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Research Article

PHYTOCHEMICAL ANALYSIS AND FREE RADICAL SCAVENGING POTENTIAL OF *BALIOSPERMUM MONTANUM* (Willd.) Muell. LEAF

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ABSTRACT

Baliospermum montanum (Willd.) Muell. is used in India for reducing oxidative stress. The main objective of the study was to investigate phytochemical and antioxidant activities to justify the use of this plant in medicines. Antioxidant activity of different concentrations of methanolic leaf extract was evaluated with the determination of total phenolic, DPPH* radical scavenging assay, and $ABTS^+$ decolouration assay. The total phenolic content was higher in this extract. The antioxidant potential of the extract was well established with DPPH*, which provide a basis for the traditional use of this plant in medicines.

Keywords: $ABTS^+$ Antioxidant, *Baliospermum montanum*, DPPH*, Free radical scavenging, Phenols.

INTRODUCTION

Plants have been used as medicinal remedies for centuries throughout the world. Medicinal plants are the sources of many nutrients and non-nutrients which have disease preventive properties. Phytochemical with antioxidant properties present in plant naturally are important to protect cells from damage caused by the free radicals and offers protection against cellular oxidation reaction (Praveen et al., 2007 and Scalper and Williamson 2000). Generation of free radicals or reactive oxygen species during the metabolism of biological system creates oxidation stress, which in turn leads to disruption of structure and function of the cell.

This oxidation stress plays an important role in diseases like cancer and aging process (Zima et al., 2001). Natural antioxidants in plants include carotenoids, phenols, flavonoids, cinnic acid, tocopherols, ascorbic acid, folic acid, benzoic acid, etc. (Walton et al., 1999) has free radical scavenging activity. These play a preventive role in human diseases and proact against allergies, ulcers, tumors, cardiovascular diseases, platelet aggregation, Parkinson's disease, arthritis, and also reduce the risk of cancer (Prakash and Gupta 2009).

Baliospermum montanum (Willd.) Muell. is an important medicinal plant, which is commonly called as Danti. The plant is a stout monoecious under shrub with many shoots from the base. The various parts of the plant like roots, leaves, and seeds are used traditionally for the treatment of various ailments. In Ayurveda, root are used to cure jaundice, leucoderma, skin diseases, wounds, and as an anthelmintic (Ravindra M and Raju R.W 2008. Leaves are found to be useful in asthma, bronchitis (Nadkarni 1988) and in treating abdominal tumor (Chopra et al, .1994). Seeds are used as purgative and in gastric complaints (Goel .et al., 1984). Decoction of stem is used to get relief from toothache (Bhatt et al., 1982).

The literature revealed the presence of number of chemical constituents like glycoterpenoids, steroids, flavonoids, etc., from the scientists Ogura. et al.,(1978), Husain. (1980), Mukherjee and Ray (1980), Agarwal (1989), Antony . et al., (2010) and Vaghasiya et al., (2011). Very little scientific information is available on *Baliospermum montanum* leaf and its therapeutic potential. The solvents and aqueous extract of only roots have been studied and found to posses anticancer, antimicrobial, free radical scavenging, immunomodulatory, hepatoprotective and anthelmintic properties. Leaves are not much explored scientifically for their biological activity (Ravindra and Raju, 2008).

The present study was to correlate the antioxidant action of DPPH* and $ABTS^+$ assay with the preliminary qualitative phytochemical screening and total phenolic content of methanolic extract of *Baliospermum montanum* leaf using different concentrations.

MATERIALS AND METHODS

Plant materials

Plant material was collected from GKVK, University of Agricultural Sciences, Bangalore, Karnataka. The leaves were washed, shade dried, and made into a fine powder. Ten grams of powdered material was extracted in 100 ml methanol by Soxhlet extractor. The extracted solvent was concentrated at a temperature below 40^{oc} and used for determination of phytochemical analysis and various antioxidant actions.

Phytochemical Screening

The preliminary qualitative Phytochemical screening for alkaloids, phenols, carbohydrates, tannins, steroids, saponins, flavonoids, cardiac glycosides, proteins, terpenoids, resins, and glycosides were carried out according to the method suggested by Harbon (1998) and Parekh and Chanda (2007).

Estimation of phenolic content

The total phenolic content of leaf extract was determined by Folin-ciocalteu method (Slinkard and Singleton 1977), using Catechol as standard. 1 ml of plant extract was mixed with 1ml of Folin-ciocalteu reagent and 3 ml of sodium carbonate solution (20%) was added. The mixture was allowed to stand for 45 min. at room temperature. The total phenol was determined by spectrophotometer at 760 nm. The values were expressed in terms of Catechol equivalent.

Determination of antioxidant activity

DPPH* method

The radical scavenging activity was studied using 1-1-diphenyl-2-picrylhydrzyl according to Blios (1958). 2 ml of various dilutions of leaf extract were mixed with 5ml of 0.1mM methanolic DPPH* solution and incubated at 37^{o}**c** for 30min. The wavelength of maximum absorbance of DPPH* was measured at 517 nm using spectrophotometer. The percentage of free radical scavenging activity of each concentration was calculated. All measurements were performed in triplicates.

$ABTS^+$ radical scavenging assay

2, 2, Azino Bis-3-ethylbenzothiazoline-6-sulphonate radical cation decoloration assay was determined as described by Re et al., 1999. $ABTS^+$ was prepared by mixing 7mM of $ABTS^+$ with 2.5 mM of potassium persulfate and incubated in dark for 18 hours at room temperature before use. The mixture was diluted with methanol to give absorbance of 0.7± 0.02 units at 734nm, using spectrophotomrter. 1 ml of diluted $ABTS^+$ solution was added to

sample extract of different aliquots. Absorbance was measured after 30min. of incubation at room temperature. The capacity of free radical scavenging was determined in triplicates.

RESULT AND DISCUSSION

The preliminary phytochemical screening of methanolic extract of *Baliospermum montanum* leaves revealed the presence of alkaloids, terpenoids, and resins in lower amount (+), Carbohydrates and cardiac glycosides in moderate amount (++), and phenols in higher amount (+++). Proteins, tannins and saponins were absent. Flavonoids were present in traces which are given in **Table 1.** Total phenolic content was higher in the extract (595µg/ml).

Table1: Phytochemical analysis of methanolic extract of *B.montanum* leaves

Carbohydrates	++ve
Proteins	-ve
Terpenoides	+ve
Cardiac glysosides	++ve
Tannins	-ve
Phenols	+++ve
Flavonoides	+ve
Resins	+ve
Glycosides	+ve
Saponins	-ve
Alkaloides	+ve

DPPH* radical scavenging activity

DPPH* was used to measure the free radical scavenging activity which was stable at room temperature and accepts electron or hydrogen free radical to form a stable diamagnetic molecule. This ability of DPPH* to undergo reduction by an antioxidant is measured in terms of decrease in its absorbance at 517 nm. As DPPH* radical reacts with a suitable reducing agent, the odd electron becomes paired and the solution loses color from purple to yellow stoichiometrically depending on the number of DPPH* radicals that have undergone reduction. Antioxidant activity in the present investigation showed a sharp fall in the absorbance of DPPH* indicating higher antioxidant activity when compared to standard Ascorbic acid. Scavenging activity of DPPH was also observed ranging from 4.56% to 71.1%. The results clearly indicate that the extract has significantly high scavenging potential that prevent free radical damage occurring in the human body (**Graph 1**).

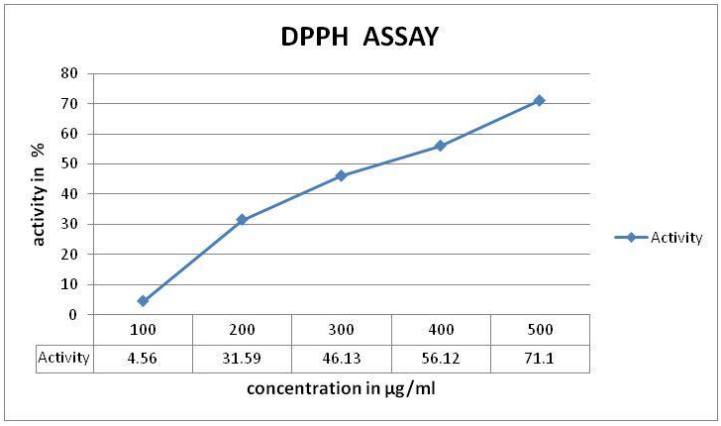

Fig.1: DPPH* radical scavenging activity

ABTS $^{+}$decolourization assay

ABTS^{+} is an excellent tool to determine the antioxidant activity. The decolourization of ABTS^{+} radical reflects the capacity of an antioxidant species to donate electrons or hydrogen atoms to inactivate the radical species. In the present study the sample showed decrease in the absorbance with moderate scavenging activity. The percentage of inhibition of ABTS^{+} radical is plotted as a function of concentration. The extract exhibited 9.3% of antioxidant activity when compared to standard Ascorbic acid. The result is presented in **Graph 2**.

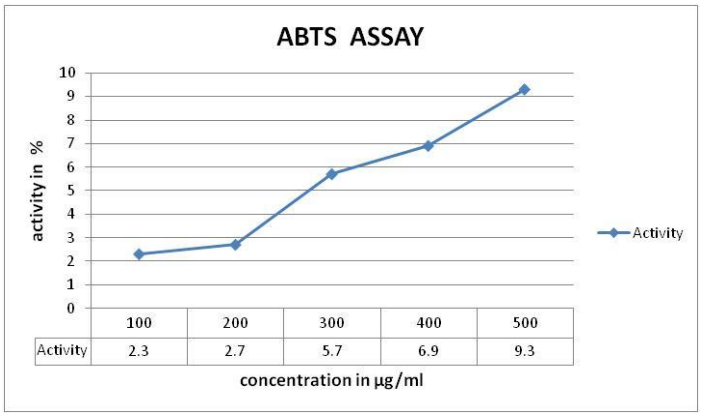

Fig. 2: ABTS $^{+}$decolourization assay.

CONCLUSION

The results of present study of plant extract showed the presence of phenols in higher amount. Some of the phenolic compounds present in natural products have higher antioxidant activity than the synthetic ones (Rice–Evan 1997 and Kandhasamy 2010). They also reinforce defence against Reactive oxygen species and prevent molecular damage (Vaya et al 1997). Among the two methods studied, DPPH* assay was found to have higher radical scavenging activity when compared to ABTS$^{+.}$ Hence it is concluded that *Baliospermun montanum* can be a potential source of new useful drug. It is suggested that further studies on phytochemical characterization of different solvent extracts and the identification of responsible bioactivity and bioactive compounds are necessary. Due to indiscriminate collection and over exploitation for medicinal use, this plant has been disappearing very fast and is having included under red list category (FRLHT 1997). Since this plant belongs to IUCN threat category, the plant requires conservation measures.

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