

Research Article

Antioxidant and Anti-inflammatory Activities of *Platanus orientalis*: An Oriental Plant Endemic to Kashmir Planes

¹Syed Irtiza, ²Gulzar A. Bhat, ^{3,4}Mudasar Ahmad, ³Hilal A. Ganaie, ³Bashir A. Ganai, ³Azra N. Kamili, ¹Seema Akbar and ^{1,3}Mudasir A. Tantry

¹Phytochemistry Laboratory, Regional Research Institute of Unani Medicine (CCRU) University of Kashmir, Srinagar, Kashmir 190006, India

²Department of Biochemistry, University of Kashmir, Srinagar, Kashmir 190006, India

³Phytochemistry Research Laboratory, Centre of Research for Development, University of Kashmir, Srinagar, Kashmir 190006, India

⁴Plant Tissue Culture Research Laboratory, Department of Botany, University of Kashmir, Srinagar, Kashmir 190006, India

Abstract

Background and Objectives: *Platanus orientalis* also called Oriental plane or Chinar or Boonyi (Platanaceae) is a large, deciduous tree, known for its longevity and spreading crown. The present work describes determination of antioxidant, anti-inflammatory and total phenol content of *Platanus orientalis*. **Materials and Methods:** Different extracts of the plant such as stem, root and leaves were prepared in different solvents such as chloroform, methanol and water. **Results:** Total phenol content in this plant reveal that methanol extract of *Platanus orientalis* root showed maximum free radical scavenging activity followed by methanol wood extract. Antioxidant activity of the plant extracts in this study reveal that it is methanol and chloroform extracts which showed above 50% inhibition in contrast to aqueous extracts. Anti-inflammatory activity of *Platanus orientalis* showed that aqueous wood extract of *Platanus orientalis* showed maximum activity in contrast to other extracts. With the help of acute toxicity test it was found that all the extracts are nontoxic at 2 g kg⁻¹ b.wt. to Wistar rats. **Conclusion:** These finding provides some biological value of different extracts of *Platanus orientalis* for the use as antioxidant and anti-inflammatory agents. However, further investigation on isolation and purification of the bio-active molecules is needed to explore the exact mechanism of action of the *Platanus orientalis* extracts.

Key words: *Platanus orientalis*, antioxidant activity, anti-inflammatory activity, total phenol content

Received: December 21, 2015

Accepted: March 03, 2016

Published: April 15, 2016

Citation: Syed Irtiza, Gulzar A. Bhat, Mudasar Ahmad, Hilal A. Ganaie, Bashir A. Ganai, Azra N. Kamili, Seema Akbar and Mudasar A. Tantry, 2016. Antioxidant and anti-inflammatory activities of *Platanus orientalis*: An oriental plant endemic to Kashmir planes. Pharmacologia, 7: 217-222.

Corresponding Author: Mudasar A. Tantry, Phytochemistry Laboratory, Center of Research for Development University of Kashmir, Srinagar, Kashmir 190006, India Tel: + 911942272274 Fax: + 911942272275

Copyright: © 2016 Syed Irtiza *et al.* This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

From ancient times, traditional system of medicine is being used for the treatment of various diseases. To maintain a good health, this system of medicine is being widely used in most developing countries¹. These plants possess active compounds that owe them their medicinal value. The active compounds produce various physiological actions in human and animal body².

The preliminary screening of extracts through Standard Operating Procedures (SOPs) is a must before leading to deepened phytochemical investigation. So this investigation is on those lines to illustrate the pharmacological evaluation of Oriental plane (*Platanus orientalis*). *Platanus orientalis* (Plantanaceae) is a native of Eastern Mediterranean region of world. It is commonly cultivated and highly valued as an ornamental tree in Kashmir³. This is a part of study, in accordance with 'Quality by design' approach of medicinal and aromatic plants (MAPs) of Western Himalayas undertaken by our centre.

Platanus is a unique living member of *Platanaceae* family. It is a small genus of trees that is known in English as plane trees. The principal use of plane trees are as ornamental trees, especially by roadsides and parks^{4,5}. The plane trees are widely planted to improve the microclimate⁶. The plane leaves commonly known in Iran as "Barge chenar", have been used in concentrated aromatic liquids, herbal remedies and Iranian traditional medicine to treat several disorders. They are used in Iranian folk and traditional medicines for treating some dermatological, gastrointestinal, rheumatic and inflammatory diseases⁷⁻⁹. Some Persian scientists and hakims such as Avicenna and Hakim Momen were also familiar with this tree and mentioned its medicinal uses like teeth pain killer and analgesic and antiinflammatory effects for knee pain and inflammation in their books^{7,8}. The plane leaves contain flavonoids, pentacyclic triterpenoids, tannins and caffeic acid⁹⁻¹³. Many pharmacological activities such as cytotoxic, cytostatic, astringent, antimicrobial and antiseptic effects have been attributed to the *Platanus* species. *Platanus orientalis* is the major species of *Platanus* in Iran and is widely distributed in northern and central parts of the country^{13,14,15}.

MATERIALS AND METHODS

Collection of plant material and preparation of the extracts:

The plant viz. *Platanus orientalis* collected from Shopian region of Kashmir in the month of May-July, 2007 and identified by Centre of Plant Taxonomy (COPT) University of Kashmir. The plant parts i.e., stem and leave, after collection

were cleaned and dried under shade for few days, cut into small pieces and then powdered in a wood grinder under sterilized conditions. The colour of the powder was stem (yellowish) leaves (dark green) and roots (reddish).

Animals: Randomly bred closed colony Wistar rats (12-16 weeks old and weighing 120-160 g) reared in animal house laboratory (CSIR-Indian Institute of Integrative Medicine Jammu) were used. Rats were housed in groups of 5-10 in plastic cages. They were given pellet diet *ad libitum* during the course of experimentation. Twelve hour light cycle was automatically controlled (on at 7.00 AM and off at 7.00 PM) and the room temperature was regulated at $26 \pm 1^\circ\text{C}$. Before the experimentation, animals were housed in these conditions for 3-4 days to become acclimatized.

Determination of antioxidant activity

General free radical scavenging-DPPH assay: This is the primary method in which the free radical i.e., DPPH which is purple in colour is reduced by the antioxidant to yellow colour based on efficacy of the antioxidant. In this method 3 mL of mixture was prepared by adding 1 mL DPPH (5% in methanol) 200-100 μL of extract and respective solvent, the mixture was incubated at room temperature for 30 min and formation of yellow coloured complex was read at 517 nm spectrophotometrically. Ascorbic acid was taken as positive control and reaction solvent with DPPH as negative:

$$\text{Percent inhibition} = \frac{\text{Absorbance of negative control} - \text{Absorbance of extract}}{\text{Absorbance of negative control}} \times 100$$

Superoxide anion scavenging-riboflavin photo oxidation

method: Univalent reduction of oxygen causes production of superoxide anion, which interacts with hydrogen peroxide leading to the generation of highly reactive and toxic hydroxyl radicals. Superoxide anion is thus the first reduction product of oxygen, which reacts with lipids, DNA and directly affects intercellular enzymes. In this method, the photo oxidation of riboflavin leads to the generation of riboflavin radicals which then oxidizes and generates superoxide radicals. The reaction mixture 1.7 mL was made by adding 300 μL of EDTA (0.1 M) 500 μL NBT (1.5 mM) 0.067 M phosphate buffer and 200 μL of different concentrations of extracts (25 mg mL⁻¹). The tubes were incubated at 37°C for 5-8 min. Then, 200 μL of riboflavin was added and were kept in sunlight for 10-12 min. Until colour change was observed (purple). The absorbance was then read at 560 nm. Reaction medium devoid of extract was taken as negative control and ascorbic acid as positive controls (C1 and C2; one having H₂O₂ and other without it):

$$\text{Percent inhibition} = \frac{\text{Absorbance of negative control} - \text{Absorbance of extract}}{\text{Absorbance of negative control}} \times 100$$

Hydroxyl radical scavenging-deoxyribose assay: The highly reactive radical i.e., hydroxyl radical (OH^\cdot) is generated by the reaction of H_2O_2 with ferrous ions, which cleaves covalent bonds in proteins, carbohydrates, causes lipid per oxidation and destroys cell membrane. The assay was done by two ways in first one, 1 mL of reaction mixture was prepared by adding 500 μL DNA (500 mg 50 mL^{-1}), 100 μL ferric nitrate (20 mM), 100 μL ascorbic acid (500 mM), different volumes of Tris HCl buffer (0.001 M) and 100 μL of different concentrations of extracts. The reaction mixture was incubated at 37°C for 24 h in water bath. Then, 1 mL TCA (20%) was added and centrifuged at 5000 rpm for 5 min. To the supernatant was added 1 mL TBA (1.68%) kept in boiling water for 20 min and cooled under tap water. In the second method, 30 μL H_2O_2 was added after ferric nitrate to make total of 1 mL volume and the rest procedure was same as first one. The reddish pink colour complex was red at 535 nm in both. Reaction medium without extract act as negative control and ascorbic acid as positive control:

$$\text{Percent inhibition} = \frac{\text{Absorbance of negative control} - \text{Absorbance of extract}}{\text{Absorbance of negative control}} \times 100$$

Determination of acute inflammatory test in Wistar rats:

The test was performed by standard procedure. Oedema was induced in Wistar rats in groups of four by injecting 0.1 mL of carrageenan (1% w/v) solution in normal saline into sub plantar region of the left hind paw after 45 min of extract administration (250 mg kg^{-1} b.wt.). Paw volume was immediately measured and after 4 h of carrageenan injection. Ibuprofen was used as positive control and normal distilled water as negative control. The inflammation was measured by volumetric differential meter.

Determination of preliminary toxicity test: The test was performed accordingly as used¹⁵. In this method few groups of animals were made, each group containing two Wistar rats and normal or negative control which was given normal double distilled water. Extract was solubilised in water and administered orally as a single dose of 5 mg kg^{-1} b.wt. These animals were observed for 72 h period. The number of deaths was expressed as percentile and preliminary toxicity test observed.

RESULTS AND DISCUSSION

Platanus orientalis contains various tannins and polyphenols, including flavonoids such as quercetin, kaempferol and their glycosides^{10,11,14}. It has been reported that these flavonoids have potent anti-inflammatory and analgesic actions on inflammation and pain¹⁶⁻¹⁸. Depending on the chemical substitutions on the flavone-skeleton, flavonoids can play a modulating, biphasic and regulatory action on inflammation and immunity. Flavonoids exert their properties both as plant extracts and as purified aglycone molecules¹⁶. Also caffeic acid is found in the plant leaves and it has been reported that this compound has anti-inflammatory activity^{19,20}. It seems these natural compounds in the polyphenolic and total extracts of the plant leaves have been partly associated with our pharmacological findings, although it is not clear whether these plant constituents are the only contributing components of this extract.

Total yield of plant extracts: Hot extraction of plant in different solvent systems yielded different amount of extracts. *Platanus orientalis* chloroform wood extract (10.12%) followed methanol root extract (8.06%). Methanol extract of *Platanus orientalis* root (≈ 400 ppm) and wood (≈ 360 ppm) showed higher polyphenol content in comparison to other extracts (Table 1).

DPPH radical scavenging assay of *Platanus orientalis*

extracts: Methanol, chloroform and aqueous extracts of *Platanus orientalis* wood, root and leaves were evaluated for their antioxidant activity. From the present study it was elucidated that the chloroform extracts of wood and root and also methanol extract of leaves showed maximum activity in contrast to control (Table 2).

Deoxyribose assay of extracts: Methanol, chloroform and aqueous extracts of *Platanus orientalis* wood, root and leaves

Table 1: Total phenolic content in different parts of plant extracts

<i>Platanus orientalis</i>	Type of extract	Mean \pm SE	Phenol content (ppm)
Wood	MeOH	0.903 \pm 0.057	360
	CHCl_3	0.183 \pm 0.012	78
	Aqueous	0.163 \pm 0.007	78
Root	MeOH	1.070 \pm 0.015	400
	CHCl_3	0.107 \pm 0.007	48
	Aqueous	0.417 \pm 0.017	157
Leaf	MeOH	0.490 \pm 0.039	200
	CHCl_3	0.113 \pm 0.015	48
	Aqueous	0.270 \pm 0.015	125

Table 2: Free radical activity determined with DPPH method of different extracts of *Platanus orientalis*

Extract	Concentration (mg mL ⁻¹)	Mean ± SE	Inhibition (%)
MeOH			
Wood	200	0.252 ± 0.001	70.28
	400	0.205 ± 0.00	75.82
Root	200	0.442 ± 0.001	47.88
	400	0.336 ± 0.003	60.38
Leaf	200	0.392 ± 0.001	53.77
	400	0.206 ± 0.001	75.70
CHCl₃			
Wood	200	0.132 ± 0.003	81.11
	400	0.142 ± 0.003	84.72
Root	200	0.277 ± 0.001	79.15
	400	0.171 ± 0.003	87.13
Leaf	200	0.974 ± 0.002	26.71
	400	0.875 ± 0.001	34.16
Aqueous			
Wood	200	1.360 ± 0.003	-26.51
	400	1.198 ± 0.001	-11.44
Root	200	1.212 ± 0.001	-12.74
	400	0.813 ± 0.001	24.37
Leaf	200	-	-
	400	1.852 ± 0.001	-72.27
Ascorbic acid	200	0.647 ± 0.003	39.81
	400	0.544 ± 0.002	49.39

Table 3: Protection of deoxyribose assay from oxidation of *Platanus orientalis* extracts

Extracts	Concentration (µg mL ⁻¹)	Mean ± SE	Inhibition (%)
MeOH			
Wood	2500	0.64 ± 0.002	7.24
	5000	0.56 ± 0.002	18.84
Root	2500	0.63 ± 0.001	8.69
	5000	0.58 ± 0.002	15.94
Leaf	2500	0.52 ± 0.001	24.64
	5000	0.46 ± 0.001	33.33
CHCl₃			
Wood	2500	0.57 ± 0.001	17.40
	5000	0.55 ± 0.001	23.20
Root	2500	0.68 ± 0.001	1.45
	5000	0.66 ± 0.002	4.35
Leaf	2500	0.58 ± 0.001	15.94
	5000	0.56 ± 0.002	18.84
Aqueous			
Wood	2500	0.72 ± 0.002	-43.47
	5000	0.71 ± 0.002	-28.98
Root	2500	0.73 ± 0.001	-57.97
	5000	0.72 ± 0.002	-43.47
Leaf	2500	0.68 ± 0.001	1.40
	5000	0.67 ± 0.002	2.90
Ascorbic acid	500	0.270 ± 0.001	60.86

were evaluated for their antioxidant activity. From the present study it was elucidated that methanol extract of all showed comparable activity (Table 3).

NBT (superoxide scavenging) assay: Methanol, chloroform and aqueous extracts of *Platanus orientalis* wood, root and leaves were evaluated for their antioxidant activity. Methanol extract of leaves showed the maximum activity (Table 4).

Table 4: Antioxidant activity of different parts of *Platanus orientalis* by superoxide scavenging assay

Extracts	Concentration (mg mL ⁻¹)	Mean ± SE	Inhibition (%)
MeOH			
Wood	1000	0.434 ± 0.001	4.64
	2000	0.299 ± 0.001	33.99
Root	1000	0.797 ± 0.001	-75.93
	2000	0.510 ± 0.001	-12.58
Leaf	1000	0.512 ± 0.001	-13.02
	2000	0.298 ± 0.001	36.2
CHCl₃			
Wood	1000	0.806 ± 0.001	-77.92
	2000	0.490 ± 0.001	-8.17
Root	1000	0.409 ± 0.001	9.7
	2000	0.400 ± 0.001	11.69
Leaf	1000	0.578 ± 0.001	-27.59
	2000	0.269 ± 0.001	40.61
Aqueous			
Wood	1000	0.431 ± 0.001	4.86
	2000	0.395 ± 0.001	12.8
Root	1000	0.468 ± 0.001	-3.31
	2000	0.461 ± 0.001	-1.76
Leaf	1000	0.531 ± 0.001	-17.21
	2000	0.202 ± 0.001	55.41
Ascorbic acid	500	0.165 ± 0.001	63.57

Anti-inflammatory activity evaluation of *Platanus orientalis* leaves extract by carrageenan induced paw oedema:

Aqueous, methanol and chloroform extracts were administrated at a dose level of 250 mg kg⁻¹ b.wt. in five group of rats four each group in which one as positive and other negative control. Present study shows methanol and chloroform solvent system extracts showed negative results. Whereas aqueous showed (6.38%) inhibition. Ibuprofen administrated at 100 mg kg⁻¹ b.wt., showed (72.76%) inhibition (Table 5).

Anti-inflammatory activity evaluation of *Platanus orientalis* roots extract by carrageenan induced paw oedema:

Aqueous, methanol and chloroform extracts were administrated at a dose level of 250 mg kg⁻¹ b.wt. in five group of rats four each group in which one as positive and other negative control. Present study shows methanol and chloroform solvent system extracts showed negative results. Whereas aqueous showed (1.88%) inhibition. Ibuprofen administrated at 100 mg kg⁻¹ b.wt., showed (75.84%) inhibition (Table 5).

Anti-inflammatory activity evaluation of *Platanus orientalis* wood extract by carrageenan induced paw oedema:

Aqueous, methanol and chloroform extracts were administrated at a dose level of 250 mg kg⁻¹ b.wt., in five group of rats four each group in which one as positive and

Table 5: Anti-inflammatory activity of different extracts of *Platanus orientalis*

Treatments (extract)	Dose (mg kg ⁻¹ b.wt.)	Initial paw volume (mL) Mean ± SE	Final paw volume (mL) Mean ± SE	Oedema Mean ± SE	Inhibition (%)
Wood					
Control	0	0.725 ± 0.025	1.825 ± 0.0408	1.100 ± 0.0408	
Aqueous	250	0.733 ± 0.033	1.600 ± 0.100	0.867 ± 0.202	21.18
MeOH	250	0.825 ± 0.063	1.825 ± 0.149	1.000 ± 0.912	9.09
CHCl ₃	250	0.725 ± 0.025	1.650 ± 0.185	0.925 ± 0.160	15.90
Root					
Control	0	0.950 ± 0.029	2.750 ± 0.131	1.325 ± 0.0125	
Aqueous	250	0.875 ± 0.025	2.175 ± 0.147	1.300 ± 0.147	1.88
MeOH	250	0.975 ± 0.025	2.425 ± 0.085	1.450 ± 0.086	-9.43
CHCl ₃	250	0.975 ± 0.025	2.325 ± 0.025	1.350 ± 0.028	-1.88
Leaf					
Control	0	0.975 ± 0.025	2.150 ± 0.087	1.175 ± 0.0629	
Aqueous	250	1.000 ± 0.058	2.100 ± 0.091	1.100 ± 0.0478	6.38
MeOH	250	0.925 ± 0.025	2.125 ± 0.125	1.200 ± 0.1225	-2.127
CHCl ₃	250	0.925 ± 0.025	2.175 ± 0.063	1.250 ± 0.0645	-6.38
Ibuprofen	100	1.100 ± 0.030	1.420 ± 0.040	0.320 ± 0.040	70.90

other negative control. Present study shows aqueous extract (21.18%) inhibition, chloroform extract (15.90%) inhibition followed by methanol extract (9.09%) inhibition. Ibuprofen administrated at 100 mg kg⁻¹ b.wt. showed (70.90%) inhibition (Table 5).

Preliminary toxicity test: This test was done for 17 extracts and sub-fractions i.e., of *Platanus orientalis* of which anti-inflammatory activity was done. The animals under consideration did not died for the stipulated time of 72 h and also not showed any conditions of increased diuresis, convulsion, ataxy, diarrhoea or weight loss.

Successful analysis of botanical compounds from the plant material is largely dependent on the type of solvent used in the extraction procedures. Traditional healers use primarily water as solvent but in present study, it was found that plant extract in organic solvent was found such chloroform and methanol provided more convenient activity as compared to aqueous extracts. In this study, it was found that it is the wood of *Platanus orientalis* which showed the whole activities in maximum, so it is to be concluded that most of the bioactive materials are concentrated in the wood whatever the solvent system. Total phenol content was found accordingly root extract > wood extract > leaves extract or in accordance with, MeOH extract > CHCl₃ extract > aqueous extract, so it is the methanol extract of *Platanus orientalis* root (~ 400 ppm) and wood (~ 360 ppm) which showed maximum phenol content. Antioxidant activity was found accordingly: Wood extract > root extract > leaves extract or as MeOH extract > CHCl₃ extract > aqueous extract. Anti-inflammatory activity of *Platanus orientalis* was observed accordingly as aqueous extract > CHCl₃ extract > MeOH extract or as wood extract > leaves extract > root extract. The results of the present study shows that, in *Platanus orientalis* wood, aqueous

extract; chloroform extract and methanol extract inhibition of inflammation is 21.18, 15.90 and 9.09%, respectively. In *Platanus orientalis* leaves, methanol and chloroform solvent system extracts showed negative results, whereas aqueous extracts showed (6.38%) inhibition. In *Platanus orientalis* root, methanol and chloroform solvent system extracts showed negative results, whereas aqueous extract showed (1.88%) inhibition. These finding provides some biological value of different extracts of *Platanus orientalis* for the use as antioxidant and anti-inflammatory agents. However, further investigation on isolation and purification of the bio-active molecules is needed to explore the exact mechanism of action of the *Platanus orientalis* extracts.

CONCLUSION

In conclusion, the pharmacological activities of the plant leaves showed moderate antioxidant and anti-inflammatory effects and further pharmacological and biological studies are needed to elucidate the mechanisms of its action.

ACKNOWLEDGEMENTS

The financial support from the Centre Council for Research in Unani Medicine (CCRUM) and Council of Scientific and Industrial Research (CSIR) New Delhi, India, as Pool Officer under the Grant No. IA-27401 is gratefully acknowledged.

REFERENCES

1. Cragg, G.M., P.G. Grothaus and D.J. Newman, 2014. New horizons for old drugs and drug leads. J. Nat. Prod., 77: 703-723.

2. Cragg, G.M. and D.J. Newman, 2013. Natural products: A continuing source of novel drug leads. *Biochim. Biophys. Acta*, 1830: 3670-3695.
3. Chopra, R., S.L. Nayar and I.C. Chopra, 1956. Glossary of Indian Medicinal Plants. 1st Edn., National Institute of Science Communications, New Delhi, India.
4. Mozaffarian, V., 1994. Plant Systematics, Dicotyledons. Vol. 2. Daneshe Emrooz Publications, Tehran, pp: 35-36.
5. Mazaffarian, V., 1996. A Dictionary of Iranian Plant Names. Farhange Moaser Publications, Tehran, pp: 418-419.
6. Pourkhabbaz, A., N. Rastin, A. Olbrich, R. Langenfeld-Heyser and A. Polle, 2010. Influence of environmental pollution on leaf properties of urban plane trees, *Platanus orientalis* L. *Bull. Environ. Contamin. Toxicol.*, 85: 251-255.
7. Ebne Sina, A., 1988. *Ghanoon Dar Teb*. Vol. 2. Soroosh Press, Tehran, pp: 119-120.
8. Tonekaboni, S.M.M., 2007. *Tohfato Momenin*. Nashre Shahr, Tehran, Pages: 200.
9. Zargari, A., 1990. Medicinal Plants. Vol. 4, Tehran University Publications Tehran, pp: 469-470.
10. Dimas, K., C. Demetzos, S. Mikatu, M. Marselos, T. Tzavaras and D. Kokkinopoulos, 2000. Cytotoxic activity of kaempferol glycosides against human leukaemic cell lines *in vitro*. *Pharmacol. Res.*, 41: 83-86.
11. Mitrokotsa, D., S. Mitaku, C. Demetzos, C. Harvala, A. Mentis, S. Perez and D. Kokkinopoulos, 1993. Bioactive compounds from the buds of *Platanus orientalis* and isolation of a new kaempferol glycoside. *Planta Med.*, 59: 517-520.
12. Mitrocotsa, D., S. Bosch, S. Mitaku, C. Dimas and A.L. Skaltsounis *et al.*, 1998. Cytotoxicity against human leukemic cell lines and the activity on the expression of resistance genes of flavonoids from *Platanus orientalis*. *Anticancer Res.*, 19: 2085-2088.
13. Ibrahim, M.A., A.A. Mansoor, A. Gross, M.K. Ashfaq, M. Jacob, S.I. Khan and M.T. Hamann, 2009. Methicillin-resistant *Staphylococcus aureus* (MRSA)-active metabolites from *Platanus occidentalis* (American sycamore). *J. Nat. Prod.*, 72: 2141-2144.
14. Emami, A., M.R. Shams Ardekani and I. Mehregan, 2004. Color Atlas of Medicinal Plants. ITMRC Publications, Tehran, Pages: 240.
15. Gupta, M., U.K. Mazumder, P. Gomathi and V.T. Selvan, 2006. Antiinflammatory evaluation of leaves of *Plumeria acuminata*. *BMC Compl. Alternat. Med.*, Vol. 6. 10.1186/1472-6882-6-36.
16. Chirumbolo, S., 2010. The role of quercetin, flavonols and flavones in modulating inflammatory cell function. *Inflamm. Allergy Drug Targets*, 9: 263-285.
17. Parveen, Z., Y. Deng, M.K. Saeed, R. Dai, W. Ahamad and Y.H. Yu, 2007. Antiinflammatory and analgesic activities of *Thesium chinense* Turcz extracts and its major flavonoids, kaempferol and kaempferol-3-O-glucoside. *Yakugaku Zasshi*, 127: 1275-1279.
18. Palanichamy, S. and S. Nagarajan, 1990. Analgesic activity of *Cassia alata* leaf extract and kaempferol 3-O-sophoroside. *J. Ethnopharmacol.*, 29: 73-78.
19. Norata, G.D., P. Marchesi, S. Passamonti, A. Pirillo, F. Violi and A.L. Catapano, 2007. Anti-inflammatory and anti-atherogenic effects of catechin, caffeic acid and trans-resveratrol in apolipoprotein E deficient mice. *Atherosclerosis*, 191: 265-271.
20. Da Cunha, F.M., D. Duma, J. Assreuy, F.C. Buzzi, R. Niero, M.M. Campos and J.B. Calixto, 2004. Caffeic acid derivatives: *In vitro* and *in vivo* anti-inflammatory properties. *Free Radical Res.*, 38: 1241-1253.