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Phytochemical Evaluation of *Chenopodium murale* Linn.

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Abstract: Phytochemical evaluation of *Chenopodium murale* Linn. whole plant, revealed the presence of two flavonoids along with two steroidal glycosides, which were identified as 3,7-Dihydroxy-3`-(4-hydroxy-3-methylbutyl)-5,6,4`-trimethoxyflavone (1), 5,7-Dihydroxy-3`-(2-hydroxy-3-methyl-3-butenyl)-3,6,4`-trimethoxyflavone (2), β-Sitosterol 3-O-β-D-glucoside (3),Stigmasterol 3-O-β-D-glucoside (4). Although these were known compounds, but isolated for the first time from this plant specie. The characterization of all these compounds was achieved by various spectroscopic methods and the results were compared with the literature.

Key words: Phytochemical evaluation, *Chenopodium murale,* flavonoids, spectroscopic methods

Plant description: *Chenopodium murale* Linn. is a medicinal plant that grows in waste places, on rubbish, by road sides and also in crops, in U.S.A., Mexico, Brazil, Argentina Republic, Barbados, Porto Rico and India (Robert and Henry, 1991). This plant belongs to the family Chenopodiaceae also called as goosefoot family that contains about 102 genera and 1400 species of annual and perennial herbs and shrubs scattered throughout the world (Marie, 1965). The genus *Chenopodium* consists of 200 species (Boulos, 1983).

Chenopodium murale is an erect annual plant, up to 60 cm in height. It is slightly mealy and its leaves are 2-8 x 1-6 cm broad, ovate, angular sides, lobed, sharply toothed, base wedge shaped and stalked long or short. Flowers are in slender spikes, forming loose dense axillary clusters. Seeds are sharply keeled, dotted and horizontal (Bamber, 1916; Davis, 1967).

Uses in traditional medicine: The importance of *Chenopodium* species is due to their wide variety of medicinal properties. Plants belonging to this genus are reported to have wide applications in folk medicines; as an anthelmintic, stomachic, antispasmodic, diaphoretic, emmenagogue, for the pain of amenorrhea, as an abortifacient and for the relief of asthma, catarrh and migraine (Watt and Breyer, 1962; Vasishita, 1989). In Mexico, *Chenopodium* is used for medicinal purpose to treat different illnesses, conditions or discomfort, e.g., sterility, digestive problems, anxiety, depression, hair loss and cough etc (Vega and Iskander, 1997). Some flavonoids isolated from *C. murale* showed antihypertensive activity (Ahmad and Elmazar, 1997). Various *Chenopodium* species have been reported to have anthelmintic properties (Lozoya and Lozoya, 1982).

Previously isolated chemical constituents: Chemical studies of members of this genus have been concerned with essential oils (Rustenbekova *et al.*, 1974; De-Paschual *et al.*, 1983); a wide variety of flavonoids (Bahrman *et al.,* 1985; Ahmad *et al.*, 2000 and El-Sayed *et al.*, 1999); sterols and steroidal oestrogens like substances (Van-Rompuy and Zeevaart, 1979; Bathory *et al.*, 1982), alkaloids and coumarins (Rizk, 1986a and b).

Newly isolated chemical constituents: During the present work, we isolated four compounds for the first time from *Chenopodium murale* L. which were identified as 3,7-Dihydroxy-3`-(4-hydroxy-3-methylbutyl)-5,6,4`-trimethoxyflavone (1), 5,7-Dihydroxy-3`-(2-hydroxy-3-methyl-3-butenyl)-3,6,4`-trimethoxyflavone (2), β-Sitosterol 3-O-β-D-glucoside (3),Stigmasterol 3-O-β-D-glucoside (4).

MATERIALS AND METHODS

Instrumentation: All melting points were recorded in glass capillary tubes using Buchi 535 melting point apparatus.

Optical rotations were measured on JASCO DIP-360 (Japan Spectroscopic Co. Ltd., Tokyo, Japan) digital polarimeter.

The Ultraviolet (UV) spectra were recorded in methanol on Hitachi UV-3200 (Hitachi Corporation, Tokyo, Japan) spectrophotometer.

The Infrared (IR) spectra were measured on JASCO IRA-1 (Japan Spectroscopic Co. Ltd., Tokyo, Japan) and a Shimadzu IR-460 (Shimadzu Corporation, Tokyo, Japan) Infrared Spectrometers.

Proton magnetic resonance (^{1}H NMR) spectra were run in $CDCl_3$, CD_3OD and $(CD_3)_2CO$ using TMS as an

internal standard at 300 MHZ, 400 MHZ or 500 MHZ on Bruker AC-300, AM-300, AM-400 or AMX-500 nuclear magnetic resonance spectrometers with aspect 3000 data systems at a digital resolution of 32 K. The ^{13}C NMR spectra were recorded in $CDCl_3$, CD_3OD and $(CD_3)_2CO$ at 75, 100 or 125 MHZ on the same instruments.

Low-resolution electron impact mass spectra were recorded on a Finnigan MAT 311 with MASPEC Data System. Peak matching, field desorption (FD) and field ionization (FI) was performed on the finnigan MAT 312 mass spectrometer. High-resolution mass measurements and fast atom bombardment (FAB) mass measurement were carried out on (Jeol) JMS HX 110 mass spectrometer. FAB source using glycerol or thioglycerol as the matric and cesium iodide (CsI) as internal standard was used for accurate mass measurements.

Chromatography: Column chromatography was carried out on silica gel 60 (E.Merck), (70-230 mesh). Precoated silica gel GF_{-254} preparative plates (20×20, 0.5 mm thick) (E. Merck) were used for preparative thick layer chromatography. Purity of the samples was also checked on the same precoated plates.

Spraying reagents: Ceric sulphate [$Ce(SO_4)_2$] reagent was used for the visualization of compounds. Ceric sulphate (0.1g) and trichloroacetic acid (0.1g) were dissolved in 4 ml distilled water. The solution was boiled and concentrated Sulphuric acid was added drop wise until the disappearance of turbidity.

Plant material: The plant was collected from Matta, District Swat, NWFP, Pakistan during the month of July 2001. The plant was identified by Mehboob-ur-Rehman, plant taxonomist Government College Matta, District Swat, NWFP, Pakistan and verified by Dr.Abdur-Rashid, Chairman Department of Botany, University of Peshawar, NWFP, Pakistan, where voucher specimens were deposited in their Herbarium.

Extraction: The shade dried plant material was chopped into small pieces and finally pulverized into fine powder. The powdered plant material (10 Kg) was soaked in methanol (3 x 10 L), with occasional shaking, at room temperature. After 15 days, the methanol soluble materials were filtered off. The filtrate was concentrated under vacuum at low temperature (40°C) using rotary evaporator. A dark greenish crude extract (253 g) was obtained.

Fractionation: The crude methanolic extract (253 g) was suspended in distilled water (500 ml) and partitioned with *n*-hexane (3 x 500 ml), chloroform (3 x 500 ml), ethyl acetate (3 x 500 ml) and *n*-butanol (3 x 500 ml) to yield the *n*-hexane (24 g), chloroform (61 g), ethyl acetate (30 g), *n*-butanol (35 g) and aqueous (73 g) fractions, respectively.

Isolation: The chloroform soluble fraction (61g) of the crude extract of *C. murale* was subjected to gravity eluted column chromatography over silica gel. Elution was carried out with *n*-hexane (100%), *n*-hexane : $CHCl_3$, $CHCl_3$ (100%), $CHCl_3$: MeOH and MeOH (100%) in increasing order of polarity to obtain six fractions i.e. *n*-hexane (100%) (Fraction A), *n*-hexane : $CHCl_3$ (7:3) (Fraction B), *n*-hexane : $CHCl_3$ (1:1) (Fraction C), *n*-hexane : $CHCl_3$ (2:8) (Fraction D), pure $CHCl_3$ (Fraction E) and $CHCl_3$: MeOH (9:1) (Fraction F), on pooling of the similar fractions on TLC profile.

The Fraction C eluted with *n*-hexane : $CHCl_3$ (1:1) was resubjected to column chromatography over silica gel using *n*-hexane: EtOAc in increasing order of polarity to obtain three major fractions i.e. *n*-hexane : EtOAc (9.5:0.5) (C-1), (9.0:1.0) (C-2) and (8.5:1.5) (C-3), respectively. The fractions (C-1), eluted with *n*-hexane : EtOAc (9.5 : 0.5) were subjected to PTLC using the same solvent system as column which yielded 3,7-Dihydroxy-3`-(4-hydroxy-3-methylbutyl)-5,6,4`-trimethoxyflavone (1) (20 mg).

The Fraction E eluted from the main column with $CHCl_3$ (100%) was subjected to repeat column chromatography over silica gel using solvent system *n*-hexane : acetone in increasing order of polarity to obtain four major fractions i.e *n*-hexane : acetone (8.5:1.5) (E-1), (8.0:2.0) (E-2), (6.5:3.5) (E-3) and (6.0:4.0) (E-4), respectively.

The Fraction E-4 was a binary mixture which was further CC using *n*-hexane : ethylacetate (4:6) as eluent and finally purified by preparative TLC using solvent system *n*-hexane: acetone (7.0:3.0) and *n*- hexane: ethylacetate: methanol (6.0:3.5:0.5) that afforded 5,7-Dihydroxy-3`-(2-hydroxy-3-methyl-3-butenyl)-3,6,4`-trimethoxyflavone (2) (16 mg).

The Fraction F obtained from the main column using $CHCl_3$: MeOH (9:1) gave a binary mixture, which was further chromatographed over silica gel using chloroform and increasing the polarity with methanol. The fractions eluted with $CHCl_3$: MeOH (9:1) yielded β-Sitosterol 3-O-β-D-glucoside (3) (10 mg) and with $CHCl_3$: MeOH (8:2) afforded Stigmasterol 3-O-β-D-glucoside (4) (15 mg).

RESULTS AND DISCUSSION

The isolation and purification of the chemical constituents were undertaken from the Chloroform soluble fraction of the crude extract during which compound 1-4 were isolated by utilizing repeat column chromatography

and preparative TLC. The structure elucidation of these isolated chemical entities was carried out through standard chemical and physical methods including UV, IR, MS, ^{1}H and ^{13}C-NMR (Mabry, Markham and Thomas, 1970; Markham and Mabry, 1975) and comparative studies from the literature.

Table 1 shows ^{1}H-NMR and Table 2 shows ^{13}C-NMR spectral data of compound 1, 2, 3 and 4 respectively.

Compound (1) was assigned the molecular formula $C_{23}H_{26}O_8$ by HR-EIMS showing $[M]^+$ peak at m/z 430.1629 (calcd for $C_{23}H_{26}O_8$, 430.1627). It gave positive Shinoda and negative Quastel test for flavonoids. The IR spectrum showed the absorption at 3380 cm^{-1} indicating the presence of hydroxyl groups, 2905 and 1190 cm^{-1} for methoxyl groups and a, β-unsaturated C=O at 1650 and 1590 cm^{-1}. The UV spectrum showed absorption at 271 and 340 nm suggesting it to be flavonoid (Voirin, 1983).

The ^{1}H NMR spectrum included signals of ABX splitting pattern of phenyl ring B (δ 7.98, 7.96 7.12), the signals for the side chain (δ 3.46, 2.67, 1.65, 1.40 and 0.98) and the signals for three methoxyl groups (δ 3.84, 3.81 and 3.69) attached at C-5, C-4' and C-6 positions respectively. The aromatic proton of ring A also showed the singlet at δ 6.56 indicating a 5,6,7-oxygenated substitution pattern. Acetylation provided a triacetate, confirming the presence of three hydroxyl groups.

The ^{13}C NMR spectrum (BB and DEPT) revealed the presence of four methyl, three methylene, five methines and eleven quaternary carbon atoms. Comparison of ^{13}C NMR data with those of literature (Itrat *et al.*, 2001) confirmed the same substitution pattern in rings A/B and the side chain.

Mass spectroscopic techniques exhibited peaks at m/z 430 $[M]^+$, 358 $[M-C_4H_8O]^+$, 357 $[M-C_4H_9O]^+$, 343 $[M-C_4H_8O-CH_3]^+$, 221 $[M-C_{10}H_9O_5]^+$, 152 $[M-C_{19}H_{18}O_5]^+$. The fragmentation pattern showed that two methoxyls and one hydroxyl group were in ring A, one methoxyl group and the side chain was in ring B and the remaining hydroxyl was on C-3.

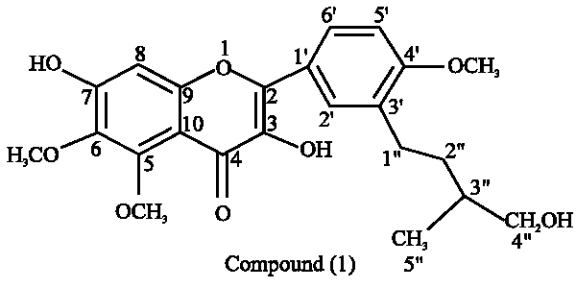
The position of side chain at C-3′ was determined by HMBC experiment in which methylene protons of side chain at δ 2.67 showed ^{2}J correlation to C-3' (δ130.8) and ^{3}J interactions with C-2' (δ 129.5), C-4' (δ156.6) and C-2" (δ 33.0) confirming its position at C-3'. The compound (1) is having a methoxyl group in ring B. Its position at C-4′ was confirmed by HMBC correlations. The protons of methoxyl at C-4' (δ 3.81) showed ^{3}J interactions with C -4' (δ 156.6). The aromatic protons of ring B showed HMBC correlations; the proton at δ 7.12 (H-5') with C-4' (δ 156.6), C-3' (δ 130.8) and C-1' (δ 122.2), the proton at δ 7.84 (H-2') showed cross peaks (^{2}J and ^{3}J) with C-4' (δ 156.6), C-2 (δ 152.0), C-1" (δ 27.4), C-6' (δ 127.9) and the proton at δ 7.78 (H-6') also correlated with C-4' (δ 156.6), C-2 (δ 152.0)

Table 1: ^{1}H-NMR spectral data of compound 1, 2, 3 and 4

^{1}H. No.	Compound No.1	Compound No.2	Compound No.3	Compound No.4
3	-	-	3.85 (m)	3.83 (m)
6	-	-	5.12 (*d*, *J*= 5.4Hz)	5.23 (*d*, *J*= 5.4Hz)
8	6.56 (*s*)	6.54 (*s*)	-	-
22	-	-	-	5.14 (*dd*, *J*= 15.2, 8.4Hz)
23	-	-	-	5.02 (*dd*, *J*= 15.2, 8.6Hz)
1′	-	-	5.33 (*d*, *J*= 7.2Hz)	4.78 (*d*, *J*= 7.4Hz)
2′	7.98 (*d*, *J*=2.3Hz)	7.90 (*d*, *J*=2.3Hz)	-	-
5′	7.12 (*d*, *J*=8.5Hz)	6.98 (*d*, *J*=8.7Hz)	-	-
6′	7.96 (*dd*, *J*=8.5, 2.3Hz)	7.99 (*dd*, *J*=8.7, 2.3Hz)	-	-
1′′	2.67 (*t*)	3.02 (*dd*, *J*=13.7,4.3Hz)	-	-
2′′	1.40 (*m*)	4.33 (*dd*, *J*=8.3, 4.3Hz)	-	-
3′′	1.65 (*m*)	___	-	-
4′′	3.46 (*d*, *J*=6.4Hz)	4.90 (*s*), 4.83 (*s*)	-	-
5′′	0.98 (*d*, *J*=6.67Hz)	1.82 (*s*)	-	-
Me-18	-	-	0.68 (s)	0.67 (s)
Me-19	-	-	1.01 (s)	1.01 (s)
Me-21	-	-	0.92 (*d*, *J*= 6.2Hz)	0.90 (*d*, *J*= 6.2Hz)
Me-26	-	-	0.83 (*d*, *J*= 6.5Hz)	0.83 (*d*, *J*= 6.5Hz)
Me-27	-	-	0.81 (*d*, *J*=6.5Hz)	0.80 (*d*, *J*=6.5Hz)
Me-29	-	-	0.84 (t, J=7.0Hz)	0.82 (*t*, *J*=7.0Hz)
Me-5′′	-	-	-	-
MeO-3	-	3.82 (*s*)	-	-
MeO-5	3.84 (*s*)	-	-	-
MeO-6	3.69 (*s*)	4.01 (*s*)	-	-
MeO-4′	3.81 (*s*)	3.91 (*s*)	-	-

Table 2: ^{13}C-NMR spectral data of compound 1, 2, 3 and 4

	Compound No.1		Compound No.2		Compound No.3		Compound No.4	
C. No.	^{13}C NMR	Multip-licity	^{13}C NMR	Multip- licity	^{13}C NMR	Multip-licity	^{13}C NMR	Multip- licity
1	-	-	-	-	38.7	CH_2	37.8	CH_2
2	152.06	-C-	156.1	-C-	29.9	CH_2	32.8	CH_2
3	138.19	-C-	138.4	-C-	80.9	CH	79.8	CH
4	178.89	-C-	179.13	-C-	43.9	CH_2	43.9	CH_2
5	159.6	-C-	152.8	-C-	142.0	C	141.5	C
6	131.3	-C-	130.0	-C-	122.1	CH	121.1	CH
7	156.4	-C-	155.0	-C-	33.0	CH_2	31.9	CH_2
8	93.9	CH	93.2	CH	32.9	CH	31.7	CH
9	152.38	-C-	152.2	-C-	50.4	CH	50.8	CH
10	105.5	-C-	106.2	-C-	37.1	C	36.9	C
11	-	-	-	-	21.5	CH_2	21.5	CH_2
12	-	-	-	-	40.8	CH_2	39.9	CH_2
13	-	-	-	-	43.0	C	43.1	C
14	-	-	-	-	56.9	CH	57.0	CH
15	-	-	-	-	25.8	CH_2	24.5	CH_2
16	-	-	-	-	29.7	CH_2	28.9	CH_2
17	-	-	-	-	56.5	CH	56.1	CH
18	-	-	-	-	12.2	CH_3	12.6	CH_3
19	-	-	-	-	19.5	CH_3	19.5	CH_3
20	-	-	-	-	37.2	CH	40.5	CH
21	-	-	-	-	19.7	CH_3	21.9	CH_3
22	-	-	-	-	39.5	CH_2	138.9	CH
23	-	-	-	-	29.5	CH_2	129.1	CH
24	-	-	-	-	50.5	CH	52.1	CH
25	-	-	-	-	26.0	CH	32.9	CH
26	-	-	-	-	18.2	CH_3	19.1	CH_3
27	-	-	-	-	20.1	CH_3	21.7	CH_3
28	-	-	-	-	23.7	CH_2	25.6	CH_2
29	-	-	-	-	11.9	CH_3	12.1	CH_3
1′	122.23	-C-	122.6	-C-	103.2	CH	102.8	CH
2′	129.51	CH	131.3	CH	74.2	CH	74.2	CH
3′	131.46	-C-	127.4	-C-	76.81	CH	76.9	CH
4′	156.6	-C-	159.6	-C-	70.2	CH	70.6	CH
5′	110.04	CH	110.3	CH	76.6	CH	76.7	CH
6′	127.9	CH	128.7	CH	62.0	CH_2	62.2	CH_2
1′′	27.47	CH_2	36.98	CH_2	-	-	-	-
2′′	33.01	CH_2	75.3	CH	-	-	-	-
3′′	35.4	CH	147.0	-C-	-	-	-	-
4′′	67.7	CH_2	110.9	CH_2	-	-	-	-
5′′	16.49	CH_3	18.07	CH_3	-	-	-	-
MeO-3	-	-	60.1	OCH_3	-	-	-	-
MeO-5	60.68	OCH_3	-	-	-	-	-	-
MeO-6	60.07	OCH_3	60.9	OCH_3	-	-	-	-
MeO-4′	55.4	OCH_3	55.6	OCH_3	-	-	-	-



Compound (1)

and C-2' (δ 129.5) confirming the substitution pattern of ring B. The position of other two methoxyls group was also confirmed by HMBC interactions; the methoxyl protons at C-5 position at δ 3.84 showed cross peak with C-5 (δ 159.6) and methoxyl protons at C-6 (δ 3.69) coupled with C-6 (δ 131.3). By comparing all these assignments with the literature (Itrat *et al*., 2001) the structure of (1) was confirmed as 3,7-Dihydroxy-3`-(4-hydroxy-3-methylbutyl)-5,6,4`-trimethoxyflavone.

Compound (2) was assigned the molecular formula $C_{23}H_{24}O_8$ by HR-EIMS showing $[M]^+$ peak at *m/z* 428.1475 (calcd for $C_{23}H_{24}O_8$, 428.1471). It gave a red colour in the Shinoda test, typical for flavonoids. Negative results in the Quastel test indicated the absence of an ortho dihydroxyl moiety. The UV spectrum with λ_{max} 272 and 338 nm also suggested it to be a flavonoid. The IR spectrum revealed the presence of the α,β-unsaturated carbonyl group (1680 and 1600 cm^{-1}), a methoxyl group (2927 and 1193 cm^{-1}) and hydroxyl groups (3430 cm^{-1}).

The ^{1}H NMR spectrum provided signals of functional groups including a chelated hydroxyl group at δ 12.94 (1H, s), methoxyl groups [δ 4.01 (3H, s, MeO-6), 3.91 (3H, s, MeO-4'), 3.82 (3H, s, MeO-3)] and an isoprenyl group comprising of a C_5-unit which was revealed to be 2-hydroxy-3-methyl-3-butenyl, two one-proton double doublet at δ 3.02 (J = 13.7, 4.3 Hz) and 2.84 (J = 13.7, 8.3 Hz) assignable to 1" protons, a one-proton double doublet at δ 4.33 (J = 8.3, 4.3 Hz) assignable to a proton at the 2"-position bearing a hydroxyl group, a three-proton singlet at δ 1.82 (CH_3 at 5") and two one-proton singlets at δ 4.90 and 4.83 assignable to an exo-methylene (CH_2 at 4"). It also exhibited an ABX system (B ring) [δ 7.99 (1H, dd, J = 8.7, 2.3 Hz), 7.90 (1H, d, J = 2.3 Hz) and 6.98 (1H, d, J = 8.7 Hz)] and one aromatic proton (A ring) [δ 6.54 (1H, s, H-8)].

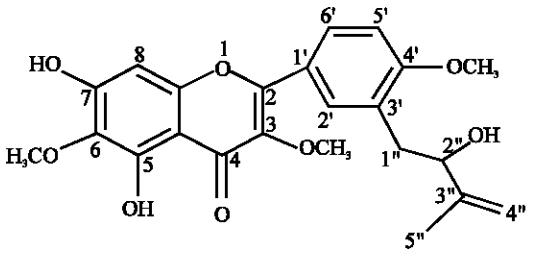
The ^{13}C NMR spectrum (BB and DEPT) of (2) corroborated the presence of four methyl, two methylene, five methines and twelve quaternary carbons. The signal at δ 93.2 was typical of C-8 carbon of 5,6,7-oxygenated flavonoids.

The EIMS gave distinct peaks for flavanones at *m/z* 428 ($[M]^+$), 358 ($[M-70]^+$), 357 ($[M-71]^+$), 343 ($[M-70-CH_3]^+$) and two typical daughter ions at *m/z* 183 ($[A_1+H]^+$) and 219 ($[B_2]^+$). This confirmed the presence of two hydroxyl groups and one methoxyl group in ring A and one methoxyl and the side chain in ring B and the remaining methoxyl group at C-3. The benzyl cleavage explained the loss of 71. a.m.u., while the loss of 70 a.m.u. was due to the *β*-cleavage of the side chain with H-transfer to the aromatic nucleus *via* a 1,6 rearrangement (Markham and Mabry, 1975).

On the basis of the analysis of its 2D NMR spectra (HMQC and HMBC), (2) was deduced to be a flavanone with isoprenylated group. The two double doublets of methylene protons of the isoprenylated moiety at δ 3.02 and 2.84 correlated with C-3' (δ 127.4), C-2" (δ 75.3), C-2' (δ 131.3), C-3" (δ 147.0) and C-4' (δ 159.6), the proton germinal to the secondary hydroxyl group at δ 4.33 correlated with C-4" (δ 110.9) and the exo methylene protons at δ 4.90 and 4.83 coupled with C-2" (δ 75.3) and C-5" (δ 18.1). The protons of methyl group at δ 1.82 showed cross-peaks (J^2 and J^3) with C-3" (δ 147.0), C-4" (δ 110.9) and C-2" (δ 75.3) confirming the position of hydroxyl and olefinic groups in the prenyl moiety which was itself assigned to C-3'. The methoxyl protons at δ 3.91 also showed correlation with C-4' (δ 159.6) providing evidence for its location at C-4' position. The other methoxyl groups at δ 3.82 and 4.01 were also confirmed by HMBC interactions showing correlations with C-3 (δ 138.4) and C-6 (δ 130.0), respectively.

The ring A proton at δ 6.54 showed cross-peaks with C-7 (δ 155.0), C-9 (δ 152.2), C-6 (δ 130.0) and C-10 (δ 106.2), on the basis of which it could be assigned to C-8. The proton of the chelated hydroxyl group at δ 12.94 also showed HMBC interactions with C-5 (δ 152.8), C-6 (δ 130.0) and C-10 (δ 106.2).

The other aromatic protons of ring B also showed HMBC interactions; the proton at δ 6.98 (H-5') coupled with C-4' (δ 159.6), C-3' (δ 127.4) and C-1' (δ 122.6), the proton at δ 7.90 (H-2') coupled with C-4' (δ 159.6), C-2 (δ 156.1), C-1" (δ 37.0), C-6' (δ 128.7) and the proton at δ 7.99 (H-6') correlated with C-4' (δ 159.6), C-2 (δ 156.1) and C-2' (δ131.3) to confirm the substitution pattern of ring B. By comparing all these data with the published values (Itrat *et al*., 2002); the structure of compound (2)was, therefore, assigned as 5,7-Dihydroxy-3`-(2-hydroxy-3-methyl-3-butenyl)-3,6,4`- trimethoxyflavone.



Compound (2)

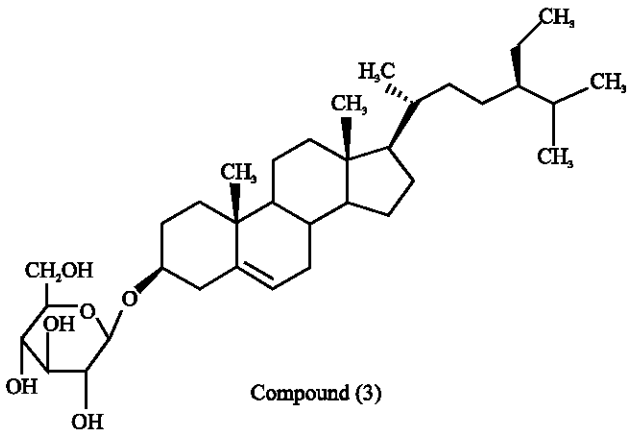
β-Sitosterol 3-O-β-D-glucoside (3) was obtained as colorless crystals from chloroform soluble portion of the methanolic extract of *C. murale* on elution with $CHCl_3$: MeOH (9:1). The HR-EIMS gave the molecular ion peak at *m/z* 576.4386 consistent with the molecular formula $C_{35}H_{60}O_6$ (calcd for $C_{35}H_{60}O_6$ 576.4389). The fragmentation pattern in the mass spectrum of compound (3) was characteristic for sterol with double bond at C-5 (Wyllie *et al*., 1977).

The IR spectrum showed absorptions bands for hydroxyl group (3452 cm^{-1}) and trisubstituted double bond (3044, 1646 and 814 cm^{-1}).

The ^{1}H-NMR data were similar to β-Sitosterol (Ian *et al*., 1976) except additional resonances at δ 5.33 (1H, d, J = 7.2 Hz) for the anomeric proton and between δ 3.82-4.42 (sugar protons geminal to hydroxyl groups).

On the basis of above evidence, comparison of ^{13}C NMR data with the published values (Herbert *et al*., 1978; Adolfo and Pomilio, 1983), co-TLC and mixed m.p. with an authentic sample, the structure of (3) was established as β–Sitosterol 3-O-β-D-glucoside.

Stigmasterol 3-O-β-D-glucoside (4) crystallized as colorless crystals from chloroform soluble fraction of the methanolic extract of *C. murale*. The molecular formula was established as $C_{35}H_{58}O_6$ by HR-FAB-MS, which

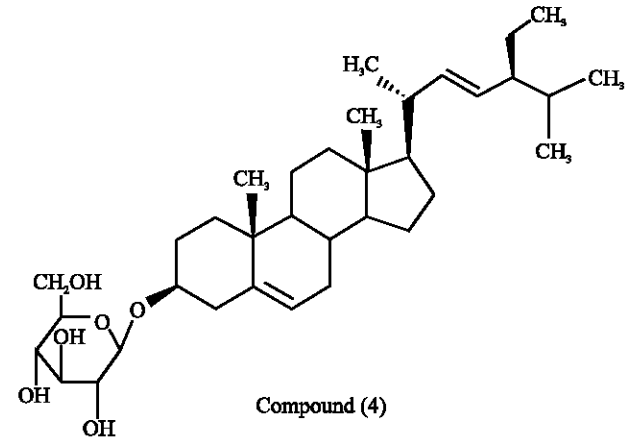


Compound (3)

showed molecular ion peak at *m/z* 574.4231 (calcd. for $C_{35}H_{58}O_6$ 574.4233). The mass spectrum showed characteristic fragmentation pattern for sterols (Wyllie *et al*., 1977).

The ^{1}H-NMR of (4) completely corresponded to the data for stigmasterol (Ian *et al*., 1976) except additional resonances at δ 5.23 (1H, d, *J* = 5.4 Hz) and between 3.84-4.44 corresponding to the sugar moiety. The ^{13}C NMR spectrum was also in agreement with the published data for Stigmasterol (Herbert *et al*., 1978) except additional peaks for sugar moiety.

On the basis of above evidence as well as co-TLC and mixed m.p with an authentic sample, the structure of compound (4) was established as Stigmasterol 3-O-β-D-glucoside.



Compound (4)

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REFERENCES

Adolfo, M.I. and A.B. Pomilio, 1983. Components of *Bauhinia candicans*, J. Nat. Prod., 46: 752-753.

Ahmed, A.G., G.T. Maatooq and M. Niwa, 2000. Two flavonoid glycosides from *Chenopodium murale*, Phytochemistry, 53: 299-303.

Ahmad, A.G. and M.M.A. Elmazar, 1997. Isolation of Hypotensive flavonoids from *Chenopodium* species growing in Egypt, Phytotherapy Research, 11: 564-567.

Bahrman, N., M. Jay and R. Gorenflot, 1985. Contribution to the chemosystematic knowledge of some species of genus *Chenopodium,* Lett. Bot., 2: 107-113.

Bamber, C.J., 1916. Plants of the Punjab, Superintendent Government Printing, Punjab, Pakistan, pp: 387.

Bathory, M., I. Toth, K. Szendrei and J. Reisch, 1982. Ecdysteroids in Spinaciaoleraceae and Chenopodium bonus, Phytochemistry, 21: 236-238.

Boulos, L., 1983. Medicinal plants of North Africa, Reference Publication, Algonac, MI 48001.

Davis, P.H., 1967. Flora of Turkey, Edinburgh University press, pp: 302.

De-Pascual, T.J., C. Torres, M.S. Gonzalez, M. Grande and I.S. Bellido, 1983. Delta-5-hydro-1-hydroxy carvomenthols from the essential oil of *Chenopodium multifidum*. Phytochemistry, 22: 2749-2752.

El-Sayed, N.H., A.S. Awaad, M.S. Hifnawy and T.J. Mabry, 1999. A flavonol triglycoside from *Chenopodium murale*. Phytochemistry, 51: 591-593.

Herbert, L.H., R.P.D. Peter and J.T. Greegg, 1978. ^{13}C nuclear magnetic resonance spectra of some C-19-hydroxy, C-5, 6 epoxy, C-24 ethyl and C-19-norsteroids. Can. J. Chem., 56: 3121-3127.

Ian, R., L.D. John, A.D.H. Clague and L. J. Mulheirn, 1976. The 220 MHZ. NMR spectra of Phytosterols. Phytochemistry, 15: 195-200.

Itrat, A., E. Anis, S. Ahmad, G. Mustafa, A. Malik, Z. Amtul and Atta-ur- Rahman, 2001. Thrombin inhibitory constituents from *Duranta repens* Linn., Helv. Chim. Acta, 84: 649.

Itrat, A., S. Ahmad, A. Malik, A. Yasin and M.I. Choudhary, 2002. Enzymes inhibitory constituents from *Duranta repens* Linn. Chem. Pharm. Bull., 50: 515.

Lozoya, J. and M. Lozoya, 1982. In: Flora medicinal de México. Primera part: plantas in digenas, Instituto Mexicano del Seguro Social, Mexico, pp: 31.

Mabry, T.J., K.R. Markham and M.B. Thomas, 1970. The systematic identification of flavonoids. New York: Springer.

Marie, C.N., 1965. In Gardens of Hawaii. Bishop Museum Press, pp: 331.

Markham, K.R. and T.J. Mabry, 1975. In J.B. Harborne, T.J. Mabry and H. Mabry, The flavonoids, Chapman and Hall. London, pp: 48-63.

Rizk, A.M., 1986a. Constituents of Plants growing in Qatar, Fitoterapia, 57: 3-9.

Rizk, A.M., 1986b. The Phytochemistry of Flora of Qatar, King print of Richmond. London, pp: 30-32.

Robert, B. and T. Henry, 1991. Medicinal Plants, Periodical Experts Book Agency-Delhi, India, pp: 216.

Rustenbekova, G.B., M.I. Goryaev and G.A. Nizhinskaya, 1974. Flavonoids of Chenopodium botrys, Khim. Prir. Soedin, Tashk., 3: 403.

Van-Rompuy, L. and J. Zeevaart, 1979. Are steroidal oestrogens natural plants Constituents. Phytochemistry, 18: 863-866.

Vasishita, P.C., 1989. In: Taxonomy of Angiosperms, Ram Chand, India, pp: 648.

Vega-Carrillo and H.R. Iskander, 1997. Int. J. Environ. Anal. Chem., Gordon and Breech, 66: 95-105.

Voirin, B., 1983. Two new isoflavones from *Ceiba pentandra*. Phytochemistry, 22: 2107.

Watt, J.M. and M.G. Breyer-Brandwijk, 1962. The Medicinal and Poisonous Plants of Southern and Eastern Africa. 2nd edn, Livingstone, Edinburgh, pp: 184-192.

Wyllie, S.G., B.A. Amos and L. Tokes, 1977. Electron Impact Induced Fragmentation of Cholesterol and related C-5 Unsaturated Steroids. J. Org. Chem., 42: 725-732.