EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 11
Issue: 12
Year: 2024

ANTI-PROLIFERATIVE ACTIVITY OF ILLISIC ACID FROM *ARTEMISA MARITIMA*

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Article Received on 25/10/2024 **Article Revised on 15/11/2024** **Article Accepted on 05/12/2024**

ABSTRACT
Artemisia is one of the largest genera of the family Asteraceae used as folklore medicine to cure various kinds of diseases reported in Ayurveda. All parts of the plants are useful as an active ingredient for the preparation of a botanical medicine. Fractionation of a defatted methanol extract of the herb *Artemisia maritima* led to the isolation of three major secondary metabolites. The structures of the compounds have been determined in combination of ^{1}H and ^{13}C NMR spectroscopy and EIMS analysis. Finally, their structure has been confirmed by single crystal X-ray diffraction study. In order to check the efficacy of anti-proliferative activity, the cell cytotoxicity of these compounds has been tested on different cancer cell lines using SRB assay. Amongst them, only the illisic acid (**3**) has been exhibited potent activity with IC_{50} 16.84 ±0.79, 14.08 ± 0.54, 10.9 ±1.4 µg/mL against the human HT-1376 (urinary bladder), HPAC (pancreas), HT-29 (prostate) cancer cell lines respectively in comparison with paclitaxel as control, whereas compound (**1**) and compound (**2**) possessed almost negligible activity.

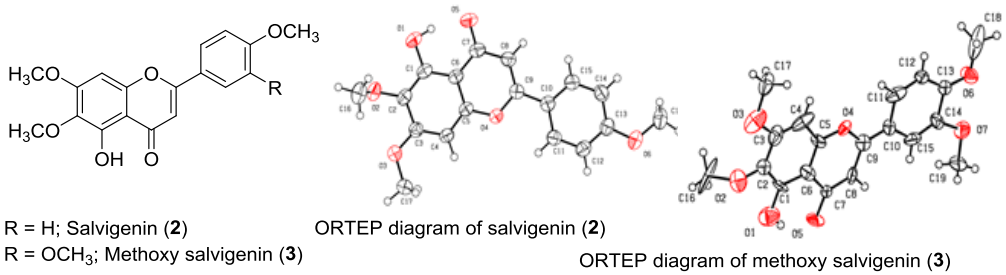
KEYWORD: *Artemisia maritima*; Asteraceae*;* terpenoid and flavoniods; structural characterization; Single crystal; X-ray diffraction study; anti-proliferative activity.

INTRODUCTION
Artemisia species (family*: Asteraceae*) are distributed across Europe and temperate Asian countries including Pakistan, India, Nepal, Tibet and China. In India, around seventeen *Artemisia* species and varieties have been reported.[1-3] These species are distributed in the Himalayan region across Jammu and Kashmir (Gurez Kistwar valleys), Himachal Pradesh (Kulu valley and Lahul & Kangara district) and Uttar Pradesh at altitudes of 2100-4200 m. This herb is a rich source of bioactive compounds[4-7] and is the source of the important anti-malarial sesquiterpenoid artemisinin.[4-8] Liu *et al.* reported the first occurrence of sesquiterpenoids from *Artemisia annua*.[8-9] Several sesquiterpenoids and pentacyclic triterpenoids have also been reported from *Artemisia* species. In this paper, we report the first chemical investigation of *Artemisia maritima* resulted isolation a bio-active terpenoid along with two flavones as major chemical constituents along with trace amount steroids, terpenoids and other flavonoids was also present which were not characterised due to availability in insufficient amount. The evaluation of anti-proliferative activity has been conducted against different cancer cell line in comparison with paclitaxel as control. It has found that illisic acid exhibited moderate anti-proliferative activity.

The most credential pharmaceutical activities of various *Artemisia* species are anti-proliferative,[8] hepato-protective,[9,10] anti-malarial activity,[10-24] cytotoxicity,[25-27] anti-inflammatory activity,[28] anti-oxidant,[29-32] anti-cancer activity, [25-27] anti-microbial,[29] anti-viral,[28] anti-tumour,[35] anti-diabetic,[36] etc. This manuscript deals with isolation, structural characterization and evaluation of anti-proliferative activity of major chemical constituent isolated from the methanol extract of whole plant of *Artemisia maritima* in comparison of paclitaxel as control using sulfo-rhodamin (SRB) assay.

RESULTS AND DISCUSSION
The phytochemical investigation of crude extract defatted methanol extract of whole plant of *Artemisia maritima,* was fractionated by column chromatographed over silica gel with gradient solvent elution with a binary mixture of solvent system, ethyl acetate in hexane followed by chloroform and binary a mixture of methanol in chloroform with gradient change of polarities. A yellow solid substance (approximately 2%

dry weight plant) was obtained while eluting with 5-10% methanol in chloroform by column chromatography over silica gel followed by gel permeation chromatography (GPC) over sephadexLH20 as a major chemical constituent (**1-3**) as major chemical constituent along with other minor constituents such as coumarin, terpenoids and steroids. In order to assign structural skeleton, the preliminary investigated on micro plate and the qualitative classification of the major compound (**1-3**), different chemical tests have been carried out such as Lieberman-Burchard test, Dragendorff's test, Shinoda test, Molish test, dilute H_2SO_4 spray test, and frothing test/foam test among others. The compound (**1**) & (**2**) showed positive Shinoda test (Mg/HCl in MeOH) gave purple colour)[37-39] and sprayed with 10% H_2SO_4 on micro plate followed by heating at 120 ^{0}C for 5 min produced yellow spot on microplate indicated that compound (**1**) and (**2**) belongs to flavonoid.[39] The sharp absorption peak appeared at 269, 333 nm in UV spectrum (methanol) confirmed the presence of benzoyl and cinnamoyl parts in these compounds.[40] This adsorption peak indicated the presence of flavone moiety in the compound (**1**) and compound (**2**). A dark greenish spot was appeared on TLC plate when sprayed with neutral $FeCl_3$ solution (ethanol) suggesting that a phenolic hydroxyl group adjacent to the carbonyl group in these compounds.[39] A bathochromic UV shifts about 35 nm was observed upon the addition of anhydrous $AlCl_3$ at an acidic to methanolic solution of both the compound (**1**) & (**2**) showed 30 nm bathochromic shift which is very stable under acidic pH. This batho chromic shift indicated 5-hyrdoxy ketone moiety of these compounds formed chelatation with $AlCl_3$.[40] Based on the above physical, chemical and spectral evidences, the structure of compound (**1**) and compound (**2**) has been assigned as salvigenin and methoxy salvigenin as depicted in figure 1. Finally, their structure was confirmed by single crystal X-ray diffraction study.[41-45] The chemical structure of these compounds is depicted below.



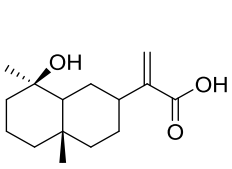
R = H; Salvigenin (**2**)
R = OCH_3; Methoxy salvigenin (**3**)

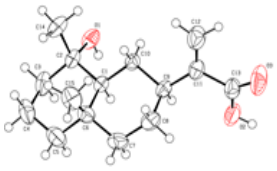
ORTEP diagram of salvigenin (**2**)

ORTEP diagram of methoxy salvigenin (**3**)

Figure 1: Chemical structure and ORTEP diagram of compounds (1) and (2).

The compound **3** performed Lieberman-Burchard test and prayed with 10% H_2SO_4 on micro plate followed by heating at 120 ^{0}C for 5 min produced crimson red colour revealed that it belongs to terpenoids.[39, 41] When the compound (**3**) treated with aqueous $NaHCO_3$ solution, an effervescence was observed due to liberation of CO_2 gas. The slow effervescence indicated the presence of carboxylic functionality present in the compound (**3**). Based on NMR spectral and MS study, the structure of the compound (**3**) is assigned as illisic acid and confirmed by single crystal X-ray diffraction study.[42-45] The chemical structure of illisic acid and its ORTEP diagram has been depicted below in figure 1.

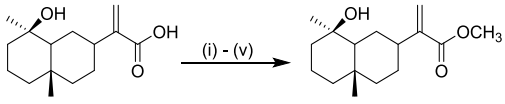




Illisic acid (**3**) ORTEP diagram of illisic acid (**3**)

Figure 2: Chemical structure and ORTEP diagram of compounds (3).

In order to establish structure activity, the compound (**3**), was methylated using methanol in presence of catalytic amount H_2SO_4 for overnight stirring to yield methylated derivative of compound (**3**). The anti-proliferative activity of this methylated derivative has been tested but it did not exhibit antiproliferative activity.



Illisic acid (**3**) Methyl ester illisic acid (**3a**)

(i). Solvent and reagent used: MeOH; (ii). Catalyst used: H_2SO_4 (iii). Stirring over night at room temperature (iv). Usual worked up (v). Purification column

Scheme 1: Methylation reaction of compounds (3).

The anti-proliferative activity of methyl ester of illisic acid (**3a**) has also been checked against different cancer cell lines under similar experimental condition, but it exhibited almost negligible activity.

ANTI-PROLIFERATIVE ASSAY

Anti-proliferative activity of major secondary metabolites (1-3) against different cancer cell lines by using sulfo-rhodamine B (SRB) assay

Anti-proliferative activity of major secondary metabolites (**1-3**) isolated from methanol extract of whole plant *Artemisia maritima* has been evaluated against human different cancer cell lines using sulfo-rhodamine B (SRB) assay and was performed at The College of Pharmacy, The Ohio State University, Ohio, Columbus, USA.[46-49] The sulfo-rhodamine B (SRB) cell

cytotoxicity assay is one of the most widely used methods to detect cell viability. This assay is independent of cell metabolic activity. The incorporated dye released from stained cells after washing is directly proportional to the cell biomass and can be measured at 460 nm. Five different types of human cancer cell lines such as urinary bladder (HT-1376), pancreatic (HPAC), prostate (DU-145), colon (HT-29) and thyroid (MDA-T32) cancer cell line were used to carry out bioassay experiment for cell cytotoxicity study (Table-1).

Sample preparation. Test samples and controls (paclitaxel) were dissolved in 100% DMSO to prepare stock solutions of 10 mg/ml. Dilutions were prepared using 10% DMSO in water and 100% water.

Cell culture. Cancer cells urinary bladder (HT-1376), pancreatic (HPAC), prostate (DU-145), colon (HT-29) and thyroid (MDA-T32)] were obtained from American Type Culture Collection, Manassas, VA, USA. Monolayer cells were cultured using T75 tissue culture flasks in Roswell Park Memorial Institute medium (RPMI) or Dulbecco's Modified Eagle Medium (DMEM), containing 10% fetal bovine serum and 1% anti-biotic-anti-mycotic from Gibco. Cells were kept at 37 °C and in an atmosphere with 5% of CO_2.

Anti-proliferative assays. Anti-proliferative activity of test samples was evaluated in triplicate on cancer cells, using a sulfo-rhodamine B (SRB) assay as reported previously in three independent experiments.

Table 1: Anti-proliferative activity of major chemical constituents isolated from methanol extract of whole plant of *Artemisia maritime.*

Experimental results of anti-proliferative activity of major constituents of methanol extract of whole plant of *Artemisia maritima* on different human cancer cell lines using sulfo-rhodamine B (SRB) assay								
Cancer cell lines used	**Salvigenin (1)**		**Methoxy-salvegenin (2)**		**Illisic acid (3)**		**Paclitaxel (control)**	
	% Inhibition	**IC_{50}±SE**	**% Inhibition**	**IC_{50}±SE**	**% Inhibition**	**IC_{50}±SE**	**% Inhibition**	**IC_{50}±SE**
HT-1376/urinary bladder	<50	-	<50	-	70.01 ± 1.79	16.84 ± 0.79	-	13.17
HAPC/pancreatic	<50	-	<50	-	65.16± 0.54	14.08 ± 0.54	-	0.051
DU-145/colon	<50	-	<50	-	<50	-	-	0.69
HT-29/prostate	<50	-	<50	-	62.0	10.9 ±1.4	-	0.028
MDTA32/thyroid	<50	-	<50	-	<50	-	-	0.028

[a] Sample tested in triplicate and in two separate experiments at **20µg/mL**
[b] % Inhibition <50 deemed inactive
[c] IC_{50} ≥20 deemed inactive

CONCLUSION AND SUMMERY OF WORK

Chemical profiling of whole plat of *Artemisia maritima* revealed that major chemical constituent presents in defatted methanol extract was salvigenin and methyl salvigenin and illisic acid along with trace number of steroids. Their structural characterization of major chemical constituents has been done by spectroscopic and spectrometric methods and confirmed by single crystal X-ray diffraction study. Three compounds (**1**-**3**) have been screened to check efficacy of anti-proliferative activity against the different human cancer cell lines using SRB assay. Amongst them, only the illisic acid (**3**) has been exhibited potent activity with IC_{50} 16.84 ±0.79, 14.08 ± 0.54, 10.9 ±1.4 µg/mL against the human HT-1376 (urinary bladder), HPAC (pancreas), HT-29 (prostate) cancer cell lines respectively in comparison with paclitaxel as control, whereas compound **2** and compound **3** possessed almost negligible activity.

EXPERIMENTAL SECTION

General experimental procedures

Melting points were determined using a Buchi M560 point apparatus. Specific rotations were obtained using a JASCO DIP 1000 digital polarimeter. UV spectra were measured on a Shimadzu UV-2100 UV-Vis spectrophotometer. NMR spectra were recorded in $CDCl_3$ or CD_3OD on a Bruker Avance 200, Varian 500 MHz, Varian 600 MHz, Varian 800 MHz spectrometers using residual $CHCl_3$/HDO as an internal standard. Chemical shifts are given in ppm (δ_C & δ_H), relative to residue $CHCl_3$/HDO (7.25 & 77.00/4.78 or 3.30 & 49.00 ppm). Mass spectra were recorded using Fission 8000 (8000 series, UK) and Shimadzu QP5050A mass spectrometer, Japan. UHPLC/MS analysis of the samples was carried out using a Vanquish UHPLC system coupled to a Q-Exactive quadrupole orbitrap mass spectrometer (Thermo Fisher Scientific, USA) at the Ohio State University, College of Pharmacy Instrumentation Facility. Silica gel 60 (Merck) was used for analytical TLC. Silica gel (230-450 mesh, Aldrich, USA) was used for column chromatography. All compounds were visualized in TLC under exposure of both long range as well as short range wavelength, using vanillin-perchloric acid-EtOH followed by heating at 110 °C for 5 min and neutral $FeCl_3$ in MeOH.

Plant material

The seed of *Artemisia maritima* (Kirmai aajwan as whole plant) was purchased from local herbal and spice market Pydhuni, Mumbai in May, 2021. The material was authenticated by Dr. Hussian Barbhuiya, Landscape and Cosmetic Section, A & SED Division, Bhabha Atomic Research Centre, Trombay, Mumbai-400085.

Extraction and Isolation

Freshly dried seeds (100 gram) of *Artemisia maritima* were powdered using mechanical grinder and extracted with analytical grade methanol (3x250 mL) at room temperature to obtain methanol extract. Solvent was removed using rota-vapor at reduced pressure maintaining water bath temperature at 40 °C to afford a brown viscous residue (~3.0 g). For preliminary investigation to check chemical composition of methanol extract, trace amount of residue was dissolved in methanol and monitored by micro TLC plate. This microplate was developed using different combinations of solvent systems, sprayed with different reagents, on exposure of UV radiation, several spots were visualized. Amongst, three spots were visualized as major components on micro plate. Based on this idea of solvent system used on microplate, the solvent system chosen for column chromatography.

This extract was fractionated by column chromatography over silica gel (500-gram, 230-400 mesh, Aldrich, USA) and eluted with a step gradient of hexane, ethyl acetate in hexane, then chloroform and mixtures of methanol in chloroform to obtain ten fractions, with the volume of each aliquot being 200-500 mL. In some cases, the volume of the aliquot collected was more than 500 mL. Fractions were monitored by TLC to examine the chemical compositions of the respective fractions. The fractions with similar chemical profiles were combined and, in some cases, further purified by using repetitive column chromatography on an open column chromatography over silica gel, and gel permeation chromatography (GPC) using sephadexLH20 with gradual solvent elution followed by preparative thin layer chromatography (PTLC).

Fraction 1 and 2 were eluted with 5-10% ethyl acetate in petroleum ether. Both the fractions were monitored by TLC. TLC profiles indicated that they were similar in composition and combined. Preliminary investigation revealed that it was lipid.

Fraction 3 was eluted with 15% ethyl acetate in petroleum ether. The volume of collected aliquot was approximately 500 ml. On removing solvent, a pale-yellow sticky mass was obtained. Preliminary investigation of residue revealed that it was also lipid.

Fraction 4 was eluted with 15-20% ethyl acetate in petroleum ether. Total volume of each collected aliquot was approximately 350 ml. It was subjected to concentrate in a small volume for crystallization. A canary yellow colour crystal separated to filter through a micro filter system and washed thoroughly using cold 10% ethyl acetate in petroleum ether for three times to obtain crystals (1 g, abundance 1% dry seed weight), mp 180 °C (lit.180-181 °C).[22-24]

Compound **1** was isolated as a yellow crystalline solid with molecular formula $C_{18}H_{16}O_6$ as deduced by EIMS at *m/z* 328 in combination with the ^1H and ^{13}C NMR spectra. Its IR spectrum showed sharp absorptions at around 3583, 1646, and 1588 cm^{-1}, which revealed the presence of –OH, >C=O and >C=C< groups. Its UV spectrum showed absorptions at 242, 276 and 334 nm due to the presence of benzoyl (band I) and cinnamoyl (band II) parts respectively[28] and it gave a positive Shinoda test hence, it was a flavone. It displayed a red shift in its UV absorption on addition of $AlCl_3$ in methanol solution, indicating the presence of a phenolic-OH group, probably located either at the C-5 or the C-3 position. The chelate was stable to acidic pH and showed a similar red shift in its UV absorption[28] indicating that the attachment of the -OH group was at C-5 and not at the C-3 position. When its UV spectrum was recorded on the addition of NaOAc and H_3BO_3 respectively no red shifts were displayed, indicating the absence of a free hydroxyl group at C-7. In ^1H NMR spectrum of compound **1**, a sharp singlet has appeared at 12.81 ppm integrating one proton due to the C_5-OH proton chelated with the adjacent carbonyl group. It also showed an A_2B_2 spin system at 7.84 & 7.02 integrating for two protons each with coupling constants 2.2 & 8.8 Hz assigned as H-1′, H-6′ and H-3′, H-5′ of the B ring protons. Two singlets at δ 6.59 and 6.55 integrating for one proton each were assigned to H-3 and H-8. Three sharp singlets at 3.97, 3.92, and 3.98 integrating three protons each were due to three methoxyl groups located at C-6 and C-7 in the A ring and C-4′ in the B ring. In ^{13}C NMR spectrum had eighteen peaks consisting of three methoxyl peaks at δ 55.80, 56.20 and 59.80, six methine and nine quaternary carbon peaks (Table 1). On comparison with spectroscopic data available in the literature, compound **1** was identified as 4′, 6, 7-trimethoxy flavones known as salvigenin. Its structure was also confirmed by an X-ray diffraction study.

Compound **1** (Salvigenin): yellow crystalline substance, mp 198 °C. ^1H NMR data ($CDCl_3$, 200 MHz): 6.55 (s 1H, H-3), 12.81 (s, 1H, OH), 6.59 (s, 1H, H-8), 7.84, (dd, 8.68 & 2.2 Hz, H-2′, H-6′). 7.02, (dd, 8.68 & 2.2 Hz, H-3′, H-5′), 3.92 (s, 3H , C_6-OCH_3), 3.97 (s, 6H, C_7-OCH_3), 3.98 (C_4'-OCH_3). ^{13}C NMR data ($CDCl_3$, 200 MHz): 132.28 (C-2), 103.75 (C-3), 182.37 (C-4) 108.28 (C-4a), 162.32 (c-5), 152.92 (c-6), 163.72 (C-7), 103.75 (C-8), 158.43 (C-8a), 123.16 (C-1′) 127.67 (C-2′), 114.20 (C-3′), 152.72 (C-4′), 114.20 (C-5′), 127.67(C-6′), 55.80 (C_6-OCH_3), 59.80 (C_7-OCH_3), 56.20 (C_4'-OCH_3). ^1H NMR see Table **2**; ^{13}C NMR data see Table **3**; IR Y_{max} (KBr): 3583, 2924, 1654, 1588, 1453, 1350, 1294, 1205, 1190, 1120,1100, 1040, 900 cm^{-1}. UV λ_{max} (MeOH): 241 (0.135), 272 (0.168) and 324 (0.184) nm. EIMS: *m/z* (%) 328 (72), 314 (78), 300 (60), 282 (100), 268 (40), 254 (13), 214 (15), 182 (19), 153 (30), 136 (57), 126 (45), 117 (34), 90 (19), 70 (15), 64 (14).

Crystal data: $C_{18}H_{16}O_6$, M_r = 328.31, crystal system triclinic, space group p-1, a = 7.0979 (8) A, b = 7.5025 (8) A, c = 14.964 (1) A, α = 76.64 (1) deg, β = 85.74 (1)

deg, γ = 81.37(1) deg, Z = 2, D_c = 1.424 mg/ m^{-3}, F (000) 344, crystal dimension 0.55 x 0.35 x 0.08 mm.

Compound **2** was isolated as a yellow crystalline solid substance with molecular formula $C_{19}H_{18}O_7$ as deduced by EIMS at *m/z* 358 in combination with its ^1H and ^{13}C NMR spectrum. In IR spectrum, it has shown sharp absorptions at 3583, 1650, and 1586 cm^{-1}. This revealed the presence of –OH, >C=C and >C=O groups. In UV spectrum of compound **2**, it has shown absorptions at 242, 276 and 334 nm due to the presence of benzoyl (band I) and cinnamoyl (band II) parts respectively and since it has also performed a positive Shinoda test hence, it was a flavone. A red shift in UV absorption upon addition of $AlCl_3$ followed by addition of acid revealed the presence of a hydroxyl group, probably located at either the C-5 or C-3 position. The strong stability of the chelate with $AlCl_3$ under acidic pH conditions showed a similar red shift in UV spectrum, thus ascribing that -OH group at the C_5 position. When UV spectra were recorded upon the addition of NaOAc and H_3BO_3 respectively no red shifts were observed. This indicated the absence of a free hydroxyl group at C-7. The ^1H NMR spectrum of compound **3** showed very similar signals to those of compound **2**, the major difference being the display of an AMX spin system in ring B of compound **3** with a one-proton *ortho* doublet at 6.96 (*J* = 8.2 Hz) a signal at 7.32 as a *meta* doublet (*J* = 2.0 Hz) and a signal at 7.50 as doublet of doublets with coupling constants 8.2 and 2.0 Hz instead of the A_2B_2 spin system in compound **1**. One additional singlet appeared at 3.85 integrating for three protons due to an extra methoxyl peak in compound **2**, replacing an aromatic proton in compound **1**. Its ^{13}C NMR spectrum (Table 2), indicates that one aryl methine resonance of compound **1** has been replaced by an oxy-aryl carbon peak in compound **2**, with slight variations in the chemical shifts of the remaining carbon signals. The presence of additional methoxy functionality in compound **2** was consistent with the difference in molecular weight from compound **1** of 30 amu. The position of the -OCH_3 group may be assigned to C-3′ of the B-ring, consistent with the observed AMX spin system. Based on the above evidence, compound **2** had been assigned the structure 3', 4′, 6, 7-tetramethoxy flavone. This identification was confirmed by comparing its ^1H and ^{13}C NMR spectroscopic data with literature data and by a single crystal X-ray diffraction study.

Compound **2** (Methoxy salvigenin): yellow crystalline substance, mp 180 °C. ^1H NMR data ($CDCl_3$, 200 MHz): 6.59 (s 1H, H-3), 12.80 (s, 1H, OH), 6.54 (s, 1H, H-8), 6.96 (dd, 8.68 & 2.2 Hz, H-2′ and H-6′), 7.51, (dd, 8.68 & 2.2 Hz, H-3′, H-5′), 3.91 (s, 3H , C_6-OCH_3), 3.96 (s, 6H, C_7-OCH_3, C_4'-OCH_3, C_3'-OCH_3). ^{13}C NMR data ($CDCl_3$, 200 MHz): 132.28 (C-2), 103.75 (C-3), 182.37 (C-4) 108.28 (C-4a), 162.32 (c-5), 152.92 (c-6), 163.72 (C-7), 103.75 (C-8), 158.43 (C-8a), 123.16 (C-1′) 127.67 (C-2′), 114.20 (C-3′), 152.72(C-4′), 114.20 (C-5′), 127.67(C-6′), 55.80 (C_6-OCH_3), 59.80 (C_7-OCH_3), 56.20 (C_4'-OCH_3). IR data, γ_{max} (KBr): 3583, 2924, 1588, 1453, 1351, 1294, 1205, 1190, 1120, 1101, 1040, 911 cm^{-1}. UV absorption data, λ_{max} (MeOH): 241 (0.135), 272 (0.168) and 324 (0.184) nm. EIMS: *m/z* (%) 358 (100), 341 (29), 313 (44), 298 (30), 270 (15), 245 (15), 214 (10), 198 (12), 180 (13), 163 (29), 148 (72), 137 (24), 108 (17), 91 (19), 91 (19), 71 (16).

Crystal data: $C_{19}H_{18}O_7$, M_r=358.33, crystal system monoclinic, space group P 21/c, a = 27.929 (8) °A, b = 8.6045 (8) °A, c = 14.382 (1) °A, *α* = 90 deg, β = 104.95 (1) deg, γ = 14.382(1) deg, Z = 8, D_c = 1.426 mg/ m^{-3}, F (000) 1504, crystal dimension 0.10 x 0.08 x 0.03 mm.

Fraction 5 was eluted with 15% methanol in chloroform. Total volume of each collected aliquot was approximately 350 ml. It was subjected to concentrate in a small volume for crystallization. A colourless rectangle crystal has been appeared. It was filtered a micro filter system and washed thoroughly using cold 10% ethyl acetate in petroleum ether for three times to obtain crystals (1 g, abundance 1% dry seed weight), mp 180 °C (lit.180-181 °C).[22-24]

Compound **3** performed Lieberman-Burchard test and prayed with 10% H_2SO_4 on micro plate followed by heating at 120 ^{0}C for 5 min produced crimson red colour[49] revealed that it belongs to terpenoids.[50] When the compound (**3**) treated with aqueous $NaHCO_3$ solution, an effervescence was observed due to liberation of CO_2 gas. The slow effervescence indicated the presence of carboxylic functionality present in the compound (**3**). Based on NMR spectral and MS study, the structure of the compound (**3**) is assigned as illisic acid and confirmed by single crystal X-ray diffraction study.

Table 1. Crystal data and structure refinement of illisic acid/compound (**3**).

Identification code:1167 (K-9); Empirical formula: $C_{15}H_{24}O_3$; Formula weight: 252.34; Temperature: 299 (2) K; Wavelength: 0.71073 A; Crystal system, space group: Orthorhombic: P, 21 21 2; Unit cell dimensions: a = 12.195 (1) A, α = 90 deg. b = 16.719 (1) A, β = 90 deg., c = 7.2182 (9) A, Y = 90 deg.; Volume: 1471.7 (2) A^3; Z, Calculated density 4, 1.139 Mg/m^3; Absorption coefficient: 0.077 mm^{-1}; F (000): 552; Crystal size: 0.48 x 0.45 x 0.16 mm; Theta range for data collection: 2.82 to 26.37 deg.; Limiting indices: -15<=h<=15, -20<=k<=15, -9<=l<=8; Reflections collected/unique: 6815/1741; [R(int) = 0.0179]; Completeness to theta = 26.37/99.7%; Absorption correction: Semi-empirical from equivalents; Max. and min. transmission: 0.9874 and 0.9613; Refinement method; Full-matrix least-squares on F^2; Data/restraints/parameters:1741/0/236; Goodness-of-fit on F^2: 1.041; Final R indices [I>2sigma(I)]: R1 = 0.0335, wR2 = 0.0849; R indices (all data): R1 = 0.0407, wR2 = 0.0882; Absolute structure parameter: none; Friedel pairs merge;

Extinction coefficient: 0.038(3); Largest diff. peak and hole: 0.136 and -0.115 e.A^{-3}

NMR spectral analysis of illisic acid/ compound (**3**): ^{1}H NMR data ($CDCl_3$, 500 MHz): δ_H 6.27 (s, 1H, olefinic H), 5.64 (s, 1H, olefinic H), 4.42 (brs, 2H, -COOH and -OH), 2.53 (dt, 1H), 1.95 (dd, 1H), 1.83 (dd, 1H), 1.64 (ddd, 1H), 1.55 (dd, 1H), 1.46 (dd, 1H), 1.43 (d, 1H), 1.40 (dd, 1H), 1.36 (dd, 1H), 1.29 (dd, 1H), 1.10 (s, 3H , CH_3), 0.96 (s, 3H, -CH_3) ^{13}C NMR Data ($CDCl_3$, 500 MHz): δ_C 171.66, 145.27, 124.49, 72.91, 55.05, 44.61, 43.44. 41.03, 40.13, 34.80, 27.15, 26.86, 22.49, 20.24, 18.89.

Fraction 6-8 was eluted with 25-30% methanol in chloroform. Total volume of each collected aliquot was approximately 500 ml. On removal of solvent using rota vapour, a negligible mass was obtained. It has not been analysed due to insufficient amount.

Fraction 9 was eluted with 50-60% methanol in chloroform. Total volume of each collected aliquot was approximately 500 ml. On removal of solvent using rota vapour, a polymeric mug was obtained. Analysis of the residue did not conduct.

Fraction 10 was eluted with 100% methanol. Total volume of each collected aliquot was approximately 1000 ml. On removal of solvent using rota vapour, a sticky hygroscopic mass was yielded. Analysis of the residue did not conduct.

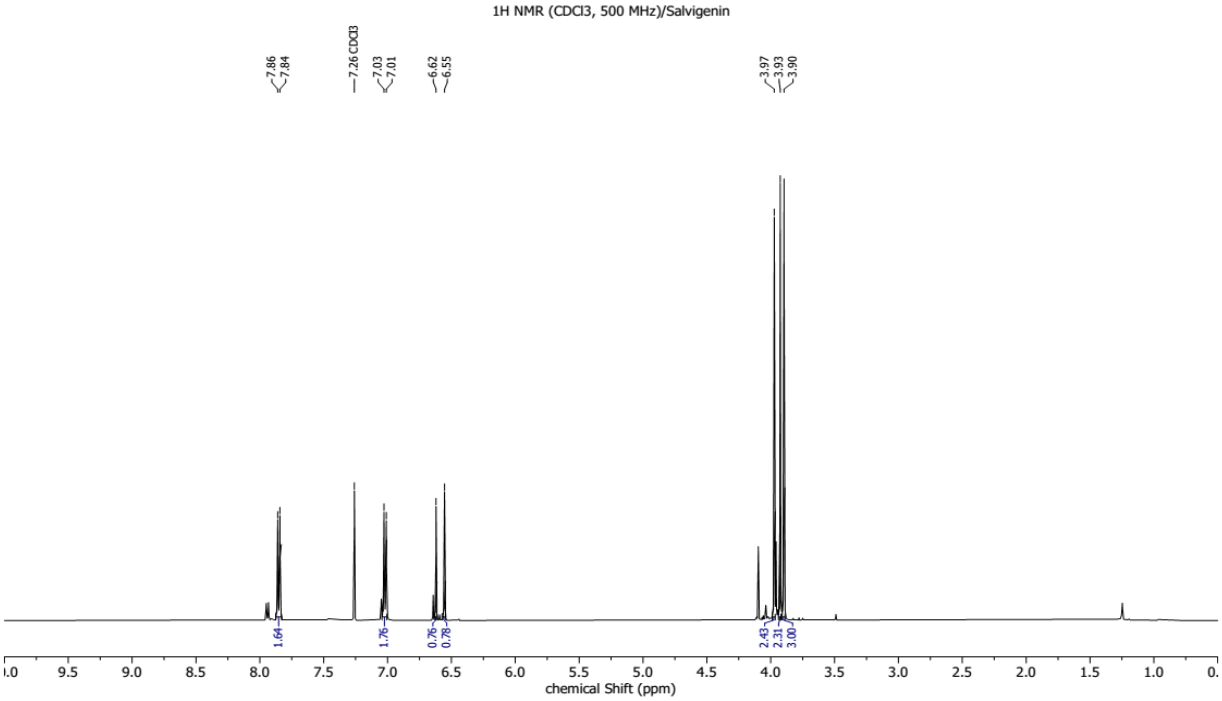


Figure S1: ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound 1 (salvigenin).

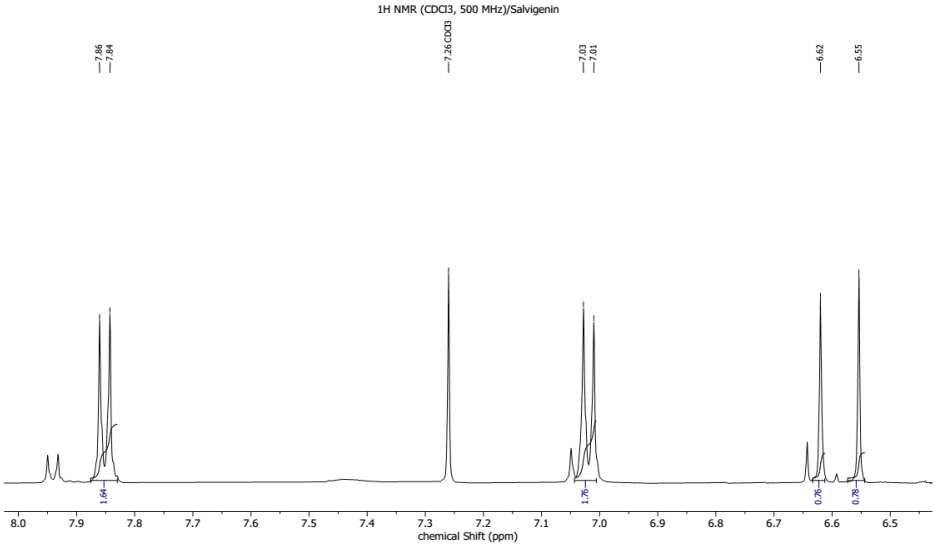


Figure S2: Expansion of ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound 1 (salvigenin).

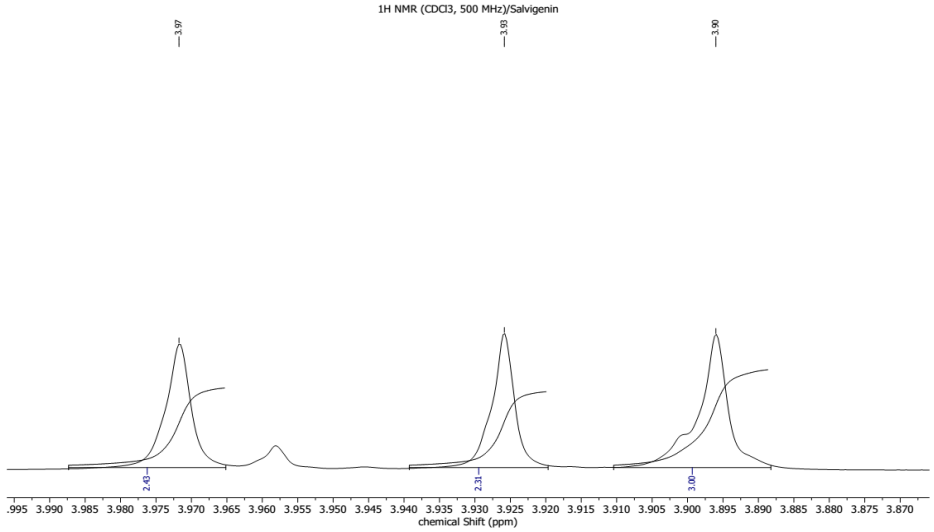


Figure S3: ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound 1 (salvigenin).

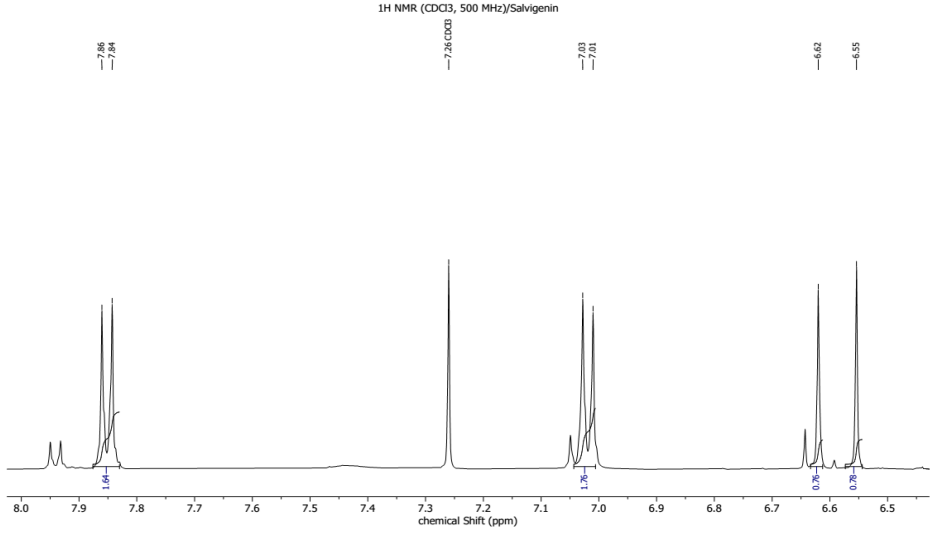


Figure S4: Expansion of ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound 1 (salvigenin).

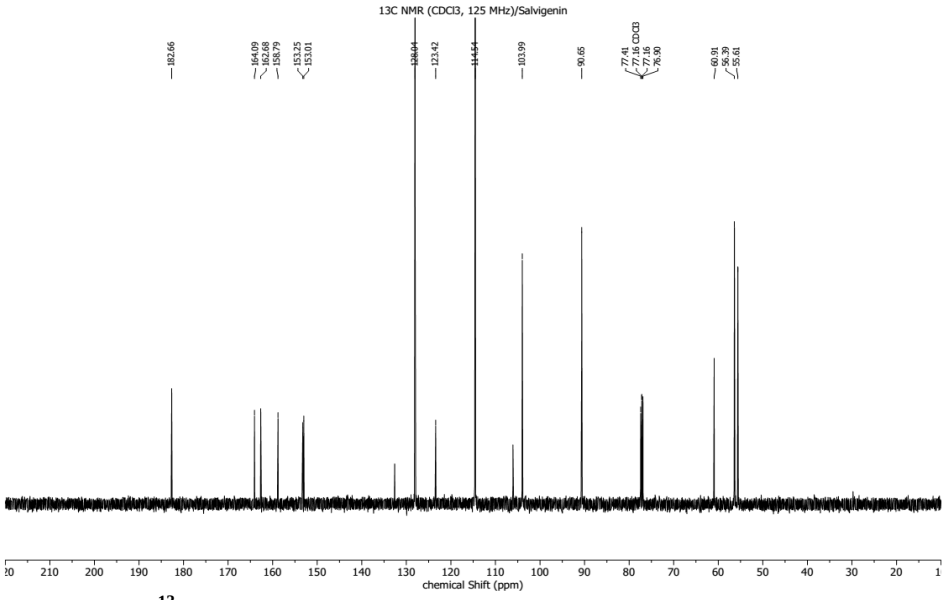


Figure S5: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of compound 1 (salvigenin).

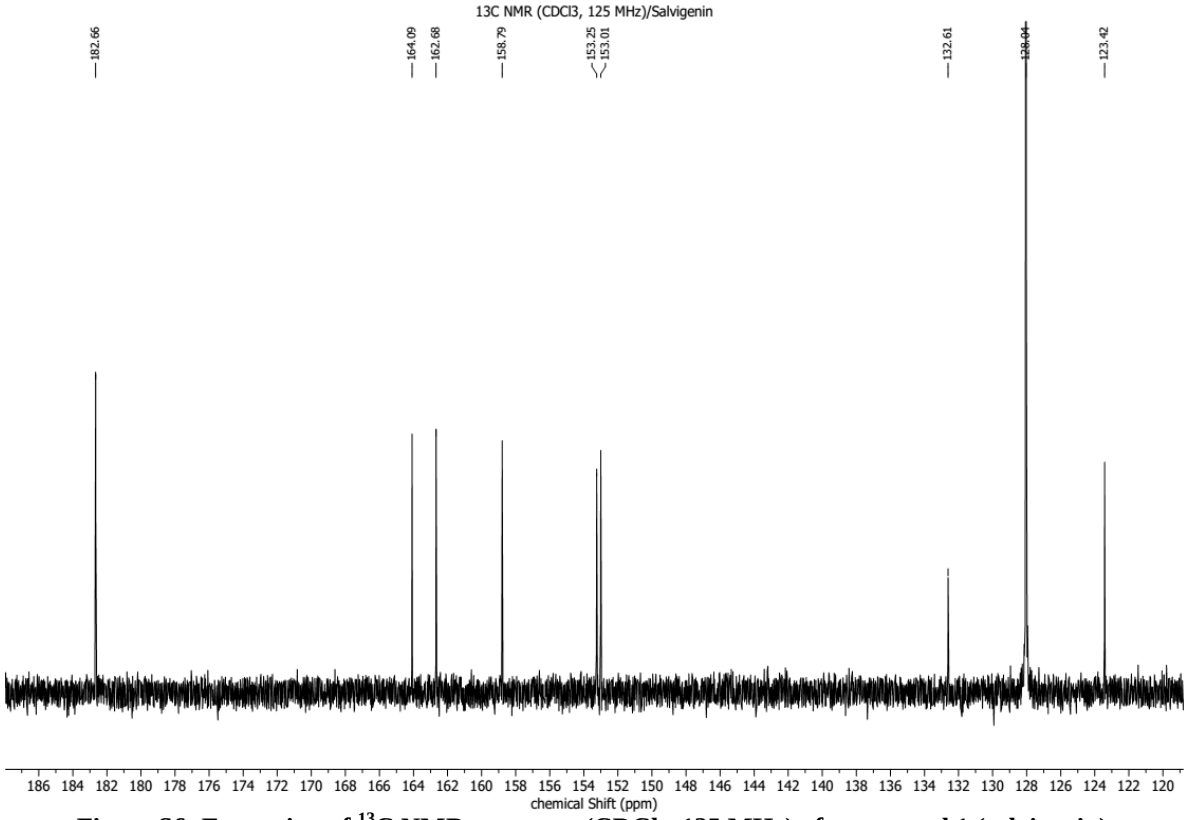


Figure S6: Expansion of ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of compound 1 (salvigenin).

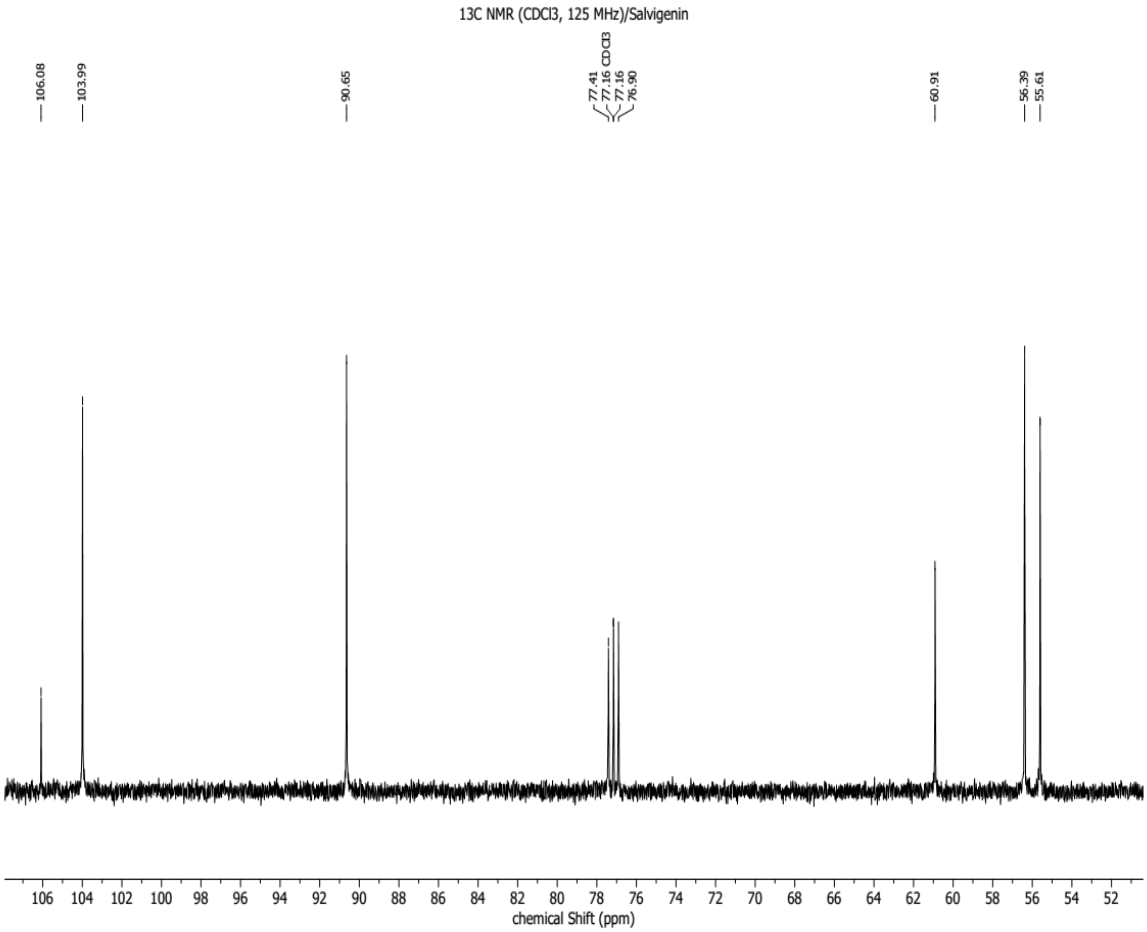


Figure S7: Expansion of ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of compound 1 (salvigenin).

Low Res + ESI (Positive):

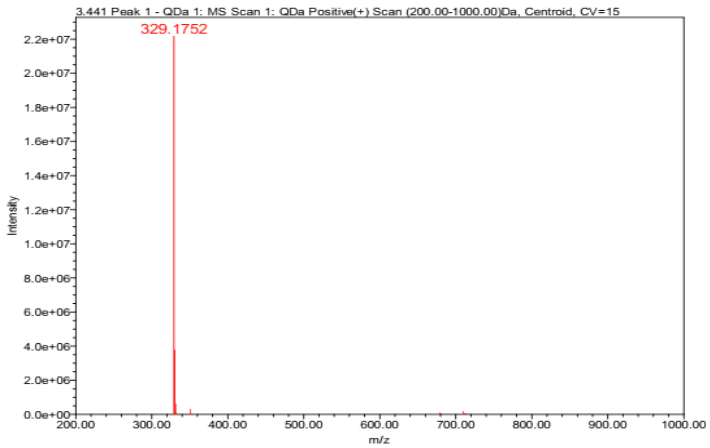


Figure S8: EIMS of compound 1 (salvigenin).

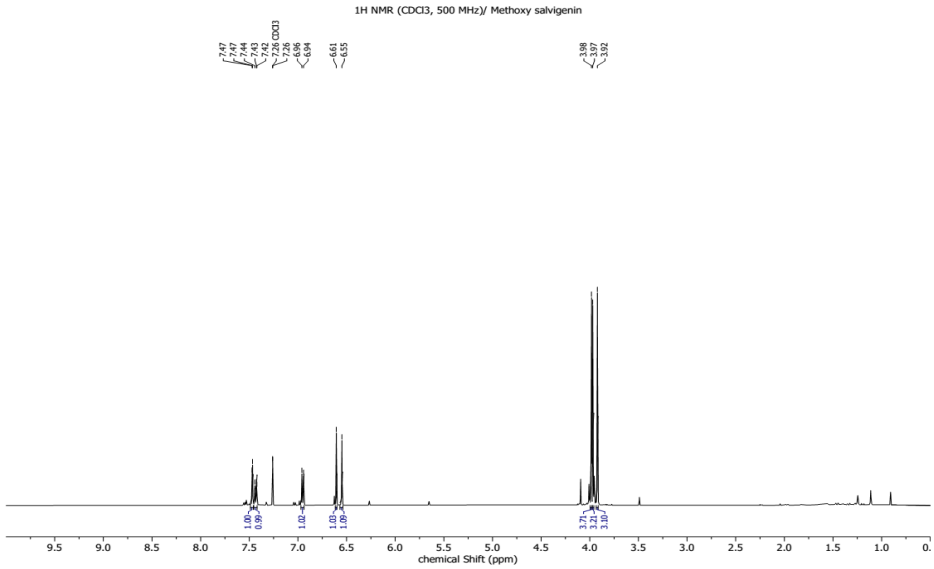


Figure S9: ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound 2 (Methoxy salvigenin).

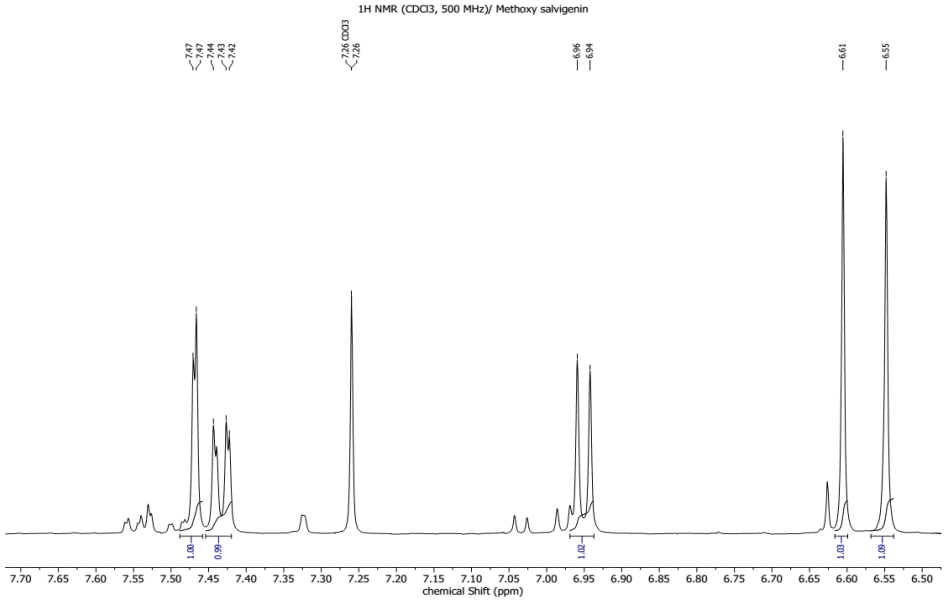


Figure S10: Expansion of ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound 2 (Methoxy salvigenin).

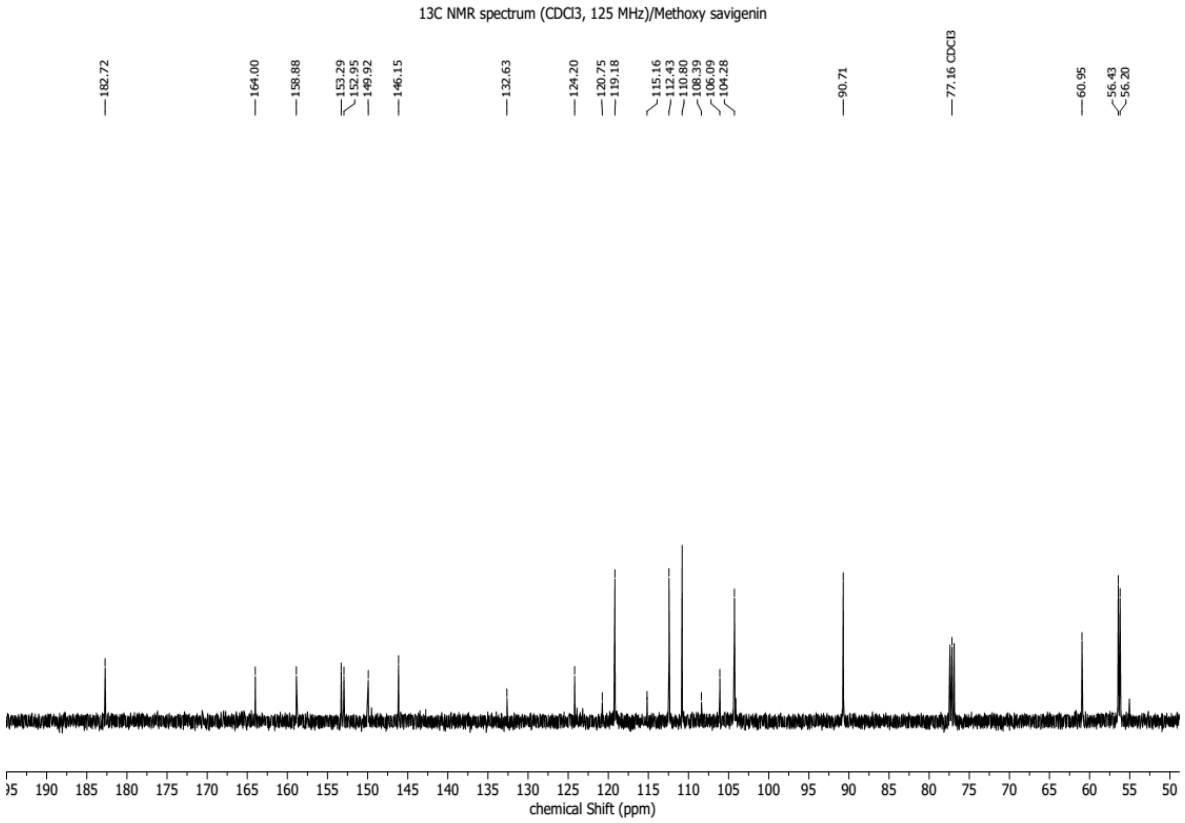


Figure S11: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of compound 2 (Methoxy salvigenin).

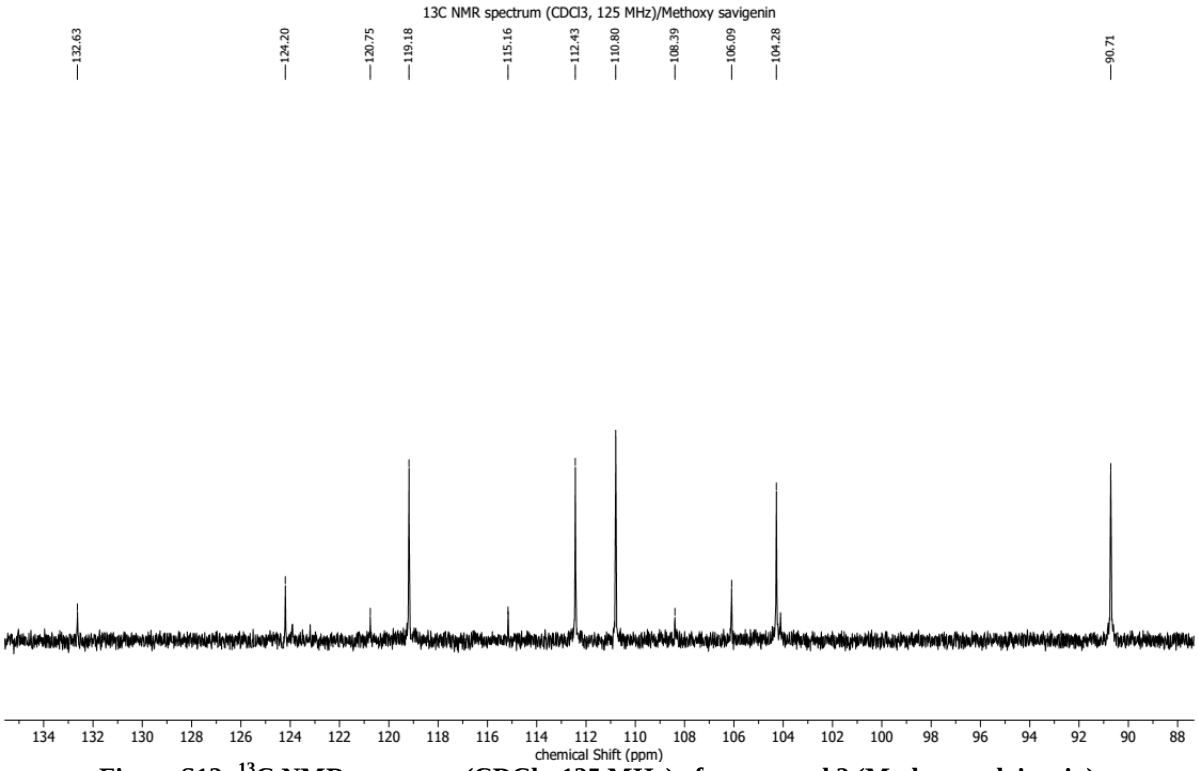


Figure S12: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of compound 2 (Methoxy salvigenin).

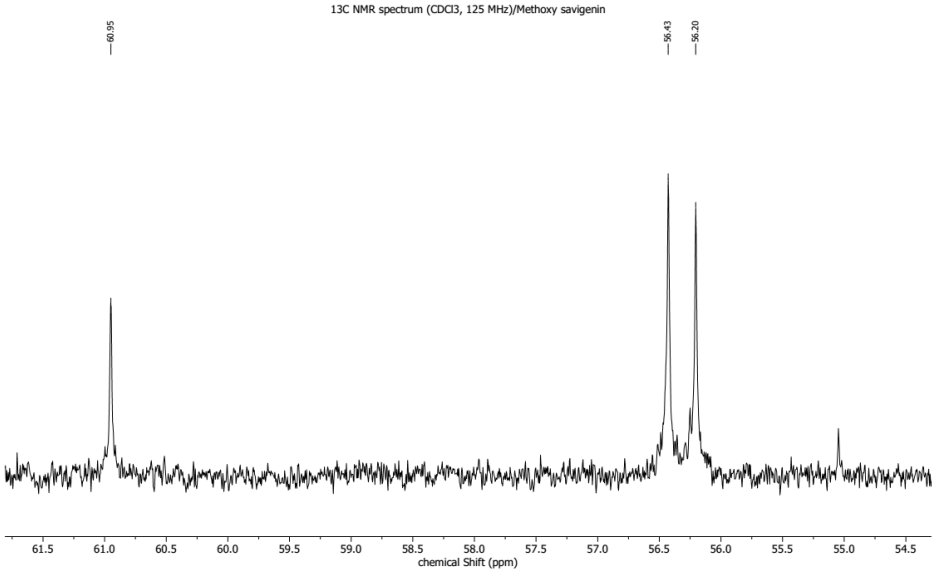


Figure S13: Expansion of ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of compound 2 (Methoxy salvigenin).

Low Res + ESI (Positive):

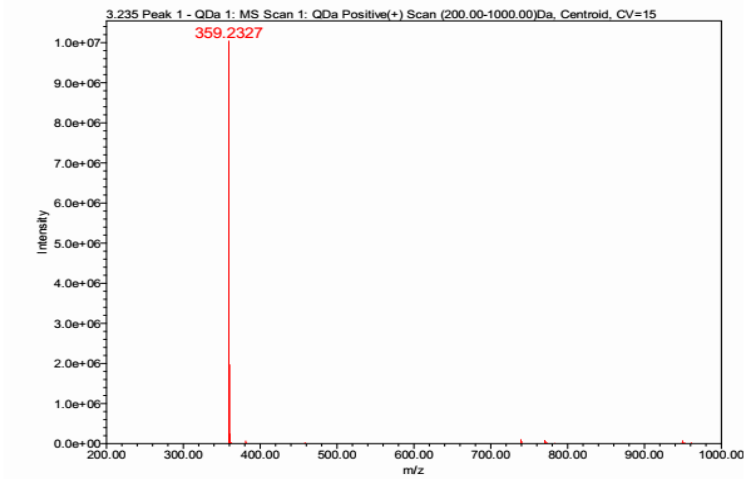


Figure S14: EIMS of compound 2 (Methoxy salvigenin).

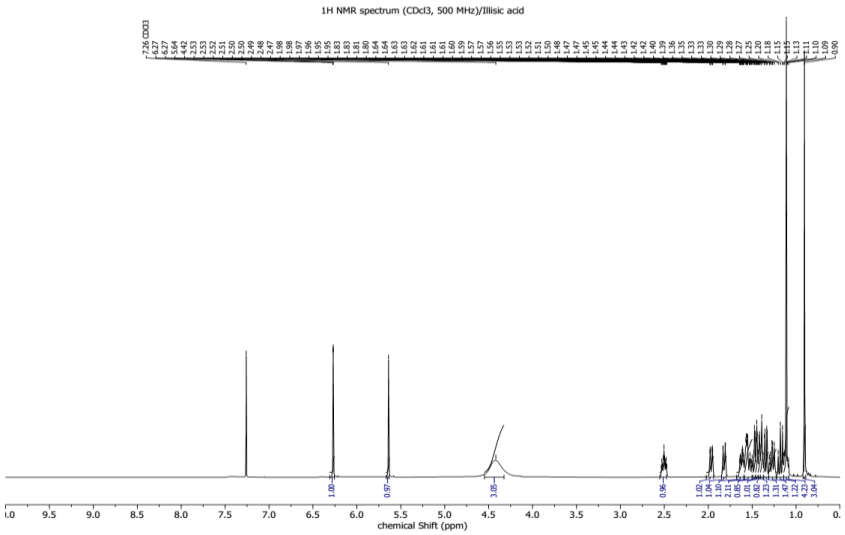


Figure S15: ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound 3 (illisic acid).

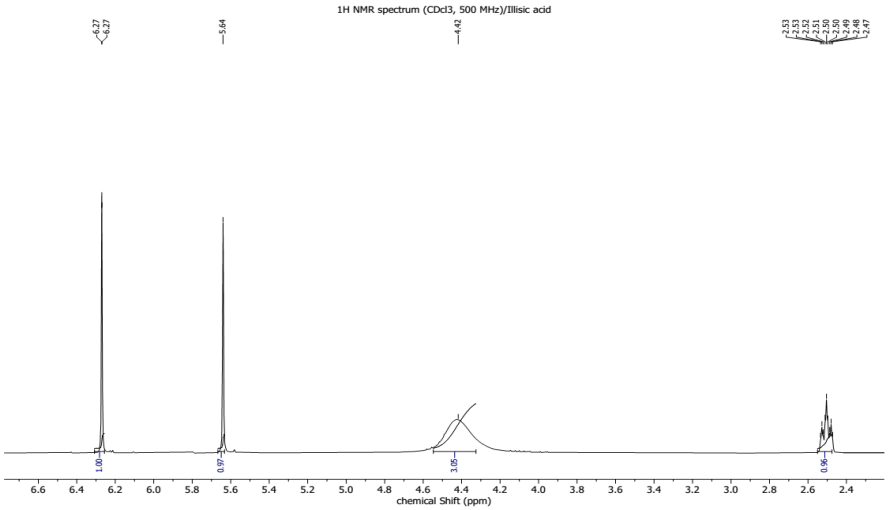


Figure S16: Expansion of ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound 3 (illisic acid).

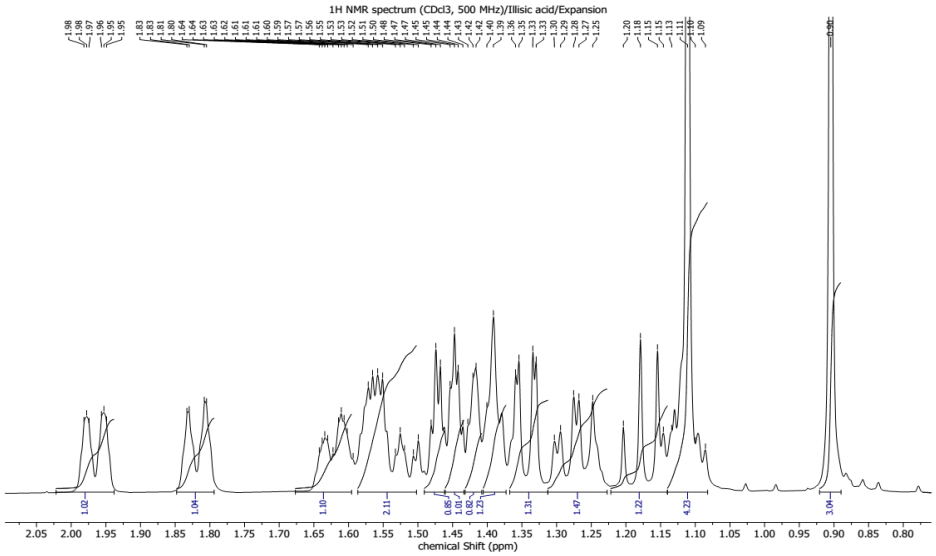


Figure S17: Expansion of ^{1}H NMR spectrum ($CDCl_3$, 500 MHz) of compound 3 (illisic acid).

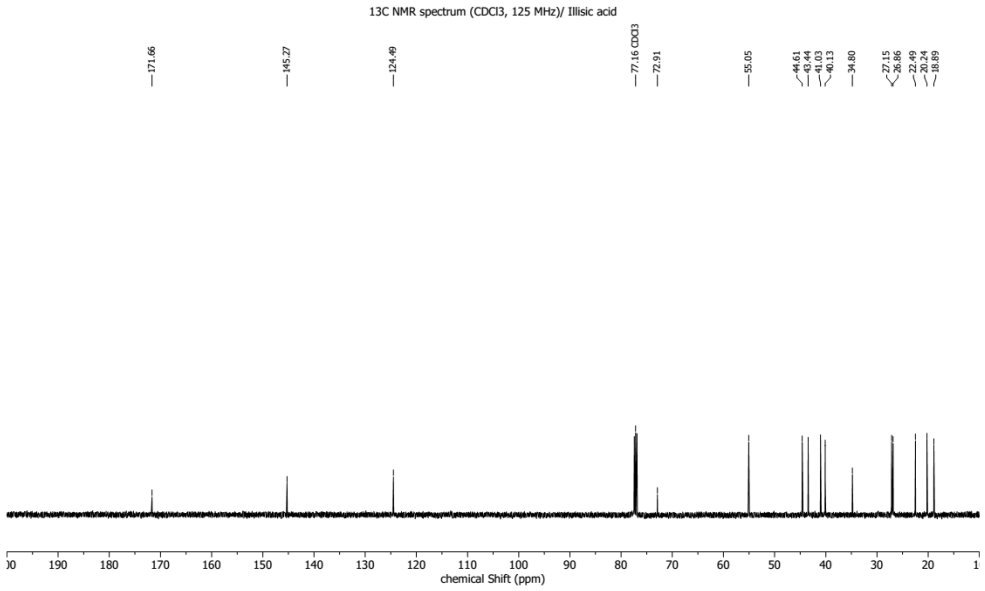


Figure S18: ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of compound 3 (illisic acid).

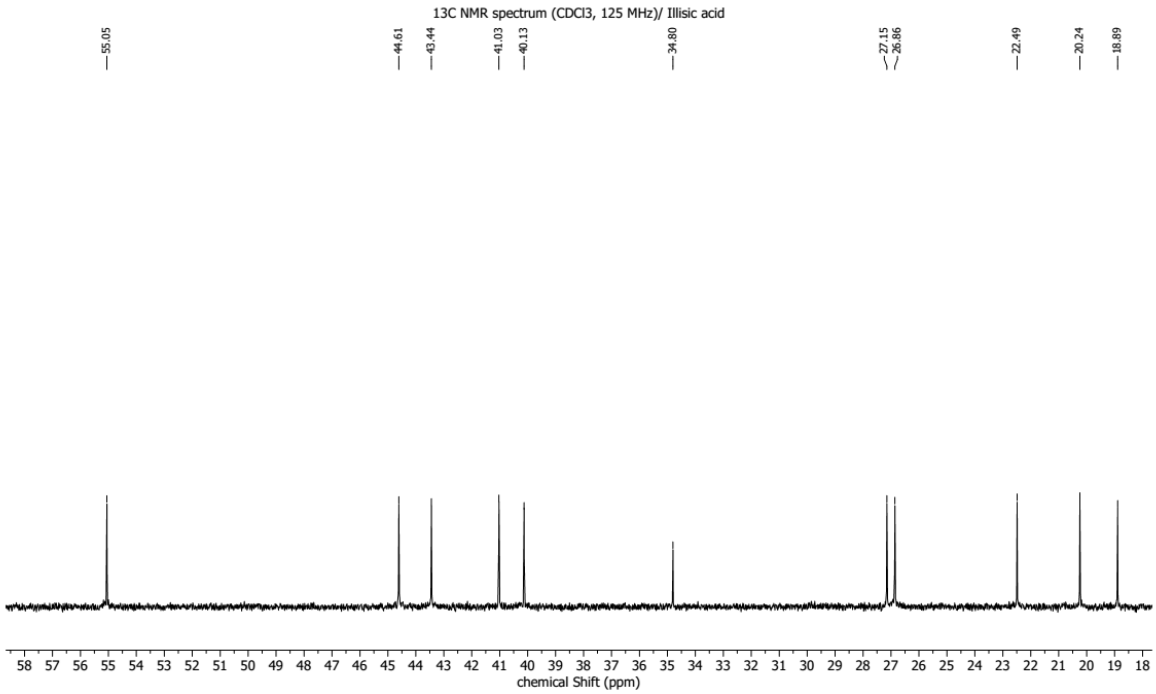


Figure S19: Expansion of ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of compound 3 (illisic acid).

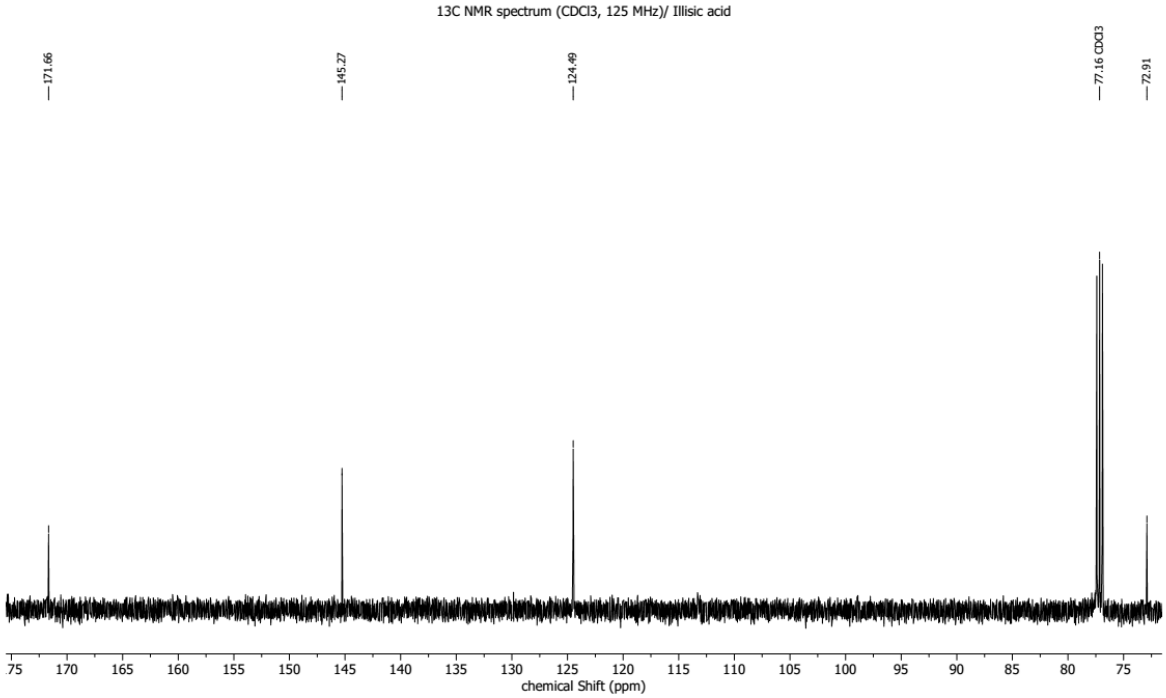


Figure S20: Expansion of ^{13}C NMR spectrum ($CDCl_3$, 125 MHz) of compound 3 (illisic acid).

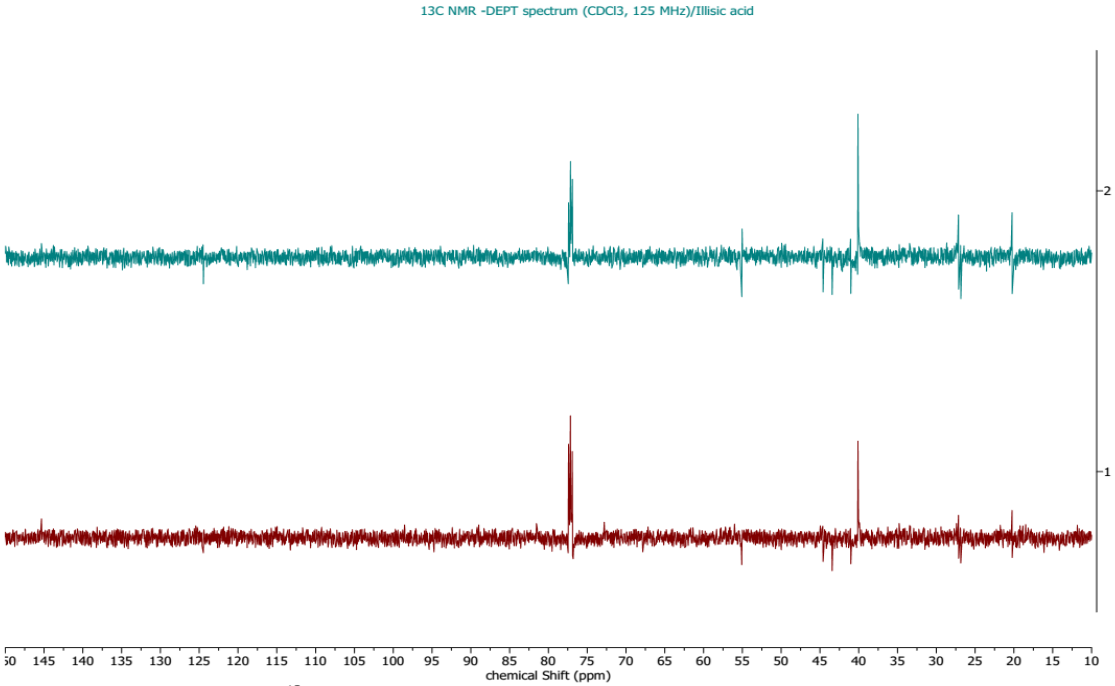


Figure S21: DEPT ^{13} C NMR spectrum ($CDCl_3$, 125 MHz) of compound 3 (illisic acid).

SUPPLEMENTARY INFORMATION

Supplementary Information is available for this paper in Annexure 1.

Annexure 1 contents supporting information (Spectroscopic data consisting ^{1}H NMR,^{13}C NMR, DEFT, EIMS, HRESIMS of compound (**1-3**) were enclosed in attached file Crystallographic data for the structures reported in this paper have been deposited with the Cambridge Crystallographic Data Centre (CCDC). Copies of the data can be obtained, free of charge, on application to Director, CCDC, 12 Union Road, Cambridge CB2, 1EZ, UK (fax: + 44-(0)1223-336033 or email: deposit@ccdc.cam.ac.uk

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ACKNOWLEDGEMENTS

We express our sincere gratitude to Prof. David G. I. Kingston, Department of Chemistry, Virginia Tech, USA, for fostering this opportunity to collaborate with Prof. Esperanza J. Carcache de Blanco, College of Pharmacy, The Ohio State University, Ohio, Columbus. We are thankful to Dr. Dmitriy Uchenik and Dr. Deepa Krisnan, OSU College of Pharmacy instrumental facility, for allowing acquisition of HR MS data. Authors are very much grateful to Head of Division, Bio-Organic Division and GD of BSG for their continuous encouragement and support.

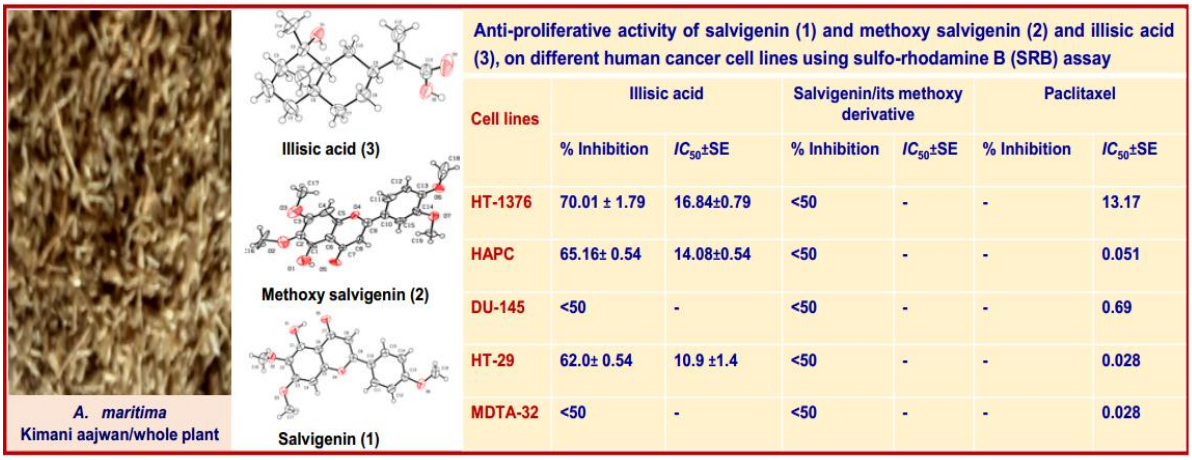
CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

AUTHOR CONTRIBUTION STATEMENT

AKB performed isolation, structural characterization, chemical transformation/reaction and drafted the manuscript. SF contributed analysis tools and analyzed the XRD data of the crystals, structure analysis and computation. IC and ESA contributed to the samples/reagents/materials/ analysis tools and analyzed anti-proliferative assay the data. EJCB supervised biological experimental, analyzed the data of anti-proliferative assay, reviewed the manuscript, and managed for acquiring HRESI-MS and LR-ESI-MS data.

GRAPHICAL ABSTRACT



Cell lines	Illisic acid		Salvigenin/its methoxy derivative		Paclitaxel	
	% Inhibition	IC_{50}±SE	% Inhibition	IC_{50}±SE	% Inhibition	IC_{50}±SE
HT-1376	70.01 ± 1.79	16.84±0.79	<50	-	-	13.17
HAPC	65.16± 0.54	14.08±0.54	<50	-	-	0.051
DU-145	<50	-	<50	-	-	0.69
HT-29	62.0± 0.54	10.9 ±1.4	<50	-	-	0.028
MDTA-32	<50	-	<50	-	-	0.028

REFERENCES

1. The Plant List. A working list of plant species: (http://www.worldfloraonline.org/taxon/wfo-4000006171. Accessed on: 26 Dec. **2022**)
2. G. V. Satyavati, A. Gupta, and N. Tandon, N. Medicinal plants of India, vol. II, New Delhi (INDIA), ICMR, 1987; 407-410.
3. A. K. Nadkarni. *Indian Materia Medica*, 1954; 1: 1129-1103, 3rd edition, Bombay Popular Prakashan, Bombay (India)
4. R. N. Chopra, S. L. Nayar and I. C. Chopra: Glossary of Indian medicinal Plants: *Council Sci. Ind. Res*., New Delhi, 1956.
5. Anonymous 1. The Wealth of India, Raw materials, CSIR, The Wealth of India: Raw materials, 1985.
6. The Wealth of India-Raw materials, vol I: A; 434-443. Published by: Publications & Information Directorate, CSIR Hillside Road, New Delhi (INDIA).
7. C. H. Drury. The useful Plants of India (with notices of their chief value in commerce, medicine & the arts), 1978; William H. Allen & Co, 13 Waterloo place, London, S. W.
8. S. Z. Taghizadeh Rabe, M. Mahmoudi, A. Ahi, S. A. Emami. Anti-proliferative effects of extracts from Iranian *Artemisia* species on cancer cell lines. *Pharm Biol*., 2011; 49: 962-969.
9. A. H. Gilani, S. Yaeesh, Q. Jamal, M. N. Ghayur. Hepatoprotective activity of aqueous–methanol extract of Artemisia vulgaris. *Phytother Res*., 2005; 19: 170-172.
10. A. F. Walker. Of Hearts & Herbs. *Biologist*, 1996; 43: 177-180.
11. V. Dhingra, V. Rao, L. Narasu. Current state of artemisinin and its derivative as anti-malarial drugs. *Life Sci*., 2000; 66: 279-300.
12. G. Li, X. Guo, Z. Jin, J. Wang, H. Hian and Z. Li., *J. Trad. Chin. Med*., 1982; 2: 125-127 and literature cited therein.
13. J. Gruenwald. PDR for herbal medicines, 1st edition, 1998, Montvale, NJ, Medicinal economical Company Inc.
14. J. A. Duke. Handbook of Medicinal Herbs, 2000; Second Edition, Boca Raton, FL: CRC Press.
15. D. L. Klayman. Qinghaosu (Artemisinin) an anti-malarial drug from China. *Science*, 1985; 228: 1049-1055 and literature cited therein.
16. D. L. Klayman, A. Lin, N. Acton, J. P. Scovill, J. M. Hoch, W. K. Milhous and A. D. Theharides. *J. Nat. Prod*., 1984; 47:715-717 and literature cited therein.
17. H. Ekiert, J. Świątkowska, P. Klin, A. Rzepiela, A. Szopa. *Artemisia annua*-Importance in Traditional Medicine and Current State of Knowledge on the Chemistry, Biological Activity and Possible Applications. Planta Med., 2021; 87: 584-599. DOI: 10.1055/a-1345-9528
18. R. S. Bhakuni, D. C. Jain, R. P. Sharma and S. Kumar. Secondary metabolites of *Artemisia annua* and their biological activity. *Current Science*, 2001; 80: 35-48.
19. G. Li, X. Guo, Z. Jin, J. Wang, H. Hian and Z. Li., *J. Trad. Chin. Med*., 1982; 2: 125-127 and literature cited therein.
20. J. Gruenwald. PDR for herbal medicines, 1st edition, 1998, Montvale, NJ, Medicinal economical Company Inc.
21. P. Newton and N. White. *Anu. Rev. Medicine*, 1999; 50: 179-192.
22. D. L. Klayman. Qinghaosu (Artemisinin) an antimalarial drug from China. Science, 1985; 228:1049-1055 and literature cited therein.
23. D. L. Klayman, A. Lin, N. Acton, J. P. Scovill, J. M. Hoch, W. K. Milhous and A. D. Theharides. *J. Nat. Prod*., 1984; 47: 715-717. and literature cited therein
24. WHO report on implementation of global malaria control strategy (1993-2000) Technical Report Series No. 839, World Health Organization, ISBN 13 9789241208390, order No. 11000839, WHO Press Editions de I'MOS-Edicones de la OMS.
25. D. B. Zhao, Y. X. W. Yang, X. H. Z. Liu and H. Q. Wang. *Zhongguo Zhong Yao Za Zhi*, 2005; 30: 1430-1432.

26. H. Lai and N. P. Singh. Cancer Letters, 1995; 91: 41-46.
27. H. Lai and N. P. Singh. Cancer Letters, 1995; 91: 41-46.
28. W. H. El-Tantawy. Biochemical effects, hypolipidemic and anti-inflammatory activities of *Artemisia vulgaris* extract in hypercholesterolemic rats. *J. Clin. Biochem. Nutr.*, 2015; 14-19.
29. S. B. Erel, G. Reznicek, S. G. Şenol, N. U. Yavasoglu, S. Konyalioglu et al. Antimicrobial and antioxidant properties of Artemisia L. species from western Anatolia. Turk J Biol., 2012; 36: 75-84.
30. N. Balasundram, K. Sundram, S. Samman. Phenolic Compounds in Plants and Agri-industrial By-products: Antioxidant Activity, Occurrence, and Potential Uses. *Food Chem*., 2006; 99: 191-203. DOI: 10.1016/j.foodchem.2005.07.042.
31. K. S. Bora and A. Sharma. Phytochemical and Pharmacological potential of *Artemisia absinthium* and *Artemisia asiatica* Nakai. *J. Pharm. Res*., 2010; 3: 325-28.
32. S. Zaman, B. M. Zahoor, S. W. A. Shah, Z. Ullah, R. Ullah, A. Alotaib. Pharmacognostic evaluation of Artemisia maritima L. a highly medicinal specie of genus Artemisia. Saudi J. Biol Sci., 2022; 29: 103419. doi: 10.1016/j.sjbs.2022.103419
33. F. Malagon, J. Vazquez, G. Delgado and A. Ruiz. *Parasitology*, 1997; 39: 3-7.
34. A. Caner, M. Dos kaya, A. D. girmenci, H. Can, S. Baykan S. Comparison of the effects of *Artemisia vulgaris* and *Artemisia absinthium* growing in western Anatolia against trichinellosis (Trichinella spiralis) in rats. *Exp Parasitol*., 2008; 119: 173–179.
35. Sun WC, Han JX, Yang WY, Deng DA, Yue XF, Antitumor activities of 4 derivatives of artemisic acid and artemisinin B in vitro. *Acta Pharmacol Sin.*, 1992; 13: 541-543.
36. G. Sravanthi, G. N. Reddy, R. Gunnam, J. V. Suresh, G. Harika. Evaluation of antidiabetic activity of ethanolic extract plant material (Leaves) Artemisia vulgaris. *World J. Pharm. Biotechnol*., 2015; 2: 16-21.
37. Shinoda teste: V. E. Tyler, L. R. Brady, J. E. Robbers. Pharmacognosy, 9th edition. Leo and Fabigar. Philadephia, 1988; 856.
38. Na/Hg teste: Bouveault-Blanc type reduction reaction.
39. F. M. Dean, ′Naturally Occurring Oxygen Ring Compounds′, Butterworths: London, UK, 1963; 288-289.
40. T. J. Mabry, K. R. Markham, M. B. Thomas, ′The Systematic Identification of Flavonoid', Springer-Verlag: New York, **1970**; 36-38.
41. Nonius. CAD4/PC Software, Version 2.0. Nonious 1993; GmbH, Solingen, Germany.
42. G. M. Sheldrick. SHELXL-97 (1997): Program for the refinement of crystal structures, University of Gottingen, Germany.
43. G. M. Sheldrick. SHELXS-97 (1997): Program for computing structure solution, University of Gottingen, Germany.
44. A. L. Spek, A. L. J. Appl. Cryst., 2003; 36: 7-13.
45. Stoe-REDU4, Data Reductions Program, Version 6.2c (1987). Stoe&Cie GmbH, Darmstadt, Germany.
46. G. D. Anaya-Eugenio, C. Y. Tan, L. H. Rakotondraibe, E. J. Carcache de Blanco. Tumor suppressor p53 independent apoptosis in HT-29 cells by auransterol from *Penicillium aurantiacobrunneum*. *Biomed. Pharmacother*., 2020; 127: 110124.
47. V. Vichai, K. Kirtikara. Sulfo-rhodamin B colorimetric assay for cytotoxicity screening. *Nat. Protoc*., 2006; 1: 1112-1116.
48. G. D. Anaya-Eugenio, E. M. Addo, N. Ezzone, M. Henkin, T. N. Ninh, Y. Ren, D. D. Soejarto, A. D. Kinghorn, Car cache de Blanco. Caspase-dependent apoptosis in prostate cancer cells and zebrafish by corchorusoside C from *Streptocaulonjuventas*. *J. Nat. Prod.*, 2019; 82: 1645-1655.