Journal of Phytology 2010, 2(3): 89-100
Phytochemistry
ISSN: 2075-6240
Available Online: www.journal-phytology.com

REGULAR ARTICLE

PHYTOCHEMICAL INVESTIGATION OF FRUITS OF *CORYLUS COLURNA* LINN.

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SUMMARY

Four chemical constituents, steroidal glycosides, namely colurnasterol glycoside, β-sitosterol glycoside, campesteryl glycoside and corylusterol glycoside (new compound) have been isolated from the fruits of Corylus colurna and their structures were established as 20-β-hydroxycampesterol-3β-D-glucopyranoside (F1), stigmast-5-en-3-O-β-D-glucopyranoside (F2), (24R)ergost-5-en-3β-D-glucopyranoside (F3) and cholest-5-en-3β, 12 β-triol-3β-D-glycopyranoside (F4) by spectral data analyses and chemical evidences.

Key words: Steroidal glycosides, namely Colurnasterol glycoside, β-Sitosterol glycoside, *Corylus colurna* Linn. (Family: Betulaceae)

Parwaiz Akhtar et al. Phytochemical Investigation of Fruits of *Corylus colurna* Linn. J Phytol 2/3 (2010) 89-100.
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1. Introduction

Corylus colurna Linn. (Family : Betulaceae), commonly known as 'Funduq' os moderate sized deciducous tree distributed in western temperate Himalayas from Kashmir to Kumaon at the altitude of 1700 – 3300 m and extensively cultivated in Turkey for nuts (Fruits).the fruits are used as a brain and intestinal tonic, aphrodisiac and expectorant and prescribed in weakness of brain and liver, gonorrhoea and palpitation. The description herein is on the isolation and elucidation of four steroidal glycosides from the fruits of the plant.

2. Results and Discussion

Compound F_1 named colurnasterol glycoside was obtained as a colourless amorphous powder from $CHCl_3$-MeOH (9:1) eluants. It gave positive test for a sterol glycoside. Its IR spectrum showed the absorption bands for hydroxyl group (3400 cm^{-1}) and unsaturation (1650 cm^{-1}). Its mass spectrum showed an ion fragment at m/z 401 [578-$C_6H_{11}O_5$ -Me]$^+$ corresponding to a sterol formula $C_{28}H_{48}O_2$. The base peak at m/z 146 was generated due to $C_{8,\ 14}$ – $C_{9,\ 11}$ fission and elimination of glucose moiety. Another prominent peaks were arose at m/z 368 [M - $C_6H_{12}O_6$ – 2 x Me]$^+$, 234 [M - $C_6H_{12}O_6$ –146 - H_2O]$^+$, 224 [$C_{8,\ 14}$ – $C_{12,\ 13}$ fission]$^+$ and 206 [224 - H_2O]$^+$ suggesting the presence of one hydroxyl group in the side chain (Scheme-1).

The ^{1}HNMR spectrum of F_1 displayed a one-proton downfield multiplet at ε 5.32 assigned to C-6 olefinic proton. A one-proton broad multiplet at ε 3-63 ($w_{1/2}$ = 16.17 Hz) was ascribed to C-3 ε carbinol proton. The methyl protons were appeared as three proton broad signal at δ 0.65 (Me-18) and 0.95 (Me-19) and three-proton doublets at δ 0.90 (J=6.12 Hz and 0.80 of (J=5.11Hz)

associated with C-26, C-27 and C-28 secondary methyl, respectively. A three proton broad signal at 1.22 was attributed to C-21 angular methyl protons attached to a carbinol carbon.

The sugar protons resonated as two one – proton doublets at δ 4.87 (J=5.2 Hz) and 4.20 (J=7.30 Hz) assigned to C-1 anomeric and proton multiplets at δ 4.43 (C-2), 3.09 (C-3) and (C-4) and a two proton broad signal at δ 4.85 ascribed to C-6 oxygenated methylene protons. The remaining methene and methylene protons appeared between δ 2.50-1.08.The presence of all methyl signals between δ 1.20-0.65 supported their attachments to saturated carbon signals at δ 76.9 (C-3) and 76.72 (C-20) and sugar carbons at δ 100.81-61.08. Alkaline hydrolysis of F_1 yielded a sterol aglycone and D-glucose. The data led to establish the structure of F_1 as 20-β-hydroxycampesterol-3β-D-glucopyranoside. This is a new phytosterol isolated from a natural or synthetic source.

Table-1. ^{1}H and ^{13}C NMR Spectral Data of Colurnasterol Glycoside (F_1)

Position	^{1}H NMR		^{13}C NMR
	Alfa	Beta	
1	1.33 m	2.38 m	36.20
2	1.80 m	1.76 m	36.24
3	3.63 br m ($w_{1/2}$ 16.17)	-	76.96
4	2.50 brs	2.08 m	40.31
5	-	-	14.44
6	5.32 m	-	121.16
7	1.93 m	2.13	29.02
8	-	1.98 m	31.39
9	1.50 m	-	49.61
10	-	-	35.47
11	1.93 m	1.50 m	20.59
12	1.10 m	1.80 m	36.83
13	-	-	41.84
14	1.13 m	-	56.18
15	1.08 m	1.55	27.77
16	1.61 m	1.55	25.46
17	1.45 m	-	55.44
18	0.65 br s	-	11.65

19	0.95 br s	-	19.07
20	-	-	76.72
21	1.22 br s	-	18.60
22	1.58 m	1.08 m	33.35
23	1.17 m	1.78 m	25.46
24	1.33 m	-	45.14
25	1.50 m	-	28.71
26	0.90 d (6.12)	-	19.67
27	0.80 d (5.10)	-	18.92
28	0.80 d (5.11)	-	22.61
1'	4.87 d (5.2)	-	100.81
2'	4.43 m	-	76.73
3'	3.09 m	-	73.45
4'	3.04 m	-	70.08
5'	4.20 d (7.30)	-	76.96
6'	4.85 br s	-	61.08

Coupling constants in Hertz are given in parentheses

Compound F_2, β-sitosterol glycoside was obtained as a colourless amorphous powder from $CHCl_3$- MeOH (9:1) eluants. It responded positively to Liebermann-Buchard test for steroids and produced honeycomb-like froth with sodium bicarbonate solution indicating saponin nature of the molecule. Its IR spectrum showed absorption bands for hydroxyl group (3450 cm^{-1}) and unsaturation (1620 cm-1). It mass spectrum had a molecular ion peak at m/z 414 [M- sugar]$^+$ corresponding to a steroidal formula $C_{29}H_{59}O$. It indicated five double bond equivalents; four of them were adjusted in the steroidal carbon skeleton and one in the olefinic linkage. The other diagnostic important peaks generated at m/z 399 [M-Me]$^+$, 396 [M-H_2O]$^+$, 381 [396-Me]$^+$, 273 [M-side chain, $C_{10}H_{21}$]$^+$, 285 [273-H_2O]$^+$, 213 [255-ring D- fission]$^+$ and 198 [213-Me]$^+$. These fragments suggested that it was a C_{29} sterol possessing one soluble bond in the steroidal carbon framework and C_{10} saturated side chain. The ion fragments at m/z 55 [$C_{1,10}$ - $C_{4,5}$ fission-H_2O], 69 [$C_{2,5}$ – $C_{5,10}$-$C_{6,7}$ fission]$^+$ 83 [$C_{2,3}$ - $C_{5,10}$ - $C_{7,8}$ fission]$^+$, 71 [$C_{1,2}$ - $C_{4,5}$ fission]$^+$, 124 [$C_{6,7}$ - $C_{9,10}$ fission]$^+$, 106 [124-H_2O]$^+$, 149 [M-124-side chain]$^+$, 138 [$C_{7,8}$ - $C_{9,10}$ fission]$^+$, 120 [138-H2O]$^+$, 276 [M-138]$^+$, and 135 (9.5) indicated the presence of the olefinic bond in ring B at C-5 and hydroxyl group in ring A which was placed at C-3 on the basis of biogenetic grounds. The ion peak at m/z 164 [$C_{8,14}$-$C_{9,11}$ fission]$^+$, 146 [164-H_2O]$^+$, 107 [M-164-side chain]$^+$, 174 [$C_{8,14}$ - $C_{12,13}$ fisson-H_2O]$^+$, 160 [174-CH_2]$^+$ and 81 [$C_{8,24}$ - $C_{12,13}$ fission-side chain]$^+$ supported the saturated nature of the ring C. The mass spectrum indicated the existence of ethyl group in side chain that was placed at C-24 on the basis of biological analogy (Scheme -2).

The ^{1}H NMR spectrum of F_2 exhibited a one-proton doublet at δ 5.31 (J=5.2 m/z)

assigned to C-6 proton. A one proton broad multiplet at δ 4, 43 with $w_{1/2}$ 18.5 Hz showed the presence of 3α - methine proton (axial) interacting with C-2 equatorial, C-2 axial and C-4 equatorial protons. Four doublets at δ 0.90 (J=6.0 Hz), 0.92 (J=6.10 Hz), δ 0.80 (J=6.6 Hz) and 0.82 (J=7.3 Hz); integrating three protons each, were ascribed corresponding to C-21, C-26, C-27 secondary methyl and C-29 primary methyl protons. The remaining two tertiary C-18 and C-19 methyl proton signals appeared as singlets at δ 0.64 and 0.95, respectively. The presence of all the methyl signals in the region δ 0.64-0.95 suggested that these functionalities were attached to saturated carbons. The remaining methylene and methine protons resonated in the region δ 2.50-1.06. In addition to these, the anomeric C-1 proton appeared at δ 4.90 as a broad signal. Two one –proton broad signals at δ 4.88 and 4.66 were ascribed to C-6 oxygenated methylene protons. These one-proton multiplets at δ 3.62, 3.08 and 3.04 were associated with H-2, H-3 and H-4, respectively. A one-proton doublet at δ 4.22 (J=5.6 Hz) was due to H-5′ proton of the glycone moiety (Table –1).

Further evidence for the structure of F_2 was provided by its ^{13}C NMR spectral data that showed the presence of 35 carbon atoms in the molecule. Signals at δ 140, 71, 121.63 and 71.73 were assigned C-5, C-6 unsaturated carbons and C-3 carbinol carbons respectively. The β-configuration of the methyl group was confirmed by the comparison of chemical shifts of carbons and protons of the side chain in the ^1H and ^{13}C NMR spectra of F_2 with β- sitosterol, stigmast-4-en-3-one, stigmast-4-en-6β-ol-3-one (Greca *et. al*., 1990) and lawsaritol (Gupta *et.al*., 1992). The H_3-29 resonance of 24-R configuration (δ 0.82) was more up shielded as compared to 24-5 resonance (δ 0.86) (Rubinstein *et.al*., 1976). The anomeric and oxygenated methylene carbons of the sugar moiety appeared at δ 100.84 and 61.10, respectively. The remaining sugar carbon signals resonated at δ 76.74 (C-2′), 73.47 (C-3′), 70.09 (C-4′) and 76.74 (C-5′). Alkaline hydrolysis of F_2 yielded D-glucose and an aglycone that was identified as β-sitosterol by direct comparison with an authentic sample (Co-TLC, mmp). On the basis of these findings, the structure of F_2 has been established as stigmast-5-en-3-O-β-D-glucopyranoside.

Table-2. ^1H and ^{13}C NMR Spectral Data of β-Sitosterol Glycoside (F_2) and β-Sitosterol

Position	^1H NMR		^{13}C NMR	
	Alpha	Beta		β-Sitosterol
1	1.38 m	2.50 m	36.86	37.33
2	1.80 m	1.78 m	29.28	31.63
3	4.43 br m ($w_{1/2}$ 18.5 Hz)	-	77.00	71.73
4	2.50 m	2.34 m	40.33	42.00
5	-	-	100.46	140.71
6	5.31 d (5.2)	-	121.18	121.16
7	1.93 m	2.08 m	33.38	31.96
8	-	1.13 m	31.38	31.81
9	1.45 m	-	49.64	51.13

10	-	-	36.22	36.43
11	1.93 m	1.50 m	20.62	21.09
12	1.13 m	1.80 m	38.34	39.79
13	-	-	42.11	42.37
14	1.15 m	-	56.21	56.75
15	1.06 m	1.80 m	23.88	24.15
16	1.61 m	1.36 m	28.74	28.75
17	1.45 m	-	55.47	56.02
18	0.64 br s	-	11.74	11.84
19	0.95 br s	-	19.10	19.46
20	-	2.34 m	36.22	36.07
21	0.90 d (6.0)	-	18.63	18.68
22	1.78 m	1.10 m	35.51	33.95
23	1.25 m	1.80 m	25.49	26.10
24	1.30 m	1.55 m	44.66	45.82
25	1.73 m	-	27.81	29.51
26	0.92 d (6.10)	-	19.62	19.77
27	0.80 d (6.6)	-	18.95	19.21
28	1.15 m	1.42 m	22.63	23.13
29	0.82 d (7.3)	-	11.67	11.04
1′	4.90 br s	-	100.84	-
2′	3.62 m	-	76.74	-
3′	3.08 m	-	73.47	-
4′	3.04 m	-	70.09	-
5′	4.22 d (5.6)	-	76.74	-
6a′	4.88 br s	-	61.10	-
6b′	4.66 br s	-	-	-

Coupling constants in Hertz are provided in parenthesis

Compound F_3 a compesteryl glycoside, was obtained as a colourless amorphous powder from $CHCl_3$ – MeOH (9:1) eluants. It responded positively to Liebermann-

Burchard and to glycoside tests indicating steroidal saponin nature of the molecule. Its IR spectra showed absorption bands at 3400, 3350 (OH) and 1645 (C=C). Its mass spectra had an ion peak at m/z 400 $[M\text{-}C_6H_{11}O_5]^+$ corresponding to $C_{28}H_{48}O$. It indicated five degrees of unsaturation; four of them were adjusted in steroidal carbon framework and one in the olefinic linkage. The important ion peaks at appearing at m/z 83 [$C_{2,3}$ - $C_{5,10}$ - $C_{7,8}$ fission]$^+$, 71 [$C_{1,10}$ - $C_{4,5}$ fission]$^+$, 111 [$C_{1,10}$ - $C_{5,10}$ - $C_{7,8}$ fission]$^+$, 69 [83-CH_2]$^+$, 106 [$C_{6,7}$ - $C_{9,10}$ fission - H_2O]$^+$, 149 [$C_{6,7}$ - $C_{9,10}$ fission - side chain]$^+$ and 120 [$C_{7,8}$ - $C_{9,10}$ fission - H_2O]$^+$ suggested saturated nature of ring A, the presence of the olefinic linkage of C-5 and the hydroxyl group in ring A, which was placed at C-3 on the basis of biological considerations. The ion peaks at m/z 192 [$C_{8,14}$ - $C_{12,13}$ fission]$^+$, 81 [400-192-side chain]$^+$, 164 [$C_{8,14}$ - $C_{9,11}$ fission]$^+$, 109 [400-164-side-chain]$^+$ and 146 [164-H_2O]$^+$ supported the saturated nature of ring C. The diagnostical ion fragments generated at m/z 385 [400-Me]$^+$, 273 [400-C_9H_{19}]$^+$, 258 [273-Me]$^+$ and 216 [258-ring D]$^+$ indicated the presence of C_9 saturated side chain (Scheme- 3).

The ^{1}H NMR spectrum of F_3 showed a one-proton down field multiplet at δ 5.32 assigned to C-6 olefinic proton, a one-proton broad multiplet at δ 3.62 ($w_{1/2}$=16.23Hz) was associated with C-3 α- carbinol proton. Four up-field doublets at δ 0.90 (J=6.10 Hz), 0.88 (J=6.0 Hz), 0.81 (J=5.05 Hz) and 0.79 (J=5.10 Hz), integrating three-proton each were ascribed to C-21, C-26, C-27 and C-28 secondary methyl protons respectively. The C-18 and C-19 tertiary methyl signals appeared as three proton singlets at δ 0.64 and δ 0.95, respectively. The appearance of all the methyl functionalities in the region δ 0.64-0.95 indicated that these groups were attached to saturated carbons. The C-27 methyl group signal is most sensitive to C-24 stereochemistry and the chemical shifts of all methyl protons are markedly influenced by the solvent system (Rubinstein *et al*., 1976). The anomeric C-1′ proton appeared as a broad signal at δ 4.88. The C-6 oxygenerated methylene protons resonated as two-proton broad signal at δ 4.84. A one-proton doublet at δ 4.22 (J=7.39 Hz) was assigned to C-5 proton. Three one-proton multiplets at δ 4.43, 3.09 and 3.04 were attributed to C-2, C-3 and C-4 protons. The remaining methine and methylene protons of the steroidal nucleus resonated between δ 2.50-1.09 (Table – 2).

The ^{13}C NMR spectrum of F_3 exhibited 28 carbon signals for steroidal molecules and 6 carbon signals for sugar moiety. The olefinic carbon signals appeared at δ 140.71 (C-5) and 121.63 (C-6). The C-3 carbinol carbon resonated at δ 71.73. A signal at δ 100-80 was assigned to C-1′ anomeric carbons. The signals at δ 76.73, 73.46, 70.10, 76.96 and 61.10 were assigned to the remaining sugar carbons (Table-2.8). The alkaline hydrolysis of F_3 yielded compesterol and β-D-glucose. On these findings the structure of F_3 has been characterized as (24R) ergost-5-en-3β-D-glucopyranoside.

Table-3 . ^{1}H and ^{13}C NMR Spectra Values of Compesteryl Glycoside (F_3) (DMSO-D)

Position	^{1}H NMR		^{13}C NMR
	Alpha	Beta	
1	1.36 d d d d (11.12,9.75,5.33,6.23)	2.50 m	38.31
2	1.81 m	1.79 m	29.26
3	3.62 br m ($w_{1/2}$ 16.23)	-	76.73
4	2.50 m	2.12 m	41.85
5	-	-	140.45

6	5.32 m	-	121.17
7	1.93 m	2.12 m	31.40
8	-	1.20 m	31.40
9	1.50 m	-	49.63
10	-	-	36.20
11	1.93 m	1.50 m	20.59
12	1.13 d d d (11.2,9.31,5.28)	1.85 m	36.82
13	-	-	42.90
14	1.12 m	-	56.18
15	1.09 m	1.50 m	22.61
16	1.61 m	1.55 m	28.32
17	1.45 m	-	55.43
18	0.64 br s	-	11.76
19	0.95 br s	-	19.08
20	-	2.34 m	35.47
21	0.90 d (6.10)	-	18.93
22	1.60 m	1.06 m	33.35
23	1.26 m	1.77 m	25.46
24	1.30 m	-	45.15
25	1.54 m	-	28.72
26	0.88 d (6.10)	-	19.69
27	0.81 d (5.05)	-	18.60
28	0.79 d (5.10 d)	-	23.85
29	-	-	-
1′	4.88 brs	-	100.80
2′	4.43 m	-	76.73
3′	3.09 m	-	73.46
4′	3.04 m	-	70.10
5′	4.22 d (7.39)	-	76.96

6a′	4.84 brs	-	61.10
6b′	4.84 brs	-	-

Coupling constants in Hertz are provided in parentheses

Compound F_4, named corylusterol glycoside, was obtained as a colourless amorphous powder from $CHCl_3$ – MeOH (9:1) eluants. It gave positive test for steroids and steroidal glycosides. Its IR spectrum exhibited the absorption bands for hydroxy groups (3440 cm^{-1}) and unsaturation (16.10 cm^{-1}). Its mass spectrum displayed an ion peak at m/z 418 $[M- C_6H_{11}O_5]^+$. It indicated five double bonds equivalents; four of them were adjusted in steroidal skeleton and one in olefinic linkage. The ion peaks at m/z 72 $[C_{1,10}- C_{4,5}$ fission$]^+$, 55 $[72-H_2O]^+$, 138 $[C=-C_{9,10}$ fission$]^+$, 123 $[138-Me]^+$, 120 $[138-H_2O]^+$, 276 $[418-138-H_2O]^+$ and 163 $[276$-side chain$]^+$ indicated the presence of one of the oxygenated carbon in ring C, which was placed at C-3 on biological analogy and the olefinic linkage at C-5. The ion peaks at m/z 125 $[C_{8,14}- C_{9,11}$ fission - side chain, $C_8H_{17}]^+$, 84 $[C_{8,14}$ – $C_{9,11}$ fission - side chain - ring D$]^+$, 111 $[C_{8,14}- C_{11,15}$ - side chain$]^+$ and 70 $[C_{8,14}- C_{11,15}$ - side chain – ring - D$]^+$, supported to the saturated nature of ring C. The existence of the diagnostic ion peaks appearing at m/z 400 $[418-H_2O]^+$, 385 $[400-Me]^+$, 305 [400-side chain, $C_8H_{12}]^+$ and 113 [C_8H_{17}, side chain$]^+$ indicated the C-8 saturated side chain (Scheme- 4).

The ^1H NMR spectrum of F_4 displayed a one-proton downfield multiplet at δ 5.32 assigned to H-6 olefinic proton. A one-proton broad multiplet at δ 3.63 ($w_{1/2}$=18.2 Hz) and one-proton double doublet at δ 3.12 (J=8.4, 5.3 Hz) were ascribed to C-3 α and C-12 β carbinol protons, respectively. Two three-proton broad signals at δ 0.64 and 0.95 attested the presence of C-18 and C-19 tertiary methyl protons, respectively. Three doublets at δ 0.91 (J=6.13 Hz), 0.84 (J=6.42 Hz) and 0.80 (J=6.21 Hz), integrated three protons each were attributed to C-21, C-26 and C-27 secondary methyl, respectively. The presence of methyl signal in the region δ 0.64-0.91 indicated the attachment of these groups to saturated carbons. The sugar proton resonated as two one-proton doublets at δ=□4.87 (J=4.82 Hz) and 4.23 (J=7.66 Hz) assigned to C-1 anomreic and C-5 protons, as one-proton double doublet at δ 4.43 (J=6.5, 6.1 Hz) attributed to C-2 proton, two one-proton multiplets at δ 3.56 and 3.01, ascribed to C-3′ and C-4′ proton and a one-proton broad signal at δ 4.86 due to C-6′ oxygenated methylene protons. The remaining methine and methylene protons appeared between δ 2.88-1.07. (Table-3)

The ^{13}C NMR spectrum of F_4 showed 35 signals in the molecule. The olefinic and carbinol carbons appeared at δ 140.44 (C-5), 121.17 (C-5), 73.05 (C-3), 76.73 (C-8) and 69.65 (C-8). The sugar anomeric carbon signal resonated at δ 100.78 (C-1) along with other sugar carbons signals at δ 76.94 (C-2), 76.46 (C-3), 70.11 (C-4), 76.73 (C-5) and 61.10 (C-6). Alkaline hydrolysis of F_4 yielded a sterol and β-D-glucose. (Table-2.9). On the basis of foregoing account, the structure of F_4 has been established as cholest-5-en-3β, 12β-triol-3β-D-glycopyranoside. This is a new steroidal saponin and its presence is being reported for the first time from a natural or synthetic sources.

Table-4. ^1H and ^{13}C NMR Spectra of Corylusterol Glycoside (F_4)

Position	^1H NMR		^{13}C NMR
	Alpha	Beta	
1	1.33 d d d (16.1, 9.7, 5.3)	2.16 d d d (12.2,5.8,3.4)	36.82

2	1.89 m	1.81 m	31.40
3	3.63 br m ($w_{1/2}$ =18.2)	-	73.05
4	2.38 d (10.3)	2.12 brs	40.32
5	-	-	140.44
6	5.32 m	-	121.17
7	2.88 d (8.5)	2.90 d (4.42)	27.77
8	-	-	76.73
9	1.50 m	-	49.60
10	-	-	36.20
11	2.16 m	1.45 m	20.58
12	3.12 d d (8.4, 5.3)	-	69.65
13	-	-	45.14
14	1.16 m	-	56.17
15	1.07 m	1.50 m	23.84
16	1.63 m	1.45 m	28.72
17	1.41 m	-	55.43
18	0.64 br s	-	11.66
19	0.95 br s	-	19.08
20	-	1.89 m	35.46
21	0.91 d (6.13)	-	18.60
22	1.61 m	1.07 m	33.52
23	1.21 m	1.78 m	25.46
24	1.18 m	1.13 m	22.61
25	1.61 m	-	29.26
26	0.84 d (6.42)	-	19.69
27	0.80 d (6.21)	-	18.93
1′	4.87 d (4.82)	-	100.78
2′	4.43 d d (6.5, 6.1)	-	76.94
3′	3.56 m	-	73.46

4′	3.04 m	-	70.11
5′	4.23 d (7.66)	-	76.73
6′a	4.86 brs	-	61.10
6′b	4.86 brs	-	-

Coupling constants in Hertz are provided in parenthesis

Table– 5. Chemical Constituents Isolated from Fruits of *Corylus colurna* Linn.*

S.No.	Common Name	Yield	mp	Molecular Formula	IUPAC Name
1.	Colurnasterol glycoside	0.34 %	242-243°	$C_{34}H_{58}O_7$	20 -Hydroxycampesterol-3□β□D-glycopyranoside (New)
2.	β□Sitosterol glycoside	0.36 %	252-253°	$C_{35}H_{60}O_6$	Stignast-5-en-3-0□β□D-glycopyranoside
3.	Compesteryl glycoside	0.54 %	239-240°	$C_{34}H_{58}O_6$	(24R)-Ergost-5-en-3β□D-glycopyranoside
4.	Corylusteryl glycoside	0.43 %	246-247°	$C_{33}H_{56}O_8$	Cholest-5-en-3β, 8β, 12β-triol-3β□D-glycopyranoside (New)

*All the compounds were obtained as steroidal glycoside in CHCl3 – MeOH (9:1) as an eluant of the column.

Two new compounds were established in the present study. They are 20 β-Hydroxycampesterol–3β-D–glycopyranoside (Phytosteroidal) and Cholest–5–en-β, 12,β-triol-β-D–glycopyranoside (Steroidal saponin). The same are reported for the first time from a natural or synthetic source.

3. Experimental Section

Melting points are uncorrected. ^1H and ^{13}C NMR spectra were recorded in $CDCL_3$ on Bruker DRX-300 at 300 M Hz and 100 M Hz, respectively, using TMS as an internal standard; mass spectra ofn Jeol D-300 (EI/CI) system; UV spectra on Bechman DU-64 model; and IR spectra on Hitachi model-270. Purity of the compounds was checked by TLC over silica gel G (Merck)> The spots were visualised by exposure to the iodine vapours, UV radiation and spraying with perchloric acid and ceric sulphate solution.

Plant material

The fruite of *C. colurna* were procured from crude market, Delhi and were examined exomorphologically to establish its botanical identity.

Extraction and isolation of chemical constituents

The air dried and coarsely powdered fruits (2 kg) was extracted exhaustively with ethanol (95%) in a soxhlet apparatus and the ethanolic extract was concentrated to get a dark brown viscous mass (22.55g). It was dissolved in minimum amount of methanol and absorbed on silica gel for column. The slurry was dried in air and loaded to Si-gel column prepared in petroleum ether. The column was eluted with petroleum ether, petroleum ether-$CHCl_3$ (9:1, 3:1, 1:1, 1:3, v/v). $CHCl_3$, $CHCl_3$-MeOH (98:2, 95:5, 9:1, 3:1, 1:1, 1:3, v/v) and MeOH. The homogeneity of the eluants was monitored by TLC and the identical fractions were combined. The concentrated fractions were left at room

temperature for crystallization to get the following compounds.

Colurnasterol glycoside (F1)

Elution of column with $CHCl_3$-MeOH (9:1) (fractions–19-20) gave colourless amorphous powder of F_1, recrystallized from $CHCl_3$-MeOH (1:1) 76 mg (0.34 % yield).
R_f value : 0.31 $CHCl_3$: MeOH (1:1) **mp**:242-243 °C (dec.) **IR ν_{max}** 3400, 2935, 2865, 1650, 1465, 1360, 1160, 1070, 1020, 895 cm^{-1},**^{1}H NMR**:Table – 1, **^{13}C NMR**: Table – 1
EIMS m/z (ret int): 578 $[M]^+$ $C_{34}H_{58}O_7$ (N.O.), 401(3.6), 368 (82.1), 302 (24.5), 234 (7,3), 224 (5.1), 206 (4.3), 155 (6.7), 146 (100), 118 (12.6), 85 (5.3), 57 (10.7), 43 (15.6)

Alkaline hydrolysis of F1

Compound F_1 (25 mg) was heated with 2N ethanolic KOH solution (10 ml) for 6 hours. The reaction mixture was acidified with dilute HCl to pH 5; extracted with $CHCl_3$ (3 x 10 ml); the organic phase was washed with water (2 x 10 ml) and dried over fused Na_2SO_4. Removal of the $CHCl_3$ layer yielded colourless amorphous powder of 21-hydroxycampesterol, m.p. 171-172°C. L.B. test positive.

The aqueous phase was concentrated and subjected to TLC. It was developed with ethyl acetate-acetic acid-water-methanol (6:1:2:1), spraying with aniline phthalate; the spot at R_f 0.41 was comparable with D-glucose.

β-Sitosterol glycoside (F2)

Elution of column with $CHCl_3$-MeOH (9:1) (fractions–21-22) furnished colourless amorphous powder of F_2, recrystallized from $CHCl_3$-MeOH (1:1) 82 mg (0.36 % yield)
R_f value : 0.30 $CHCl_3$: MeOH (1:1) **mp**: 252-253 °C (dec.) **UV γ_{max}**, (MeOH) 205 nm (log e 4.15), **IR ν_{max}** :3450, 2965, 2875, 1810, 1620, 1460, 1375, 1055, 955, 785 cm^{-1},**^{1}H NMR** :Table – 2, **^{13}C NMR** :Table – 2

EIMS m/z (ret int)

576 $[M]^+$ $C_{35}H_{60}O_6$ (N.O.) 414(3.1), 400 (3.0), 396 (32.1), 381 (3.4), 276 (3.7), 273 (3.0), 255 (6.2), 231 (3.0), 213 (5.5), 198 (4.9), 174 (4.9), 173 (5.7), 164 (5.5), 160 (1.9), 159 (6.4), 149 (10.6), 146 (18.9), 138 (8.0), 135 (9.5), 133 (16.1), 121 (10.3), 120 (6.4), 119 (6.4), 109 (16.9), 106 (15.2), 105 (15.1), 95 (20.1), 120 (10.3), 95 (22.6), 83 (15.9), 81 (44.3), 71 (24.2), 69 (41.6), 67 (19.7), 55 (52.3), 43 (100.0).

Alkaline hydrolysis of F2

To compound F_2 (30 mg) was mixed with ethanolic KOH solution (20 %, 10 ml) and the solution refluxed for 6 hours. It was acidified with dilute HCl to Congo red and extracted with $CHCl_3$ (3 x 10 ml), the organic phase washed with water (2 x 10 ml), dried (Na_2SO_4) and evaporated to secure colourless amorphous powder of β-sitosterol, m.p.130-140°C. CO-TLC comparable.

The aqueous phase was concentrated and subjected to silica gel TLC with standard spot of β-D-glucose. After developing the plate in ethyl acetate-acetic acid-water-methanol (6:1:2:1 v/v) and spraying with aniline phthalate reagent, the sugar was identified as β□D-glucose, R_f 0.4, TLC comparable.

Campesteryl glucoside (F3)

Elution of the column with $CHCl_3$-MeOH (9:1) (fractions – 25-30) furnished colourless amorphous powder of F_3, recrystallized from $CHCl_3$-MeOH (1:1) 121mg (0.54 % yield)
R_f value : 0.36 $CHCl_3$: MeOH (1:1) **mp**: 239-240 °C (dec.),**IR ν_{max}**:3400, 3350, 2935, 2870, 1645, 1465,1379, 1162, 1095, 1025, 895 cm^{-1},**^{1}H NMR**:Table – 3, **^{13}C NMR**: Table – 3
EIMS m/z (ret int) :400 $(M)^+$ $C_{28}H_{48}O$ (N.O.) (61.7), 357 (5.3), 273 (4.9), 258 (8.4), 216 (10.4), 192 (5.6), 162 (15.6), 160 (10.5), 149 (36.2), 146(14.4), 136 (18.9), 127 (9.0), 120 (10.4), 111 (15.8), 109 (15.3), 106 (15.5), 5 (33.2), 83 (46.3), 81 (36.2), 71 (49.2), 69 (75.2), 57 (83.2), 55 (76.8), 43 (100).

Alkaline hydrolysis of F3

Compound F_3 (25 mg) was refluxed with ethanolic KOH solution (20 % 10 ml) for 6 hours. The reaction mixture was acidified with dilute HCl to Congo red, extracted with solvent ether (3 x 10 ml), the organic phase was washed with water (2 x 10 ml) and dried (Na_2SO_4). Evaporation of the solvent yielded campesterol, m.p. 155-157°C (lit. m.p. 157-158°), $[\beta]_D$-32.5° (0.2, $CHCl_3$).

The aqueous phase was concentrated and spotted on a silica gel TLC plate. After development of the plate with ethyl acetate-acetic acid-water-methanol (6:1:2:1 v/v) and spraying with aniline phthalate reagent, the spot at R_f 0.4 was comparable with D-glucose.

Corylusterol glycoside (F4)

Elution of the column with $CHCl_3$-MeOH (9:1) (fractions – 31-33) afforded colourless amorphous powder of F_4, recrystallized from $CHCl_3$-MeOH (1:1) 98 mg (0.43 % yield)

R_f value : 0.38 $CHCl_3$: MeOH (1:1) **mp**: 246-247 °C (dec.)**UV λ_{max}**, 217 nm (log e 5.3), **IR λ_{max}** :3440, 2915, 2850, 1610, 1465, 1395, 1310,1080, 1035, 800, 720 cm^{-1},**^{1}H NMR**:Table– 4, **^{13}C NMR**:Table – 4

EIMS m/z (ret int):580 $[M]^+$ $C_{33}H_{56}O_8$ (N.O.), 418 (12.5), 400 (24.4), 385 (5.8), 305 (4.0), 276 (4.6), 163 (14.8), 138 (6.9), 136 (14.9), 125 (8.3), 123 (12.1), 120 (10.8), 113 (5.9), 111 (16.7), 95 (23.6), 84 (9.1), 72 (21.5), 70 (52.7), 55 (53.6), 43 (100).

Alkaline hydrolysis of F4

Compound F_4 (30 mg) was refluxed with ethanolic 2N KOH solution (10ml) for 5 hours. The reaction mixture was acidified with dilute HCl to pH 5, extracted with $CHCl_3$ (3 x 10 ml), the $CHCl_3$ layer was washed with water (2 x 10 ml) and dried (Na_2SO_4). Evaporation of the solvent gave colourless amorphous mass of dihydroxycholesterol, m.p. 166-167°, L.B. test positive.

The aqueous phase was concentrated and subjected to TLC and then developed in ethyl acetate-acetic acid-water-methanol (6:1:2:1). Spraying the plate with aniline phthalate reagent gave a spot (R_f 0.4) comparable with D-glucose.

Acknowledgement

The authors are thankful to the Head, Instrumentation Centre, RSIC, CDRI, Lucknow, for screening NMR and mass spectra, and to the Director, Central Council for Research in Unani Medicine, New Delhi for necessary facilities.

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