

PHYTOCHEMICAL SCREENING OF PLANT *BRYONOPSIS LACINIOSA* LINN. SEED

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ABSTRACT

Traditional medicines are used by about 60% of the world's population. It has prompted man to explore his immediate natural surroundings and try many plants to develop a variety of therapeutic agents. The herbal drug market itself is growing at a rate of 20-30% annually. The growth rate of this in the global market is encouraging for the manufacturers to produce more pure herbal drugs. Bryonopsis laciniosa Linn is one such herbal plant which has many therapeutic actions. It is most commonly available plant throughout India. Traditionally it is used as Antipyretic, Analgesic, Anti-inflammatory, Anti-microbial. As per literature, there is no extensive work done on Pharmacognostic and Phyto-chemical screening of the plant SEED so, it was planned for this study. Phyto-chemical screening was done for identification of active chemical constituents. Results concluded that, the identified compound contains steroids and when compared with standards it was revealed that compound is β -sito sterol. It also contains some bitter principles as reported.

INTRODUCTION

Since ancient time mankind are depended upon plant kingdom to meet all their needs. It has prompted them to explore natural surroundings and try many plants, animal products to develop a variety of therapeutic agents. Medicinal plants play an important role in preventive and curative treatments despite of modern medicines. They generate income to the people by different means and hence are considered as an important National resource. The ancient civilization of India, China, Greece, Arab and other countries of the world developed their own systems of medicine independent of each other but all of them were predominantly plant-based.

World Health Organisation (WHO) estimated that 80% of the population in developing countries is depended on the plant drugs. Even the modern Pharmacopoeia contains at least 25% drugs derived from plants. About 90% of medicinal plants used by the industries are collected from the wild. Demand for herbal drugs is increasing throughout the world due to growing recognition of natural plant waste products. In India, plants have been traditionally used for human health care and also for food and textile industries. The herbal drug market itself is growing at a rate of 20-30% annually. The growth rate of this market is encouraging for the manufacturers to produce more pure herbal drugs. Several health care systems of medicines are being practice in Indian sub continents like Ayurveda, Siddha, Unani, etc which are based on medicinal plants and herbal drugs [1, 2]

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Taxonomy of the Plant- *Bryonopsis laciniosa*

Domain: Eukaryota

Kingdom: Plantae

Research Article



Subkingdom: Viridaeplantae
 Phylum: Tracheophyta
 Subphylum: Euphyllophytina
 Infraphylum: Radiatopses
 Class: Magnoliopsida
 Subclass: Dilleniidae
 Superorder: Violanae
 Order: Cucurbitales
 Family: Cucurbitaceae
 Subfamily: Cucurbitoideae
 Tribe: Benincaseae
 Genus: Bryonia
 Vernacular Names:
 Telugu: Lingadonda, English: Lollipop Plant,
 Hindi: Shivlingi, Sanskrit: Baja,
 Tamil: Aiveli

Biological Source

Dried seeds are obtained from the plant *Bryonopsis laciniosa* belonging to family Cucurbitaceae. Other Species of *Bryonopsis laciniosa* are *Bryonia alba*, *Bryonia acuta*, *Bryonia callosa*, *Bryonia cretica*, *Bryonia geminate*, *Bryonia epigaea*, *Bryonia aspera*.

Distribution

It is commonly available plant in India and also found in many parts of the world like USA.

Description

It is common herb found all over India. It is herbaceous, slender, extensive, monoecious climber. Leaves are palmately 3-7 lobed, membranous and glabrous. Flowers are creamy white in axillary fascicles. Seeds are ovate, creamy-white or pale yellow, minutely scorbiculate.

Reported Chemical Constituents: Bryonin

Reported Therapeutic Uses:

- Leaves and seeds are used for treatment of fever with flatulence in Ayurveda.
- It is used as tonic.
- Plant is useful in inflammation. It is also used in stomach ache.
- It is used as Antipyretic, Anti-inflammatory, Anti-microbial and Analgesic.



Leave, Friuts and Dried seeds of *Bryonopsis laciniosa*

MATERIALS AND METHODS

Bryonopsis laciniosa Seeds, Floroglucinol HCl, Alcohol, Water, Dessicator, Flat bottom dish, Glass-stopper, Conical flask, Soxhlet extractor.

Collection and authentication of the plant

Fruits were collected in the month of January from the Manikanta Farmhouse in Kodakandla OF Medak Dist, Andhra Pradesh and seeds were removed from and it was authenticated by B. Amarendhar Reddy M.Sc Professor in Dept of botany in Sai Goutami College, Ibrahimpatnam R.R dist.Telangana.

Pharmacognosic Study

A.Macroscopy

Macroscopic Features and Organoleptic features viz. colour, odour, taste, texture, shape and size of *Bryonopsis* seeds were observed and the results are tabulated in Table no.1.

B. Microscopy

Transverse Section of Seed

A thin cross section of seed was taken. Then it was stained with Phloroglucinol and HCl.The details of transverse section observed are described in results and photograph of the same is shown in figure no.1

Powder Macroscopy

The dried seed powder was boiled and Then stained with equal quantity of Phloroglucinol and HCl and observed for the microscopic features under low power (10x)The microscopic characters observed are described in results and photograph of the same is shown in Figure No.2, 3, 4.

Determination of Extract Value

About 6.0g of seed powdered was weighed accurately in glass-stopper conical flask macerated with 100ml of solvent for 6hrs shaking frequently and then allowed to stand for 18hrs.It was filtered and 25ml of the filtrate was transferred to tarred flat bottom dish and evaporated to dryness over water-bath. Residue was dried at 105⁰c for 6hrs cooled in desiccator for 30min and weighed. The content of extractable matter was calculated in mg/g of air dried material.

Solvents used for determination of extractive value- Water, Alcohol & 50% Alcohol and % extractive value was calculated with reference to shade dried powdered seeds.

Determination of Total Ash

The ash remaining following ignition of medical plant was determined by Total Ash Method. About 2-4g of powdered seeds of 25 g of the shade dried powdered seeds of *Bryonopsis laciniosa* was weighed accurately in a



previously ignited and tarred crucible. The material was spread in an even layer and ignited by gradually increasing the heat to 500-600 c until it became white, indicating the absence of carbon. It was then cooled in a desiccator and weighed. The content of total ash was calculated in mg per gm of air-dried material. Results are tabulated in table no.2

Determination of Moisture Content

An excess of water in medicinal plant materials will encourage microbial growth, the presence of fungi or insects, and deterioration following hydrolysis. Limits for water content should therefore be set for every given plant material. This is especially important for materials that absorb moisture easily or deteriorate quickly in presence of water.

Preliminary Phytochemical Screening

The dried powdered seeds of *Bryonopsis laciniosa* were subjected to systematic phyto-chemical screening by total alcoholic extraction and hydro-alcoholic extraction and then investigation by qualitative chemical identification tests, thin layer chromatographic techniques.

Total Alcoholic Extraction

5 g of the shade dried powdered seeds of *Bryonopsis laciniosa* was extracted with 95% alcohol in a Soxhlet extractor. The liquid extract was concentrated and then evaporated to dryness in tarred china-dish. The residue was then weighed and the percentage yield of alcoholic extract is reported in table no.3

Hydroalcoholic Extraction

25 g of the shade dried powdered seeds of *Bryonopsis laciniosa* as macerated with 50% alcohol for 7 days with occasional shaking to get the hydro-alcoholic extract. The hydro alcoholic extract was concentrated and then evaporated to dryness in tarred china-dish. The residue was then weighed and the percentage yield of hydro-alcoholic extract is reported in table no.3

About 4.0g of coarsely powdered seeds were taken in a glass-stopper conical flask, macerated with 100ml of the solvent for 6 hours, shaking frequently and then allowed to stand for 18 hours. It was filtered and 25ml of the filtrate was transferred to a tarred flat-bottomed dish and evaporated to dryness over a water-bath. The residue was dried at 105°C for 6 hours, cooled in a desiccator for 30 minutes and weighed.

The content of extractable matter was calculated in mg per g of air-dried material. Solvents used for determination of extractive value-water, alcohol & 50% alcohol. Percentage of extractive values was calculated with reference to the shade-dried powdered leaves. Results are tabulated in table no.2

Qualitative Chemical Test

Test for carbohydrates

Molish's test

Treat the extract slowly with few drops of alcoholic-naphthol. Add 0.2 ml of concentrated H₂SO₄ slowly through the sides of the test tube, purple to violet colour ring appears at the junction.

Test for Reducing Sugars

Benedict's Test

Treat the extract solution with few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) and upon boiling on water bath, reddish brown precipitates forms if reducing sugars are present.

Fehling's Test

Equal volume of Fehling's A (copper sulphate in distilled water) and Fehling's B (Potassium tartarate and sodium hydroxide in distilled water) reagents are mixed along with few drops of extract solution, boiled, a brick red precipitate of cuprous oxide forms, if reducing sugars are present.

Test for Tannins

Ferric Chloride Test

Extract solution gives blue green colour with FeCl₃.

Test for Proteins & Amino Acids

Biuret Test

Extract solution with 2 ml of Biuret's reagent appears, violet or pink colour upon gentle heating.

Ninhydrin Test

Amino acids and proteins when boiled with 0.2% solution of Ninhydrin (Indane 1, 2, 3 trione hydrates) violet colour appear.

Test for Steroids And Triterpenoids

Liebermann-Burchard Test

Extract treated with few drops of acetic anhydride, boil and cool, concentrated sulphuric acid is added from the side of the test tube, shows brown ring at the junction of two layers and upper layer turns green which shows the presence of sterols and formation of deep red colour indicates the presence of triterpenoids.

Salkowski's Test

Treat extract in chloroform with few drops of concentrated sulphuric acid, shake well and allow to stand for some time, red colour appears in the lower layer indicates the presence of sterols and formation of yellow coloured lower layer indicates the presence of triterpenoids.



Test for glycosides

General test for the presence of glycosides

Test I

Extract 200 mg of drug by warming in a test tube with 5 ml of dilute (10%) sulphuric acid on water bath at 100 c for two minutes, centrifuge or filter, pipette out supernatant or filtrate. Neutralize the acid extract with 5% solution of sodium hydroxide (noting the value of NaOH added). Add 0.1 ml of Fehling's solution A and B until alkaline (test with pH paper) and heat on water bath for 2 minutes. Note the quantity of red precipitate formed and compared with that formed in Test II.

Test II

Extract 200 mg of the drug using 5 ml of water instead of sulphuric acid and boil on water bath. After boiling add equal volume of water to the volume of NaOH used in the above test. Add 0.1 ml of Fehling's A and B until alkaline (red litmus change to blue) and heat on water bath for two minutes. Note the quantity of the red precipitate formed. Compare the precipitates of test II with test I. If the precipitate in test II is greater than in test I, then Glycosides may be present. Since test I represent the amount of free reducing sugar already present in the crude drug, where test-II represents the Glycosides after acid hydrolysis.

Test for Alkaloids

Mayer's Test: (Potassium mercuric iodide solution).

The extract/sample shows cream colour precipitates.

Dragendoff's Test: (Potassium bismuth iodide solution).

The extract/sample shows reddish brown precipitate.

Wagner's Test: (Solution of iodine in potassium iodide).

The extract/sample shows reddish brown precipitate.

Hager's Test: (saturated solutions of Picric acid)

The extract/sample yellow colour precipitate.

Test for Flavonoids

Shinoda Test (Magnesium hydrochloride reduction test)

To the extract solution add few fragments of magnesium ribbon and concentrated hydrochloric acid drop wise, pink scarlet, crimson red or occasionally green to blue colour appears after few minutes.

Chromatographic Studies

Thin layer chromatographic (TLC) studies were carried out for various extracts for steroids to confirm their presence in the extract.

TLC is mode of liquid chromatography in which extract is applied as a small spot to the origin of the thin sorbet layer supported on a glass plate. The mobile phase move through stationary phase by capillary action sometimes assisted by gravity or pressure. TLC separation

takes place in the open layer with each component having the same total migration time but different migration distance. Mobile phase consists of a single solvent or a mixture of solvent. Silica gel G is used as a stationary phase.

Procedure

Slurry of silica gel G was prepared in distilled water poured over a glass plate to form a thin film. The prepared plates were allowed for setting (air-drying). After setting, the plates were kept in an oven at 100-120 c (30 minutes) for activation. The extract was dissolved in alcohol or any other suitable solvent and spotted over an activated plate (1 cm above from the bottom). It was then kept in previously saturated developing chamber containing mobile phase [petroleum ether: acetone 90 : 10 [carr-price reagent] and allowed to run 3/4th of the height of the plate. The developed plate was removed, air-dried, sprayed with reagent and heated in an oven at 120 c for about 15 minutes. For visualizing of the spots. The R_f was calculated using the following formula.

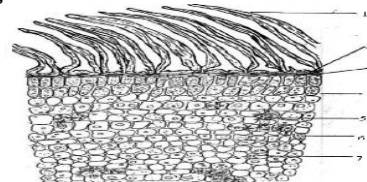
$$R_f = \frac{\text{Distance travelled by the solute front}}{\text{Distance travelled by the solvent front}}$$

The results are tabulated in table no. 5.

RESULTS

In the present study an attempt has made to standardize Bryonosis laciniosa Linn seeds along with its phyto-chemical and pharmacological investigations. It reveals the following data.

Figure 1. Transverse section of seed



- 1.Lignified trichomes
- 2.Epidermal cells
- 3.Parenchyma
- 4.Endosperm
- 5.Plasmodesma
- 6.aleuroena grains
- 7.Oil globulins

Powder Characteristics



Figure 2. Trichomes in Bryonopsis laciniosa

The powdered seeds of *Bryonopsis laciniosa* have shown the presence of the trichomes.

Trichomes: These are covering trichomes which are unicellular, uniseriate with acute apex



Phytochemical Investigations

The respective extracts were weighed and percentage extractive values were determined and reported in following table. Percentage extractives and physical characteristics of the various extracts of *Bryonopsis laciniosa*.

TLC Profile for Steroids

Stationary Phase: Silica Gel G
Mobile Phase: Petroleum Ether: Acetone [90:10]
Visualizing Agent: Carr-Price Reagent.
Colours of the spots: yellowish brown

Table 1. Macroscopic characteristics of Dried Leaves

S.no	Characters	Observation
1	Color	Dark brown
2	Odour	Unpleasant
3	Taste	Bitter
4	Size	Seeds Ca. 5×3mm, grey, belted attenuate with raised projections on both sides.
5	Shape	ovoid, with thickened, corrugated, margins;

Table 2. Physico-Chemical Parametres

Parameter	Percentage (%)
Extractive Values	
• Alcohol soluble	15.26% w/w
• Hydro alcoholic	38.67% w/w
• Water soluble	13.89% w/w
Ash Values Total ash	12.9% w/w
Moisture content	0.04% w/w

Table 3. Percentage extractives and physical characteristics of the various extracts of *Bryonopsis laciniosa*

Extract	%Dry weight in grams	Color	Odour	Constituency
Alcoholic	55.2	Dark brown	characteristic	Sticky
Hydro-alcoholic	38.67	Light brown	Characteristic	Sticky

Table 4. Qualitative Chemical Analysis of various extracts of *Bryonopsis laciniosa*

Phyto-constituents	Alcoholic extract	Hydro-alcoholic
Sugars	-ve	-ve
Tannins	-ve	-ve
Proteins & AA's	-ve	-ve
Steroids	+ve	-ve
Glycosides	-ve	-ve
Alkaloids	-ve	-ve
Flavonoids	-ve	-ve
Bitters	+ve	+ve

Table 5. TLC Profile of *Bryonopsis laciniosa*

Extract	No of spots	R _F value
Alcoholic	1 spot	0.48
β-sito sterol Std	1 spot	0.46

CONCLUSION

Phytochemical screening was done for identification of active chemical constituents. Results concluded that identified compound contains steroids and when compared with standard it was revealed that compound is β-sito sterol and compound also contains

some bitter principles as reported. Many pharmacological activities done on this plant has shown the best results so further pharmacological investigation can be done and further phytochemical screening can be done for isolation of active constituents.



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