Evaluation of Phytoconstituents of *Bryonopsis laciniosa* fruit by UV-Visible Spectroscopy and FTIR analysis

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ABSTRACT

Objectives: To Investigate the phytochemicals present in *Bryonopsis laciniosa* fruit. **Methodology:** Qualitative, Quantitative screening, Compound Identification by UV-Visible method and identification of functional group of the active chemical components were followed by standard procedures. **Result:** The results showed the presence of phytonutrients like reducing sugar, terpenoids, triterpenoids, aminoacids, anthroquinone, polyphenols, glycosides, anthocyanins, tannins, coumarins, emodins, saponins, total alkaloids, total flavonoids, lignin and serpentine. These substances may be responsible for the health related properties of the plant which are based on antioxidant,anticancer, antipyretic, antiaphoretic, antidiabetic, anti-inflammation, antiheamatisum, antimicrobial and antiviral activity. **Conclusion:** This study supports the popular use of *Bryonopsis laciniosa* fruit in preparation of various pharmaceutical formulations for human welfare.

Key words: *Bryonia laciniosa,* FTIR, Phytochemical screening, Phytonutrients, UV-Vis.

INTRODUCTION

India is sitting on a gold mine of well-recorded and traditionally well-practiced knowledge of herbal medicines, therefore, any scientific data on such plant derivatives could be of clinical importance Medicinal plants are the richest bio-resource of drugs of traditional system of medicine, modern medicines, nutraceuticals intermediates and chemical entities for synthetic drugs Medicinal plants are of great importance to the health of individuals and communities They are generally used in traditional medicine for the treatment of many ailments[1]

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Many plants contain a variety of phytopharmaceuticals, which have found very important applications in the fields of agriculture, human and veterinary medicine. The use of plants as therapeutic agents in addition to being used as food is age long and there is a great awareness in the use and significance of these medicinal floras (WHO 2002). This has led to intensified efforts on the documentation of medicinal plants.[2] Phytochemicals are natural bioactive compounds found in plants that functions along with dietary fiber and nutrients which protect against various diseases. Vegetables, fruits and nuts are the rich sources of phytochemicals, help in slow aging and reduce the risk of many diseases, including diabetes, heart diseases, infections, cancer etc.[3] Some important phytochemicals are alkaloids, flavonoids, tannins, saponins that posses antifungal,antib acterial,anticancer,anti-inflammatory.[4] The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drug for curing of various diseases The knowledge of the chemical constituents of plants

would further be valuable in discovering the actual value of folk medicines.[5]

Bryonopsis laciniosa (*Linn)* is annual slender plant in the cucurbitaceae family native throughout India from the Himalayas to Ceylon, tropical Africa, Australia. *Bryonopsis laciniosa* is a highly valuable medicinal cucurbit commonly known as lollipop climber and it is called as "Shivlingi" in India. It is one of the most versatile medicinal plant having a wide spectrum of Biological activity[6]

Stem is much branched, slender, grooved and glabrous. Tendrils are slender, scabrous above, smooth, margin denticulate. Flowers monoeicious, often male and female clustered together. Pedicels shorter in male flowers. Fruits barriers, spherical yellowish-green or green-white, 6-stipped, 12-17 mm thick up to 2 cm across. Seeds ovoid, with thickened, corrugated, margins. It is bitter and aperients, and is considered to have tonic properties.[6] Plant flowers and fruits during the period from August to December

Bryonopsis laciniosa leaves and fruits are cooked as vegetables.[7] Leaves and seeds are anti inflammatory and febrifuge. They are used to treat flatulence, fever and reduce inflammation. The seeds are used in Homeopathy and Ayurveda as a tonic for females and they rejuvenate female reproductive system and promotes conception of child. In males the seeds promote spermatogenesis and increase sperm count. The seeds are also used for snake bite. Seeds are antibacterial and anti-fungal. In Homeopathy, a tincture made from the roots are the lollipop plant is prescribed for the treatment of inflammation of uterus, vaginal disorders and other urinary genital proplems.[8] A juice made from the leaves can be applied for pains and joints. Whole plant is used to treat ailments such as asthma, cough and bronchitis .Fruits are used as aphrodisiac, tonic. Sharp, cutting, lancinating or tearing pain, inflammation with muscular tension are cured by this plant The aim of this study is to determine the phytochemicals, in the *Bryonopsis laciniosa (Linn)* fruit, which may provide an insight in its use in traditional medicine.

MATERIALS AND METHODS

Collection of Bryonopsis laciniosa fruit

Bryonopsis laciniosa (Linn) is an annual scan dent herb and is widely spread in india The fruits ware collected from Ramanathapuram Distrct. (Identification: These samples were authenticated by Dr. V. Ramachandran. Associate professor, Department of Botany, Bharathiyar University, Coimbatore. A Voucher specimen (Number:BU/Dept BOT/Bl/16.06.2014) has been deposited at the Herbarium, Bharathiyar University, Coimbatore, Tamil Nadu, India. Each specimen was washed under running tap water, labeled, weighed and annotated with the date of collection. Each specimen dried at 37^{0}C for 15 days, powdered and stored in air tight container.

Preparation of Fruit extract

2 gm of dried finely powdered fruit was taken in a beaker. 30 ml of distilled water and 70 ml of methanol was added. The mixture was shacked by continuous stirring at room temperature for 30 minutes and kept for 2 days. Then the solvent was allowed to evaporate and the extract was used for the phytochemical analysis.

Phytochemical Screening

Preliminary phytochemical tests were carried out in the ethanolic extract of *Bryonopsis laciniosa* fruit using standardized procedures to identify the constituents as described.[9]

Quantitative analysis of secondary metabolites by HPLC Analysis

Sample preparation

The extraction was carried out using 2 ml of fermented broth with 50 mL of 95% ethanol under 80 KHz, 45°C in ultrasonic extraction device for 30 min, repeated twice. The extract was collected and filtered; the filtrate was dried at 50°C under reduced pressure in a rotary evaporator. The dried crude extract was dissolved in 100 ml mobile phases. After filtering through a filter paper and 0.45 mm membrane filter (Millipore), the extract was injected into HPLC.

HPLC conditions

Samples were analyzed using an RP-HPLC method,[10] Shimadzu Corp., Kyoto, consisting of a LC-10ATVp pump, SCL 10A system controller and a variable Shimadzu SPD- 10ATVp UV VIS detector and a loop injector with a loop size of 20 µl. The peak area was calculated by a CLASSVP software. Reverse-phase chromatographic analysis was carried out in isocratic conditions using a C-18 reverse phase column (250×4.6 mm i.d., particle size 5 µm, Luna 5 µ C-18; phenomenex, Torrance, CA, USA) at 25°C. The gradient elution of solvent A [water-acetic acid (25:1 v/v)] and solvent B (methanol) had a significant effect on the resolution of compounds. As a result, solvent gradients were formed, using dual pumping system, by varying the

proportion of solvent a [water-acetic acid (25:1, v/v)] to solvent B (methanol). Solvent B was increased to 50% in 4 minutes and subsequently increased to 80% in 10 minutes at a flow rate of 1.0 mL/min. Detection wavelength was 280 nm.

UV visible and FTIR Spectroscopic analysis

The extracts were examined under visible and UV light for proximate analysis. For UV and FTIR spectrophotometer analysis, the extracts were centrifuged at 3000 rpm for 10 minutes and filtered through Whatmann No. 1 filter paper by using a high pressure vacuum pump. The sample is diluted to 1:10 with the same solvent. The extracts were scanned in the wavelength ranging from 260-900 nm using Perkin Elmer Spectrophotometer and the characteristic peaks were detected. FTIR analysis was performed using Perkin Elmer Spectrophotometer system, which was used to detect the characteristic peaks ranging from 400-4000 cm^{-1} and their functional groups. The peak values of the UV and FTIR were recorded. Each and every analysis was repeated twice for the spectrum confirmation.[11]

RESULTS AND DISCUSSION

In this present study the *Bryonopsis laciniosa(Linn)* fruit extract was Subjected to phytochemical screening and it is represented in Table 1. Saponins, flavonoids are found to be abundant while terpenoids, triterpenoids, proteins, anthocyanins, coumarins are in moderate amount. Alkaloids, reducing sugar, amino acids, tannins, polyphenols, glycosides, emodins are present in trace amount. Selectively total alkaloids, total flavonoids, tannin, lignin, serpentine and glycosides were estimated quantitatively and given in Table 2. The fruit are rich in the flavonoid content when compared to glycosides, alkaloids, tannins, lignins and serpentine.

The fruit of *Bryonopsis laciniosa* appear to be rich in secondary metabolites, widely used in traditional medicine to combact and cure the various ailments. Flavonoids are found to be abundant in the fruit and they protect against allergies, Platelet aggregation, microbial infections, Ulcers, hepatotoxins and tumors.[12] Flavonoids reduce the risk of estrogen induced cancers by interfering with the enzymes that produce estrogen.[13] They act as powerful protective agent against gastrointestinal infections, inflammations, Odema and inhibit the synthesis of Prostaglandin E2, F2 and thromboxane B2. They are powerful antioxidants and free radical scavengers, which prevent oxidative cell damage, and have strong anticancer activity They also inhibit microbes which are resistant to antibiotics. Presence of Saponins are responsible for the, foaming activity and cell membrane permeabilizing activity. Especially Saponins have haemolytic property, induce cytotoxic effect, expectorant action, antitumor, antimutagenic activities and can reduce the risk of cancers by preventing the cancer cells from growing. It also used to stop bleeding and in treating wounds.[14] Traditionaly saponins are used as detergents, foaming, surface active agents, pesticides, molluscides and have relationship with oxytocin which controls the onset of labor of women and the subsequent release of milk.[15] Saponin have the ability to modulate the cell mediated immune system as well as enhance antibody production.[16] Terpenoids posses membrane distruption and inhibitory effect against fungi and bacteria and also have inflammatory, analgesic, anticancer, antimicrobial, antiviral, anti ulcer, hepaticidal and antitumor activities.[17] Anthocyanins help the immune system and protect the body against influenza virus. Various studies have been

Table 1: Preliminary phytochemical Screening of *Bryonopsis laciniosa* fruit extract

Name of the Compound	Observation
Tannins	+
Phlobatannins	---
Saponin	+ + +
Flavonoids	+ + +
Steroids	---
Terpenoids	+ +
Triterpenoids	+ +
Alkaloids	+
Carbohydrates	+
Amino acids	+
Anthroquinones	---
Polyphenols	+
glycosides	+
Proteins	+ +
Anthocyanins	+ +
Coumarins	+ +
Emodins	+

(+) Presence; (--) Absence ++ = Medium, +++ = High concentrations.

Table 2: Quantitative analysis of Phytochemicals of *Bryonopsis laciniosa* fruit extract

Name of the Compound (mg Kg-1)	Observation
Total Alkaloids	0.86 ± 0.05
Total Flavonoids	2.36 ± 0.16
Tannin	0.35 ± 0.02
Lignin	0.42± 0.02
Glycoside	0.06 ±0.01
Serpentine	0.13 ±0.01

Table 3: UV-VIS Peak Values of Extract of *Bryonopsis laciniosa* fruit

Wave length (nm)	Absorption peak
208.04	2.76
271.94	0.72
881.96	0.04

proved that coumarins have strong antioxidant effect due to its ability to show both antimutagenic as well as anticarcinogen effect Many coumarin derivatives act as free radical scavengers[18] Non toxicity and high activity of several Coumarins was observed in the inhibition of carcinogenesis produced by benzo[*a*]pyrene.[19] Generally Coumarins are highly potent anti inflammatory agent and directed against cell-adhesion molecule and they play as effective anticoagulants by inhibiting the function of Vitamin K which is essential for prothrombin biosynthesis.[20] Alkaloids posses plasmolytic, anticholinergic, analgesic, stimulants, antimalarials and anesthetic activity and reduces the fever and headache.[21] Phenols are highly effective anticoagulants, antioxidants, immune enhancers, hormone modulators, and they modify the prostaglandin pathways, protect platelets from clumping and inhibits the enzymes which stimulates the inflammation.[22] Saponins and Glycosides shows antimicrobial activity.[23] Glycosides are cardio protective and used to treat the cardiac arrhythmia and congestive heart failure Tannins are moderately present and posses haemostatic activity, potential biological antioxidant, anti diarrheal, anti hemorrhoidal, antiviral, antiparasitic,[24] antibacterial, antifungal, proton precipitating agent and effective metal ion chelator.[25] Apart from this tannins posses astringency property i.e. faster the healing of wounds and inflamed mucous membrane. The potential anti mutagenic and anti carcinogenic activity has been related to their anti oxidative property, which is important in protecting cellular damage including lipid peroxidation. Moderate amount of polyphenols posse's cellular support and form the integral part of the cell wall. They are anti apoptotic, anti aging, anti carcinogenic, anti inflammatory, anti atherosclerotic, cardiovascular protective and prevent from oxidative stress as well as inhibition of angiogenesis and cell proliferation.[26] Emodins has anti angiogenetic effect[27] Plenty of pharmaceutical studies indicates that emodins have anti inflammatory, anticancer and antimicrobial activity anti-cancer, anti-microbial and anti-inflammatory effects.[28]

The UV-Visible spectra were performed to identify the compounds containing σ- bonds, π-bonds, and lone pair of electrons, chromophores and aromatic rings The profile showed the peaks at 208.04, 271.94 and 881.96 nm with the absorption 2.76,0.72 and 0.04 respectively Table 3 and Figure 1. The result confirms the occurrence of peaks at 208-881 nm reveals that the absorption bands are due to the presence of flavonoids, phenol and its derivatives in the bryonopsis laciniosa fruit.[29]

FTIR Spectrum identified the functional group of the active chemical components present in the fruit based on the peak value in the region of infra red radiation. When the fruit extract was passed in to the FTIR, the functional group of the components was separated based on its ratio. The peak values and the functional groups were represented in

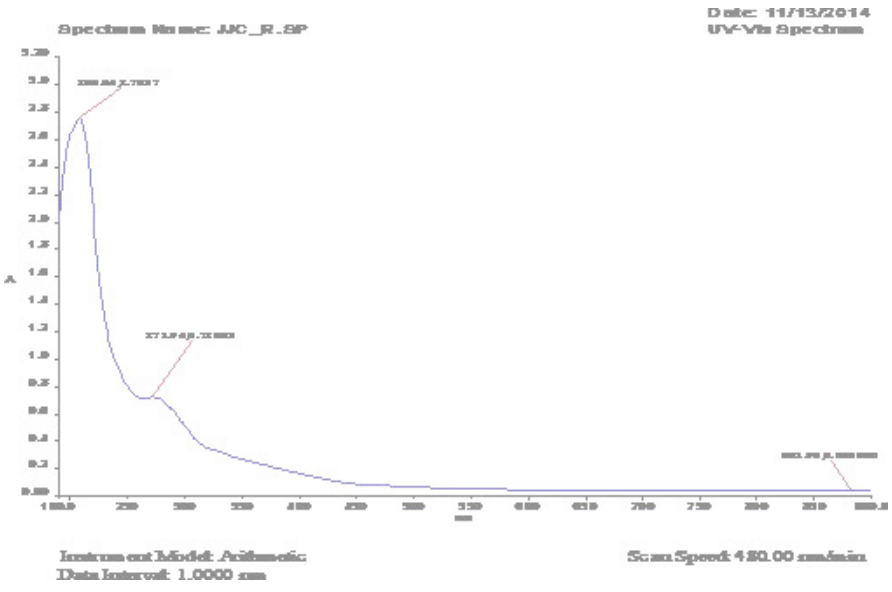

Figure 1: UV-Vis Spectral analysis of Bryonopsis laciniosa fruit extract

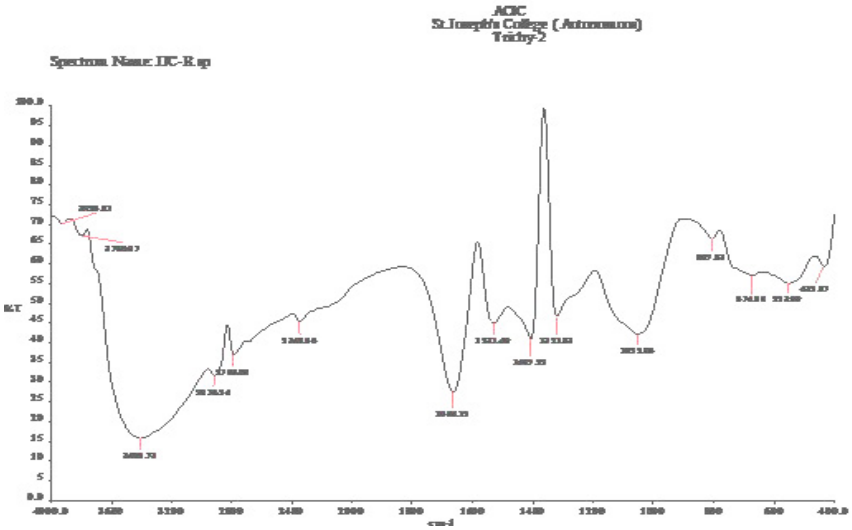

Figure 2: FTIR analysis of Bryonopsis laciniosa fruit extract

Table 4: FTIR Peak Values of Extract of *Bryonopsis laciniosa* fruit

Peak Values	Functional groups
3926.81	Unknown
3796.97	Unknown
3409.73	Unknown
2916.24	Alkanes
2788.08	Deuterated R- OH
2349.66	Unknown
1665.12	Alkenes
1531.40	Ketones
1407.22	Aromatics
1321.61	Alcohols,carboxylic acids,esters,ethers
1052.66	Aliphatic Amines
807.83	Aromatic P-substituted
674.88	Halogen Compound
553.90	Sulfur compounds
432.87	Unknown

extract was passed in to the FTIR, the functional group of the components was separated based on its ratio. The peak values and the functional groups were represented in Table 4 and Figure 2. The results of the FTIR confirmed the presence of Alkenes, Alkenes, Ketones, Aromatics, Carboxylic acids, Esters, Ethers, Aliphatic amines, Halogen compounds, Sulphur compounds and Aromatic P substituted compounds.

CONCLUSION

The results obtained in present study reveals that *Bryonopsis laciniosa* fruit extract posses, various, phytochemical,

Table 4 and Figure 2. The results of the FTIR confirmed the presence of Alkenes, Alkenes, Ketones, Aromatics, Carboxylic acids, Esters, Ethers, Aliphatic amines, Halogen compounds, Sulphur compounds and Aromatic P substituted compounds.

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 anti inflammatory, anticancer, antimicrobial, antioxidants, antidiarrheal, antihemorrhoidal 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.

CONFLICTS OF INTEREST

Authors do not have any conflict of interest.

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