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Chemical constituents in essential oils of *Pterocarpus soyauxii* leaf, leaf stalk and stem bark

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ABSTRACT

The Nigerian essential oils of fresh *Pterocapus soyauxii* leaf, leaf stalk and stem bark were isolated by hydro-distillation using the adapted all glass Clevenger's apparatus designed to British Pharmacopeia specifications. Chemical compositions of the plant parts were characterized using Gas Chromatography-Mass Spectroscopy (GC-MS). The leaf essential oil contained 12 compounds, out of which 10 were identified accounting for 93.91% of it. Leaf stalk oil had 20 major compounds, which were characterised; they constitute 95.47% of the oil. 11 compounds make up 78.61% of stem bark oil, out of which 7 were characterised, responsible for 76.30% of it. Prominent compounds in the leaf oil were cembrene (43.59%), a monocyclic diterpenoid, eremophilene (29.72%), and azulene derivatives (6.62%), which are polycyclic aromatic compounds. Leaf stalk oil was dominated by hexadecane (32.97%), cis- and trans- β-ocimene (11.60 and 7.74% respectively) and heptanol (8.39%). Major compounds in stem bark oil were neophytadiene (22.11%), 2-heptanol (19.27%) and 3,7,11,15-Tetramethyl-2-hexadecene-1-ol (14.35%). Other prominent compounds in the stem bark oil are 4-propyl-cyclohexene (4.83%), 3-Eicosyne (7.63%), 3,7,11-trimethyl-14-(1-methylethyl-[S-(E,Z,E,E)]-1,3,6,10-cyclodecatetraene (5.10%) and methyl-Z-5,8,11,14,17-Eicosapentaenoate (3.01%). Notable compounds of biological importance and in appreciable amounts in the oils include phytol (6.93%), squalene (1.14%) and ambrial (1.97%). Fragmentation patterns in the mass spectrum of some unidentified compounds are also presented which are unique features of the oils.

Interesting classes of compounds in the three oils include monoterpenes, sesquiterpenes, diterpenes, naphthalenes, alcohols and hydrocarbons.

Keywords: *Pterocarpus soyauxii*, Terpenoids, Fabaceae, Neophytadiene, non-ubiquitous, GC-MS, retention index, alcohol, phytol, hydro-distillation.

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INTRODUCTION

Pterocarpus soyauxii a Fabaceae is commonly known as 'African padauk' and African coral wood. It is native to central and tropical West Africa, found also in Eastern part of Nigeria, Congo-Kinshasa and southern Angola. [1] Traditionally, it is referred to as Osun in Nigeria, N'gula in Zaire, Mbel (Gabon), Kisese (Congo) and Tacula (Angola).

Pterocarpus soyauxii belongs to family Fabaceae, subfamily Faboideae (which is extremely large), tribe Dalbergieae. The tree grows between 27 to 55 m, with a diameter of about 140 to 200 cm. It grows around sea level up to 500 m, preferring deep and well-drained soil of roughly 150-170 cm of annual rainfall [2]

Pterocarpus soyauxii is a useful multipurpose tree utilized in the production of good-quality timber, dye, vegetables and for medicinal purposes [2]. It is the source of the commonly called true barwood dye. In Africa nowadays, the dye is still used to colour red fabrics, fibres and clothings including the tail-like ornaments made from raffia fibre in Cameroon, which is worn on the back by women of the Bulu people [3].

Leaves of *Pterocarpus soyauxii* have high ascorbic acid (vitamin C) content even after cooking and therefore have high nutritional value [4]. Bark of *Pterocarpus soyauxii* showed antifungal activity against some pathogenic fungi and therefore can be used as treating skin infections. [5]

The powdered wood, baked with a slice of lime is used in Gabon on wounds when mixed with palm oil, raffia oil or vegetable butter from seeds of *Tieghemella africana* Pierre, to treat skin diseases, ringworm and yaws [6]. Probably due to its blood-red colour and the associated symbolism, it is also used in ritual ceremonies for circumcision, initiation, marriage, delivery and widowing. The bark contains a kino type resin 'dragon's blood' which is very astringent and used to ward off skin parasites in ethno-veterinary medicine. In Gabon, the resin is used (usually in combinations with parts

of other plant species) as an enema to treat dysentery, toothache, gonorrhoea and excessive menstruation [7]. In Congo and the Central African Republic, a bark decoction is drunk to treat dysmenorrhoea, uterine haemorrhage, dysentery and haemorrhoids.

A pulp obtained by scraping the inner surface of the bark is applied as a wet dressing against inflammations, oedemas, incipient hernia and whitlow [6]. Decoctions, draughts or vapour-baths of leaves and bark are taken against broncho-pulmonary infections.

Heartwood of *Pterocarpus soyauxii* contains the biflavonoids santalin A, santarubin A and santarubin B; isoflavonoids including pterocarpin, formononetin and prunetin. Other flavonoids reported include isoflavanequinone, claussequinone, isoflavanes vestitol and mucronulatol [8].

We report the essential oil composition of the leaf, leaf stalk and stem bark of *Pterocarpus soyauxii*, which has not been reported in literature before now.

MATERIALS AND METHOD

Sample collection and preparation:

Fresh samples of *Pterocapus soyauxii* were collected from Forest Research Institute of Nigeria (FRIN), and were authenticated. Voucher samples have been deposited in the forest herbarium, Ibadan with voucher number FHI.112031. The plant was separated into leaf (1.0 Kg), leaf stalk (0.7 Kg) and stem bark (1.3 Kg) parts.

Each sample was crushed separately and hydro-distilled for 3 hours in an adapted all glass Clevenger apparatus designed to Bristish Pharmacopeia specifications. Oils were collected under iced condition with 1.5 ml distilled n-hexane, which the instrument corrected for. The oils were refrigerated prior to analysis.

GC-MS Analysis:

Gas Chromatography-Mass Spectrometric (GC-MS) analysis was performed on an Agilent Technology 7890 GC-MS system, with an HP5 MS column [30 m by 0.25 mm (i.d) by 0.25 µm film thickness]. Temperature was programmed from 80 ^{0}C to 240 ^{0}C at 10^0 per minute. Helium was used as carrier gas.

Identification of Compounds

Identification of the essential oil constituents were based on their retention indices (determined with reference with a homologous series of n-alkanes) and by comparison of their mass spectra fragmentation patterns with certified standards (NIST).

RESULTS AND DISCCUSSION

Three essential oils were obtained from fresh *Pterocapus soyauxii* leaf, leaf stalk and stem bark by hydro-distillation using the adapted all glass Clevenger's apparatus designed to British Pharmacopeia specifications.

0.58, 0.87 and 0.94 g of colourless oils were obtained respectively from the leaf, leaf stalk and stem bark. Leaf stalk essential oil had the highest yield of 0.124%, while stem bark had 0.072% and the leaf essential oil had the lowest yield of 0.058%. Each of the oils had characteristic aroma as shown in Table 1. The chromatograms of these essential oils are shown in Figures 1 to 3.

Table 1: Yield of the Essential Oils procured from *Pterocarpus soyauxii* plant parts

Plant part	Weight of sample (g)	Weight of Essential oil procured (g)	% Yield of Essential oil procured	Physical examination (colour and smell)
Leaf	1000	0.58	0.058	Colourless oil with a herbal smell
Leaf stalk	700	0.87	0.124	Colourless oil with slight woody smell
Stem bark	1300	0.94	0.072	Colourless oil with strong woody smell

Leaf essential oil contained 12 compounds, out of which 10 were identified accounting for 93.91% of it. Cembrene is a major constituents of leaf oil (43.59%), also known as 3,7,11-trimethyl-14-(1-methylethyl)-[S-(E,Z,E,E)]- 1,3,6,10-cyclotetradecatetraene which is a monocyclic diterpene. It acts as a trail pheromone for termites [9]. Cembrenes are biosynthesized by macrocyclization of geranylgeranyl pyrophosphate and it is central to wide variety of other natural products found both in plants and animals [10]. Another interesting compound in leaf oil is eremophilene (29.72%) which is, 1,2,3,5,6,7,8,8a-octahydro- 1,8a-dimethyl-7-(1-methylethenyl)- [1R-(1α,7β,8a.α)] naphthalene, a sesquiterpene. Of interest also in the leaf oil is 2,4,5,6,7,8-hexahydro-1,4,9,9-tetramethyl-[3aR-(3a.α.,4β.,7α)]3H-3a,7-methanoazulene (5.20%), an isomer of naphthalene. Napthalene is important in the production of dyes, also useful as insecticide [11]. The leaf oil has naphthalene derivatives in 36.28% abundance. Other seven compounds in appreciable amount are presented in table 2.

Leaf stalk oil had 20 major compounds, which were characterised; they constitute 95.47% of it. The leaf stalk oil is dominated by hexadecane (32.97%), cis-β-ocimene (11.60%) and (S)-2-heptanol, (8.39%). Trans-β-ocimene (7.74%) is also in appreciable amount. Neophytadiene (4.28%), a notable compound, is a good analgesic, antipyretic, anti-inflamatory, antimicrobial and antioxidant. [12]. Phytol (6.93%) an acyclic diterpenoid is known to have antioxidant, antibacterial, antifungal, antiviral activities, and is a precursor for the manufacture of synthetic forms of vitamin E and K1. [13,14] Insects such as *Sumac flea* beetle are reported to use phytol and its derivative: phytanic acid as chemical deterrents against predation. [15] Squalene (1.14%) a triterpene and important precursor of steroids is a known anticancer agent as well as antioxidant and detoxifier. [16] The other compounds identified in the leaf stalk oil are presented in table 3.

11 compounds were predominant in the stem bark oil, constituting 78.61% of it, out of which 7 were identified, responsible for 76.30% of it. Stem bark essential oil is characterised with abundance of neophytadiene (22.11%) a sesquiterpene, which was also characterised in the leaf stalk oil, also 2-heptanol (19.27%) and 3,7,11,15-Tetramethyl-2-hexadecene -1-ol (14.35%). Other notable compounds in the stem bark oil are 4-propyl-cyclohexene (4.83%), 3-Eicosyne (7.63%), 7,11-trimethyl-14-(1-methylethyl-[S-(E,Z,E,E)]-1,3,6,10-cyclodecatetraene (5.10%) and methyl-Z-5,8,11,14,17-Eicosapentaenoate (3.01%) (Table 4).

Most of the characterized compounds in the oils are non-ubiquitous, but interesting compounds in appreciable amounts, which may be responsible for the vast ethno-medicinal and traditional uses of *Pterocarpus soyauxii*. Notable among the non-ubiquitous compounds are

cembrene, eremophilene, naphthalene, phytol, squalene, azulene and 3-Eicosyne, which are unique features of the three oils of *Pterocarpus soyauxii* (Fig. 4). We present the fragmentation patterns for 6 unidentified (u.i.) compounds, which are in appreciable amounts in leaf and stem bark oils (Tables 2 and 4; Figures 5a & 5b). They are likely new compounds.

Table 2: Chemical composition of the Essential Oil from *Pterocarpus soyauxii* Leaf

Peak No[a]	MS (Base peak + most abundant peaks)[b]	Identified compound[c]	% TIC[d]	Retention time (minutes)[e]	RI (calculated)[f]
1	204,189,105,119,91	2,4,5,6,7,8-hexahydro-1,4,9,9-tetramethyl- [3aR- (3a.α.,4β.,7α)] 3H-3a, 7-methanoazulene	5.23	10.43	802
2	108,93,121,77,55	1,4,6- Trimethyl- 1,2,3,3a,4,7,8,8a octahydro-4,7-ethanozulene	1.39	11.21	1075
3	137,81,69,55,67	Ambrial	1.97	15.49	1488
4	93,81,105,107,119	Cembrene	43.59	17.32	1531
5	161,91,78,81,91	Eremophilene	29.72	17.52	1536
6	93,81,90,107,55	3,7,11-trimethyl-14-(1-methylethyl -, [S-(E,Z,E,E)] 1,3,6,10 cyclodecatetraene	1.52	17.20	1528
7	81,79,55,121,69	1,5-diethyl-3-methyl 1-2-methylene-(1α,3α,5α) cyclohexane	3.31	18.04	1547
8	121,93,136,67,92	1,7,11-trimethylcyclotetradeca-1,3,7,11-tetraene (1E,3E,7E,11E)-4-Isopropyl	1.53	18.33	1554
9	95,55,69,71,81	4-methyle-5-[3,3,3-trifluoropropionylpropyl]- Imidazole	0.62	18.94	1568
10	55,69,81,67,109	(E)-3-methyl-5-(1R,4aR,8aR)-5,5,8a-trimethyl-2-methylenedecahydronaphthalen-1-yl) pent-2-en-1-ol	5.03	19.65	1915
11	95,55,81,137,79	u.i.	3.12	20.39	1932
12	245,163,91,55,69	u.i.	2.98	21.89	1967

[a]Elution according to the retention time from GC (see Fig.1); [b]m/e value of base peak first stated and other most prominent ions; see identification of component; [d]Total ion concentration in %; [e]Retention time in minutes; [f]Retention index determined with reference to homologous series of n-alkanes; u.i.-unidentified, but with mass spectrum.

Table 3: Chemical composition of the Essential Oil from *Pterocapus soyauxii* Leaf stalk

Peak No[a]	MS (Base peak + most abundant peaks)[b]	Identified compound[c]	% TIC[d]	Retention time (minutes)[e]	RI (calculated)[f]
1	55,56,83,57,70,	(S)-2-Heptanol,	8.39	3.13	303
2	71,55,93,61,67	5-cyclopropylidene 1-pentanol;	1.05	6.01	504
3	59,121,93,79,67	1-methylene-4-(1-methylethenyl)-Cyclohexane (136.2)	0.67	7.40	717
4	57,71,55,56,70	Isobutyl pentyl Oxalate	0.70	12.90	1116
5	57,71,55,56,85	Hexyl pentyl ether	0.65	10.08	1145
6	57,82,55,56,69	(E)- 2-Nonen-1-ol	0.98	14.29	1149
7	68,57,82,55,95	E-6-Octadecen-1-ol acetate	9.96	15.84	1496
8	81,57,82,68,95	6,10-dimethyl,Dodeca-1,6-dien-12-ol	2.67	16.16	1504
9	81,82,57,68,95,55,	Neophytadiene	4.28	15.86	1497
10	81,93,105,106,91	cis-β-Ocimene	11.60	17.20	1528
11	81,91,67,55,79	trans-β-Ocimene	7.74	17.39	1532
12	79,69,121,54,53	1,1- carboxaldehyde 3-cyclohexene	0.80	17.96	1546
13	93,68,121,54,53	1.1-methyl-5-(1-methylethenyl)-Cyclohexene	0.98	18.27	1553
14	56,79,95,67,93	3-Methylene-1,6-heptadiene	0.78	18.33	1554
15	57,71,97,85,69	2-Tetradecanol	0.83	10.53	1912
16	71,57,55,81,88	Phytol	6.93	19.79	1918
17	57,91,85,55,52	Hexadecane	32.97	22.67	1985
18	57,91,85,51,52	carbonic acid prop-1-en-2-yl tetradecyl ester	1.15	25.56	2385
19	149,57,167,55,70	Mono (2-ethylhexyl) phthalate	1.20	26.18	2400
20	69,81,41,95,121,137	Squalene	1.14	28.95	2781

[a]Elution according to the retention time from GC (see Fig.2); [b]m/e value of base peak first stated and other most prominent ions; see identification of component; [d]Total ion concentration in %; [e]Retention time in minutes; [f]Retention index determined with reference to homologous series of n-alkanes; u.i.-unidentified.

Table 4: Chemical composition of the Essential Oil from *Pterocarpus soyauxii* Stem bark

Peak No[a]	MS (Base peak + most abundant peaks)[b]	Identified compound[c]	% TIC[d]	Retention time (minutes)[e]	RI (calculated)[f]
1	55,83,56,70,57	2-Heptanol	19.27	3.16	305
2	56,69,57,55,54	u.i	0.57	6.06	506
3	55.83,69,56,57	u.i	0.62	13.84	1139
4	68,57,55,95,82,81	Neophytadiene	22.11	15.86	1497
5	81,57,68,55,95	4-propyl-cyclohexene	4.83	16.17	1504
6	81,82,68,94,57	3-Eicosyne	7.63	16.43	1510
7	93,81,90,107,55	3,7,11-trimethyl-14-(1-methylethyl -, [S-(E,Z,E,E)] 1,3,6,10 cyclodecatetraene	5.10	17.20	1528
8	71,55,79,95,67	u.i	0.57	17.34	1531
9	79,91,67,105,161	methyl-Z-5,8,11,14,17-Eicosapentaenoate	3.01	17.39	1533
10	81,69,57,109,91	u.i	0.55	19.56	1913
11	71,57,55,81,82	3,7,11,15-Tetramethyl-2-hexadecene -1-ol	14.35	19.83	1919

[a]Elution according to the retention time from GC (see Fig.3); [b]m/e value of base peak first stated and other most prominent ions; see identification of component; [d]Total ion concentration in %; [e]Retention time in minutes; [f]Retention index determined with reference to homologous series of n-alkanes; u.i.-unidentified.

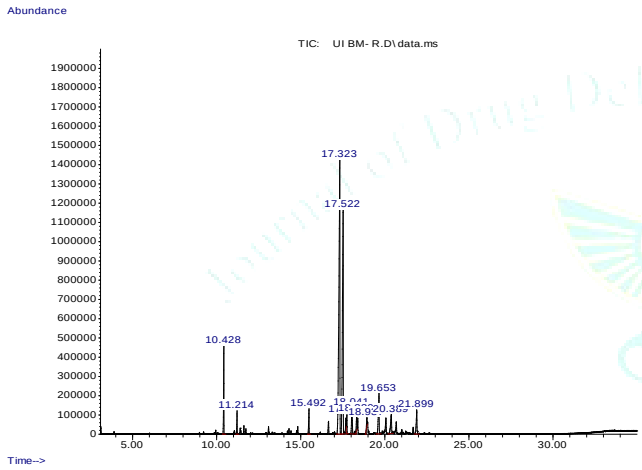


Figure 1: Gas chromatogram of the leaf essential oil of *Pterocarpus soyauxii* using Agilent Technology 7890 GC-MS system, with an HP5 MS column [30 m by 0.25 mm (i.d) by 0.25 µm film thickness]. Temperature was programed from 80 ^{0}C to 240 ^{0}C at 10^0 per minute. Helium was a carrier gas. [See Table 2 for its identified compounds]

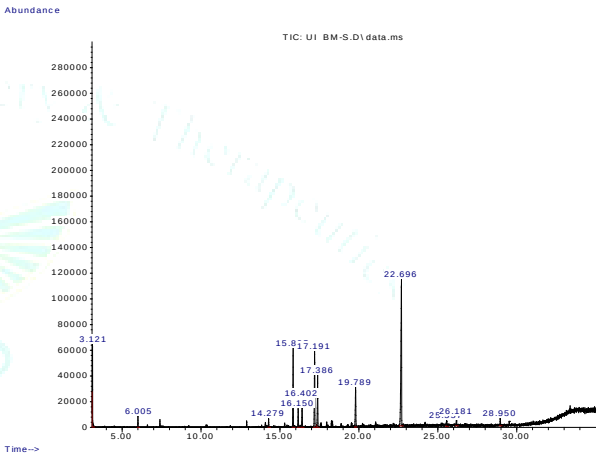


Figure 2: Gas chromatogram of the leaf stalk essential oil of *Pterocarpus soyauxii* using Agilent Technology 7890 GC-MS system, with an HP5 MS column [30 m by 0.25 mm (i.d) by 0.25 µm film thickness]. Temperature was programed from 80 ^{0}C to 240 ^{0}C at 10^0 per minute. Helium was a carrier gas. [See Table 3 for its identified compounds]

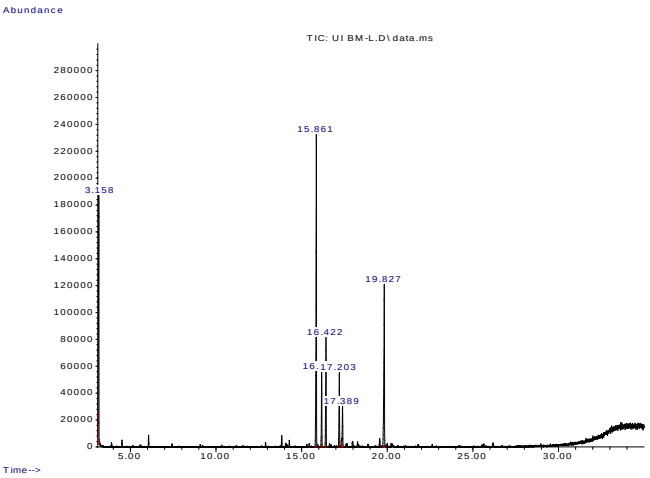
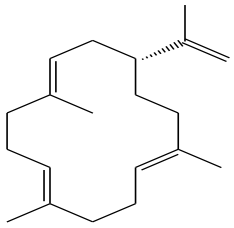
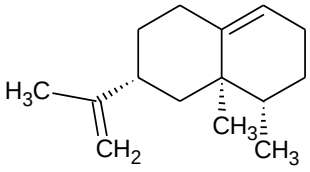


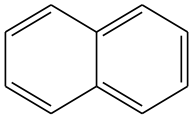
Figure 3: Gas chromatogram of the stem-bark essential oil of *Pterocarpus soyauxii* using Agilent Technology 7890 GC-MS system, with an HP5 MS column [30 m by 0.25 mm (i.d) by 0.25 µm film thickness]. Temperature was programed from 80 ^{0}C to 240 ^{0}C at 10^0 per minute. Helium was a carrier gas. [See Table 4 for its identified compounds].



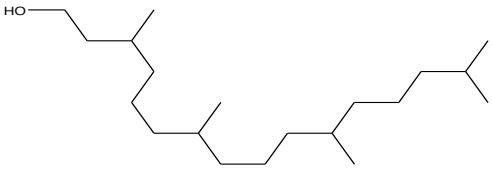
Cembrene



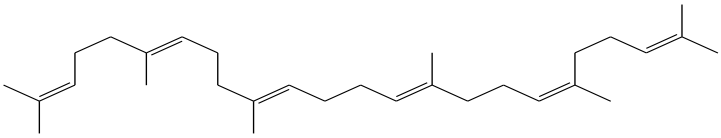
Eremophilene



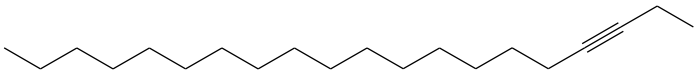
Naphthalene



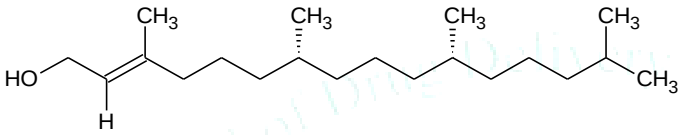
Phytol



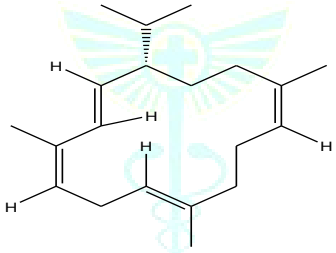
Squalene



3-Eicosyne



3,7,11,15-Tetramethyl-2-hexadecene -1-ol



3,7,11-trimethyl-14-(1-methylethyl)-[S-(E,Z,E,E)]- 1,3,6,10-Cyclotetradecatetraene

Figure 4: Chemical Structures of Interesting Non-ubiquitous Compounds in the Essential oils of *Pterocarpus soyauxii*.

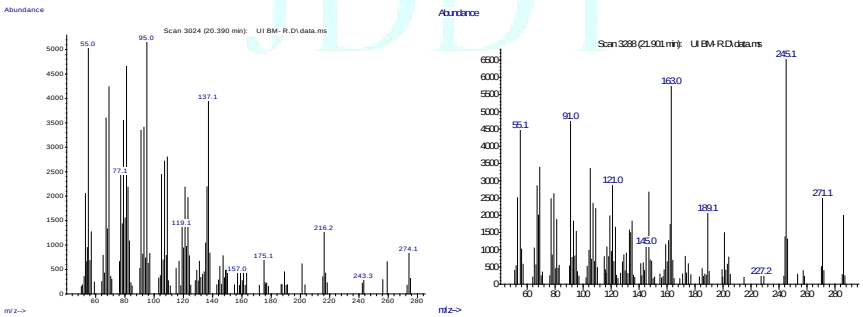


Figure 5a: Mass spectrum of two unidentified (u.i.) compounds in leaf oil

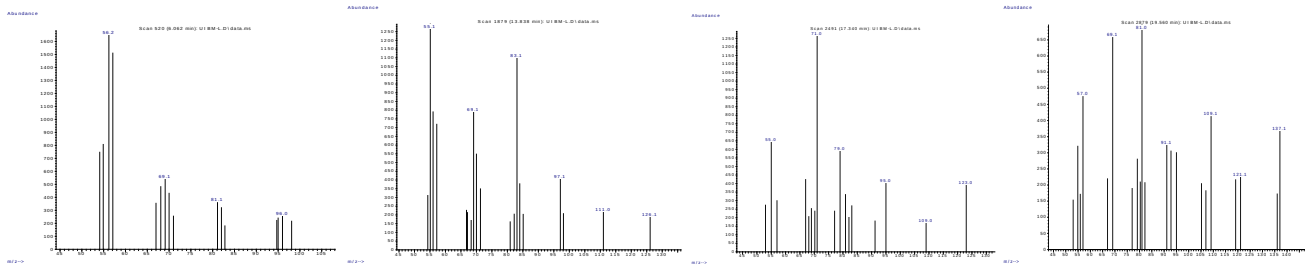


Figure 5b: Mass spectrum of four unidentified (u.i.) compounds in stem bark oil

SUMMARY AND CONCLUSION

In this study we report details on the essential oil compositions of *Pterocarpus soyauxii* leaf, leaf stalk and stem bark, which were characterised using GC-MS.

We identified 10 compounds in the leaf essential oil, which accounted for 93.91% of it. Leaf stalk oil had 20 major compounds, which were characterised; they constitute 95.47% of the oil. 7 compounds were identified in the stem bark oil, responsible for 76.30% of it.

The major compounds in each of the oils are presented, which were supported by their reported and established biological activities. Also non-ubiquitous compounds of interest are presented. Fragmentation patterns of six unidentified (u.i.) compounds in appreciable amount were given from their mass spectrum, which are unique features of these oils and may likely be new compounds.

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