In vitro antimicrobial activities of different solvent extracted samples from *Iris germinica*

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Abstract: **A**ntibacterial and antifungal activities of different solvents extracted samples of *Iris germinica* were carried out through disc diffusion assay. For this purpose five different solvent extracts were prepared with two concentrations (1 and 2 mg disc^{-1}) and their antimicrobial activity was tested using disc diffusion assay against eight pathogenic bacteria viz. *Staphylococcus aureus*, *B. subtilis*, *Bacillus atrophaeus, Escherichia coli, Pseudomonas aeruginosa*, *Erwinia carotovara, Klebsiella pneumoniae*, *Salmonella typhi* and one fungal specie (*Candida albicans*). Butanol and ethyl acetate fraction were more effective to control the growth of different pathogens followed by chloroform, hexane and aqueous fractions respectively. *C. albicans, S. aureus E. carotovara, B. atrophaeus* and *E. coli* were comparatively susceptible inhibited by all extracts of *I. germinica* compared with the rest of microbes. Maximum activity was shown by ethyl acetate extracted samples against *B. atrophaeus* followed by the same solvent against *E. carotovara*. Butanol extracted samples were effective against *B. subtilis* showing 62% reduction in growth at 1 or 2mg disc^{-1} concentration. From these results it can be concluded that different solvent extracted samples from the leaves of *I. germinica* possess varying degree of antimicrobial against different micro-organisms and can be a good sources of antibiotics for the treatment of certain bacterial and fungal diseases.

Keywords: *Iris germinica*, antibacterial activity, antifungal activity, disc diffusion assay.

INTRODUCTION

Medicinal plants are rich sources of antimicrobial agent (Bakht *et al*., 2011 a, b, c and d; 2012; 2013 a,b; 2014 a, b,c; 2015; Parveen and Bakht, 2013; Nasir *et al*., 2015; Ullah *et al*., 2015; Zakir *et al*., 2015; Malik *et al*., 2015). It is reported that more than 400, 000 plant species of tropical origin possesses medicinal properties (Lopez *et al.,* 2001; Odugbemi, 2006). Plants are used for the treatments of different illnesses and serve as a source of many potent and power drugs. The different plant parts used included root, stem, flower, leaves and modified plant organs. Infectious diseases are the world's major threat to human health and a serious concern for health professionals and common people around the world. The development of multi drug resistance by the microorganisms to the available antimicrobial agents has further complicated the situation and therefore scientists around the globe are investigating the antimicrobial compounds in different plant species (Choudhary *et al.,* 2005; Khan and Zakia, 2014; Ahmad *et al*., 2015; Ashraf *et al*., 2015; Karabuluti and Sule, 2015).

The genus *Iris* belongs to the family *Iridaceae* and consists of more than 300 species. Plants of this family possess antioxidant activity and also are used in the traditional medicines to treat cold, flu, malaria, toothache, bruises and burns (Lin *et al.,* 2002; Hacıbekiroğlu and Kolak, 2012; Amin *et al.,* 2013). Some compounds and extracts of *Iris* species have been reported to possess anti-inflammatory, pesticidal, cytotoxic, hypolipidemic, antibacterial, antiulcer, antioxidant and anticholinesterase activities (Atta *et al*., 2003; Rigano *et al.,* 2006; Hacıbekiroğlu and Kolak, 2011). *I. germanica* is grown in almost all the countries of the world; it is also grown as an ornamental plant. *I. germanica* is also found in northern areas of Pakistan (Choudhary *et al.,* 2005). *Iris* species is reported to have enormous medicinal significance against viruses, bacteria and cancer. The leaves of *I germanica* are the best way of ascorbic acid and vitamins and its oil is used in cosmetics and perfume. Extract of *I. germanica* reduces smooth muscle activity *in vivo*, agitate respiration, and demonstrates fundamental anti-serotonin activity (Zain *et al.,* 2012). The juice obtained from the fruit of *I. germanica* is used to eradicate the freckles of skin. The roots of the plant are utilized in dropsy and act as anti-spasmodic, stimulants, diuretic, aperients, gall bladder diseases, as a constituent for blood purifier and medicine for venereal diseases (Hanawa *et al.,* 1991; Abbas *et al.,* 2004). The aqueous extracts of the *I. germanica* roots has been reported as an enema or topically rubbing the oil on arthritic limbs (Adams *et al.,* 2009).

MATERIALS AND METHODS

Plant materials

Leaves of *I. germinica* were collected from the Botanical Garden of Islamia College University Peshawar, KPK

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Pakistan and adjacent areas of Peshawar city. The leaves were washed with distilled water to remove the dust and dirt particles and dried in shade at room temperature for one week. The dried plant materials were grinded by tissue homogenizer to fine powder (Infinigen™ Tissue Mixer Mill, ACTGene.

Preparation of crude extract

Powdered leaves of *I. germinica* (500g) was macerated in four liters of methanol (Sigma-Aldrich) and kept at room temperature for 7 days at room temperature. The solution was stirred six times a day during this period for thorough mixing and the solution was then filtered (Whatman ™ Whatman UK). One litre of fresh methanol was added to the remaining leaf material and filtered again through Wattman filter paper and this process was repeated thrice. The filtered solution was evaporated with the help of a rotary evaporator (Rotavapor R-R 210/R215; BUCHIL Labortechnik AG). Methanol was separated at 45°C under vacuum pressure and a semi-solid extract was obtained (crude extract).

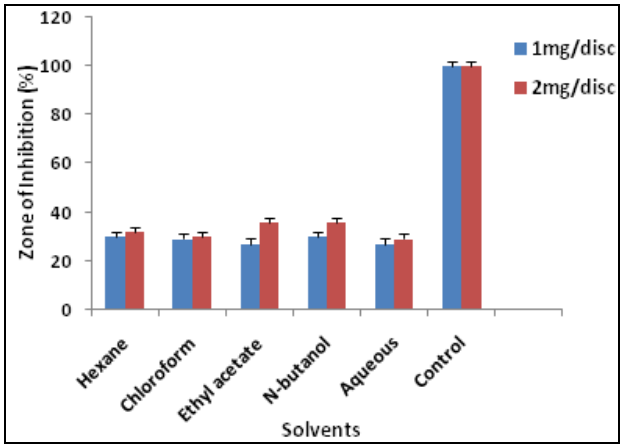

Fig. 1: Antifungal activity of hexane, chloroform, ethyl acetate, N-butanol and aqueous extracted samples from *I. germinica* against *C. albicans* by disc diffusion assay (Bar shows LSD value at P<0.05).

Crude extract fractionation

The crude extracts obtained was dissolved in 200ml hexane (Sigma-Aldrich) poured into a separatory and compounds soluble in ethyl acetate were collected and this process was repeated three times with ethyl acetate. All fractions of ethyl acetate were combined and semisolid hexane fraction was removed by evaporation. The hexane fraction was dried in water bath (45ºC). Similar procedures were adopted for chloroform, ethyl acetate, N-butanol and aqueous.

Culture media and its preparation

Nutrient agar media (HiMedia Laboratories Pvt. Ltd.) was used for the culturing and growth and nutrient broth was used for shaking incubation and standardization of different microorganisms. Media was prepared as described in Bakht *et al.* (2011 a).

Microorganisms tested

Antimicrobial activity of different solvent extracted samples were tested against *P. aeruginosa*, *S. typhi*, *K. Pneumoniae*, *E. carotovora*, *S. aureus*, *B. subtilis*, *B. atrophaeus*, and *C. albicans.*

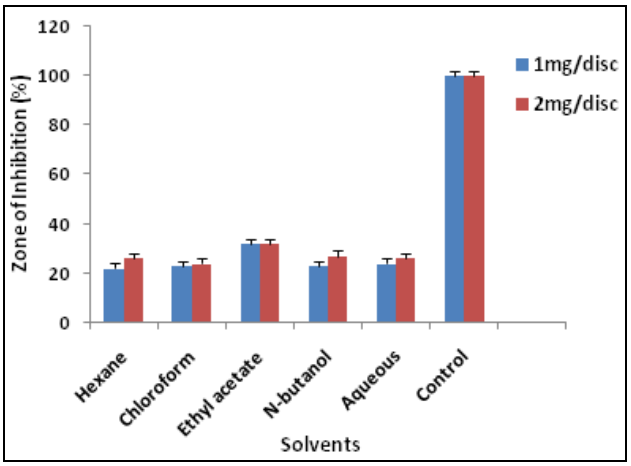

Fig. 2: Antibacterial activity of hexane, chloroform, ethyl acetate, N-butanol and aqueous extracted samples from *I. germinica* against *S. aureus* by disc diffusion assay (Bar shows LSD value at P<0.05).

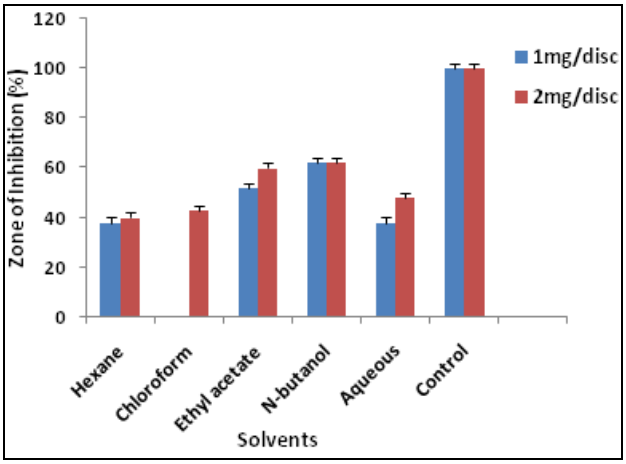

Fig. 3: Antibacterial activity of hexane, chloroform, ethyl acetate, N-butanol and aqueous extracted samples from *I. germinica* against *B. subtilis* by disc diffusion assay (Bar shows LSD value at P<0.05).

Disc diffusion susceptibility method

The antibacterial activity of different solvent extracted samples of *I. germinica* was carried by disc diffusion assay as described in Bauer *et al.* (1966) and antifungal activity by Ramdas *et al.* (1998). Different antibiotics (Arithromycine, Ciprofloxacin at 50µg concentrations for Gram-positive and Gram-negative bacteria; 50µg Clotrimazol for fungus were aseptically placed over the seeded agar plates. The plates were incubated at 37ºC for 24 hours and the diameter of the inhibition zones (in mm) were measured. The experiments were conducted in triplicate and the zone of inhibitions was determined by the following formula.

$$\text{Inhibition \%} = \frac{\text{Zone of sample}}{\text{Zone of control}} \times 100$$

STATISTICAL ANALYSIS

Data are presented as mean values of three replicates. MSTATC computer software was used to carry out statistical analysis (Russel and Eisensmith, 1983). The significant difference among means was compared using Least Significant Difference (LSD) test (Steel *et al*., 1997).

RESULTS

Results indicated that hexane and ethyl acetate extracted samples were more effective against *C. albicans* than the other tested extracts (fig. 1). Hexane extracted samples inhibited the growth of *C. albicans* measuring 30% at one mg disc^{-1}or 32% at two mg disc^{-1} concentration. Ethyl acetate measured growth reduction of 27% and 36% in *C. albicans* at low and high concentration respectively. Minimum activity was recorded by aqueous extracted samples against *C. albicans* at 1mg disc^{-1} concentration (27%). Our results showed that ethyl acetate extracted samples were found to be more effective against *S. aureus* compared with other extracts under study recording 32% reduction in growth at either concentration (fig. 2). Butanol extracted samples inhibited the growth *S. aurous* by 26% at one mg disc^{-1} while at two mg disc^{-1} concentration its activity was slightly reduced (27%). Hexane, chloroform and aqueous extracted samples showed less activity against *S. aureus* at both concentrations.

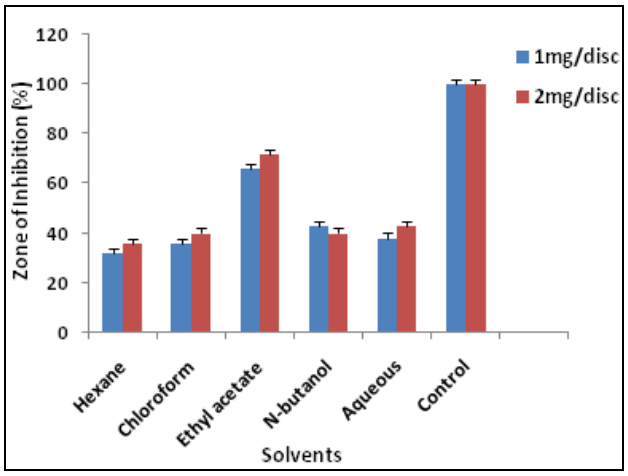

Fig. 4: Antibacterial activity of hexane, chloroform, ethyl acetate, N-butanol and aqueous extracted samples from *I. germinica* against *B. atrophaeus* by disc diffusion assay (Bar shows LSD value at P<0.05).

Butanol fraction was more potent to control the activity of *B. subtilis* followed by ethyl acetate than other fractions (fig. 3). Butanol extracted samples inhibited the growth of *B. subtilis* by 62% at both 1 and 2mg disc^{-1} concentration. Butanol fractions inhibited the activity of *B. subtilis* by 62% at one and two mg disc^{-1}. Similarly, ethyl acetate extracted samples reduced the growth of *B. subtilis* by 60% at two mg disc^{-1} concentration. Hexane, chloroform or aqueous fractions were comparatively less effective against *B. subtilis*. The data also revealed that ethyl acetate extracted samples showed maximum inhibition in the growth of *B. atrophaeus* followed by butanol and chloroform extracts (fig. 4). Ethyl acetate extracted samples showed maximum activity against *B. atrophaeus* among all the extracts and micro-organisms (72% at higher concentration). Minimum activity was revealed by hexane-extracted samples against the same microbe.

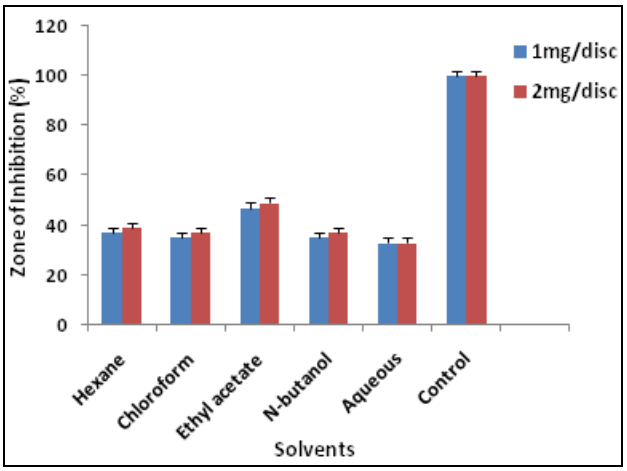

Fig. 5: Antibacterial activity of hexane, chloroform, ethyl acetate, N-butanol and aqueous extracted samples from *I. germinica* against *E. coli* by disc diffusion assay (Bar shows LSD value at P<0.05).

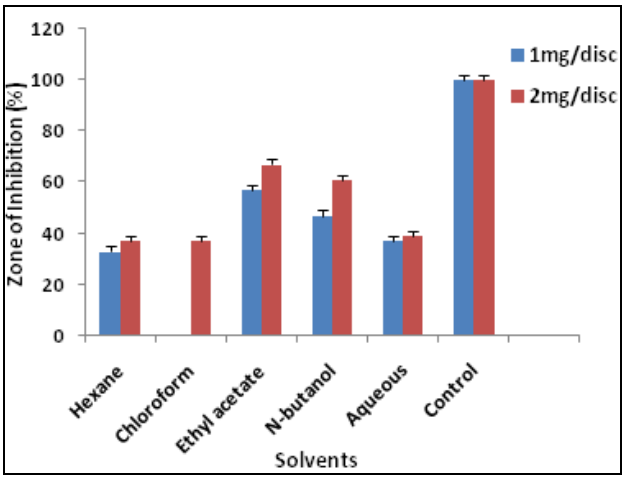

Fig. 6: Antibacterial activity of hexane, chloroform, ethyl acetate, N-butanol and aqueous extracted samples from *I. germinica* against *E. carotovora* by disc diffusion assay (Bar shows LSD value at P<0.05).

Data regarding the activity of chloroform, ethyl acetate, butanol, hexane and aqueous extracted samples against *E. coli* is showed in fig. 5. Ethyl acetate fractions were more

potent to reduce the growth of *E. coli* when compared with other tested extracts. Ethyl acetate extracted samples inhibited the growth of *E. coli* by 47% at lower concentration (one mg disc^{-1}) and 49% at two mg disc^{-1}. Hexane fractions recorded 37% growth inhibition of *E. coli* at lower concentration and 39% at higher concentration. Chloroform and butanol extracted samples showed similar antibacterial activity against *E. coli* at both concentrations. Aqueous extracted samples were found to be least effective against *E. coli* (fig. 5). *In vitro* antimicrobial activity of chloroform, ethyl acetate, butanol, hexane and aqueous extracted samples revealed ethyl acetate extracted samples reduced the activity of *E. carotovara* effectively (57% and 67% ZI at lower and higher concentration respectively) compared with other extracts under study. Butanol extracted samples were second in their activity and reduced the growth *E. carotovara* by 47% and 61% at one and two mg disc^{-1} respectively. Chloroform fractions revealed no activity against *E. carotovara* at lower concentration, however, at higher concentrations, it reduced the growth of *E. carotovara* by 37% (fig. 6).

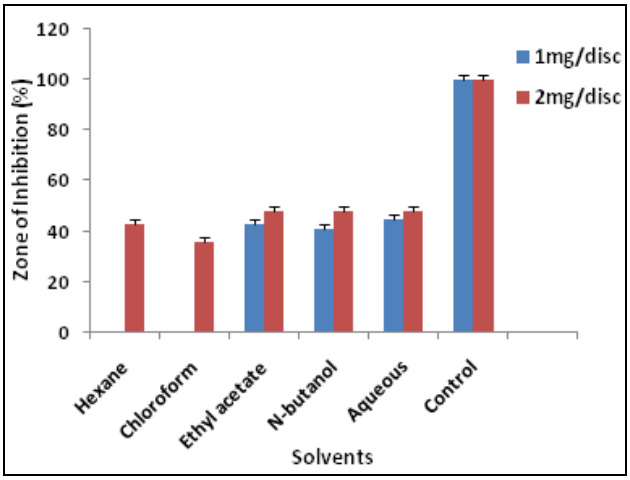

Fig. 7: Antibacterial activity of hexane, chloroform, ethyl acetate, N-butanol and aqueous extracted samples from *I. germinica* against *S. aureus* by disc diffusion assay (Bar shows LSD value at P<0.05).

The results indicated that aqueous extracted samples inhibited the activity of *S. typhi* by 48% at higher concentration than lower (45%) (fig. 7) followed by ethyl acetate extracted samples inhibiting the activity of 43% at lower concentration (1 mg disc^{-1}) and 48% at higher concentration. The data also revealed that hexane and chloroform fractions showed no activity against *S. typhi* at lower concentrations; however, at higher concentration these extracts reduced the growth of *S. typhi* by 43% and 36% respectively (fig. 7). Growth inhibition of *K. pneumonia* was effectively inhibited by ethyl acetate extracted samples followed by butanol at both concentrations; however, higher concentration (2mg disc^{-1}) was more effective than lower concentration (1mg disc^{-1}). The data further showed that chloroform fractions showed no activity against *K. pneumonia* at either concentration whereas aqueous fraction reduced the growth of *K. pneumonia* at higher concentration only (fig. 8). Our results also indicated that ethyl acetate extracted samples from *I. germinica* showed maximum activity (47% and 57% at 1 and 2mg disc^{-1} respectively) against *P. aeruginosa* followed by butanol and hexane extracted samples respectively (fig. 9). Chloroform and aqueous extracted samples showed no activity against *P. aeruginosa* at lower concentration, however, at higher concentration, were effective to inhibit the growth of *P. aeruginosa* by 35% and 39% respectively.

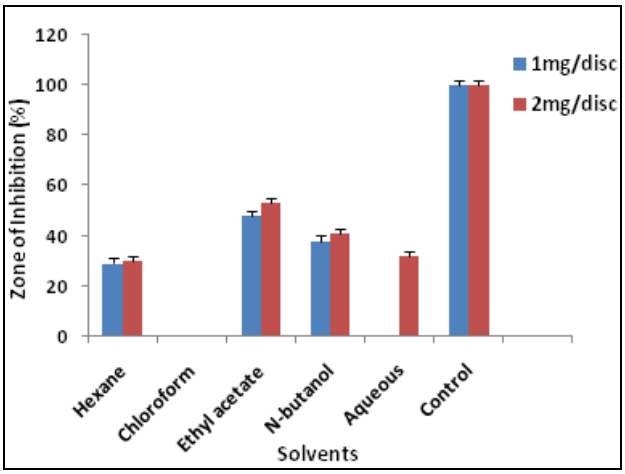

Fig. 8: Antibacterial activity of hexane, chloroform, ethyl acetate, N-butanol and aqueous extracted samples from *I. germinica* against *K. pneumoniae* by disc diffusion assay (Bar shows LSD value at P<0.05).

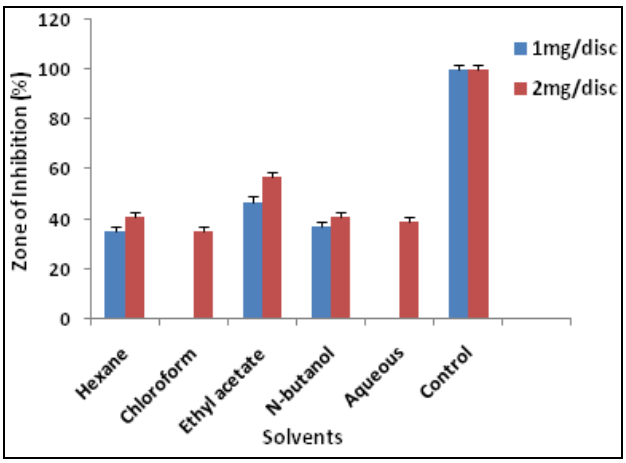

Fig. 9: Antibacterial activity of hexane, chloroform, ethyl acetate, N-butanol and aqueous extracted samples from *I. germinica* against *P. aeruginosa* by disc diffusion assay (Bar shows LSD value at P<0.05).

DISCUSSION

In the present study *I. germinica* leaves were evaluated for their antimicrobial activity against gram positive, gram-negative bacterial species and a fungus. Our results showed that the different solvent extracted samples from

the leaves of *I. germinica* were active against all the tested microbes. *C. albicans, S. aureus, E. carotovara, B. atrophaeus* and *E. coli* were comparatively susceptible inhibited by all extracts of *I. germinica* compared with the rest of microbes. Antimicrobial activity of different solvent extracted samples from the leaves extract of *I. germinica* was independent of gram strain. Wang *et al.* (2008) revealed that medicinal plant extracts showing zone of inhibition greater than 6 mm are considered to antimicrobial activity. Our results showed that ethyl acetate and butanol fraction were more effective to control the growth of different pathogens followed by chloroform, hexane and aqueous fractions respectively. Ethyl acetate extracted samples were found to be efficient to control the growth of *B. atrophaeus* and *E. carotovora* compared with other microbial strains. Similar results are also reported by Bakht *et al.* (2012). Our results also indicated that the antimicrobial activity of butanol extracted samples against *B. subtilis, B. atrophaeus, E. coli, E. carotovora, S. typhi* and *K. pneumonia*. These results agree with Bakht *et al.* (2011a, b, c and d). The present study also showed that chloroform extracted sample revealed no activity against *B. subtilis*, *E. carotovora*, *S. typhi, K. pneumonia* and *P. aeruginosa* at lower concentration. However, however, the same extract was comparatively active at higher concentration except *K. pneumonia* against which no activity was recorded even at higher concentration. From these result it can be concluded that ethyl acetate and butanol extracted samples form the leaves of *I. germinica* showed good inhibitory activity against the tested microbes compared with other solvent extracted samples.

REFERENCES

Abbas D, Mohammad S, Yash K, Maureen B, Lutfun N, Masoud M and Satyajit DS (2004). Two isoflavones from *Iris songarica* Schrenk. *Daru. J. Pharmacet. Sci.,* **12**: 54-57.

Adams M, Berset C, Kessler M and Hamburger M (2009). Medicinal herbs for the treatment of rheumatic disorders-a survey of European herbals from the 16th and 17th century. *J. Ethnopharmacol.,* **121**: 343-359.

Ahmad N, Zebta KS, Javid H and Razia P. (2015). Phytochemical, antibacterial and antioxidant investigation of *Alhagi maurorum* Medik. *Pak. J. Bot*., **47**: 121-124.

Amin A, Sajid HW, Taseem AM, Shoiab B, Asrar HW, Javid IM, Qazi PH and Raies AQ (2013). Investigating the pharmacological potential of *Iris kashmiriana* in limiting growth of epithelial tumors. *Pharmacog. J*., **5**: 170-175.

Ashraf A, Raja AS, Farooq A, Shaukat AS and Khalid MA. (2015). Chemical composition and biological activities of leaves of *Ziziphus mauritiana* L. native to Pakistan. *Pak. J. Bot.,* **47**: 367-376.

Atta UR, Nasim S, Baig I, Jalil S, Orhan I, Sener B and Choudhary MI (2013). Anti-inflammatory isoflavonoids from the rhizomes of *Iris germanica*. *J. Ethnopharmacol.,* **86**: 177-180.

Bakht J, Tayyab M, Ali H, Islam A and Shafi M (2011a). Effect of different solvent extracted samples of *Allium sativum* on bacteria and fungi. *Afr. J. Biotechnol.,* **10**: 5910-5915.

Bakht J, Islam A, Tayyub M, Ali H and Shafi M (2011b). Anti-microbial potentials of *Eclipta alba* by disc diffusion method. *Afr. J. Biotechnol.,* **10**: 7668-7674.

Bakht J, Ali H, Khan MA, Khan A, Saeed M, Shafi M, Islam A and Tayyab M (2011c). Antimicrobial activities of different solvents extracted samples of *Linum usitatissimum* by disc diffusion. *Afr. J. Biotechnol.,* **10**: 19825-19835.

Bakht J, Islam A and Shafi M (2011d). Antimicrobial potential of *Eclipta alba* by well diffusion method. *Pak. J. Bot.,* **43**: 161-166.

Bakht J, Azra and Shafi M (2012). Anti-microbial activity of *Nicotiana tobaccum* using different solvent extracts. *Pak. J. Bot.,* **44**: 459-463.

Bakht J, Khan S and Shafi M (2013a). Anti-microbial potentials of fresh *Allium cepa* against gram positive and gram negative bacteria and fungi. *Pak. J. Bot.,* **45**: 1-6.

Bakht J, Azra and Shafi M (2013b). Anti-microbial potential of different solvent extracts of tobacco (*Nicotiana rustica*) against gram negative and positive bacteria. *Pak. J. Bot.,* **45**: 643-648.

Bakht J, Shehla K and Shafi M (2014a). *In vitro* anti-microbial activity of *Allium cepa* (dry bulbs) against Gram positive and Gram-negative bacteria and fungi. *Pak. J. Pharma. Sci.,* **27**: 139-145.

Bakht J, Shaheen S and Shafi M (2014b). Antimicrobial potentials of *Mentha longifolia* by disc diffusion method. *Pak. J. Pharmacet. Sci*., **27**: 939-945.

Bakht J, Gohar N and Shafi M (2014c). *In vitro* antibacterial and antifungal activity of different solvent extracted samples of *Alhagi maurorum*. *Pak. J. Pharmaceu. Sci*., **27:** 1955-1961.

Bakht J, Fatema S, Shafi M. (2015). Screening of *Vinca rosea* for their antibacterial and antifungal activity by disc diffusion assay. *Pak. J. Pharmacet Sci.,* **28**: 833-839.

Bauer AW, Kirby WMM, Sherris JC and Turck M (1966). Antibiotic susceptibility testing by standardized single disk method. *Am. J. Clin. Pathol.,* **45**: 493-496.

Choudhary MI, Naheed S, Jalil S, Alam JM and Rahman AU (2005). Effects of ethanolic extract of *Iris germanica* on lipid profile of rats fed on high-fat diet. *J. Ethnopharmacol.,* **98**: 217-220.

Hacıbekiroğlu I and Kolak U (2011). Antioxidant and anticholinesterase constituents from the petroleum ether and chloroform extracts of *Iris suaveolens. Phytother. Res.,* **25**: 522-529.

Hanawa F, Tahara S and Mizutani J (1991). Isoflavonoids produced by *Iris pseudacorus* leaves treated with

cupric chloride. *Phytochem.,* **30**: 157-163.

Karabuluti G and Sule O. (2015). Antifungal activity of *Evernia prunastri, Parmelia sulcata, Pseudevernia furfuracea* var. Furfuracea. *Pak. J. Bot.,* **47**: 1575-1579.

Khan AY and Zakia L. (2014). Screening of medicinally natural extracts for their antibacterial activity against *Salmonella* species. *Pak. J. Bot.,* **46**: 2269-2275.

Lin J, Puckree T and Mvelase TP (2002). Anti-diarrhoeal evaluation of some medicinal plants used by Zulu traditional healers. *J. Ethnopharmavol.,* **79**: 53-56.

Lopez A, Hudson B and Towers GHN (2001). Antiviral and antimicrobial activities of Colombian medicinal plants. *J. Ethnopharmacol.,* **77**: 189-196.

Malik R, Tasveer ZB, Muhammad FS, Uzma Y, Muhammad IH and Imran AK. (2015). Antimicrobial activity of *Nerium oleander* L. and *Nicotiana tabacum* L.: A comparative study. *Pak. J. Bot.,* **47**: 1587-1592.

Nasir A, Dawood A and Bakht J (2015). Antimicrobial activity of different solvent extracted samples from the flowers of medicinally important *Plumeria obstusa. Pak. J. Pharma. Sci.,* 28(1): 195-200.

Odugbemi, T (2006). Medicinal plants as antimicrobials In: Outline and pictures of Medicinal plants from Nigeria. University of Lagos Press, Nigeria, pp.53-64.

Parveen G and Bakht J. (2013). Antimicrobial activity of turmeric extract and its potential use in food industry. *J. Food Sci. Technol.,* **52**: 2272-2279.

Ramdas K, Suresh G, Janardhana N, Masilamani S (1998). Antifungal activity of 1,3-disubstituted symmetrical and unsymmetrical thioureas. *Pest Sci.,* **52**: 145-151.

Rigano D, Grassia A, Formisano C, Basile A, Sorbo S and Senatore F (2006). Antibacterial and allelopathic activity of methanolic extract from *Iris pseudopumila* rhizomes. *Fitoter.,* **77**: 460-462.

Russel DF and Eisensmith SP (1983). MSTAT-C. Crop Soil Science Department, Michigan State University USA.

Steel RGD, Torrie JH and Dickey DA (1997). Principles and procedures of statistics. *A Biometrical Approach*, 3rd Ed. McGraw Hill Book Co. Inc., New York USA, pp.172-177.

Ullah R, Bakht J and Shafi M. (2015). Antibacterial and anti-oxidant potential of *Periploca hyaspidis*. *Bangladesh J. Pharmacol.,* **10**: 645-651.

Wang H, Zhao M, Yang B, Jiang Y and Rao G (2008). Identification of polyphenols in tobacco leaf and their antioxidant and antimicrobial activities. *Food Chem.,* **107**: 1399-1406.

Zakir UD, Anwar AS and Bakht J, Inam U, Saleem J. (2015). *In vitro* anti microbial, antioxidant activity and phytochemical screening of *Apium graveolens*. *Pak. J. Pharma. Sci.,* **28**: 1699-1704.