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Research Article

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Antioxidant potential of indigenous medicinal plant *Bacopa monnieri*: An *in vitro* evaluation

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ABSTRACT

Bacopa monnieri has been long used in Indian traditional medicine to treat cognitive disorders and for memory enhancement. Current study was aimed at exploring the antioxidant potential of ethanolic and methanolic leaf extracts of Bacopa monnieri by utilizing in vitro methods. This was achieved by performing catalase, peroxidase, and DPPH assays and also by the determination of total phenolic content. It was found that the methanolic extract of the leaves possessed significantly higher antioxidant levels in comparison to the ethanolic extract. This would help explain the known medicinal properties of Bacopa monnieri in diseases involving oxidative stress. It also shows that methanol is a better solvent than ethanol for extraction of the active components of Bacopa monnieri.

Key words: *Bacopa monnieri*, Antioxidant, DPPH, Catalase assay.

INTRODUCTION

Oxidative stress plays a major role in the establishment and progression of several disorders [1]. Acute and chronic diseases involving oxidative damage have been studied extensively in animal models, cell lines and human subjects [2],[3]. Metabolic disorders such as diabetes and obesity are known to be associated with mechanisms of oxidative stress and generation of reactive oxygen species. Obesity is known to cause other metabolic syndromes such as diabetes, hypertension, dyslipidemia and also cancer and cardiovascular disorders. Studies have proven increased levels of systemic oxidative stress in cases of obesity [4]. Models of drug induced toxicity have also shown that generation of reactive oxygen species and reactive nitrogen species tend to cause oxidative damage to cellular membranes and macromolecules ultimately resulting in tissue injury and damage [5].

There are several medicinal plants such as rosemary, turmeric, pepper, ginger and also Chinese herbs with significant antioxidant property [6]–[9]. The active antioxidant compounds found are flavonoids, isoflavones, flavones, saponins, flavanols, phenols and anthocyanins[10]. Previous studies have proven that food and beverages rich in phenolic content reduce the risk of heart disease [11]. Flavonoids have been found to inhibit the release of histamine and expression of pro-inflammatory cytokines in mammals [12]. Isoflavanoids have the ability to bind with estrogen receptor of human mammary tumour cells and therefore inhibit the breast carcinoma.

Bacopa monnieri(Figure 1) is an ayurvedic plant which is used by medical practitioners in India for many decades. Also known as Brahmi, Aindri, *Lysimachia monnieri* L. Cent., *Graticola monnieri* L., *Herpestis monniera* L.

Kunth., Water hyssop, Thyme-leafed gratiola, Indian Pennywort and Jalabrahmi. It comes under the family of Scrophulariaceae and in genus *Bacopa*. It is an herbal plant with purple flowers found in wet, sandy areas and in tropical regions. Stems and leaves of the plant are extensively used for medicinal purposes.

Figure 1: Aerial parts of *Bacopa monneri*

Bacoside A is the major constituent of the *Bacopa monnieri*[13]. This plant also tends to show cell protective and antioxidant effects because of the presence of the components like flavanoids, saponins, anthocyanins, isoflavanoids and phenols [14]. It can be used as a nerve tonic and believed to increase the memory function of brain and traditionally been used for the treatment of epilepsy, asthma, anemia, ulcers, tumors, anxiety, depression, and various neuropharmacological disorders whereas it has also been used for anti-inflammatory, antipyretic, astringent, laxative, cough, poisoning, and blood disorders [15]–[18]. The present study is aimed to evaluate the *invitro* antioxidant potential of ethanolic and methanolic herbal extract of *Bacopa monieri*.

EXPERIMENTAL SECTION

2.1 Chemicals

All chemicals and reagents used in current study were purchased from Sigma Aldrich Pvt. Ltd., India.

2.2 Plant materials

Bacopa monnieri plant material was collected from Vellore district of Tamil Nadu, India. The leaves of *Bacopa monnieri* were used for the preparation of methanolic and ethanolic extract. The leaves were washed with water and allowed to shade dry and was finely powdered.

2.3 Preparation of plant extract

2.3.1 Methanolic extract

About 5 g of finely powdered leaves of *Bacopa monnieri* was dissolved in 50ml of methanol and incubated in a conical flask for 24 hours at 25°C with constant shaking. Whatmann filter paper no.1 was used to filter the extract. The extract was concentrated using a rotary vaporizer at 40°C under low pressure. The residue was kept in a hot air oven, evaporated and stored at 4°C for further use.

2.3.2 Ethanolic extract

About 5 g of finely powdered leaves of *Bacopa monnieri* was dissolved in 50 ml of ethanol and incubated it in a conical flask for 48 h at 25°C with constant shaking. Whatmann filter paper no.1 was used to filter the extract. The extract was concentrated using a rotary vaporizer at 40°C under low pressure. The residue was kept in a hot air oven and evaporated and stored at 4°C for further use.

2.3.3 Dilutions

Both the extracts were serially diluted to the concentrations of 1:2, 1:4, 1:8, 1:16 and 1:32 in order to determine the optimal concentration with maximum antioxidant potential.

2.4 Antioxidant potential

2.4.1 Assay of catalase activity

The catalase activity was assayed following the method of [19].

2.4.2 Assay of peroxidase activity

Peroxidase activity was estimated by the method of [20]. To 1 ml of extract (of varying dilutions), 3 ml of pyrogallol and 0.5 ml of hydrogen peroxide were added and 3 ml pyrogallol mixed with 1 ml extract served as the blank. The absorbance was measured every 30 s for a total period of 3 min.

2.4.3 DPPH assay

DPPH radicals scavenging activity was estimated by the method of [21]. 1 ml of extract (serially diluted) and 0.5ml of DPPH (0.2 mM) were mixed together. The absorbance was measured at 520 nm against a blank made by mixing methanol or ethanol with DPPH.

2.4.4 Total phenolic content

Total phenolic content was estimated by the method of[22]. 1ml Folin's reagent was added to 1 ml extract (of varying dilutions) and after 2 min of incubation 1ml sodium carbonate was added. The mixture was incubated for 2 h at room temperature. Folin's reagent and sodium carbonate were mixed at a ratio of 1:1 to serve as the blank. The absorbance was measured at 650 nm.

2.4.5 Statistical analysis

The data obtained was analyzed and expressed as mean ± S.D.

RESULTS AND DISCUSSION

3.1 Antioxidant potential of the plant extract of *B. monnieri*

3.1.1 Total phenolic content

Phenolic compounds are large group of plant secondary metabolites and an essential component of human diet.They consist of an aromatic ring having one or more hydroxyl substituents. It has antioxidant properties because of the ability to scavenge free radicals, donate hydrogen atom or electron. Flavonoids are the largest group of plant phenolics[23]. According to the present study, total phenolic content of methanolic extract was 2.2 ± 0.84mg GAE/g and that of ethanolic extract was 1.88 ± 0.77mg GAE/g (Figure 2). Phenolic content was found to be higher in methanolic extract when compared to ethanolic extract.

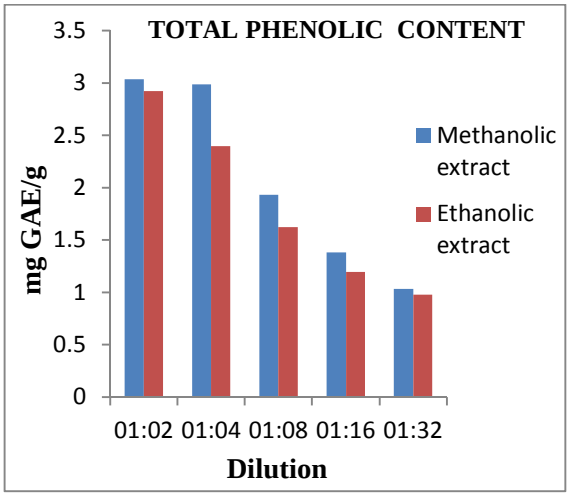

Figure 2: Total phenolic content

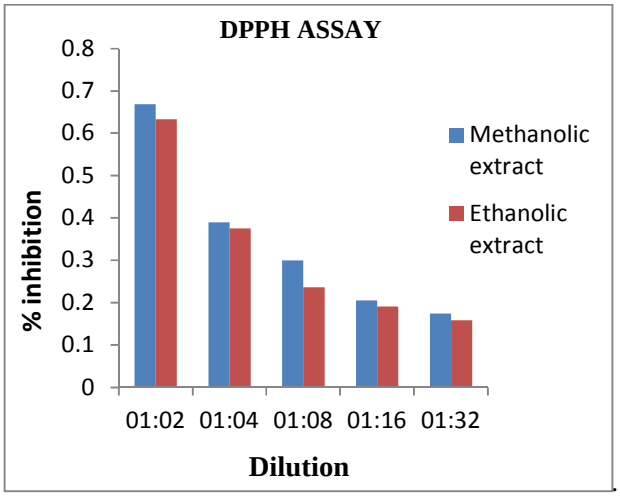

Figure 3: DPPH scavenging activity

3.1.2 DPPH scavenging activity

DPPH radical scavenging activity is widely used method for testing the antioxidant activity of plant extract. DPPH is a stable free radical and this assay is based on reduction of DPPH. When antioxidants react with DPPH, the purple

colour is reduced to yellow coloured stable compound. Hence, DPPH is used to evaluate the antioxidant activity [24]. In the present study, the scavenging activity of the methanolic extract was 0.34±0.19 µg/mL and that of ethanolic extract was 0.34±0.21µg/mL (Figure 3). Hence, higher DPPH scavenging activity was showed by methanolic extract.

3.1.3Catalase activity

Catalase is an antioxidant enzyme that defends against oxidative stress. Hydrogen peroxidase formed by many metabolic processes is actively converted to less dangerous substances with the help of catalase [25]. In this study, the catalase activity of methanolic extract was 0.554 ± 0.1099 and that of ethanolic extract was 0.5006 ± 0.831 (Figure 4). Methanolic extract showed higher catalase activity.

3.1.4 Peroxidase assay

Peroxidases are enzymes found in bacteria, fungi, plants and animals. They are involved in scavenging of reactive oxygen species and are rich source of hydrogen peroxide [26]. These enzymes have a significant role in lignin biosynthesis, detoxification of hydrogen peroxide and hormone generation. From the study, the peroxidase activity of methanolic extract was 0.26±0.06 and that of ethanolic extract was 0.1±0.03 (Figure 5). Methanolic extract showed high peroxidase activity.

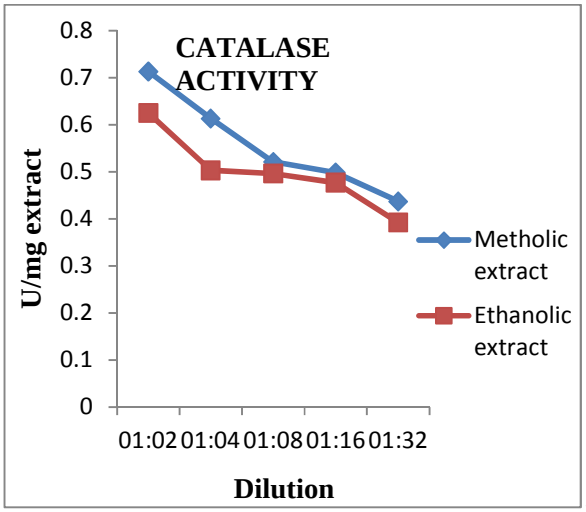

Figure 4: Catalase activity

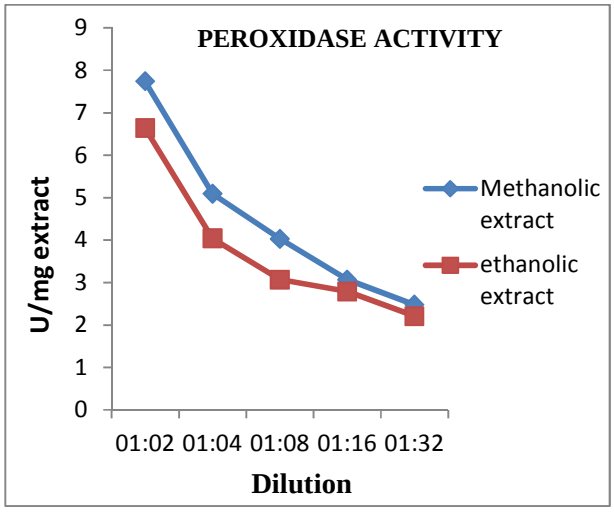

Figure 5: Peroxidase activity

CONCLUSION

From the results obtained in the present study it is concluded that methanolic extract of leaves of *B. monnieri* showed high antioxidant activity when compared to the ethanolic extract. The antioxidant activities are due to high phenolic content, free radical scavenging activity and reducing power. This *invitro* study reveals that this plant is a good source of natural antioxidants and could be useful in preventing oxidative stress to a great extent.

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