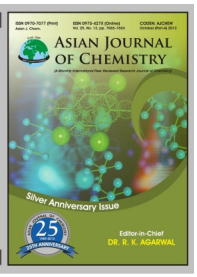
Asian Journal of Chemistry; Vol. 25, No. 18 (2013), 10519-10525

ASIAN JOURNAL OF CHEMISTRY

http://dx.doi.org/10.14233/ajchem.2013.15811



Antioxidant, Antimicrobial, Cytotoxicity and HPLC Studies of *Sophora mollis*

HUMERA IRAM[1], NASIR RASOOL[1,*], MUHAMMAD RIAZ[1], MUHAMMAD ZUBAIR[1], UMER RASHID[2,*], IFTIKHAR HUSSAIN BUKHARI[1], RASOOL BAKHSH TAREEN[3] and MD. SAIFUL ISLAM[4]

[1]Department of Chemistry, Government College University, Faisalabad-38000, Pakistan
[2]Institute of Advanced Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia
[3]Department of Botany University of Balochistan, Quetta, Pakistan
[4]Department of Chemistry, Faculty of Sciences, Universiti Putra Malaysia, 43400 UPM, Serdang, Selangor, Malaysia

*Corresponding author: Tel: +92 332 7491790; Tel: +60 38 9467393; E-mail: nasirhej@yahoo.co.uk; umer.rashid@yahoo.com

(*Received*: 21 May 2013; *Accepted*: 25 November 2013) AJC-14439

The present study was undertaken to evaluate the antioxidant activities of plant extract/fractions. Total phenolic contents value ranged from 5.69 to 39.36 for stem and 5.50 to 118.8 GAE (mg/100 g) for leaves while total flavonoid contents value ranged from 6.81-261.00 for stem and 26.16-91.04 CE (mg/100 g) for leaves of *S. mollis*. The highest total phenolic contents value was showed by ethyl acetate fraction (118.80 mg/100 g) of leaves and ethyl acetate fraction (261.00 mg/100 g) of stem showed highest total flavonoid contents value. The lowest IC_{50} value was exhibited by ethyl acetate fraction (17.77 µg/mL) of leaves. The maximum % inhibition of peroxidation in linoleic acid system was observed in ethyl acetate fraction (91.54 %) of leaves. The methanolic extract and its different fractions were explored to antimicrobial activity by using disc diffusion method. The maximum antibacterial activity was shown by *n*-butanolic fraction against *Bacillus cereus*. From the present investigation, the haemolytic effect of plant was found to be in the range of 1.40 to 14.31 % for stem extract/fractions from 2.34 to 16.56 % for leaves extract/ fractions of *S. mollis*. From HPLC analysis of some extract and fractions of plant different phenolic constituents were identified. From this research work it is concluded that this plant could be used as natural antioxidant and antimicrobial agents and medicinally very important.

Key Words: *Sophora mollis*, Antioxidant activity, Antimicrobial activity, Haemolytic activity, HPLC analysis.

INTRODUCTION

Sophora mollis (*S. mollis*) belongs to Papilionaceae family[1]. Genus *Sophora* contains 45 species which consists of small trees and shrubs. Genus *Sophora* is rich in flavonoids and alkaloids which include sophocarpine, sophoramine, oxymatrine, sophoridine and matrine. These alkaloids have various pharmacological effects and mainly used to relief from many diseases and chief effects are sedative, antipyretic, anti-hepatitis B virus inotropic and anti tumor effects[2,3]. Numerous species of genus *Sophora* are traditionally used to treat various diseases like sore throat, dermatitis, dysentery, fever, eczema, inflammation, asthma, gastrointestinal hemorrhage, allergy and diarrhea[4]. The seeds of *S. mollis* are frequently used to heal skin diseases and also helpful to kill vermin like cockroach, flee, rats, lice, pests and parasites[5]. Extract of freshly grinded leaves are used to treat sore eyes and also helpful in relieving severe headache. Leaves and branches of *S. mollis* are also utilized by livestock as food. *S. mollis* is best soil binder and increase fertility. Roots of *S. mollis* enhanced the growth of hair and also impart dark colour to hairs. This plant is used locally as green fodder, fuel and pesticide, therefore the plant frequently cutdown[5]. From the aerial parts of *S. mollis* eight compounds were isolated and named as (E)-phytyl epoxide, 7,11,15-trimethyl-3-methylenehexadecane-1,2-diol, loliolide, scopoletin, hexacosanol, octacosanol, β-sitosterol and daucosterol[6] and six new prenylated isoflavanones; sophoronols A-F, together with eight phenolic constituents were isolated from the roots of *S. mollis*[6,7] where sophoronol C and sophoronol E showed moderate antiplasmodial activity. Some authors[8-14] have studied chemical analysis and biological properties of various plants, more work is needed to explore the un explored plants. Because of many traditional and medicinal uses of this plant, it was further studied to evaluate phytochemical screening and various biological activities of different extract/fractions of the plant *S. mollis*.

EXPERIMENTAL

The *S. mollis* plant was collected from Quetta and Ziarat valley. The plant was further authenticated and identified by Dr. Rasool Bukhsh Tareen Department of Botany, University

of Baluchistan, Quetta, Pakistan, where a voucher specimen (SM-RBT-05) was deposited in the collection/herbarium.

Plant extracts and fractions preparation: The different parts of *S. mollis* were washed with cold water to remove dust and other extraneous matter. The shaded dried parts were grinded into powdered form. For phytochemical and antioxidant screening some powdered plant material was kept in clean airtight bottles to avoid moisture. For the preparations of methanolic extract 1000 g of leaves and 250 g stem powder was soaked in methanol solvent separately. It was filtered after 3 days. This methanolic extract was concentrated and kept for evaporation. The procedure was repeated three times to get the extract. Fractions were done by using different solvents having different polarities. In methanolic extract first of all fraction was done with *n*-hexane then chloroform was added followed by ethyl acetate and at the end *n*-butanol was added for fractionation[15].

Qualitative phytochemical analysis: Analysis for identification of major phytoconsitituents in *S. mollis* plant was carried using standard phytochemical procedures as method described by Sofowora[16] for detection of alkaloids, steroids and saponins. The detection of flavonoids, glycosides, tannins and phlobatanins done by reported method[17]. All these tests were also carried out with the extract/fractions of *S. mollis*. The solution of all these plant extract/fractions was made in their respective solvent.

Quantitative phytochemical analysis: Quantitative phytochemicals were analyzed by following different procedures such as alkaloids were determined using[18], flavonoids, total phenols, saponins and tannins were tested as method described by Edeoga *et al.*[19].

Antioxidant activity of different extracts/fractions of *S. mollis*

Analysis of total phenolic contents (TPC): The amount of total phenolic content in the different extracts of *S. mollis* were examined using Folin Ciocalteu's reagent[20]. Amounts of TPC were calculated using a calibration curve for gallic acid (20-200 ppm). The results were expressed as gallic acid equivalents (GAE) mg/100 g of dry plant matter. All samples were performed thrice and averaged values were used for each sample.

Analysis of total flavonoids contents (TFC): The quantity of flavonoids was determined as described by Dewanto *et al.*[21]. Flavonoids amount were estimated spectrophotometerically. Absorbance of reaction mixture was measured at 510 nm. Catechin equivalents (mg/100g of dry plant powder) were used as standard to estimate total flavonoid contents (TFC) of samples. All samples were performed thrice and averaged values were used for each sample.

DPPH free radical scavenging assay: The 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) assay was evaluated as described earlier[22,23]. The antioxidant activity of different extracts/fractions of *S. mollis* were examined by calculating their scavenging abilities to DPPH radical spectrophotometrically.

Percentage inhibition of peroxidation in linoleic acid system: By following the method given in literature[24,25], the antioxidant activity of different extracts/fractions of *S. mollis* were estimated using peroxidation of linoleic acid in terms of % inhibition. The synthetic antioxidant such as butylated hydroxytoluene (BHT) was used as positive control. The highest oxidation level was examined at 360 h (15 days) in the sample. The sample used as blank had no antioxidant element. The absorbance of samples was measured at 500 nm. Inhibition (%) of linoleic acid oxidation was investigated with the following equation:

$$\text{Inhibition (\%)} = 100 - \left[\frac{\text{(Abs. increase of sample at 360 h}}{\text{Abs. increase of control at 360 h}} \times 100\right]$$

Analysis of total ascorbic acid content: Ascorbic acid content in each plant extract and fraction was determined by the method described by Pollard *et al.*[26]. 50 µg of each plant extract was homogenized in 10 mL of 4 % oxalic acid and centrifuged for 15 min at 5000 rpm. The supernatant was collected and added bromine water drop wise with constant stirring until a yellow colour appeared. The excess bromine was excluded in air by blowing with a pipette. Then 25 mL oxalic acid (4 %) was mixed with 2 mL brominated extract and adjusted to 30 mL with distilled water. This was allowed to react with 1 mL of 2 % DNPH followed by 1-2 drops of 10 % thiourea, then filtered and used. Control was prepared as described above without extract and incubated at 37 ºC for 3 h. The orange red crystals were dissolved by adding 7 mL H_2SO_4 (80 %). The absorbance was calculated at 540 nm by using UV-visible spectrophotometer and the results were expressed as milligram ascorbic acid equivalent per gram of dry weight.

Analysis of reducing power: The procedure reported by Yen *et al.*[27] was used to evaluate the reducing power of different extracts/fractions of plant with little modifications. 1 mg extract/fractions were mixed with 0.2 M sodium phosphate buffer (5 mL) of pH 6.6 and 5 mL potassium ferricyanide (1 %). Then heated the mixture at 50 ºC for 20 min; 10 % trichloroacetic acid (5 mL) was added and centrifuged at 980 rpm at 5 ºC for 10 min. Now its upper layer (5 mL) was dissolved in 5 mL distilled water and finally 0.1 % freshly prepared ferric chloride (1 mL) was added. The absorbance was calculated at 700 nm and a result for each sample was recorded in triplicate.

Antimicrobial assay

Antimicrobial assay by disc diffusion method: Antimicrobial activity of different extracts/fractions of *S. mollis* were investigated against three bacterial strains *i.e.*, *Bacillus cereus, Escherichia coli* and *Nitrospira* and three fungal strains *i.e.*, *Aspergillus niger*, *Aspergillus flavus* and *Candida albicans* by following disc diffusion method. Nutrient agar and potato dextrose agar (Oxoid) 28 g/L were suspended in distilled water for bacteria and fungi, respectively, mixed well and distributed homogenously. The medium was sterilized by autoclaving for 15 min at 121 ºC. Inoculum (100 µL/100 mL) was added to the medium and poured in sterilized petri plates. After this, small discs (wick paper) 6 mm in diameter were laid flat on growth medium containing extract (50 µL). Novidate and fungone (50 µL/disc; oxoid) were used as positive control for bacteria and fungi, respectively, while respective solvent 50 µL/disc served as negative control. The petri plates were incubated at 37 ºC for 24 h, for the growth of bacteria and at 28 ºC for 48 h, for the growth of fungus. The extracts having

antimicrobial activity showed clear zones. The zones of inhibition were measured by using zone reader[28]. The minimum inhibitory concentration (MIC) of different extracts/fractions of *S. mollis* was evaluated by resazurinmicrotitre-plate assay reported by Sarker *et al.*[29] with little modifications.

Haemolytic activity: Haemolytic activity of different extract/fractions of *S. mollis* were checked according to the method described by Riaz *et al.*[25].

Preparation of phenolic extract for HPLC: The extraction of phenolic compounds was undertaken by method of Demiray *et al.*[30] with small modifications. The phenolic extracts were prepared of different fractions (*n*-butanol, ethyl acetate and chloroform) of both parts (stem and leaves) of *S. mollis* for determination of efficiency of solvent type with increase polarity. Took 0.05 g of each fraction and 20 mL of respective solvent was added in it. The mixtures were shaken for 1 h in a SKIR-601 refrigerated incubator shaker (Korea) at room temperature. Then, the mixtures were centrifuged at 9000 rpm in a Universal Hettich Zenrifugen (Tuttlingen, Germany) at 4 ºC for 5 min. The supernatant was recovered and used for the determination of phenolic compounds.

Analysis of phenolic contents by HPLC: The content of phenolics in different fractions (*n*-butanol, ethyl acetate and chloroform) of *S. mollis* was analyzed on a Shim-Pack HPLC (C-18) system. The conditions utilized were as follows: C-18 column CLC-ODS, 4.6 mm × 250 mm, 5 µm; mobile phase was composed of solvent A (water (pH 2.27) with 6 % acetic acid) and solvent B (100 % acetonitrile); injection volume 20 µL, gradient elution from 15-100 % B; run time 45 min and flow rate was 1 ml/min. The chromatograms were examined at 280 nm with a LC gradient detector. The phenolic compounds were recognized by comparing retention times and UV absorption spectra with those of pure standards.

RESULTS AND DISCUSSION

Yield % of extract and fractions: The yield % of *S. mollis* extract/fractions of stem were in the range of 0.16-8.11 g/100 g and the range for the extract/fractions of leaves of *S. mollis* were 0.08-3.50 g/100 g (Table-3). The methanolic extracts of both stem and leaves showed highest yield and *n*-hexane fractions showed lowest % yield. By comparing the results of stem and leaves extracts/fractions, it is revealed that the methanolic extract of stem demonstrated maximum yield (8.11 %) while minimum by *n*-hexane fraction of leaves (0.08 %). The maximum % yield obtained with methanol is in agreement with reported results[31]. The amount of materials extracted from a plant depends upon the amount and nature of the solvent used, mixing of the different solvents during extraction procedure and nature of the sample[32-34].

Qualitative phytochemical analysis: The extract/fractions of *S. mollis* and results of stem and leaves fractions were given in Table-1. Phytochemical analysis revealed that alkaloids, glycosides, tannins, flavonoids and saponins were present while terpenoids, phlobatannin, steroids and anthraquinones were absent in *S. mollis*. Phenols, alkaloids, flavonoids and saponins all are polar that's why showed no results in *n*-hexane fraction of plant[35]. The genus *Sophora* is rich in alkaloids and flavonoids. Alkaloids constituted the bulk of compounds like quinolizidine alkaloids, lupine alkaloids particularly matrine, sophoramine and sophoridine[36] along with flavonoids and saponins[37].

Quantitative phytochemical analysis: Quantitative phytochemical evaluation of *S. mollis* extract/fractions were showed in Table-2. Crude yield (%) of alkaloids and phenols was highest in methanol fraction (1.86 and 3.00 %, respectively) of *S. mollis* stem while lowest yield of alkaloids by *n*-hexane fraction (0.23 %) of stem and lowest yield of phenolic contents by 80 % methanol extract (0.02 %) of leaves of *S. mollis*. The methanolic extract of leave showed highest yield of flavonoids (0.80 %) and saponins (4.02 %). Ethyl acetate and aqueous extract of *S. mollis* stem showed highest crude yield of tannin (0.13 %). The secondary metabolites of plants exert a wide range of biological activities on physiological systems. The quantitative analysis of phytochemicals revealed

TABLE-1
QUALITATIVE ANALYSIS OF THE PHYTOCHEMICALS OF DIFFERENT EXTRACTS/AND FRACTIONS OF *S. mollis*

Plant part	Phytochemicals	H1	H2	H3	H4	H5	H6	H7	H8
Stem	Alkaloids	+++	+++	+++	+++	++	–	+	+
	Glycosides	+++	+	+	++	–	–	+	+
	Tannins	+++	+	–	++	+	–	+	+
	Flavonoids	+++	++	+	+++	+	–	–	+
	Saponins	+++	++	++	+++	–	–	–	+
	Terpenoid	–	–	–	–	–	–	–	–
	Phlobatannin	–	–	–	–	–	–	–	–
	Steroids	–	–	–	–	–	–	–	–
	Anthraquinones	–	–	–	–	–	–	–	–
Leaves	Alkaloids	+++	++	+	++	+	–	+	+
	Glycosides	++	+	–	+++	–	–	+	+
	Tannins	+++	+	+	++	–	+	–	+
	Flavonoids	+++	++	+	+++	+	–	+	+
	Saponins	+++	++	+	+++	+	–	+	+
	Terpenoid	–	–	–	–	–	–	–	–
	Phlobatannin	–	–	–	–	–	–	–	–
	Steroids	–	–	–	–	–	–	–	–
	Anthraquinones	–	–	–	–	–	–	–	–

+++ = Very strong presence of constituents; ++ = Strong presence of constituents; + = Presence of constituents; – = Absence of constituents; H1 = Methanol; H2 = 80 % Methanol (80:20 %; methanol: water); H3 = *n*-butanol H4 = Ethyl acetate; H5 = Chloroform; H6 = *n*-hexane; H7 = Aqueous; H8 = Dry plant.

TABLE-2
QUANTITATIVE ANALYSIS OF PHYTOCHEMICALS IN DIFFERENT FRACTIONS OF *S. mollis*

Plant part	Extract/fractions	Alkaloids (%)	Flavonoid (%)	Saponin (%)	Phenols (%)	Tannin (%)
Stem	Methanol	1.86 ± 0.05	0.70 ± 0.02	2.90 ± 0.10	3.00 ± 0.0899	0.10 ± 0.0006
	80 % Methanol	0.97 ± 0.03	0.52 ± 0.02	1.95 ± 0.05	1.56 ± 0.0037	0.07 ± 0.0006
	n-Butanol	0.85 ± 0.05	0.43 ± 0.03	2.00 ± 0.15	0.11 ± 0.0010	0.08 ± 0.0007
	Ethyl acetate	1.66 ± 0.06	0.60 ± 0.03	2.52 ± 0.08	2.71 ± 0.0283	0.13 ± 0.0004
	Chloroform	1.46 ± 0.06	0.38 ± 0.02	1.50 ± 0.05	1.73 ± 0.0320	0.11 ± 0.0006
	n-Hexane	0.23 ± 0.02	0.21 ± 0.01	0.52 ± 0.03	0.04 ± 0.0007	0.08 ± 0.0005
	Aqueous	0.76 ± 0.04	0.13 ± 0.03	0.98 ± 0.13	0.09 ± 0.0006	0.13 ± 0.0007
Leaves	Methanol	1.65 ± 0.06	0.80 ± 0.03	4.02 ± 0.13	2.45 ± 0.0060	0.10 ± 0.0006
	80 % Methanol	1.23 ± 0.03	0.60 ± 0.02	3.02 ± 0.10	0.02 ± 0.0006	0.05 ± 0.0004
	n-Butanol	1.00 ± 0.02	0.41 ± 0.03	2.03 ± 0.15	0.19 ± 0.0012	0.04 ± 0.0005
	Ethyl acetate	1.25 ± 0.04	0.71 ± 0.02	3.57 ± 0.08	2.14 ± 0.0056	0.06 ± 0.0008
	Chloroform	1.01 ± 0.03	0.49 ± 0.01	2.43 ± 0.08	0.66 ± 0.0031	0.06 ± 0.0005
	n-Hexane	0.24 ± 0.02	0.11 ± 0.01	0.55 ± 0.05	0.03 ± 0.0007	0.08 ± 0.0005
	Aqueous	0.64 ± 0.04	0.22 ± 0.02	1.10 ± 0.10	1.62 ± 0.0049	0.04 ± 0.0002

Results were expressed as means of three replicates ± SD.

that stem and leaves of *S. mollis* was rich in alkaloids, flavonoids, phenols, saponins and tannins. The presence of various phytoconstituents especially alkaloids and flavonoids for *S. japonica* has been reported earlier[38].

Antioxidant analysis

Total phenolic and flavonoid contents: The amounts of total phenolic and flavonoid contents (TPC and TFC, respectively) ranged from 5.69-39.36 for stem and 5.50-118.8 GAE (mg/100 g) for leaves while 6.81-261.00 for stem and 26.16-91.04 CE (mg/100 g) for leaves of *S. mollis*, respectively (Table-3). The ethyl acetate fraction showed significant TPC and TFC values in both stem and leaves. The highest TPC value was showed by ethyl acetate fraction of leaves (118.8 mg/100 g) and ethyl acetate fraction of stem showed highest TFC value (261.0 mg/100 g). The minimum TPC and TFC values were shown by *n*-hexane fraction. Phenolics and flavonoids are polar compounds so, extracted in polar solvent such as methanol extract, *n*-butanol and ethyl acetate fractions. The amount of TPC and TFC extracted from plant depends upon the ability of a definite solvent to dissolve compound.

DPPH Radical scavenging activity: The DPPH radical scavenging IC_{50} of *S. mollis* ranged from 25.61-205.21 and 17.77-109.60 µg/mL for stem and leaves, respectively (Table-3). When DPPH (1,1-diphenyl-2-picrylhydrazyl) scavenged by various samples, it is reduced to DPPH-H form through hydrogen donation so this procedure is effectively used for determining free radical scavenging activity of plant extracts. The lowest IC_{50} value was exhibited by methnol extract (25.61 µg/mL) of stem and ethyl acetate fraction (17.77 µg/mL) of leaves. The IC_{50} value of methanolic extract of *S. interrupta* (30 µg/mL) was reported earlier[39]. The free radical scavenging activity depends upon the nature and quantity of secondary metabolites of the plant.

Inhibition % of peroxidation in linoleic acid oxidation: The inhibition % of linoleic acid oxidation ranged from 11.13 to 86.99 % for stem fractions and 22.34 to 91.54 % for leaves fractions of *S. mollis* (Table-3). Inhibition of linoleic acid was affected by different solvents. BHT showed maximum value of inhibition % (94.07 %). The maximum inhibition % was showed by ethyl acetate fraction of both stem and leaves. Comparatively leaves fractions showed better inhibition then stem fractions.

Ascorbic acid content: The aqueous fraction of leaves of *S. mollis* had highest amount of total ascorbic acid content (TAC). The results ranges from 0.021 to 0.048 mg/g for stem

TABLE-3
YIELD (%), TOTAL PHENOLIC CONTENTS (TPC), TOTAL FLAVONOID CONTENTS (TFC), IC_{50}, % INHIBITION OF PEROXIDATION IN LINOLEIC ACID SYSTEM AND TOTAL ASCORBIC ACID CONTENT (TAC) OF *S. mollis* EXTRACT AND FRACTIONS

Plant part	Extract/fractions	Yield % (g/100 g)	TPC (mg/100 g)	TFC (mg/100 g)	IC_{50} (µg/mL)	Inhibition (%)	TAC (mg/g)
Stem	Methanol	8.11	22.09 ± 0.06	109.18 ± 0.10	25.61 ± 0.04	68.69 ± 1.34	0.040 ± 0.0005
	80 % Methanol	1.73	38.4 ± 0.08	30.01 ± 0.05	39.11 ± 0.06	63.85 ± 1.35	0.029 ± 0.0006
	n-Butanol	0.58	39.36 ± 0.12	28.58 ± 0.09	34.83 ± 0.05	24.89 ± 1.28	0.021 ± 0.0005
	Ethyl acetate	2.50	17.05 ± 0.08	261.00 ± 1.73	36.69 ± 0.03	86.99 ± 0.89	0.048 ± 0.0009
	Chloroform	0.84	6.55 ± 0.05	11.97 ± 0.06	139.60 ± 1.61	17.51 ± 1.60	0.038 ± 0.0008
	n-Hexane	0.16	5.69 ± 0.04	6.81 ± 0.05	205.21 ± 1.99	11.13 ± 1.34	0.022 ± 0.0006
	Aqueous	1.84	9.95 ± 0.04	72.52 ± 0.14	89.16 ± 0.57	60.24 ± 1.63	0.032 ± 0.0008
Leaves	Methanol	3.50	14.85 ± 0.05	67.66 ± 0.21	19.04 ± 0.03	89.27 ± 2.03	0.032 ± 0.0005
	80 % Methanol	1.48	14.39 ± 0.05	40.96 ± 0.17	21.80 ± 0.02	83.09 ± 1.65	0.028 ± 0.0006
	n-Butanol	0.37	10.85 ± 0.08	32.82 ± 0.15	37.90 ± 0.03	78.39 ± 1.46	0.043 ± 0.0007
	Ethyl acetate	1.17	118.8 ± 0.13	91.04 ± 0.25	17.77 ± 0.04	91.54 ± 2.35	0.047 ± 0.0008
	Chloroform	0.18	13.96 ± 0.03	29.58 ± 0.18	102.23 ± 0.73	23.04 ± 1.63	0.057 ± 0.0006
	n-Hexane	0.08	5.50 ± 0.03	26.16 ± 0.13	109.60 ± 0.76	25.02 ± 1.64	0.038 ± 0.0005
	Aqueous	0.12	6.62 ± 0.02	76.51 ± 0.23	100.84 ± 0.93	22.34 ± 1.82	0.098 ± 0.0008
	BHT	–	–	–	8.96 ± 0.03	94.07 ± 1.48	–

Results were expressed as means of three replicates ± SD.

and from 0.028 to 0.098 mg/g for leaves were presented in Table-3. Ascorbate was a potent reducing agent which is capable of scavenging number of reactive oxygen species (ROS) rapidly.

Reducing power: The reducing potential of different extracts/fractions of *S. mollis* were investigated at 0.0, 2.5, 5.0, 7.5 and 10 mg/mL concentrations and their absorbance ranges from 0.31 to 1.85 nm. The control (ascorbic acid) showed maximum value of reducing power (1.85 nm). Other results of reducing power were recorded in Figs. 1 and 2. The methanolic extract of *S. interrupta* exhibited the strong antioxidant activity due to presence of reductants and the power of extract was increased with quantity of sample already reported[39].

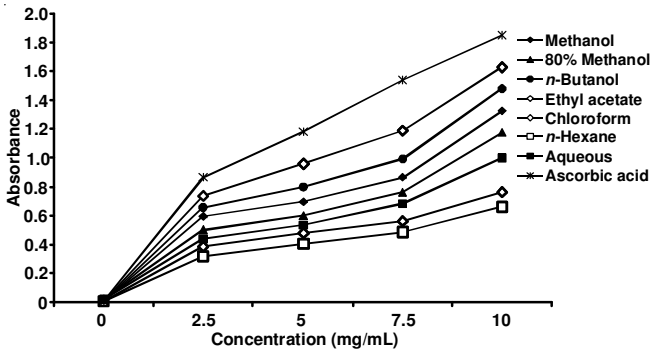


Fig. 1. Reducing potential of stem extract/fractions of *S. mollis*

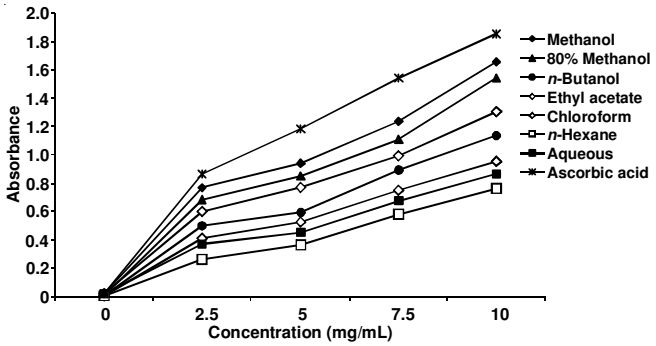


Fig. 2. Reducing potential of leaves extract/fraction of *S. mollis*

Antimicrobial activity: The plant *S. mollis* exhibited significant antimicrobial activity against all examined strains (Table-4). *n*-Butanol fraction (18.72 mm) of stem and ethyl acetate fraction (11.37 mm) of leaves showed maximum antibacterial activity against *B. cereus*. Ethyl acetate fraction of stem (12.51 mm) and leaves (10.34 mm) showed maximum antibacterial activity against *E. coli*. Methanolic extract of stem and 80 % methanol extract of leaves showed considerable activity against *N. spira*. Ethyl acetate fraction of stem showed strong inhibitory activity against *A. flavus* and *A. niger* with highest inhibition zones (13.52 and 11.22 mm) while in leaves absolute methanol and aqueous fraction showed significant results (13.57 and 12.35 mm) against *A. flavus* and *A. niger,* respectively. *n*-Butanol fraction (10.42 mm) for stem and methanol extract (13.43 mm) for leaves of plant were showed considerable activity against *C. albicans*. Some flavonoid derivatives from *S. flavescens*, including quercetin, sophoraflavanone G and kaempferol, have been previously demonstrated evidence to antimicrobial and antimalarial activity[40]. The antimicrobial effect of the medicinal plants is well acknowledged[41]. The results of different studies furnish evidence that medicinal plants might be used as potential sources of new antibacterial agents even against some antibiotic-resistant strains[42].

Minimum inhibitory concentration (MIC): The range of MIC values was found to be 0.023 to 0.078 mg/mL for stem extract and fractions of plant against *B. Cereus* (Table-5). The lowest MIC value exposed by *n*-butanol fraction of stem, the same fraction showed maximum antibacterial activity against *B. cereus*. Similarly ethyl acetate fraction of leave exposed highest antibacterial activity against *B. cereus* and its MIC value was lowest as 0.064 mg/mL. Methanolic and 80 % methanolic extract of stem and leaves showed lowest MIC values (0.039 and 0.063 mg/mL), respectively as these extracts exhibited maximum activity against *Nitrospira*. Similar results were demonstrated by fungal strains (Table-5).

Haemolytic activity: The methanolic extract of both stem and leaves showed maximum haemolytic activity 14.31 and 16.56 %, respectively (Table-6). The 80 % methanol, *n*-butanol, ethyl acetate, *n*-hexane and aqueous fractions of *S. mollis* plant

TABLE-4
ANTIMICROBIAL ACTIVITY BY ZONE OF INHIBITION (ZI) IN mm OF DIFFERENT FRACTIONS OF *S. mollis*

Plant part	Extract/fractions	Bacterial strains			Fungal strains		
		B. cereus	*E. coli*	*N. spira*	*A. niger*	*A. flavus*	*C. albicans*
Stem	Methanol	12.08 ± 0.46	11.8 ± 0.26	11.3 ± 0.27	11.53 ± 0.32	10.55 ± 0.12	10.24 ± 0.10
	80 % Methanol	15.37 ± 0.27	9.48 ± 0.45	–	–	–	9.49 ± 0.39
	n-Butanol	18.72 ± 0.54	–	9.53 ± 0.13	12.54 ± 0.22	9.03 ± 0.4	10.42 ± 0.45
	Ethyl acetate	15.09 ± 0.31	12.51 ± 0.14	10.4 ± 0.16	13.52 ± 0.37	11.22 ± 0.37	10.28 ± 0.23
	Chloroform	10.16 ± 0.34	10.7 ± 0.54	8.47 ± 0.3	9.88 ± 0.28	8.42 ± 0.31	–
	n-Hexane	8.42 ± 0.7	8.56 ± 0.76	–	–	7.58 ± 0.33	8.42 ± 0.70
	Aqueous	–	10.69 ± 0.52	–	8.58 ± 0.27	8.78 ± 0.52	–
Leaves	Methanol	11.17 ± 0.7	9.19 ± 0.56	–	13.57 ± 0.36	11.22 ± 0.14	13.43 ± 0.30
	80 % Methanol	–	–	11.47 ± 0.19	–	9.29 ± 0.27	–
	n-Butanol	9.36 ± 0.36	–	9.52 ± 0.35	–	12.35 ± 0.25	8.39 ± 0.22
	Ethyl acetate	11.37 ± 0.37	10.34 ± 0.11	–	11.36 ± 0.32	11.37 ± 0.37	12.65 ± 0.22
	Chloroform	–	9.06 ± 0.35	–	–	9.31 ± 0.28	7.57 ± 0.04
	n-Hexane	–	–	8.56 ± 0.4	8.24 ± 0.24	–	–
	Aqueous	8.53 ± 0.26	–	–	–	10.49 ± 0.1	–
Standard	Novidate/fungone	21.77 ± 0.49	25.26 ± 0.36	22.92 ± 0.43	20.74 ± 0.55	19.03 ± 0.6	23.82 ± 0.67

Results were expressed as means of three replicates ± SD.

TABLE-5
ANTIMICROBIAL ACTIVITY BY MINIMUM INHIBITORY CONCENTRATION (MIC) OF *S. mollis*

Plant part	Extract/fractions	Bacterial strains			Fungal strains		
		B. cereus	*E. coli*	*N. spira*	*A. niger*	*A. flavus*	*C. albicans*
Stem	Methanol	0.078 ± 0.004	0.039 ± 0.005	0.039 ± 0.004	0.152 ± 0.005	0.359 ± 0.009	0.156 ± 0.004
	80 % Methanol	0.040 ± 0.01	0.637 ± 0.035	–	–	–	1.180 ± 0.006
	n-Butanol	0.023 ± 0.004	–	0.158 ± 0.006	0.153 ± 0.006	0.036 ± 0.003	0.073 ± 0.001
	Ethyl acetate	0.039 ± 0.005	0.016 ± 0.001	0.039 ± 0.003	0.019 ± 0.001	0.019 ± 0.001	0.149 ± 0.004
	Chloroform	0.041 ± 0.004	0.022 ± 0.004	0.590 ± 0.044	1.179 ± 0.004	0.076 ± 0.003	0.144 ± 0.003
	n-Hexane	0.035 ± 0.004	0.073 ± 0.002	–	–	0.144 ± 0.003	0.287 ± 0.005
	Aqueous	–	0.074 ± 0.003	–	0.295 ± 0.004	0.574 ± 0.017	–
Leaves	Methanol	0.082 ± 0.003	0.157 ± 0.005	0.600 ± 0.041	0.015 ± 0.001	0.077 ± 0.001	0.325 ± 0.004
	80% Methanol	–	–	0.063 ± 0.002	–	.041 ± 0.006	–
	n-Butanol	0.299 ± 0.031	–	1.187 ± 0.040	–	0.016 ± 0.002	0.288 ± 0.004
	Ethyl acetate	0.064 ± 0.004	0.013 ± 0.003	–	0.153 ± 0.005	0.636 ± 0.014	0.019 ± 0.001
	Chloroform	–	0.016 ± 0.002	–	–	0.089 ± 0.006	1.188 ± 0.004
	n-Hexane	–	–	0.074 ± 0.004	0.295 ± 0.004	–	–
	Aqueous	0.159 ± 0.008	–	–	–	0.292 ± 0.004	–
Controls	Novidate/fungone	0.97 ± 0.013	0.46 ± 0.021	0.39 ± 0.007	0.48 ± 0.008	0.86 ± 0.017	0.25 ± 0.015

TABLE-6
HAEMOLYSIS % BY EXTRACTS AND FRACTIONS OF *S. mollis* STEM AND LEAVES

Extract/fractions of plant	Haemolysis % of RBCs	
	Stem	Leaves
Methanol	14.31 ±0.05	16.56 ± 0.05
80 % Methanol	8.12 ± 0.05	8.7 ± 0.04
n-Butanol	3.4 ± 0.05	4.03 ± 0.05
Ethyl acetate	2.06 ± 0.02	2.5 ± 0.02
Chloroform	10.77 ± 0.03	15.02 ± 0.04
n-Hexane	3.74 ± 0.03	4.27 ± 0.05
Aqueous	1.4 ± 0.02	2.34 ± 0.04

also exhibited considerable haemolytic activity. The aqueous fractions of stem of plant showed less haemolytic effect (1.40 %). The seeds of *S. mollis* contain an alkaloid cytosine which resembles to nicotine and similarly toxic[43].

HPLC analysis of *S. mollis*: Different phenolic acids were identified from different fractions (*n*-butanol, ethyl acetate and chloroform) of *S. mollis* stem and leaves (Table-7). Caffiec acid, chlorogenic acid, ferrulic acid, gallic acid, *m*-coumeric acid, *p*-coumeric acid, syringic acid and vanillic acid were identified in different fractions of plant. Maximum number of phenolic acids were found in ethyl acetate fraction of leaves, while in chloroform fraction of stem no phenolic acid was found. The most of the plants showed antioxidants properties due to presence of phenolic compounds in these medicinal plants.

Conclusion

It is concluded from present investigation that all the extracts/fractions of *S. mollis* stem and leaves showed considerable biological activities but the methanol and ethyl acetate fraction of stem and leaves of plant exhibited good antioxidant and antimicrobial activity. HPLC data indicated that more phenolic acids were extracted from leaves fractions of plants than stem fractions of plants and maximum five phenolic acids were extracted from methanolic extract of leaves. This plant might be used as raw material for therapeutic purposes in future. The cytotoxicity of *S. mollis* extract/fractions was examined by haemolytic activity against human RBCs and the lysis (%) of RBCs minor was observed.

TABLE-7
HPLC ANALYSIS OF DIFFERENT FRACTIONS OF *S. mollis* FOR PHENOLIC CONTENTS

Plant part	Phenolic acids	*n*-Butanol (ppm)	Ethyl acetate (ppm)	Chloroform (ppm)
Stem	Caffiec acid	1.89	–	–
	Chlorogenic acid	0.28	–	–
	Ferrulic acid	–	11.16	–
	Gallic acid	1.15	–	–
	Syringic acid	1.04	0.70	–
Leaves	Caffiec acid	16.69	1.16	–
	Chlorogenic acid	–	6.62	–
	Ferrulic acid	25.63	–	11.94
	Gallic acid	1.42	1.45	–
	m-Coumeric acid	–	–	6.64
	p-Coumeric acid	–	–	2.18
	Syringic acid	–	4.05	–
	Vanillic acid	74.23	5.80	–

– = Not detected.

ACKNOWLEDGEMENTS

The authors are highly thankful to Dr. Raja Adil Sarfraz Hi Tech Laboratory University of Agriculture, Faisalabad, Pakistan for providing the facility regarding the analysis of phenolics in samples by HPLC.

REFERENCES

1. T. Nishihara, K. Watabe, T. Ichikawa, M. Kondo and M. Fujii, *Chemosphere*, **14**, 1133 (1985).
2. D. Zhang and Y. Hamauzu, *Food Chem.*, **88**, 503 (2004).
3. K. Zhou and L. Yu, *LWT-Food Sci. Technol.*, **39**, 1155 (2006).
4. K.H. Kang, Z.J. Qian, B. Ryu, F. Karadeniz, D. Kim and S.K. Kim, *Phytother. Res.*, **26**, 1555 (2012).
5. H.J.M. Bowen and V.P. Guinn, *CRC Crit. Rev. Anal. Chem.*, **10**, 127 (1981).
6. Q.-F. Zhang, Z.-R. Zhang and H.-Y. Cheung, *Food Chem.*, **115**, 297 (2009).
7. D.Q. Fei, M. Arfan, J. Rafiq and K. Gao, *Chem. Nat. Comp.*, **6**, 747 (2009).
8. E.M. Abdallah and A.E. Khalid, *Int. J. Chem. Biochem. Sci.*, **1**, 1 (2012).
9. S. Adeel, F. Rehman, T. Gulzar, I.A. Bhatti, S. Qaiser and A. Abid, *Asian J. Chem.*, **25**, 2739 (2012).
10. K. Ahmad, Z.I. Khan, M. Ibrahim, M. Ashraf and M. Hussain, *J. Plant Nutr.*, **33**, 496 (2010).

11. F. Batool, S. Adeel, M. Azeem, A.A. Khan, I.A. Bhatti, A. Ghaffar and N. Iqbal, *Radiat. Phys. Chem.*, **89**, 33 (2013).
12. A. Fardous, S. Gondal, Z.A. Shah, K. Ahmed, Z.I. Khan, M. Ibrahim, A. Ejaz, W. Ahmed, S. Ullah and E.E. Valeem, *Pak. J. Bot.*, **42**, 2411 (2010).
13. F. Rehman, S. Adeel, M. Zuber, I.A. Bhatti, S. Qaiser and M. Shahid, *Radiat. Phys. Chem.*, **81**, 1752 (2012).
14. K. Rehman, S. Ashraf, U. Rashid, M. Ibrahim, S. Hina, T. Iftikhar and S. Ramzan, *Pak. J. Bot.*, **45**, 391 (2013).
15. M. Riaz, N. Rasool, I. Bukhari, M. Shahid, F. Zahoor, M. Gilani and M. Zubair, *Afri. J. Microbiol. Res.*, **6**, 5700 (2012).
16. A. Sofowora, *J. Bioactive Agents*, **21**, 134 (1993).
17. G.E. Trease and W.C. Evans, Pharmacognosy, Brailliar Tiridel, London, England, edn. 11, pp. 1-795 (1989).
18. J.B. Harborne and C.A. Williams, *Phytochemistry*, **55**, 481 (1992).
19. H.O. Edeoga, D.E. Okwu and B.O. Mbaebie, *J. Biotechnol.*, **4**, 685 (2005).
20. A. Chaovanalikit and R.E. Wrolstad, *J. Food Sci.*, **69**, 67 (2004).
21. V. Dewanto, X. Wu, K.K. Adom and R.H. Liu, *J. Agric. Food Chem.* **50**, 3010 (2002).
22. B. Bozin, D. Micica, N. Simin and G. Anackov, *J. Agric. Food Chem.*, **54**, 1822 (2006).
23. K. Rizwan, M. Zubair, N. Rasool, M. Riaz, M. Zia-Ul-Haq and D.V. Feo, *Int. J. Mol. Sci.*, **13**, 6440 (2012).
24. S. Iqbal, M.I. Bhanger and F. Anwar, *Food Chem.*, **93**, 265 (2005).
25. M. Riaz, N. Rasool, I.H. Bukhari, M. Shahid, M. Zubair, K. Rizwan and U. Rashid, *Molecules*, **17**, 14275 (2012).
26. S.J.T. Pollard, F.E. Thompson and G.L. McConnachie, *Water Res.*, **29**, 337 (1995).
27. G.C. Yen, P.D. Duh and D.Y. Chuang, *Food Chem.*, **70**, 437 (2000).
28. T. Mckee and J.R. Mckee, Biochemistry, Brown Publishers, Dubuque, IA, USA, edn. 1, pp. 1-50 (1996).
29. S.D. Sarker, L. Nahar and Y. Kumarasamy, *Methods*, **42**, 3214 (2007).
30. S. Demiray, M.E. Pintado and P.M.L. Castro, *World Acad. Sci. Eng. Technol.*, **54**, 312 (2009).
31. M.N. Bari, M. Zubair, K. Rizwan, N. Rasool, I.H. Bukhari, S. Akram, T.H. Bokhari, M. Shahid, M. Hameed and V.U. Ahmad, *J. Chem. Soc. Pak.*, **34**, 990 (2012).
32. F. Aslam, N. Rasool, M. Riaz, M. Zubair, K. Rizwan, M. Abbas, T.H. Bukhari and I.H. Bukhari, *Int. J. Phytomed.*, **3**, 567 (2011).
33. S.N. Hsu, S. Yonekura, Y. Lwai, T. Uemura, H.M. Robertson, C.H. Lee and A. Chiba, *A Drosophila Research Conference*, **47**, 609 (2006).
34. M. Zubair, S. Hassan, K. Rizwan, N. Rasool, M. Riaz, M. Zia-Ul-Haq and V. De Feo, *The Scientific World J.*, **2013**, 1 (2013).
35. B. Tepe, *Food Chem.*, **92**, 89 (2005).
36. M. Imran, J. Iqbal, S. Iqbal and N. Ijaz, *Turk. J. Biol.*, **31**, 67 (2007).
37. M.S. Iqbal, I.H. Bukhari and M. Arif, *Appl. Organomet. Chem.*, **19**, 864 (2005).
38. M.S. Iqbal, A.H. Khan, B.A. Loothar and I.H. Bukhari, *Med. Chem. Res.*, **18**, 31 (2009).
39. J.R. Ferraro, Low-frequency Vibrations of Inorganic and Coordination Compounds, John Wiley, New York, USA, pp. (1971).
40. A. Burneau and C. Carteret, *Phys. Chem. Chem. Phys.*, **2**, 3217 (2000).
41. C.F. Baes and R.E. Mesmer, The Hydrolysis of Cations, Wiley-Interscience, New York, USA, pp. 267-274 (1976).
42. S.F. Montanher, E.A. Oliveira and M.C. Rollemberg, *J. Hazard. Mater.*, **117**, 207 (2005).
43. I.V. Sheveleva, A.N. Kholomeydik, A. Voit and L.A. Zemnukhova, *Chem. Plant Raw Mater.*, **4**, 171 (2009).