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Research Article
Phytochemical Screening of Root Extract of *Momordica boivinii* and Isolation of Two Steroids

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Abstract

Background and Objective: *Momordica boivinii* (*M. bovinii*) is one of the plant species widely used in traditional medicine in Sidama zone, Southern part of Ethiopia, for treatment of several human illnesses but their chemical constituents are not studied before. Therefore, the aim of the present study was to conduct phytochemical screening tests on extracts of the root of this plant species and also to isolate compounds from the extracts. **Materials and Methods:** The root material of *M. boivinii* was extracted with four solvent systems [n-hexane, dichloromethane, dichloromethane/methanol (50:50% by volume) and methanol] using maceration technique and sequential extraction approach. All the extracts were subjected to phytochemical screening tests following standard procedures reported in literatures. Finally, the dichloromethane/methanol (50:50% by volume) extract was subjected to column chromatographic separation. **Results:** The yields of the extracts were 1.1, 1.8, 7.5 and 5.4 g for n-hexane, dichloromethane, dichloromethane/methanol (50:50% by volume) and methanol, respectively. The preliminary phytochemical analysis revealed the presence of alkaloids, saponins, flavonoids, phenols, steroids, glycosides, terpenoids and tannins in the crude ectracts of dichloromethane and dichloromethane:methanol (50:50 by volume). The crude extract of n-hexane showed the presence of alkaloids, saponins, steroids, glycosides and terpenoids. However, flavonoids, phenols and tannins were not detected. On the other hand, the crude extract of methanol revealed the presence of secondary metabolites listed above except tannins. Column chromatographic separation of the dichloromethane/methanol (50:50% by volume) extract afforded two compounds (labeled as compound MB-1 and MBC-1). The structures of the compounds were elucidated to be stigmasterol and methyl ester of betulinic acid-3-trans-caffeate based spectroscopic (IR, ^{1}H-NMR and ^{13}C-NMR) data and comparison with literature reports. This is the first report of isolation of the compounds from *M. boivinii*. **Conclusion:** The findings of the study validate the use of the plant in traditional medicine. However, biological activity test is recommended to get comprehensive information about the potential of the plant as source of modern drugs.

Key words: *Momordica boivinii*, sequential extraction, phytochemical screening, steroids, stigmasterol, betulinic acid-3-trans-caffeate

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The genus *Momordica* consists of approximately 825 species of annual and perennial plants that are distributed in tropical areas such as East Africa, Caribbean and Southern America[1]. The species in the genus of *Momordica* are widely used to treat several alignments in different parts of the world due to the presence of variety of phytoconstituents. Different plant parts (leaves, roots and fruits) of the species are used in traditional medicinal uses in areas where these parts are available in abundance. For instance, leaf decoction has been used as anti-fungal, anti-inflammatory, anti-malarial, anti-parasitic, anti-septic, anti-tumor, antidotal, antipyretic tonic, appetizing, antibilious, carminatives, digestive stimulant, febrifuge, menstrual stimulator, blood circulation, immunity, control fever, blood impurities, liver diseases, skin ailments, vermifuge, purgative, colic, topically for sores, wounds, infections, for worms and parasites; as an antiviral for measles, hepatitis, laxative and in addition, it has been effectively used to treat diabetes and cancer[2-7].

One of the *Momordica* species that is widely found in East Africa is *Momordica boivinii* (*M. bovinii*) (Fig. 1). It is distributed in Botswana, Ethiopia, Kenya, KwaZulu-Natal, Malawi, Mozambique, Namibia, Northern Provinces, Somalia, Swaziland, Tanzania, Uganda, Zambia and Zimbabwe[8]. Though there are no scientific research reports (studies), it is well known that its different parts are widely used in traditional medicines for treatment of human diseases in countries where the plant is available. Its roots and leaves are used by Midzichenda tribes (Kenya) to treat spiritual ailments[9]. A report showed that stems and leaves of *M. boivinii* are used in the treatment of stomach problem[10]. Taking the plant with *Cissus faucicola* and other herbs has also been reported to be used in the treatment of oestrogen sufferings[11]. Solution of aqueous extract of this plant has also been reported to show anti-HIV activity[12]. In Ethiopia, the plant is also used in traditional medicine. For instance, its leaf and fruit are used to treat pneumonia[13]. Fresh roots and leaves are reported to be effective in the treatment of gonorrhea and intestinal parasites[14]. Its leaves is mixed with vegetables used as an appetite stimulant; fruit juice of *M. boivinii* mixed with *Allium sativum* (locally known as nech shunchurt) and chewing of its leaves also used to treat diabetes, diarrhoea, skin problem, hypertension, menstrual problems, abdominal pain, intestinal pain, abortion, breast cancer and anthrax (personal communication with healers).

A number of researchers reported biological activities of crude extracts from different parts of *Momordica* species[13,15-22] as well as isolation of pure compounds[23-34]. Moreover, the biological/pharmacological properties of the different parts of these species are attributed to the presence of phytochemicals such as flavonoids, saponins, terpenoids, coumarins, alkaloids, proteins, cardiac glycosides, anthraquinones, anthocyanins, steroids and phenols in its leaves and fruits[7,29,30,35-42]. To the best of authors knowledge, there are no reports on phytochemical study and isolation of compounds from roots and other parts of *M. boivinii.* Therefore, the aim of the present study was to carry out phytochemical screening on root extract of *M. boivinii* so as to detect its chemical composition and perhaps substantiate the traditional use of the plant. The phytochemical screening tests were carried out following standard procedures reported in literature.

MATERIALS AND METHODS

Chemicals: The n-hexane, dichloromethane and methanol were used for gradient extraction; ethyl acetate and n-hexane were used for column elution and pre-coated Thin Layer Chromatography (TLC) (silica gel, UV254) plates were

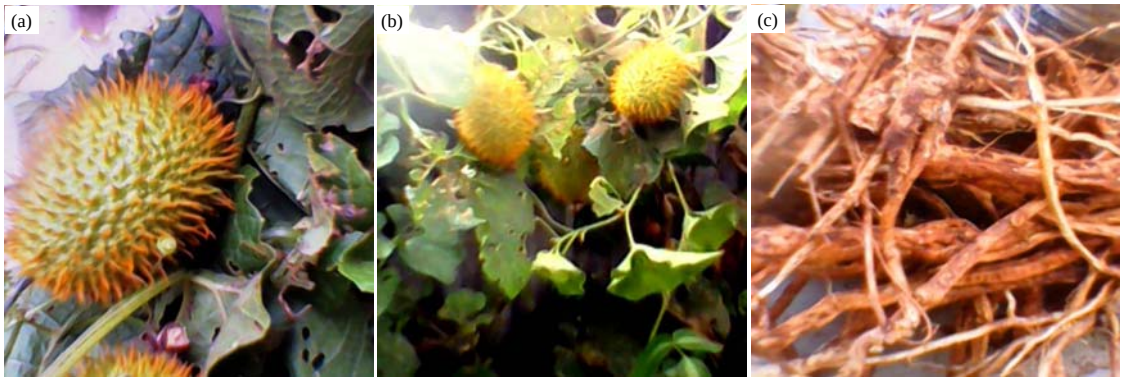


Fig. 1(a-c): Aerial part of *M. boivinii*
Photo by Filippo T., 29 September, 2016

used for chromatographic analyses. The reagents such as Dragendorff's, dilute sodium hydroxide, ferric chloride, concentrated hydrochloric acid, concentrated sulphuric acid glacial acetic acid, acetic anhydride and chloroform were used to determine phytochemicals in the crude extracts. $CDCl_3$ was used as solvent for spectroscopic analysis. The chemicals used in this study were all of analytical grades and were purchased from Ranchem Co. Ltd. Agents in Addis Ababa, Ethiopia.

Equipments: Rotary evaporator (Heidolph, UK) for concentration of crude extracts, Grant (GLS 400) thermostatic bath shaker (for maceration of plant materials) was used. Oven (model N50L, GENLAB, WIDNES, ENGLAND), Analytical Balance ADAM (AFP-110L), UV chamber (Uvitec), ^{1}H-NMR, ^{13}C-NMR and DEPT-135 were recorded using Bruker 400 MHz spectrometer for characterization of the isolated compounds. Infrared (IR) spectra were obtained from Perkin Elmer BX infrared spectrometer (400-4000 cm^{-1}).

Collection of plant material: The root of *M. boivinii* was collected in August 29, 2016 from Dale district, Sidama Zone, SNNPR region, Ethiopia. The area is about 317 km far from Addis Ababa (in Southern direction on the highway to Kenya). It is also 42 km from Hawassa University in the same direction. The plant (species) was authenticated by botanist Reta Regassa, Department of Biology, Hawassa College of Teachers' Education, Ethiopia and was given voucher number of MB/0034.

Preparation of plant specimen: The collected plant material (root) was chopped to small pieces and air-dried for 30 days without exposing to sun light and was then milled to suitable size for extraction using mortar and pestle.

Extraction: The roots of *M. boivinii* (500 g) were extracted sequentially using n-hexane, dichloromethane, dichloromethane/methanol (50:50% by volume) and methanol and maceration techniques. The mixture was subjected to continuous shaking for 48 h. The solution was filtered using Whatmann No.1 filter paper and the residual solvent in each gradient extract was removed using rotary evaporator under reduced pressure. The crude extracts of each solvent was dried, weighed and stored refrigerator till further analyses (phytochemical screening and chromatographic isolation). The percent yields of the extracts were calculated using the Eq.:

$$\text{Percentage yield} = \frac{\text{Mass of the extract}}{\text{Mass of the plant material used for extraction}} \times 100$$

Phytochemical screening tests: Phytochemical screening tests were carried out on the crude extract of n-hexane, dichloromethane, dichloromethane/methanol (50:50% by volume) and methanol using standard procedures reported in literature[43-46] to detect the presence of some secondary metabolites namely steroids, terpenoids, saponins, flavonoids, tannins, alkaloids, phenols and glycosides.

Isolation and characterization of compounds: Among the above four crude extracts, the crude extract of dichloromethane/methanol (50:50% by volume) showed the best TLC profile. Then, 6.5 g of dichloromethane/methanol (50:50% by volume) extract was adsorbed onto silica gel (20 g) and subjected to column chromatographic isolation. The column was then eluted with n-hexane:ethyl acetate solvent system (with gradual increase in polarity). A total of 71 fractions (each 30 mL) were collected. The collected fractions were concentrated using rotary evaporator. The spots on the TLC plates were visualized using UV light (at 254 and 365 nm) followed by iodine vapor. Fractions were tested using TLC and those with the same TLC profiles were combined. The column chromatographic separation led to isolation of two compounds (compound MB-1 and MBC-1). The structural elucidations of the compounds were carried out based on data obtained from spectroscopic (UV, IR and NMR) data. All the spectroscopic analyses were done at the Department of Chemistry, Addis Ababa University, Ethiopia.

RESULTS AND DISCUSSION

All the crude extracts of *M. boivinii* were subjected to preliminary identification of phytoconstituents. The structural elucidation of the isolated compounds was performed based on the spectroscopic data (UV-Vis, FTIR and NMR) data and comparison with literature reports.

Masses of crude extracts: As discussed above, the extraction of the plant material (root) was carried out using different solvents (of different polarities) and in sequential extraction approach. It was started with n-hexane (least polar) and followed by dichloromethane and dichloromethane/methanol (50:50% by volume) (intermediate polarity) and methanol (the most polar). The amounts of extracts were 1.1, 1.8, 7.5 and 5.4 g for n-hexane, dichloromethane, dichloromethane/methanol (50:50% by volume) and methanol, respectively (Table 1). The resulted amount of crude extract of dichloromethane/methanol was found to be higher than the other crude extracts.

Phytochemical screening: Preliminary phytochemical screening tests were performed as per standard procedure, the various phytoconstituents on n-hexane, dichloromethane, dichloromethane/methanol (50:50% by volume) and methanol root extracts of *M. boivinii* following standard report. The results of the tests are presented in Table 2. Three secondary metabolites (flavonoids, phenols and tannins) were not detected in n-hexane extract. Tannins were also not detected in methanol extract. The other two extracts (dichloromethane extract and dichloromethane/ methanol (50:50% by volume extract) were found to contain all the secondary metabolites tested in the present study (Table 2).

Secondary metabolites are known to elicit several biological/pharmacological activities such as antiulcer, anti-inflammatory, antioxidant, cytotoxic, antitumor and antidepressant activities (e.g., phenols)[47], antimicrobial, cytotoxicity, anti-inflammatory, antitumor, oestrogenic, anti-allergic, antioxidant and vascular activities (e.g., flavonoids)[47,48], astringents, against diarrhoea, as diuretics, against stomach and duodenal tumors and as anti-inflammatory, antiseptic, antioxidant and haemostatic pharmaceuticals (e.g., tannins)[47,49], antihypertensive effects antiarrhythmic effect, antimalarial activity and anticancer actions (e.g., alkaloids)[47,50], anticarcinogenic, antimalarial, anti-ulcer, hepaticidal, antimicrobial or diuretic activity and the sesquiterpenoid antimalarial drug artemisinin and anticancer (e.g., terpenoids and steroids)[50,51]. There are also reports that show antimicrobial activities of extracts of *Momoridca* species. For instance, extracts obtained from different parts of *Momordica* species such as *M. charantia* have been found to show antimicrobial[35,52-55], antioxidant[52,56,57] and antidiabetic[58,59] activities. These biological/ pharmacological activities believed to be due to the above secondary metabolites[25,60]. In the present study, the aforementioned secondary metabolites were detected in the root extracts of *M. boivinii*. This supports medicinal uses of the roots and also suggests the potentials of the different parts of the plant to be rich sources of compounds that could be used as drug candidates. However, further biological activity test is needed on the root extracts and also on the extracts from other parts of the plant as such studies may lead to drug discovery and development.

Isolation and characterization of compounds: Two compounds (MB-1 and MBC-1) were isolated in the present study. The structures of these compounds are proposed based on the spectral data and comparison with literature reports.

Structural elucidation of compound MB-1: Compound MB-1 was obtained as a white amorphous solid (73.4 mg). Its R_f value was determined to be 0.4 in n-hexane:ethyl acetate (80:20%). Analysis of its IR spectrum (Appendix 1) revealed a broad band at 3435 cm^{-1} indicating the presence of hydroxyl functional group. The strong band at 2924 cm^{-1} represents C-H stretch of alkenes whereas the weak band around 2800 cm^{-1} could be attributed to C-H stretching of methyl groups. The band at 1631 cm^{-1} could be attributed to C=C bond. The observed data suggested that compound MB-1 could be an alcohol with at least one C=C double bond[18].

In the ^{1}H-NMR spectrum (Appendix 2) of compound MB-1, the singlet peaks at δ 0.75, 0.81, 0.86, 0.94, 1.01 and 1.27 (Table 3) indicate the presence of protons of six methyl (-CH_3) groups whereas the peaks at δ 5.20 and 5.40 indicate the presence of olefinic protons in compound MB-1. The patterns of peaks in the ^{13}C-NMR spectrum (Appendix 3) of compound MB-1 suggest that the compound could be stigmasterol-type compound. The peaks/signals at δ 140.80, 121.70, 139.60 and 117.30 could be assigned to C-5, C-6, C-22, C-23 double bonds, respectively, of stigmasterol-type compounds (Table 3). The

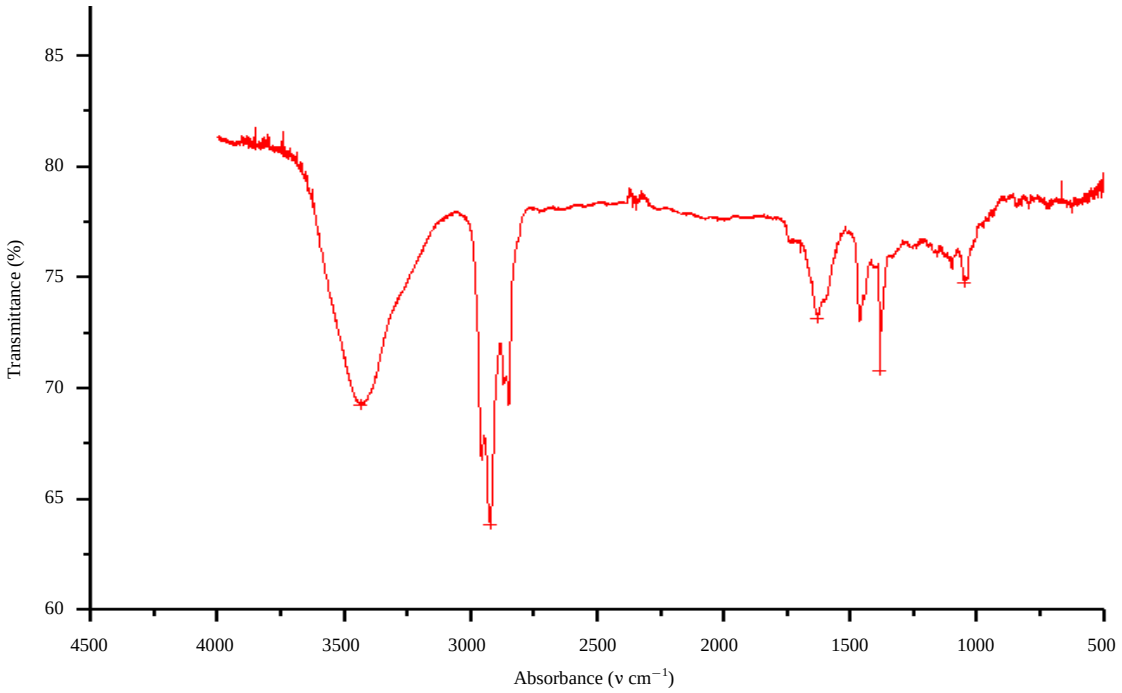
Table 1: Mass of extracted matter in each gradient extract

Solvent used for extraction	Mass of crude extract (g)	Yield (%)
n-hexane	1.1	0.22
Dichloromethane	1.8	0.32
Dichloromethane/methanol (50:50% by volume)	7.5	1.50
Methanol	5.4	1.08

Table 2: Phytochemical analyses results of *M. boivinii* root extract

Classes of phytochemicals	Extracts			
	n-hexane	Dichloromethane	Dichloromethane/methanol (50:50% by volume)	Methanol
Alkaloids	+	+	+	+
Saponins	+	+	+	+
Flavonoids	-	+	+	+
Phenols	-	+	+	+
Steroids	+	+	+	+
Glycosides	+	+	+	+
Terpenoids	+	+	+	+
Tannins	-	+	+	-

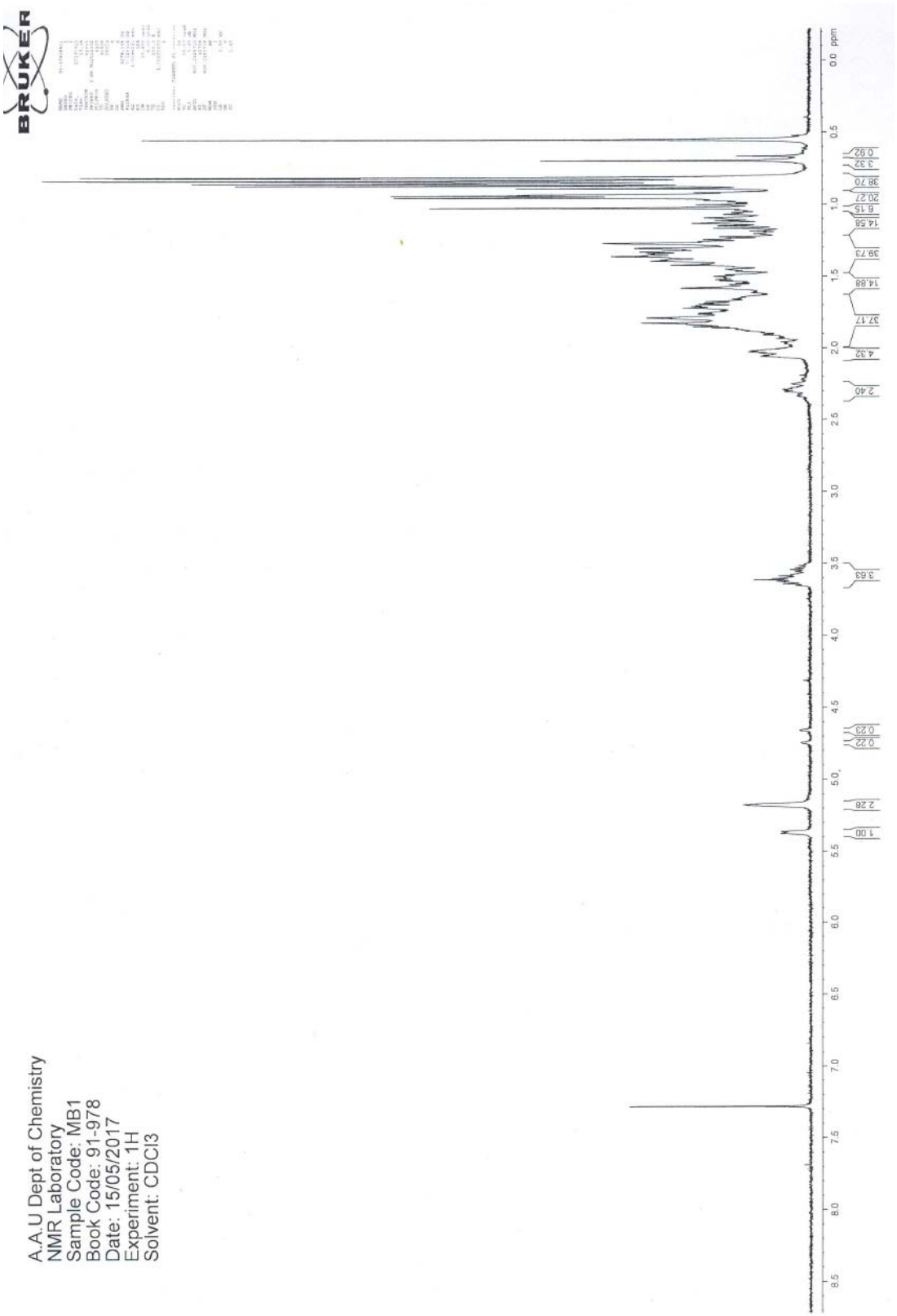
+: Present, -: Not detected



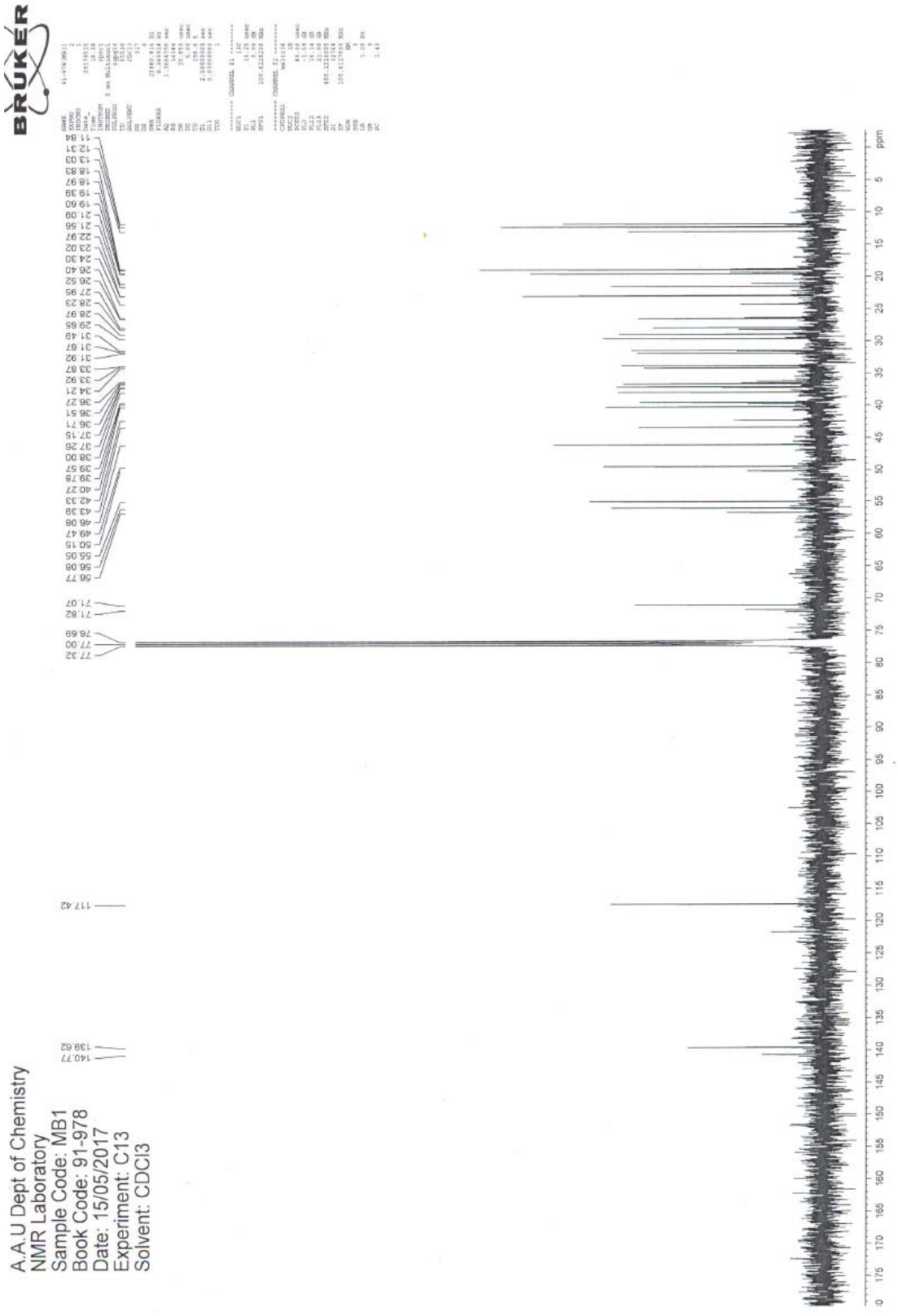
Appendix 1: IR spectrum of compound MB-1

Table 3: The ^{13}C-NMR and ^{1}H-NMR data of compound MB-1 and reported NMR data of stigmasterol[62-64]

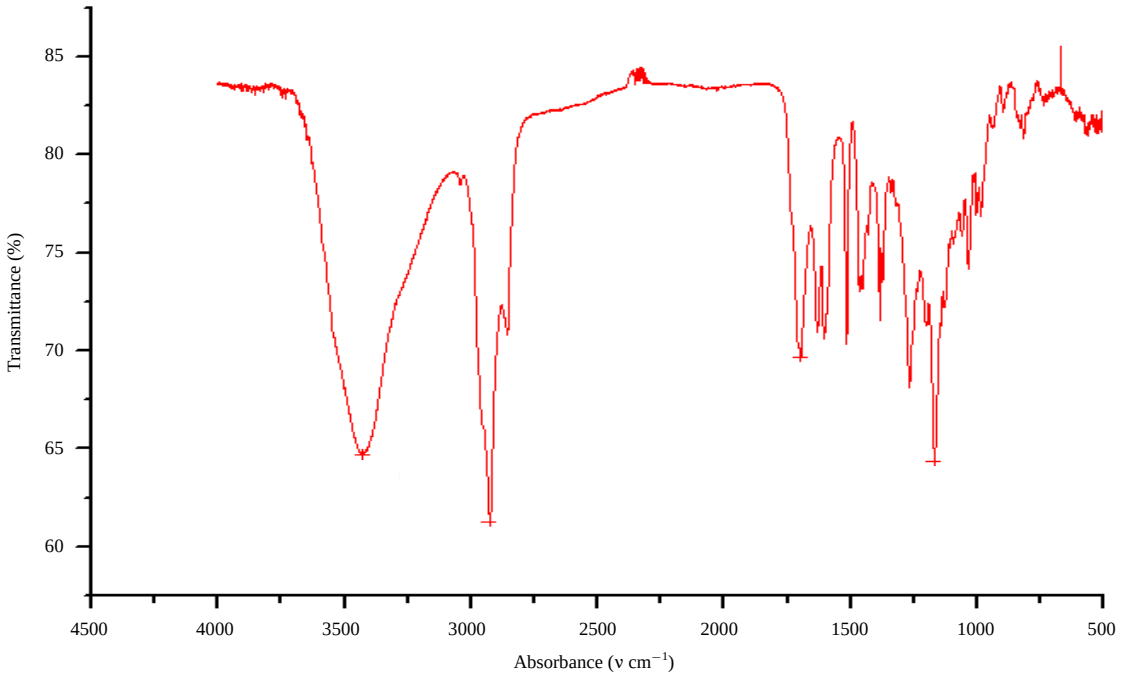
Carbon atoms	^{13}C-NMR data of compound MB-1	Reported ^{13}C-NMR data of stigmasterol	^{1}H-NMR data of compound MB-1	Reported ^{1}H-NMR data of stigmasterol	Nature of carbon
C-1	37.2	37.2			CH_2
C-2	31.7	31.6			CH_2
C-3	71.8	71.7	3.6 (tdd, 1H)	3.51(tdd, 1H)	CH
C-4	42.3	42.2			CH_2
C-5	140.8	140.8			C=C
C-6	121.7	126.6	5.40 (t, 1H)	5.31(t, 1H)	C=CH
C-7	31.5	31.6			CH_2
C-8	29.7	31.8			CH
C-9	50.2	50.0			CH
C-10	36.3	36.2			C
C-11	24.3	21.1			CH_2
C-12	39.6	39.6			CH_2
C-13	42.3	42.1			C
C-14	56.8	56.8			CH
C-15	24.3	24.3			CH_2
C-16	29.0	28.8			CH_2
C-17	56.1	55.8			CH
C-18	11.8	12.2	1.27 (d, 3H)	1.03 (s, 3H)	CH_3
C-19	21.6	19.9	0.75 (d, 3H)	0.71 (s, 3H)	CH_3
C-20	40.3	40.4-40.5			CH
C-21	22.0	21.0	1.01 (d, 3H)	0.91 (d, 3H)	CH_3
C-22	139.6	138.2	5.20 (m, 1H)	4.98 (m, 1H)	C=C
C-23	117.4	129.2-129.6	5.20 (m, 1H)	5.14 (m, 1H)	C=C
C-24	55.1	51.1-51.3			CH
C-25	31.7	31.9			CH
C-26	21.1	21.2	0.86 (d, 3H)	0.80 (d, 3H)	CH_3
C-27	19.4	19.1	0.94 (d, 3H)	0.82 (d, 3H)	CH_3
C-28	26.5	25.4-25.5			CH_2
C-29	12.3	12.3-25.3	0.81 (t, 3H)	0.83 (t, 3H)	CH_3



Appendix 2: ^{1}HNMR spectrum of compound MB-1 in $CDCl_3$



Appendix 3: ^{13}C-NMR spectrum of compound MB-1 in $CDCl_3$



Appendix 4: IR spectrum of compound MBC-1 in $CDCl_3$

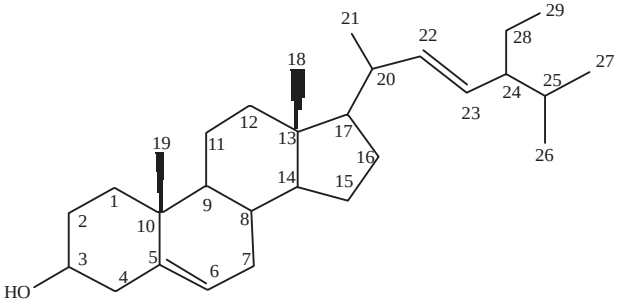
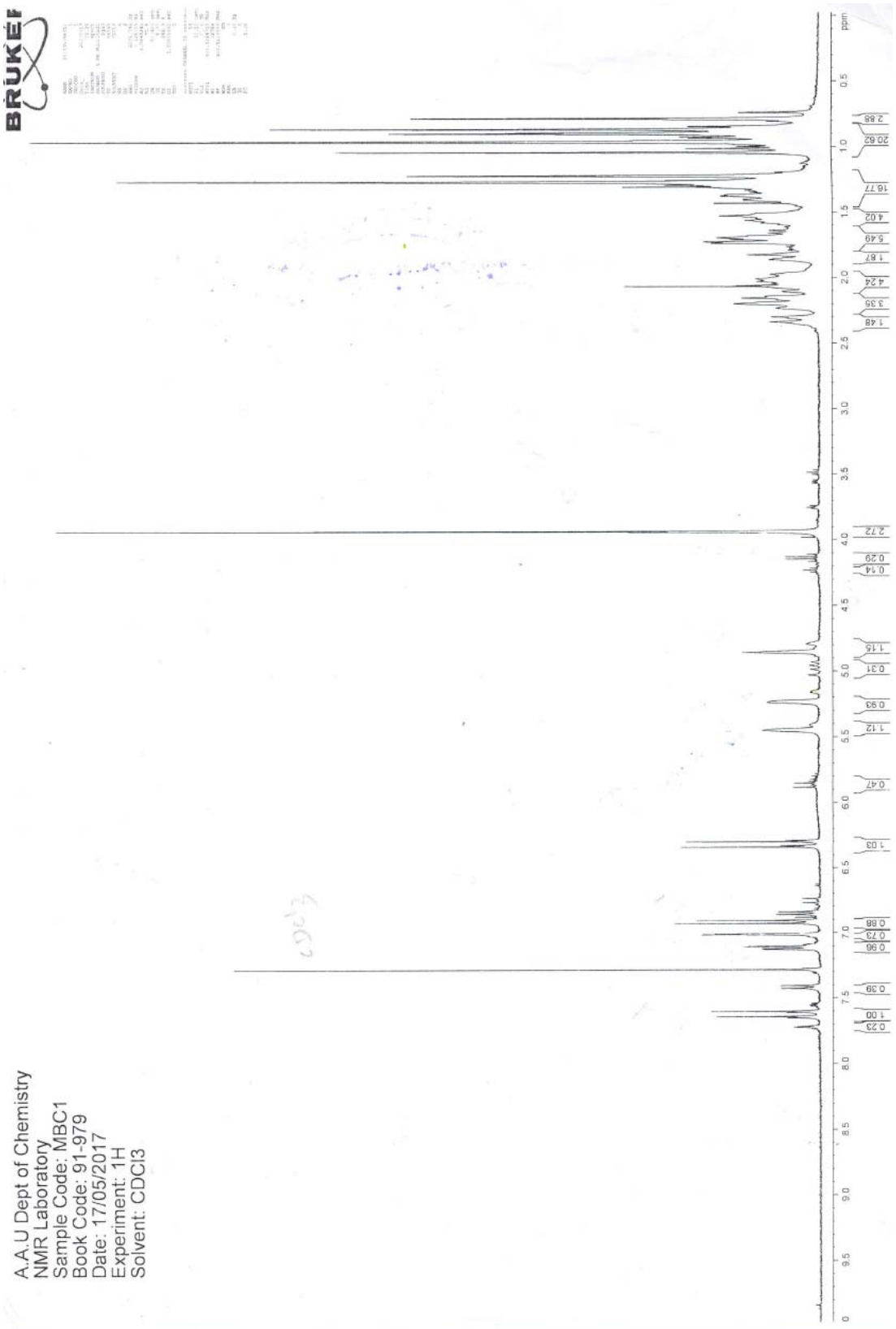


Fig. 2: Proposed structural of compound MB-1 (the same with that of stigmasterol)

peak 71.8 can also be assigned to methine at C-3 bearing a hydroxyl group[61]. The ^{13}C-NMR (Appendix 3) showed that a total of 29 and 26 signals, respectively, that could be assigned to six methyl, nine methylene, eleven methine and three quaternary carbon atoms. Thus, the observed NMR data of compound MB-1 were found to be consistent with the reported NMR data of stigmasterol (or 24-Ethyl-cholesta-5, 22-dien-3beta-ol) (Fig. 2, Table 3)[62-64].

Structural elucidation of MBC-1: A greenish crystalline compound (37.5 mg, labeled as MBC-1) was obtained from the column chromatographic separation that was eluted with hexane:ethyl acetate (94:6%) solvent system. Its R_f value was determined to be 0.45 in n-hexane:ethyl acetate (90:10%) solvent system. Its IR spectrum (Appendix 4) showed a strong band around 3429 cm^{-1} indicating the compound has an alcohol or alcohol -OH functional group. The strong band at 2926 cm^{-1} represents C-H stretch of methylene whereas, the weak band (shoulder-type) around 2800 cm^{-1} indicated the C-H stretching of methyl groups. The band at 1679 cm^{-1} indicate the presence of a,b-unsaturated carbonyl functional group and bands around 1600 and 1500 cm^{-1} (aromatic and olefin C=C bonds). The observed data suggested that compound MBC-1 could bear an alcohol/phenol functional group and also at least one C=C double bond and aromatic ring.

^{1}H-NMR spectrum (Appendix 5) showed symmetric doublet of doublet peaks at δ 6.4 and 7.6 indicating the presence of trans-olefinic hydrogen that is conjugated with aromatic ring. Moreover, peaks at 7.1, 7.0 and 6.9 could be attributed to three protons of tri-substituted aromatic/benzene ring. On the other hand, a singlet (and intense) peaks at δ 3.9 indicate the presence of methoxy group whereas a singlet peak at δ 4.8 indicates the presence of alcohol or phenol hydroxyl (-OH) group of an alcohol. The broad singlet peaks at δ 5.3 and 5.5 indicate the presence of germinal olefinic hydrogen atoms. The ^{1}H-NMR spectrum also revealed a broad range of signals (from δ 0.7-2.5) that could be attributed to methine, methylene and methyl protons

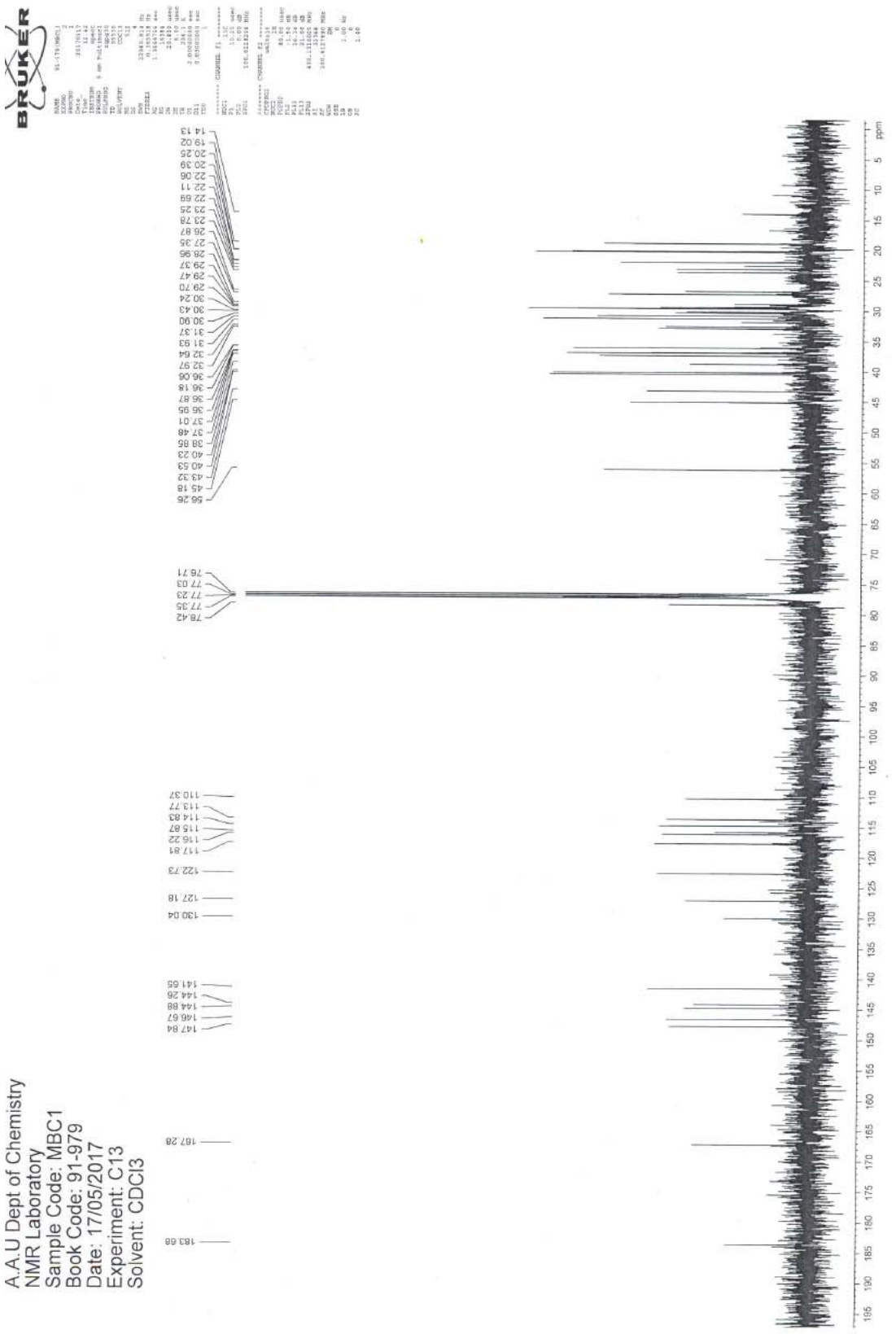


Appendix 5: ^{1}H-NMR spectrum of compound MBC-1 in $CDCl_3$

Table 4: The ^{13}C-NMR, DEPT-135 and ^{1}H-NMR data of compound MBC-1 and reported NMR data of betulinic acid-3-trans-caffeate[65-67]

Carbon atoms	^{13}C-NMR data of compound MBC-1 (ppm)	Reported ^{13}C-NMR data of Betulinic acid-3-trans-caffeate[65-67]	^{1}H-NMR of compound MBC-1 (ppm)	Reported ^{1}H-NMR data of Betulinic acid-3-trans-caffeate[65-67]	Nature of carbon
C-1	38.6	39.2	1.70 (m)	1.73(m)	CH_2
C-2	23.9	24.6	1.66 (m)	1.68 (m)	CH_2
C-3	78.4	81.2	4.2 (t)	4.57-5.1(t)	CH
C-4	37.5	38.3	-	-	C
C-5	56.3	55.7	0.90 (m)	0.91(m)	CH
C-6	19.0	19.0	1.52 (m)	1.53, 146 (m)	CH_2
C-7	33.0	35.1	1.4 (m)	1.39 (m)	CH_2
C-8	40.2	42.7	-	-	C
C-9	43.3	50.7	1.4 (m)	1.43 (m)	CH
C-10	31.9	34.6			C
C-11	22.1	21.8	1.13 (m)	1.12 (m)	CH_2
C-12	22.1	26.4	1.26 (m)	1.74 (m)	CH_2
C-13	36.9	39.0	2.38 (m)	239 (m)	CH
C-14	40.5	43.3			C
C-15	30.2	30.5	1.6 (m)	1.59 (m)	CH_2
C-16	31.9	32.9	2.26 (m)	2.26 (m)	CH_2
C-17	60.5	56.8	-	-	C
C-18	45.2	49.5	1.6 (m)	1.59 (m)	CH
C-19	43.3	47.1	3.5 (m)	3.0(m)	CH
C-20	147.8	150.0	-	-	C
C-21	32.6	31.4	-	1.93, 1.37(m)	CH_2
C-22	37.0	37.9	1.92 (m)	1.93, 1.37 (m)	CH_2
C-23	28.9	28.3	0.85 (s)	0.86 (s)	CH_3
C-24	19.0	16.3	0.87 (s)	0.89(s)	CH_3
C-25	20.3	16.9	0.90 (s)	0.93 (s)	CH_3
C-26	16.30	16.4	0.96 (s)	0.97 (s)	CH_3
C-27	14.1	14.9	0.96 (s)	0.97 (s)	CH_3
C-28	183.7	177.8	-	-	C
C-29	110.4	110.0	-	4.60, 4.57	C
C-30	20.4	19.6	1.6 (s)	1.68 (s)	CH_3
C-31	56.1	-	3.9 (s)	-	OCH_3
1′	127.2	128.0	-	-	CH
2′	122.7	122.6	7.0 (s)	7.07 (d)	C
3′	115.9	115.8	-	-	C
4′	146.7	146.1	-	-	CH
5′	144.3	143.9	6.9 (d)	6.86 (d)	CH
6′	114.8	114.6	7.1 (d)	6.99 (dd)	C
7′	144.9	144.3	7.6 (d)	7.53 (d)	CH
8′	116.2	116.9	6.3 (d)	6.24 (d)	CH
9′	167.3	167.7			C

of triterpene skeleton. ^{13}C-NMR spectrum (Appendix 6) of compound MBC-1 suggested that the compound could be a triterpene with 31 carbons. Further investigation of ^{13}C-NMR spectrum also revealed the characteristic signals for no-conjugated ester (δ183.7), vinyl carbons (quaternary carbon at δ147.8 and CH_2 at δ110.4) as well as an oxygen-bearing methine group (δ78.4) (Table 4). Additional aromatic moiety peaks were observed at δ127.2, 122.7, 115.9, 146.7, 144.3 and 114.8. Comparison of the NMR spectral data of compound MBC-1 with data reported in literature[65-67] suggested that the compound has similar skeleton with that of betulinic acid-3-trans-caffeate. The only difference between compound MBC-1 and betulinic acid-3-trans-caffeate was that in the spectrum of compound MBC-1, there is no band of carboxylic acid functional group (in the range of 2400-3400 cm^{-1}). Moreover, there was no peak around 12 ppm in the ^{1}H-NMR spectrum of compound MBC-1. This could indicate that, though it has similar skeleton with betulinic acid-3-trans-caffeate, compound MBC-1 has no carboxylic acid functional group or it is not betulinic acid-3-trans-caffeate. Instead, both ^{1}H-NMR and ^{13}C-NMR showed the presence of methoxy group bonded to carbonyl functional group or presence of $COOCH_3$. Based on the spectral data and literature reports, compound MBC-1 is suggested to be methyl ester of betulinic acid-3-trans-caffeate (Fig. 3).



Appendix 6: ^{13}C-NMR spectrum of compound MBC-1 in $CDCl_3$

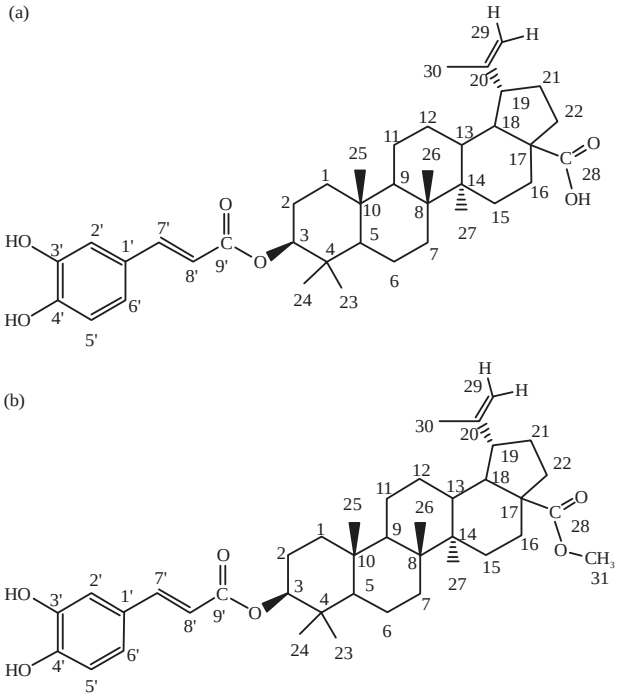


Fig. 3(a-b): Chemical structures of (a) Betulinic acid-3-trans-caffeate and (b) Proposed structure of compound MBC-1

CONCLUSION

Phytochemical screening tests revealed that *M. boivinii* roots contain alkaloids, saponins, flavonoids, phenols, steroids, glycosides, terpenoids and tannins. However, further investigations are suggested to evaluate biological activities of root extracts and also extracts from its leaves and fruits. Moreover, two compounds (Stigmasterol and methylester of betulinic acid-3-trans caffeate) were identified and their structures were determined based on spectroscopic data and comparison of the data with literature reports. The results suggested the potential of the plant in discovery of new drugs for treatment of human diseases.

SIGNIFICANCE STATEMENT

The study conducted to analyze the phytoconstituents of *M. boivinii* roots and found that it contain some active compounds like alkaloids, saponins, flavonoids, phenols, steroids, glycosides, terpenoids and tannins which confirm the secondary metabolites presence within the plant root. This study would help other researchers in ameliorating the secondary metabolites in detail in future with their medical perspective. Thus, the best theory on it may be arrived at.

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