Phytochemical investigation of *Hyoscyamus albus*

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Abstract: The work presented in this paper illustrates the isolation and structure elucidation of secondary metabolites of *Hyoscyamus albus*. Two new natural source and three known compounds were isolated from the *Hyoscyamus albus*. Among the isolated compounds, grivilloside H (1) and betulaplatoside (2) were isolated for the first time while scopolamine (3), *β*-sitosterol (4) and stigmasterol (5) have been reported previously from the same plant. The structures of all the isolated compounds were established by using modern spectroscopic technique (UV, IR, NMR, and EI-MS) and by comparing with those available in literature.

Keywords: *Hyoscyamus albus*, grivilloside H, betulaplatoside, UV, IR, NMR, and EI-MS.

INTRODUCTION

Plants play a key role in the field of medicines as well as in the process of physical and ritual purification (Moerman, 1996). In the ancient past, plants were used for food, poisoning and medicinal purposes. After time, people became familiar with the uses of plants and transferred the knowledge of herbal remedies from generation to generation (Gurib-Fakim, 2006). Natural herbal products are considered highly beneficial to human life. These natural resources provide food, shelter and are also used for the treatment of many kinds of diseases (Okigbo *et al.*, 2009). According to WHO report, 80% of the world population is dependent on natural medicines, but sometimes, herbal treatment may harm patients and the wrong use of herbs may cause death. Thus the investigation of medicinal plants as remedies is essential to aware the people about the use of herbs in therapeutic amounts (Fransworth *et al*., 1985). More than 60% of anticancer agents are derived from natural sources including land plants, marine plants and microorganisms (Cragg *et al*., 1997: Newman *et al.*, 2003). Hartwell in 1982 reported over three thousand plant species that are used as anticancer agents (Hartwell *et al.*, 1982). Graham *et al* added more than three hundred plant species to Hartwell's list in 2000 (Graham *et al.*, 2002). There are large numbers of plant species which are not phytochemically investigated.

Hyoscyamus is highly diversified genus of the family solanaceae, comprising 20 species, which are distributed worldwide. The genus is commonly known as henbanes (Mohy-ud-dint *et al.,* 2010). The genus *Hyoscyamus* is a rich source of tropane alkaloids which show pharmacological activities such as antispasmodic, mydriatic, anticholinergic and antiemetic effect (Herborane and Baxter, 1993). The plant *H. albus* is commonly used as a medicinal plant in Europe. It was mostly used to assist cultivating prophecies by oracles and soothsayers. It was known by different names and terms like dragon plant, Zeus bean, ancient earth oracle, Zeus-Ammon, Apollo's plant etc. (Ratsch and Christian. 2005). The burned seeds were used as ritual incense and the leaves as wine additive. On the inhalation of smoke of its seeds and drinking wine of its leaves, soothsayers and prophetesses usually called upon a deity "Apollo". The seeds or the whole *H. albus* plant is still used in Moroccas incense (Ratsch and Christian, 2005: Voogelbreinder, 2009). *In vivo* activities of the crude methanol extract from the leaves of *H. albus* have shown analgesic and anti-pyretic potential (*Benhouda* & Yahiai, 2014). Mahmood *et al* investigated new tropane alkaloid 2, 3-dimethylnonacosane along with four known compounds, scopolamine, atropine, hyoscine and hyoscyamine from the aerial parts of *H. albus* (Mahmood *et al*., 2014). Hygrine and norhygrine, a tropane alkaloid; were also reported from the roots and aerial parts of various species of genus *Hyoscyamus* (Parr *et al*., 1990). The anticholinesterase potential of scopolamine and hyoscyamine, isolated from the various species of genus *Hyoscyamus* have been reported by Schmeller *et al* (Schmeller *et al*., 1995).

Keeping in view, the importance of medicinal plants, here we report the isolation and characterization of two new source and three known compounds from *Hyoscyamus albus*.

MATERIALS AND METHODS

Distilled solvents (methanol, n-hexane/petroleum ether, chloroform, n-butanol and Ethyl acetate) were used for the extraction, fractionation and isolation purposes. Rotary evaporator (BUCHI Rotavapor R-200) was involved in each step. IR Prestige-21 spectrophotometer (Shimadzu) was used to record the IR spectral data of the compounds in the range 4000-400cm^{-1}. Bruker AV 100 - 600 MHz NMR spectrophotometers were used for

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recording NMR spectra. Deuterated solvents like $CDCl_3$ and CD_3OD are used for the analysis. Mass spectrometer model MAT312 was used for Electron Impact Mass Spectrometric (EIMS) analysis.

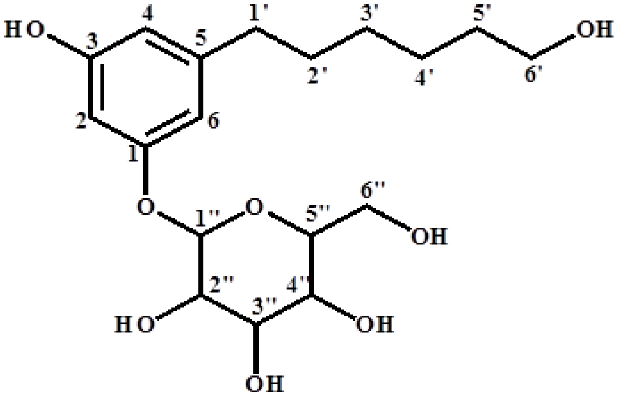

Fig. 1: Structure of Grevilloside H

Chromatographic conditions

For TLC, Aluminum sheets, pre-coated with silica gel (60 F_{254}, E. Merck) were used while the developed chromatograms were visualized by UV at 254 and 366 nm or spraying with Dragendorff's reagent. "*n*-hexane-acetone (8:2 and 7:3)", solvent system were used to examine the *Rf* values.

Plant material

The aerial parts of *H. albus* were collected from its natural high altitude habitat in Swat, KP, Pakistan, in the month of April 2012. The plant was identified by Prof. Dr. M. Nisar, Plant taxonomist, Botany department, University of Malakand and a voucher specimen no. H.UOM.BG-162 was deposited at the herbarium of Botany Department, University of Malakand. The plant material was shade dried, ground and properly stored for further process.

Extraction and isolation

Shade dried powder plant material (6 kg) was extracted with methanol (80%) three times (3x10L) and then filtered. The combined extracts were concentrated on rotary evaporator to get 400 g gummy solid mass. The crude methanolic extract was loaded on column, using silica gel with elution started from *n*-hexane (non-polar system), *n*-hexane-chloroform gradients up to 20% methanol-chloroform that afforded fifteen (15) sub fractions. Sub fractions F3, F8 and F9 on repeated flash column chromatography (FCC) resulted in the isolation of previously reported compounds, scopolamine (1), *β*-sitosterol (2) and stigmasterol (3) while fractions F10 and F35 on FCC afforded two new natural source compounds, grivilloside H (4) and betulaplatoside (5). The structures of all the isolated compounds were established on the basis of spectroscopic data and comparing with those available in literature.

RESULTS

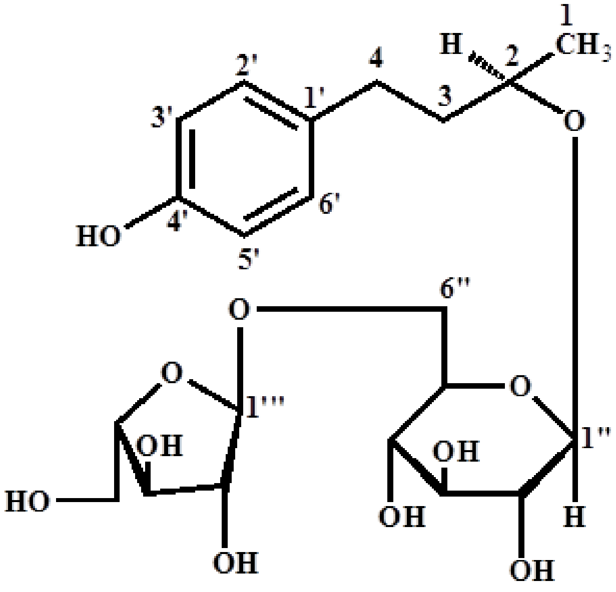

Fig. 2: Structure of Betulaplatoside

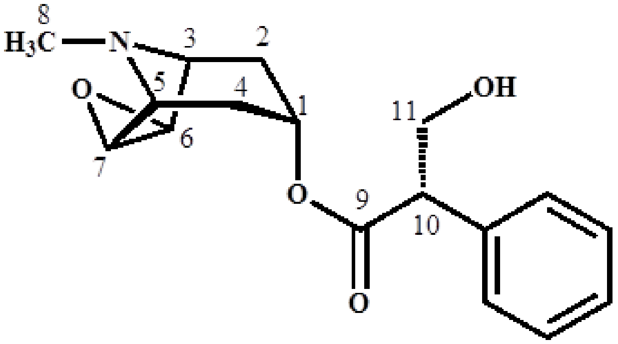

Fig. 3: Structure of scopolamine

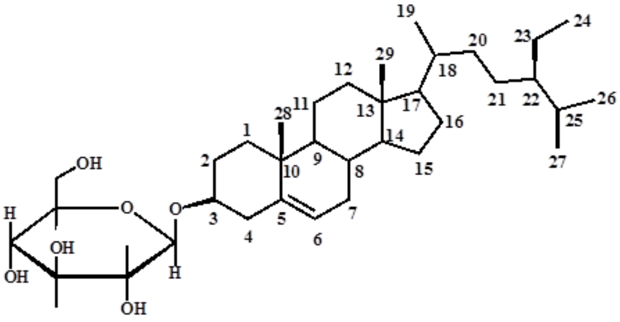

Fig. 4: Structure of *β*-Sitosterol-3-O-*β*-D-glucopyranoside

DISCUSSION

Compound 1

The compound 1 was obtained from the chloroform soluble fraction F10 in the form of amorphous powder. The structure of compound 1 was obtained from detailed spectroscopic analysis. The EIMS spectrum show molecular ion peak (M+) at m/z 372.414, suggesting as $C_{18}H_{28}O_8$ molecular formula for compound 1. The ^1H –

Table 1: ^{13}C-NMR data of compound Grevilloside H (125 MHz in CDCl$_3$, δ = ppm)

C. No	Multiplicity	^{13}C-NMR(δ)	C. No	Multiplicity	^{13}C-NMR(δ)
1	C	160.2	4`	CH$_2$	25.8
2	CH	102.5	5`	CH$_2$	33.6
3	C	159.3	6`	CH$_2$	63.0
4	CH	130.7	1``	CH	102.3
5	C	146.4	2``	CH	75.0
6	CH	109.3	3``	CH	78.2
1`	CH$_2$	36.9	4``	CH	71.5
2`	CH$_2$	32.3	5``	CH	78.1
3`	CH$_2$	30.1	6``	CH$_2$	62.6

Table 2: ^{13}C-NMR data of betulaplatoside (125 MHz in CDCl$_3$, δ = ppm)

C. No	Multiplicity	^{13}CNMR(δ)	C. No	Multiplicity	^{13}CNMR(δ)
1	CH$_3$	22.2	3``	CH$_2$	78.1
2	CH	77.4	4``	CH$_2$	72.1
3	CH$_2$	40.1	5``	CH	76.5
4	CH$_2$	31.7	6``	CH$_2$	68.2
1`	CH	134.6	1```	CH	110.0
2`,6`	CH	130.4	2```	CH$_2$	83.2
3`,5`	CH	116.1	3```	CH$_2$	79.0
4`	C	156.3	4```	CH	86.0
1``	CH	104.3	5```	CH$_2$	63.1
2``	CH$_2$	75.4			

Table 3: ^{13}C-NMR data of compound 3 (150 MHz in CDCl$_3$, δ= ppm)

C. No	Multiplicity	^{13}C-NMR(δ)	C. No	Multiplicity	^{13}C-NMR(δ)
1	CH	57.80	10	CH	54.24
2	CH$_2$	30.95	11	CH2-OH	63.98
3	CH	63.86	1	C	135.61
4	CH$_2$	30.77	2’	CH	128.97
5	CH	57,93	3’	CH	128.03
6	CH	56.30	4’	CH	127.95
7	CH	55.85	5’	CH	128.03
8	N-CH3	42.22	6’	CH	128.97
9	C	171.81			

NMR data of compound 1 showed signals methine, methylene and methyl protons. A doublet of one proton integration was observed at δ 4.85 (J = 8 Hz) which suggest the presence of anomeric acetal group in compound 1. In the down field region of the same ^{1}H-NMR spectrum, six doublet of doublet peaks appear at δ 6 .43, 6.38, 6.30, 3.89,3.70 and 3.43 (J = 2.2 Hz, 12.2 Hz & 8.9 Hz) were due to the resonance of H-6, H-2, H-4 ,H-6″a, H-6″ and H-2″protons. Similarly, the two triplets signals appear at δ 3.53 and 2.50 (J = 7Hz) were due to methylene protons at a position H-6’and H-1’. While, in the upfiled region, two quintets peaks appear at 1.60 and 1.52 (J = 6 & 7 Hz) were due to methylene protons at a position H-2’and H-5’. Three multiplets at δ 3.43, 3.41 and 1.37-1.35 were due to methine and methylene protons at a position H-5”, (H-3”, H-4”) and (H-3’, H-4’) respectively.

The^{13}C-NMR spectrum (Broad Band, DEPT) (table 1) of compound 1 exhibited eighteen signals, including seven methylene, eight methine and three quaternary carbon atoms. In the spectrum the signals appeared at δ 160.2, 102.5, 159.3, 110.7, 146.6, 109.3 were due to aromatic carbon C-1, C-2, C-3, C-4, C-5 and C-6 respectively. Similarly, the aliphatic moiety gives peaks at δ 36.9, 32.3,30.1, 25.8, 33.6 and 63.0 at carbons position number C-1`, C-2`, C-3`, C-4`, C-5`and C-6`. While the six signals appear at δ 102.3, 75.0, 78.2, 71.5, 78.1 and 62.6 at a position of carbon number C-1``, C-2``, C-3``, C-4``, C-5`` and C-6`` were due to β-glucopyranose. Thus on the basis of spectral analysis as well as literature data, the compound 1 was identified as Grevilloside H (Yamashta *et al.*, 2010).

Table 4: ^{13}C-NMR data of the compound 4 (150 MHz in $CDCl_3$, δ= ppm)

C. No	Multiplicity	^{13}C-NMR(δ)	C. No	Multiplicity	^{13}C-NMR(δ)
C 2	CH_2	37.6	C 19	CH_3	11.79
C 2	CH_2	31.5	C 20	CH	36.39
C 3	CH	72.1	C 21	CH_3	19.30
C 4	CH_2	42.3	C 22	CH_2	34.09
C 5	C	141.1	C 23	CH_2	26.08
C 6	CH	121.8	C 24	CH	46.00
C 7	CH_2	31.8	C 25	CH	29.10
C 8	CH	31.8	C 26	CH_3	18.98
C 9	CH	50.2	C 27	CH_3	19.29
C 10	C	36.6	C 28	CH_2	23.00
C 11	CH_2	21.5	C 29	CH_3	12.08
C 12	CH_2	39.9	C $1^{/}$	CH	101.79
C 13	C	42.4	C $2^{/}$	CH	73.11
C 14	CH	56.8	C $3^{/}$	CH	76.30
C 15	CH_2	24.4	C $4^{/}$	CH	75.63
C 16	CH_2	29.3	C $5^{/}$	CH	79.11
C 17	CH_2	56.2	C $6^{/}$	CH_2	61.51
C 18	CH_3	40.6			

Table 5: ^{13}C-NMR data of compound 5 (150 MHz in $CDCl_3$, δ= ppm)

C. No	Multiplicity	^{13}C-NMR(δ)	C. No	Multiplicity	^{13}C-NMR(δ)
1	CH_2	37.06	19	CH_3	21.07
2	CH_2	32.01	20	CH	34.02
3	CH	72.01	21	CH	26.03
4	CH_2	42.04	22	CH_2	26.03
5	C	141.01	23	CH_2	25.04
6	CH	12.08	24	CH	12.01
7	CH_2	31.08	25	CH	29.06
8	CH	31.08	26	CH_3	20.02
9	CH	50.02	27	CH_3	19.08
10	C	36.06	28	CH_2	18.08
11	CH_2	21.05	29	CH_3	12.02
12	CH_2	39.06	$1^{/}$	CH	101.79
13	C	42.02	$2^{/}$	CH	73.11
14	CH	56.08	$3^{/}$	CH	76.30
15	CH_2	24.04	$4^{/}$	CH	75.63
16	CH_2	29.03	$5^{/}$	CH	79.11
17	CH_2	56.02	$6^{/}$	CH_2	61.51
18	CH_3	40.06			

Compound 2

The compound 2 was obtained from sub fraction F35 as amorphous powder. The molecular formula $C_{21}H_{32}0_{11}$ of compound 2 was established from its EIMS spectrum with molecular ion peak at m/z 477 (M+). ^{1}H –NMR spectrum of the compound 2 showed one singlet, eleven doublets, six triplets and five multiplets signals for methine, methylene and methyl protons. In the downfield region of the spectrum a strong intense peaks of one singlet and six doublet were due to hydroxyl substituent proton appear at δ 5.0 (aromatic OH) and 2.0 at C-2``,C-3``,C-4``,C-2```,C-3``` and C-5```. Similarly, the five doublet peaks were assigned to methine proton appear δ 6.95, 6.68 and 5.18 at a position (H-2`, 6`), (H-3`, 5`) and H-1```. In the same spectrum's, five multiplets peaks appear at δ 3.01, 1.75, 2.55, 4.06 and 3.91 were assigned to H-2, H-3, H-4, H-5``and H-4``` respectively while a prominent doublet peak at δ 1.21 at a position H-1 correspond to methyl proton and the two broad doublet signals appear at δ 3.63 and 3.79 at a position of proton number H-6`` and H-5``` correspond to methylene proton. A six triplets integration resonating a methine proton appear at δ 5.03 having a coupling constant J = 7.9 Hz, 3.73, 3.40, 3.98 and 3.65 were assigned to H-1``, H-2``, (H-3``, 4``), H-2``` and H-3``` due to β-D-glucopyranosyl respectively.

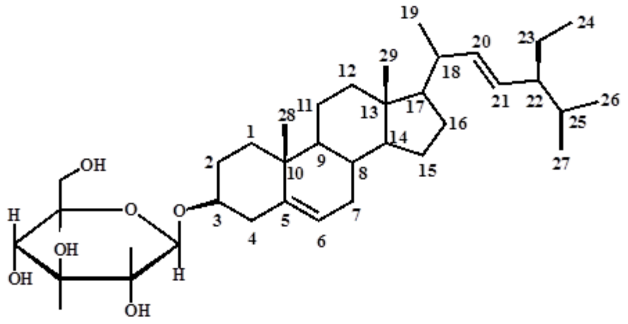

Fig. 5: Structure of Stigmasterol-3-O-β-D-glucopyranoside

The ^{13}C-NMR spectrum (Broad Band, DEPT) (table 2) of compound 2 showed twenty one signals, including one methyl, four methylene, fourteen methine and two quaternary carbon atoms.The signals in the spectrum appeared at δ 134.6, 130.4, 116.1 and 156.1 were assigned to aromatic carbon C-1`, (C-2`, 6`), (C-3`, 5`) and C-4` respectively while the signals appear at δ 104.3, 75.4, 78.1, 72.1, 76.5, 68.2, 83.2, 110.0 , 79.0, 86.0 and 63.1 were assigned to glucoses moiety at a position C-1``, C-2``, C-3``, C-4``, C-5``, C-6``, C-1```, C-2```, C-3```, C-4```and C-5```respictevily. Similarly, signals appear at δ 22.2, 77. 4, 40.1 and 31.7 were due to C-1, C-2, C-3 and C-4 were assigned to aliphatic moiety. On the basis of above spectral analysis and literature data the compound 2 is recognized as Betulaplatoside (Matsuda *et al.*, 1998).

Compound 3

The compound 3 was purified from sub fraction F3 of chloroform soluble part of crude methanolic extract. The EI-MS data of the compound 3 exhibited the molecular ion peak (M^{+}) at *m/z* 303. The other prominent peaks are at 154, 138, 137, 136, 120, 108, and 103. The molecular formula $C_{17}H_{21}NO_4$ was deduced from HREI-MS. The signals for methine, methylene and methyl protons are shown by ^{1}H–NMR spectrum. In the downfield region of the spectrum five multiplets peaks appear in the range of δ 7.19-7.37 were due to aromatic proton. In the spectrum a broad signal appears at δ 3.76 was due to methylene proton having hydroxyl substituent. Similarly, the two axial methylene protons at carbon 2 and 4 give a multiplet at δ 2.08. In the same spectrum a quartet appeared at δ 3.11 (*J*=1.92 Hz) was assigned to H-5 proton. While, in the down field region a triplet at δ 5.01 (*J*=5.4 Hz) was due to H-3 proton. Two doublets of each proton's integration resonating at δ 3.36, and δ 2.64, with coupling constant *J* =3.0 and 2.97 Hz were assigned to H-6 and H-7 methine protons. While the doublets appear at δ 1.55 and 1.32 (*J*=15.3 and 15.5) were due to equatorial protons at C-2 and 4 respectively. A singlet of three protons integration resonating at δ 2.44 was assigned to N-methyl protons. A doublet of doublet of one proton integration resonating at δ 4.15 (*J* =2, 8.5 Hz) was assigned to C-10, methine proton.

In^{13}C-NMR spectrum (Broad Band, DEPT) (table 3) of the compound exhibited seventeen signals, including one methyl, three methylene, eleven methine and three quaternary carbon atoms. In the spectrum the signals appeared at δ 135.61, 128.97, 128.03, 127.95, 128.03 and 128.97 were assigned to aromatic carbon C-1` C-2`, C- 3`, C-4`, C-5` and C-6` respectively. The signal at δ 42.22 corresponds to N-CH_3. While the signals appear at δ 63.98 was due to the methylene carbon having oxygen substitution (CH_2-OH). In the spectrum's down field region signal appears at δ 171.83 was due to the carbonal carbon of ester. Based on the above spectral analysis as well as literature data the structure of this compound deduced as scopolamine (Doerk-schemitz *et al*., 1994)

Compound 4

The compound 4 was isolated by repeated column chromatography from the crude methanolic extract (fraction F8) of *Hyoscyamus albus*, as white needle and its structure is derived based on detailed spectroscopic data. The EI-MS data showed molecular ion peak (M^{+}) of the compound 4 appeared at m/z 414.17 The other prominent peaks are at 395, 384, 355, 293, 283, 255, 219, 163, 147, 95, 58 and their molecular formula deduced as $C_{29}H_{50}O$.The ^{1}H -NMR data of compound 4 showed twenty nine signals which including nine methine, eleven methylene, six methyl signals and three quaternary carbons. The two singlets each of three protons integration in the ^{1}H-NMR spectrum resonating at δ 0.71 and 1.03 in a position H-29 and H-28. The other prominent peaks of three doublets correspond to methyl protons integration resonating at δ 0.80, 0.82 and 0.91 having a coupling constant *J* = 6.2, 6.6 and 6.4 Hz at a position H-19, H-26 and H-27 respectively. Similarly, the triplet at δ 0.83 was assigned to methyl proton at a position H-24. The proton corresponding to the H-3 of a sterol moiety was appeared as a triplet of doublet of doublets at δ 3.51. In the down field region of the spectrum showed an intense peaks of multiplets resonating at δ 1.3, 1.7, 5.44, 1.47, 1.5, 1.5, 1.5, 1.47 and 1.46, at a position H-1, H-2, H-6, H-7, H-8, H-9, H-11, H-12 and H-15 respectively. The spectrum resonating a broad peak at δ 3.24 - 3.45 for glucoses proton. A broad doublet signal appear at δ 2.33 having a coupling constant *J* = 8.41 Hz, was assign to methylene proton at a position of carbon number C-4. While the broad intense peaks showed a proton integration at δ 3.68 (*J* = 11.7, 5.71 Hz), 3.86 (*J* = 11.71, 2.4 Hz), 4.58 (*J* = 7.51 Hz) in a position Hb-6, Ha-6$^{/}$ and H-1$^{/}$ corresponds to sugar moiety.

In^{13}C-NMR spectrum (Broad Band, DEPT) (table 4) of this compound exhibited twenty nine signals comprised of eleven methylene, six methyl, three quaternary carbons and nine methine. The HMQC spectrum determines ^{1}H-

^{13}C correlation, while the long ranges ^{1}H-^{13}C correlation were established through HMBC technique. The signals appeared in the spectrum at δ 101.79, 61.51, 73.11, 75.63, 76.30 and 79.11 were assigned to anomeric carbon of suger moiety C-1$^{/}$ C-6$^{/}$, C- 2$^{/}$, C-4$^{/}$, C-3$^{/}$ and C-5$^{/}$ respectively. Prominent signals at δ 141.02, 36.31 and 42.29 were assigned to the quaternary carbons C-5, C-10 and C-13 respectively. While the signals appear at δ 11.79, 19.89, 19.30, 12.08, 18.98 and 19.29 were due to the methyl carbon having position of carbon number C-28, C-29, C-19, C-24, C-26 and C-27. The spectrum's down field region signals appear at δ 121.08, 72.01, 3.08, 50.02, 34.02, 26.03, 22.10, 56.02 and 56.08 were due to methine carbons at a position of carbon number C-6, C-3, C-8, C-9, C-20, C-22, C-25, C-17 and C-14 respectively. The above spectral analysis as well as literature data of compound 4 is recognized as β - Sitosterol-3-O-β-D-glucopyranoside (Pateh *et al*., 2009).

Compound 5

The compound 5 was isolated from the sub-fraction F9 in the form of white crystals. The structure of the compound was elucidated from various spectral analysis. The EIMS spectrum showed molecular ion peak (M^{+}) of the compound 5 appeared at m/z 302 and the molecular formula deduced from it as $C_{29}H_{48}O$. The ^{1}H –NMR data of this compound exhibited signals for methine, methylene, methyl and quaternary carbons. The two singlet's each of three protons integration at δ 1.05 and 0.7 was due to the resonance H-19 and H-18. Three doublets correspond to methyl protons integration resonating at δ 1.0, 0.88 and 0.89 having a coupling constant J = 6.2, 6.6 and 6.4 Hz at a position H-21, H-26 and H-27 respectively. In the down field region spectrum, three multiplets peaks of methine protons appear at δ 5.33, 4.62 and 4.61 at a position H-6, H-22 and H-23. Similarly, the triplet at δ 0.9 was assigned to methyl proton at a position H-29. In the down field region the spectrum showed an intense peaks of multiplets resonating at δ 1.3, 1.7, 3.59, 1.47, 1.5, 1.5, 1.5, 1.47 and 1.46, at a position H-1, H-2, H-3, H-7, H-8, H-9, H-11, H-12 and H-15 respectively. The glucoses proton spectrum resonating a broad peak at δ 3.24 - 3.45. While a broad doublet signal appear at δ 2.33 having a coupling constant J = 8.41 Hz, was assign to methylene proton at a position of carbon number C-4. The broad intense peak showed a proton integration at δ 3.68 (J =11.7, 5.71 Hz), 3.86 (J =11.71, 2.4 Hz), 4.58 (J =7.51 Hz) in a position Hb-6, Ha-6$^{/}$ and H-1$^{/}$ corresponds to sugar moiety.

In^{13}C-NMR spectrum (Broad Band, DEPT) (table 5) of the compound exhibited twenty nine signals including eleven methylene, six methyl, three quaternary carbons and nine methine. The signals appeared in the spectrum at δ 101.79, 61.51, 73.11, 75.63, 76.30 and 79.11 were assigned to anomeric carbon of suger moiety C-1\` C-6\`, C- 2\`, C-4\`, C-3\` and C-5\` respectively. The prominent signals at δ 141.02, 36.31 and 42.29 were assigned to the quaternary carbons C-5, C-10 and C-13 respectively. While the signals appear at δ 11.79, 19.89, 19.30, 12.08, 18.98 and 19.29 were due to the methyl carbon having position of carbon number C-19, C-18, C-21, C-29, C-26 and C-27. In the down field region of the spectrum signal appear at δ 122.01, 72.10, 32.08, 50.29, 36.39, 46.00, 29.10, 37.61 and 59.08 were due to methine carbons at a position of carbon number C-6, C-3, C-8, C-9, C-20, C-24, C-25, C-17 and C-14 respectively. The above spectral analysis as well as literature data of compound 5 is recognized as Stigmasterol-3-O-β-D-glucopyranoside (Pateh *et al*., 2009).

CONCLUSION

The present research work will be helpful in the utilization of *Hyoscyamus Albus* as a source of useful bioactive compounds. Moreover, the five isolated compounds may be useful for their therapeutic importance.

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