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Pharmacognostic Standardization of the Leaf and Stem bark of Millingtonia hortensis Linn. (Bignoniaceae)

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ABSTRACT

Millingtonia hortensis Linn. (Bignoniaceae) is a medicinal plant used for the treatment of various diseases such as fever, asthma, and microbial infections. This study aimed to investigate the quality control parameters of the leaf and stem bark of Millingtonia hortensis for proper authentication and to prevent adulteration. The macroscopic and microscopic characteristics, phytochemical, physicochemical, fluorescence properties, and heavy metal content of the leaf and stem bark of M. hortensis were determined using WHO-approved standard protocols and other published methods. The macroscopic and microscopic results showed imparipinnate compound leaves and oppositely arranged leaflets which are deltoid in shape with serrated margins. The outer stem bark is rough and brittle with fissures and ridges. The microscopic characteristics of the leaf show anomocytic stomata and wavy-walled epidermal cells. Saponins, flavonoids, and alkaloids were detected in both leaf and stem bark. The physicochemical results were within published acceptable limits. Heavy metals such as chromium and arsenic were not detected in both leaves and stem bark. The results of this study establish the identity, purity, and safety of M. hortensis.

Key words: *Standardization, Millingtonia hortensis, Heavy metals, Macroscopic evaluation, Quality control, microscopy*

INTRODUCTION

Standardization and quality control of herbal medicines and raw materials are important to ensure purity, safety, and efficacy [1-5]. Such quality indices emphasized by the World Health Organization (WHO) include macroscopic and microscopic examination, extractive value, moisture content, and ash value determinations, as well as phytochemical analysis [6, 7].

Millingtonia hortensis (Bignoniaceae), commonly known as the Indian cork tree, is a tall, erect ornamental tree that is indigenous to South-East Asia [8] and common in West Africa including Ghana where it is known as oshí'shiu among Ga's [9]. The useful plants of west tropical Africa, Vol 1). It is fast-growing and evergreen and is used to slow down afforestation [10].

The leaves and flowers are used in folkloric medicine as a cholagogue, antipyretic, and tonic [11]. The stem bark is also used for the treatment of lung disease, asthma, and also as an antimicrobial agent [12].

The methanol extract of the leaves has been shown to have antioxidant activity and antibacterial activity against *Micrococcus luteus* [8]. The antimicrobial activity of the essential oil extracted from the flowers has also been investigated against organisms such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Bacillus subtilis* [13]. The hepatoprotective effects [14] and the larvicidal properties [15] of the flower extract have also been established. Extracts of the stem bark have been investigated for their anthelmintic activities as well [16].

Phytochemical investigations have revealed the presence of glycosides, alkaloids, flavonoids, and phenols in different extracts of the stem bark [16, 17]. GC-MS analysis of the methanol leaf extract revealed the presence of flavones, isoquinolines, and coumarins [8]. From flower extracts, flavonoids, and glycosides such as hispidulin, hortensin, scutellarin, salidroside, and 2-phenethyl rutinoside have been detected [18].

In this study, we report on the quality control profile of the leaf and stem bark of *M. hortensis* from Ghana to aid in identification and also to ensure purity.

MATERIALS AND METHODS

Plant collection and preparation

Fresh leaves and stem bark of *M. hortensis* were collected from the Campus of the University of Ghana (N 05° 39'11.6, W 00° 11'09.3), Legon, Ghana. The samples were authenticated at the herbarium of the Plant Development Department, Center for Plant Medicine Research, Mampong-Akuapem, Ghana. The plant parts collected were pressed and processed following standard practices [19], and voucher specimens numbered CPMR 4898 have been deposited at the CPMR medicinal plants herbarium.

The leaves and stem bark were dried at room temperature (25^0C) for fourteen days, pulverized into a coarse powder, and kept in air-tight containers until ready for use. Fresh leaves were used for the microscopic examination.

Macroscopic evaluation

The morphological characteristics of the leaves and stem bark of *M. hortensis* were examined and described. For the leaves, features such as leaf type, the shape of lamina, apex, margin, base, venation, and texture were observed and described. The stem bark was also described using parameters such as the color of the outer and the inner bark, texture, fracture, and slash.

Microscopic evaluation

Freehand sections of the fresh leaf lamina were made, placed in a test tube containing chloral hydrate, and boiled in a water bath for four hours to clear all pigment. After cooling, the cleared leaf sections were examined microscopically for surface characteristics such as epidermal cell type, venation details, and stomata [6, 20]. Quantitative leaf parameters such as stomatal number, stomatal index, vein islet number, and veinlet termination number were as well determined [6]. The powdered samples of the leaf and stem bark were mounted and observed for the presence of features including stone cells, calcium oxalate crystals, and xylem vessels. All microscopic observations were made under low power (x10) and high power (x40) magnifications using the Leica optical microscope.

Physicochemical analyses

The physicochemical analyses of the powdered leaf and stem bark were performed by following already published protocols [6, 21]. Moisture content was determined using the loss on drying method. Petroleum ether-soluble, 70% ethanol-soluble, and water-soluble extractives, as well as total ash, water-soluble ash and acid-insoluble ash values, were also determined.

Preliminary phytochemical analysis

Qualitative tests for secondary metabolites such as alkaloids, saponins, terpenoids, flavonoids, and others were performed following standard methods [22, 23].

Fluorescence analysis

Each powdered leaf and stem bark of M. hortensis was treated with different solvents and observed under natural daylight, short ultraviolet wavelength (254 nm), and long ultraviolet wavelength (365 nm). Solvents used to constitute the samples include distilled water, 1N H2SO4, 1N HCl, glacial acetic acid, 1N NaOH, 70% ethanol, ethyl acetate, and chloroform [24].

Heavy metal analysis

Energy Dispersive X-ray Fluorescence (ED XRF) was employed to determine the presence of heavy metals in the leaf and stem bark powders of M. hortensis. Each powdered plant material was sieved with a mesh of aperture size 180 µm to produce uniform particles. Each powdered sample was then irradiated using an Olympus Vanta M Portable ED-XRF (VMR) analyzer. The measurements were done in triplicates [25].

RESULTS AND DISCUSSION

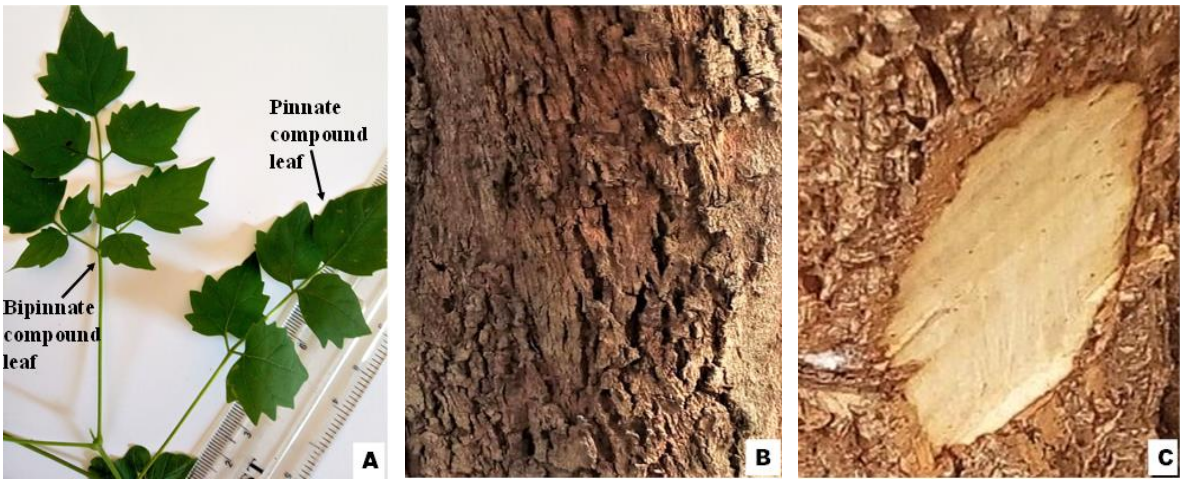
Macroscopic description

Morphological and microscopic assessments of crude drugs serve as quick tools for the identification of the specific crude drug [26]. Also, microscopy reveals additional minute details of the crude drug, thus preventing adulteration [27].

In this study, the leaves of M. hortensis are observed to be pinnate to bipinnate compound, with a single leaflet occurring at the apex of the rachis (imparipinnate). The leaves are oppositely arranged 3-5 foliate and stipulate. Each compound leaf bears oppositely arranged leaflets which are dark green on the dorsal surface and light green on the ventral surface. The leaflet is deltoid in shape with acute to acuminate apex, serrated margin, glabrous surface, obtuse to the asymmetrical base, and pinnately reticulate venation **(Figure 1a)**. These observations are similar to reports from published literature [11, 28].

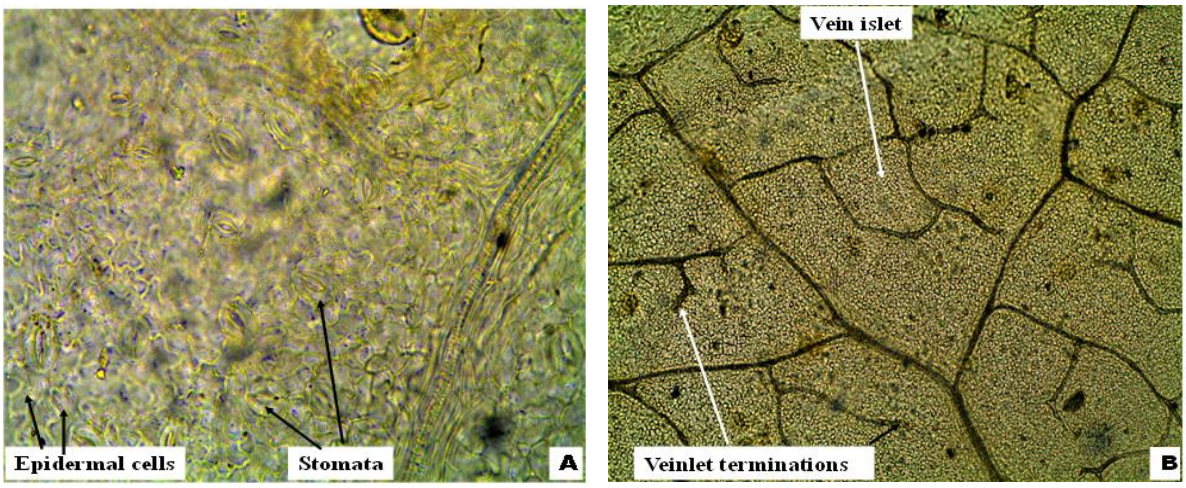
The outer bark of the stem is uniformly brown in color, rough, scaly, and brittle with irregularly outlined fissures and ridges **(Figure 1b)**. The slash is light brown to pale yellow with a smooth texture **(Figure 1c)** [12].

The cleared lamina surface is characterized by wavy-walled epidermal cells with evenly distributed stomata. The guard cells of each stoma are surrounded by four similar-sized epidermal cells, depicting anomocytic stomata **(Figure 2a)**. Similar findings are reported by Khan, (2020) [28]. The venation pattern is observed to be randomly reticulated with moderately developed areoles and unbranched veinlet terminations **(Figure 2b)**. Results of the leaf constants which include vein islet number, veinlet termination number, stomatal number, and stomatal index are detailed in **Table 1**. Stomatal index values are particularly useful in the detection of adulteration since these are relatively constant and not affected by factors such as leaf size, age of the plant, and environmental conditions [26]. Aggregated stone cells (brachysclereids) were also present in the stem bark powder of M. hortensis **(Figure 3)**.



a) **b)** **c)**

Figure 1. Leaves and stem bark of M. hortensis
a) Compound leaves; b) Outer bark; c) Slash



a) **b)**

Figure 2. Microscopic features of the leaf surface of M. hortensis
a) Epidermal cells and stomata; b) Venation pattern, vein islets, and veinlet terminations

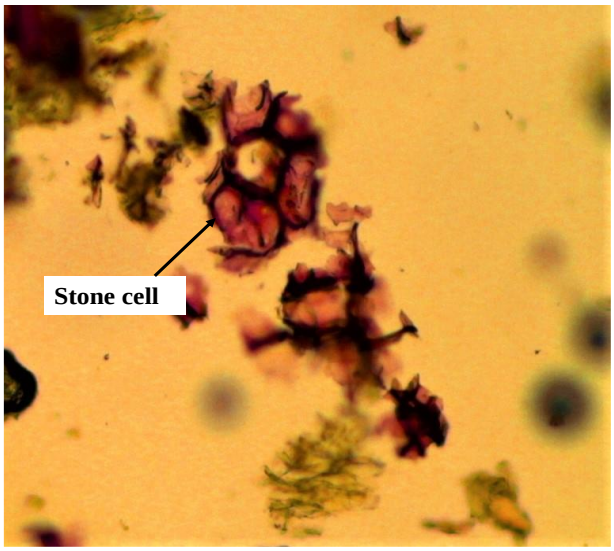


Figure 3. Powdered microscopy of stem bark of M. hortensis showing stone cells (brachysclereids) stained reddish-pink with phloroglucinol in concentrated hydrochloric acid

Table 1. Quantitative microscopy of *Millingtonia hortensis* leaves

Quantitative parameter	Average Values
Vein islet number (per mm^2)	5.66±1.41
Veinlet termination number (per mm^2)	7.00±1.49
Stomatal number (per mm^2)	7.22±1.64
Stomatal index (%)	13.80±1.62

Physicochemical properties

The results of the physicochemical analyses revealed a higher moisture content of 9.32±0.060 %w/w in the stem bark of M. hortensis than in the leaves. However, both values fall within the acceptable limit of 10% w/w for crude plants, suggesting a low likelihood of microbial attack [29]. High moisture content in herbal products is associated with microbial growth [27]. Ash values are indicative of the purity of the plant material and possible contamination by inorganic matter [27]. Total ash represents the material remaining after ignition. However, its value alone is not enough to reflect the quality of the material [7]. Water-soluble ash values can be used to detect already extracted or exhausted plant materials [30] Acid insoluble ash represents the amounts of siliceous matter present [7]. The highest extractive values were recorded for 70% ethanol, 17.26±0.10 %w/w, and 12.4±0.22 %w/w for both leaves and stem bark respectively. This may be attributable to the higher amount of medium polar

phytoconstituents that are soluble in aqueous-alcohol in both leaf and stem bark of M. hortensis. Details of the physicochemical results are presented in **Table 2**.

Table 2. Physicochemical properties of Millingtonia hortensis leaves and stem bark

Parameters	Leaf	Stem bark
Moisture content (%w/w)	5.73±2.04	9.32±0.060
Total ash (%w/w)	16.50±0.30	15.00±0.55
Water-soluble ash (%w/w)	2.00±0.51	8.00±1.52
Acid insoluble ash (%w/w)	3.25±0.20	3.00±0.0
Petroleum ether-soluble extractive (%w/w)	1.33±0.32	4.08±0.16
70% Ethanol-soluble extractive (%w/w)	17.26±0.10	12.4±0.22
Water-soluble (%w/w)	4.8±0.82	4.0±0.13

Preliminary phytochemical analysis

The preliminary phytochemical analysis showed the presence of constituents such as saponins, flavonoids, and alkaloids **(Table 3)**. Findings are consistent with published literature [16, 17].

Table 3. Preliminary phytochemical results

Parameters	Leaf	Stem bark	Test
Reducing sugars	+	+	Fehling's test
Saponin	+	+	Frothing test
Tannins	-	-	Ferric chloride test
Flavonoids	+	-	Alkaline reagent test
Alkaloids	+	+	Dragendorff's test
Phenols	+	-	Lead acetate test
Anthracene glycosides	-	-	Borntrager's test

Key: + (Detected) ; - (Not detected)

Fluorescence analysis

Various chemical constituents present in plant drugs fluoresce under UV light when extracted with different solvents or reagents [22]. This is useful in recognizing adulterants in liquid preparations. The fluorescence characteristics of the powdered leaf and stem bark in different reagents under visible and UV light are presented in **Tables 4 and 5**.

Table 4. Results of fluorescence analyses of Millingtonia hortensis leaves

Powdered sample + solvent	Visible light	Short UV wavelength (254 nm)	Long UV wavelength (365 nm)
Distilled water	Olive green	Dark green	Dark green
1N H2SO4	Light green	Light green	Purple
1N HCl	Burgundy	Dark green	Purple
Glacial acetic acid	Light green	Pale yellow	Pale yellow
1N Acetic acid	Yellowish green	Yellowish green	Yellowish green
1N NaOH	Light green	Light green	Dark green
Ethanol	Lemon green	Dark green	Yellowish green
Ethyl acetate	Light green	Colorless	Light orange
Chloroform	Light green	Straw-colored	Light orange

Table 5. Results of fluorescence analyses of Millingtonia hortensis stem bark

Powdered sample + solvent	Visible light	Short UV wavelength (254 nm)	Long UV wavelength (365 nm)
Distilled water	Brown	Dark brown	Dark brown
1N H2SO4	Dark brown	Colorless	Greenish brown
1N HCl	Brown	Dark brown	Greenish brown

Glacial acetic acid	Light yellow	Colorless	Colorless
1N Acetic acid	Brown	Colorless	Light green
1N NaOH	Brown	Pale yellow	Brown
Ethanol	Brown	Dark brown	Dark brown
Ethyl acetate	Brown	Colorless	Straw-colored
Chloroform	Brown	Colorless	Straw-colored

Heavy metal analysis

Heavy metals can be detrimental to one's health when in excess amounts. Chromium, nickel, mercury, and arsenic were not detected in both the leaf and stem bark of M. hortensis **(Table 6)**. Copper occurred in a much higher quantity in the stem bark (117±2.65 ppm) than in the powdered leaf (24.33±1.53 ppm). However, the detected elements were within acceptable limits [31]. The presence of these elements in the various plant parts may contribute to the general well-being of the plant and its therapeutic benefits [26, 32].

Table 6. Average heavy metal composition of the leaf and stem bark of M. hortensis

Element	**Concentration (ppm)**	
	Leaf	**Stem bark**
Zinc (ppm)	74.00±2.65	23.00±0.00
Lead (ppm)	1.00±1.73	Not detected
Mercury (ppm)	Not detected	Not detected
Cadmium (ppm)	19.33±1.53	17.67±2.89
Copper (ppm)	24.33±1.53	117.00±2.65
Nickel (ppm)	Not detected	Not detected
Arsenic (ppm)	Not detected	Not detected
Chromium (ppm)	Not detected	Not detected

CONCLUSION

Medicinal plants contribute considerably to the provision of primary healthcare to rural communities and play a significant part in modern drug discovery. In many parts of the world, they are used as bulk ingredients in indigenous medicines [29]. The pharmacognostic standardization of the leaf and stem bark of Millingtonia hortensis provides information on its identity, quality, and purity and helps to stem out adulteration and its detrimental effect.

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REFERENCES

1. Alam F, Saqib QN. Pharmacognostic study and development of quality control parameters for fruit, bark and leaf of Zanthoxylum armatum (Rutaceae). Anc Sci Life. 2015;34(3):147.
2. Faller EM, Hernandez MT, Hernandez AM, Gabriel JR. Emerging Roles of Pharmacist in Global Health: An Exploratory Study on their Knowledge, Perception and Competency. Arch Pharm Pract. 2020;11(1):40-6.
3. Soboleva MS, Loskutova EE, Kosova IV, Amelina IV. Problems, and the Prospects of Pharmaceutical Consultation in the Drugstores. Arch Pharm Pract. 2020;11(2):154-9.

4. Saraswat N, Sachan N, Chandra P. A Detailed Review on The Rarely Found Himalayan Herb Selinum Vaginatum: Its Active Constituents, Pharmacological Uses, Traditional and Potential Benefits. Pharmacophore. 2020;11(2):40-52.
5. Arifin Z, Milanda T, Suwantika AA. Cost-effectivity of standardized-herbal medicine for DHF inpatients in a Primary Health Center. J Adv Pharm Educ Res. 2019;9(4):19-23.
6. WHO. Quality control methods for medicinal plant materials: Updated edition. Geneva: WHO Press; 2011.
7. Pradhan N, Gavali J, Waghmare N. WHO (World Health Organisation) Guidelines for standardization of herbal drugs. Int Ayurvedic Med J. 2015;3(8):2238-43.
8. Sivaraj C, Aswitha V, Srinidhi M, Saraswathi K, Arumugam P. Antibacterial, antioxidant activities and GC-MS analysis of leaves extract of Millingtonia hortensis L. Pharma Innov J. 2019;8(1):513-21.
9. Burkill HM. The useful plants of West Tropical Africa. Vol. 1. Families AD. Royal Botanic Gardens; 1985.
10. Hegde S, Hedge S, Souza LD. In vitro Propagation of an Omamental Tree Millingtonia hortensis L. f. Die Gartenbauwiss. 1995;60(6):258-61. Available from: https://www.jstor.org/stable/43390204.
11. Nagaraja MS, Padmaa MP. Millingtonia hortensis Linn.–a review. Pharmacology online. 2011;2:597-602.
12. Kumari A, Sharma RA. A review on Millingtonia hortensis Linn. Int J Pharm Sci Rev Res. 2013;19(2):85-92.
13. Sittiwet C. Anti-microbial activities of Millingtonia hortensis Linn. flowers essential oil. J Pharmacol Toxicol. 2009;4(1):41-4.
14. Babitha S, Banji D, Banji OJ. Antioxidant and hepatoprotective effects of flower extract of Millingtonia hortensis Linn. on carbon tetrachloride induced hepatotoxicity. Journal of Pharmacy & Bioallied Sciences. 2012;4(4):307-12.
15. Thongpoon C, Poolprasert P. Phytochemical and Mosquito Larvicidal Properties of Millingtonia hortensis L.f. In: International Conference on Agriculture, Ecological and Medical Sciences (AEMS-2014). London; 2014. p. 32-6.
16. Chumbhale DS, Chaudhari SR, Upasani CD. Preliminary Phytochemical Analysis and in vitro Anthelmintic Activity of Millingtonia Hortensis Linn. Int J Pharm Chem Biol Sci. 2016;6(3):304-8.
17. Karthiya V, Vijayalakshmi A. Pharmacognostic and Preliminary Phytochemical Analysis of Millingtonia hortensis L. and Tecoma stans L. Int J Recent Sci Res. 2018;9(11B):29563-6.
18. Hase T, Kawamoto Y, Ohtani K, Kasai R, Yamasaki K. Cyclohexylethanoids and related glucosides from Millingtonia hortensis. Phytochemistry. 1995;39(1):235-41.
19. Martin GJ. Ethnobotany-A "'People and Plants'" Conservation Manual. London: Chapman and Hall; 1995.
20. Ash A, Ellis B, Hickey LJ, Johnson K, Wilf P, Wing S. Manual of Leaf Architecture: Morphological description and categorization of dicotyledonous and net-veined monocotyledonous angiosperms. Smithsonian Institution; Washington, DC: Leaf Architecture Working Group; 1999. p. 1-67.
21. Khandelwal KR. Practical pharmacognosy. 19th Editi. Vol. 68, The Analyst. Pragati Books Pvt Ltd; 2008.
22. Gunavathy SK, Sherine HB. Preliminary Phytochemical Investigation, Fluorescence analysis, and Determination of Ash Content of leaf extracts. Int J Pharm Biol Sci. 2019;9(2):1053-61.
23. Evans WC. Trease and evans' pharmacognosy E-book. Elsevier Health Sciences; 2009.
24. Ranjith D. Fluorescence analysis and extractive values of herbal formulations used for wound healing activity in animals. J Med Plants Stud. 2018;6(2):189-92.
25. Oppong Bekoe E, Dodoo KB, Kitcher C, Gordon A, Frimpong-Manso S, Schwinger G. Pharmacognostic Characteristics and Mutagenic Studies of Alstonia boonei De Wild. Res J Pharmacogn. 2020;7(1):7-15.
26. Mireku-Gyimah NA, Sarpong K, Amponsah IK, Mensah AY, Dickson RA. Comparative Pharmacognostic Studies Of Two Ghanaian Medicinal Plants: Saba senegalensis and Saba thompsonii. Int J Pharm Sci Res. 2018;9(4):1451-61.
27. Prakasia PP, Nair AS. Pharmacognostic and physicochemical standardization of leaves of Glycosmis pentaphylla (Retz.) DC. Pharma Innov J. 2016;5(9):23-30.
28. Khan D. Surface micromorphology of Millingtonia hortensis Linn. f. cultivated in Dubai, UAE. Int J Biol Biotechnol. 2020;17(2):411-32.
29. Wangchuk P, Yeshi K, Vennos C, Mandal SC, Kloos S, Nugraha AS. Three medicinal Corydalis species of the Himalayas : Their ethnobotany, pharmacognosy, phytochemistry, and pharmacology. J Herb Med. 2020;23(May 2019):100384. doi:10.1016/j.hermed.2020.100384
30. Menpara D, Chanda S. Phytochemical and pharmacognostic evaluation of leaves of Pongamia pinnata L. (Fabaceae). Pharma Comm. 2014;4(2):3-7.

31. Jabeen S, Shah MT, Khan S, Hayat MQ. Determination of major and trace elements in ten important folk therapeutic plants of Haripur basin, Pakistan. J Med Plants Res. 2010;4(7):559-66.
32. Rajurkar N, Damame M. Mineral content of medicinal plants used in the treatment of diseases resulting from urinary tract disorders. Appl Radiat Isot. 1998;49(7):773-6.