Assessment of Pharmacognostic and Preliminary Physicochemical Investigations of Ethnomedicinal Plant *Leea Asiatica* (L.) Ridsdale of Valmiki Tiger Reserve, West-Champaran District, Bihar

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ABSTRACT

*Leea asiatica* (L.) Ridsdale is synonymously known as *Phytolacca asiatica* L. and *Leea crispa* L. *Leea asiatica* is a perennial shrub or small tree in the family Vitaceae. According to a survey, any report was not available on macroscopic, microscopic, and physicochemical investigations of *Leea asiatica* (L.) Ridsdale. The present study was aimed to evaluate macroscopic, microscopic characters, and physicochemical investigations of *Leea asiatica* (L.) Ridsdale. All parameters were established according to the Pharmacognostical standards procedure. Leaves were found to be petiolate, leaflets 5-7, oblong or elliptic-ovate. Stems are soft wooded, erect, and pubescent. Transverse sections of the midrib of leaf displayed a single layer of the epidermis, glandular, and nonglandular trichomes. Powder microscopy of the leaf and stem showed the presence of rosette crystals of calcium oxalate, trichomes, and starch grains. Physicochemical studies like ash values, extractive values, and LOD of *Leea asiatica* leaves have also been established. The present studies have established parameters for the correct identification of *Leea asiatica*. The present investigation was carried out to focus on pharmacognostic and preliminary physicochemical investigations of plant, *Leea asiatica* (L.) Ridsdale, which will assist in standardization for authenticity, quality, and identification of herbal products.

INTRODUCTION

Plants are being used for many ethnomedicinal and non-ethnomedicinal purposes of humans since ancient times (Aati et al., 2019). India is recognized the world over as a rich source of aromatic and medicinal plants (Kumar and Jnanesha, 2016). The northern region of India is very rich in plant biodiversity as well as in ethnic diversity (Roy et al., 2015). Vitaceae (the grape family) has comprised 14 genera and 900 known species distributed across the world, basically in the tropical and subtropical regions of Asia- China, India, Nepal, Bhutan, Thailand, Bangladesh, Cambodia, Myanmar, Laos,
Vietnam (Soejima and Wen, 2006). Eleven species of the family Vitaceae are monogeneric, which are distributed mostly in the tropical, subtropical and evergreen forests of India (Soejima and Wen, 2006; Zhang et al., 2015).

Leea asiatica (L.) Ridsdale is a traditional Indian medicinal plant of Valmiki Tiger Reserve, West-Champaran District, Bihar, India which belongs to the family Vitaceae, which is most closely allied to the great economically important grape family, Vitaceae (Singh and Singh, 2014; Mishra et al., 2016). Leea asiatica (L.) Ridsdale, is a shrub to small tree that is widely distributed in moist deciduous and evergreen forests up to 1800m in altitude at the India-Nepal border in the West Champaran district of Bihar, India on the side of river Gandak. It is a photoautotroph. This medicinal plant is traditionally used by the tribal community other than tharu in this landscape are Oraon, Munda, Hohra, and Bhuiya. In tropical and subtropical India, where people living in villages of West-Champaran District, Bihar viz. Bettiah, Bagaha, and Narkatiaganj are biodiversity-rich areas and forests have been using indigenous plants as medicines for ages (Prasad and Singh, 2014; Singh et al., 2001). The leaves, stems, and roots of Leea asiatica (L.) Ridsdale, was used as a folk medicine in the treatment of asthma, constipation, cough and cold, snake-bite, parasitic intestinal worms, blood coagulation, bone fracture, diabetes, hepatic disorder, osteoarthritis, hair fall, wounds, and other oxidative stress-related disorders for the people living in remote and backward areas of West-Champaran District, Bihar (Sen et al., 2014; Jain and Pachaya, 2015).

This medicinal plant is used by the traditional medicine practitioners and local communities of the district West-Champaran of Bihar with enormous potentials have not been scientifically validated for their pharmacognostic, phytochemical and pharmacological evaluation (Dwivedy and Singh, 2016). Based on the above literature review the present work deals with the assessment of pharmacognostic and preliminary physicochemical analysis of the plant Leea asiatica (L.) Ridsdale. Obvious pharmacognostic and preliminary physicochemical analysis were found to be useful evidence for further scientific investigations of this medicinal plant (Karthika et al., 2018). The medicinal plant Leea asiatica (L.) Ridsdale (Family: Vitaceae) commonly called Kumali/ Basant jari/ Nagashya/ Nanli in Hindi (ENVIS, 2020). The fresh stem was used for making a basket (Non-ethnomedicinal use). A search of the traditional and ethnomedicinal literature revealed that this plant is very safe and effective for medicinal uses (Ahmed and Azam, 2001).

Figures 1, 2, and 3: Photographs of Leea asiatica (L.) Ridsdale plant parts.

**Plant taxonomy**

Kingdom: Plantae
Phylum: Magnoliophyta
Class: Eudicots
Order: Vitales
Family: Vitaceae
Genus: Leea
Species: Lasiatica
Binomial name: Leea asiatica (L.) Ridsdale. (Bais, 2013; Hao et al., 2015)

**Synonyms** Leea crispa L., Phytolacca asiatica L., Leea aspera EDGE.

**Vernacular names**

Hindi: Kumali, Basant jari, Nagashya, Nanli
Sanskrit: Kakjangha
MATERIALS AND METHODS

Plant materials

The fresh medicinal plant was collected from moist deciduous and evergreen forests of Valmiki Tiger Reserve, West-Champanar District, Bihar, India with the help of a local tribe of Village- Bagha in the period of September to November 2017. The plant specimen was authenticated by Dr. Narendra Kumar, Scientist, CSIR- Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow – 226015, India (Specimen No. CIMAP/Bot-Pharm./2018/10).

The fresh leaves and stems were separated and cleaned to remove unwanted materials. The fresh leaves and stems were air-dried at room temperature for about one week. The dried leaves and stems were coarsely powdered in a blender and were used for further analysis.

Instruments and chemicals

UV chamber (DESAGA, Germany), compound microscope, stage micrometer, camera lucida, drawing...
sheets, digital pH meter (Systronics India Ltd, Gujarat), glass slides, coverslips, watch glass and other common glasswares are used for the present study. The photomicrographs were made using a digital scanning electron microscope (SEM; Labline Scientific Instruments, Mumbai, India). Chemicals like phloroglucinol, glycerin, Conc. HCL, Dilute HCL, Conc. H₂SO₄. Chloral hydrate solution and organic solvents, standard buffer tablet (pH 4, 7 and 9.2) and sodium hydroxide, etc. were obtained from Ranbaxy Fine Chemicals Ltd., Mumbai, India.

Pharmacognostic Investigations

Macroscopical investigation

Macroscopical characters were examined with the naked eyes to determine the color, shape, odor, margin, texture, base symmetry, etc. of the plant parts as per the requirement of Indian Herbal Pharmacopoeia (Patil et al., 2005; Wallis, 1985).

Microscopical investigation

For microscopic studies, thin-hand sections of the leaves and stems were taken, cleared with chloral hydrate solution and stained as per the standard protocol. The photomicrographs of the thin-hand sections of the leaves and stems were made using a digital scanning electron microscope (SEM; Labline Scientific Instruments, Mumbai, India) (Brain and Turner, 1975; Johansen, 1940).

Quantitative microscopical analysis

The quantitative microscopical parameters of the leaf of Leea asiatica like the stomatal number, stomatal index, palisade ratio, vein islet number, and vein termination number were determined on epidermal strips (Patil et al., 2005; Wallis, 1985).

Powder microscopy of leaf and stem

For microscopical studies, the air-dried powdered drug was treated with NaOH, mounted on glycerin after staining with safranin, and powder characteristic was observed using the compound microscope and camera lucida (Trease and Evans, 2002; Kulkarni and Gokhale, 2015).

Physico-chemical Investigations

Physicochemical studies of the air-dried powdered drug have been conducted to its high medicinal properties. The following physic-chemical analysis such as ash value, foreign matter, loss on drying (LOD), foaming index, swelling index, extractive value and pH were performed by Indian Pharmacopoeia and the WHO-recommended parameters from Leea asiatica leaf powder. Fluorescence study was carried out as per the method advocated by Chase & Pratt, Harborne, Kokoski (IP, 1996; WHO, 1998).

Ash value

The analysis of the purity of the drug has been based on ash value. For the analysis of ash values, the leaf powder was screened for the following tests (Evans and Trease, 2009; WHO, 1998).
Table 1: Macroscopy of the leaf of *Leea asiatica*

| S. No. | Observations          | Characters                |
|-------|-----------------------|---------------------------|
| 1     | Shape and Structure   | Ovate to elliptic slightly hairy |
| 2     | Colour                | Green                     |
| 3     | Odour                 | Characteristic            |
| 4     | Taste                 | Astringent                |
| 5     | Size                  | 10-16 x 3-6 cm            |
| 6     | Touch                 | Smooth                    |

**Total ash**

Accurately weighed about 2g of the air-dried powdered drug and taken separately in a tarred silica crucible, which was previously ignited and weighed. The powder was spread as a fine layer on the bottom of the crucible. The powder was incinerated gradually by increasing the temperature to 500-600°C until it became white. The crucible was cooled in a desiccator and weighed. The process was repeated to obtain constant weight. Then, the percentage of total ash value was calculated according to the reference of the ground air-dried powder.

**Water soluble ash**

The ash obtained as described above was boiled with 25 ml of distilled water for 5 minutes. The insoluble matter was collected on tarred silica crucible, washed with hot water, ignited, cooled and weighed. The process was repeated to obtain constant weight. Subtracted the weight of insoluble matter from the weight of total ash; this difference in the weight represents the water-soluble ash. Then, the percentage of water-soluble ash value was calculated according to the reference of the ground air-dried powder.

**Acid insoluble ash**

The ash obtained as described above was boiled with 25 ml of 2N HCl for 5 min. The insoluble matter was collected on tarred silica crucible, washed with hot water, ignited, cooled and weighed. The process was repeated to obtain constant weight. Calculated the percentage of acid-insoluble ash according to the reference of the ground air-dried powder.

**Sulfated ash**

Accurately weighed about 2g of air-dried powdered drug and taken in a tarred silica crucible, which was previously ignited and weighed. Then ignite gently first until the powder was thoroughly charred. The residue was cooled and moistened with 1ml of Conc. H$_2$ SO$_4$, heated gently until the white fumes were no longer evolved and then ignited at 800°C ± 25°C until free from carbon. The crucible was allowed to cool, few drops of Conc. sulphuric acid was added and again heated. The ignition was carried out as before, allowed to cool and weighed. The process was repeated to obtain constant weight. Calculated the percentage of sulfated ash according to the reference of the ground air-dried powder.

**Foreign matter**

Accurately weighed about 100g of air-dried powdered drug and taken separately in a tarred silica crucible, which was spread out in a thin layer. The foreign matter was grouped either by visualization, using a magnifying lens or with the help of a suitable sieve according to the requirements. Its percentage in the air-dried sample was calculated (WHO, 1998).

Table 2: Quantitative microscopical parameters of the leaf of *Leea asiatica*

| S. No | Leaf Constants/ Parameters | Observed value |
|-------|-----------------------------|----------------|
| 1     | Stomatal number (mm$^{-2}$)  |                |
|       | Upper epidermis             | 01=01          |
|       | Lower epidermis             | 32-41=36.5     |
| 2     | Epidermal cells (mm$^{-2}$)  |                |
|       | Upper epidermis             | 227-295=261    |
|       | Lower epidermis             | 456-523=489.5  |
| 3     | Stomatal Index (%)          |                |
|       | Upper epidermis             | 0.38           |
|       | Lower epidermis             | 6.939          |
| 4     | Palisade ratio              | 21-27          |
| 5     | Vein islet no.              | 5-8            |
| 6     | Vein termination no.        | 2-3            |

**Loss on Drying**

Accurately weighed, 3g of air-dried powdered drug in a dried and tarred porcelain dish which was earlier dried in an oven at 100-105°C to constant weight. Cooled in a desiccator and calculated the loss of weight of air-dried material (WHO, 1998).

**Foaming Index and Swelling Index**

The foaming and swelling ability were carried out as per the official methods prescribed in WHO guidelines (WHO, 1998).

**Extractive value (Cold maceration method)**

Five grams of air-dried powdered drug were cold macerated with 100 ml of different solvents ranging from non-polar to polar solvents in a separate conical flask, plugged with cotton wool and the kept on a rotary shaker at 120 rpm for twenty-four hours. It was then filtered and the filtrate was evaporated to dryness at 105°C. The percentage of the extractable
Table 3: Determination of physicochemical parameters of Leea asiatica leaves

| S.No. | Parameters          | Values               |
|-------|---------------------|----------------------|
| 1     | Ash Values          |                      |
| A.    | Total ash          | 9.82±0.48 % w/w      |
| B.    | Acid insoluble ash | 1.11±0.03 % w/w      |
| C.    | Water soluble ash  | 0.78±0.16 % w/w      |
| D.    | Sulfated ash       | 15.09±0.06 % w/w     |
| 2     | Foreign matter analysis | 7.34 % w/w       |
| 3     | Loss on drying     | 7.23±0.83% w/w       |
| 4     | Swelling Index     |                      |
| 5     | Foaming Index      |                      |
| 6     | Extractive Values  |                      |
|       | Extract Cold       | Successive Extraction| Colour of extract |
|       | Maceration (% w/w) | (% w/w)              |                      |
| A.    | Petroleum ether    | 0.69                 | 1.07                | Brown |
| B.    | Chloroform         | 0.98                 | 1.40                | Brick red |
| C.    | Ethyl              | 1.79                 | 2.89                | AcetateGreen |
| D.    | Methanol           | 12.47                | 5.87                | Green |
| E.    | Ethanol            | 7.32                 | 9.25                | Yellow |
| F.    | Aqueous            | 16.05                | 8.47                | Light Yellow |
| 7     | pH                 |                      |
| A.    | 1% solution        | 6.32                 |                      |          |
| B.    | 10% solution       | 7.12                 |                      |          |
| 8     | Solubility of extract | > Hot water> Cold water> Methanol> Ethanol> Ethylacetate> Chloroform | |

matter was calculated according to the reference of the sample taken (WHO, 1998).

**Solubility of extract**

The solubility study of plant extract was identified by various solvents based on polarity gradience.

**pH value**

A digital pH meter was used for the analysis of the pH of 1% and 10% aqueous solution of air-dried powdered drug.

**UV Fluorescence analysis**

Fluorescence nature of air-dried powdered drug was observed by treating with different organic solvents under daylight and UV light. The color of fluorescence was recorded (Chase and Pratt, 1949; Kokoski et al., 1958).

**RESULTS AND DISCUSSION**

**Pharmacognostic Investigations**

**Leaves**

Raw drug sample was dried leaves of *Leea asiatica* L. It is a rigid shrub or small tree growing up to 2–16 m tall (Figure 1). The upper dorsal surface of the leaf was smooth, dark-greenish, prominent veins and lower surface or ventral surface was less in green color, less prominent veins and prominent midrib (Figure 2). The leaves were 2 or 3 pinnae bearing 5-7 leaflets, ovate to elliptic, acute apex, serrate-dentate, acuminate having petioles of 3 cm, subcoriaceous, sparsely hairy above, with globose glands; petiolate equal base. Leaves were identified as compound, bipinnate to tripinnate; rachis 6-14 cm long; leaflets 10-16 cm long and 3-6 cm wide (Figure 3); margin serrate to dentate, glabrous with hairs; midrib raised above; secondary nerves 12-19 pairs. Flower, size 5-6 mm, pale green in terminal and axillary branched cymes. Fruit berry, purple-black; usually 4–6-seeded, 5x3 mm. As shown in Table 1.

**Stems**

Stems were pubescent, erect. It usually grows from 15 cm tall in between two nodes, 0.5 cm diameter (Figure 3). Angular stems were swollen above the nodes and internodes. Stems having longitudinal longitudinal striations. The fresh stem was light in color and becomes dark after drying; outer surface dark green in color; inner surface yellowish-white (creamish). Stems having splinter fractures in outer portion while inner portion having fibrous fractures.
Table 4: Fluorescence analysis of powdered leaf treated with different reagents

| S. No | Treatment                              | Day light | UV light | 254nm | 365nm |
|-------|----------------------------------------|-----------|----------|-------|-------|
| 1     | Powder                                 | Pale-green| Grey     | Green |
| 2     | Powder + 1N NaOH(aqueous)              | Brown     | Black    | Black |
| 3     | Powder + 1N NaOH(alcoholic)            | Green     | Black    | Green |
| 4     | Powder + 1N Hydrochloric acid          | Pale-brown| Black    | Green |
| 5     | Powder + 50% Sulphuric acid            | Pale-brown| Black    | Dark Green |
| 6     | Powder + 50% Nitric acid               | Pale-brown| Black    | Green |
| 7     | Powder + Picric acid                   | Green     | Black    | Dark Green |
| 8     | Powder + Acetic acid                   | Brown     | Black    | Dark Green |
| 9     | Powder + Ferric chloride               | Green     | Black    | Dark Green |
| 10    | Powder + Con. Nitric acid              | Brown     | Black    | Green |
| 11    | Powder + Nitric acid + Ammonia         | Brown     | Black    | Dark Green |
| 12    | Extract with methanol                  | Greenish yellow | Black    | Green |
| 13    | Extract with ammonia                   | Pale-brown| Black    | Dark Green |
| 14    | Extract with iodine solution           | Brown     | Black    | Dark Green |

Microscopical Investigation

Transverse section of leaf

The transverse section of the leaf through midrib was showed a single layer of the epidermis, followed by a collenchymatous layer; the upper side of the midrib shows a prominent lobe composed of mainly collenchymatous cells. Vascular bundles were six in number and conjoint. Thick-walled fibrous cells were present outer side of the vascular bundle. Rosette crystals, secretory cells were present in the midrib region. Glandular and non-glandular trichomes were present. Starch grains were absent. Lamina was showed uniseriate, smooth-walled upper epidermis followed by single layer palisade cells. Spongy parenchymatous was located towards the lower side of lamina. Rosette crystals were present in the laminar region. Petiole was showed trichomes, collenchymatous cortex, closed vascular bundle, druses or rosette crystals, and raphid crystals (Figure 4).

Surface Preparation/ Stomata

The leaf surface preparation of Leea asiatica was showed the presence of anisocytic stomata, wavy walled epidermal cells and vein arrangement (Figure 5).

Transverse section of stem

The transverse section of the stem was showed single layer epidermis with numerous covering and few glandular trichomes, followed by cortex layer; collenchyma cells were present in the cortex. Cluster crystals were present in the epidermis. Collenchyma having rosette crystals. The vascular bundle system was closed and surrounded by thick-walled fiber cells and the secretory cells were present. The large center region occupied by parenchymatous pith cells. Medullary rays were present in between vascular bundles. Starch grains were present in the pith region. Rosette crystals and secretory cells were present (Figure 6).

Quantitative microscopical analysis

The quantitative microscopical parameters of the leaf of Leea asiatica were determined on epidermal strips and the results were listed in Table 2.

Powder microscopy of leaf

The powder was a light green color with characteristic odor and taste. Leea asiatica leaf powder was examined under the microscope and it reveals the presence of lignified cells and parenchymatous cells containing starch grains. Calcium oxalate crystals, fibers, tannins and stone cells were present (Figure 7).

Powder microscopy of stem

The fine powder was yellow colored with faint odor. The diagnostic features Leea asiatica (L.) Ridsdale stem powder showed cluster crystals, rosette crystals, lignified fibers from xylem, lignified cork, tannin contents from cortex, simple starch grains, druses or rosette types of calcium oxalate crystals from cortex and medullary rays, lignified parenchyma cells, stone cells and pitted vessel. Raphides were present in parenchymatous cells (Figure 8).

Physico-chemical Investigations

The study of various physicochemical parameters of
Leea asiatica leaf powder was performed and the results were listed in Tables 3 and 4.

The district has been blessed by nature with one of the richest vegetation of ethnomedicinal plants from which the crude drugs can easily be produced. Information from the ethnic communities of the area on traditional herbal medicine has always played a vital role in the discovery of new drugs. Ethnopharmacologically, the aerial parts of the plant were reported to possess good wound healing activity, anti-inflammatory, anthelmintic, antioxidant, hepatoprotective and antidiabetic activity in the traditional system of medicine.

In the present study, the pharmacognostic and preliminary physicochemical investigations of plant Leea asiatica were established. These characteristic parameters may be used as standards for setting up a monograph of the plant. Leea asiatica is an evergreen large shrub or small tree of family Vitaceae growing up to 2–16m. In the present investigation, the macroscopic features of the leaves are ovate to elliptic with serrate to dentate margins. Leaves are 2-3 pinnate bearing 5-7 leaflets, with petioles 3 cm long. Fruits are berry and purple-black, bearing 4-6 seeds. Flowers are pale green in the terminal and axillary branched cymes. Stems are pubescent, erect, and noded. Microscopically leaf consists of single layer epidermis, conjoint vascular bundles, rosette crystals, secretory cells, glandular and non-glandular trichomes. Lamina is uniserial and contains single layer palisade cells. The leaf epidermal cells are irregular shapes and contain anisocytic stomata. The stem consists of multicellular covered and glandular trichomes, starch grains and closed vascular bundles. The leaf powder was characterized by microscopical observation. The physicochemical analysis provides standards helpful in judging quality control of the powdered crude drug. Fluorescence analysis may help to check and verify the identity of the drug.

CONCLUSIONS

In conclusion, standardization is an essential measure for quality, purity and sample identification. Macroscopical and microscopical evaluations of the leaf and stem reported in the current study are the important criteria for identification and authentication of the plant drug. Thus, pharmacognostic and preliminary physicochemical investigations of Leea asiatica will provide useful information for its identification and for further investigations on the isolation of specific phytochemicals, which having specific pharmacological action. The pharmacognostic and preliminary physicochemical investigations established in this study can serve as a means for assessing the quality and purity of Leea asiatica used in West-Champaran folklore medicine for the treatment of hepatic disorders and diabetes. So, the data provided in the present research work may provide standards helpful for drug identification and botanical standardization of Leea asiatica.

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Conflict of Interest

The authors declare that there is no conflict of interests regarding the publication of this paper.

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