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PHYTOCHEMICAL ANALYSIS AND IN VITRO ANTIOXIDANT ACTIVITY FROM THE EXTRACT OF BACOPA MONNIERI (L.) PENNEL – A MULTIPURPOSE MEDICINAL PLANT

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ABSTRACT

Bacopa monnieri, an important medicinal plant belonging to the family of Scrophulariaceae, which has been valued for centuries in Ayurvedic medicine, was extracted with ethyl acetate and 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 photochemical screening reveals the presence of many therapeutically important compounds such as glycosides, alkaloids, saponins, phenols, proteins and carbohydrates. Since the phenolic compounds have remarkable antioxidant activities, our present work aims at evaluating the antioxidant activities by the three *in vitro* models such as DPPH free radical scavenging activity, ferric thiocyanate (FTC) and Thiobarbituric acid (TBA) 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.

KEY WORDS

Phytochemical, Antioxidant Activity, DPPH, FTC, TBA, *Baccopa monnieri*.

INTRODUCTION

Many medicinal plants are the sources of bioactive compounds which have protective or disease preventive properties[1,2]. Plants contain a wide variety of free radical scavenging molecules, such as phenolic compounds, nitrogen compounds, vitamins, terpenoids and other endogenous metabolites, which are rich in antioxidant activity[3]. The intake of antioxidant activity has been associated with reduced risk of cancer, diabetes, respiratory problems and other disease associated with ageing[4].

Bacopa monnieri is a small, creeping herb with numerous branches, succulent, rooting at the nodes, with numerous prostrate branches, each 10-30 cm long. *Bacopa monnieri* which occurs naturally in India and has a long history of being used in the traditional Ayurvedic medicine in the treatment of a number of disorders, particularly those involving anxiety, intellect and poor memory. Traditionally, it was used as a brain tonic to enhance memory development, learning, and concentration. Phytochemical analysis of *Bacopa monnieri* plant extract revealed the presence of various bioactive compounds such as alkaloids, Carbohydrates, Saponins, Proteins and amino acids and Phenol.

MATERIALS AND METHODS

Collection of Plant Material:

The fresh *Bacopa monnieri* plant was collected from in and around Thanjavur District of TamilNadu, India.

Extraction and Preparation of Extract:

The plants were collected, shadow dried and finely powdered. The 10g of dried powdered plant material was extracted with 100ml of ethyl acetate and methanol in conical flask in shaking condition.

The extract was decanted into reweighed glass vials. The process was repeated 3 times with the same material but with the fresh solvent. The solvent was removed by condensation. The extracted residues were weighed and redissolved in different solvents to yield 10mg/ml solutions ready for further analysis.

***PHYTOCHEMICAL ANALYSIS*:**

The Phytochemical Screening was carried out using the standard methods[5,6,7,8,9,] to detect the bioactive compounds like alkaloids, carbohydrates, Glycosides, Saponins, phenols, proteins and amino acids.

IN VITRO ANTIOXIDANT ACTIVITY:

The *in vitro* antioxidant activity of plant extract was carried out using Radical Scavenging Activity (RSA) Nenadis and Tsimidou (2002), Ferric Thiocyanate method[10] and Thiobarbituric acid test[11.]

The RSA activity of different extracts was determined using DPPH (1, 1-diphenyl-2-picrylhydrazyl) assay according to Nenadis and Tsimidou (2002), with small modification. The decrease of the absorption at 517nm of the DPPH solution after addition of the anti oxidant (plant extract) was measured in a cuvette containing 2960 µl of 0.1 mM ethanolic DPPH solution was mixed with 40 µl of 20-200 µg/ml of plant extract. Blank containing 0.1 mM ethanolic DPPH solution without plant extract and vortexed thoroughly, the set up was left at dark room temperature. The absorption was monitored after 20 min. Ascorbic acid (AA) and Butylated hydroxytoluene were used as references. The ability to scavenge DPPH

radical was calculated by the following equation.

$$\% \text{ of DPPH radical scavenging activity (\%RSA)} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} X100$$

$Abs_{control}$ is the absorbance of DPPH radical + ethanol; Abs_{sample} is the absorbance of DPPH radical + leaf extract. Measurements were performed in triplicate. Absorbance values were corrected for radical decay using blank solutions.

According to the Ferric Thiocyanate method, the leaf samples of 4mg in 99.5% ethanol were mixed with 2.51% linoleic acid in 99.5% ethanol (4.1 ml), 0.05 M phosphate buffer, pH 7 (8ml) and distilled water (3.9 ml) and kept in a screw cap container under dark conditions at 40 ° C. To 0.1 ml of this solution, 9.7 ml of 75% ethanol and 0.1 ml of 30% ammonium thiocyanate were added. After 3 minutes, 0.1 ml of 2 M ferrous chloride in 3.5% of HCl was added to the reaction mixture and absorbance of red color was measured at 500 nm each 24 hr until one day after absorbance of the control reached maximum. The control and the standard were subjected to the same procedure as the sample except for the control, where there was no addition of sample, and for the standard 4 mg of sample were replaced with 4 mg of α- tocopherol or BHT.

The samples as prepared for the FTC method were used in TBA test. To 1ml of sample solution, 2 ml each of 20%aqueous trichloroacetic acid were added. This mixture was then incubated in a boiling water bath for 10 min. After cooling, it was centrifuged at 3000 rpm for 20 min and the absorbance of supernatant was measured at 532 nm.

RESULTS AND DISCUSSION

PHYTOCHEMICAL ANALYSIS:

The present Phytochemical analyses of plant extract shows positive result for alkaloids, carbohydrates, saponins, proteins, amino acids and phenols (Table-1)

Table-1

Compounds	Test	Result
Alkaloids	Mayer's	+
Carbohydrates	Fehling's	+
	Benedict's	+
Glycosides	Borntrager's	-
Saponins	Foam	+
Proteins & amino acids	Millon's	+
Phenol	Ferric Chloride	+
	Lead acetate	-

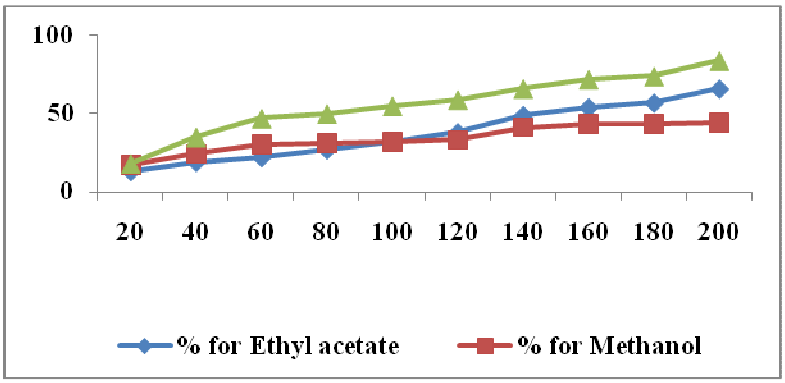
+ ***: Indicates the presence***
- ***: Indicates the absence***

IN VITRO ANTIOXIDANT ACTIVITY:

Radical Scavenging Activity

DPPH radicals react with suitable reducing agent, the electrons become paired off and the solution looses color stoichiometrically depending on the number of electrons taken up. 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 as the concentration increased for each individual of *Bacopa monnieri*. Highest DPPH radical scavenging activity detected in the methonolic extract of *Bacopa monnieri* (Graph-1)

Graph-1
RADICAL SCAVENGING ACTIVITY USING DPPH ASSAY



X axis – Concentration in µg/ml ; Y axis – % Scavenging activity.

FERRIC THIOCYANATE (FTC) AND THIOBARBITURIC ACID (TBA) METHOD **:**

The antioxidant activity of methanolic extract is measured to inhibit lipid peroxidation (LPO) by FTC and TBA method (Graph 2&3).The tested plant extracts showed strong antioxidant activity to inhibit LPO by FTC and TBA method which is indicated by their low absorbance values. The FTC method measures the amount of peroxide produced during the initial stages of lipid oxidation. Then in the later stage of lipid oxidation, peroxide decomposes to form carbonyl compounds that are measured by the TBA method. At a given concentration higher activity was recorded in the extracts of Baccopa monnieri by the standard antioxidant of α- tocopherol or BHT. In general the antioxidant by FTC method is lesser than the TBA method. It mentioned that the amount of peroxide in the initial stage of lipid per oxidation is less than the amount of peroxide in the secondary stage

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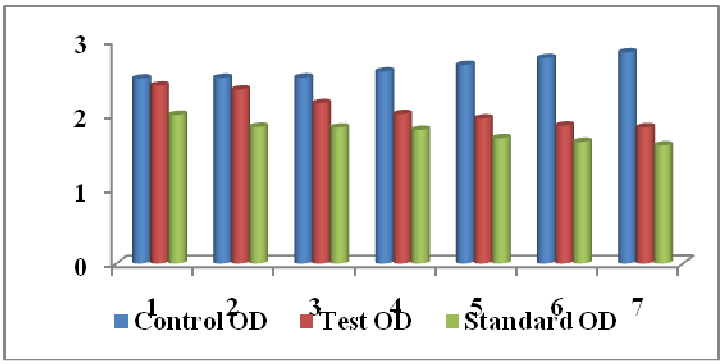
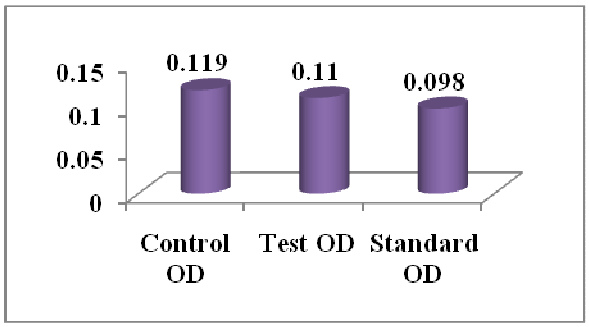
Graph-2
Ferric Thiocyanae (FTC) Method



X axis – Number of days ; Y axis – Optical Density.

Graph-3
Thiobarbituric Acid (TBA) Test



***X axis – Particulars ; Y axis – Optical Density*.**

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 values in treating disorders arising out of mental stress, primarily because of the presence of vital anti oxidants. The abundance availability and ease of use have propelled large scale commercial use of the plant *Bacopa monnieri* for medicinal and research purpose

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