Journal of Pharmacognosy and Phytochemistry

Available online at www.phytojournal.com

E-ISSN: 2278-4136
P-ISSN: 2349-8234
JPP 2019; 8(3): 4228-4244
Received: 10-03-2019
Accepted: 12-04-2019

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Isolation of compounds from root bark extracts of *Moringa stenopetala* and evaluation of their antibacterial activities

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Abstract
This study was conducted to isolate compounds from root bark extracts of *M. stenopetala* and evaluating its antibacterial activity. The root bark was extracted with different solvent systems (Petroleum ether, chloroform, and acetone) using maceration technique. The acetone crude extract was subjected to column chromatographic separation. Four compounds (labeled as MOST-1, MOST-2, MOST-3, and MOST-5) were obtained in the process. The compounds were found to be stigmastereol, ursolic acid, tasnemoxide and oleic acid respectively, based on their spectral analyses (IR, ^{1}H-NMR, ^{13}C-NMR and DEPT-135) data and comparison with data reported in literatures. The antibacterial activity of the compounds reveled that they show good antibacterial activities against the four bacterial strains used in the experiment. However, further tests (on more bacterial strains) are recommended in order to make decision on potential of the isolated compound as lead compounds and also to support the claims made by traditional healers on medicinal use of the plant used in the study.

Keywords: *Moringa stenopetala*, Ursolic acid, stigasterol, Oleic acid, disc diffusion, Tasnemoxide

Introduction
Moringa stenopetala (*M. stenopetala*) is one the 14 species of moringa. *M. stenopetala* is one of the well-known trees in southern part of Ethiopia (especially in Konso, Arbamicnh and Wolayta areas). The tree (locally known as *Haleko* or *Shiferaw*) is well known for its medicinal uses and nutritional value [1]. Some of the medicinal uses of its different parts to treat malaria, hypertension, stomach pain, leishmaniasis and also to expel retained placenta in women who have just given birth [1-4]. This made it imperative to conduct scientific investigations in search of lead compounds that could be used in drug discovery programs of pharmaceutical companies and academic institutes. This involves isolation of compounds, and subsequent test of their biological activities. There are several literature reports in this regard. For instance, a compound with antimicrobial (antibacterial and antifungal) activities 4-(α-L-rhamnosyloxy) benzyl isothiocyanate (1) (Figure 1) was isolated from seeds of *M. stenopetala* [5]. Isolation of other compounds such as benzyl isothiocyanate (2), isobutyl isothiocyanate (3), benzene-1-isocyano-2-methyl (4), cycloprpane pentyl (5), methyl 9-octadecenoat (6), methyl palmitate (7), nonanoic acid (8), δ-cadinene (9), 5,5-dimethyloxazolidine-2-thione (10) (Figure 1) were reported from seed extract of the plant [5, 6]. Reports also showed isolation of diverse group of unsaturated fatty acids such as oleic acid (11), and saturated acids such as behenic acid (12) and palmitic acid (13) as well as high levels of β-sitosterol (14), stigmasterol (15), and campesterol (16) from n-hexane and chloroform: methanol (1:1) extracts of seed oils of the plant [7]. Moreover, defatted and shell-free seeds of were found to contain the glucosinolates such as 4-(α-L-rhamnopyranosyloxy)-benzylglucosinolate (17) [8]. Similar reports showed isolation several compounds from the leaves and roots of *M. stenopetala*. Compounds such as 4-(α-l-rhamnopyranosyloxy)-benzylglucosinolate (17), 4-(4'-O-acetyl-L-rhamnosyloxy)-benzylisothiocyanate (18), 4-(4'-O-acetyl-L-rhamnosyloxy)-benzaldehyde (19), rutin (20), quercetin 3-*O*-rhamnoglucoside (21) and 5-caffeoylquinic acid (22) have been isolated from the leaves [9] whereas benzyl glucosinolate (23), cholest-5-en-3-ol (cholesterol) (24), palmitic acid (13), n-octacosane (25), Oleic acid (26), 1, 3-dilinoleoyl-2-olein (27) and 1, 3-dioleoyl-2-linolein (28) (Figure 1) are some of the compounds isolated from roots and root wood of the plant [9, 10]. Biological activity tests of the isolated compounds showed promising potentials to be used as drug candidates in the development of drugs for treatment human diseases such as antitrypanosomasis [11, 12], hypertension [6], bacterial infection [7] and leishmaniasis [10].

In continuation of our previous effort to explore potential drug candidate for antibacterial activity from *M. stenopetala* [7, 10], we carried out isolation of compounds from root bark of this

plant species and evaluation of their antibacterial activities. Thus, this paper discusses isolation and characterization of compounds, and also evaluation of antibacterial activities of the isolated compounds.

Results and Discussions

Structural elucidation of isolated compounds

Four compounds (MOST-1, MOST-2, MOST-3 and MOST-5) were isolated from the acetone crude extract of root bark of *M. stenopetala*. The structural elucidation of the compounds was done by comparing the observed spectral and melting point data with the reported data of these compounds in literature.

Compound MOST-1

It was obtained as white amorphous powder. Analysis of IR (KBr) spectrum (Supplementary material 1) of compound MOST-1 showed a band at 3430 cm^{-1} that indicates the presence of hydroxyl functional group attached to an alkyl or an aromatic carbon. The strong band at 2937 cm^{-1} represents C-H stretch of alkenes whereas the weak bands at 2862.9 cm^{-1} could be attributed C-H stretching of methyl groups. The band at 1637.8 cm^{-1} could also be attributed to C=C bond stretch. The observed IR data suggested that compound MOST-1 could be an alcohol possessing at least one C=C double bond in its chain [13-15]. In the ^{1}H-NMR spectrum of compound MOST-1 (Supplementary material 2), the peaks at δ 0.69, 0.71 0.82, 0.86, 0.94, 1.02 and 1.27 indicated the presence of protons of six methyl (-CH_3) groups whereas the peaks at δ 5.01, 5.16 and 5.36 indicated the presence of olefinic protons in compound MOST-1. The ^{13}C-NMR spectrum (Supplementary material 3) of the compound MOST-1 showed signals at δ140.75, 121.71, 138.34, and 129.26 (Table 2) that could be assigned to two double bonds in stigmasterol-type skeleton compound reported in literature. The ^{1}H-NMR signals at δ 3.54 and ^{13}C-NMR peaks at δ 71.78 suggested compound MOST-1 to a have hydroxyl group attached to C-3 of pentacyclic triterpene [14, 16]. The ^{13}C-NMR and DEPT-135 spectra (Supplementary material 3 and 4) showed that the compound possesses 29 and 26 signals, respectively, that can be assigned to six methyl, nine methylene, eleven methane and three quaternary carbon atoms in stigmasterol-type moiety [14]. The observed ^{1}H-NMR data (Table 1) and ^{13}C-NMR and DEPT-135 data (Table 2) of compound MOST-1 were found to be consistent with the reported spectral data of Stigmasterol [14-16]. Moreover, the observed melting point of compound MOST-1 (157-158 ^{o}C) was comparable to the reported melting point value of Stigmasterol (i.e., 169-171°C) [17]. These data indicates that compound MOST-1 can be proposed to be Stigmasterol (Figure 2).

Compound MOST-2

Compound MOST-2 (40 mg) was obtained as a colorless crystalline solid compound by combining the fractions 63-66 that were eluted with 6% ethyl acetate in petroleum ether. The R_f value of the compound was determined as 0.41 in petroleum ether-ethyl acetate (90:10 % by volume). The IR spectrum (Supplementary material 5) of compound MOST-2 showed absorption bands that can be attributed to tri-substituted double bond at 1466 and 959 cm^{-1}, sharp band for carbonyl group at 1712 cm^{-1} and broad band for hydroxyl group at 3429 cm^{-1}. The band at 2928 cm^{-1} represents C-H stretch of alkenes whereas the bands at 2850 cm^{-1} indicated C-H stretching of methyl groups [18, 19]. Its ^{1}H-NMR spectrum (Supplementary material 6) revealed presence of five tertiary methyl groups at δ 0.89, 0.94, 1.01, 1.17, 1.25 and 1.26; and two secondary methyl group peaks at δ 0.89 and 0.92. A doublet of one proton at δ 2.34 and a triplet of one proton at δ 5.35 could be assigned to methine and olefinic protons, respectively. These data suggest that compound MOST-2 might have an urs-12-ene skeleton. The peak at δ 3.49 can be assigned to one methine proton that suggests compound MOST-2 to have one hydroxyl group [14]. The IR and ^{1}H-NMR spectra and comparison with literature reports suggested that compound MOST-2 to be an acid with ursane skeleton, and is most likely ursolic acid [20]. The ^{13}C-NMR spectrum (Supplementary material 7) also accounted for 30 carbons. The peaks at δ 179.41 indicated the presence of a carbonyl group whereas the two peaks at δ 121.70 and 140.68 could be attributed to sp^2 (or olefininc) carbons. The prominent peaks at higher field of δ 29.09, 15.76, 18.23, 18.23, 24.29, 18.77 and 21.08 were attributed to methyl carbons of C-23, C-24, C-25, C-26, C-27, C-29 and C-30, respectively (Table 4). The DEPT-135 spectrum (Supplementary material 8) experiment also indicated seven methyl, nine methylene, seven methane and seven quaternary carbons. Therefore, the comparison of spectral data obtained from IR, ^{1}H-NMR (Table 3), ^{13}C-NMR and DEPT-135 data (Table 4) with literature reports suggested that the compound MOST-2 is most likely ursolic acid (3-beta-hydroxyurs-12-en-28-oic acid) (Figure 3) [20-22]. The observed melting point of compound MOST-2 (279 -281^{o}C) was also found to be comparable to the reported melting point value of Ursolic acid (i.e., 283 - 285 °C) [20].

Compound MOST-3

Compound MOST-3 (25 mg) was isolated from the fractions 98-101 eluted with 8% ethyl acetate in petroleum ether. Its R_f value of was found to be 0.39 in petroleum ether-ethyl acetate (90:10% by volume). IR spectrum (Supplementary material 9) of compound MOST-3 indicated that it has carbonyl group (1712 cm^{-1}). The bands near and ranging 2928-2850 cm^{-1} could be attributed to C-H stretching of CH_3 and CH_2, along with weaker bands near 1465 cm^{-1} from C-H bending of CH_2 and CH_3, absorption at 1062 cm^{-1} from C-C bending and long chain band (four or more CH_2 groups in an open chain) at 722 cm^{-1} in the IR spectrum of compound MOST-3 indicated that the compound could contain an alkane group and carbonyl functional group. The ^{1}H-NMR spectrum of compound MOST-3 showed resonances peaks for a six methyl groups, eight methylenes, four methines and six quaternary carbons (Table 5). The IR, ^{1}H-NMR spectral data together with literature reports suggested the compound MOST-3 to be most likely Tasnemoxide-type compound reported in literature [23]. This suggestion was also confirmed by the observed ^{13}C-NMR spectrum (Supplementary material 10). The spectrum revealed the presence of a total of 25 carbon atoms. The peaks at δ 178.68 indicate carboxylic acid whereas peaks at δ 121.75 and 141.56 are could be attributed to a disubstituted double bond. The peaks at δ76.7 and 81.41 could be attributed to protons on an endoperoxide ring. The peak at δ 51 could also be attributed to methoxy group of an ester functional group. The DEPT-135 spectrum (Supplementary material 11) also indicated a total of twenty four carbons that include six methyl carbons, eight methylene groups, four methane and six quaternary carbons. However, the reports showed the presence of three types of Tasnemoxides namely Tasnemoxide A (29), Tasnemoxide B (30) and Tasnemoxide C (31) isolated from the Red Sea sponge *Diacarnus erythraenus* (Figure 3) [23, 24]. The ^{1}H-NMR

data (Table 5), ^{13}C-NMR and DEPT-135 data (Table 6) and comparison with literature reports suggested that the compound MOST-3 is most likely 31(Figure 4).

Compound MOST-5

Compound MOST-5 was obtained from the fractions 111-138 that were eluted with 16% ethyl acetate in petroleum ether. It is a fine brown solid compound (36 mg). Its R_f value was found to be 0.23 in petroleum-ethyl acetate (90:10% by volume) solvent system. In the IR spectrum (Supplementary material 12) of compound MOST-5, the bands at 2918 cm^{-1} and 2850 cm^{-1} indicated the C-H stretch of olefinic group and alkyl groups. The band at 1706 cm^{-1} indicated C=O stretch of carbonyl groups. This band could be attributed to C=O stretch of carboxylic acid whereas the intense absorption band at 1292 cm^{-1} indicates C-O stretching. The absorption bands at 1464 and 1292.4 cm^{-1} indicate characteristic bending of C-H bending of CH_2 and CH_3, respectively. Thus, the observed carbonyl group (C=O) and C-O stretching bands in the IR spectrum (Appendix 13) indicates that the compound MOST-5 is most likely a carboxylic acid.

In the ^{1}H-NMR spectrum (Supplementary material 13) of compound MOST-5 triplet peak at δ0.88 indicated presence of protons of methyl (-CH_3) groups; the peaks at δ 1.28 and 1.67 indicate protons of aliphatic methylene (-CH_2) group; a peak at δ 2.02 indicated presence of protons of a methylene group that is bonded to C=C bond. On the other hand, the peak at δ2.35 indicates presence of protons of methylene that is directly bonded to a carboxylic acid group whereas the peak at δ 5.37 indicates presence of olefinic protons in the structure. The ^{1}H-NMR spectral data were found to be consistent with the reported data of oleic acid (Table 7) [7]. The ^{13}C-NMR spectrum (Supplementary material 14) of compound MOST-5 was analyzed to identify the natures of carbon atom in the compounds. It showed peaks at δ130.04 and 129.74 indicated C=C bonds; a single peak at 179.84 ppm indicate quaternary carbon atom (of carbonyl carbon) of carboxylic acid. On the other hand, the chemical shift values in the range of δ 14.13 to 34.01 indicated presence of methyl (-CH_3) and methylene (-CH_2) carbons (Table 8). The DEPT-135 spectrum (Supplementary material 15) of the compound MOST-5 also showed single peaks that indicate the presence of methyl (-CH_3) carbon at δ 14.13, methylene carbons at δ 22.70 to 34.01 and olefinic methine carbons at δ130.04 and 129.74 (Table 8). Comparing the observed NMR data (Table 7 and Table 8) with literature reports [12, 25, 26] indicates that the chemical structure of compound MOST-5 to be identical with that of oleic acid (Figure 5). The compound was previously reported by our group from acetone extract of root wood of *M. stenopetala* [7].

Evaluation of antibacterial activities of the isolated compounds

After characterizing the four isolated compounds, their antibacterial activities were evaluated in order to assess their potential as lead compounds in the search for effective antibacterial agents and also to support the claim of traditional use of *M. stenopetala*. The evaluation was carried out against *S. aureus* (ATCC25903), *E. coli* (ATCC 25722), *P. aeruginosa* (DSMZ 1117) and *S. thyphimurium* (ATCC 13311) using a standard procedure (*See Experimental Section*). The activity was evaluated by measuring the inhibition zone of each strain used in the experiment (Table 9).

As discussed above, the activities of the compounds were evaluated by measuring the inhibition zone of each strain used in the experiment. The first isolated compound MOST-1 (stigmasterol) has showed good activity against *S. thyphimurium* (14 mm) and *E. coli* (16 mm) the activity of this compound was observed to be little activity against *S. aureus* (8 mm) and *P. aeruginosa* (10 mm) (Table 9). These data (or its trend) was consistent with similar studies carried in our previous studies [17, 27] and other reports revealing antibacterial activity of stigmasterol [28]. The next compound MOST-2 (ursolic acid) has shown almost similar activities against *S. aureus* (11 mm), *S. thyphimurium* (12 mm), and *E. coli* (12 mm*)* but its effectiveness to inhibit growth of *P. aeruginosa* was weak (9 mm) (Table 9). The finding is consistent with reports previous study results that revealed antibacterial activity of Ursolic acid [29-32]. The compound MOST-3 (Tasnemoxide C) is one of cytotoxic cyclic norsestertepene peroxides that showed moderate cytotoxicity against three cancer cell lines [24]. Evaluation of its antibacterial activity this compound revealed that it has relatively higher activity against *S. aureus* (12 mm). But it showed relatively lower activity against *P. aeruginosa* (9 mm), *S. thyphimurium* (8 mm) and *E. coli* (10 mm) (Table 9). When compared to other compounds used in the study, compound MOST-5 (Oleic acid) showed relatively superior antibacterial activities against all the tested bacterial strain used in the study (Table 9). This finding was consistent with previous reports demonstrating antibacterial activity of oleic acid [7, 33, 34].

In conclusion, four compounds (MOST-1, MOST-2, MOST-3 and MOST-5) were isolated from acetone extracts of root barks of *M. stenopetala*. The compounds were characterized, and identified as stigmasterol, ursolic acid, tasnemoxide C and olieic acid, respectively. To the best of our knowledge, this is the first report of isolation of uric acid and tasnemoxide C from *M. stenopetala.* Evaluation of antibacterial activities of the isolated compounds against four bacterial strains (*S. aureus*, *P. aeruginosa*, *S. thyphimurium* and *E. coli.*) showed that the compounds showed comparable activities to each other. Further antibacterial activity tests are recommended on large number of bacterial strains and other microorganisms in order to support the traditional/medicinal use of *M. stenopetala*.

Materials and Methods

Collection of plant material

The root bark of *M. stenopetala* was collected in November, 2012 from Arbaminch College of Teachers' Education, Arbaminch town. The town is found 470 km South of Addis Ababa, Ethiopia. The sample was recognized by Dr. Remesh Moochikkal (a botanist) at The Department of Biology, College of Natural Sciences, Jimma University. The plant material (sample) was labeled and given a Voucher number (MTG/00190).

Preparation of plant material

The collected plant material was chopped into small pieces and air-dried under shade on a plastic material for a period of forty days. Then the dried material was milled to suitable size (to facilitate extraction) with a grinding machine at The College of Agriculture and Veterinary Medicine, Jimma University. The prepared sample was stored under refrigerator below 4 ^{0}C until it was used for extraction.

Extraction

For the extraction process, 1,200 g of powdered plant material was used. It was sequentially extracted with petroleum ether, chloroform and acetone using maceration technique for 72 hours with continuous shaking. The extracted matter was filtered using Whatmann No.1 filter paper. Residual solvent in each gradient extract was removed using Rotary evaporator under reduced pressure.

Isolation and characterization of compounds

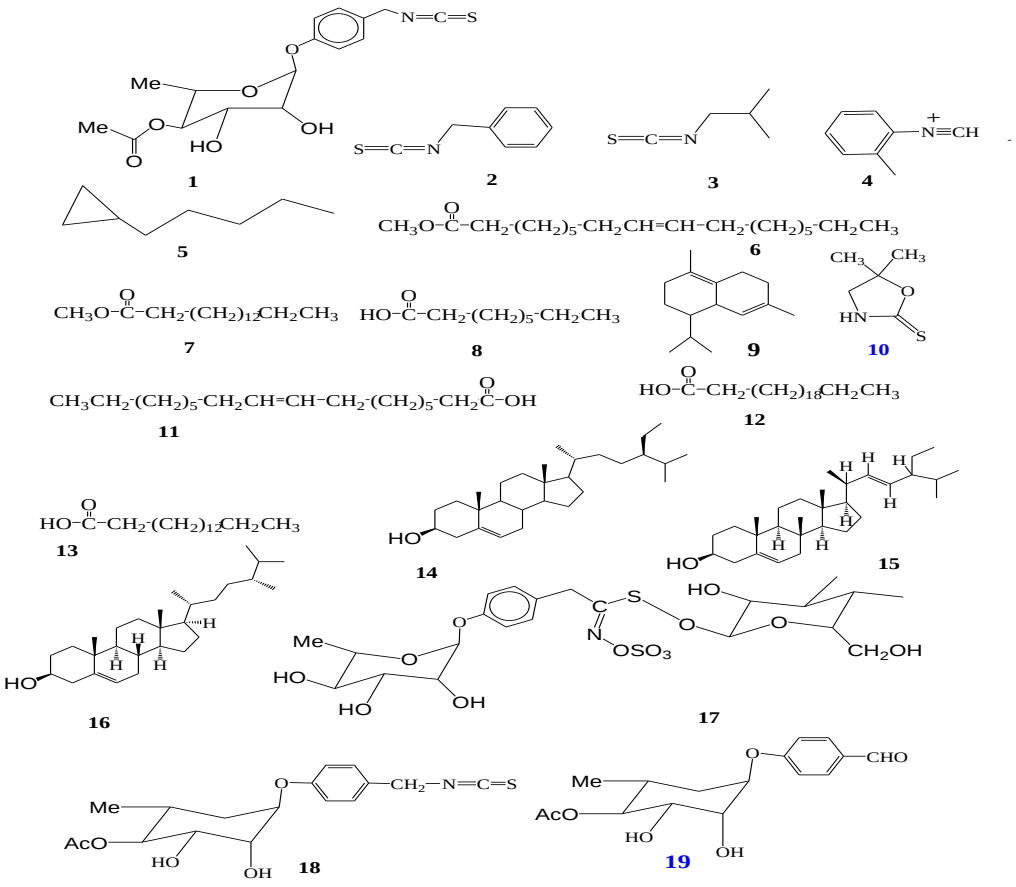
The acetone extract (10 g) was adsorbed onto 20 g of silica gel that was activated in an oven at a temperature of 120 ^{0}C for 3 hours. The adsorbed crude extract was then subjected to column chromatographic isolation. In the isolation process, the column was first eluted with 100% percent petroleum ether. The elution was then followed by a mixture of petroleum ether and ethyl mixture with gradual increase in polarity (or percent of ethyl acetate in the mixture). The A total of 280 fractions (each 30 ml) were collected. The collected fractions were concentrated using rotary evaporator (at 40 ^{o}C). The fractions were monitored by TLC. The spots on the TLC plates were visualized using UV light (at 254 nm and 365 nm) followed by iodine vapor. Fractions of same TLC profile were combined, and were concentrated to remove the solvent completely using rotary evaporator. Pure compounds were obtained from fractions (collected eluents) using a mixture of petroleum ether: ethyl acetate in 98:2 (MOST1), 94:6 (MOST 2), 92:8, 88:12, 84:16, 82:18, 80:20, 76:24 and 74:26 % (by volume). The structures of the compounds were elucidated based on combined spectral data which include Infrared, Nuclear Magnetic Resonance (^{1}H-NMR, ^{13}C-NMR and DEPT-135) spectra data and melting point values as well as comparison of these data with data reported in literature. All spectroscopic analyses were carried out at Department of chemistry, Addis Ababa University, Ethiopia.

Antibacterial activity tests of isolated compounds

The tests were carried out against *Staphylococcus aureus* (ATCC25903), *Escherichia coli* (ATCC 25722), *Pseudomonas aeruginosa* (DSMZ 1117) and *Salmonella thyphimurium* (ATCC 13311) obtained from The Department of Biology, Jimma University. Literature reported standard were used to carry out the antibacterial activity tests of the compounds (Nascimento *et al*, 2000). A cell suspension of each microorganism (used in the experiment) equivalent to McFarland 0.5 turbidity standard was obtained by preparing 1% V/V of H_2SO_4 and 1% W/V $BaCl_2$ then 95.5 ml of 1% V/V of H_2SO_4 mixed with 0.5 ml of 1% $BaCl_2$W/V for comparison of the turbidity to a cell suspension of each organism in order to have a suspension containing approximately 1-2 x 10^8 CFU ml^{-1} [35]. The bacterial suspensions were spread over solid Mueller Hinton agar plates with a sterile swab and each crude extract 50 µL of 100 mg ml^{-1} concentration dissolved in DMSO were impregnated on Whatmann No.1 filter paper disc (diameter 6 mm) using micropipette. At the same time ciprofloxacine was used as positive control and DMSO as negative control. The plates were then left for 5 minute till the extract diffuse in the medium and then incubated at 37 ^{o}C for 24 hours. After 24 hours incubation zone of inhibition (in diameter) was measured using ruler and mean was recorded [36]. Ciprofloxacin, which is a broad spectrum antibiotic, was used for comparison. Similar procedures were used for evaluation of antibacterial activities of the pure compounds.

Author's contribution and competing interests

All the three authors contributed equally to the study with respect to planning, execution, interpretation of data. LA prepared the manuscript. There are no competing interests.



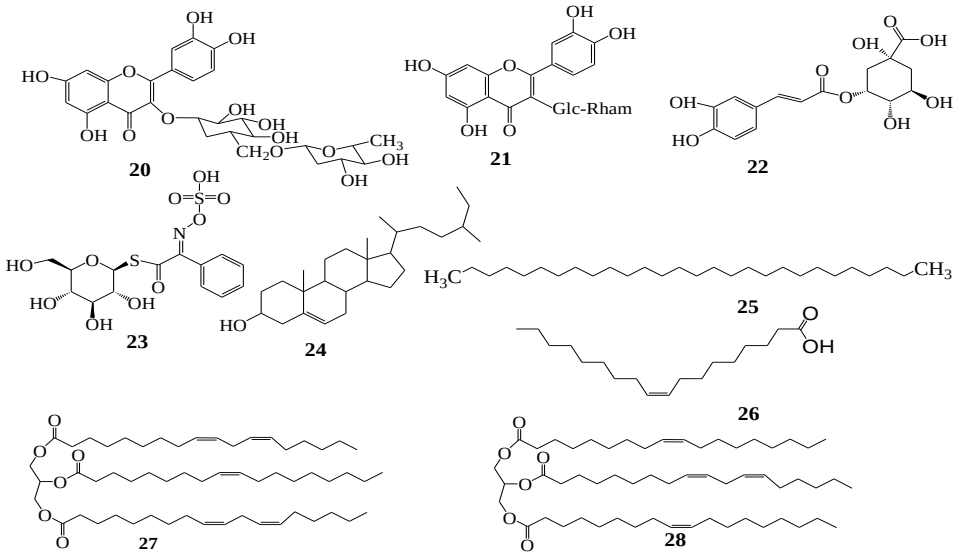


Fig 1: The chemical structures of some compounds isolated from *M. stenopetala*.

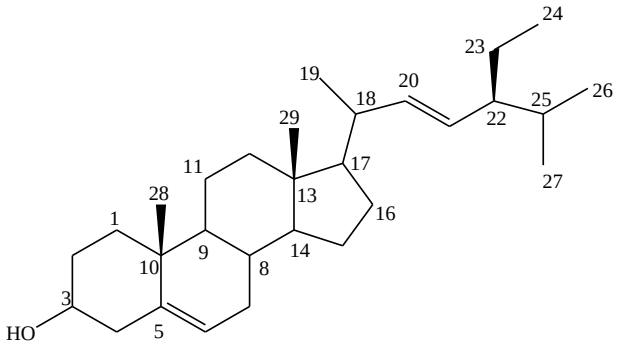


Fig 2: The proposed chemical structures compound MOST-1 (or Stigmasterol, 15)

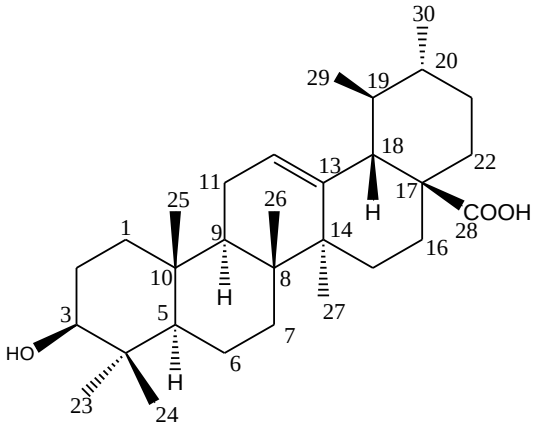


Fig 3: The proposed structure of compound MOST-2 (or Ursolic acid**)**

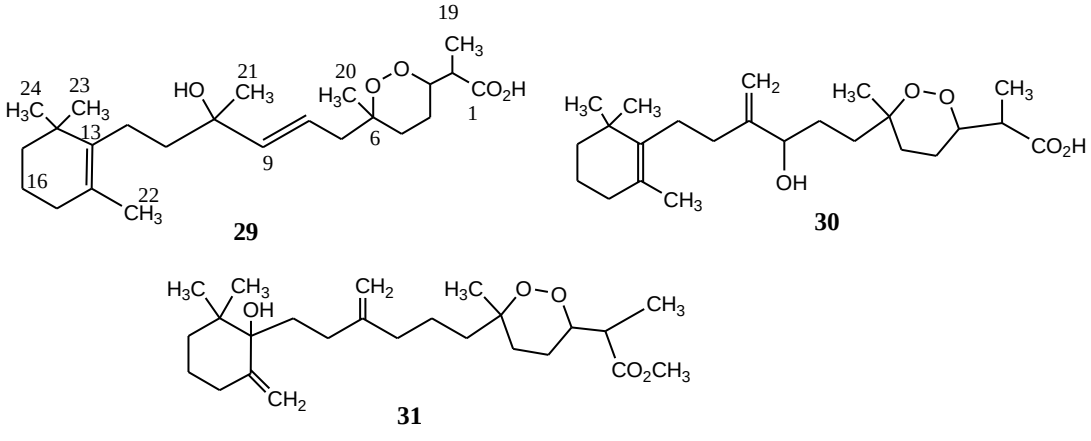


Fig 4: The proposed chemical structures of Tasnemoxide class of compounds

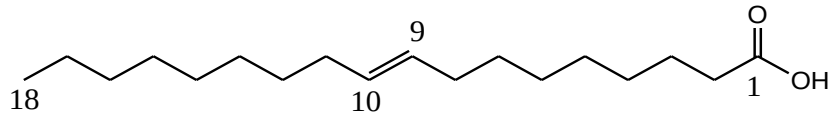


Fig 5: The proposed chemical structure of compound MOST-5 (or oleic acid)

Table 1: ^{1}H-NMR data of compound MOST-1 along with reported ^{1}H-NMR data of Stigmasterol [14-16]

H	^{1}H-NMR data of MOST-1 (ppm)	Reported ^{1}H-NMR data of Stigmasterol (ppm)
H-3	3.54	3.52
H-6	5.36	5.34
Me-18	0.69	0.69
Me-19	1.02	1.01
Me-21	0.94	1.02
Me-26	0.86	0.79
Me-27	0.82	0.85
Me-29	0.71	0.80
H-20	5.01	
H-21	5.16	

Table 2: ^{13}C-NMR and DEPT-135 spectral data for compound MOST-1 and literature reported data of Stigmasterol [14-16]

C	^{13}C-NMR of compound MOST-1 (ppm)	DEPT-135 of compound MOST-1 (ppm)	Literature reported ^{13}C-NMR data of Stigmasterol	Nature of carbon
1	37.26	37.26	37.3	CH_2
2	31.62	31.62	31.6	CH_2
3	71.78	71.78	71.8	CH
4	42.32	42.26	42.3	CH_2
5	140.75	-	140.8	C
6	121.71	121.71	121.7	CH
7	31.90	31.90	31.9	CH_2
8	31.62	31.62	31.9	CH
9	50.13	51.25	51.2	CH
10	36.51	-	36.5	C
11	20.23	20.49	21.1	CH_2
12	39.78	39.78	39.7	CH_2
13	42.32	-	42.3	C
14	56.77	56.77	56.9	CH
15	24.31	24.31	24.4	CH_2
16	28.26	28.26	28.4	CH_2
17	56.06	56.06	56.1	CH
18	11.87	11.87	11	CH_3
19	21.24	21.24	21.2	CH_3
20	39.78	40.53	40.5	CH
21	21.24	21.09	21.2	CH_3
22	138.34	138.34	138.3	CH
23	129.26	129.27	129.3	CH
24	50.13	50.13	51.2	CH
25	31.90	31.92	31.9	CH_2
26	21.09	19.41	21.2	CH_3
27	20.23	20.26	19	CH_3
28	25.43	25.43	25.4	CH
29	12.27	11.99	12.1	CH_3

Table 3: ^{1}H-NMR ($CDCl_3$, 400MHz) data of compound MOST-2 with the reported data of Ursolic acid [20, 22]

^{1}H	^{1}H-NMR data of MOST-2 (ppm)	^{1}H-NMR data of Ursolic acid reported
H-3	3.49	3.49
H-12	5.35	5.52
H-8	2.34	2.68
H-23	1.28	1.27
H-24	0.92	0.91
H-29	1.04	1.03
H-25	1.61	1.08
H-26	1.50	1.05
H-27	1.25	1.25
H-29	1.01	1.03
H-30	0.89	0.98

Table 4: The ^{13}C-NMR and DEPT-135 data of compound MOST-2, and reported ^{13}C-NMR data of Ursolic acid [20, 22]

Carbon	^{13}C-NMR data of compound MOST-2	DEPT-135 data of compound MOST-2	Reported ^{13}C-NMR data of Ursolic acid	Nature of carbon
1	39.77	39.77	39.8	CH_2
2	29.09	29.27	27.8	CH_2
3	76.73	75.74	79.7	CH
4	39.68	-	40.0	C
5	56.06	56.76	56.7	CH
6	19.37	19.37	19.5	CH_2
7	34.08	34.08	34.3	CH_2
8	39.77	-	40.8	C
9	50.13	50.13	49.2	CH_2
10	37.24	37.24	38.1	CH
11	24.29	23.05	24.0	CH_2
12	121.70	121.70	126.9	CH
13	140.68	-	139.6	C
14	42.31	-	43.2	C
15	29.72	29.72	28.8	CH_2
16	25.62	24.73	25.3	CH_2
17	50.13	-	48.6	C
18	56.76	56.76	54.4	CH
19	40.53	39.07	40.4	CH
20	39.77	39.68	40.4	CH
21	29.72	29.72	31.8	CH_2
22	38.82	38.82	38.1	CH_2
23	29.49	29.47	29.2	CH_3
24	18.77	18.77	16.4	CH_3
25	18.23	18.23	16.0	CH_3
26	18.68	18.68	17.7	CH_3
27	24.29	23.05	24.3	CH_3
28	179.41	-	181.7	Carbonyl carbon
29	18.23	18.97	17.8	CH_3
30	21.08	21.11	21.6	CH_3

Table 5: ^{1}H-NMR data of compound **MOST-3** and literature reported ^{1}H-NMR data of Tasnemoxide C [24].

^{1}H	^{1}H-NMR data of MOST-3 (ppm)	^{1}H-NMR reported data of Tasnemoxide C
H-1	-	-
H-2	2.66 (m)	2.65 (m)
H-3	4.19 (m)	4.09 (m)
H-4	1.65 (m)	1.70 (m)
H-5	1.63 (m)	1.63 (m)
H-6	-	-
H-7	1.65 (m)	1.62 (m)
H-8	1.52 (m)	1.52 (m)
H-9	2.02 (m)	2.00 (m)
H-10	-	-
H-11	2.04 (m)	2.03 (m)
H-12	1.51(m)	1.57 (m)
H-13	-	-
H-14	-	-
H-15	2.35 (m), 2.20 (m)	2.32 (m), 1.95 (m)
H-16	2.37 (m), 1.85 (m)	2.34 (m), 1.98 (m)
H-17	1.33 (m)	1.52 (m)
H-18	-	-
H-19	1.51 (m)	1.52 (m)
H-20	1.27 (d)	1.23 (d)
H-21	5.16 (s), 5.14 (s)	5.03 (s), 5.01 (s)
H-22	4.98 (s)	4.97 (s)
H-23	0.86 (s)	0.97 (s)
H-24	0.83 (s)	0.87 (s)
OCH_3	3.55 (s)	3.68 (s)

Table 6: ^{13}C-NMR and DEPT-135 data of compound MOST-3 and reported ^{13}C-NMR data of Tasnemoxide C [24].

Carbon	^{13}C-NMR data of compound MOST-3 (ppm)	DEPT-135 data of compound MOST-3 (ppm)	Reported ^{13}C-NMR of of Tasnemoxide C	Nature of carbon
1	178.68	-	174.2	C
2	42.27	42.09	42.9	CH

3	81.41	81.65	81.3	CH
4	23.07	22.71	23.4	CH_3
5	31.91	31.63	30.1	CH_2
6	76.70	-	79.7	C
7	39.78	39.77	39.4	CH_2
8	24.31	24.31	24.0	CH_2
9	27.23	26.05	26.2	CH_2
10	155.04	-	155.5	C
11	31.63	31.63	31.1	CH_2
12	24.71	24.75	24.8	CH_2
13	91.73	-	90.1	C
14	141.56	-	147.8	C
15	31.91	33.87	34.0	CH_2
16	19.41	19.03	19.0	CH_3
17	39.78	39.67	39.9	CH_2
18	42.33	-	42.7	C
19	14.13	14.13	13.5	CH_3
20	22.71	22.71	20.9	CH_3
21	121.75	114.28	114.9	CH
22	107.01	108.30	108.7	CH
23	23.07	24.31	22.7	CH_3
24	22.71	22.71	22.0	CH_3
OCH_3	51.0		51.8	(CH_3)

Table 7: ^{1}H-NMR data of compound MOST-5 and reported ^{1}H-NMR data oleic acid [7].

H	^{1}H-NMR data of compound MOST-5 (ppm)	^{1}H-NMR data reported for of oleic acid
H-1	-	-
H-2	2.35	2.34
H-3	1.63	1.63
H-4	1.28	1.29
H-5	1.28	1.29
H-6	1.61	1.29
H-7	1.32	1.32
H-8	2.03	2.01
H-9	5.35	5.34
H-10	5.37	5.36
H-11	2.04	2.01
H-12	1.65	1.29
H-13	1.67	1.29
H-14	1.69	1.29
H-15	1.69	1.32
H-16	0.92	1.32
H-17	0.90	1.27
H-18	0.88	0.88

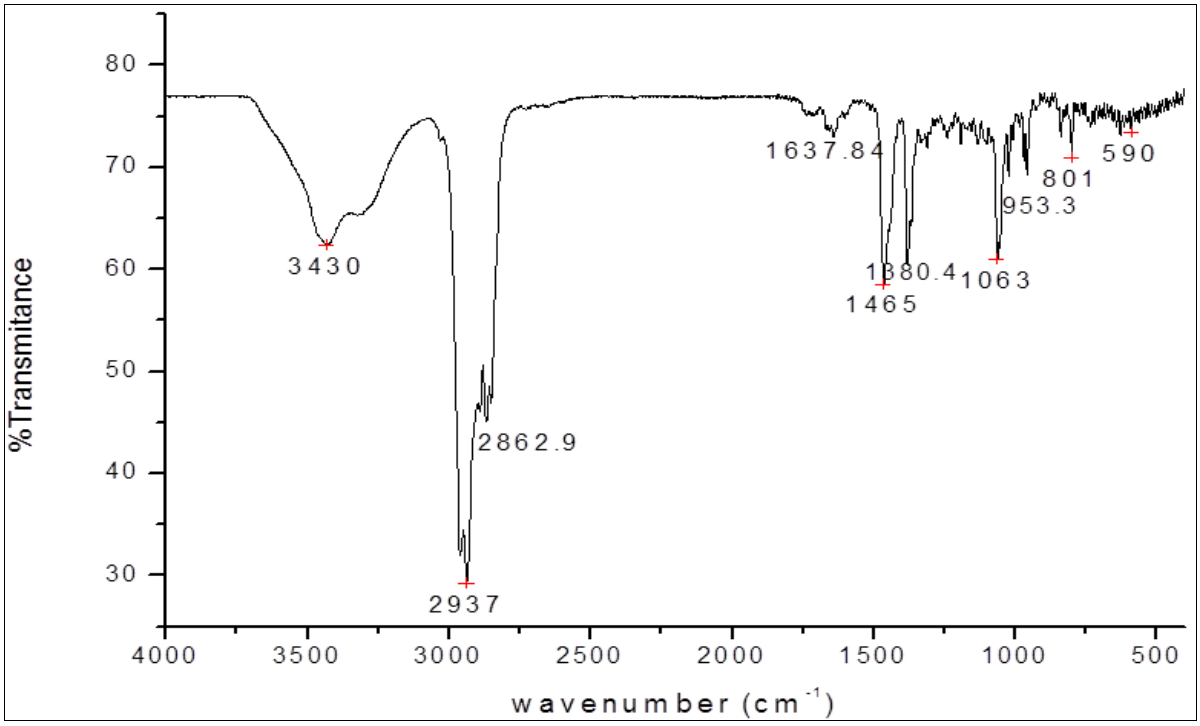
Table 8: The ^{13}C-NMR and DEPT-135 data of compound MOST-5 and that of Oleic acid [7, 37]

Carbon	^{13}C-NMR data of compound MOST-5 (ppm)	DEPT-135 data of compound MOST-5 (ppm)	Reported ^{13}C-NMR data of Oleic acid (ppm)	The reported DEPT-135 data of Oleic acid	Nature of Carbon
1	179.84	-	180.5	180.5	C=O
2	34.01	34.01	34.12	34.1	CH_2
3	24.69	24.69	24.66	24.68	CH_2
4	29.25	29.15	29.14	29.1	CH_2
5	29.33	29.07	29.07	29.0	CH_2
6	29.44	29.45	29.05	29.44	CH_2
7	29.60	29.68	29.68	29.6	CH_2
8	27.16	27.16	27.16	27.1	CH_2
9	129.74	129.74	129.7	129.7	CH
10	130.04	130.04	130.0	130.0	CH
11	27.22	27.16	27.22	27.2	CH_2
12	29.53	29.60	29.78	29.706	CH_2
13	29.37	29.33	29.33	29.338	CH_2
14	29.44	29.37	29.53	29.536	CH_2
15	29.25	29.25	29.33	29.256	CH_2
16	31.94	31.94	31.92	31.9	CH_2
17	22.70	22.70	22.68	22.7	CH_2
18	14.13	14.13	14.07	14.1	CH_3

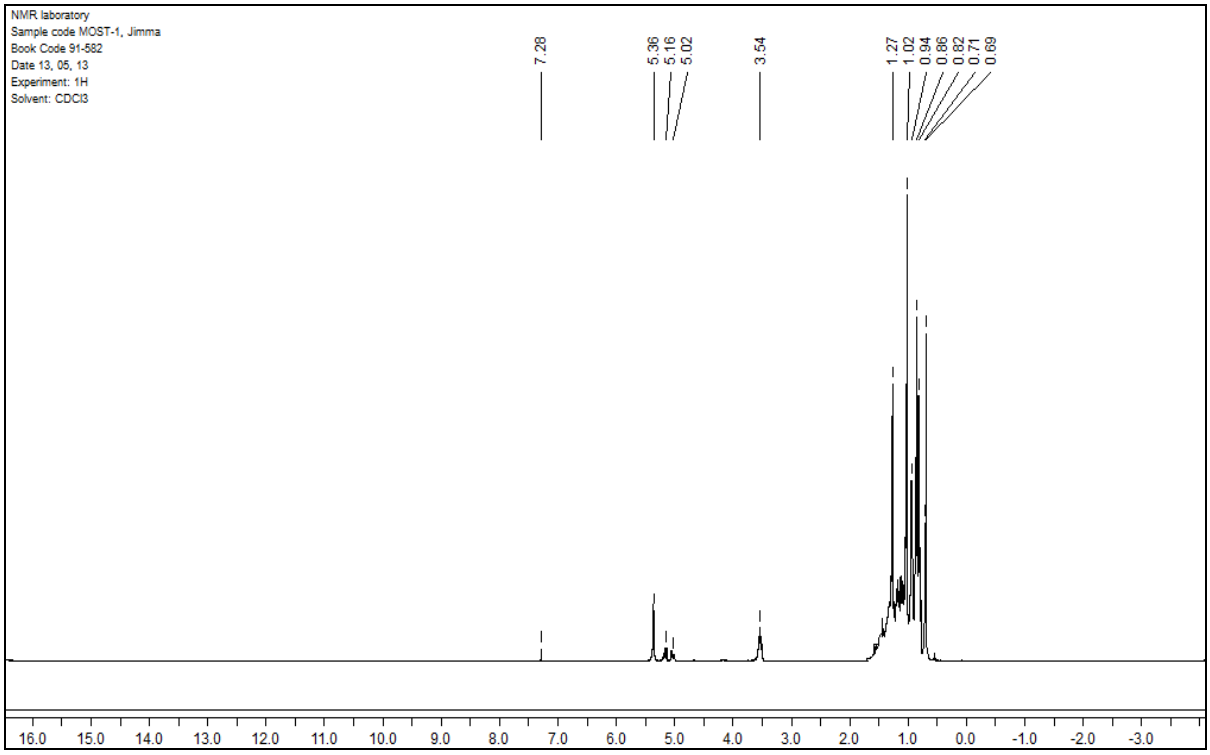
Table 9: The Antibacterial activity test results (growth inhibition zone in mm) of the isolated compounds against the four bacterial strains used in the study

Bacterial strains used	Growth inhibition zone (mm)					
	Compound MOST-1	**Compound MOST-2**	Compound **MOST-3**	**Compound MOST-5**	**Ciprofloxacin**[#]	**DMSO**[##]
E. coli	16	12	10	15	25	-
P. aeruginosa	10	9	9	15	26	-
S. thyphimurium	14	12	8	14	26	-
S. aureus	8	11	12	16	25	-

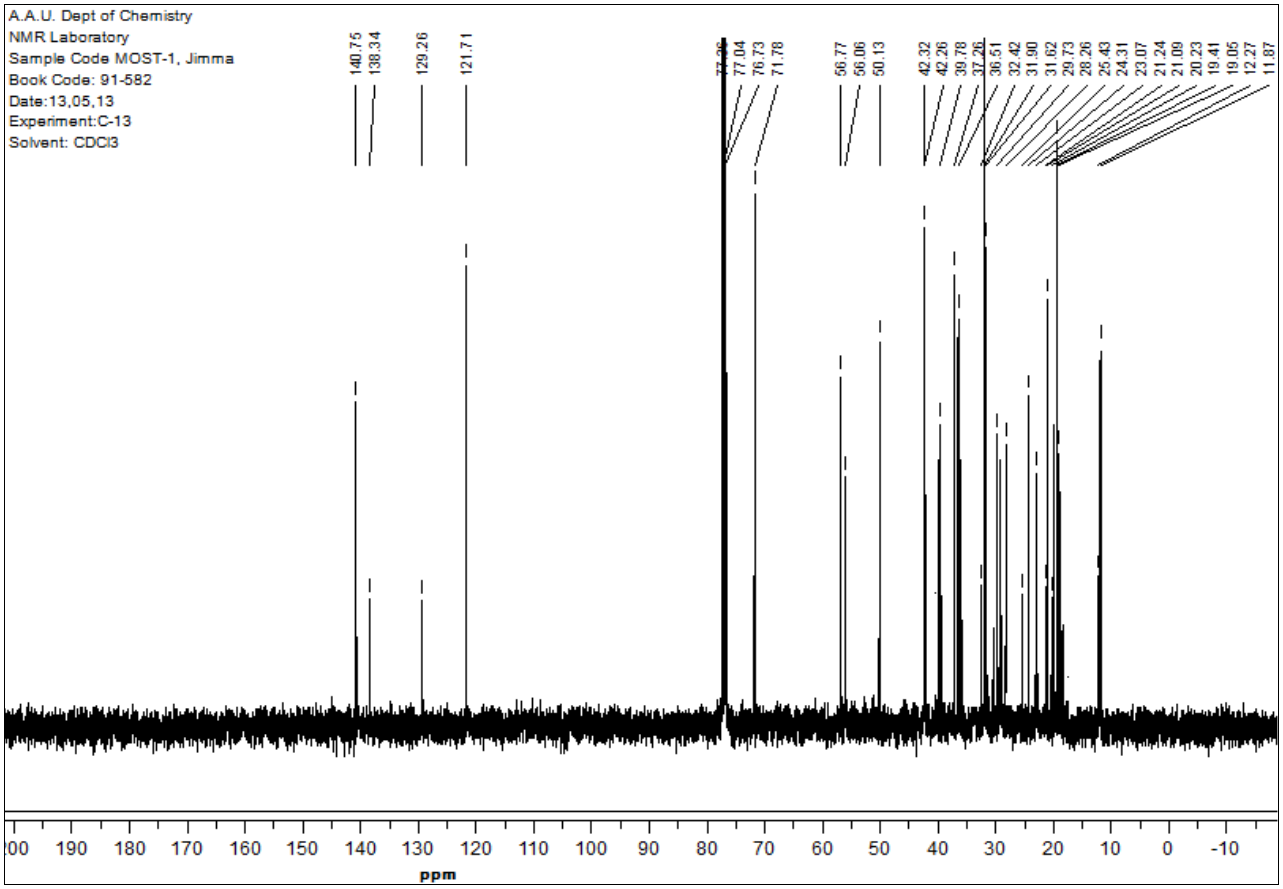
[#] = positive control; [##] = Negative control



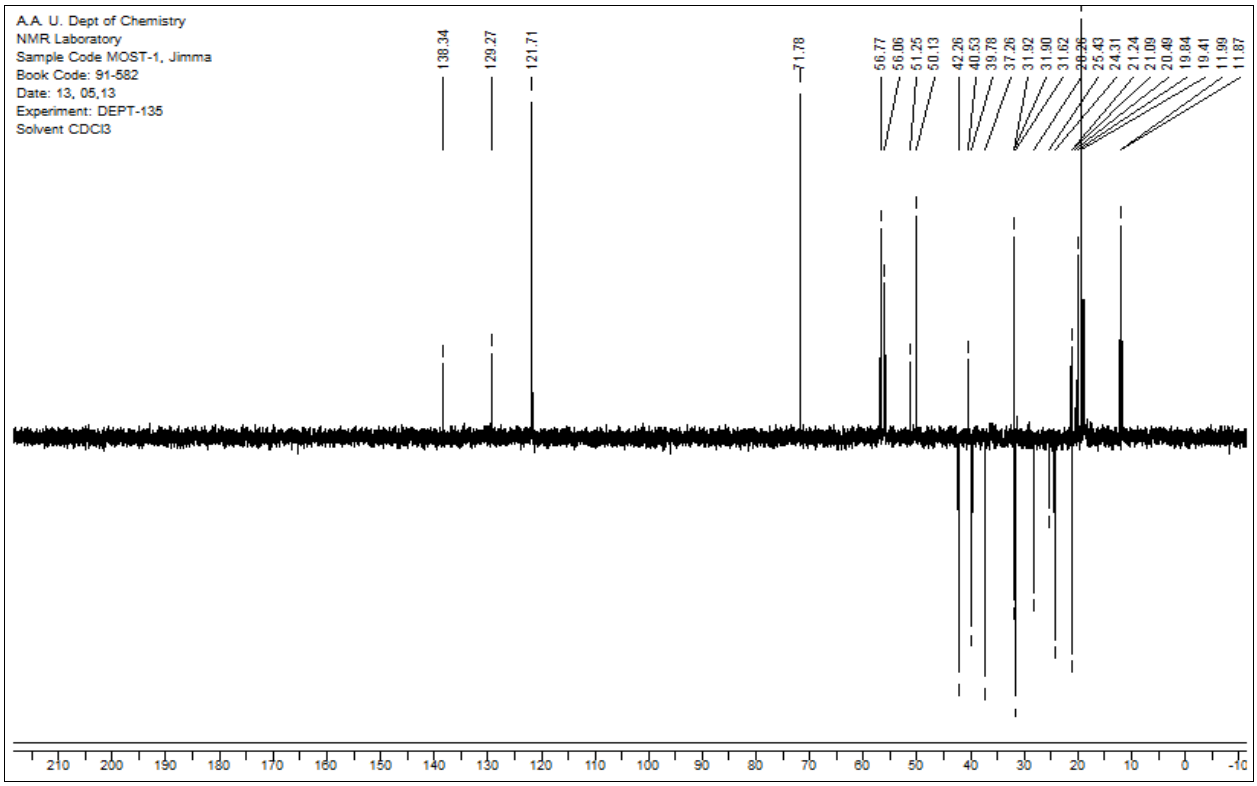
Supplementary material 1: The IR spectrum of compound MOST-1



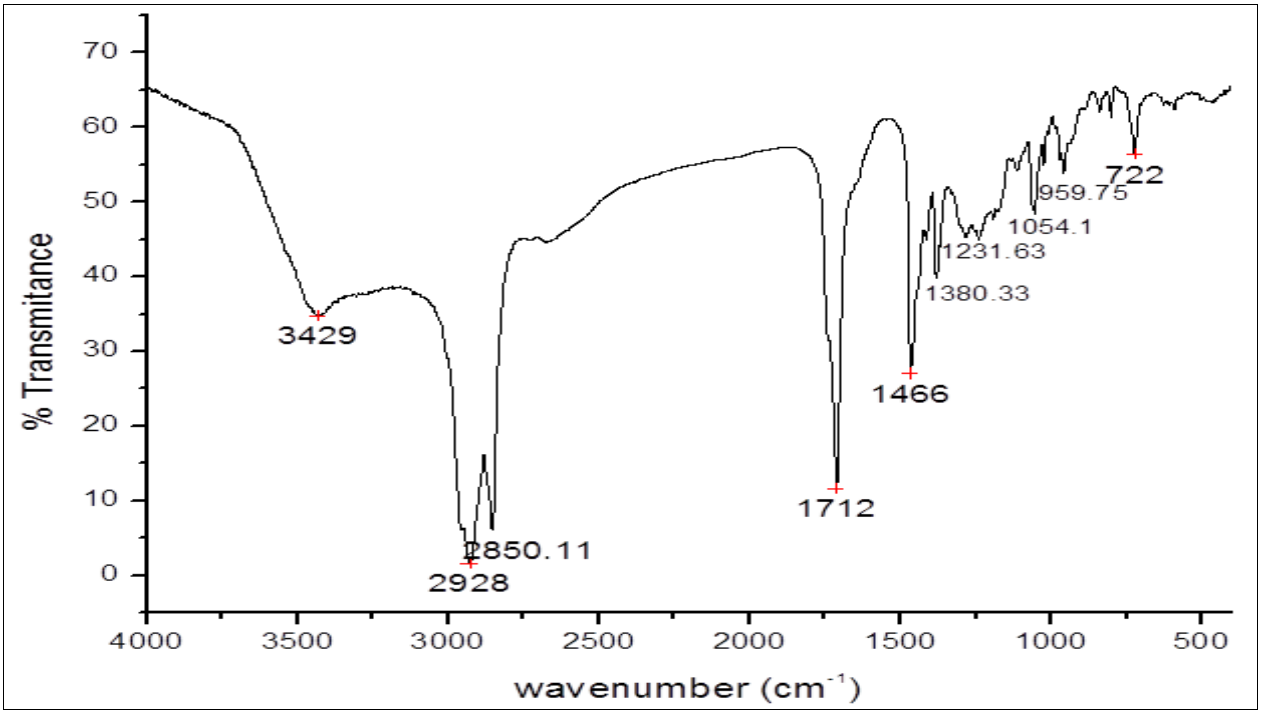
Supplementary material 2: The [1]H-NMR spectrum of compound MOST-1



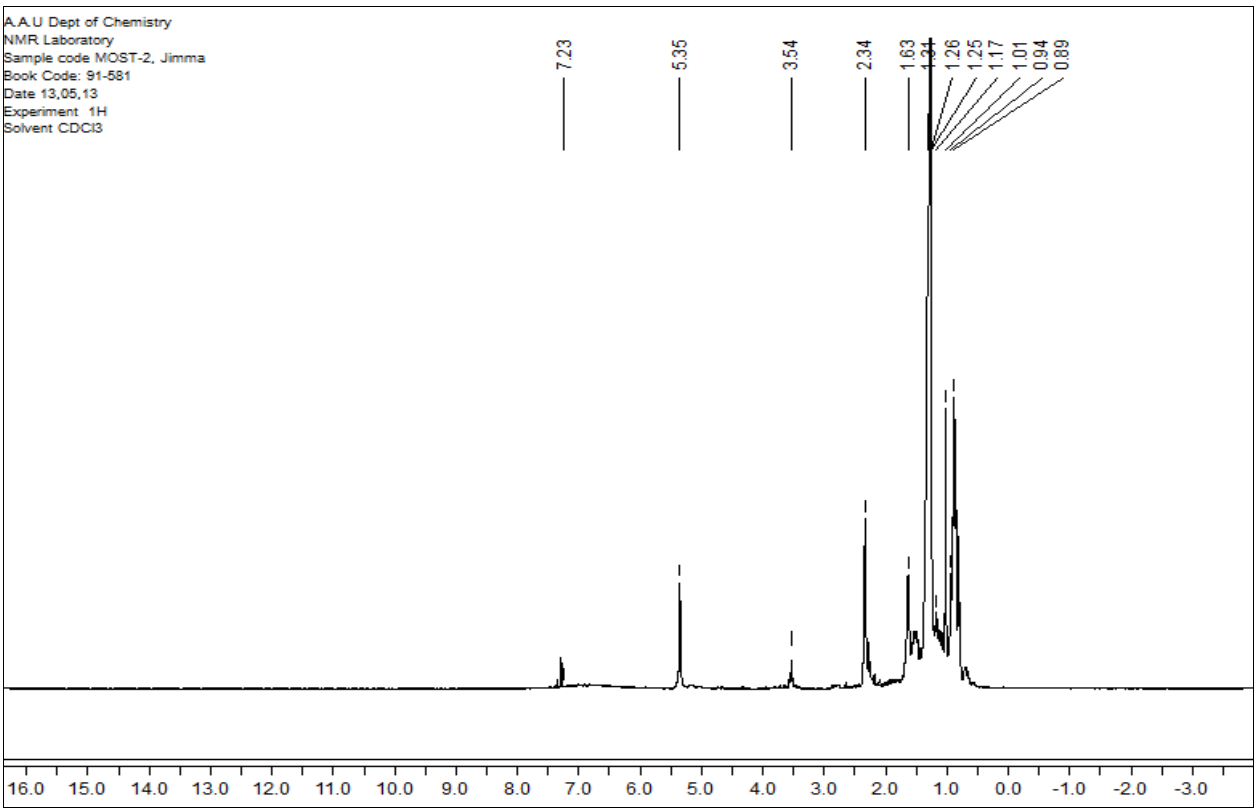
Supplementary material 3: The ^{13}C-NMR spectrum of compound MOST-1



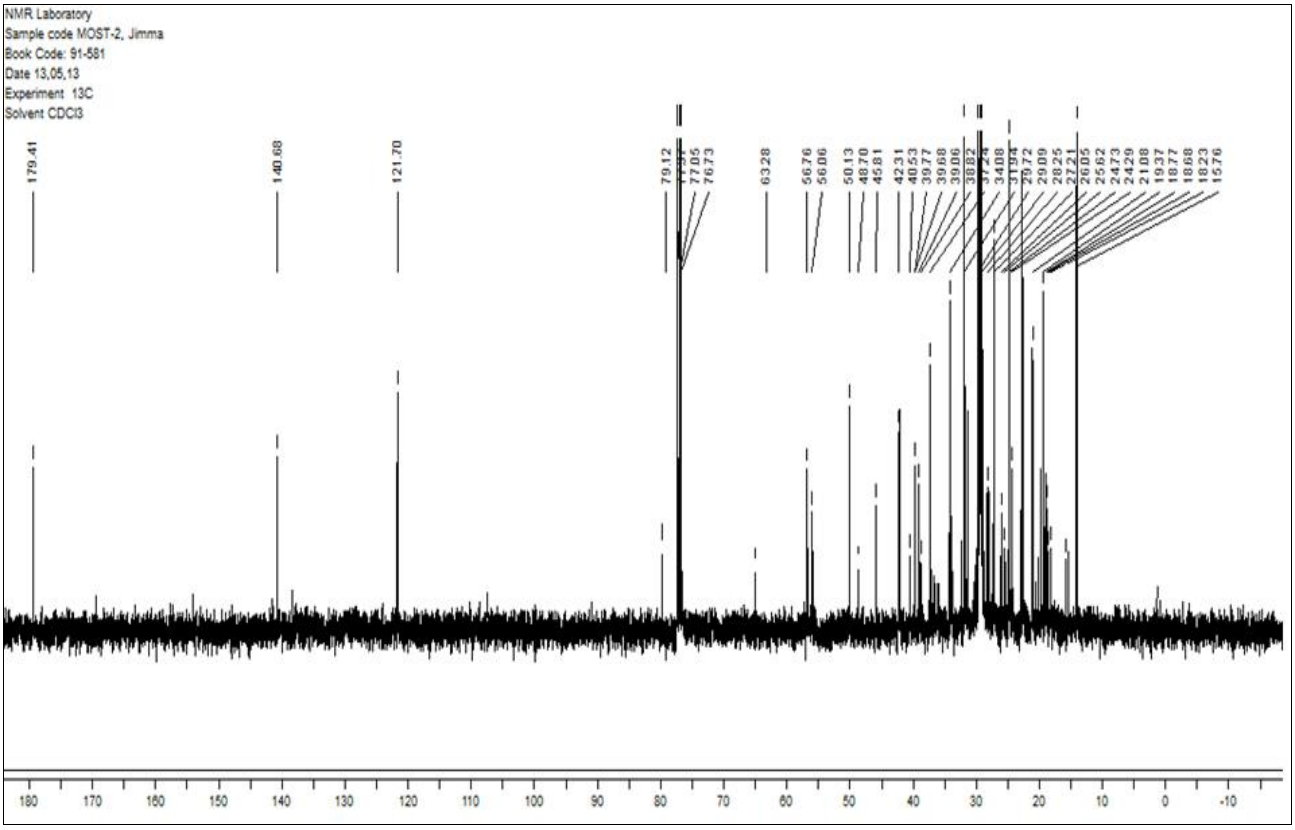
Supplementary material 4: The DEPT-135 spectrum of compound MOST-1



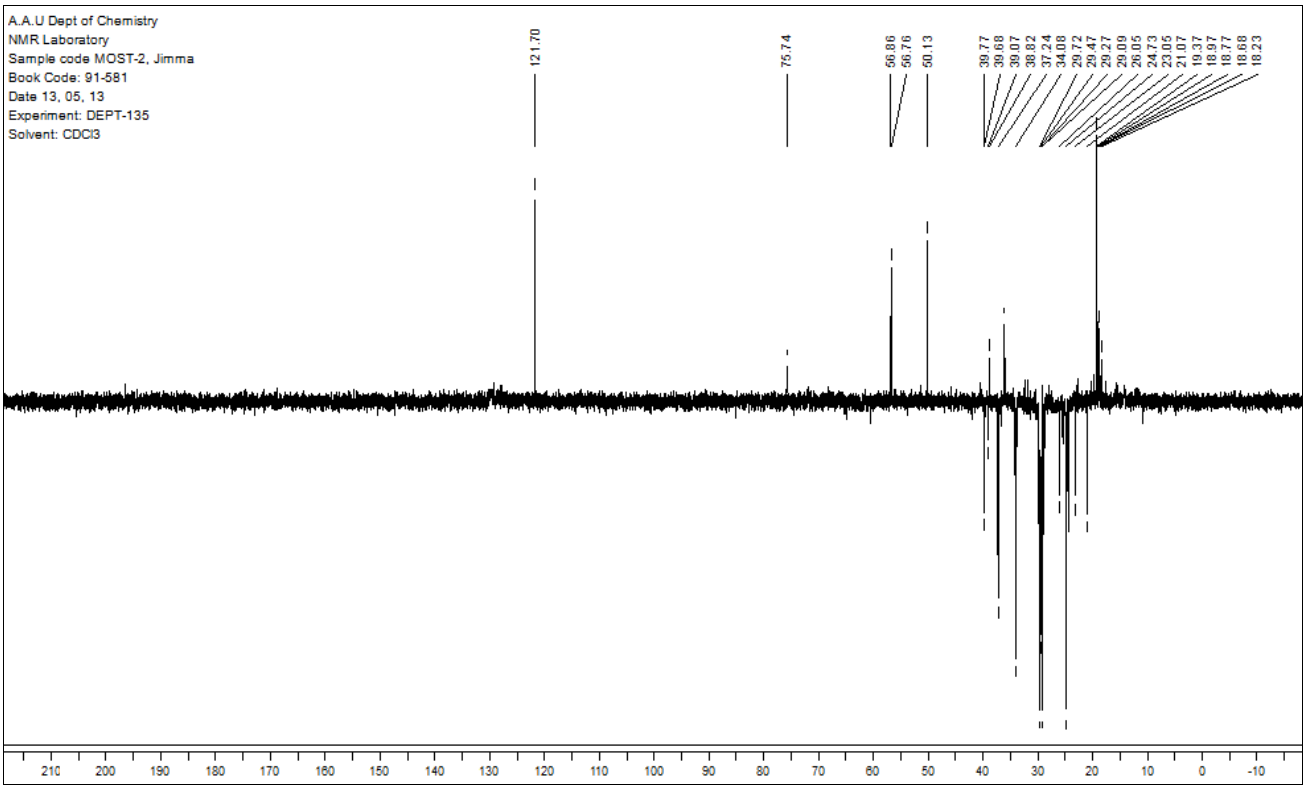
Supplementary material 5: The IR spectrum of compound MOST-2



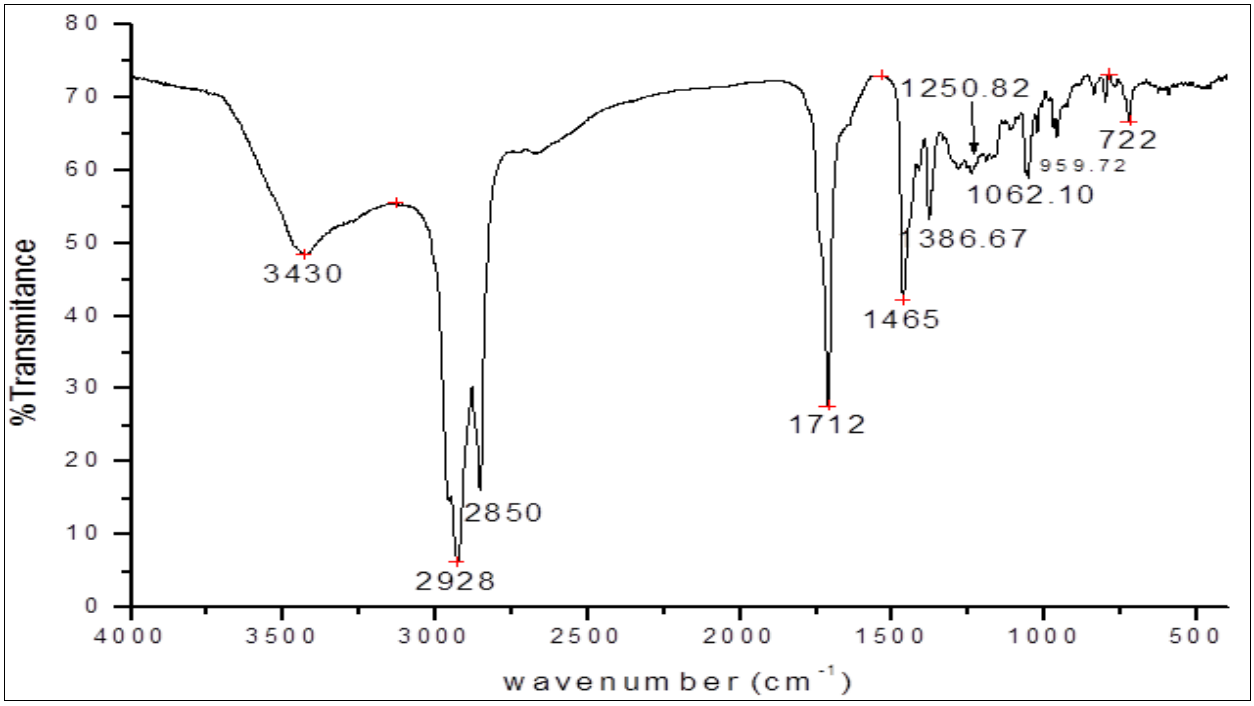
Supplementary material 6: The ^{1}H-NMR spectrum of compound MOST-2



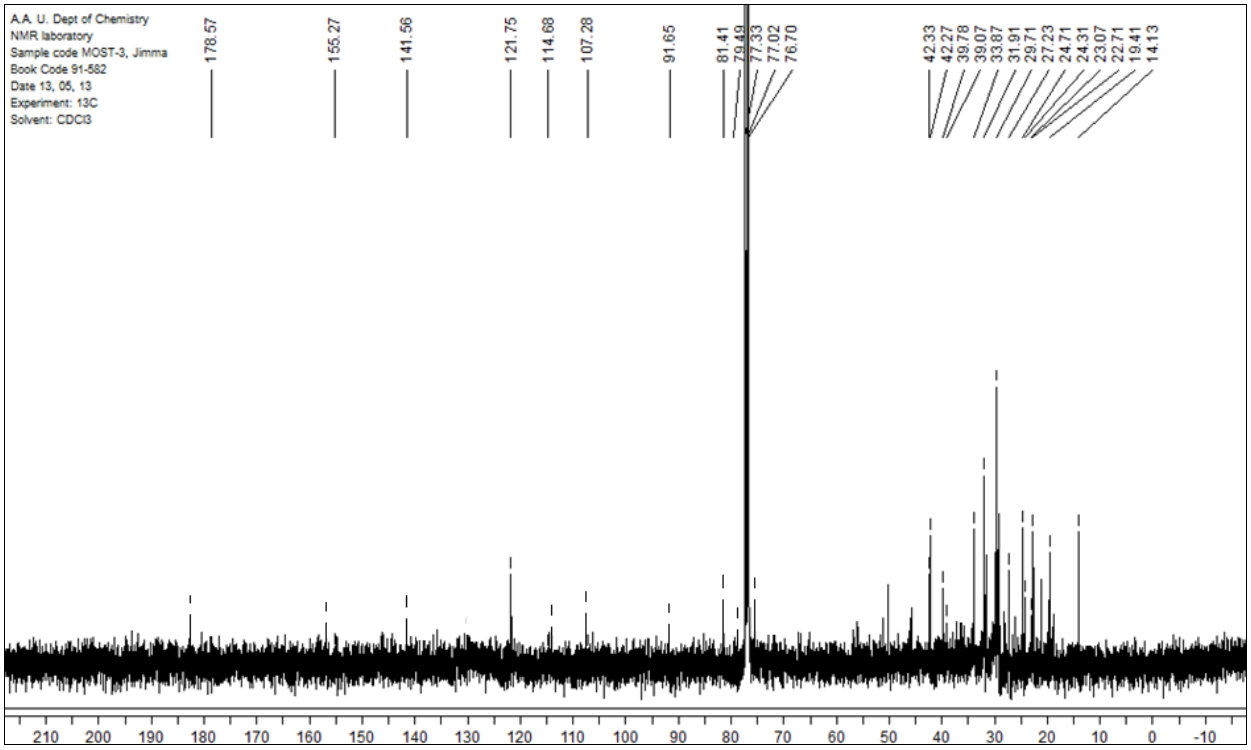
Supplementary material 7: The ^{13}C-NMR spectrum of compound MOST-2



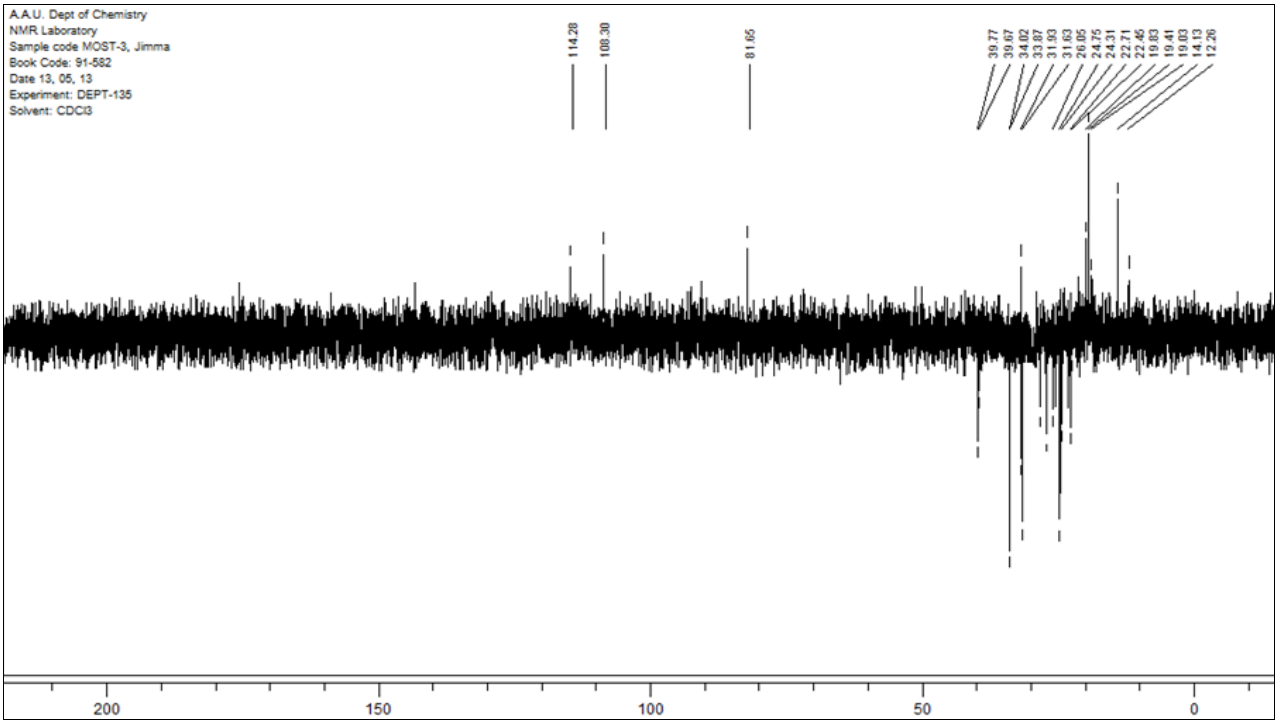
Supplementary material 8: The DEPT-135 spectrum of compound MOST-2



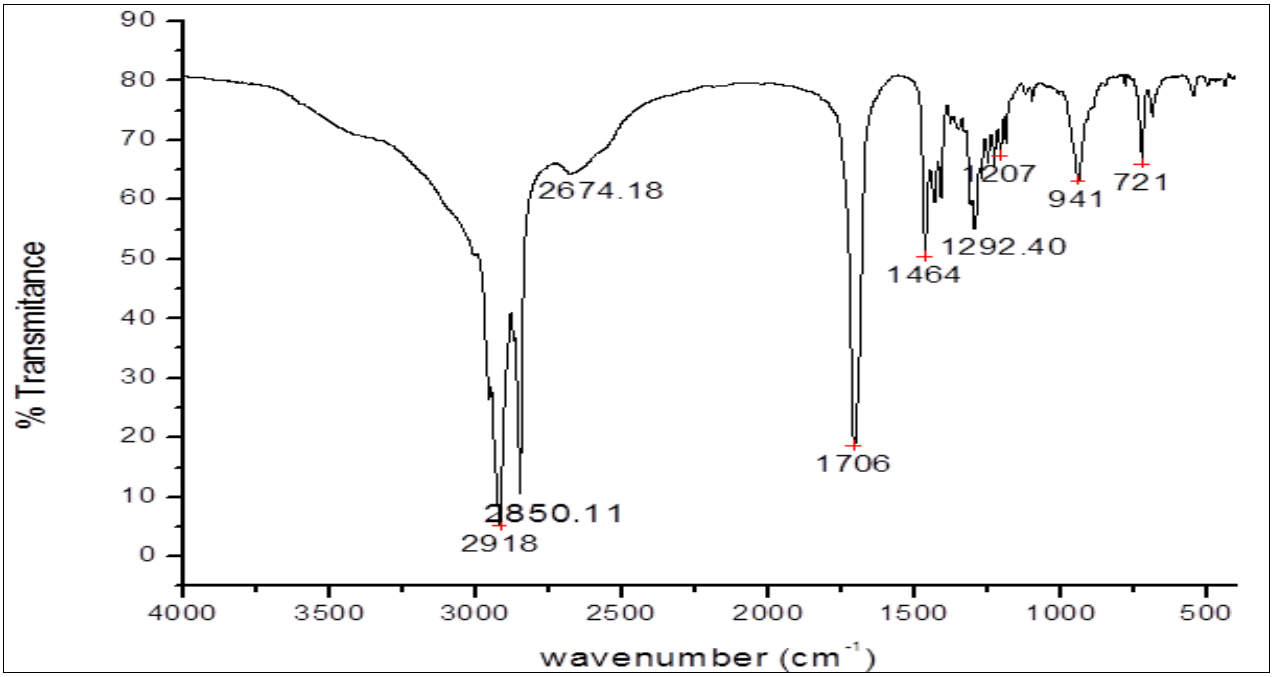
Supplementary material 9: The IR spectrum of compound MOST-3



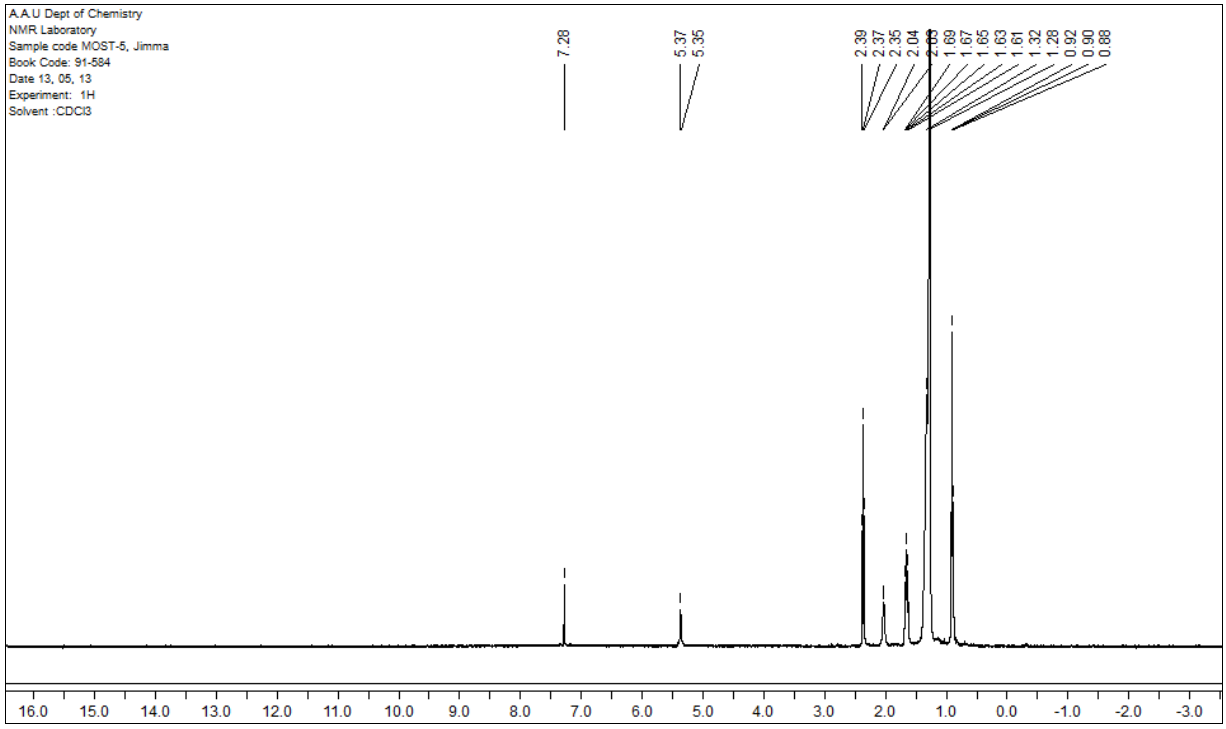
Supplementary material 10: The ^{13}C-NMR spectrum of compound MOST-3



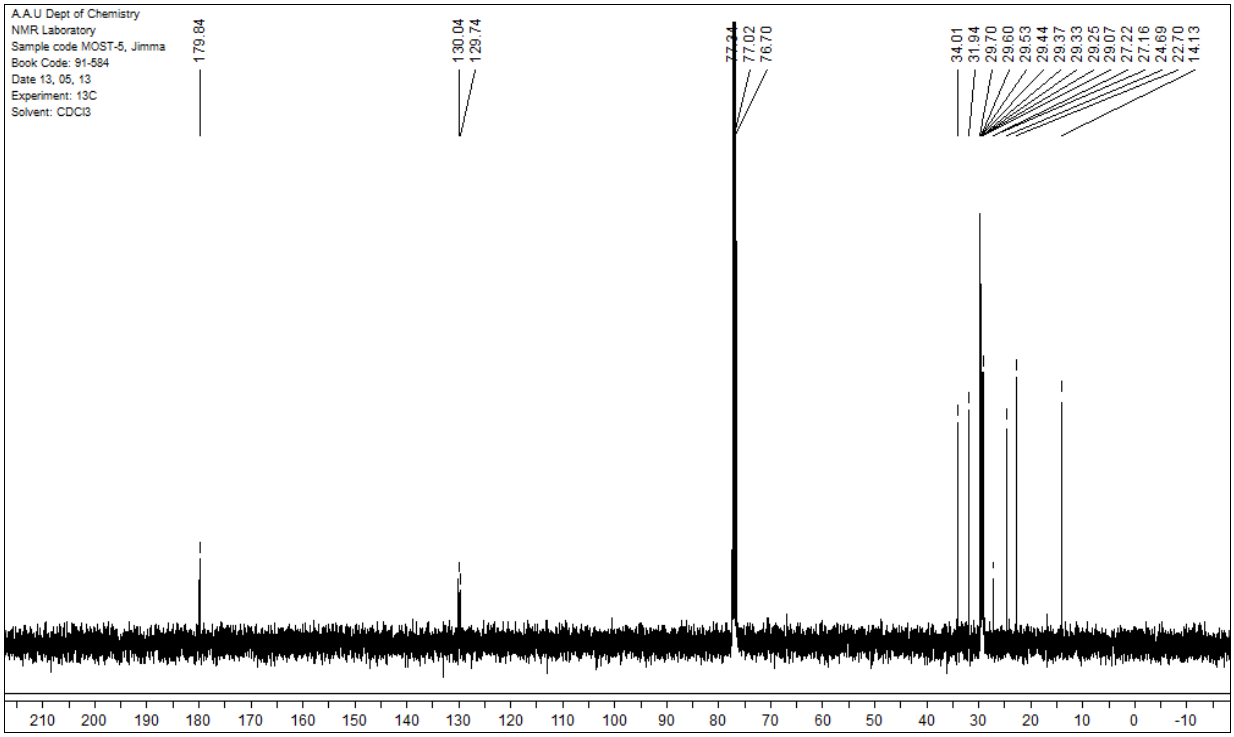
Supplementary material 11: The DEPT-135 spectrum of compound MOST-3



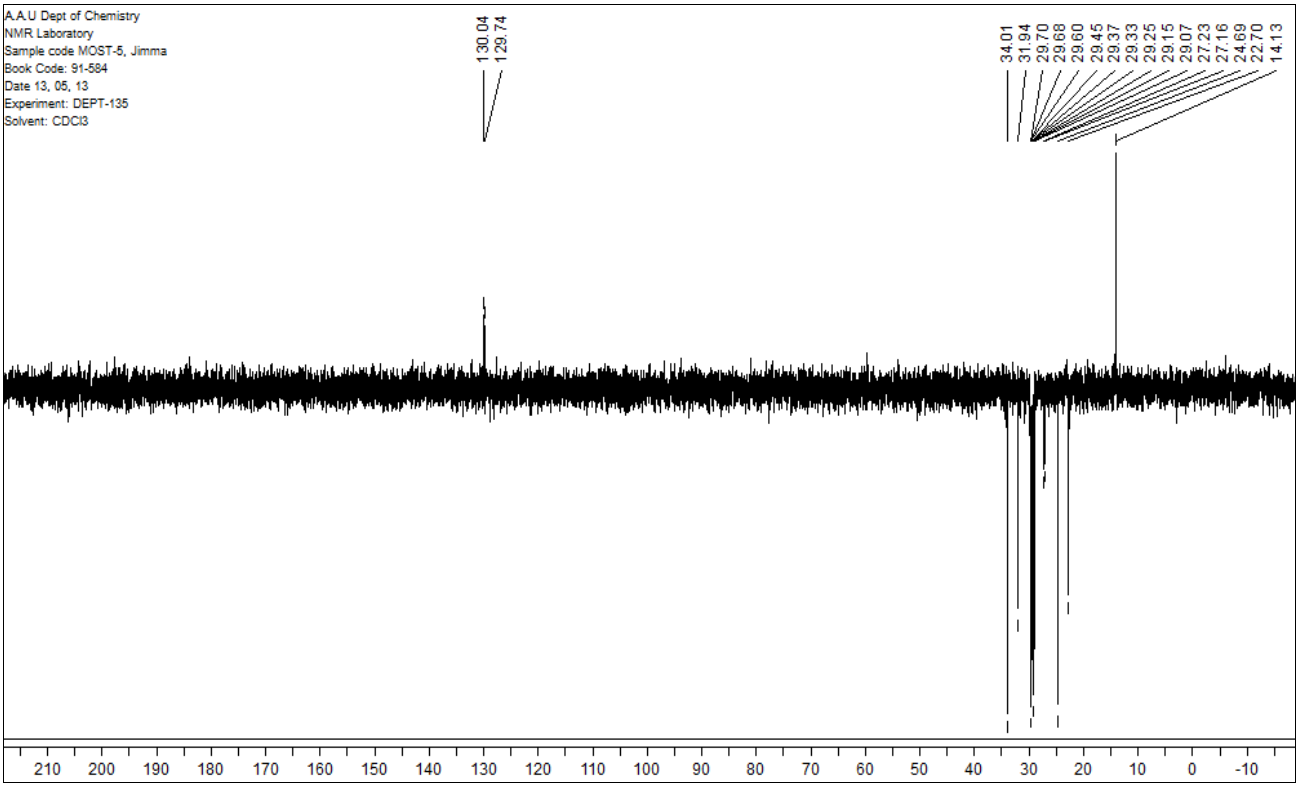
Supplementary material 12: IR spectra of compound MOST-5



Supplementary material 13: The ^{1}H-NMR spectrum of compound MOST-5



Supplementary material 14: The ^{13}C-NMR spectrum of compound MOST-5



Supplementary material 15: The DEPT-135 spectrum of compound MOST-5.

Acknowledgement
The authors would like to acknowledge Jimma University, Ethiopia, for material and financial support to carry out the research (in 2012/2013 Academic Year), and The Department of Biology, College of Natural Sciences, Jimma University, Ethiopia.

Conflicts of Interest
The authors declare no conflict of interest.

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