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IN-VITRO ANTI-OXIDANT ACTIVITY OF METHANOLIC EXTRACT OF *BALIOSPERMUM MONTANUM*

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ABSTRACT
The methanolic extracts of root of Baliospermum montanum were screened for antioxidant activity against DPPH, hydrogen peroxide and Nitric oxide free radicals. The methanolic extract of *Baliospermum montanum* (MEBM) has scavenged the DPPH, Nitric oxide and Hydrogen peroxide radicals in dose dependent manner. The experimental results were compared with the standard antioxidant ascorbic acid. The methanolic extract exhibited concentration dependant DPPH, Nitric oxide and Hydrogen peroxide radical scavenging activity with IC_{50} values of 226, 272.8 and 246.84 µg/ml respectively. The in-vitro studies reveal that the methanolic extracts of *Baliospermum montanum* possesses a significant antioxidant activity.

KEYWORDS: Ascorbic acid, DPPH, Nitric oxide, Hydrogen peroxide.

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INTRODUCTION

Baliospermum montanum plant is locally known as Danti. It is distributed common in fields, waste places and in shady habitats and in semi-evergreen forests throughout most India up to 1000m altitude in the Himalayas and 1800m in the western ghats. The black, thick barked roots are used in Ayurvedic medicines either alone or in combination with other plant drugs. It is used as anthelmentic, diuretic and useful in treating skin diseases, wounds, piles, jaundice, dropsy and enlarged spleen. It is an ingredient of the Ayurvedic preparations *Dasamula panchakoladhi kashayam* , used for treating ascites. The powdered roots are taken internally to relieve indigestion among the Paudi Bhuinya in northern Orissa[1].

The tribals of Madhya Pradesh and Andhra Pradesh (Karim Nagar) of India are using leaves of Danti for the treatment of asthma [2,3] and headache [4]. Decoction of stem is used to get relief from toothache [5,6]. The roots of the plant are practiced as laxative [6,7] in dropsy, jaundice, anasarca [3,7] , in rheumatism, anemia [8] and also in the treatment of jaundice, skin diseases, helminthic infections, leucoderma and piles.

MATERIALS AND METHODS

Plant Material

The roots of *Baliospermum montanum* (Specimen no. K.U/10/2008) were collected from Warangal, Andhra Pradesh, India. It was authenticated by Prof. V. S. Raju, Dept of Botany, Kakatiya University, Warangal, India.

Preparation of extract

The roots were cleaned, shade dried and coarsely powdered. The coarse powder of roots was then exhaustively extracted by maceration process using methanol as solvent. After extraction, the methanolic extract was concentrated by air-dried and it is preserved in a vacuum desiccator.

Chemicals

1,1-Diphenyl-2 picryl hydrazyl (DPPH), Nitric oxide, H_2O_2 was obtained from Sigma Aldrich Co., St.Louis, USA, Methanol (Merck, Mumbai), Phosphate buffer Saline (PBS) was obtained from Himedia, Mumbai, India, Ascorbic acid was purchased from SD fine chemicals Ltd., Mumbai, India. All chemicals used were of analytical grade.

Scavenging of Diphenyl Picryl Hydrazyl (DPPH) radicals

The free radical scavenging activity of MEBM was measured by DPPH method [9]**.** Ascorbic acid was used as a reference standard. 0.1mM solution of DPPH in ethanol was prepared and 1ml of this solution was added to 3ml of different concentrations (50, 100, 200, 300, 400 µg/ml) of MEBM solution. After 30 min, absorbance was measured at 517 nm. The percentage inhibition was calculated by comparing the absorbance values of the control and test samples. The capability to scavenge the DPPH radical was calculated using the following equation:

$$\% \text{ DPPH scavenging activity} = \frac{(A_{control} - A_{test})}{A_{control}}$$

Where $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the extract was expressed as IC_{50}. The IC_{50} value was defined as the concentration (in µg /ml) of extract that inhibits the formation of DPPH radicals by 50%.

Determination of nitric oxide radical (NO^-) scavenging activity

In this method nitric oxide generated from sodium nitroprusside was measured by the Greiss reaction. Sodium nitroprusside in aqueous solution at physiological P^H spontaneously generates nitric oxide [10,11,12] which interacts with oxygen to produce nitrite ions that can be estimated by using Greiss reagent.

Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide [11,12] . Sodium nitroprusside (5mM) in phosphate buffer saline (PBS) was mixed with 3ml of different concentrations (50, 100, 200, 300, 400 µg/ml) of the extract dissolved in the suitable solvent systems and incubated at 25^0C for 150mins. The samples from the above were reacted with Greiss reagent (1% sulphanilamide, 2%H_3PO_4 and 0.1% naphthylethylenediamine dihydrochloride). The absorbance of the chromophore formed during the diazotization of nitrite with sulphanilamide and subsequent coupling with naphthylethylenediamine was read at 546nm and referred to the absorbance of standard solutions of potassium nitrite, treated in the same way with Greiss reagent. Ascorbic acid was used as a reference compound. The % inhibition was calculated as:

$$\% \ NO^- \text{ scavenging activity} = \frac{(A_{control} - A_{test})}{A_{control}}$$

Scavenging of Hydrogen Peroxide (H_2O_2) radicals

The ability of MEBM to scavenge hydrogen peroxide was determined according to the method [13] . A 40mM solution of H_2O_2 was prepared in phosphate buffer (P^H 7.4). Extract of selected concentrations (50, 100, 200, 300, 400 µg/ml) in distilled water were added to a H_2O_2 solution (0.6ml, 40mM). Absorbance of H_2O_2 at 230nm was determined 10mins later against a blank solution containing phosphate buffer with out H_2O_2. Ascorbic acid was used as a standard antioxidant. The % of H_2O_2 scavenging of both extracts and standard compounds were calculated.

$$\% \ H_2O_2 \text{ scavenging activity} = \frac{(A_{control} - A_{test})}{A_{control}}$$

Where $A_{control}$ is the absorbance of the control reaction and A_{test} is the absorbance in the presence of the sample of the extracts. The antioxidant activity of the extract was expressed in IC_{50} values.

RESULTS AND DISCUSSIONS

The reduction capability of DPPH radicals was determined using absorbance of 517nm. Fig.1 shows that the % scavenging activity increases steadily with increasing the concentration of methanolic extract and reference standard ascorbic acid. And the IC_{50} values were found to be 226 µg/ml for methanolic extract and 74.5 µg/ml for reference ascorbic acid respectively.

Nitric oxide radical (NO-) generated from sodium nitroprusside at physiological PH was found to be inhibited by extracts. Fig. 2 illustrates that the methanolic extract inhibited the NO- radical which is comparable with that of reference ascorbic acid. The IC_{50} value was found to be 272.8 µg/ml for methanolic extract and 94 µg/ml for ascorbic acid.

Hydrogen peroxide itself is not only very reactive, but it can be some times be toxic to cell as it provides hydroxyl radical in the cells [14]. Thus, removing H_2O_2 as well as O is very important for protection of food system. The results of H_2O_2 radical scavenging activity was represented fig. 3. The methanolic extract inhibited H_2O_2 radical in a concentration dependant manner and the results are comparable with that of standard reference ascorbic acid. The IC_{50} values were found to be 246. 84 µg/ ml and 84.5 µg / ml for methanolic extract and reference ascorbic acid.

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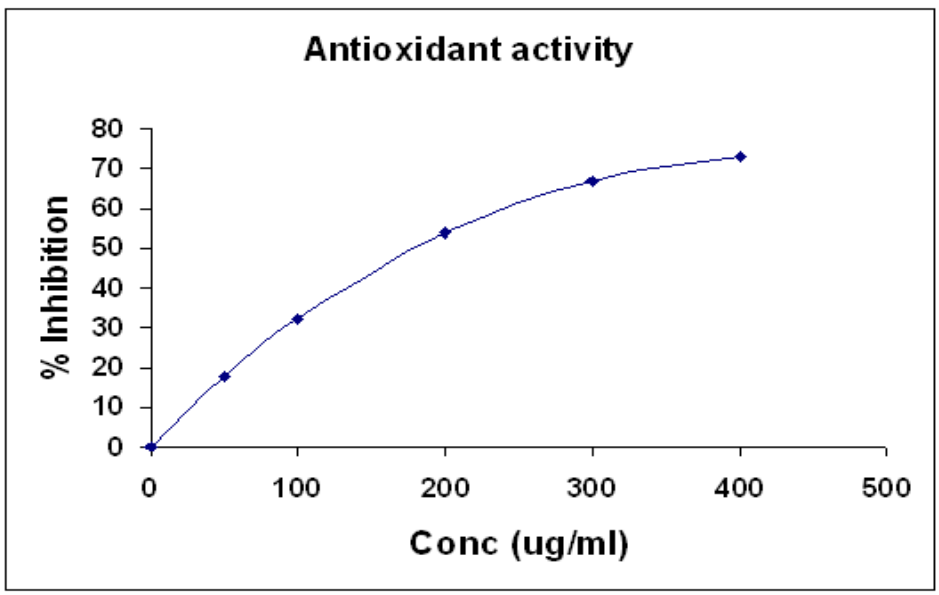

Figure 1: DPPH free radical scavenging activity of methanolic extract of BM

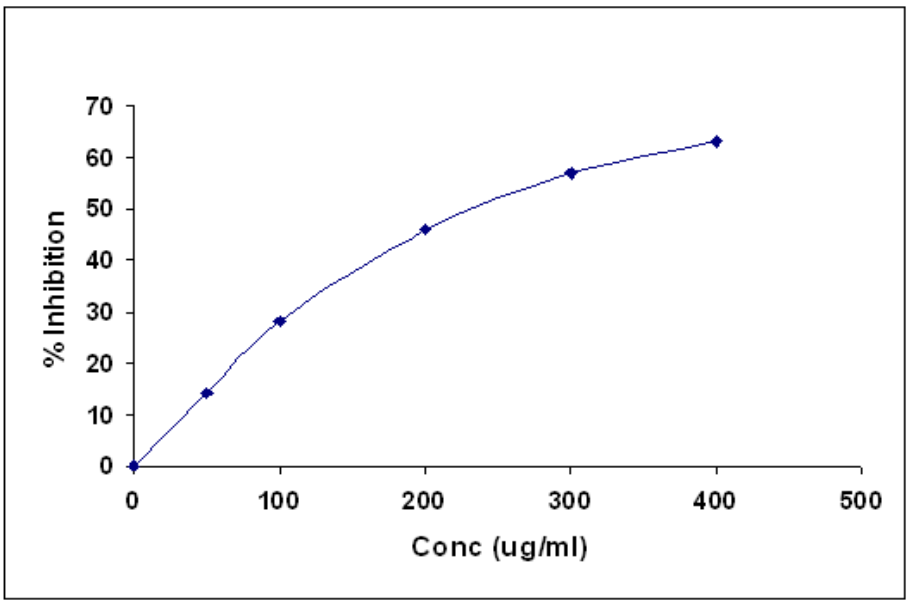

Figure 2: Nitric oxide scavenging activity of methanolic extract of BM

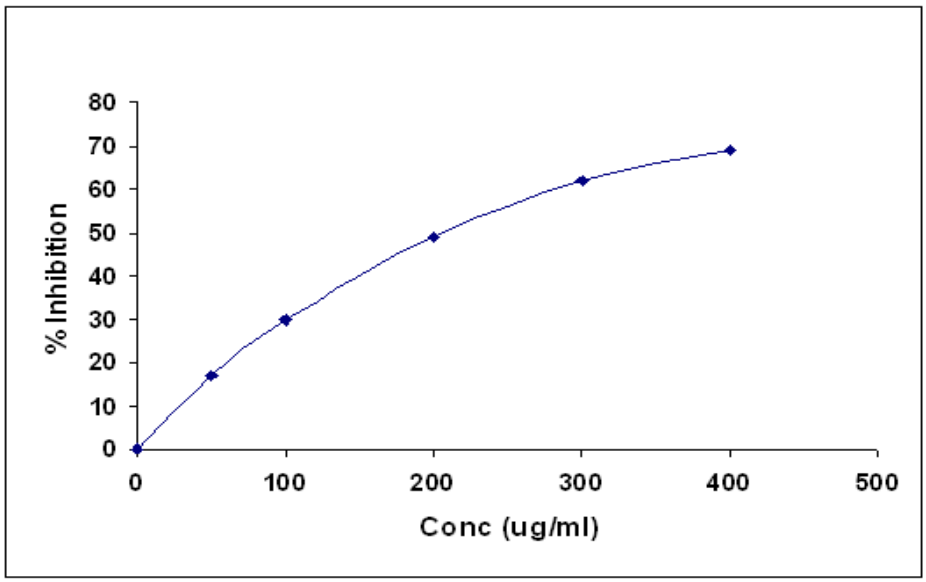

Figure 3: Hydrogen Peroxide scavenging activity of methanolic extract of BM

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