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Pharmacognostic and phytochemical evaluation of *Blepharis repens*

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Abstract
Blepharis repens (Vahl.) Roth, (Synonym–Acanthus repens Vahl, *Blepharis molluginif olia*pers) belonging to family Acanthaceae, commonly known as Hadsan (in Marathi). The plant has natural distribution indeciduous forest. There are about 130 species distributed throughout tropical and subtropical region of the world. In Maharashtra this genusis represented by two species: *Blephari srepens* (Vahl) Roth. And *B. Madras petansis* (L.) Roth. Mostly all parts are medicinally used. In present study Pharmacognostic and Phytochemical Evaluation were carried out.

Keywords: Pharmacognostic, phytochemical, *Blepharis repens*

Introduction
Acanthaceae is one of the large pantropical family of about 229 genera and 3450 species. Family Acanthaceae have been reviewed by several authors from embryological point of view, in order to solve various taxonomic problems [1].
Plant-derived substances have recently become of great interest owing to their versatile applications. Medicinal plants are the richest bio-resource of drugs of traditional systems of medicine, modern medicines, nutraceuticals, food supplements, folk medicines, pharmaceutical intermediates and chemical entities for synthetic drugs [2].
Plants have played a significant role in maintaining human health and improving the quality of human life for thousands of years and have served humans well as valuable components of medicine, seasonings, beverages, cosmetics and dyes. *Blepharis repens* (Vahl.) Roth, (Synonym –Acanthus repens Vahl, *Blepharis molluginif oliapers*) belonging to family Acanthaceae, commonly known as Hadsan (in Marathi) the decoction of leaves used in treatment of old persistent fever and the paste is used for fractured bones [3].
Blepharisrepens (Vahl.) Roth, is a plant of medicinal importance and no pharmacog-nostical studies are available on this species, so the present investigations was undertaken. Which is a traditional medicinal herb of the family Acanthaceae. This plant is used traditionally to treat bone fractures, skin diseases, urinary discharges and allergies. Whole plant is highly medicinal, used against chronic fever and as a diuretic, aphrodisiac and expectorant, and also for urinary discharges. Stem powder is also consumed to curebone fracture. The plant has natural distribution indeciduous forest. There are about 130 species distributed throughout tropical and subtropical region of the world. In Maharashtra this genusis represented by two species: *Blephari srepens* (Vahl) Roth. and*B. Madras petansis* (L.) Roth [4].
The plant *Blepharis repens* a rare medicinal species, belongs to the Family Acanthaceae. This leaves may contain cystoliths, calcium and carbonate. Flat branches (phyllodes) are heated and tied in case of joint-ache. The phyllodes are jointed like the knees. Leaves are roasted and then extract is obtained. This extract is drunk as a remedy against flatulence. Roots are employed as antidote on snake – bite. Fruits are roasted and applied on swellings. Stem powder is consumed to cure bone fracture. The availability of the plant is very low. The importance of the shoot part will emergence for the *in vitro* approaches [5].

Plant profile
Name: *Blepharis repens* (Vahl) Roth [3]
Common name: Hadsan (in Marathi) [3] Hariduhachaga (in kannada) [13]
Kingdom: Plantae – Plants
Order: lamiales
Family: Acanthaceae
Genus: *Blepharis*
Species: *repens*
Synonym: *Acanthus repens* Vahl

Chemical constituents: May be Cystoliths, Calcium and Carbonate [5].

Uses: treat bone fractures, skin diseases, urinary discharges and allergies [4]. It is used in joint ache and roots are employed as antidote on snake-bite [5]

Fig 1: *Blepharis repens* (Vahl) Roth

Experimental Work

Collection of Plant Material

The plants of *Blepharis repens* were collected from forest of Yavatmal district, Maharashtra, India. The collected plants was carefully examined for infected parts and were removed accordingly. Only fresh parts were taken for the analysis. These plant parts were dried in the shade till all its moisture gets evaporated.

Authentication of plant

The herbarium sheet was prepared and submitted to Botany department of Rashtrasant Tukdoji Maharaj Nagpur University, Nagpur, and authentified with preference no. 10227.

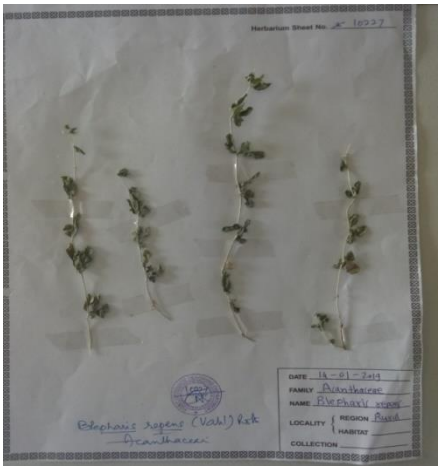

Fig 2: Authentification of *Blepharis repens* (Vahl) Roth.

Extraction

Ethanolic extract

About 200g of powder of *Blepharis repens* (Vahl) Roth. Were dried in shade under normal environmental condition and such powder drug was extract into maceration method and successive extraction was carried out with ethanol solvent.

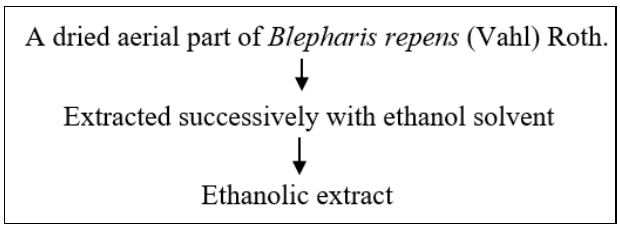

Fig 3: Scheme of Successive Extraction.

Preliminary Test for Phytoconstituents

Test for Carbohydrates

Test for Reducing Sugars

A. **Molisch's test:** To 2-3 ml aqueous extract, add few drop of alpha- napthol solution in alcohol, shake and add conc. sulphuric acid from side of test tube.
B. **Fehling's test:** Mix 1 ml Fehling's A and 1 ml Fehling's B solution, boil for one minute, Add equal volume of test solution. Heat in boiling water bath for 5-10 min.
C. **Benedict's test:** Mix equal volume of Benedict's reagent and test solution in test tube. Heat in boiling water bath for 5 min.

Test for Monosaccharide

A. **Barfoed's test:** Mix equal volume of Barfoed's reagent and test solution. Heat for 1-2 min. In boiling water bath and cool.

Test for Tannins

A. **Gelatin test:** To a solution of tannin, aqueous solution of Gelatin and sodium chloride were added.
B. **Ferric chloride:** Drug solution + 5% ferric chloride.
C. **Acetic acid:** Drug solution + acetic acid solution.
D. **Bromine water:** Drug solution + Bromine water.

Test for Alkaloids

A. **Dragendroff's test:** To 2-3 ml filtrate, add few drops Dragendroff's reagent.
B. **Wagner's test:** 2-3 ml filtrate with few drops Wagner's reagent.
C. **Hager's test:** 2-3 ml filtrate with Hager's reagent.

Test for Flavonoids

A. **Lead acetate test:** To small quantity of residue, add lead acetate solution.
B. **Sulphuric acid test:** solution + sulphuric acid (66% or 80%).

Test for Saponin Glycosides

A. **Foam test:** Shake the drug powder vigorously with water.

Tests for Steroids

A. Salkowaski reaction: To 2 ml of extract, add 2 ml chloroform and 2 ml conc. H2SO4. Shake well.

Test for Triterpenoids

A. Test solution + 5 ml conc. Sulphuric acid from side of test tube [11]

Determination of Extractive Value, Ash Value & Loss on Drying

Extractive Value

Ethanol-soluble extractive value

Macerate 5 g of the air-dried drug, coarsely powdered, with 100 ml of ethanol of the specified strength in a close flask for

24 hour, shaking frequently during the first 6 hour and allowing to stand for 18 Hour. Thereafter, filter rapidly taking precaution against loss of ethanol, evaporate 25 ml of the filtrate to dryness in a tarred flat-bottomed shallow dish, dry at 105 °C and weigh. Calculate the percentage of ethanol-soluble extractive with reference to the air-dried drug.

Water – soluble extractive value

Add 5 g to 50 ml of water at 80 °C in a stoppered flask. Shake well and allow to stand for 10 minutes, cool, add 2 g of kieselguhr and filter. Transfer 5 ml of the filtrate to a tarred evaporating dish, 7.5 cm in diameter, evaporate the solvent on a water-bath, continue drying for 30 minute, finally dry in a steam oven for 2 hour and weigh the residue. Calculate the percentage of water-soluble extractive with reference to the air-dried drug.

Petroleum ether - soluble extractive value

Macerate 5 g of the air-dried drug, coarsely powdered, with 100 ml of petroleum ether of the specified strength in a close flask for 24 hour, shaking frequently during the first 6 hour and allowing to stand for 18 Hour. Thereafter, filter rapidly talking precaution against loss of petroleum ether, evaporate 25 ml of the filtrate to dryness in a tarred flat-bottomed shallow dish, dry at 105 °C and weigh. Calculate the percentage of petroleum ether-soluble extractive with reference to the air-dried drug.

Ethyl acetate - soluble extractive value

Macerate 5 g of the air-dried drug, coarsely powdered, with 100 ml of ethyl acetate of the specified strength in a close flask for 24 hour, shaking frequently during the first 6 hour and allowing to stand for 18 hour. Thereafter, filter rapidly talking precaution against loss of ethyl acetate, evaporate 25 ml of the filtrate to dryness in a tarred flat-bottomed shallow dish, dry at 105 °C and weigh. Calculate the percentage of ethyl acetate-soluble extractive with reference to the air-dried drug.

Ash Value

Total ash

Take about 2g, accurately weighed, of the ground drug in a tarred platinum or silica dish previously ignited and weighed. Scatter the ground drug in a fine even layer on the bottom of the dish. Incarnated by gradually increasing the heat-not exceeding dull red heat- until free from carbon, cool and weigh.

If a carbon free ash cannot be obtained in this way, exhaust the charred mass with hot water, collect the residue on an ash less filter paper, increate the residue and filter paper, add the filtrate, evaporate to dryness and ignite at low temperature. Calculate the percentage of ash with reference to the air dried drug.

Sulphated ash

A silica crucible was heated to redness for 10 minutes, allowed to cool in desiccators and weighed. 1 g of substance was accurately weighed and transferred to the crucible. It was ignited gently at first, until the substance was thoroughly charred. Then the residue was cooled and moistened with 1 ml concentrated sulfuric acid, heated gently until white fumes are no longer evolved and ignited at 800° ± 25 °C until all black particles have disappeared.

The ignition was conducted in a place protected from air currents. The crucible was allowed to cool, and a few drops of concentrated sulfuric acid were added and heated. Ignited as before, allowed to cool, and weighed. The operation was repeated until two successive weighing does not differ by more than 0.5 mg. calculate the percentage of Sulphated ash with reference to the air dried drug [12].

Loss on Drying (LOD) by Gravimetric Method

Weigh about 1.5 g of the powdered drug into a weighed flat and thin porcelain dish. Dry in the oven at 100˚c or 105˚c, until two consecutive weighing do not differ by more than 0.5 mg. Cool in a desiccator and weigh. The loss in weight is usually recorded as moisture.

Results and Discussion

Extractive Value: The percentage (%) yield obtained after successive extraction of *Blepharis repens* (Vahl.) Roth, was found to be:

Extractive Value

Sr.no.	Extractives	Extracts%yield w/w (Test)	Extracts%yield w/w (Reference)
1	Alcohol soluble extract	7.5%	5.3%
2	Water soluble extract	5%	4.5%
3	Petroleum ether soluble extract	4.5%	1.24%
4	Ethyl acetate soluble extract	4%	2.1%

The Extracts% yield of test was found to be greater than Extracts% yield of reference [7].

Ash Value: The% ash value of *Blepharis repens* (Vahl) Roth, was found to be:

Ash Value

Sr.no.	Ash value	% Ash value
1	Total Ash	8.5%
2	Sulfonated Ash	12%

Loss on drying (LOD): The% LOD of *Blepharis repens* (Vahl) Roth, was found to be:

Loss on drying (LOD)

Sr.no.	Loss on drying (LOD)	% LOD
1	By Gravimetric Method	83.34%

Chemical Tests for Alcohol Soluble Extract
Test for Carbohydrates
Test for Reducing Sugars

Test for Reducing Sugars

Sr.no	Test	Observstion	Inference
A	**Molisch's test:**-To 2-3 ml aqueous extract, add few drop of alpha-napthol solution in alcohol, shake and add conc. sulphuric acid from side of test tube.	Violet ring is formed at the junction of two liquids.	Test was positive
B	**Fehling's test:** Mix 1 ml Fehling's A and 1 ml Fehling's B solution, boil for one minute, Add equal volume of test solution. Heat in boiling water bath for 5-10 min.	First yellow, then brick red ppt. is observed.	Test was positive
C	**Benedict's test:** Mix equal volume of Benedict's reagent and test solution in test tube. Heat in boiling water bath for 5 min.	solution appears green	Test was positive

Test for Monosaccharide

Sr.no	Test	Observation	Inference
A	**Barfoed's test:** Mix equal volume of Barfoed's reagent and test solution. Heat for 1-2 min. In boiling water bath and cool.	Red ppt was not observed.	Test was negative

Test for Tannins

Sr.no	Test	Observation	Inference
A	**Gelatin test:** To a solution of tannin, aqueous solution of Gelatin and sodium chloride were added.	White buff coloured ppt was observed.	Test was positive
B	**Ferric chloride:** Drug solution + 5% ferric chloride.	Deep blue-black colour was not observed.	Test was negative
C	**Acetic acid:** Drug solution + acetic acid solution.	Red coloured solution was not observed.	Test was negative
D	**Bromine water:** Drug solution + Bromine water.	Decolouration of Bromine water was not observed.	Test was negative

Test for Alkaloids

Sr.no	Test	Observation	Inference
A	Dragendroff's test: To 2-3 ml filtrate, add few drops Dragendroff's reagent.	Orange brown ppt. was found.	Test was positive
B	Wagner's test: 2-3 ml filtrate with few drops Wagner's reagent.	Reddish brown ppt. was observed	Test was positive
C	Hager's test: 2-3 ml filtrate with Hager's reagent.	Yellow ppt. was observed.	Test was positive

Test for Flavonoids

Sr.no	Test	Observstion	Inference
A	Lead acetate test: To small quantity of residue, add lead acetate solution.	Yellow colored ppt was observed.	Test was positive
B	Sulphuric acid test: solution + sulphuric acid (66% or 80%).	Deep yellow solution was not observed.	Test was negative

Test for Saponin Glycosides

Sr.no	Test	Observation	inference
A	Foam test: Shake the drug powder vigorously with water.	Persistent foam was observed.	Test was positive

Test for Steroids

Sr.no	Test	observation	Inference
A	Salkowaski reaction: To 2 ml of extract, add 2 ml chloroform and 2 ml conc. H2SO4. Shake well.	Chloroform layer and acid layer does not show any florescence.	Test was positive

Test for Triterpenoids

Sr.no	Test	observation	Inference
A	Test solution + 5 mL conc. Sulphuric acid from side of test tube.	Greenish blue colour was observed.	Test was positive

Chemical Test for Various Extractives

Sr.no	Tests	Petroleum ether	Water	Ethyl acetate
1	**Carbohydrates**			
	a) Molisch's test	+	+	+
	b) Fehling's test	+	+	-
	c) Benedict's test	-	+	-
	d) Barfoed's test	-	-	-
2	**Tannins**			
	a) Gelatin test	+	+	+
	b)Ferric chloride	-	-	-
	c)Acetic acid	-	-	-
	d)Bromine water	+	-	-
3	**Alkaloids**			
	a) Dragendroff's test	+	+	-
	b) Wagner's test	-	+	+
	c) Hager's test	-	+	-
4	**Flavonoids**			
	a) Lead acetate test	+	+	+
	b) Sulphuric acid test	+	+	+
5	**Steriods**			
	a) Salkowaski reaction	+	+	+
6	**Saponin glycosides**			
	a) Foam test	+	+	-
7	**Triterpenoids**	+	+	-

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