Research Article

Physicochemical and Fluorescence Analysis of leaves of *Alternanthera brasiliana* (L). Kuntze and *Alternanthera bettzickiana* (Regel) Voss

Kasthuri O R[1*], Ramesh B[2]

[1]Assistant Professor, Department of Biochemistry, Navarasam Arts and Science College for Women, Arachalur, Erode, Tamilnadu, India.
[2]Associate Professor, Department of Biochemistry, PSG College of Arts and Science, Coimbatore 641014, Tamilnadu, India.
***Corresponding author's E-mail:** kasthure@gmail.com

Received: 30-05-2018; **Revised:** 22-06-2018; **Accepted:** 12-07-2018.

ABSTRACT

The present study was carried out to analyse the physicochemical and fluorescence profile of leaf extracts of *Alternanthera brasiliana* (*A brasiliana*) and *Alternanthera bettizicikiana* (*A bettizicikiana*). Medicinal plants have been used as a major source of treatment for various forms of human ailments. The pharmacognostic evaluation of the plant material was an important parameter to detect any adulteration or improper handling of drugs. In the present study, ash values of leaves of *A brasiliana* and *A bettizicikiana* were analysed. Extractive yields of six different solvent extracts (petroleum ether, chloroform, acetone, ethanol, hydroethanol and water) of leaves of *A brasiliana* and *A bettizicikiana* were estimated. Fluorescence analysis was carried out on the solvent extracts and leaf powder. In both leaf studied, the total ash values were found to be higher followed by acid insoluble and water soluble ash. Extractive yields were high in the hydroethanol solvent extracts of the two leaf extracts studied. The highest percentage of extractive yield, total ash, water soluble ash and acid insoluble ash were found in *A bettizicikiana* when compared with *A brasiliana*. Fluorescence analysis of leaf extracts and leaf powder of *A brasiliana* and *A bettizicikiana* showed characteristic coloration with various chemicals.

Keywords: *Alternanthera brasiliana, Alternanthera bettizicikiana*, Ash value, Flourescence, Extractive yield.

INTRODUCTION

Natural products either as pure compounds or as standardized plant extracts act as vast source for the development of new medicines[1]. The use of herbal medicines has been on the rise in recent years, because of low prices and of a common perception among people that herbal medicines have little side effects. They are also believed as "being natural in origin, herbs are safe." These medicines are providing long-term effectiveness against various chronic illness[2]. Medicinal herbs have been known for centuries and are highly valued all over the world as a rich source of therapeutic agents for prevention and treatment of diseases and ailments[3]. Plants, besides providing nutrition, are an important source of chemical compounds such as secondary metabolites, which can be used for medicinal purposes[4].

Reliable methodologies are required for the correct identification, standardization and quality assurance of herbal drugs[5]. For confirmation of the identity and determination of quality and purity of the crude drugs, the major and reliable criteria are pharmacognostical parameters[6]. Complete information of the crude drug can be obtained by using a simple and reliable tool, Pharmacognosy[7]. These studies are valuable in the identification and authentication of plant material. Correct identification and quality assurance of the starting materials are an essential prerequisite to ensure the reproducible quality of herbal medicine. This will contribute to the safety usage and efficacy[8]. Ash value determination can be used for the detection of low-grade products, exhausted drugs and excess of sandy or earthy matter present in it. This property was more applicable to powdered drugs[9]. Determinations of extractive values are primarily useful for the evaluation of crude drug and for determination of exhausted or adulterated drugs[10].

Amaranthaceae is a cosmopolitan family consisting of 64 genera and about 800 species, mostly abundant in tropical regions of America, Africa and India[11]. The genus *Alternanthera*, an important representative of the family *Amaranthaceae* was established by Forsskal in 1775. The genus comprises approximately 80 species which are widespread in the tropical and subtropical regions of New World. *Alternanthera brasiliana* and *Alternanthera bettzickiana* are herbaceous plants belonging to the family Amaranthaceae. The genus is widespread with cosmopolitan distribution[12]. *A brasiliana* is native to different countries such as Brazil, Australia, and India. It is commonly known in Brazil as Penicillin, Brazilian Joy Weed, grows easily on poor, and deforested soil[13]. *A bettzickiana* Regel. is a species of flowering plant, known as red calico plant. The plant is used as an edible vegetable in Southeast Asia[14]. The present work was carried out to analyse the pharmacochemical properties and fluorescence characteristics of *A brasiliana* and *A bettzickiana.*

MATERIALS AND METHODS

Collection of plant materials

Whole plant of *A brasiliana* and *A bettzickiana* were collected from SKM Siddha and Ayurvedha, Erode. The plant specimens were identified and botanically

authenticated by Dr G.V.S.Murthy, Botanical Survey of India, and Coimbatore with voucher number BSI/SRC/5/23/2015/Tech/100 for *Alternanthera brasiliana* (L). Kuntze – AMARANTHACEAE and BSI/SRC/5/23/2015/Tech/101 for *Alternanthera bettzickiana* (Regel) Voss – AMARANTHACEAE. The leaves of *A brasiliana* and *A bettzickiana* were separated and were air dried thoroughly under shade (at room temperature) for 2- 3 weeks to avoid direct loss of phytoconstituents from sunlight. They were coarsely powdered using a mechanical grinder. The powdered leaf samples were stored in air tight and light resistant containers to be used for further analysis.

Preparation of the different solvent extracts from the leaves of *A brasiliana* and *A bettzickiana* using soxhlet apparatus

The shade dried coarsely powdered leaf samples of (250 g) *A brasiliana* and *A bettzickiana* were extracted with solvents of increasing polarity like petroleum ether, chloroform, acetone, ethanol, hydroethanol and water by using hot continuous percolation process (Soxhlet) and the different extracts were concentrated by using rotary vacuum evaporator (Buchi) at 50 °C, dried in a vacuum dessicator and stored at – 20 °C till further use.

Ash value Determination

Total ash, Acid Insoluble and Water soluble ash content were done as per standard procedure[15].

Determination of total ash

Three grams of the powdered leaf samples of *A brasiliana* and *A bettzickiana* were accurately weighed separately in silica crucible, which was previously ignited and weighed (350 °C for 1 hour). Dried leaf materials were spreaded like a fine layer on the bottom of the crucible. The crucible was incinerated at a temperature not exceeding 450 °C in a muffle furnace (Nabertherm) until it was white, indicating they are free from carbon. The crucible was cooled and weighed. The percentage of total ash was calculated with reference to the air-dried powder.

Determination of acid insoluble ash

The ash obtained as described in the determination of total ash was boiled with 25 ml of 2 N HCL for 5 minutes. The insoluble ash was collected on an ashless filter paper and washed with hot water. The insoluble ash was transferred to pre-weighed silica crucible and ignited for 15 minutes at a temperature not exceeding 450 °C. The percentage of acid insoluble ash was calculated with reference to the air dried powder.

Determination of water soluble ash

The ash obtained in the determination of total ash was boiled for 5 minutes with 25 ml of water. The insoluble matter was collected on an ash less filter paper and washed with hot water. The insoluble ash was transferred into a pre-weighed silica crucible and ignited for 15 minutes at a temperature not exceeding 450 °C. The procedure was repeated to get the constant weight. The weight of the insoluble matter was subtracted from the weight of the total ash. The difference in weight was considered as the water soluble ash. The percentage of water soluble ash was calculated with reference to the air-dried powder.

Determination of extractive values

The coarsely powdered leaf samples of *A brasiliana* and *A bettzickiana* were subjected to extraction. The extractive yield was expressed as:

$$\text{Extraction yield (\%)} = \frac{\text{Weight of the Dry extract (g)}}{\text{Weight of the Sample used for extraction}} * 100$$

Fluorescence analysis of leaf extracts

The petroleum ether, chloroform, acetone, ethanol, hydroethanol and water extracts of leaves of *A brasiliana* and *A bettzickiana* were subjected to fluorescence analysis in daylight and in UV- light (365 nm).

Fluorescence analysis of leaf powder

A small quantity (1 gm) of dried and finely powdered leaf of *A brasiliana* and *A bettzickiana* were treated with freshly prepared aqueous NaOH, alcoholic NaOH, 1N HCl, conc. H_2SO_4(1:1), conc. HNO_3(1:1), ammonia, iodine, 5%$FeCl_3$ and acetic acid. They were subjected to fluorescence analysis in daylight and in UV- light (365 nm). The colours observed in different radiations were recorded[16].

RESULTS AND DISCUSSION

Medicinal plants have been used over the millennia for human welfare in the promotion of health. The World Health Organization (WHO) estimates that 80% of the people living in developing countries are almost completely dependent on the traditional medicine as therapeutic remedies for their primary health care needs[17]. All over the world, herbal medicines as potential source of therapeutics aids has attained a significant role in health system of both humans and animals not only in the diseased condition but also as potential material for maintaining proper health.

Ash values

Ash value is a validity parameter to assess the degree of purity and in evaluating the quality of crude drugs[18]. Ash constitutes the inorganic residues obtained after complete combustion of a drug. It indicated the presence of various impurities like carbonate, oxalate and silicate. Total ash represents the total content of physiological ash and non-physiological ash. Water soluble ash was the content of total ash soluble in hot water and acid insoluble ash was represented by the non-physiological ash especially sand and soil[19].

The purpose of ashing plant material was to remove all traces of organic matter that might otherwise interfere in analytical determination.

In the present investigation, the ash value of the leaf powder of *A brasiliana* and *A bettzickiana* were evaluated and the results were given in Table 1.

Table 1: Ash Value

	Alternanthera brasiliana	***Alternanthera bettzickiana***
Total ash	10.98%	11.11%
Acid insoluble ash	0.29%	1.66%
Water soluble ash	0.10%	0.52%

The total ash value represents both physiological and non-physiological ash. Physiological ash is the ash inherent in the plant due to biochemical processes and the non-physiological ash is the contaminants from the environment. These may be carbonates, phosphates, nitrates, sulphates, chlorides and silicates of various metals which were taken up from the soil[20]. For the evaluation of purity of drugs, total ash value was particularly important. A high percentage of total ash value revealed the presence of inorganic constituents and very low value of acid insoluble ash indicated the presence of negligible amount of siliceous matter.

The acid insoluble ash was a part of total ash that was insoluble in dilute HCl. Water soluble portion of the total ash constitutes the water soluble ash[21]. Water soluble ash can be used as an important indicator for the presence of exhausted material[22].

In this evaluation, in both leaf extracts, total ash value was higher followed by acid insoluble ash and water soluble ash. The total ash, acid insoluble ash and water soluble ash were found to be higher in *A bettzickiana* than *A brasiliana*.

Appreciable amount of ash values obtained for leaf powder of *A brasiliana* and *A bettzickiana* implied that the leaf powder of the two plants had higher organic content and fairly low inorganic content.

Extractive values of leaf extracts of *A brasiliana* and *A bettzickiana*

Determination of extractive values aids in the evaluation of chemical constituents present in the crude drug. This value also helps in the estimation of specific constituents soluble in a particular solvent. The formations of the bioactive principle of the medicinal plants are influenced by number of intrinsic and extrinsic factors. High alcohol soluble and water soluble extractive values are indicatives for the presence of polar substance like phenols, tannins and glycosides[23]. Six different solvent extracts of leaf samples of *Alternanthera brasiliana* and *Alternanthera bettzickiana* were analysed to estimate the percentage yield of individual extracts (Table 2).

Table 2: Extractive values of leaf extracts of *Alternanthera brasiliana* and *Alternanthera bettzickiana*

EXTRACT	***Alternanthera brasiliana***	***Alternanthera bettzickiana***
Petroleum ether	3.32%	4.52%
Chloroform	3.95%	4.16%
Acetone	4.56%	5.02%
Ethanol	10.25%	12.68%
Hydroethanol	16.73%	18.42%
Water	11.68%	14.61%

The extractive yield was found to be abundant in hydroethanol leaf extracts of *A brasiliana* and *A bettzickiana* than other extracts. Next to hydroethanol extract, the water and ethanol extracts obtained good yield of percentage. The petroleum ether, chloroform and acetone extracts of *A brasiliana* and *A bettzickiana* showed least yield in the leaves of both plants. Due to the high polarity of hydroethanol, most of the chemical constituents of extracts would be dissolved in it, thus the percentage yield was increased tremendously than other solvents. In the hydroethanol leaf extracts, *A bettzickiana* showed a highest extractive yield when compared to *A brasiliana.*

In the present study, the higher percentage of extractive values of crude drugs in hydroethanol, water and ethanol extracts implied that hydroethanol, water and ethanol are better solvents for extraction than petroleum ether, chloroform and acetone. The variation in the extractive values may be possible due to the presence of specific compound according to the solubility, soil condition, atmospheric condition and water content of the sample.

Extractive values of a number of medicinal plants with different solvents has been carried out by many workers[24, 25].

Fluorescence analysis of leaf extracts of *A brasiliana* and *A bettzickiana*

Fluorescence is the phenomenon exhibited by various chemical constituents present in the plant material under UV light. This can be used to characterize the crude drugs[26]. It is also one of the pharmacognostic procedures useful in the identification of authentic samples and recognizing adulterants[27].

Fluorescence studies helps in the identification of drugs that are more or less difficult to distinguish. In a mixture of drugs of two or more species, fluorescence studies helps to identify a particular drug by the use of estimates of intensity of fluorescence. The comparison of the unknown should be made with a sample of known identity.

The fluorescence analysis of petroleum ether, chloroform, acetone, ethanol, hydroethanol and water extracts of leaves of *A brasiliana* and *A bettzickiana* were observed

under visible light and also under UV light (365 nm) and the results were recorded in Table 3.

Table 3: Fluorescence analysis of leaf extracts of *A brasiliana* and *A bettzickiana*

Solvent	*Alternanthera brasiliana*		*Alternanthera bettzickiana*	
	Visible Light	UV Light (365nm)	Visible Light	UV Light (365nm)
Petroleum ether	Dark green	Blackish green	Dark green	Blackish green
Chloroform	Dark green	Dark green	Dark green	Dark green
Acetone	Green	Blackish brown	Dark green	Brownish green
Ethanol	Green	Dark brown	Light green	Brownish green
Hydroethanol	Green	Light green	Light yellow	Bright yellow
Water	Green	Light green	Light brown	Dark brown

A correlation exists between a compound present in the drugs and their fluorescent behaviour under different conditions. Fluorescent study of petroleum ether, chloroform, acetone, ethanol, hydroethanol and water extracts of *A brasiliana* and *A bettzickiana* leaves showed characteristic colouration under visible light and UV light (365 nm). Florescence analysis of different extracts of *A brasiliana* and *A bettzickiana* leaves gives a clue whether these extracts are in adulteration, thus can be used as a diagnostic tool for testing the adulteration. Such studies were done previously in *Morinda tinctoria*[28] and *Abutilon indicum*[29].

Color variation was observed in visible light and UV light and it can be used as a standard parameter for quality control of the drug. The quality control is necessary if plant products are to fill the needs for cheap and reliable medicines or when natural products are to be used as template for new drug molecules.

Fluorescence analysis of dried leaf powder of *A brasiliana* and *A bettzickiana*

The fluorescent colour is specific for each compound. Plant materials give different coloration when treated with various chemicals. Some plant constituents showed characteristic fluorescence in the visible range in daylight. The ultra violet light produces fluorescence in many natural products which do not visibly fluoresce in daylight. If substance themselves are not fluorescent, addition of different reagents results in the conversion into fluorescent derivatives or decomposition products. Crude drugs are often assessed qualitatively in this way and fluorescence analysis was an important parameter for pharmacognostic evaluation of crude drugs[30]. The color formation with respect to the particular reagents was noted and was aid in the determination of quality and purity of the leaf powder.

For fluorescence analysis, the powdered samples of leaves of *A brasiliana* and *A bettzickiana* were treated with various chemical agents and were observed under visible light as well as under UV light (356 nm). The results obtained are reported in Table 4.

Table 4: Fluorescence analysis of dried leaf powder of *A brasiliana* and *A bettzickiana*

	Alternanthera brasiliana		*Alternanthera bettzickiana*	
	Visible Light	UV Light (365nm)	Visible Light	UV Light (365nm)
Plant+1N NaOH Aqueous	Yellow	Bright yellow	Light green	Green
Plant+1N NaOH Alcoholic	Light yellow	Bright yellow	Green	Dark green
Plant+1N Hcl	Light green	Dark green	Green	Dark green
Plant+H_2SO_4(1:1)	Light yellow	Green	Light green	Dark green
Plant+HNO_3(1:1)	Dark yellow	Yellow green	Light yellow	Yellow green
Plant+Ammonia	Yellow green	Yellow green	Green	Dark green
Plant+Iodine	Light green	Brown	Light green	Dark brown
Plant+5%$FeCl_3$	Light green	Brown	Light green	Brown
Plant+Acetic acid	Light green	Green	Green	Dark green

The crude drug when viewed under UV light showed different fluorescence at different wavelengths. This is due to the presence of different phytochemical constituents in the drug [31]. Flavones which are light yellow in aqueous condition, under UV light, turns to bright yellow under alkaline conditions. Phytosterols, when treated with 50% H_2SO_4 shows green fluorescence under UV light. Coumaric acid appears yellowish green in alkaline condition under UV radiation. Terpenoids, exhibits yellow green fluorescence under short UV light[32]. Berberin showed light yellow colour of fluorescence[33].

The results of the fluorescence analysis of various extracts and powder of *A brasiliana* and *A bettzickiana* leaves showed characteristic colouration on treatment with various chemical reagents. The major bioactive compounds present in the crude drugs of *A brasiliana* and *A bettzickiana* were found to be flavones, sterols, terpenoids and berberin.

Proper control of starting material was most essential one for ensuring the reproducible quality of herbal drugs. In recent years there has been a great emphasis in the

standardization of medicinal plants because of therapeutic benefits. Pharmacognostical studies are more reliable, accurate and inexpensive means for the identification and evaluation of plant drugs. According to World Health Organization (WHO), the macroscopic and microscopic description of a medicinal plant was the first step towards establishing its identity and purity and should be carried out before any tests are undertaken[34].

Pharmacognosy is a simple and reliable foot, by which complete information of the crude drug can be obtained. The present investigation revealed the ash values, extractive yields and fluorescence characteristics of leaves of *A brasiliana* and *A bettzickiana*.

CONCLUSION

Pharmacochemical analysis ofmedicinal plants are a parameter of quality control. So it becomes a necessary step in the study of pharmacognostic characteristic of the plant before its use in the field of research and also in pharmaceutical formulation. From this study, it may be concluded that the analysis showed the purity of *A brasiliana* and *A bettzickiana* leaf powders. Moreover, hydroethanol followed by water and ethanol extracts yielded good results. So these extracts can be used for further studies of this plant material as potential source in pharmaceutical preparations.

Acknowledgment: The authors are thankful to the Management, PSG College of Arts and Science, Coimbatore, for providing with all the facilities required in this work.

REFERENCES

1. Sasidharan S, Chen Y, Saravanan D, Sundram KM, Latha LY, Extraction, isolation and characterization of bioactive compounds from plants extracts, African Journal of Traditional Complementary and Alternative Medicines, 8(1), 2011, 1-10.
2. Amorozo MCM, Use and diversity of medicinal plants in Santo Antonio do Leverger, MT, Brasil Acta Bot Brasilica, 16, 2002, 189-203.
3. Pandya K , Maniar K, Soni H, Bhatt S, Patel P, Solanki B, Gurav N, Standardization of Anti-diabetic ayurvedic herbomineral formulation, International Journal of Pharmaceutical Sciences Review and Research, 10(1), 2011, 174-186.
4. Sathishkumar R, Lakshmi PTV, Annamalai A, Effect of drying treatment on the content of antioxidants in Enicostemma littorale Blume, Research Journal of Medicinal plant, 3, 2009, 93-101.
5. Shazia Sultana, Mir Ajab Khan, Mushtaq Ahmad, Asghari Bano, Muhammad Zafar and Zabta Khan Shinwari, Authentication of herbal medicine neem (*azadirachta indica a.juss*.) by using taxonomic and pharmacognostic techniques, Pak J Bot 43, 2011, 141-150.
6. Bhattacharya S, Zaman KM, Pharmacognostical Evaluation of *Zanthoxylum nitidum* Bark, International Journal of Pharm Tech Research, 1, 2009, 292-298.
7. Nivedithadevi D, Somasundaram R, Pharmacognostical and Qualitative Phytochemical Studies on the aerial parts of Tephrosla purpurea (L)., International Journal of Research in Biological Sciences, 2, 2012, 48-53.
8. Arunkumar S, Muthuselvam M, Analysis of Phytochemical Constituents and Antimicrobial Activities of Aloe vera L. against Clinical Pathogens, World Journal of Agricultural Sciences, 1, 2009, 572-576.
9. Prajapati N Chetankumar, Patel M, Physio-chemical and Phytochemical Evaluation of *Leptadenia reticulata* Roots, International Journal of Research in Pharmaceutical and Biomedical Sciences, 3, 2012, 1791- 1797.
10. Khandelwal KR, Practical Pharmacognosy Techniques and Experiments. Nirali Prakashan, India, 4th ed, 1998.
11. Hussien M Alwadie, Morphology and Distribution of Three Genera of Amaranthaceae in the South Western Area of Saudi Arabia, J King Saud Univ, 18, 2005, 51-62.
12. Zafra-Stone S, Yasmin T, Bagchi M, Chatterjee A, Vinson J A, Bagchi D, Berry anthocyanins as novel antioxidants in human health and disease prevention, Mol Nutr Food Res, 51, 2007, 675-83.
13. Brochadoa CO, Almeidaa AP, Barretoa BP, Costaa LP, Ribeiroa LS, Peireira RLC, Flavonol Robinobiosides and Rutinosides from Alternanthera brasiliana (Amaranthaceae) and their effects on lymphocyte proliferation in vitro, J Braz Chem Soc, 14, 2003, 449-451.
14. Hundiwale JC, Patil AV, Kulkarni MV, Patil DA, Mali RG, A current update on phytopharmacology of genus *Alternanthera,* J Pharmacol Res, 5, 2012, 1924-1929.
15. Ayurvedic Pharmacopoeia of Indi, Part- 1 Appendix Vol.1, 2001, 206-214.
16. Kokoski J, Kokoski R, Salma FJ, Fluorescence of powdered vegetable drugs under ultraviolet radiation, J Am Pharm Ass, 47, 1958, 715-717.
17. Okwu DE, Phytochemicals and vitamin content of indigenous spices of Southeastern Nigeria, J Sustain Agric Environ, 6, 2004, 30-37.
18. Kokate CK, Purohit AP, Gokhale SB, Analytical pharmacognosy. In: Pharmacognosy. 42nd ed, Pune, Nirali Prakashan; 2008, 19-20.
19. Ghildiyal SK, Sharma CM, Gairola S, Effect of cold stratification on the germination of seeds of chirpine (*Pinus Roxburgii* Sargent) from Indian Himalayan Region, Nature and Science, 7, 2009, 36-43.
20. Ehiabhi OS, Omoregie EH, Comfort OE, Folashade KO, Phytochemical and proximate analyses and thin layer chromatography fingerprinting of the aerial part of *Chenopodium ambrosioides* Linn. (Chenopodiaceae), J Med Plant Res, 6, 2012, 2289-2294.
21. Kokate CK, Purohit AP, Gokhale SB, Pharmacognosy, Nirali Prakashan, 38th edition, 2007.
22. Verma ML (1988): Pharmacognostic studies on certain species of the family Asteraceae. Ph.D. Thesis. Department of Botany, U.O.R., Jaipur, India.
23. Baravalia Y, Nagani K, Chanda S, Evaluation of pharmacognostic and physicochemical parameters of

Woodfordia fruticosa Kurz. Flowers, Pharmacognosy Journal, 2, 2011, 13-18.

24. Devika R, Sajita U, Phytochemical and pharmacological investigation of *Phyllanthus niruri* – A preliminary screening, J Phytol Res, 20, 2007, 133-135.
25. Mohanty A, Das C, Dash S, Sahoo DC, Physico-chemical and antimicrobial study of polyherbal formulation, International Journal of Comprehensive Pharmacy, 1, 2010, 1-3.
26. Dominic VJ, Manju Madhavan, Pharmacognostic and preliminary phytochemical screening of the rhizome of *Anaphyllum wightii* schott, an endemic and threatened genus of the Western ghats, Journal of Herbal Medicine and Toxicology, 6, 2012, 55 – 60.
27. Tyler VE, Brady LR and Robbers JE, Pharmacognosy, Lea & Febiger, Philadelphia, 1976, 24.
28. Parekh J, Darshana J, Sumitra C, Efficacy of aqueous and methanol extracts of some medicinal plants for potential antibacterial activity, Journal of Biology, 29, 2005, 203-210.
29. Atish KS, Narayanan N, Satheesh Kumar N, Rajan S, Pulok KM, Phytochemical and therapeutic potentials of *Morinda tinctoria* Roxb. (Indian mulberry), Oriental Pharmacy and Experimental Medicine, 9, 2009, 101-105.
30. Zhao Z, Liang Z, Guo P, Macroscopic identification of Chinese medicinal materials: Traditional experiences and modern understanding, J Ethnopharmacol, 131, 2011, 556-561.
31. Reddy M and Chaturvedi A. Pharmacognosticaly studies of *Hymenodictyon orixence* (Roxb.) Mabb. leaf. Int J Ayurveda Res, 1, 2010, 103-105.
32. Harborne JB. Phytochemical methods. Chapman & Hall, New York, 1976, 1-288.
33. Evans WC. In: Trease and Evans' Pharmacognosy. Singapore, Harcourt Baraco and Company Asia Pvt. Ltd., 1996, 431–437.
34. Anonymous, Quality Control Methods for Medicinal Plant Materials (An authorized publication of World Health Organization, Geneva), New Delhi, AITBS Publishers & Distributors (Regd.), 2002.

Source of Support: Nil, **Conflict of Interest:** None.