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In Vitro Antioxidant Activity of *Bryonopsis Laciniosa* Fruit Extract

KEYWORDS: Bryonia laciniosa, antioxidant activity, fruit, free radicals.

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ABSTRACT *Antioxidant activity of methanolic extract of Bryonopsis laciniosa fruit was carried out for proving its effect in free radical mediated diseases including diabetes, cardiovascular disease, cancer etc. The methanolic extract was screened for in vitro antioxidant activity by DPPH radical scavenging assay, Total antioxidant assay, Superoxide anion scavenging activity, Nitric oxide scavenging activity, Fe2+ chelating activity assay at different concentrations. Bryonopsis laciniosa fruit extract showed a marked antioxidant activity and it is due to the essential phytochemicals especially flavonoids and polyphenols present in it. The results of the present study showed that the Bryonopsis laciniosa(Linn) fruit extract contains active biocompounds which participate in various pathophysiology of diseases including cancer, cardiovascular diseases ,diabetic, ageing etc. This work has gathered experimental evidence on the Bryonopsis laciniosa(Linn) fruit as natural antioxidant for its capacity to scavenge reactive oxygen and nitrogen species and protect cells/organism from oxidative damage and thus could be effective against oxidative stress.*

INTRODUCTION

The adverse effects of oxidative stress on human health have become a serious issue. Under stress, our body produce more reactive oxygen species (ROS) .This imbalance leads to cell damage (1,2) and many health problems (3). A lack of antioxidants, which can quench the reactive free radicals, facilitates the development of degenerative diseases, including cardiovascular diseases, cancer, neurodegenerative diseases, Alzheimer's disease and inflammatory diseases (4,5). The World Health Organization (WHO) has estimated that 80% of the earth's inhabitants rely on traditional medicine for their primary health care needs and most of this therapy involves the use of plant extracts and their active components (6). Plant and its products are rich sources of phytochemical and have been found to possess a variety of biological activities including antioxidant potential (7). The majority of the active antioxidant constituents are flavonoids, isoflavones, flavones, anthocyanins, coumarins, lignans, and isocatechins. In addition to the above compounds found in natural foods, vitamins C and E, beta-carotene, and tocopherol are known to possess antioxidant potential (2). In recent years, intensive research on natural antioxidants derived from plants has grown due to their potential health-benefits in the search for replacements of synthetic antioxidants. Various parts are traditionally used as food or medicine and may contribute as potential sources for new natural antioxidants. (8,9).Phytochemicals exerting antioxidant actions are largely being recognized as of benefiting human health and disease prevention. These benefits may exhibit a wide range of medicinal properties, including anti-inflammatory, anticarcinogenic, antiviral, anti-allergic and immune-stimulating agents (4). These protective effects have been mostly ascribed to their free radical scavenging, metal chelating and chain breaking effects. Therefore, the aim of this study was designed for the evaluation of antioxidant activity in order to identify new sources of natural antioxidants and to investigate antioxidant properties

Bryonopsis laciniosa is a well known herb spread throughout India from Himalayas to Ceylon, Mauritius, tropical Africa, Malaya, Philippines, Australia and is one of the most versatile medicinal plants having a wide spectrum of biological activity. Stem is much branched, slender, grooved, glabrous. Tendrils are slender, scabrous above, smooth, beneath, margin denticulate, undulate or subcrenulate. Flowers monoeicious, often male and female clustered together. Fruits barriers, spherical yellowish-green or green-white, Seeds ovoid, with thickened, corrugated, margins. It is bitter and aperients, and is considered to have tonic properties(10). Plant flowers and fruits during the period from August to December. *Bryonopsis laciniosa* 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 Seeds are antibacterial and anti-fungal. In Homeopathy, a tincture made from the roots of the lollipop plant is prescribed for the treatment of inflammation. 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 ,serous inflammation, pain in serous cavities, with muscular tension.

MATERIALS AND METHODS

Collection of *Bryonopsis laciniosa* fruit

Bryonopsis laciniosa (Linn) fruits were collected from Ramanathapuram District, Tamil Nadu. The collected fruits were identified and authenticated by Botanist, 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.

Preparation of Fruit extract

2g of dried finely powdered *Bryonopsis laciniosa* fruit was taken in a beaker. 30ml of distilled water and 70ml of methanol was added to it. The mixture was shaken by continuous stirring at room temperature for 30 minutes and

Volume : 5 | Issue : 9 | September 2015 | ISSN - 2249-555X

kept for 2 days. Then the solvent was allowed to evaporate and the extract was used for *in vitro* antioxidant studies.

Preparation of extract

Different concentrations of *Bryonopsis laciniosa* (20, 40, 60 and 80 µg/ml) were chosen for *in vitro* antioxidant activity. L-Ascorbic acid was used as the standard.

***In vitro* antioxidant activity**

DPPH radical-scavenging activity was determined by Shimada, *et al.,* method (11). The total antioxidant activity of the extract was evaluated by the phosphomolybdenum method (12). The superoxide anion radicals scavenging activity was measured by Liu *et al*. Method (13). Nitric oxide scavenging activity was determined according to Garrat method (14). The chelating activity of the extracts for ferrous ions (Fe2+) was measured according to Dinis method (15). The Fe^{3+} reducing power of the extract was determined by Oyaizu method (16).

RESULTS AND DISCUSSION

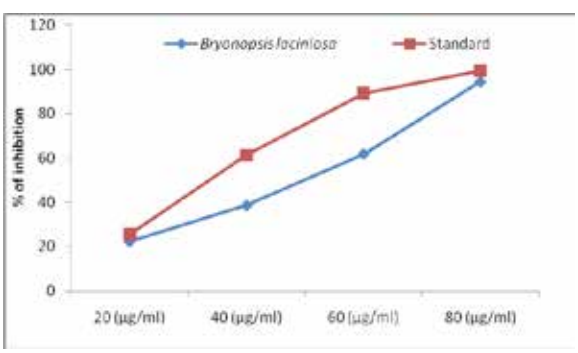
The results of our previous study showed that the *Bryonopsis laciniosa(Linn)* fruit extract contains essential phytochemicals, inorganic elements and vitamins(8,9). These phytochemicals exhibited greatest antioxidant activity. DPPH Scavenging activity , superoxide anion scavenging, nitric oxide scavenging and hydroxyl radical scavenging activities and metal chelating activity (ion chelator and ion reducing power) which participate in various pathophysiology of diseases including cancer, diabetic, ageing etc(8,17). This work has gathered experimental evidence on the *Bryonopsis laciniosa(Linn)* fruit as natural antioxidant for its capacity to scavenge reactive oxygen and nitrogen species and protect cells/organism from oxidative damage and thus could be effective against oxidative stress.

1. DPPH Assay

Table-1. % of DPPH radical scavenging activity of methanolic extract of *Bryonopsis laciniosa* at different concentrations

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
Bryonopsis laciniosa	22.36±1.56	38.65±2.70	61.82± 4.32	94.5±6.61	45.61
Standard (Ascorbic acid)	25.6±2.04	61.26±4.90	88.98±7.11	99.34±7.94	37.34

Values were expressed as Mean ± SD for triplicates



Graph – 1 % of DPPH Radical scavenging activity of methanolic extract of *Bryonopsis laciniosa* at different concentrations

DPPH radical scavenging activity of *Bryonopsis laciniosa(Linn)* fruit extract and standard ascorbic acid are presented in table1 and graph.1. The DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants (**18**). Recently, the use of the DPPH• reaction has been widely diffused among food technologists and researchers, for the evaluation of free radical scavenging activity on extract from plant, food material or on single compounds. In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The proton transfer reaction of the DPPH• free radical by a scavenger (A-H) causes a decrease in absorbance at 517 nm, which can be followed by a common spectrophotometer set in the visible region. The effect of antioxidants on DPPH• is thought to be due to their hydrogen donating ability (**19**). The half inhibition concentration (IC_{50}) of fruit extract and ascorbic acid were 45.61µg ml^{-1} and 37.34µg ml^{-1} respectively**.** The radical-scavengers are free radical inhibitors and primary antioxidants. The degrees of free radical-scavenging activities are attributed to the extent of phenolic nature (20) In the present study, a higher degree of radical-scavenging activity of the fruit extract exhibited a significant dose dependent inhibition of DPPH activity. The prominent free radical scavenging activity is due to synergistic antioxidant activity of various components present in the fruit extract. The fruit extract contains Nonaldehyde, Nonanoic acid, 2-methyltetracosane, Ascorbic acid, Hexadecanoic acid, 9-Octadecenoic acid which already reported to posses high free radical scavenging and antioxidant activity (8). The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentration. The DPPH assay of *Bryonopsis laciniosa(Linn)* fruit extract is near to standard ascorbic acid.

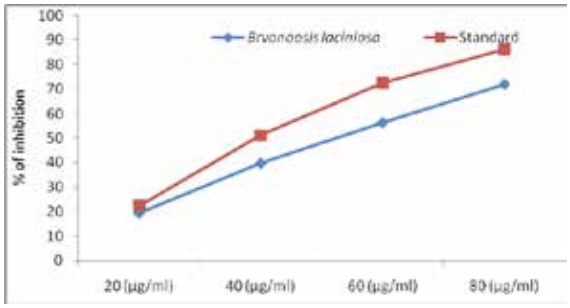
2. Total antioxidant activity

Table-2. % of Total antioxidant activity of methanolic extract of *Bryonopsis laciniosa* at different concentrations.

Parameters	**20** (µg/ml)	40 (µg/ml)	**60** (µg/ml)	80 (µg/ml)	**IC_{50}** (µg/ml)
Bryonopsis laciniosa	19.38±1.35	39.68 ±2.58	56.25±3.93	71.88± 5.03	53.70
Standard (Ascorbic acid)	22.35± 1.80	51.23± 4.09	72.54± 5.80	86.35± 6.91	42.41

Values were expressed as Mean ± SD for triplicates

Volume : 5 | Issue : 9 | September 2015 | ISSN - 2249-555X



Graph – 2 . % of Total antioxidant activity of methanolic extract of *Bryonopsis laciniosa* at different concentrations

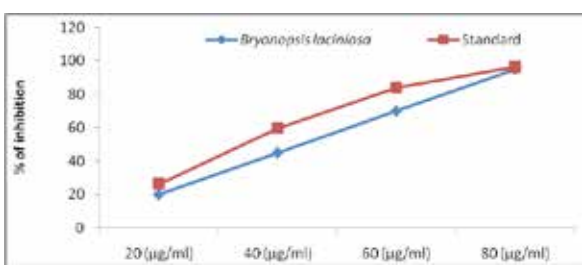
The total antioxidant capacity of the methanol extract of *Bryonopsis laciniosa(Linn)* fruit is given in table 2 and graph 2. The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/ Mo (V) complex with a maximal absorption at 695 nm. This assay is successfully used to quantify vitamin E in fruit,(21). Vitamin E is a powerful antioxidant that helps to reduce the atherosclerosis and posses strong anticancer activity by increasing apoptosis (22) and the profound antioxidant activity of *Bryonopsis laciniosa(Linn)* fruit could be attributed to flavonoids,saponins,terpinoids,triterpinoids,,antho cyanins,tannins,polyphenols,emodins,coumarins,lignin and serpentine content. (8). Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing concentration of the fruit extract. The half inhibition concentration (IC_{50}) of fruit extract and ascorbic acid were 53.70µg ml^{-1} and 42.41µg ml^{-1} respectively**.**

3. Nitric oxide scavenging activity

Table -3. % of Nitric oxide scavenging activity of methanolic extract of *Bryonopsis laciniosa* at different concentrations

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
Bryonopsis laciniosa	20± 1.4	45 ±3.15	70± 4.9	95± 6.65	44
Standard (Ascorbic acid)	26.21 ± 2.04	59.62± 4.65	84.23 ± 6.56	96.45 ± 7.52	35.89

Values were expressed as Mean ± SD for triplicates



Graph-3 . % of Nitric oxide scavenging activity of methanolic extract of *Bryonopsis laciniosa* at different concentrations

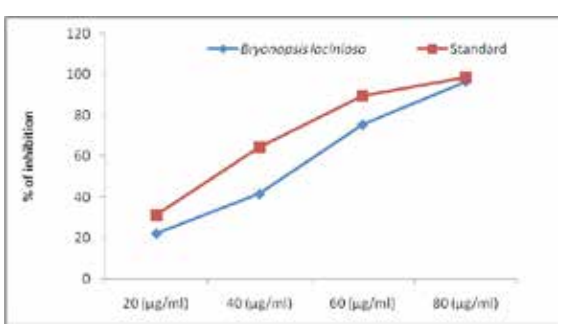
Bryonopsis laciniosa(Linn) fruit extract inhibits the nitric oxide radical scavenging activity in dose dependent manner and the results are given in table-3 and graph- 3. The IC_{50} being 44.0µg ml^{-1} and ascorbic acid was 35.89µg ml^{-1} respectively**.** Nitric oxide (NO) is a potent pleiotropic mediator of physiological processes such as smooth muscle relaxation, neuronal signaling, inhibition of platelet aggregation and regulation of cell mediated toxicity. It is a diffusible free radical which plays many roles as an effector molecule in diverse biological systems including neuronal messenger, vasodilation and antimicrobial and antitumor activities (23,24). Flavonoids are found to be abundant in the fruit and they protect against allergies, Platelet aggregation, microbial infections,Ulcers,hepatotoxins and tumors(8, 25). It also participate in muscle regulation,membrane stabilization,nerve co ordination,ion transport and amino acid activationAdequate amount prevents from peripheral vasodilation,cardiac diseases, depressed skeletal muscle contraction,leukemia and cancer (9 , 26].

4. Superoxide Radical scavenging activity

Table 4- % of Superoxide Radical scavenging activity of methanolic extract of *Bryonopsis laciniosa* at different concentrations

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
Bryonopsis laciniosa	22.09±1.54	41.43 ±2.90	75.32 ±5.27	96.42±6.74	43.29
Standard (Ascorbic acid)	31.25 ± 2.50	64.23 ± 5.13	89.54 ± 7.16	98.51 ± 7.88	31.62

Values were expressed as Mean ± SD for triplicates



Graph- 4. % of Superoxide Radical scavenging activity of methanolic extract of *Bryonopsis laciniosa* at different concentrations

The superoxide anion radical scavenging activities of the extract from *Bryonopsis laciniosa(Linn)* fruit assayed by the PMS-NADH system were shown in Table 4 and graph 4. Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, which are very harmful to the cellular components in a biological system (23). The superoxide scavenging activity of *Bryonopsis laciniosa(Linn)* fruit was increased markedly with the increase of concentrations. The half inhibition concentration (IC_{50}) of *Bryonopsis laciniosa(Linn)* fruit was 43.29µg ml^{-1} and ascorbic acid were 31.62µg ml^{-1}**.** These results suggested that *Bryonop-*

Volume : 5 | Issue : 9 | September 2015 | ISSN - 2249-555X

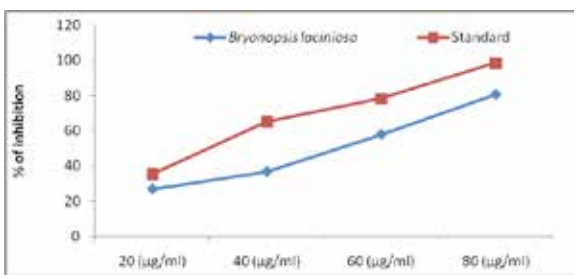
sis laciniosa(Linn) fruit had notably superior superoxide radical scavenging effects. The results obtained in this study thus suggest that the scavenging activity is due to identified phytochemicals ,minerals and vitamins (8.9). Therefore, the data generated from these experiment provide the chemical basis for the wide use of this plant as therapeutic agent for treating various ailments including diabetes, cancer, microbial infections, inflammations etc

5. Iron chelating activity

Table 5- % of Iron chelating activity of methanolic extract of *Bryonopsis laciniosa* at different concentrations

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)	IC_{50} (µg/ml)
Bryonopsis laciniosa	26.92±1.88	36.76± 2.57	58± 4.06	80.76±5.65	49.37
Standard (Ascorbic acid)	35.23 ± 2.81	65.21 ± 5.28	78.51± 6.28	98.65 ± 7.89	31.17

Values were expressed as Mean ± SD for triplicates



Graph 5- % of Iron chelating activity of methanolic extract of *Bryonopsis laciniosa* at different concentrations

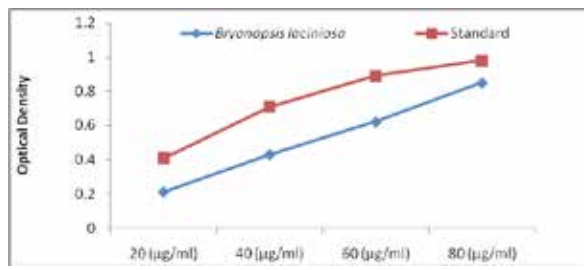
Ferrozine can make complexes with ferrous ions. In the presence of chelating agents, complex (red colored) formation is interrupted and as a result, the red color of the complex is decreased. Thus, the chelating effect of the coexisting chelator can be determined by measuring the rate of color reduction. The formation of the ferrozine– Fe^{2+} complex is interrupted in the presence of methanolic extract of *Bryonopsis laciniosa(Linn)* fruit, indicating that they have chelating activity with an IC_{50} of 49.37µg ml^{-1} and ascorbic acid was 31.17µg ml^{-1} (Table 5 and graph 5). Ferrous ion can initiate lipid peroxidation by the Fenton reaction as well as accelerating peroxidation by decomposing lipid hydroperoxides into peroxyl and alkoxyl radicals (**27,28**). Metal chelating activity can contribute in reducing the concentration of the catalyzing transition metal in lipid peroxidation. Furthermore, chelating agents that form bonds with a metal are effective as secondary antioxidants because they reduce the redox potential and thereby stabilize the oxidized form of the metal ion **(29).** Thus, *Bryonopsis laciniosa(Linn)* fruit demonstrate a marked capacity for ion binding, suggesting their ability as a peroxidation protector that relates to the ion binding capacity.

6.Reducing power assay

Table 6- Reducing power assay of methanolic extract of *Bryonopsis laciniosa* at different concentrations

Parameters	20 (µg/ml)	40 (µg/ml)	60 (µg/ml)	80 (µg/ml)
Bryonopsis laciniosa	0.21±0.014	0.43±0.030	0.62±0.043	0.85±0.059
Standard (Ascorbic acid)	0.41± 0.03	0.71 ± 0.05	0.89± 0.07	0.98 ± 0.08

Values were expressed as Mean ± SD (Optical density) for triplicates



Graph- 6- Reducing power assay of methanolic extract of *Bryonopsis laciniosa* at different concentrations

For the measurements of the reducing ability, the Fe^{3+}–Fe^{2+} transformation was investigated in the presence of *Bryonopsis laciniosa(Linn)* fruit. The reducing capacity of a compound may serve as a significant indicator for its potential antioxidant activity. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition-metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstruction, reductive capacity and radical scavenging **(30,31).** The results depicts the reductive effect of *Bryonopsis laciniosa(Linn)* fruit (8). Similar to the antioxidant activity, the reducing power of *Bryonopsis laciniosa(Linn)* fruit increased with increasing dosage. All the doses showed significantly higher activities than the control indicating that *Bryonopsis laciniosa(Linn)* fruit consist of hydrophilic polyphenolic compounds that cause the greater reducing power.

Conclusion

This work has gathered experimental evidence on the *Bryonopsis laciniosa(Linn)* fruit as natural antioxidant for its capacity to scavenge reactive oxygen and nitrogen species and protect cells/organism from oxidative damage and thus could be effective against oxidative stress.The fruit of *Bryonopsis laciniosa* appeared to have the highest antioxidant activities. The profound antioxidant activity of *Bryonopsis laciniosa* could be attributed to known Phytochemicals and other known compounds. Thus, it can be concluded that *Bryonopsis laciniosa(Linn)* fruit can be used as an accessible source of natural antioxidants with consequent health benefits which might be helpful in preventing the progress of various oxidative stress mediated diseases including aging, diabetis,cardiovascular disease, cancer etc.

Volume : 5 | Issue : 9 | September 2015 | ISSN - 2249-555X

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