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ISOLATION, PHYTOCHEMICAL INVESTIGATION AND PHARMACOLOGICAL SCREENING FOR SEED EXTRACT OF BRYONIA LACINIOSA LINN BELONGING TO FAMILY CUCURBITACEAE

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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 -sito sterol. It also contains some bitter principles as reported.

KEYWORDS: Traditionally it is used as Antipyretic, Analgesic, Anti-inflammatory, Anti-microbial.

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]

Taxonomy of the Plant

Bryonopsis laciniosa

Domain:	Eukaryota
Kingdom	Plantae
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 seeeds are obtained from the plant Bryonopsis laciniosa belonging to family Cucurbitaceae. Other Species of Bryonopsis lacinioa are Bryoniaalba, Bryoniaacuta, Bryoniacallosa, Bryoniacretica, Bryonia geminate, Bryoniaepigaea, Bryoniaaspera.[3]

Distribution

It is commonly available plant in India and also found in many parts of the world like USA.[3]

Description

It is common herb found all over India. It is herbaceous, slender, extensive, monoecious climber. Leaves are palmately 3-7 lobed, membraneous and glabrous. Flowers are creamy white in axillary fascicles. Seeds are ovate, creamy-white or pale yellow, minutely scorbiculate.[4]

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, Antimicrobial and Analgesic.[6]

MATERIALS AND METHODS

Bryonopsis laciniosa Seeds, Floroglucinol HCI, Alcohol, Water, Dessicator, Flat bottom dish, Glassstopper, 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 SaiGoutami College, Ibrahimpatnam R.R dist.Telangana.[6]

Pharmacognosic Study

A.Macroscopy Macroscopic: Features and Organoleptic features viz. colour, odour, taste, texture, shape and size of Bryonopsis laciniosa seeds were observed.[5]

B. Microscopy

Transverse Section of Seed A thin cross section of seed was taken. Then it was stained with Phloroglucinol and HCI.The details of transverse section observed.[5]

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.[1, 2]

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 1050 c 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 desiccators and weighed. The content of total ash was calculated in mg per gm of air-dried material.[7]

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.

Sr. no.	Plant names	Part	Colour	Nature	% yield w/w
1	**shivlingi seeds**	**Fruit**	Dark brown	Solid	20.1

Preliminary Photochemical 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.[8]

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.[7]

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 flatbottomed dish and evaporated to dryness over a waterbath. The residue was dried at 1050C 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.

Qualitative Chemical Test

Test for carbohydrates

Molish's test

Treat the extract slowly with few drops of alcoholic-napthol. 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.[9]

Test for Steroids AndTriterpinoids

Libermann-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.[9]

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.[10]

Sr. no.	Plant names	Flavonoids			
		Shinoda's test	Lead acetate solution.	NaOH test	5% Ferric chloride test
1	shivlingi seeds	--	--	--	--

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.

Sr. no.	Plant names	Alkaloids			
		Dragendroff's test	Wagner's test	Hager's test	Mayer's test
1	Bryonia	++	++	++	++
Sr. no.	Plant names	Carbohydrates			
		Molisch's test	Barfoed's test	Benedict's test	
1	Bryonia	++	--	++	

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.

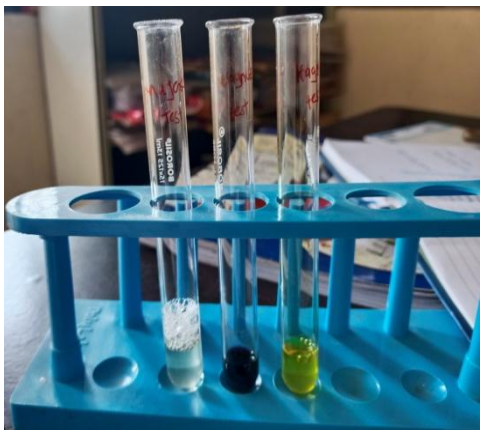
PHYTOCHEMICAL EVALUATION

PHYTOCHEMICAL SCREENING

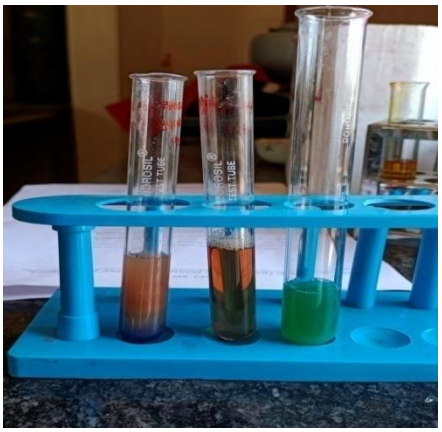
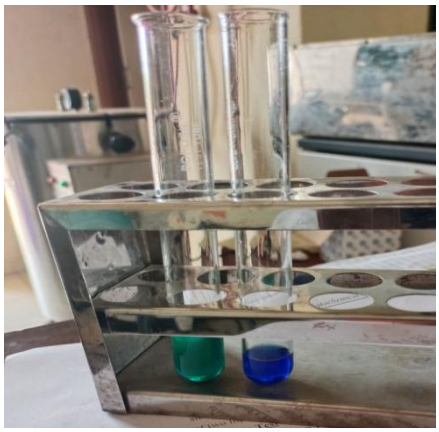
Table no. Phytochemical screening.

Sr. no.	Plant names	Cardiac Glycosides			
		Balget's test	Keller killiani test	Legal's test	
1	shivlingi seeds	++	++	+	
Sr. no.	Plant names	Steroids			
		Salkowaski test	Liebermann Burchard test	Sulphur test	
1	shivlingi seeds	--	++	++	
Sr. no.	Plant names	Tannins			
		5% Ferric chloride test	10% lead acetate	Dil. Iodine	Pot. Permagnate
1	shivlingi seeds	++	++	++	++
Sr. no.	Plant names	Protein			
		Millon's test	Xanthoproteic test	Biuret test	Ninhydrin test
1	shivlingi seeds	++	++	--	--

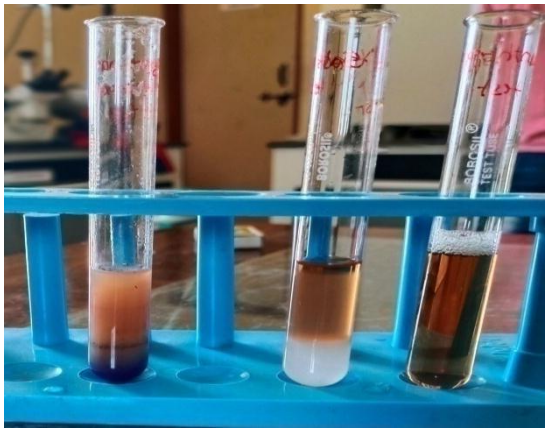
Alkaloids



Steroids

Cardiac Glycosides




Tannins

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 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 minutesFor visualizing of the spots.

The Rf was calculated using the following formula.

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

Sr. no	Name of plant	Mobile Phase	Ratio	RF value
1	shivlingi seeds	Toluene :Ethyl acetate	7:3	
2	shivlingi seeds	Benzene : Ethyl acetate	7:3	
3	shivlingi seeds	Butanol :Acetic acid :water	6:3:1	

Pharmacological Activity

1. Antifungal activity against Candida albicans: Antifungal activity

Stock solution for antifungal activity: For antifungal study each compound was dissolved in DMSO at a concentration of 5mg/ml and 10mg/ml stored in a refrigerator till further used Antifungal activities of the sample A were evaluated by means of agar well diffusion assay. The assay was carried out according to the method of (Hufford et al., 1975). Sabouraud dextrose agar (Hi media) was used for the growth of fungus. Media with acidic pH (pH 5.5 to 5.6) containing relatively high concentration of glucose (40%) is prepared by mixing (SDA) Sabouraud dextrose and distilled water and autoclaved at 121 o C for 15 minutes. Twenty five ml of molten (45 0 C) SDA medium was aseptically transferred into each 100mm×15mm sterile Petri dish.

For counting of spore (fungi) were suspended in normal saline to make volume up to1ml and then counted with help of heamocytometer (neubar chamber). Once the agar was hardened, 6mm wells were bored using a sterile cork borer. Then concentration of 100µland 200µlwas placed in each the well and the plates were incubated for 72 hour at 29 o C. Two wells in each petri dish were supplemented with water and reference antifungal drug fluconazole (1mg/ml) dissolved in water serve as negative and positive control respectively. The antifungal activitywas measured as the diameter (mm) of clear zone of growth inhibition. (Umadevi et al., 2003).

Table No.: 01 Antifungal Activity of Sample 01 against*candidaalbicans.*

	SAMPLES	**CONC. (mg/ml)**	**ZONE IN DIAMETER(mm) against *Candida albican***
	Control	-	00
	Standard (fluconazole)	1	30
1	Sample -A	100µl	17
2	Sample -A	200µl	27

The antifungal activity of the Sample A studied on *Candida albicans*, in which Sample A showed good activity.

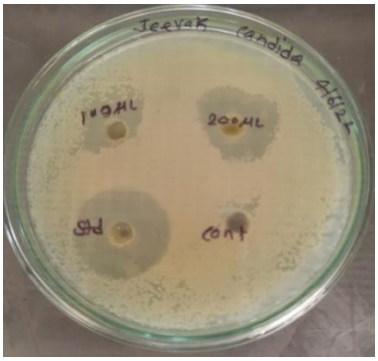


Fig .No. 01: Antifungal Activity of Sample 01 against*candidaalbicans.*

2. Antioxidant activity by DPPH (96 well method) Procedure.

Determination of Anti-oxidant activity

Antioxidant activity in the **Sample A** compounds was estimated for their free radical scavenging activity by using DPPH (1, 1-Diphenyl-2, Picryl-Hydrazyl) free radicals (George *et al.*, 1996).

100µL of **Sample sssA** (100µL) compounds were taken in the micro titer plate. 100µL of 0.1% methanolic DPPH was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively and read the plate on Elisa plate reader at 490nm.

Radical scavenging activity was calculated by the following equation.

DPPH radical scavenging activity (%) = [(Absorbance of control - Absorbance of test sample) / (Absorbance of control)] x 100

Table no.01 Effects of different compounds Sample A against DPPH.

Sr. no.	compounds (1 mg/ml)	ABS (OD)	Mean	Percentage of DPPH radical scavenging
	Control	3.163		
		3.188	**3.152**	
		3.105		
	Std Ascorbic acid	2.527		
		2.475	**2.499**	**20.71**
		2.496		
	Sample A	2.642		
		2.636	**2.625**	**16.71**
		2.601		

At the concentration of 100µL, compound showed good antioxidant property.

3. Anti-inflammatory activity

In vitro anti-inflammatory activity by Protein denaturation method

The reaction mixture (1 mL) consisted of 0.1 mL of egg albumin (from fresh hen's egg), 0.5 mL of phosphate buffered saline (PBS, pH 6.4) and 0.4 mL of Essential oil similar volume of double-distilled water served as control. Then the mixtures were incubated at (37^0c ±2) in an incubator for 15 min and then heated at 70^oc for 5 min. After cooling, their absorbance was measured at 660 nm by using vehicle as blank. Ibuprofenat concentration 2 and 4 mg/ml) was used as reference drug and treated similarly for determination of absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula,

% inhibition = absorbance of control - absorbance of test / absorbance of control x 100

Table no.01: In vitro anti-inflammatory activity of Sample A by Protein denaturation method.

Sample code	Concentration	Anti-inflammatory activity	
		Absorbance at 660nm	% inhibition
Blank		**1.10**	
Ibuprofen	**2mg/ml**	0.33	70.00
Ibuprofen	**4mg/ml**	0.35	68.18
04	**2mg/ml**	0.40	63.63
04	**4mg/ml**	0.46	58.18

According to the table, it indicates that the sample A showed good anti-inflammatory activity.

Fig. no. 01: In vitro anti-inflammatory activity of Sample 01 Protein denaturation method.

CONCLUSION

The results obtained in present study reveals that Bryonopsis laciniosa fruit extract posses, various, phytochemical, constituents like Flavonoids, saponins, terpinoids, triterpinoids, reducing, sugar, proteins, anthocyanins, tannins, polyphenols, emodins, glycosides, coumarone, lignin and serpentine. Therefore screening intimates, presence of many bioactive chemical constituents which act as antiinflammatory, antimicrobial, antioxidants agents It is suggested that further work should be carried out to isolate, purify and possibly characterize the active constituents responsible for the activity of the plant. Scientific validation is necessary before being put in to practice.

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