)	$C_{19}H_{38}O_2$	21.34
18.675	3,3-Dimethylnonadecane	$C_{21}H_{44}$	9.34
19.526	Eicosanoic acid, methyl ester (arachidic acid, methyl ether)	$C_{21}H_{42}O_2$	2.01
23.394	Squalene	$C_{30}H_{50}$	4.17
	Total		99.99%
	SA		57.42%
	USA		28%
	*Other compounds		14. 57%

*Other compounds = a derivative of phenol, diterpene alcohol, hydrocarbon and triterpenes

The chemical composition of root is shown in Table 3 and Figure 3 the major components are palmitic acid, ethyl ester(28.43%), 17-methyl stearic acid, methyl ester(12.06%), Oleic acid(10.71%), alpha.-Amyrin(11.87%), Stigmast-7-en-3-ol, (3,beta.,5.alpha.,24S) (6.78%), ethyl elaidate (5.89%). To the best of our knowledge the content of petroleum ether extract of root and stem bark have not previously been reported.

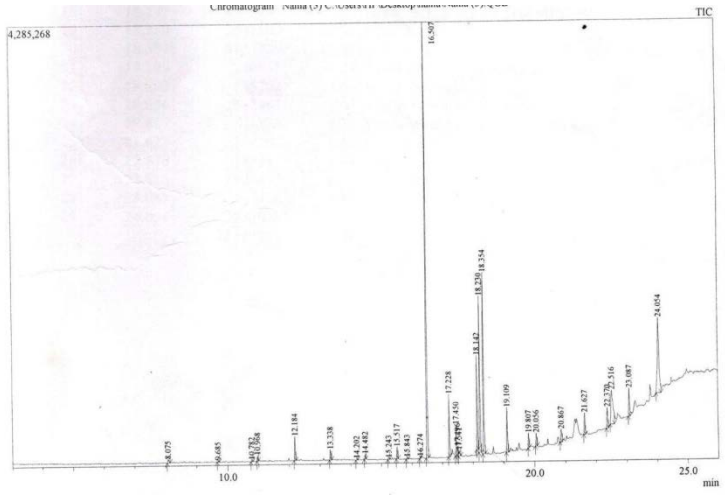

Fig.3: Gas Chromatogram of the Petroleum ether extract of Sterculia setigera root.

Table 3: result of GC-Ms analysis of petroleum ether extract of Sterculia setigera root

R_t in min.	Compound name	Chemical form.	Area %
8.075	Benzaldehyde,4-methoxy	$C_8H_8O_2$	0.38
10.968	Heptanedioic acid,diethyl ester(diethyl pimlate)	$C_{11}H_{20}O_4$	0.38
12.184	Octanedioic acid, diethyl ester (Diethylsuberate)	$C_{12}H_{22}O_4$	1.56
13.338	nonanedioic acid (Azelaic acid)	$C_9H_{16}O_4$	0.73
14.202	Nonanoic acid,9,9-diethoxy-,ethyl ester	$C_{15}H_{30}O_4$	0.11
14.482	Tetradecanoic acid, ethyl ester (myristic acid,ethyl ester)	$C_{16}H_{32}O_2$	0.38
15.243	14-methyl-Hexadecanoic acid, ethyl ester	$C_{19}H_{38}O_2$	0.14
15.517	Pentadecanoic acid, ethyl ester	$C_{17}H_{34}O_2$	0.74
15.843	Hexadecanoic acid, methyl ester	$C_{17}H_{34}O_2$	0.14

16.274	9-hexadecenoic acid, ethyl ester(palmitelaidic acid)	$C_{18}H_{34}O_2$	0.16
16.507	Hexadecanoic acid, ethyl ester(palmitic acid,ethyl ester)	$C_{18}H_{36}O_2$	28.43
17.228	9-Octadecenoic acid(Z)-, ethyl ester	$C_{20}H_{38}O_2$	4.2
17.450	Heptadecanoic acid, ethyl ester (margaric acid)	$C_{19}H_{38}O_2$	2.11
17.496	9,12-Octadecadienoic acid (Z,Z)- ,methyl ester (linoleic acid, methyl ester)	$C_{19}H_{34}O_2$	0.51
17.541	9-Octadecenoic acid, methyl ester,(E)-	$C_{19}H_{36}O_2$	0.47
18.142	9-Octadecenoic acid, ethyl ester,(E)-	$C_{20}H_{38}O_2$	5.89
18.230	9-octadecenoic acid(Z)(Oleic acid)	$C_{18}H_{34}O_2$	10.71
18.354	Octadecanoic acid, 17-methyl-, methyl ester	$C_{20}H_{40}O_2$	12.06
19.109	13-docosenoic acid, ethyl ester,(Z) (erucic acid, ethyl ester)	$C_{24}H_{46}O_2$	3.24
19.807	9- Octadecenoic acid, 12-hydroxy-,[R-(Z)]-(Ricinoleic acid)	$C_{18}H_{34}O_3$	1.01
20.056	Methyl 19-methyl-eicosanoate	$C_{22}H_{44}O_2$	1.03
20.867	Pentacontanoic acid, ethyl ester	$C_{52}H_{104}O_2$	1.33
21.627	Octadecanoic acid, ethyl ester (stearic acid,ethyl ester)	$C_{20}H_{40}O_2$	1.64
22.370	Docosanoic acid, ethyl ester(Behenic acid,ethyl ester)	$C_{24}H_{48}O_2$	1.64
22.516	Stigmast-7-en-3-ol, (3,beta.,5.alpha.,24S)-	$C_{29}H_{50}O$	6.78
23.087	Tetracosanoic acid , ethyl ester	$C_{26}H_{52}O_2$	2.13
24.054	Alpha.-Amyrin	$C_{30}H_{50}O$	11.87
	Total		99.77
	SA		54.55
	USA		26.19
	*Other compounds		19.03

Key: *Other compounds= aldehyde, triterpenes,sterols

Conclusion

This study shows that the main constituents of petroleum ether extracts in the tested parts were fatty acids derivatives. Since the parts tested contain numerous bioactive compounds including palmitic acid, stearic acid and oleic acid, therefore, the fatty acid can be further used to evaluate the various biological activities. Regarding the high amount of unsaturated fatty acids, it seems

that Sterculia setigera seeds may be a good dietary source for unsaturated fatty acids especially linoleic acid(omega 6)(25.53%).

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