Pak. J. Bot., 47(SI): 313-319, 2015.

ANTIMICROBIAL, PHYTOTOXIC, HEAMAGGLUTINATION, INSECTICIDAL AND ANTIOXIDANT ACTIVITIES OF THE FRUITS OF *SARCOCOCCA SALIGNA* (D. DON) MUEL.

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Abstract

Crude methanol extract and different fractions of the fruits of *Sarcococca saligna* (D. Don) Muel were investigated for various pharmacological/biological activities; antibacterial, antifungal, phytotoxic, haemagglutination, insecticidal and antioxidant activities. The results revealed that the plant possess good antibacterial activity (65.3 and 63.0%) against Methicillin Susceptible *Staphylococcus aureus* (MSSA) and *Salmonella typhi*, respectively while rest of the fractions along with crude methanolic extract (Crd. MeOH Ext.) were found moderately active or inactive against the tested pathogens. Low antifungal activity was observed for A, D, H and J fractions while other test samples were found inactive against the test fungi. Moderate phytotoxic activity was shown by C, D, G, H, K and L fractions at higher concentration (1000µg/ml). Moderate insecticidal activity was shown by G, H and I fractions against *T. castaneum* and G, H, I, K and L against *R. dominica*. A concentration dependent Diphenyl Picryl Hydrazine (DPPH) free radical scavenging activity was shown by the test samples. Fruits of the plant lack phytolactines as no haemagglutination activity were observed against human erythrocytes.

Keywords: Antibacterial, Antifungal, Phytotoxic, Haemagglutination, Insecticidal, Antioxidant, *Sarcococca saligna*

Introduction

Plants in general and medicinal plants in particular have a vital importance in a country's economy. Much effort has been made to promote the traditional medicine especially WHO is promoting the use of traditional medicines because low income South Asian countries depends mostly on traditional medicine (Oliyiowola, 1984). More than eight thousand species of medicinal plants exist in South Asia and approximately two thousands of the medicinal species exist in Pakistan and are widely distributed in northern region of country. Our country has a rich store of phyto medicinal flora which is still undiscovered. This huge store of medicinal flora attains much more importance when matters come to country like Pakistan where there is poverty. By the year 2050, it is being expected that the Global market would rise to 5.0 trillion (Karki, 2002).

Plants are the richest source of drugs by bringing new chemotherapeutic agents. Traditional medicines are the economical source of therapy to the population (Kaufman *et al.,* 1999). Several plants show antibacterial activities (Shah *et al.,* 2014). On micro level in Pakistan the import bill of medicinal plants is just US$ 6.0 million. On the macro level, 30% of the total medicines are being prepared from herbs. It is the need of the day to let the medicinal flora play its role in the local and global market. Therefore, the medicinal plants of northern area of Pakistan have great potential in local and global market and it is important to explore the natural resources of phytomedicine of this area. In continuation to our reported work on exploring the medicinal flora of Khyber Pakhtunkhwa, for the current study we select the fruit of *S. saligna* belongs to Buxaceae family. All plants of this family are ever green trees, rarely perennial herbs and shrubs. It comprises of four genera, *Sarcococca*, *Pachysandra*, *Buxus* and *Simondsia* and about 100 species. It is mostly distributed in tropical and temperate regions. In last three decades during various phytochemical investigations maximum numbers of steroidal alkaloids have been reported from the various plants of Buxaceae family (Rehman & Chaudhary, 1997; Devkota *et al.,* 2008). In the folk system of medicine local people used *S. saligna* for the cure of different diseases like skin infection and malaria (Rai *et al.,* 2006). Locally the aqueous extract of *S. saligna* is used as calmative and antipyretic and the leaves of this plant have potential application for fever and rheumatism. This plant is also used for the treatment of rheumatic fever and pain (Nasir & Ali, 1972). It is used for the treatment of syphilis, pain, liver diseases, the gastrointestinal tract hyperactive states, infection and inflammation (Khan, 1999*;* Chatterjee *et al.,* 1965*;* Kiamuddin *et al.,* 1970; Kohli *et al.,* 1967). The antitumor, antiulcer, antidiarrheal, antisecretory and acetyl cholinesterase inhibitory activities of the plant have been reported (Rahman *et al.,* 2000). The chloroform ($CHCl_3$) fraction of *S. saligna* showed significant antibacterial activity (80%) against *Staphylococcus aureus*, while its crude methanolic extract showed good (77%) antimicrobial activity. The *n*-hexane fraction of plant showed good (60%) insecticidal activity against *C. analis*, against *T. castaneum* this fraction showed moderate activity of 40%. Weak (+) heamagglutination activity was shown by crude extract of the plant against O^{-ive}, *n*-hexane against A^{-ive} and O^{-ive} and $CHCl_3$ against O^{-ive} at dilution of 1:2, respectively (Bashir *et al.,* 2010). Crude extracts, fractions and compounds isolated from Sarcococca genus are potent Acetyl and

butyryl-cholinesterases inhibitors (Devkota *et al.,* 2008*;* Choudhary *et al.,* 2004). Seventeen steroidal alkaloids, along with five synthetic derivatives from the plant *S. hookeriana*, have shown potent to mild anti-leishmanial properties (Devkota *et al.,* 2007). Some steroidal alkaloids which were isolated from *S. hookeriana* showed moderate *in-vitro* antiplasmodial activity against W2 strain of *P. falciparum* (Devkota *et al.,* 2008). Crude Extract and compounds that were obtained from *S. saligna* showed a dose dependent spasmolytic activity in the rabbit jejunum preparations and have also relaxed high K^+ (80 mM) induced contraction. This was indicative of calcium channel-blocking mechanism (Khalid *et al.,* 2004*;* Gilani *et al.,* 2005). The dichloromethane extract of *S. coriacea* was found to be cytotoxic, while the methanol extract showed a polygalacturonase inhibition (Paudel *et al.,* 2003).

Material and Methods

Collection of plant: The fruits of *S. saligna* were collected from hilly area of Abbotabad, Khyber Pakhtunkhwa (Pakistan) in the month of September 2011. The collected fruits were identified by Prof. Dr. Farrukh Hussain, plant taxonomist, Department of Botany, University of Peshawar.

Extraction: The collected fruits were shade dried, chopped into small pieces and grounded to fine powder, using an electric grinder. The powdered material (1.5 Kg) was soaked in commercial grad methanol for 10 days, twice at room temperature with occasional shaking. After 10 days the material was filtered and the filtrate was concentrated at 40°C under vacuum by using a rotary evaporator. A blackish Crd. MeOH Ext. of 200g was obtained.

Fractionation: The Crd. MeOH Ext. was fractionated in different solvents (*n*-hexane, $CHCl_3$, Ethyl acetate, Acetone and Methanol).

Crd. MeOH Ext. was coat over silica, packed into a column and fractionated in various organic solvent in different combinations and in increasing order of polarity. A total of 12 different fractions were obtained. 5 gram of Crd. MeOH Ext. was reserved for pharmacological screening.

Antibacterial activity: Due to the increasing incidents of infections and evolution of the new pathogens which shows resistance to present antibiotics, various decoctions of plants materials are used for the treatment of injuries and burns relief. In the tropical region of the world one half of the deaths are occurs due to infections, especially (Muhammad *et al.,* 2008). We performed our screening keeping in view the clinical significance of various pathogens.

Test organisms: *E. coli*, Methicillin resistant *Staphylococcus aureus*, MSSA, *Pseudomonas aeruginosa* and *S. typhi* were used, available at Center of Biotechnology and Microbiology, University of Peshawar.

The antibacterial activity of the test sample against the above mentioned pathogens was performed as per reported procedure (Bashir *et al.,* 2010).

1. Nutrient broth medium was prepared, autoclaved and poured into sterile test tubes and to check the sterility incubate for 24 hrs at 37°C.
2. After incubation, the test organism were inoculated to the sterile nutrient broth and incubated for 24 hrs at 37°C.
3. On the same day of experiment nutrient agar medium was prepared and poured into the sterilized petri dishes. For sterility test these plates were incubated for 24 hrs at 37°C.
4. After sterility check of the medium, bacterial lawn was prepared by using 18-24 hrs old bacterial culture(s).
5. Well (6 mm) were made with the help of sterile borer in the petri dishes.
6. Stock solution of test samples at a concentration of 3mg/ml was prepared in DMSO. 100µl from the stock solution was transferred into the respective wells and incubated at 37°C for 24 hrs.
7. DMSO (<1%) was used as negative control and Amoxicillin as positive control.
8. On the next day zone of inhibitions were measured in mm and percent zone of inhibition was measured by following formula.

$$\% \text{ inhibition} = \frac{\text{Zone of Inhibition of Sample}}{\text{Zone of Inhibition of Standard}} \times 100$$

After the measurement of the zone of inhibition we preceded the experiment to determine the MIC_{50}as per our reported procedure (Bashir *et al.,* 2010).

Minimum inhibitory concentration (MIC_{50}): With slight modification following steps were carried for determining the MIC_{50}.

1. Transfer 4ml of the prepared nutrient broth into the sterile test tube and incubate at 37°C for 24 hrs.
2. After 24 hrs the test pathogen was inoculated in test tubes containing sterilized medium.
3. From the stock solution different concentrations were poured to the tubes containing test pathogens.
4. After the incubation at 37°C for 24 hrs the results were recorded.

Antifungal activity

Test Organism: Antifungal screening was performed against various fungal species including; *Aspergillus oryzae, Fusarium fumigatus, Fusarium solanum, Aspergillus flavus, Aspergilus niger* and *Penicillium notatum*.

The antifungal activity of the various fractions and Cr. MeOH Ext. of the fruits were performed according to Bashir *et al.,* 2010.

1. Stock solutions of the test samples were prepared at concentration of 24mg/ml in DMSO.

2. SDA medium was prepared, autoclaved, 4ml was poured into sterilized test tubes and when cool upto 50°C, 66.6µl from the stock solutions. To make the slants the test tubes were kept in slant position. In order to check the sterility the slants were incubate at 28±1°C for 24 hrs.
3. After 24 hrs the 7 days old fungal culture were inoculated into the test tubes and incubated for 5-7 days at 28±1°C.
4. Miconazole were as positive control and DMSO as negative control.

The results were measured at 7th day by measuring the linear growth inhibition. The percent inhibition was measured by using the following formula in comparison with positive and negative control.

$$\% \text{ inhibition} = \frac{\text{Linear growth in test sample (mm)}}{\text{Linear growth in standard (mm)}} \times 100$$

Haemagglutination activity: Haemagglutination activity of Cr. MeOH Ext. and various fractions of fruit were performed as per reported procedure of Bashir *et al.,* 2010

Phosphate buffer was prepared by dissolving 0.47g of Na_2HPO_4 and 0.453g of KH_2PO_4 in 50ml of distilled water. These are mixed in the ratio of 3:7 (KH_2PO_4: Na_2HPO_4). A stock solution of test sample was prepared by dissolving 1mg of test sample in 1ml of DMSO. In the phosphate buffer different dilution; 1:2, 1:4, 1:8, 1:16 were made from the stock solution. From a healthy person, blood samples were collected on the day of experiment and centrifuged. The red blood cells (RBC's) were collected for the next steps and the plasma was discarded. In the phosphate buffer, 2% of RBC □s suspension was prepared. From each dilution 1ml was taken in a test tube and 1ml of RBC □s suspension was added to the sample. The tubes were incubated at 37°C for 30 min. After incubation the positive and negative results were examined by rough granules and smooth button formation.

Phytotoxic activity: The test samples were screened for phytotoxic activity according to Prof. McLaughlin protocols as following.

1. Stock solutions were prepared by dissolving 20mg of test sample in 1ml of methanol.
2. E-medium was prepared for the growth of *L. minor***.**
3. 10, 100, 1000µg/ml from the stock solutions were poured to flask and allow to evaporate the organic solvent at room temperature.
4. After evaporation of organic solvent, 20ml of E-media was poured to all flasks.
5. Sixteen healthy plants of *L. minor* were selected and put into respective flasks.
6. Results were recorded after 7 days of incubation at 28 ±1°C in growth chamber.

Insecticidal activity

Test samples preparation: For preparation of the stock solutions, 200mg of the test samples were dissolved in 3ml of methanol.

Rearing technique: In breeding media (sterile) the above mentioned pests were reared in plastic bottles in the laboratory, under controlled conditions of humidity and temperature. Insects of uniform size and age were selected for experimental work.

Procedure: For determining the insecticidal activity, the contact toxicity assay (Bashir *et al.,* 2010) was used. The assay was performed as following:

On first day, Petri plates (90mm) were sterilized and filter papers were cut according to the size of Petri dishes. The filter papers were kept in the Petri dishes and stock solutions of test samples were introduced using micropipette. The Petri plates were left overnight for methanol evaporation.

On the second day, 16 healthy insects of small and equal size from each specie were selected and transferred to the labeled plates using a clean brush. The plates were incubated for 24hr at 27°C with 50% relative humidity in growth chamber.

After incubation for 24 hrs, results were recorded by counting the number of survivals in each plate. The percent mortality was calculated by using the following formula:

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

Estimation of radical scavenging activity using DPPH assay: The hydrogen atom or electron donation abilities of the consequent extracts/fractions and standards were measured from the bleaching of the purple-colored methanol solution of DPPH experiments were carried out according to the method of Rauf *et al.,* 2012. 1mM solution of DPPH radical solution in methanol was prepared and 1ml of this solution was mixed with 3ml of sample solutions in methanol (containing 20-250µg/ml). The reaction solution without DPPH was used as a blank test and ascorbic acid (20-250µg/ml), was used as positive control. The solution was kept for 30 min and the absorbance was measured at 517 nm. Decreasing of the DPPH solution absorbance indicates an increase of the DPPH radical-scavenging activity. Scavenging of free radicals by DPPH as percent radical scavenging activities was calculated as follows.

$$\% \text{ DPPH} = \frac{\text{Control absorbance} - \text{extract absorbance}}{\text{Control absorbance}} \times 100$$

The extract concentration showing 50% inhibition (EC_{50}) was calculated from the graph of percent DPPH against extract concentration.

Results and Discussions

Antibacterial activity: One of the major problem faces by the science in current situation is resistance to antimicrobial drugs. Finding new antimicrobials will help us to get rid of such problems (Freeman, 1997). Crd. MeOH Ext. and various fractions of *S. saligna* fruits were screened for possible antimicrobial activity against *E. coli,* MRSA, MSSA, *P. aeruginosa* and *S. typhi* (Tables 1 & 2).

The order of antibacterial activity against *E. coli* was; E=L (48%) > A=D=J=K (37%) > L (33.3%) > C=I (26%) and B (22.2%). G and H fractions were found inactive against *E. coli*. Against MRSA; A=D=G=I (53.3%) > B=E=H (46.6%) >Cr. Ext. (33.3%) >J=K=L (26.6%) >F (20.0%) >C (13.5%). Against *MSSA*; F (65.3) > A (58%) > D=E=K=L (57%) > B=C (53.8%) > J (50%) > H=I (46%) > G (38.4%). Against *P. aeruginosa*; H (59.2%)> A=D=L (55.5%) > C (52%) > K (51.85%) >I (44.4%) > E=J (37%) and B=F=G were found inactive against *P. aeruginosa*. Against *S. typhi*; H (59.2%) >A (55.5%) >C (52%)>K (51%)>F (48.1%) > D=G=I (44.4%) > E (40%)>L (37%).

The above results revealed that fraction E and L showed moderate inhibitory effect while rest of the fractions except G and H, showed low antibacterial activity against *E. coli.* Fraction A, D, G and I showed moderate activity while rest of the fractions possess low antibacterial activity against MRSA. Fraction F showed good antibacterial effect while other fractions were not that much active against MSSA. Fraction A, D, H, K and L were moderately active and the other fractions showed low or found inactive against the *P. aeruginosa.* Fraction C showed moderate while rest of the fractions showed low inhibitory effect against *S. typhi.*

Minimum inhibitory concentration (MIC_{50}): The MIC_{50} values indicate the potency of a test sample, lower the MIC_{50} values higher will be the potency and vice versa. MIC_{50} values (mg/ml) of the various fractions of *S. saligna* (with moderate antibacterial activities) were calculated by using Microsoft XL sheet from three different reading (Table 2).

Antifungal activity: All the test samples of *S. saligna* fruits were screened for the antifungal activity against *A. oryzae, F. fumigatus, F. solani, A. flavus, A. niger* and *P. notatum* (Table 3). Amphotericin-B and Miconazole served as standard drugs. The fractions A, D, H and J showed low percent linear inhibition (20%) against *F. fumigatus*. while the rest of the fractions were inactive against *F. fumigatus*. All the fractions were inactive against *A. oryzae, F. solani, A. flavus, A. niger* and *P. notatum*. The results indicate that *S. saligna* fruits contain no antifungal agents.

Table 1. Percent antibacterial activity of crude methanolic extract and various fractions of *S. saligna* fruits.

Name of Bacteria	Zone of Inhibition (Percent)												
	Cr. Ext	A	B	C	D	E	F	G	H	I	J	K	L
E. coli	26.0	37.0	22.2	26.0	37.0	48.0	33.3	0.0	0.0	26.0	37.0	37.0	48.0
MRSA	33.3	53.3	46.6	13.5	53.3	46.6	20.0	53.3	46.6	53.3	26.6	26.6	26.6
MSSA	34.6	58.0	53.8	53.8	57.6	57.6	65.3	38.4	46.0	46	50.0	57.6	57.6
P. aeruginosa	37.0	55.5	0.0	52.0	55.5	37.0	0.0	0.0	59.2	44.4	37.0	51.0	55.5
S. typhi	37.0	51.8	55.5	63.0	44.4	40.0	48.1	44.4	48.0	48.0	48.0	51.8	37.0

Table 2. MIC_{50} values (mg/ml) of the Cr. Ext and different fractions of *Sarcococca saligna* fruits.

Name of Bacteria	Cr. Ext	A	B	C	D	E	F	G	H	I	J	K	L
E. coli	---	---	---	---	---	---	---	---	---	---	---	---	6.9
MRSA	---	7.2	8.6	---	7.0	8.9	---	7.2	8.2	7.1	---	---	---
MSSA	---	6.1	6.6	6.7	6.5	6.6	5.9	---	7.5	7.4	6.9	6.4	6.6
P. aeruginosa	---	5.1	---	5.6	5.2	---	---	---	6.7	5.9	---	7.3	5.9
S. typhi	---	6.2	2.5	6.9	6.7	6.1	3.2	5.9	5.6	5.8	5.7	7.5	---

Table 3. Antifungal activity of the Cr. Ext and various fractions of *Sarcococca saligna* fruits.

Names of Fungi	Percent linear growth inhibition														
	-ive Control	+ive Control	Cr. Ext	A	B	C	D	E	F	G	H	I	J	K	L
A. oryzae	--	100	--	--	--	--	--	--	--	--	--	--	--	--	--
F. fumigatus	--	100	--	20	--	--	10	--	--	--	20	--	10	--	--
F. solani	--	100	--	--	--	--	--	--	--	--	--	--	--	--	--
A. flavus	--	100	--	--	--	--	--	--	--	--	--	--	--	--	--
A. niger	--	100	--	--	--	--	--	--	--	--	--	--	--	--	--
P. notatum	--	100	--	--	--	--	--	--	--	--	--	--	--	--	--

Phytotoxic activity: The herbicides which are obtained from plants are often environment friendly. Therefore it is sensible to search for the herbicides which are plant □s origin. *L. minor* is sensitive to bioactive compound, that's why it is used for detection of phytotoxic compounds. Phytotoxic compounds are accepted antitumor and plant growth stimulant (Pusztai, 1998). All the test samples of *S. saligna* fruits were screened for the possible phytotoxic activity against the *L. minor* (Table 4).

The fraction A showed low phytotoxic activity (25 and 6.25%) at concentration of 1000 and 100µg/ml, respectively. Fraction B showed 31.25 and 12.5% growth regulation at 1000 and 100µg/ml. The fraction C showed moderate phytotoxicity (50%) against *L. minor* at higher concentration while at 100µg/ml, low phytotoxic activity (31.25%) was observed. A moderate phytotoxicity (43.75%) was observed for fraction D at higher concentration and low activity (25%) was shown by this fraction at 100µg/ml. Fraction E and F and I showed low phytotoxic effect at all concentrations. The fractions G and H showed moderate growth regulation (50 and 56%, respectively) at higher concentration while the same fractions showed low activity at lower concentrations. A low phytotoxic effect was shown by fraction J at all concentrations. Fractions K and L showed moderate activity (43.75 and 56.25%, respectively) at higher concentration while low activity (18.75 and 31.25%) was observed at low concentration. Parquet was used as standard plant growth inhibitor.

Heamagglutination activity: Certain plants, in their different parts contain haemagglutinin, which can be used as blood typing reagents in the same way as agglutinins from animal source. By using lectins we can study the sugar components on the cancerous cell and normal cell surface (Lis & Sharon, 1986). Lectins are the one of the most important toxic and show resistance to absorption when ingested (Pusztai 1998). By different mechanism it negatively affects the nutrient utilization. The plants source agglutinins are available in large quantities and are economical there for it has advantages our animal source.

Each sample of *S. saligna* fruits were test for heamagglutination activity against RBC's of all blood groups (Table 5). The fraction A showed weak agglutination against O^{-ive} blood group at 1:2 dilutions. Similarly, rest of the fractions showed no activity against the erythrocytes isolated from the human various blood groups. The above results indicate that *S. saligna* fruits contain no lectins.

Insecticidal activity: The insecticidal activity of various fractions of *S. saligna* fruits was performed, using impregnated filter paper method, against *C. analis*, *R. dominica* and *T. castaneum* (Table 6).

These results revealed that fractions H, I and L showed moderate insecticidal activity (40%), E, F, G, J and K showed low while remaining fractions were found inactive against *T. castaneum*. A moderate insecticidal activity (40%) was shown by fractions G, H, I, K and L against *R. dominica* .The rest of the fractions showed low or no activity against the tested insect. fractions C, D, E, F, I, K and L showed low insecticidal activity (20%) against *C. analis* except A, B, G, H and J which were found inactive. Organic solvent was used as negative control and Permethrin (copex) was used as positive control. Positive control showed 100% while negative control showed no insecticidal activity.

Table 4. Percent growth regulation of Crd. MeOH Ext. and various fractions of *S. saligna* against *L. minor*.

Concentration (µg/ml)	Percent growth regulation													
	Cr. Ext	A	B	C	D	E	F	G	H	I	J	K	L	Control
1000	25	25	31.25	50.0	43.75	18.75	37.5	50.0	56.25	31.25	37.5	43.7	56.25	100
100	12.5	6.25	12.5	31.25	25	6.25	18.75	37.5	37.5	18.75	18.75	18.75	31.25	100
10	---	---	---	6.25	6.25	---	6.25	12.5	12.5	---	6.25	---	6.25	100

Table 5. Heamagglutination activity of the Crd. MeOH Ext. and various fractions of *S. saligna* fruits

Blood Group	AB^{-ive}, AB^{+ive}, O^{+ive}, O^{-ive}, A^{-ive}, A^{+ive}, B^{-ive}, B^{+ive}			
Dilution	1:2	1:4	1:8	1:16
Crude extract	-	-	-	-
A	-	-	-	-
B	-	-	-	-
C	-	-	-	-
D	-	-	-	-
E	-	-	-	-
F	-	-	-	-
G	-	-	-	-
H	-	-	-	-
I	-	-	-	-
J	-	-	-	-
K	-	-	-	-
L	-	-	-	-

(-) No activity, (+) Weak, (++) Moderate, (+++) Strong.

Table 6. Insecticidal activity of Crd. MeOH Ext and various fractions of *Sarcococca saligna* fruits.

Name of insect	% Mortality													
	+ive Control	Cr. Ext	A	B	C	D	E	F	G	H	I	J	K	L
T. castaneum	100	--	--	--	--	--	20	20	20	40	40	30	20	40
R. dominica	100	--	--	--	20	6	6	20	40	40	40	--	40	40
C. analis	100	--	--	--	20	20	20	20	--	--	20	--	20	20

*Permethrin at concentration 235.9 µg/cm^2 was used as standard drug

Diphenyl Picryl hydrazine (DPPH) radical scavenging activity: Antioxidants are secondary metabolites found naturally in plants such as vegetables and fruits. Plants produce a very notable array of antioxidant compounds that includes ascorbic acid, benzoic acids, cinnamic acids, carotenoids, flavonoids, folic acid, tocopherols and tocotrienols to prevent oxidation of the susceptible substrate (Krishnaiah *et al.,* 2007).

Keeping in view the importance of antioxidants, the test samples were screened for DPPH free radical scavenging at different concentrations; 20, 40, 60, 80, 100ppm (Tables 7 & 8).

The results showed that at concentration 20ppm the Crd. MeOH Ext. and all fractions showed low percent radical scavenging activity. At the concentration 40ppm fraction D, J and L showed moderate antioxidant activity (43, 41 and 40%, respectively). The rest of the fractions and Crd. MeOH Ext. showed low radical scavenging activity at same concentration. When the concentration increased to 60ppm, fractions D (57%), J (55%), L (53%), I (51%), G (47%), B (45%), H (43%) and C (40%) showed moderate activity while other fractions E (39%), K(38%), A = F(32%) and Crd. MeOH Ext. showed low percent radical scavenging activity (29%). At the concentration of 80ppm, good antioxidant activity was observed for fraction J and L (65%), G (63%) and I (60%). Moderate activity was observed for fraction B (58%), C and H (54%), E and K (48%), A and F (45%) while Crd. MeOH Ext. (30%) showed low percent radical scavenging activity. As we increased the concentration to 100ppm fractions B, C, D, F. G, H, I, J and L showed good antioxidant activity ranging from 61 to 73%. At the same concentration moderate antioxidant activity was observed for fraction E (56%), K (55%), A (53%) and Crd. MeOH Ext. (50%). The extract concentration showing 50% inhibition (EC_{50}) was calculated from the graph of percent radical scavenging assay against extract concentration. Fractions J, I, D, H, L, G and B (40.55, 40.74, 42.5, 45.48, 56.61 and 58.61µg/ ml) showed moderate EC_{50} values, while rest of the fractions showed low EC_{50} values.

Table 7. DPPH free radical scavenging assay of the Crd. MeOH Ext. and various fractions of *S. saligna* fruits

Concentration (ppm)	Cr. Ext	A	B	C	D	E	F	G	H	I	J	K	L	Standard (vit C)
20	10	10	22	14	39	18	12	15	21	29	32	14	27	38
40	20	22	34	26	40	25	22	30	34	39	40	25	41	53
60	29	32	45	40	57	39	32	47	43	51	55	38	53	68
80	30	45	58	54	60	48	45	63	54	60	65	48	65	80
100	50	53	61	62	70	56	65	73	63	71	69	55	73	83

Table 8. EC_{50} Values of the crude methanolic extract and various fractions of *S. saligna* fruits.

Test Sample	Cr. Ext	A	B	C	D	E	F	G	H	I	J	K	L
EC_{50}	90	84.5	58.4	73.5	42.5	76.6	77.5	56.6	45.5	40.7	40.5	78.6	45.5

Conclusion

The present work revealed that fruit of *S. saligna* contains potent antimicrobial property as fraction F showed good antibacterial activity against MSSA (65.3%) and fraction C also possess good antibacterial activity against *S. typhi* (63.0%) with a respective MIC_{50} of 5.9 and 6.9 mg/mL. The fruit extracts showed no significant antifungal activity which reveals that fruits do not contains potent antifungal constituents. The fruit extracts was shown to lack phytolectins as there was no agglutination of RBC's. The extracts also showed good phytotoxic activity at higher concentrations. The extracts possess good insecticidal activity against *T. castaneum* and *R. dominica*. A concentration dependent DPPH free radical-scavenging activity was also shown by the Crd. MeOH Ext. and various fractions of the fruits of *S. saligna*.

Acknowledgement

The authors are very thankful to Higher Education Commission (HEC), Islamabad, Pakistan, for financial support under National Research Program for Universities (NRPU), project number 1979.

References

Bashir, A., A. Sadiq, B. Shumaila, K. Ibrar, A. Achyut and M.I. Choudhary. 2010. Anti-inflammatory and enzyme inhibitory activities of a crude extract and a pterocarpan isolated from the aerial parts of *Vitex agnus-castus*. *Biotechnol. J.*, 5: 1207-1215.

Bashir, A., Sadia, A. Sadiq, B. Shumaila and K. Ibrar. 2010. Biological screening of aerial parts of *Sarcococca saligna*. *J. Med. Plants Res*., 4(22): 2404-2410.

Chatterjee, A., B. Das, C.P. Dutta and K.S. Mukerjee. 1965. Steroidal alkaloids of *Sarcococca Pruniformis* Lindle. *Tetrahedron Lett*., 1: 67.

Choudhary, M.I., K.P. Devkota, S.A. Nawaz, F. Shaheen and A.U. Rahman. 2004. Cholinesterase-Inhibiting New Steroidal Alkaloids from *Sarcococca hookeriana* of Nepalese Origin. *Helv. Chim. Acta.*, 87(5): 1099-1108.

Conservation, Cultivation and trade of medicinal herbs and species in paper presented in international workshop on health challenges of 21st century and traditional medicines in SAARC Region November 4-6, 2004. Islamabad, Pakistan.

Devkota, K.P., B.N. Lenta, P.A. Fokou and N. Sewald. 2008. Terpenoid alkaloids of the Buxaceae family with potential biological importance. *Nat. Prod. Rep*., 25: 612-630.

Devkota, K.P., M.I. Choudhari, R. Ranjit, Samreen and N. Sewald. 2007. Structure activity relationship studies on antileishmanial steroidal alkaloids from *Sarcococca hookeriana*. *Nat. Prod. Res*., 21(4): 292-297.

Freeman, C.D. 1997. Antimicrobial resistance; implications for the clinician. All of Critical Care Nursing. 20: 21-35.

Gilani, A.H., M.N. Ghyur, A. Khalid, Z.U. Haq, M.I. Choudhari and A.U. Rahman. 2005. Presence of antispasmodic, anti-diarrheal, anti-secretory, calcium antagonist and acetyl-cholinesterase inhibitory steroidal alkaloids in *Sarcococca saligna*. *Plant Med*., 71(2): 120-125.

Karki, M. 2002. Medicinal and aromatic plants programme in. IDRC/SARO, New Delhi, India.

Kaufman, P.B., L.J. Csake, S. Warber and J.A. Duke, Brielmann III. Eds. 1999. Natural Products from Plants: CRC press, Bocs Raton, FL. PP. 27-34.

Khalid, A., Z.U. Haq, M.N. Ghayur, F. Feroz, A.U. Rahman, A.H. Gilani and M.I. Choudhary. 2004. Cholinesterase inhibitory and spasmolytic potential of steroidal alkaloids. *J. Steroid Biochem. Mol. Biol*., 92: 477-484.

Khan, M.R. 1999. Isolation and identification of the chemical constituents of *Sarcococca saligna.* Ph.D. Thesis, University of Punjab, Lahore.

Kiamuddin, M., Hye and K.M.A. Humayun. 1970. Pharmacological activity of an alkaloid from *Sarcococca saligna*. *Pak. J. Sci. Indust. Res.,* 13: 59.

Kohli, J. M., A. Zama and A.R. Kidwai. 1967. Separation and characterization of alkaloids of *Sarcococca pruniformis*. *Tetrahedron*, 23: 3829-3835.

Krishnaiah. D., R. Sarbatly and A. Bono. 2007. Phytochemical antioxidants for health and medicine – A move towards nature. *Biotech. Mol. Bio. Rev*., 1(4): 097-104,

Lis, H. and N.P. Sharon. 1986. The lectins: Properties, functions and applications. (Eds.): I.E. Liener, N. Sharon and I.J. Goldstein. Academic Press, New York. pp. 294-327.

McLaughlin, J.L. 1988. Bioassay for discovery of antitumoral, antiviral agents from natural sources" in Proceedings of NIH Workshop, Bethesda, Md, USA. pp. 22.

Muhammad, N., K. Inamullah, A. Bashir, A. Ihsan, A. Waqar and M.I. Choudhary. 2008. Antifungal and antibacterial activities of *Taxus wallichiana* Zucc. *J. Enz. Inh. Med. Chem.*, 23(2): 256-260.

Nasir, E.S. and S.I. Ali. 1972. *The Flora of Pakistan*. Fakhri Printing Press, Karachi. pp. 457.

Olayiwola, A. 1984. "WHO's Traditional Medicine Programme: Progress and Perspectives," World Health Organization Chronicle, 38(2):76-81.

Paudel, A.N., P. Rai, M.D. Manandhar, M.I. Choudhary, K. Masuda and A.U. Rahman. 2003. 23-Hydroxy-24-methylenecycloartanol, a new cycloartane triterpenoid from the aerial parts of *Sarcococca coriacea* of Nepalese origin. *ACGC Chem. Res. Commun*. 16: 19-27.

Pusztai, A. 1998. Biological effects of dietary lectins. In: *Recent Advances of Research in Anti-nutritional factors in Legume seeds*, (Eds.): J. Huisman, A.F.B. van der Poel and I.E. Liener. Pudoc: Wageni gen, The Netherland. pp. 17-29.

Rahman, A.U. and M.I. Chaudhary. 1997. Diterpenoid and steroidal alkaloids. *Nat. Prod. Report*., 14(2): 191-203.

Rahman, A.U., M.I. Choudrhary, M.R. Khan, S. Anjum, A. Farooq and M.Z. Iqbal. 2000. New steroidal alkaloids from *Sarcococca saligna*. *J. Nat. Prod.*, 63: 1364-68.

Rai, N.P., B.B. Adhikari, A. Paudel, K. Masuda, R.D. Mekelvey and M.D. Manandhar. 2006. Phytochemical constituents of the flowers of *Sarcococcacoriacea* of Nepalese origin. *J. Nepal Chem. Soc.*, 21: 1-7.

Rauf, A.M., G. Qaisar, S. Uddin, N.M. Akhtar and M. Qaisar. 2012. *In-vitro* antifungal activity of three selected Pakistani medicinal plants. *Middle-East J. Med. Plants Res*., 1: 09-13.

Shah, M.A., S.M. Abdullah, M.A. Khan, G. Nasar and I. Saba. 2014. Antibacterial activity of chemical constituents isolated from *Asparagus racemosus*. *Bangladesh J. Pharmacol*., 9: 1-3.

(Received for publication 26 August 2014)