Journal of Medicinal Plants Research Vol. 6(31), pp. 4653-4659,15 August, 2012
Available online at http://www.academicjournals.org/JMPR
DOI: 10.5897/JMPR12.016
ISSN 1996-0875 ©2012 Academic Journals

Full Length Research Paper

Chemical composition and antifungal, phytotoxic, brine shrimp cytotoxicity, insecticidal and antibacterial activities of the essential oils of *Acacia modesta*

Bashir Ahmad*, Ibrar Khan, Shumaila Bashir and Sadiq Azam

Pharma Biotech Research Laboratory, Centre for Biotechnology and Microbiology, University of Peshawar, Khyber Pakhtunkhwa, Pakistan.

Accepted 1 March, 2012

Many bioactive compounds are produced by the medicinal plants that have important pharmacological activities. The present study describes the chemical composition, antifungal, phytotoxic, brine shrimp cytotoxic, insecticidal and antibacterial activities of essential oils from *Acacia modesta*. The oils were extracted from the *n*-hexane fraction of the aerial parts of the plant and were analyzed by gas chromatograph and mass spectrometer (GC-MS). The results revealed 38 components from the oil of *A. modesta*. The oils exhibited moderate antifungal activity (40%) against *Microsporum canis* and low against *Fusarium solani* (25%). At the concentration of 1000 and 100 µg/ml, the oils showed moderate phytotoxic activity of 50 and 40%, respectively, against *Lemna minor*. The oils were highly cytotoxic at the concentration of 100 µg/ml killing all the shrimps in the experiment. At the concentration of 10 µg/ml only 2 and at concentration of 1 µg/ml, only 8 shrimps survived out of 30. The oils showed no antibacterial and insecticidal activity against the test organisms.

Key words: Essential oil, *Acacia modesta*, antibacterial, antifungal, phytotoxic, insecticidal brine shrimp cytotoxicity.

INTRODUCTION

Presently a tremendous pressure is being faced by the food industry from consumers for using chemical preservatives to prevent the growth of food borne and spoiling microbes. Worldwide the consumers want to reduce or eliminate the chemically synthesized additives from food. One of the new approaches is the use of essential oils form plant's origin. Essential oils are gaining popularity both in industry and scientific research because of their antifungal, antioxidant and antibacterial activities and can be used in foods as natural additives (Pattnaik et al., 1997). Major losses in agriculture production are caused by fungi for example *Aspergillus* spp. causing spoilage of mangoes while *Fusarium* spp. causing spoilage during food production.

The aflotoxins of *Aspergillus flavus* and *Aspergillus parasiticus* contaminate cotton seed, corn, peanuts and tree nuts during harvesting or storage (Wilson and Payne, 1994). The most common fungal disease of wheat and barley is Fusarium head blight caused by *Fusarium oxysporum*. This results in economic loss that is, reduction of grain quality and yield. The seeds contaminated by the mycotoxins are rejected in the market place (Parry et al., 1995). The identification of novel antifungal drugs is the need of the era due to increase of fungal diseases in human, animals and plants caused by drug resistant fungi. The scientific community is focusing on medicinal plants to combat fungal infectious diseases (Eloff et al., 2005). Increasing awareness of ecology has led to the development of highly effective and selective biopesticides that are non-toxic to humans and animals (Bowen et al., 2000). The pesticide *Plutella xylostella* (Lepidoptera: Plutellidae) is a world scale, catastrophic, resistant vegetable pest that occurs for several generations in a year.

*Corresponding author. E-mail: bashirdr2001@yahoo.com. Tel: 929216701. Ext. 3070.

The active components isolated from fermented products of *Paecilomyces cicadae* (Miquel) Samson have played a key role in ecological and environmental protection (Chen and Liu, 1993). Multiple therapeutic activities have been reported for *P. cicadae* in recent years (Takano et al., 2005; Jin et al., 2006, 2005; Kiho et al., 1990; Wang et al., 2001). *Acacia modesta* (Phulai) or Palosa (Pushto), belonging to genus *Acacia,* Sub-family Mimosaceae of family Leguminosae is being used traditionally for the treatment of various ailments. To relieve the body weakness of women after childbirth, the gum obtained from the bark is mixed with Desi ghee, almond and wheat flour and fed as a tonic. Z*huble sharbat*; One teaspoonful of gum dissolved in a glass of water, is used as a health tonic.

It is used as a source of medicinal gum (by 27%), has commercial value (44%), cure of cough (by 11%) and use a tooth brush (locally called Miswak by 18%) (Hussain et al., 2006; Qureshi et al., 2007).The antibacterial efficacy of *A. modesta* extract against *Lactobacillus* (Gram positive), strain of bacteria which cause dental carriers, has been established (Asghar et al., 2003). 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 (Singh et al., 1975). Gum is used as a sex tonic (Mahmood et al., 2004) and restorative (Qureshi et al., 2007). Therefore the current study was carried out to find the chemical composition and antifungal, Phytotoxic, Brine shrimp cytotoxicity, Insecticidal and antibacterial activities of the essential oils of *A. modesta*.

MATERIALS AND METHODS

Plant material

Acacia 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, KhyberPukhtoonKhwa, Pakistan.

Extraction

The collected plant material was dried in shade, chopped into small pieces and grinded to powder, using electric grinder. The powder (8 kg) was soaked in methanol for 15 days, twice, at room temperature, with occasional shaking. The collected materials were filtered and concentrated, at 40°C, under vacuum; by rotary evaporator giving a blackish crude extract. From the crude extract *n*-hexane fraction was separated by fractionation and from the *n*-hexane fraction, essential oil was extracted by column chromatography with *n*-hexane.

Identification by GC-MS

1 µl aliquot of oil, dissolved in diethyl ether and adjusted to 1000 ppm was injected to GC-MS (QP-2010, Shimadzu Co., Kyoto, Japan). The length of the column (DB-5MS) was 30 m, i.d. and thickness was 0.25 mm and 0.25 µm ((Agilent Technologies, J&W Scientific Products, Folsom, CA). helium was used as a carrier gas. The operating condition of GC oven temperature was maintained as: initial temperature 50°C for 5 min, programmed rate 5°C/min up to final temperature 280°C with isotherm for 5 min. The temperature of injector and detector were set at 250 and 280°C, respectively. The components of essential oil were identified by comparing their retention time and mass fragmentation pattern with standards and their quantity using peak area measurements.

Antibacterial activity

The antibacterial activity of essential oil was carried out against *Escherichia coli*, *Bacillus subtilis*, *Shigella flexenari*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*. Ampicillin was used as a standard drug. Essential oil was dissolved in Tween 80 and the antibacterial activity was determined by disc diffusion method (Ahmad et al., 2011). The culture of the test organisms were grown for 24 h at 37°C in nutrient broth and then transferred to sterile nutrient agar plates of 9 cm diameter. Filter paper disc (6 mm diameter) were soaked in samples and ampicillin and placed on the surface of nutrient agar plates. The plates were incubated for 24 h at 37°C and zone of inhibition was measured in mm.

Antifungal activity

The antifungal activity of the oils was determined against *Candida albicans*, *A. flavus*, *Microsporum canis*, *Fusarium solani* and *Candida glaberata*. The cultures of these organisms were grown on Sabouraud dextrose agar (SDA) for seven days as we need seven days old cultures. Essential oil was dissolved in Tween 80 and the antifungal activity was determined following Bashir et al. (2009). 4 ml of SDA and one ml volume of each diluted solution was mixed and put in the test tubes. The spores from a mycelial disk were then introduced into the in the test tubes and incubated for 7 days at 27°C. Test tubes supplemented with Miconazole and Tween 80 served as positive and negative controls.

Phytotoxic activity

The phytotoxic activity of the oil was carried against *Lemna minor* at the concentration of 1000, 100 and 10 µl/ml which was prepared from the stock solution of 20 µl/ml following (McLaughlin, 1988). E-media was introduced into the flask with 16 healthy *L. minor* plants. The flasks were incubated in growth chamber at 28±1 for seven days. Results were taken after seven days of incubation.

Insecticidal activity

The insecticidal activity of the essential oil was carried out against *Rhyzopertha dominica, Tribolium castaneum* and *Callosobruchus analis*. Essential oil was dissolved in *n*-hexane and the insecticidal activity was determined by the contact toxicity assay (Ahn et al., 1995). Filter paper was cut according to the size of Petri dishes and sample was introduced into it. The plates were left for evaporation of *n*-hexane. 10 healthy insects of small and equal size from each specie were selected and transferred to the labeled plates with the help of a clean brush. The plates were then incubated for 24 h at 27°C with 50% relative humidity in growth chamber.

Brine shrimp cytotoxicity

The cytotoxic activity of the essential oils of *A. modesta* was

determined against brine shrimp as our reported procedure (Karaman et al., 2003). The eggs of the brine shrimp were hatched in artificial sea water, prepared with a commercial salt mixture (Instant Ocean, Aquarium System, Inc., Mentor, OH, USA) and double distilled water. Essential oil dissolved in *n*-hexane was transferred to vials and *n*-hexane was allowed to evaporate. To each vial 1 ml of sea water and 10 larvae were added. The final volume of each vial was adjusted to 5 ml with sea water. The vials were incubated under illumination at 26±1°C for 24 h. After incubation period brine shrimps that survived were counted using a magnifying glass.

RESULTS

Chemical composition of essential oils

The *n*-hexane fraction of the aerial parts of *A. modesta* was subjected to column chromatography and using pure *n*-hexane as mobile phase, the essential oils were collected. The oils were analyzed by GC-MS and the results are presented in Table 1. Thirty eight components were identified in the oils of *A. modesta*.

Antifungal activity

The antifungal activity of the oils was determined against *C. albicans*, *A. flavus*, *M. canis*, *F. solani* and *C. glaberata*. The results are mentioned in Figure 1. The sample showed moderate activity (40%) against *M. canis* and low activity against *F. solani* (25%) and *A. flavus* (5%). The sample was inactive against *C. albicans* and *C. glaberata*.

Phytotoxic activity

The phytotoxic activity of the sample (oils) was determined against *L. minor*. The results are given in Figure 2. At the concentration of 1000 and 100 µg/ml, the oils showed moderate activity of 50 and 40%, respectively. At the concentration of 10 µg/ml, the sample showed low activity of 25% against *L. minor*.

Brine shrimp (*Artemia salina*) Lethality bioassay

The cytotoxic activity of the oils of *A. modesta* was carried out against Brine shrimp (*Artemia salina*). The results are depicted in Figure 3. The results indicate that the oils of *A. modesta* are highly toxic at the concentration of 100 µg/ml. out of 30 shrimps, not a single shrimp survived. At the concentration of 10 µg/ml, only 2 survived out of 30. 8 shrimps, out of 30, survived at the concentration of 1 µg/ml. The LD_{50} value was 0.1887 µg/ml. The upper and lower limit was 0.7390 and 0.0002, respectively. These results indicate that the oils obtained from *A. modesta* are highly cytotoxic. These should not be utilized by human beings and animals. On the other side they can be used as a cytotoxic agent, when required.

Insecticidal activity

The insecticidal activity of the oils of *A. modesta* was carried out against *T. castaneum*, *R. dominica* and *C. analis*. The results are mentioned in Table 2. The oils obtained from *A. modesta* showed no activity against any of the test insects. This means that these oils do not have insecticidal property.

Antibacterial activity

The oils isolated from *A. modesta* were screened for antibacterial activities against *Escherichia coli*, *Bacillus subtilis*, *Shigella flexenari*, *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Salmonella typhi*. The results are depicted in Table 3. The results indicate that the oils of *A. modesta* were inactive against all the bacterial strains used in the research.

DISCUSSION

The present study describes the chemical composition, antifungal, phytotoxic, brine shrimp cytotoxic, Insecticidal and antibacterial activities of essential oils from *A. modesta*. The oils extracted from the *n*-hexane fraction of the aerial parts revealed 38 components. The oils exhibited moderate antifungal activity (40%) against *M. canis* and low against *F. solani* (25%). Previous research on the antifungal activities of essential oils has demonstrated that they have varying degrees of growth inhibition against some agriculture pathogenic fungal species (Alvarez et al., 2001). At the concentration of 1000 and 100 µg/ml, the oils showed moderate phytotoxic activity of 50 and 40%, respectively, against *L. minor*. The oils of *Origanum acutidens* showed potent phytotoxic effect against *Amaranthus retroflexus, Chenopodium album* and *Rumex crispus* (Saban et al., 2008). The oils were highly cytotoxic at the concentration of 100 µg/ml killing all the shrimps in the experiment. At the concentration of 10 µg/ml only 2 and at concentration of 1 µg/ml, only 8 shrimps survived out of 30. The oils of *Stachys germanica* showed 77% of inhibition at a concentration of 100 µg/ml against C32 cell line (Filomena et al., 2009). In our study the oils showed no antibacterial and insecticidal activity against the test organisms.

ACKNOWLEDGEMENTS

The authors are thankful to the Higher Education Commission of Pakistan for funding the research. The

Table 1. Essential oil components of aerial parts of *A. modesta.*

S/N	Component	Retention time	Factor
1	Benzyl alcohol	5:41.3	66.8
2	Benzyl urethane	8:44.6	62.9
3	Tridecane	12:41.2	66.9
4	Benzenepropanoic acid, á-hydroxy-, methyl ester	15:36.7	71.1
5	L-Phenylalanine, N-[N-(trifluoroacetyl)-L-alanyl]-, methyl ester	15:39.3	61.6
6	Tetradecane	15:53.6	74.6
7	5-Benzofuranacetic acid, 6-ethenyl-2,4,5,6,7,7a-hexahydro-3, 6-dimethyl-á-methylene-2-oxo-, methyl ester	17:37.6	60.3
8	Pentadecane	18:58.2	73.1
9	Phenol, 2,4-bis (1, 1-dimethylethyl)-	20:25.2	77.3
10	1-Decanol, 2-hexyl-	21:47.1	81.7
11	Propanoic acid, 2-methyl-, 1- (1,1-dimethyl)-2-methyl-1,3-propanediyl ester	21:54.9	79
12	5-Benzofuranacetic acid, 6-ethenyl-2,4,5,6,7,7á-hexahydroxy-3,6-dimethyl-á-methylene-2-oxo-, methyl ester	23:24.6	59.5
13	Colchicine, N-desacetyl-N-retinoyl	27:13.4	64.2
14	9-Hexadecenoic acid	27:14.7	72.5
15	Acetamide, N-methyl-N-[4-[4-methoxy-1-hexahydropyridyl]-2-butynyl]-	27:16	72.7
16	Acetamide, N-methyl-N-[4-[4-methoxy-1-hexahydropyridyl]-2-butynyl]-	27:17.3	73.7
17	11-Octadecenal	27:19.9	72.6
18	10,13-Eicosadienoic acid, methyl ester	28:28.8	65.9
19	8,11-Eicosadienoic acid, methyl ester	28:30.1	66.4
20	Ethanol,2- (9-octadecenyloxy)-,(Z)-	28:31.4	69.1
21	Pentadecanoic acid,14-methyl-, methyl ester	30:53.1	75.6
22	Cyclopropanepropionic acid, 2- [(2-decylcyclopropyl) methyl ester	32:21.5	60.3
23	3', 8,8'-Trimethoxy-3-piperidyl-2,2'-binaphtthyl-1,1',4,4'-tetrone	32:24.1	62.9
24	Cyclopropane octanoic acid, 2-[(2-pentylcyclopropyl)methyl]-, methyl ester, trans, trans-	32:25.4	61.8
25	4H-1-Benzopyran-4-one,5,7-dihydroxy-2-(4-hydroxyphenyl)3-methoxy-	32:30.6	65.2
26	Oxiraneoctanoic acid, 3-octyl-, cis	32:31.9	64
27	9, 15-Octadecadienoic acid, methyl ester, (Z,Z)-	34:57.4	70.9
28	9,15,octadecadienoic acid, methyl ester, (Z,Z)-	35:01.3	70.4
29	7,10,octadecadienoic acid, methyl ester	35:06.5	70.7
30	10-H-Phenothiazine, 2-chloro-6-methoxy-	35:09.1	68
31	Nonanoic acid, 9-(3-hexenylidenecyclopropylidene)-2-hydroxy-1-(hydroxymethyl) ethyl ester	35:11.7	68.2
32	4H-1-Benzopyran-4-one-,2(3,4-dihydroxyphenyl)-6,8-di-á-D-glucopyranosyl-5,7-dihydroxy-	35:16.9	68.1
33	Methyl 9,12-epithio-9,11-octadecanoate	35:18.2	68.3
34	Thiocyanic acid, (2-benzothiazolythio) methyl ester	37:07.4	60
35	1,2-Benzenedicarboxylic acid, diisooctyl ester	47:37.8	78.4
36	Card-20 (22)-enolide, 3-[(4,6-dideoxy-3-O-methylhexopyranos-2-ulos-1-yl) oxy]-6, 11, 14- trihydroxy-12-oxo-, (3á, 11á)-	51:35.7	67.4
37	1' H-Androst-16-eno [17,16-b] indol-3-ol, 1'-methyl-, acetate (ester), (3á, 5á)-	51:38.3	53.8
38	1H-2,8a-Methanocyclopenta [a] cyclopropa [e] cyclodecen-11-one, 5, 6- bis (acetoloxy)-1-[(acetoloxy) methyl]- 1a, 2,3,4,5,5a, 6,9,10,10a-decahydro-5a-hydroxy-1,4,7,9,tetramethyl-	51:42.2	52

Table 2. Insecticidal activity of the oils of *A. modesta.*

Name of the insect	Mortality (%)		
	Control (+ ve)	Control (- ve)	Sample
Tribolium castaneum	100	0	0
Rhyzopertha dominica	100	0	0
Callosbruchus analis	100	0	0

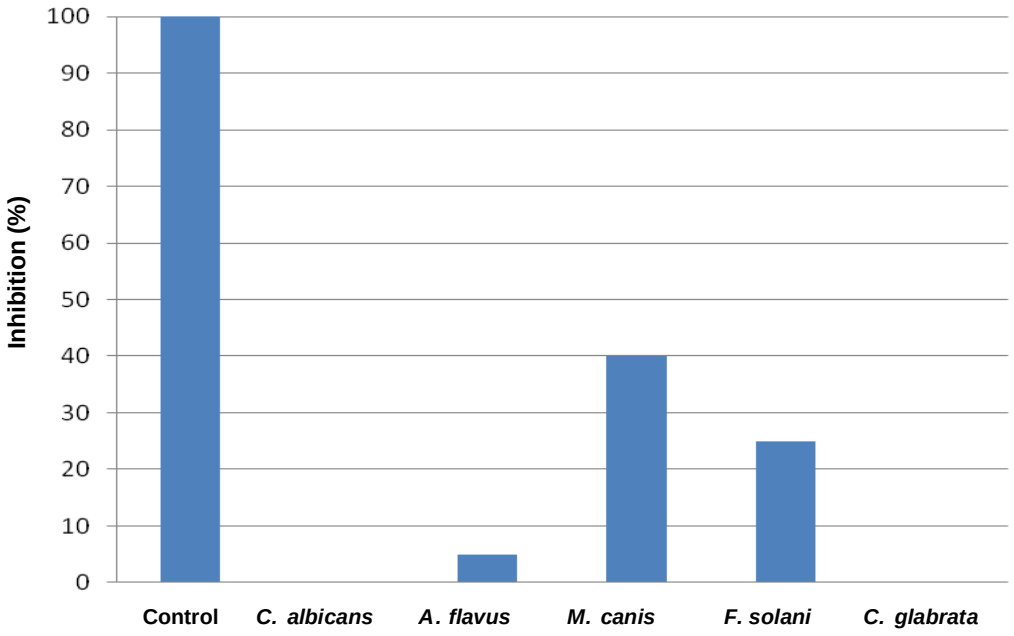

Figure 1. Antifungal activity of the oils of *A. modesta*.

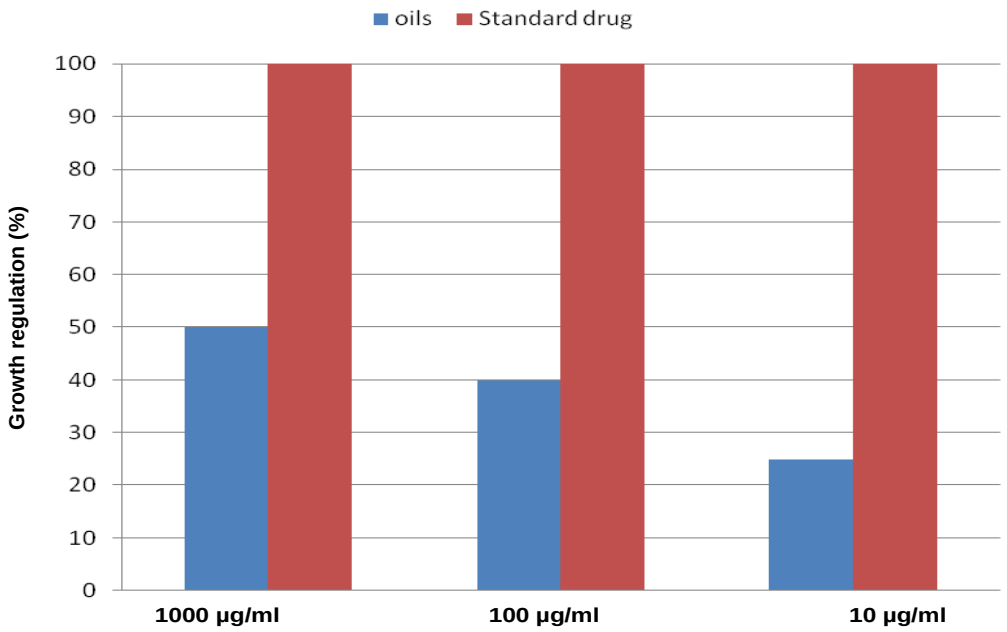

Figure 2. Phytotoxic activity of the oils of *A. modesta* against *L. minor*.

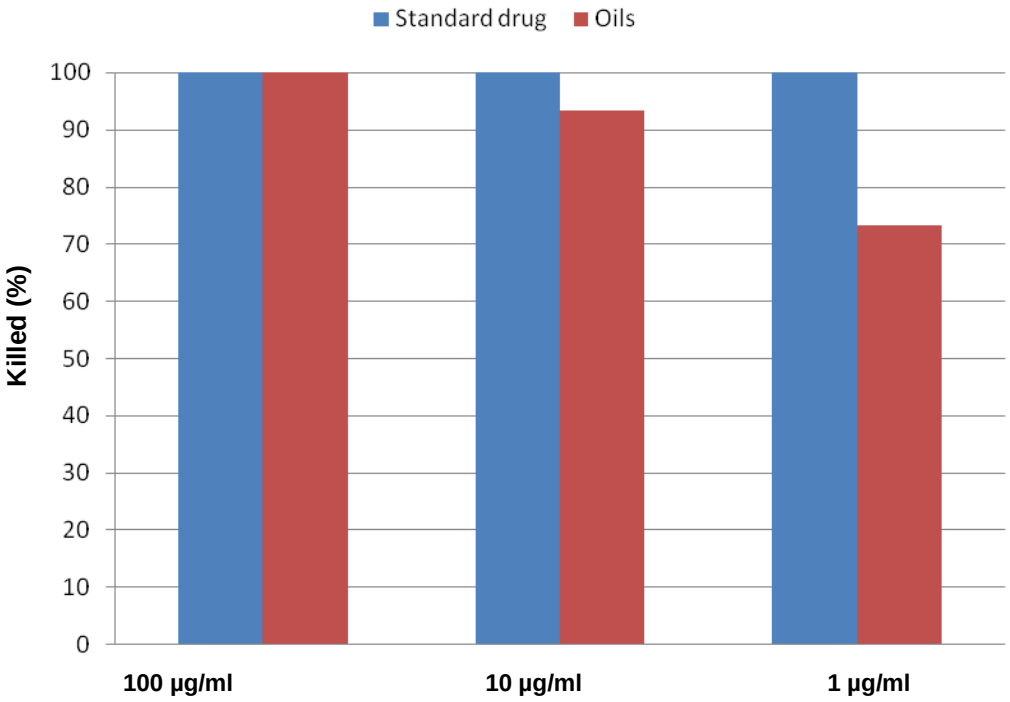

Figure 3. Brine shrimp (*A. salina*) cytotoxicity of the oils of *A. modesta.*

Table 3. Antibacterial activity of the oils of *Acacia modesta*.

Name of bacteria	Zone of inhibition of sample (mm)	Zone of inhibition of standard drug (mm)
Escherichia coli	0	35
Bacillus subtilis	0	36
Shigella flexenari	0	35
Staphylococcus aureus	0	43
Pseudomonas aeruginosa	0	32
Salmonella typhi	0	40

authors are also thankful to Prof. Dr. Abdur Rasheed for identification of plants.

REFERENCES

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, Repub. Korea, p. 495.

Asghar R, Ahmad M, Zafar M, Akram A, Mahmood J, Hassan M (2003). Antibacterial Efficacy of *Acacia modesta Wall* (Miswak) against Dental Pathogen, Pakistan. J. Biolo. Sci. 6(24):2024-2025.

Alvarez-Castellanos PP, Bishop CD, Pascual-Villalobos MJ (2001). Antifungal activity of the essential oils of flowerheads of garland chrysanthemum (*Chrysanthemum coronarium*) against agricultural pathogens. Phytochemistry, 57:99–102.

Bowen D, Blackburn M, Rocheleau T, Grutzmacher C, ffrench Constant RH (2000). Secreted proteases from photorhabdus luminescens: separation of extracellular proteases from the insecticidal Tc toxin complexes. Insect Biochem. Molecu. Biol. 30:69-74.

Chen ZA, Liu GY (1993). Studies on cultivation of *Paecilomyces cicadae* and its pharmacological function. Actu Mycolog. Sin. 12:138-144.

Eloff JN, Famakin JO, Katerere DRP (2005). Isolation of an antibacterial stilbene from *Combretum woodii* (Combretaceae) leaves. Afr. J. Biotechnol. 4:1167–1171.

Filomena C, Federica M, Carmen F, Daniela R, Felice S, Nelly AA, Franco P (2009). Comparative chemical composition, free radical-scavenging and cytotoxic properties of essential oils of six Stachys species from different regions of the Mediterranean Area. Food, Chem. 116(4):898-905.

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.

Jin LQ, Lu JX, Yan JZ (2006). Effects of Paecilomyces cicadidae total polysaccharides on macrophages of old rats. Chinese, J. Pathophysiol. 22:116-119.

Jin ZH, Chen YP, Deng YY (2005). The mechanism study of Cordyceps soboliferu mycelium preventing the progression of glomerulosclerosis. Chinese, J. Integrat. Tradit. West. Nephrozogy 6:132-136.

Karaman I, Şahin F, Gulluce M, Outçu H, Şengül M, Adiuzel A (2003). Antimicrobial activity of aqueous and methanol extracts of *Juniperus*

oxycedrus L. J. Ethnopharmacol. 85:231–235.
Kiho T, Nagai K, Miyamoto I, Watanabe T, Ukai S (1990). Polysaccharides in fungi. XXV. Biological activities of two galactomannans from the insect-body portion of Chin hub (fungus: Cordyceps cicadae). Yakugaku Zasshi 1104:286-288.
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.
McLaughlin JL (1988). Brine shrimp, crown gall tumors: Simple bioassays for the discovery of plant antitumor agents. Proceedings of NIH workshop, Bioassay for discovery of Antitumoral, Antiviral agents from natural sources, Bethesda. October 18-19, 22.
Parry DW, Jenkinson P, McLeod L (1995). *Fusarium* ear blight (scab) in small grain cereals – a review. Plant Pathol. 44:207–238.
Pattnaik S, Subramanyam VR, Bapaji M, Kole CR (1997). Antibacterial and antifungal activity of aromatic constituents of essential oils. Microbios, 89:39–46.
Qureshi RA, Ahmad M, Ghufran MA (2007). Indigenous knowledge of some important wild plants as folk medicines in the area of Chhachh (Distt. Attock) Punjab, Pakistan. Elect. J. Environ. Agricult. Food Chem. 6(11):2500-2511.
Saban K, Ahmet C, Hakan O, Ramazan C, Memis K, Ebru M (2008). Antifungal, phytotoxic and insecticidal properties of essential oil isolated from Turkish *Origanum acutidens* and its three components, carvacrol, thymol and *p*-cymene. Bioresour. Technol. 99(18):8788-8795.
Singh KN, Chandra V, Barthwal KC (1975). Letter to the editor: Hypoglycemic activity of *Acacia Arabica.* Indi. J. Physiol. Pharmacol. 19(3):167-168.
Takano F, Yahagi N, Yahagi R, Takada S, YamaguchiM, Shoda S, MuraseT, Fushiya S, Ohta T (2005). The liquid culture filtrates of *Paecilomyces tenuipes* (Peck) Samson (= Isaria japonica Yasuda) and *Paecilomyces cicadae* (Miquel) Samson (= Isaria sinclairii (Berk.) Llond) regulate Thl and Th2 cytokine response in murine Peyer's patch cells *in vitro* and *ex vivo.* Internat. Immunopharmacol. 5:903-916.
Wang Y, Zhao XJ, Tang FD (2001). Primary exploring on pharmic effect of Cordyceps cicadae. Zhejiang J. Chin. Tradit. Med. 36:219-220.
Wilson DM, Payne GA (1994). Factors affecting *Aspergillus flavus* group infection and aflatoxin Contamination of crops. In: D.L. Eaton and J.D. Groopman, Editors. The Toxicology of Aflatoxins, Academic Press, San Diego pp. 309–325.