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PHYTOCHEMICAL SCREENING AND *IN VITRO* ANTIOXIDANT POTENTIAL OF HYDROALCOHOLIC EXTRACT FROM THE AERIAL PARTS OF *BRYONOPSIS LACINIOSA*

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ABSTRACT

The aim of present study was to estimate the total phenolic and flavonoid content and also to evaluate in-vitro antioxidant potential of hydroalcoholic extract of aerial parts of *Bryonopsis laciniosa (HAEBL).* Antioxidant activity was assessed by using 2, 2- diphenyl-1-picryl-hydrazyl (DPPH) assay, ferric reducing power, Hydrogenperoxide radical scavenging assay, nitric oxide scavenging assay, superoxide radical scavenging assay and hydroxyl radical scavenging assay using ascorbic acid as standard antioxidant. The data obtained from all these models clearly suggested that the antioxidant activity of *HAEBL* was significant and dose dependent. The extract was found to scavenge effectively the free radicals such as peroxides, superoxides and hydroxyl radicals. The total phenolic and flavonoid contents were determined by established methods and were found to be 126.1 mg GAE/g and 262.6 mg of CE/g in gallic acid and catechin equivalents respectively. The antioxidant activities may be attributed to the presence of significant amounts of phenolic and flavonoid compounds.

KEYWORDS: ***Bryonopsis laciniosa*, Hydroalcoholic extract, Phytochemical Screening, TLC, Total phenolic content, Total flavonoid content, Antioxidant activity.**

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INTRODUCTION

The traditional medicine all over the world is nowadays revealed by an extensive activity of researches on different plant species and their therapeutic principles. Plants contain phytochemicals with various bioactivities including antioxidant, anti-inflammatory and anticancer activities. Currently, about 25% of the active components from plants were identified that are used as prescribed medicines.[1] There is an increasing interest in natural antioxidants *e.g.* polyphenols, present in medicinal and dietary plants, which might help in preventing oxidative damage. Free radicals in the body contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia and reperfusion injury of many tissues, central nervous system injury, gastritis, cancer and AIDS.[2-3] Different parts of plants such as seeds, leaves and bark of stem and root known to contain substantial amounts of phytoconstituents such as phenolics, flavonoids and tannins having the ability to inhibit the free radicals that are excessively produced, hence can act as antioxidants.[4] Recently there has been an upsurge of interest in the therapeutic potentials of medicinal plants as antioxidants in reducing free radical induced tissue damages. The continued search among plant secondary metabolites for natural antioxidants has gained importance in recent years because of the increasing awareness of herbal remedies as potential sources of phenolic oxidants.[5] Besides, well known and traditionally used natural antioxidants from tea, wine fruits, vegetables, spices and many other plant species have been investigated in the search for novel antioxidants.[6-7] However, there is dearth of information on the efficacy and more importantly, the toxicity of these remedies.[8] There is therefore, the need for more scientific validation of these claims. *Bryonopsis laciniosa* Linn. belongs to the family *Cucurbitaceae* locally known as 'shivlingi' is distributed throughout India. It is an annual climber with bright red fruits and is reported to be highly medicinal.[9] Locally in India its seeds are being used for promoting conception in women. Ayurvedic literature indicated that the entire plant is a bitter tonic, hepatoprotective, anti-pyretic, laxative and also used to correct the metabolic abnormalities.[10-12] From seeds, a bitter principle bryonin has been reported.[13]Occurrence of punicic acid, goniothalamine, and glucomannan has been reported.[14-16] From seeds, saponin molecules are identified by thin layer chromatography and antibacterial, antifungal, anti-inflammatory and diuretic activities were investigated.[17] Cytotoxic, analgesic, anti-pyretic and anti-diabetic activities have also been reported for various extracts of -this plant.[18-19] The literature survey has revealed that antioxidant potential of the aerial parts of this plant has not been reported. Free radicals arising from metabolism or environmental sources interact continuously in biological systems and their uncontrolled generation correlates directly with molecular level of many diseases.[20] Therefore, this study was designed to evaluate the *In-vitro* antioxidant potential of aerial parts of *Bryonopsis laciniosa* by using different methods.

MATERIALS AND METHODS

Plant Collection and Authentication

The plant selected was collected from Dundigal, a suburb of Hyderabad, Telangana state, India in the month of January 2017. The plant was identified and authenticated by Prof. P. Jayaraman,Plant Anatomy Research Centre (PARC), West Tambaram, Chennai and a specimen copy was maintained in Department of Pharmacognosy, MLR Institute of Pharmacy, Dundigal, Hyderabad (Authentication number: PARC/2017/4068).

Preparation of Extracts

The aerial parts were shade dried and coarsely powdered. About 500gm of powdered drug was extracted by maceration with 70% alcohol. After 72 hrs of maceration, it was filtered and then concentrated by using rotary flash evaporator to yield a semisolid mass. Percentage yield was calculated on the air dried basis and preserved in desiccator.[21-22]

Preliminary Phytochemical Screening

Chemical tests were carried out for identification of bioactive chemical constituents like alkaloids, carbohydrates, glycosides, saponins, phenolic compounds, phytosterols, proteins, amino acids, flavonoids, and tannins by adopting standard procedures.[23-25]

Thin Layer Chromatography

Extract was dissolved in 70% alcohol to form a sample solution. The solvent system was made from toluene: ethyl acetate: formic acid in the ratio of 5:4:1 (v/v). The iodine was used as the visualizing agent to detect the spot. A meter rule was used to measure the distance moved by the solvent and distance moved by spot, from which the retention factor (R_f values) of the various spots was calculated.[26-27]

$$Rf = \frac{Distance\ Travelled\ by\ Solute}{Distance\ Travelled\ by\ Solvent\ Front}$$

Total Phenolic Content [28]

Total phenolic content of the extract was determined by Folin ciocalteu reagent according to Singleton and Rossi using gallic acid as a standard. 0.1ml (100 µg) of sample solution was made up to 3ml using distilled water. About 0.5ml of Folin ciocalteau reagent was added, mixed thoroughly and incubated for 3 min at room temperature. Subsequently 3ml of 20% Na_2CO_3 was added, mixed thoroughly and incubated in boiling water bath for 1 min. The absorbance was measured at 650nm. The amount of total phenolic content was expressed as gallic acid equivalents (GAE, mg gallic acid/g sample) through the calibration curve of gallic acid.

Total Flavonoid Content [29]

Total flavanoid assay was measured by the aluminum chloride colorimetric method. An aliquot (1ml) of extract

or standard solution of catechin (10, 20, 30, 40 and 50µg/ml) was added to 10ml volumetric flask containing 4ml of distilled water. To the flask was added 0.3ml 5% $NaNO_2$. After 5 min, 0.3 ml 10% $AlCl_3$ was added. At 6^{th} min, 2 ml of 1M NaOH was added and the total volume was made up to 10 ml with distilled water. The solution was mixed well and the absorbance was measured against prepared reagent blank at 510 nm. Total flavonoid content was expressed as mg catechin equivalents (CE)/ g of extract.

In-vitro antioxidant activity

The antioxidant activity of the hydroalcoholic extract of aerial parts of *Bryonopsis laciniosa* was determined by using various in vitro assays such as 2, 2- diphenyl-1-picryl-hydrazyl (DPPH) assay, ferric reducing power, hydrogenperoxide radical scavenging assay, nitric oxide scavenging assay, superoxide radical scavenging assay and hydroxyl radical scavenging assay.

DPPH radical scavenging activity

The free radical scavenging activity by plant extract was done according to the method reported by Gyamfi *et al.*.[30] Fifty micro liters of the plant extract in methanol, yielding 100µg/ml respectively in each reaction was mixed with 1ml of 0.1mM DPPH in methanol solution and 450µl of 50mM Tris-HCl buffer (pH 7.4). Methanol (50µl) only was used as control of experiment. After 30 min of incubation at room temperature the reduction of the DPPH free radical was measured reading the absorbance at 517nm. L-ascorbic acid was used as control. All the tests were performed in triplicate and percentage of inhibition was calculated by comparing the absorbance values of the control and test samples. The percent inhibition was calculated from the following equation,

$$\%\ inhibition = \frac{(\mathrm{Abs_{control}} - \mathrm{Abs_{sample}})}{(\mathrm{Abs_{control}})} \times 100$$

Where $Abs_{Control}$ is absorbance of control and Abs_{Sample} is absorbance of test sample.

Ferric Reducing Power

The reducing power was determined according to the method of Oyaizu.[31] Different concentrations of the extract (50, 100 ,150 ,200, 250 µg/ml) ware prepared in methanol and mixed with phosphate buffer (2.5 ml , 0.2M, pH 6.6) and potassium ferric cyanide { $K_3Fe(CN)_6$} (2.5ml , 1%). The mixture was incubated at 50°C for 20 min and tricholoroaceticacid (2.5ml of 10%) was added to the mixture ,which was then centrifuged at 3000rpm for 10min. the upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml)and $FeCl_3$ (0.5ml , 0.1%) and the absorbance was measured at 700nm. Increased Absorbance of the reaction mixture indicated increased reducing power. All the tests were performed in triplicates and ascorbic acid was used as reference standard.

Hydrogen Peroxide Scavenging Activity

The H_2O_2 scavenging ability of the extract was determined according to the method of Ruch *et al.*[32] A solution of H_2O_2 (40mM) was prepared in phosphate buffer (pH 7.4). 100, 200,300,400,500 µg/ml concentrations of extract in 3.4ml Phosphate buffer were added to H_2O_2 solution (0.6ml, 40mM). The absorbance was recorded at 230nm. Ascorbic Acid was used as Standard.

Nitric Oxide Scavenging Activity

Nitric oxide radical scavenging activity was determined according to the method reported by Garrat .[33] Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide, which interacts with oxygen to produce nitrite ions, which can be determined by the use of the Griess-Illosvoy reaction. 2ml of 10mM sodium nitroprusside in 0.5ml phosphate buffer saline (pH 7.4) was mixed with 0.5ml of extract at various concentrations and the mixture incubated at 25°C for 150min. From the incubated mixture 0.5ml was taken out and added into 1.0ml sulfanilic acid reagent (33% in 20% glacial acetic acid) and incubated at room temperature for 5 min. Finally, 1.0 ml naphthyl ethylenediamine dihydrochloride (0.1% w/v) was mixed and incubated at room temperature for 30 min and absorbance was measured at 540 nm.

Superoxide Radical Scavenging Activity[34]

To the reaction mixture containing 0.1 ml of NBT (1 mg/ml solution in DMSO) and 0.3 ml of the extracts, the compound and standard in dimethyl sulphoxide (DMSO), 1 ml of alkaline DMSO (1 ml DMSO containing, 5 m MNaOH in 0.1 ml water) was added to give a final volume of 1.4 ml and the absorbance was measured at 560 nm.

Hydroxyl Radical Scavenging Activity[35]

The degradation of deoxyribose generated by Fenton reaction was measured spectrophotometrically in the presence and absence of test compound. The final reaction mixture in each test tube consisted of 0.3 ml each of deoxyribose (30 mM), ferric chloride (1mM) , EDTA (1mM), hydrogen peroxide (20mM), in the phosphate buffer having pH 7.4 and 0.3 ml of test compound at different concentrations. The test tubes were incubated for 30 min at 37°C after incubation , trichloro acetic acid (0.5 ml , 5%) and thiobarbituric acid (0.5 ml , 1%) were added and the reaction mixture was kept in boiling water bath for 30 min. it was then cooled and the absorbance was measured at 532 nm. The results were expressed as a % of scavenging of hydroxyl radical.

STATISTICAL ANALYSIS

The data is expressed as the mean ± SD analyzed by one-way analysis of variance (ANOVA) test using statistical program SPSS10.

RESULTS AND DISCUSSION

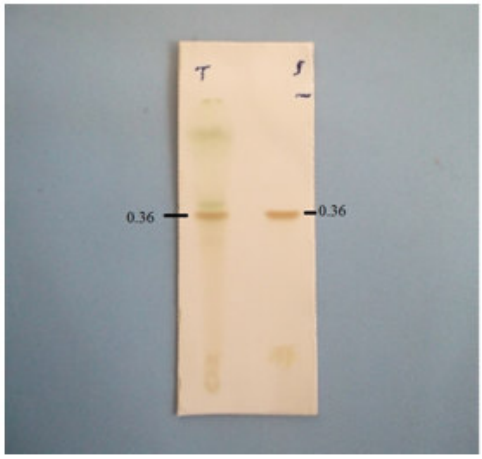
Preliminary Phytochemical Screening

The preliminary phytochemical screening of aerial parts

of *Bryonopsis laciniosa* revealed the presence of flavonoids, phenolic compounds, steroids and tannins.

Thin Layer Chromatography

Hydroalcoholic extract was subjected to thin layer chromatography by taking Quercetin as reference standard. The extract showed the spot with R_f value 0.36 which is corresponding with quercetin as shown in [Figure 1].



T- Test Sample (Hydroalcoholic Extract); S- Standard Quercetin

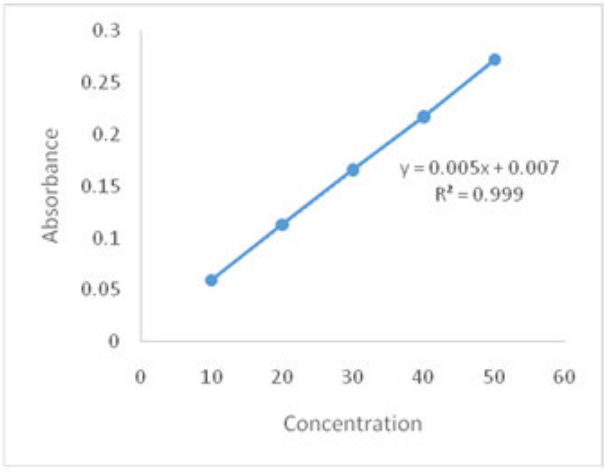
Figure 1
TLC Profile of Hydroalcoholic Extract of Bryonopsis laciniosa

Total Phenolic Content

Total phenolic content was calculated using the standard curve of gallic acid (standard curve equation: Y=0.003x+0.1543, R^2=0.9997) and it was found to be 126.1 mg GAE/g in terms of gallic acid equivalent. Results are shown in [Table 1 and Figure 2].

Table 1
Data showing absorbance of various concentration of Gallic acid

Standard Gallic acid Calibration curve	
Concentration (µg/ml)	**Absorbance**
10	0.184
20	0.214
30	0.244
40	0.273
50	0.304
60	0.331
70	0.364
Sample	
Concentration (100µg/ml)	**Absorbance**
HAEBL (70% Alcohol)	0.194



From the Standard Graph of Gallic Acid, The total phenol concentration present in the extract was found to be: 126.1 mg GAE / g of extract.

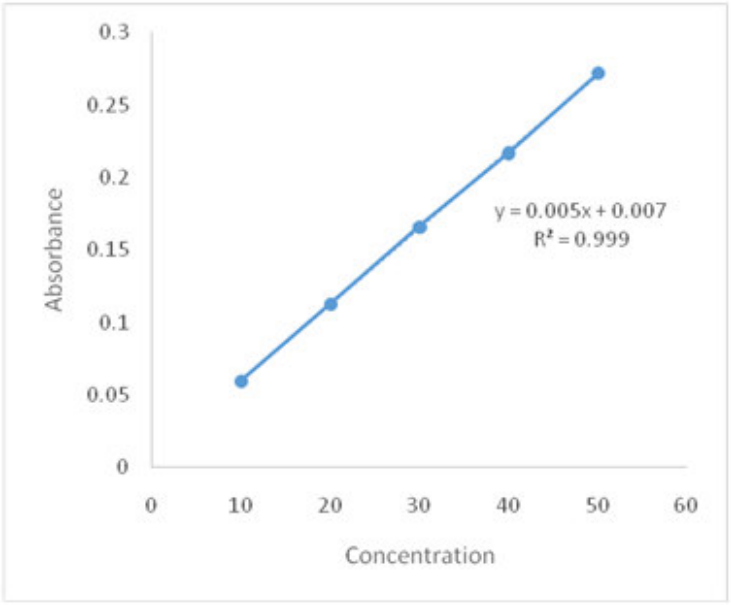
Figure 2
Gallic acid Standard curve

Total Flavonoid Content

The total flavonoids content of Hydroalcoholic extract of *Bryonopsis laciniosa* was found to be 262.6 mg of CE/g in terms of catechin equivalent. Total flavonoid content was calculated using the standard curve of catechin (standard curve equation: Y=0.0053x+0.0072, R^2=0.9999) [Table 2 and Figure 3].

Table 2
Data showing absorbance of various concentration of Catechin

Catechin Standard curve	
Concentration (µg/ml)	**Absorbance**
10	0.060
20	0.113
30	0.166
40	0.217
50	0.272
Sample	
Concentration (100µg/ml)	**Absorbance**
HAEBL (70% Alcohol)	0.135



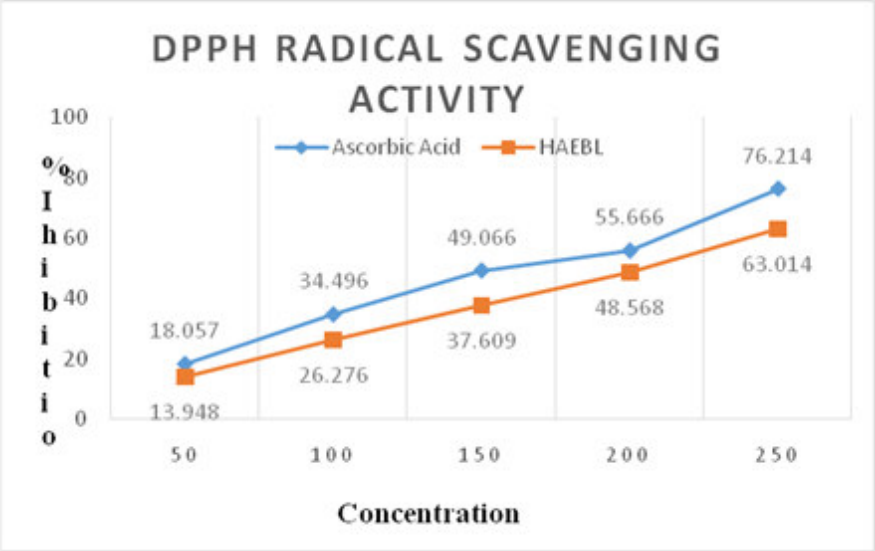
From the Standard Graph of Catechin, The total flavonoid concentration present in the extract was found to be: 262.6 mg of CE / g of extract.

Figure 3
Standard curve of Catechin

In vitro antioxidant activity

DPPH radical scavenging activity

In the DPPH radical scavenging assay DPPH radicals was used as a substrate to evaluate free radical scavenging activity of hydroalcoholic extract of aerial parts of *Bryonopsis laciniosa*. It involves reaction of specific antioxidant with a stable free radical 2,2-diphenyl-1-picrylhydrazyl DPPH*. As a result, there is a reduction of DPPH concentration by antioxidant, which decreases the optical absorbance of DPPH, this is detected by spectrophotometer at 517 nm. The scavenging activity of hydroalcoholic extract of *Bryonopsis laciniosa* was dose-dependent. The absorbance increases with increase in the concentration from 50 to 250 µg/ml. The percentage of inhibition was found as 13.94 at 50µg/ml and 63.01 at 250 µg/ml respectively. From the results it is known that the species, *Bryonopsis laciniosa* possess hydrogen donating capabilities for hydroalcoholic extract and does scavenging free radicals. Results are expressed in [Figure 4].

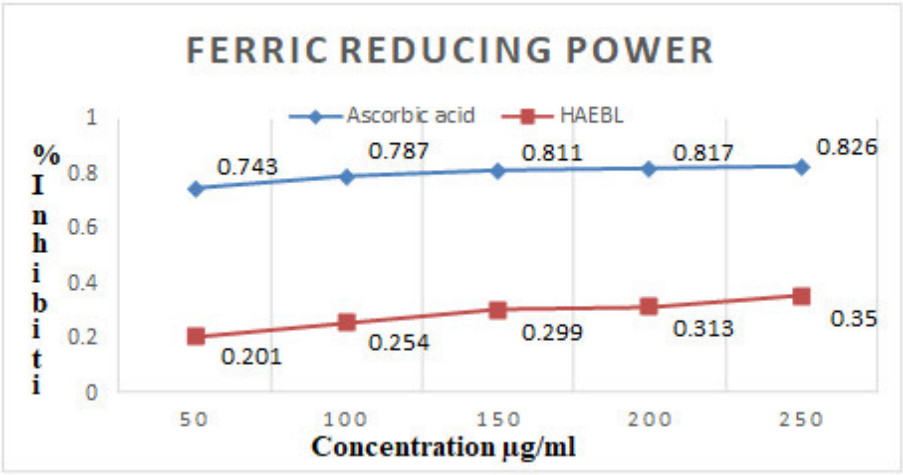


Ascorbic acid was used as reference standard. HAEBL – Hydroalcoholic Extract of Bryobopsis laciniosa
Values were performed in triplicates and represented as mean ± SD.

Figure 4
DPPH Radical Scavenging Activity of Hydroalcoholic extract of Bryonopsis laciniosa

Ferric Reducing Power

The reducing power has been used as one of the important antioxidant capabilities for medicinal plants. The reducing power of hydroalcoholic extract of *Bryonopsis laciniosa* was increased consistently with the increase in the volume of extract from 50µg/ml to 250µg/ml with percentage inhibition from 0.201 to 0.350.From the above it can be inferred that the increase in ferricreducing activity was more [Figure 5].

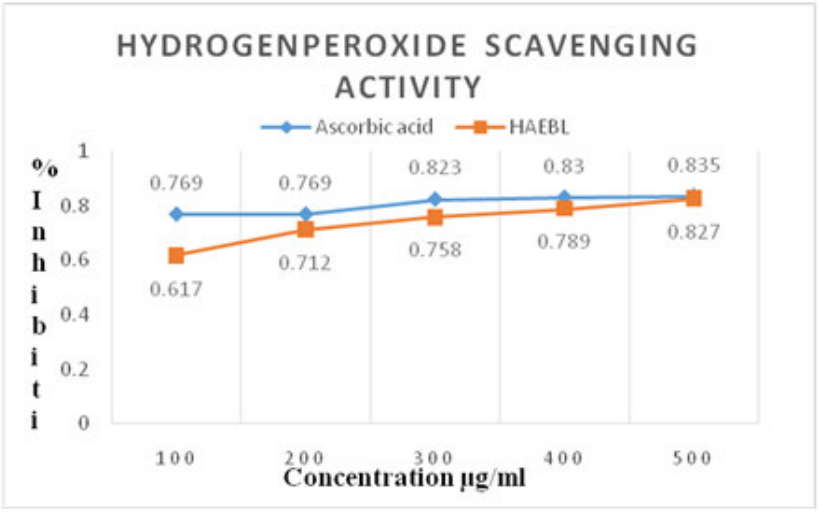


Ascorbic acid was used as reference standard. HAEBL – Hydroalcoholic Extract of *Bryobopsis laciniosa*
Values were performed in triplicates and represented as mean ± SD.

Figure 5
Ferric Reducing Power of Hydroalcoholic extract of Bryonopsis laciniosa

Hydrogen Peroxide Scavenging Activity

Hydrogen peroxide is a weak oxidizing agent and a few enzymes are inactivated directly by oxidation of essential thiol (-SH) groups. H_2O_2 can rapidly cross cell membranes, once inside the cell, hydroxyl radical are formed probably by the reaction of H_2O_2 with Fe^{2+} and possibly Cu^{2+} ion which may be the origin of many of its toxic effects hence it is biologically advantageous for cells to control the accumulation of H_2O_2 in allowed amount. *HAEBL* has demonstrated hydrogen peroxide putrefaction activity in a concentration dependent manner. The decomposition of H_2O_2 by the extract may perhaps result from its antioxidant and free radical scavenging activity. The activity was higher and was comparable to that of standard i.e. ascorbic acid [Figure 6].

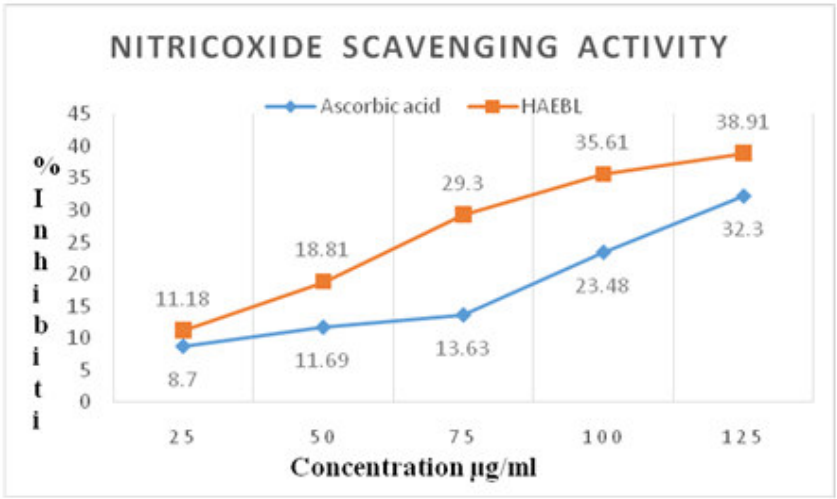


Ascorbic acid was used as reference standard. HAEBL – Hydroalcoholic Extract of Bryobopsis laciniosa
Values were performed in triplicates and represented as mean ± SD.

Figure 6
H_2O_2 scavenging activity of Hydroalcoholic extract of Bryonopsis laciniosa

Nitric Oxide Scavenging Activity

Active oxygen species and free radicals are involved in a variety of pathological events. In addition to ROS, Besides ROS, the role of nitric oxide is manifested in cancer and many pathological conditions including inflammation. The oxidation of tyrosine residue of protein, peroxidation of lipids, and degradation of DNA and oligonucleosomal fragments indicate the potential determination of oxidative damage. Nitric Oxide or reactive nitrogen species such as NO_2, N_2O_4, N_3O_4, nitrate and nitrite are very reactive, which are formed during its reaction with oxygen or superoxide. These formed compounds alter the structure and function of many cellular components Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this damage. Hydroalcoholic extract of *Bryonopsis laciniosa* shows increase in nitric oxide, as shown in the [Figure 7]. The nitric oxide scavenging activity was dose dependent. The values were comparable to that of the standard i.e. ascorbic acid.

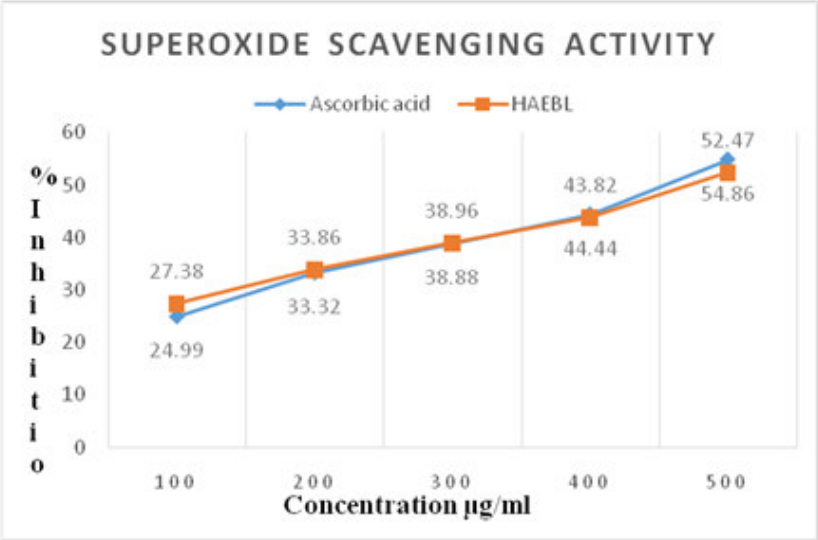


Ascorbic acid was used as reference standard. HAEBL – Hydroalcoholic Extract of Bryobopsis laciniosa
Values were performed in triplicates and represented as mean ± SD.

Figure 7
Nitric oxide scavenging activity of Hydroalcoholicextract of Bryonopsis laciniosa.

Superoxide Radical Scavenging Activity

Superoxide radicals are known to be very harmful to the cellular component. Super oxide free radical was formed by alkaline DMSO which reacts with NBT to produce colored diformazan. Hydroalcoholic extract of *Bryonopsis laciniosa* scavenges super oxide radical and thus inhibits formazan formation. The above graph illustrates increase scavenging of superoxide radicals in dose dependent manner due to the scavenging ability of the *Bryonopsis laciniosa* extract.IC_{50} value of ascorbic acid is 456.57 µg/ml. From the [Figure 8**]** it can be inferred that the extract has better super oxide scavenging ability when compared comparable with that of standard ascorbic acid.

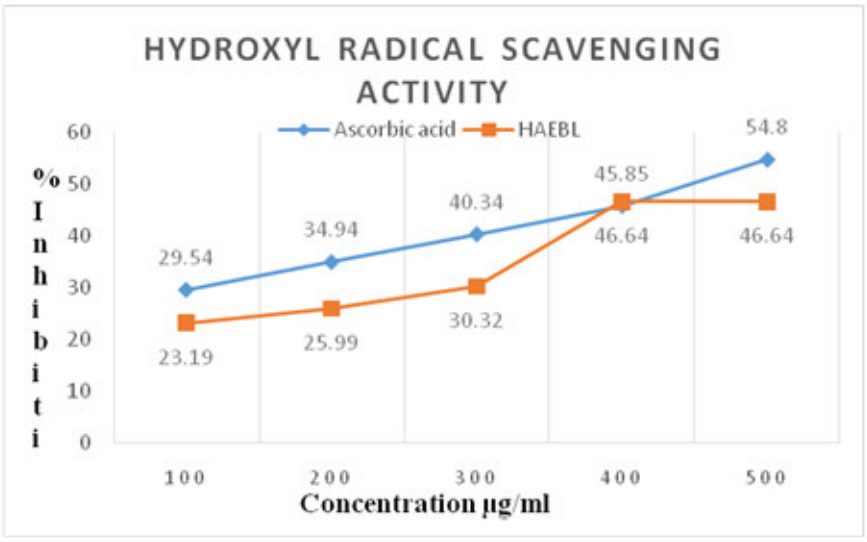


Ascorbic acid was used as reference standard. HAEBL – Hydroalcoholic Extract of Bryobopsis laciniosa
Values were performed in triplicates and represented as mean ± SD.

Figure 8
Superoxide scavenging activity of Hydroalcoholic extract of Bryonopsis laciniosa

Hydroxyl Radical Scavenging Activity

Hydroxyl radical is highly reactive oxygen centered radical formed from the reaction of various hydro peroxides with transition metal ions. It attacks proteins, DNA, polyunsaturated fatty acids in membranes and most biological molecule it contacts and is known to be capable of abstracting hydrogen atoms from membrane lipids and brings about peroxidic reaction of lipids. Activity of the hydroalcoholic extract of *Bryonopsis laciniosa* on hydroxyl radical has been shown [Figure 9]**.** The plant extract exhibited concentration dependent scavenging activity against hydroxyl radial generated in a Fenton reaction system.



Ascorbic acid was used as reference standard. HAEBL – Hydroalcoholic Extract of Bryobopsis laciniosa
Values were performed in triplicates and represented as mean ± SD.

Figure 9
Hydroxy Radical scavenging activity of Hydroalcoholic extract of Bryonopsis laciniosa

CONCLUSION

The phytochemical screening of hydroalcoholic extract of aerial parts of *Bryonopsis laciniosa* showed the presence of flavanoids and phenols which are considered to be responsible for antioxidant activity. The quantification of these bioactive molecules has indicated they are present in significant amounts. The study for their anti-oxidant potential has provided substantial positive data pointing towards the evidence of antioxidant activity. The data obtained from ferric reducing power, hydrogen peroxide radical assay, nitric oxide radical scavenging, and superoxide radical scavenging assay clearly suggested that the antioxidant activity of HABL was dose dependent. The extract was also found to scavenge the free radicals such as peroxides, superoxides and hydroxyl radicals. So these findings of present study suggest that this plant is a

potential source of natural antioxidant. Further studies are warranted for the isolation and characterization of antioxidant compounds, and also *in vivo* studies are needed for understanding their mechanism of action as antioxidants.

CONFLICT OF INTEREST

Conflict of interest declared none.

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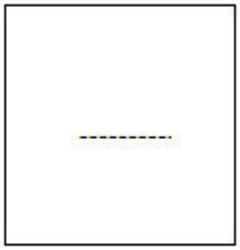

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