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Content

Free radical scavenging activity of aqueous extract of roots of *Baliospermum montanum* Muell-Arg

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Free radical scavenging potential of the aqueous extract of roots of *Baliospermum montanum* Muell-Arg, a medicinal plant, was evaluated by using diphenyl-picryl-hydrazyl (DPPH) assay and nitric oxide (NO) scavenging assay. The *in vitro* antioxidant activity of the plant extract was determined by DPPH and NO scavenging activity method. In the DPPH method, antioxidants present in the plant extract reacted with DPPH, which is a stable free radical, and converted it to 1,1-diphenyl-1,2-picryl, hydrazine. The results were expressed as IC_{50}. Ascorbic acid, which was used as a standard, showed an IC_{50} of 19.90 ± 2.3 µg/ml, whereas the plant extract showed an IC_{50} of 26.22 ± 0.8 mg/ml. In the second method, NO generated from sodium nitroprusside in aqueous solution at physiological pH interacted with oxygen to produce nitrite ion, which was measured at 540 nm. Ascorbic acid, the standard, showed an IC_{50} of 5.62 ± 2.5 µg/ml; the plant extract showed an IC_{50} of 36.66 ± 2.8 µg/ml. In the present study, aqueous extract of *B. montanum* showed remarkable and concentration-dependent free radical scavenging activity comparable to that of a standard such as ascorbic acid in the studied models; significant results were observed in the estimated parameters, thereby indicating that *B. montanum* has promising free radical scavenging activity.

Key words: *Baliospermum montanum*, DPPH assay, free radical scavenging, nitric oxide assay

INTRODUCTION

Molecular oxygen is an essential component for all living organisms, but the formation of various reactive intermediates of molecular oxygen metabolizes the cells aerobically, thus eventually leading to a process termed as oxidation. Oxidation is one of the destructive processes, where it breaks down and damages various molecules. On the one hand, oxidation *in vitro* involves the participation of oxygen, while, on the other hand, most of the biological oxidation *in vivo* occurs in the absence of oxygen resulting in biological metabolism (Halliwell, 1989). Free radicals are chemical species of atoms or molecules that possess an unpaired electron on their outermost orbit. These free radicals are highly unstable and can, therefore, react with other molecules by giving out or accepting single electrons (Crastes, 1990). Although the human body continuously produces free radicals, it possesses several defense systems, which are constituted of enzymes and radical scavengers (Naik, 2003). *Baliospermum montanum* Muell-Arg (Euphorbiaceae) has been reported to possess anti-inflammatory, tonic, diuretic, purgative and stimulant properties. In Indian ethnomedicine, this plant is locally known as Danti, and its roots have long been used as Ayurvedic remedy for jaundice (Varies, 1994). Free radicals are believed to be involved in bacterial and parasitic infections, lung damage, inflammation, reperfusion injury, cardiovascular disorder and neoplastic diseases (Roy, 1994). Antioxidant agents of natural origin have attracted special interest because they can protect human body from free radicals (Osawa, 1990). Hence, the present study was aimed at evaluating the free radical scavenging activity of the aqueous extract of roots of *B. montanum*.

MATERIALS AND METHODS

Plant Material

Fresh roots of *B. montanum* were collected from local area of Belgaum and authenticated by the Botanical Survey of India, Pune. A voucher specimen (BST/WC/Tech 277) has been preserved at K.L.E.S's College of Pharmacy, Belgaum.

Preparation of Extract

Fresh air-dried roots were powdered and passed through mesh no. 40 to get appropriate powder of the drug. Then, 100 gm of powdered roots was extracted with 500 ml of distilled water for 24 h below 50°C. The extract was filtered with Whatman no.2 filter paper. The filtrate was collected and water was evaporated under reduced pressure using vacuum evaporator. The extract was subjected to qualitative chemical tests for steroids, terpenoids, flavonoids, alkaloid, etc. (Evans, 1997; Harborne, 1998).

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Free Radical Scavenging Activity

Diphenyl-picryl-hydrazyl assay

The free radical scavenging capacity of the aqueous extract of *B. montanum* was tested by its ability to bleach the stable 2,2-diphenyl 2-picryl-hydrazyl (DPPH) radical (Bonina and Saija, 1998). A stock solution of DPPH (1.5 mg/ml of methanol) was prepared such that 75 µl of it in 3 ml methanol gave initial absorbance of 0.9. This stock solution was used to measure the antiradical activity. Decrease in absorbance in the presence of aqueous extract of *B. montanum* at different concentrations was noted after 15 min. IC_{50} was calculated from percentage inhibition. Ascorbic acid was used as reference standard.

Nitric oxide scavenging activity

The interaction of aqueous extract of *B. montanum* with nitric oxide (NO) was assessed by the nitrite detection method. The chemical source of NO was sodium nitroprusside (10 mM) in 0.5 M phosphate buffer, pH 7.4, which spontaneously produced NO in an aqueous solution. NO interacted with oxygen to produce stable products, leading to the production of nitrites. After incubation for 60 min at 37°C, Griess reagent (α-napthyl-ethylenediamine 0.1% in water and sulphanilic acid 1% in H_3PO_4 5%) was added. The same reaction mixture without the aqueous extract of sample but with equivalent amount of distilled water served as control (Green *et al.*, 1982). Ascorbic acid was used as positive control.

RESULTS AND DISCUSSION

Recently much attention has been focused on reactive oxygen species and free radicals, which play an important role in the genesis of various diseases such as inflammation, cataract, liver cirrhosis and ischemia/reperfusion injury (Halliwell, 1994). Herbal drugs containing radical scavengers are gaining importance in the prevention and treatment of such diseases. Phenolic compounds and flavonoids are the major constituents in most plants reported to possess antioxidant and free radical scavenging activity (Larson, 1988). Preliminary phytochemical screening of aqueous extracts gave positive tests for flavonoids, tannins and steroids. The free radical scavenging capacity of aqueous extract of roots of *B. montanum* was tested by its ability to bleach the stable DPPH radical. This assay provided information on the reactivity of the test compound with a stable free radical, since its odd electron DPPH gives strong absorption band at 517 nm in visible spectroscopy (deep violet colour). As this electron becomes paired off in the presence of a free radical scavenger, the absorption vanishes and the resulting decolourization is stoichiometric with respect to the number of electrons taken up. It showed excellent antiradical activity by inhibiting DPPH radical with an IC_{50} value of 26.22 µg/ml (Table 1). The scavenging activity was comparable to that observed with ascorbic acid.

Nitric oxide exhibits numerous physiological properties and it is also implicated in several pathological states (Moncada and Parmer, 1991). It is an important second messenger, acts as a neurotransmitter and plays an important role in the defense against pathogens as well as in the control of blood pressure. NO is produced in various cells including neurons, endothelial cells and neutrophils by three isoforms of NO synthase enzyme (encoded by a unique gene), from nitrogen of the guanidine group of L-arginine and from molecular oxygen (Sessa and Harrison, 1993). The interaction of NO with other radicals leads to the formation of more hazardous radicals such as peroxynitrite anion and hydroxyl radical. In fact, NO reacts more rapidly with superoxide than the latter dose with superoxide dismutase. Aqueous extract significantly decreased with IC_{50} value 36.22 µg/ml, in a dose-dependent fashion, the concentration of nitrite after spontaneous decomposition of sodium nitroprusside, indicating that aqueous extract may contain compounds able to scavenging NO (Table 2).

However, the specificity of this assay has been questioned because nitrite is one final product of the reaction of NO with oxygen, through intermediates such as NO_3, N_2O_4 and N_2O_3 (Marcocci *et al.*, 1994); therefore, the decrease in nitrite production could also be due to an interaction of the extract with other nitrogen oxides. Our results indicate that aqueous extract of roots of *B. montanum* possesses free radical scavenging activity. Further studies are needed to better characterize the important active constituents responsible for the free radical scavenging activity.

Table 1: Antiradical activity of aqueous extract of roots of *Baliospermum montanum* observed with DPPH assay

Sample	Concentration (mg/ml)	% inhibition ± SD	IC_{50} (mg/ml) *r**
Aqueous extract	4	15.30 ± 2.0	
Aqueous extract	8	20.33 ± 3.3	
Aqueous extract	12	26.42 ± 3.9	26.22 (0.9516)
Aqueous extract	16	38.40 ± 2.8	
Aqueous extract	20	45.26 ± 3.9	
Aqueous extract	24	52.42 ± 3.6	
Ascorbic acid		19.90 ± 2.3	

N = 3; Values are means ± SD, *r** - regression coefficient

Table 2: *In vitro* nitric oxide scavenging activity of aqueous extract of roots of *Baliospermum montamum*

Sample	Concentration (mg/ml)	% inhibition ± SD	IC_{50} (mg/ml) *r**
Aqueous extract	4	23.15 ± 2.2	
Aqueous extract	8	27.24 ± 3.3	
Aqueous extract	12	38.30 ± 1.7	36.22 (0.9516)
Aqueous extract	16	46.52 ± 2.5	
Aqueous extract	20	57.56 ± 2.7	
Aqueous extract	24	59.59 ± 2.7	
Ascorbic acid		5.62 ± 2.5	

N = 3; Values are means ± SD, *r** - regression coefficient

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