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Pharmacognostic and Preliminary Physio - Phyto chemical investigations on the leaves of *Blepharis boerhaaviafolia*

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Abstract:
Extraction of bioactive compounds from medicinal plants permits demonstration of their physiological activity. The plant selected for the present study is *Blepharis boerhaaviafolia* (*Acanthaceae)* which is used traditionally for wounds, ulcers, nasal hemorrhage, asthma, throat inflammation, ascitis, liver and spleen disorders. The present study was aimed to evaluate the parameters to determine the quality of the plant *Blepharis boerhaaviafolia*. These studies comprises of detail macroscopy, histology, quantitative, physical constants-ash, extractive values, and fluorescence characteristics. The study contributes to the development of standardization parameters of herbal drugs used in Indian system of medicine.

Keywords:*Blepharis boerhaaviafolia*, Preliminary Phytochemical Screening, Pharmacognostical studies.

Introduction

Herbs are used as medicine since time immemorial. Many of the natural products in plants of medicinal value offer us new sources of drugs which have been used effectively in traditional medicine. There in an increased consciousness regionally and globally in production and use of plants with healing property. *Blepharis boerhaaviafolia* (*Acanthaceae)* is a prostrate herb rooting at nodes, internodes elongate creeping, flowering and fruiting time is September and January. Propagation by seeds. Leaves commonly sold in Indian market, are reported to be useful in wounds, ulcers, nasal hemorrhage, asthma, throat inflammation, ascitis, liver and spleen disorders. Root is considered dysmenorrhoea. Seeds are considered to be expect deobstruent and useful in strangury and conjunctivitis[1,2,3]. Considering the various uses of these plants we have proposed to evaluate the pharmacognostic and phytochemical parameters of the plants.

Material and Methods

Leaves of *Blepharis boerhaaviafolia* were collected from Tirunelveli dist (Tamil Nadu) .The plant was authenticated by Dr. Chelladurai, survey of medicinal plants unit, Palayamkottai. The voucher specimen was kept at Dept. of Pharmacognosy, K.M.College of pharmacy.

Macroscopy:

Stems are short, 15cm long, rigid usually branching from the base. Leaves are opposite or 4-nately whorled andelliptic – ovate ,margin entire or distily toothed in the upper half, apex acutely apiculate ,simple usually in two pairs at the same node, glaucous below,base acute to cuneate, sessile. Flowers are clustered at the nodes, white with pink nerves. Bracteoles are spathulate1cm.Outer calyx lobes 4, outer calyx lobes unequal, 1 and 1.5 cm each, inner ones 0.5 cm stamens. The ovary size is 2 × 1.5 mm. Seeds are two, sub orbicular, flattened [4].

Histology:

Transverse section of leaves of *Blepharis boerhaaviafolia* is clothed with dense unbranched , long filiform epidermal trichomes. The midrib and lateral veins are prominent both on the upper and lower sides of the leaves. The vascular strands of the midrib and lateral veins are single and collateral, lamina is bifacial, anamocytic stoma.

Microscopy:

Miroscopy of Stem(T.S):

A Transverse section of stem showed the following characters. The outer epidermis consisited of single layer of tangentially elongated parenchymatous cells. It was covered by a thin cuticle.

Multicellular uniseeriate covering trichomes were present. Cortex Just below the epidermal cells, three layers of collenchymatous and four layers of parenchymatous cells were seen. Below this Endodermis was seen. Pericycle made up of 4-5 layers of cells followed by phloem was seen. Phloem was composed of 2-3 layers of small sized polyhedral closely arranged parenchymatous cells. Below this cambium was seen. Xylem vessels were large and arranged in radial rows. It consisted of metaxylem and protoxylem. Lignified xylem parenchyma was also seen. Pith was made up of somewhat rounded parenchymatous cells with large intercellular space.

Microscopy of Stem: (L.S):

Epidermis was made up of single layer of compactly arranged rectangular cella with a thin cuticle. This was followed by a zone of cortex consisting of 2-3 layers of elongated collenchymatous cells and four layers of parenchymatous cells were seen. Then single layer of endodermis was present. Trichomes were multicellular uniseriate covering trichomes. Phloem was composed of small sized closely arranged parenchymatous cells. Below this xylem vessels and pith were seen.

Microscopy of Root (T.S):

Epidermis was made up of one layer of cork cells covered by cuticle, unicellular covering trichomes were seen. Cortex layer was composed of 8-9 layers of parenchymatous cells, which were irregularly arranged. Pericycle was made up of 1-2 layers. Pholem was seen composed of 1-2 layers. Xylem vessels were present, varying in size and accompanied by inter xylary parenchymatous cells, which were lignified. Pithas made up of somewhat rounded parenchymatous cells with intercellular space.

Microscopy of Root :(L.S)

Epidermis was made up of one layer of cork cells. The cortex composed of three to four layers of elongated parenchymatous cells that were irregularly arranged was seen. Below this endodermis was present. Pericycle was made up of two to three layers. Pholem was composed of one to two layer of elongated parenchymatous cells. Xylem vessels were reticulated and pitted. Below and in between the xylem vessels three to four layers of elongated parenchymatous cells were seen.

Microscopy of Leaf: (T.S)

It consisted of the following parts

Midrib:

The midrib was having a slight projection on the upper side and the lower side was wider with vascular strand.

a) Upper epidermis:

Upper epidermis is composed of tangentially arranged single layer of subrectangular cells with smooth cuticle. Below this the projection area with three layers of collenchymatous cells with smooth cuticle. Below this the projection area with three layers of cellenchymatous tiisue were seen. Trichomes ere mlticellular, uniseriate covering trichomes.

Palisade layer:

Palisade parenchyma is composed of elongated and more or le cylindrical cells, which are close together with long axes of the cells perpendicular to the epidermis.

b) Vascular Bundle:

Lignified pericyclic sclerenchymatous cells of one to two layer present above and below the xylem vessel. Vasuclar bundle was surrounded by phloem cells.

c) Lower Epidermis:

It consisted of single layer of tangentially arranged subrectangular cells with smooth cuticle. Just above the lower epidermis, two layers of collenchymatous cells were present.

Lamina:

a) Upper epidermis:

Upper epidermis was composed of tangentially arranged single layer of

subrectangular cells with smooth cuticle. Trichomes were multicellular, uniseriate covering trichomes.

b) Mesophyll:

Upper palisade layer was single layered. Spongy parenchyma was made up of loosely arranged parenchymatous cells.

c) **Lower epidermis**:

It was made up of tangentially arranged single layer of subrectangular cells with smooth cuticle.

Quantitiative Microscopy

The length and width of the trichome and length and width of the fibre, vein islet number, vein terminal number, stomatal number, stomatal index were determined on fresh leaves of *Blepharis boerhaaviafolia* [5-,8]Table 3,4,5,6,7,8 **.**

Table 1 : Loss on drying, Ash values

S l. no	Parameters	Values % w/w
1	Loss on drying	4
2	Crude fibre content	4.5
	Ash value	
3	A. Total ash	16.72
4	B. Acid insoluble ash	6.37
5	C. Water-soluble ash	9.1
6	D. Sulphated ash	8.1

Table 2 – Extractive values

Sl. no	Solvents	Extractive Values (%)w/w
1.	Petroleum ether	2.2
2.	Chloroform	12.5
3.	Methanol	13.5
4.	Solvent hexane	9
5.	Ethanol	15
6.	Water	14.5

Flourescence analysis

When physical and chemical parameters are inadequate as it often happens with the powdered drugs, the plant material may be identified from their adulterants on basis of fluorescence study. The powder of leaves was examined under daylight and ultra violet light using 254nm and 366nm [9,10].The observed character were recorded. Table 9

Table 3 : Data of Length and Width of Trichomes

S.No	Length in (µ)	Width in (µ)	Length in (µ)	Width in (µ)
1.	19	5	9	3
2.	16	4	7	3
3.	14	4	9	3
4.	15	4	9	2
5.	17	5	8	2
6.	14	4	8	2
7.	17	4	7	3
8.	17	4	9	2
9.	18	5	6	3
10.	19	5	9	3
11.	15	4	8	3
12.	17	4	9	2
13.	16	4	7	2
14.	21	5	7	2
15.	20	5	6	3
16.	19	5	8	3
17.	19	5	9	2
18.	18	5	6	2
19.	17	4	7	2
20.	16	4	6	2
Total	344	89	154	49
Aver age	17.2	4.45	7.7	2.45

Behaviour of leaf powder with different chemical reagents

Behaviour of leaf of *Blepharis boerhaaviafolia* with different chemical reagents was performed to detect the occurrence of phytoconstituents along with colour changes under ordinary daylight by standard method . Table 10

Table 4 : Data of Length and Width of Fibers

S.No	Length in Microns	Width in Microns
1.	81	5
2.	54	4
3.	70	4
4.	56	4
5.	61	3
6.	49	3
7.	46	5
8.	45	3
9.	49	3
10.	52	4
11.	59	4
12.	64	5
13.	72	5
14.	48	3
15.	71	5
16.	75	5
17.	68	4
18.	60	4
19.	53	5
20.	52	5
Total	1185	82
Average	59.25	4.1

Table 5 : Data of Vein- islet number of the leaf

Observation Number	Vein- islet number
1	20
2	22
3	18
4	21
5	17
Total	98
Average	19.6

Preliminary phytochemical investigation [11,12,13,14]

The qualitative chemical test of various extracts of *Blepharis boerhaaviafolia* was carried out using standard procedure Table 11

Table 6 : Data of Vein- terminal number of the leaf

Observation Number	Vein- islet number
1	25
2	23
3	28
4	30
5	26
Total	132
Average	26.4

Table 7 : Data of Stomatal number of Upper and Lower epidermis of the leaf number of the leaf

Observation Number	Upper epidermis	Lower epidermis
1	97	112
2	81	126
3	86	91
4	95	95
5	83	101
Total	442	525
Average	88.4	105

Table 8 : Data of Stomatal index of Upper and Lower epidermis of the leaf number of the leaf

Observation Number	Upper epidermis	Lower epidermis
1	24.36	25.6
2	21.20	26.1
3	23.1	24.7
4	21.3	22.1
5	20.4	22.6
Total	110.36	121.1
Average	22.07	24.22

Quantitative standards

Total carbohydrate content in leafs of *Blepharis boerhaaviafolia* by phenol-sulphuricacid method [15] was estimated to be 13.25%w/w. Similarly protein content [16], was found to be, 0.857%w/w respectively. Table 12

Table 9 – Fluorescence characteristics of powder leaves of *Blepharis boerhaaviafolia*

Sl.no	Treatment	Day light	UV light 254 nm	UV light 366nm
1.	Powder	Green	Dark Green	Dark Green
2.	Powder treated with distilled water	Green	Dark Green	Blackish green
3.	Powder +CHCL3	Dark Green	Dark Green	Green
4.	Powder +acetone	Yellowish Green	Pale Green	Pale Green
5.	Powder + Conc. HCL	Pale Green	Yellowish Green	Yellowish Green
6.	Powder +1N HCL	Light Green	Green	Green
7.	Powder +50% HNO3	Pale Green	Pale Green	Pale Green
8.	Powder +50% H2SO4	Light Green	Colorless	Pale Green
9.	Powder +1N NaoH in methanol	Buff	Buff Green	Pale Green
10.	Powder +1N NaoH in water	Dark Green	Green	Light Green

Table 10: Behaviour of leaf extract of *Blepharis boerhaaviafolia*

Reagent	Colour / ppt	Constitituent
Powder	Green	-
Powder + con. H2so4	Brown	Carbohydrate present
Powder + aqueous Fecl3	Bluish black	Tannin present
Powder + Iodine solution	No black	Starch absent
Powder + Aqs. Hgcl2	Blue	Alkaloids present
Powder + picric acid	Yellow	Alkaloids present
Powder + Mg Hcl	Mangoe colour	Flavonoids present
Powder + aqueous AgNo3	Ppt is not formed	Protein absent
Powder + ammonia solution	Pink colour	Cardiac glycoside present
Powder + Aqs. KOH	Pink colour	Cardiac glycoside present
Powder + Aqs. Na nitrite	Red colour	Phytosterols present
Powder + Water (shaking)	Foam is produced	Saponins present

Table –11 Phytochemical analysis

Name of the Constituents	Petroleum ether extract	Benzene extract	Chloroform extract	Ethyl Alcohol extract	Aqueous extract
Fixed oil		--	-	-	-
Carbohydrates	-	-	+	+	+
Protein			+	+	+
Tannin	-	-	+	+	+
Alkaloids	-	-	+	+	+
Sterol	-	-	+	+	+
Flavanoids	-	-	+	+	+
Glycoside	-	-	+	+	+
Saponins	-	-	+	+	+

Table- 12 Results of qualitative estimations of leaves of *Blepharis boerhaaviafolia*

Sl.no	Estimation	Results in %w/w
1.	Tannin content	3
2.	Total saponin content	9.2
3.	Flavonoid content	2.40
4.	Total sugar content	14.31
5.	Chlorophyll content	0.741
6.	Total alkaloid content	5.50

Determination of Saponin

According to the results obtained from positive foaming test and high foaming index of leafs of *Blepharis boerhaaviafolia* study was carried out for the estimation of total saponin content [17,18,19].

Physico chemical standards:

Physico chemical parameters of the powdered drug such as loss on drying, ash value, extractive value and crude fibre content were performed according to the standard method.

Quantitative analysis:

Estimation of chlorophyll, flavonoids, sugar, alkaloids, saponins, tannins also carried out using *Blepharis boerhaaviafolia* leaves.[20-22]

HPTLC fingerprinting of different extracts of *Blepharis boerhaaviafolia* [23]

Chloroform extract:

10µl of 1mg/ml solution of chloroform extract in methanol was applied on the silica gel GF 254 HPTLC plates (10×10). Toluene: Chloroform: Acetone (4:2.5:3.5) was used as the mobile phase. Table 13**.**

Results and Discussions

Physio- Chemical standards

The percentage of loss on drying, total ash, acid insoluble ash ,water soluble ash, crude fiber content and sulphated ash extractive values of extracts are obtained by employing standard method of analysis and described in Table 1and 2 .The loss on drying is 4% and the crude fiber content is 4.5%. The total ash content is 16.72%, the acid insoluble ash content is 6.37% and water soluble ash content is 9.1% and sulphated ash content is 8.1%..The percentage yield of petroleum ether, chloroform, methanol ,solvent hexane, ethanol and aqueous extract is 2.2%, 12.5%, 13.5%, 9%,15% and 14.5%

Preliminary phytochemical investigation

Preliminary phytochemical screening of the *Blepharis boerhaaviafolia* plant powder was done per standard methods and results are presented in the Table 11. Chloroform, Ethanol and Aqueous extracts shows the presence of carbohydrate, glycoside, phytosterols ,alkaloids, tannins, steroids ,flavanoids and saponin. The medicinal properties exhibited by this species are due to the presence of alkaloids, flavanoids and glycosides.

Fluorescence characteristics

Blepharis boerhaaviafolia leaf extract powder treated with1N NaOH in methanol shows buff colour in ordinary light , black green and pale green colour in 254 and 365 nm. In 1N NaOH in water dark green in visible , green and light green colour in 254 and 365 nm. In 1N HCl it shows light green in visible light and green colour in 254 and 365 nm. In 50% HNO_3 pale green colour in ordinary light and also in 254 and 365 nm. In 50% H_2SO_4 it shows light green colour in visible light and colourless, pale green colour in 254 and 365 nm. In distilled water it shows green in visible light and dark green, blackish green colour in 254 and 365 nm. In chloroform it shows dark green in visible light and dark green, green colour in 254 and 365 nm. The result of fluorescence analysis is shown in the Table 9.

Behaviour of leaf powder with different chemical reagents

Blepharis boerhaaviafolia leaf powder treated with Con H_2SO_4 it shows no blue colour. Aqueous $FeCl_3$ it gives no bluish

Table- 13 HPTLC of Chloroform extract

Mobile phase	sample	Rf values	
		UV 254nm	UV 366nm
Toluene: chloroform : acetone (4:2.5:3.5)	Ethanolic extract	0.30, 0.94, 0.97	0.10, 0.14, 0.25, 0.30, 0.40, 0.57, 0.70, 0.85, 0.98

black colour. Leaf powder treated with iodine it gives blue colour. Aqueous mercuric chloride it gives blue colour. Powder is treated with picric acid it gives yellow colour. Magnesium hydrochloride it gives mango colour. Powder is treated with ammonia solution, Aqueous KOH, Aqueous $NaNo_3$ it gives pink colour. Powder is treated with water and shake it, foam is produced in Table 10.

Determination of qualitative estimation

The total saponin content is 9.2%, tannin content 3%, flavanoids content 2.40%, total sugar content 14.31%, chlorophyll content 0.741%, total alkaloidal content 5.50% and the foaming index is more than 1000 in Table 12

HPTLC fingerprinting of different extracts of *Blepharis boerhaaviafolia*

After development the plates were scanned in ultraviolet range at 254 nm and 366 nm, 3 spots with Rf 0.30, 0.94,0.97 were observed under 254nm and 9 spots with Rf 0.10,0.14,0.25,0.30,0.40,0.57,0.70,0.85,0.98 under 366 nm in Table-13.

Conclusion

The comparative and multidisciplinary approach to the study of *Blepharis boerhaaviafolia* does help in understanding their identification taxonomical determination, and medicinal importance.The adulterants in drugs obtain from *Blepharis boerhaaviafolia* can be identified by this investigation. Adulterants if any can be easily identified using these parameters.

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