Preliminary Pharmacognostic and Phytochemical Investigation of *Blepharis sindica*-T. Anders Seeds

**Abstract**

**Background:** *Blepharis sindica* – T. Anders of family Acanthaceae is an important medicinal plant which is mainly used as an invigorating tonic, given to cattle to increase milk production and its roots are used for urinary discharge and dysmenorrhea. It is commonly known as “Bhaṅgārī”. It is extensively used traditionally as *Vājikāraka* and *Vṛya* (Aphrodisiac) by *vaidyas* of Rajasthan and Gujarat. The seeds of *Blepharis sindica* T. Anders are the parts of the plant valued for their medicinal value. **Aims:** The present investigation deals with the pharmacognostic studies of the root, stem, seed and seed powder. **Materials and Methods:** The study includes macroscopy, microscopy, preliminary phytochemical analysis and physicochemical evaluation using methods given in Indian Ayurvedic Pharmacopoeia. **Results:** Physicochemical parameters such as total ash value, acid insoluble ash value and water soluble ash value were determined to be 21.449 ± 2.629%w/w, 0.986%w/w, 18.746%w/w respectively. Preliminary phytochemical analysis of extracts were carried out. Different extracts contain carbohydrate, protein, alkaloids, phenols, saponins and steroids prominently. Calcium, zinc, potassium and iron were also present. These secondary metabolites are the active constituents and may be responsible for *B. sindica*’s pharmacological activities. Chief characters of transverse section of stem include ringed vascular bundles, radial striped pith rays and parenchymal pith. Seed shows presence of scalariform tracheids and lignified walls. **Conclusions:** The result of the present study can be useful for the identification and preparation of a monograph of the plant.

**Keywords:** Bhangari, Blepharis sindica, Pharmacognostic studies, preliminary phytochemical

**Introduction**

*Blepharis sindica* – T. Anders; commonly known as Bhaṅgārī; is an endangered species whose natural population is decreasing due to unscientific exploitation and habitat destruction.[1] It is mainly distributed in South Western India, Indus delta region of India and Southern Pakistan.[2] It is a small, dichotomously branched, woody annual. Stem is short, sometimes almost zero, branches ash colored, slender, terete, clothed with very short hairs. Leaves are sessile in a whorl of 4, unequal, 2.5 – 6 × 0.2 – 0.4 mm, linear, acute, usually with a few small spinous teeth near the base, finely apiculate, rough with short stiff hairs, margins recurved, midrib prominent strong beneath. Flowers are in strobilate sub sessile hairy spikes 2.5–7.5 cm long, in the forks of the branches, solitary or few (5–7) on the top of very short stout woody peduncle, usually numerous in the lowest fork, solitary in the upper. Flowers are blue or pinkish in color. Calyx is divided almost to base, softly hairy on both surfaces. Corolla is 8–13 mm long one lipped, the lip 3 lobed; mid lobe quadrate, slightly larger than the lateral lobes which are rounded at apex, tube 4 mm long, brown, constricted below the limb; 4 didynamous stamens. Fruits are capsules 8 × 5 mm, ellipsoid, compressed, narrowed at both ends glabrous and shining brown. Seeds are 4 mm. long, compressed, densely clothed with thick hygroscopic hairs which are longer than the nucleus. Flowering and fruiting period is from August to January. Seed and whole plant is used for its medicinal properties.[3] The seeds are boiled in milk and taken as tonic. It is also given to cattle to increase milk production.[4] Compressed seeds with densely clothed hygroscopic hairs are used in the preparation of herbal medicines and it is used as aphrodisiac.[5] A decoction is used to wash wounds and powder of dried plant material is applied locally on infections.[6] The powder of seeds is also

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used in the treatment of earache.[7] Its roots are used for urinary discharge and dysmenorrhea. Powdered plant is applied locally on the infections of genitals and on the burns.[8] The seeds are also used for the purpose of Pumsavana Karma by vaidyas of Rajasthan. The seeds are mainly marketed in herbal markets of Rajasthan such as Jaipur, Jodhpur under the trade name Bhāṅgārī, Uṭtīṅganā or Uccaṭā. Seeds are mainly used in the preparation of herbal medicines and it is used as aphrodisiac.[9]

Materials and Methods

The plant specimen was collected from natural habitat of Gudhamalani, Barmer (Rajasthan) [Figure 1]. The taxonomic identification of the plant was confirmed by Dr. J. P. Parmar, Scientist “E” and Head of office, Botanical Survey of India, Jodhpur Rajasthan vide Letter No. BSI/AZRC19014/SE-1/Estt./549, dated 27.01.2010 and the voucher specimen of the plant were deposited in the crude drug museum of the Department of Dravyaguna Vigyan, National Institute of Ayurveda, Jaipur. Prior to all analysis, all the raw materials were cleaned to remove any foreign materials and dust. The samples were subjected to organoleptic, microscopic and phytochemical study so as to generate inputs that can be considered for laying down standards in respect of this plant. The seeds were dried in shade, powder in an electric grinder, passed through 120 mesh sieves and stored in an airtight container for organoleptic, physicochemical and phytochemical studies. Physicochemical and preliminary phytochemical screening of the seeds was carried out on the coarse powder according to the standard method. The microscopy of root, stem, seed and seed powder was done. The various parameters analyzed are as under.

Organoleptic characters

The organoleptic evaluation refers to the color, odour, taste, texture, etc. The organoleptic characters of the dried seeds were evaluated based on the method described by Ayurvedic pharmacopoeia of India.[10] To determine the average weight of a seed of Blepharis sindica T. Anders 10 seeds were selected randomly from the homogeneous sample. They were weighed separately on an electronic balance keeping them on a pre-weighed Whatman’s filter paper. Each value was noted; the mean weight was calculated. The same procedure was followed to determine the average weight of the fruit.

Microscopical studies

The root, stem, seed, seed powder was subjected for microscopic study. The stem and root were soaked in 70% alcohol for 24 hours to take freehand sections cleared with chloral hydrate solution and water, stained with saffrarin according to standard prescribed methods. The seed powder was stained with phloroglucinol and concentrated HCl obtaining observations through an image analyzer.[11,12]

Physicochemical and phytochemical parameters

Physicochemical and phytochemical screening were carried out as per the guidelines given in Pharmacopoeia of India 2006.[13] Physicochemical parameters such as moisture content (loss on drying at 105°C), water soluble extractive value, alcohol soluble extractive value, total ash value, acid soluble ash value and water soluble ash were calculated. The organic substances of the Blepharis sindica-T. Anders show their solubility in various solvents in different quantities. To determine extractive values, seven solvents were selected according to their polarity: petroleum ether, toluene, chloroform, ethyl acetate, acetone, methanol and water separately for two different samples collected in different seasons. Coarsely powdered air dried drug material is accurately weighed and taken in a glass stopper conical flask. Solvent is added to the flask and the flask is attached to a reflux condenser and boiled for 6 hours on a water bath. After six hours, the flask is allowed to cool and the content is filtered through filter paper. The filtrate is transferred to a pre-weighed flat bottomed dish and evaporated to dryness on a water bath. The dish is then kept in an oven for six hours for the contents to get dried fully. The dish is cooled by placing it in a desiccator for 30 minutes and weighed without delay. The residual mass remaining on the filter paper is dried as such and is collected fully. This mass in again put into the conical flask and added with the next solvent according to polarity and fitted with reflux condenser, and the extract is prepared in the same method used above. This procedure is repeated with all the seven solvents. The content of the extractable matter is calculated in mg per g of air dried material. Preliminary tests were carried out on ethanolic and water extract for presence/absence of phytoconstituents such as alkaloids, flavonoids, tannins, resins, carbohydrates, protein and saponins.[14,15] Prepared ash was used for inorganic analysis. 5 g seeds were taken in a 500ml measuring cylinder and distilled water was added to it up to a level of 500 ml. It was then shaken well for 30 seconds and kept standing for 24 hours. During this period, it was shaken

Figure 1: Dried plant of Blepharis sindica T. Anders showing dried fruits and stem
gently thrice. After 24 h., total increase in water level was measured. This increase was presented as swelling index for seeds of *Blepharis sindica* T. Anders [Figure 2].

The water and ethanolic extract of the drug was subjected to thin layer chromatographic analysis. T.L.C. plates precoated with 0.25 mm layer of silica gel GF 254 with fluorescent indicator (Merck’s). It was cut in to sizes of 8 × 2 cm. Ethanolic extract was applied on the plate above 1 cm from base line. The separated spots were visualized under UV 366 nm. The qualitative evaluation of the plate was done by determining the migratory behavior of the separated substances given in the form of Rf. Values.¹⁶

**Results**

**Organoleptic characters of seed**

- **Touch (sparśa)** - velvety smooth (snīgdha, mṛdu)
- **Colour (rūpa)** - yellow blackish whitish (kiñcit śyāma pītabha śveta varṇa)
- **Taste (rasa)** - Sweet - pungent (madhura – tikta)
- **Smell (gandha)** - Somewhat oily (mṛttikā evaṃ viśiṣṭa tailavat).

**Microscopy**

The transverse section of stem showed epidermis compactly arranged of elongated, transparent and devoid of chloroplast parenchymatous cells. The vascular strand is in the form of ring; obtusely wedged vascular bundles are present around the central pith, inner to the pericycle. Each vascular bundle consists of phloem on the outside and xylem towards inner side. Phloem lies towards pericycle on the outer side of vascular bundle. Xylem is towards the pith; the inner portion of vascular bundles. Xylem consists of two parts: smaller protoxylem (of narrow bundles) and larger metaxylem of broader elements. The xylem is in endarch position which means that the protoxylem lies at the tip of metaxylem towards the pith or centre of stem. The medullary or pith rays are in the form of radial strips which are present between adjacent vascular bundles, connecting the pith with pericycle and cortex. The pith is made up of polygonal oval or rounded parenchyma cells which enclose intercellular spaces [Figure 3]. The transverse section of root showed narrow, superficial, less distinct periderm comprising of two or three layers of cells. The epidermis (rhizodermis) has disintegrated. The medullary rays are formed opposite to protoxylem points. Primary xylem is distinguished by its central position. Secondary xylem is on the inner side. As compared to primary xylem the vessels of secondary xylem are broader. Secondary xylem cylinder is wide and dense [Figure 4]. The transverse section of the seed showed the presence of hairy structure throughout its surface. Anemochory means the dispersal of fruit and seeds through the agency of wind or water currents. This hairy structure helps in its dispersal by water [Figure 5]. The seed powder is yellow blackish whitish, having characteristic odour and having sweet-pungent taste. Under microscope it showed elongated tracheids, angular dead cells with hard lignified walls, wide lumen and narrow end walls. The thickening deposited like a spiral or helix is spiral tracheids. The ladder like thickening appearance are scalariform tracheids.
because they are laid down in the form of transverse bands [Figure 6].

**Physicochemical and phytochemical studies**

The ash value determines the quantity of the inorganic material present in the drug so it gets great significance. Higher ash value is suggestive of thermo non-labile/heat stable or inorganic constituents. The average percentage of total ash of seeds of *Blepharis sindica* T. Anders is $21.449 \pm 2.629\%$ w/w. This may be due to presence of high amount of iron (Fe) and zinc (Zn) and Calcium (Ca). The acid insoluble part of ash was found 0.986% w/w whereas water-soluble part was 18.746% w/w [Table 1].

The status of ten inorganics was examined, out of which four viz. Calcium, zinc, potassium and iron were found present. Rest of the elements such as phosphorus, sulphur, cobalt, mercury, nickel, silver were absent [Table 2]. Different extracts contain carbohydrate, protein, alkaloids, phenols, saponins and steroids prominently [Table 3]. This drug is traditionally used to treat male infertility. These organic groups are of great medicinal values out of which carbohydrate, proteins, and steroids are particularly known to play very important role in the maintenance of male reproductive physiology. In successive extraction the maximum extractive value was found in methanol 16.468%; whereas in water, it was less (3.983%). As far as water-soluble extractive value estimation by cold method extraction is concerned, the maximum, extractive value was 46.42%. On this basis, it can be said that the seeds of *Blepharis sindica* T. Anders contain mostly polar constituents, which can be extracted to their maximum limit under the influence of water by cold maceration only.

TLC data is summarized in Table 4.

**Discussion and Conclusions**

This study is in line with the quality parameters prescribed in the Ayurvedic Pharmacopeia of India and also standards set by other international agencies. This work provides qualitative and quantitative standards for the identification of *Blepharis sindica* T. Anders and from this study, it is concluded that pharmacognostic and phytochemical studies on *Blepharis sindica* T. Anders will be highly useful in determining qualitative and quantitative standards which can ascertain the identity, quality and purity of this plant drug.

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Nil.
Table 2: Qualitative analysis of inorganic matters in the *Blepharis sindica* T. Anders seeds

| Mineral        | Precipitate/colour observed                                                                 | Status of presence |
|----------------|---------------------------------------------------------------------------------------------|--------------------|
| Calcium        | Flocculent, white precipitate (sparingly soluble in ammonium chloride)                        | ++                 |
| Iron           | Red colour appeared                                                                         | +                  |
| Phosphorus     | The diagnostic yellow colour could not be found                                             | -                  |
| Potassium      | Formation of KClO<sub>4</sub> crystals observed                                              | +                  |
| Sulphur        | The white precipitate of BaSO<sub>4</sub> could not be seen                                  | -                  |
| Cobalt         | No precipitate seen                                                                         | -                  |
| Mercury        | No precipitate                                                                              | -                  |
| Nickel         | No precipitate                                                                              | -                  |
| Silver         | No precipitate                                                                              | -                  |
| Zinc           | White precipitate observed                                                                  | ++                 |

*: Presence of component, ++: Strong presence of component, –: Absence of the component

Table 3: Observation of qualitative analysis of organic matter in seeds of *Blepharis sindica* T. Anders

| Tests/method                | n-hexane | Benzene | Toluene | DCM  | Ethyl acetate | Acetone | Methanol | Water |
|-----------------------------|----------|---------|---------|------|---------------|---------|----------|-------|
| Carbohydrate (Molisch’s reagent) | +        | +       | +       | +    | +             | -       | +        | +     |
| Tannin (Vanillin)           | +        | -       | -       | -    | -             | -       | -        | -     |
| Protein (Ninhydrin)         | -        | +       | -       | -    | -             | +       | +        | +     |
| Saponin                     | -        | -       | -       | -    | -             | +       | +        | -     |
| Phenol (FeCl<sub>3</sub>)   | -        | -       | -       | -    | -             | +       | +        | +     |
| Glycoside (Keller–Kiliiani) | -        | -       | -       | -    | -             | +       | +        | -     |
| Glycoside (Legal)           | -        | -       | -       | -    | -             | +       | +        | -     |
| Alkaloid (Dragendorff)      | -        | +       | -       | -    | -             | +       | +        | -     |
| Alkaloid (Mayer’s)          | +        | +       | -       | -    | -             | +       | +        | -     |
| Fat                         | +        | +       | -       | -    | -             | -       | -        | -     |
| Steroid                     | -        | ++      | -       | +    | ++            | +       | +        | -     |
| Reducing sugar (Fehling’s solution) | +    | +       | -       | -    | -             | +       | +        | +     |

*: Presence of component, ++: Strong presence of component, –: Absence of the component

Table 4: Thin layer chromatographic data of *Blepharis sindica* T. Anders

| Extract | Mobile phase | Visualized under          | Total run (cm) | Spots seen at | R<sub>f</sub> value |
|---------|--------------|---------------------------|----------------|---------------|---------------------|
| Water   | MeOH + DW (2:3) | Iodine chamber            | 5              | 3.0           | 0.600               |
|         |               |                           |                | 3.5           | 0.700               |
|         |               |                           |                | 4.4           | 0.880               |
| Ethanol | MeOH + DCM (8.5:1.5) | Ultraviolet chamber      | 5.8            | 0.2           | 0.034               |
|         |               |                           |                | 1.2           | 0.206               |
|         |               |                           |                | 2.2           | 0.379               |

Conflicts of interest

There are no conflicts of interest.

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