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Antioxidant activity, phenol and flavonoid content of *A.racemosus* Willd. a medicinal plant grown using different organic manures

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ABSTRACT

Antioxidant activity, phenol and flavonoid content were determined in the ethanol extract of roots of *Asparagus racemosus* grown under organic regime in the Experimental Garden of the Life Sciences Department, Dibrugarh University, Assam, during three consecutive years 2007 to 2010. Organic regimes were provided by treating the soil with bulky organic manures- cowdung, compost, and vermicompost without using mineral or chemical fertilizer. The roots were harvested from 18 months old plants and subjected to estimation of phenol and flavonoid content, and for antioxidant activities. Total phenol and flavonoid content and DPPH radical scavenging activity of the extracts were spectrophotometrically determined. Catechol, Quercetin, and ascorbic acid were taken as standard in case of phenol, flavonoid content and antioxidant activity respectively. The total phenol and total flavonoid content was highest in the plants from vermicompost treated soil. The DPPH radical scavenging activity was highest in the plants from compost treated soil. There observed a relationship between phenol and flavonoid content but failed to show relationship between phenolic content and antioxidant activity of the ethanol extracts of the plants grown in different organic regimes. Effect of growing conditions on production of secondary metabolites by medicinal plants has been revealed by the present study.

Key words: *A. racemosus*, organic manure, antioxidant, phenol, flavonoid.

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INTRODUCTION

Medicinal plants have been playing a vital role on the health and healing of man since down of human civilization. In spite of tremendous development in the field of allopathic medicines during the 20th century, plants still remain one of the major sources of drugs in modern as well as in traditional system of medicine. Medicinal plants are source of certain bioactive molecules which act as antioxidants and antimicrobial agents [1-4]. There is an upsurge in demand of plant materials containing phenolics as they retard oxidative degradation of lipids and thereby improving quality and nutritional value of food [4-6].

Free radicals are responsible for several disorders in human body [7-8]. Oxidative process is one of the most important routes for producing free radicals in food, drug, and even in living systems. The free radicals in the human body have adverse effects on its immune system[9]. Consumption of natural oxidants as free radical scavengers may become necessary to improve the depleted immune system [7, 10-12]. It is reported that the antioxidant constituents of plant materials provide protection from coronary heart disease and cancer[13] and protect the body from damage caused by free radical induced oxidative stress[14-15].

Recently, more attention has been given in medicinal plants of therapeutic potentials as antioxidants in reducing free radical induced tissue injury. Many plants have been investigated in the search for novel antioxidants [16-24]. The synthetic antioxidants have restriction for use, as they are suspected to be carcinogenic. Therefore, the importance of searching for and exploiting natural antioxidants has increased greatly in present years [25].

A. racemosus is one of the most important medicinal plant used in indigenous system of medicine (ISM). Roots and leaves are the sources of drug. Roots are useful in nervous disorders, dyspepsia, diarrhea, dysentery, tumours, inflammations, burning sensations, throat infections, tuberculosis, cough, bronchitis, gonorrhea, leucorrhoea, leprosy, epilepsy, fatigue, hyperacidity, haemorrhoids, cardiac debility, hypertension etc[26]. In Ayurveda it is used as a galactagogue to increase milk production during lactation[27].

Organic farming was recommended by the UNO [28-29] as the system ensures safety products for human and environmental health [30]. The organically produced crops are the safe and neat sources of nutrients [31-32]. Organically grown medicinal plants by using compost, produced best results in many investigations [33-39]. A high content of total phenols was recorded in certain crops grown organically than the crops grown by conventional farming[40]. Further, the organically grown herbal drugs are not only readily acceptable in global market but also fetch premium prices than those grown by conventional farming.

The aim of this study was to investigate the antioxidant activity, phenol and flavonoid content of a potential medicinal plant *Asparagus racemosus* grown under organic farming regime using three different organic manures- cowdung, compost and vermicompost.

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MATERIALS AND METHODS

The experimental plots were treated with three organic manures viz. cowdung, compost and vermicompost that provided different organic regimes where *A.racemosus* plants were grown separately in the medicinal plant germplasm repository of the Department of Life Sciences, Dibrugarh University, Assam, India. The plant was botanically authenticated, a voucher specimen (DUL.Sc.2529) of the plant has been deposited to the herbarium of the Dept. of Life Sciences, Dibrugarh University, Dibrugarh, Assam, and India. Fresh roots harvested from18 month's old plants were cut into small pieces and ground with a pestle and mortar in the measured volume of solvents (80: 20 ethanol –water). The extract was filtered through Whatmann No. 1 filter paper. Each extract was prepared just before the analysis for prevention of any degradation. Folin-Ciocalteau reagent and all other chemicals used were Merck products.

DPPH radical scavenging activity [41]

Antioxidants react with 1, 1- diphenyl -2-picryl-hydrazyl (DPPH) radical and convert it to 1, 1- diphenyl -2-picryl hydrazine. The degree of change in colour from purple to yellow can be used as a measure of the scavenging potential of antioxidant extracts. Aliquots of extract solutions were taken and made up the volume to 3ml with methanol. 0.15ml of freshly prepared DPPH solution was added, stirred and left to stand at room temperature for 30 minutes in dark. The control contains only DPPH solution in methanol instead of sample while methanol served as the blank (negative control). Absorbance was noted at 517 nm by using UV-Vis spectrophotometer. The capacity of scavenging free radicals was calculated as follows:

Scavenging activity (%) = {(Control abs.- sample abs.)/Control abs.} X 100.

IC_{50} value was calculated from the plotted graph of scavenging activity against the concentrations of the samples. IC_{50} is defined as the total antioxidant necessary to decrease the initial DPPH radical by 50%. Triplicate measurements were carried out and IC_{50} was calculated for all the extracts based on the percentage of DPPH radicals scavenged. Ascorbic acid was used as the reference compound (positive control) with concentrations 20 to 500 µg/ml.

Determination of total phenolics

The total phenolic contents of extracts of *A.racemosus* were determined according to the method described by Malik and Singh [42]. Aliquots of the extracts were taken in a 10 ml glass tube and made up to a volume of 3 ml with distilled water. Then 0.5 ml folin ciocalteau reagent (1:1 with water) and 2 ml Na_2CO_3 (20%) were added sequentially in each tube. The tubes with solution were warmed for 1 minute, then cooled. A blue color was developed in each tube because the phenols undergo a complex redox reaction with phosphomolibdic acid in folin ciocalteau reagent in alkaline medium which resulted in a blue colored complex.

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Absorbance was measured at 760 nm. A standard calibration plot was generated at 760 nm using known concentrations of catechol. The concentrations of phenols in the test samples were calculated from the calibration plot and expressed as mg catehchol equivalent of phenol/g of sample.

Determination of total flavonoids

The aluminum chloride method was used for the determination of the total flavonoid content of the extracts [25]. Aliquots of extract solutions were taken and made up the volume 3ml with methanol. Then 0.1ml $AlCl_3$ (10%), 0.1ml Na-K tartarate and 2.8 ml distilled water were added sequentially. The solution mixture was vigorously shaken. Absorbance at 415 nm was recorded after 30 minutes of incubation. A standard calibration plot was generated at 415 nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /g of sample.

RESULTS AND DISCUSION

Total phenol and total flavonoid content and the antioxidant activity of ethanol extracts of *A. racemosus* roots are shown in table 1 and table 2. Total phenol content in terms of catechol equivalent (the standard curve equation: y =0.0966x, r^2 = 0.9878) were between 1.12mg /g and 6.9 mg /g dry material while total flavonoid content (the standard curve equation: y = 0.0148x, r^2 = 0.975) in terms of quercetin equivalent were between 20.25mg/g and 40.8mg/g dry wt. Similar result on phenol content of Asparagus extract in different cultivars was obtained by Rodriguez et al. [43].

Table-1: Total phenol & flavonoid content of *A. racemosus* root grown in different organic manure regimes

Root sample	Phenol content (mg catechol equivalent/g dry material)	Flavonoid content (mg quercetin equivalent /g dry material)
Cowdung treated soil	3.68	23.5
Compost treated soil	1.12	20.25
Vermicompost treated soil	6.9	40.8

Table-2: Antioxidant activity of *A. racemosus* root grown in different organic manure regimes

Root sample	Antioxidant activity IC_{50} in ug/ml (lower IC_{50} value indicate higher antioxidant)
Cowdung treated soil	1201.97
Compost treated soil	822.82
Vermicompost treated soil	1038.15

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It is observed that phenol and flavonoid content of the roots differ with difference in its growing condition provided by the use of different organic manures. Highest phenol and flavonoid content were noted in the extracts of plants grown in the soil treated with vermicompost followed by the plants from the cowdung treated soil than the plants from the compost treated soil (Table 1). It has been recognized that the production of secondary metabolite in plants is related to its growing condition. The phenolic compounds act as free radical terminators [44] and mechanism of action of flavonoids are through scavenging or chelating process [8, 45]. The antioxidant activity of the plants varied considerably in terms of IC_{50} value (Table 2). The highest antioxidant activity was noted in the extracts of plants grown in compost treated soil followed by the plants from vermicompost treated soil than the plants from cowdung treated soil. There was no relation between total phenolic content and antioxidant activity; but there are some reports [23, 43], which showed correlation between antioxidant activity and phenolics content of certain medicinal plants. The results of the present study supported the findings of some other investigators in certain other medicinal plants [1, 46]. The reason for lacking correlation between phenolics content and antioxidant activity in the present study may be due to the presence of some other phytochemicals such as ascorbic acid, tocoferol and pigments as well as the synergistic effects among them [1]. These phytochemicals as a whole contribute to the total antioxidant activity of the extracts. The result also showed that the percentage of antioxidant activity of the ethanol extracts increases with increasing concentration of the extracts in 200 µl to 1200 µl in all the samples (Fig1) and the results are in agreement with findings of others [4].

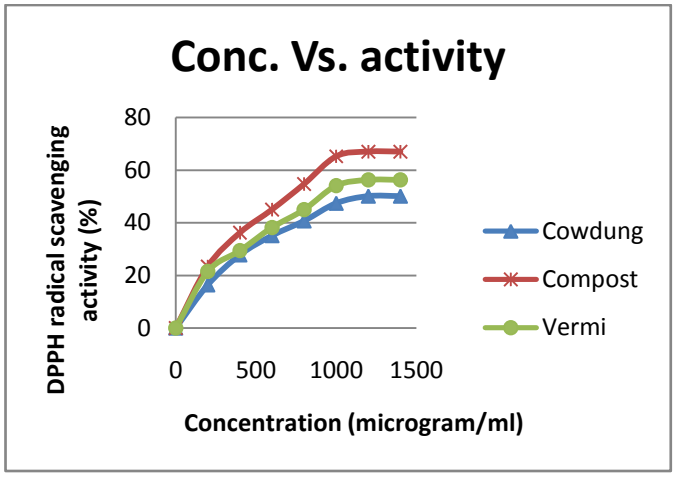

Fig1 : Conc. of ethanol extract Vs. DPPH free radical scavenging activity of *A. racemosus* root grown in different organic manure regimes

In the present study, the extracts of plant roots grown in compost regime showed highest antioxidant activity. Plants grown in vermicompost regime showed highest total phenol and flavonoid content supporting the idea of the significance of agronomical conditions of growing areas on the quantity of secondary metabolites of the root. Detail work by using different methods and by using more types of organic manures will be the aim of further investigation. Further, studies on other medicinal plants would be of great importance.

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