



Original Research Article

Total Polyphenolic Contents and Antioxidant Activity of Leaf, Bark and Root of *Adina cordifolia* Benth. & Hook

Pratibha Baral^{1*}, Ashutosh Dubey¹, Salil Tewari², Pashupat Vasmatkar¹, A.K. Verma¹

¹Department of Biochemistry, College of Basic Sciences and Humanities, G.B.P.U.A &T Pantnagar, Udham Singh Nagar, Uttarakhand, India

²Department of Agroforestry, College of Agriculture, G.B.P.U.A &T Pantnagar, Udham Singh Nagar, Uttarakhand, India

*Corresponding Author: Pratibha Baral, Department of Biochemistry, College of Basic Sciences and Humanities, G.B.P.U.A &T Pantnagar, Udham Singh Nagar, Uttarakhand

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ABSTRACT

Adina cordifolia comes under the family Rubiaceae. It has been used in folklore medicine since times immemorial. It is medicinal plant used for the treatment of chronic cough, jaundice, stomachache and several other disease. The methanolic extract of leaf, bark and root were first time subjected to different antioxidant assays such as radical scavenging assay, total antioxidant assay and estimation of polyphenolic contents. Methanolic extract of leaf showed the highest total antioxidant activity i.e., 1.43 ± 0.087 . The IC_{50} values of methanolic extract of leaf for DPPH radical scavenging activity and nitric oxide scavenging activity was found to 48.4 and 110.5 $\mu\text{g/ml}$ respectively. Polyphenolic contents were also found higher in methanolic extract of leaf. The active antioxidant compounds were found higher in methanolic extract of leaf which showed a direct correlation between the total polyphenols extracted and its anti-oxidant activity.

Keyword: *Adina cordifolia*; methanolic extract; scavenging activity; polyphenols

INTRODUCTION

Reactive oxygen species (ROS) helps in many biological system, it prevent disease by assisting the immune system, mediate signaling and playing essential role in apoptosis, besides

these benefits they may harmful if they produce in higher amount. Uncontrolled production of these ROS can damage the tissue, important macromolecule of cells and sometimes it can cause carcinogenesis or

cardiac vascular disease [1]. Antioxidant are the substance which inhibit or delay the oxidation of substrate even if they are present in very less quantity [2,3]. One possible mechanism for this could be scavenging of ROS. Antioxidant compound can be recycled or their oxidation product are less harmful and further converted into harmless substance. Antioxidant including phenolics compound (eg- flavonoids, phenolic acid and tannins) have diverse biological activity such as anti-inflammatory, anticarcinogenic and antiatherosclerotic [4]. The antioxidant properties of extract were evaluated by estimated their bioactive compound like total phenolics, total flavonols, tannins content and proanthocyanidins. On other hand they were also evaluated by determining percentage scavenging such as DPPH, nitric oxide and H₂O₂ etc. Synthetic antioxidants like butylated hydroxytoulene (BHT) and butylated hydroxyanisole (BHA) commonly used in processed food, have side effect and are carcinogenic [5,6]. The natural resources of both potential and established antioxidants are vast. Some antioxidant compounds are extracted from easily available sources, such as agricultural and horticultural crops like maize, buckwheat, grapevine, carrots, beetroot, citrus hesperidia, hops, apples, berries, tea leaves etc. or medicinal plants such as pine, skullcap, sage, rosemary, tormentil, and many others.

Adina cordifolia commonly known as haldu, belongs to family Rubiaceae. It is a large deciduous threatened tree species, scattered in lowland and lower hills of sub Himalayan area of India and some other country like Burma, Thailand, srilanka, Indochina and rarely found in Malasiya. *Adina cordifolia* has been used in oriental medicine since ancient times as an essential component of various febrifuge prescription [7] due to having hepatoprotective activity [8]. Leaf of *Adina cordifolia* is having antidiabetic activity [9] and antifertility properties [10]. Bark of *Adina cordifolia* is having anti-inflammatory, and antinociceptive

activity [11]. Cadambine [12] and triterpenoids [13] were isolated from the bark and stem bark and 3,4,5,7-tetraacetyl quercetin was isolated from the heartwood of *Adina cordifolia* [14]. Bark and root of *Adina cordifolia* is exhibit strong antiamebic activity due to the presence of bioactive compound such as 7-hydroxycoumarin and 7- β -D glucosylcoumarin [15]. Stem has been evaluated for antiulcer potential [16]. However the antioxidant activity in *Adina cordifolia* has not yet been characterized so the present study was aimed to investigate the antioxidant activity of methanolic extract

MATERIAL AND METHODS

Preparation of crude plant extract

The sample of leaf, bark and root of *Adina cordifolia* were collected from Agroforestry Research Centre, G. B. Pant University of Agriculture & Technology, Pantnagar Uttarakhand, in the month of February, 2013. The leaves, root and bark of *Adina cordifolia* were washed thoroughly in running water, dried in hot air oven at 35-40 °C for two days of leaves and seven days of root and bark. The dried plant materials were ground in mechanical grinder. The powdered samples were then used for extraction and stored in an air tight container for further use. Powdered plant material, 25 g was dissolve in 250 mL methanol in 500 mL conical flask. The flask was kept on rotator shaker at 37 °C temperature for 24 h. After shaking, the extract was filtered by Whatman No.1 filter paper and filtrate was evaporated in rotavapour at 65 °C. The dried methanolic extract was weighed and dissolved in methanol to prepare different concentration of stock solutions (1mg/ml).

2, 2-Diphenyl 1- picryl hydrazyl (DPPH) free radical scavenging activity

The 2, 2-diphenyl-1-picrylhydrazine (DPPH) radical scavenging assay was determined by Blois [17] with some modification. The

antioxidant activity was then measured by the decrease in absorption at 517nm. In this method various concentrations of plant sample extracts (20, 40, 60, 80 and 100 µg/ml) were mixed with 1 ml of DPPH solution (0.1 mM of DPPH in methanol). The mixture was incubated at room temperature for 30 min and the absorbance was measured at 517 nm in UV-Visible spectrophotometer. The control and standard were subjected to the same procedure except for the control, where there was no addition of the sample and for the standard sample, there was plant extract replaced. A lower absorbance indicates higher radical scavenging power. DPPH radical scavenging activity was calculated by following equation.

$$\text{DPPH radical scavenging activity (\%)} = [1 - (A_t / A_0)] \times 100$$

(Where A_t is the absorbance of the sample and A_0 is the absorbance of the control at 517 nm).

Nitric oxide scavenging

The nitric oxide scavenging activity of methanolic extract was determined as described by Ebrahimzadeh [18]. A volume of 2 ml of 10mM sodium nitroprusside prepared in 0.5 mM phosphate buffer saline (pH 7.4) was mixed with 1 ml of various concentration of plant extract and ascorbic acid (Standard). The mixture was incubated at 25 °C for 150 min. An aliquot of 0.5 ml of the solution was added to 0.5 mL of Griess reagents [(1.0 mL of sulfanilic acid reagent (0.33% prepared in 20% glacial acetic acid at room temperature for 5 min with 1 ml of naphthyethylenediamine chloride (0.1% w/v)). The mixture was incubated at room temperature for 30 min. The absorbance was measured at 540 nm. The amount of nitric oxide radical was calculated using the equation: NO radical scavenging activity = [(Abs control - Abs sample) / (Abs control)] x100, where Abs control was the absorbance of NO radical + methanol; Abs sample was the absorbance of NO radical + sample extract or standard.

Estimation of Tannins

Tannins estimation assay was performed according to method describe by Attarde [19]. For this tannic acid was taken as standard, 1mL of various concentration of tannic acid solution and 1mL of methanolic plant extracts (100µg/mL) were mixed with 1mL of Folin-ciocalteu reagent and 4mL of (7.5%w/v) sodium carbonate. The absorption was measured after 30 min. at 20 °C at 740nm.

Estimations of Proanthocyanidins

Proanthocyanidins were determined by the vanillin- HCl method describes by Sun [20] with some modifications. Various concentration of 1 ml of catechin was used as standard solution. This catechin solution and 1 mL of methanolic extract were mixed with 4% of vanillin methanol solution. After this 1.5 mL of HCl was added in each test tube then the test tubes were incubated for 20 min at room temperature and absorbance was taken at 500 nm.

Determination of Total antioxidant capacity

The total antioxidant capacity was measured by spectrophotometric method of Prieto [21]. Ascorbic acid was used as standard. Reagent solution (0.6M H_2SO_4 , 28mM sodium phosphate, 4mM ammonium molybdate mixture) 1 ml were added into 1 ml of various concentration of standard (20, 40, 60, 80, and 100 µg/ml) and 1 ml of single concentration of methanolic plant extracts. The tubes were incubated for 90 min at 95°C. The mixture was cooled to room temperature and the absorbance was measured at 695 nm against blank.

Determination of Total flavonols

Total flavonols were estimated using the method of Kumaran and Karunakaran [22]. A volume of 2 ml of the plant extract was mixed with 2 ml of $AlCl_3$ prepared in ethanol and 3 ml of 50 g/L sodium acetate solution. The mixture

was incubated at 20 °C for 2.5 h after which the absorbance was measured at 440 nm. Extract sample were evaluated at a final concentration of 0.1 mg/mL. various concentration of quercetin were taken as standard.

RESULT AND DISCUSSION

Plants have been the basis of traditional medicines throughout the world for thousands of years and continue to provide new remedies to humankind; a great deal of effort has therefore focused on using available experimental techniques to identify natural antioxidants from plants. The DPPH radical scavenging activity of methanolic extracts of

leaf, bark and root of *Adina cordifolia* is shown in Fig 1. Among the extracts tested, leaf extract had higher scavenging activity (IC_{50} value of 48.4 $\mu\text{g/ml}$) followed by bark (IC_{50} value 56.1 $\mu\text{g/ml}$) and root (IC_{50} value 63.4 $\mu\text{g/ml}$). The antioxidant activity of putative antioxidants have been attributed to various mechanisms, among which are prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging [23-24]. DPPH radical is a stable free radical and accepts an electron or hydrogen radical to become a stable diamagnetic molecule [25].

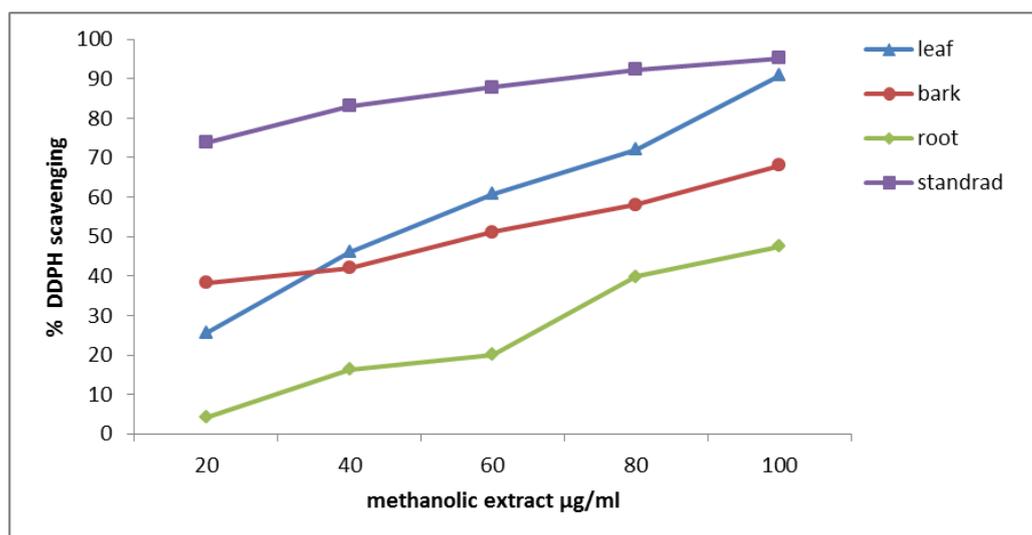


Fig. 1: Scavenging effects of DPPH radical of the methanolic extracts of leaf, bark and root of *Adina cordifolia* at different concentrations

Nitric oxide (NO) is an important chemical mediator generated by endothelial cells, macrophages, neurons etc and is involved in the regulation of various physiological processes (26). Nitric oxide radical generated from the sodium nitropruside and measured by the Greiss reduction. Sodium nitropruside at physiological pH spontaneously generates nitric oxide, which thereby interacts with oxygen to produce nitrate ions that can be estimated by use of Greiss reagents [27]. The methanolic extract of leaf of *Adina cordifolia* exhibit high nitric oxide scavenging activity leading to the

reduction of the nitrite concentration in the assay medium as compare to bark and root extracts. The NO scavenging capacity was concentration dependent with 100 $\mu\text{g/ml}$ scavenging most efficiently. The methanolic extracts in SNP solution significantly inhibited the accumulation of nitrite, a stable oxidation product of NO liberated from SNP in the reaction medium with time compared to the standard ascorbic acid. The present study shows that methanolic extract of leaf has a potent nitric oxide scavenging activity as compare to bark and root. The IC_{50} value of

methanolic extracts were found to be 110.5, 125.7 and 163.2 $\mu\text{g/ml}$ for leaf, bark and root

respectively. Whereas the IC_{50} value of ascorbic acid was found to be 30.75 $\mu\text{g/ml}$ (Fig 2).

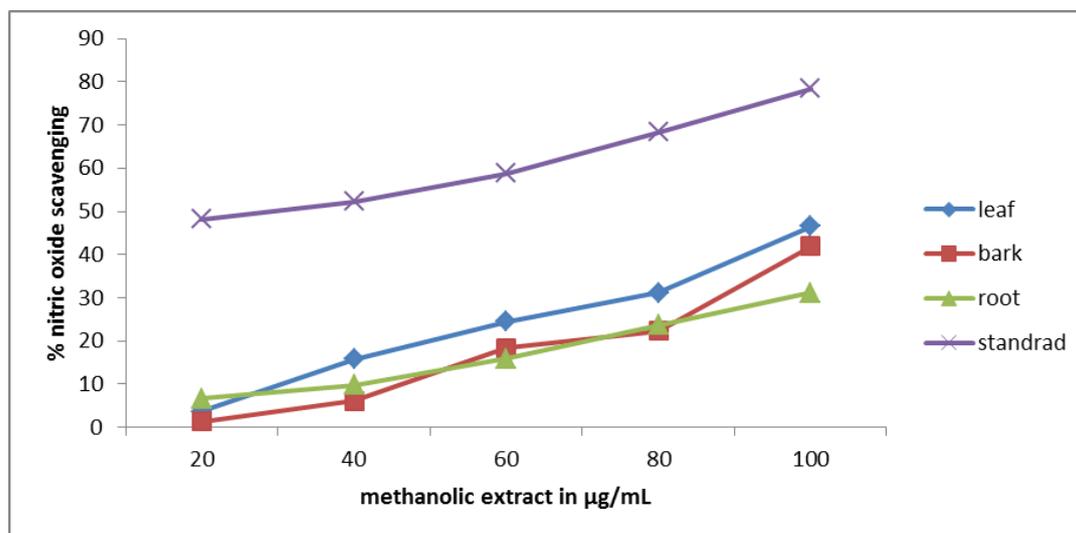


Fig 2 : Scavenging effects of nitric oxide radical of the methanolic extracts of leaf, bark and root of *Adina cordifolia* at different concentrations

The therapeutic effects derived from several medicinal plants have been attributed to the presence of phenolic compounds such as flavanoids, phenolic acid, proanthocyanidins, diterpenes and tannins [28]. These compounds exhibit antioxidant activity by inactivating free radicals or by preventing the decomposition of hydroperoxides into free radicals [29]. Phenolic compounds are known to inhibit various types of oxidizing enzymes. These potential

mechanisms make the diverse group of phenolic compounds an interesting target in the search for health beneficial phytochemicals [30].

A significant level of phenolic compounds in methanolic extracts of leaf, bark and root of *Adina cordifolia* were present. Leaf extract had a higher amount of poly phenolic contents than bark and root extract (Table 1 and Fig 3).

Table 1: Polyphenolic compound in leaf, bark and root of *Adina cordifolia*

Plant part of <i>Adina cordifolia</i>	Flavonol	Proanthocyanidines	Tannins
Leaf	0.054 \pm 0.012	0.136 \pm 0.015	0.034 \pm 0.009
Bark	0.031 \pm 0.025	0.024 \pm 0.0005	0.032 \pm 0.0005
Root	0.032 \pm 0.024	0.002 \pm 0.00	0.02 \pm 0.006

(Mean values \pm standard error of 3 replicates)

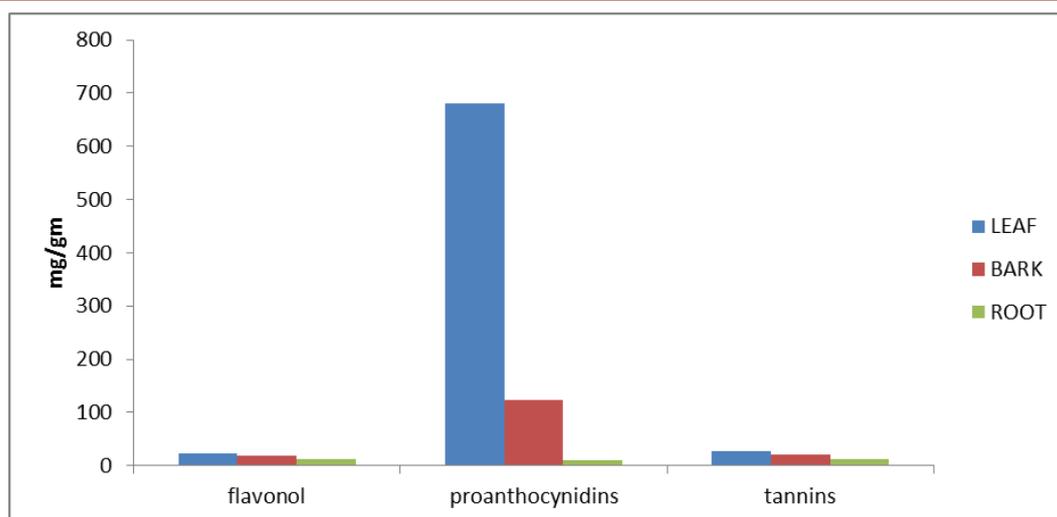


Figure: 3 Polyphenolic contents of methanolic extract of leaf, bark and root

Table 2: Total antioxidant activities of leaf, root and bark of *Adina cordifolia*

Plant part of <i>Adina cordifolia</i>	Total antioxidant activity
Leaf	1.43±0.087
Bark	0.838±0.010
Root	0.374±0.109

Total antioxidant capacity of methanolic extracts of *Adina cordifolia* are expressed as the number of equivalents of ascorbic acid (Table 2). The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing concentration of the plant extract leaf extracts seems to be having a higher capacity than the bark and root extracts. The antioxidant activity of the plant part sample of *Adina cordifolia* are in the order: leaf > bark > root.

CONCLUSION

The results of this study showed that the methanolic extracts of *Adina cordifolia* leaf had higher total antioxidant, polyphenolic contents, DPPH radical and Nitric oxide scavenging than bark and root extracts. So leaves of *Adina cordifolia* can be used as easily accessible

source of natural antioxidants and as a possible food supplement or in pharmaceutical industry. It can be used in stabilizing food against oxidative deterioration. Further study are require for the isolation and identification of individual polyphenolic compounds and also in vivo studies are needed for understanding their mechanism of action as an antioxidant better.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no competing interests.

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