Biological screening of *Scrophularia nodosa* extract and its fractions

Mansoor Ahmad*[1], Noor Muhammad[1], Mehjabeen[2], Noor Jahan[3], Syed Mahboob Alam[4], Manzoor Ahmad[5] and Obaidullah[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 & Technology, Karachi, Pakistan
[3]Department of Pharmacology, Dow College of Pharmacy, Dow University of Health Sciences, Karachi, Pakistan
[4]Department of Pharmacology, Jinnah Post Graduate Medical Center, Karachi, Pakistan
[5]Department of Chemistry, University of Malakand, KPK, Pakistan
[6]Department of Pharmacy, University of Peshawar, Peshawar, Pakistan

Abstract: Biological screening of *Scrophularia nodosa* crude extract and its fractions (hexane, chloroform, ethyl-acetate, *n*-butanol and aqueous) was carried out on phytotoxicity, cytotoxicity, antibacterial, antifungal and analgesic activities. Crude extract and its fractions produced 50-100% phytotoxicity at 1000µg/ml concentration whereas 25-77% phytotoxicity was observed at 10µg/ml concentration. The fractions exhibited significant antibacterial and antifungal effects. The non-toxic results of this plant were recorded in Brine Shrimps Bioassay method at all concentrations. Similarly no significant insecticidal activity was observed in crude extracts and fractions. Analgesic activity results of *S. nodosa* in mice were found highly significant in crude extract as compared to fractions. In writhing test crude extract at 500 mg/kg showed 65.6% highest inhibitory response in mice.

Keywords: *S. nodosa*, antioxidant, analgesic, phytotoxic, hemagglutination

INTRODUCTION

Scrophularia nodosa, is used to treat skin diseases such as eczema, acne and psoriasis. The leaves are employed in burns and swellings (Font Quer, 1990). In Homeopathic System of Medicine it is used in skin disease, breast tumors, painful swelling, colic below navel bleeding, tubercular testis and protruding *piles* (Boericke 1927). This species is considered to possess bacteriostatic (Kolodynska and Wieniaski, 1966; Swiatek, 1973; Schaubenger and Paris, 1977) and anti-inflammatory properties (Paris and Moyse, 1976; Schaubenger and Paris, 1977). The herb and root have been used to treat cancer of the fleshy parts. In 1963 Woo isolated an antipyretic compound, *p*-methoxy cinnamic acid from the ethanol extract of the roots of *Scrophularia* whereas in 1966 Karimova *et al.* studied the chemical composition of the members of *Scrophulariaceae* including *S. nodosa* contain saponins and tannins.

MATERIAL AND METHODS

Plant material, preparation of crude extract and fractionation

Shade-dried *S. nodosa* (25 kg) was ground and extracted with methanol at room temperature (Ahmad *et al.,* 2011 and 2012). The extract was filtered and solvent was evaporated under reduced pressure to obtain a thick gummy mass. It was fractionated into *n*-hexane, chloroform, ethyl acetate, *n*-butanol and aqueous fractions. All these extracts were evaluated for phytotoxicity, brine shrimp lethality, insecticidal, urease inhibition, antibacterial, antifungal and analgesic activities.

Phytotoxic activity

Phytotoxic activity was determined by using the modified protocol of *Lemna minor* (Atta-ur-Rahman, 1991).

Brine shrimp lethality bioassay

In this method, artificial "sea water" was prepared by dissolving 3.8 g sea salt per liter of double distilled water and filtered. The procedure was used according to Meyer *et al*. (1982). The data were analyzed with a Finney computer program to determine the LD_{50} values.

Insecticidal activity

The fractions and crude extract of *S. nodosa* were evaluated against different insects viz., *Tribolium castaneum*, C*allosbruchus analis* and *Rhyzopertha dominica* (Collins 1998, Ahmad *et al*., 2011). The percentage mortality was calculated by the formula:

$$\text{Percentage Mortality} = 100 - \frac{\text{No. of insects alive in test}}{\text{No. of insects alive in control}} \times 100$$

Antimicrobial assay

The antibacterial and antifungal activity was evaluated by the agar-well diffusion method (Kavanagh *et al*., 1963, Mehjabeen *et al.,* 2011 and Jahan *et al*., 2010).

2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay

The antioxidant activity of crude extract and fraction of *S. nodosa* were determined according to method described by Yamaguchi *et al*. (1998).

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

Enzyme (Urease) inhibition activity

Urease assay was performed by a reported method (Ahmad *et al.*, 2008; Akhtar *et al*., 2008). The percentage inhibition was calculated from the formula:

$$\% \text{ inhibition} = 100 - \frac{\text{optical density of test}}{\text{optical density of control}} \times 100$$

Jack bean urease was used as the standard inhibitor.

Hemagglutination activity

The blood was obtained from Husaini Blood Bank Karachi and erythrocytes were obtained as reported earlier (Muhammad, 2006). The hemagglutination activity was investigated serially in different concentration of crude extract against human erythrocytes of blood groups A, B, AB, and O ($Rh^+$and Rh^-). Weak, moderate and strong agglutinations were determined on the basis of the extent of granular deposition.

Analgesic activity

Acetic acid induced writhing test and tail flick was performed according to the modified method of Koster *et al*., 1959, Turner 1971 and Ahmad *et al*., 2011 respectively.

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

Phytotoxic bioassay

Phytotoxicity of crude extract and its four fractions were carried out at three different concentrations i.e., 1000, 100 and 10µg/ml (table 1).

Brine shrimp lethality bioassay

LD_{50} measurements of crude extract and fractions were evaluated against *Artemia salina* brine-shrimp eggs (table 2).

Table 2: *In vitro* cytotoxic bioassay of different fractions of *S. nodosa* crude extract and fraction by brine shrimp lethality bioassay

Samples	Conc.	No. of survivors	Control	Results
n-hexane		30		-
Chloroform		28		+
ethylacetate	1000	25	30	+
n-butanol		30		-
Aqueous		29		-
Crude extract		29		-
n-hexane		30		-
Chloroform		30		-
ethylacetate	100	29	30	-
n-butanol		30		-
Aqueous		30		-
Crude extract		30		-
n-hexane		30		-
Chloroform		30		-
ethylacetate	10	30	30	-
n-butanol		30		-
Aqueous		30		-
Crude extract		30		-

Table 1: *In vitro* phytotoxic bioassay of *S. nodosa* crude extract and fractions

Samples	Conc.	No. of fronds samples	Control	% Growth regulation	Conc. of std. drug (µg/ml)
n-hexane		20		50	
Chloroform		04		90	
ethylacetate	1000	0	40	100	0.015
n-butanol		16		60	
Aqueous		0		100	
Crude extract		04		90	
n-hexane		10		75	
Chloroform		22		45	
ethylacetate	100	29	40	27.5	0.015
n-butanol		17		57.5	
Aqueous		23		42.5	
Crude extract		28		30	
n-hexane		09		77.5	
Chloroform		09		27.5	
ethylacetate	10	29	40	27.5	0.015
n-butanol		30		25	
Aqueous		29		27.5	
Crude extract		30		25	

Insecticidal activity

The results of insecticidal activity are presented in table 3.

Table 3: Insecticidal activity of *S. nodosa* crude extract and fractions

Sample	% Mortality		
	C. analis	*T.castaneum*	*R.dominica*
n-hexane	50	0	0
Chloroform	40	0	10
Ethylacetate	20	0	0
n-butanol	20	0	0
Aqueous	10	0	10
Crude extract	0	0	0
+ ve Control	100	100	100
- ve Control	0	0	0

Concentration of test sample 1572.7 µg/cm^2
Concentration of Standard Drug 235.9 µg/cm^2
Standard drug (+ve control): Permethrin (Coopex),
(-ve Control): solvent

Antimicrobial bioassay

The antibacterial study was performed against six bacteria viz., *Escherichia coli, Bacillus subtilis, Shigella flexenari, Staphylococcus aureus, Pseudomonas aeruginosa* and *Salmonella typhi.* The dose was given in a single concentration (1mg/ml) (table 4a).

The fungicidal activity of these extracts was performed against six fungi viz. *Trichophyton longifusus, Candida albicans, Aspergilus flavus, Microsporum canis, Fusarium solani* and *Candida glaberata* (table 4b).

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity

Fig. 1 and table 5 represent the percentage of radical scavenging activity of the crude extract and fractions from *S. nodosa.* The ethyl-acetate fraction showed the highest antioxidant activity with (70%), followed by *n*-butanol (65%) and chloroform fractions (50%). The standard antioxidant 3-*t*-butyl-4-hydroxyanisole showed maximum (92.5%) activity.

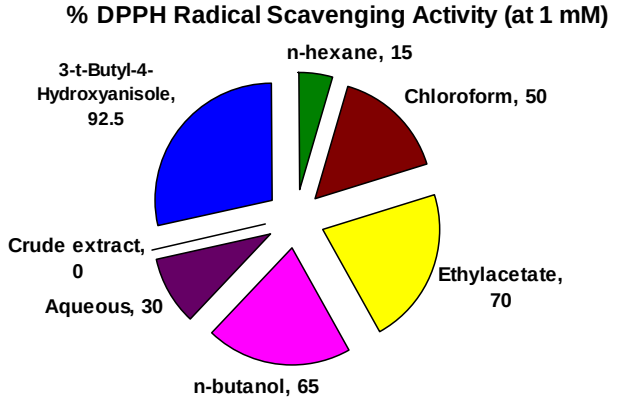

Fig. 1: Represents the % of radical scavenging activity of *S. nodosa* extract and fractions.

Enzyme (Urease) inhibition activity

During enzyme inhibition studies the crude extract and fractions of selected plant were screened against urease (jack bean) enzyme. The results deduced from current investigations are displayed in table 5; fig. 2. The results indicated that aqueous and chloroform fraction showed

Table 4a: Antibacterial bioassay of *S. nodosa* crude extract and fractions

Bacterial species	Zone of inhibition in mm, disc size 6mm						
	n-hexane	Chloroform	Ethylacetate	*n*-butanol	Aqueous	Crude extract	Standard
E.coli	-	-	-	-	-	-	Gentamycin (15)
B. subtilus	-	11	12	9	12	-	Imipenam (22)
S. flexenari	-	-	-	-	-	-	Pan resistant (0)
S. aureus	-	11	8	8	13	-	Noroxin (17)
P. aeruginosa	-	-	-	-	-	-	Pan resistant (0)
S. typhi	8	12	8	8	-	-	Velosef (18)

Dose of extract: 500µg/disc; Dose of standard drug 40 µg/disc

Table 4b: Antifungal bioassay of *S. nodosa* crude extract and fractions

Fungal species	Zone of inhibition in mm, disc size 6mm						
	n-hexane	Chloroform	Ethylacetate	*n*-butanol	Aqueous	Crude extract	Standard
T. longifysis	9	13	12	8	15	10	Miconazole (18)
C. albicans	-	-	-	-	-	-	Miconazole (22)
A. flavus	-	11	11	-	16	14	Amphotericin(18)
M. canis	16	9	-	16	15	11	Miconazole (19)
F. solani	-	14	-	13	-	-	Miconazole (16)
C. glabarata	9	14	-	13	-	-	Miconazole (10.8)

Dose of extract: 500µg/disc; Dose of standard drug 40 µg/disc

Table 5: Antioxidant and urease inhibition activities of *S. nodosa* crude extract and fractions

Sample 200µg/ml	% DPPH radical scavenging activity (at 1 mM)	% inhibition of urease activity
n-hexane	15 (minor)	0 (inactive)
Chloroform	50(intermediate)	10(minor)
Ethylacetate	70(high)	50(high)
n-butanol	65(high)	70(high)
Aqueous	30(minor)	72(high)
Crude extract	0(inactive)	0(inactive)
Jack bean urease	-	21.01±0.51 (IC50±SEM)
3-*t*-Butyl-4- Hydroxyanisole	92.5(high)	-

maximum and minimum inhibition respectively, while crude extract and *n*-hexane fraction had no activity. These results revealed that *S. nodosa* can be a source of natural inhibitor of this enzyme.

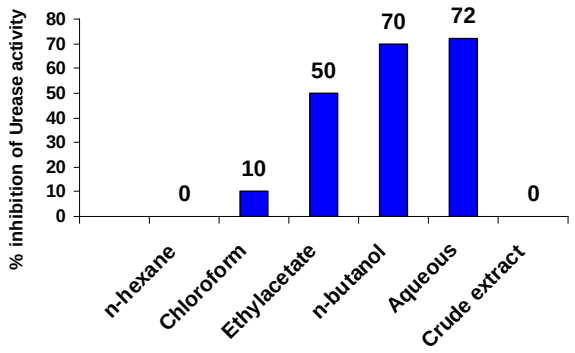

Fig. 2: Represents the percentage of urease inhibition activity of *S. nodosa* extract and fractions.

Hemagglutination activity

The hemagglutination activity of the crude extract derived from *S. nodosa* was tested against various groups of human erythrocytes; the results are shown in table 6. The extract possessed a high (+++) or medium (++) agglutination effect against all human blood groups at 5mg/ml concentration except blood group O^+ and O^- where it is low and very low respectively . However, no agglutination properties were exhibited by this extract at 0.625 and 0.3125 mg/ml. 1.25 mg/ml concentration of samples showed very low activities in blood groups A^+, B^+, B^- and AB^- (table 6).

Analgesic activities

In tail flick results maximum response was produced by CE 500 mg/kg at 90min. The crude extract of *S. nodosa* has more analgesic activity as compare to its fractions (table 7). In writhing test, maximum percentage of inhibition of writhes was observed with CE 500mg/kg (65.6%, Aspirin: 68.9%). CE 300 mg/kg and ethyl-acetate fractions inhibitory response was 54.5 and 5.6% respectively (table 8 and fig. 3).

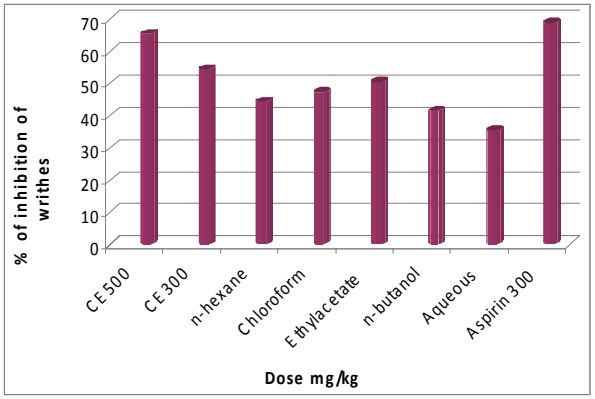

Fig. 3: Represents the percentage inhibition of acetic acid induced writhing of *S. nodosa* extract and fractions.

DISCUSSION

In phytotoxicity assay ethyl-acetate and aqueous fractions was found to be highly active, having 100% plant growth inhibition at 1000µg/ml. *n*-hexane fraction showed maximum activity 77.5% at lower concentration (10µg/ml) as compared to activity at 100 (75%) and 1000µg/ml (50%) concentration respectively. The activity remained same (27.5%) in ethyl acetate fraction at two different concentrations i.e., 100µg/ml and 1000µg/ml. In Brine shrimps bioassay it is evident from the results that very weak lethality is present in crude extract as well as their fractions. The crude extract did not exhibit any insecticidal activity against any of insects. Similarly all fractions had no activity against *T. castaneum*, low activity against *R. dominica* and moderate activity against *C. analis*.

Crude extract showed no activity at all against any microorganism while *n*-hexane fraction showed activity against *S. typhi* only. Similarly all fractions had no activity against *P. aeruginosa* and *S. flexenari.* The data indicated that the chloroform, ethyl-acetate, *n*-butanol and aqueous fractions were found to be most effective and exhibiting different zone of inhibitions against *B. subtilis*, S. *aureus* and *S. typhi*.

Table 6: *In vitro* hemagglutination activity of crude extract of *S. nodosa*

Blood group	Dose of drug(mg/ml)				
	5	2.5	1.25	0.625	0.3125
A^+	++	+	+	-	-
B^+	+++	+	+	-	-
O^+	++	-	-	-	-
AB^+	+++	++	-	-	-
A^-	++	+	-	-	-
B^-	+++	++	+	-	-
O^-	+	+	-	-	-
AB^-	+++	+	+	-	-

+++ = high activity, +++ = medium activity, ++ = low activity, + = very low activity

Table 7: Analgesic activity of *S. nodosa* extract and its fractions

Dose mg/kg	0min	30min	60min	90min	120min	150min	180min	210min
	(Tail flick time in sec.)							
Control	2.1±0.44	2.2±0.37	2.1±0.24	2.0±0.14	1.9±0.15	2.1±0.25	2.3±0.12	2.3±0.19
CE 300	2.2±0.25	3.9±0.32	4.5±0.21	4.6±0.52	5.2±0.02	5.5±0.29	5.0±0.35	4.2±0.35
CE 500	2.3±0.45	4.8±0.19	5.4±0.28	6.6±0.35	5.7±0.31	5.4±0.26	5.2±0.31	4.5±0.35
n-hexane	1.9±0.17	2.3±0.21	2.5±0.15	3.1±0.23	3.4±0.19	3.0±0.25	3.0±0.19	2.5±0.21
Chloroform	2.2±0.19	2.7±0.23	3.3±0.23	3.8±0.21	4.0±0.31	3.9±0.22	3.6±0.17	3.1±0.19
Ethylacetate	1.9±0.15	2.4±0.19	2.9±0.21	3.3±0.19	3.8±0.25	3.7±0.32	3.5±0.19	2.8±0.25
n-butanol	2.1±0.18	2.6±0.32	3.1±0.32	3.9±0.26	4.1±0.31	4.0±0.28	3.7±0.17	2.9±0.32
Aqueous	2.3±0.18	2.3±0.18	2.9±0.19	3.2±0.24	3.5±0.21	3.6±0.16	3.2±0.19	2.6±0.17
Diclofenic	1.6±0.16	2.3±0.15	3.3±0.15	3.82±0.19	4.26±0.19	3.4±0.22	3.0±0.21	2.6±0.15

Dose of fractions of *S. nodosa*: 300 mg/kg, * significant result, ** highly significant result when compared with standard drug Aspirin at $P < 0.05$ with ± S.E.M

Table 8: Acetic acid induced writhing test of *S. nodosa* extract and fractions

Treatment Dose(mg/kg)	Mean No. of writhes	% of inhibition
Control (0.5ml saline)	124±2.96	-
CE500	42.7±3.12	65.6**
CE300	56.4±2.13	54.5*
n-hexane	85.2±2.16	44.4*
Chloroform	65.2±3.01	47.4*
Ethylacetate	61.3±2.32	50.6*
n-butanol	72.5±3.32	41.5*
Aqueous	80.1±3.56	35.4
Aspirin 300	38.5±2.01	68.9**

Dose of fractions of *S. nodosa*: 300 mg/kg.
*significant result.
**highly significant result when compared with standard drug Aspirin at $P < 0.05$ with ± S.E.M.

The antifungal activity results indicated that crude extract and fractions of *S. nodosa* are not active against the tested fungal strain *C. albicans* while aqueous fractions was most effective against *T. longifusus*, *A. flavus* and *M. canis.* It was further observed that ethyl-acetate fraction was weakly active while chloroform and *n*-butanol fraction was strongly active against tested microorganisms. The crude extract of *S. nodosa* exhibited antifungal activity against *T. longifusus*, *A. flavus* and *M. canis.*

In the past decade natural antioxidants have generated considerable attention in preventive medicine. Consequently, much attention has been directed toward the discovery of new natural antioxidants, including herbal products, aimed at quenching biologically harmful radicals (Ismail *et al.*, 2009). DPPH radicals have been widely used to evaluate the antioxidant properties of natural products as well as plant extracts (Wang *et al.*, 2003). The tested samples reduced the stable radical DPPH to the yellow-colored diphenylpicrylhydrazine. The ethyl-acetate fraction had maximum activity and crude extract had no activity.

The results of urease inhibition activity indicated that aqueous and chloroform fraction showed maximum and

minimum inhibition respectively, while crude extract and *n*-hexane fraction had no activity. These results revealed that *S. nodosa* can be a source of natural inhibitor of this enzyme.

It is well known that the hemagglutination activity is mainly related to a group of proteins called lectins (Benevides *et al.,* 1999) which are valuable tools for the separation and characterization of glycoconjugates and glycopeptides, histochemistry of cells and tissues, and the study of cell differentiation (Gabius and Gabius, 1993). This preliminary investigation revealed that *S. nodosa*, possessing hemagglutination activity, might contain some valuable phytolectins that may find applications in the above-mentioned areas.

Analgesic activity of *S. nodosa* is possibly due to presence of flavonoids (Ahmad *et al.*, 2012) these flavonoids produces their analgesic effect by interfering the prostaglandins (Hossinzadeh *et al.*, 2002).

CONCLUSION

On the basis of above-mentioned results it is concluded that *Scrophularia nodosa* may be a good source of antimicrobial, antioxidant and analgesic agent.

REFERENCES

Ahmad M, Mehjabeen, Zia-Ul-Haq M and Noor Jahan (2011). Determination of LD_{50} and ED_{50} by dose response relationship and assessment of toxicological and non-toxicological behaviour of *Ipomoea hederacea*. *J. Pharm Res*., **4**(4): 1176-1178.

Ahmad M, Jahan N, Mahayrookh, Mehjabeen, Rehman AB, Ahmad M, Obaidullah and Mohammad N (2011). Differential inhibitory potencies of alcoholic extract of different parts of *Dryopteris chrysocoma* on inflammation in mice and rats. *Pak. J. Pharm. Sci.*, **24**(4): 559-563.

Ahmad M, Muhammad N, Mehjabeen, Jahan N, Ahmad M, Obaidullah, Mahmood Qureshi M and Jan SU (2012). Spasmolytic effects of *Scrophularia nodosa* extract on isolated rabbit intestine. *Pak. J. Pharm. Sci*., **25**(1): 267-275.

Ahmad M, Muhammad N, Ahmad M, Lodhi MA, Mehjabeen, Noor Jahan, Khan Z, Ranjit R, Shaheen F, and Choudhary MI (2008). Ureaese inhibitor from *Datisca cannabina* Linn. *J. Enzym. Inhib. Med. Chem.*, **23**(3): 386-390.

Akhtar T, Hameed S, Khan KM and Chaudhary MI (2008). Syntheses, urease inhibition and antimicrobial studies of some Chiral 3-Substituted-4-amino-5-thioxo-1*H*,4*H*-1,2,4-triazoles. *Med. Chem.*, **4**(6): 539-543.

Atta-ur-Rahman (1991). Studies in Natural Product Chemistry. Elsevier Science Publishers, Netherlands, **9**: 383-384.

Collins PJ (1998). Resistance to grain protectants and fumigants in insect pests of stored products in Australia. *In:* Banks HJ, Wright EJ and Dameevski KA (Eds.). CSIRO Stored Grain Research Laboratory, Presented in Australian Post Harvest Technical Conference, pp.55-57.

Font Quer (1990). Plantas Medicinales *El Dioscórides Renovado*, (12th Ed.), Labor SA, Barcelona, p.608.

Hossinzadeh H, Ramezani M, Fadishei M and Mahmoudi M (2002). Anti-inflammatory and acute toxicity effects of *Zhumeria majdae* extracts in mice and rats. *Phytomedicine*, **9**: 135-141.

Ismail M, Ibrar M, Iqbal Z, Hussain J, Hussain H, Ahmad M, Ejaz A and Choudhary MI (2009). Chemical constituents and antioxidant activity of *Geranium wallichianum. Rec. Nat. Prod.* **3**(4): 193-197.

Karimova SG, Smirnova SG and Nasyrov KM (1966). Chemical composition and pharmacology of the Scrophulariaceae. *Mater Konf Tiziol Biokhim Farmakol Uchestiem Prakt Urachei Ufa*, 153-155.

Kavanagh F (1963). Analytical Microbiology. Academic Press, London, pp.125-141.

Kolodynska and Wieniaski (1966). Synthesis of hydroxicinnamides I. Synthesis of sinapamide and caffeic amides. *Dissertations of Pharmacy and Pharmacol.*, **18**(1): 54-61.

Koster R, Anderson M and Beer EJ (1959). Acetic acid for analgesic screening. *Federation Proceeds*, **18**: 412-416.

Mehjabeen, Ahmad M, Jahan N, Zia-ul-Haq M, Alam SM, Wazir A, and Hassan S (2011). Antimicrobial screening of some plants of medicinal importance. *Pak. J. Bot*., **43**(3): 1773-1775.

Meyer NB, Ferrigni RN, Putnam EJ, Jacobsen BL, Nichols ED and McLaughlin IJ (1982). Brine shrimp: A convenient general bioassay for active plant constituents. *J. Planta Medica*, **45**: 31-35.

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.

Jahan N, Ahmad M, Mehjabeen, Zia-ul-haq M, Alam S M, Qureshi M (2010). Antimicrobial screening of some medicinal plants of Pakistan. *Pak. J. Bot*., **42**(6): 4281-4284.

Paris RR and Moyse H (1976). *Matiére Medicale*, Vol.I (2nd Ed.), Masson, Paris, p.73.

Schaubenger P and Paris F (1977). Guides des Plantes Mddicinales. Delachux & Niestle SA, Paris, p.262.

Swiatek L (1973). Phenolic acids of underground parts of *Scrophalaria nodosa. Pol. J. Pharmacol. Pharm.*, **25**(5): 461-464.

Turner RA (1971). Screening methods in pharmacology. Academic Press, New York, pp.100-113.

Wang SY, Chang HN, Lin KT, Lo CP, Yang NS and Shyur LF (2003). Anti-oxidant properties and

phytochemical characteristics of extracts from *Lactuca indica. J. Agric. Food Chem.*, **51**: 1506-1512.

Boericke W (1927). Pocket manual of homeopathic Materia Medica (9th Ed.), B. Jain Publishers Pvt. Ltd., India, p.577.

Woo WS (1963). Pharmacologically active component in *Scrophularia* root, *p*-methoxycinnamic acid. I. Identification of *p*-methoxicinnamic acid and its antipiretic action. *Yakhak Hoeiji.*, **7**: 55-57.

Yamaguchi T, Takamura H, Matoba T and Terao J (1998). HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1-diphenyl-2-picrylhydrazyl. *Biosci, Biotechnol. Biochem.*, **62**: 1201-1204.

Vol. 25, No.2, April 2012

Vol. 25, No.2, April 2012

Vol. 25, No.2, April 2012

Vol. 25, No.5, April 2012

April 2012 Vol. 25, No.2, Pages 295-492

April 2012 Vol. 25, No.2, Pages 295-492

April 2012 Vol. 25, No.2, Pages 295-492

April 2012 Vol. 25, No.2, Pages 295-492