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TLC and GC-MS analysis of fermented wood *"Nikhra"* petroleum ether fraction of Combretaceae spp. *Combretum hartmannianum* and *Terminalia laxiflora*

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Abstract

This study aims to analysis fractions (petroleum ether, chloroform, methanol and aqueous) fermented wood Nikhra of Combretaceae spp (*Combretum hartmannianum* and *Terminalia laxiflora*) analysis it by using chromatographic and spectroscopic analysis. Petroleum ether Nikhra fraction analysis with TLC and spray TLC with vanillin H_2SO_4 (pink) (B1, B2), Rf values (0.84, 0.81), were expected to be phenolic, with vanillin HCL (red) compounds spots (B2) with Rf values (0.81) was expected to be catechin and with vanillin H_3PO_4, blue-violet zones compounds spots (A1, A2, A3), (B1, B2, B3, B4), with Rf values (0.88, 0.78, 0.67), (0.84, 0.81, 0.67, 0.59), respectively were expected to be lignans. Petroleum ether fermented wood*"Nikhra"* fraction was 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. GC-MS analysis gave a spectrum of fragrance aromatic compounds (phenolics) in the petroleum ether Nikhrafractions of *T. laxiflora*, was Lup-20(29)-en-3-ol, acetate, (3β) and Tetracosamethyl-cyclododecasiloxane, main terponoids compounds were eicosamethylcyclodecasiloxane. Main fragrance aromatics compound in the petroleum ether Nikhra fraction of *C. hartmannianum* was 2-tert-Butyl-5-(hydroxtmethyl)-4-formylfuran, and main terponoids compound was Tetracosamethylcyclododecasiloxane.

Key words: *Combretaceae, Combretum hartmannianum, Terminalia laxiflora,* Gas Chromatography, Thin-layer chromatography (TLC)

Introduction

The resinous heartwood of *C. hartmannianum* and *T. laxiflora*, trees are usually used in Sudanese fragrances, the scented heart wood is used as perfume and the root bark is used to treat wound and strains, the macerated stem bark of *T. laxiflora* serves as antiseptic to wash mouth in order to resist gingivitis and thrush serves as wound dressing, diuretic management, pile and yaws treatment(Ivory coast), anti-skin inflammation, sores and ulcers treatment (Sierra Leone), eye lotion(Gambia), hair perfume, severe jaundice and chewing stick (Cameroon) across other African

countries (Abbiw, 1990; Daniel, 1990; Batawila *et al.*, 2005). The barks decoction of *T. laxiflora* is used for malaria (Doka and Yagi, 2009). *Dokhan* and *Bakhour* are a traditional processes used by Sudanese married women to make their own perfume, rarely single female use them for medical purpose (Mariod *et al*., 2014). The present study, is designed to analyze fractions (petroleum ether, chloroform, methanol and aqueous) of fermented wood Nikhra-Combretaceae spp (*Combretum hartmannianum* and *Terminalia laxiflora*) by using chromatographic and spectroscopic analysis.

Materials and Methods

Collection of plant materials

Natural fermented hardwood *"Nikhra*" of *Combretum hartmannianum* and *Terminalia laxiflora* were collected from Kordofan state, Sudan. They were, carefully, washed, oven-dried for 1 h at 50°C and ground into a fine powder.

Plant materials preparation and extraction

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

Thin Layer Chromatographic (TLC) analysis

TLC was performed on a pre-coated silica gel TLC plates grade F254 (E-Merck, Darmstadt, Germany) to determine the number of compounds present in petroleum ether *Nikhra* fraction. Sample was spotted at 1.0 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 vapor by wetting a filter paper lining. The chromatogram was visualized under UV light (366 nm and 254 nm), Natural Product Reagent (NPR), H_2SO_4, HCL and H_3PO_4 acid reagent spray. The R_f values of the compounds were calculated using the following formula.

R_f = distance travelled by the compound/Distance travelled by the solvent front

GC-MS Analysis

Petroleum ether 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 is 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) (Table 1). Polyphenols (phenolic acids, flavanoids) has been detected using NPR (366 nm). 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 H_2SO_4 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 H_2SO_4 (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 wood of *T. laxiflora* and *C. hartmannianum* by vanillin H_2SO_4, they showed typical pink and purple zones of phenolic. Accordingly compounds spots (B1, B2), R_f values (0.84, 0.81), 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 the petroleum ether fraction of fermented wood of *T. laxiflora* and *C. hartmannianum* by vanillin HCL, they showed typical red zone of catechin, accordingly compounds spots (B2) with R_f value (0.81) 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 wood of *T. laxiflora* and *C. hartmannianum* by vanillin H_3PO_4, they showed typical red to blue-violet and brown zones of lignans. Accordingly, compounds' spots (A1, A2, A3), (B1, B2, B3, B4), with R f values (0.88, 0.78, 0.67), (0.84, 0.81, 0.67, 0.59) were respectively expected to be lignans (Table 1).

Table 1. TLC profile of the petroleum ether fraction of fermented wood of *T. laxiflora* (A 1-4) and *C. hartmannianum* (B 1-8) sprayed by NPR, Vanillin H_2SO_4, Vanillin HCL, and Vanillin H_3PO.

Spot No	R_f Value		UV Reaction		Reaction to diagnostic reagents				Expected Metabolite			
	254 nm	**366 nm**	**254 nm**	**366 nm**	**NPR 366**	**Van H_2SO_4**	**Van HCL**	**Van H_3PO_4**	**NPR 366 nm**	**Van H_2SO_4**	**Van HCL**	**Van H_3PO_4**
A1	0.88	-	Quenching	Yellow	Yellow	-	-	Red	-	-	-	Lignan
A2	0.78	-	Quenching	Blue	Blue	-	-	Red	Phenolic	-	-	Lignan
A3	0.67	-	Quenching	Blue	Blue	-	-	Red	Phenolic	-	-	Lignan
A4	0.13	-	Quenching	Blue	Blue	-	-	-	Phenolic	-	-	-
B1	0.84	0.64	Quenching	Yellow	Yellow	Yellow	-	Red	Phenolic		-	Lignan
B2	0.81	0.55	Quenching	Yellow	-	purple	Red	Red	-	Terpernoid	Catechin	Lignan
B3	0.67	0.45	Quenching	Blue	Blue	-	-	Red	Phenolic	-	-	Lignan
B4	0.59	-	Quenching	Blue	Blue	-	-	Red	Flavanoid	-	-	Lignan
B5	0.38	0.23	Quenching	Yellow	Yellow	-	-	-	Flavanoid	-	-	-
B6	0.30	0.21	Quenching	Yellow	Yellow	-	-	-	Flavanoid	-	-	-
B7	0.24	0.16	Quenching	Blue	Yellow	-	-	-	Flavanoid	-	-	-
B8	0.18	0.09	Quenching	Blue	Yellow	-	-	-	-	-	-	-

GC-MS Analysis

The chemical composition of *Nikhra* petroleum ether fractions of *T. laxiflora*, and *C. hartmannianum* were 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.

Main fragrance aromatic compounds (phenolics) in the petroleum ether Nikhra fraction of *T. laxiflora*, (Fig. 1) was Lup-20(29)-en-3-ol, acetate, (3β) which representing 15.71% and Tetracosamethyl-cyclododecasiloxane repeated in different concentrations the highest one was (3.02%) (Fig. 2), total compounds (34.56%) and main terponoids compounds was eicosamethylcyclodecasiloxane (2.69%) total fragrance aromatic compounds (10.08%) (Fig. 3).

Main fragrance aromatics compounds in the petroleum ether Nikhra raction of *C. hartmannianum* was 2-tert-Butyl-5-(hydroxtmethyl)-4-formylfuran (7.73%) total fragrance aromatic compounds (11.85%) (Fig. 4), and main terponoids compounds was Tetracosamethylcyclododecasiloxane (2.36%) total fragrance aromatic compounds (7.54%) (Fig. 5).

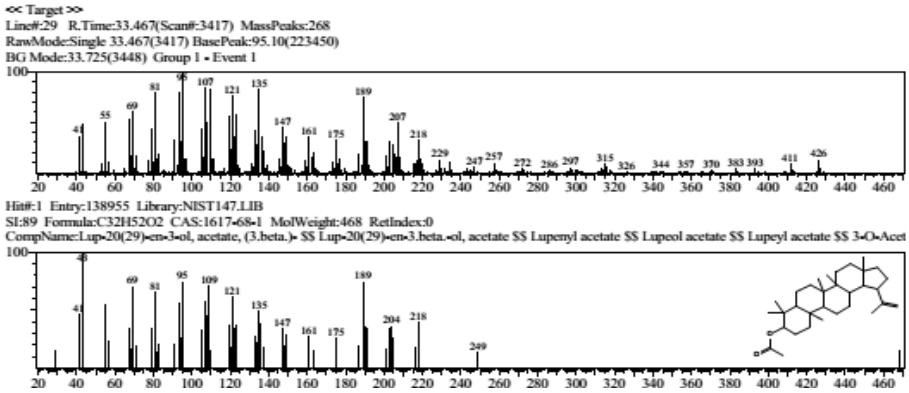

Fig. 1. Fragrant aromatic compounds (phenolics) in the petroleum ether Nikhra fraction of *T. laxiflora* (Lup-20(29)-en-3-ol, acetate,(3.beta-)

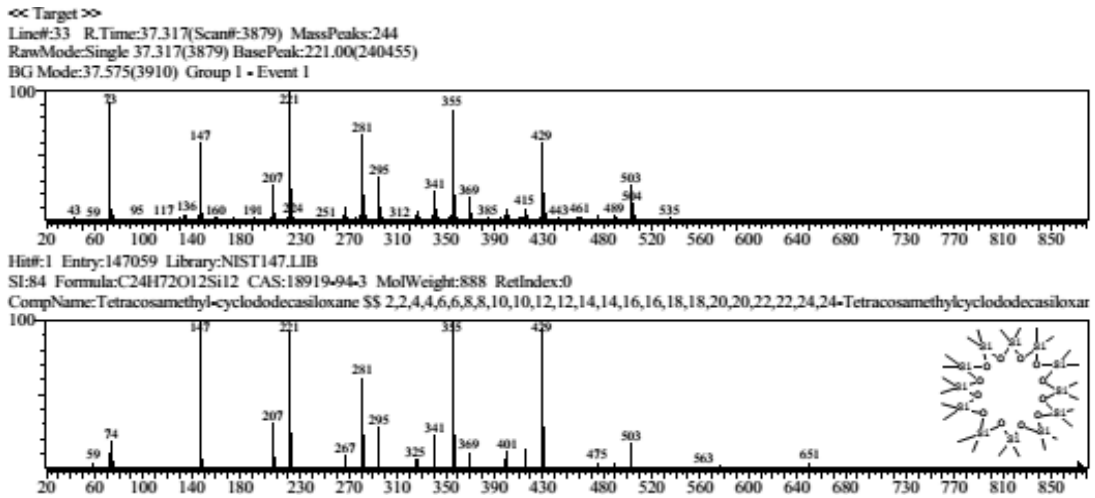

Fig. 2. Fragrant aromatic compounds (phenolics) in the petroleum ether Nikhra fraction of *T. laxiflora* (Tetracosamethyl-cyclododecasiloxane).

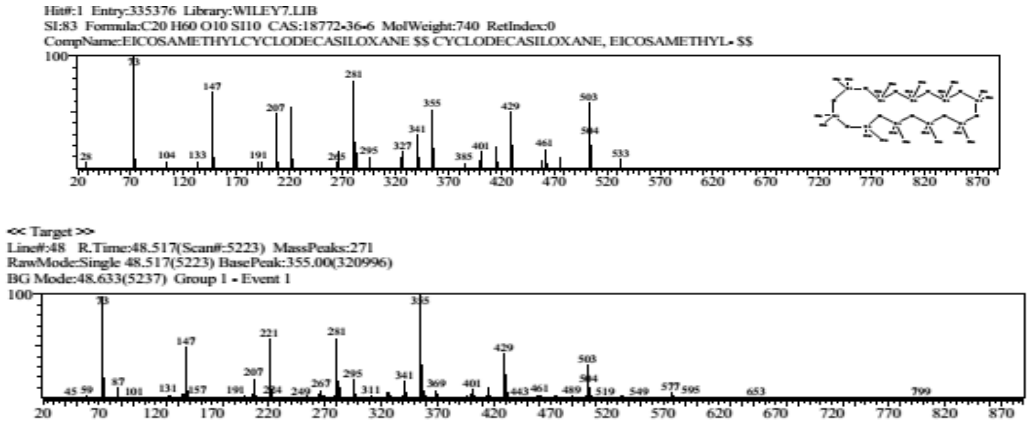

Fig. 3. Fragrant aromatic compounds (terpenoids) in the petroleum ether Nikhrafraction of *T. laxiflora* (Eicosamethylcyclodecasiloxane).

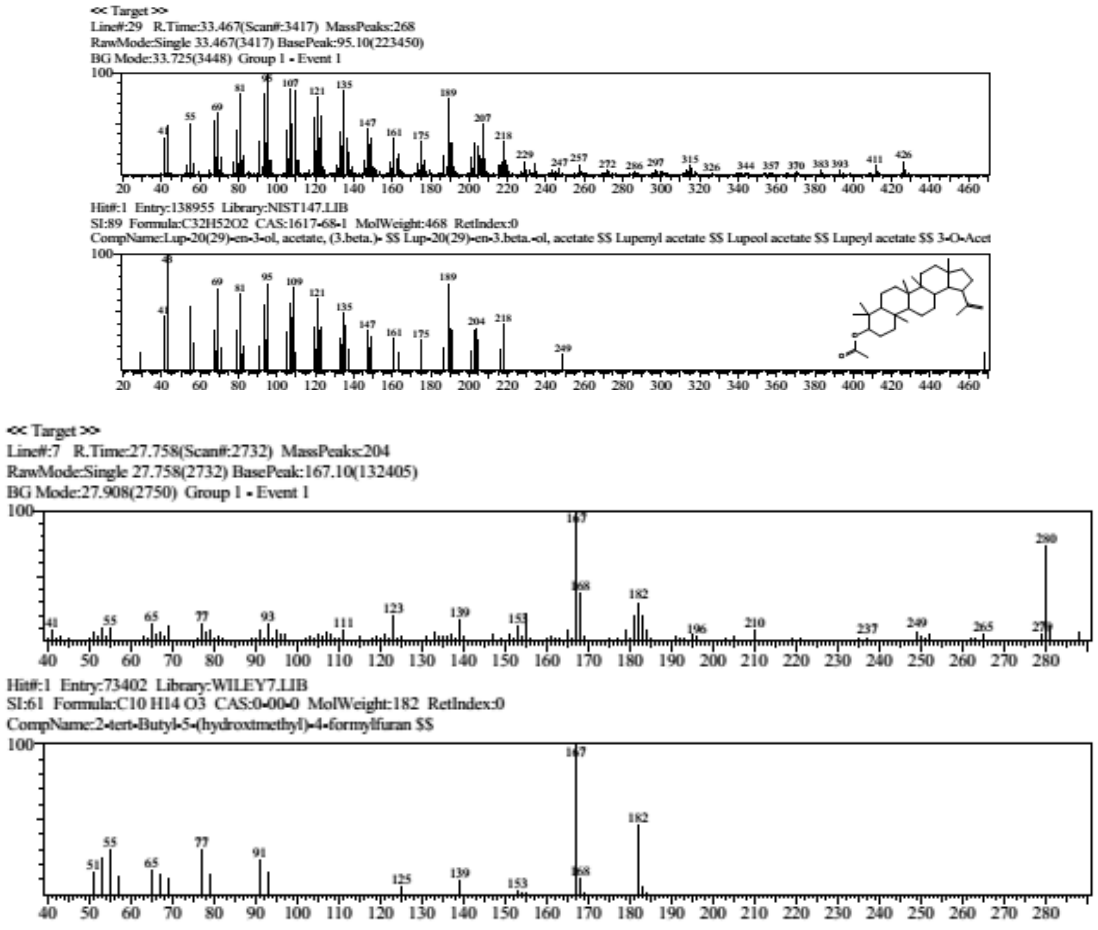

Fig. 4. Fragrant aromatic compounds (phenolics) in the petroleum ether Nikhrafraction of *C. hartmannianum* (2-tert-Butyl-5-(hydroxtmethyl)-4-formylfuran).

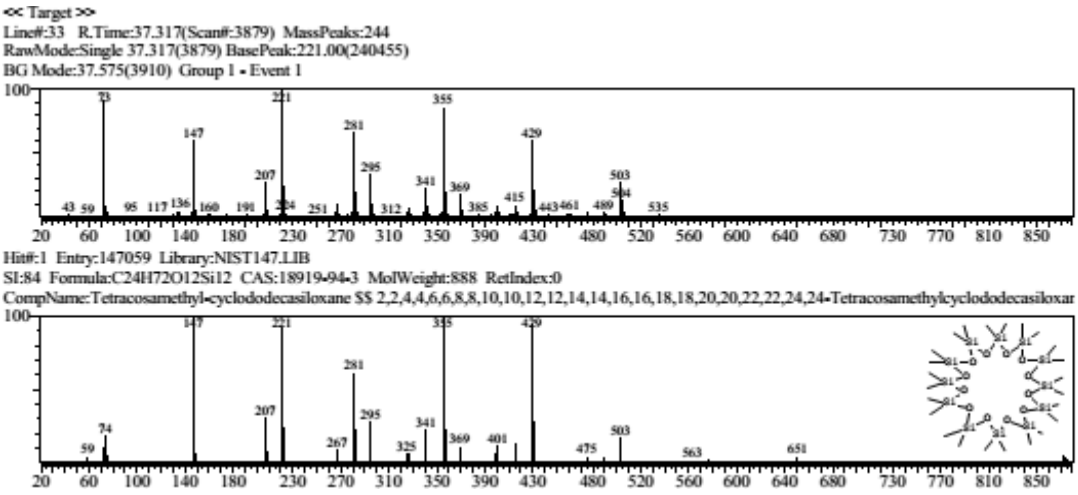

Fig. 5. Fragrant aromatic compounds (terpenoids) in the petroleum ether Nikhrafraction of *C. hartmannianum* (Tetracosamethyl-cyclododecasiloxane)

We observed that petroleum ether Nikhra fraction of *T. laxiflora* have many fragrance aromatics compounds in high concentrations opposite of the petroleum ether Nikhra fraction of *C. hartmannianum* has only two fragrance aromatics compounds in low concentrations.

CONCLUSIONS

Polyphenolics and terpenoids were expected to be responsible for the fragrances in the petroleum ether Nikhra fraction. Fragrance in the petroleum ether Nikhra 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 H_2SO_4) and lignans (van H_3PO_4). GC/MS analysis of the petroleum ether Nikhra fraction revealed that the total fragrant compounds phenolics and terponoids for *T. laxiflora* (44.64%), while those of *C. hartmannianum* which is mostly used for treatment health problems were (19.39%).

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