ISCA Journal of Biological Sciences _____ ISSN 2278-3202
Vol. **1(2),** 32-37, June **(2012)** *ISCA J. Biological Sci.*

Evaluation of Antimicrobial Properties, Phytochemical Contents and Antioxidant Capacities of Leaf Extracts of *Punica granatum* L.

Hegde Chaitra R.[1], M. Madhuri[1], Swaroop T. Nishitha[1], Das Arijit[1*], Bhattacharya Sourav[1] and K.C. Rohit[2]
[1]Dept. of Microbiology, A Div. of Centre for Advanced Studies in Biosciences, Jain University, Bangalore, INDIA
[2]Sapthagiri College of Engineering, Bangalore, INDIA

Available online at: www.isca.in
(Received 21st May 2012, accepted 26th May 2012)

Abstract

The numerous side effects associated with the use of allopathic drugs have led to renewed level of interest in Ayurvedic medicines. Punica granatum L. is a fruit-bearing deciduous shrub, belonging to the family Lythraceae and is cultivated throughout Asia, Middle-East and the Mediterranean region. The shrub has been known to possess several medicinal and curative properties. The present investigation focuses on antimicrobial properties, phytochemical analysis and antioxidant potential of leaf extracts of Punica granatum L. The methanolic extract inhibited Staphylococcus aureus, Bacillus cereus, Salmonella typhi and Proteus mirabilis, whereas, the chloroform, ethyl acetate and aqueous extracts exhibited moderate inhibitory effects against the test bacteria. On the other hand, only methanolic extract demonstrated antifungal activity against Aspergillus niger, Aspergillus flavus, Trichophyton rubrum, Candida albicans and Cryptococcus sp. *The phytochemical screening of the methanolic extract of the leaves revealed the presence of carbohydrates, reducing sugars, sterols, glycosides, phenolics, tannins, flavonoids, proteins and saponins, whereas, gums were not detected. Total antioxidant potential of the methanolic and aqueous extracts were found as 2.26 and 1.06 mg of ascorbic acid equivalent per ml of the extract, respectively. The results indicated that the methanolic extract of the leaves are pharmacologically more active than the other extracts.*

Keywords: *Punica granatum* L., antimicrobial, phytochemical analysis, antioxidant potential.

Introduction

For thousands of years, the practice of Ayurvedic medicine has alleviated illnesses and attributed overall positive health[1]. The Indian subcontinent has a rich flora of various plants used in traditional medical treatments[2]. These plants contain different bioactive ingredients used to cure diseases or relieve pain[3]. The medicinal properties of these plants could be based on the antioxidant, antimicrobial, antipyretic and/or analgesic effects of different phytochemicals present in them[4]. Recently, the side effects associated with the use of allopathic drugs have resulted in an increased demand for the phytopharmaceutical products of Ayurveda[1].

Punica granatum L., commonly known as pomegranate, is a fruit-bearing deciduous shrub or small tree, native to Asia and belongs to the family Lythraceae[5]. The leaves are shiny and about 7.6 cm long[6]. Different parts of the plant such as bark, leaves, immature fruits and fruit rind have medicinal significance[7]. *P. granatum* has been extensively used as a traditional medicine in many countries for the treatment of dysentery, diarrhea, helminthiasis, acidosis, hemorrhage and respiratory pathologies[8]. Additionally, this plant is reported to have excellent antibacterial, antifungal, antiprotozoal and antioxidant properties[9-11]. Numerous phytochemical constituents have been reported to be present in different parts of the pomegranate plant making it pharmacologically precious[12].

Therefore, the present study was undertaken with the objectives to assess the antimicrobial properties, phytochemical contents and antioxidant capacities of the leaf extracts of *P. granatum* L.

Material and Methods

Source of Plant Material: Leaves of pomegranate plant (*Punica granatum* L.) was obtained from the local vendors of *K.R. Market*, Bangalore, India.

Preparation of Leaf Extracts: The leaves of the plant were carefully removed and thoroughly washed with distilled water to remove dust particles. They were dried in shade and finely powdered using an electric blender. Five grams of powdered material was subjected to cold extraction with chloroform, ethyl acetate, methanol and water separately. The extracts were centrifuged at 5000 rpm for 30 min at 4˚C and evaporated to dryness under controlled temperature (35-40˚C). Each residue was reconstituted with 25 ml of respective solvent. The extracts were stored in air tight containers under refrigeration. These extracts were used for antimicrobial assay, phytochemical analysis and antioxidant properties.

Source of Microorganisms: The test bacterial pathogens included *Staphylococcus aureus, Bacillus cereus, Escherichia coli, Proteus mirabilis* and *Salmonella typhi.* The test fungal pathogens comprised of *Aspergillus niger, Aspergillus flavus,*

Trichophyton rubrum, Candida albicans and *Cryptococcus* sp. All the bacterial and fungal pathogens were clinical isolates obtained from the Department of Microbiology, Genohelix Biolabs, Bangalore.

Assay of Antibacterial activity: Antibacterial activities of the extracts were studied by agar well diffusion method[13]. Test cultures of the bacterial pathogens were prepared by transferring a loop full of bacteria from nutrient agar slants into Mueller Hinton broth and incubated at 37˚C. Lawn cultures of the test pathogens were prepared by swabbing sterile Mueller Hinton agar plates with 24 hrs old bacterial broth. Wells were punched with a sterile cork borer (6 mm internal diameter) and 35 µl of the extract was added to each well. Controls were maintained with respective solvents. Ampicillin and streptomycin (50 mg/ml) were used as standard antibiotics for gram positive and gram negative bacteria, respectively. Following incubation at 37˚C for 24 hrs, diameters of the inhibitory zones were measured to the nearest millimeter.

Assay of Antifungal activity: Antifungal activities of the extracts were studied by agar well diffusion method. Suspensions of fungal pathogens were prepared by transferring a loop full of fungi from Sabouraud dextrose agar slants into Sabouraud dextrose broth. Lawn cultures of the test pathogens were prepared by swabbing sterile Sabouraud dextrose agar plates with the fungal suspensions. Wells were punched with a sterile cork borer (6 mm internal diameter) and 35 µl of the extract was added to each well. Controls were maintained with respective solvents. Fluconazole (20 mg/ml) was used as the standard antifungal. Following incubation at 27˚C for 48 hrs, diameters of the inhibitory zones were measured to the nearest millimeter.

Phytochemical Assessment of the Leaf Extract: Qualitative screening for the presence of various phytochemical compounds was performed using the methanolic extract. Presence of carbohydrates and reducing sugars was determined by Molish's test, Benedict's test and Fehling's test[14]. Presence of glycosides was detected by Borntrager's test[15]. Alkaloids in the extracts were evaluated by Mayer's test. The presence of phytosterols was indicated by Salkowski's test. Deoxy sugars were detected by Killer Kiliani's test. The saponins were analyzed by Froth's test[16]. The occurrence of phenolic compounds and tannins were confirmed by ferric chloride test and gelatin test, respectively[17]. The presence of flavonoids was investigated by lead acetate test. The occurrence of amino acids in the extract was assessed by Ninhydrin's test[18] while the possibility of gums was studied by conducting borax test.

Determination of Total Antioxidant Capacity: The total antioxidant capacities of the aqueous and methanolic extracts were determined by the phosphomolybdenum assay[19], based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate-Mo (V) complex in acidic condition. 0.1 ml of each extract was combined with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The reaction mixture was incubated at 95ºC for 90 min. After cooling to room temperature, the absorbance of the solution was measured at 695 nm using a UV-visible spectrophotometer (Sanyo Gallenkamp, Germany). 0.1 ml methanol was used as the blank. The total antioxidant capacity was expressed as the number of gram equivalents of ascorbic acid per ml of the extract.

Results and Discussion

Medicinal plants have always been the sources of biologically active compounds used for the treatment of various infectious diseases[20, 21]. *P. granatum* L. is widely grown in many tropical and subtropical countries[22]. For centuries, this plant has been used in traditional medicine in Middle East, Asia and Africa for the treatment of various diseases[23].

Antibacterial activity of Leaf Extracts: Chloroform, ethyl acetate, methanolic and aqueous extracts of the leaves of *P. granatum* L. were tested against several gram positive and gram negative bacterial pathogens. The chloroform and ethyl acetate extracts exhibited maximum inhibition against *S. aureus* (10 ± 0.05 mm and 16 ± 0.20 mm, respectively). The methanolic extract was found to exhibit better inhibition against the gram positive bacteria than the gram negative ones, with the highest inhibitory zone against *B. cereus* (30 ± 0.32 mm). The significant results of the antibacterial activities of the leaf extracts have been clearly presented in table 1. In a previous study, pomegranate extract at a higher concentration (1%, v/v) was found to completely inhibit the growth of *Staphylococcus aureus* FRI 722 and subsequent enterotoxin production[24]. The effectiveness of the pericarp extract of *P. granatum* against bovine strains of *S. aureus* has been reported earlier[25]. The ethyl acetate extract revealed a zone of 16 ± 0.20 mm in the present study, whereas, the highest activities were previously found in the ethyl acetate fraction when tested against different *S. aureus* strains[26]. The peel ethanol extract of pomegranate has also been found to inhibit different species of *Salmonella* including *S. typhi* ATCC 19943, *S. paratyphi* A, *S. enteritidis* and *S. typhimurium* with inhibitory zones ranging between 9.6 ± 0.5 mm to 18.6 ± 1.1 mm[8].

On the other hand, the aqueous leaf extract was moderately effective against *B. cereus* and *E. coli*. Mathabe *et al*.[27] reported that methanol, ethanol, acetone and water extracts obtained from pomegranate were active and effective against the tested microorganisms (*S. aureus, E. coli, S. typhi, Vibrio cholerae, Shigella dysenteriae, S. sonnei, S. flexneri, S. boydii*), showing inhibition zones between 12-31 mm.

Table-1
***In vitro* antibacterial activities of the leaf extracts of *Punica granatum* L. showing diameters of the inhibitory zones (in mm)**

Bacterial pathogens	E1	E2	E3	E4	Antibiotic
S. aureus	10 ± 0.05*	16 ± 0.20	28 ± 0.24	-	42 ± 0.12[a]
B. cereus	9 ± 0.05	-	30 ± 0.32	10 ± 0.00	-
E. coli	-	-	13 ± 0.10	8 ± 0.05	25 ± 0.15[s]
P. mirabilis	-	-	15± 0.14	-	-
S. typhi	8 ± 0.05	7 ± 0.05	14 ± 0.18	-	20 ± 0.21[s]

Keys: E1=chloroform extract; E2=ethyl acetate extract; E3= methanolic extract; E4=aqueous extract; *=mean of triplicate; ± standard deviation; [a]ampicillin; [s]streptomycin; -=no zone

Antifungal activity of Leaf Extracts: The results of antifungal study revealed that only the methanolic extract of pomegranate leaves effectively inhibited the phytopathogenic molds and the dermatophytes. The activity of methanolic extract against *A. niger* has been reported earlier to be between 8.0 and 23 mm[9]. The highest zone was obtained against *C. albicans* (17 ± 0.22 mm). A very similar result was observed in a study which reported the antifungal activity of methanolic leaf extract against *C. albicans* with a zone diameter of 15 mm[28]. The significant results of the antifungal activities of the leaf extracts have been presented in table 2. Another study reported the antifungal activity of gallagic acid and punicalagin, isolated from pomegranate peel extract, against *Cryptococcus neoformans* with IC_{50} values lower than 15 µg/ml[29].

Table-2
***In vitro* antifungal activities of the leaf extracts of *Punica granatum* L. showing diameters of the inhibitory zones (in mm)**

Fungal pathogens	E1	E2	E3	E4	Fluconazole
A. niger	-	-	10 ± 0.05*	-	14 ± 0.34
A. flavus	-	-	12 ± 0.14	-	23 ± 0.26
T. rubrum	-	-	15 ± 0.07	-	22 ± 0.38
C. albicans	-	-	17 ± 0.22	-	21 ± 0.23
Cryptococcus sp	-	-	14 ± 0.08	-	22 ± 0.13

Keys: E1=chloroform extract; E2=ethyl acetate extract; E3= methanolic extract; E4=aqueous extract; *=mean of triplicate; ± standard deviation; -=no zone

Phytochemical assessment of Leaf Extract: In general, plants produce phytoalexins as a defensive tool in response to microbial invasion[30]. In this context, detailed research on the phytochemistry and pharmacology of traditionally valued plant products is essential as this may lead to the discovery of new medicine of therapeutic importance. Different phytochemical tests were conducted with the methanolic extract of the leaves of pomegranate since better antimicrobial properties have been found to be associated with the methanolic extract. Our study revealed the presence of carbohydrates, reducing sugars, deoxy sugars, sterols, glycosides, phenolic compounds, tannins, saponins and flavonoids, as outlined in table 3. Similar findings have been reported by other workers[31,32]. The antimicrobial action of the methanolic extract of the leaves of this plant may be attributed to the presence of numerous bioactive compounds in them. A recent work on the phytochemical analysis has reported the presence of tannins (punicalin and punicafolin), flavonoids, glycosides, including luteolin and apgenin in the leaves of pomegranate[7]. The occurrence of gallotannins in the leaves has also been reported. According to a previous report the antibacterial activity of pomegranate is due to the presence of tannins such as ellagitannins and flavonoids[33]. Tannins have been reported to prevent the microbial growth by precipitating microbial proteins[34]. Secondary metabolites like flavonoids are synthesized by plants in response to microbial infection[35]. The growth of many molds, yeasts, bacteria and viruses are inhibited by tannins. On the contrary, amino acids and gums were not detected in the methanolic extract in our study.

Table-3
Analysis of phytochemical contents of the methanolic extract of *Punica granatum* L. leaves

Phytochemical Tests	Compounds Detected	E1
Molish's test	Carbohydrates	+
Benedict's test	Reducing sugar	+
Fehling's test	Reducing sugar	+
Mayer's test	Alkaloids	-
Salkowski's test	Sterols	+
Killer Kiliani's test	Deoxy sugars	+
Borntrager's test	Glycosides	+
Froth's test	Saponins	+
Ferric chloride test	Phenolic compounds	+
Gelatin test	Tannins	+
Lead acetate test	Flavonoids	+
Ninhydrin's test	Amino acids	-
Borax test	Gums	-

Keys: E1=methanolic extract of the leaves; +=positive; -= negative

Total Antioxidant Capacities of the Leaf Extracts: The term antioxidant denotes a compound which can delay or inhibit the oxidation of biomolecules by inhibiting the initiation or propagation of oxidative chain reactions and thus prevents damage done to the body's cell by oxygen, i.e. reactive oxygen species (ROS). Researches on antioxidants reveal that many

phytonutrients, particularly phenolic compounds, may protect the human body against damage caused by ROS. Regular consumption of fresh fruits and vegetables has been reported to have potential health benefits due to the presence of a wide variety of antioxidant phenolic compounds in them[36]. Antioxidant (mainly phenolic) compounds from plant extracts can act by either free radical scavenging, singlet oxygen quenching, chelating of transitional metal such as iron, as well as reducing agents and activator of antioxidative defence enzyme systems to suppress radical damage in biological system[37]. Apart from promoting good health, antioxidants have been widely used in food industry to increase the shelf life of foods. Synthetic antioxidants such as butylated hydroxy toluene (BHT) and butylated hydroxy anisole (BHA) are widespread food additives used to prevent deterioration; however their use is increasingly restricted due to their potential health risks and toxicity. Moreover, there is a growing awareness among consumers regarding the safety of food additives[38]. Therefore, different plant extracts are being investigated as source of safe, cheap and effective antioxidants. In the present study, the total antioxidant potential of the methanolic and aqueous leaf extracts was found as 2.26 and 1.06 mg ascorbic acid equivalent per ml of the extract, respectively. Antioxidant study conducted previously on *P. granatum* suggests a high value (96%) of antiradical activity in 50 µg/ml methanolic extract. Furthermore, in the same study, the total flavonoids and the total phenolic content of the leaf methanolic extract of *P. granatum* were evaluated as 54.360 mg rutin equivalent/g extract and 242.26 mg tannic acid equivalent/g extract, respectively[11].

Conclusion

Among the various solvents used in this study, the methanolic extract of *Punica granatum* leaves has been found to possess good antibacterial and antifungal properties against some bacterial pathogens and dermatophytic yeasts, respectively. The extract is also rich in various phytochemical components. Furthermore, due to the presence of good antioxidant potential in this plant it is suggested that pomegranate may be included in the diet for a healthy lifestyle.

Acknowledgement

We wish to extend our sincere gratitude to Dr. R. Chenraj Jain, Chairman, Jain Group of Institutions, Bangalore; Dr. N. Sundararajan, Vice-Chancellor of Jain University, Bangalore; Prof. K. S. Shantamani, Chief Mentor, JGI and Dr. S. Sundara Rajan, Director of Genohelix Biolabs, A Division of Centre for Advanced Studies in Biosciences, Jain University, for providing us with the laboratory facilities required for this research work. We are also thankful to the entire supporting staff of the laboratory whose help has been invaluable for the successful completion of our research work.

References

1. Samy R.P., Pushparaj P.N. and Gopalakrishnakone P.A., Compilation of bioactive compounds from Ayurveda, *Bioinformation*, **3,** 100–110 **(2008)**

2. Ballabh B. and Chaurasia O.P., Traditional medicinal plants of cold desert Ladakh--used in treatment of cold, cough and fever, *J. Ethnopharmacol.,* **112(2),** 341-349 **(2007)**

3. Okigbo R.N., Eme U.E. and Ogbogu S., Biodiversity and conservation of medicinal and aromatic plants in Africa, *Biotechnol. Mol. Biol. Rev.*, **3(6),** 127-134 **(2008)**

4. Adesokan A.A., Yakubu M.T., Owoyele B.V., Akanji M.A., Soladoye A. and Lawal O.K., Effect of administration of aqueous and ethanolic extracts of *Enantia chlorantha* stem bark on brewer's yeast-induced pyresis in rats, *Afri. J. Biochem. Res.,* **2(7),** 165-169 **(2008)**

5. Altuner E.M., Investigation of antimicrobial activity of *Punica granatum* L. fruit peel ash used for protection against skin infections as folk remedies especially after male Circumcision, *Afr. J. Microbiol Res*., **5(20),** 3339-3342 **(2011)**

6. Qnais E.Y., Elokda A.S., Abu Ghalyun Y.Y. and Abdulla F.A., Antidiarrheal activity of the aqueous extract of *Punica granatum* (Pomegranate) peels, *Pharm. Biol*., **45(9),** 715–720 **(2007)**

7. Arun N. and Singh D.P., *Punica granatum*: a review on pharmacological and therapeutic properties, *IJPSR*, **3(5),** 1240-1245 **(2012)**

8. Choi J.G., Kang O.H., Lee Y.S., Chae H.S., Oh Y.C., Brice O.O., Kim M.S., Sohn D.H., Kim H.S., Park H., Shin D.W., Rho J.R. and Kwon D.Y., In vitro and in vivo antibacterial activity of *Punica granatum* peel ethanol extract against *Salmonella*, *Evid. Based Complement, Alternat. Med.,* 1-8 **(2011)**

9. Dahham S.S., Ali M.N., Tabassum H. and Khan M., Studies on antibacterial and antifungal activity of pomegranate (*Punica granatum* L.), *American-Eurasian J. Agric. and Environ. Sci.,* **9(3),** 273-281 **(2010)**

10. Inabo H.I. and Fathuddin M.M., In vivo antitrypanosomal potentials of ethyl acetate leaf extracts of *Punica granatum* against *Trypanosoma brucei brucei,* Adv. Agr. Bio., **1,** 82-88 **(2011)**

11. Moussa A.M., Emam A.M., Diab Y.M., Mahmoud M.E. and Mahmoud A.S., Evaluation of antioxidant potential of 124 Egyptian plants with emphasis on the action of *Punica granatum* leaf extract on rats, *IFRJ*, **18,** 535-542 **(2011)**

12. Prakash C.V.S. and Prakash I., Bioactive chemical constituents from pomegranate (*Punica granatum*) juice, seed and peel-a review, *Int. J. Res. Chem. Environ.,* **1(1),** 1-18 **(2011)**

13. Bauer A.W., Kirby W.M.M., Sherris J.C. and Turck M., Antibiotic susceptibility testing by a standardized single disk method, *Amer. J. Clin. Pathol.*, **45,** 493-496 **(1966)**

14. Ramkrishnan S. and Rajan R., Text Book of Medical Biochemistry, Orient Longman, New Delhi, India **(1994)**

15. Evans W.C., Trease and Evans Pharmacology, (14th Edn), Harcourt Brace and Company, Asia. Pvt. Ltd., Singapore **(1997)**

16. Kokate C.K., Practical Pharmacognosy, (4th Edn), Vallabh Prakashan Publication, New Delhi, India **(1999)**

17. Mace M.E., Histochemical localization of phenols in healthy and diseased tomato roots*, Phytochem.,* **16,** 915-925 **(1963)**

18. Yasuma A. and Ichikawa T., Ninhydrin-Schiff and alloxan-Schiff staining; a new histochemical staining method for protein, *J. Lab. Clin. Med.,* **41(2),** 296–299 **(1953)**

19. Prieto P., Pineda M. and Aguilar M., Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: specific application to the determination of vitamin E, *Anal. Biochem.,* **269,** 337-341 **(1999)**

20. Dev S., Ethnotherapeutics and modern drug development: the potential of Ayurveda, *Curr. Sci.,***73(11),** 909-928 **(1997)**

21. Cox P.A. and Balick M.J., The ethnobotanical approach to drug discovery, *Sci. Am.,* **270,** 60-65 **(1994)**

22. Yasoubi P., Barzegar M., Sahari M.A. and Azizi M.H., Total phenolic contents and antioxidant activity of pomegranate (*Punica granatum* L.) peel extracts, *J. Agric. Sci. Technol.,* **9,** 35-42 **(2007)**

23. Rathinamoorthy R., Udayakumar S. and Thilagavathi G., Antibacterial efficacy analysis of *Punica granatum* L. leaf, rind and *Terminalia chebula* fruit extract treated cotton fabric against five most common human pathogenic bacteria, *Int. J. of Pharm. and Life Sci.,* **2(10),** 1147-1153 **(2011)**

24. Braga L.C., Leite A.A., Xavier K.G., Takahashi J.A., Bemquerer M.P., Chartone-Souza E. and Nascimento A.M., Synergic interaction between pomegranate extract and antibiotics against *Staphylococcus aureus*, *Can. J. Microbiol.*, **51,** 541-547 **(2005)**

25. Silva M.A.R., Higino J.S., Pereira J.V., J.P. Siqueira-Júnior and M.S.V. Pereira, Antibiotic activity of the extract of *Punica granatum* Linn, Over bovine strains of *Staphylococcus aureus, Brazilian J. Pharmacog*., **18(2),** 209-212 **(2008)**

26. Machado T.B., Pinto A.V., Pinto M.C.F.R., Leal I.C.R., Silva M.G., Amaral A.C.F., Kuster R.M. and Netto-dosSantos K.R., In vitro activity of Brazilian medicinal plants, naturally occurring naphthoquinones and their analogues, against methicillin-resistant *Staphylococcus aureus*, *Int. J. Antimicrob. Agents***, 21,** 279-284 **(2003)**

27. Mathabe M.C., Nikolova R.V., Lall N. and Nyazema N.Z., Antibacterial activities of medicinal plants used for the treatment of diarrhoea in Limpopo Province, South Africa, *J. Ethnopharmacol*., **105,** 286-293 (**2005)**

28. Elmanama A.A., Alyazji A.A. and Gheneima N.A.A., Antibacterial, antifungal and synergistic effect of *Lawsonia inermis*, *Punica granatum* and *Hibiscus sabdariffa, Annals of Alquds Medicine,* **7,** 33-41 **(2011)**

29. Reddy M.K., Gupta S.K., Jacob M.R., Khan S.I. and Ferreira D., Antioxidant, antimalarial and antimicrobial activities of tannin-rich fractions, ellagitannins and phenolic acids from *Punica granatum* L., *Planta Med.,* **73,** 461- 467 **(2007)**

30. Glazebrook J. and Ausubel F.M., Isolation of phytoalexin-deficient mutants of *Arabidopsis thaliana* and characterization of their interactions with bacterial pathogens, *Proc. Natl. Acad. Sci. USA*, **91(19),** 8955–8959 **(1994)**

31. Egharevba H.O., Kunle O.F., Iliya I., Orji P.N., Abdullahi M.S., Okwute S.K. and Okogun J.I., Phytochemical analysis and antimicrobial activity of *Punica granatum* L. (fruit bark and leaves), *New York Science Journal*, **3(12),** 91-98 **(2010)**

32. Jain P. and Nafis G., Antifungal activity and phytochemical analysis of aqueous extracts of *Ricinus communis* and *Punica granatum, JPR,* **4(1),** 128-129 **(2011)**

33. Wang R., Ding Y., Liu R., Xiang L. and Du L., Pomegranate: Constituents, bioactivities and pharmacokinetics, *Fruit, Veg. Cereal Sci. Biotech.,* **4(2),** 77-87 **(2010)**

34. Prasad R.N., Viswanathan S., Devi J.R., Nayak V., Swetha V.C., Archana B.R., Parathasarathy N. and Rajkumar J., Preliminary phytochemical screening and antimicrobial activity of *Samanea saman*, *J. Med. Plants Res.*, **2,** 268-270 **(2008)**

35. Jamine R., Daisy P. and Selvakumarb B.N., *In vitro* efficacy of flavonoids from *Eugenia jambolana* seeds against ESAYL-producing multidrug-resistant enteric bacteria, *Res. J. Microbiol.*, **2,** 369-374 **(2007)**

36. Sumino M., Sekin T., Ruanagrungsi N., Igarashi K. and Ikkegami F., Ardisiphenols and other antioxidant principles from the fruits of *Ardisia colorata*, *Chem. Pharm. Bull*., **50,** 1484-1487 **(2002)**

37. Lodovici M., Guglielmi F., Meoni M. and Dolara P., Effect of natural phenolic acids on DNA oxidation *in vitro*, *Food Chem. Toxicol.*, **39(12),** 1205-1210 **(2001)**

38. Valentao P., Fernandes E., Carvalho F., Andrade P.B., Seabra R.M. and Bastos M., Antioxidative properites of carbon *Cynara cardunculus* L. infusion against superoxide radical hydroxy radical and hypochlorous acid, *J. Agric. Food Chem*., **50,** 4989-4993 **(2002)**