



Antioxidant activity, phenol and flavonoid content of a medicinal herb *Andrographis paniculata* (Burm.F.) Nees grown using different organic manures

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Received on: 05-10-2010; Revised on: 14-12-2010; Accepted on: 09-02-2011

ABSTRACT

Andrographis paniculata is a value added medicinal herb. A study was conducted on the phenol and flavonoid content and antioxidant activity of the ethanol extract of the plants grown in the Experimental Garden of the Life Sciences Department, Dibrugarh University, Assam, during three consecutive years 2008 to 2010. Plant was grown under organic regimes by treating the soil with bulky organic manures- cowdung, compost, and vermicompost without using mineral or chemical fertilizer. The herbs were harvested just before flowering and subjected to estimation of total phenol and flavonoid content, and for antioxidant activities using ethanol extract. 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 total phenol, flavonoid content and antioxidant activity respectively. IC_{50} value was also calculated for the samples. The total phenol content of the extracts was noted in the order of treatment, cowdung >compost >vermicompost and total flavonoid was vermicompost >compost >cowdung. The DPPH radical scavenging effect of the extracts was noted in the order of treatment cowdung >compost >vermicompost. Further, there observed a relationship between phenolic content and antioxidant activity of the ethanol extracts of the plants grown in different organic regimes; greater amount of phenol content leads to more potent radical scavenging activity.

Key words: *Andrographis paniculata*, organic manure, antioxidant, phenol, flavonoid.

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].

It has also been reported that there may be a correlation between antioxidant capacity and phenolics content of drugs^[26,8, 23]. The flavonoids are group of phenolic compounds having bioactive properties like free radical scavenging activity, inhibition of hydrolytic and oxidative enzymes and anti-inflammatory action^[27]. However, Khkonen et al., 1999^[17] stated that antioxidant activity does not necessarily correlate with high amounts of phenolics. One of the easiest, rapid and sensitive method for the antioxidant screening of plant extracts is free radical scavenging assay, using 1, 1-diphenyl – 2 picryl hydrazyl (DPPH) stable radical by spectrophotometric technique. In presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases^[21].

Andrographis paniculata is one of the most important medicinal plant used in indigenous system of medicine (ISM). The most important alkaloid produced by the herb is andrographolide. The bitter annual herb has hepatoprotective properties and forms an important ingredient of various herbal and pharmaceutical formulations.

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.

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

MATERIALS AND METHODS

The experimental plots were treated with three organic manures viz. cowdung, compost and vermicompost that provided different organic regimes where *A.paniculata* 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.2528) of the plant has been deposited to the herbarium of the Dept. of Life Sciences, Dibrugarh University, Dibrugarh, Assam, India. The leaves were collected just before flowering of the plant. Plant materials were air shade dried and taken for analysis. Accurately weighed powdered sample was ground with a pestle and mortar in the measured volume of solvents (80: 20 ethanol –water). The extract was filtered through Whatman No. 1 filter paper. Each extract was prepared just before the analysis for prevention of any degradation. Folin-Ciocalteu 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 μ g/ml.

Determination of total phenolics:

The total phenolic contents of extracts of *A.paniculata* 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 ciocalteu 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 ciocalteu reagent in alkaline medium which resulted in a blue colored complex. 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 catechol 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^[43]. 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 DISCUSSION

Total phenol and total flavonoid content and the antioxidant activity of ethanol extracts of *A. paniculata* leaves are shown in Table 1 & Table 2. Total phenol content in terms of catechol equivalent (the standard curve equation: $y = 0.0966x, r^2 = 0.9878$) were between 147 mg /g and 102 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 430mg/g and 440mg/g dry wt. The phenolic compounds act as antioxidant agents. It is observed that phenol and flavonoid content of the leaves differ with difference in its growing condition provided by the use of different organic manures. Highest phenol content was noted in the extracts of plants

Table-1: Total phenol & flavonoid content of *A. paniculata* leaf grown in different Organic manure regimes

Leaf sample	Phenol content (mg catechol equivalent/g dry material)	Flavonoid content (mg quercetin equivalent /g dry)
Cowdung treated soil	147	430
Compost treated soil	127	438
Vermicompost treated soil	102	440

Table-2: antioxidant activity of *A. paniculata* leaf grown in different organic manure regimes

Leaf sample	Antioxidant activity
	IC ₅₀ in ug/ml (lower IC ₅₀ value indicate higher antioxidant)
Cowdung treated soil	432.80
Compost treated soil	708.92
Vermicompost treated soil	811.54

grown in the soil treated with cow dung followed by the plants from the compost treated soil than the plants from the vermicompost treated soil (Table 1). On the other hand, highest flavonoid content was noted in the extracts of plants grown in the soil treated with vermicompost followed by the plants from the compost treated soil than the plants from the cow dung treated soil (Table 1). It has been recognized that the production of secondary metabolite in plants is related to its growing condition. The antioxidant activity of the herbs grown in these conditions was also varied considerably in terms of IC₅₀ value (Table 2). The highest antioxidant activity was observed in the extracts of plants grown in the soil treated with cow dung followed by the plants from compost treated soil than the plants from vermicompost treated soil. The phenolic compounds act as free radical terminators^[44] and mechanism of action of flavonoids are through scavenging or chelating process^[8,45]. In general, extracts with high antioxidant activity show high phenolics content. The results revealed that the antioxidant activity has correlation with the phenolics content of the herbs. It was also noted that the percentage of activity of the ethanol extracts increases with increasing concentration of the extracts in 200 µl to 1000 µl in all the samples (Fig1). The present results show affinities with the findings of other investigators in certain other medicinal plants^[4]. The extracts of the herbs had overall good antioxidant activity and showed in terms of percentage by the herbs as in cow dung treated soil > compost treated soil > vermicompost treated soil. Herbs from cow dung regime showed significant antioxidant response (over 89% inhibition) on 1000 µl ethanol extract.

Phenols and flavonoids contribute to quality and nutritional value in terms of modifying

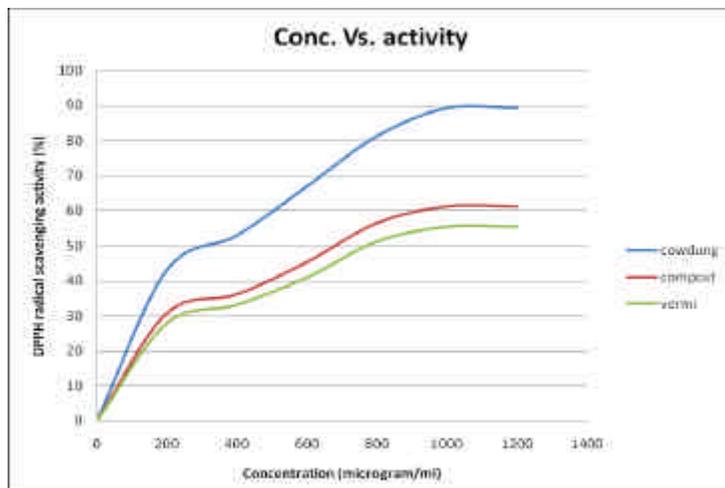


Fig 1: Conc. of ethanol extracts Vs. DPPH free radical scavenging activity of *A. paniculata* leaf grown in different organic manure regimes

colour, taste, aroma and flavour. As a whole the antioxidants are vital substances which possess the ability to protect body from damage by free radical induced oxidative stress. The present study revealed that the extracts of plant leaves grown in cowdung regime exhibit highest antioxidant activity. Detail work on the benefit by using more types of organic manures and different methods is required. Moreover, studies on other medicinal plants would be of great importance.

ACKNOWLEDGEMENT

Authors are thankful to the U.G.C. for financial support to the first author and Dibrugarh University, Assam for providing necessary facilities.

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Source of support: Nil, Conflict of interest: None Declared