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Research Article

Development of Quality Control Parameters for Standardization of *Cyperus scariosus* R.Br. Roots

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ABSTRACT

Cyperus scariosus R.Br. (Family: Cyperaceae) is perennial and delicate slender sedge found wildly in damp or marshy areas. The present study was undertaken for the development of quality control parameters of *Cyperus scariosus* R.Br. roots. The study includes determination of various standardization parameters like morphological characters, microscopic studies, histochemical detection and physicochemical evaluation of *Cyperus scariosus* R.Br. roots. The morphological studies exhibited the organoleptic and surface characteristics of the roots. The microscopic study showed the presence of various characteristic features of roots like cortex, lignified endodermis, pericycle, scalariform xylem vessels, tracheids and phloem. Physicochemical constants such as moisture content, ash values, extractive values, swelling index and foaming index were established. Preliminary phytochemical analysis of root extracts revealed the presence of alkaloids, anthraquinone glycosides, phenols, flavonoids, tannins, saponins, steroids and terpenoids. These pharmacognostic features established in this study would helpful for the establishment of pharmacopoeial standards to facilitate quality control and correct identification of *Cyperus scariosus* R.Br. roots, also to minimize adulteration and substitution.

Keywords: *Cyperus scariosus*, Pharmacognostical, phytochemical, physicochemical.

INTRODUCTION

Traditional medicines embrace a holistic approach to health since time immemorial and it commonly involves the use of herbal remedies[1,2,3]. The most important reason behind applying herbal therapy is the belief that it promotes healthier living without adverse effects. Therefore, It has become essential for healthcare professionals to facilitate better understanding of the factors associated with the quality of these herbal products and to ensure that all herbal medicines are safe to use[4]. The more effective a herbal drug, the greater its chances of exploitation and depletion[5]. Hence, to meet the growing demand of that herbal drug, it is adulterated with low grade materials resulting decreased therapeutic efficacy of the drug. In some cases the drug is completely substituted. Sometimes, closely related species are known by the same vernacular or common names due to which substitution of these plants become very easy[5,6]. The lack of documentation and rigorous quality control has also become a key obstacle hindering the acceptance of herbal drugs in developed countries[7]. Keeping all these backdrops in mind, the development of standards becomes extremely important for accurate identification, authentication, and standardization of herbal medicines[8]. Pharmacognostical standardization is an effective tool to lay down standardization parameters to ensure the authentication of plants and prevention of adulteration[5,6]. These studies are not only beneficial in authentication but also ensure reproducible quality of herbal products in commerce[6]. Standardization and quality control of plants as well as herbal products are also essential for the worldwide acceptance of these drugs in modern system of medicine. Hence, there is own set of guidelines accepted by each country for quality control of the herbal medicine[9]. *Cyperus scariosus* R.Br., a perennial, delicate slender sedge belonging to family Cyperaceae, is commonly known as "nagarmotha" or "nutgrass." It is widely distributed in India and found in various parts of the country, especially in damp or marshy areas like around rivers and waterfalls. Nagarmotha is also found in South Africa, China and Pacific Islands. The plant is well known for its uses in the traditional systems of medicine[10]**.** The plant has been proved for various pharmacological activities such as antimicrobial[11,12], antinociceptive, hypoglycaemic[13], hepatoprotective[14], hypolipidemic[15], anti depressant[16], hypotensive and spasmolytic activities[17]. In spite of its high medicinal value, the pharmacognostical standardization of its root is not reported till date. Hence, the present study was carried out for the determination of various pharmacognostical standardization parameters such as morphological characters, microscopic studies, histochemical detection, heavy metal detection and physicochemical evaluation of *Cyperus scariosus* R.Br. roots. Preliminary phytochemical screening was also carried out for determination of various phytoconstituents in the plant roots.

MATERIAL AND METHODS

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Figure 1: *Cyperus scariosus* R. Br. Roots.

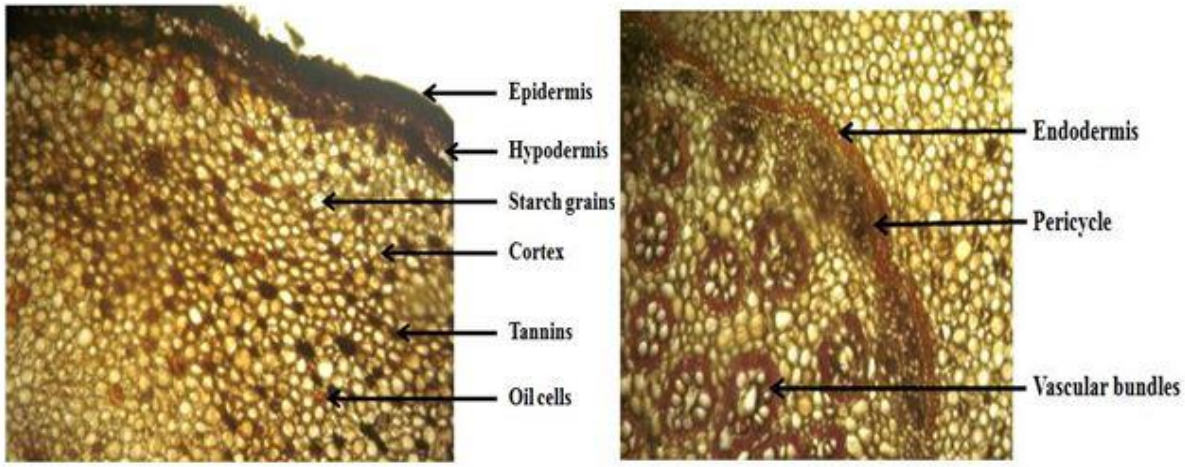

Figure 2: Transverse section of *Cyperus scariosus* R.Br. roots.

Table 1: Histochemical detection of cell wall and its contents in *Cyperus scariosus* R. Br. Roots.

Test	Result
Cellulose cell wall	+
Lignified cell wall	+
Suberized cell wall	+
Aleurone grains	+
Calcium carbonate	-
Calcium oxalate	-
Fats	+
Mucilage	-
Starch	+
Tannins	+

+ =present; - =absent

Plant collection and authentication

Cyperus Scariosus R. Br. roots were procured by Nature and Nurture Healthcare Pvt. Ltd. (New Delhi) in March, 2015. The roots of this plant have been selected for present work. The plant was authenticated by Dr. Sunita Garg, Chief Scientist, Raw Material Herbarium and Museum, CSIR-National National Institute of Science Communication and Informaion Resources, New Delhi, vide reference no, NISCAIR/RHMD/Consult/2015/2835/28 dated 16.06.2015. The plant was identified as *Cyperus scariosus* R. Br.

Morphological studies

The morphological characters color, odour, taste, shape, size, surface characteristics and texture of the roots were studied by naked eye and with the help of dissection microscope.

Microscopical studies

The dried roots were soaked in water to make it smooth enough for transverse section. Transverse section of root was cut by free hand sectioning. The transverse sections were cleared with chloral hydrate, stained with phloroglucinol and concentrated hydrochloric acid, mounted in glycerol and studied under microscope. For powder microscopy, small amount of root powder was taken on slide and mounted with phloroglucinol, concentrated hydrochloric acid and glycerine. The slides were observed under light microscope (Carl Zeiss Primo star, Germany). Histochemical studies were also done by using various reagents to detect cell wall and its contents in the plant roots[18].

Table 2: Physicochemical characters of *Cyperus scariosus* R. Br. Roots.

S.no.	Parameter	Value
1.	Foreign organic matter	1.83 ± 0.15 %w/w
2.	Loss on drying	2.6 ± 0.08 % w/w
3.	Swelling index	0.00 ml/g
4.	Foaming index	Less than 100
5.	Extractive values	
	Ethanol extractive value	
	Hot extraction method	7.9 ± 0.09 % w/w
	Cold maceration method	7.33 ± 0.11 % w/w
	Aqueous extractive values	
	Hot extraction method	14 ± 0.15 % w/w
	Cold maceration method	8.26 ± 0.17 % w/w
6.	Ash values	
	Total ash	5.83 ± 0.14 % w/w
	Water soluble ash	1.33 ± 0.10 % w/w
	Acid insoluble ash	1.66 ± 0.23 % w/w
	Sulphated ash	5.74 ± 0.18 % w/w

Values in % w/w are expressed as mean ± SEM; n=3

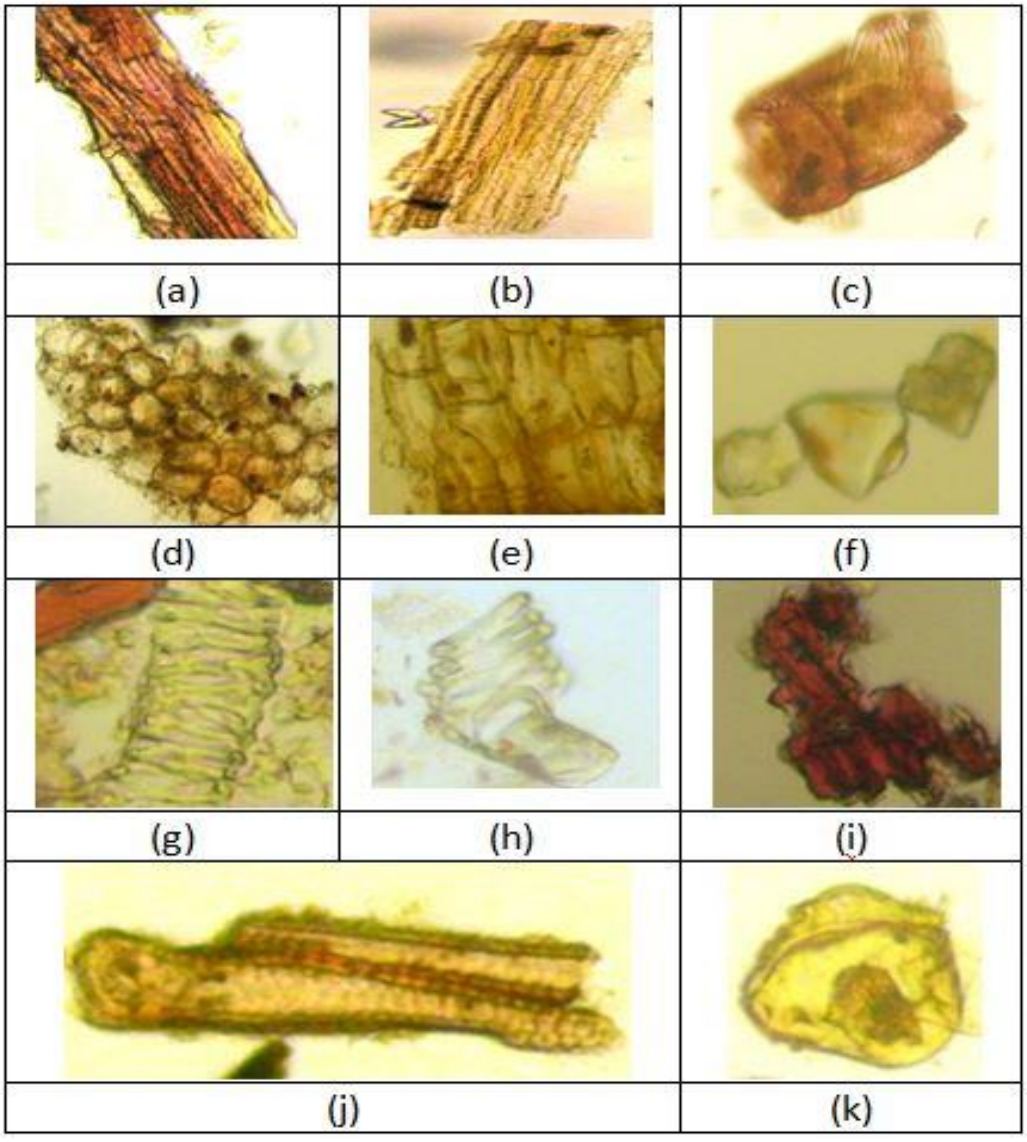

Figure 3: Powder microscopy of *Cyperus scariosus* R. Br. Roots.

Table 3: Elemental analysis of *Cyperus scariosus* R.Br. roots.

Metal	Concentration (ppm)
Lead	0.893
Cadmium	0.035
Zinc	0.360
Arsenic	0.000
Copper	0.697
Iron	0.508
Mercury	0.000
Magnesium	10.548

Physicochemical parameters

Physicochemical parameters of the plant roots were studied using standard procedures[18,19]. These parameters include foreign organic matter, ash values, extractive values, swelling index, foaming index and loss on drying.

Elemental analysis

Elemental analysis was performed using nitric-perchloric acid digestion method using the procedure recommended by the AOAC (1990). One gram of root sample was taken and boiled gently with 10 ml of concentrated nitric acid for 30-45 min. The mixture was cooled down and 5 ml of 70% perchloric acid was added to it. The mixture was boiled gently until the appearance of dense white fumes. The mixture was cooled down and boiled further with 20 ml of distilled water to release the fumes. After cooling, the solution was filtered through Whatman No. 42 filter paper[20,21]. Samples so obtained were analysed in Atomic Absorption Spectroscope (AAS) (GBC 932 plus). An atomic absorption spectrophotometer with hollow cathode lamp for lead (Pb), cadmium (Cd), copper (Cu), arsenic (As), zinc (Zn), mercury (Hg), iron (Fe) and magnesium (Mg) was used. The instrument was calibrated by using standard solutions of As, Hg, Pb, Cd, Cu, Zn, Fe and Mg at various wavelengths 193.7, 253.7, 283.5, 228.8, 324.8, 213.9, 248.3, 242.1 nm respectively. The standard calibration curves were then prepared. The instrument was optimized as per requirement and results were obtained in ppm levels.

Prelminary phytochemical screening

The powdered roots of *Cyperus scariosus* R.Br. (100g) was defatted by extracting with petroleum ether and then successively extracted with ethanol and water. Each time the powdered drug was extracted with one solvent, it was dried below 50^{o} C before extracting with the next solvent. The ethanol extract was prepared by using soxhlet apparatus. The extract obtained from soxhalation was concentrated by distilling off the solvent and recovering the same. The total aqueous extract was prepared by cold maceration method. The drug was macerated with distilled water for 24 hours and then filtered. The marc obtained was again macerated with distilled water and filtered. The filtrates were combined. The ethanol and aqueous extracts were then evaporated to dryness to obtain dried extracts and were kept in dessicator. The prepared extracts were subjected to various chemical tests for the presence of different phytoconstituents[22].

Determination of total phenol content

The amount of total phenol content was determined by Folin-ciocateu reagent method. 1ml of ethanol extract was taken and 0.5 ml of Folin-ciocalteu reagent (0.5 N) was added to it. This solution was incubated at room temperature for 15 minutes. Then 2.5 ml saturated sodium carbonate was added and further incubated in dark for 40 minutes at room temperature. Absorbance was measured at 725 nm. Gallic acid was used as standard. The total phenolic content was expressed as mg of gallic acid equivalents/g of dry weight[23,24].

Determination of total flavonoid content

The total flavonoid content was determined by aluminium chloride method[25]. 1 ml of ethanol extract was mixed with 4 ml of water and 0.3 ml of sodium nitrite solution (5%). Then, 0.3 ml of aluminium chloride (10%) was added after 5 minutes. The solution was kept for 5 minutes and then, 2 ml of 1M NaOH was added to it. The solution was diluted to a final volume of 10 ml and absorbance was measured at 510 nm. Total flavonoid content was determined by using calibration curve of quercetin and expressed as mg of quercetin equivalents/g of dry weight.

RESULTS AND DISCUSSION

Morphological studies

Cyperus scariosus R. Br. consisted of tuberous roots with a few hairy rootlets and striations on external surface. The roots were oblong shaped with fibrous fracture. The roots were black or brown in colour externally and buff coloured internally. The roots were bitter in taste and had a muddy odour. The roots are shown in figure 1. The organoleptic characteristics revealed by morphological studies of the roots, can be used as basis for identification of the plant.

Microscopical characters

Transverse section of the root shows an outermost layer of characteristic undulating epidermis with tabular cells. The cork is absent in the plant roots. Below epidermis, 3-4 layered hypodermis is present which possess sclerenchymatous cells. Cortex is broad with thin walled parenchyma cells embedded at places with starch grains and oil cells. Some cortical parenchyma cells are also filled with brown coloured condensed tannins. Endodermis consists of two layers of tangentially elongated and lignified cells. The endodermis separates the outer cortex from stele which consists of pericycle, ground parenchyma tissue and scattered vascular bundles. Pith is absent in stele. The parenchyma cells show the feature similar to cortical cells and are filled with tannin material. Large number of vascular bundles is embedded in parenchyma tissues which are concentric with phloem enclosing the xylem. The transverse section of root is shown in figure 2. In powder microscopy, *Cyperus scariosus* roots showed the presence of parenchyma cells, sclerenchyma cells, scalariform xylem vessels, lignified fibres, non-lignified fibres, tracheids, starch grains and oil cells which are shown in figure 3. Histochemical studies showed the presence of cellulose cell wall**,** lignified cell wall, suberized cell wall, aleurone grains, starch and tannins. The results are shown in Table 1. These microscopic studies will be useful in differentiation of the plant from other related species.

Table 4: Preliminary phytochemical screening of *Cyperus scariosus* R. Br. root extracts.

Plant constituent	Ethanol extract	Aqueous extract
Carbohydrates		
Molish test	+	+
Fehling's test	+	-
Benedict's test	+	+
Barfoed test	+	-
Test for pentose sugar	+	-
Test for hexose sugar	-	-
Alkaloids		
Dragondroff's reagent	+	-
Meyer's reagent	+	-
Wagner;s reagent	+	-
Hager's reagent	+	-
Anthraquinone glycosides		
Modified Borntrager's test	+	-
Cardiac glycosides		
Keller Killiani test	-	-
Legal test	-	-
Coumarin glycosides		
Fluorescence test	+	-
Cynophoric glycosides		
Sodium picrate test	-	-
Saponins		
Foam test	+	-
Flavonoids		
Ammonia test	+	-
Shinoda test	+	-
Vanillin HCl test	+	-
Phenolic compounds and tannins		
Matchstick test	-	+
Chlorgenic acid test	-	+
Ferric chloride test	+	+
Lead acetate test	+	+
Gelatin test	-	+
Steroids and terpenoids		
Salkovaski Test	+	-
Libermann Burchard's test	+	-

+= present, -= absent

(a) Lignified fibres (b) Non-lignified fibres (c) Lignified vessels (d), (e) Parenchyma cells (f) Starch grains (g) Xylem vessels (h) Vessel elements (i) Sclerenchyma cells (j) Tracheids (k) Oil cell

Physicochemical analysis

The results of different standardization parameters such as foreign organic matter, ash values, extractive values, swelling index, foaming index and loss on drying are given in the Table 2. These physicochemical parameters will be useful to identify the authenticity of the plant even in its crushed or powdered form.

Elemental analysis

The elemental contents Pb, Cd, Cu, As, Zn, Hg, Fe and Mg were analysed in the powdered roots and were found to be in limits. The results are shown in the Table 3.

Prelminary phytochemical screening

Preliminary phytochemical analysis of *Cyperus scariosus* R. Br. root extracts showed the presence of alkaloids, anthraquinone glycosides, phenols, flavonoids, steroids, terpenoids, saponins and tannins. The results of various tests performed for preliminary phytochemical screening of root extracts are shown in Table 4.

Determination of total phenolic content

The total phenolic content in ethanol extract was estimated by Folin Ciocalteu's method using gallic acid as standard. The gallic acid solutions of concentration (20-100 ppm) confirmed to Beer's Law at 725 nm with a regression co-efficient (R^2) = 0.998. The equation of standard curve is y = 0.011x + 0.085. Total phenolic content was found to be 0.47±0.06 mg of gallic acid equivalents/g of dry weight.

Determinationof total flavonoid content

The total flavonoid content in ethanol and aqueous extracts were estimated by Folin Ciocalteu's method using quercetin as standard. The quercetin solutions of concentration (20-100 ppm) confirmed to Beer's Law at 510 nm with a regression co-efficient (R^2) = 0.998. The equation of standard curve is y = 2.100x -0.196. Total flavonoid content was found to be 0.86±0.04 mg of quercetin equivalents/g of dry weight.

CONCLUSION

The study was undertaken to develop the quality control parameters of *Cyperus scariosus* R. Br. roots. These results obtained from pharmacognostical and preliminary phytochemical studies would serve as a diagnostic tool for the standardization of the roots to facilitate quality control and identification of the plant material and to minimize adulteration and substitution. These parameters can also be used for the further scientific investigation of the roots.

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REFERENCES

1. Gurib-Fakim A. Medicinal plants**:** Traditions of yesterday and Drugs of Tomarrow. Mol aspets Med, 2006; 27(1): 1-93.
2. Chopra A, Doiphode VV. Ayurvedic medicine: Core concept, Therapeutic principles and current relevance. Med Clin N AM, 2002; 86 (1): 75-89.
3. Wah CL, Hock SH, Yun TK. Current scientific status and regulatory control of traditional/herbal medicinal products: Globalization challenges. Pharm Eng, 2012; 32 (6): 1-16.

4. Ekor M. The growing use of herbal medicines: issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol, 2014; 4: 1-10.
5. Amponsah IK, Mensah AY, Otoo A, Mensah MLK, Jonathan J. Pharmacognostic standardisation of *Hilleria latifolia* (Lam.) H. Walt. (Phytolaccaceae). Asian Pac J Trop Biomed, 2014; 4(12): 941-946.
6. Chanda S. Importance of pharmacognostic study of medicinal plants: an overview. J Pharmacogn Phytochem, 2014; 2(5): 69-73.
7. Baragi UC, Baragi PC, Vyas MK, Shukla VJ. Standardization and quality control parameters of *Dashanga Kwatha* Ghana tablet: An *Ayurvedic* formulation. Int J Ayurveda Res, 2011; 2(1): 42-47.
8. Datta D, Chandola H, Agarwal SK, Shukla VJ, Pandya PN. Pharmacognostical and analytical study of Tulsi-Amla-Yasti Ghrita. Ayu, 2012; 33(2): 274-278. Ahmad S. 2007. Introduction of Plant Constituents and their Tests. Jamia Hamdard, Hamdard Nagar, New Delhi.
9. Ahmad I, Ahmad Khan MS, Cameotra SS. Quality Assessment of Herbal Drugs and Medicinal Plant Products. Encycl Anal Chem, 2014: 1-17.
10. Srivastava RK, Singh A, Srivastava GP, Lehri A, Niranjan A, Tewari SK, Kumar K, Kumar S. Chemical Constituents and Biological Activities of Promising Aromatic Plant Nagarmotha (Cyperus scariosus R.Br.): A Review. Proc Indian Natl Sci Acad, 2014; 80: 525-536.
11. Lahariya AK, Rao JT. *In vitro* antimicrobial studies of the essential oil of *Cyperus scariosus* and *Ocimum basilicum*. Indian Drugs. 1979; 16: 150-152.
12. Deshmukh SK, Jain PC, Mycotoxicity of some essential oils against six dermatophytes. Symp. Recent Adv Stud Essent Oils, 1985: 34.
13. Alam MA, Jahan R, Rahman S, Das AK, Rahmatullah M. Antinociceptive and anti-hyperglycemic activity of methanol leaf extract of *Cyperus scariosus*. Pak J Pharm Sci, 2011; 24 (1): 53-56.
14. Gilani AH, Janbaz KH. Studies on protective effect of *Cyperus Scariosus* extract on acetaminophen and CCl_4-induced hepatotoxicity. General Pharmac, 1995; 26 (3): 627-631.
15. Chawda HM, Mandavia DR, Parmar PH, Baxi SN, Tripathi CR. Hypolipidemic activity of a hydroalcoholic extract of *Cyperus scariosus* Linn. root in guinea pigs fed with a high cholesterol diet. Chin J Nat Med, 2014; 12(11): 819-826.
16. Ramesh S, Rao BM, Mahesh V, Prabhaker T, Swamy P, Nagaraju P. Pharmacological study of anti-depressant like activity of *Cyperus Scariosus* oil in mice. Int Res J Pharm App Sci, 2012; 2(5): 139-142.
17. Gilani AH, Janbaz KH, Zaman M, Lateef A, Tariq SR, Ahmad HR. Hypotensive and spasmolytic activities of crude extract of *Cyperus scariosus*. Arch Pharm Res, 1994; 17(3): 145-149.
18. World Health Organization. 2011. Quality control methods for herbal materials. (Updated edition of quality control methods for medicinal plant materials, 1998). Malta: World Health Organization.
19. Indian Pharmacopoeia 2007, Vol. 1. Ghaziabad, India: The Indian Pharmacopoeia Commission, Government of India.
20. Hseu ZY. Evaluating heavy metal contents in nine composts using four digestion methods. Bioresource Technol, 2004; 5: 53-59.
21. AOAC.1990. AOAC official methods of analysis. 15th ed. Association of Official Analytical Chemists. Arlington: Virginia 84-85.
22. Ahmad S. 2007. Introduction of Plant Constituents and their Tests. Jamia Hamdard, Hamdard Nagar, New Delhi.
23. Kujala TS, Loponen JM, Klika KD Pihlaja K. Phenolic and betacyanins in red beetroot (*Beta vulgaris*) root: distribution and effects of cold storage on the content of total phenolics and three individual compounds. Journal Agr Food Chem, 2000; 48, 5338–5342.
24. Siddhuraju P. Antioxidant activity of polyphenolic compounds extracted from defatted raw and dry heated Tamarindus indica seed coat. LWT - Food Sci Technol, 2007; 40: 982-990.
25. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents on mulberry and their scavenging effects on superoxide radical. Food Chem, 1999; 64: 555-559.