REPORT

In vitro antibacterial and antifungal activity of different solvent extracted samples of *Alhagi maurorum*

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Abstract: This research work was conducted at the Institute of Biotechnology and Genetic Engineering (IBGE), The University of Agriculture Peshawar, KPK Pakistan. The aim of the study was to determine the anti-microbial activity of different samples extracted from *Alhagi maurorum* plant using different solvents. Plant material was collected from the local areas of Peshawar valley during the month of April. The anti-microbial potential of all the six samples were determined against seven bacterial strains, three-gram positive (*B. atrophus*, *B. subtilis* and *S. aureus*) and four-gram negative strains (*E. coli*, *P. aeruginosa*, *K. pneumonia* and *S. typhi*) and one fungal specie (*C. albicans*) using disc diffusion susceptibility assay. All the extracted samples were applied in concentration of 1 and 2 mg/disc. Analysis of the data showed that butanol extracted samples were the most effective fraction and inhibited the growth of almost all the tested microbes while hexane extracted samples were the least effective. Other extracts (ethyl acetate, chloroform, methanol and water) showed variable activity against the tested microbes at both concentrations. The most resistant bacterial strain was *P. aeruginosa*, which showed resistance to most of the extracts while the most susceptible bacterial specie was *K. pneumonia*, the growth of which was inhibited by all six extracts. The anti-fungal activity was revealed by ethyl acetate (2 mg/disc) and butanol fractions only. The rest of extracts were ineffective in controlling the growth of *C. albicans* even at high concentration (2 mg/disc).

Keywords: Anti-microbial activity, *Alhagi maurorum*, disc diffusion assay, solvents

INTRODUCTION

Life is the result of cellular processes; any malfunction may results in infection. A number of agents are responsible for the dysfunction of the normal processes; however, the most important are the microorganisms which cause many infectious diseases. For example, *S. aureus* (gram positive) may causes cellulitis, abscesses, pneumonia and osteomyletis. *E. coli* (gram negative), *K. pneumonia* (gram negative) and *aeruginosa* (gram negative) result in urinary tract infections, bacteremia and pneumonia. *S. typhi* is the major cause of typhoid fever, paratyphoid fever and bacteremia. *Candida albicans* (Fungus) causes the genital tract disorder (vaginitis). Number of chemicals is used both *in vitro* and *in vivo* for controlling the growth of microorganisms Chemo-therapeutic agents or antibiotics can be classified into different groups on the basis of their mode of action (Park and Strominger, 1957; Trucco and Pardee, 1958). However, the widespread and injudicious use of these synthetic antibiotics leads to drug resistance in the microbial species (Tenover, 2006). Plants contain variety of therapeutic agents, which can be used to treat different illnesses (Kong *et al*., 2003). Moreover, these are less toxic, easily available, and more effective with broad spectrum activity (Chin *et al.*, 2006; Bakht *et al*., 2011 a, b, c and d; 2012; 2013 a,b; 2014a,b, Parveen and Bakht, 2013; Nasir *et al*., 2015). Apart from their potential against various disorders, plants posses a number of phytochemicals that can reduce the growth of microorganisms (Cowan, 1999; Bakht *et al*., 2011 a, b, c and d; 2012; 2013 a,b; 2014a,b). A number of methods are used to determine the sensitivity of microorganisms by various samples extracted from different plants. Some of these methods are disc diffusion assay (Bauer *et al.*, 1996), tube dilution method (Berhge and Vlietinck, 1991) and agar well diffusion method (Shahidi and Bonjar, 2004).

Alhagi maurorum is a spiny, perennial shrubby plant belongs to family fabaceae (papilionaceae). It is also called pea family, which is a sub-family of leguminoseae. It is commonly called as camel thorn. Besides this, Al-agool, aqool, and Persian manna plant etc. are the other common names used for *Alhagi maurorum*. It is reported that this plant has great medicinal value and the whole plant is traditionally used for the treatment of various disorders. Generally the aqueous extract of the aerial parts of the plants are used for the treatment of systemic disorders, however, ethanolic extract is also used in some cases. It is reported that the extract of *Alhagi maurorum* showed significant anti-inflammatory (Zakaria *et al.*, 1999), anti-nociceptive activity and anti-ulcerorgenic

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activity (Amani *et al.,* 2006). The extract of the plant is used for the treatment of rheumatism, piles and gastrointestinal disorders. It is also used as medicinal herb for its gastroprotective, diuretic, diaphoretic, expectorant, laxative, anti-diarrheal, antiseptic properties and remedy for bilharziasis, liver disorders and urinary tract infections. An aqueous extract of the whole plant is used to treat heartburn resulted from gastric reflux. Phytochemical analysis of *Alhagi* species revealed that a number of medicinally important compounds like fatty acids, cumarins, alkaloids, sterols and vitamins (Kudliki *et al.,* 1991; Kalhoro *et al.,* 1997). Many other compounds found in *Alhagi* species include triterpenes, tannins, carbohydrates and flavanone glycosides (alhagitin and alhagidin and proanthocyanidins). Keeping in view the importance of *Alhagi maurorum* as medicinal plant, the present study was carried out with following objectives.

(1). To determine the anti-bacterial activity of different solvent extracted samples from *Alhagi maurorum* against various bacterial species (gram positive and gram negative) through disc diffusion assay.

(2). To determine the anti-fungal activity of different solvent extracted samples from *Alhagi maurorum* against a fungal specie (*Candida albicans*) through disc diffusion assay.

MATERIALS AND METHODS

Experimental material

The present research work was conducted at the Institute of Biotechnology and Genetic Engineering (IBGE), The University of Agriculture Peshawar, KPK Pakistan. Aerial part of the plant (*Alhagi maurorum*) at the pre-flowering stage was collected from different areas of Peshawar valley during the month of April. Plant material was washed with water to remove dust and dirt and kept at room temperature for two weeks to be shaded dried.

Crude extract preparation

The dried plant material was chopped into small pieces and grinded into fine powder using tissue homogenizer (Infinigen™ Tissue Mixer Mill). About 130 g of powder was placed into a large container (extraction drum). Methanol was added to the extraction drum in such a way that the whole plant material was completely dipped into methanol. The extraction drum was covered with aluminum foil and placed at room temperature for a week. During this period of time the plant material was regularly shaken thrice a day. After a week, the solution was filtered through Whatman No.1 (Whatman ™) and more methanol was added to the remaining plant material and the same process was repeated thrice. The filtrate was dried by evaporating methanol using rotary evaporator (Rotavapor R-R 210/R215; BUCHIL Labortechnik AG) fixed at 45ºC. The extracted plant material was isolated and dried in the china dish at 45ºC.

Fractionation of crud extract

From the extracted plant material about 2g of the sample was separated as crude methanol extract. The crude methanol extract was stored at room temperature in a glass vial to be tested for anti-microbial activity. The remaining sample was dissolved in water, transferred to a separating funnel and calculated amount of distilled hexane was added, shaked vigorously and allowed to stand for about 10 minutes. The upper layer of hexane was collected and the lower aqueous layer was again fractioned with fresh hexane and the same process was repeated thrice. The collected three fractions of hexane were dried under reduced pressure using rotary evaporator. The remaining viscous hexane fraction was transferred into pre-weighted glass vial and dried at 45ºC using water bath, weighed and stored for further use. The remaining aqueous portion was again poured into separating funnel and the same process was repeated with ethyl acetate, chloroform and butanol. Finally the remaining aqueous portion was collected, dried under reduced pressure, weighted and stored in glass vial for further us. In this way six different fractions i.e. crude methanol, hexane, ethyl acetate, chloroform, butanol and water were obtained from the powdered material of *Alhagi maurorum* plant.

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*. (2011a,b,c,d).

Microbial strains

The anti-microbial potential of the different fractions extracted from *Alhagi maurorum* plant was determined against different bacterial and fugal species (Table 1).

Disc susceptibility diffusion assay

The antibacterial activity of different solvent extracted samples of *Alhagi maurorum* was tested by disc diffusion assay according to the methods of Bauer *et al*. (1996) and antifungal activity by Ramdas *et al*. (1998). Two concentrations of the extracts (1 and 2 mg/disc) in volume of 6 and 12µl were applied to the disc. Antibiotic and antifungal drugs used as positive control for Gram positive, Gram-negative bacteria and fungus were Erythromycin, Ciprofloxacin and Clotrimazole respectively.

For Gram positive bacteria: Erythromycin 50 µg 6 μl^{-1}
For Gram negative bacteria: Ciprofloxacin 50 µg 6 μl^{-1}
For Fungi: Clotrimazole 50 µg 6 μl^{-1}

RESULTS

The data indicated that hexane extracted samples of *Alhagi maurorum* were almost inactive against *E. coli*, *P. aeruginosa*, *B. atrophus*, *B. Subtilis* and *S. typhi* using

disc diffusion assay (figs. 1-5). The growth of these bacterial species was not reduced by hexane extracted samples applied at low (1 mg/disc) or high concentration (2mg/disc) recording no zone of inhibition. Similarly, hexane extracts did not show any anti-fungal activities against *C. albicans* revealing 0% ZI at both concentrations (fig. 6). Fig. 7 shows the antimicrobial activities of hexane extracted samples against *S. aureus*. The results indicated that hexane extracted samples were ineffective against *S. aureus* when used at low concentration (1 mg/disc) measuring zero percent zone of inhibition. However, when applied at high concentration i.e. 2 mg/disc, it reduced the growth of *S. aureus* and recorded 25% zone of inhibition. The results shown in fig. 8 revealed that *K. pneumonia* was the most susceptible bacterial strain to hexane extracted samples both at low and high concentrations revealing 21% ZI at low and 24% zone of inhibition at high concentration.

Results concerning the anti-microbial potential of ethyl acetate extracted samples indicated that *P. aeruginosa* was the highly resistant strain when tested by disc diffusion assay. The growth of *P. aeruginosa* was not inhibited by ethyl acetate extracted samples at both concentrations measuring no zone of inhibition (fig. 2). Similar trend was also observed for *E. coli* and *S. typhi* and *C. albicans* at low concentration (1 mg/disc) producing zero percent zone of inhibition (fig. 5 and 6). However, when applied at high concentration (2 mg /disc), it reduced the growth of these microbes. The growth of *E. coli* was reduced by 19%, *S. typhi* by 29% and *C. albicans* by 38%. Ethyl acetate extracted samples were more effective against *B. atrophus*, *B. Subtilis* and *S. aureus* and *K. pneumonia* at both concentration showing 35%, 30%, 25% and 29% ZI at low concentration and 46%, 36%, 31% and 36% ZI respectively at high concentration respectively (figs. 3, 4, 7 and 8).

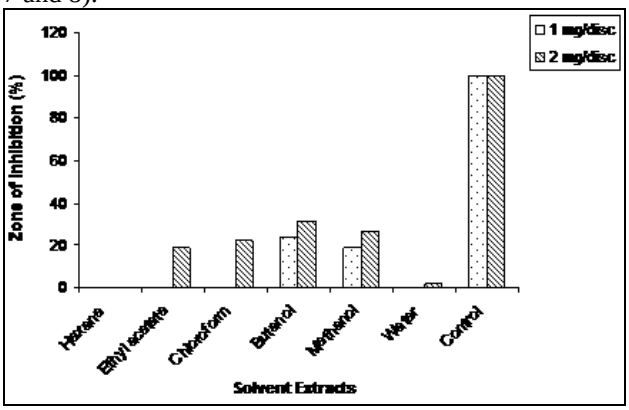

Fig. 1: Antibacterial activity of hexane, ethyl acetate, chloroform, butanol, ethanol and wáter extracted samples from *Alhagi maurorum* against *E. coli* by disc diffusion assay.

Data of chloroform-extracted samples indicated that this extract was very effective against all gram positive bacterial strains (*B. atrophus*, *B. subtilis* and *S. aureus*) (figs. 3, 4 and 7). Among gram positive strains, *B. atrophus* was the most susceptible strain to chloroform extracted samples at both concentrations recording 42% ZI at 1 mg/disc and 65% ZI at 2 mg/disc. Likewise, growth inhibition of *S. aureus* was also increased with increase in extract concentration. At lower concentration (1 mg/disc), the growth of *S. aureus* was reduced by 28% while at higher concentration (2 mg/ disc) 31% zone of inhibition was measured. Concentration of chloroform extracts had little effect on the growth inhibition of *B. subtilis* and measured 30% and 33% ZI at 1 and 2 mg/disc respectively. Among the gram-negative bacterial strains, *K. pneumonia* was the most susceptible at both concentrations recording 26% and 38% ZI at 1 and 2 mg/disc respectively. However, growth of *E. coli* was reduced by 22% when 2 mg chloroform extract was applied per disc (fig. 1). Among gram negative bacteria, *P. aeruginosa* and *S. typhi* were totally resistant to the chloroform extracted samples at both concentrations (figs. 5 and 8). The data further revealed that chloroform extracts had no anti-fungal activity against *C. albicans* when applied at both concentrations and therefore no zone of inhibition was observed (fig. 6).

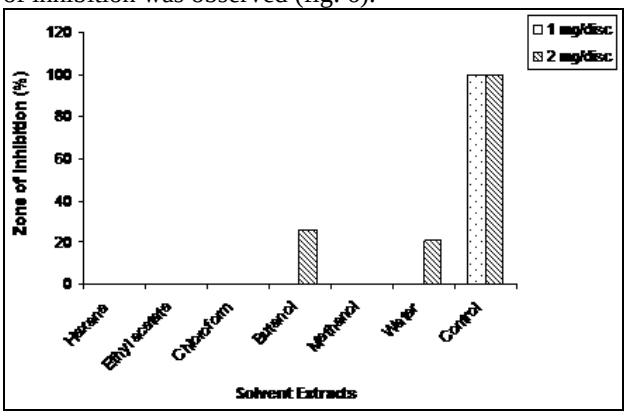

Fig. 2: Antibacterial activity of hexane, ethyl acetate, chloroform, butanol, ethanol and wáter extracted samples from *Alhagi maurorum* against *P. aeruginosa* by disc diffusion assay.

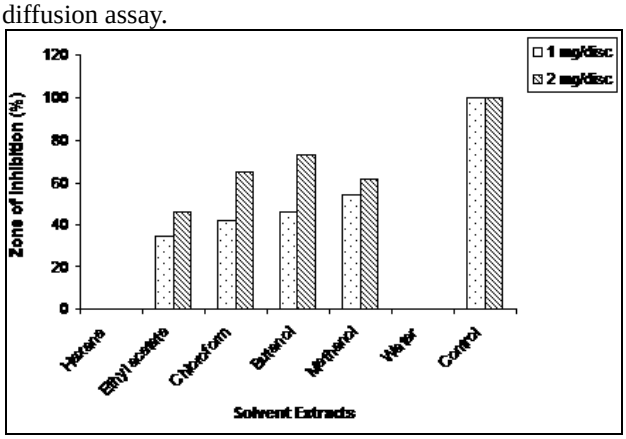

Fig. 3: Antibacterial activity of hexane, ethyl acetate, chloroform, butanol, etanol and wáter extracted samples from *Alhagi maurorum* against *B. atrophus* by disc diffusion assay.

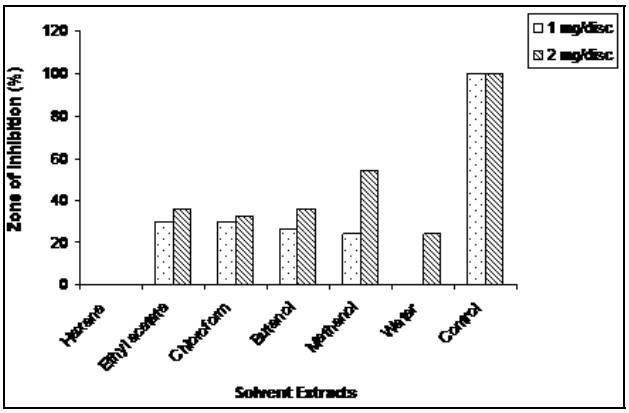

Fig. 4: Antibacterial activity of hexane, ethyl acetate, chloroform, butanol, ethanol and wáter extracted samples from *Alhagi maurorum* against *B. subtilis* by disc diffusion assay.

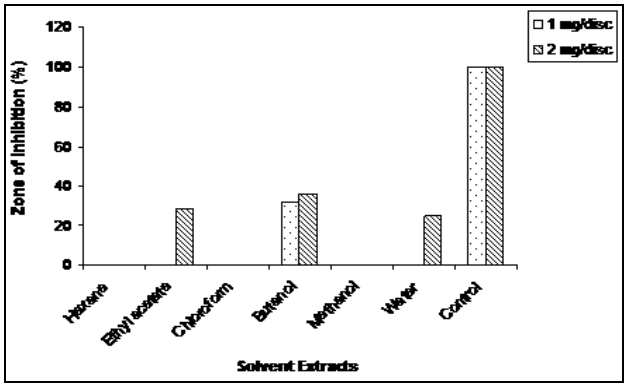

Fig. 5: Antibacterial activity of hexane, ethyl acetate, chloroform, butanol, ethanol and wáter extracted samples from *Alhagi maurorum* against *S. typhi* by disc diffusion assay.

The data also indicated that butanol extracted samples were the most active fraction among all extracts against all the tested microbial strains at both concentrations. Gram negative bacterial species were susceptible to butanol extracts at both concentrations; however, the most susceptible strain was *B. atrophus* recording 46% and 73% ZI at 1 and 2 mg/disc respectively (fig. 3). Similar reduction in the growth of *B. subtilis* and *S. aureus* was also noted (27% and 25% ZI at 1 mg/disc and 36% and 34% ZI respectively at 2 mg/disc) (fig. 4 and 7). Butanol extracted samples were also effective against *C. albicans* at low and high concentrations (fig. 6). The percent of growth reduction increased with increase in extract concentration. The growth of *C. albicans* was reduced by 31% at 1 mg/disc and 46% zone of inhibition was recorded at 2mg/disc using disc diffusion assay. The data also suggested that butanol extracted samples were active against the gram-negative strains at both concentrations; however, the most susceptible strain was *K. pneumonia* (fig. 8). Butanol extracts reduced the growth of *K. pneumonia* by 43% and 48% at 1 and 2 mg/disc respectively. Similarly, the growth of *E. coli* and *S. typhi* was inhibited by 24% and 32% at 1 mg/disc respectively, however, was unable to control the growth of *P. aeruginosa* at 1 mg/disc concentration (fig. 2). Similarly, zone of inhibition was increased by 32% and 36% for *E. coli* and *S. typhi* respectively at 2 mg/disc (figs. 1 and 5). The data also suggested that the growth of *P. aeruginosa* was reduced by 26% at 2 mg/disc.

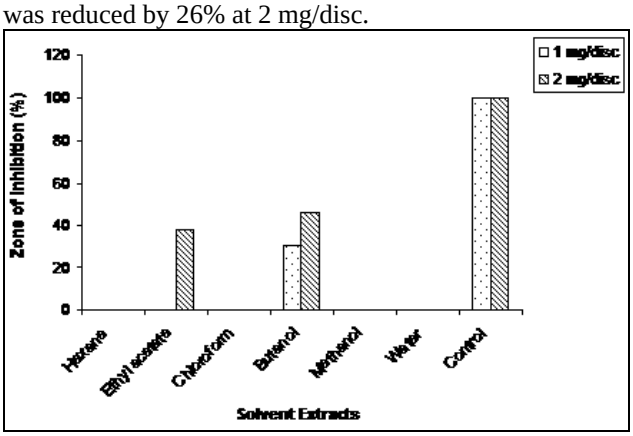

Fig. 6: Antibacterial activity of petroleum ethyl acetate, chloroform, butanol, ethanol and wáter extracted samples from *Alhagi maurorum* against *C. albicans* by disc diffusion assay.

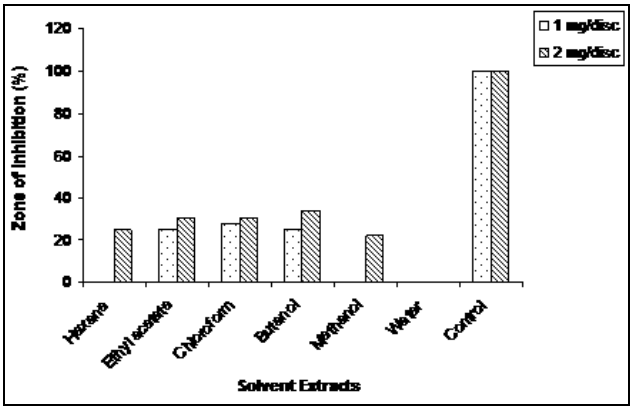

Fig. 7: Antibacterial activity of hexane ethyl acetate, chloroform, butanol, ethanol and wáter extracted samples from *Alhagi maurorum* against *S. aureus* by disc diffusion assay.

Crude methanol extracts was unable to control the growth of two-gram negative strains (*S. typhi* and *P. aeruginosa*) at both concentrations using disc diffusion assay measuring no zone of inhibition (figs. 2 and 5). Similarly, crude methanol extracts was ineffective in controlling the growth of *C. albicans* even at higher concentration (2 mg/disc) and thus no zone of inhibition was recorded (fig. 6). Data revealed that crude methanol extracted samples were very effective against all gram-positive strains and two gram negative strains. In case of gram negative species, growth inhibition by crude methanol extracts increased with increase in the concentration of sample. The growth of *E. coli* was restricted by 19% and 27% at 1

and 2 mg/disc respectively (fig. 1). Similarly, the growth of *K. pneumonia* was reduced by 21% and 24% at 1 and 2 mg/disc respectively (fig. 8). Among the three-gram positive strains, *B. atrophus* was the most susceptible, however, reduction in its growth slightly increased with increase in concentration of sample (crude methanol) measuring 54% and 62% ZI at 1 and 2mg/disc respectively, while growth inhibition of *B subtilis* increased with increase in the concentration. Therefore, crude methanol extracts reduced the growth of *B. subtilis* by 24% and 54% at 1 and 2 mg/disc respectively. However, in case of *S. aureus* the crude methanol extract was ineffective at low concentration and thus zero percent of zone of inhibition was observed and growth of the same microbe was reduced by 22% when applied at 2mg/ disc using disc diffusion assay.

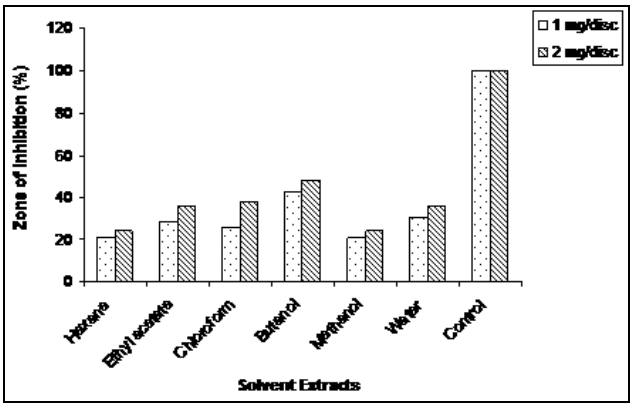

Fig. 8: Antibacterial activity of hexane ethyl acetate, chloroform, butanol, ethanol and wáter extracted samples from *Alhagi maurorum* against *K. pneumonia* by disc diffusion assay.

Water extracted samples were effective against all the gram-negative strains; however, the most susceptible one was *K. pneumonia*. The growth of *K. pneumonia* was restricted by 31% at 1 mg/ disc. It was also noted that water extract was not effective at low concentration against any other species and 36% reduction in the growth of *K. pneumonia* was measured at 2mg/ disc. The growth of other gram negative strains i.e. *E. coli* by 22%, *P. aeruginosa* by 21% and *S. typhi* by 25% at 2 mg/disc was reduced using disc diffusion assay. Water extracted sample was effective only in controlling the growth of *B. subtilis* at high concentration (2 mg/disc) recording 24% zone of inhibition. *B. atrophus* and *S. aureus* were totally resistant to the water-extracted samples at both concentrations measuring no zone of inhibition. *C. albicans* also showed resistance to water extracts even at high concentration (2mg/disc) and as a result no zone of growth inhibition was recorded.

DISCUSSION

Hexane extracted samples of *Alhagi maurorum* did not affect the growth of *E. coli, P. aeruginosa, B. atrophus, B. Subtilis* and *S. typhi* and *C. albicans* at the tested concentrations. However, the growth of *C. albicans* is reported to be inhibited by hexane extracted samples of *Alhagi maurorum* at much higher concentration i.e. 6mg/ml (Abd-Ellatif *et al.*, 2011). Our results also showed that hexane extracted samples did not reduce the growth of *S. aureus* at low concentration (1 mg/disc), however, at high concentration i.e. 2 mg/disc, it inhibited the growth of *S. aureus.* Similar reduction in the growth of *S. aureus* was observed by Rahman and Rashid (2008) with increasing concentration. *K. pneumonia* was the most susceptible strain to hexane extracted samples at both concentrations. Similar results were also reported by Rahman and Rashid (2008). *P. aeruginosa* was the highly resistant microbe towards ethyl acetate extracted samples at both concentrations. Similar trend of resistance was also noted for *E. coli* and *S. typhi* and *C. albicans* at low concentration of ethyl acetate extracted samples. However, when tested at high concentration, it inhibited the growth of all these microbes. Ethyl acetate extracted samples were more effective against *B. atrophus, B. Subtilis* and *S. aureus* and *K. pneumonia* at both concentrations.

Chloroform-extracted samples were very effective against all gram positive bacterial strains (*B. atrophus, B. subtilis* and *S. aureus*). Among these strains, *B. atrophus* was the most susceptible strain to chloroform extracted samples at both concentrations. Similarly, the reduction in the growth of *S. aureus* increased with increase in extract concentration. On the other hand, concentration of chloroform extracts had smaller effects on the growth inhibition of *B. subtilis*. Among the gram-negative bacterial strains, *K. pneumonia* was the most susceptible at both concentrations However, the growth of *E. coli* was reduced by chloroform extracted samples at higher concentration. *P. aeruginosa* and *S. typhi* were completely resistant to chloroform extracted samples at both concentrations. The data also revealed that the chloroform extracts did not show any anti-fungal activity against *C. albicans* when applied at both concentrations. Butanol extracted samples on the other hand were the most active extracts among all against all the microbial strains at both concentrations. Butanol extracted samples were also effective against *C. albicans* at low and high concentrations. Our results also suggested that butanol extracted samples were effective against the gram-negative strains at both concentrations and the most susceptible strain was *K. pneumonia*. Similarly, the growth of *E. coli* and *S. typhi* was reduced by butanol extracted at 1 mg/disc, however, was unable to control the growth of *P. aeruginosa* at the same concentration.

Crude methanol extracted samples were very effective against all gram-positive strains and two gram negative strains. In case of gram negative species, growth inhibition by crude methanol extracts increased with increase in its concentration. These results agree with

Table 1: Microbial strains tested for susceptibility to *Alhagi maurorum* extracts

Microbial species	Gram strain type	Details of the microbial strains used
Bacillus cereus	Positive	Clinical isolate obtained from Microbiology Laboratory of Quaid-I-Azam University Islamabad Pakistan
Bacillus subtilis	Positive	Clinical isolate obtained from Microbiology Laboratory of Quaid-I-Azam University Islamabad Pakistan
Candida albicans	Fungus	Clinical isolate obtained from Hayatabad Medical Complex Peshawar KPK Pakistan
Erwinia carotovora	Negative	Plant Pathology Department KPK Agricultural University Peshawar Pakistan
Escherichia coli	Negative	ATCC # 25922
Kleibsiella pneumoniae	Negative	Clinical isolate obtained from Microbiology Laboratory of Quaid-I-Azam University Islamabad Pakistan
Pseudomonas aeruginosa	Negative	ATCC # 9721
Salmonella typhi	Negative	Clinical isolate obtained from Microbiology Laboratory of Quaid-I-Azam University Islamabad Pakistan
Staphylococcus aureus	Positive	ATCC # 6538

The different microbial strains, from the stock culture, were freshened on the nutrient agar medium using streak method. The micro-organisms were then inoculated in agar broth medium and placed in shaking water bath (GLSC-SBR-04-28) at 37ºC and 200 rpm for 24 hr.

Rahman and Rashid (2008). Among the gram positive strains, *B. atrophus* was the most susceptible, however, its growth reduction slightly increased with increase in concentration of sample (crude methanol). Similarly, the growth of *B. subtilis* was reduced more with increase in the concentration. In case of *S. aureus*, the crude methanol extracts was ineffective at low concentration, however, reduced its growth when applied at 2mg/ disc using disc diffusion assay. Increase in growth reduction of *S. aureus* was also observed with increase in concentration (Rahman *et al.*, 2011).

Crude methanol extracts was unable to affect the growth of two-gram negative strains (*S. typhi* and *P. aeruginosa*) and *C. albicans* at both concentrations. Abd-Ellatif *et al.* (2011) reported that the growth of *C. albicans* can be inhibited by the methanol extracts of *Alhagi maurorum* at much higher concentration i.e. 6 mg/ml. Aqueous extracted samples were very effective against all tested gram-negative strains at higher concentration and the most susceptible one was *K. pneumonia*. Water extracted sample was effective against *B. subtilis* at high concentration (2 mg/disc) only. *B. atrophus*, *S. aureus* and *C. albicans* were resistant to the water-extracted samples at both concentrations.

REFERENCES

Abd-Ellatif S, Abdel-Rahman SM and Deraz SF (2011). Promising anti-fungal effect of some folkloric medicinal plants collected from El-Hammam habitate, Egypt against dangerous pathogenic and toxinogenic fungi. *ARPN J. Agric. and Biol. Sci.,* **5**: 25-32.

Amani AA, Maitland DJ and Soliman GA (2006). Anti-ulcerogenic activity of *Alhagi maurorum*. *Pharmacet. Biol.,* **44**: 292-296.

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 *Eclipt 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). Anti-microbial 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). Anti-microbial 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 potential 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 Gram positive bacteria. *Pak. J. Bot.,* **45**: 643-648.

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

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

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. Pharmacet. Sci*., **28:** (In press).

Bauer AW, Kirby WM, Sherris JC and Turek M (1996). Antibiotic susceptibility testing by standardized single disc method. *Am. J. Clin. Pathol.,* **44**: 493-496.

Berhge DAV and Vlietinc AG (1991). Methods in plant biochemistry assays for bioactivity, Academic Press, London UK. pp. 47-69.

Chin Y, Balunas MJ, Chai B and Kinghorn AD (2006). Drug discovery from natural sources. *AAPSJ.,* **8**: 239-253.

Cowan MM (1999). Plant products as anti-microbial agents. *Clin. Microbiol. Rev.,* **12**: 564-582.

Kalhoro MA, Kapadia Z and Badar Y (1997). Physicochemical studies of indigenous medicinal plants. *Bangldsh. J. Sc. Industl. Res.*, **32**: 418-421.

Kong JM, Goh NK, Chia LS and Chia TF (2003). Recent advances in traditional plant drugs and orchids. *Acta Pharma. Sin.*, **24**: 7-21.

Kudliki WP, William D, Kramer SK, Makhamed BG and Iskakov BK (1991). Eukaryotic protein synthesis initiation factor 2. *Eur. J. Biochemist.,* **197**: 623-629.

Park JT and Strominger JL (1957). Mode of action of penicillin. *Sci.*, **125**: 99-101.

Parveen G and Bakht J (2013). Antimicrobial activity of turmeric extract and its potential use in food industry. *J. Food Sci. Technol*., DOI 10.1007/s13197-013-1195-4.

Rahman MS and Rashid MA (2008). Anti-microbial activity and cytotoxicity of *Eclipta prostarta* Oreintal. *Pharma. Exp. Med.,* **8**: 47-52.

Rahman SMA, Sawsan AA, Sahar FD and Ashraf AK (2011). Antibacterial activity of some wild medicinal plants collected from western Mediterranean coast, Egypt: Natural alternatives for infectious disease treatment. *Afr. J. Biotechnol*., **10**: 10733-10743.

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

Shahidi BGU, Aghighi S and Karinii N (2004). Anti-bacterial and anti-fungal survey in plants used in indigenous herbal medicine of South Coast regions of Iranian. *J. Biol. Sci.*, **4**: 405-412.

Tenover FC (2006). Mechanism of anti-microbial resistance in bacteria. *The Am. J. Med.*, **119**: 3-10.

Trucco RE and Pardee AB (1958). Synthesis of E. coli cell wall in the presence of penicillin. *J. Biol. Chemist.,* **230**: 435-446.

Zakaria M, Islam NM, Radhakrishnan MN, Chen R, Ismail HB, Chan A and Habibullah KM (1999). Pharmacological evaluation of anti-inflammatory activity of *Alhagi maurorum*. *J. Pharma. Pharmacol.,* **51**: 118.