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Research Article

Chemical Composition from *Cucumis prophetarum L*

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Abstract

Petroleum ether extract of root part of *Cucumis prophetarum L.* afforded one compound 4,4- dimethyl-Stigmast-5-22E, 24-trien-3 β -ol which has structural similarity with Stigmast-5-22E-dien-24S-3 β -ol and Propheterin. More over the ^{1}H-NMR and ^{13}C-NMR spectrum of the isolated compound matched with the literature values of previously reported data's whereas, the methanol extract afforded one compound 17-octahydro-17-((E)-6-hydroxy-6-methylhept-3-en-2-yl)-9,13-dimethyl-6H-cyclopenta [a] phenanthren-7-yl acetate. The structural elucidation of these compounds was conducted using ^{1}H-NMR, ^{13}C-NMR, UV, IR and DEPT spectroscopic methods.

Keywords: Cucurbitaceae; *Cucumis prophetarum L.*; Cucurbitacins; Spectroscopy

Introduction

Cucumis prophetarum L. belongs to the family of Cucurbitaceae, the plants of the family are collectively known as cucurbits [1-3].

Previous studies revealed that cucurbitacins were isolated as constituents of *Cucumis prophetarum L.*: such as Cucurbitacin B, isocucurbitacin B, dihydrocucurbitacin B, cucurbitacin E, isocucurbitacin E, dihydrocucurbitacin E, isocucurbitacin D, dihydroisocucurbitacin D, cucurbitacin I, dihydrocucurbitacin I, cucurbitacin Ql, and dihydrocucurbitacin Ql, Cucurbitacin P, Cucurbitacin O [1-6].

Cucurbitacins are highly oxygenated tetracyclic triterpenoids, the name cucurbitane has been proposed for the hydrocarbon skeleton of cucurbitacins and characterized as 19-(10→9 β)-abeo-10 α -lanost-5-ene also known as 9 β -methyl-19-nor lanosta-5-ene [7-11] (Figure 1). Cucurbitacins have received a great deal of attention because of their cytotoxic, anti-proliferative, anti-inflammatory, analgesic, antimicrobial, anti-helminthic hepato-protective, cardiovascular, and anti-diabetic effects, antioxidant activity and anticancer effects *in vitro* and *in vivo* [12-15].

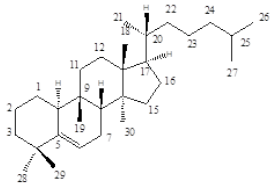

Figure 1: Basic structure of Cucurbitacins (19(10→9 β)-abeo-10 α -lanost-5-ene).

Moreover, as cited by Abdulrhman et al. the antidiabetic and antioxidant activity of the different fractions of fruits of *Cucumis prophetarum L.* has been reported [16].

Cucumis prophetarum L. Linnaeus subsp. dissectus (Naud.) type: Ethiopia; which is locally called as yemder-inbuyi used in traditional medicine for the treatment of rabies and while giving birth to a child, it helps to remove the placenta quickly. There is no report on the isolation and characterization of natural products from the plant of Ethiopian origin. Therefore, the aim of this work was extraction, isolation, and structural elucidation of components from the root of *Cucumis prophetarum L.* with the help of spectroscopic techniques.

Materials and Methods

Electrical grinder (KIkA® WERKE), Rotary evaporator, digital measuring balance, Shaker, Erlenmeyer flask, conical flask, TLC

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plate (pre-coated aluminum sheet, 20 × 20 cm) silica gel (60 F_{254}), 254 nm UV lamp, were used. Melting points were measured in glass capillaries using Thiele tube. Techniques employed for purification of compounds were column chromatography (CC) which was performed using silica gel (230-400 mesh size) stationary phase and organic solvent as mobile phase. Iodine was also employed for visualization purpose in which a few iodine crystals were placed in a TLC tank and warmed for a few minutes then spots appeared on a TLC plate when placed in a tank for one minute [17].

^{1}H and ^{13}C-NMR spectra were recorded using a Bruker 400 MHz Advance NMR spectrometer at 22°C, in deuterated chloroform ($CDCl_3$) using tetramethylsilane (TMS) as the internal standard; chemical shifts are given in (ppm) values. The UV-Vis spectra were determined using spectrophotometer (200-400 nm) at room temperature. The IR spectra were measured on a spectrometer (400-4000 cm^{-1}) in KBr pellets.

Plant materials

The root part of the plant *Cucumis prophetarum L.* (500 g) was collected from North Gondar, Ethiopia. The plant was botanically authenticated in the Department of Biology, College of Natural and Computational Science, University of Gondar.

Coding system

In CP-1 and CP-4 C- stands for the genus name Cucumis and, P- stands for the species name prophetarum. The number behind CP indicates fraction number while it was isolated by using column chromatography.

Drying and pulverizing

The root part of *Cucumis prophetarum L.* plant was thoroughly washed, chopped in to small pieces and dried at room temperature under shade. The dried root sample was then pulverized by using electrical grinder in to fine part, weighs using digital measuring balance and stored at room temperature.

Extraction

The air dried and powdered roots of *Cucumis prophetarum L.* (500 g) were macerated in Petroleum ether (2.5 liter) for 7 days. The Petroleum ether extract was evaporated in a rotary evaporator under reduced pressure and it results a semi solid yellow residue (5.0 g, 1%).

Second extract after Petroleum ether was taken by soaking the plant in methanol (2.5 liter) for 7 days, and the solvent was filtered and evaporated in a rotary evaporator under reduced pressure to yield green semisolid residue (10 g, 2%).

Isolation of CP-1 and CP-4

The isolation was carried out by column chromatography. The Petroleum ether (5.0 g), and the methanolic extract (10 g) were subjected to TLC which revealed the presence of several compounds. In order to separate individual constituents, the petroleum ether and methanolic extracts were subjected to column chromatography over silica gel (65 g, 70 g) (230-400 mesh). Firstly, each extract was mixed with 5 g of silica gel separately, then the mixture was evaporated to dryness. The cotton wool was used as a support for the column. The column was packed by wet packing method. The slurry of the adsorbent was prepared in the same solvent that was used in the chromatographic process. The slurry was added to the column gradually up to approximately one third of the column height and then the sample mixture was loaded at the top [18]. The gradient elution method was followed. The elution was successfully carried out with increasing polarities of a mixture of acetone: petroleum ether from (1:3) to (3:3). Five fractions were collected; Thin Layer Chromatography profiling was done simultaneously in different solvent system. The fraction that has similar Rf value was pooled and concentrated to give total of three and nine fractions for petroleum ether and methanolic extract respectively. Again, the concentrated fractions applied to Column chromatography in which the elution was carried out with acetone: petroleum ether (2:3) solvent system with simultaneously increasing polarity. The fractions eluted were subjected to TLC profiling again using several solvent systems including acetone: petroleum ether (3:3), chloroform: petroleum ether (1:2), the fractions giving single spot were regarded as pure. Moreover, and these fractions were concentrated using rotary evaporator. The structure of purified fraction was elucidated by spectral studies (UV-VIS, IR, ^{1}H-NMR, ^{13}C-NMR and DEPT) and by comparing the data from the literature.

Results and Discussion

Two compounds were isolated and characterized from the petroleum ether and methanol extract CP-1 and CP-4 respectively. Structural elucidation of the compounds was deduced with the help of spectroscopic techniques including UV-Vis, IR, ^{1}H-NMR, ^{13}C-NMR and DEPT-135 and with comparison of spectroscopic data obtained in the literature for related compounds. The characterizations of the two compounds are described below.

Characterization of CP-1

Compound CP-1 (50 mg, 0.01%) was a light-yellow crystal, from petroleum ether extract and isolated by repeated column chromatography with acetone-petroleum ether (3:1) solvent system and concentrated under reduced pressure using rotavapor. The Rf value was 0.65 and its melting point was determined to be 174-175°C. Its spectral studies (characterization of compound by UV-VIS, IR, ^{1}H-NMR, ^{13}C-NMR and DEPT) and by comparing the data from the literature.

UV λ max spectrometer (Appendix 1) 263 nm suggesting that there is Cisoid dienes that has two alkyl substituents.

The IR λ max cm^{-1} spectrum (Appendix 2) comprised of 3400 cm^{-1}, 2931 cm^{-1}, 2868 cm^{-1}, 1663 cm^{-1}, 1041 cm^{-1}. Thus the IR spectrum showed absorption band for hydroxyl group so it revealed the presence of OH groups (3400 cm^{-1}), The strong bands at 2931 cm^{-1} and 2868 cm^{-1} arose due to the presence of alkyl groups and to an olefinic system (C=C) at 1663 cm^{-1}, It also revealed the presence of and to C-O stretching at 1041 cm^{-1}.

^{1}H-NMR (400 MHz $CDCl_3$) spectrums (Appendix 3) showed six methyls resonated at δ 0.81 (3H, s), δ 0.83 (3H, d), δ 0.83 (3H, d), δ 0.93 (3H, d), δ 1.05 (3H, s) and δ 1.36 (3H, d). The signal resonated at δ 2.03 (^{1}H, m) accounted for the proton in carbon-25. Four olefinic signals, each of one proton resonated at δ 4.23 (^{1}H, m), δ 5.04 (^{1}H, dd), δ 5.06 (^{1}H, dd), δ 5.17 (^{1}H, s) and their associated carbons are resonated at δ 117.4 (C-6), 138.2 (C-22), 129.4 (C-23) and 117.4 (C-28) respectively which indicated the three double bonds in the molecule. Carbinylic proton absorbed at δ 3.61 (^{1}H m) while bonded with C-3 which resonated at δ 71.07, all this indicating a hydroxyl group at C-3 position.

^{13}C-NMR ($CDCl_3$, 400 MHz) (Appendix 4) δ: 139.5 (C-5), 138.2 (C-22), 129.4 (C-23), 129.4 (C-24) 117.4 (C-28), 117.4 (C-6), 71.0 (C-3), 55.8 (C-14), 55.1 (C-17), 51.2 (C-9), 43.3 (C-13), 43.2 (C-4), 40.2 (C-20), 39.5 (C-12), 37.1 (C-1), 36.7 (C-10), 31.9 (C-8), 31.9 (C-25), 31.8 (C-2), 31.4 (C-7), 29.1 (C-16), 25.4 (C-15), 21.5 (C-30), 21.4 (C-31), 21.3 (C-21), 21.1 (C-11), 20.9 (C-27), 19.0 (C-19), 18.9 (C-26), 12.3 (C-18), 12.0 (C-29), Hydroxyl group substitution at C-3 was confirmed by signal at δ 71.07 while olefinic carbons C-5 and C6 were resonated at δ 139.5 and 117.4, C-22 and C-23 were resonated at δ 138.20 and 129.46, and C-24 and C-28 were resonated at δ 129.42 and 117.43 respectively, thus the ^{13}C-NMR spectrum of compound CP-1 exhibited thirty one carbon signals (Table 1).

DEPT-135: (Appendix 5) The spectra revealed the presence of 26 carbon signals due to eleven methine, seven methylene, eight methyl and, five quaternary carbons. The structure of the isolated compound was elucidated by detailed ^{1}H and ^{13}C-NMR spectroscopy and by comparison of the spectral data with the literature results, thus the compound isolated (CP-1) is derivative of Stigmast-5-22E-dien-24S-3 β -ol (Table 2). Moreover, it has structural similarities with Propheterin, previously isolated compound from *Cucumis prophetarum L* (Figure 2).

Table 1: Proton decoupled ^{13}C NMR and DEPT spectra for compound CP-1.

Position	^{13}C-NMR for compound CP-1	DEPT-135 δ in ppm	Remark
1	37.1	37.1	CH_2
2	31.8	31.8	CH_2
3	71	71.07	CH
4	43.2	-	Quaternary C
5	139.5	-	Quaternary C
6	117.4	117.4	CH
7	31.4	31.4	CH_2
8	31.9	31.9	CH
9	51.2	51.2	CH
10	36.7	-	Quaternary C
11	21.1	21.1	CH_2
12	39.5	39.5	CH_2
13	43.3	-	Quaternary C
14	55.8	55.8	CH
15	25.4	25.4	CH_2
16	28.5	28.5	CH_2
17	55.1	55.1	CH
18	12.3	12.3	CH_3
19	19	19	CH_3
20	40.2	40.2	CH
21	21.3	21.3	CH_3
22	138.2	138.2	CH
23	129.4	129.4	CH
24	129.4	-	Quaternary C
25	31.9	31.9	CH
26	20.9	20.9	CH_3
27	18.9	18.9	CH_3
28	117.4	117.4	CH
29	12	12	CH_3
30	21.5	21.5	CH_3
31	21.4	21.4	CH_3

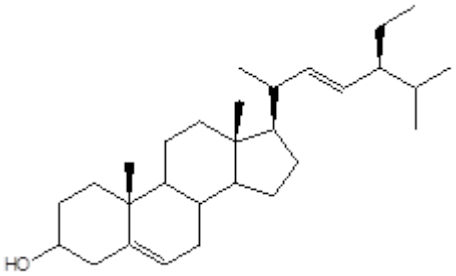

Figure 2: Structure of Stigmast-5-22E-dien-24S-3 β -ol.

The comparison of ^{13}C-NMR spectra of compound CP-1 (Figure 3) with Stigmast-5-22E-dien-24S-3 β -ol reported in the literature

showed a good agreement except Cp-1 has two substituted methyl groups at carbon-4 which resonated at δ 21.3 (C-30) and 21.1 (C-31) and the new compound (CP-1) has unsaturation at carbons 24-28 (Δ24-28), so the carbon-24 and carbon-28 in compound CP-1 resonates at δ 129.4 and 117.4 respectively, whereas in Stigmasterol carbon-24 and carbon-28 resonates at δ 51.2 and 25.4 respectively (Table 2). Furthermore, it has structural and spectroscopic data similarity with propheterin except CP-1 has unsaturation at carbon 22-23 (Δ22-23).

Table 2: Comparisons of Proton decoupled ^{13}C NMR spectra of compound CP-1 with literature value of Stigmasterol.

Position	^{13}C-NMR for compound CP-1	Literature value of Stigmast-5-22E-dien-24S-3 β -ol
1	37.1	37.3
2	31.8	31.6
3	71	71.8
4	43.3	42.3
5	139.5	140.8
6	117.4	121.7
7	31.4	31.9
8	31.9	31.9
9	51.2	51.2
10	36.7	36.5
11	21.1	21.1
12	39.5	39.7
13	43.3	42.3
14	55.8	56.9
15	25.4	24.3
16	29.1	28.3
17	55.1	56.1
18	12.3	12.4
19	19	19.4
20	40.2	40.5
21	21.3	21.2
22	138.2	138.5
23	129.4	129.3
24	129.4	51.2
25	31.9	31.9
26	20.9	21.1
27	18.9	19
28	117.4	25.4
29	12	12.1
30	21.5	-
31	21.4	-

The comparative study of its ^{13}C NMR spectroscopic date with the reported data revealed its identity as derivative of Stigmast-5-22E-dien-24S-3 β -ol (Figure 2). Therefore CP-1 is derivative of Stigmasterol.

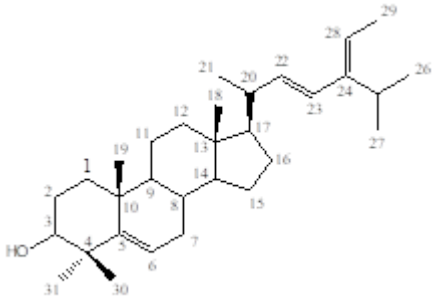

Figure 3: The proposed structure of Structure of CP-1.

Characterization of CP-4

The compound CP-4 (55 mg, 0.011%) was a light green solid, from methanol extract and isolated by repeated column chromatography with acetone: petroleum ether (3:1) solvent system and was concentrated under reduced pressure using rotavapor. The Rf value was 0.73. Its structure was elucidated by spectral studies (characterization of compound by IR, ^{1}H-NMR, ^{13}C-NMR and DEPT spectrums).

The IR λ max cm^{-1} spectrum of the compound CP-4 (Appendix 6), revealed the absorption band at 3407 cm^{-1} which showed the strong O-H stretching that indicated the presence of a hydroxyl group. The strong absorption band at 2928 cm^{-1} and 2856 cm^{-1} showed the presence of the C-H stretching for sp3 hybridized carbon. The strong absorption at 1714 cm^{-1} showed the presence of carbonyl group. The absorption peak at 1465 cm^{-1} reveals the presence of aromatic group. Strong C-O stretch confirms by the peak at 1075 cm^{-1}.

The ^{1}H-NMR (400 MHz $CDCl_3$) spectrums (Appendix 7) showed six 3H signals which resonated at δ 0.844 (3H, s), δ 0.861 (3H, s), δ 0.879 (3H, s), δ 0.886 (3H, d), δ 0.905 (3H, s) and δ 2.042 (3H, s) indicating the presence of six methyls groups (Table 3).

Table 3: Proton decoupled ^{13}C NMR and DEPT spectra for compound CP-4.

Position	^{13}C -NMR for compound CP-4	DEPT-135 δ in ppm	Remark
1	128.7	128.7	CH
2	125.7	125.7	CH
3	125.7	125.7	CH
4	128.7	128.7	CH
5	132.3	-	Quaternary C
6	29.6	29.6	CH_2
7	68.1	68.1	CH
8	30.3	30.3	CH
9	31.9	-	Quaternary C
10	136.4	-	Quaternary C
11	207.5	-	Quaternary C
12	38.6	38.6	CH_2
13	29.8	-	Quaternary C
14	30.9	30.9	CH
15	24.8	24.8	CH_2
16	23.7	23.7	CH_2
17	50.4	50.4	CH
18	19.7	19.7	CH_3
19	22.6	22.6	CH_3
20	29.6	29.6	CH
21	14	14	CH_3
22	130.9	130.9	CH
23	129.5	129.5	CH
24	34	34	CH_2
25	76.7	-	Quaternary C
26	28.9	28.9	CH_3
27	27.1	27.1	CH_3
1'	167.8	-	Quaternary C
2'	22.9	22.9	CH_3

The peaks from δ 2.298 to δ 1.238 accounted for the methine and methylene protons. The peak at δ 2.790 (^{1}H, t) integrated for the proton at carbon-8, the peak at δ 4.202 (^{1}H, t) accounted for the proton at carbon-7 which is directly attached to oxygen. Two olefinic signals, each of one proton resonated at δ 5.357 (^{1}H, t) and δ 5.344 (^{1}H, q). In the aromatic ring the signal for the equivalent protons H-1 and H-4 appeared to be a doublet (δ 7.100), the signal for the H-2 and H-3 protons to be split into a double by the H-1 and H-4 protons respectively, and each peak of the doublet to be split into another doublet by the adjacent protons, forming a doublet of doublets resonating at δ 7.69 and δ 7.53.

^{13}C-NMR ($CDCl_3$, 400 MHz) (Appendix 8) δ : 207.532 (C-11), 167.840 (C-1'), 136.460 (C-10), 132.372 (C-5), 130.927 (C-22), 129.543 (C-23), 128.787 (C-1), 128.787 (C-4), 125.754 (C-2), 125.754 (C-3), 76.795 (C-25), 68.161 (C-7), 50.427 (C-17), 38.696 (C-12), 34.005 (C-24), 31.924 (C-9), 30.913 (C-14), 30.338 (C-8), 29.872 (C-13), 29.698 (C-20), 29.618 (C-6), 28.911 (C-26), 27.193 (C-27), 24.819 (C-15), 23.718 (C-16), 22.979 (C-2'), 22.692 (C-19), 19.721 (C-18), 14.053 (C-21) Hydroxyl group substitution at C-25 was confirmed by signal at δ 76.795 while equivalent aromatic carbons C-1 and C-4 resonate at same chemical shift δ 128.787 whereas two equivalent aromatic carbons C-2 and C-3 appear at the same chemical shift δ 125.754 (Table 3). The spectrum of CP-4 exhibited twenty-nine carbon signals indicating eleven methine, five methylene, six methyl and, seven quaternary carbons atoms (Figure 4).

The DEPT-135 spectra (Appendix 9) revealed the presence of 22 carbon signals indicating eleven methine, five methylene, six methyl and, seven quaternary carbons atoms.

Hence the structure of the isolated compound was elucidated by detailed IR, ^{1}H, ^{13}C-NMR and DEPT spectra.

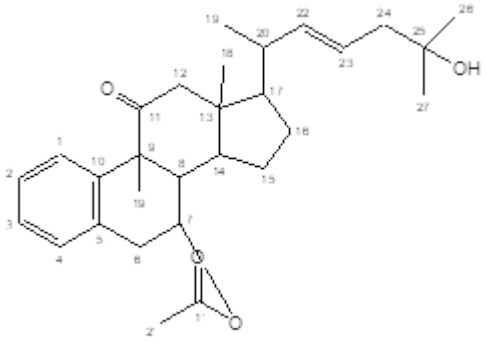

Figure 4: The proposed structure of Structure of CP-4.

Conclusion

The genus *Cucumis prophetarum L.* used in traditional medicine in Middle East, North Africa and Ethiopia, Moreover the secondary metabolites such as class of Cucurbitacins isolated from the genus exhibits anti-inflammatory, analgesic,

antimicrobial, anti-helminthic hepato-protective, cardiovascular, and anti-diabetic effects, antioxidant activity and anticancer effects. In the present investigation, isolation and purification of the chemical constituents from *Cucumis prophetarum L.*, provided 4,4- dimethyl-stigmast-5-22E, 24-trien-3 β -ol and 17-octahydro-17-((E)-6-hydroxy-6-methylhept-3-en-2-yl)-9,13-dimethyl-6H-cyclopenta [a] phenanthren-7-yl acetate (first reported compounds from this species) respectively, whose structures were elucidated by spectroscopic studies as well as comparison with published results [7,8,11,12,14-16]. The finding of this study is helpful for bioassay-guided isolation of bioactive metabolites from the plant.

Conflict of Interest

The authors declare that they have no conflict of interest.

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