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Phytochemical Analysis, Estimation and Antioxidant Activity in *Bacopa monnieri*(L.)

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***Abstract*: *Bacopa monnieri*, an important medicinal plant belonging to the family of Scrophulariceae, which has been valued for centuries in Ayurvedic medicine, was extracted with methanol solvents for the purpose of preliminary screening. The different qualitative chemical tests were performed on the extracts to detect the various phyto constituents or antioxidants present in them. The phytochemical screening reveals the presence of many therapeutically important compounds such as glycosides, alkaloids, saponins phenols. Since the phenolic compounds have remarkable antioxidant activities, our worl aims at evaluating the antioxidant activities, by the in vitro models such as DPPH method. From the present investigative phytochemical analysis of Bacopa monnieri plant extract it is revealed that the antioxidant activity of the plant material is due to the presence of phenolic compounds.**

***Keywords*: Antioxidant, Baccopa monnieri, DPPH.**

1. Introduction

The present study is designed for the phytochemical estimation and antioxidant activity of *Bacopa monnieri (L.)* Bacopa monnieri (L.) also known as Water Hyssop (vernacular-Bramhi), it belongs to family scrophulariaceae and an important nerving herb in Ayurvedic medicine. It is found in throughout India, and also found in Vietnam, China, Taiwan, Sri Lanka, Nepal, and southern states of USA [1]. It is a mall creeping herb with numerous branches with purple flowers and small fleshy, oblong leaves. Generally, it grows in wet and sandy areas, near the water stream. The timing of flowering and fruiting is in summer. Whole plant parts are used for various medicinal purposes. Traditionally it has been used in treatment of anxiety, anger, insomnia, enhancement of memory (development, learning, and enhancement) [2]. *Bacopa monnieri* L**.** (Brahmi) is a well-known memory booster, which propagates vegetatively and rarely by seed. Plant extract is used in improving memory anxiety, attention deficit hyperactivity disorder (ADHD), allergic condition, irritable and bowel syndrome. It is also used in backache, hoarseness, mental illness, epilepsy, joints pain and sexual performance in both male and female. It is also effective against digestive problem, skin disorder. The role of Bacopa monnieri in neuro protective disease such as Alzheimer's disease, Prions disease and Parkinson's disease affects the neurons in the human brain which is chronic and incurable condition which targets mostly the aging population. Progressive deterioration of neurons, sensory information transmission disruption and movement control are major symptoms of above disease. Extract of Bacopa monnieri shows a significant neuroprotective effect against Alzheimer's disease by stabilizing the structural and functional integrity of the membrane [3]. Patients which are treated with Bramhi shows overwhelming oxidative stress as well as Alzheimer's disease. Historically this plant was used approximately 6th century AD, but now practitioners of Ayurveda recognized it.

2. Material and Methods

A. Plant materials

The plant material of *Bacopa monnieri* was collected from the Morabadi, Ranchi in 2018. The Botanical identity of the plant was confirmed by professor Hanuman Prasad Sharma, Designation with address and compare it with the authentic sample present in the Herbarium of Taxonomy Department of Ranchi University, Ranchi. The leaves were plucked from carefully and washed properly in running water. Leaves were dried in shade and then dried in oven at 60 degree. After 2-3 weeks, when leaves were dried completely, they were ground with the help of mixture grinder into powder form. The powder was stored in dry place until used for further experiment [4]

B. Extraction of plant material

The prepared powder of shade dried plant was soaked with methanol. 10gm powder of leaf of *Bacopa monnieri* was completely soaked in 60 ml of methanol for 3 days. The flasks were covered by Alunminium foil to avoid evaporation and then kept in rotatory shaker at room temperature. After 3 days the solution were filtered by using Whattman filter paper No.1. The filtrate were collected in a beaker and kept them in incubator at 37 degree for the evaporation of the solvent. The prepared extract of *B. monnieri* was stored in an air tight container at 4 degree Celsius for further study.

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C. Phytochemical screening

The phytochemical screening was carried out according to the procedure as per standard methods described by Harborne and Evans [5]. Screening was carried out on the aqueous extract of powdered sample to identify the constituents. In this process different reagents are used to know the presence of main group of natural constituents. The different phytochemicals in various extracts was identified by colour reaction with different reagents. Table 1 showing the different type of secondary metabolite of plant extract.

D. Phytochemical estimation

1) Determination of total phenolic content (TPC)

The total phenolic content in methanolic extract was determined by Folin-ciocalteau reagent using this method of Chen et. al. (2013) [6]. This experiment was done by preparing stock solution of Galic acid. For this 1000 µg/ml Galic acid standered stock solution was prepared by dissolving galic acid in methanol. First of all, 5.0 ml of 50% folin-ciocalteau reagent was mixed with 1ml of galic acid solution. Then mixture was left for 5min. After that 4.0 ml of 7.5% sodium carbonate(Na_2CO_3) aquous solution was added to the mixture properly and shaked it. The mixture was incubated for 30 min in dark at room temperature. Absorbance of all samples were taken at 765nm with the help of spectrometer. The same procedure was repeated with the methanolic extract of A. squamosa & A. reticulate. Galic acid was used for preparing standered curve by preparing 1ml of aliquots of 1.0, 2.5, 5.0, 10, 15, 20, 25, 50, 100 µg/ml of galic acid solutions. The result was expressed in milligrams, Gallic acid equivalents per gram of extract (mg GAE/g extract). Total phenolic compound extract was determined by applying the following equations:

$C = C_1 \times V/m$

Where; C= Total content of phenolic compound in mg/g, in GAE (Galic acid equivalent),

C_1 = Concentration of Galic acid established from the calibration curve in mg/ml,

V= Volume of extract in ml,

M= Weight of plant extract in gm.

2) Determination of total flavonoid content

The total flavonoid content was estimated by aluminium chloride method [7]. Quercetin was used as a standered in this method where flavonoids content were measured as quercetin equivalent. The curve calibration of quercetin was drawn for estimation of flavonoids. 1ml aliquots of quercetin (1, 10, 20, 40, 60, 80, 100, 200, 300, 400, 500, 600 µg/ml) was taken into 15ml of volumetric flask, containing 4ml of distil water, 0.3ml of 5% $NaNO_3$ added to the flask, solution was left for 5 min, then 0.3ml of 10% $AlCl_3$ was added and distil water was added to made the volume up to10ml. Same process was done with the plant extract. The Absorbance was taken at 510 nm using UV-visible spectrometer.

3) Determination of total tannins content

The total tannins were determined by Folin-ciocalteu method. Colometric estimation of tannins is based on the measurement of blue colour which is formed by the reduction of phophotungto molybdic acid by tannin like compounds in alkaline medium. About 0.1ml of the sample extract was added in a volumetric flask of 10 ml containing 7.5 ml of distil water and 0.5 ml of FCR. 1 ml of 35% of Na_2Co_3 solution was taken and diluted with 10 ml of distils water. This mixture was shaken properly and kept at room temperature for 30 min. A set of reference of standered soln of Tannic acid (20, 40, 60, 80, 100µg/ml) were prepared in same manner. Absorbance was measured at 700 nm. The total tannic acid content was expressed as mg of Tannic acid equivalent per gram of extract.

4) Determination of total Saponin content

The total saponin content in the extract was determined on dry weight basis [8]. In this method 2 gram of plant sample was mixed with 20ml of 20% aqueous ethanol and heated over water bath (55˚C) for 4 hour with continuous stirring. Then the mixture was filtered and residue was re-extracted with another 20ml 20% ethanol. This extract was reduced to 4ml over water bath at 90˚C and transferred to 250ml separatory funnel. To this 10ml of diethyl ether ($(C_2H_5)_2O$) was added and shaken vigorously. Then the aqueous layer was recovered and 6ml n-butanol was added. This extract was washed twice with 10ml of 5% aqueous sodium chloride (NaCl) and heated on water bath for evaporation. Samples were dried in the oven to a constant weight; the saponin content was calculated in percentage value.

5) Determination of total alkaloid content

For estimation of alkaloids [8], 5gm plant sample was mixed with 20ml of 10% acetic acid in ethanol, incubated for 4 hour and filtered. The filtrate was kept on water bath to make it concentrated or to make its volume 1/4th the original volume. To this, drop by drop concentrated ammonium hydroxide was added to precipitate alkaloid. This solution was left to settle and the precipitate was collected in a filter paper. The collected precipitate was washed with dilute ammonium hydroxide solution and dried in oven at 40˚C, until a constant weight was obtained. Then alkaloid precipitate was calculated in mg/gm of the dried plant material.

6) Determination of antioxidant activity through DPPH radical scavenging activity

1,1-Diphenyl-2-picryl-hydrazyl (DPPH) assay gives an account on the free radical scavenging ability [9]. About 1 mL of DPPH solution (0.1 mmol/L) prepared in methanol was added to 3 mL of test or standard (gallic acid) solution at different concentration (1-64 µg/mL). The mixture was incubated in dark at 30 °C for 30 min and the absorbance was measured at 517 nm and percentage inhibition was calculated. A control reaction was carried out without the test sample. Control was also carried out to determine the absorbance of DPPH, before interacting with the extract. The percentage of inhibition of extract was calculated using the following equation:

$$DPPH\ scavenging\ activity\ (\%) = AB - AA/AB \times 100$$

Where, AB = absorbance of DPPH in MeOH;

AA = Sample=absorbance of DPPH+ sample extract or standard in MeOH.

3. Result

A. Phytochemical Screening

Table 1

Phytochemical	Observation	Present/Absent
Phenol	Reddish black	Present
Tannin	Brownish black ppt	Present
Flavanoid	Yellow colour	Present
Alkoloid	Turbidity obtained	Present
Saponin	Foam formation	Present
Phlobatannin	Red ppt	Present
Anthraquinone	Pink colour not observed	Absent
Terpenoid	Reddish brown colour	Absent
Cardiac glycoside	Ring formed	Present

Table 1 represents the preliminary phytochemical screening test result of significant secondary metabolities. This reveals the presence of phenol, flavonoids, tannins, saponin and alkaloids in the methanolic extract of B. *monneri.*

B. Phytochemical estimation

1) Phenol estimation

The mean absorbance (λ_{max}) of standard gallic acid and Tannic acid is presented in Table 2 and 3 respectively. Table 4 shows the contents of total phenols that were measured by Folin-ciocalteu reagent in terms of gallic acid equivalent. The result of total phenol content was calculated from the regression equation of the standard plot (y = 0.017x + 0.089, R^2 = 0.998, Fig 1). The phenol content in 1 gm methanolic extract of *Bacopa monnieri* (L.) was 44.210 mg GAE/gm (gallic acid equivalent).

The tannin content of the plant extracts was also determined using the Folin-ciocalteu reagent expressed in terms of Tannic acid correspondent from the regression equation of the standard graph (y = 0.0057x + 0.0358; R^2 = 0.9886, Fig. 2). The values obtained for the concentration of the tannin content are expressed in mg of TAE / gm of extract. The tannin content in *Bacopa monnieri* was 9.661 mg of TAE / gm (Table 4).

The alkaloid and saponin per gm of dried root sample from the *Bacopa monnieri* plant were found to have 18.307 mg and 2.01 mg respectively (Table 4).

The total flavonoid content was calculated from the standard graph regression equation (y = 0.0001x + 0.0282; R^2 = 0.9878) and is expressed as quercetin equivalents (QE, Fig. 3). The total flavonoid content recorded at 1 mg/ml of methanolic plant extract was 74.052 mg of QE/gm (Table 4).

Table 2
Absorbance of standard compound, gallic acid in 765 nm wavelength

Concentration (µg/ml)	Absorbance (Mean) λ_{max} =765 nm
10	0.234
20	0.462
40	0.76
80	1.484
100	1.774

Table 3
Absorbance of standard compound (Tannic Acid)

Concentration (µg/ml)	Absorbance (Mean) λ_{max} =700 nm
10	0.088
20	0.188
40	0.323
80	0.512
100	0.547

Table 4
The total phenolic, total flavonoids, tannins, saponins and alkoloids content present in methanolic extracts of *Bacopa monnieri*(L.)

Parameters	Unites	Methanol Extract
Total phenolic content	mg of GAE/gm of extract	44.210
Tannins content	mg of TAE/gm of extract	9.661
Alkoloids content	mg/gm of dry material	18.307
Saponins content	mg/gm of dry material	2.01
Total flavonoid content	mg of QE/gm of extract	74.052

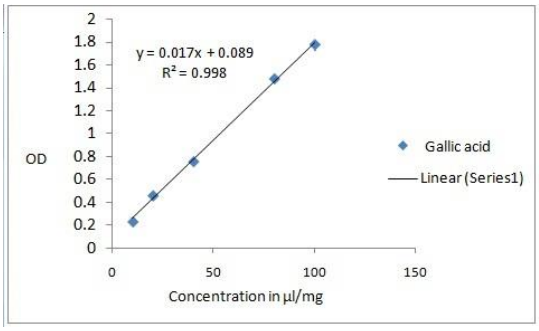

Fig. 1. Calibration curve of standard gallic acid for determination of total phenolic content in *Bacopa monnieri* (L)

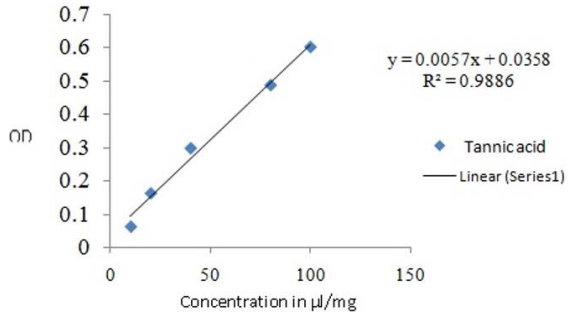

Fig. 2. Calibration curve of standard tannic acid for determination of total tannins content in *Bacopa monnieri* (L)

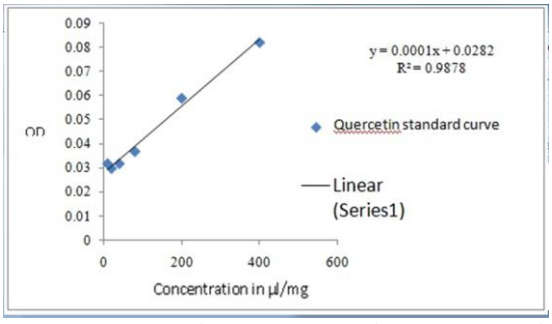

Fig. 3. Calibration curve of standard quercetin for determination of total flavonoid content in *Bacopa monnieri* (L)

Table 5
DPPH radical scavenging activity of *B. monneri* Leaf

Concentration (µg/ml)	Per cent radical scavenging activity	
20	Plant Extract of *B. monneri*	Gallic acid (standard)
40	20.38	30.24
60	40.63	45.38
80	53.38	63.81
100	61.05	68.64
200	70.19	71.38

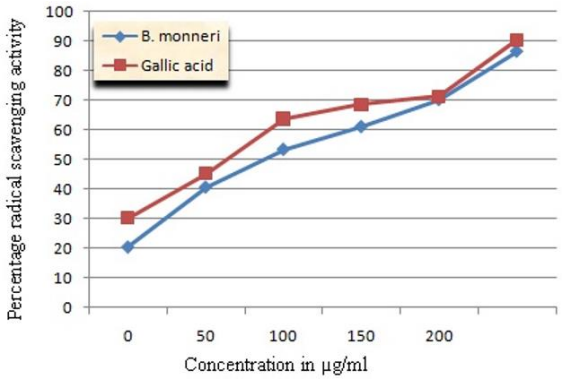

Fig. 4. A comparative graph of per cent radical scavenging activity in *B. monneri* with gallic acid

4. Discussion

The phytochemical screening shows the highest percentage of alkaloid content i.e. 74.052 mg of QE/gm of extract then phenolic content is also high; it is 44.21 mg of QE/gm of extract. The participation of reactive oxygen species in the etiology and pathophysiology of human diseases such as neurodegenerative disorders, inflammation, viral infection, autoimmune pathologies and digestive system disorders such as gastrointestinal inflammation and gastric ulcers was already evident. To understand the role of these reactive oxygen species in several disorders and potential antioxidant.

The reduction capability of the DPPH radical is determined by the decrease in its absorbance at 517 nm induced by antioxidants. The scavenging effects of extract increased with their concentrations to similar extents. From the present result it may be postulated that *Bacopa monnieri* Plant extract reduces the radicals to the corresponding hydrazine when it reacts with the hydrogen donor in the antioxidant principles. The activity increased for each individual of *Bacopa monnieri.* Highest DPPH radical scavenging activity detected in the methonolic extract of *Bacopa monnieri.*

5. Conclusion

As proven time and again, traditional Indian medicine has been significant in curing a number of physical and mental disorders. On these lines the plant species of *Bacopa monnieri* is found to have immense medicinal commercial use of the plant *Bacopa* values in treating disorders because of the presence of vital anti-oxidants. The abundance availability and ease of use have propelled large scale *monnieri* for medicinal and research purpose.

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