

Full Length Research Paper

Screening of *Acacia modesta* for haemagglutination, antibacterial, phytotoxic and Insecticidal activities

Bashir Ahmad^{1*}, Ibrar Khan¹, Sadiq Azam¹, Shumaila Bashir², Jamshaid Ahmad¹,
Farrukh Hussain³

¹Pharma Biotech Research Lab, Centre of Biotechnology and Microbiology, University of Peshawar Khyber Pakhtunkhwa, Pakistan.

²Department of Pharmacy, University of Peshawar, Khyber Pakhtunkhwa, Pakistan.

³Centre of Plant Diversity, University of Peshawar, Khyber Pakhtunkhwa, Pakistan.

Accepted 17 January, 2011

Plants or their parts are used as folk medicines in many parts of the world to cure various infectious diseases such as bronchitis, urinary tract infections, diarrhea, cutaneous abscesses and parasitic diseases. The crude methanolic extract and various fractions of the aerial parts of *Acacia modesta* were screened for haemagglutination, antibacterial and phytotoxic insecticidal activities. The crude methanolic extract of the plant showed weak haemagglutination activity against A^{+ive}, B^{-ive} and O^{+ive} at dilution of 1:2. The ethyl acetate (EtOAc) fraction showed weak activity against B^{-ive}, A^{-ive} and O^{+ive} at dilution of 1:2. The n-hexane and EtOAc fractions showed good antibacterial activity against *Klebsiella pneumoniae* (66.66 %) and (61.90%), respectively. All the fractions showed moderate activity against *Escherichia coli* except chloroform (CHCl₃). Aqueous fraction showed no activity against *Streptococcus pneumoniae*. Low phytotoxic activity was shown by the test samples at concentration of 1000 and 100 µg/ml against *Lemna minor* L. At 10 µg/ml, no phytotoxic activity was shown by the test samples. The n-hexane fraction showed good (60%), EtOAc and aqueous fractions, low (20%), while the crude methanolic extract and CHCl₃ fractions were inactive against *Callosobruchus analis*.

Key words: *Acacia modesta*, haemagglutination, antibacterial, phytotoxic, insecticidal, MIC₅₀

INTRODUCTION

Ancient civilization considered plant extracts to be significant for various ailments (Grabely and Thiericke, 1999). There are about 2 500 000 species of higher plants in the world and most of them are not studied for their pharmacological activities (Jeevam et al., 2004). More than 50% of all the drugs in the world, today, are from natural products and their derivatives. About 25% are contributed by the higher plants (Cragg and Newman, 2005). Many potent drugs have been derived from flowering plants, for example pilocarpine to treat glaucoma and dry mouth, which is derived from *Pilocarpus* spp. from *Rauwolfia* spp, reserpine and other hypertensive and tranquilizing alkaloids have been derived (Newman et al., 2000). *Acacia* is the common name for the plants of the genus *Acacia* of the family Mimosaceae. *Acacia* is a large genus with 900 species

(Hutchinson, 1964; Nasir and Ali, 1973) approximately, 700 of which are native of Australia.

The remainder occurs mainly in tropical and sub-tropical regions of Africa, Asia and America. The wood of *Acacia* tree is in some cases very valuable, though very small in making railway carriages, wheels, handles, furniture and is the best for making charcoal (Gohl, 1975). The genus *Acacia* are popular for their gums. The *acacia* trees of Pakistan that produces gums are *A.arabica*, *A. catechu*, *A. churnea*, *A. farnesiana*, *A. jacquemontii*, *A. leucophloae*, *A. modesta*, *A. senegal* and *A. auriculiformis* (National research council, 1979). The gum Arabica is used for the treatment of low blood pressure caused by hemorrhage or surgical shock. In plastic surgery grafting of the destroyed peripheral nerves has been successfully carried out with 50% gum Arabic adhesive (Osol and Farrar, 1995). The *A.concina* leaves are taken orally in malaria (Bora et al., 2007). Similarly, the bark and leaves of *A. farnesiana* are crushed and boiled and is inhaled by the malarial patient (Bora et al.,

*Corresponding author. E-mail: bashirdr2001@yahoo.com.

2007). The n-hexane, EtOAc, ethanol and water extract of *Parkia bicolor* A. Chev exhibited a concentration dependent antimicrobial activity against *Staphylococcus aureus*, *Bacillus cereus*, *E. coli*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Candida utilis* (Ajaiyeoba, 2002). The antimicrobial activity of the extracts of *A. nilotica* was assayed against *Streptococcus viridans*, *S. aureus*, *E. coli*, *Bacillus subtilis* and *Shigella sonnei* using the agar well diffusion method. The plant extract exhibited antimicrobial activity against all the test microorganisms. The minimum inhibitory concentration (MIC) of the stem bark extract of the plant ranged between 35 and 50 mg/ml while the minimum bactericidal concentration (MBC) ranged between 35 and 60 mg/ml (Banso, 2009). *Acacia modesta* (Phulai) locally called Palosa (Pushto) belong to genus *Acacia*, Sub-family Mimosaceae of family Leguminosae is being used traditionally for various purposes. The gum obtained from the bark is mixed with butter, almond and wheat flour and fed to women after childbirth as a tonic to relieve her body weakness. One teaspoon full of gum is dissolved in a glass of water; a preparation called *Zhuble sharbat* and is taken 3 times a day as a health tonic. The ash from the wood or branches is used as narcotic ingredient (Hussain et al., 2006; Qureshi et al., 2007). It has commercial value (44%), source of medicinal gum (27%), used as tooth brush (locally called Miswak 18%) and cure of cough (by 11%) (Hussain et al., 2006; Qureshi et al., 2007). *A. modesta* extract has significant antibacterial efficacy against *Lactobacillus* (gram positive) strains of bacteria which cause dental carriers (Asghar et al., 2003). In normal rats fed on a diet containing powdered seeds of *A. modesta* and other *Acacia* species, the blood sugar level was lower than in rats fed with a standard semi-purified casein-glucose-starch diet, no such differences are noted in alloxan diabetic animals (Singh et al., 1975). Gum is restorative (Qureshi et al., 2007) and sex tonic (Mahmood et al., 2004). The objectives of the present study were to screen the crude methanolic extract and various fraction of *A. modesta* for haemagglutination, antibacterial, phytotoxic and insecticidal activities.

MATERIALS AND METHODS

Plant material

The plant *A. modesta* (aerial parts) was collected from the Northern region of Pakistan. The plant was identified by Prof. Dr. Abdur-Rashid, Department of Botany, University of Peshawar, Khyber Pakhtunkhwa, Pakistan.

Extraction

The plant material was kept in shade for drying. After drying they were chopped into small pieces and grinded to powder, using an electric grinder. The powdered material (8 kg) was soaked in commercial grade methanol for 15 days, twice, at room

temperature, with occasional shaking. Each time the material was filtered and the filtrate was concentrated, at 40°C, under vacuum, by rotary evaporator. A blackish crude methanolic extract (950 g) was obtained.

Fractionation

The crude methanolic extract (950 g) was suspended in distilled water (500 ml) and partitioned with n-hexane (3 × 500 ml), CHCl₃ (3 × 500 ml), EtOAc (3 × 500 ml), respectively to yield the n-hexane (250 g), CHCl₃ (190 g), EtOAc (55 g) and aqueous (360 g) fractions, respectively. 50 g of the crude extract were left for biological/pharmacological activities.

Haemagglutination assay

Against the human erythrocytes, haemagglutination activity of the crude methanolic extract and various fractions of *A. modesta* was performed following the method of Bashir et al. (2010). Fresh blood was centrifuged and 2% erythrocytes suspension was prepared in phosphate buffer (pH 7). 1 mg/ml of the test sample prepared in DMSO served as stock solution and from it different dilutions, that is, 2, 4, 6, 8, 10 and 12 were made in phosphate buffer (Table 1). From each dilution 1 ml was added to 1 ml of 2% RBC's suspension and incubated at 37°C for 30 min. Rough granular precipitate and smooth button formation indicates positive and negative results respectively. The intensity of the positive result was determined from the extent of deposition.

Antibacterial activity

Percent zone of inhibition

The crude methanolic extract and various fractions of *A. modesta* were screened for possible antibacterial activity against *E. coli*, *P. aeruginosa*, *S. aureus*, *Staphylococcus epidermidis*, *Salmonella typhi*, *Bacillus pumilus*, *K. pneumoniae*, *Streptococcus pneumoniae* and *Enterobacter aerogenes* employing agar well diffusion method (Bashiret et al., 2009). On the sterile nutrient agar plates, 18 h old culture from nutrient broth was transferred and spread on it to make bacterial lawn. Wells were made in the plates using a sterile 6 mm borer. The test samples were prepared by dissolving 3 mg of the extract in 1 ml of DMSO, serving as stock solution. From each stock solution 100 µl was introduced in the respective well and incubated for 24 h at 37°C. Amoxicillin was used as positive and DMSO as negative control. Percentage zone of inhibition was measured in mm in comparison with positive control.

Minimum inhibitory concentration (MIC)

The experiment was proceeded to measure the MIC₅₀ of the test samples. From the stock solutions (3 and 4 mg/ml) different concentrations that is, 0.9, 1.5, 2.1, 2.7 and 3.2 mg/ml were used to measure MIC against the test organisms. 4 ml of media was taken in test tube and incubated for 24 h to check sterility of the media. After 24 h the test organism and test samples were inoculated in already labeled test tubes. The results were recorded after 24 h of incubation at 37°C on the basis of percentage clarity of the test tubes.

Phytotoxic bioassay

Phytotoxic activity of the crude methanolic extract and various

Table 1. Haemagglutination activity of the crude methanolic extract and various fractions of *A. modesta*.

Blood group	Crude methanolic extract				n-hexane				Chloroform				Ethyl acetate				Aqueous			
	1:2	1:4	1:6	1:8	1:2	1:4	1:6	1:8	1:2	1:4	1:6	1:8	1:2	1:4	1:6	1:8	1:2	1:4	1:6	1:8
AB ⁻	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
B ⁻	+	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	+	-	-	-
A ⁻	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-
B ⁺	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
A ⁺	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
AB ⁺	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
O ⁺	+	-	-	-	+	-	-	-	-	-	-	-	++	+	-	-	-	-	-	-
O ⁻	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

- = no agglutination, + = weak, ++ = moderate, +++ = strong.

fractions of *A.modesta* were determined against *Lemna minor* L following the method of Bashiret et al. (2009). The stock solutions of the test samples were prepared in methanol (20 mg/ml). From the stock solutions, 1000, 100 and 10 µg/ml were transferred to sterilized conical flasks and left overnight for evaporation of methanol. 20 ml of E-media were then added to the conical flasks and 16 *Lemna minor* plants with a rosette of three fronds, were introduced into each flask. Paraquat and methanol served as positive and negative control respectively. All the flasks were then incubated in growth chamber at 28 (±1) for 7 days. The results were determined by counting the number of plants damaged and growth inhibition was calculated with reference to negative control.

Insecticidal activity

The insecticidal activity of the test samples was determined following the contact toxicity assay (Ahn et al., 1995). To start with, 200 mg of the test samples was dissolved in 3 ml of methanol and applied to filter paper kept in Petri plates (90mm diameter). The Petri plates were left overnight so that the solvent get evaporated. Next day, 10 healthy test organisms (*Tribolium castaneum*, *Rhizopertha dominica* and *Callosbruchus analis*) were transferred with the help of a brush to the respective plate. The whole setup was left for 24 h and then mortality count was done.

Permethrin (235.71 µg/cm₂) was used as reference insecticide.

RESULTS AND DISCUSSION

Lectins have become a well-established means for understanding varied aspects of cancer and metastasis in the past few years. Lectins can be used for cell adhesion and localization, tumor cell recognition (surface markers), signal transduction across membranes, augmentation of host immune defense, mitogenic stimulation, cytotoxicity and apoptosis (Mody et al., 1995). The haemagglutination activity of the test samples (crude methanolic extract and various fractions of *A.modesta*) was determined against human RBC's. The results are shown in Table 1. Against AB^{-ive}, AB^{+ive}, O^{-ive}, B^{+ive}, all the results were negative at all dilutions. The crude methanolic extract and aqueous fraction showed weak haemagglutination activity against A^{+ive} at dilution of 1:2. Against B^{-ive}, crude methanolic extract and EtOAc fraction exhibited weak haemagglutination activity at dilution of 1:2. Against A^{-ive}, EtOAc fraction

presented weak haemagglutination activity at dilution of 1:2 showed. The crude methanolic extract and n-hexane fractions showed weak haemagglutination activity at dilution of 1:2 against O^{+ive}. The EtOAc fraction showed moderate and low haemagglutination activity at dilution of 1:2 and 1:4 respectively against O^{+ive}. The seeds of *Adenanthera pavonina* (a member of family Mimosaceae) showed no haemagglutination activity against erythrocytes of human blood (Prakashkumar, 1998). However in our research some of the positive results indicated the plant could be a source of lectins. An important cause of postoperative morbidity and mortality is the surgical site infections (SSIs).

Most SSIs are caused by skin-derived bacteria such as coagulase-negative *staphylococci* (Dohmen, 2008). The main problem is that many of these pathogenic bacteria have become resistant to the available antibiotics for example, *S. aureus* has become resistant to several antibiotics to which it was previously susceptible. Some of the antibiotics to which it is now resistant are Pencillin G, macrolides, lincosamides, tetracyclines and gentamicin (Ayliffe, 1997). As

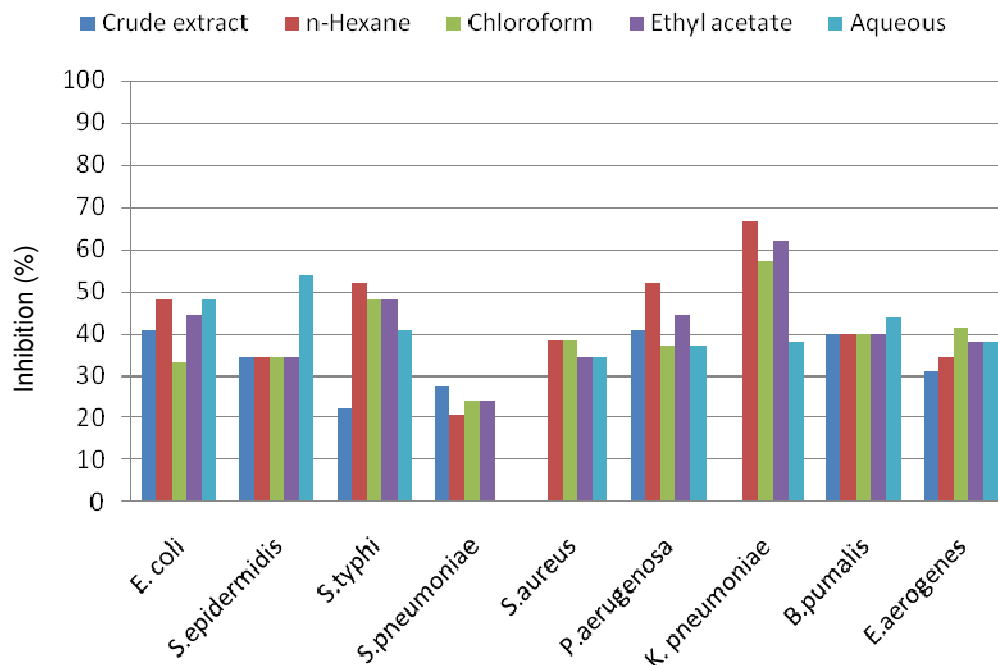


Figure 1. Antibacterial activities of crude methanolic extract & various fractions of *A. modesta*.

plants could be a rich source of diverse compounds, *A. modesta* was screened with intent for new antimicrobial compounds.

The results of the antibacterial activity are given in Figure 1. The crude methanolic extract showed moderate activity against *E. coli* (40.74%), *P. aeruginosa* (40.74%) and *B. pumilus* (40%). It showed low activity against *S. epidermidis* (34.61%), *S. typhi* (22.22%), *S. pneumoniae* (27.58%) and *E. aerogenes* (31.03%) while no activity was observed against *S. aureus* and *K. pneumoniae*. The n-hexane fraction showed good activity against *K. pneumoniae* (66.66%), moderately active against *E. coli* (48.14%), *S. typhi* (51.85%), *P. aeruginosa* (51.85%) and *B. pumilus* (40%). It showed low activity against *S. epidermidis* (34.61%), *S. pneumoniae* (20.68%), *S. aureus* (38.46%), and *E. aerogenes* (34.48%). Moderate activity was shown by the CHCl_3 fraction against *S. typhi* (48.14%), *K. pneumoniae* (57.14%), *B. pumilus* (40%) and *E. aerogenes* (41.37%). CHCl_3 fraction showed low activity against *E. coli* (33.33%), *S. epidermidis* (34.61%), *S. pneumoniae* (24.13%), *S. aureus* (38.46%) and *P. aeruginosa* (37.03%). Good activity was shown by the EtOAc fraction against *K. pneumoniae* (61.90%). This fraction showed moderate activity against *E. coli* (44.44%), *S. typhi* (48.14%), *B. pumilus* (40%) and *P. aeruginosa* (44.44%). Low activity was shown by this fraction against *S. epidermidis* (34.61%), *S. pneumoniae* (24.13%), *S. aureus* (34.61%) and *E. aerogenes* (37.93%). The aqueous fraction showed moderate activity against *E. coli* (48.14%), *S. epidermidis* (53.84%), *S. typhi* (40.74%) and *B. pumilus*

(44%), low activity against *S. aureus* (34.61%), *P. aeruginosa* (37.03%), *K. pneumoniae* (38.09%) and *E. aerogenes* (37.93%) and no activity against *S. pneumoniae*. The experiments were extended for the determination of MIC_{50} of the test sample.

The data of MIC_{50} is shown in Figure 2. Similar approach was utilized in our previous work for exploring various fractions of *Vitex agnus castus* for MIC_{50} against *E. coli*, *S. epidermidis*, *S. pneumoniae*, *P. aeruginosa*, *K. pneumoniae*, *B. Pumalis*, *E. aerogenes*, *S. aureus*, and *S. typhi* (Bashir et al., 2010). Against *S. aureus*, *B. cereus*, *E. coli*, *P. aeruginosa*, *Aspergillus niger* and *Candida utilis*, the n-hexane, EtOAc, ethanol and water extract of *Parkia bicolor* A. Chev were tested for antimicrobial activity (Ajaiyeoba, 2002). The crude methanolic extracts of different plants were tested against gram-positive and gram-negative bacteria for new bioactive compounds (Shahidi, 2004). Significant activity was shown by n-hexane and EtOAc fraction against *K. pneumoniae* with low values of MIC_{50} . Lemna bioassay is an important tool for determining the cytotoxic effect of the plant extracts and compounds. The phytotoxic activity of the crude methanolic extracts of *Rumex hastatus*, *Rumex dentatus*, *Rumex nepalensis*, *Rheum australe*, *Polygonum persicaria* and *Polygonum plebejum* (Family Polygonaceae) were determined in our previous work using Lemna bioassay (Hameed et al., 2009).

Herbicides, originating from plant's origin are often environment friendly. Therefore search for plant's origin herbicides, is sensible. The results of the phytotoxic activity of the crude methanolic extract and various

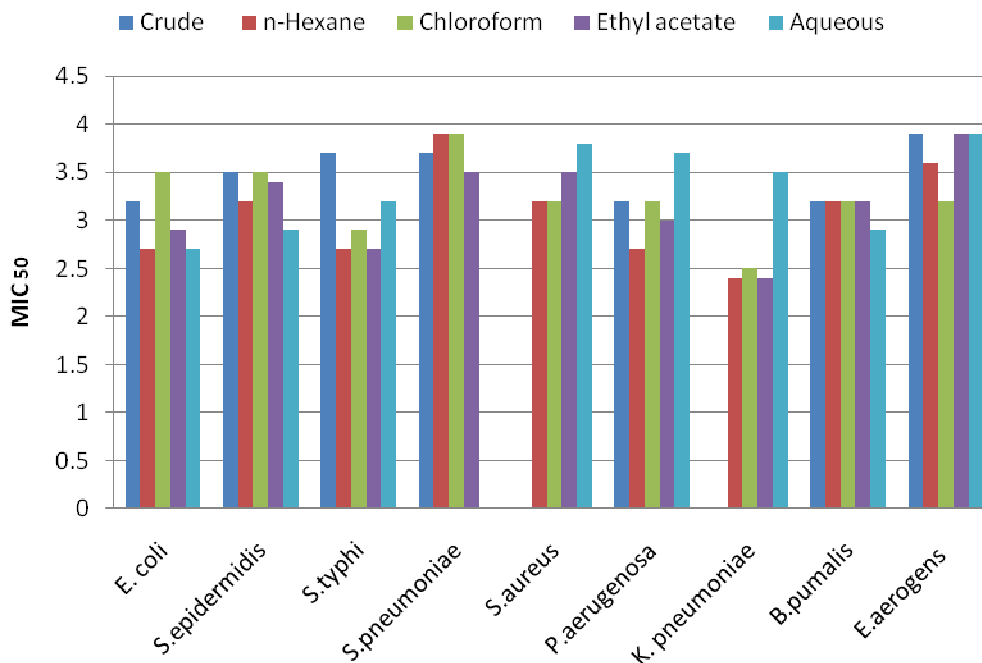


Figure 2. MIC₅₀ values of crude methanolic and various fractions of *A. modesta*.

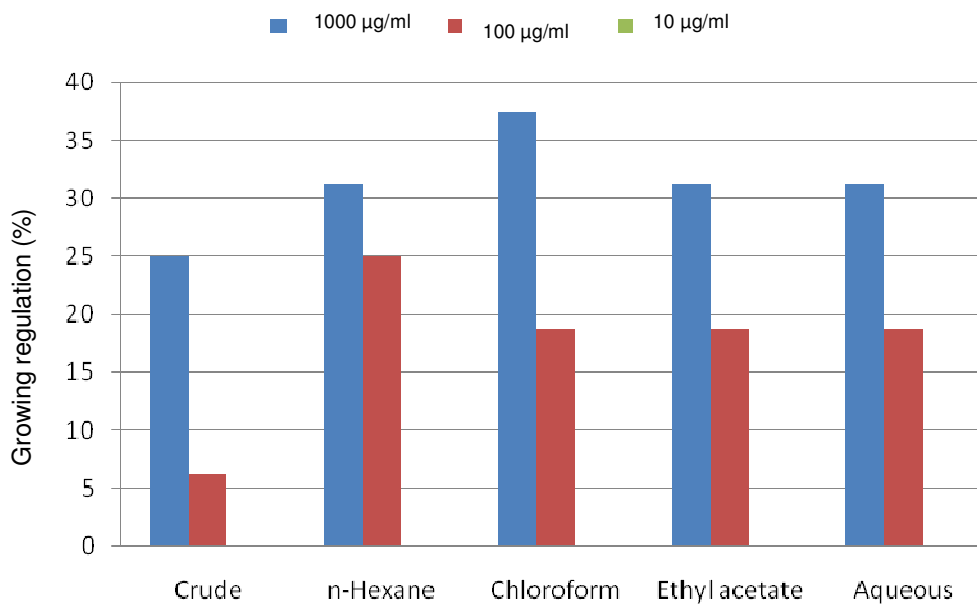


Figure 3. Phytotoxic activity of the crude methanolic extract and various fractions of *A. modesta*.

fractions of *A. modesta* against *Lemna minor* L are given in Figure 3. The crude methanolic extract, n-hexane, CHCl_3 , EtOAc and aqueous fractions showed low growth inhibition of (25 and 6.25%), (31.25 and 25%), (37.5 and 18.75%), (31.25 and 18.75%) and (31.25 and 18.75%), respectively at concentrations of 1000 and 100 $\mu\text{g/ml}$,

respectively. At concentration of 10 $\mu\text{g/ml}$ no phytotoxic activity was observed. The results indicated that this specie of *Mimosaceae* lacks phytotoxic agents.

The synthetic insecticides are a great threat to the environment due to their toxic effect. Therefore, environment friendly insecticides from the natural

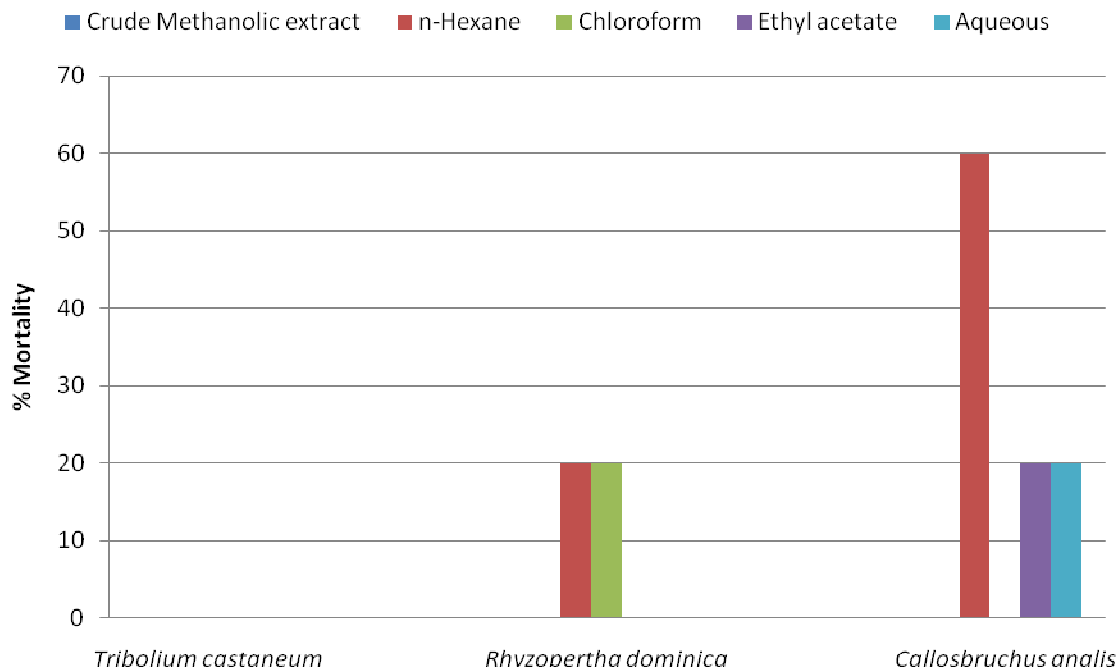


Figure 4. Insecticidal activity of crude extract and fractions of *Acacia modesta* by contact toxicity method:

resources should be searched (Suszkiw, 1998). Against *T. castaneum*, *R. dominica* and *C. analis*, the insecticidal activity of crude methanolic extract and various fractions of *A. modesta* was determined. The results are mentioned in Figure 4. The crude methanolic extract and various fractions were inactive against *T. castaneum*. Low activity (20%) was shown by the n-hexane and CHCl_3 fractions against *R. dominica*, while rests of the test samples were inactive against it. The n-hexane fractions showed good (60%), EtOAc and aqueous fraction showed low (20%) activity, while the crude methanolic extract and CHCl_3 fractions were inactive against *C. analis*.

ACKNOWLEDGEMENT

The authors are thankful to Higher Education Commission (HEC), Pakistan for funding the research.

REFERENCES

- Ahmad B, Ali N, Bashir S, Choudhary MI, Azam S Khan I (2009). Parasitocidal, antifungal and antibacterial activities of *Onosma griffithii* Vatke. Afr. J. Biotechnol., 8(19): 5084-5087.
- Ahmad B, Ali N, Bashir S, Choudhary MI (2009). Biological activities of aerial parts of *Tylophora hirsuta* Wall. Afr. J. Biotechnol., 8: 4627-4631.
- Ahn YJ, Kim GH, Cho KY (1995). Bioassay system for insecticidal compounds. In: Proceedings of the third symposium on the biochemical methodology for the research and development of the bioactive substances, held at Seoul, Republic of Korea, pp. 495-506.
- Ajaiyeoba, EO (2002). Phytochemical and antibacterial properties of *Parkia Biglobosa* and *Parkia Bicolor* leaf extracts. Afr. J. Biomed. Res., 5(3): 125-129.
- Asghar R, Ahmad M, Zafar M, Akram A, Mahmood J, Hassan M (2003). Antibacterial Efficacy of *Acacia modesta* Wall (Miswak) against Dental Pathogen. Pak. J. Biol. Sci., 6(24): 2024-2025.
- Ayliffe G (1997). The progressive intercontinental spread of methicillin-resistant *Staphylococcus aureus*. Clin. Infect. Dis., 24: 74-79.
- Bashir A, Sadiq A, Shumaila B, Ibrar K, Achyut A, Muhammad IC (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.
- Bora U, Sahu A, Saikia AP, Ryakala VK, Goswami P (2007). Medicinal plants used by the people of Northeast India for curing Malaria. Phytother. Res., 21: 800-804.
- Banso A (2009). Phytochemical and antibacterial investigation of bark extracts of *Acacia nilotica*. J. Med. Plant. Res., 3(2): 082-085.
- Cragg GM, Newman DJ (2005). Biodiversity: A continuing source of novel drug leads. Pure. Appl. Chem., 77(1): 7-24.
- Dohmen PM (2008). Antibiotics resistance in common pathogens reinforces the need to minimize surgical site infections. J. Hospit. infec., 70(2): 15-20.
- Grabely S, Thiericke R (1999). Bioactive agents from natural sources: trends in discovery and application. Adv. Biochem. Eng. Biotech., 64: 101-154.
- Gohl B (1975). Tropical feeds information summaries and nutritive values. F.A.O, Agriculture studies No. 96, Food and Agriculture Organization (FAO), United Nations, Rome, Italy, pp. 148-159.
- Hutchinson I (1964). The genera of flower plants. Oxford University press, London. Vol.1.
- Hussain F, Badshah L, Dastagir G (2006). Folk medicinal uses of some plants of South Waziristan, Pakistan. Pak. J. Plant. Sci., 12(1): 27-39.
- Hussain F, Hameed I, Dastagir G, Nisa S, Khan I, Ahmad B (2009). Cytotoxicity and Phytotoxicity of some selected medicinal plants of Family *Polygonaceae*. Afr. J. Biotechnol., 9(5): 770-774.
- Jeevam RA, Bhakshu L Venkata R (2004). *In vitro* antimicrobial activity of certain medicinal plants from Eastern Ghats, India, used for skin diseases. J. Ethnopharm., 90: 353-357.
- Mody R, Joshi SH, Chaney W (1995). Use of lectins as diagnostic and therapeutic tools for cancer. J. Pharm. Toxicol. Meth., 33(1): 1-10.

- Mahmood T, Khan MA, Ahmad J, Ahmad M (2004). Ethno medicinal studies of Kala Chitta Hills of district Attock. *Asian J. Plant. Sci.*, 3(3): 335-339.
- Newman DJ, Cragg GM, Snader KM (2000). The influence of natural products upon drug discovery. *Nat. Prod. Rep.*, 17(3): 215-234.
- Nasir E, Ali SI (1973). *Flora of West Pakistan*, National herbarium, Pakistan Agriculture Research Council, Islamabad, 39: 6.
- National Research Council (1979). *Tropical Legumes, Resources for the future*, National Academy of Science, Washington D.C, USA, P. 141.
- Osol A, Farrar GE (1995). *The Dispensatory of the United States*, Lippincott, J.F, Philadelphia, 25th edition, P. 1.
- Prakashkumar R, Pushpangadan P, Vijayakumar T (1997/1998). Search for lectins in seeds of tropical trees of Kerala, India. *Biol. Planta.*, 40(1): 155-158.
- Qureshi RA, Ahmad M, Ghufuran MA (2007). Indigenous knowledge of some important wild plants as a folk medicines in the area of Chhachh (Distt. Attock) Punjab, Pakistan. *Elec. J. Env. Agri. Food. Chem.*, 6(11): 2500-2511.
- Singh K.N, Chandra V, Barthwal KC (1975). Letter to the editor: Hypoglycemic activity of *Acacia Arabica*. *Indian. J. Physiol. Pharmacol.*, 19(3): 167-168.
- Shahidi BGH (2004). Evaluation of antibacterial properties of Iranian Medicinal plants against *Micrococcus luteus*, *Serratia marcescens*, *klebsiella pneumoniae* and *Bordetella bronchoseptica*. *Asian J. Plant. Sci.*, 3: 82-86.
- Suszkiv J (1998). The formosan termite: a formidable foe. *Agric. Res.*, 46: 4-9.