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PHARMACOGNOSTIC AND PHYTOCHEMICAL EVALUATION OF *NYCTANTHES ARBOR-TRISTIS* STEM

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ABSTRACT: Objective: To study detailed pharmacognostic characters of the stem of *Nyctanthes arbor-tristis* (Oleaceae), along with their physicochemical parameters, fluorescence analysis, and phytochemical screening. **Methods:** The pharmacognostic characters were determined in terms of macroscopy, microscopy, powder microscopy, physicochemical analysis, fluorescence analysis and preliminary phytochemical investigation of the plant stem. **Results:** The microscopic study shows the general characteristic of the stem. Physico-chemical investigation shows the total ash, acid insoluble ash, water-soluble ash were 8.69 ± 0.17% w/w, 0.21 ± 0.11% w/w, and 3.92 ± 0.05% w/w respectively. Phytochemical analysis revealed the presence of various phytochemical groups like alkaloids, glycosides, steroids, phenolic, tannins constituents. **Conclusion:** It can be concluded that the established pharmacognostic profile of *Nyctanthes arbor-tristis* stem will help develop pharmacopoeial standards for correct identification and quality control.

INTRODUCTION: India has a rich heritage of traditional medicine and traditional health care systems that have been flourishing for many centuries. Nowadays, the prevalent use of traditional medicines in developed countries is trending, and it has become more popular throughout the world. Traditionally, important large shrub of tropical and subtropical regions of the world have been used to counteract disease [1].

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Nyctanthes arbour-tristis L. (Oleaceae) commonly known as Parijat or Harsinghar, a large hardy shrub or small tree up to 5-10 m in height, widely occurring in outer Himalayan ranges from Kashmir to Nepal and throughout India up to 1000-1500 m altitude [2].

The name 'Nyctanthes' has been coined from two Greek words 'Nykhta' means night and 'anthos' means flower. It is also planted in gardens due to its highly fragrant flowers [3]. It is a shrub or small tree, with drooping branches and quadrangular branchlets. Leaves are opposite, ovate, acute or acuminate, entire slight cuneate. Flowers are small, 3-7 in the head, arranged in trichotomous cymes, delightfully fragrant, sessile, slender, and hairy; corolla glabrous, orange colored and lobes are

white [4, 5]. Fruits are capsules of 1-2 m in diameter, long and broad, compressed, 2 celled separating into 2 flat one-seeded carpels, reticular veined and glabrous [6]. Different parts of this plant are used in folk-medicines [7, 8]. The leaves are bitter, useful in chronic fever [9], malarial fever [10], obstinate sciatica, constipation, hemorrhoids [11] and eczema [12]. The flowers are astringent, stomachic, and useful in dyspepsia, greyness of hair and baldness [11]. The plant elaborates different classes of organic compounds of medicinal importance including alkaloids, terpenes, steroids, β-sitosterol, glycosides, iridoid glycosides, arbortristoside-A, B, C, D, E [13, 14, 15, 16, 17, 18].

Different parts of this plant are used in Indian systems of medicine for various pharmacological actions like as anti-leishmaniasis, anti-viral, anti-fungal, antibacterial, anti-pyretic, antihistaminic, anti-malarial, antioxidant, hepatoprotective, and anti-inflammatory activities[19]. The literature survey and scientific data revealed that no systematic pharmacognostical parameter had been carried out on the stem of *Nyctanthes arbor-tristis* Linn. till date. Hence, the objective of the present study is to evaluate various pharmacognostic parameters such as macroscopy, microscopy, physicochemical and phytochemical evaluations of the *Nyctanthes arbor-tristis* Linn. (Stem).

MATERIALS AND METHODS:

Chemicals and Instruments: Phloroglucinol, glycerin, hydrochloric acid, potassium hydroxide and all other chemicals used in the study were of analytical grade.

Plant Material: The stem of *Nyctanthes arbor-tristis* was collected from Dehradun, Uttarakhand, India in August 2016 and authenticated by Dr. S. K. Srivastava, Botanical Survey of India, Northern Regional Center, Dehradun, where a voucher specimen (specimen no. 116216) has been deposited.

Macroscopic and Microscopic Analysis: Macroscopic studies were done using a simple microscope. The color, shape, size, taste, and odor of the stem were determined. Microscopic study of the fresh stem was carried out by preparing a thin transverse section and staining it with concentrated hydrochloric acid: phloroglucinol (1:1). Photographs sections were carefully taken. The dried stem was powdered and treated with 5% KOH solution followed by staining with concentrated hydrochloric acid - phloroglucinol (1:1) for 5 min and mounted in 50% glycerine solution [20, 21, 22].

Physiochemical Analysis: Physiochemical parameter such as ash values (total ash, acid insoluble ash) and extractive values (water soluble, alcohol soluble extractives) were determined using the powdered drug. The moisture content was detected by loss on drying method [23, 24, 25].

Fluorescence Analysis: For the fluorescence analysis of stem powder it was treated with various chemicals and was observed exclusively to different wavelengths of ultraviolet (254 nm and 365 nm) and visible light for observing characteristic color presentation [22, 26, 27].

Pre-liminary Phytochemical Screening: Pre-liminary phytochemical screening was qualitatively tested for the presence of phytochemicals as per described standard methods [22, 28, 29, 30].

RESULTS:

Macroscopic Characteristics: Organoleptic characters of stem depicted that the stem was woody having light grey to greenish in color with characteristic odor and taste. The stem was 1-10 m tall, erect and branched.

Microscopic Characteristics:

Stem Microscopy: Section of stem appears quadrangular and revealed the following tissues: The epidermis was single, layered consisting of rectangular cells with a thick continuous cuticle on the epidermis along with many multicellular hairs. Cortex was several cells deep below the four protruded comers while only a few layers deep at the other places just beneath the epidermis. It was differentiated into collenchyma and parenchyma. Many intercellular spaces were present, and the region extended up to the vascular tissue. Vascular bundles were present in the cortex; each protrudes bulb containing one. The pointed xylem end was faced towards the outer side in each of the conical bundle. In other words, the vascular bundle was conjoint, collateral, open an exarch.

The microscopy revealed that the endodermis was not well developed. The pericycle was observed in

the form of sclerenchymatous patches. The vascular system was composed of primary phloem, secondary phloem, cambium, primary xylem, and secondary xylem. Crushed primary phloem was irregularly present in patches below the pericycle. The secondary phloem consisted of sieve tubes, companion cells, phloem parenchyma and was present in the form of a continuous ring. Cambium was present as one to three cells thick continuous layer in between phloem and xylem. Secondary xylem was present just inner to the cambial ring and consisted of mainly thick walled woody parenchyma and fibers. Tracheids and vessels were also observed. Primary xylem was situated just near the pith in a way facing its protoxylem towards the center. Pith was found to be thin-walled and parenchymatous as shown in **Fig. 1**.

Stem Powder Microscopy: Microscopic observation of *Nyctanthes arbor-tristis* stem indicated the presence of parenchyma cells, collenchyma cells, fiber, xylem vessels, and calcium oxalate crystal as shown in **Fig. 2**.

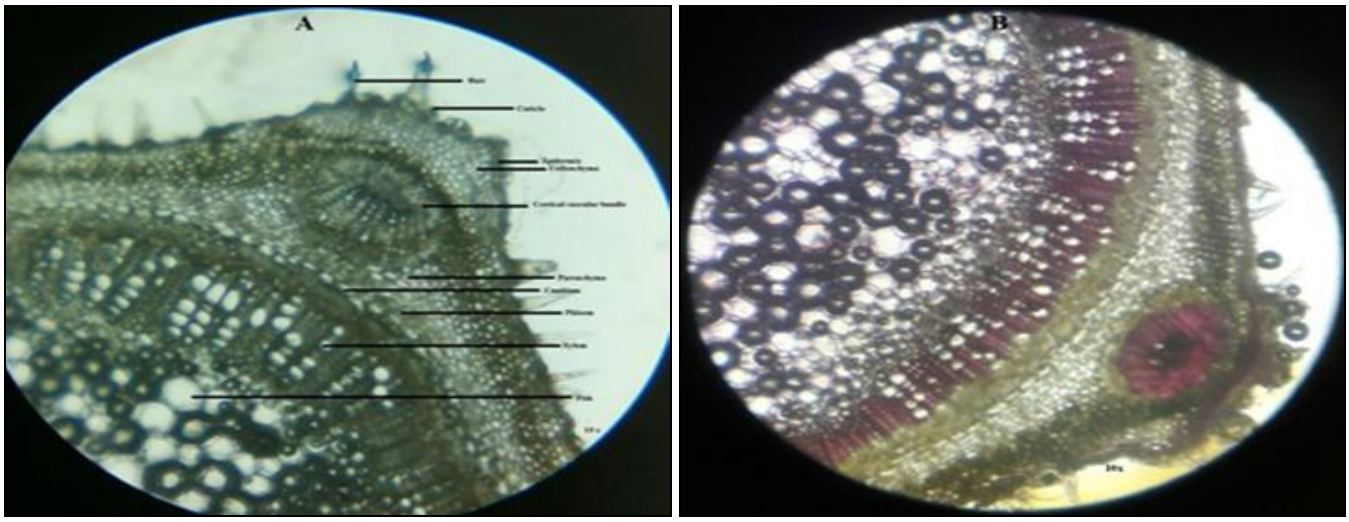

FIG. 1: TRANSVERSE SECTION OF STEM OF *NYCTANTHES ARBOR-TRISTIS* VIEWED AT 10x; A- WITHOUT STAINING REAGENT, B- WITH STAINING REAGENT

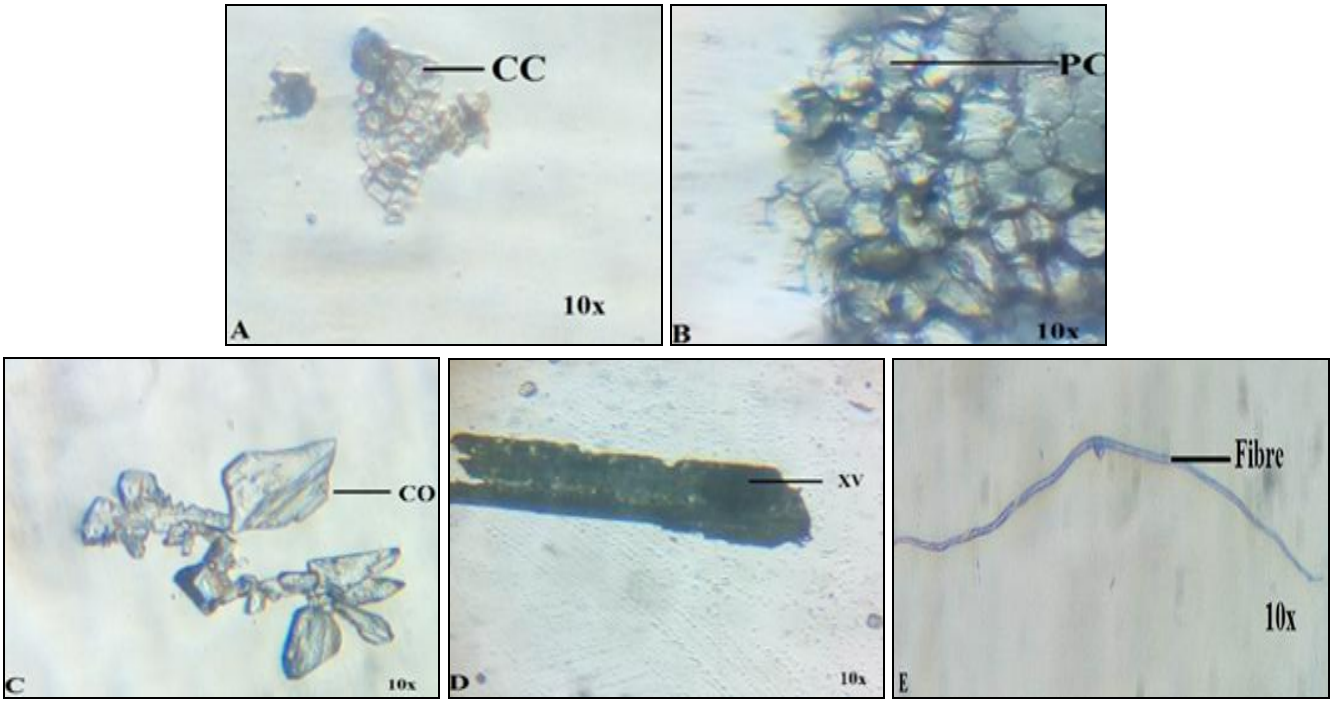

FIG. 2: POWDERED CHARACTERISTICS OF THE STEM PARTS OF *NYCTANTHES ARBOR-TRISTIS* SHOWING A: COLLENCHYMA CELL (CC) B: PARENCHYMA CELLS (PC) C: CALCIUM OXALATE CRYSTALS (CO) D: XYLEM VESSELS (XV) E: FIBER

Physicochemical Parameters: Ash value of the drug gives an idea about the earthy matter or inorganic composition and other impurities present along with the drug. Various physicochemical parameters such as total ash, acid insoluble ash, and water soluble ash of *Nyctanthes arbor-tristis* stem were found to be 8.69 ± 0.17, 0.21 ± 0.11 and 3.92 ± 0.05% w/w, respectively. However, 15.93 ± 0.46% w/w alcohol soluble and 18.31 ± 0.46% w/w water soluble extractives were observed. The moisture content of stem powder was nearly 5.49 ± 0.02% w/w **Table 1**.

Fluorescence Analysis: Fluorescence analysis of stem powder was carried out after treating it with several solvents and chemicals. Fluorescence was observed at 254 and 365 nm comparing its change of color in visible light. The observations are presented in **Table 2**.

Pre-liminary Phytochemical Screening: Pre-liminary phytochemical screening of *Nyctanthes arbor-tristis* is shown in **Table 3**.

TABLE 1: PHYSICOCHEMICAL CONSTANTS FOR *NYCTANTHES ARBOR-TRISTIS* STEM

S. no.	Physicochemical parameter	Values (% w/w)
1	Moisture content	5.49 ± 0.02
2	Total ash	8.69 ± 0.17
3	Acid insoluble ash	0.21 ± 0.11
4	Water soluble ash	3.92 ± 0.05
5	Alcohol soluble extractive	15.93 ± 0.46
6	Water soluble extractives	18.31 ± 0.46

TABLE 2: FLUORESCENCE ANALYSIS OF *NYCTANTHES ARBOR-TRISTIS* STEM

Treatment	Visible light	Under UV light	
		Short wavelength (254 nm)	The long wavelength (365 nm)
Powder	Brown	Light brown	Green
Powder + Methanol	Brown	Light brown	Yellowish green
Powder + 70% ethanol	Brown	Light brown	Green
Powder + Pet. ether	Light brown	Light green	Green
Powder + 50% H_2SO_4	Brown	Greenish brown	Brownish
Powder + 50% HCl	Dark Brown	Green	Green black
Powder + 1N NaOH (aq.)	Light brown	Dark brown	Brownish black
Powder + 1N NaOH (alc.)	Light brown	Dark green	Greenish black
Powder + 50% HNO_3	Brown	Light brown	Light green
Powder + 5% KOH	Brown	Purplish green	Dark purplish green
Powder + Ammonia	Brown	Green	Black
Powder + Picric acid	Yellowish brown	Green	Dark brown

TABLE 3: PHYTOCHEMICAL SCREENING *NYCTANTHES ARBOR-TRISTIS* STEM

S. no.	Class of constituents	PEE	CE	EAE	EE	AE
1	Amino acids	-	-	-	-	-
2	Proteins	-	-	-	-	-
3	Carbohydrates	-	-	-	+	+
4	Steroids	+	+	-	-	-
5	Triterpenoids	+	+	-	-	-
6	Alkaloids	-	+	-	+	+
7	Glycosides	-	+	-	+	+
8	Saponins	-	-	-	-	-
9	Flavonoids	-	-	+	+	-
10	Tannins	-	-	-	+	+
11	Phenolic compounds	-	+	+	+	+

PEE- Pet. ether extract, CE- Chloroform extract, EAE- Ethyl acetate extract, EE- Ethanol extract, AE- Aqueous extract, (-) Negative, (+) Positive

DISCUSSION: The wide use of herbal drugs in traditional medicines and herbal formulations, standardization that has been made to an important measure for ensuring and justifying the quality, purity, and authenticity of the crude drugs [31]. Standardization purpose, morphological and microscopic analysis is one of the simplest and cheapest methods to start with establishing the correct identification of the source materials [32, 33]. As there is no pharmacognostic work available on this medicinally potent plant, the present work was undertaken to lay down the standards which could be useful for establishing its authenticity. Organoleptic and microscopic studies are useful for

identifying parameters for authentication of the drug [34, 35]. Physicochemical studies act as a reliable method for detecting adulteration. Physicochemical constants like total ash, acid insoluble ash, and water soluble ash can serve as a valuable source of information which is usually helpful in the evaluation of purity and quality of a crude drug.

The earthly matter or inorganic composition and other impurities which are present along with the drug are determined by the ash values. Acid-insoluble ash usually indicates the contamination with silicon material like earth and sand. Water-soluble ash was used for the estimation of the amount of inorganic elements [36]. The extractive values give an idea about the chemical constitution of the drug [33].

Fluorescence analysis is an alternative rapid useful method for the identification of authentic samples and recognizing adulterants. In this analysis, the crude drugs may be examined as such, in solution or as extracts and in powdered form [37]. Fluorescence characteristics enable the identification and differentiation of plant materials from their adulterants when physical and chemical methods are scarce. Various chemical constituents present in the plant material exhibit fluorescence on absorbing light. Fluorescence is shown by some of the constituents even in the visible range in daylight. The ultraviolet light produces fluorescence in many natural products *viz.* alkaloids like berberine, which does not show fluoresce in the daylight. With the aid of different reagents, the nonfluorescent substances can easily be transformed into their fluorescent derivatives or decomposition products [33].

Phytochemical evaluation and chemo-profiling are useful for the quality assessment of plant materials. Phytochemical compounds in the plant are known to have various therapeutic importance. For instance saponins, terpenoids, flavonoids, tannins, steroids, and alkaloids have anti-inflammatory effects. Glycosides, flavonoids, tannins, and alkaloids have hypoglycemic activities [38, 39]. Flavonoids possess the hepatoprotective, and antioxidant activities [40]. The saponins have hypocholesterolemic and antidiabetic properties [41]. In the animal studies, terpenoids tend to decrease blood sugar level. Steroids, as well as triterpenoids, exhibit the analgesic properties [42]. The steroids and saponins are responsible for CNS activities [43].

CONCLUSION: The present study was focused on establishing pharmacognostic standards for the identification and authentication of the *Nyctanthes arbor-tristis*. Therefore, the outcomes of the above findings will serve as a promising source for laying down pharmacopoeial standards for future studies and research.

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CONFLICT OF INTEREST: We declare that we have no conflict of interest.

REFERENCES:

1. Mukherjee PK: Quality control of herbal drugs- An Approach to Evaluation of Botanicals Business Horizons, New Delhi, Edition 1st, 2002.
2. Nadkarni KM: Indian Materia Medica, Bombay: Bombay Popular Prakashan Pvt. Ltd., 1982; 3: 1.
3. Kirtikar KR and Basu BD: Indian Medicinal Plants. Sri Satguru Publications, Delhi, Vol. IV, 2000
4. Saxena RS, Gupta B and Lata S: Tranquillizing, the antihistaminic and purgative activity of *Nyctanthes arbor-tristis* leaf extract. J Ethnopharmacol 2002; 81: 321-325.
5. Ratnasooriya WD, Jayakody JRAC and Hettiarachchi ADI: Sedative effects of hot flower infusion of *Nyctanthes arbo-tristis* on rats. Pharm Biol 2005; 43: 140-146.
6. Bhosale AV, Abhyankar MM, Pawar SJ, Shoeb K and Patil N: *Nyctanthes Arbor-tristis*. A pharmacognostic review. Research J Pharmacognosy and Phytochemistry 2009; 1(2): 91-97.
7. Gacche R and Dhole N: Antioxidant and possible anti-inflammatory potential of selected medicinal plants prescribed in the Indian traditional system of medicine. Pharmaceutical Biology 2006; 44(5): 389-95.
8. Khare CP: Indian medicinal plants: an illustrated dictionary: Springer Science & Business Media 2008.
9. Gogoi R and Borthakur SK: Notes on herbal recipes of Bodo tribe in Kamrup district, Assam. Ethnobotany 2001; 13: 15-23.
10. Changkija S: Applied Ethnobotany: A Case Study among the Kharias of Central India. Asian Folklore Studies 1999; 57(2): 397-9.
11. Warrier PK and Nambiar V: Indian medicinal plants: a compendium of 500 species: Orient Blackswan 1993.
12. Bhatt DC, Mitaliya KD and Mehta SK: Observations on ethnoveterinary herbal practices in Gujarat. Ethnobotony 2001, 13: 91-95.
13. Kapoor LD: Survey of Indian plants for saponins, alkaloids and flavonoids, II, Lloydia 1971; 34: 94.
14. Agrawal J and Pal A: *Nyctanthes arbor-tristis* Linn-A critical ethnopharmacological review. Journal of Ethnopharmacology 2013; 146(3): 645-58.

15. Khatune NA, Islam ME, Abdur Rahman MA, Mosaddik MA and Haque ME*: In-vivo* cytotoxic evaluation of a new benzofuran derivative isolated from *Nyctanthes arbor-tristis* L. on ehrlich ascite carcinoma cells (EAC) in mice. J Med Sci. 2003; 3(2): 169-73.
16. Mathuram V and Kundu AB: A reinvestigation of the structures of arbortristosides A and B from *Nyctanthes arbor-tristis*. J Nyctanthes arbor-tristis Prod 1991; 54(1): 257-60.
17. Stuppner H, Muller EP, Mathuram V and Kundu AB: Iridoid glycosides from *N. arbor-tristis*. Phytochemistry 1993; 32(2): 375-78.
18. Gupta P, Bajpai SK, Chandra K, Singh KL and Tandon JS: Antiviral profile of *Nyctanthes arbortristis* L. against encephalitis-causing viruses. Indian J Exp Biol 2005; 43(12): 1156-1160.
19. Jain PK and Pandey A: The wonder of Ayurvedic medicine - *Nyctanthes arbor-tristis*. Int J Herb Med 2016; 4(4): 9-17.
20. Shah BN and Seth AK: Pharmacognostic studies of the *Lagenaria siceraria* (Molina) Standley. International Journal of Pharm Tech Research 2010; 2(1): 121-4.
21. Kokate CK: Practical Pharmacognosy, Vallabh Prakashan, New Delhi, Edition 4th, 1994.
22. Khandelwal KR: Practical Pharmacognosy, Nirali publication, Pune, Edition 18th, 2007.
23. Government of India: The Ayurvedic pharmacopeia of India. New Delhi: Ministry of Health and Family Welfare, Department of Indian Systems of Medicines and Homeopathy, Edition 1st, 1996.
24. WHO: Quality control for medicinal plant material. New Delhi: AITBS Publishers, 1998.
25. WHO: Quality control methods for medicinal plant material. Geneva: WHO, 1992.
26. Kokoski CJ, Kokoski RJ and Slama FJ: Fluorescence of powdered vegetable drugs under ultraviolet radiation. J Amer Pharma Asso 1958; 10: 715-717.
27. Joshi BC and Uniyal S: Establishment of quality control protocols and antioxidant activity of *Urtica dioica* L. Journal of Conventional Knowledge and Holistic Health 2017; 1 (1): 1-6.
28. Farnsworth NR: Biological and phytochemical screening of plants. J Pharm Sci 1966; 55: 225-276.
29. Harborne A: Phytochemical methods a guide to modern techniques of plant analysis: Springer science & business media, Edition 3rd, 1998.
30. Trease GE and Evans WC: Pharmacognosy. Harcourt brace & Co. Asia, Pvt. Ltd., W.B. Saunders Company Ltd., Edition 15th, 2002.
31. Verma S and Singh SP: Current and future status of herbal medicines. Vet World 2008; 1(11): 347-350.
32. Gopalkrishnan B, Chiranjeev R. Quality Standardization of flowers of *Nyctanthes arbor-tristis* Linn. International Journal of Pharmacognosy and Phytochemical Research 2017; 9(10): 1314-1317.
33. Sheikh N, Desai T and Patel R: Pharmacognostic Evaluation of *Epilobium hirsutum* Linn. Pharmacognosy Journal 2016; 8 (3): 226-29.
34. Zhao Z, Liang Z and Guo P: Macroscopic identification of Chinese medicinal materials: traditional experiences and modern understanding. J Ethnopharmacol 2011; 131: 556-61.
35. Vilash V, Suja SR, Latha PG and Rajasekharan S: Physicochemical evaluation and pharmacognostical standardization of *Pellionia heyneana* Wedd. Leaf Pharmacognosy Journal 2016; 8(6). 551-56.
36. Bello H, Mohammed Z and Katsayal U: Pharmacognostic evaluation of the root *Cassia sieberiana* DC: A promising ethnomedicinal plant. Journal of Pharmacognosy and Phytochemistry 2016; 5(3): 270-75.
37. Hudson N, Baker A and Reynolds D: Fluorescence analysis of dissolved organic matter in natural, waste and polluted waters-a review. River Research and Applications 2007; 23 (6): 631-49.
38. Sharma B, Balomajumder C and Roy P: Hypoglycemic and hypolipidemic effects of flavonoid-rich extract from *Eugenia jambolana* seeds on streptozotocin-induced diabetic rats. Food Chem Toxicol 2008; 46(7): 2376-2383.
39. Orhan I, Kupeli E, Sener B and Yesilada E: Appraisal of anti-inflammatory potential of the clubmoss, *Lycopodium cuvatum* L. J Ethnopharmacol 2007; 109: 146-150.
40. Kumar RS, Manivannan R and Balasubramaniam A: Anti-oxidant and hepatoprotective activity of ethanol extract of *Indigofera trita* Linn. on CCl_4 induced hepatotoxicity in rats. Journal of Pharmacology and Toxicology 2008; 3: 344-350.
41. Rupasinghe HP, Jackson CJ, Poysa V, Di Berado C, Bewley JD and Jenkinson J: Soyasapogenol A and B distribution in soybean (*Glycine max* L. Merr) about seed physiology, genetic variability and growing location. J Agric Food Chem 2003; 51: 5888-5894.
42. Srivastava M, Kumar A and Pal M: Phytochemical investigation on *Jatropha curcas* seed cake. Int J Pharm Life Sci 2010; 1(6): 357-362.
43. Salna KP, Sreejith K, Uthiralingam M, Prince MA, John Milton MC and Fleming AT: Comparative study of phytochemicals investigation of *Andrographis paniculata* and *Murraya koenigii*. IJPPS 2011; 3(3): 291-292.

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