ANTIOXIDANT ACTIVITY OF MEIZOTROPIS PELLITA
A CRITICALLY ENDANGERED AND ENDEMIC PLANT OF HIMALAYAN REGION

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ABSTRACT

The present study evaluates the antioxidant activity of methanolic extract of leaves of a critically endangered plant, Mezotropis pellita, commonly known as ‘patwa’ by the local inhabitants of Patwadangar. Methods used to evaluate the antioxidative potential of the sample includes 1,1-diphenyl-2-picryl-hydrazy (DPPH) free radical scavenging assay and β-carotene–linoleic acid systems. The synthetic antioxidants viz butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA) were used as reference. The scavenging activity was calculated for different concentrations of extract. The activity was expressed in terms of percent scavenging activity (%SA) for DPPH assay and it was found to be 76.10%, some four times higher than the synthetic antioxidant BHT & BHA at same concentration (4mg/ml). For β-carotene–linoleic acid systems, activity was expressed in terms of percent antioxidation activity (%AA) and it was found to be 33.30%, slightly less than the synthetic antioxidant at same concentration (5mg/ml). The activity was positively correlated to the concentration of the extract. The results obtained suggests that the essential oil extracted from Mezotropis pellita possess strong antioxidant properties and can be a promising natural substitute for synthetic antioxidant in pharmaceutical industries. Furthermore, deciphering the pharmaceutical importance of the plant may initiate some concrete steps for protection of this endangered plant which otherwise have gained negligible importance till the date.

Key Words: Antioxidant, β-carotene, DPPH, Extract, Mezotropis pellita

The highly reactive and transient derivatives of oxygen, attributed to as Reactive Oxygen Species (ROS), are class of reactive molecules, generated, either as byproducts of various biochemical reactions taking place in the body or from exogenous sources(1). Some of these ROS have been reported to play important role in energy production, phagocytosis, regulation of cellular growth, intracellular signaling and synthesis of biologically important compounds(2). Majority of ROS, however, are perversive and their rapid and prolonged production beyond the steady state concentration may lead to oxidative damage of biomolecules including amino acids, proteins, lipids and DNA (3,4). It is well established that oxidative damage plays major role in the induction of several chronic and degenerative diseases including atherosclerosis, heart disease, ageing, diabetes, immunosuppression, cancer, neurodegenerative diseases and many other (5).

Antioxidants are the compound that act against the ROS and thus minimizes the oxidative damages. The prefix "anti" means against, in opposition to, or corrective in nature. Human body has an inbuilt antioxidative mechanism and many biological functions such as anti-mutagenic, anti-carcinogenic and anti-aging responses arise from this property (6,7). Antioxidants work by converting the ROS to harmless stable molecules and thus prevent the possible damages. The antioxidant nature of any compound is due, mainly to their redox potential which help them to act as reducing agent, hydrogen donators, singlet oxygen quencher and metal chelator (8,9,10). Recently there has been a great upsurge in search of natural antioxidant of plant origin because they have wide applicability in preventive medicines and food industries without associated side effects. (11). The role of phytochemicals as antioxidants may be attributed, in-part, to lipid soluble vitamin A and E, the water soluble vitamin C and to a wide range of amphipathic molecules, collectively known as phenolic compounds.

Till the date many plant species have been investigated in search of novel antioxidants (12). Natural antioxidants from tea, wine, fruits, spices and vegetables are already being used commercially in the form of different formulations ranging from green...
tea to capsules (13), still there is an ever increasing demand for search of new candidate plants as better alternative and with potent activity. The Indian sub-continent is very rich in biodiversity with unlimited plant resource yet to be explored for various phytochemical activities. In this regards the present study investigates the antioxidant potential of *Meizotropis pellita* (Fabaceae), commonly known as ‘Patwa’ by the local inhabitants of Patwadangar located in Kumaun hills of lower Himalayas some 12 KMs away from Nainital (Uttar) and 1530 meters above the sea level. (Tiwari L.M.). *M. pellita* is an endangered and endemic shrub with stout, woody perennial root stock which erects shoots upto six feet height and broad leaves. The strict objective of this study was, therefore, not only to decipher the medicinal values of this plant but to also bring importance to this plant for erecting protective efforts towards its conservation.

**MATERIALS AND METHODS**

**Chemicals**

BHA (butylated hydroxyanisole), BHT (butylated hydroxytoluene), β- Carotene, DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical, Linoleic acid were purchased from Hi-media™ (India). Methanol and ethanol were obtained from E. Merck (Germany). All the chemicals used were of analytical grade.

**Plant materials**

Fresh leaves, stems and roots of *Meizotropis pellita* (patwa) were taken from three different sites of patwadangar (12 Km. from Nainital) during August 2009. The authentication of the sample was done on the basis of literature available, conformation from local people and from The Department of Botany, Kumaun University (DSB Campus), Nainital. The plant materials were gently washed, air dried and ground to fine powder using mortar and stored in sealed plastic bags until used.

**Extract Preparation**

10gm of dried powder of each plant part was mixed in 100ml of ethanol (100%) in a conical flask and was kept on a rotator shaker for 72 h. The extracts were then filtered using Whatman No. 1 filter paper. The filtrates were collected in a fresh flask and the above process was repeated twice. The final filtrates were then evaporated separately on a rotary shaker at 80 rpm till the ethanol evaporated completely. The dried fine powder was weighed and dissolved in methanol (1mg/ml) and were stored at 4°C for further use.

**Determination of total phenolic content**

Total phenolic content of the plant extracts was determined using Folin Ciocalteu method (14,15). Briefly, 0.1 ml of methanolic extract was mixed with 2% w/v sodium carbonate solution and mixed thoroughly by vortexing. After 10 minutes 0.1 ml of 50% Folin Ciocalteu reagent was added and incubated for 1hr; finally the absorbance was read at 750nm against blank. The same procedure was followed for gallic acid at known concentrations to construct standard calibration curve and used to determine total phenolic content which was expressed as Gallic Acid Equivalents (GAE) in milligrams per grams of extract (GAmg/gm).

**Determination of antioxidant activity**

β-Carotene bleaching test assay was carried out as per the method developed by Wettasinghe & Shahidi (18). Briefly, 0.5 mg of β-carotene was added to 1 ml of chloroform (HPLC grade) and mixed by gentle shaking. Exactly 1 ml of β carotene-chloroform solution was pipetted into a round-bottom rotary boiling flask containing 25 mg linoleic acid and 0.2 ml Tween 40 (100%). Chloroform was evaporated.100 ml of distilled water was added and shaken vigorously to form an emulsion. Emulsion aliquots (4 ml) were transferred into tubes containing 200 µl of sample extracts. As soon as the emulsion was added to each tube, zero time absorbance was read at 470 nm against blank. The tubes were placed in a water bath at 50°C for 2hrs. Oxidation of the emulsion was monitored by reading of absorbance every 30 minutes for 2hrs against blank solution containing all ingredients except β-Carotene. BHA was used as synthetic references. Antioxidant activity (AA) was calculated as percent of inhibition relative to the control using the following equation:

\[
AA = \left[1 - \left( \frac{A_t - A_i}{A_{t0} - A_i} \right) \right] x 100
\]

\[
AA = \text{antioxidant activity, } A_i = \text{initial absorbance of the sample; } A_t = \text{absorbance of sample after incubation (120 min) at 50°C; } A_{t0} = \text{initial absorbance}
\]
of control; \(A_t\) = absorbance of control after incubation (120 min) at 50°C.

**DPPH – radical scavenging activity**

Free radical scavenging activity was determined using the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical according to the method described by Blois (AO8.4). Briefly, to 1 ml of different concentrations of methanolic extracts of stem, roots and leaves 1 ml of DPPH (0.1 mM) was added. The mixture was shaken vigorously and allowed to stand at room temperature for 30 minutes in dark. The absorbance was measured at 517 nm in a spectrophotometer. Decrease in absorbance of the reaction mixture indicated higher free radical scavenging activity. An equimolar solution of DPPH was used as blank. BHA was used as standard. The DPPH radical concentration was calculated using the following equation:

\[
\text{DPPH}^* \text{ scavenging effect (\%)} = 100 - \left( \frac{A}{A_0} \right)
\]

\(A_0\) was the absorbance of the control reaction and \(A\) was the absorbance in the presence of the sample extract.

**RESULTS AND DISCUSSIONS**

Total antioxidant activity of many plants has already been investigated earlier. The antioxidant activity of the plant extract is attributed to many different types of phytochemicals including phenolics and its derivatives. (AO3.18-19). The activity varies with the physiological conditions of plant, developmental stages, geographical locations and the parts of plants analyzed. The extracts prepared showed appreciable phenolic contents. The highest phenolic content was found in stem extract (70.21±5.4 mg/gm) followed by leaf (44.10±4.2) and root (34.80±5.6) extracts respectively. The antioxidant activity as measured by β-Carotene bleaching assay was found to be correlated to phenolic content and also to the concentrations employed for measurement, over a wide range. Mean percent antioxidant activity (AA %) was found to be 61.90±6.9%, 36.30±30 and 33.70±4.5% for stem, leaves and roots respectively.
The DPPH assay measures the ability of the extract to donate the hydrogen to the DPPH radical resulting in bleaching of the DPPH solution. The greater the scavenging action, the higher is the antioxidant activity. The plant extract was able to reduce the stable, purple colored radical DPPH into yellow colored DPPH. In the present study the scavenging activity of leaf extract was found to be highest (76.10±2.4%) followed by stem and root extract (42.25±51% & 19.80±28%) respectively.

The present study thus shows that extracts of Meizotropis may be potentially used as good source of antioxidants. Further, research can be continued to determine specific phenolic constituents and other compounds responsible specifically for the activity. The in vivo studies are also to be done to know the effect of natural antioxidants in living organisms. Also, with a number of other assays may be performed for other useful properties.

REFERENCES


