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PHARMACOGNOSTICAL AND PHYTOCHEMICAL EVALUATION OF DHARUHARIDRA (BERBERIS ARISTATA)

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Abstract

Daruharidra had been mentioned for its varied benefits in the classical literature of *Ayurveda.* So the study was undertaken on the bases of classical references in ayurvedic literature. *Daruharidra* stem was studied for its preliminary pharmacognostical and phytochemical aspect. The study was conducted on ethyl alchohol extract of daruharidra stem (Berberisaristata).

Key words: Daruharidra, Berberisaristata, pharmacognostical, phytochemical ethyl alchohol extract

Introduction:

Ayurveda, the knowledge of life science bestowed health and longevity inthe form of preventive and curative measures. The curative aspects are mainly covered by *Dravyachikitsa* (Treatment using drugs). As diseases are born with human there is always a search for safest and curative drugs.

In the present era, the attraction towards *Ayurveda* is increasing day by day due to less unwanted side effects. On account of increasing urbanization, increasing demand of medicine for population, shortage of authentic material and also tendency of profiteering, there is a need for statutory control and development of pharmacopoeial standards.The stem of *Berberisaristata* Linn.were taken for present study. Analysis of samples was done to evolve sutable parameters for checking the quality.

On scientific background the present drug *Daruharidra* was subjected to different studies to know its external and internal structure and different chemical constituents in the selected part of plant. Most of ayurvedic drugs or formulations are known for their safety and efficacy. Hence literary research is done to specify a medicine

that can act as a *pittasaraka,vranaropaka*, and *vrunashodaka*. Lot of drugs are mentioned as *vrunashodaka* and *ropaka* in ayurvedic classics. The selected drug *Daruharidra* is having *ushnaveerya* and *tiktha* and *kashaya rasa* properties.By literary survey *Daruharidra* is mentioned from *niganthukala* and some ayurvedic modern books mentioned that, it has *pitthasaraka* and *vrunarupaka* action.

On the scientific backround the present drug *daruharidra* stem (Berberisaristata) was subjected for different studies to know its pharmacognostical charecters chemical constitution in the selected part of the plant.

OBJECTIVES:

Pharmacognostical and preliminary phytochemical analysis of *daruharidra* stem.

REVIEW OF LITERATURE:

Charakokta Ghana: Lekhaneeya, Arshogna, Kandugna[1]

Sushrutokta Ghana :Haridradi ,Mustadi ,Lakshadi[2]

NIGHANTU KALA:

Nighantu	Vargas
Bhavaprakash nighantu[3]	*Haritakyadivarga*
Raja nighantu[4]	*Pippalyadivarga*
Dhanvantari nighantu[5]	*Guduchyadivarga*
Kaiyadeva nighantu[6]	*Oushadivarga*
Shodala nighantu[7]	*Guduchyadivarga*
Saligram nighantu[8]	*Astavarga*
Hrudayadipika nighantu[9]	*Tripadavarga*
Astanga nighantu[10]	*Haridradivarga*

METHOD OF COLLECTION:[11]

The roots of these species are collected in fairly large quantities in Chamba district of Himachala Pradesh and in Tehri – Garhwal of Uttar Pradesh during August-September and are sold in drug markets of Chamba, Dehradun and Haridwar.

Barberry bushes bloom from February to June attracting bees for the pollen and nectar. The stems of berberis are yellowish brown, cylindrical, more or less knotty hard and tough. Collects

after rainy season, with the bark intact they are cut into pieces of varying length and a maximum diameter of 45mm. The concentration of berberin is more in lower altitudes, so they collect from lower altitudes in wet condition and dried under shade. Kept in moisture free bottles.

SANSKRIT NAME: *Daruharidra*

BOTANICAL NAME WITH MEANING:[12]

Berberis aristata Linn Berberis = belonging to the berberis family and aristata = furnished with an elongated projecting bristle, in connection with the costa (rib).

FAMILY: Berberidaceae

BOTANICAL CLASSIFICATION:[13]

Class	- Dicotyledons
Sub class	- polypetalae
Series	- Thalami florae
Cohort	- Ranales
Order	- Berberidaceae
Family	- Berberidaceae
Genus	- Berberis
Species	- aristata.

ETYMOLOGICAL DERIVATION OF SYNONYMS: [14,15,16,17,18,]

1. *Katamkateri – Katamkatam ugradoshavarakam gunam irshyati iti katamkateri.*

It eliminates even the bodily vitiated *doshas* or severely deranged *dosha* which may cause *srotorodha* [obstruction in various channels].

2.*Kaleyaka – kalayati doshan kaliyakaha.*

It removes the *doshas* or *rogas.*

3 *Daruharidra – i*

yam haridra daruni bhavati.

Harihi pingaha pitova va drurasya.

This type of turmeric has its origin in the form of a stem.

4. *Darvi – dirayate dru vidarane.*

It will do doshanivarana.

5. *Pachampacha – Atyartham pachati iti pachampacha.*

It is a very good digestive.

6. *Parjani –*

i) *piparti rogeabyaha parjani.*

It protects body from diseases.

ii) *Param palakam swasthyam janayati va.*

It restores good health.

7. *Pitadru*

i) pito dru skandoasya.

Its branches/stems are yellow coloured.

ii) *Pitam dravati dru gatau.*

It provides yellow extract.

8. *Haridravaha – Harihi pingaha pitova dru asya.*

Its stems are yellow or yellowish green in colour.

a. *Daruharidra – Darupradhana haridravarna*.

Daruharidra is a plant having yellow wood and flowers.

b. *Katamkateri – Patranam kantakitvat.*

The plant has spines on margins of leaves.

c. *kantakini – patre kantakayukta.*

The plant has spines on margins of leaves.

d. *Kusumbala – Kusumbavat varnam latiti.*

Making yellow dye like *kusumbha.*

e. *Krimihara – Krimin haratiti.*

It is anthelmintic[*krimigna]*.

f. *Darvi –Darupradhana aushadihi*.

The important part of the plant is wood [*darvi]* which is used as a drug.

g. *Pachampacha – Pakanantaram Pachati Dhatupakam Karoti yakrit iti.*

It improves liver functions.

h. *Parjanya – Meghagame phalagamat.*

It gives fruits during rainy season.

i.*Pitadaru – Pitam darvasya.*

It is having yellow wood and flowers

HABITAT:[19]

Himalaya from chota Bangal to Nepal 6,500 – 10,500ft. Berberry bushes grow on the Nilgiris and all over the temperate Himalayas from Bhutan to Kunwar.

It is distributed in temperate and subtropical parts of Asia, Europe and America; mostly it is found in Nepal. Grown in Nilgiris and all over temperate Himalayas, about 77 species are recorded from India.

MORPHOLOGY:[20]

Habit- A large deciduous shrub 1.8 – 3.6m high. Twigs whitish or pale yellowish brown deeply furrowed rough.

Leaves – 3.8 – 10 by 1.5 -3.3 cm, obovate or elliptic, entire or spinous toothed. Base gradually narrowed, dark green above, pale beneath.

Petiole – 0-4 mm.

Inflorescence – A simple drooping racem, 2.5 – 7.5cm long. Densely-flowered.

Fruit- 7- 10mm long ovoid blue, black, distinct style. Fruits called as zarishka.seeds are of 3/8cm.

Stem – Stem has swethaba or peetabhavarna, it has thorns, it is cylindrical, more or less knotty, hard and tough, corky. Diameter of 20cm or 8 inch. Stem is devoid of pith. if present has yellow colour with shiny nature.

Flower – Bisexual, complete has yellow colour, diameter of ½ cm.

MATERIALS AND METHODS:

A] PHARMACOLOGICAL STUDY:

Aim: The aim of this study was to analyze morphological, microscopical evaluation of Daruharidra stem. [*Berberisaristata*linn]

1] MORPHOLOGICAL STUDY:

Materials: The materials collected for the study were

Drug:*Berberisaristata*linn.

Parts used: stem

Collection of materials: The stem of *Berberisaristata* were collected from shimla [Himachal]

Equipments: sense organs.

METHODS:

1] Organoleptic method 2] Extra features.

a] Organoleptic method: In this method the colour, taste, size, shape, odour, characteristics of stem of Daruharidra were studied with the help of sense organs.

b] Extra features: The special characteristics of stem were studied.

2] MICROSCOPICAL STUDY:[21]

Materials: The materials collected for the study were,

Drug: stem of Daruharidra (*Berberisaristata*Linn).

Equipments: Compound microscope, eye piece, glass slides, cover slips, watch glass, camel hair brush, filter paper, blades.

Chemicals: glycerin, Iodine, saffron staining solution.

METHODS:

1] Section method

2] Staining method.

1] SECTION METHOD:

The fresh stem collected kept in water for 24 hours.

The sample held vertically in between thumb and fore finger.

With the help of new blade, thin transverse sections horizontally were taken.

10 – 15, sufficient sections were taken, thick and oblique sections were rejected.

With the help of mountain hair brush, the section transferred to watch glass containing water.

2] STAINING METHOD:

Thin sections were selected.

The thin sections selected in watch glass are added with staining solution.

The thin section of the sample was taken and transferred it, on a slide with help of mountain hair brush.

Drop of water was added.

Then the section covered with the cover slip.

The section focused under microscope and arrangements of cells were studied.

B]MATERIALS FOR PHYTOCHEMICAL STUDY:

Aim: To know the chemical constituent in a trial drug, subjecting to different tests like extraction, preliminary phytochemical test.

1] Solubility of *Berberisaristata* Linn.

Materials: Fine powder of Daruharidra [*Berberis aristata*Linn] stem.

Solvents :

1) Benzene
2)Petroleum ether
3)Ethyl acetate
4)Distlled water
5)Ethyl alcohol
6)solvent ether
7)Acetone
8)Toluene
9)Chloroform
10)Xylene
11)Carbon tetrachloride
12)Methane.

METHODOLOGY:[22]

A different solvent mixed with fine powder of stem of *Berberis aristata*linn and was in different funnels using different filter papers. The solvent giving minimum residue was selected. Because this solvent is may having maximum solubility.

EXTRACTION:

MATERIAL:

Drug: Coarse powder of stem *Berberis aristata*linn.

Equipments: Soxhlet apparatus of 1000ml round bottom flask, water condenser with distillation apparatus, Beaker 500ml, measuring cylinder, Thermostat(heater) stand, Electronic weighing machine, Filter paper, Water bath, Boiling chips etc.

Chemicals: Ethyl alcohol.

Methods: The coarse powder of *Berberis aristata* Linn stem was subjected to exhaustive extraction by soxhlet apparatus with ethyl alcohol. After extraction the solvent was distilled off, to obtain semisolid extract then kept in the water bath and weights of each batch extracts were recorded.

DETERMINATION OF P^H:[23]

MATERIAL:

Drug – Extract of stem of *Berberis aristata* Linn

Equipment – Digital calibrate P^H.

METHOD:

50ml distilled water was taken in a beaker, digital P^H was immersed up to

the maximum immersion level. Allowed the reading to stabilize and screw driver was used to turn the P^H calibration trimmer to read 7.0.

Then 0.5gms of *Berberis aristata* Linn extract was added to 50 ml of distilled water in a beaker, stirred well with glass rod gently, at uniform suspension digital P^H meter was immersed observed the maximum immersion level and reading was recorded.

DETERMINATION OF MOISTURE CONTENT (LOSS ON DRYING):[23]

AIM: Determination of the moisture(water drying off) content from the drug.

MATERIALS:

1] Drug :*Berberis aristata* Linn.

2] Weighing apparatus.

3] Tared Evaporating dish.

4] Hot air oven.

5] Dessicator.

PROCEDURE:

Take about 5gms of *Berberis aristata* Linn stem powder without preliminary drying and placed in a tared evaporating dish. Avoid the use of high speed mills for preparing the samples, after placed the drug in the tared evaporating misolid extract then kept in the water bath and weights of each batch extract were recorded.

Sodium nitrate5%

Sodium hydroxide 17% Remove evaporating dish from hot air oven, Kept in a dessicator for itself cooled and weighed at one hour interval, it is repeated for 2-3 successive constant weight was recorded.

Moisture content – 100 w/w.

Wt. of Tared evaporating dish - in gms. (A)

Wt. of sample – 10gms. (B)

$$\text{Moisture content} = \frac{\text{wt. of petridish} + \text{wt. of sample} - \text{concurrent values}}{\text{Wt. of sample}} \times 100$$

2]PRELIMINARY PHYTOCHEMICAL TEST:[24]

MATERIALS:

Drug: Extractive sample of stem of *Berberis aristata* Linn.

Equipments: Test tube, Test tube holder, Test tube stand, Spirit lamp, pipette, Glass rods, Beaker 50ml, 250ml conical flask, water bath, Burner, stand.

Chemicals:

10% conc. H_2SO_4
Chloroform solution
Acetic anhydride
Sulphur powder
Soda lime
Millions reagent
Mercuric sulphate 10%
Sulphuric acid 1%
Cu SO_4 10%
Tannic acid
Acetyl chloride
Zinc chloride
Wagners reagent
Hagers reagent
Dragendroffs reagent
Ammonium Renicate
Molischs reagent
Barfords reagents
Benedicts reagent
Saponin
Ferric chloride fragments
Pieces of magnesium ribbon
Conc. HCl
Zinc dust
Sodium hydroxide
10% Lead acetate
Bromine water
Ferric chloride
Lead acetate.

METHODS:

1] TEST FOR STEROLS:

a) Salkowskis test: 2ml extract, 2ml chloroform and 2ml conc.H_2SO_4 were added, shaked and allowed to stand.

b) Sulphur test: A pinch of sulphur was added to the extract.

2] TEST FOR PROTEINS:

Preparation of test solution: 0.5 gm of sample was added in 100ml water and heated. This solution was used for following test.

a) Biuret test: 3ml Test solution, 4% soda lime and few drops 1% $CuSo_4$ were mixed and allowed to stand.

b) Millons test: 3ml Test solution and 5ml millions reagent was added.

c) Xanthoprotein test: 3ml test solution and 1ml cons.H_2So_4 was added, boiled then added NH_4OH.

3] TEST FOR TRITERPENOIDS:

a) Liebermann-Buchards test: 2ml of extract was mixed with 2ml chloroform, Acetic anhydride,and cons.H_2SO_4 was added from the sides of the test tube.

b) Tschugajew test: 2ml of Acetyl chloride and pinch of Zinc chloride were added to the extract and boiled in water bath.

4] TEST FOR ALKALOIDS:

Preparation of test solution: The benzene extract was evaporated to residue; diluted HCl was added and shacked well then filtered by using filtrate. The following tests were performed.

Mayers Test: 2ml filtrate and few drop of Mayers reagents i.e. potassium mercuric iodide, mixed and allowed to stand.

Wagners Test: 2ml filtrate and Wagners reagents mixed and allowed to stand.

HagersTest : 2ml filtrate and few drops Hagers reagents mixed and allowed to stand.

Dragendroffs test: 2ml filtrate with Dragendroffs reagent mixed and allowed to stand.

5] TEST FOR CARBOHYDRATES:

a) Molischs test: 2ml extract with few drops of molischs reagent were taken and shaked then 2ml of cons.10%H_2SO_4 added slowly to the sides of the test tube.

b) Barfords test (test for monosacrides): Equal volume of sample solution and Barfords reagents were taken boiled 2 minutes in the water bath.

6] TEST FOR SAPONINS:

a) Foam test: The drug extract shaked vigorously with water.

b) Heamolysis test: The drug extract was added to 1 drop of blood on glass slide.

7] TEST FOR FLAVONOIDS:

a) Ferric chloride test: 2ml extract and few drops of Ferric chloride solution were mixed and allowed to stand.

b) Lead acetate test: 2ml extract was mixed with two drops of 10% lead acetate.

c) Bromine water test: 2ml extract was mixed with few drops bromine water and allowed to stand.

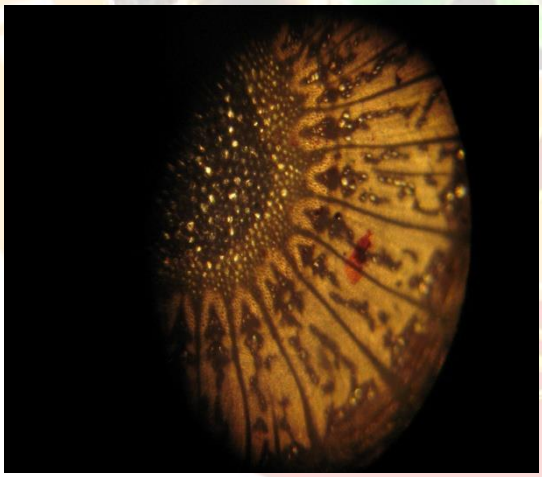
8] TEST FOR TANNINS:

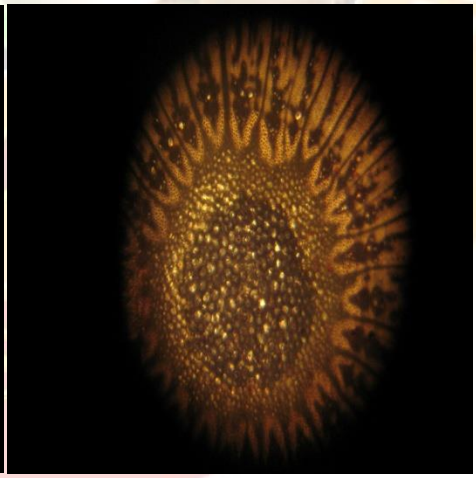
a) Ferric chloride test: 2ml extract and few drops of Ferric chloride solution was mixed and allowed to stand.b) Lead acetate test: 2ml extract was mixed with two drops of 10% lead acetate.c) Bromine water test: 2ml extract was mixed with few drops bromine water and allowed to stand.

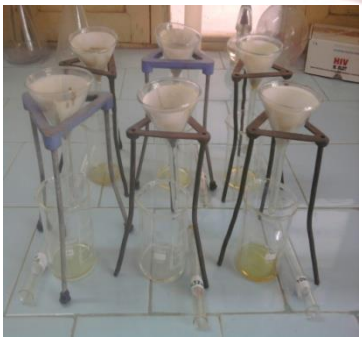
DARUHARIDRA SHRUB

BERRIES OF DARUHARIDRA

DARUHARIDRA STEM COURSE POWDER





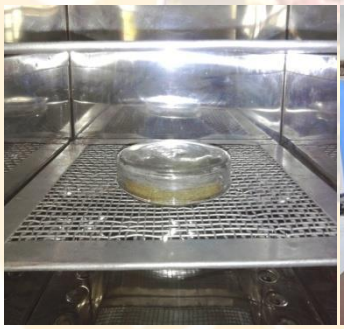


SOLUBILITY TEST OF DARUHARIDRA STEM FINE POWDER


PRELIMINARY PHYTOCHEMICAL TEST



EXTRACTION OF DARUHARIDRA STEM POWDER



DETERMINATION OF MOISTURE CONTENT P^H OF DARUHARIDRA EXRACT

RESULTS:

A] RESULTS OF PHARMACOGNOSTICAL STUDY:

1] MORPHOLOGICAL STUDY:

Table No.1: T.S. Results of morphological study

Colour	Pale yellowish-brown
Taste	Bitter
Size	Bark about 0.4-0.8cm, 40-50mm of thickness of stem.
Shape	Cylindrical, more or less knotty hard and tough.
Odour	Pleasant.

Nature of stem	Closely and rather deeply furrowed, rough, brittle.
Touch	Hard and rough.

2] MICROSCOPICAL STUDY:

After section method and staining process method, under microscope the following constituents were seen phloem, parenchymatous cells, secondary phloem, xylem, prismatic crystals of calcium oxalate.

B] RESULTS OF PHYTOCHEMICAL STUDY:

1] SOLUBILITY TEST:

Table No.2: T.S. Results of Solubility test.

Solvents	Soluble	Sparingly	Insoluble
Distilled water	-	+	-
Solvent ether	-	-	+
Petroleum ether	-	+	-
Acetone	-	-	+
Benzene	-	-	+
Toluene	-	-	+
Chloroform	-	+	-
Ethyl alchohol	+	-	-
Xylene	-	-	+
Carbon tetrachloride	-	-	-
Methanol	-	+	-
Butyl alchohol	-	+	-

2] EXTRACTION:

Table No.3: T.S. Result of Extraction.

Stem powder of Berberisaristata Linn.	Solvent	Extract
Coarse Stem powder	1000ml ethyl alcohol	40gms

150gms.		

3] RESULTS OF P^H VALUE:

Table No.4: T.S. Result of P^H value.

Distill water normal P^H	7.0
Acidic media P^H	0 – 7
Alkaline media P^H	7 – 14
Berberisaristata Linn extract P^H	6.5

4] RESULTS OF MOISTURE CONTENT:

Weight of petridish - 143.45gm.

Drug powder - 10gm.

Wt. of petridish + Drug powder – 153.45gm.

After heating concurrent value - 152.89gms.

$$\text{Moisture content} = \frac{\text{wt. of petridish + wt. of sample – concurrent values}}{\text{Wt.of.sample.}} \times 100$$

$$= \frac{153.45 - 152.89}{10} \times 100$$

Moisture Content = 5.6 w\w

RESULTS OF PRELIMINARY PHYTOCHEMICAL TEST:

Table No.5: T.S. Results of preliminary phytochemical test.

TESTS	RESULTS
1] Test for carbohydrates:	
Benedict`s test: Molish`s test: Barford`s test:	+ ve + ve - ve
2] Test for Proteins:	
Biuret test: Ninhydrin test(for amino acids): Million`s test:	ve + ve ve
3] Test for Fats and oils:	
Saponification test: Filter paper test:	ve ve
4] Test for Steroids:	

Salkowski reaction:	Ve
5] Test for volatile oils:	
Filter paper test:	Ve
6] Test for saponins:	
a) Foam Test:	ve
7]Test for Flavonoids:	
a)Shinoda test:	- ve
8]Test for Alkaloids:	
a)Mayer`s Test: b)Hager`s Test: c)Wagner`s Test:	+ ve + ve + ve
9]Test for Tanins and Phenols:	
Bromine water: Dilute HNO_3:	+ ve + ve

DISCUSSION:

Pharmacognostic study: The stem is rough, britle for touch and has pale yellowish brown colour.this brown colour is probabaly because of presence of berberin.It is bitter in taste.The microscopical study of stem of *Daruharidra* showed the arrangement of the xylem and phloem and parenchymatous cells and prismatic crystals of calcium oxalate.

Phloem fibres arranged in tangential rows consisting of 1-4 cells. A few cells of rhytidoma also contain prismatic crystals of calcium oxalate ; xylum fibres numerous, lignified, large,thick walled with wide lumen and pointed tips, xylem rays quite distinct,straight,multiseriate consisting of radially arranged rectangular cells.each ray contains 30-50 cells high,8-12 cells wide.A few ray cells containing yellowish brown contents.

Phytochemical study :Before carrying out the phytochemical test,the drug was subjected for its solubility in different solvants like ethyl alchohol,chloroform,distill water etc.the maximum solubility was observed in 90% ethyl alchohol. Hence Daruharidra stem powder extraction is done in the same, and the extraction is used for further phytochemical analysis in the laboratory of Dravyaguna dept. extracts were subjected to various preliminary phytochemical tests for detection of the phytoconstituents. It shows presence of alkaloids,

carbohydrates, protiens, tannins and phenols.

CONCLUSION:

The TS of stem of *Daruharidra* (*Berberis aristata*) showed the arrangement of the xylem, phloem and parenchymatous cells and prismatic crystals of calcium oxalate cells, A few ray cells containing a yellowish brown contents phytochemical analysis of ethyl alchoholextact of daruaridra stem shows presence of alkaloids, carbohydrates, protiens and tannins and phenols.

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