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Pharmaceutical Standardization

Preliminary pharmacognostic and phytochemical standardization of *Dhataki* [*Woodfordia fruticosa* (L.) Kurz.] leaves

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Abstract

Background: *Woodfordia fruticosa* (L.) Kurz., known as *Dhataki*, is an important medicinal plant used in Ayurveda. Recent studies on leaf showed that it contains important chemical constituents responsible for biological activities. The ethnic folk from India and Nepal are using the leaf to treat ulcers, rheumatism, fever, hemoptysis and as a disinfectant. It is also reported to be used in perfume, leather and textile industries. **Aim:** To investigate preliminary pharmacognostical and phytochemical parameters of leaf to standardize the drug. **Materials and Methods:** Identification of plant was done as per the standard guidelines given in the floras. Macro and microscopic evaluation performed as per the routine laboratory procedures. Phytochemical, physico-chemical, florescence analysis, behavior of powdered drug have been conducted as per the WHO guidelines. **Results:** Unique arrangement of the vascular bundle in mid rib region is observed. Aqueous and alcoholic extracts showed the presence of alkaloids, phenols, tannins, and flavonoids. **Conclusion:** The findings of this study will be helpful in the identification of *Dhataki* leaf.

Key words: *Dhataki*, pharmacognosy, physico-chemical, *Woodfordia fruticosa*

Introduction

Woodfordia fruticosa (L.) Kurz. belongs to family Lythraceae. In Ayurveda, it is known as *Dhataki*, *Tamrapushpi*, *Bahupushpi*, *Dhaity*, *Dhavani* and *Dhai*. Flowers are used to prepare *Asavas* and *Arishtas* and effective in leucorrhea, dysmenorrhea, dysuria and hematuria.[1-3]

Although, in Ayurveda, usage of leaves has not been reported; tribal people are using leaves to treat fever,[4,5] hemoptysis[6] rheumatism[7] and as disinfectant.[8,9] Leaves are being used to treat ulcers,[10] for milk enhancement in livestock[11] and in perfume, leather and textile industries.[12]

The leaves contain flavonoids,[13] essential oil[14] and phenolic compounds, responsible for antimicrobial and antiradical activity.[15,16]

Keeping in view the medicinal importance of *Dhataki* leaves as reported by the ethnic communities of India and Nepal; the study is aimed to standardize the leaf using pharmacognostic and phytochemical parameters.

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Materials and Methods

Collection of sample

Drug required for the study was collected from pollution free area; thoroughly washed under running tap water, and allowed water to drain from leaves. Leaves were shade dried, and powder was prepared with the help of grinding mill. Powder was sifted through 60 mesh sieve and preserved in air tight containers for further studies.

Preparation of herbarium

Plant samples of *W. fruticosa* were identified and authenticated with the help of The Flora of British India[17] and Flora of Maharashtra state.[18] Plant specimen was authenticated by comparing herbarium specimen available at Botanical Survey of India. Herbarium was prepared and deposited in the herbarium section of Institute with a voucher specimen no. 2578.

Preparation of wet sample

Freshly collected and thoroughly washed leaves were kept in a glass bottle containing a solution of (Formalin: Glacial acetic acid: 70% Ethyl alcohol; [10:5:85].[19]

Figure 1: Leaf

Figure 2: Habit

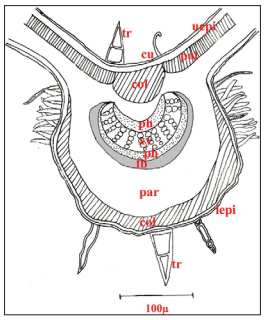

Figure 3: Diagrammatic transverse section of midrib. Col: Collenchyama, cu: Cutical, fb: Fibers, lepi: Lower epidermis, pal: Palisade, ph: Phloem, tr: Trichome, uepi: Upper epidermis

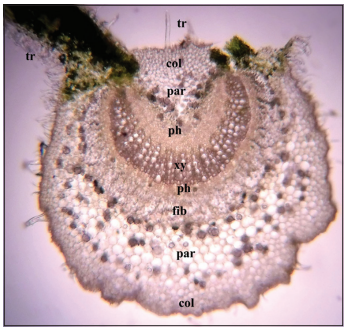

Figure 4: Transverse section passing through midrib. Col: Collenchyma, fib: Fibres, par: Parenchyama, ph: Phloem, tr: Trichome, xy: Xylem

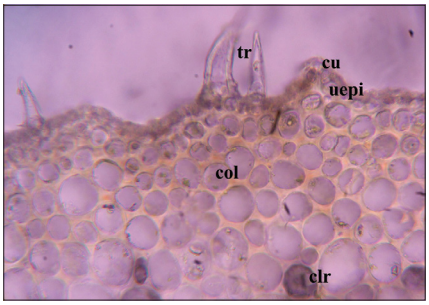

Figure 5: Upper epidermis with collenchyma. Clr: Cluster crystals of calcium oxalate, col: Collenchyma, cu: Cuticle, tr: Trichome, uepi: Upper epidermis

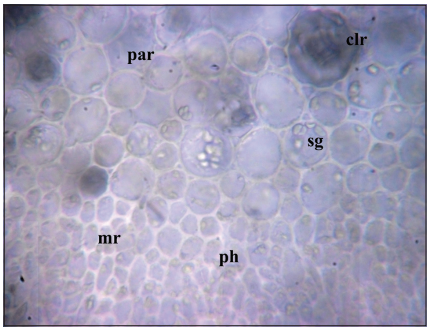

Figure 6: Parenchyma. Clr: Cluster crystals of calcium oxalate, mr: Medullary rays, par: Parenchyma, ph: Phloem, sg: Starch grains

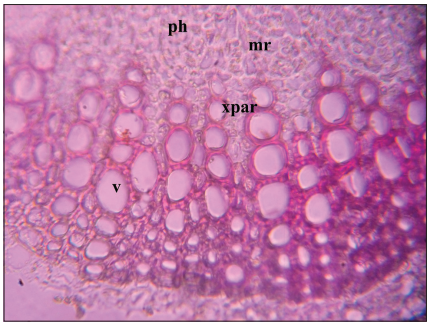

Figure 7: Xylem. mr: Medullary rays, ph: Phloem, xpar: Xylem parenchyma, v: Vessel

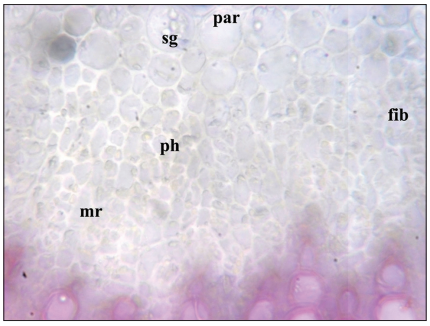

Figure 8: Phloem. Fib: Fibres, mr: Medullary rays, ph: Phloem, par: Parenchyma, sg: Starch grains

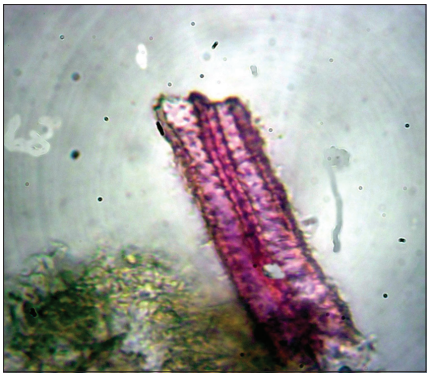

Figure 9: Pitted vessel

Microscopy

Free hand section of midrib, lamina were taken with the help of platinum razor blades. Sections were treated with phloroglucinol, followed by HCl, Iodine, and Sudan red-III for identification of various cellular details. Peelings of upper and lower epidermii were prepared to study types of stomata, stomatal index, stomatal number and palisade ratio. Camera lucida drawings were made with the help of prism type of camera lucida. Micrometry was performed as per the standard procedure.[20] Microphotographs were captured with the help of Samsung digital camera attached to the microscope.

Phytochemical and physico-chemical analysis

Dried leaf powder was used for the analysis of physico-chemical parameters such as total ash, acid insoluble ash, loss on drying, swelling index, foaming index, pH, extractive values and phytochemical tests for carbohydrates, alkaloids, tannins, phenols, saponin, and flavonoids. Fluorescence analysis and behavior of powder drug were done as per the standard guidelines. To study the behavior of powder drug with different chemical reagents, pinch of powder was kept on the surface of reagent and behavior was recorded.[20-22]

Figure 10: Spiral vessel

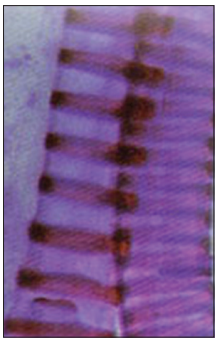

Figure 11: Annular vessel

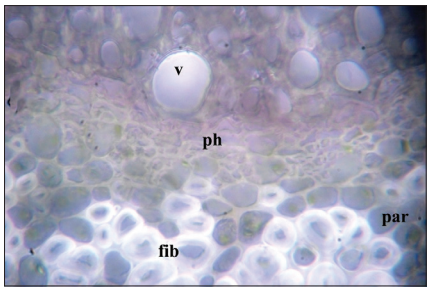

Figure 12: Lower phloem and fiber. Fib: Fibre, par: Parenchyma, ph: Phloem, v: Vessel

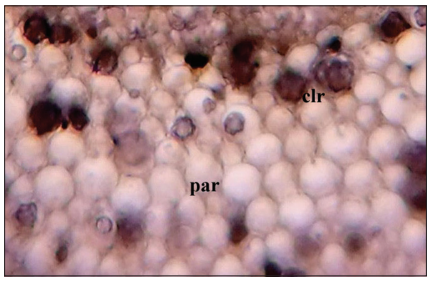

Figure 13: Ground tissue parenchyma. Clr: Cluster crystals of calcium oxalate, par: Parenchyma

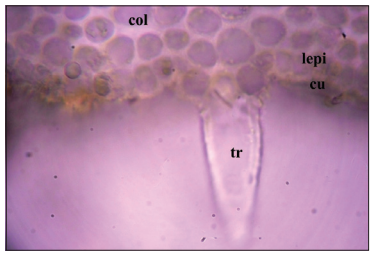

Figure 14: Lower epidermis with collenchyma and trichome. Col: Collenchyma, cu: Cutical, lepi: Lower epidermis, tr: Trichome

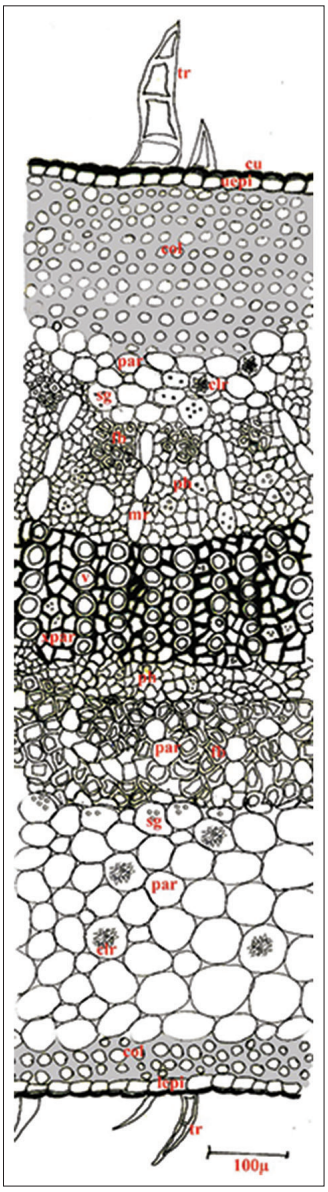

Figure 15: Detailed diagrammatic transverse section of Midrib. Clr: Cluster crystals of calcium oxalate, col: Collenchyma, cu: Cutical, fb: Fibers, lepi: Lower epidermis, mr: Medullary rays, par: Parenchyma, ph: Phloem, sg: Starch grains, tr: Trichome, uepi: Upper epidermis

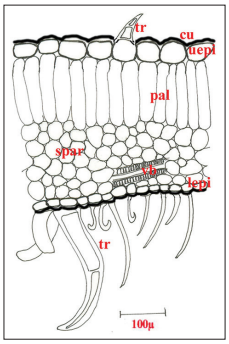

Figure 16: Diagrammatic transverse section of lamina. Cu: Cutical, lepi: Lower epidermis, pal: Palisade, spar: Spongy parenchyma, tr: Trichome, uepi: Upper epidermis, vb: Vascular bundles

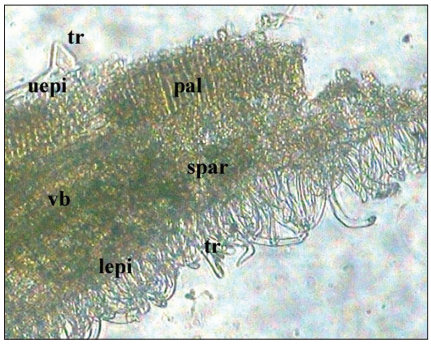

Figure 17: Transverse section of lamina. Lepi: Lower epidermis, pal: Palisade, spar: Spongy parenchyma, tr: Trichome, uepi: Upper epidermis, vb: Vascular bundles

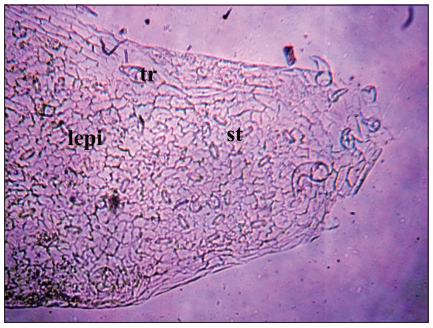

Figure 18: Lower epidermis in surface view. Lepi: Lower epidermis, st: Stomata, tr: Trichome

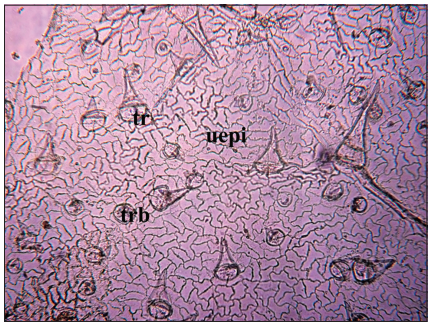

Figure 19: Upper epidermis in surface view. Uepi: Upper epidermis, tr: Trichome, trb: Trichome base

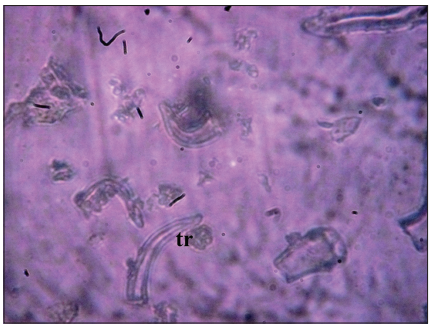

Figure 20: Fragments of trichomes. Tr: Trichome

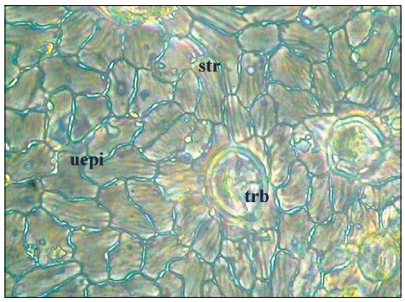

Figure 21: Upper epidermis with striated cutical. Str: Striations, trb: Trichome bases, uepi: Upper epidermis

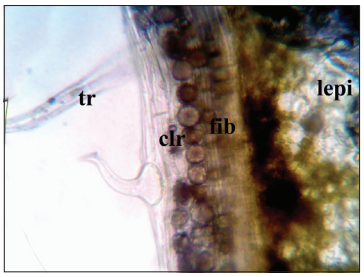

Figure 22: Fibers containing cluster crystals of calcium oxalate. Clr: Cluster crystals of calcium oxalate, fib – fibers, lepi: Lower epidermis, tr: Trichome

Results and Discussion

Macroscopy

Leaves are simple, opposite to sub-opposite, sessile, exstipulate, dorsiventeral, 3.5-8 cm long and 1.22.5 cm wide, lanceolate, oblong to lanceolate, ovate to lanceolate, acute, apices slightly curved, oblique, rounded or curved at base, upper surface smooth, pubescent, dark green, lower surface pubescent, glabrous, light green, nigro-punctate, prominent intra-marginal nerves in 6–12 pairs, taste slightly astringent and odor characteristic [Figures 1 and 2].

Microscopic evaluation

Transverse section passing through midrib

Midrib is dorsally convex and ventrally flat convex with notch in the center [Figures 3 and 4]. Transverse section showed tangentially elongated, squarish to rectangular cells of upper and lower epidermis, measuring 13.2 µm to 44 µm and 6.4 µm to 20.2 µm length and width, respectively. The upper and lower epidermii are covered with thick cuticle and numerous simple, curved uni to multicellular trichomes. Trichomes are 22 µm to 250.8 µm in length and 3 µm to 9 µm in width. 10-12 layers of collenchyma are present below the upper epidermis and 3-4 layers are present above the lower epidermis. Collenchyma is composed of circular to oval shaped cells measuring 22–35 µm in diameter [Figure 5]. Collenchyma is followed by 4–5 layers of parenchyamatous cells embedded with cluster crystals of calcium oxalate and starch grains [Figure 6]. Centre is occupied by arc of endarch, bi-collateral, closed vascular bundle traversed with uni to bi-seriate medullary rays [Figure 7]. Phloem is 15–17 layered, contains cluster crystals of calcium oxalate, and simple, oval starch grains; 5–10 groups of fibers are traversed in the region [Figure 8]. Xylem is composed of pitted, spiral, and annular vessels, parenchyma, and uni to bi-seriate xylem rays followed by 4–5 layers of thin walled irregularly shape parenchyma. 5–6 layers of fibers are present traversed with parenchymatous cells [Figure 12]. Ground tissues are composed of 6–7 layers of oval to oblong cells embedded with cluster crystals of calcium oxalate and few simple, oval starch grains [Figure 13]. Underneath this, 2–3 layers of collenchyma present [Figure 14]. Cellular details of section passing through midrib are presented in the form of the line drawing obtained with the help of camera lucida [Figure 15].

Transverse section passing through lamina

Section passing through lamina shows tangentially elongated squarish to rectangular cells of upper and lower epidermis shows simple, curved, uni to multicellular trichome and are covered with thick cuticle. Underneath upper epidermis, single layer of cylindrical palisade cells containing chlorophyll pigments are present. It is followed by 4–6 layers of spongy parenchyma embedded with chlorophyll pigments, clusters of calcium oxalate. It is traversed with obliquely cut vascular strand [Figures 16 and 17].

Powder microscopy

Powdered drug showed groups of epidermal cells with anomocytic and anisocytic types of stomata, trichome and trichome base in surface view [Figures 18 and 19]. Fragments of crystalloid fibers were also found. Presences of fragments of simple, curved, unicellular trichome were also seen [Figure 20]. Annular, spiral and pitted vessels measuring average length and breadth of 1795.86 µm, 130.53 µm; 890 µm, 218.53 µm; and 1224.66 µm, 121.46 µm respectively are observed [Figures 9-11,]. Powder also shows upper epidermis with striations in surface view [Figure 21], fragment of collenchyma cells, cluster crystals of calcium oxalate, isolated starch grains, fragment of parenchymatous cells containing cluster crystals of calcium oxalate [Figures 22 and 23].

Physico-chemical content

Powder shows average ash value 5.05%, acid insoluble ash 0.05%, extractive values in different solvent, water soluble extractive 15%, ethyl alcohol soluble extractives 25.94%, petroleum ether soluble extractives 2.52%, chloroform soluble extractive 5.20%, carbon tetra chloride soluble extractives 3.51%, ethyl acetate soluble extractives 5.04%, acetone soluble extractive 17.7% and methyl alcohol soluble extractives 25.11%. Aqueous solution of leaves is acidic in nature and showed 4.6 pH and 4.6 foaming index. Animocytic and anisocytic types of stoma are found in the leaves with 14.29% stomatal index, 4.6 stomata per millimeter square and 1:6 palisade ratio [Table 1].

Phytochemical content

Aqueous and alcoholic extracts were subjected to different chemical tests for presence of different phytochemicals in leaf powder. Aqueous extract of leaf showed presence of carbohydrates, alkaloids, tannins, phenols, saponin, and flavonoids. Whereas, all the above except saponin were tested positive in alcohol extract [Table 2].

Fluorescence analysis

Extracts obtained from different solvents were observed under ultra violet (UV) light at 254 nm and 366 nm wavelength,

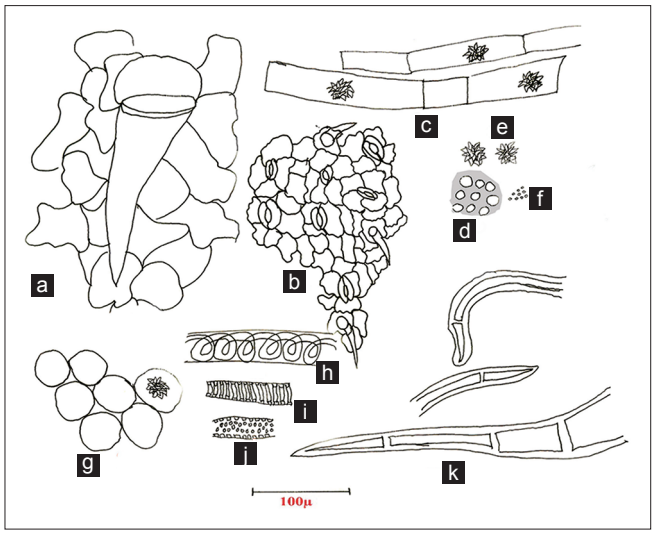

Figure 23: Powder characters. (a) Upper epidermis in surface view; (b) lower epidermis in surface view; (c) fibers containing cluster crystals of calcium oxalate; (d) fragment of collenchyma; (e) cluster crystals of calcium oxalate; (f) starch grains; (g) parenchyma cells containing crystals; (h) spiral vessel; (i) annular vessel; (j) pitted vessel; (k) fragment of trichomes

and under day light. Extract showed remarkable change in color under UV light. Under ordinary light, ethyl alcohol, ethyl acetate, acetone, methyl alcohol extracts showed green color, umber color was observed in carbon tetra chloride and distilled water extracts, whereas luteous (light yellow) and citrus (dull light green) color was observed in petroleum ether and chloroform extracts respectively. Under short UV light, herbage green color was displayed from the extracts of ethyl alcohol, petroleum ether and distilled water, dark purple color was observed from extracts of chloroform, acetone, and methyl alcohol. Light yellow and dark violet color reflected from extracts of carbon tetra chloride and ethyl acetate. Ethyl alcohol extract showed blood red color under long UV light, dull to dark purple color was exhibited with the extract of petroleum ether, chloroform and distilled water [Table 3].

Behavior of powdered drug

Powder drug exhibited a unique behavior with a particular solvent or reagent. Powder floats on the surface of almost all solvents used in the study except glacial acetic acid solutions. In sulfuric acid, hydrochloric acid, nitric acid, ferric chloride, powder turns reddish black, green, orange yellow, dark green respectively. Powder turns brownish yellow in the solution of sodium hydroxide and potassium hydroxide.

Discussion

Pharmacognostic and phytochemical investigation showed that the cellular structure of the leaf having diagnostic characters such as two types of stomata with striations on the upper epidermis. Presence of bi-collateral, closed and end arch vascular bundle in the midrib region is a unique feature of leaves. Presence of alkaloids, saponin and tannins and phenolic compounds in both alcoholic and aqueous extracts and could be used to prepare new herbal drug to treat various diseases. However, quantitative estimation of alkaloids, tannin, phenol, flavonoids contents are need to be establish as these compounds are being utilized in pharmaceutical industries. Drug showed a remarkable difference in florescence studies and with various chemical reagents. Seasonal variation in the chemical contents of leaf needs to be investigated in future to utilize the drug in the medicine.

Table 1: Physico-chemical constants of *Woodfordia fruticosa* leaf

Parameters	Result (%w/w)
Total ash	5.05
Acid insoluble ash	0.05
Loss on drying at 110°C	14.35
Foaming index	4.6
Swelling index	0.0
pH	4.6
Stomatal index	14.29
Stomatal number	4.6
Palisade ratio	1:6
Extractive values	
Ethyl alcohol	52.94
Petroleum ether	2.52
Chloroform	5.2
Carbon tetra chloride	3.51
Ethyl acetate	5.3
Acetone	17.7
Absolute alcohol	25.11
Distilled water	15

Table 2: Phytochemical components of *Woodfordia fruticosa* leaf

Natural product	Test performed	Result	
		Water extractive	Alcohol extractive
Carbohydrate	Molish's test	Positive	Positive
Sugar	Fehling test	Negative	Negative
Proteins	Million's test	Negative	Negative
	Biuret test	Negative	Negative
Amino acid	Ninhydrin test	Negative	Negative
	Tryosine test	Negative	Negative
Alkaloids	Dragendorff's test	Positive	Positive
Tannins and phenolic compounds	5% $FeCl_3$	Positive	Positive
	Lead acetate solution test	Negative	Negative
Starch	Iodine test	Negative	Negative
Saponin	Foam test	Positive	Negative
Flavonoids	Shinoda test	Positive	Positive
Mucilage	Ruthenium red	Negative	Negative

Conclusion

Pharmacognostic and preliminary phytochemical investigations of leaf showed unique diagnostic characters, which could be helpful to identify the leaf drug of *Dhataki*.

Table 3: Fluorescence behavior of different extracts of *Woodfordia fruticosa* leaf

Extractives	Day light	UV 254 nm	UV 366 nm
Ethyl alcohol	Dark green	Dark herbage green	Blood red
Petroleum ether	Light yellow	Dark herbage green	Dull purple
Chloroform	Dull light green	Dark purple	Dark purple
Carbon tetra chloride	Amber	Light yellow	Yellow
Ethyl acetate	Dark green	Dark violet	Shiny pink
Acetone	Dark green	Dark purple	Shiny pink
Methyl alcohol	Herbage green	Dark purple	Dark purple
Distilled water	Amber	Herbage green	Dark green

UV: Ultra violet

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हिन्दी सारांश

आयुर्वेदिय पादप धातकी (वुडफोर्डिया फ्रुटिकोझा (लिन) कुर्ज) का भैषजज्ञानीय तथा पादपरासायनिक मानकीकरण

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वुडफोर्डिया फ्रुटिकोझा (लिन) कुर्ज, यह एक महत्वपुर्ण आयुर्वेदिक वनस्पती है, जो आयुर्वेद मे धातकी के नाम से जानी जाती है, चूंकि इस वनस्पती की पत्तियों मे महत्वपूर्ण रासायनिक योगिक होते हैं जो किगठिया, अलसर तथा यकृत सम्बधी बिमारियों का निदान करते हैं तथा प्रतिजैविक क्रिया भी दर्शाते हैं। भविष्य में इन पत्तियों से प्रतिजैविक औषधीयाँ बनाई जा सकती है। भारतीय आयुर्वेदिय फार्माकोपीया तथा विश्व स्वास्थ्य संगठन के दिशानिदेशों के अनुसार इस वनस्पती के पत्तों का भैषजज्ञानीय तथा विभिन्न पादप रासायनिक पहलुओं का अध्ययन करके मानकीकरण किया गया। इस अध्ययन के द्वारा यह प्रेक्षित किया गया कि, पत्ते मे अद्वितीय वेस्कुलर गुच्छ की रचना पायी गयी जो एनडार्च, बायकालेटरल तथा क्लोज पद्धति की है जो पत्ते के केन्द्र स्थान मे पाई गयी है। फ्लोएम मे कैलशियम ऑक्झायलेट के क्लस्टर क्रिस्टल तथा स्टार्च के कण पाये गये। इस में तीन विभिन्न प्रकार के वैसल पायी गई जो पिटेड, स्पायरल तथा एनुलर प्रकार की है। मूलउतकों में कैलशियम ऑक्झायलेट के कलस्टर क्रिस्टल तथा स्टार्च के कण पाये गये। इस पत्राचार के माध्यम से भौतिक–रासायनिक नियतांक, पादपरासायनिक संग्रह, फ्लुओरोसेस विश्लेषण तथा औषधी का विभिन्न रसायनों के साथ व्यवहार दर्शाया गया। धातकी के पत्तों का मानकीकरण के सबंध मे यह प्रतिवेदन है।