



Research Journal of
**Medicinal
Plant**

ISSN 1819-3455



Academic
Journals Inc.

www.academicjournals.com

Antioxidant and Anti-Ulcerogenic Activity of Wild *Punica granatum* Ethanolic Seed Extract

¹N.S. Gill, ¹S. Dhawan, ¹A. Jain, ¹R. Arora and ²M. Bali

¹Rayat School of Pharmacy, S.B.S. Nagar, Punjab Technical University, Ropar-144533, India

²Quest Group of Institutes, Jhanjeri, Punjab Technical University, Mohali, India

Corresponding Author: Naresh Singh Gill, Rayat School of Pharmacy, SBS Nagar, Punjab Technical University, Ropar-144533, India Tel: +91-8146991679

ABSTRACT

These seeds have been traditionally used for various diseases like dysentery, diarrhoea, piles, keeping in mind of their traditional value our present study was to explore wild *Punica granatum* seeds for its antioxidant and antiulcer potential. The phytochemical screening of the ethanolic seed extract indicates the presence of triterpenoid, sterols, alkaloids, flavonoids, tannins and coumarin glycoside. Antioxidant activity of the wild *Punica granatum* ethanolic seed extract was measured by 1,1-diphenyl-2-picrylhydrazyl and hydrogen peroxide free radical scavenging method. Further it was evaluated for its anti-ulcer activity by pyloric ligation model in wistar albino rats. Gastric volume, ulcerative index and free and total acidity were measured in this model. The extract showed maximum scavenging activity i.e., 82.80 ± 0.267 and 74.91 ± 0.235 at $100 \mu\text{g mL}^{-1}$ by 1,1-diphenyl-2-picrylhydrazyl and hydrogen peroxide method respectively as compared to the standard (ascorbic acid) in case of quantitative analysis and showed yellow coloration with 1,1-diphenyl-2-picrylhydrazyl scavenging radical in case of qualitative analysis. In pyloric ligation method, the extract showed optimum percentage inhibition of 74.51% at $100 \mu\text{g mL}^{-1}$ which was comparable to the standard (ranitidine). The present study concludes that wild *Punica granatum* ethanolic seed extract possesses significant antioxidant and anti-ulcer activities.

Key words: *Punica granatum*, antioxidant activity, 1,1-diphenyl-2-picrylhydrazyl, antiulcer activity, pyloric ligation induced ulcer

INTRODUCTION

Nature has given a complete storehouse of remedies to mankind for the treatment of various diseases (Makhija *et al.*, 2011). Plants provide a source of inspiration for the novel drug compounds. They play a vital role in the development of new drugs as they become the base for the medicines of plant origin and have made large contributions to human health and well being (Priyadarsini *et al.*, 2010). The natural plant medicine is the mainstay of about 75-80% of the world's population for their primary healthcare as it has better compatibility with the humans with lesser side effects (Jayaraj, 2010). After following ethno medicinal use of the plants, it has been discovered that 14-28% of higher plant species are use medicinally and 74% are pharmacologically active plant derived components (Das *et al.*, 2010). The information and knowledge of medicinal and traditional plants has been gathered and accumulated in different medicinal systems such as Ayurveda, Unani and Siddha. In India, 2500 plant species are used as traditional healers and

around 100 species of plants are regular sources of medicine. There is a great demand for natural medicines even in this modern era. It continuously provides mankind with new remedies and lead them with a disease free and healthy life (Muthu *et al.*, 2006). The excess of free radicals in humans causes various diseases, such as atherosclerosis, Parkinson's disease, heart failure, myocardial infarction, Alzheimer's disease (Rakesh *et al.*, 2010).

An antioxidant is a substance capable of preventing the oxidation of other molecules (Bhalodia *et al.*, 2011). Oxidation reactions produce free radicals which start chain reactions that damages cells. By removing free radical intermediates the antioxidants terminate these chain reactions (Hamid *et al.*, 2010). Antioxidants prevent tissue damage in various human diseases (Khanahmadi *et al.*, 2010). As plant based antioxidants are safe so they are preferred over synthetic ones (Chanda *et al.*, 2011).

The presence of free radicals further leads to ulceration. Ulcer is caused by the disruption of the gastric mucosal defense and repair systems (Gregory *et al.*, 2009). It is among the major disease of gastrointestinal tract. The plant derived medicines are more popular as they have lesser adverse effects (Gupta *et al.*, 2010).

Wild *Punica granatum* belonging to family Punicaceae possesses various pharmacological activities (Yoganandam *et al.*, 2010). In traditional medicine, this plant has been used for the treatment of various diseases. The ripe fruit is used as tonic, astringent, laxative, diuretic. Bark, leaves and fruit rind are used to prevent dysentery, diarrhoea, piles (Bagri *et al.*, 2010). Its flowers are used in the treatment of stomach ache. Fruit juice is used to cure edema, leprosy, cough (Al-Yahya, 2005). The main attention was given to bark, rind, flowers and leaves but the seed part has not yet explored. Thus, the present study was conducted to evaluate the wild *Punica granatum* seeds as therapeutic agent.

MATERIALS AND METHODS

Plant material: Wild *Punica granatum* seeds were purchased on 1st September 2010 from Parwanoo (HP) India. The healthy looking seeds were authenticated from Botanical and Environmental Science Department, Guru Nanak Dev University, Amritsar (Punjab) and the voucher specimen number is 0394/Herb. The seeds were cleaned, washed, dried and carefully grinded as a coarse powder under favourable conditions with temperature not exceeding 20°C and were kept in tightened light-protected containers.

Extraction: The powdered seeds were extracted with ethanol by simple maceration process for 24 h. The solvent was completely removed using rotary evaporator at 40-45°C under reduced pressure. The crude extract was defatted with hexane. Extract obtained was stored in the refrigerator at 0-4°C to prevent any degradation. This extract was used for various investigations such as antioxidant and antiulcer activity.

Phytochemical screening: The wild *Punica granatum* ethanolic seed extract was subjected to different phytochemical investigations for the presence of various constituents such as alkaloids, carbohydrates, flavonoids, proteins, saponins, sterols, triterpenoids, coumarin glycosides, anthraquinones and tannins according to standard procedures (Gill *et al.*, 2011a).

Drugs and chemicals: 1,1-diphenyl-2-picrylhydrazyl and hydrogen peroxide were obtained from Sigma Chemical Co. Ranitidine as a free sample was obtained from Ranbaxy Laboratories,

Gurgaon. The other solvents and chemicals like hexane, chloroform, ethanol, sodium hydroxide and Topfer's reagent were of analytical grade and purchased from SD Fine Chem Limited, Mumbai.

Animals: The animal study was carried out from 1st Feb-10th May 2011. Wistar albino rats were divided into six groups, each group comprising six rats (36 animals) in the size of 150-250 g of either sex purchased from Punjab Agriculture University, Ludhiana. The animals were acclimated to standard animal house conditions and maintained under day/night cycles with an average temperature of $24.0 \pm 1.0^\circ\text{C}$ and relative humidity 55-65%. They were allowed free access to standard dry pellet diet (Hindustan Lever, India). The experimental protocol was approved by the IAEC (Institutional Animal Ethical Committee) of CPCSEA, (Committee for the Purpose of Control and Supervision of Experiments on Animal) with registration no. 874/ac/05/CPCSEA.

Qualitative scavenging activity on 1,1-diphenyl-2-picrylhydrazyl radical: The wild *Punica granatum* ethanolic seed extract (2 mg) was diluted with 1 mL of the solvent, then small quantity of the diluted extract was carefully loaded individually on TLC plates (20×10 cm). These were allowed to dry. Hexane-ethyl acetate (9.5:0.5) was used as mobile phase. Once dried, the plates were sprayed with 0.2% solution of radical-DPPH (1,1-diphenyl-2-picrylhydrazyl) in methanol (Gill *et al.*, 2010a). Compounds with radical-scavenging activity showed a yellow-on-purple spot due to the discoloration of DPPH.

Quantitative evaluation of the 1,1-diphenyl-2-picrylhydrazyl free radical scavenging activity: The antioxidant activity of the ethanolic seed extract was carried out by 1,1-diphenyl-2-picrylhydrazyl method. About 1 mL solution of the extract ($50-100 \mu\text{g mL}^{-1}$) was added to 1.5 mL of freshly prepared methanolic solution of 1,1-diphenyl-2-picrylhydrazyl (0.05 mM) and the volume was made up with methanol upto 5 mL. Mixtures were shaken vigorously and kept in the dark place for 30 min. It was analyzed at 517 nm by ultraviolet spectrophotometer using methanol as blank. The 1 mL of 0.05 mM 1,1-diphenyl-2-picrylhydrazyl diluted with 4 mL^{-1} of methanol was used as control (Krishna *et al.*, 2009). Ascorbic acid was used as a reference standard. All the readings were taken in triplicate and their mean value was taken in consideration. Inhibition of DPPH radical was calculated using the equation:

$$I(\%) = 100 \times \frac{A_0 - A_s}{A_0} \quad (1)$$

where, A_0 is the absorbance of the control and A_s is the absorbance of the test sample

Free radical scavenging activity using hydrogen peroxide: The wild *Punica granatum* ethanolic seed extract was evaluated for their hydrogen peroxide radical scavenging activity. Solution of 0.6 mL hydrogen peroxide (43 mM) and 1 mL of different concentrations ($50-100 \mu\text{g mL}^{-1}$) of the extract was mixed. Then 2.4 mL of 0.1 M phosphate buffer of pH 7.4 was added. The resulting solution was kept for 10 min and the absorbance was recorded at 230 nm using ultraviolet spectrophotometer against a blank (without hydrogen peroxide) (Gill *et al.*, 2010b). Ascorbic acid was used as a reference standard. All the readings were taken in triplicate and their mean value was taken in consideration. The percentage of scavenging of hydrogen peroxide was calculated using the following formula:

$$\text{Scavenging activity (\%)} = \frac{V_c - C_t}{V_c} \times 100 \quad (2)$$

where, V_c is absorbance of control and V_t is absorbance of test sample.

Anti-ulcer activity

Experimental design for pyloric ligation induced ulcer (PL): Animals were divided into 6 groups, each comprising of 6 rats.

- Group I** : (Normal control group): rats were subjected to administration of 1 mL normal saline, p.o. 1 h before ulcer induction
- Group II** : (PL control group): rats were subjected to pyloric ligation for induction of ulcer
- Group III** : (Ranitidine+PL group): rats were subjected to administration of ranitidine (50 mg kg⁻¹, p.o.) 1 h before pyloric ligation
- Group IV-VI** : (EEPG 50, 75, 100 mg kg⁻¹, p.o. + PL): rats were subjected to administration of low, medium and higher doses of EEPG for seven consecutive days and 1 h before induction of ulcer on the day of experiment

Pyloric ligation induced peptic ulcer: Ethanolic extract of wild *Punica granatum* seeds (50, 75 and 100 mg kg⁻¹) was administered for a period of seven days. After the last dose the rats were kept for 24 h fasting with free access to water. The abdomen was opened and pylorus was ligated under anaesthesia without causing any damage to its blood vessels (Ramachandran *et al.*, 2011). The stomach was replaced carefully and the abdominal wall was closed with sutures. The animals were deprived of water during the postoperative period. After 4 h of ligation, stomachs were dissected out and contents were collected into clean tubes. The gastric volume, pH, free acidity and total acidity were determined.

Anti-ulcer activity parameters

Estimation of gastric volume and free and total activity changes: Four hours after ligation, stomachs were dissected out and contents were collected into measuring cylinder to measure the volume of gastric contents.

Determination of free and total acidity: The gastric contents were centrifuged and subjected to titration for estimation of free and total acidity. The supernatant liquid (1 mL) was pipette out and diluted to distilled water (10 mL). The solution was titrated against 0.01 N sodium hydroxide using Topfer's reagent (Srikanth and Muralidharan, 2009) as indicator, to the endpoint when the solution changed to orange colour. The volume of sodium hydroxide needed was taken as corresponding to the free acidity. Titration was further continued by adding 1% solution of phenolphthalein till it gained pink colour. The volume of sodium hydroxide was noted and was taken as corresponding to the total acidity. The sum of the two titrations was total acidity (Raj Kapoor *et al.*, 2002).

Acidity was expressed as:

$$\text{Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality} \times 100 \text{ mEq / L} / 100 \text{ g}}{0.1} \quad (3)$$

Estimation of gastric ulcerative index changes: The stomach was opened along the greater curvature and it was washed with running tap water. Then the ulcerative area was counted by placing it on a flat wooden plate (Takagi *et al.*, 1969). The ulcer index was determined by using the formula:

$$\text{Ulcer Index} = \frac{10}{X} \quad (4)$$

where, X = Total mucosal area/Total ulcerated area

Percentage ulcer protection was calculated using the formula:

$$\text{Ulcer protection (\%)} = \frac{U_c - U_t}{U_c} \times 100 \quad (5)$$

where, U_c = Ulcer index of treated group and U_t = Ulcer index of disease control group.

Statistical analysis: All the biochemical results were expressed as Mean±Standard Error of means (SEM). Data were analyzed by Turkey's multiple range tests using Sigma Stat Version-3.5 software. A probability value of $p < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

The phytochemical screening of wild *Punica granatum* ethanolic seed extract indicates the presence of triterpenoid, sterols, alkaloids, flavonoids, tannins and coumarin glycoside as shown in Table 1. Thus, ethanolic extract was analyzed for antioxidant by qualitative and quantitative methods. In case of qualitative method, the TLC plate showed yellow coloration with 1,1-diphenyl-2-picrylhydrazyl scavenging radical. In case of quantitative method, it was evaluated by 1,1-diphenyl-2-picrylhydrazyl and hydrogen peroxide methods. The highest scavenging activity was found to be 82.80 ± 0.267 at $100 \mu\text{g mL}^{-1}$ by 1,1-diphenyl-2-picrylhydrazyl method as shown in Table 2 and 74.91 ± 0.235 at $100 \mu\text{g mL}^{-1}$ by hydrogen peroxide method as shown in Table 3 as

Table 1: Phytochemical screening of wild *Punica granatum* ethanolic seed extract

Chemical constituents	Ethanol
Flavonoids	+
Amino acids	-
Triterpenoids	++
Sterols	++
Alkaloids	+
Tannins	++
Coumarin glycosides	+

-: Absence, +: Minor presence of chemical constituent, ++: Maximum presence of chemical constituents

Table 2: Antioxidant activity of wild *Punica granatum* ethanolic seed extract by 1,1-diphenyl- 2-picrylhydrazyl method

Concentration ($\mu\text{g mL}^{-1}$)	Inhibition (%)	
	Seed extract	Ascorbic acid
50	60.63 ± 0.181	62.33 ± 0.164
75	69.13 ± 0.215	74.64 ± 0.242
100	82.80 ± 0.267	85.31 ± 0.293

Values are the average of triplicate experiments and represented as Mean±SEM

Table 3: Antioxidant activity of wild *Punica granatum* ethanolic seed extract by hydrogen peroxide method

Concentration ($\mu\text{g mL}^{-1}$)	Seed extract (% inhibition)	Ascorbic acid (% inhibition)
50	59.60 \pm 0.114	61.18 \pm 0.152
75	68.34 \pm 0.192	72.53 \pm 0.228
100	74.91 \pm 0.235	81.66 \pm 0.271

Values are the average of triplicate experiments and represented as Mean \pm SEM

Table 4: Effect on gastric volume, free acidity and total acidity in pyloric ligation induced ulcers

Groups (mg kg^{-1})	Gastric volume ($\text{mL } 100 \text{ g}^{-1}$)	Free acidity (mEq L^{-1})	Total acidity (mEq L^{-1})
Normal	1.28 \pm 0.076	32.22 \pm 0.464	59.29 \pm 1.494
PL	2.86 \pm 0.046	62.39 \pm 3.914	109.15 \pm 2.452
Ranitidine (50)	1.33 \pm 0.020 ^a	27.41 \pm 1.942 ^a	58.51 \pm 1.635 ^a
EEPG (50)	2.30 \pm 0.017 ^b	37.64 \pm 2.852 ^b	74.27 \pm 2.353 ^b
EEPG (75)	2.12 \pm 0.039 ^b	34.26 \pm 1.527 ^b	67.46 \pm 1.874 ^b
EEPG (100)	1.46 \pm 0.054 ^a	26.94 \pm 0.813 ^a	60.16 \pm 2.112 ^a

Effect of ethanolic extract of wild *Punica granatum* seeds on ulcerative parameter in pyloric ligation model. Data are represented as Mean \pm SEM. Statistical analysis was done by one-way ANOVA followed by turkey test. $\alpha = p < 0.05$ as compared to PL control group, $\beta = p < 0.05$ as compared to ranitidine treated group

Table 5: Determination of ulcerative index and percent inhibition in pyloric ligation induced ulcers

Groups (mg kg^{-1})	Ulcerative index	% Inhibition
Normal	0.0 \pm 0.0	0.0
PL	5.1 \pm 0.211	0.0
Ranitidine (50)	1.1 \pm 0.142 ^a	78.40
EEPG (50)	2.4 \pm 0.674 ^b	52.94
EEPG (75)	1.9 \pm 0.632 ^b	62.74
EEPG (100)	1.3 \pm 0.542 ^a	74.51

Effect of ethanolic extract of wild *Punica granatum* seeds on ulcerative parameter in pyloric ligation model. Data are represented as Mean \pm SEM. Statistical analysis was done by one-way ANOVA followed by turkey test. $\alpha = p < 0.05$ as compared to PL control group, $\beta = p < 0.05$ as compared to ranitidine treated group

compared to the standard (ascorbic acid). The various studies have been done on fruit rind (Hasan *et al.*, 2009), fruit aril, juice (Ricci *et al.*, 2006) and flowers of *Punica granatum* plant (Kaur *et al.*, 2006). There is no reported study related to the *Punica granatum* seeds of this species. The free radicals are responsible for lipid peroxidation and mucosal damage. ROS degrades epithelial membrane components, modify cellular and DNA components (Demir *et al.*, 2003). 1,1-diphenyl-2-picrylhydrazyl reacts with the antioxidants and accepts a hydrogen atom due to which it gets changed into 1,1-diphenyl-2-picrylhydrazine and therefore shows decrease in absorbance (Gill *et al.*, 2011c). In case of wild *Punica granatum*, the seed extract showed maximum scavenging activity at 100 $\mu\text{g mL}^{-1}$ which may be through free radical scavenging mechanism so the extract was further evaluated for antiulcer activity by pyloric ligation model.

In the pyloric ligation induced ulcer model, the animals were treated with seed extract (50, 75 and 100 $\mu\text{g mL}^{-1}$). There is accumulation of gastric secretions (2.86 mL) in the disease control group due to pyloric ligation. Free and total acidity estimated in the disease control group were 62.39 \pm 3.914 and 109.15 \pm 2.452 mEq mL⁻¹, respectively. The extract at the dose of (100 $\mu\text{g mL}^{-1}$) has shown significant reduction in gastric volume (1.46 mL), free acidity (26.94 \pm 0.813 mEq mL⁻¹) and total acidity (60.16 \pm 2.112 mEq mL⁻¹) as shown in Table 4. The

extract showed optimum percentage inhibition of 74.51% at 100 $\mu\text{g mL}^{-1}$ as shown in Table 5 which were comparable to the ranitidine treated group. The antiulcer activity has been done previously on the flower (Ahmad *et al.*, 2008), bark (Shu *et al.*, 2009) and rind part (Ajaikumar *et al.*, 2005) of *Punica granatum*. The seed part has not been explored for antiulcer activity. Pylorus ligation induced ulcers are due to the accumulation of gastric acid and pepsin which leads to auto-digestion of gastric mucosa (Gill *et al.*, 2011b). These factors cause the development of upper gastrointestinal damage which includes ulcers, lesions and haemorrhage (Goswami *et al.*, 2011).

The decrease in the ulcerative index suggests the ability of the extract to protect the gastric mucosa against free radical mediated tissue injury. Thus the action of the extract may be through free radical scavenging mechanism.

CONCLUSION

The results of the present study suggest that the wild *Punica granatum* ethanolic seed extract possesses antioxidant and antiulcer potential. It has muco-protective activity when compared with that of the reference drug ranitidine.

ACKNOWLEDGMENT

Thanks to Professor A.C. Rana and all the faculty members of Rayat Institute of Pharmacy for their support. We are highly indebted to Rayat and Bahra Educational and Research Trust for providing us facilities to carry out the project.

REFERENCES

- Ahmad, M.P., M.S. Alam, M.A. Ahmad, S. Ahmad and A.K. Najmi, 2008. Anti-ulcer activity of *Punica granatum* L flowers in rats. Indian J. Pharmacol., 40: S142-S148.
- Ajaikumar, K.B., M. Asheef, B.H. Babu and J. Padikkala, 2005. The inhibition of gastric mucosal injury by *Punica granatum* L. (pomegranate) methanolic extract. J. Ethnopharmacol., 96: 171-176.
- Al-Yahya, M.A., 2005. Preliminary phytochemical and pharmacological studies on the rind of pomegranate (*Punica granatum* L.). Pak. J. Biol. Sci., 8: 479-481.
- Bagri, P., M. Ali, V. Aeri, S. Sultana and M. Bhowmik, 2010. Evaluation of Anti-inflammatory and Analgesic activity of *Punica granatum* Linn. Int. J. Drug Dev. Res., 2: 698-702.
- Bhalodia, N.R., P.B. Nariya, R.N. Acharya and V.J. Shukla, 2011. Evaluation of *in vitro* antioxidant activity of flowers of *Cassia fistula* Linn. Int. J. PharmTech Res., 3: 589-599.
- Chanda, S., R. Dave and M. Kaneria, 2011. *In vitro* antioxidant property of some Indian medicinal plants. Res. J. Med. Plant, 5: 169-179.
- Das, K., R.K.S. Tiwari and D.K. Shrivastava, 2010. Techniques for evaluation of medicinal plant products as antimicrobial agent: Current methods and future trends. J. Med. Plant Res., 4: 104-111.
- Demir, S., M. Yilmaz, M. Koseoglu, N. Akalin, D. Aslan and A. Aydin, 2003. Role of free radicals in peptic ulcer and gastritis. Turk. J. Gastroenteriol., 14: 39-43.
- Gill, N.S., K. Dhiman, J. Bajwa, P. Sharma and S. Sood, 2010a. Evaluation of free radical scavenging, anti-inflammatory and analgesic potential of *Benincasa hispida* seed extract. Int. J. Pharmacol., 6: 652-657.
- Gill, N.S., R.K. Bansal, M. Garg, S. Sood, A. Muthuraman and M. Bali, 2010b. Evaluation of antioxidant, anti-inflammatory and analgesic potential of *Citrullus lanatus* seed extract in rodent model. Internet J. Nutr. Wellness, Vol. 9. No. 2.

- Gill, N.S., J. Bajwa, K. Dhiman, P. Sharma and S. Sood *et al.*, 2011a. Evaluation of therapeutic potential of traditionally consumed *Cucumis melo* seeds. Asian J. Plant Sci., 10: 86-91.
- Gill, N.S., J. Bajwa, P. Sharma, K. Dhiman and S. Sood *et al.*, 2011b. Evaluation of antioxidant and antiulcer activity of traditionally consumed *Cucumis melo* seeds. J. Pharmacol. Toxicol., 6: 82-89.
- Gill, N.S., K. Dhiman, P. Sharma, J. Bajwa and S. Sood *et al.*, 2011c. Evaluation of free radical scavenging and antiulcer potential of methanolic extract of *Benincasa hispida* seeds. Res. J. Med. Plant, 5: 596-604.
- Goswami, M., M. Kulshreshtha, C.V. Rao, S. Yadav and S. Yadav, 2011. Anti-ulcer potential of *Lawsonia inermis* L. leaves against gastric ulcers in rats. J. Applied Pharm. Sci., 1: 69-72.
- Gregory, M., K.P. Vithalrao, G. Franklin and V. Kalaichelavan, 2009. Anti-Ulcer (Ulcer-Preventive) activity of *Ficus arnottiana* Miq. (Moraceae) leaf methanolic extract. Am. J. Pharmacol. Toxicol., 4: 89-93.
- Gupta, J.K., N. Upmanyu, A.K. Patnaik and P.M. Mazumder, 2010. Evaluation of Antiulcer activity of *Leucas lavandulifolia* on mucosal lesion in rat. Asian J. Pharm. Clin. Res., 3: 118-120.
- Hamid, A.A., O.O. Aiyelaagbe, L.A. Usman, O.M. Ameen and A. Lawal, 2010. Antioxidants: Its medicinal and pharmacological applications. Afr. J. Pure Applied Chem., 4: 142-151.
- Hasan, R., M. Hossain, R. Akter, M. Jamila and M.E.H. Mazumder *et al.*, 2009. Antioxidant, antidiarrhoeal and cytotoxic properties of *Punica granatum* Linn. Lat. Am. J. Pharm., 28: 783-788.
- Jayaraj, P., 2010. Regulation of traditional and complementary medicinal products in Malaysia. Int. J. Green Pharm., 4: 10-14.
- Kaur, G., Z. Jabbar, M. Athar and M.S. Alam, 2006. *Punica granatum* (pomegranate) flower extract possesses potent antioxidant activity and abrogates Fe-NTA induced hepatotoxicity in mice. Food Chem. Toxicol., 44: 984-993.
- Khanahmadi, M., S.H. Rezazadeh and M. Taran, 2010. *In vitro* antimicrobial and antioxidant properties of *Smyrniium cordifolium* Boiss. (Umbelliferae) extract. Asian J. Plant Sci., 9: 99-103.
- Krishna, K.L., K. Mruthunjaya and J.A. Patel, 2009. Antioxidant and hepatoprotective activity of leaf extract of *Justicia gendarussa* burm. Int. J. Biol. Chemi., 3: 99-110.
- Makhija, I.K., L. Richard, S.P. Kirti, K. Saleemullah, M. Jessy and S. Annie, 2011. *Sphaeranthus indicus*: A review of its chemical, pharmacological and ethnomedicinal properties. Int. J. Pharmacol., 7: 171-179.
- Muthu, C., M. Ayyanar, N. Raja and S. Ignacimuthu, 2006. Medicinal plants used by traditional healers in Kancheepuram District of Tamil Nadu, India. J. Ethnobiol. Ethnomed., 2: 43-43.
- Priyadarsini, R., K. Tamilarasi, A. Karunambigai and S.G. Devi, 2010. Antioxidant potential of the leaves and roots of *Solanum surattense*. Plant Arch., 10: 815-818.
- Raj Kapoor, B., R. Anandan and B. Jayakar, 2002. Anti-ulcer effect of *Nigella sativa* Linn. against gastric ulcers in rats. Curr. Sci., 82: 177-179.
- Rakesh, S.U., P.R. Patil and S.R. Mane, 2010. Use of natural antioxidants to scavenge free radicals: A major cause of diseases. Int. J. Pharm Tech. Res., 2: 1074-1081.
- Ramachandran, S., G. Poovi and M.D. Dhanaraju, 2011. Evaluation of gastric and duodenal antiulcer activity of famotidine formulation in experimental animals. J. Pharmacol. Toxicol., 6: 189-195.
- Ricci, D., L. Giamperi, A. Bucchini and D. Fraternali, 2006. Antioxidant activity of *Punica granatum* fruits. Fitoterapia, 77: 310-312.

- Shu, L., Z. Qixin, Z. Ying, S. Jingchuan and Y. Tao, 2009. Effects and mechanisms of the acetone extract obtained from pomegranate bark on the experimental gastric damages. *Pharmacol. Clin. Chinese Materia Medica*, Vol. 3, <http://www.google.com.pk/search?q=Evaluation+of+in+vitro+antioxidant+activity+of+flowers+of+Cassia+fistula+Linn.+&hl=en&sa=N&tab=sw&aq=f&aqi=&aql=&oq=>
- Srikanth, J. and P. Muralidharan, 2009. Antiulcer activity of *Morinda Citrifolia* Linn fruit extract. *J. Sci. Res.*, 1: 345-352.
- Takagi, K., S. Okabe and R. Saziki, 1969. A new method for the production of chronic gastric ulcer in rats and the effect of several drugs on its healing. *Jpn. J. Pharmacol.*, 19: 418-426.
- Yoganandam, G.P., K. Ilango and S. De, 2010. Evaluation of anti-inflammatory and membrane stabilizing properties of various extracts of *Punica granatum* L. (Lythraceae). *Int. J. PharmTech. Res.*, 2: 1260-1263.