



## Pharmacognostical and analytical profile of *Utigan* (*Blepharis maderaspatensis* (L.) B. Heyne ex Roth.) seed

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### Abstract

**Introduction:** *Blepharis* is the largest genus which includes 126 species distributed among three subgenera (*Blepharis*, *Ebracteata Volllesen*, and *Acanthodium*). Seeds of *Utigan* (*Blepharis maderaspatensis*) have been useful in *Mutrakruccha* (dysuria), *Svasa* (Cough), *Kasa* (Asthma), as a *Vajikaran* (Aphrodisiac), and eye disorder.

**Aim and Objective:** To evaluate the pharmacognostical and analytical study of the seed of *B. maderaspatensis*.

**Materials and Methods:** The pharmacognostical study includes details on macroscopic and microscopic characteristics and analytical study of the seed of *B. maderaspatensis* evaluated as per standard procedures.

**Result and Observation:** The results shows seeds are 5 to 7 mm in length and 3 to 4 mm in width, and 0.3 to 0.6  $\mu\text{m}$  in diameter and are creamish white in colour. The transverse section of seed shows an outer layer of the irregular shape of cells of the epidermis filled with blackish content and covered with cuticle, bearing very mucilaginous multiseriate trichomes which are straight at the base. The result of analytical study shows presence of high amount of saponin, carbohydrates, steroids, terpenoids and amino acids. HPTLC study of the seed shows total 9 different Rf value at short as well as at long ultra-violet wavelength respectively. The FT-IR analysis of seed shows the functional groups such as alcohols & phenols, alkanes, amines, and carboxylic derivatives.

**Conclusion:** The above reported morphological, microscopical, and analytical study of *B. maderaspatensis* may help for identification, to prevent adulteration and helpful for generation the monograph of the peculiar one.

**Keywords:** acanthaceae, *Blepharis maderaspatensis*, FT-IR, HPTLC, seed, *Utigan*

### Introduction

Acanthaceae is a family of dicotyledon types of flowering plants containing about 2500 species, mostly tropical herbs and, shrubs or sometimes epiphytes <sup>[1]</sup>. In the acanthaceae family, *Blepharis* is the largest genus which includes 126 species distributed among three subgenera (*Blepharis*, *Ebracteata Volllesen*, and *Acanthodium*) <sup>[2]</sup>.

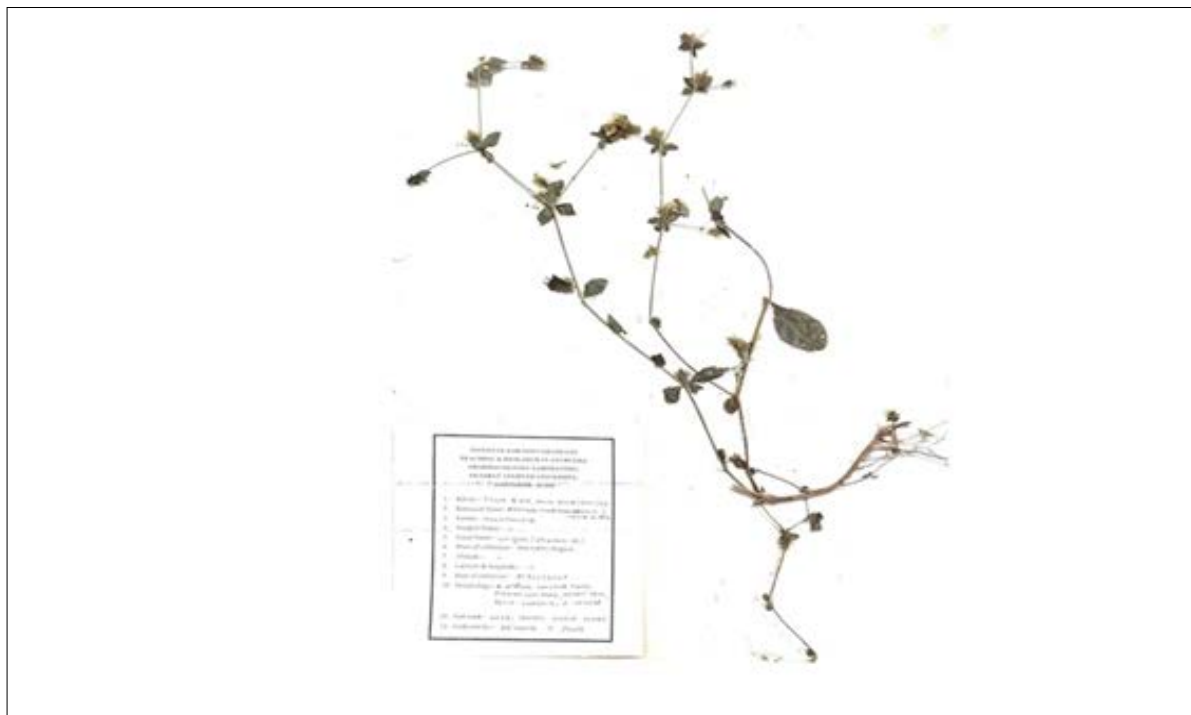
*B. maderaspatensis* as the species name suggested *maderaspatensis* indicates it was first found in Madeira Islands in the Atlantic Ocean, near tropical Africa, South Africa, Madagascar, and extended to Asia <sup>[3]</sup>. Out of 126 species of *Blepharis*, seven species have been used for its ethnomedicinal properties. Therefore, it is necessary to evaluate for its authentication and prevent of the adulteration of other drugs or similar species in the preparation of formulation and use as a medicine. The identification of drugs plays a pivot role and, for that pharmacognostical study and quality of the drug, quality control study is compulsory before preparation and usage of herbal medicine/formulation.

Among the seven species of *Blepharis*, *B. maderaspatensis* is found useful with its remarkable ethano-medicinal properties. The plant is used in various ailments such as *Daha* (Burns), fracture of bones <sup>[4]</sup> and, has been used in the disease of Central Nervous System <sup>[5]</sup>. The *Panchang* of the *B. maderaspatensis* is used to cure *Mutrakrachcha* as urinary tract-related problems <sup>[6]</sup>. The plant in local language is known as *Utigan* <sup>[7]</sup> and the seeds of *Utigan* have been useful in *Mutrakruccha* (dysuria), *Svasa* (Cough), *Kasa* (Asthma); and as *Vajikaran* (Aphrodisiac) <sup>[7]</sup>. Till date, no any scientific data are available on different parts of the plant and specifically the chief characteristics of *B. maderaspatensis* for its identification and authentication. Further no any work reported on Pharmacognosy of plant such as macroscopy, microscopy and quality standards on seed of *B. maderaspatensis*. So, in the present study, an endeavour attempted for the evaluation of pharmacognostical and phytochemical characteristics of the seed of *B. maderaspatensis*.

### Material and Methods

*Blepharis maderaspatensis* (L.) B. Heyne ex Roth. was identified and collected from its natural habitat near Gandhinagar (22° 18' 33.9300" N 72° 8' 10.4280" E) Gujarat, India from January to April 2020. The

authentication of the raw drugs was done based on the morphological features, organoleptic characters and powder microscopy of an individual plant. The voucher specimen has submitted been to Pharmacognosy, IPGT & RA (Vide no.: Ph.M. 6298/2020/21). The voucher specimen has been verified from the Botanical Survey of India (BSI) Kolkata. (Vide no.: DYJ-01) (Plate-1).



**Fig. 1(a)**



**Fig. 1(b)**



**Fig. 1(c)**

**Plate 1:** Herbareium and Species authentication of Utigan Seed

### Chemicals and Reagents

All the chemicals which used during the study were of analytical grade and were purchased commercially.

### Macroscopic study

The specimens were observed as such with naked eyes or with the help of dissecting microscope. Organoleptic characters of the seed part were observed, such as color, odor, taste, texture, fracture. The seed was studied for a macroscopic study, compared the characters and observations which have given in various floras, and available antiquity books and noted to confirm the identification and authentication of the plant [8].

### Microscopic study

The seed was evaluated for detailed microscopic study. The transverse sections (T. S.) of the *Utigan* seed was taken, and observed the various parts of sections and tissues as well as their parts of cell contents. The transverse section of *Utigan* was stained with Phloroglucinol + HCl to observe the lignified portions such as fibers, crystals, epithelial tissue, and stone cells. The sections were observed in a Carl Zeiss trinocular electron microscope [9], and photos of sections are given at the end of the pharmacognostical and analytical study.

### Powder microscopy

*Utigan* was powdered and sieved through the mesh no. 80#. The powder specimens were observed under the microscope first with plain water and stained with Phloroglucinol + Conc. HCl and iodine to observe the lignified elements. The microphotographs are taken under a Carl Zeiss Binocular microscope attached with a camera [9].

### Physicochemical parameters

The physicochemical study of seed powder of *Utigan* sample was carried out using various physicochemical parameters such as loss on drying, ash value, acid-insoluble ash, pH, water-soluble extractive value, and alcohol soluble extractive value as mentioned in Ayurvedic Pharmacopeia of India (2001) [10]. All the physicochemical parameters were performed in terms of triplicate values.

### Qualitative tests

A stock solution of *Utigan* in the concentration of 1% (w/v) of each extract was obtained using methanol and water. These extracts were tested for the presence of active phytochemicals by the standard methods [11].

### High Performance Thin Layer Chromatographic (HPTLC)

The methanol extract of seed powder of *Utigan* was used for an evaluation of the HPTLC study. The solvent system used for the study is Toluene: Ethyl acetate (7:3). The conditions for the HPTLC using CAMAG Linomat V, and the plate was scanned with CAMAG TLC Scanner 3.

### Fourier transform infrared spectroscopy (FT-IR)

FT-IR is an important technique for the identification and characterization of functional groups. The Infrared spectrum of an organic compound provides a unique fingerprint, which was distinguished from the absorption patterns of all other molecules. Infrared Spectroscopy gives information on the vibrational and rotational modes of motion that occurred in a sample. FTIR (Thermo Scientific Nicolet 1S5 FTIR) was used to analyze the *Utigan* powder (*Churna*). The room had kept at a controlled ambient temperature (25°C) and relative humidity (30%). And FTIR spectra were evaluated by the KBr pellet technique. The spectrum of *Utigan* powder and recorded in the 400–4000  $\text{cm}^{-1}$  range with a resolution of 4  $\text{cm}^{-1}$ .

### Observation and Result

#### Macroscopy of seed

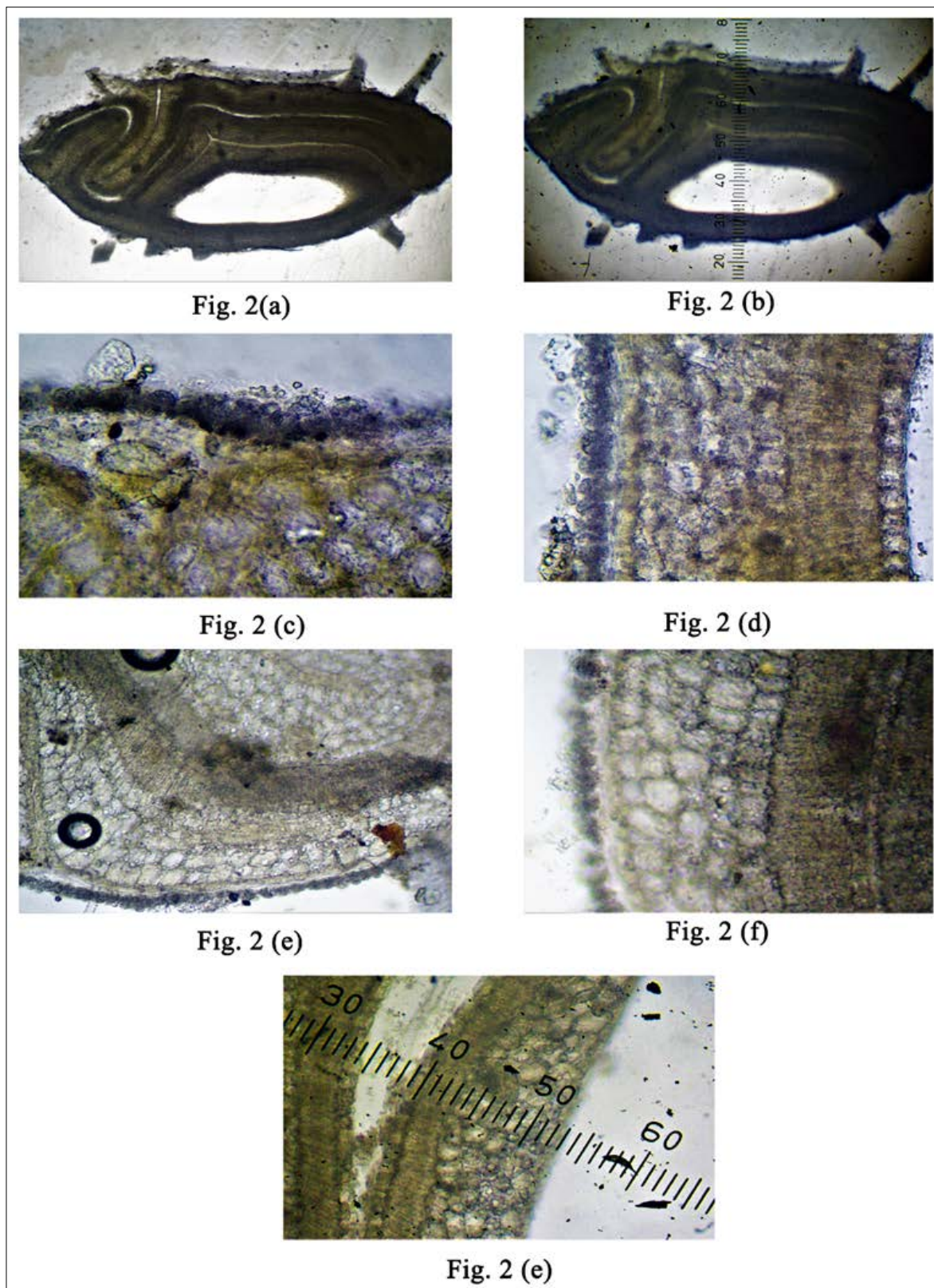
The seed of *B. maderaspatensis* is 5 to 7 mm in length and 3 to 4 mm in width, and 0.3 to 0.6 in diameter, and flattened. They are the obtuse apex, broad-based and bristly hairs, pointed towards apex getting stretched outwardly. The seeds are highly mucilaginous in nature. The *Utigan* seed are creamish white in colour and compressed types of seeds are observed.

#### Microscopic study of seed

The transverse section of a whole seed measures about 0.5cm x 0.4 cm. The transverse section of the seed measures 9.2  $\mu\text{m}$  x 4.5  $\mu\text{m}$ . The diagrammatic section of seed shows irregular margin bearing long-oppressed trichomes, flattened base of the seed, and occupying an area of the seed section covered with peripheral hairy testa.

The detailed transverse section of *B. maderaspatensis* shows an outer layer of globular to irregularly shaped squarish cells of epidermis packed with granular blackish contents of unknown nature, covered with thin cuticle and bearing at place very long mucilaginous multiserrate shaggy trichomes which are slightly bend at base, characterized with broad, conical, thick walled, multicellular base, terminating with very long ribbed cylindrical upper portion and embedded with minute crystals of calcium oxalate. The individual cells get easily split apart on moistening with water and obtain CO<sub>2</sub> bubbles with acid. Cotyledon consists of upper and lower epidermis

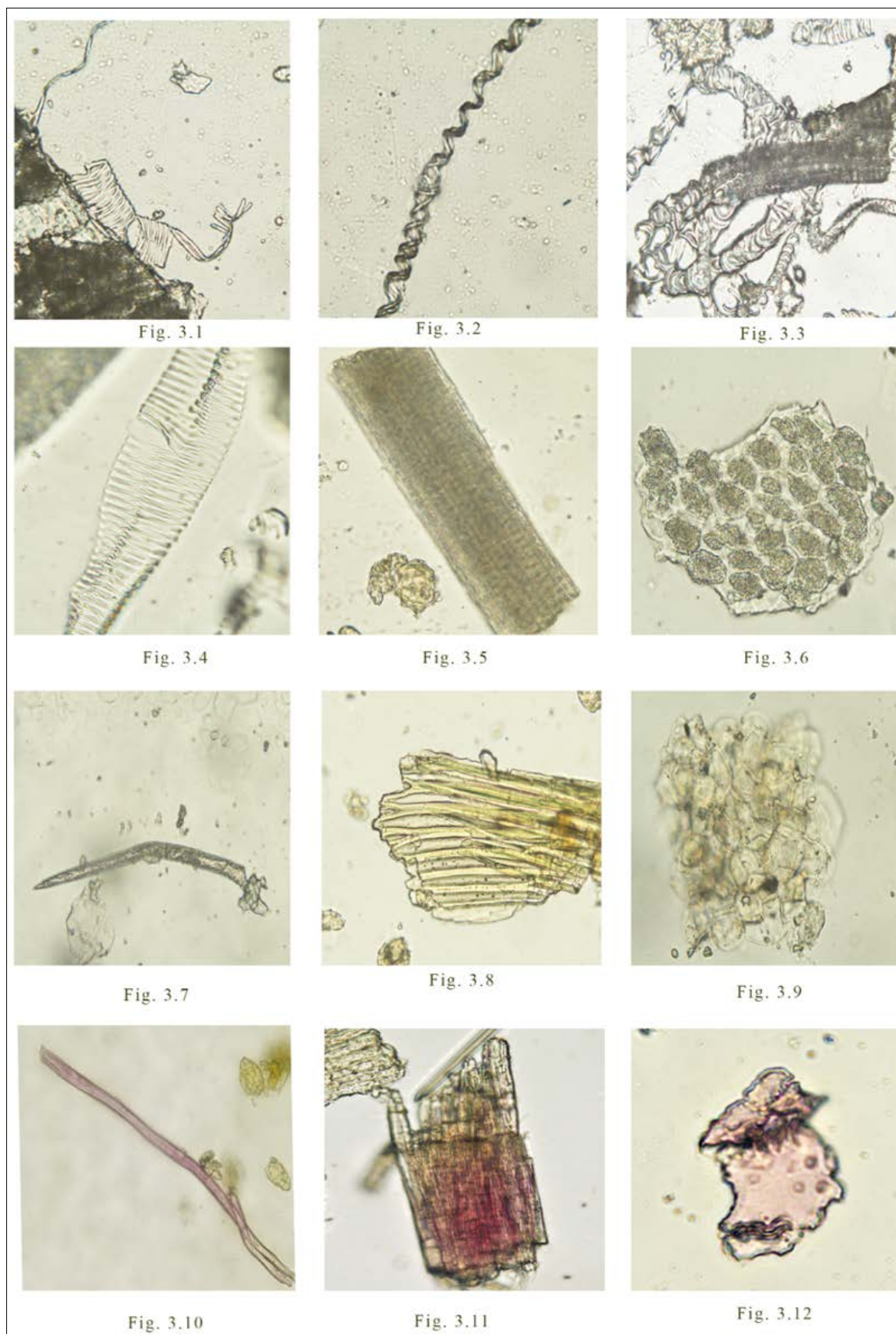
followed by palisade cells. Mesophyll cells present below the upper epidermis and consists oil globules. Centrally located oval shaped radicle and detached from the cotyledon. The images for the transverse section of *Utigan* seed are shown in Fig. 2.



**Fig 2:** Transverse section of Utigan Seed

#### **Powder microscopy of seed**

The powder of *B. maderaspatensis* is brownish cream in color, highly mucilaginous in nature, and *Tikta* in *Rasa*. The diagnostic characters of the powder showed scalary form vessel, spiral vessel, pitted vessel, epicarp cells, brown contents with aleurone grains; a group of fibers, group of sclarides and mesocarp cells with starch grains. The powder contains mucilage-containing cells, crystal fibers, and warty simple trichomes shown in Fig. 3.



**Fig 3:** Powder microscopy of Wigan seed

#### **Physico-chemical analysis**

The physicochemical analysis of the powder is shown in table no. 1. All the physicochemical parameters were performed in terms of triplicate values.

**Table 1:** Physicochemical parameters of seed of *B. maderaspatensis*

Physicochemical Parameters	Results (Mean ± SD)
Loss on drying (% w/w)	11.33±0.16
Ash value at 450°C (% w/w)	3.45±0.75
Acid insoluble Ash at 450°C (% w/w)	2.28±0.85
Alcohol soluble extractive (ASE) (% w/w)	26.34±2.96
Water soluble extractive (WSE) (% w/w)	14.67±4.00
pH of 10% w/v aqueous solution	7.00 ± 0.001

**Qualitative analysis**

Detailed results of the qualitative analysis of seed of *Utigan* have shown in table 2. Carbohydrates, steroids, terpenoids, amino acids, flavonoids, saponin, and proteins are present in the water extract of seed of *Utigan*. In methanol extract of seed of *Utigan*, all the phytoconstituents are present except protein and flavonoids.

**Table 2:** Qualitative parameter of Seed of *B. maderaspatensis*

Phytoconstituents	Tests	Water Extract	Methanol Extract
Carbohydrate	Molisch test	+	+
Steroids & terpenoids	Salkowski test	+	+
Amino acid	Ninhydrin test	+	+
Flavonoids	Shinoda's test	+	-
Tannin & phenol	5% FeCl <sub>3</sub>	-	-
Alkaloids	Mayer's reagent	-	-
Saponin	Foam test	+	+
Cardiac Glycoside	Keller-killiani test	-	-
Proteins	Biuret test	+	-
Mucilage	Ruthenium red	-	-

+ = Present, - = Absent

**HPTLC analysis**

The construction of chromatographic fingerprints plays an important role in the quality control of complex herbal medicines [12]. The methanol extract of seed of *Utigan* showed 9 peaks at 254nm and its R<sub>f</sub> value is 0.03, 0.11, 0.19, 0.39, 0.51, 0.59, 0.63, 0.67, 0.77 while, 9 peaks have been obtained at 366 nm wavelength and its R<sub>f</sub> value is 0.03, 0.09, 0.40, 0.50, 0.71, 0.83, 0.86, 0.90, 0.94.

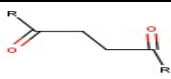

**Table 3:** HPTLC analysis of *B. maderaspatensis* seed powder

Solvent System	Sample (Methanol extract)	254 nm (Short UV)		366nm (Long UV)	
		Number of Spots	R <sub>f</sub> Value	Number of Spots	R <sub>f</sub> Value
Toluene: Ethyl acetate (7:3)	Seed	9	0.03, 0.11, 0.19, 0.39, 0.51, 0.59, 0.63, 0.67, 0.77	9	0.03, 0.09, 0.40, 0.50, 0.71, 0.83, 0.86, 0.90, 0.94

**FT-IR spectroscopy analysis**

The FTIR spectra of a seed of *Utigan* have been shown in Fig. 4. The functional groups obtained from FTIR spectra analysis shows the different kinds of functional groups such as alcohols & phenols, alkanes, amines, carboxylic derivatives, aromatic compounds, and aliphatic peroxides (Table 4). All the obtained functional groups of the *Utigan* seed are based on the stretching of different kinds of bonds such as stretching, bending, and rotation.

**Table 4:** Functional groups of *Utigan* seed powder from FTIR graph

Wave number (cm <sup>-1</sup> )	Assignment	Functional group
3306.89	O-H Stretch	Alcohols and Phenols
2925.46	C-H Stretch	Alkanes
2854.38	C-H asym./sym. Stretch	Methylene C-H asym./sym. stretch
2360.39	=NH <sub>2</sub> <sup>+</sup> , -NH <sup>+</sup>	Amide bands
1867.85		Five membered ring anhydrides <sup>b</sup> Aromatic combination bands
1745.79		Alkyl Carbonate <sup>b</sup> Aromatic combination bands

1651.96	C=C Stretch	Alkenyl <sup>b</sup> Aromatic combination bands
1541.19	C=O (Amide 1 Band) N=H ( 2i-Amide 2 Band)	Alkanes Carboxylic acid and derivatives
1456.26	$\alpha$ - CH <sub>2</sub> Bending	Aldehyde and ketones
1396.26, 1338.57	O-H Stretch	Phenol or tertiary alcohol
1240.79	C-N Stretch O-C Stretch	Amines Carboxylic acid and derivatives
1075.49	C-O Stretch Si-O-Si Stretch	Alkyl-substituted ether Organic siloxane or silicone
934.18	C-H Stretch P-O-C Stretch	Aromatic C-H out-of-plane bend Aromatic phosphates
898.34	C-H Stretch	Vinyl C-H out-of-plane bend Aromatic
837.21	C-O-O-C <sup>a</sup> Stretch	Peroxide
717.74, 701.72	(-CH <sub>2</sub> ) <sub>n</sub> Stretch C-Cl Stretch	Methylene (-CH <sub>2</sub> ) <sub>n</sub> rocking Aliphatic chloro compounds
475.23, 447.84, 411.96	S-S Stretch	Aryl disulfides

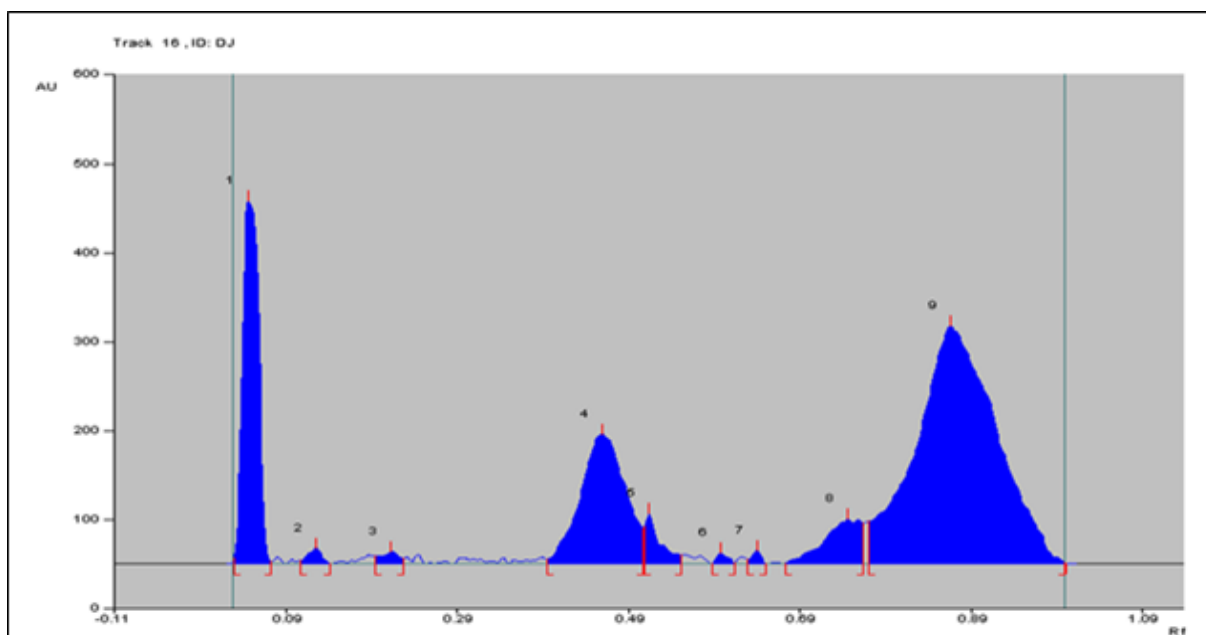


Fig. 4.1

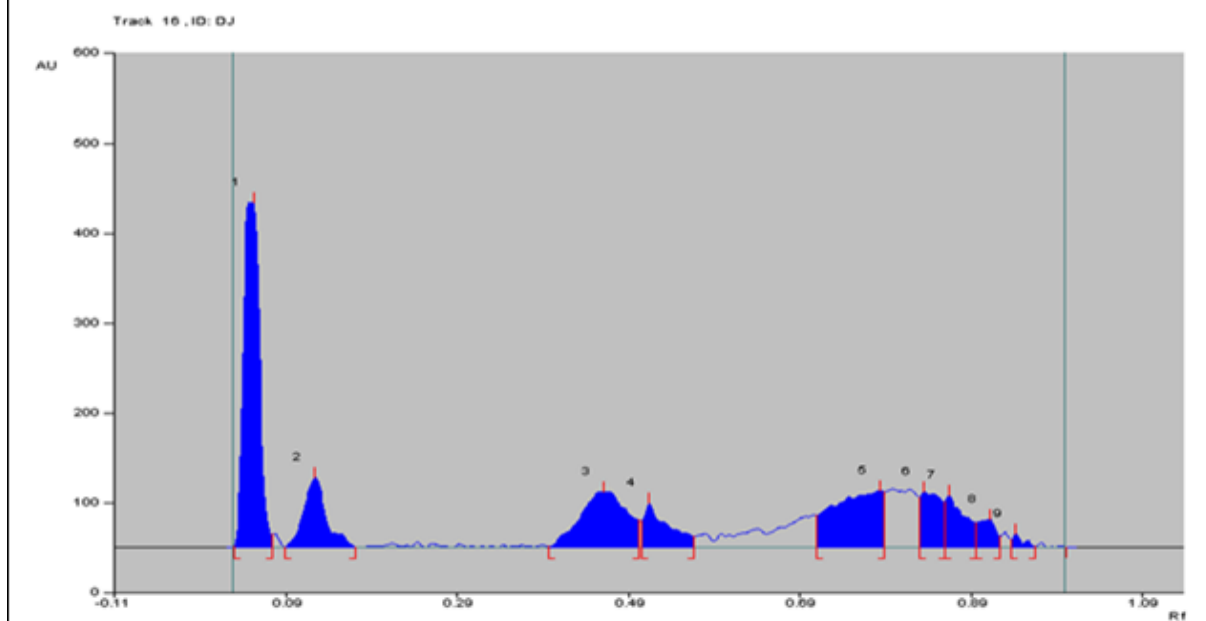
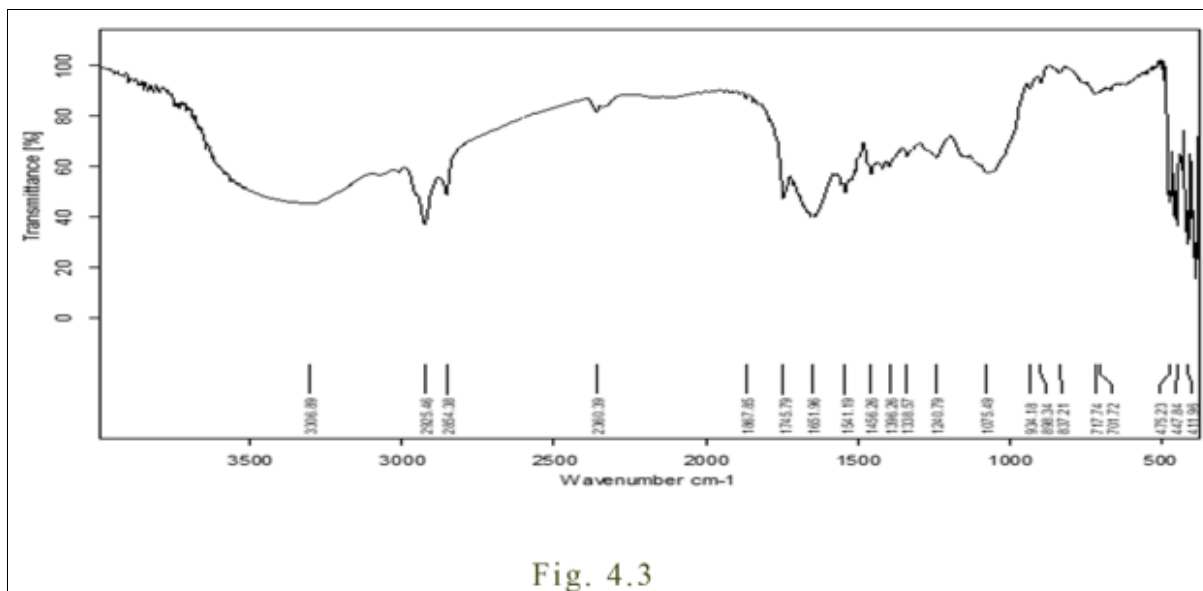


Fig. 4.2



**Fig 4:** Instrumental analysis of Utigan seed

### Discussion

*Blepharis maderaspatensis* (L.) B. Heyne ex Roth, belongs to family Acanthaceae. It is perennial herb with simple leaves, four at a node; blue to yellowish flowers with compressed seeds with bristly hairs which are main characteristics for the identification of the *B. maderaspatensis*. On the outer side, seeds are obtuse apex and broad in a base with a somewhat rough surface in nature. The seed of the *Utigan* is highly mucilaginous in nature. The plant is morphologically similar to *Blepharis persica*. The whole seed measures about 0.5cm x 0.4 cm. T.S. of seed showed a layer of the irregular shape of cells of epidermis filled with blackish content and bearing very mucilaginous multiseriate trichomes and straight at the base.

The physicochemical analysis and standardization of plant are used for the set of standards for crude drug and a finished product. The LOD of the seed powder suggest that drug has quite moisture content which, might correlate with the role of mucilaginous nature of the seed. The pH of the sample suggested that seed powder is neutral in pH. The data showed higher compatibility with the non-polar solvents as the extractive value is higher in alcohol soluble extractive. The qualitative analysis shows the presence of primary and secondary metabolites almost similar in both water and methanol extracts except flavonoids and proteins that are not present in the methanol extract of the seed powder of *Utigan*.

The HPTLC analysis of the seed powder of *Utigan* showed nine different spectra at 254 nm and 366 nm. This data could further lead towards the primary and secondary metabolites present in the peculiar seed part. There is still need to do research on seed of *Utigan* for identification of chemical moieties.

The FT-IR spectroscopy showed the various kinds of bonds such as alcohol, phenols, alkanes in seed powder of *Utigan* from which the probable chemical compound present in the plant will be identified and isolated in the vicinity.

### Conclusion

The present research study identified the morphological and microscopical characteristics of *Utigan* (*B. maderaspatensis*) and set of analytical quality standards which may help in identification and authentication of plant. It may be useful in prevention of adulteration and for generation the monograph of the peculiar one.

### Conflict of Interest

There is no conflict of interest.

### Acknowledgement

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### References

1. Xu Z, Chang L. Acanthaceae, In: Identification and control of common weeds, Singapore: Springer publication, 2017, 3.
2. The Plant Lists, <http://www.theplantlist.org/1.1/browse/A/Acanthaceae/Blepharis>.
3. Vijayalakshmi S, Kripa KG. Therapeutic uses of plants of genus *Blepharis*- a systematic review. International Journal of Pharmaceutical Biological Sciences, 2016;7(4):236-243.
4. Patwardhan B, Warude D, Pushpangadan P, Bhatt N. Ayurveda and traditional Chinese medicine: a comparative overview. Evidence Based Complement Alternative Medicine, 2005;2(4):465-473.

5. Sandhya S, Vinod KR, Kumar S. Herbs used for brain disorders. *Journal of Drugs and Medicine*,2010;2(1):38-45.
6. Mathur A, Joshi H. Ethnobotanical studies of the Tarai region of Kumaun, Uttarakhand. India. *J. Plants People Applied Research*,2013;11:175-203.
7. Thakar JI. *Vanaspatishastra: Kathiyavad na barda dungar ni jadibutti*. 2nd ed., Pravin Prakashan Pvt. Ltd.: Rajkot (India), 1998, 554.
8. Evans WC, Trease. *Pharmacognosy*. 16<sup>th</sup> ed., New York (USA): Elsevier Publication, 2016, 563-65.
9. Khandelwal KR, Author. *Practical Pharmacognosy*. Pune (India): Nirali Prakashan, 2008, 8.
10. Anonymous. *The Ayurvedic Pharmacopoeia of India, Part I, Vol. VI, 1st ed.*, New Delhi (India): The Controller of Publication, 2001, 139-140.
11. Harborne AJ. *Phytochemical methods a guide to modern techniques of plant analysis*. Springer Science & Business Media, 1998.
12. Gong F, Liang YZ, Xie PS and Chau FT. Information theory applied to chromatographic fingerprint of herbal medicine for quality control. *Journal of Chromatography A*,2003;1002(1):25-40.