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Characterisation of Chemical Compounds from the Root and Leaf Extract of *Abrus precatorius*

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ABSTRACT

A total of five secondary metabolites were isolated and purified from dichloromethane root and leaf extracts of *Abrus precatorius* using different chromatographic methods. The structures were elucidated using different spectral data and were confirmed to be 5,7-Dimethoxyflavanone (**1**), Xanthoxylin (**2**), Hexadecanoic acid (**3**) successfully isolated from the root extract, and Beta-eudesmol (**4**) and Squalene (**5**) successfully isolated from the leaf extract. 5,7-Dimethoxyflavanone **(1)** and xanthoxylin **(2)** were reported for the first time from the root extract and this contributed to the pharmacological importance of *A. precatorius.*

Keywords: 5,7-Dimethoxyflavanone, *Abrus precatorius*, beta-eudesmol, hexadecanoic acid, squalene, xanthoxylin

INTRODUCTION

Numerous efforts by researchers have been directed towards the provision of empirical proof to back the use of tropical plants in traditional medicinal practice (Wakawa *et al*., 2021). Focus on medicinal plant research has increased worldwide and evidence abounds in the immense potentials of medicinal plants used in various traditional systems. Various medicinal plants have been studied using different scientific approaches and results from these studies have revealed the potentials of medicinal plants in pharmacology (Wakawa & Hauwa, 2013). These medicinal plants are of great importance to the health of the individuals and communities to larger extend, and nutritional benefits are derived from these plants since they are commonly used as vegetables. The use of these medicinal plants in most communities is commonly referred to as traditional medicine.

The use of traditional medicine at the primary health care level is widespread and plant-based treatments are being recommended for curing/managements of various diseases by traditional medical practitioners all over the world. The phytochemicals present in the fruits, vegetables and medicinal plants are gaining attention day-by-day for their active role in the treatment of various human diseases. *Abrus precatorius* (Linn) (Fabaceae) is a lean woody creeper and it is a widely distributed tropical medicinal plant with several therapeutic properties (Akinloye & Adalumo, 1981; Molgaard *et al*., 2001). It is widely found in Africa, India and many other parts of the world. In West Tropical Africa, *A. precatorius* leaves have been employed to sweeten food, treat stomach complaints, treat fevers, cough and cold (as decoction). The leaves are casually chewed, and the vine is sometimes sold as a masticatory in Curacao (Morton, 1981). The plant is also traditionally used to treat tetanus, and to prevent rabies (Bhutia & Maiti, 2011). Glycyrrhizin (a natural sweetener), a compound obtain from the leaves and roots extract, is an important phytoconstituent of liquorice which is widely used in the pharmaceutical and food industries (Karwasara *et al.,* 2010), and it is reported to exhibit anti-HIV (Hirabayashi *et al*., 1991), immunomodulatory (Yoshida *et al*., 2006), anti-ulcerative and anti-inflammatory properties. This work is also in line with the view to discover more plants and plant materials with scientific proof that can be used in the treatment of diseases as well as material that can serve as potential sources of new product for food, and to

larger extend increase the value for the medicinal plant.

MATERIALS AND METHODS

Plant Material

Fresh plant materials were collected from uncultivated farm land in Garkida, Gombi Local Government Nigeria. They were authenticated in Modibbo Adama University Yola. The plant materials were given a voucher specimen number as HWP/AP/2014-11/001 (*Abrus precatorius* leaves) and HWP/AP/2014-11/002 (*A. precatorius* root). The fresh leaves were carefully plucked and washed under running tap water. They were then air dried and then spread in the laboratory to dry at room temperature until they were fully dried. The root was also washed under running tap water. It was then cut into smaller pieces, spread in the laboratory and allowed to dry at room temperature until they are fully dried.

Sample Preparation

The dried plant materials (leaves and root) were ground into fine powder form using laboratory mortar and pestle, and electric grinder. The powdered samples (mesh 30) were packed into clean, dry sample containers and were labelled appropriately and kept for further use. Extraction was carried out by the conventional solvent extraction method described by Fasihuddin *et al*. (2010) with slight modification. This was achieved by soaking the ground plant materials in solvents in the order of increasing polarity. A total of two kilograms of the dried and ground powdered samples were separately extracted using cold soaking method with hexane. The samples were soaked in the hexane with the ratio of 1:3 (sample: hexane) in five litres Erlenmeyer flasks at room temperature for five days. The resulting hexane solution was then filtered using Whatman filter paper No. 4 and the residue was then re-extracted with fresh hexane and filtered. Both extracts were combined and evaporated to dryness with a rotary evaporator (Heidolph Laborota 4000 efficient) under reduced pressure below 50 ^{o}C to obtain the hexane crude extract. The residues were re-extracted using similar procedure with dichloromethane, followed by ethyl acetate and methanol to obtain respective crude extracts. The dry weight and percentage yield of each crude extract was determined (simple percentage).

Isolation and Identification of Secondary Metabolites

Combination of the following methods were used for the isolation and identification of secondary metabolites.

Column chromatography

The column was prepared using methods as described by Firdous *et al.* (2013). Sample loading via wet-packing method and column elution with suitable solvent systems with increasing polarity was used as described by Fasihuddin *et al*. (2010) and Patra *et al*. (2012). The procedure was repeated using different solvent systems, based on increasing polarity. Samples from the column fractions were examined using TLC plates in few suitable solvent systems until fraction with single spot that appeared on TLC plate was treated as possible pure secondary metabolite as described by Patra *et al.* (2012).

Chromatotron

Chromatotron was also used for the separation of the substances and was observed by illuminating the disk with short-wave UV light, so that the desired fractions can be collected. The layer was covered with a fused silica disk, as normal glass is not transparent to short-wave UV light. The sample was then collected at time interval into vails and TLC analysis of the different fractions collected was performed (Varsha & Sonal, 2015)

Vacuum-liquid chromatography

Vacuum liquid chromatography was used for separation in step gradient elution of compounds in which the flow of compounds was activated by vacuum to enhance separation. Successive addition of eluting solvent in different ration combination was done, and band of fractions were collected into several conical flasks (John & Bruce, 1986)

At the end of the combined isolation methods used, 11.6 mg of compound **1** (5,7-dimethoxyflavanone) was obtained in combined solvent system Hexane: $CHCl_3$ (7:3) and Hexane: EA (4:1), 9.8 mg of compound **2**

(Xanthoxylin) in hexane: EA (7:3), 6.4 mg of compound **3** (Hexadecanoic acid) in Hexane: $CHCl_3$ (9:1), 7.3 mg of compound **4** (Beta-Eodesmol) in Hexane: $CHCl_3$ (3:2) and DCM: EA (5:1) and 12.62 mg of compound **5** (Squalene) in Hexane: $CHCl_3$ (7:3) and Hexane: EA (4:1).

RESULTS AND DISCUSSION

Isolation and Purification of Secondary Metabolites from the Root Extract

Compound 1 (5,7-Dimethoxyflavanone)

5,7-Dimethoxyflavanone **(1)**; White crystalline; melting point 144 – 146 ^oC; IR V_{max} cm^{-1}: 3013, 2953, 1670, 1606, 1459, 1262, 1214, 1111, 821, 763, 701; MS *m/z* (% rel. int): 356 (1), 342 (2), 287 (4), 286 (8), 285 (44), 284 (14), 268 (7), 256 (6), 226 (2), 208 (24), 209 (7), 193 (6), 182 (20), 180 (100), 153 (8), 152 (42), 137 (38), 121 (7), 104 (10), 81 (6), 77 (11), 44 (10): ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 7.41– 7.43 (2H, d, H2'/H-6'), 7.37 - 7.36 (2H, m, H3'/H-5'), 6.11 (1H, t, H-8), 6.04 (1H, d, H-6), 5.36 (1H, d, H-2), 3.84 (3H, m, 5-OMe), 3.72 (3H, m, 7-OMe), 2.96 (1H, d, H-3a), 2.79 (1H, d, H-3b), 2.12 (1H, m, H-4'). ^{13}C-NMR (500 MHz, $CDCl_3$) δ (ppm): 189.49 (C-4), 165.84 (C-7), 164.47 (C-5), 162.30 (C-8a), 138.65 (C-1), 138.65 (C-1'), 125.56 (C-2'/C-6'), 125.56 (C-3'/C-5'), 125.56 (C-4'), 105.86 (C-4a), 93.64 (C-6), 92.76 (C-8), 78.78 (C-2), 55.66 (7-OMe), 55.13 (5-OMe), 45.60 (C-3), 44.21 (C-11).

Mass spectrum and IR spectrum of compound 1 is shown in Figure 1 and Figure 2, respectively. Based on the table of ^{1}H-NMR characteristics absorption and ^{1}H-NMR peaks splitting pattern as reported by Janice (2008), the proton signals were all integrated and were assigned to every proton NMR of Compound 1 as the proposed chemical structure. The result showed that ^{1}H-NMR spectrum of Compound 1 exhibited nine proton resonates. Two doublet proton signals were observed at δ 6.04 (1H, d) and δ 5.36 (1H, d) indicating the presence of aromatic and a methine protons in the structure, therefore assigned to H-6 and H-2, respectively. Another doublet proton signal was observed at δ 6.11 (1H, d) and was assigned to H-8 indicating the presence of methine group of the structure. Also, a doublet proton signal was observed at δ 7.41-7.37 (2H, d) which was assigned to H-2'/H-6', and a multiplet proton signal appeared at δ 7.37-7.36 (2H, m) and this was assigned to H-3'/H-5', these two proton signals indicate protons that are attached to symmetry carbon attached to the structure of Compound 1. Two multiplet proton signals were observed at chemical shift δ 3.84 (3H, m) and δ 3.72 (3H, m) both indicating the presence of methoxy group and were assigned to 5-OCH_3 and 7-OCH3. Two identical proton signals, a doublet at δ 2.96 (1H, d) and a multiplet at δ 2.79 (1H, m) can be seen to indicate the existence of methylene group and were assigned to H-3a and H-3b, respectively. The chemical shift at δ 2.12 (1H, m) a multiplet proton signal was observed which appear to be attached to symmetry carbon of the structure and was assigned to H-4'. The result is in congruent with the published work of Yenji *et al*. (2009) as shown in Table 1.

The result of the ^{13}C-NMR spectrum of Compound 1 was analyzed based on the table of ^{13}C-NMR characteristics absorption reported by Janice (2008). A total of 17 carbon resonates were observed in the spectrum. The downfield region showed three signals at δ 162.30, δ 164.47 and δ 165.84 which were identified as methine carbons and were assigned to C-8a, C-5 and C-7, respectively. Another signal observed at δ 189.49 was assigned to C-4 which was identified as C=O group. A signal appeared at δ 138.65 and was assigned to C-1', while the peaks signal appeared at δ 125.56 which indicated the presence of methine carbons of the structure and were assign to C-2', C-3', C-4', C-5' and C-6'. Five signals appeared at the upfield region, two signals at δ 105.86 and δ 78.78 which indicated the presence of methine carbons and were assigned to C-4a and C-2, respectively. Two other methine signals were observed at δ 93.64 and δ 92.76 which were assigned to C-6 and C-8, respectively. Two signals which appeared at δ 55.13 and δ 55.66 which were identified as OMe-group were assigned to 5-OMe and 7-OMe, respectively. Another signal which appeared at the upfield region at δ 45.60 and was assigned to C-3 which indicated the presence of methylene carbon in the chemical structure of Compound 1. Chemical shift of every carbon NMR for Compound 1 is shown in Table 2 and comparison with NMR data of similar compound reported by Yenji *et al.* (2009).

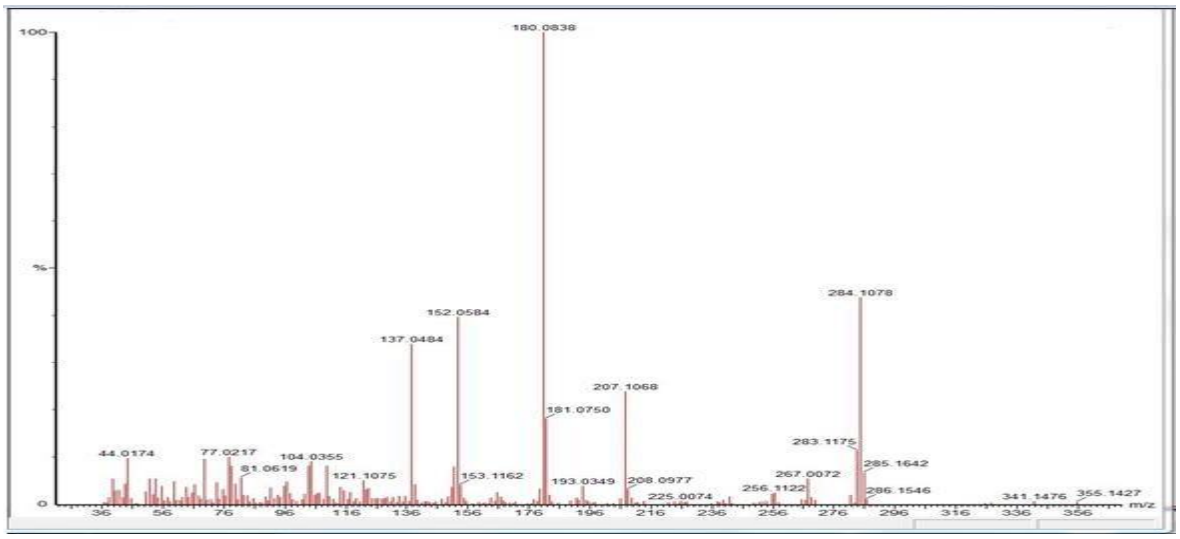


Figure 1. Mass spectrum of Compound 1 (5,7-Dimethoxyflavanone)

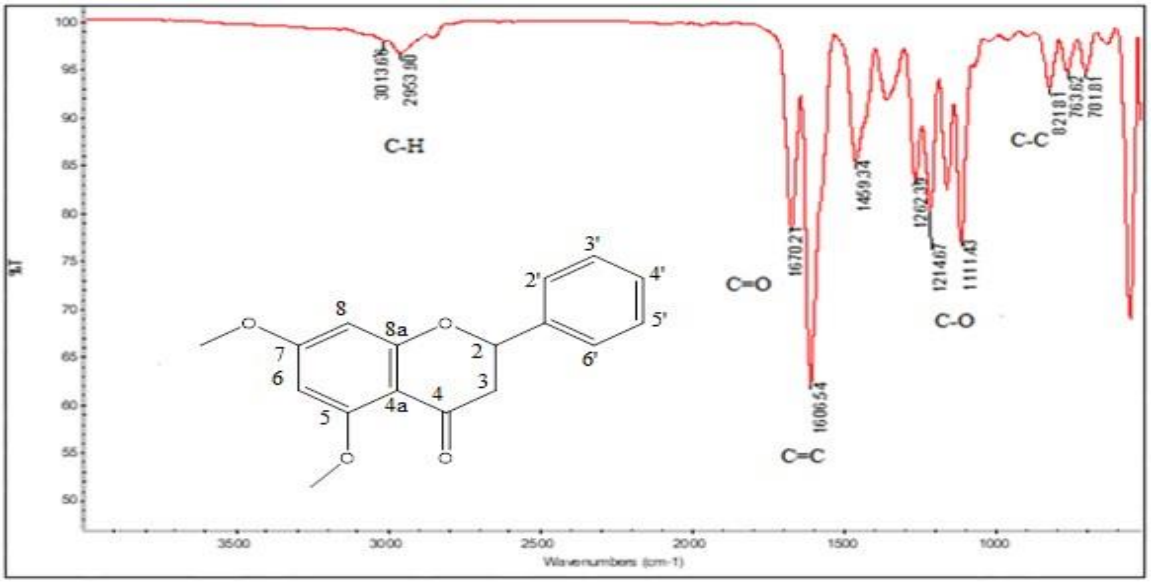


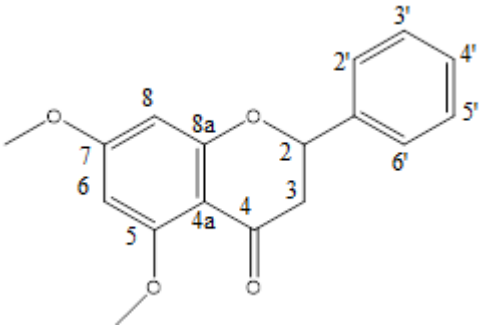
Figure 2. IR Spectrum of Compound 1 (5,7-Dimethoxyflavanone)

Table 1. Proton NMR signal of Compound 1 and for 5,7-dimethoxyflavanone reported by Yenji *et al.* (2009)

Proton assigned to Compound 1	Proton chemical shift (ppm) of Compound 1	Proton assigned to 5,7-dimethoxyflavanone (Yenji *et al*., 2009)	Proton chemical shift (ppm) of 5,7dimethoxyflavanone (Yenji *et al*., 2009)
H-2	5.36 (1H, d, *J* = 13.0, 2.2 Hz)	H-2	5.40 (1H, dd, *J* = 13.1, 2.9 Hz)
H-3a	2.96 (1H, d, *J* = 13.1 Hz)	H-3a	3.00 (1H, dd, *J* = 16.5, 13.1 Hz)
H-3b	2.79 (1H, m)	H-3b	2.79 (1H, dd, *J* = 16.5, 2.9 Hz)
H-6	6.04 (1H, d, 3.2 Hz)	H-6	6.08 (1H, d, *J* = 2.2 Hz)
H-8	6.11 (1H, d, 3.2 Hz)	H-8	6.15 (1H, d, *j* = 2.2 Hz)
5-OMe	3.84 (3H, m)	5-OMe	3.88 3H, s)
7-OMe	3.72 (3H, m)	7-OMe	3.81 (3H, s)
H-3'/H-5'	7.37 - 7.36 (2H, m)	H-2'/3'/4'/ 5'/6'	7.37 – 7.45 (5, m)
H-2'/H-6'	7.41- 7.37 (2H, d)		
H-4'	2.12 (1H, m)		

Table 2. Carbon NMR signal of Compound 1 and for 5,7-dimethoxyflavanone reported by Yenji *et al.* (2009)

Carbon assigned to compound 1	Carbon chemical shift (ppm) of compound 1	Carbon assigned to 5,7-dimethoxyflavanone (Yenji *et al*., 2009)	Carbon chemical shift (ppm) of 5,7-dimethoxyflavanone (Yenji *et al*., 2009)
C-1’	138.65	C-1’	138.7
C-2	78.78	C-2	79.2
C-3	45.60	C-3	45.6
C-4	189.49	C-4	189.2
C-5	164.47	C-5	165.0
C-6	93.64	C-6	93.2
C-7	165.84	C-7	166.0
C-8	92.76	C-8	93.5
C-8a	162.30	C-8a	166.0
C-4a	105.86	C-4a	162.3
C-3’/5’	125.56	C-3’/5’	128.8
C-2’,6’	125.56	C-2’/6’	126.1
C-4’	125.56	C-4’	128.6
5-OMe	55.13	5-OMe	56.1
7-OMe	55.66	7-OMe	55.6



Structure of 5,7-Dimethoxyflavanone **(1)**

5,7-Dimethoxyflavanone (**1)** is a major active constituent of many herbal plants, such as *Kaempferia paviflora*, *Piper caninum*, and *Leptospermum scoparium* and has demonstrated many beneficial pharmacological effects *in vitro*, including anti-infammatory, antioxidant, cardioprotective effects, as well as chemopreventive and chemosensitizing properties (Bei & An, 2016).

It was found to possess a comparable effect to aspirin on the rat paw edema model, and it showed no inhibition on cotton pellet-induced granuloma formation. On the rat pleurisy model, 5,7-dimethoxyflavanone (**1)** exhibited an antiexudative effect, interfered with leukocyte migration, and markedly inhibited prostaglandin biosynthesis. In addition, 5,7-dimethoxyflavanone (**1)** caused marked lowering of the rectal temperature of rats. Tep-Areenan *et al*. (2010) investigated the vascular effects of 5,7-dimethoxyflavanone (**1**) isolated from the rhizomes of *Kaempferia parviflora* on rat isolated aortic rings and its possible mechanisms. They observed that 5,7-dimethoxyflavanone (**1)** (1 – 100 µM) caused concentration-dependent relaxations in aortic rings precontracted with methoxamine, thus the study demonstrated that 5,7-dimethoxyflavanone (**1)** causes endothelium-dependent relaxation that is partly mediated by NO-cGMP and cyclooxygenase pathways.

Compound 2 (Xanthoxylin)

Xanthoxylin **(2)**; white crystalline; melting point 86 - 88 ^{o}C; IR V_{max} cm^{-1}: 3003, 2943, 1708, 1585, 1322, 1038, 947; MS *m/z* (% rel. int): 251 (1), 198 (2), 197 (5), 196 (49), 181 (100), 166 (14), 154 (4), 138 (13),123 (7), 110 (6), 95 (12), 79 (4), 69 (11), 53 (6), 43 (10): ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 14.02 (1H, OH), 6.04 – 6.07 (1H, d, H-3), 5.90 – 5.93 (1H, m, H-5), 3.86 (3H, s, 6-OMe), 2.60 (3H, m, 4-OMe), 2.15 (3H, s, H-8); ^{13}C-NMR (500 MHz, $CDCl_3$) δ (ppm): 203.48 (C-7), 167.62 (C-4), 166.24 (C-2), 162.79 (C-6), 105.86 (C-1), 93.64 (C-3), 90.59 (C-5), 55.62 (C-9), 32.87 (C-10), 31.10 (C-8).

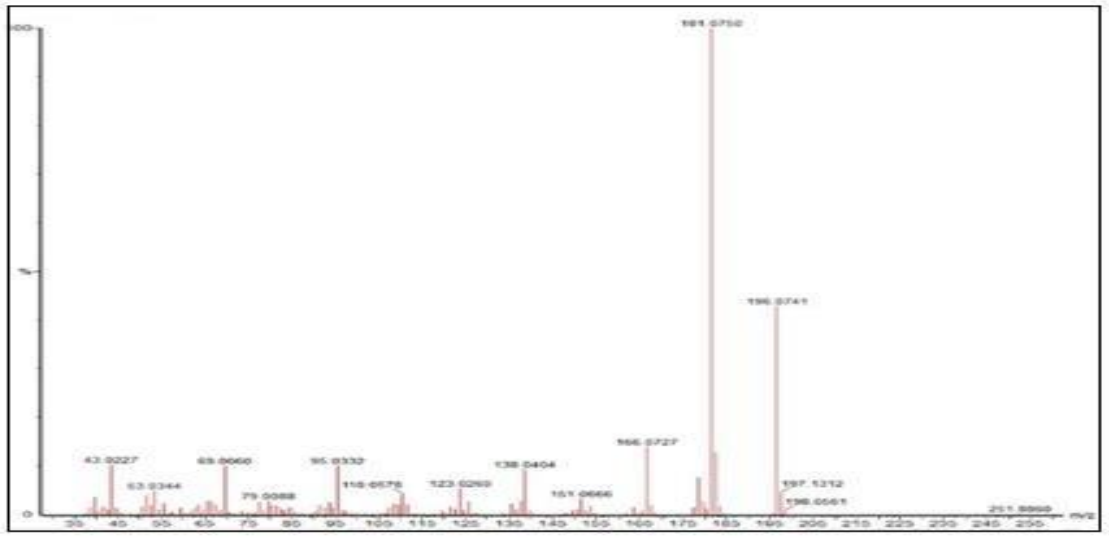


Figure 3. Mass spectrum of Compound 2 (Xanthoxylin)

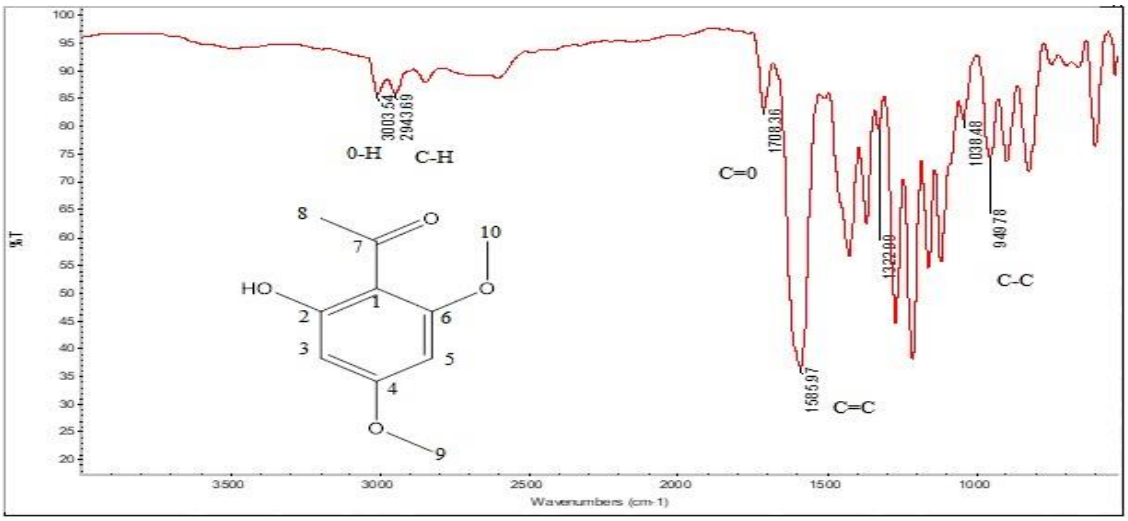


Figure 4. IR Spectrum of Compound 2 (Xanthoxylin)

Mass spectrum and IR spectrum of Compound 2 shown in Figure 3 and Figure 4, respectively. NMR analysis was further performed for the elucidation of the chemical structure of Compound 2 based on the table of ^{1}H-NMR characteristics absorption and ^{1}H-NMR peaks splitting pattern as reported by Janice (2008), the proton signals were all integrated and were assigned to every proton NMR of Compound 2 as the proposed chemical structure. The ^{1}H-NMR spectrum of Compound 2 exhibited 6 proton resonates. A singlet proton signal was observed at δ 14.02 (1H, s) indicating the presence of an OH group (hydroxy) of the structure. A singlet proton signal was observed at δ 3.86 (3H, s) indicating the presence of a methoxy group and was assigned to 6-OMe. A singlet proton signal was observed at δ 2.15 (3H, s) which correspond with a methyl group and was assigned to H-8. A proton signal was observed at chemical shift δ 5.90 – 5.93 (1H, d), a doublet which represent a methine group present in the structure of the compound and was assigned to H-5, and a multiplet at δ 2.60 (3H, m) which indicated a methoxy group and was assigned to 4OMe. At chemical shift at δ 6.04 – δ 6.07 (1H, d) a doublet proton signal was observed as to a methine group of the structure and was assign to H-3, as shown in Table 3 and it is in agreement with the published work of Zhu (2014).

Table 3. Proton NMR signal of Compound 2 and xanthoxylin reported by Zhu (2014)

Proton assigned to Compound 2	Proton chemical shift (ppm) of Compound 2	Proton assigned to xanthoxylin (Zhu, 2014)	Proton chemical shift (ppm) of xanthoxylin (Zhu, 2014)
H-3	6.04 – 6.07 (1H, d, *J* = 2.59)	H-3	6.07 (1H, d, *j* = 2.1 Hz))
H-5	5.90 – 5.93 (1H, m)	H-5	5.93 (1H, d, *j* = 2.3 Hz)
H-8	2.15 (3H, s)	H-8	2.57 (3H, s)
4-OMe	2.60 (3H, m)	4-OMe	3.82 (3H, s)
6-OMe	3.86 (3H, s)	6-OMe	3.85 (3H, s)
OH	14.02	-	-

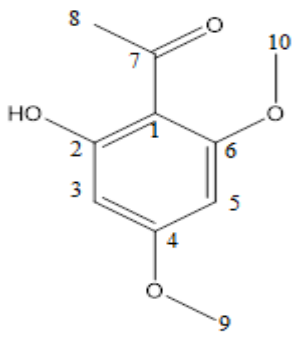
Note: '-' indicates no data available

From the result of the ^{13}C-NMR spectrum of Compound 2, every carbon NMR signal observed was assign to the proposed chemical structure of Compound 2 which is based on the table of ^{13}C-NMR characteristics absorption reported by Janice (2008). A total of 10 carbon resonates were observed in the ^{13}C-NMR spectrum of Compound 2. At the downfield region signals were observed at δ 167.62, δ 166.24 and δ 162.79 and were assigned to C-4, C-2 and C-6, respectively. A signal was observed at δ 203.48 and was assigned to C-7 which was identified as a carbon attached to C=O group. A signal was observed at δ 105.86 and was assigned to C-1, while the two peak signals that were observed at δ 93.64 and δ 90.59, which indicated the presence of methine groups of the structure were assigned to C-3 and C-5, respectively. At the upfield region, two signals were observed at δ 55.62 and δ 32.87 that were identified as methoxy groups were assigned to C-9 and C-10, respectively. A signal was observed at the upfield region at δ 31.10 and was assigned to C-8 which indicated the presence of a methyl group in the chemical structure of Compound 2. Chemical shift of every proton and carbon NMR for Compound 2 is shown in Table 4 and comparison was made with NMR data of similar compound reported by Zhu (2014).

Table 4. Carbon NMR signal of Compound 2 and xanthoxylin reported by Zhu (2014)

Carbon assigned to Compound 2	Carbon chemical shift (ppm) of Compound 2	Carbon assigned to xanthoxylin (Zhu, 2014)	Carbon chemical shift (ppm) of xanthoxylin (Zhu, 2014)
C-1	105.86	C-1	106.3
C-2	166.24	C-2	166.1
C-3	93.64	C-3	93.6
C-4	167.62	C-4	167.6
C-5	90.59	C-5	90.6
C-6	162.79	C-6	162.9
C-7	203.48	C-7	203.3
C-8	31.10	C-8	31.6
C-9	55.62	C-9	55.7
C-10	32.87	-	-

Note: '-' indicates no data available



Structure of Xanthoxylin **(2)**

Xanthoxylin (**2**) is a compound containing a methoxy group attached to the benzene ring of a phenol moiety. It has been found to induced melanogenesis (increase melanin content and mRNA expression of regulatory melanogenesis protein), increased dendrites and activated tyrosinase and Mift expression in mouse melanoma cells (Moleephan *et al*., 2010). In another study, Pinheiro *et al.* (1999) reported that xanthoxylin (**2**) and its derivatives contain a fungistatic effect against filamentous fungi and dermatophytes. Jee-Hyun *et al*. (2012) reported on the effect of xanthoxylin (**2**) on toothache. In a study on the determination of flavonoids and xanthoxylin (**2**) in toothache-tree containing toothpaste, they reported xanthoxylin **(2)** to exhibit good preservative ratio which shows to

be very chemically stable. Several pharmacological activities have been reported including neurotransmitter-mediated contractions in nonvascular smooth muscles (Moleephan *et al*., 2010), antifungal (Lima *et al.*, 1995), antispasmodic (Filho *et al*., 1995), antioedema (Cechinel-Filho *et al.*, 1996) and inhibitor of prostaglandin synthetase and 5-lipoxygenase (Valenciennes *et al.*, 1999).

Compound 3 (Hexadecanoic acid)

Hexadecanoic acid **(3)**; colorless (oily) compound; melting point; 39 - 40 ^{o}C; IR V_{max} cm^{-1}: 3521, 2935, 2852, 1733, 1218, 982, 853; MS *m/z* (% rel. int): 414 (1), 355 (2), 340 (1), 326 (1) 281 (3), 256 (720), 228 (6), 227 (9), 213 (40), 199 (13), 171 (21), 157 (22), 143 (11), 129 (39), 115 (20), 97 (26), 82 (42), 72 (100), 60 (76), 43 (90), 41 (66): ^{1}H-NMR (500 MHz, Acetone D6) δ (ppm): 7.99 (1H, s, OH), 2.11 (2H, s, H-2), 1.94 (2H, s, H-3), 1.25 (24H, s, H-4 – H-15), 0.84 (3H, s, H-16); ^{13}C-NMR (500 MHz, $CDCl_3$) δ (ppm): 179.85 (C-1), 38.35 (C-2), 31.95 (C-14), 29.79 (C-4 – C-13), 24.04 (C-3), 22.84 (C-15), 14.24 (C-16).

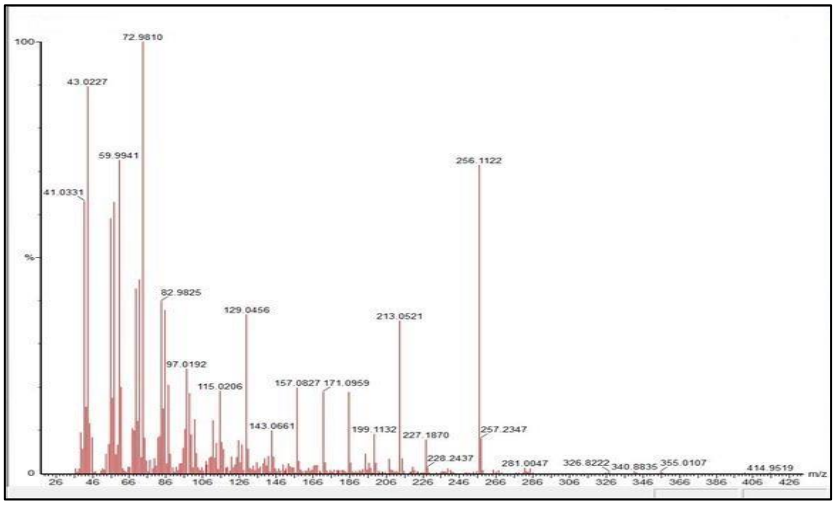


Figure 5. Mass spectrum of Compound 3 (Hexadecanoic acid)

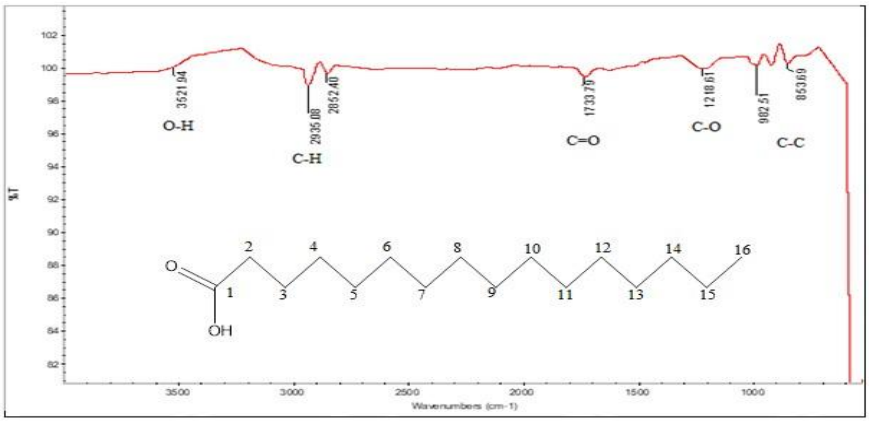


Figure 6. IR Spectrum of Compound 3 (Hexadecanoic acid)

Mass spectrum and IR spectrum of Compound 3 is shown in Figure 5 and Figure 6, respectively. NMR analysis of Compound 3 was performed to further elucidate the chemical structure. The proton NMR of the compound were integrated and assigned to the proposed chemical structure which is based on the table of ^{1}H-NMR characteristics absorption and ^{1}H-NMR peaks splitting pattern reported in organic chemistry by Janice (2008). The ^{1}H-NMR spectrum of Compound 3 exhibited 5 proton resonates of characteristic of aliphatic carboxylic acid at region between δ 0.80 to δ 2.50. The spectrum indicated the presence of a long chain methylene protons representing fourteen methylene groups overlapped as broad

peak at δ 1.25 (24H, s) which was identified as singlet of the long chain that was integrated for one proton and assigned H-4 - H-15. Two singlet protons were observed at a chemical shift δ 1.94 (2H, s) and δ 2.11 (2H, s) which were also identified as protons of two methylene group and were assigned to H-3 and H-2, respectively. A singlet proton was observed at a chemical shift of δ 0.84 (3H, s) at the terminal carbon which was identified as the terminal methyl group at C-16 and assigned to H-16. At a chemical shift of δ 7.99 (1H, s) a singlet proton was observed which was identified as a demonstrative of an OH in COOH group, as shown in Table 5 and it is in congruent with the published work of Keat *et al*. (2010).

Table 5. Proton NMR signal of Compound 3 and hexadecanoic acid reported by Keat *et al*. (2010)

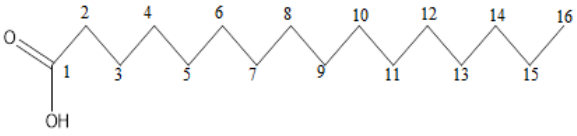
Proton assigned to Compound 3	Proton chemical shift (ppm) of Compound 3	Proton assigned to hexadecanoic acid (Keat *et al*., 2010)	Proton chemical shift (ppm) of hexadecanoic acid (Keat *et al*., 2010)
H-2	2.11 (2H, s)	H-2	2.32 (2, t, *J* = 7.3 Hz)
H-3	1.94 (2H, s)	H-3	1.60 (2H, m)
H-4 – H-15	1.25 (24H, s)	H-4 – 14	1.26 (22H, br, s)
-	-	H-15	1.27 (2H, m)
H-16	0.84 (3H, s)	H-16	0.85 (3H, t, *J* = 7.3 Hz)
OH	7.99 (1H, s)	-	-

Note: '-' indicates no data available

The result for the ^{13}C-NMR spectrum of Compound 3 is shown in Table 6. Every carbon NMR is assigned to the proposed chemical structure which is based on the table of ^{13}C-NMR characteristics absorption reported in organic chemistry by Janice (2008). The ^{13}C-NMR spectrum of Compound 3 indicated the presence of 7 carbon resonates in the chemical structure. A recognizable signal was observed at a chemical shift of δ 14.24 which indicated the presence of terminal methyl carbon attached to a methylene carbon and is therefore assigned to terminal carbon C-16. A methylene carbon resonate was also observed at δ 22.84 which indicated the presence of alpha and beta carbon and was assigned to C-15. A methylene carbon resonate was observed at δ 31.95 and was assigned to C-14 which also indicated the presence of alpha and beta carbon. A long peak signal observed at δ 29.79 which contain alpha and beta carbon were identified as a long chain of methylene carbons of to the structure and was assigned to C-4 to C-13. Two methylene resonates containing alpha and beta carbon to the acidic group were observed at δ 24.04 and at δ 38.35 and were assigned to C-3 and C-2, respectively. The spectrum shown a recognizable peak identified as carbonyl group at δ 179.85 which was assigned to C-1 double bonds of the acidic group. The chemical shift of ^{13}C-NMR for Compound 3 is shown in Table 6 and the comparison was made with ^{13}C-NMR data of similar compound reported by Keat *et al.* (2010).

Table 6. Carbon NMR signal of Compound 3 and hexadecanoic acid reported by Keat *et al*. (2010)

Carbon assigned to Compound 3	Carbon chemical shift (ppm) of Compound 3	Carbon assigned to hexadecanoic acid (Keat *et al.*, 2010)	Carbon chemical shift (ppm) of hexadecanoic acid (Keat *et al.*, 2010)
C-1	179.85	C-1	179.2
C-2	38.35	C-2	34.0
C-3	24.04	C-3	24.7
C-4 – C-13	29.79	C-4 – C-13	29.0 – 29.7
C-14	31.95	C-14	31.9
C-15	22.84	C-15	22.7
C-16	14.24	C-16	14.1



Structure of Hexadecanoic acid (**3**)

Hexadecanoic acid (**3)** has been reported to caused autolysis of membranous structure (Wang *et al.*, 2010; Ajoku *et al.*, 2015), inhibited phagocytic activity and nitric oxide production of certain cells (Sarkar *et al*., 2006), reduced levels of tumour necrosis factor-alpha,

prostaglandin E_2 (PGE2) and interleukin-10 (IL-10) without affecting ATP levels (Cai *et al*., 2005). Hexadecanoic acid (**3)** has been reported to have a moderate antibacterial activity against *Salmonella* group, moderate antifungal activity against *Microsporum canis* and good antioxidant activity (Mahmood *et al*., 2009). This fatty acid has been reported as one of the constituents that was isolated from two weed species of *Spermacoce* (Rubiaceae) (Keat *et al*., 2010).

Isolation and Purification of Secondary Metabolites from the Leaf Extract

Compound 4 (Beta-eudesmol)

Beta-eudesmol **(4)**; white crystalline; melting point 76 – 78 ^{o}C; IR V_{max} cm^{-1}: 3391, 2914, 1728, 1565, 1412; MS *m/z* (% rel. int): 222 (8), 204 (22), 189 (34), 175 (9), 161 (30), 149 (51), 133 (18), 122 (26), 109 (33), 93 (35), 81 (32), 59 (99), 41 (20); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 8.13 (CHO), 5.32 (2H, s, H15), 4.71 (1H, d, H-5), 2.72 (2H, d, H-3), 2.16 (1H, d, H-7), 1.62 (2H, m, H-6), 1.59 (8H, m, H-1/H-12/H13), 1.59 (2H, m, H-8), 1.59 (2H, M, H-9), 1.48 (2H, m, H-2), 0.87 (1H, s, H-14); ^{13}C-NMR (500 MHz, $CDCl_3$) δ (ppm): 150.97 (C-4), 107.64 (C-15), 75.34 (C-7), 73.91 (C-11). 49.88 (C-5), 43.88 (C-1), 40.24 (C-9), 37.60 (C-3), 35.96 (C-10), 31.99 (C-6), 26.28 (C-8), 24.99 (C-12), 24.59 (C-13), 23.23 (C-2)16.23 (C-14).

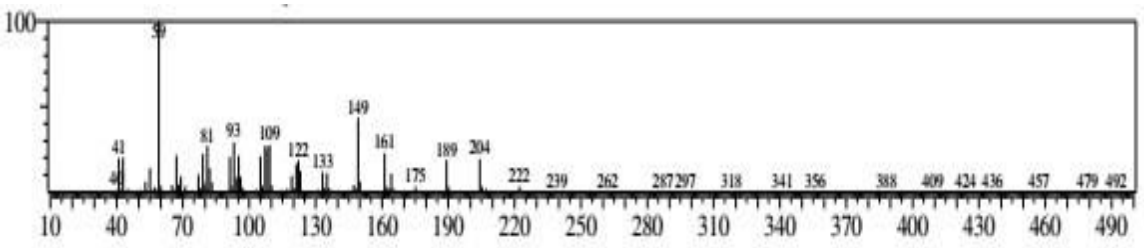


Figure 7. Mass spectrum of Compound 4 (Beta-eudesmol)

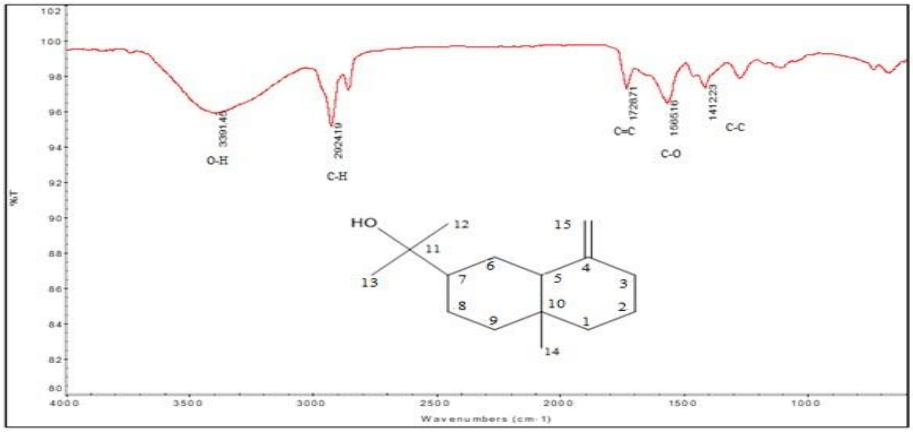


Figure 8. IR spectrum of Compound 4 (Beta-eudesmol)

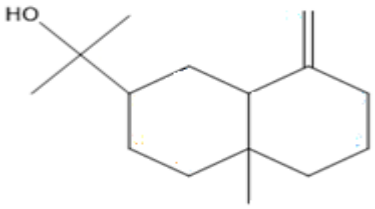
Mass spectrum and IR spectrum of Compound 4 is shown in Figure 7 and Figure 8, respectively. NMR analysis was done to further elucidate the chemical structure of the Compound 4. The proton NMR of Compound 4 was all integrated and assigned to the proposed chemical structure which is based on the table of ^{1}H-NMR characteristics absorption and ^{1}H-NMR peaks splitting pattern reported in organic chemistry by Janice (2008). The ^{1}H-NMR spectrum of Compound 4 was shown to exhibit nine proton resonates. A singlet proton signal was observed at δ 0.87 (3H, s) which was identified as a methyl group and was assigned to H-14. Two doublet proton signals were observed at a chemical shift δ 2.72 (2H, d) which indicated the presence of methine group in the structure and δ 2.16 (1H, m) and are therefore assigned to H-3 and H-7, respectively. A singlet proton signals was observed at chemical shift of δ 5.32 (2H, s) and δ 4.71 (1H, s) which was assigned to H-15 and H-5, respectively. Two multiplet proton signals appeared at δ 1.48 (2H, m) and δ 1.62 (2H, m) and were assigned to H-2 and H-6,

respectively, which indicated the presence of methine group in the compound chemical structure. At a chemical shift of δ 1.59 (H12, m) multiplet proton was observed which consist of 12 protons containing the 2H of H-1, the two methyl protons of H-12 and H-13 and 2H of H-8/H-9 each, was assigned to H-1. A signal was observed at δ 8.13 (1H) and was assigned to OH as shown in Table 7, and the result is in agreement with the published work of El-Askary *et al*. (2003).

Table 7. Proton NMR signal of Compound 4 and Beta-eudesmol reported by El-Askary *et al.* (2003)

Proton assigned to Compound 4	Proton chemical shift (ppm) of Compound 4	Proton assigned to Beta-eudesmol (El-Askary *et al*., 2003)	Proton chemical shift (ppm) of Beta-eudesmol (El-Askary *et al*., 2003)
H-1	1.59 (8H, m)	H-1	1.74 (2H, m, *J*= 13.3)
H-2	1.48 (2H, m)	H-2	1.60 (2H, m)
H-3	2.72 (2H, d, *J* = 15.2 Hz)	H-3	2.61 (2H, dd, *J*= 13.1)
H-5	4.71 (1H, d, *J* = 13.2 Hz)	H-5	4.71 (1H, s)
H-6	1.62 (2H, m)	H-6	1.65 (2H, m)
H-7	2.16 (1H, m)	H-7	1.38 (1H, m)
H-8	1.59 (2H, m)	H-8	1.58 (2H, m)
H-9	1.59 (2H, m)	H-9	1.62 (2H, m)
H-14	0.87 (3H, s)	H-14	0.86 (3H, s)
H-15	5.32 (2H, d, *J* = 13.1 Hz)	H-15	5.05 (2H, s)
OH	8.13 (1H)	OH	9.0 (1H)

The result for the ^{13}C-NMR spectrum of Compound 4 is shown in Table 8. Every carbon NMR of Compound 4 was assigned to the proposed chemical structure which is based on the table of ^{13}C-NMR characteristics absorption reported in organic chemistry by Janice (2008). The ^{13}C-NMR spectrum of Compound 4 indicated the presence of 15 carbon resonates in the chemical structure. Signals observed at δ 24.99, δ 24.59 and δ 16.23 indicated the presence of methyl carbon and are therefore assigned to C-12, C-13 and C-14, respectively. Six signals observed at δ 43.88, δ 23.23, δ 37.60, δ 31.99, δ 26.28 and at δ 40.24 were assigned to C-1, C-2, C-3, C-6, C-8 and C-9, respectively, which indicated the presence of methylene group in the structure. A signal was observed at δ 73.91 and was assigned to C-11 which indicated the presence of two methyl groups. A signal at δ 35.96, δ 49.88 and 75.34 were assigned to C-10, C-5 and C-7, respectively. A signal was observed at δ 107.6 indicating a methylene group and was assigned to C-15. A carbon signal also appeared at δ 150.97 and was assigned to C-4 which is bonded to C-15 indicating the presence of double bond in the chemical structure. The chemical shift of every proton and carbon NMR for compound 4 is and the comparison was made with NMR data of similar compound reported by El-Askary *et al*. (2003).



Structure of Beta-eudesmol (**4**)

Beta-eudesmol (**4)** has been found to have effect on the behavior of the formosan subterranean termite (*Coptoterames formosanus shiraki)* by significantly reducing their ability to construct tunnel in sand and their consumption on filter paper placed on sand, thus resulting in decreased feeding activity (Ibrahim *et al*., 2010). In another study, Nakamura *et al.* (1988) reported on the new method that can be used to determine the critical micelle concentration (CMC) of anionic surfactants by capillary electrophoresis, which is based on the measurement of the migration time of Beta-eudesmol (**4)** as a marker compound, using various concentration of the desired surfactants in a phosphate buffer. Thus, it was found to enhance the determination of the anionic surfactants using small volumes of the surfactant's solutions.

Table 8. Carbon NMR signal of Compound 4 and Beta-eudesmol reported by El-Askary *et al*. 2003)

Carbon assigned to Compound 4	Carbon chemical shift (ppm) of Compound 4	Carbon assigned to Beta-eudesmol (ElAskary *et al*., 2003)	Carbon chemical shift (ppm) of Beta-eudesmol (El-Askary *et al*., 2003)
C-1	43.88	C-1	34.9
C-2	23.23	C-2	21.9
C-3	37.60	C-3	31.7
C-4	150.97	C-4	152.1
C-5	49.88	C-5	75.5
C-6	31.99	C-6	31.0
C-7	75.34	C-7	43.5
C-8	26.28	C-8	22.3
C-9	40.24	C-9	36.2
C-10	35.96	C-10	37.8
C-11	73.91	C-11	72.8
C-12	24.99	C-12	26.8
C-13	24.59	C-13	27.9
C-14	16.23	C-14	19.9
C-15	107.64	C-15	107.6

Compound 5 (squalene)

Squalene **(5)**; light orange; -6.9°C; IR V_{max} cm^{-1}: 2973, 1666, 879, 646; MS *m/z* (% rel. int): 203 (3), 191 (4), 175 (5), 161 (6),149 (10),137 (20), 121 (18), 109 (14), 95 (24), 81 (68), 69 (100), 55 (8), 41 (40), 39 (2); ^{1}H-NMR (500 MHz, $CDCl_3$) δ (ppm): 5.07 – 5.17 (6H, d, H-4), 1.94 – 2.07 (20H, d, H-30), 1.67 (6H, m, H-25), 1.58 – 1.59 (18H, d H-1); ^{13}C-NMR (500 MHz, $CDCl_3$) δ (ppm): 132.53 (C-10/C-15), 130.98 (C-6/C-9), 130.51 (C-2/C-23), 129.52 (C-7/C-18), 128.61 (C-11/C14), 121.37 (C-3/C-22), 38.91 (C-9/C-16), 37.32 (C-5/C-20), 32.04 (12/C-13), 29.78 (C-4/C-21), 27.33 (C-8/C17), 22.77 (C-1/C-24), 19.54 (C-27/C-28), 18.85 (C-25/C-30), 14.20 (C26/C-29).

Mass spectrum and IR spectrum of Compound 5 is shown in Figure 9 and Figure 10, respectively. NMR analysis was performed to further elucidate the chemical structure of Compound 5. The proton NMR of the compound were integrated and assigned to the proposed chemical structure which is based on the table of ^{1}H-NMR characteristics absorption and ^{1}H-NMR peaks splitting pattern reported by Janice (2008). The ^{1}H-NMR spectrum exhibited four proton resonates. A multiplet proton was observed at δ 1.67 (6H, m) which was identified as methyl group and was assigned to H-25. At a chemical shift δ 1.58 – 1.59 (18H, d) a doublet proton signal was observed containing 18 protons including the methyl protons of the terminal methyl carbon and was assigned to H-1. A doublet proton signal was observed at δ 5.07 – 5.17 (6H, d) and was assigned to assigned to H-3 which identified as internal vinyl group in the compound chemical structure. At a chemical shift of δ 1.94 – 2.07 (H20, d) a doublet proton signal was observed which consist of 20 protons which was identified to contain the methyl protons of a methyl carbon was assigned to H-30 and the result is in congruent with the published work of Inte *et al.* (1998) as shown in Table 9.

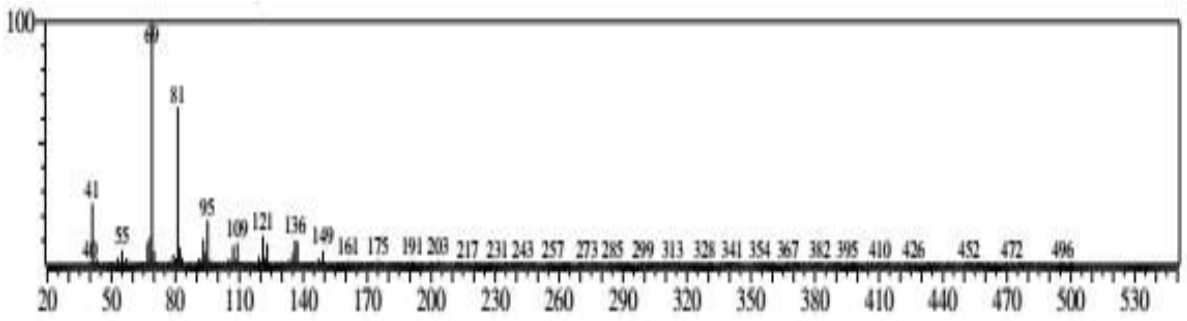


Figure 9. Mass spectrum of Compound 5 (squalene)

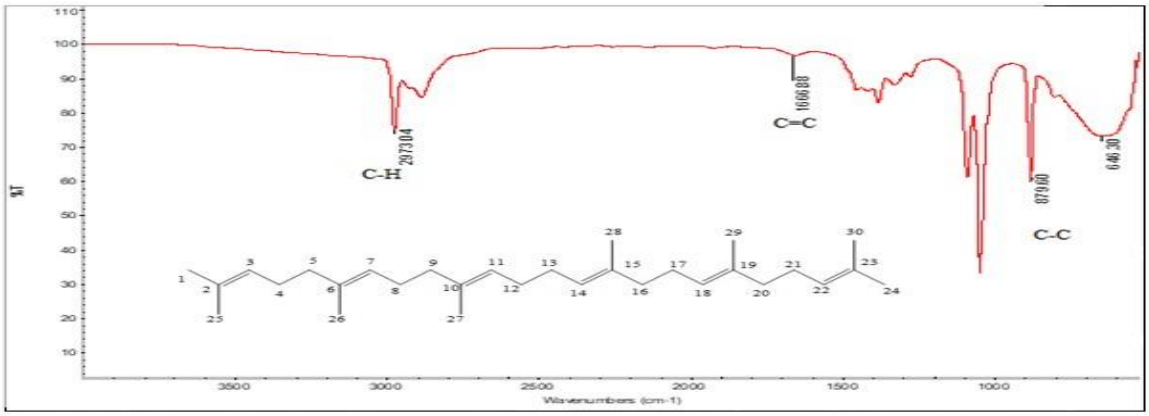


Figure 10. FT-IR Spectrum of Compound 5 (squalene)

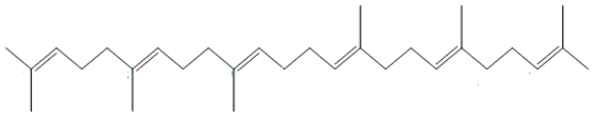
Table 9. Proton NMR signal of Compound 5 and squalene reported by Inte *et al.* (1998)

Proton assigned to Compound 5	Proton chemical shift (ppm) of Compound 5	Proton assigned to squalene (Inte *et al.*, 1998)	Proton chemical shift (ppm) of squalene (Inte *et al.*, 1998)
H-1	1.58 – 1.59 (18H, d, 9.2 Hz)	H-1	1.60 (18H)
H-3	5.07 -5.17 (6H, d, *J* = 13.1 Hz)	H-3	5.09 – 5.15 (6H)
H-25	1.67 (6H, m)	H-25	1.68 (6H)
H-30	1.94 – 2.07 (20H, d, *J*= 9. 1 Hz)	H-31	2.01 – 2.07 (20H)

Every carbon NMR is assigned to the proposed chemical structure which is based on the table of ^{13}C-NMR characteristics absorption reported by Janice (2008). The ^{13}C-NMR spectrum of Compound 5 indicated the presence of 15 carbon signals in the chemical structure. Six signals were observed at the upfield region where signals at δ 132.53, δ 130.98, δ 130.51 and δ 129.52 indicated the existence of C-ethylene carbon and are therefore assigned to C-10/C-15, C-6/C-19, C-2/C-23 and C-7/C-18, respectively. Whereas signals at δ 128.61 and δ 121.37 indicated the presence of double bond (C=C) and were assigned to C-11/C-14 and C-3/C-22, respectively. Five signals identified as methylene groups were observed at δ 32.04, δ 38.91, δ 27.33, δ 37.32, and at δ 29.78 and were assigned to C-12/C-13, C-9/C16, C-8/C-17, C-5/C-20 and C-4/C-21, respectively. A signal was observed at δ 22.77 and was assigned to C-1/C-24 which indicated a methyl group. Three other signals were observed at δ 19.54, δ 18.85 and δ 14.20 which were also identified as methyl group and were assigned to C-27/28, C-25/30 and C-26/29, respectively. Thus, the spectrum indicated signals corresponding to eight methyl groups (CH_3), ten methylene groups (CH_2) and ten methine groups (CH). The chemical shift of every carbon NMR for Compound 5 was assigned and the comparison with NMR data of similar compound reported by Inte *et al.* (1998) and Nam *et al.* (2017) as shown in Table 10.

Table 10. Carbon NMR signal of Compound 5 and squalene reported by Nam *et al.* (2017)

Carbon assigned to Compound 5	Carbon chemical shift (ppm) of Compound 5	Carbon assigned to squalene (Nam *et al*., 2017)	Carbon chemical shift (ppm) of squalene (Nam *et al*., 2017)
1/24	22.77	C-1	25.71
2/23	130.51	C-2	131.26
3/22	121.37	C-3	124.42
4/21	29.78	C-4	26.78
5/20	37.32	C-5	39.74
6/19	130.98	C-6	134.90
7/18	129.52	C-7	128.28
8/17	27.33	C-8	26.67
9/16	38.91	C-9	39.77
10/15	132.53	C-10	135.11
11/14	128.61	C-11	124.32
12/13	32.04	C-12	28.29
25/30	18.85	C-13	17.69
26/29	14.20	C-14	16.05
27/28	19.54	C-15	16.01



Structure of squalene (**5**)

Squalene-((E)-2,6,10,15,19,23-hexamethyl-2,6,10,18,22-tetracosahaxene) (**5)** is a naturally occurring acyclic symmetric triterpene that has been found to be a key intermediate in the biosynthesis of sterols (Psomiadou & Tsimidou, 1999), and it is synthesized and converted to cholesterol in the human body. Squalene (**5)** plays a major role in medicine, it has a major involvement in the reduction of cancer risk, particularly cancer of the pancreas and colon in rodent (Newmark, 1997; Rao *et al*., 1998; Smith *et al*., 1998). It has been shown to be useful at the surface of the skin where it plays a role as protective barrier against UV radiation (Auffray, 2007). The traditional source of squalene (**5)** is primarily from shark (*Centrophorus squamos)* and whale (*Physeter microcephalus*) liver oil (Jahaniaval *et al.,* 2000) and is known to contain effective medicinal properties. Squalene (**5)** exhibits an excellent antioxidant activity and can be found naturally in human skin. It has been isolated from *A. lakoocha* and shown to exhibit significant antioxidant activity based on free radical scavenging activity and its antioxidant effect makes it a good cardio-protective and anti-peroxidative compound (Biswas & Chakraborty, 2013). It has been reported to significantly suppress colonic ACF formation and crypt multiplicity which supports the findings that it possesses chemopreventive activity against colon carcinogenesis (Ragasa *et al*., 2014). Squalene (**5)** has been reported to increase the stability of various emulsions (pharmaceutical formulations and vaccines) (Reddy & Couvreur, 2009; Fox, 2009), and it is appreciated in many cosmetics as emollient agent in creams and capillary serums (James *et al*., 2010). However, the use of squalene (**5)** in cosmetic application is limited by the uncertainty of its availability as a result of international concern for the protection of marine animals. Similarly, the presence of similar compounds like cholesterol in oils from marine animal liver can make squalene purification difficult (He *et al*., 2002).

CONCLUSION

Crude extracts of the leaf and root of *A. precatorius* was obtained after successive extraction using four solvents, namely hexane, dichloromethane, ethyl acetate and methanol. The leaf and root extracts of *A. precatorius* were purified using column chromatography, chromatotron and vacuum-liquid chromatography to obtain pure compounds and the structures of the pure compounds isolated were elucidated using various spectroscopic information especially MS, NMR and FT-IR. Five compounds identified as 5,7-Dimethoxyflavanone (**1**), Xanthoxylin (**2**), Hexadecanoic acid (**3**) successfully isolated from the root extract, while Beta-eudesmol (**4**) and Squalene (**5**) successfully isolated from the leaf extract. Although all the pure compounds isolated in this work are known compounds, the land mark of this work is the isolation of 5,7-dimethoxyflavanone **(1)** and xanthoxylin **(2)** from the root extract of *A. precatorius* for the first time. These compounds have been shown by various researchers to exhibit biological activities; thus, this work will advance the cause of the importance of this medicinal plant.

ACKNOWLEDGEMENTS

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