Antimicrobial, antioxidant potential and phytochemical screening of *Fagonia olivieri*

Anwar Ali Shad[1*], Zakir U Din[1], Jehan Bakht[2] and Saleem Jan[3]
[1]Department of Agricultural Chemistry, The University of Agriculture Peshawar, KPK Pakistan
[2]Institute of Biotechnology and Genetic Engineering, The University of Agriculture Peshawar, KPK Pakistan
[3]Department of Chemistry, University of Science and Technology Bannu, KPK Pakistan

Abstract: The present paper investigates antioxidant, antimicrobial and photochemical screening different extracts of *Fagonia olivieri*. Analysis of the data indicated that the subject plant contained a good amount of flavonoids, tannins, saponins, terpenoids and steroids. Maximum concentrations of phenolic compounds was found in methanol fraction (29.0±6.12 mg GAE/g) while minimum (22.10±6.31mg GAE/g) in methylated spirit fraction. Similarly, ethanol fraction contained higher concentration of flavonoid content (135.4±7.63mg Quercetin/g) followed by methanol fraction (138.4±2.96 mg Quercetin/g). Analysis of the data revealed that maximum antioxidant activity was recorded in mthylated spirit fraction (IC_{50}= 10.69±1.66) followed by methanol fraction (IC_{50}= 9.10±0.76) while no activity was noted in hexane fraction. The data indicated good antibacterial and antifungal activity against *S. typhi*, *S. aureus*, *P. aeruginosa* and *A. flavus*.

Keywords: Phyto-chemistry screening, antibacterial activity, antifungal activity, anti-oxidant activity, *Fagonia olivieri*

INTRODUCTION

It has been estimated that 60% of world population both in developing and developed countries use native plants for their basic health care needs. Besides many adverse effects, the injudicious utilization of synthetic antibiotics for treating different diseases has resulted in the acquisition of increased resistance by different microorganisms. It is reported that root, fruit, stem, flower, modified plant organs and twigs exudates obtained from different plant species contain different medicinal properties which can be utilized as herbal medicines and preservative (Cowan, 1999; Kaur and Arora, 2009; Parveen and Bakht, 2015). These days many plants are under investigation for their phytochemistry, antioxidant and antimicrobial properties. A number of modern drugs have been isolated from natural sources including plant (Cowan, 1999; Duraipandiyan and Ignacimuthu, 2009). Different plant fractions possessing antimicrobial activity are playing a vital role in the treatment of different diseases caused by various pathogens (Bakht *et al*., 2011 a, b, c, d, 2012; 2013 a, b; 2014; a, b,c; 2015; Nasir *et al*., 2015; Ullah *et al*., 2015; Zakir *et al*., 2015; Chaun *et al*., 2015; Bilal *et al*., 2016; Wajid *et al*., 2016 a, b; Amjad *et al*., 2016; Anwar *et al*., 2016). The therapeutic behavior of is due to the presence of different bio-active secondary metabolites in these native plant species grown widely in different ecological regions of the world (Krishnaiah *et al*., 2007). The ethno-medicinal investigation of such plants could be very helpful in understanding their therapeutic efficiency and nutraceutical uses (Zavala *et al*., 2009; Zavala *et al*., 2011). Pakistan is among very few countries in the world containing rich flora to be used as foods and drugs. These plants are routinely used for common ailments through traditional knowledge.

Fagonia olivieri is the member of family Zygophyllaceae comprised of twenty five genera and two hundred and forty species (Mabberley, 1987). It is biennial to perennial, thin dense glandular or hairless green herb, up to 48cm tall. Stems are abundantly branched and woody at the bases. In Pakistan, the family of Zygophyllaceae is composed of eight genera and twenty two species widely grown in the dry mountainous part of Khyber Pukhtun Khwa and Baluchistan provinces. Fagonia has popularity in folk medicine and used for the treatment of internal and external disease. It is reported that extract of *Fagonia olivieri* is used for the treatment of diabetes and cough, cancer and many other illnesses (Barkatullah and Hussain, 2009). The aqueous extract of this plant shows a vast range of bio-pharmacological activities including anti-microbial, hematological, endo-crinological, anti-inflammatory, anti-tumor, anti-oxidant, anti-pyretic and prophylactic capabilities and as blood purifier (Sharma *et al*., 2009; Qureshi, 2010; Sajid *et al*., 2011; Rashid *et al*., 2013). Fagonia is used as a preventive syrup for the treatment of inflammation of the urinary tract and it is useful in the treatment of cold, rheumatoid and arthritis and as insect repellents and its extract is also used as an anthelmintic. The phyto-chemical investigation of Fagonia showed the presence of tarpenoids, tannins, saponnins and total poly-phenols (Sharawy and Alshammari, 2009; Sharma *et al*., 2010; Sajid *et al*., 2011; Anil *et al*., 2012).

**Corresponding author:* e-mail: anwaralishad@aup.edu.pk

MATERIALS AND METHODS

Plant materials

Plants of *Fagonia olivieri* were collected from various locations of Peshawar, Bannu and Nizampur (Nowshera) natural habitats of KPK province of Pakistan. Plants were dried at room temperature (25ºC) under shade. The dried samples were grinded by tissue homogenizer to fine powder (Infinigen™ Tissue Mixer Mill, ACTGene), sealed in plastic bags and stored at 4ºC until analyzed.

Extractions

The powdered samples were macerated in aqueous methanol, ethanol, hexane and methylated spirit (Sigma-Aldrich) and kept for one week at room temperature. The solution was stirred four times a day for thorough mixing and then filtered (Whatman ^{TM} Whatman UK). One litre each of fresh solvents were added to the plant material and filtered again through Wattman filter paper and this process was repeated three times. The filtered solution was evaporated by rotary evaporator (Rotavapor ^{R}-R 210/R215; BUCHIL Labortechnik AG).

Phyto-chemical screening

Determination of tannins

Half gram of crude extract of the subject plant was boiled in twenty ml of water and allowed to cool and filtered. One percent of ferric chloride was added and the development of blue black or brownish green color was noted which showed the existence of tannins (Trease and Evans, 1989).

Determination of saponnins

For the estimation of saponnins, 2grams of powdered sample was boiled in 20ml distilled water for half an hour, allowed to cool and filtered. Five ml distilled water was mixed with ten ml of the filtrate and constantly shaken until a persistent froth appeared. Few drops of olive oil were mixed with the froth and shaking continued till the formation of emulsion (Safowara, 1993).

Determination of flavonoids

For the flavonoids determination, crude extract of the subject plant was prepared and filtered as discussed previously and five ml of diluted ammonia and known amount of sulphuric acid (conc.) was mixed with each crude extract. The presence of flavonoids in the sample was confirmed by the appearance of yellow color (Sofowara, 1993).

Determination of terpeniods

For the identification of terpeniods, a mixture containing 5ml of plant sample and 2ml of chloroform was prepared. Three ml of H_2SO_4 (conc) was added to the mixture for the formation of a layer. Terpeniod presence was established by the appearance of reddish brown color (Harborne, 1973).

Determination of steroids

For the estimation of steroids, approximately 20g of sample was soaked in ethanol in a volumetric flask and boiled for 10 minutes. The extract was filtered and the ethanol fraction was extracted and separated. The remained crude solid sample was dissolved in 3ml chloroform followed by the addition of acetic anhydride (4.5ml) and sulfuric acid (0.5ml). The presence of steroids was confirmed by change of color of the crude extract from violet to green (Sofowara, 1993).

Determination of total flavonoid content

The total flavonoid content of the subject plant was evaluated by colorimetric method using quercitin as a standard. About one milliliter of the crude sample was treated with 4ml distilled water and 300µl each of $NaNO_2$ and $AlCl_3$ were poured into this mixture. The mixture was warmed for 5minutes and NaOH was added to this mixture in such quantity so that the total volume of the mixture became 10ml. Absorbance was measured by spectrophotometer and total flavones are represented as quercitin equivalents (QE) in mg/g of dry crude extract.

Determination of antioxidant activity by ABTS protocol

ABTS (2,2-azinobis-3-ethylbenzothiazoline-6-sulphonate) assay was carried out as described by Arnao *et al*. (2001) with few modifications. The stock solutions contained 7.4mM ABTS*+ and 2.6mM potassium per sulfate. The two stock solutions were mixed in equal amount to prepare working solution and kept for twelve hours at room temperature in the dark. Sixty ml of methanol was added to 1mL ABTS solution to get an absorbance of 1.170.02 units at 734 nm. Fractions prepared from aerial parts of *Fagonia olivieri* were added to ABTS*+ solution for 2h in a dark condition and measured at 734 nm. The standard curve was linear between 25 and 600mM Trolox. All the values obtained were compared with trolox (standard).

Determination of antioxidant activity by DPPH protocol

Antioxidant activity by DPPH was determined as described in Lee *et al*. (2003) with certain modifications. The powdered aerial parts of *Fagonia olivieri* were extracted with methanol for 40-48 hrs and solvent was separated through vacuum evaporator. One ml DPPH was mixed with ethanol and poured into 2.5ml of each fraction. Antioxidant activity was measured by spectrophotometer at 517nm at 30 minutes of the reaction. All readings/data obtained was compared with trolox (standard). Radical scavenging activity was measured as follow

% radical scavenging activity = (control OD – sample OD/control OD) X 100.

IC_{50} value is the concentration of the sample required to scavenge 50% DPPH free radical.

Table 1: Total flavonoid and phenolic contents of *Fagonia olivieri*

Plant Extracts	Total flavonoid contents	Total phenolic contents
Methanol	135.4±7.63	29.0±6.12
Ethanol	138.4±2.96	23.0±2.28
Methalted spirit	117.0±3.32	22.10±6.31
Hexane	-	25.63±5.387

±= Standard deviation

Table 2: Antioxidant Potential of *Fagonia olivieri* using DPPH, ABTS and FRAP protocol

Plant Extracts	IC_{50} Value		
	DPPH	ABTS (TEAC)	FRAP* (mmol/L)
Methanol	0.82±0.095	0.98±0.20	9.10±0.76
Ethanol	1.98±0.31	1.18±0.16	7.85±1.54
Methalted spirit	1.92±0.062	1.11±0.19	10.69±1.66
Hexane	-	-	-
Trolox	1.04±0.05	1.08±0.14	-
Quercitin	0.05	0.30	-

*Ferric reducing activity (expressed as mmole of $FeSO_4$ equivalent/litre of extract).
±= Standard deviation

Table 3: Antibacterial assay of different crude fractions of *Fagonia olivieri* (MIC; average Value ±SD, µg/ml)

Plant Extracts	*E. coli*	*P. aeroginosa*	*S. typhus*	*B. subtilis*	*S. aureus*
Methanol	10.03± 0.25	10.9± 5.7	8.7± 1.5	10.0 ± 1.5	0.5± 0.2
Ethanol	0.74± 0.15	0.67± 0.25	0.12± 0.04	1.18± 0.04	0.5± 0.2
Methalted spirit	01± 0.1	4.04± 0.95	5.38± 1.06	10.1± 1.06	0.51± 0.11
Hexane	-	-	-	-	-
Ciprofloxacin	0.06	1.5	0.25	0.5	0.25

Ciprofloxacin (standard drug),
±= Standard deviation
- = No activity

Table 4: Antifungal assay at 5 mg/ml of different crude fractions of *Fagonia olivieri* (MIC; average Value ±SD, µg/ml)

Plant Extracts	*T. longifusus*	*C. albicans*	*C. glaberata*	*A. flavus*	*F. solani*
Methanol	33.34±8.63	-	-	23.67± 2.52	32.34± 8.08
Ethanol	27.34±2.51	-	-	21±1.74	25±4.58
Methalted spirit	26.7±4.05	-	-	21.3± 4.25	45.6±7.45
Hexane	-	-	-	-	-
Amphotericine B	0.5±0	0.5±0	0.5±0	0.5 ± 0	0.5± 0
	-	-	-	-	-

Amphotericine B (standard drug)
±= Standard deviation
- = No activity

Determination of antioxidant activity by FRAP protocol

In this protocol the antioxidant activity of the subject plant was determined according to the methods of Benzie and Strain (1996). The solution for ferric reducing antioxidant power (FRAP) was prepared by mixing of 20ml ferric chloride hexa hydrate (BDH), 10ml of TPTZ (tripyridy1-s-triazine, Fluka Chemicals, Switzerland) and 300 mM acetate buffer (Riedel- de Haen, Germany). Plant samples were systematically added to 1.9ml of FRAP reagent, shaken for few minutes and absorbance was measured at 593nm through spectrophotometer. The difference in measurement at D*A*593nm between the final reading and the M1 reading was calculated for each sample and the same procedure was adopted for the standard solution.

Disc diffusion susceptibility method

The antibacterial activity of different solvent extracted samples of *Fagonia olivieri* was carried by disc diffusion assay as described in Bauer *et al.* (1998) and antifungal activity by Ramdas *et al.* (1998). Different antibiotics (Ciprofloxacin at 50µg concentrations for Gram-positive and Gram-negative bacteria; 50µg Amphotercin B for fungus were aseptically placed over the seeded agar

plates. The plates were kept at 37ºC for 24 hours and the diameter of the inhibition zones was measured.

Minimum inhibitory concentration (MIC) measurements

MIC was measured according to Khan *et al*. (2007). Briefly, extracts of *Fagonia olivieri* was dissolved in 2 ml distilled water and added with 2 drops tween-80 for complete dissolution. The suspension of each test organisms was prepared by approximately 10^7 per ml and 1 drop of this suspension was mixed with each broth dilution. The tubes were observed for the growth after 18-24 h incubation at 37°C. MIC of the plant extract was noted when no growth was observed. Bacterial activity was noted in tubes having concentration of the extract below the inhibitory level and the broth showed turbid (cloudy). Distilled water having 2 drops of tween-80, Ciprofloxacin and Amphotercin B were used as negative and positive controls respectively.

Microorganisms tested

The selected bacterial strains for the current study were *Bacilus subtilis*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Escherchia coli* and *Salmonella typhi*. The fungal strains for the current investigation were *Trichophyton longifusus*, *Aspergillus flavus*, *Fusarium solani*, *Candida glaberata* and *Candida albicans*.

STATISTICAL ANALYSIS

The experiment was repeated in triplicate and MSTAT computer software was used for the analysis of the data. Standard deviation was calculated for each sample (Steel *et al.,* 1997).

RESULTS

Phytochemical analysis

The present investigation measured concentration of different phytochemicals. The data revealed that *Fagonia olivieri* contained appreciable amount of tannins, sopannins, terpeniods and steroids. The subject plant was also investigated for total poly-phenol and flavanoid contents. The data indicated that methanol fraction showed higher phenolic content (29.0±6.12mg Gallic Acid Equivalent/g) while 22.10±6.31mg GAE/g was found in methylated spirit extract. The results also revealed that ethanol fraction of the *Fagonia olivieri* contained maximum level of flavonoid content (138.4±2.96mg Quorcetin/g) followed by methanol fraction (135.4±7.63mg Quercetin /g). However, hexane fraction showed negligible value for flavonoid content for this investigated plant (table 1).

Anti-oxidant assay

Antioxidant activity was measured by three different methods for certainty. These methods included DPPH, ABTS and FRAP. When measured by DPPH, the data revealed that antioxidant activity was highest in ethanol and methylated fractions (IC_{50}= 1.18±0.19 and 1.11±0.16, respectively) when compared with the standard trolox and quercetin (IC_{50}= 1.18±0.19 and 1.11±0.16 respectively) (table 2). Free radical scavenging activity was also determined by FRAP method. Analysis of the data revealed that maximum antioxidant activity was recorded in mthylated spirit fraction (IC_{50}= 10.69±1.66) followed by methanol fraction (IC_{50}= 9.10±0.76) while no activity was noted in hexane fraction. Ethanol and methylated spirit fraction exhibited higher antioxidant activity (IC_{50}= 1.18±0.19 and 1.11±0.16 respectively) when measured through ABTS protocol.

Antimicrobial activity

Antimicrobial activity of crude fractions (methanol, ethanol, methylated spirit and hexane) of *Fagonia olivieri* was also investigated by disc diffusion assay against five each bacterial strains (*S. aureus, E. coli, P. aeruginosa, S. typhi* and *B. subtilis*) and fungal strains (*T. longifusus, C. albicans, C. glaberata, F. solani* and *A. flavus* (tables 3 and 4). Results indicated that methanolic fraction was less effective against *E. coli, S. typhi, B. subtilis* and *P. aeruginosa* showing MIC value of 10.03±0.25, 10.9±0.7, 8.7±1.5 and 10.0±1.5µg/ml respectively as compared to other fractions. The ethanolic fraction was more effective against *S. typhi* (MIC=0.12±0.04µg/ml), *S. aureus* (MIC=0.5±0.2µg/ml), *P. aeruginosa* (MIC= 0.67±0.25µg/ml). *Fagonia olivieri* was also evaluated against antifungal activity using different strains of fungi. The data showed that the ethanolic fraction was more effective against *A. flavus* (MIC=21±1.74µg/ml) followed by methylated spirit fraction against the same fungus (MIC= 21.3±4.25µg/ml). Hexane on the other hand did not show any activity against all the fugal strains under test (table 4).

DISCUSSION

Phyto-chemical screening of the tested plant indicated the existence of tannins, sopannins, terpeniods and steroids in aqueous ethanol extracts. The data also indicated that methanol fraction showed higher phenolic content and lower concentration was measured in methylated spirit extract. Similarly, our results also revealed that ethanol fraction contained maximum level of flavonoid followed by methanol fraction. However, hexane fraction on the other hand showed negligible value of flavonoid. Antioxidant activity was determined by three different protocols for certainty. These methods were DPPH, ABTS and FRAP. Antioxidant activity by DPPH revealed that this parameter was highest in ethanol and methylated fractions when compared with the standard trolox and quercetin. Free radical scavenging activity determined by FRAP showed that maximum antioxidant activity was noted in mthylated spirit fraction followed by methanol

fraction while zero activity was noted in hexane fraction. Ethanol and methylated spirit fraction measured higher antioxidant activity when measured through ABTS protocol. It has been reported that methanol extract of *Fagonia cretica* contain saponins, alkaloids, cardiac glycosides, flavonoids and tannins (Sajid *et al*., 2011) and *Fagonia indica* (Sharma *et al*., 2009; Anil *et al*., 2012). Similarly, Hamidi *et al.* (2014) confirmed the existence of bioactive chemicals: Ethyl Palmitate, Phenol2,6-bis (1,1-dimethylethyl)-4-methyl, n-Hexadecaonoic acid and 9,12,15- Octadecatrienoic acid, (Z,Z,Z). Peter *et al*. (1983) discovered that these compounds had antimicrobial, antioxidant and anticancer activities.

The search for novel antibiotic compounds requires proper and systemic screening of different extracts obtained from various medicinal plants. The microbes tested in the present investigation cause different illnesses in human beings. For example, *E. coli* causes infections of urinary tract, lungs, gall bladder, skin lesions and diarrhea (Black, 1996; Adams and Moss, 2000). Similarly, *S. aureus* causes boil, ulcers, food poisoning, toxic shock and pneumonia etc. Antimicrobial activity of different samples (methanol, ethanol, methylated spirit and hexane) of *Fagonia olivieri* was also investigated by disc diffusion assay against five each bacterial (*S. aureus, E. coli, P. aeruginosa, S. typhi* and *B. subtilis*) and fungal strains (*T. longifusus, C. albicans, C. glaberata, F. solani* and *A. flavus*. Our results indicated that methanolic fraction was less effective against *E. coli, S. typhi, B. subtilis* and *P. aeruginosa* as compared to other fractions. The ethanolic fraction showed more activity against *S. typhi, S. aureus* and *P. aeruginosa*. Immune compromised patients are always at high risk from persistent opportunistic fungal infections (Groll *et al*., 1996). The antibacterial activities in different extracts are due to the presence of different phytochemicals in *Fagonia olivieri.* Presence of different secondary metabolites such as tannins saponins, terpenoids, steroids, flavonoids and phenolic compounds in different extracts of *Fagonia olivieri* might have contributed towards diversified antibacterial activities. Aspergillus species are major cause of fungal infections (Bodev *et al*., 1992). The present study screened different extracts of *Fagonia olivieri* for their antifungal activity against different strains of fungi. The data showed that the ethanolic fraction of *Fagonia olivieri* was more effective against *A. flavus* followed by methylated spirit fractions and methanolic fraction. Hexane fraction on the other hand did not show any activity against all the fugal strains under test. These results agree with Sharma *et al*. (2009), Qureshi (2010) and Sajid *et al*. (2011).

CONCLUSION

Fagonia olivieri contained appreciable amount of saponnins, tannins, flavonoids, terpeniods, steroids, total phenolic and flavonoid which signify its nutraceutical importance. Different extracts from the subject plant showed good antioxidant activity, anti-bacterial and anti-fungal activity against different microbes. The result of the present investigation signifies the medicinal importance of *Fagonia* olivieri and as a source of therapeutic agent.

REFERENCES

Adams MR. and Moss MO (2000). Food Microbiology. 2nd edn. Cambridge, UK: Royal Society of Chemistry. pp. 15.

Amjad U, Arshad I, Bakht J, Khalid N and Naushad A (2016). *In vitro* antimicrobial activities of different solvent extracted samples from *Iris germinica*. *Pak. J. Pharmaceut. Sci.*, **29**: 145-150.

Anwar AS, Seemab A Bakht J, Saleem J and Khan AZ. (2016). Antimicrobial potentials and phytochemical analysis of desert cotton (*A. Javanica*) and flax (*L. Ustitatissimum*). *Pak. J. Pharmaceut. Sci.*, **29**: 861-868.

Anil P, Nikhil B, Manoj G and Prakash NB (2012). Phytochemicals and biological activities of *Fagonia indica*. *Intl. Res. J. Pharm.*, **3**: 56-59.

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. *Afri. J. Biotechnol.*, **10**: 5910-5915.

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

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

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

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). Antimicrobial activity of *Nicotiana tobaccum* using different solvent extracts. *Pak. J. Bot.*, **44**: 459-463.

Bakht J, Khan S and Shafi M (2013 a). Antimicrobial 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 (2013 b). Antimicrobial 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 (2014). *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). 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. Pharmacet. Sci*., **27**: 1955-1961.

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

Bilal MK and Bakht J (2016). Anti-fungal, anti-yeast, anti-oxidant and HPLC analysis of different solvent extracted samples from *Calmus aromaticus* leaves. *Bangladesh J. Pharmacol.,* **11**: 91-100.

Chaun RZ, Wajid K, Bakht J and Nair MG (2015). New inti-inflammatory sucrose esters in the natural sticky coating of tomatillo (*Physalis philadelphica*) an important culinary fruit. *Food Chem.,* **196**: 726-732.

Barkatullah IM and Hussain F (2009). Ethnobotanical studies of plants of Charkotli Hills, Batkhela district, Malakand, Pakistan. *Front. Biol. China.,* **4**: 539-548.

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

Benzie IFF and Strain JJ (1996). The ferric reducing ability of plasma (FRAP) as a measure of "Antioxidant Power": The FRAP assay. *Anal. Biochem.,* **239**: 70-76.

Black JG (1966). Microbiology: principles and application. New York: Prentice Hall, p. 260.

Bodey G, Bueltmann B, Duguid W, Gibbs D, Hanak H and Hotchi M (1992). Fungal infections in cancer patients: an international autopsy survey. *Eur. J. Clin. Microbiol. Infect. Dis*., **11**: 99-109.

Cown MM (1999). Plant products as antimicrobial agents. *Clin. Microbiol. Rev*., **12**: 564-582.

Duraipandiyan V and Ignacimuthu S (2009). Antibacterial and antifungal activity of Flindersine isolated from the traditional medicinal plant, *Toddalia asiatica* (L.) Lam. *J. Ethnopharmacol*., **123**: 494-498.

Groll AH, Shah PM, Mentzel C, Schneider M, Nuebling G and Huebner K (1996). Trends in the postmortem epidemiology of invasive fungal infections at a university hospital. *J. Infect*., **33**: 23-32.

Hamidi N, Lazouni HA, Moussaoui A, Ziane L, Djellouli M and Belabbesse A (2014). Ethnopharmacology, Antibacterial and antioxidant activities, phytochemical screening of bioactive extracts from the aerial parts of *Fagonia longispina*. *Asian J. Natl. Appl. Sci*., **3**: 53-63.

Harborne JB (1973). Phytochemical methods, London. Chapman and Hall, Ltd. pp.49-188.

Kaur GJ and Arora DS (2009). Antibacterial and phytochemical screening of *Anethum graveolens*, *Foeniculum vulgare* and *Trachyspermum ammi. BMC Complement. Alternat. Med.,* **9**: 30

Khan WA, Seas C, Dhar U, Salam MA and Bennish ML (1997). Treatment of shigellosis V. Comparison of azithromycine and ciprofloxacin: A double-blinded, randomized, control trial. *Ann. Intern. Med*., **126**: 697-703.

Krishnaiah D, Sarbatly R and Bono A (2007). Phytochemical antioxidants for health and medicine: A move towards nature. *Biotechnol. Mol. Biol. Rev*., **1**: 97-104.

Mabberley DI (1987). The plant book. Cambridge, New York: Cambridge University Press.

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**: 195-200.

Parveen G and Bakht J (2015). Antimicrobial activity of turmeric extract and its potential use in food industry. *J. Food Sci. Technol.,* **52**: 2272-2279.

Peter YM and Georgi YP (1983). Furanoid diterpenes from *Teucrium polium*. Phytochem., **22**: 2791-2793.

Qureshi R, Bhatti GR and Memon RA (2010). Ethno medication uses of herb from Northern part of Nara deesert, Pakistan. *Pak. J. Bot*., **42**: 839-851.

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

Sajid B, Alia E, Rizwana K, Uzma S and Alamgeer HMI (2011). Phytochemical screening and antimicrobial activity of *Fagonia cretica* plant extracts against selected microbes. *J. Pharma. Res.,* **4**: 962-963.

Sharma S, Joseph L, George M and Gupta V (2009). Analgesic and anti-microbial activity of *Fagonia indica*. *Pharma.*. **3**: 623-632.

Sharrma S, Gupta V and Sharma G (2010). Phytopharmacology of Fagonia indica (L): A Review. *J. Natura. Consci.,* **1**: 143-147.

Sharawy SM and Alshammari AM (2009). Checklist of poisonous plants and animals in Aja Mountain, Ha'il Region, Saudi Arabia. *Aust. J. Basic & Appl. Sci*., **3**: 2217-2225.

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

Trease GE and Evans WC (1989). Pharmacognsy. 11th edn. Brailliar Tiridel Can. Macmillian publishers.

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

Vieira RF and Skorupa LA (1993). Brazilian medicinal plants gene bank. *Acta Horti*., **330**: 51-58.

Wajid K, Bakht J and Shafi M (2016a). Antimicrobial potential of different solvent extracted samples from *Physalis ixocarpa*. *Pak. J. Pharmacet. Sci.,* **29**: 467-475.

Wajid K, Bakht J and Shafi M (2016b). Evaluation of polyphenol content in different parts of *Physalis ixocarpa*. *Pak. J. Bot.,* **48**: 1145-1151.

Zavala JFA, Gonzalez-Aguilar GA and Sanchez LDT (2009). Enhancing safety and aroma appealing of fresh-cut fruits and vegetables using the antimicrobial and aromatic power of essential oils. *J. Food Sci*., **74**: R81-R94.

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

Zavala JFA, Vega-vega V, Dominguez CR, Palafox-Carlos, Villa-Rodriguez JA, Davila-Avina JE and Gonzalez-Aguilar GA (2011). Agro-industrial potential of exotic fruit byproducts as a source of food additives. *Food Res. Intl*., **44**: 1866-1874.