SHORT COMMUNICATION

Screening of solvent dependent antibacterial activity of *Prunus domestica*

Zahra Yaqeen[1], Naim-ul-Hasan Naqvi[2], Tehmina Sohail[1], Zakir-ur-Rehman[1], Nudrat Fatima[1], Hina Imran[1*] and Atiq-ur-Rehman[1]

[1]Pharmaceutical Research Centre, PCSIR Labs Complex, Karachi, Pakistan
[2]Baqai Institute of Pharmaceutical Sciences, Baqai Medical University, Karachi, Pakistan

Abstract: Fruit of *Prunus domestica* was extracted in ethanol. The ethanol extract was further extracted with two solvents ethyl acetate and chloroform. The crude ethanol extract and two fractions (ethyl acetate and chloroform) were screened for their antibacterial activity using the agar well diffusion method .They were tested against nine bacteria; five Gram positive bacteria (*Staphylococcus aureus, Streptococcuc intermedius, Bacillus cereus, Bacillus pumilus*) and four Gram negative bacteria (*Eschrichia coli*, *Proteus mirabilis Shigella flexneri, Salmonella typhi* and *Klebsiela pneumoniae*). The susceptibility of microorganisms to all three fractions was compared with each other and with standard antibiotic (Ampicillin) Among all fractions ethyl acetate exhibited highest antibacterial activity (average zone of inhibition 34.57mm ± 1.3)while ethyl alcohol exhibited least antibacterial activity (average zone of inhibition 17.42mm ± 3.3). Minimum inhibitory concentration of ethanol, ethyl acetate and chloroform fractions was found in the range of 78ug/ml to 2500ugl/ml against gram positive and gram negative bacteria

Keywords: *P. domestica,* antibacterial activity, gram-positive bacteria, gram-negative bacteria, minimum inhibitory concentration.

INTRODUCTION

The present scenario of emergence of multiple drug resistance to human pathogenic organisms has necessitated a search for new antimicrobial substances from alternative sources including plants. It has been reported that in 1996, sales of botanical medicines increased by 37% over 1995 (Klink 1997). In this connection, different parts of plants, herbs and spices have been used for many years for prevention of infections. Medicinal plants might represent an alternative treatment in non severe cases of infectious diseases.

They can also be a possible source for new potent antibiotics to which pathogen strains are not resistant (Fabricant and Farnsworth 2001).

Prunus domestica (Rosaceae) commonly known as Plum Alu-Bukhara, Alucha found commonly in Pakistan, India, Afghanistan and Persia (Gupta 2003; Nadkarni 1976; Narayan and Kumar 2003). Many pharmacological activities are reported for blood circulation, measles, digestive problems, anticancer, anti-diabetes, anti-obesity, cardiovascular problems, dyspepsia, nausea, vomiting, thirst, in bilious fevers, headache, jaundice and hepatitis, leucorrhea, miscarriage, antioxidant, anti-hyperlipidemic, anxiolytic, asthma and laxative (Ferrel 1998; Qaiser and Naveed 2011; Qureshi *et al.,*1988; Soni *et al.,* 2011). The major chemical constituents present in *P. domestica* are carbohydrates, amino acids, vitamin A, vitamin B complex, vitamin K, potassium, calcium, magnesium, zinc, copper, manganese, selenium, boron and dietary, fibers, pectin, hemicellulose, cellulose, lignin. sorbitol, glucose, fructose and sucrose, malic, citric, tartaric, benzoic and boric acids, benzaldehyde, linalool, ethyl nonanoate, methyl cinnamate and γ-decalactone, benzaldehyde, 2-furancarboxyaldehyde, ethyl cinnamate, chlorogenic acid, neochlorogenic acid, caffeic acid, coumaric acid, rutin and proanthocyanidin, melanodins (Lombardi-Boccia *et al.,* 2004; Parmar *et al.,*1992; Qaiser and Naveed 2011).

Antimicrobials of plant origin have massive therapeutic potential. They are effective in treatment of many infectious diseases and minimized many of the side effects including allergic reactions, hypersensitivity and immune-suppression these are often associated with synthetic antimicrobial drugs. The medicinal effects of plant materials result from the combinations of secondary metabolites such as alkaloids, steroids, tannins and phenol compounds. These metabolites are capable of producing different physiological action in body (Joshi *et al.,* 2009).

Keeping in view the reported pharmacological and biological activities of oil components of *Prunus domestica* (Azhar Mahmood *et al.,* 2009) and medicinal importance of plants, it was planned to screen and

**Corresponding author:* e-mail: dr_hinaimran@yahoo.com

compare antibacterial activity in crude ethanol extract and different fractions of fruit part of the plant against wide range of gram positive and gram negative bacteria.

MATERIALS AND METHODS

Plant material

The dried fruit part of *Prunus domestica* was purchased from local market of Karachi, Pakistan. Properly identified by Dr. Beena Naqvi, Plant Taxonomist, Food and Marine Research Center, PCSIR Labs Complex, Karachi. Plant specimens were submitted in Herbarium bearing Voucher No. LGK-089-2010 and PDK-090-2010.

Preparation of plant extracts

Dried fruit part of *P. domstica* was washed to remove any dust particles and allow to air dry at room temperature. The dried fruit (200g) was soaked in 70% ethanol (1.5 Liter) and kept at room temperature for 3 days. After every 24hours mixture was stirred by using a sterilized glass rod. The socked material was then filtered and evaporated on rotary evaporator at 45ºC under reduced pressure. Recovered solvent was again used for percolation for another three days. The process was repeated three times to obtain residue which was lyophilized to get crude extract. Half of extract was used for further fractionation. Crude extract was mixed with 100 ml water and 100ml ethyl acetate in a separating funnel and left for 24 hours. Next day aqueous and ethyl acetate layers were collected separately. Ethyl acetate layer was evaporated to obtain ethyl acetate fraction. Aqueous layer was again treated with 100ml chloroform and the above mentioned process was repeated to obtain chloroform fraction. The crude extract and fractions were stored at 4ºC for determination of antibacterial activity (Fatima 2008).

Preparation of solution

The crude ethanol and fractioned extracts were dissolved in 6% dimethylformamide (DMF) to give strength of 40mg/ml from which further dilutions were made in the same solvent. Ampicilin was used as reference standard (positive control) in same strength/concentration while 6% DMF (Hussain *et al*., 2009; Sahu *et al.,* 2008) used as negative control.

Microorganisms used

The said activity was assessed against gram positive (*Staphylococcus aureus, Streptococcuc intermedius, Bacillus cereus, Bacillus pumilus)* and gram negative (*Eschrichia coli, Proteus mirabilis, Shigella flexneri, Salmonella typhi* and *Klebsiela pneumoniae*) microorganisms. All microorganisms used in the present study were the clinically isolated. These microorganisms were biochemically confirmed by standard method. All these organisms were inoculated on tryptic soya agar slants and stored at 4ºC.

Preparation of McFarland standard

0.5 McFarland standard was prepared by mixing 0.6ml of 1% barium chloride solution ($BaCl_2.2H_2O$) in 99.4ml of 1% sulphuric acid solution (H_2SO_4) and stored in the dark place at 25 ºC. Exactly 0.5 McFarland gives an equivalent density of bacteria 1×10^8 CFU (Chessbrough, 2000).

Inoculums preparation by direct colony suspension method

Small colonies of the test organisms were taken directly from the plates and mixed with sterile water in test tubes. The suspensions were adjusted to match the 0.5 McFarland's standard by adding distilled water (Isu and Onyeagba 2002).

Antibacterial activity assay

The antibacterial activity was assessed by agar well diffusion method (Ahmed *et al.,* 1998). According to this method 0.1 ml inoculums (10^6 CFU/ ml) of gram positive and gram negative bacteria was thoroughly mixed with 20 ml of molten sterile tryptic soya agar and poured in to pre-sterilize Petri dishes under sterile condition. All plates were left to set at 4ºC for 30-40 minutes. Holes of 6 mm diameter were made in the center of each seeded plates. Holes were then filled aseptically with 0.1 ml of test solution (crude ethanol and fractioned extracts). Standard disc of antibiotic Ampicillin (10ug) served as positive antibacterial control. Negative control was done by DMF. These plates were kept in incubator at 37ºC ± 1ºC for 24 hours. After incubation zones of inhibition for each fraction were measured by Vernier caliper.

Minimum inhibitory concentration (MIC) evaluation

The MIC was determined on crude ethanol extract, ethyl acetate and chloroform fractions of *P. domestica.* Two fold serial dilutions of all fractions were made each inoculum was prepared in nutrient broth and density was adjusted to 0.5 McFarland standards ($1x10^8$ CFU/ml). All test tubes were mixed wit 50ul of each inoculum except negative control tube. These tubes were kept in incubator at 37ºC and MIC was recorded after incubation period. The MIC is the lowest concentration of extract at which the microorganism tested does not demonstrate visible growth (Reiner 1982).

STATISTICAL ANALYSIS

The data are analyzed as mean $\pm$ S.E.M and compared by applying one way Anova Sigma Plot software version 11.0. The *p* value less than 0.5% is considered as significant.

RESULTS

The study not only gives a preliminary account of the antibacterial substances in the crude extract of *P. domestica* but also compares the activity of extract in

Table 1: Antibacterial activity exhibited by different fractions of *P. domestica* against gram positive and gram negative bacteria

Zone of Inhibition; dia. (mm)												
S. No.	Name of organisms	Ethanol Conc. mg/ml			Ethyl acetate Conc. mg/ml			Chloroform Conc. mg/ml			Ampi-cillin (mg/ml)	Negative Control (DMF)
		40	20	10	40	20	10	40	20	10		
1	*S.aureus*	14.10 ± 0.17	10.3 ± 0.26	6.1 ± 0.1	33.5 ± 0.5	31 ± 1.0	24.16 ± 0.7	25.16 ± 0.7	18.9 ± 0.17	16.5 ± 0.5	27.43 ± 0.81	8.5 ± 0.50
2	*St. intermedius*	15.16 ± 0.76	--	--	35.5 ± 0.5	29.16 ± 0.7	24 ± 1.3	11.6 ± 1.5	--	--	26.5 ± 0.5	7.1 ± 0.36
3	*B. pumilus*	17.1 ± 1.2	13.83 ± 0.2	--	36.1 ± 0.7	30.8 ± 1.4	27.33 ± 1.5	24.16 ± 0.7	20.83 ± 0.7	17.8 ± 0.28	26.16 ± 0.76	8.3 ± 0.76
4	*B. cereus*	12 ± 0.5	10 ± 0.45	--	33.33 ± .57	29.5 ± 0.5	24.16 ± 1.0	24.43. ± .0.8	20.1 ± 0.36	17.5 ± 0.5	27.86 ± 0.23	8 ± 0.50
5	*S.typhi*	19.5 ± 0.5	15 ± 0.5	12.33 ± 0.76	33.66 ± 0.7	30.10 ± 1	25.16 ± 0.7	25.16 ± 0.7	20 ± 0.5	17.16 ± 0.7	27.46 ± 0.50	7.4 ± 0.6
6	*Sh. flexneri*	21.83 ± 0.76	17.16 ± 1.0	13.33 ± 0.76	36.8 ± 1.6	31.8 ± 1.3	23.33 ± 1.5	24.16 ± 0.7	19.16 ± 0.7	17.5 ± 0.86	26.83 ± 0.76	7.8 ± 0.76
7	*K. pneumoniae*	17.10 ± 0.36	12.96 ± 0.45	--	33.3 ± 0.5	30.16 ± 0.7	23.83 ± 0.28	24.5 ± 0.5	19.33 ± 0.57	16.5 ± 0.86	25.9 ± 0.51	8.5 ± 0.50
8	*P.mirabilis*	18.06 ± 0.3	15 ± 0.5	--	35.16 ± 0.7	29.5 ± 0.5	26 ± 1.5	27.16 ± 0.7	23.16 ± 1.04	17.5 ± 0.8	27.5 ± 0.50	8.1 ± 0.76
9	*E. coli*	22 ± 0.2	16.15 ± 0.28	12.5 ± 0.40	33.8 ± 0.28	31.16 ± 1.6	23.66 ± 1.5	24.66 ± 0.7	21.33 ± 1.1	18 ± 0.5	28.16 ± 0.50	7.7 ± 0.70
	Avarage	17.42 ± 3.3	12.26 ± 5.2	7.74 ± 3.8	34.57 ± 1.3	30.28 ± 0.98	24.67 ± 1.2	23.34 ± 4.5	19.14 ± 4.02	15.38 ± 5.7	27.08 ± 0.77	7.9 ± 0.48

Table 2: Minimum inhibitory concentration of different fractions of *P. domestica*

S. No.	MIC µg/ml			
	Name of Organisms	Ethanol	Ethyl Acetate	Chloroform
1	*S. aureus*	1250	156	312
2	*St. intermedius*	2500	156	1250
3	*B. pumilus*	1250	78	312
4	*B. cereus*	1250	156	312
5	*S. typhi*	625	156	312
6	*S. flexaneriae*	625	156	312
7	*K. pneumoniae*	1250	156	312
8	*P. mirabilus*	1250	78	312
9	*E. coli*	625	156	312

different solvents. Crude ethanol extract of *P. domestica* and its fractions (ethyl acetate and chloroform) exhibited a varied degree of antimicrobial activity against some gram positive and gram negative microorganisms such as *Staphylococcus aureus, Streptococcuc intermittence, Bacillus cereus, Bacillus pumilus, Eschrichia coli, Proteus mirabilis Shigella flexneri, Salmonella typhi* and *Klebsiela pneumoniae*. The results of antimicrobial activity are presented in table 1. DMF is taken as negative control, having antibacterial activity up to 10mm zone of inhibition. Hence a zone of inhibition of 10mm and less is considered as no activity. The ethyl acetate fraction exhibited maximum antibacterial activity with zone size of 36.8±1 against *Sh. flexneri* as compared to crude ethanol extract and chloroform fraction as shown in fig. 1. Chloroform fraction and crude ethanol extract showed maximum antibacterial activity (27.16±0.7 and 22±0.2) against *P. mirabilis* and *E. coli* respectively as represented in figs. 2 and 3. The minimum inhibitory conc. (MIC) was determined as the least conc. of crude ethanol extract of *P. domestica* and its fractions (ethyl acetate and chloroform) which inhibited the growth of tested microorganisms. Among all fractions, ethyl acetate exhibited least MIC value 78ug/ml against *B. pumilus* and *P. mirabilis* represented in table 2 and fig. 4.

DISCUSSION

Herbs and different plants have been used for many thousands of years in folklore medicines therefore it is necessary to evaluate these botanicals scientifically. Plant extracts are valuable source of antibacterial agent. This study shows that plant extract inhibited bacterial growth but their effectiveness varied. The antibacterial activity

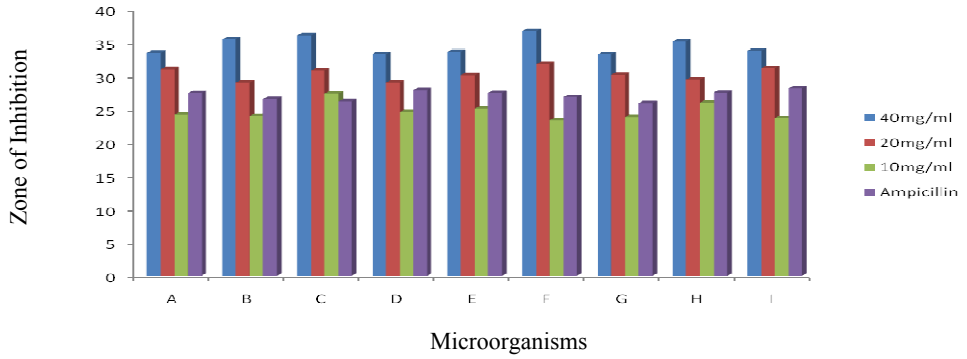

Fig. 1: Antibacterial activity of Ethyl acetate fraction of *P. domestica*
A = S.aureus, B = St. intermedius, C = B. pumilus, D = *B. cereus,* E = *S.typhi,* F = *Sh. Flexneri,* G = *K. Pneumoniae,* H = *P.mirabilis,* I = *E. coli*

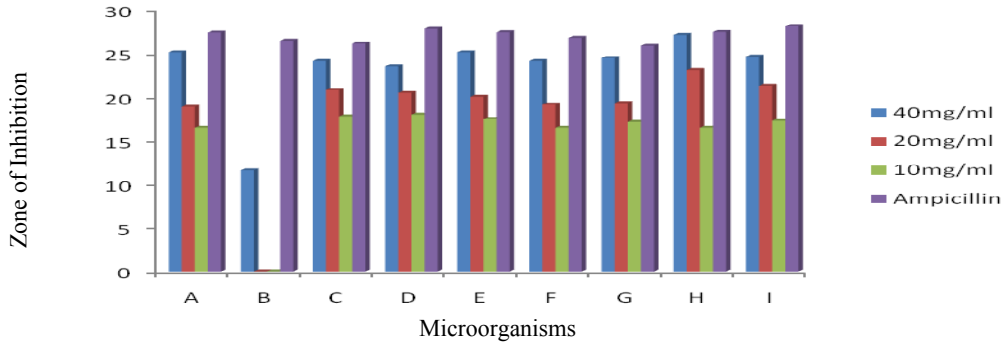

Fig. 2: Antibacterial activity of Chloroform fraction of *P. domestica.*

has been recognized due to the presence of different natural compounds including flavonoids, glycosides, abscisic acid, lignans, carotenoid, pigments, quinic acid, bipyrrole, dihydroflavonols and carbohydrates (Baranowski *et al.,* 2004; Gross and Eckhardt 1981; Kayano *et al.,* 2004; Kikuzak *et al.,* 2004; Raynal *et al.,* 1991). Herbal extracts and their constituents are hydrophobic in nature that ruptures the lipids of the bacterial cell membrane and mitochondria; due to this cell structure is changed and becomes more permeable. Extra leakage of vital molecules and ions from bacterial cells will cause the death (Joshi *et al*., 2009).

Among all fractions, ethyl acetate fraction having maximum antimicrobial activity with average zone of 34.57mm±1.3 (P<0.001) while ethanol extract showed minimum antimicrobial activity with average zone of 17.42mm ± 3.3 (P<0.05) at 40mg/ml concentration. In ethyl acetate fraction maximum activity was observed by *Sh. flexnari* (36.8mm) as shown in fig. 1. These findings confirmed by other workers who reported potent antibacterial activity against food borne pathogens (Seyhun *et al.,* 2009). These results also validate the traditional medicinal use of *P. domestica* in diarrhea, nausea, and vomiting (Said 1969). The chloroform fraction was also found effective against tested organisms with 23.34mm ± 4.5 average zone of inhibition (P<0.001) except *St. intermedius* which showed mild sensitivity (12mm) at 40mg/ml concentration. Moderate antibacterial activity was observed at 20 and 10 mg/ml with 19.28mm ± 3.4 and 15.36mm ± 5.4 average zone of inhibition respectively (P<0.05) except *St. intermedius* which was found resistant at these concentrations as presented in fig. 2.

Crude ethanol extract exhibited moderate activity with average zone of inhibition 17.42mm ± 3.3 at 40mg/ml concentration against all organisms used (P<0.05) except *E. coli* and *Sh. flexanari* which showed remarked sensitivity with inhibition zone of 22mm and 21.83mm respectively as shown in fig. 3. These results are significant (P<0.001) than control values. Mild activity was observed at 20mg/ml concentration with average zone size 12.26mm ± 5.2 against all microorganisms tested (P<0.05). At 10mg/ml concentration, no activity was observed except *S. typhi* and *Sh. flexanari* which showed mild sensitivity with 12.33mm, 13.33mm zone of inhibition respectively (P<0.05) The presence of antibacterial activity in *P. domestica* was supported by previous workers. (Mahmood *et al.,* 2009) who reported moderate antibacterial activity against salmonella group in oil fractions of *P. domestica*.

Table 2 and fig. 4 represent the minimum inhibitory

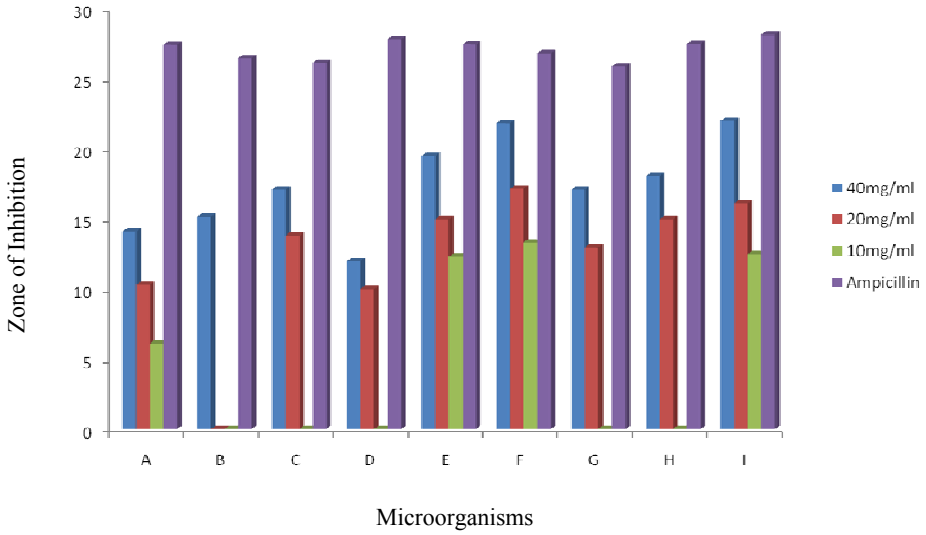

Fig. 3: Antibacterial activity of crud ethanol extracts of *P. domestica.*

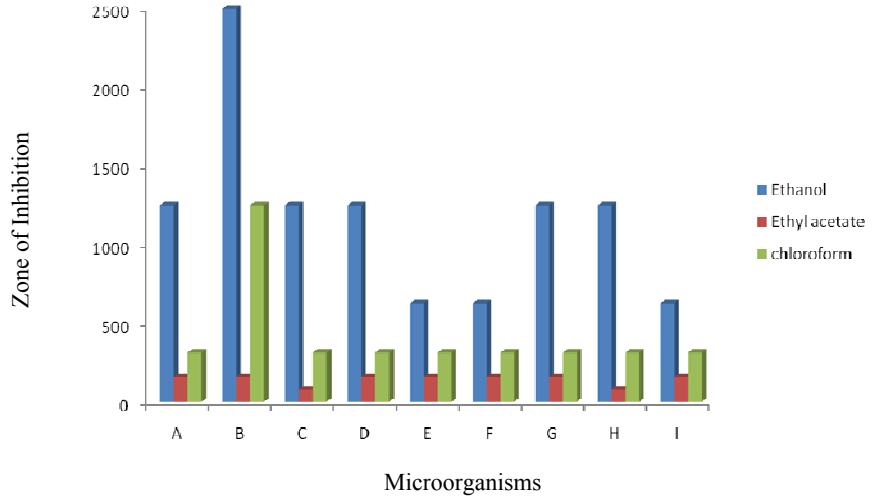

Fig. 4: Minimum inhibitory concentration of *P. domestica.*

concentration of ethanol, ethyl acetate and chloroform extracts against gram positive and gram negative bacteria. Among all fractions, least MIC value (78ug/mL) was shown by ethyl acetate fraction against *Bacilus pumilus* and *Proteaus mirabilus*. The MIC value of ethanol fraction and chloroform fraction against all microorganisms used was found to be in the range of 625-2500ug/mL and 156-312ug/ml respectively.

CONCLUSION

The crude ethanol extract along with ethyl acetate and chloroform fraction showed varying degree of antimicrobial activity against the microorganisms used. The ethyl acetate fraction was found most effective among all fractions. Antibacterial activity of ethyl acetate fraction was greater than traditional antibiotic used to treat infections caused by the pathogenic microorganisms tested. It is also concluded that selection of proper solvent can enhance the specific activity. Some new antibiotic compounds could be obtained from this plant therefore further work is required to explore the other metabolites to find antibacterial activity.

REFERENCES

Ahmed I, Mehmood Z and Mohammad F (1998). Screening of some Indian medicinal plants for their antimicrobial properties. *J. Ethanopharmacol.*, **62**: 183-193.

Alade PI and Irobi ON (1993). Antimicrobial activities of crude leaf extract of *Acalypha wilkensiana*. *J. Ethanopharmacol.*, **39**: 171-174.

Baranowski R, Kabut J and Baranowska I (2004). Analysis of mixture of catechins, flavones, flavanones, flavonols, and anthocyanidins by RP-HPLC. *Analytical Letters,* **37**: 157-165.

Chessbrough M (2000). District Laboratory Practice in Tropical Countries. Part 2, Cambridge University Press, United Kingdom, p.222.

Fabricant DS and Farnsworth NR (2001). The value of plants used in traditional medicine for drug discovery; *Environ Health Perspect Suppl*, **109**: 69-75.

Fatima N (2008). Detection of antiulcerogenic activity in *Coriandrum sativum* and *Glycyrrhiza glabr*a along with other antiulcer herbal drugs available in local market. Ph.D. Thesis, University of Karachi, Pakistan, pp.41-43.

Ferrel V (1998) Alternative cancer remedies - Facts from histories and medical researches. Pilgrims Books, USA, pp.117-130.

Gross J and Eckhardt G (1981). Structures of persicaxanthin, persicachrome and other apocarotenols of various fruits. *Phytochem*. **20**: 2267-2269.

Gupta VK (2003). Wealth of India. CSIR, New Delhi, India, pp.406-409.

Hussain AA, Mohammad AA, Ibrahim HH and Abbas AH (2009). Study the biological activities of *Tribulus Terrestris* extracts. *World Acad. Sci. Engi. and Tech*., **57**: 433-435.

Isu NR and Onyeagba RA (2002). Basic Practical in Microbiology, 2nd edition. Fasmen Communication, Okigwe, pp.25-45.

Joshi B, Lekhak S and Sharma A (2009). Antibacterial property of different medicinal plants: *Ocimum sanctum, Cinnamomum zeylanicum, Xanthoxylum armatum* and *Origanum majorana*. *Kathmandu Univ. J. Sci. Engineer. Tech.,* **5**: 143-150.

Kayano SI, Kikuzaki H, Ikami T, Suzuki T, Mitani T and Nakatani N (2004). A new bipyrrole and some phenolic constituents in prunes (*Prunus domestica* L.) and their oxygen radical absorbance capacity (ORAC). *Biosci. Biotech.* and *Bioche.,* **68**: 942-944.

Kikuzaki HS, Kayano S, Fukutsuka N, Aoki A, Kasamatsu K, Yamasaki Y, Mitani T and Nakatani N (2004). Abscisic acid related compounds and lignans in Prunes (*Prunus domestica* L.) and their oxygen radical absorbance capacity (ORAC). *J. Agric. Food Chem*., **52**: 344-349.

Klink B (1997). Alternative medicine: Is natural really better? *Drug Top*., **141**: 99-100.

Lombardi-Boccia G, Lucarini M, Lanzi S, Aguzzi A and Cappelloni M (2004). Nutrient and antioxidant molecules in yellow plums (*Prunus domestica* L.) from conventional and organic productions: A comparative study. *J. Agric. Food Chem.,* **52**: 90-94.

Mahmood A, Rehana A and Shaheen K (2009). Phytochemical screening and biological activities of the oil components of *Prunus domestica*. *J. Saud. Chem. Soc.,* **13**: 273-277.

Nadkarni KM (1976). Indian Materia Medica, Bombay Popular Prakashan, India, p.1015.

Narayan DP and Kumar U (2003). Agro's Dictionary of Medicinal Plants, 1st ed. Agrobios, Jodhpur, India, p.275

Parmar VS, Vardhan A, Nagarajan GR and Jain R (1992). Dihydroflavnoids from *P. domestica*. *Phytochemistry,* **31**: 2185-2186.

Qaiser J and Naveed A (2011). The pharmacological activities of prunes: The dried plums. *J. Med. Plants. Research*., **5**: 1508-1511.

Qureshi IH, Yaqeenuddin, Yaqeen Z, Mirza M and Qureshi S (1988). Evaluation of antiemetic action of *P. domestica* Linn. *Pak. J. Sci. Ind. Res.*, **31**: 774-776.

Raynal J, Mourgues J and Conte T (1991). Pectic substances from plum fruits (*Prunus domestica L.*) cultivar d'ente: Fractional extraction and general characteristics. Lebensmittel-Wissenschaft und – *Technologie France,* **24**: 263-265.

Reiner R (1982). Detection of antibiotics activity: In: Antibiotics: An Introduction, Roche Scientific Service, Switzerland, pp.21-25.

Sahu SK, Banerjee M, Samantray A, Behera C and Azam MA (2008). Synthesis, analgesic, anti-inflammatory and antimicrobial activities of some novel pyrazoline derivatives. *Tropi. J. Pharma. Res*., **7**: 961-963.

Said HM (1969) Hamdard Pharmacopeia of Eastern Medicine, Hamdard National Foundation, Karachi, pp.170-172.

Seyhun Y, Yurdugul and Faruk B (2009). Studies on antimicrobial activity and certain chemical parameters of freeze-dried plums. *Pak. J. Nutr*., **8**: 1434-1441.

Soni M, Mohanty PK and Jaliwala YA (2011). Hepatprotective activity of fruits of *P. domestica. Int. J. Pharma. and Bio. Sci*., **2**: 439-453.