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MORPHO-ANATOMICAL AND PHYTOCHEMICAL SCREENING OF AERIAL PARTS OF *BORRERIA HISPIDA* K.SCH.

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ABSTRACT

Borreria hispida K. Sch. (syn. *Spermacoce hispida* L.) popularly known as 'Nattaichuri' in Tamil and 'Shaggy button weed' in English, belongs to the family Rubiaceae. The plant is noted for its immense medicinal prospective. So the increasing demand for herbal medicines has inevitably led to maintaining the quality and purity of herbal raw materials and finished products. WHO acknowledged that pharmacognostical standards should be proposed as a protocol for the authentication and quality assurance of herbal drugs. The goal of the present evaluation is carried out the morpho-anatomical and phytochemical standardization of aerial parts above said plant.

KEYWORDS: *Borreria hispida*, morpho-anatomical studies, phytochemical, aqueous and ethanolic extracts.

INTRODUCTION

Nature has been a potential source of many therapeutic agents for thousands of years and an impressive number of modern drugs have been derived from plants. It is estimated that roughly 1500 plant species in Ayurveda, 1200 plant species in Siddha have been used for drug preparation. Though the Indian traditional systems of medicine are time-tested and practiced successfully from time immemorial, there is lack of standardization with regard to identification of crude drugs, methods of preparation and quality of finished products. Pharmacognosy in a broad sense, embraces the knowledge of the history, distribution, cultivation, collection, selection, preparation, commerce, identification, evaluation, preservation and use of drugs and economic substances that affect the health of men and other animals. *Borreria hispida* K. Sch. (syn. *Spermacoce hispida* L.) popularly known as 'Nattaichuri' in Tamil and 'Shaggy button weed' in English, belongs to the family Rubiaceae. *Borreria hispida* is a procumbent herb; stem quadrangular, hirsute, hispid, with usually long internodes. Leaves sub sessile, 1- 3.5 cm long, oblong or elliptic, often rounded at the tip, scabrid, pubescent. Flower very small, 4-6 in a whorl within the stipular cup. The calyx-teeth are linear-lanceolate. The corolla is pale blue or white and it is 5 to 10mm in length. The fruit is hairy capsule about 5mm in length. The seeds are oblong; granulate, opaque, usually variable and 3mm or less in length.[1]

The plant is noted for its immense medicinal prospective. All the parts of the plant have an ethno medicinal importance.[2] Seeds of this plant are crushed into a paste and taken orally to treat stomach problems.[3] Seeds have been recommended as a substitute for coffee. The seed extract of the plant has been used as a remedy for the treatment of internal injuries of nerves, kidney, anti-diabetic, anti-hyperlipidemia. The plant has been used by the tribes living in the forest regions in the Western Ghats of Kerala since ancient times as it is known to remove signs of old age, purify blood and improve vitality. It has been also reported that *Borreria hispida* is an effective natural drug for the treatment of hypertension and has hepatoprotective function[4], anti-inflammatory[5], antioxidant[6-7], analgesic[8], hypolipidemic[9], anti-diabetic[7], anti-hypertensive[10], anti-fungal[11] and anti-cancer activities.[12] With this backdrop, in the present study, an attempt has been made for morpho-anatomical and phytochemical standardization of the plant for contribution in the quality control herbal drug and increase in the knowledge of plant and its family.

MATERIALS AND METHODS

The plant material was collected from *Borreria hispida* K.Sch. was collected from Vellakovil, Tiruppur district, Tamil Nadu. The plant was identified and authenticated by the taxonomists at the PG and Research Department of Botany, Vellalar College for Women, Erode and the herbarium specimen has been deposited at the college for further reference. The shade dried aerial plant parts were ground into a coarse powder with the help of a blender. The powder was stored in an airtight container and kept in a cool, dark and dry place until further analysis.

Morphological studies were performed by using simple microscope.[13] The fresh aerial plant parts to determine the color, odour, taste, shape, size, texture, etc. For micro characterization, free hand sections of about 10-20µm thickness of stem, rachis and leaflet cleared with chloral

hydrate solution and stained with aqueous safranin (0.5%) solution. After washing, the stained sections were mounted on clean micro slides and examined. The preparation was further observed in an image analyzer (Nikon S700) and the anatomical peculiarities were photo documented.[14] The dried material was subjected to powder microscopy studies, which includes the identification of organoleptic characters, behaviour of the powder with different chemical reagents/solvents according to methods described in Indian pharmacopoeia.[15] Phytochemical reporting of the plant, 10gm of the dried powder was subjected to cold extraction with ethanol and aqueous extracts for 7 days; the extract concentrated and then carried out preliminary phytochemical following the method of.[16-18]

RESULTS AND DISCUSSION

Macroscopic Analysis

The macroscopical characters of the plant can serve as diagnostic parameters to provide the standards, to identify the crude drug and to avoid adulteration of drugs.[19] *Borreria hispida* is a procumbent herb; stem quadrangular, hirsute, hispid, 15-35cm long. Leaves sub sessile, 1- 3.5cm long, oblong or elliptic, often rounded at the tip, scabrid, pubescent. Flower very small, 4-6 in a whorl within the stipular cup. The calyx-teeth are linear-lanceolate. The corolla is pale blue or white and it is 5 to 10mm in length (Plate 1). The fruit is hairy capsule about 5mm in length. The seeds are oblong, granulate, opaque, usually variable and 3mm or less in length.

Microscopic Analysis

Anatomical features of plants have been considered as highly dependable guide lines for diagnosis of fragmentary plant.[20,21] The anatomical studies were made from very thin sections of stem and leaf showed the following features.

Stem

The stem is irregular in outline, showing ridges. Epidermis is single layered and coated with a thick cuticle and composed of spherical or ovoid cells (Plate 2.1). Some of the epidermal cells develop trichomes which are eglandular hairs and are 2-celled (Plate 2.2). Cortex is differentiated into outer layer consists of 2 rows of parenchymatous cells that are ovoid in shape, middle cortex is made up of three to four layers of chlorenchyma cells and the inner layer consists of five rows of cells irregularly elongated with various size and shape. Endodermis is elongated barrel-shaped and single layered. Secondary phloem occurs in continuous sheath encircling the xylem and is multilayered. Secondary xylem vessels are hexagonal, multilayered and radiating. Primary xylem is seen towards the center. The pith is large and consists of hexagonal, parenchmatous brown colour cells. Dense deposits of calcium oxalate crystals (Plate 2.3) and starch grains are seen in the pith and cortex region. Similarly, Soosairaj *et al.*,[22] observed ridges on stem, trichomes, cortex, endodermis, xylem and phloem and pith region showed the presence of brown colour cells deposits on *Spermacoce ocymoides*, *S. articularis* and *S. pusilla* a feature which is very distinctly characterized in all stem sections.

Leaf

The leaf is dorsiventral with a thick midrib. The midrib has a deep groove on the adaxial side and semicircular on the abaxial side (Plate 3.1). The epidermal cells are rectangular to irregular in shape with straight to slightly wavy anticlinal walls on both adaxial and abaxial side of leaf. In the leaf there are two kinds of trichome (large and small), all of them are unicellular and they were present all over leaf surface. They are frequently present, easily observable and have often been found to have variation in patterns which correlate with other features of the taxa under investigation.[23] In contrast, Soosairaj *et al.*,[22] noticed trichomes at the leaf edge in *Spermacoce ocymoides* and *S. pusilla* and in *S. articularis* they were present all over leaf surface. Also, in *Spermacoce articularis* and *S. latifolia* there was only one type of trichome. The present research, the vascular bundle of the midrib is crescent shaped. The bundle is collateral with xylem facing the adaxial side and phloem facing the abaxial side. Xylem elements are thick walled and compactly arranged. Patches of bast fibres are seen in the phloem. The lamina is smooth. The adaxial epidermis is fairly thick, the cells being rectangular. The abaxial epidermis is thin and the cells are smaller and rectangular. The palisade is bilayered with compactly arranged cylindrical cells. In contrast, Devi Priya and Siril[24] noticed single layered palisade cells in *Rubia cordifolia*. The spongy parenchyma cells are in 5to6 layers; they are irregularly shaped and loosely arranged with rosette of calcium oxalate crystals and trace bundles (Plate3.2).

Powdered-drug Analysis

Microscopic observations of trichomes and crystal characters of medicinal plants have been reported to act as biomarkers to identify the plant even in the powder form.[25] The leaf powder consists of thin and small fragments of epidermal peelings, two kinds (large and small) of epidermal trichomes which are unicellular and eglandular (Plate 4.1) and rosette crystals. Calcium oxalate crystals are abundant with powder. The crystals are in the form of thin pointed needles, which are originally in the form of thick bundles called raphides. Due to breaking of the raphides the needles are scattered in the powder (Plate 4.2). The needles are either uniformly thin or spindle shaped. Broken needles are also seen in the powder. The individual needles are thin and pointed. Similarly, Deoda *et al.*[26] observed raphides in the root powder of *Rubia cordifolia.*

The stem powder showed thick pieces of epidermis, consisting of rectangular cells arranged in compact parallel rows and their walls are thick (Plate 4.3). The vessel elements with are seen. Another characteristic feature of the powder is xylem bundles which are seen in the powder as short, thick or thin bundles. These bundles

consist of broken pieces of xylem elements, especially vessels. Powder also contains broken fragment vessel walls which possess spiral thickening (Plate 4.4).

Organoleptic evaluation

The results of organoleptic study offer a scientific basis for the traditional use of *Borreria hispida* which possessed characters like dark green colour, characteristic odour and taste is showed in Table 1. The aerial plant part powder when treated with various chemicals exhibited green, yellow colour shades showed in Table 2. Comparable to the present study, Devi Priya and Siril[24] noticed yellowish to reddish black colour on treatment of the stem and leaf powder of *Rubia cordifolia* with various reagents.

Preliminary phytochemical screening

In the present study, the aerial plant parts were shade dried and powdered materials were extracted with ethanol and water using cold percolation. Obtained extracts were dried, weighed and the percent yield was calculated as depicted in Table 3. Phytochemical analysis consists of identifying in a plant, chemical compounds showing pharmacological interest. In the present investigation, the qualitative screening of the ethanol and water extracts revealed the presences of a wide range of phytoconstituents are given in (Table 4). The results showed the presence of proteins and carbohydrates, amino acids, alkaloids, flavonoids, tannins, phenolic compounds, triterpenoids, steroids, coumarins, saponins, terpenoids, quinines anthraquinines and fixed oils and absence of glycosides. This is similar to the reports of Deoda *et al.*[26] and Devi Priya and Siril[24] in *Rubia cordifolia.* Jayachandran *et al.*[27] reported the presence of various phytoconstituents in the petroleum ether, benzene, chloroform and methanol extracts of root of *Spermacoce ocymoides*. The pharmacological action of the crude drug largely depends on the metabolites present in it.

Plate-1.

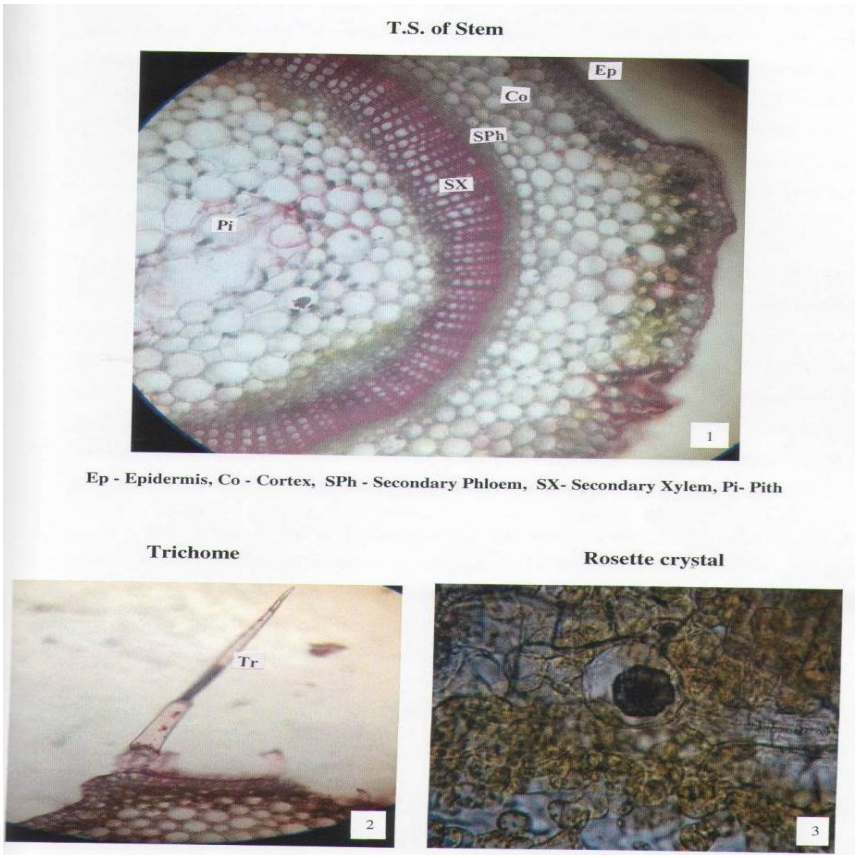

Plate-2.

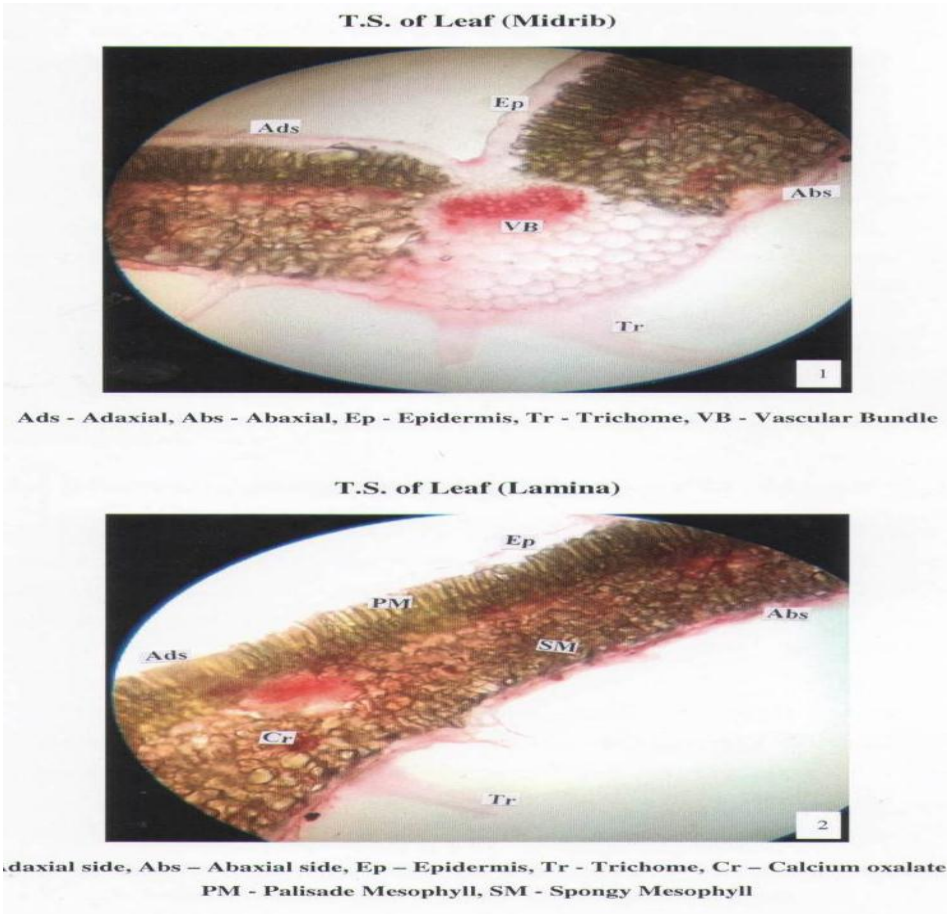

Plate-3.

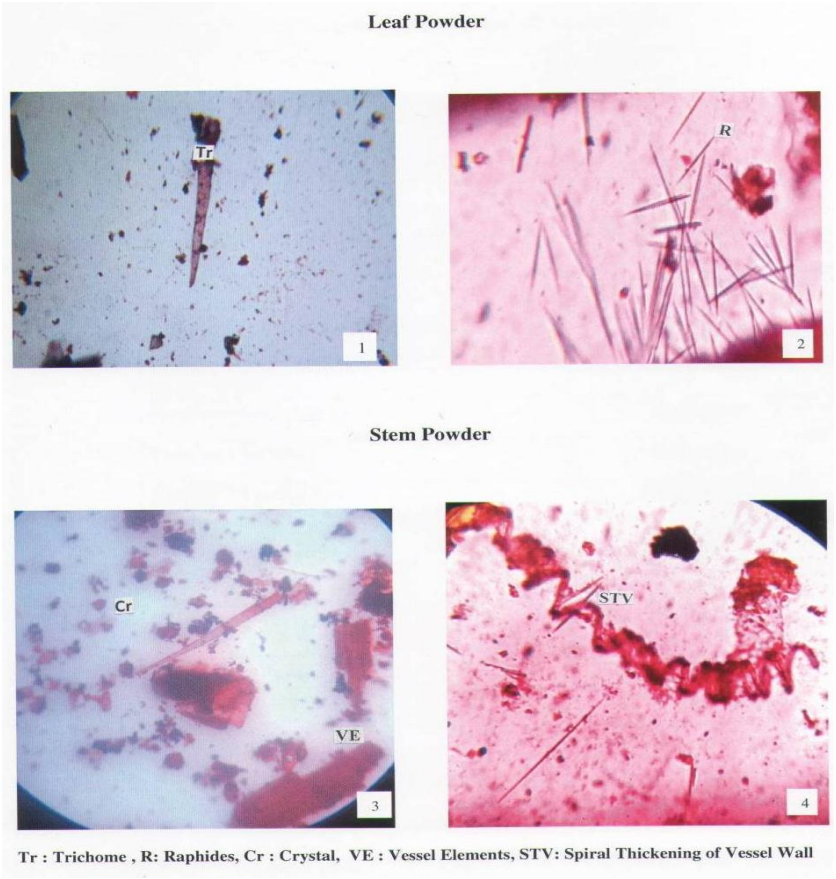

Plate-4.

Table 1: Organoleptic evaluation of aerial plant parts.

S. No.	Features	Observation
1	Nature	Coarse powder
2	Color	Dark Green
3	Odour	Characteristic
4	Taste	Acid

Table 2: Behavior of the powder with different chemical reagents.

S. No.	Treatment	Observation
1.	Powder Untreated	Green colour
2.	Powder + Conc. HCl	Green colour
3.	Powder + Conc.HNO_3	Dark green
4.	Powder + H_2SO_4	Dirty green
5.	Powder + Ferric chloride	Green colour
6.	Powder + Acetic acid	Greenish white
7.	Powder + Ammonia solution	Grass green
8.	Powder +KOH solution	Light green
9.	Powder + NaOH	Light green
10.	Powder + Distilled water	Pale green

Table 3: Percentage yield of plant powder.

S. No.	Extract	Colour	Consistency	Percentage Value (%)
1.	Ethanol	Dark green	Solid	32.4%
2.	Distilled water	Light green	Solid	14.6%

Table 4: Preliminary phytochemical screening of powder of aerial plant parts.

S. No.	Chemical constituents	Chemical tests	Solvents	
			Ethanol	Water
1.	Carbohydrates	Molisch's Reagent	+	-
		Fehling's Reagent	+	-
2.	Proteins and Amino acids	Biuret Reagent	+	+
		Ninhydrin	+	-
3.	Alkaloids	Mayer's Reagent	-	-
		Wagner's reagent	+	+
4.	Flavonoids	Extract + $FeCl_3$	+	+
		Extract + NaoH	-	+
5.	Tannins	Extract + $FeCl_3$	+	+
6.	Phenols	Extract + $FeCl_3$	+	+
		Extract + Lead acetate	+	+
7.	Terpenoids	Extract + Chloroform + Conc. H_2SO_4	+	+
8.	Triterpenoids	Libermann - Burchard's test	+	+
9.	Steriods	Salkowski Test	+	+
10.	Coumarin	Extract + 10% NaOH	+	+
11.	Saponins	Foam Test	+	+
12.	Quinine	Extract + Conc. H_2SO_4	+	+
13.	Anthraquinine	Borntrager's Reagent	-	+
14.	Glycosides	Anthrone + H_2SO_4	-	-
15.	Fixed oil	Spot test	+	-

"+" Present.
" –" Absent.

CONCLUSION

The modern pharmacognosy utilizes characteristics of analytical, phytochemical and certain physical constant values over the traditional science of taxonomy in plant systematics. Most of the botanical, chemical, physical and microbial techniques employed in pharmacognosy are applicable to the analysis of drugs and therefore, used by public analysts, forensic scientists and quality control chemists associated with industries.

REFERENCES

1. Gamble JS. Flora of Presidency of Madras. Volume I. Botanical Survey of India, Calcutta, India., 2005; 123-124.
2. Orwa. Spermacoce hispida botanical information. Agroforestry Database., 2009.
3. Chellaiah M, Muniappan A, Nagappan R, Savarimuthu I. Medicinal plants used by traditional healers in Kanchepuram district of Tamil Nadu, India. Journal of Ethnobiology and Ethnomedicine, 1999; 2: 43.
4. Karthikeyan M, Wawdhane SS, Kannan M, Rajasekar S. Hepatoprotective activity of ethanolic extract Spermacoce hispida against carbon tetrachloride (CCl_4) induced hepato-toxicity on albino wistar rats. International journal of Pharma Research and Development, 2011; 2(11): 45-52.
5. Parthasarathy G. Evaluation of anti-inflammatory activity of methanolic extract of Spermacoce hispida L. Int. J. of Pharm. Sci., 2010; 3: 1516-1517.
6. Surveswaran S, Cai Y, Corke H, Sun M. Syatematic evalution of natural phenolic antioxidants from 133 Indian medicinal plants. Food Chem., 2007; 102: 938-953.
7. Kaviarasan K, Kalaiarasi P, Pugalendi V. Antioxidant efficacy of flavonoids rich fraction from Spermacoce hispida in hyperlipidemic rats. Journal of Applied Biomedicine, 2008; 6: 165-176.
8. Sundhararajan R and Ravichandran V. Anti-nociceptive activity of ethanolic extract of Spermacoce hispida L. Int. Res. Phar. Sci., 2012; 3(1): 173-175.
9. Sivaelango G and Senthil Kumaran P. Anti-hyperlipidaemic activity of Spermacoce hispida ethanolic extract in Triton WR-1339induced hyperlipidaemic rats. Journal of Applied Pharmaceutical Sciences, 2012; 95-98.
10. Arnold N and Schmidt J. Plant constituents of traditional medicinal plants. 2nd Science report, Leibniz Institute of Plant Biochemistry, Leibniz., 2004; 23-24.
11. Mahalingam RV, Ambikapathy, Paneer selvam A. Studies on antifungal activities of some medicinal plants against Ceratocytis paradoxa causing pineapple disease. World Journal of Pharmacy and Technology, 2011; 1(7): 10-13.
12. Rathi MA, Meenakshi P, Guru Kumar D, Arul Raj C, Sunitha M and Gopalakrishnan VK. Leaves of Spermacoce hispida as a novel cancer therapeutic – An in vitro study. Research Journal of Pharmacy and Technology, 2011; 4(8): 1288-1291.

13. Evans WC, Trease and Evans, Pharmacognosy. (15thed) Saunders, Elsevier., 2009; 519-521.
14. Anonymous. Indian Pharmacopia. Ministry of Health and Family Welfare, Govt. of India, Controller of Publication, New Delhi, 1985; 310.
15. Wallis TC. Text book of Pharmacognosy. 5thed, EBS Publications, New Delhi, India., 1985; 111-117.
16. Harbone JB. Phytochemical methods. A guide to modern Techniques of plant analysis. Chapman and Hall, London., 1973; 267-270.
17. Trease GE and Evans WE. Phamacognosy, 15th Edition, W.B. Saunders Company Limited, London., 2002; 585.
18. Kokate CK, Purohit AP and Gokhale SB. Text book of Pharmacognosy. 27th edition, Nirali Prakashan, Pune, India., 2004.
19. Dinesh Kumar, Karunesh Kumar, Sunil Kumar, Tarun Kumar, Ajay Kumar, Om Prakash. Pharmacognostic evaluation of leaf and root bark of Holoptelea integrifolia Roxb. Asian Pacific Journal of Tropical Biomedicine, 2012; 169-175.
20. Metcalfe CR and Chalk L. Anatomy of the Dicotyledons, Systematic Anatomy of the leaf and stem. Clarendon Press, Oxford., 1979.
21. Kokoski CJ, Kokoski RJ, Salma FJ. Fluorescence of powdered vegetable drug under ultraviolet radiation. J. Am. Pharm. Assoc., 1958; 47: 715-717.
22. Soosairaj S, Kala A, Raja P and Dhatchanamoorthy N. Micromorphological assessment and stem anatomy of Spermacoce species of Tamil Nadu, India. Life Sciences Leaflets, 2013; 8: 19-28.
23. Cutler DF, Juncales. In Metcalfe, C.R. & L. Chalk (eds.) Anatomy of the monocotyledons. Claredon Press, Oxford., 1969.
24. Devi Priya M and Siril EA. Pharmacognostic studies on Indian Madder (Rubia cordifolia L.). Journal of Pharmacognosy and Phytochemistry, 2013; 1(5): 112-119.
25. Baral B and Maharjan BJ. Assessment of antimicrobial and phytochemical potentials of high altitudinal nepalese lichens. Journal of Microbiology and Biotechnology Food Science, 2011; 1(2): 98-112.
26. Deoda RS, Dinesh Kumar, Prasad VK, Kavita NY, Santosh SB and Manohar JP. Pharmacognostic and biological studies of the roots of Rubia Cordifolia Linn. International Journal of Drug Development and Research, 2011; 3(3): 148-158.
27. Jeyachandran M, Anantha Kumar T and Gandhimathi S. Phytochemical investigation of ethnomedicinal Spermacoce ocymoides roots. Journal of Pharmacognosy and Phytochemistry, 2013; 2(3): 86-88.