Contents lists available at ScienceDirect

Asian Pacific Journal of Tropical Biomedicine

journal homepage:www.elsevier.com/locate/apjtb

Document heading doi:10.1016/S2221-1691(12)60295-0

Pharmacognostic, Physicochemical and Phytochemical Investigation of *Mangifera indica* L. var. Kesar leaf

Kalpna Rakholiya and Sumitra Chanda*

Phytochemical, Pharmacological and Microbiological Laboratory,Department of Biosciences, Saurashtra University, Rajkot-360 005, Gujarat, India

ARTICLE INFO

Article history:
Received 25 June 2012
Received in revised from 5 July 2012
Accepted 7 August 2012
Available online 28 August 2012

Keywords:
Mangifera indica
Leaf
Pharmacognostic study
Physicochemical analysis
Solubility

ABSTRACT

Objective: The aim of the present study was to investigate the morphological and microscopical characters of *Mangifera indica* L. leaf collected from Gujarat region and its phytochemical and physicochemical analysis. **Methods:** Microscopic characters and powder analysis was done under microscope. The physiochemical properties such as loss on drying, total ash value, acid insoluble ash value, water soluble ash value, pH, solubility and extractive values of *M. indica* were carried out. **Results:** The upper epidermis was irregular, wavy with thick epidermal layer; and prismatic, rosette and cluster types of calcium oxalate crystals were found. In phytochemical analysis, cardiac glycosides and tannins showed maximum amounts. **Conclusions:** The present study provides pharmacognostical, physicochemical and phytochemical details of the *M. indica* leaf which are useful in laying down standardization and pharmacopoeia parameters.

1. Introduction

India has a rich heritage of traditional medicine constituting with its different components like Ayurveda, Siddha and Unani. The development of these traditional systems of medicines with the perspectives of safety, efficacy, and quality will help not only to preserve the traditional heritage but also to rationalize the use of natural products in healthcare[1] . Pharmacognostic study is the preliminary step in the standardization of crude drugs. The detailed of Pharmacognostic evaluation gives valuable information regarding the morphology, microscopical and physical characteristics of the crude drugs.

Mangifera indica L. (Anacardiaceae) is one of the important tropical fruits in the world and India contributes major part of the world production. Mango is considered as a king of fruits in Indian delicacy. There are many traditional medicinal uses for the different parts of *M. indica* throughout the globe. The ripe pulp posses numerous therapeutic uses including ripe pulp used as rheological properties[2], unripe pulp used as antibacterial activity against food borne bacteria[3]. Mango pulp contains vitamins, organic acids, carbohydrates, amino acids, polyphenols and certain volatile compounds[4]. The leaves possess antibacterial activity[5], antiulcerogenic action[6], hypoglycemic activity[7], atherogenicity[8]. Seed kernel possess anti-diarrhoeal activity[9], effectiveness for dyslipidemia[10]. Bark and stem possess immunomodulatory activity[11], anti-inflammatory and neuroprotective activity[12].

Authentication and standardization are prerequisite steps especially for herbal drugs and their formulations in traditional systems of medicine[13]. Hence, in this work we make an attempt for standardization of *Mangifera indica* to study the morphological and anatomical features,

*Corresponding author: Phytochemical, Pharmacological and Microbiological Laboratory, Department of Biosciences, Saurashtra University, Rajkot-360 005, Gujarat, India
E-mail: svchanda@gmail.com

physicochemical constants and qualitative phytochemical analysis were done.

Materials and methods

Plant collection and Extraction

The leaf of *M. indica* was collected in May, 2010 from Gujarat region, India. The leaves were separated, washed thoroughly with tap water, shade dried, homogenized to fine powder and stored in air tight bottle. For physicochemical investigation, 10 g of dried powder was extracted by individual cold percolation method using different solvents with different polarities. The solvent was evaporated to dryness and the dried crude extracts were stored in air tight bottle at 4 ℃. The methanol extract was used for the solubility study.

Pharmacognostic studies

Macroscopic characteristics

For morphological observations, fresh leaf approx. 18-20 cm in length were used. The macromorphological features of the leaf were observed under magnifying lens[14].

Microscopic characteristics

Free hand section of leaf was taken and stained by safranine to confirm its lignifications. Powder microscopy was also carried out and the specific diagnostic characteristics were recorded[15].

Physicochemical parameters

The physicochemical parameters like total ash value, loss on drying, water soluble ash value, acid insoluble ash value, petroleum ether, methanol, acetone and water soluble extractive values, pH value, solubility, etc. were determined as per WHO guidelines[16].

Phytochemical analysis

The crude powder of *M. indica* leaf was subjected to qualitative phytochemical analysis[17,18].

Statistical analysis

All experiments were repeated at least three times. Results are reported as Mean ± S.E.M. (Standard Error of Mean).

Results

Macroscopic characteristics

The fresh leaf of *M. indica* was pale green in color, 19.5 cm long, 1.5 cm wide, smooth surface, sharp spine at the apex and venation was reticulate (Figure 1).

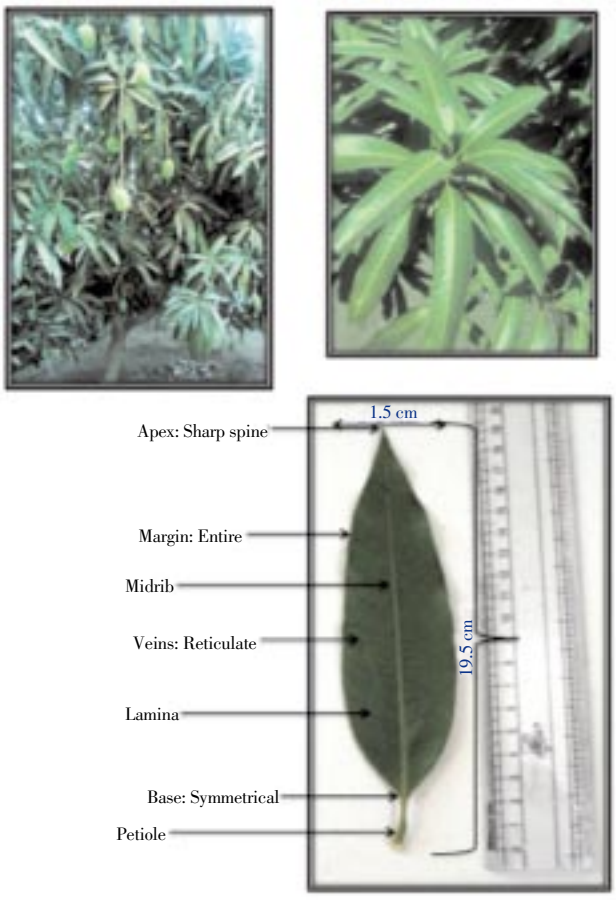

Figure 1. Macroscopic characteristics of *M. indicaleaf*

Microscopic characteristics

Transverse section of leaf is shown in Figure 2, leaf was dorsiventral. In lamina, the epidermis was covered with a single layer of cuticle. The upper and lower epidermis is single layered and in between single layer of palisade tissue and spongy parenchyma were found. Epidermal cells were found wavy thick and irregular in shape (Figure 4). Prismatic, rosette and cluster types of calcium oxalate crystals were found (Figure 3).

Powder study

The crude powder of leaf was green in color with characteristic odour and astringent taste. Microscopic study of powder showed various characters such as paracytic stomata, covering trichomes, xylem vessels, and rosette and prisms type crystals (Figure 5) .

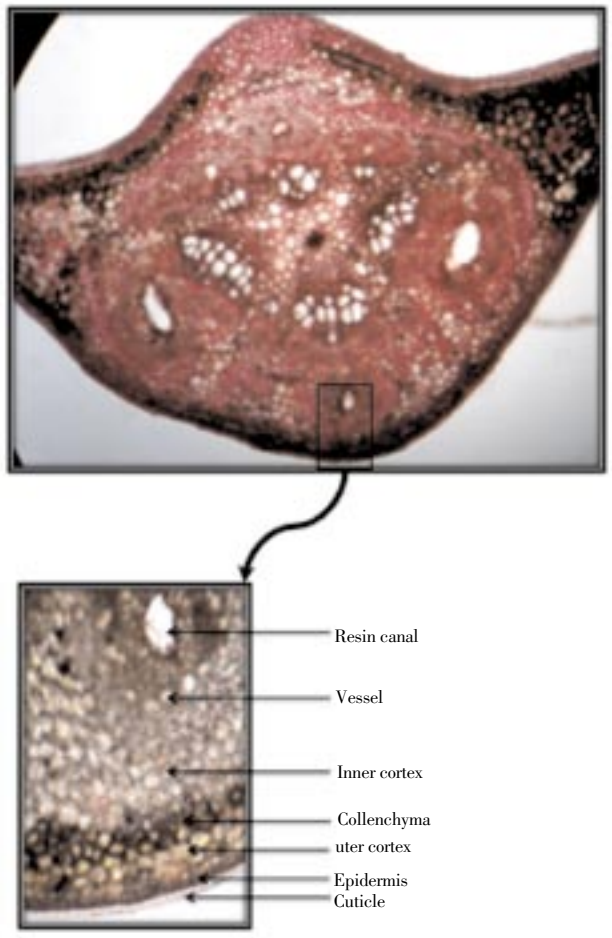

Figure 2. Photomicrographs of microscopic characteristicss of *M. indicaleaf*

Physicochemical parameters

Physicochemical characterization of powder of *M. indica* leaf is shown in Table 1. The physical constant evaluation of the drugs is an important parameter in detecting adulteration or improper handling of drugs. The leaf showed less moisture content; it was only 8.9%; hence it would discourage bacterial and fungal growth. The ash value was determined by three different forms viz., total ash, water soluble ash and acid insoluble ash. The total ash was found in range between 9.6%, while water soluble ash and acid insoluble ash was 8.0% and 7.3% respectively. pH of methanol extract was 4.43 (Table 1).

The extractive value of *M. indica* leaf is shown in Table 1. The maximum extractive value was found in methanol solvent minimum was in petroleum ether. The methanol extract of *M. indica* leaf was evaluated for its solubility in 10 solvents with varied polarities. The extract was highly soluble in dimethyl sulfoxide (DMSO) and dimethyl formamide (DMF), but less soluble in non polar solvents (Table 2).

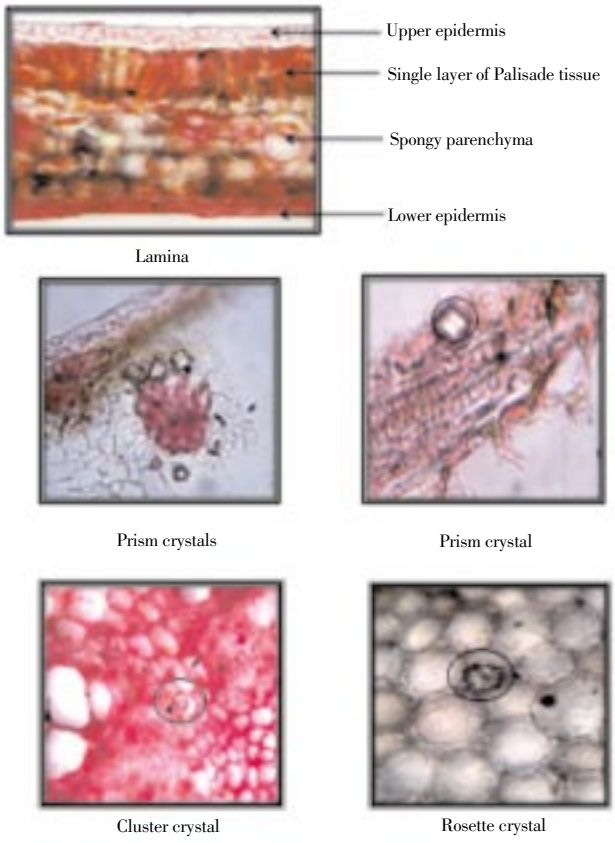

Figure 3. Photomicrographs of microscopic characteristics of *M. indicaleaf*

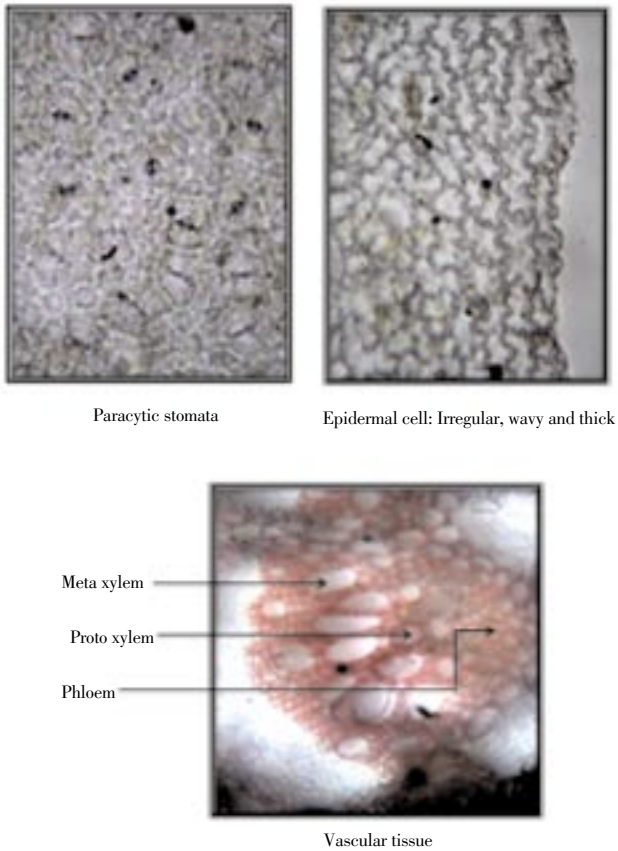

Figure 4. Photomicrographs of microscopic characteristics of *M. indicaleaf*

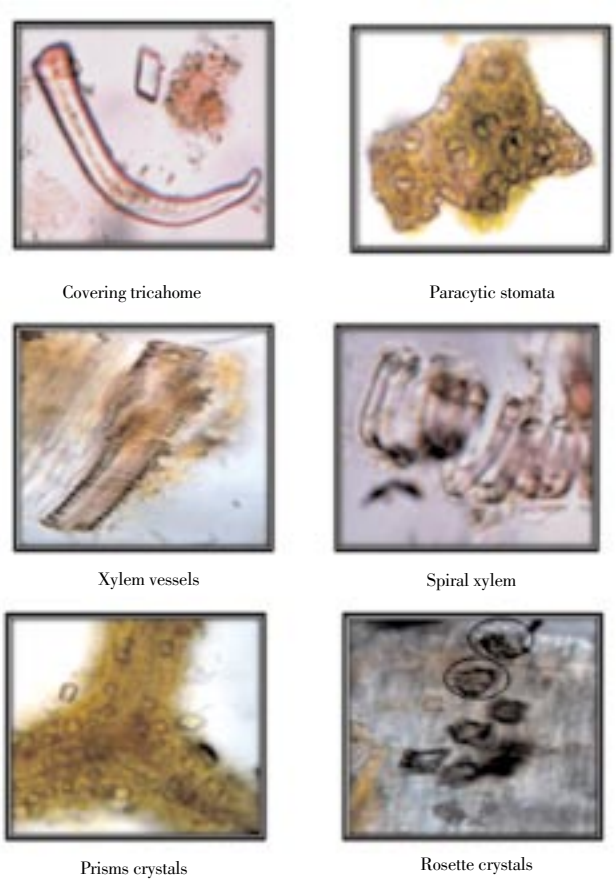

Figure 5. Photomicrographs of microscopic characteristics of *M. indicaleaf*

Table 1
Physicochemical parameters of M. indica leaf

No.	Parameters	Values
1	Loss on drying	8.9 ± 0.25
2	Total ash	9.6 ± 0.58
3	Water soluble ash	8.0 ± 0.54
4	Acid insoluble ash	7.3 ± 0.23
5	Petroleum ether soluble extractive value	1.49 ± 0.60
6	Acetone soluble extractive value	5.17 ± 0.93
7	Methanol soluble extractive value	14.76 ± 0.87
8	Aqueous soluble extractive value	9.74 ± 0.42
9	pH	4.43

Table 2
Solubility of *M. indica* leaf methanol extract in different solvents

Solvents		Solubility (mg ml^{-1})
Non polar solvents	Petroleum ether	11
	Hexane	7
	Chloroform	10
	Toluene	6
	Ethyl acetate	8
Polar solvents	Acetone	51
	Methanol	346
	Dimethyl sulfoxide (DMSO)	465
	Dimethyl formamide (DMF)	440
	Water	165

Phytochemical analysis

The results of qualitative phytochemical analysis of the crude powder of *M. indica* leaf are shown in Table 3. Leaf had maximum cardiac glycosides and tannins while saponins and triterpenes were in less amount.

Table 3
Qualitative phytochemical analysis of M. indica leaf

Phytochemicals	Test	Crude powder
Alkaloids	Dragendroff's test	++
	Mayer's test	−
	Wagner's test	+
Flavonoids	Alkaline reagent	−
Tannins	FeCl3 test	+++
Phlobatanins	HCl test	−
Triterpenes	H2SO4 test	+
Steroids	Liebermann-Burchard test	−
Saponins	Frothing test	+
Cardiac glycosides	Keller-kilianni test	+++

−: Absent; +: Less present; ++: Moderate present; +++: High present

Discussion

Indian systems of medicine uses majority of the crude drugs that are of plant origin. It is necessary that standards have to be laid down to control and check the identity of the plant and ascertain its quality before use. A detailed pharmacognostic evaluation therefore is highly an essential prerequisite. According to World Health Organization (WHO) the macroscopic and microscopic description of a medicinal plant is the first step towards establishing its identity and purity and should be carried out before any tests are undertaken[19].

Mangifera indica, widely used in traditional medicines has tremendous medicinal potential owing to its multifaceted biological functions. The salient diagnostic characteristics of leaf were paracytic stomata, xylem vessels, prism and cluster type of calcium oxalate crystals. These characters can be used for standardization of drugs and also used for preparation of plant monographs. Similar study is reported by various workers in other plants like Actinodaphne hookeri Meissn[20], Oxystelma esculentum (L.f.) R.br. Ex Schltes[21], Datura fastuosa Linn[22], Manilkara hexandra (Roxb.) Dubard[23], Polyalthia longifolia var. Pendula[24], Vitex trifolia Linn.[25], *Punica granatum* L.[26], Citrus paradisi Var. Duncan[27], *Cissus quadrangularis* L.[28], Psidium guajava L.[29], and *Cayratia trifolia* (Linn.)[30].

Ash values are used to determine quality and purity of crude drug. It indicates presence of various impurities like carbonate, oxalate and silicate. The water soluble ash is used to estimate the amount of inorganic compound present in drugs. The acid insoluble ash consist mainly silica and indicate contamination with earthy material. Moisture content of drugs could be at minimal level to discourage the growth of bacteria, yeast or fungi during storage. The extractive values are useful to evaluate the chemical constituents present in the crude drug and also help in estimation of specific constituents soluble in a particular solvent[31]. Acid insoluble ash measures the amount of silica present, especially sand. Water soluble ash is

the water soluble portion of the total ash[24, 32]. Less amount of these three parameters indicate that the inorganic matter and silica were less in *M. indica*.

As there is no pharmacognostical work on record of this traditionally much valued drug, the present work was taken up with a view to lay down standards, which could be useful to establish the authenticity of this medicinally useful plant. Macro and micro morphological standards discussed here can be considered as identifying parameters to authenticate the drug.

Acknowledgement

The authors thank Prof. S.P. Singh, Head, Department of Biosciences, Saurashtra University for providing excellent research facilities; and University Grants Commission, New Delhi, India for providing financial support in the form of a Major Research Project (Sanction letter No. F No. 37-524/2009 (SR) dated 11th January, 2010).

References

[1] Mukherje PK, Wahile A. Integrated approach towards drug development from Ayurveda and other system of medicines, *J Ethnophannacol* 2006; **103**:25-35.

[2] Dak M, Verma RC, Jaaffrey SNA. Effect of temperature and concentration on rheological properties of "Kesar" mango juice. *J Food Eng* 2007; **80**:1011-1015.

[3] Gupta C, Garg AP, Uniyal RC. Antibacterial activity of Amchur (Dried Pulp of Unripe *Mangifera indica*) extracts on some food borne bacteria. *J Pharm Res* 2008; **1**:54-57.

[4] Pino JA, Mesa J, Munoz Y, Marti MP, Marbot R. Volatile components from mango (Mangifera indica L.) cultivars. *J Agr Food Chem* 2005; **53**: 2213- 2223.

[5] Doughari JH, Manzara S. In vitro antibacterial activity of crude leaf extracts of *Mangifera indica* Linn. *Afr J Microbiol Res* 2008; **2**:067-072.

[6] Severi JA, Lima ZP, Kushima H, Brito ARM, Campaner dos Santos L, Vilegas W, Lima AH. Polyphols with antiulcerogenic action from aqueous decoction of mango leaves (Mangifera indica L.). *Molecules* 2009; **14**:1098-11.

[7] Aderibigbe AO, Emudianughe TS, Lowal BA. Evaluation of antidiabetic action of *Mangifera indica* in mice. *Phytother Res* 2001; **15**:456-458.

[8] Muruganandan S, Srinivasan K, Gupta S, Gupta PK, Lala J. Effect of mangiferin on hyperglycemia and atherogenicity in streptozotocin diabetic rats. *J Ethnopharmacol* 2005; **97**:497-501.

[9] Sairam K, Hemalatha S, Kumar A, Srinivasan T, Ganesh J, Shankar M, Venkataraman S. Evaluation of anti-diarrhoeal activity in seed extracts of *Mangifera indica*. *J Ethnopharmacol* 2003; **84**:11-15.

[10]Anila L, Vijayalakshmi NR. Flavonoids from Emblica officinalis and *Mangifera indica* - effectiveness for dyslipidemia. *J Ethnopharmacol* 2002; **79**:81-87.

[11]Makare N, Bodhankar S, Rangari V. Immunomodulatory activity of alcoholic extract of *Mangifera indica* L. in mice. *J Ethnopharmacol* 2001; **78**:133-137.

[12]Lemus-MolinaY, Maria VS, Rene D, Carlos M. *Mangifera indica* L. extract attenuates glutamate-induced neurotoxicity on rat cortical neurons. *NeuroToxicology* 2009; **30**:1053-1058.

[13]Nagani KV, Kevalia J, Chanda SV. Pharmacognostical and phytochemical evaluation of stem of Cissus quadrangularis L. *Int J Pharm Sci Res* 2011; **2**:2856-2862.

[14]Tyler V, Brady L, Robbers J. Pharmacognosy, Varghese Company, India, 1977; p. 103-141.

[15]Khandelwal KR. Practical Pharmacognosy, 19th ed. Nirali Prakashan, Pune, India. 2008; p. 49-70.

[16]WHO. Quality Control Methods for Medicinal Plant Materials. (An authorized publication of World health organization, Geneva). A.I.T.B.S. Publishers & Distributors, New Delhi, 2002.

[17]Harborne JB. Phytochemical methods. 2nd Ed. London: Chapman & Hall, 1973.

[18]Parekh J, Chanda S. Antibacterial and phytochemical studies on twelve species of Indian medicinal plants. *Afr J Biomed Res* 2007; **10**:175-81.

[19]WHO. Quality control methods for medicinal plants. Geneva. 2002; pp. 28-31.

[20]Prajapati DD, Patel NM, Patel SS, Patel MS, Savadi RV, Akki KS, Mruthunjaya K. Pharmacognostic studies on Actinodaphne hookeri Meissn leaves. *J Pharm Res* 2008; **1**:48-54.

[21]Poornima N, Umarajan KM, Babu K. Studies on anatomical and phytochemical analysis of Oxystelma esculentum (L.f.) R.br. Ex Schltes. *Bot Res Int* 2009; **2**:239-243.

[22]Nivedhitha S, Gobinath M, Muthusamy P, Rao KM, Sivakrishna B. Pharmacognostical evaluation on roots of Datura fastuosa Linn. *J Pharm Res* 2010; **3**:2689-2692.

[23]Chanda S, Nagani K, Parekh J. Assessment of quality of Manilkara hexandra (Roxb.) Dubard leaf (Sapotaceae): pharmacognostical and physicochemical profile. *Phcog J* 2010; **2**:520-524.

[24]Dave R, Nagani K, Chanda S. Pharmacognostic studies and physicochemical properties of the Polyalthia longifolia var. *pendula leaf*. *Phcog J* 2010; **2**:572-576.

[25]Thenmozhi S, Sundaram RS, Kumar JP, Bihari CG. Pharmacognostical and phytochemical investigation on leaves of Vitex trifolia linn. *J Pharm Res* 2011; **4**:1259-1262.

[26]Bapodara M, Nagani K, Chanda S. Pharmacognostic and Physicochemical study of Punica granatum L. *leaf*. *Phcog J* 2011; **3**:29-32.

[27]Gupta V, Ghaiye P, Bansal P, Shri R. Pharmacopoeial standards and pharmacognostical studies of leaves of Citrus paradisi Var. Duncan. *J Pharm Res* 2011; **4**:1084-1086.

[28]Nagani KV, Kevalia J, Chanda SV. Pharmacognostical and phytochemical evaluation of stem of Cissus quadrangularis L. *Int J Pharm Sci Res* 2011; **2**:2856-2862.

[29]Kaneria M, Chanda S. Phytochemical and pharmacognostic evaluation of leaves of Psidium guajava L. (Myrtaceae). *Phcog J* 2011; **3**:41-45.

[30]Kumar D, Gupta J, Kumar S, Arya R, Kumar T, Gupta A. Pharmacognostic evaluation of Cayratia trifolia (Linn.) leaf. *Asia Pac J Trop Biomed* 2012; 6-10.

[31]Thomas S, Patil DA, Patil AG, Chandra N. Pharmacognostic evaluation and physicochemical analysis of Averrhoa carambola L. fruit. *J Herb Med Toxicol* 2008; **2**:51-54.

[32]Vaghasiya Y, Nair R, Chanda S. Antibacterial and preliminary phytochemical and physico-chemical analysis of Eucalyptus citriodora Hk leaf. *Nat Product Res* 2008; **22**:754-762.