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Full Length Research Paper

Biological screening of the aerial parts of the *Sarcococca saligna*

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The crude methanolic extract of the aerial parts of *Sarcococca saligna* (Ss.Cr) and its various fractions were screened *in vitro* for possible antibacterial, antifungal, phytotoxic, haemagglutination and insecticidal activities. The chloroform ($CHCl_3$) fraction and Ss.Cr **showed significant (80.76%) and good (76.92%) antibacterial activity against *Staphylococcus aureus,* respectively, while rest of the fractions showed moderate and low activity against the tested bacterial strains. The Ss.Cr, *n*-hexane and $CHCl_3$ fractions of the plant showed low antifungal activity against *Fusarium oxysporum*, while rest of the fractions and Ss.Cr were found inactive against the tested microorganisms. Similarly, low phytotoxic activity ranging from 6.25 to 18.75% was shown by the test samples, at higher concentrations (1000 µg/ml). The *n*-hexane fraction of the plant showed good insecticidal activity (60%) against *C. analis* and moderate insecticidal activity (40%) against *T. castaneum*. The $CHCl_3$ and ethyl acetate (EtOAc) fractions showed moderate activity (40%) against *T. castaneum and C. analis respectively.* Weak (+) heameagglutination activity was shown by Ss.Cr against O^{-ive}, *n*-hexane against A^{-ive} and O^{-ive} and $CHCL_3$ against O^{-ive} at dilution of 1:2, respectively. No haemagglutination activity was shown by EtOAc and aqueous fractions.**

Key words: *Sarcococca saligna*, antibacterial, antifungal, phytotoxic, haemagglutination, insecticidal activities.

INTRODUCTION

On the earth, out of 4,22,127 plant species, about 35,000 to 70,000 species are used as medicinal plants (Hasan et al., 2007). In the third world countries, 20,000 plants species are believed to be used medicinally (Mukherjee, 2004). In Pakistan there are approximately 6000 flowering plants species and 700 of these species have medicinal value (Shinwari et al., 2006; Stewart, 1972). From the research work it has been confirmed that 500 species are known for their active constituents and upto 250 to 300 species have been entered to herbal market of Pakistan (Williams and Ahmad, 1999). More than 75% of population is cured by traditional medicines prescribed by more than 50,000 traditional herb practitioners in Pakistan (Gill, 2003).

Sarcococca saligna, a small shrub with scaly buds belongs to family *Buxaceae*, is a small family of 4 genera and about 100 species, distributed throughout the northern regions of Pakistan and Kashmir at altitude of 5000 to 9000 ft in tropical and temperate regions. The family is represented here by 2 genera and 3 species (Nasir and Ali, 1972; Kiamuddin et al., 1970). In traditional medicine the plants of this family are widely used. Aqueous extract of *S. saligna* is used as antipyretic and calmative (Rizwana et al., 2007). The plants of this family are rich sources of terpenoidal alkaloids. Compounds of this family have shown interesting biological activities such as cholinesterase inhibition as well as antibacterial and antileishmanial activities. 3, 20-diamino-5α-pregnane or 3/20-amino-5α-pregnane skeleton is found commonly in the genus *Sarcococca*. *Sarcococca ruscifolia* extracts has been reported to have antiulcer, anti-gastritis and antitumor activities (Chatterjee et al., 1965). Locally the leaves of the plant are used for the treatment of fever and rheumatism while the aqueous

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extract is used as antipyretic and calmative. The plant is found to be extensively used in the indigenous system of medicine for the treatment of pain and rheumatic fever, hyperactive states of the gastrointestinal tract, liver diseases, syphilis, infections, fever, pain, inflammations, rheumatism, as a laxative, blood purifier and for the relief of muscular pain (kohli et al., 1967; Ahmad et al., 2003; Rong et al., 2005; Singh and Gurjaran 2004; Harrington et al., 2005). The cardio-suppressant, vasodilator and tracheal relaxant activities of the aqueous-methanolic extract of *S. saligna* have been reported (Ghayur and Gilani, 2006). *Salignine*, a tertiary alkaloid has been isolated from the leaves of *S. saligna*, potentiates the contractile effect of endogenous acetylcholine on the isolated rat diaphragm by reversibly inhibiting the acetyl cholinesterase activity. The methanolic extracts exhibited ganglion-blocking activity by decreasing or abolishing the effects of nicotine on blood pressure and the smooth muscles of isolated guinea pig ileum. The acute toxicity of this alkaloid in mouse was found to be at 40 mg/kg, which is less than the standard pysostigmine or neostigmine (Nianhe, 2000). One of the most common causes of dementia in the elderly population is Alzheimer's disease. In treatments for Alzheimer's disease the enzyme acetyl cholinesterase has been targeted. Alkaloids isolated from *S. saligna* significantly inhibit acetyl and butyrylcholinesterase enzyme, suggesting discovery of inhibitors for nervous-system disorders (Ojha et al., 2003).

The aim of the current work was to screen the crude methanolic extract and fractions of *Sarcococca saligna*, *in vitro* for antibacterial activity against nine bacterial species, Antifungal activity against six fungal strains, phytotoxic assay against *Lamina minor* L, Haemagglutination against human RBCs and insecticidal activities against three insect species.

MATERIALS AND METHODS

Plant material

Aerial parts of *S. saligna* (Buxaceae) were collected from Hazara division, KPK, Pakistan, in December - January 2007. The plant was identified by Professor Dr. Habib Ahmad, Plant Taxonomist, Hazara University, KPK, Pakistan.

Extraction

The shade dried plant materials were chopped into small pieces and grinded to fine powder by using electric grinder. The powdered plant materials of the plant (6 kg) were soaked in commercial grade methanol for 15 days at room temperature with occasional shaking. After 15 days the methanol soluble materials were filtered off. All filtrates were combined and concentrated, under vacuum at 40°C using a rotary evaporator till we obtained a blackish crude extract of about 800 g.

Fractionation

The Ss.Cr (750 g) of was suspended in distilled water (450 mL) and partitioned with *n*-hexane (3 x 450 ml), $CHCl_3$ (3 x 450 mL) and EtOAc (3 x 450 ml) to yield *n*-hexane (60 g), $CHCl_3$ (110 g), EtOAc (170 g) and aqueous (210 g) fractions. 50 gram of Ss.Cr was reserved for pharmacological / biological screenings.

Antibacterial activity

The test samples (Ss.Cr and its fractions) were screened for possible antibacterial activity against various human pathogens including *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus, Staphylococcus epidermidis, Salmonella typhi, Bacillus pumalis, Klebsella pneumoniae, Streptococcus pneumoniae* and *Enterobacter aerogenes* as per our reported procedure (Ahmad et al., 2009). Test organisms were inoculated to 10 ml nutrients broth (Sigma-Aldrich, Germany) and incubated at 37°C. After 24 h 0.6 ml from the test organism culture was added to 60 ml of molten agar at 45°C, mixed and transfered to a sterile petri dish (for the 9 cm petri dish, 0.2 ml of the culture was added to 20 ml of agar). The agar plates were allowed to harden and wells were dug in the plates at equal distance. Agar plugs were removed from the plates to open the wells. Stock solutions of the samples were prepared at concentration of 3 mg/ml in sterile dimethyl sulfoxide (DMSO, < 1%). 100 µl from the stock solutions of the test samples were loaded to their respective wells. Amoxicillin and DMSO were used as positive and negative controls, respectively. For better diffusion of the test samples to media the plates were left for two to three hours undisturbed inside the laminar air flow and incubated for 24 h at 37°C. The zones of inhibition were measured (mm) after incubation.

Antifungal activity

Antifungal activity of the test samples was evaluated by agar tube dilution method as per our reported procedure (Bashir et al., 2007) against the tested microorganisms. Stock solutions of the test samples were prepared at a concentration of 24 mg/ml in sterile (autoclaved) (DMSO, Merck). Sabouraud Dextrose Agar (SDA, Sigma-Aldrich, Germany) was prepared by mixing 32.5 g sabouraud, 4% glucose agar and 4.0 gm of agar-agar in 500 ml distilled water and the mixture was mixed thoroughly with magnetic stirrer. Then 4 ml amount was dispensed into sterile test tubes. The non-solidified SDA media was mixed with stock solution (66.6 µl) giving the final concentration of 400 µg of the extract per ml of SDA. Tubes were then allowed to solidify in the slanted position at room temperature. An agar surface streak of the selected microorganisms was employed for non-mycelial growth. Other media supplemented with DMSO and standard antifungal drugs served as negative and positive control respectively. Inhibition of fungal growth was observed after 7 days of incubation at 28 ± 1°C.

Phytotoxic activity

The phytotoxic activity of the test samples was carried out against *Lemna minor* L. as per our reported procedure (Ahmad et al., 2009). Stock solutions of the test samples were prepared in methanol at concentration of 20 mg / ml and E- medium was prepared for the growth of *L. minor*. 10, 100 and 1000 µg / ml from the stock solution were introduced to three flasks, one for each sample and left at room temperature till the organic solvent was evaporated. 20 ml of the E- medium and sixteen healthy plants with a rosette of three fronds were added to all flasks and incubated at 28 ± 1°C for seven days. Paraquat at a concentration 0.015 µgm / ml was used as standard growth inhibitor and three flasks containing E-medium and *Lemna minor* L only. Results were noted after seven days of incubation.

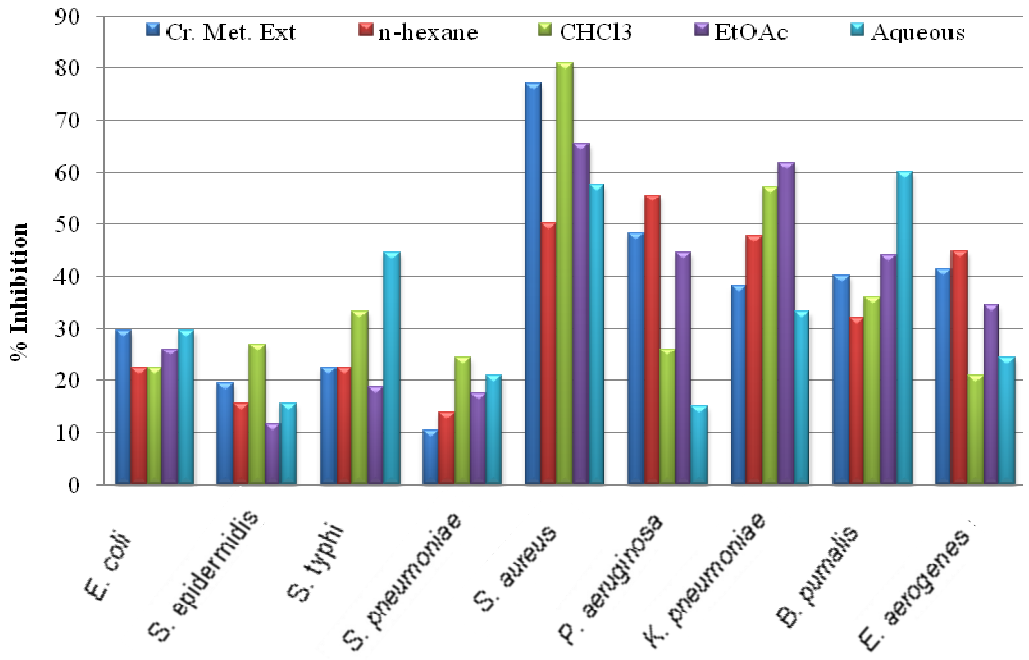

Figure 1. Antibacterial activities of Ss.Cr and its various fractions against the test organisms.

Insecticidal activity

The insecticidal activity of the extract was determined by direct contact application using filter paper method (Ahn et al., 1995). Describing the procedure, 3 ml of the test sample (1 mg/ml) was applied to filter papers (90 mm diameter). After drying, each filter paper was placed in the separate Petri dish along with 10 adults of each *Tribolium castaneum, Rhizopertha dominica, Callosbruchus analis.* Permethrin at concentration 235.71 µg/cm^2 was used as reference insecticide. All these were kept without food for 24 h after which mortality count was done.

Haemagglutination activity

Haemagglutination activity of the crude extract and various fractions of the plant were tested against human erythrocyte of all blood groups (ABO) (Naqvi et al., 1992). Stock solution of the test sample was prepared at concentration of 1 mg / ml and each solution was serially diluted (1:2, 1:4, 1:8 and 1:16) with phosphate buffer pH 7.4. Fresh blood was collected from healthy volunteers, centrifuged and separated the erythrocytes. 2% erythrocyte suspension was prepared in phosphate buffer (pH 7.4) of all blood groups. For activity 1 ml of the test sample dilution was taken with 1 ml of 2% erythrocyte and incubated at 25°C. After incubation the results were noted. Smooth button formation in bottom indicated negative while a rough granular deposition at bottom showed positive activity. The intensity of haemagglutination was determined from extent of deposition.

RESULTS AND DISCUSSION

Antibacterial activity

The Ss.Cr and its various fractions were screened for antibacterial activities against the selected micro-organisms and the results obtained are summarized in Figure 1. The Ss.Cr presented significant activity against *S.aureus* (76.92%), moderate against *P. aeruginosa* (48.14%), *E. aerogenes* (41.37%) and *B. pumalis* (40%) while low activity was observed against *K. pneumoniae* (38.09%), *E.coli* (29.62%), *S.typhi* (22.22%) *S. epidermidis* (19.23%) and *S. pneumoniae* (10.34%). The *n*-hexane fraction of the plant showed moderate activity against *S.aureus* (50%), *P. aeruginosa* (55.55%), *K. pneumoniae* (47.61%) and *E. aerogenes* (44.82%) and low activity was observed against *B. pumalis* (32%) *E. coli* (22.22%), *S. epidermidis* (15.38%), *S. typhi* (22.22%) and *S. pneumoniae* (13.79%). The $CHCl_3$ fraction was significantly active against *S.aureus* (80.76%). It showed moderate activity against *K. pneumoniae* (57.14%) and low activity was observed against *P. aeruginosa* (25.92%), *E. aerogenes* (20.68%), *B. pumalis* (36%), *E. coli* (22.22%), *S. epidermidis* (26.92%), *S.typhi* (33.33%) and *S. pneumoniae* (24.13%). The EtOAc fraction showed good activity against *S.aureus* (65.38%) and *K. pneumoniae* (61.90%), moderate activity was observed against *P. aeruginosa* (44.44%) and *B. pumalis* (44%) and low activity was shown by the fraction against *E.coli* (25.92%), *S. epidermidis* (11.53%), *S. typhi* (18.51%), *S. pneumoniae* (17.24%) and *E. aerogenes* (34.48%). The aqueous fraction of the plant showed good antibacterial activity against *B. pumalis* (60%), moderate activity against *S.typhi* (44.44%) and *S.aureus* (57.69%) and showed low activity against *E.coli* (29.62%), *S. epidermidis* (15.38%), *S. pneumoniae* (20.68%), *P. aeruginosa* (14.81%), *K. pneumoniae* (33.33%) and *E. aerogenes* (24.13%).

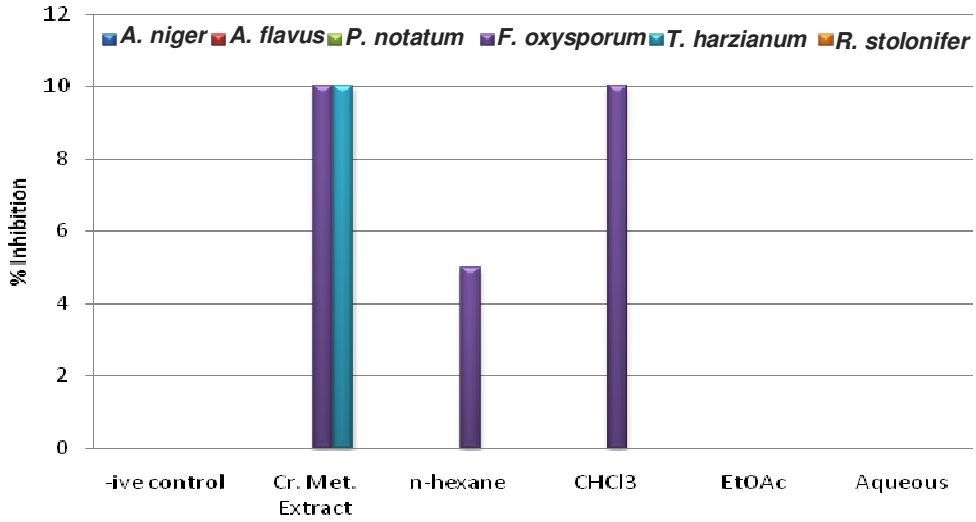

Figure 2. Antifungal activity of Ss.Cr and its various fractions against the test organisms.

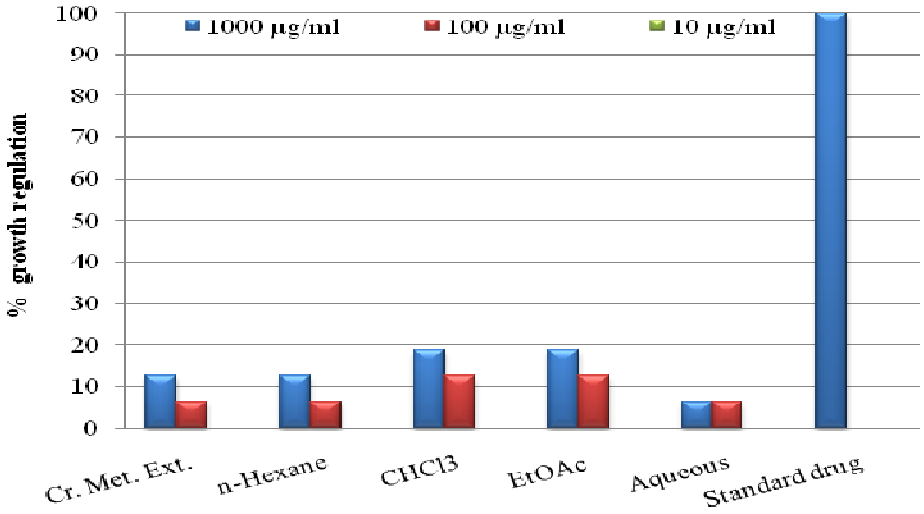

Figure 3. Phytotoxic activity of Ss.Cr and its various fractions against *Lemna minor* L.

The above results indicated that the $CHCl_3$ fraction of the plant showed significant activity up to 80.76% as compared to the standard, which revealed that this fraction of the plant contains potent antibacterial constituents. This fraction can also be subjected to activity guided isolation of the natural active constituents.

Antifungal activity

The Ss.Cr and its various fractions were screened for their antifungal activity; the results are summarized in Figure 2.

The results indicated that against the *Fusarium oxysporum*, Ss.Cr, *n*-hexane and $CHCl_3$ fraction showed low activity of 10, 5 and 10% respectively. The EtOAc and aqueous fractions of the plant were inactive against *F. oxysporum*. The remaining test samples were found inactive against *A. niger, A. flavus, P. notatum, T. harzianum* and *R. stolonifer.*

The aforementioned results indicate that *Sarcococca saligna* contains no antifungal agents.

Phytotoxic activity

The test samples were screened for their possible phytotoxic effect against *Lemna minor*. The results are summarized in Figure 3. The Ss.Cr showed 12.5 and 6.25% growth regulation at concentration of 1000 and 100 µg/mL respectively. Low phytotoxic (12.5 and 6.25%) effect was observed for the *n*-hexane fraction at higher

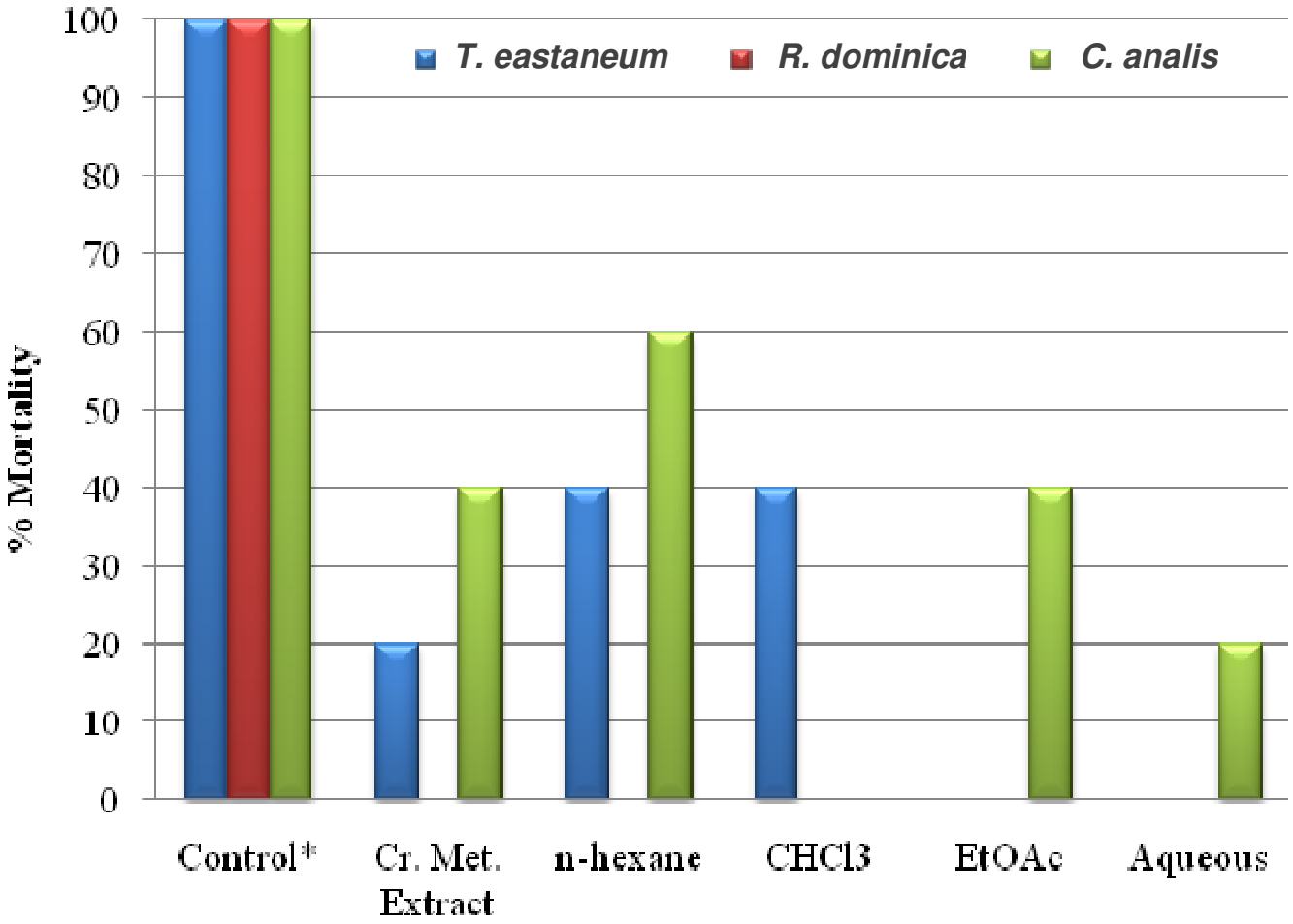

Figure 4. Insecticidal activity of Ss.Cr and its various fractions.

doses. Similarly the $CHCl_3$ and EtOAc fractions of the plant give 18.75 and 12.5% growth regulation at concentration of 1000 and 100 µg/mL respectively. 6.25% growth regulation was observed for the aqueous fraction of the plant at concentration of 1000 µg/mL respectively.

From the above results it is concluded that the test samples posses no phytotoxic activity against the *Lemna minor.*

Insecticidal activity

The Ss.Cr and its various fractions of *Sarcococca saligna* were screened for insecticidal activity against the important insects; *T. castaneum*, *R. dominica* and *C. analis*. The results are summarized in Figure 4, which indicates that the Ss.Cr of the plant showed low activity (20 and 40%) against *T. castaneum* and *C. analis* respectively, while showing no activity against *R. dominica*. The *n*-hexane fraction showed good (60 %) insecticidal activity against *C. analis* and moderate activity (40%) was observed against *T. castaneum*, while showing no activity against *R. dominica*. The $CHCl_3$ fraction showed moderate activity (40%) against *T. castaneum* and no activity against *R. dominica*, *C. analis*. The EtOAc fraction showed moderate (40 %) insecticidal activity against *C. analis* and no activity was observed against *R. dominica*, *T. castaneum.* The aqueous fraction of the plant showed low activity (20%) activity against *C. analis* and was found inactive against rest of the insects. The results showed that some of the fractions of the plant possess good insecticidal activity which indicates that the plant contains insecticidal constituents.

Haemagglutination activity

The test samples were screened for possible haemagglutination activity against the human RBC's. The results are given in Table 1. Weak (+) heameagglutination activity was observed for the Ss.Cr against O^{-ive}, *n*-hexane against A^{-ive} and O^{-ive} and $CHCL_3$ against O^{-ive} at dilution of 1:2 respectively, while inactive against the rest of the blood groups. The EtOAc and aqueous fractions

Table 1. Haemagglutination activity of the Ss.Cr and its various fractions.

Blood group	Crude methanolic extract				*n*-hexane				$CHCl_3$				EtOAc				Aqueous			
	1:2	1:4	1:8	1:16	1:2	1:4	1:8	1:16	1:2	1:4	1:8	1:16	1:2	1:4	1:8	1:16	1:2	1:4	1:8	1:16
AB^-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B^-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A^-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B^+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A^+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
AB^+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O^+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-		-	-	-
O^-	+	-	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-

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

of the plant showed no haemagglutination activity against any blood group. These results showed that the crude extract, *n*-hexane and $CHCl_3$ fractions of the plant can be used as a source for phytolectins.

Majority of the plants protect themselves from herbivores, microbial pathogens and invertebrate pests, by production and accumulation of various chemicals. These chemicals secondary metabolite produce during secondary metabolism. These secondary metabolites are not needed in the primary metabolic function. The presence of these secondary metabolites in different parts of plants prevents feeding by insects, attack of fungi, bacteria and viruses. A few of these secondary metabolites can perform both functions, that is, anthocyanins and monoterpenes when present in flowers acts as insect attractants and antimicrobial and insecticidal when present in leaves (Wink, 1999).

The present work revealed that the plant could be used for isolation of active constituents as the plant showed significant activity against *Staphylococcus aureus*, good insecticidal activity.

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