Spasmolytic effects of *Scrophularia nodosa* extract on isolated rabbit intestine

Mansoor Ahmad[1*], Noor Muhammad[1], Mehjabeen[2], Noor Jahan[3], Manzoor Ahmad[4], Obaidullah[5], Mahmood Qureshi[1] and Syed Umar Jan[6]

[1]Research Institute of Pharmaceutical Sciences, Department of Pharmacognosy, University of Karachi, Karachi, Pakistan
[2]Department of Pharmacology, Federal Urdu University of Arts, Science and Technology, Karachi-75300, Pakistan
[3] Department of Pharmacology, Dow College of Pharmacy, Dow University of Health Sciences, Karachi, Pakistan
[4]Department of Chemistry, University of Malakand, Pakistan, [5]Department of Pharmacy, University of Peshawar, Peshawar Pakistan
[6]Department of Pharmacy, University of Balochistan, Quetta Pakistan

Abstract: *Scrophularia nodosa* (figwort), an indigenous medicinal plant grows in moist and cultivated waste ground. It contains saponins, cardioactive glycosides, flavonoids, resin, sugar and organic acids. It is traditionally used for anti-inflammatory purpose and in skin disorders. It has diuretic and cardiac stimulant properties. The present studies were carried out on crude extract of *Scrophularia nodosa* and its *n*-hexane, chloroform, ethyl acetate, *n*-butanol and aqueous fractions. During phytochemical studies seven known compounds of flavonoid nature were isolated from the chloroform fraction of crude extract of *S. nodosa.* The structures of these compounds were elucidated by spectroscopic (UV, IR, Mass (EIMS, HREIMS) and NMR (^{1}H-NMR, ^{13}C-NMR, DEPT, and ^{1}H-^{1}H, COSY, HMQC, HMBC and NOESY) techniques. Compound 1 was identified as 5, 4`-hydroxy-3, 6, 7-trimethoxyflavone, compound 2 as 5-hydroxy-3,6,7,4′-tetramethoxyflavone, compound 3 as Centaurein, compound 4 as 5-hydroxy-7,8,2′,3′,4′-pentamethoxyflavone (Serpyllin), compound 5 as Kaempferol 7-O-α-L-rhamnopyranoside, compound 6 as sakuranetin 4′-*O* (6″-*O*-*α*-L-rhamnopyranosyl)-*β*-D-glucopyranoside (Vitexoside) and compound 7 as Spinoside. Crude extract and its fractions were tested on isolated rabbit intestine (*in vitro*) for their effects. The results of crude extract and its fractions in different doses showed the decrease in normal movement of the smooth muscles of rabbit intestine (jejunum). The chloroform fraction showed maximum relaxant effect (77.37%) at 15mg/ml dose and aqueous fraction showed 38.56% spasmogenic response which was not present in the crude extract. Further study was carried out on different fractions to investigate the possible mechanism of action of *S. nodosa* extract. For this purpose spasmolytic effect of different fractions were compared with agonist and antagonist activities of standard drugs including adrenaline, atropine andacetylcholine ($1x10^{-2}$, $1x10^{-4}$ and 10^{-6} M conc.). It is concluded that the chemical constituents present in *S. nodosa* having spasmolytic action are possibly acting through muscarinic receptors.

Keywords: *Scrophularia nodosa*, scrophulariaceae, spasmolytic, spectroscopy.

INTRODUCTION

Scrophularia nodosa (Scrophulariaceae) locally known as figwort belongs to genus *Scrophularia.* The species belonging to this genus have been used as anti-inflammatory drugs, for scabies, tumours and sepsis, as diuretic and shows bacteriostatic properties (Galindez *et al.*, 2002, Karimova *et al*., 1967). *S. nodosa* and respective species contain cinnamic acid, caffeic acid, vanillic acid, sugars (Swiatek 1970), cinnamoylaubucin (Swann and Melville 1972), phenol, carboxylic, flavones, methylated flavone derivatives of cinnamic acid (de Santos Galindez *et al*., 2002), iridoid glycosides and phenylethanoid glycosides (Weinges and Von der Eltz 1978; Miyase *et al*., 1999). Due to the presence of several compounds *S. nodosa* has pharmacological importance. Therefore, we have investigated the extract of *S. nodosa* for *in vitro* biological activities to evaluate its phytomedicinal potential.

**Corresponding author:* e-mail: herbalist53@yahoo.com

MATERIAL AND METHODS

S. nodosa (whole plant) was collected from Matta, Swat District, Khyber Pukhtoon Khah Pakistan, during September 2001. Mr. Mehboob ur Rehman of Jahan Zeb Post Graduate College, Saidu Sharif (Swat District) identified the plant (voucher, specimen OG-01/2002). Shade-dried whole plant (*S. nodosa*) was ground in a chopper and percolated with ethanol at room temperature. The ethanol extract was filtered and evaporated under vacuum to obtain a thick gummy mass. Its *n*-hexane, chloroform, ethyl acetate, *n*-butanol and aqueous fractions were prepared. All these fractions and the crude extract were tested for smooth muscle activities at different doses (1, 5, 10, 15, 20, 25mgs). The vehicle (distilled water) used for solubilization of drugs had no effect on tissue activity in the control experiments. HPLC (High Performance Liquid Chromatography), FTIR (Fourier Transform Infrared Spectroscopy), FTNIR (Fourier Transform Near-Infrared) and UV (Ultraviolet Spectrophotometry) were also performed on crude extract of *S. nodosa*.

HPLC, FTIR, FTNIR and UV identification of crude extract of S. nodosa

FTIR and FTNIR were performed according to the modified methods described by Andrei *et al.,* (2006). UV spectra of the crude extract (500 mg) was taken in 500 ml round bottom flask, after adding 50 ml methanol it was refluxed for 30 minutes and then cooled down to room temperature. It was filtered in 100 ml volumetric flask using Whatman filter paper 40 and volume was made up by adding methanol. A Lambda 20 Perkin Elmer was used to record the UV spectra.

For HPLC analysis, crude extract (2 gm) of *S. nodosa* was dissolved in 10 ml of methanol with the help of sonication at 40 °C for 30 min. The sample was filtered through 0.45 mm filter paper with the help of sunnix filtering assembly. HPLC was performed on a SHIMADZU HPLC (Detector: SPD 20A, Pump: LC 20AT, Auto-sampler: SIL 20A, System Controller : CBM 20A and HPLC Column Manufacture : Waters µBondapak C18 3.9X300mm). The system was operated at room temperature (20°C), the injection volume was 20 micro liters and the detection wavelength was 225 and 325 nm (Muhammad 2006).

Isolation of compounds

The chloroform fraction (40 g) of the extract was subjected to column chromatography over a silica gel column (800 g, 70-230 mesh, Merck) using *n*-hexane with gradient of chloroform up to 100% and methanol up to 20%. Thirteen fractions were collected. The initial fractions which were prominent in hexane contained mostly terpenes, whereas the chloroform rich fractions mostly contained flavonoids. The flavonoids were isolated by using repeated column chromatography (CC; flash silica gel, 230-400 mesh) preparative thin layer chromatography (TLC; silica gel 60 GF254) chloroform and hexane as a mobile phase. Fraction no. 6 of the first column was loaded on silica gel (flash silica 230-400 mesh) and eluted with 10% chloroform-*n*-hexane mobile phase, which yielded five compounds. These compounds were again loaded on silica gel column chromatography using mobile phase of *n*-hexane: chloroform (85:15) to purify compounds as nsn-1 (10 mg), nsn-2 (12 mg), nsn-3 (15 mg), nsn-4 (17 mg) and nns-5 (8 mg). Fraction 10 obtained from first column, contained compounds nsn-6 and nsn-7, was loaded on a silica gel column chromatography using a system of *n*-hexane:ethylacetate (70:30) to purify the compounds nsn-6 (13 mg) and nsn-7 (15 mg). While other fractions contained very less amount therefore were not worked out (Sticher *et al.,* 1982, Ahmad *et al.,* 1985; and Ahmad 1986).

Smooth muscle activity (spasmolytic activity)

Animals were sacrificed by a blow on the back of the head, the abdomen was cut open and a piece of jejunum was taken out. Segments 2 cm long were suspended in Tyrode's solution aerated with a mixture of 95% oxygen and 5% carbon dioxide, and maintained at 37°C. The spontaneous intestinal movements were recorded using Harvard transducers and Harvard Student Oscillograph. Crude extract and its fractions were dissolved in 1 ml of distilled water and thereafter, it was added to the organ bath after an equilibration period (Mehjabeen *et al.,* 2004 and National Research Council, 1996).

STATISTICAL ANALYSIS

The results were expressed as mean±S.E.M. All statistical comparisons were made by means of Student's *t*-test and a *P* value smaller than 0.05 was regarded as significant.

RESULTS

Basic phytochemical screening showed the presence of flavonoids, saponins, and cardiac glycosides in the crude extract. While UV, FTIR, FTNIR and HPLC analysis further elaborated the presence of different chemical constituents in the crude extract of *S. nodosa* (fig. 1a-e).

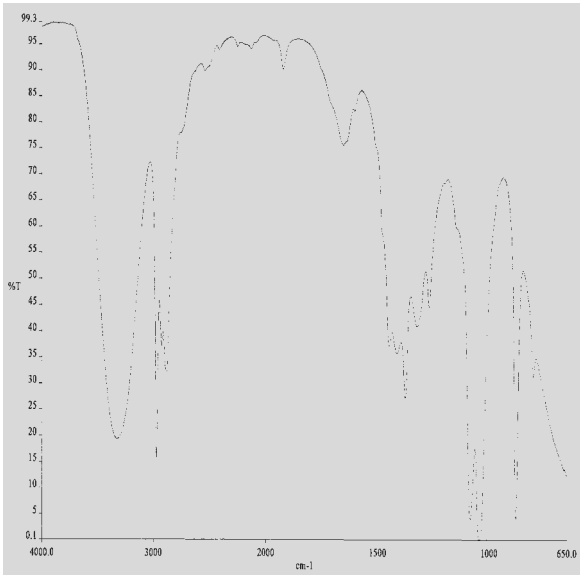


Fig. 1a: FTIR spectrum of *S. nodosa* crude extract.

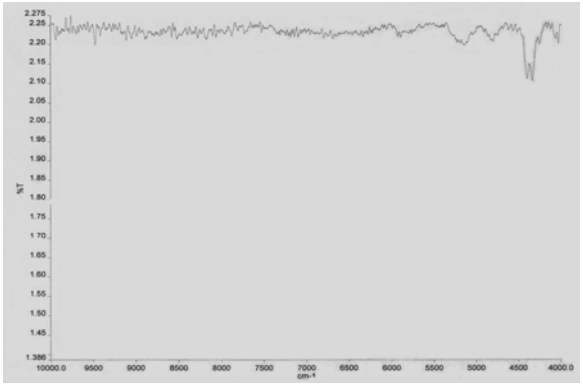


Fig. 1b: FTNIR spectrum of *S. nodosa* crude extract.

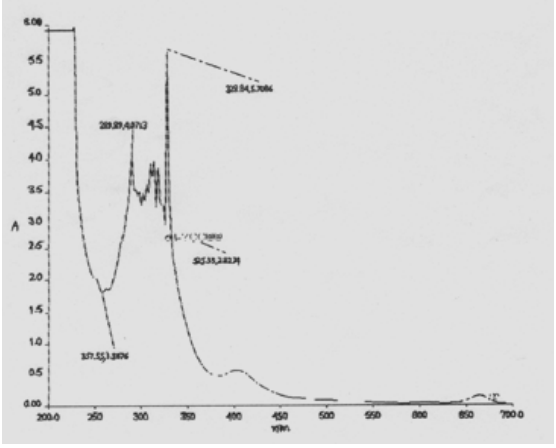


Fig. 1c: UV analysis of *S. nodosa* crude extract

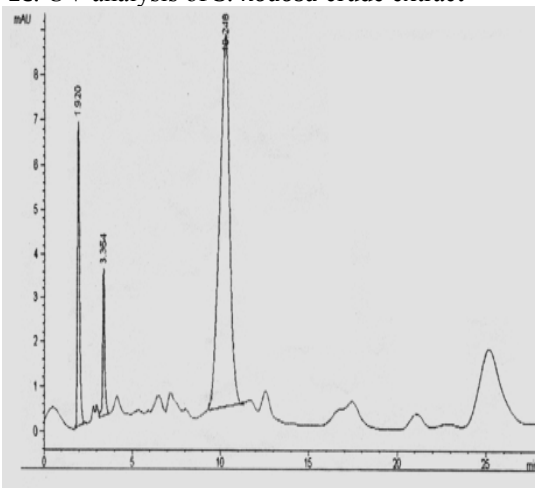


Fig. 1d: HPLC chromatogram of *S. nodosa* crude extract at 325 nm.

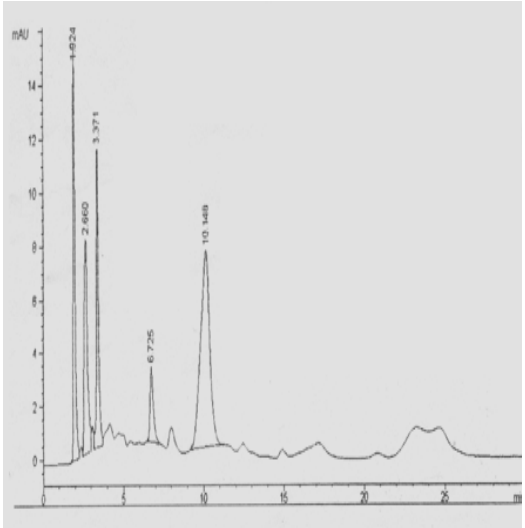


Fig. 1e: HPLC chromatogram of *S. nodosa* crude extract at 225 nm.

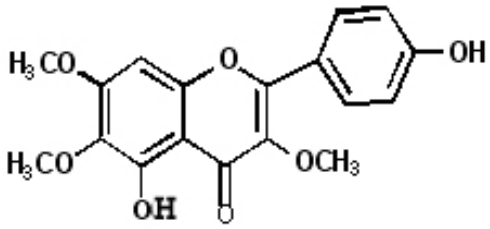
The whole plant of *Scrophularia nodosa* was extracted with ethanol. The ethanolic extract was portioned by $CHCl_3$. The $CHCl_3$-soluble fraction on silica gel column chromatography yielded thirteen sub-fractions. A series of chromatographic techniques on these sub-fractions resulted in isolation of seven known compounds (nsn-1-7) for the first time from this plant (fig. 2). The structure of the compounds were elucidated through extensive spectroscopic studies including EIMS, HREIMS, ^{1}H-NMR, ^{13}C-NMR, DEPT, AND 2D NMR techniques including ^{1}H-^{1}H, COSY, HMQC, HMBC and NOESY (tables A1 and A2).

The results of smooth muscle activity of crude extract of *S. nodosa* presented in table 1 and graph 1. There was a gradual decrease in the smooth muscle acitivity and the response was prominent at 25 mg/ml concentration. At a dose of 1 mg/ml there was a slight decrease in muscle activity. Similarly, all the effects of different fractions were tested at the conc. of 5, 10 and 15 mg/ml (table 2; graph 2).

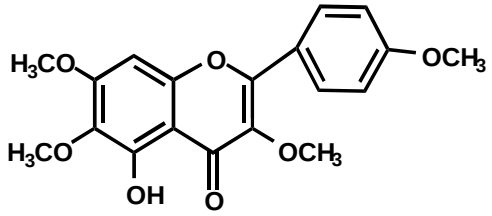
DISCUSSION

In present study the results were also compared with the published data and the compound 1 was identified as 5, 4`-hydroxy-3, 6, 7-trimethoxyflavone, reported previously from *Brickellia pendula* (Flores and Herran 1958), compound 2 was 5-hydroxy-3,6,7,4′-tetramethoxyflavone in complete agree-ment to reported literature from *Dodonaea lobulata* (Dawson *et al*., 1966), compound 3 was identified as centaurein, all the physical and spectral data was complete as reported previously from *Centaurea jacea* (Flamini *et al*., 2001; Changa *et al.,* 2007). Compound 4 was identified as 5-hydroxy-7,8,2′,3′,4′-pentamethoxy-flavone which has earlier reported from *Andrographis serpyllifolia* (Govindachari *et al*., 1968). Compound 5 was obtained as yellow amorphous powder and identified as reported compound Kaempferol 7-O-α-L-rhamnopyranoside, earlier reported from *Cephalocereus senilis* (Liu *et al.,* 1994). Compound 6 (Vitexoside) was isolated as amorphous white solid and was assigned the structure sakuranetin 4′-*O* (6′′-*O*-*α*-L-rhamnopyranosyl)-*β*-D-glucopyranoside. Previously this compound was reported from *Vitex negundo* (Ul-Haq *et al.,* 1994). Compound 7 was isolated from the chloroform soluble fraction of *Scrophularia nodosa.* It was recognized as flavonoid glycoside. Previously this compound was reported from *Amaranthus spinosus* (Ul-Haq *et al.,* 2004).

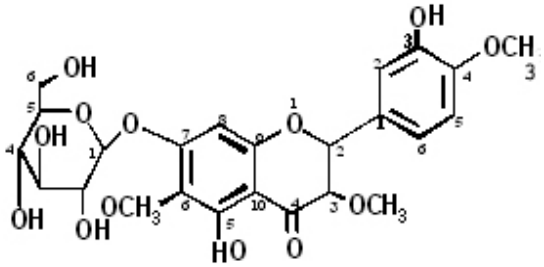
The results of smooth muscle activity on isolated rabbit intestine indicated that the crude extract of *S. nodosa* had relaxing activity (spasmolytic effect). The fractions reflect the activity which was present in its crude extract but the existence of the effect was quite changed. The ethyl-acetate fraction showed almost same effect, as appeared in 1 and 5 mg/ml doses of the crude extract. The chloroform fraction exhibited first or initial relaxing response followed by a contracting effect, this mild contraction of muscle was not observed in the crude



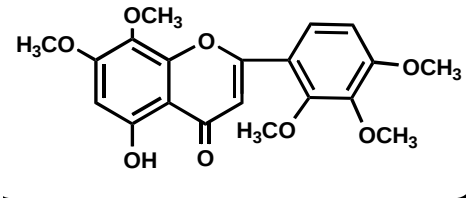
5, 4`-dihydroxy-3, 6, 7-trimethoxyflavone (1)



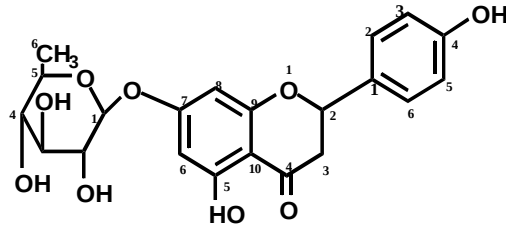
5-hydoxy-3, 6, 7, 4′-tetramethoxyflavone (2)



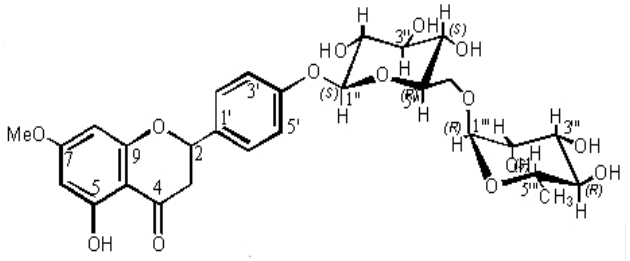
Centaurein (3)



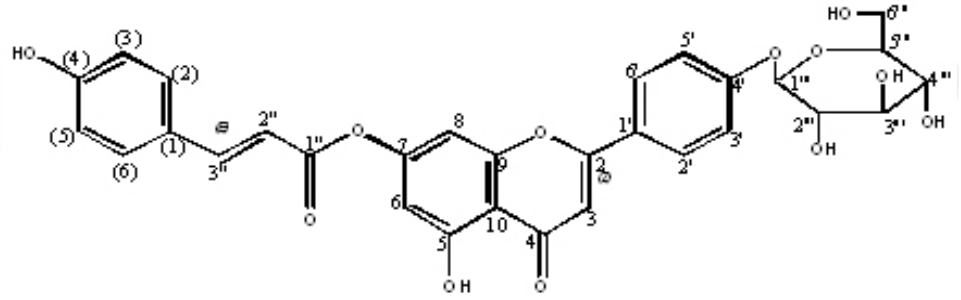
Serpyllin (4)



Kaempferol 7-O-α-L-rhamnopyranoside (5)



Vitexoside (6)



Spinoside (7)

Fig. 2: Chemical structures of flavonoids isolated from chloroform fraction of *S. nodosa* crude extract.

extract of *S. nodosa*. The *n*-hexane fraction of this plant did not show any effect or had negligible activity, whereas *n*-butanol fraction showed slight relaxing effect at the dose of 15 mg/ml. The aqueous fraction showed initial relaxation followed by contraction of tissue of intestine at the dose of 15 mg/ml. These results showed aqueous and chloroform fractions had mild contraction effect on intestinal smooth muscle which was not observed in the crude extract (graph 2).

Further interesting results were observed when these fractions were treated and compared with standard drugs acetylcholine, atropine and adrenaline. Graphs 3-6 show the effects of smooth muscle activity of fractions of *S. nodosa* compared with standard drugs on isolated rabbit intestine. The doses of all fractions weres of 15 mg/ml each.

With all fractions when the intestinal tissue was post treated with adrenaline $1x10^{-2}$M they completely blocked the muscle rhythmic activity. When the same procedure was repeated with low concentration of adrenaline (1×10^{-6}M), aqueous fractions (15mg/ml) continued spasmogenic effect (graph 3).

Table A1: Characterization of compounds isolated from *S. nodosa*

Characteristics of Compounds	(1)	(2)	(3)	(4)
MP	Yellow crystals 221-222°C	Yellow crystals 173-174°	Yellow powder 190-200°C $[\alpha]_D^{25}$: -76.6^0	Bright yellow needles 170°C
UV	UV (MeOH) $\lambda_{max:}$ 357and272nm	UV (MeOH) $\lambda_{max:}$ 355 and 272 nm	UV (MeOH) $\lambda_{max:}$ 349, and 258 nm	UV (EtOH) λ_{max} 325-310 and 272.5 nm
IR	IR ($CHCl_3$), V_{max} cm^{-1}: 3335, 1650, 1600, 1370, and 888,	IR ($CHCl_3$): V_{max} cm^{-1}: 3510, 1730, 603, 1355, and 865	(KBr) ν_{max} Cm^{-1} 3400, 3100, 1660, 1620, and 1582-1464	IR (CHC IR ($CHCl_3$) V_{max} cm^{-1} 3510, 1660, and 1600
EI-MS*m/z*(rel. Int.)	$[M]^+$ 344 (100), 29 (32), 196 (24),121 (33)	$[M]^+$ 358 (100), 357 (44), 343 (72), 340 (29), 329 (5), 327(4.5), 196 (8), 135 (30), 133 (25)	360 (100), 359, (38), 345 (7.6), 317 (15.6), 178, (4), 163 (3.1)	$[M]^+$388(11), 358(100), 357 (25),343 (94), 329 (20), 327, (11), 313 (13), 312 (19), 196(8), 153 (13), 135 (30), 196(8), 153 (13), 135 (30)
HR-EI-MS *m/z*	344.0862 (calcd. for $C_{18}H_{16}O_7$, 344.0896)	358.1059,(calcd. for.$C_{19}H_{18}O_7$ 358.1052)	521.4475 [M-H]+ (calcd. for $C_{24}H_{25}O_{13}$ 521.4475)	388.2719 388.2719 (calcd. for. $C_{20}H_{20}O_8$ 388.2713)
^{1}H-NMR	($CDCl_3$, 400 MHz) δ 8.03 (2H, d, *J* = 8.7 Hz, H-2` and H-6`), 7.09 (2H, d, *J* =8.7 Hz, H-3` and H-5`), 6.49 (1H, s, H-8), 3.87, 3.68 and 3.60 (3H each, 3xOCH_3).	($CDCl_3$, 400 MHz) δ 12.60 (1H, s, H-5), 8.06 (2H, d, *J* = 9.1 Hz, H-2′ and 6′),7.01 (2H, d, *J* = 9.1 Hz, H-3′ and H-5′),6.48 (1H, s, H-8), 4.01, 3.98, 3.94, 3.82(3H each, s, OCH_3 at C-6, C-4′, C-7, and C-3).	(CD_3OD, 300 MHz) δ7.79 (1H, d, *J* = 2.2 Hz, H-3′), 7.58 (1H,dd, *J* = 8.5 2.2 Hz, H-6`),6.87 (1H, d, *J* =8.5 Hz, H-6′),6.86 (1H, s, H-8), 3.89(3H, s,OMe), 3.78(3H, s,OMe), 3.75(3H,s, OMe), 5.33 (1H, d, *J* =7.2 Hz, H-1″), 3.91(1H,m, H-2″), 3.67(1H, m, , H-3″), 3.62 (1H,m,H-4″), 3.57 (1H, m, H-5″), 3.37-3.45(2H, m, H-6″)	^{1}H-NMR ($CDCl_3$, 400 MHz) δ 12.71 (1 δ 12.71 (1H, s, H-5), 7.68, and 6.82 (1H each, d, *J* = 9.1 and 6.82 (1H each, d, *J* = 9.1 Hz, H-5′ and H-6′), 6.45 (1H, s, H-3), 6.92 (1H, s, H-6), 3.99, 3.98, 3.95, 3.94, 3.91 (3H each, s, 5xOCH_3) H-8 or
^{13}C-NMR	($CDCl_3$, 125 MHz) δ 178.7 (C-4),155.1 (C-5), 157.8 (C-4`),154.1 (C-7), 152.5(C-9), 152.1 (C-2), 138.1 (C-3), 132.1(C-6), 131.1 (C-2` and 6`), 122.3 (C-1`),116.4(C-3` and 5`),106.1(C-10), 94.6 (C-8),60.3, 60.5 and 55.9	($CDCl_3$, 125 MHz) δ 179.2 (C-4), 161.1 (C-7), 158.2 (C-5), 156.0 (C-4′),152.4 (C-9), 152.3(C-2), 138.7 (C-3), 132.7 (C-6),130.1(C-2′and C-6′),122.8122.8 (C-1′),114.1(C-3′and 5′),106.6(C-10), 90.3 (C-8), 60.8, 60.12 (OCH_3-6, OCH_3-3) 56.3 and 55.4 (OCH_3-7 and OCH_3-4′)	(CD_3OD,75 MHz) δ179.3 (C-4), 158.8 (C-2), 158.1 (C-7), 153.6 (C-3`),153.2 (C-9), 151.8 (C-5), 148.5 (C-4′), 139.7 (C-3), 133.7 (C-6), 123.5 (C-1′),120.2 (C-6″), 114.4 (C-2′),112.2 (C-5′), 108.0 (C-10), 101.4 (C-1″), 95.5 (C-8), 77.5 (C-5″), 77.4 (C-3″), 73.5 (C-2″), 69.4 (C-4″),62.9 (C-6″), 61.5 (C-6, OMe), 60.5 (C-3, OMe), 56. (C-4′, OMe)	^{13}C-NMR ($CDCl_3$, 125 MHz) δ 182.6 (C-δ 182.6 (C-4), 163.9 (C-2), 158.7 (C-7), 153.2 (C-5), 153.1(C-9), 152.8 (C-4), 152.3 (C-3), 149.3 (C-2), 132.7 (C-8), 120.1(C-6), 110.9 (C-10), 108.8 (C-5), 106.1(C-1), 104.5 (C-3), 90.6 (C-6), 60.9 (C-3′, OCH_3), 60.2(C-2′, OCH_3), 56.4 (C-4′, OCH_3), 56.2 (C-8, OCH_3),56.1 (C-7, OCH_3)

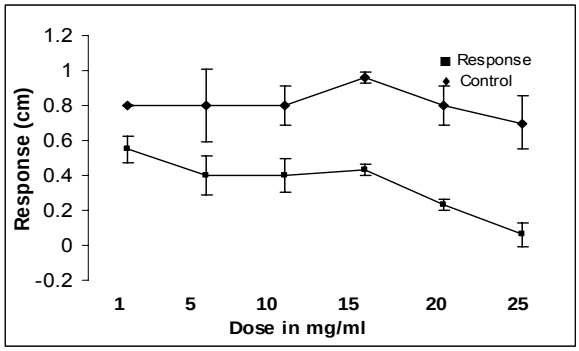
With atropine 1x10^{-4}M the contractile effect of aqueous fraction was slightly decreased (graph 4). Pre-treated tissue with atropine 1x10^{-4}M followed by administration of 15 mg/ml aqueous fraction produced contraction but the extent was low (graph 4). When the same process was repeated with high concentration of atropine i.e. 1x10^{-2}M, it did not completely block the activity of the muscles (graph 4).

Pretreated tissue with low conc. of atropine 1x10^{-4}M showed that the effect of the drug still appeared and tissue activity was not blocked (graph 4). On the basis of the above result, it is probably said since atropine did not produce its full response but adrenaline produced, thus it might be due to the involvement of muscarinic receptor. Pretreated tissue with atropine 1x10^{-2}M and then hexane fraction showed that the effect of standard drug atropine was continued and drug did not show any effect (graph 5).

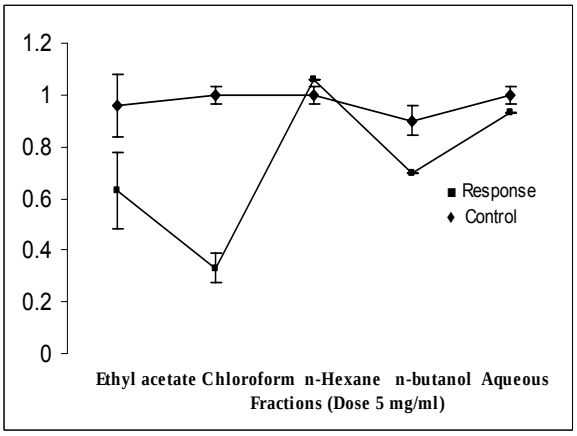
Table A2: Characterization of compounds isolated from *S. nodosa*

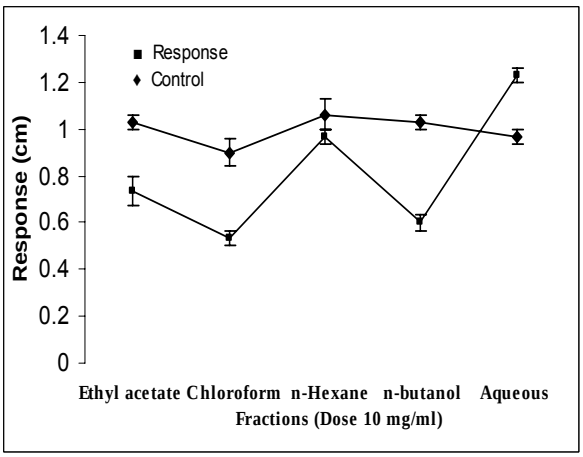
Characteristics of Compounds	(5)	(6)	(7)
MP(Melting point)	Yellow amorphous powder	Amorphous white solid	Amorphous white solid
UV	(MeOH) $\lambda_{max:}$ 367, 322, 265, and 250 nm	(MeOH) λ_{max} 340, 313, 273 nm	λ_{max}MeOH nm: 225, 269,318
IR	IR (KBr) ν_{max} Cm^{-1}3400, 3100, 1660, 1620, and 1582-1464	ν_{max} ($CHCl_3$):3200-3500,2840, 1668, 1580, 1370 cm^{-1}(-)	ν_{max} KBR cm^{-1}: 3390 (OH), 1685 (ester CO), 1654 (CO) $[\alpha]_D^{25}$ +14 $[\alpha]_D^{25}$ +14.5 (c= 0.025)
EI-MS*m/z*(rel. Int.)	433 $[M+H]^+$ (21), 432 $[M]^+$(100), 286 $[M\text{-rha}]^+$ (17),153 (2),	-	-
HR-EI-MS *m/z*	432.378 (calcd. for. $C_{21}H_{20}O_8$ 432.381)	-	-
HRFABMS	-	[M-H] 593.2567, consistent with $C_{28}H_{33}O_{14}$	(negative) *m/z*: 577.1335 $[M-H]^+$(calcd. for $C_{30}H_{25}O_{12}$: 577.1346)
^{1}H-NMR	(CD_3OD, 300 MHz) δ 12.49 (1H, br s, OH-5),8.10 (2H, d, *J* = 9.1 Hz, H-2′, H-6′), 6.94 (2H, d, *J* = 8.8 Hz, H-3′, H-5′),6.83(1H, d, *J* = 2.1 Hz, H-8), 6.43 (1H, d, *J* =2.3 Hz, H-6), 5.55 (1H, br s, H-1 of rha), and(3H, d, *J* = 5.9 Hz, CH_3 of rha)	(C_5D_5N, 400 MHz): δ 5.50, dd, *J* = 3, 13 Hz, H-2, δ 3.20, dd, *J* = 13, 17 Hz, δ 2.70 dd, *J* = 3, 17 Hz, H_2-3, δ 6.61, d, *J* = 2.1 Hz, H-6, δ 6.50, d, *J* = 2.1, H-8, δ 7.60, d, *J*=8.8 Hz, H-2′, 6′, δ 7.04 d, *J* = 8.8 Hz, H-3′, 5′, δ 5.64 d, *J* = 7.2 Hz, H-1″, δ 5.40, brs, H-1‴, δ 4.10 – 4.65 (H-2″, H-2‴ H-3″, H-3‴, H-4″, H-4‴, H-5″, H-5‴, H-6″), δ 1.55, d, *J* = 6.8 Hz, H_3-6‴	(300 MHz, DMSO-d_6) δ δ; 5.15 (1H, d, *J* = 7.1 Hz, H-1‴), 6.32 (1H, d, *J* = 15.8 Hz, H-2″), 6.46 (1H, d, *J* = 1.9 Hz, H-6), 6.64 (2H, d, *J* = 8.7 Hz, H-(3), H-(5), 6.79 (1H, d, *J* = 1.9 Hz, H-8), 6.82 (1H, s, H-3), 6.90 (2H, d, *J* = 8.7 Hz, H-3′, H-5′), 7.35 (2H, d, *J* = 8.7 Hz, H-(2), H-(6), 7.45 (1H, d, *J* = 15.8 Hz, H-3″), 7.91 (2H, d, *J*= 8.7 Hz, H-2′, H-6′), 12.91 (1H, s, 5-OH)
^{13}C-NMR	(CD_3OD, 75 MHz) δ176.0 (C-4), 161.3 (C-7), 160.3 (C-5), 159.3 (C-4′),155.7 (C-9), 147.4 (C-2),136.0 (C-3), 129.6 (C-6′),129.5 (C-2′), 121.4 (C-1′),115.4 (C-3′), 115.3 (C-5′),104.6 (C-10), 98.7 (C-6),98.3 (C-1″), 94.2 (C-8),71.5 (C-4″), 71.2 (C-3″), 70.0 (C-2″), 69.8 (C-5″),17.8 (C-6″)	(C_5D_5N, 100 MHz) 79.2 (C-2), 43.0 (C-3), 196.9(C-4), 164.4 (C-5), 97.8 (C-6), 166.0 (C-7), 96.4 (C-8), 163.4 (C-9), 104.2 (C-10), 131.1 (C-1`), 128.6 (C-2`), 114.5 (C-3`), 161.4 (C-4`), 114.5 (C-5`), 128.6 (C-6`), 101.4(C-1``), 74.5 (C-2``), 77.5 (C-3``), 72.0 (C-4``), 78.3 (C-5``), 69.7 (C-6``), 102.4 (C-1```),72.7 (C-2```), 71.2 (C-3```), 74.0 (C-4```),67.2 (C-5```), 18.5 (C-6```), 55.2 (OCH_3)	(75 MHz, DMSO-d_6) 161.4 (C-2), 102.9 (C-3), 181.8 (C-4), 156.8 (C-5), 99.4 (C-6), 164.2 (C-7), 94.4 (C-8), 161.1 (C-9), 105.3 (C-10), 120.8 (C-1`), 128.5 (C-2), 128.5 (C-2`), 115.9 (C-3`), 162.7 (C-4 162.7 (C-4`), 115.9 (C-5`), 128.5 (C-6), 128.5 (C-6`), 124.8, 130.0, 115.6, 159. 115.6, 159.7, 115.6, 130.0, 166.4 (C-1 166.4 (C-1``), 113.6 (C-2``), 144.9 (C-3 144.9 (C-3``), 99.4 (C-1```), 73.0 (C-2`` 73.0 (C-2```), 76.2 (C-3```), 70.0 (C-4`` 70.0 (C-4```),77.0 (C-5```), 62.0(C-6```)

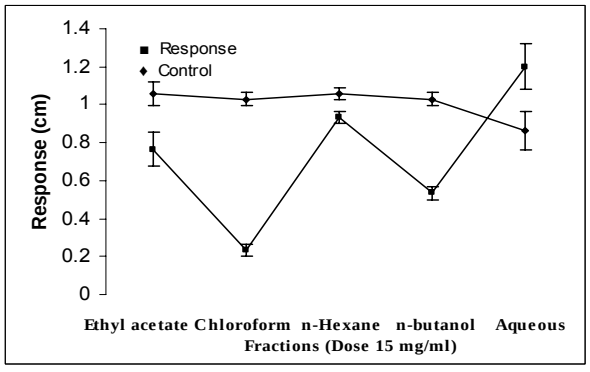
The post treated effect of atropine was also not produced with ethyl-acetate fraction (Graph5). *n*-Butanol fraction showed less potent antagonist effect when pretreated with acetylcholine $1x10^{-2}$M conc. (Graph 6), while the inhibitory response of chloroform fraction was easily overcome by acetylcholine $1x10^{-2}$M conc. (Graph 6) with its full response.



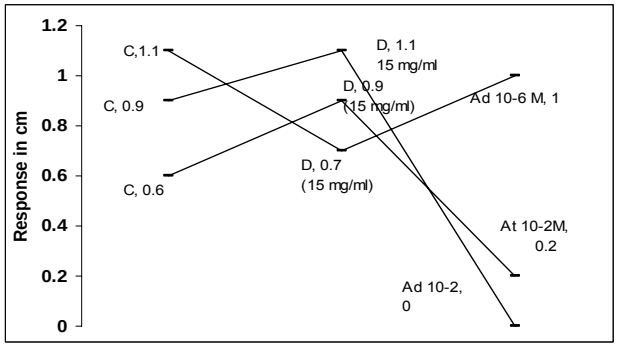
Graph 1: Dose response of crude extract of *S. nodosa* on isolated intestine.



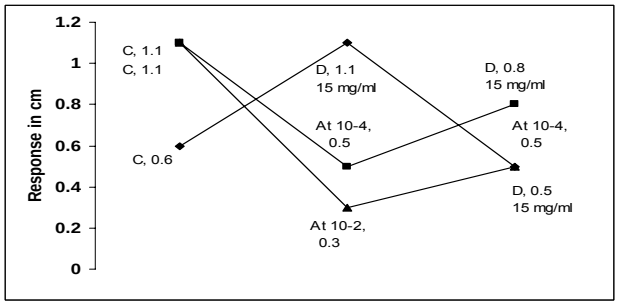




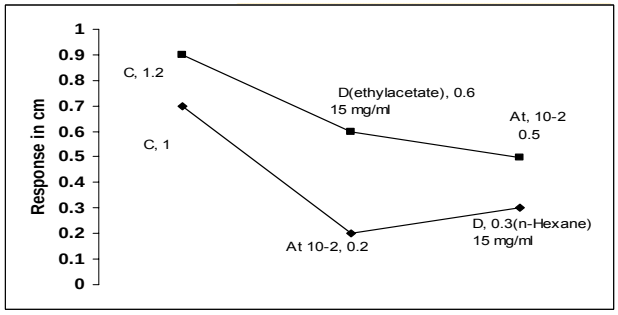
Graph 2: Dose response of different fractions of *S. nodosa* on isolated intestine.



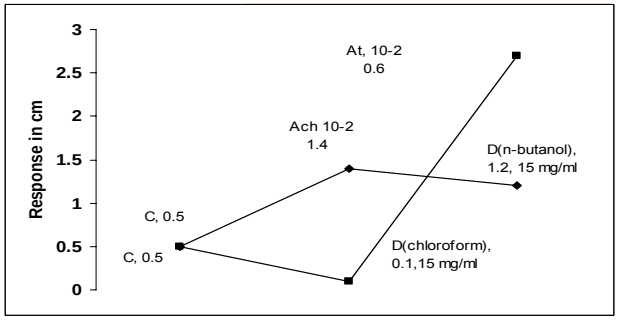
Graph 3: Effect of aqueous fraction of *S. nodosa* post-treated with atropine 1 x 10^{-2} M and adrenaline 1 x 10^{-2} M and 1 x 10^{-6}M.



Graph 4: Effect of aqueous fraction of *S. nodosa* pre and post-treated with atropine 1 x 10^{-2} M and 1 x 10^{-4} M.



Graph 5: Effect of *n*-Hexane and ethyl acetate fraction of *S. nodosa* pre and post treated with atropine 1 x 10^{-2} M.



Graph 6: Effect of *n*-Butanol and Chloroform fraction of *S. nodosa* pre and post treated with acetylcholine 1 x 10^{-2} M concentration.

CONCLUSION

The results of smooth muscle activity of *S. nodosa* indicated that the possible mechanism of action may be

Table 1: Effect of crude extract of *S. nodosa* on isolated rabbit intestine.

Dose (mg/ml)	Control (cm)	Response (cm)	% of response	t- value
01	0.8 ±0.00	0.55 ± 0.076	31	3.289
05	0.8 ±0.207	0.4 ± 0.115	50	1.690
10	0.8 ± 0.115	0.4 ± 0.099	50	2.649
15	0.96 ± 0.032	0.43 ± 0.032	55	11.856
20	0.8 ± 0.115	0.23 ± 0.032	71	4.764
25	0.7 ± 0.152	0.06 ± 0.066	91	3.842

The results are expressed in ± S.E.M, at P ≤ 0.05, *Significant, **highly significant.

Table 2: Effects of different fractions of *S. nodosa* on isolated rabbit intestine.

Fractions	Dose (mg/ml)	Control (cm)	Response (cm)	% of response	t- value
Ethyl acetate	05	0.966 ± 0.145	0.63 ± 0.120	34.78	1.8
	10	1.03 ± 0.032	0.733 ± 0.061	28.83	0.490
	15	1.06 ± 0.061	0.766 ± 0.088	27.73	0.294
Chloroform	05	1± 0.057	0.33 ± 0.032	67	10.468**
	10	0.9 ± 0.057	0.533 ± 0.032	40.77	5.66
	15	1.03±0.032	0.233±0.032	77.37	18.11**
n-Hexane	05	1± 0.00	1.06± 0.032	6	1.935
	10	1.06±0.066	0.966±0.032	8.86	1.287
	15	1.06 ± 0.032	0.933 ± 0.032	11.98	2.886
n-Butanol	05	0.9 ± 0.00	0.7 ± 0.057	22.22	3.508
	10	1.03 ± 0.032	0.6 ± 0.032	41.74	9.77
	15	1.03 ± 0.032	0.533 ± 0.032	48.25	11.295*
Aqueous	05	1 ± 0.00	0.933 ± 0.032	6.7	2.093
	10	0.966 ± 0.032	1.23 ± 0.032	27.33	-6
	15	0.866 ± 0.132	1.2 ± 0.152	38.56	1.661

The results are expressed in ± S.E.M, at P ≤ 0.05, * Significant, **highly significant

due to the involvement of muscarinic receptor. Furthermore, these results clearly indicated the presence of spasmolytic constituents in crude extract *S. nodosa* which may be in high concentration in chloroform fraction and spasmogenic effect in aqueous fraction. Isolation of seven known compounds also provided a additional support to its spasmolytic and other biological activites. To the best of our knowledge, no data has been reported on the pharmacological screening of the *S. nodosa* obtained from Pakistan. The present investigation will provide a broad base for the possibility of pharmacological and phytochemical studies on *S. nodosa*.

REFERENCES

Ahmad M (1986). Naturally occurring acteoside from *Buddleja davidii*. *J. Pharm. Univ. Kar*., **4**(2): 65.

Ahmad M. and Salama O (1985). Isolation of rutin from the fresh leaves of *Syringa vulgaris*. *J. Pharm. Univ. Kar*., **4**(1): 9.

Andrei A, Bunaciu1 Hassan Y, Aboul-Enein and Şerban F (2006). FT-IR Spectrophotometric analysis of acetylsalicylic acid and its pharmaceutical formulations. *Can. J. Anal. Sci. Spectrosc.*; **51**(5): 253-259.

Changa SL, Chiang YM, Changa CLT, Yeh HH, Shyur LF, Kuo YH, Wu TK and Yang WC (2007). Flavonoids, centaurein and centaureidin, from *Bidens pilosa*, stimulate IFN-γ expression. *J. Ethnopharmacol.*, 112: 232–236.

Dawson RM, Jarvis MW, Jefferies PR, Payne TG and Rosich RS (1966). Acidic constituents of *Dodonaea lobulata***.** *Aust. J. Chem.*, **19**(11): 2133-2142.

De Santos Galíndez J, Lanza AMD, Matellano LF (2002). Biologically active substances from the genus *Scrophularia* . *Pharm Biol*; **40**(1):45–59.

Flamini G, Antognoli E and Morelli I (2001). Two flavonoids and other compounds from the aerial parts of *Centaurea bracteata* from Italy. *Phytochemistry,* **57**: 559-564.

Flores SE and Herrán J (1958). The structure of pendulin and penduletin: A new flavonol glucoside isolated from *brickelia pendula*. *Tetrahedron*, **2**(3-4): 308-315.

Govindachari TR, Parthasarathy PC, Pai BR and Kalyanaraman PS (1968). Chemical investigation of

Andrographis serpyllifolia: Isolation and structure of serpyllin, a new flavone. *Tetrahedron,* **24**(24): 7027-7031.

Karimova SG, Smirnova SG, Nasyrov KM (1967). Chemical composition and pharmacology of the Scrophulariaceae. From Ref. Zh., Farmakol. Khimoter. Sredstva. Toksikol. *Chem. Abst.* 1967; **67**:20384-20385.

Liu Q, Liu M, Mabry TJ and Dixon RA (1994). Flavonol glycosides from *Cephalocereus senilis. Phytochemistry*, **36**(1): 229-231.

Mehjabeen, Jahan N, Ahmad M, Chishti KA, Shaista H and Rehman AB (2004). Comparative Pharmacological study on red and black fruits and leaves of these two plants of *Solanum nigrum* (Black nightshade). *I. Chem., Pharm., Med., J.,* **2**: 81-96.

Miyase T and Mimatsu A (1999). Acylated iridoid and phenylethanoid glycosides from the aerial parts of Scrophularia nodosa. *J. Nat. Prod*., **62**(8): 1079-1084.

Muhammad N (2006). *Phytomedical Investigation and Standardization of Some Indigenous Medicinal Plants of Malakand Division, Datisca Cannadina, Gratiola Officianalis and Scrophularia Nodosa.* Ph.D. thesis, University of Karachi, Karachi.

National Research Council (1996). Guide for the Care and Use of Laboratory Animals. National Academy Press, Washington, USA, pp.1-7.

Sticher O, Ahmad M, Salama O and Winkler T (1982). Two new secoiridoid glucosides from *Syringa vulgaris*. *Planta Medica*, **45**: 151.

Swiatek L (1970). Pharmacobotanical investigations of some Scrophulariaceae species. *Diss. Pharm. Pharmacol*., **22**: 321-328.

Swann K and Melville C (1972). Iridoid content of some Scrophularia species. *J. Pharm Pharmacol*., **24**: 170P.

Ul-Haq A, Malik A, Khan AU, Shah MR and Muhammad P (2004). Spinoside, new coumaroyl flavone glycoside from *Amaranthus spinosus*. *Arch. Pharm. Res*., **27**(12): 1216-1219.

Ul-Haq A, Malik A and Khan S (1994). Flavonoid Glycoside and long chain ester from the roots of *Vitex negundo*. *Pol J. Chem.*, **78**(10): 1851-1856.

Weinges K and Von der Eltz H (1978). Natural products from medicinal plants. XXIII. Iridoid glycosides from *Scrophularia nodosa* L. *Justus Liebigs Ann Chem*., **12**: 1968-1973.