

1 **Antioxidant and free radical scavenging activity comparison in various plant parts of**
2 ***Justicia adhatoda* L.**

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Abstract

There are many medicinal plants that have various medicinal properties in their different parts. The medicinal plants are major backbone of pharmaceutical industries. In this article we compare the antioxidants properties from various plants parts (root, stem, leaf, flower and bark) of the most important medicinal plant, *Justicia adhatoda* L. Various plant parts showed the good amount of antioxidant properties. These results enhance the medicinal properties of this plant due to the presence of good amount of antioxidants; among all the plant parts leaves and flowers showed maximum natural antioxidants, hence the study could be saying that this plant has good efficacy of antioxidants.

Keywords: Antioxidants, Medicinal plant, *Justicia adhatoda* L.

Introduction

Plants utilization as medicine is a very common in tribal areas where, people use different types of plants for their alternative medicine. As cost of these medicinal plants are much cheaper than that of those artificial medicines and there is no side effects in using these medicinal plants. Human are using plant as a medicine not only for their own treatment for various ailments but also use for domestic animals too. Active compound that produced during the secondary metabolism are usually responsible for the medicinal properties of the medicinal plants that are used throughout the world for various purposes, including treatment of various infectious diseases (Singh, 2015). There are about 2000 ethnic groups in the world, and almost every group has own unique traditional medicinal knowledge and experience (Liu et al., 2000). The plants having strong antioxidant properties that prevent from oxidative stress. Nose (2000) defined the antioxidants as 'any substance, when present at low concentrations compared with that of an

oxidisable substrate that significantly delays or prevents oxidation of the substrate'. Antioxidants are essential substance which has power over the capacity to defend the body from reparation caused by complimentary fundamental induced oxidative stress (Masuda et al., 2003; Kanta and Sharma, 2018). Free radicals are continuously produced in the human body, as they are essential for energy supply, detoxification, chemical signaling and immune function. Antioxidant compounds from plants, particularly polyphenols, can inhibits the propagation of free radical reactions and protect the human body from different diseases (Perron and Brumaghim, 2009; Lizcano et al., 2010). Increased level of free radical cause oxidative damage to biological macromolecules and disrupt the balance of cellular oxidation-reduction (Dowling and Simmons, 2009). The free radicals act as a toxic for cellular system, and it increases the chance of susceptible to disease causing pathogen (Sharma and Sharma, 2017a, b). Antioxidants such as phenolic compounds, can inhibits the propagation of free radical reactions and protect the living being from different diseases (Perron and Brumaghim, 2009; Lizcano et al., 2010). Major sources of natural antioxidant are fruits (Arshiya, 2013), vegetables (Parashar et al., 2014), grains (Karrar, 2014), green (Thasleema, 2013) and black tea (Perron and Brumaghim, 2009), coffee (Nardini et al., 2002) and herbs and spices (Alok et al., 2014). The study done on medicinal plants strongly supports the idea that plant constituents with antioxidant activity are capable of exerting protective effects against oxidative stress in biological systems (Kanta et al. 2018). In this study, *Justicia adhatoda* L. selected for screening of antioxidant that belongs to family acanthaceae and widely spread throughout the tropical region of south-east Asia, a common small evergreen, sub-herbaceous bush plant. In India, it is distributed throughout the country especially in the lower Himalaya up to 1300 meters above sea level. Botanically, leaves are simple, opposite, ovate-lanceolate, acute and shiny; flowers white, capsule shape. In Ayurveda

this plant is known as the 'Vasaka' that is well known medicinal plant used by the tribal/indigenous people which is used in treatment of various ailments or disorders including; leprosy, blood disorders, heart troubles, fever, vomiting, jaundice, tumours, leucoderma, mouth troubles, cough, cold, whooping-cough, asthma, bronchitis, tuberculosis, sore eyes and gonorrhea, antispasmodic, expectorant and blood-purifying qualities (Shrivastava et al., 2006; Maurya and Singh, 2010; Bajpai et al., 2015). On the basis of its wide use of medicine our experimental work focused on comparative antioxidant activities of various plant parts such as flower, leaf, stem, bark and root.

Materials and Methods

Justicia adhatoda L. was selected on the basis of availability, seasonality, multiple medicinal uses from the campus of BSI (Botanical Survey of India), Northern Regional Centre, Kaulagarh Road Dehradun, Uttarakhand. Plant tissues from different plant parts (flowers, leaves, bark, stem and roots) were homogenized in a chilled pestle and mortar. The homogenate was centrifuged at 12000 g for 20 min at 4 °C. The resulting supernatant was used as crude enzyme extract. Various antioxidants such as catalase (CAT) (Kato and Shimizu, 1987), peroxidase (PO) (Kar and Mishra, 1975), polyphenol oxidase (PPO) (Soliva et al., 2001), malondialdehyde (MDA) (Heath and Packer, 1968) and ascorbic acid (AA) (Thimmaiah, 1999) were examined by using this enzyme extract. 2, 2'-Diphenyl picryl hydrazyl (DPPH) free radical scavenging activity (Singh et al., 2005) and total phenolic contents (Singleton and Rossi, 1965) were measured on the basis of standard curve with in various concentrations of DDPH methanolic solution and gallic acid equivalent respectively. Whole experiment was done in laboratory conditions with three replicates ($n = 3$) of each treatments statistical analysis of mean values \pm SE that were subjected to one factorial analysis of variance (ANOVA).

88 Results and Discussions

89 The antioxidants evaluated and comparatively analyzed from different plant parts of
 90 *Justicia adhatoda* L. Catalase (CAT), Peroxidase (PO) and Polyphenol oxidase (PPO) that were
 91 measure in unit mg^{-1} fresh weight (U mg^{-1} fresh wt.); Melondialdehyde (MDA) and Ascorbic
 92 acid (AA) were measured in $\mu\text{g g}^{-1}$ fresh wt. which have been presented in table 1. Maximum
 93 CAT activity (91.56 ± 4.15) recorded in leaves followed by flowers (45.23 ± 3.11) while least in
 94 roots (17.09 ± 2.63); similar as CAT, maximum and minimum PO recorded in leaves (1.87 ± 0.61)
 95 and roots (0.32 ± 0.44) respectively while bark showed second largest amount (1.03 ± 0.51) of PO.
 96 In the observation of PPO bark showed maximum (9.21 ± 2.49) followed by flowers (7.76 ± 1.44)
 97 and roots (5.22 ± 1.61) while leaves (1.04 ± 0.87) showed least amount of PPO activity. In MDA
 98 evaluation leaves have maximum activity (79.24 ± 3.21) while roots (48.56 ± 2.18) showed
 99 minimum; for AA measurements stem had maximum (2850 ± 1.22) followed by roots
 100 (2130 ± 1.06) and bark (1970 ± 1.37) along with least activity in flowers (230 ± 1.56). The total
 101 DPPH % free radical scavenging activity ranges from 86.84 ± 2.40 to 93.85 ± 2.51 [86.84 ± 2.40
 102 (stem) < 90.36 ± 2.83 (leaves) < 90.64 ± 1.44 (bark) < 93.23 ± 2.15 (roots) < 93.85 ± 2.51 (flowers)]
 103 (fig. 1). Similarly, the total phenolic content ($\mu\text{g/mgGAE}$) range from 29.51 ± 2.88 to 69.83 ± 1.34
 104 [29.51 ± 2.88 (stem) < 50.32 ± 2.93 (bark) < 59.22 ± 1.47 (roots) < 62.57 ± 2.01 (leaves) <
 105 69.83 ± 1.34 (flowers)] in various plant parts (fig. 2).

106 The plant showed strong antioxidant and reducing power ability. Antioxidant enzymes
 107 are defensive against oxidative stresses that have been studied extensively in higher plants
 108 (Polidoros and Scandalios, 1999). CAT, the first reported antioxidant enzyme is the most universal
 109 oxidoreductase, which scavenges H_2O_2 to O_2 and H_2O that metabolize the peroxide (Qureshi et
 110 al., 2007; Garg and Manchanda, 2009). PO is an oxidoreductase that is directly involved in the

plant physiological functions such as hormone regulation, defence mechanisms, indolacetic degradation and lignin biosynthesis (Serrano-Martínez et al., 2008). These properties of antioxidant found in every parts of plant that make them medicinally important similar to this current study in which each plant parts shows good efficacy of antioxidants (Yen and Duh, 1993; Saran et al., 2019). The medicinal properties of plants generally due to the presence of secondary metabolites as alkaloids, phenols, tannin etc. which are present in various plant parts (Palombo, 2006). Phenolic compounds have redox properties that can impart antioxidant properties to the plants where they act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal chelators (Cook and Samman, 1996; Kumar et al., 2013). The phenolic compounds are predominantly associated with antioxidant activities that scavenge free radicals which are generally produced in human body, in this scenario various *in vitro* models have been widely used to investigate antioxidants potential of plant materials (Porto et al., 2000; Rajurkar et al., 2012; Pant et al., 2015). The plant extracts generally rich in antioxidants and other essential phytoconstituents earlier presented in various antioxidant models (Farhan et al., 2012; Amari et al., 2014). The DPPH interactions with antioxidants neutralize the free radicals where antioxidants transfer electron or hydrogen atoms to DPPH (Archana et al., 2005). Many earlier studies on the leaves (Rao et al., 2013; Saran et al., 2019; Bajpai et al., 2015) and flowers (Naqvi et al., 2013) of *Justicia adhatoda* L. were determined that are similar to this study. Nutshell, on the basis of current results each plant part could be medicinally important due to the presence of antioxidant; among all the plant parts leaves and flowers showed maximum natural antioxidants, hence the study could be saying that this plant has good efficacy of antioxidants.

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Table 1.

Plant Parts	Antioxidant Enzymes				
	Catalase	Peroxidase	Polyphenol	Malondialdehyde	Ascorbic
	(CAT)	(PO)	oxidase	(MDA)	Acid (AA)
			(PPO)		
	(U mg ⁻¹ fresh wt.)			(µg g ⁻¹ fresh wt.)	
Flower	45.23±3.11	0.68±0.24	7.76±1.44	62.11±2.59	0230±1.56
Leaf	91.56±4.15	1.87±0.61	1.04±0.87	79.24±3.21	1550±0.99
Stem	32.11±2.02	0.49±0.35	3.72±0.43	65.33±0.66	2850±1.22
Bark	39.88±3.85	1.03±0.51	9.21±2.49	51.45±1.99	1970±1.37
Root	17.09±2.63	0.32±0.44	5.22±1.61	48.56±2.18	2130±1.06

Figures

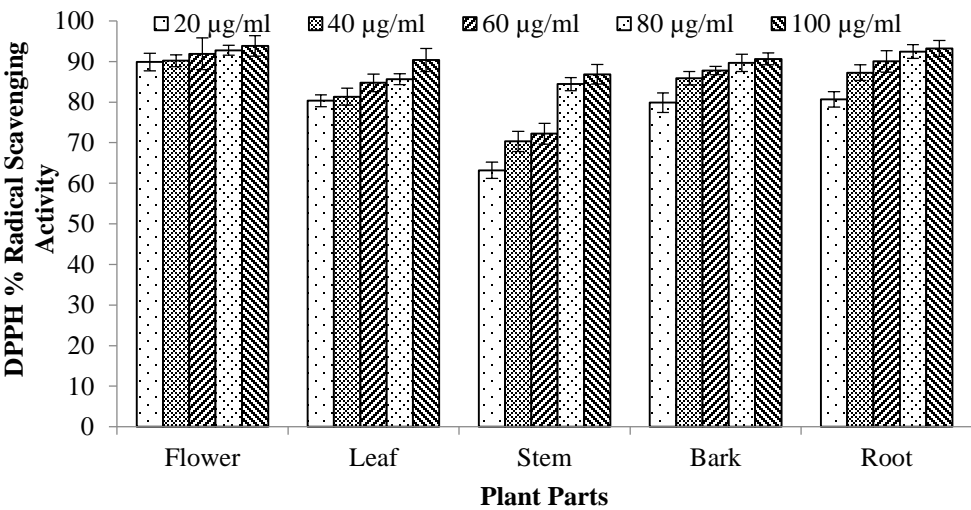


Fig. 1. DPPH % radical scavenging activity with various concentrations (µg/ml) in various plant parts of *J. adhatoda*

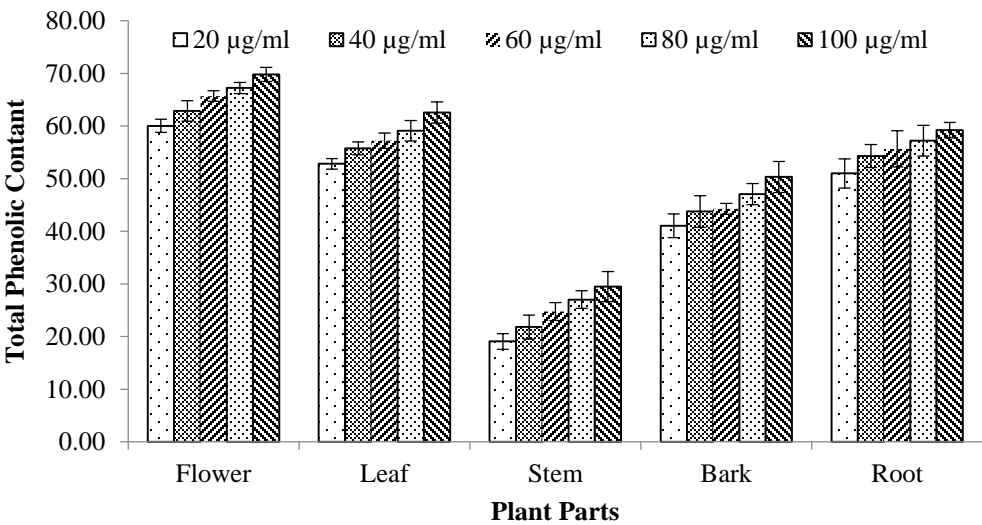


Fig. 2. Total phenolic contents with various concentrations (µg/ml) in various plant parts of *J. adhatoda*