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TLC and GC-MS analysis of petroleum ether fraction of fermented wood "*Nikhra*" of *Acacia seyal*

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Abstract

This study aims to detect organoleptically *Acacia seyal* fractions (petroleum ether, chloroform, methanol and aqueous) fermented wood "*Nikhra*" of the fractions accumulating the strongest sweet fragrance these fragrances were mainly accumulated in the petroleum ether fermented wood "*Nikhra*" fraction and analysis fraction by using chromatographic and spectroscopic analysis. Petroleum ether fermented wood "*Nikhra*" fraction analysis with TLC and spray TLC with vanillin H₂SO₄ (pink) (C₁, C₂, C₃, C₄, C₅, C₆, C₇), R_f values (0.92, 0.86, 0.71, 0.64, 0.57, 0.50, 0.36), were expected to be phenolic, with vanillin HCl (red) compounds spots (C₅, C₆, C₇) with R_f values (0.57, 0.50, 0.37) was expected to be catechin and with vanillin H₃PO₄, blue-violet zones compounds spots (C₆, C₇), with R_f values (0.50, 0.36), were expected to be lignans. Petroleum ether fermented wood "*Nikhra*" fraction was divided into two types of compounds classes aromatic and non-aromatic by <http://research.easybib.com> and hence compounds were classified to phenolics and terpenoids compounds by GC/MS. Fragrant aromatics or terpenoids were targeted in this part of study. GC-MS analysis gave a spectrum of fragrance aromatic compounds (phenolics) in the petroleum ether fermented wood "*Nikhra*" fractions of *A. seyal*, was Petadecanoic acid (5.64%) and Tetracosamethyl-cyclododecasiloxane (4.17%) total fragrance aromatic compounds (44.57%), and main terpenoids compounds was Octadecanoic acid (2.52) % total fragrance aromatic compounds (11.87%).

Keywords: *Acacia seyal*, Gas Chromatography (GC), Thin-layer chromatography (TLC)

Introduction

The resinous heartwood of *A. seyal* trees are usually used in Sudanese fragrances. The wood of *A. seyal* is pale yellow to medium brown, with localized pinkish-brown patches and some dark mahogany-red heartwood in larger or older individuals. *A. seyal* wood has potential in rural areas as timber. *A. seyal*, also produces a gum which, in spite of being of an inferior quality than that of *A. senegal*, is still marketed in Sudan 36.000-40.000 tons. The gum is edible when fresh, with a slightly acidic taste. *Talh* gum is attractive because of its clarity and solubility, gum is mixed with soot and powdered Nubian sandstone for black and red ink (Kimaro *et al.*, 2011). Phytochemically *A. seyal* was characterized with high contents of proteins, phenolics, flavonoids and anthocyanins. The bark contains 18-30 % tannins and is a source of red dye (Orwa *et al.*, 2012). The bark of *A. seyal* is the most valuable part of *A. seyal*. It is, extensively, used for feeding cattle, goats and sheep during the dry season. In human medicine *A.*

seyal leaves, gum and bark are used in phytotherapy for haemorrhage, colds, diarrhoea, gastro-intestinal disorders, jaundice, biliary diseases, syphilis, and headaches and as emollient, astringent, for burns and ophtalmia (Orwa *et al.*, 2012).

Materials and Methods

Collection of plant materials

Fermented hardwood "*Nikhra*" of *A. seyal* were collected in March 2011 from Kordofan state, Sudan. They were, carefully, washed, oven-dried for 1 h at 50°C and put in the shade in an aerated place till complete drying, then were ground into a fine powder.

Plant materials preparation and extraction

A hundred g of ground powder of each plant was extracted using methanol and a soxhlet apparatus. The methanolic extract was fractionated, sequentially, using solvents of increasing polarity namely petroleum ether, chloroform, and aqueous. Fractions were dried using an evaporator and stored at 4°C for further analysis (Fyhrquist *et al.*, 2002).

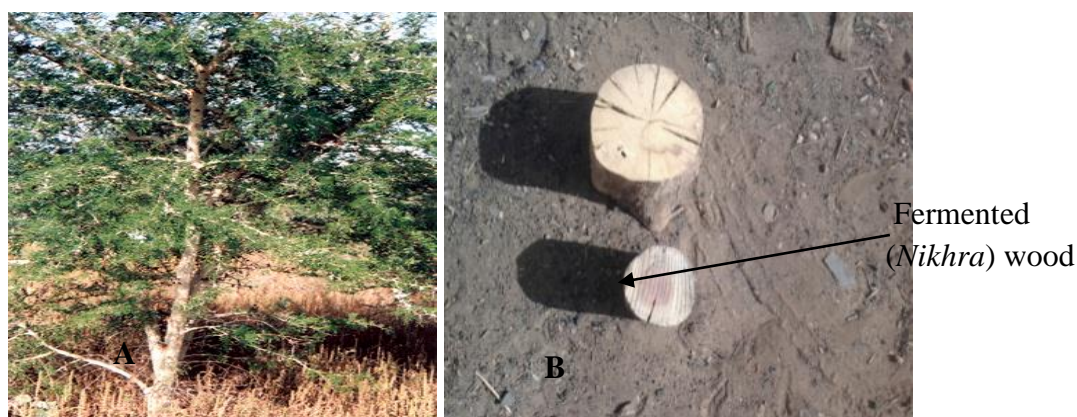


Figure 1. (A).Tree of *Acacia seyal* (*Talh*) (B). Fermented wood (*Nikhra*) of *Acacia seya*

Thin layer Chromatographic analysis

TLC was performed on a pre-coated silica gel TLC plates grade F254 (E-Merck, Darmstadt, German) to determine the number of compounds present in petroleum ether fermented wood "*Nikhra*" fraction. sample was spotted at 1 cm from the bottom of silica gel plates using capillary tubes. Development of the chromatogram was done in closed tanks, in which the atmosphere has been saturated with eluent vapour by wetting a filter paper lining. The chromatogram was visualized under UV light (366 nm and 254 nm), Natural Product Reagent (NPR), H₂SO₄, HCL and H₃PO₄ acid reagent spray. The R_f values of the compounds were calculated using the following formula.

$R_f = \text{distance travelled by the compound} / \text{Distance travelled by the solvent front}$

GC-MS Analysis

Petroleum ether fermented wood "*Nikhra*" fraction was subjected to GC MS analysis to identify the various bioactive compounds present. The sample was analyzed in GCMS-QP2010 Plus from Delhi

University (DU) India. ACQ Mode Scan: 40m/z to 600m/z, Column flow is 1.21mL/min and total flow is 16.3ml/min. Flow control with linear velocity which 40.9cm/sec. The identification of compounds was done using computer matching of mass spectra with those of standards (WILEY8. LIB. and NIST11.library).

RESULTS AND DISCUSSION

Thin layer Chromatographic analysis

The presence of flavonoids was confirmed by their color change from quenching fluorescence (254nm) to yellow or orange color for flavonoid and prominent blue color in case of flavonoidal acids or other phenolic acids (366 nm) after spraying with Natural Product Reagent (NPR). Polyphenols were mainly accumulated in the petroleum ether fraction has been detected using NPR. Fluorescence behavior of flavonoids in response to NPR is structure dependent. Flavonoids e.g. quercetin and myrecitin develops orange color and those of kaempferol and isorhamntin yellow to green colors. Flavones glycosides of luteolin develops orange colors and those apigenin yellow to green (Wagner and Bladt, 1996).

Vanillin H₂SO₄ is a universal reagent that detects components of the petroleum ether fraction, terpenoids, phenols etc., typical pink to purple colors were developed upon spraying with vanillin H₂SO₄ (heat 110°C). All phenolic at UV 254 nm show prominent quenching, and they give blue fluorescence at UV 366 nm (Wagner and Bladt, 1996). After spraying fraction of the petroleum ether of fermented (F) wood of *A. seyal* by vanillin H₂SO₄, they showed typical pink and purple zones of phenolic. Accordingly compounds spots (C1, C2, C3, C4, C5, C6, C7), R_f values (0.92, 0.86, 0.71, 0.64, 0.57, 0.50, 0.36), were expected to be phenolic.

Vanillin HCL is specific reagent that detects components of catechin. All catechin at UV 254 nm show prominent quenching, and they give blue fluorescence at UV 366 nm (Wagner and Bladt, 1996). After spraying fraction of the petroleum ether fraction of fermented (F) wood of *A. seyal* by vanillin HCL, they showed typical red zone of catechin, accordingly compounds spots (C5, C6, C7), with R_f values (0.57, 0.50, 0.37), was expected to be catechin. Lignans are formed by oxidative coupling of p-hydroxyphenylpropeue units, often linked by an oxygen bridge. They are found in fruits, foliage, heartwood and roots. All lignans at UV 254 nm show prominent quenching, and they give blue fluorescence at UV 366 nm (Wagner and Bladt, 1996). After spraying the petroleum ether fraction of fermented (F) wood of *A. seyal* by vanillin H₃PO₄, they showed typical red to blue-violet and brown zones of lignans, accordingly compounds spots (C6, C7), with R_f values (0.50, 0.36), respectively were expected to be lignans.

Gas chromatography mass spectrometry (GC/MS) of petroleum ether fraction.

The chemical composition of *Nikhra* petroleum ether fractions of *A. seyal* was analyzed by GC/MS. The compounds identified by matching their fragmentation patterns in mass spectra with those stores in NIST library with the help of HPCHEM software published mass spectra. Petroleum ether fractions were divided into two types of compounds classes aromatic and non-aromatic and hence compounds were classified to phenolics and terpenoids compounds by GC/MS. Fragrant aromatics or terpenoids were targeted in this part of study by <http://research.easybib.com>.

Anjaneyulu, and Rao (2000) chemically examined the hexane extract of the roots of *Excoecaria agallocha* they isolated eleven diterpenoids, while Nakanishi et al. (1984) isolated fragrant sesquiterpenes as major constituents from the wood of *Aquilarza malaccenszs* and identified as agarofuran, (-)-lo-epl-y-eudesmol and oxo-agarospirol.

In all petroleum ether fractions Area% represent the concentrations of corresponding compound, main fragrance aromatic phenolics and terpenoids compounds in the petroleum ether fractions of *A. seyal* “*Nikhra*” are presented in Table (1 and 2).

Table 1: Chemical composition of fragrant aromatic compounds (phenolics) in the petroleum ether fractions of *A.seyal* fermented wood “*Nikhra*”.

Science name	Peak #	tr(min)	Area%	Mol Weight	Structure assigned (MS data comparison NIST27)
<i>A. seyal</i>	1	13.024	0.73	130	Cyclononasiloxane,tetradecamethyl-
	2	13.225	0.37	78	1,3-Cyclohexadiene,5-(1,5-dimethyl-4-hexenyl)-2-methyl-,[S-
	3	13.550	0.3	118	Phenol,3,5-bis(1,1-dimethyl)-
	5	14.725	0.39	58	Hexadecane
	7		2.700	192	Benzene,1,2,4,5-tetrachloro-3,6-dimethoxy-
	9	17.625	1.19	189	Octadecamethylcyclononasiloxane
	11	19.724	0.87	165	Eicosamethylcyclodecasiloxane
	12	19.858	5.64	178	Petadecanoic acid
	15	21.909	0.99	186	Tetracosamethylcyclododecasiloxane
	19	23.134	1.91		Hexadecanamide
	21	24.097	1.77	247	Cyclononasiloxane,octadecamethyl-
	22	24.953	0.57	156	Gingerol
	25	26.260	2.83	251	1H-Purin-6-amine,[(2-fluorophenyl)methyl]-(CAS)
	26	28.367	2.45	213	1H-Purin-6-amine,[(2-fluorophenyl)methyl]-(CAS)
	28	30.398	3.11	203	Cyclononasiloxane,octadecamethyl-
	29	30.811	2.47	235	E,E,Z-1,3,12-Nonadecariene-5,14-diol
	32	32.334	3.39	199	Cyclodecasiloxane,eicosamethyl-
	33	32.626	0.87	256	Squalene
	34	34.475	3.85	188	Cyclodecasiloxane,eicosamethyl-
	35	37.11	4.00	190	Tetracosamethyl-cyclododecasiloxane
	37	40.498	4.17	234	Tetracosamethyl-cyclododecasiloxane
Total			44.57		

Table.2: Chemical composition of fragrant aromatic compounds (Terpenoids) in the petroleum ether fractions of *A. seyal* fermented wood “*Nikhra*”.

Science name	Peak #	tr(min)	Area%	Mol Weight (<i>m/z</i>)	Structure assigned (MS data comparison NIST27)
<i>seyal</i>	6	7.942	0.39	40	o-Xylene
	8	9.550	2.04	69	Alpha-pinene,(-)
	9	10.167	0.24	44	Camphene
	10	11.258	0.39	47	beta.-Phellandren
	13	13.625	0.23	43	dl-Limonene
	14	13.733	0.10	47	1,8-Cineole
	21	31.108	0.10	49	Zingiberene
	23	34.183	0.30	55	Heptadecane
	24	35.933	0.25	74	methyl2-(4-methoxy-phenoxy)propanoate
	27	39.525	1.02	216	Octadecamethylcyclononasiloxane
	35	43.442	2.52	242	Octadecanoic acid
	36	43.692	1.82	213	Octadecanamide
	43	46.000	0.58	288	Eicosamethylcyclodecasiloxane
	44	46.217	0.47	184	Octadecanamide
	47	48.283	1.42	155	Tetracosamethyl-cyclododecasiloxane
	Total		11.87		

Balaban (2004) studied the tannin composition of wood of *Ceratonia siliqua* using GC-MS. The fractioned methanolic extracts revealed phenolic compounds (gallic acid), flavonoids (catechin), methyl inositol and chalcone. Main fragrance aromatics compounds (phenolics) in the petroleum ether fractions of *A.seyal* was petadecanoic acid (5.64%) (Fig.1) and tetracosamethyl-cyclododecasiloxane (4.17%) total fragrance aromatic compounds (44.57%) (Table 1; Fig2), and main terpenoids compounds was Octadecanoic acid (2.52) % total fragrance aromatic compounds (11.87%) (Table 2; Fig. 3).

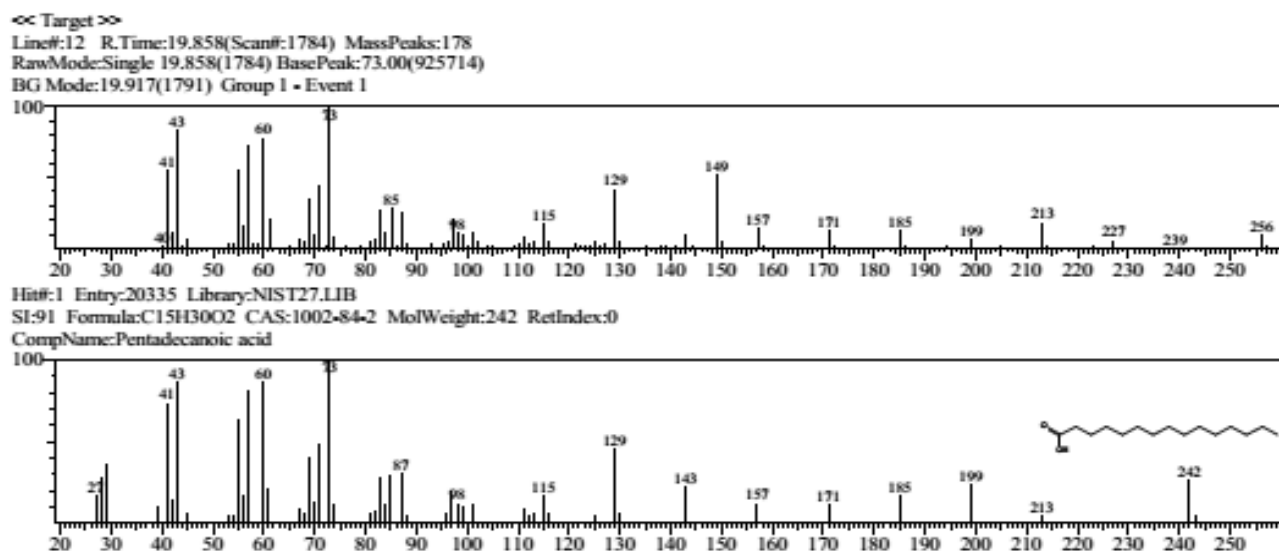


Figure 1. Fragrant aromatic compounds (phenolics) in the petroleum ether fractions of *A.seyalNikhra* (Petadecanoic acid).

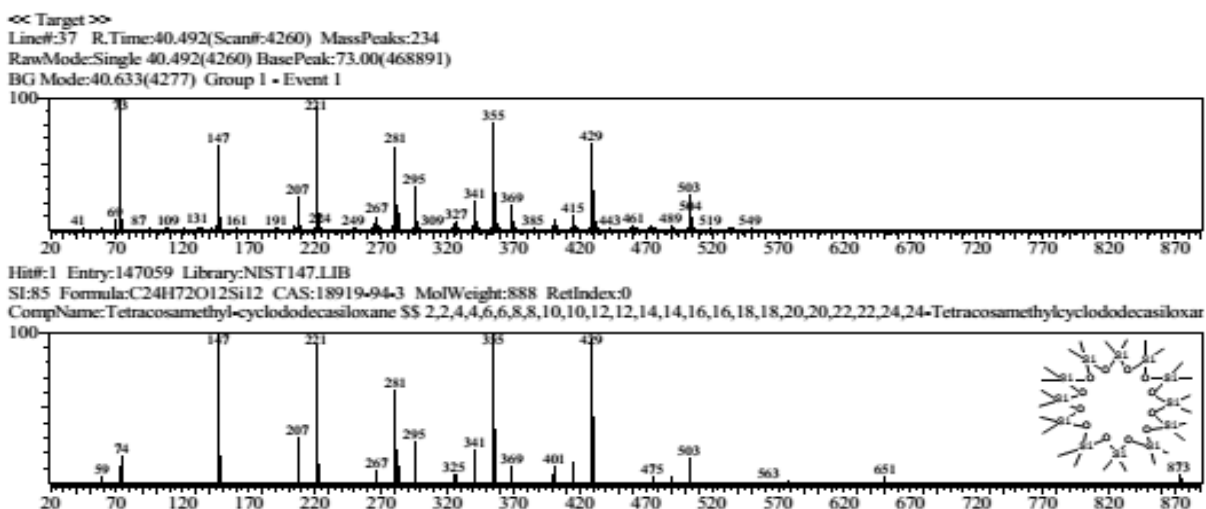


Figure 2. Fragrant aromatic compounds (phenolics) in the petroleum ether fractions of *A.seyalNikhra* (Tetracosamethyl-cyclododecasiloxane).

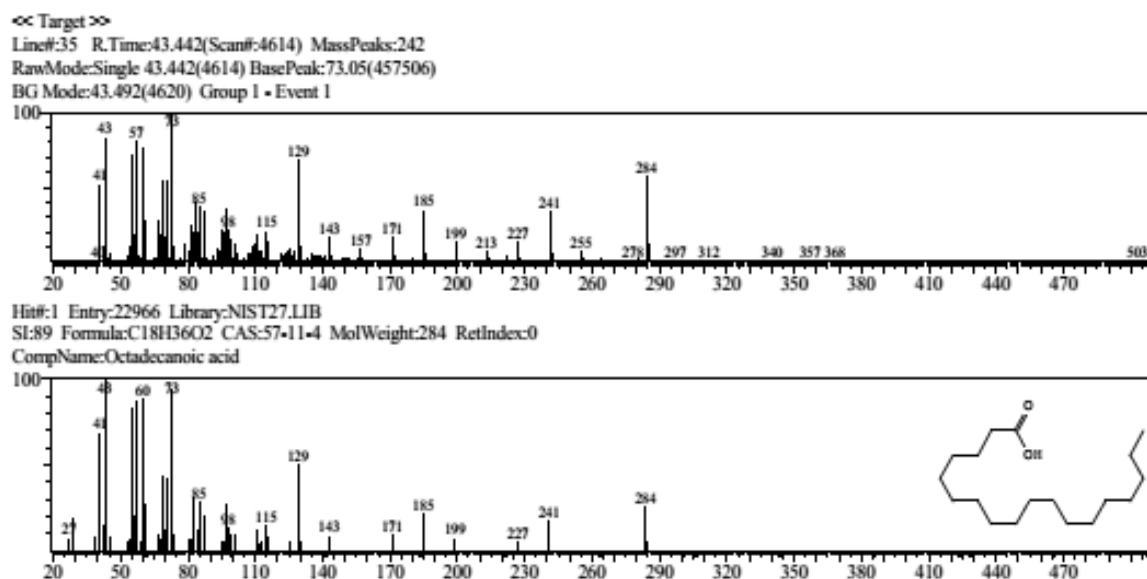


Figure 3. Fragrant aromatic compounds (terpenoids) in the petroleum ether fractions of *A.seyal* *Nikhra*(Octadecanoic acid).

CONCLUSIONS

Organoliptic survey of fragrance in different fractions of *A.seyal* was ensured stable and strong fragrances; these fragrances were mainly accumulated in the petroleum ether fractions. *Nikhra* Fragrance in the petroleum ether fraction which have different scents were proved to be polyphenols by TLC after spraying with NPR, specific reagent for detects components: catechin (van HCL), terponoids (van H₂SO₄) and lignans (van H₃PO₄). GC/MS analysis of the petroleum ether fraction revealed that the total fragrant compounds, phenolics and terponoids, for *A. seyal* was 56.44%.

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