1   **Antioxidant and free radical scavenging activity comparison in various plant parts of**

2   ***Justicia adhatoda* L.**

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20   **Abstract**

21   There are many medicinal plants that have various medicinal properties in their different

22   parts. The medicinal plants are major backbone of pharmaceutical industries. In this article we

23   compare the antioxidants properties from various plants parts (root, stem, leaf, flower and bark)

24   of the most important medicinal plant, *Justicia adhatoda* L. Various plant parts showed the good

25   amount of antioxidant properties. These results enhance the medicinal properties of this plant due

26   to the presence of good amount of antioxidants; among all the plant parts leaves and flowers

27   showed maximum natural antioxidants, hence the study could be saying that this plant has good

28   efficacy of antioxidants.

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

30   **Introduction**

31   Plants utilization as medicine is a very common in tribal areas where, people use different

32   types of plants for their alternative medicine. As cost of these medicinal plants are much cheaper

33   than that of those artificial medicines and there is no side effects in using these medicinal plants.

34   Human are using plant as a medicine not only for their own treatment for various ailments but

35   also use for domestic animals too. Active compound that produced during the secondary

36   metabolism are usually responsible for the medicinal properties of the medicinal plants that are

37   used throughout the world for various purposes, including treatment of various infectious

38   diseases (Singh, 2015). There are about 2000 ethnic groups in the world, and almost every group

39   has own unique traditional medicinal knowledge and experience (Liu et al., 2000). The plants

40   having strong antioxidant properties that prevent from oxidative stress. Nose (2000) defined the

41   antioxidants as 'any substance, when present at low concentrations compared with that of an

42   oxidisable substrate that significantly delays or prevents oxidation of the substrate'. Antioxidants

43   are essential substance which has power over the capacity to defend the body from reparation

44   caused by complimentary fundamental induced oxidative stress (Masuda et al., 2003; Kanta and

45   Sharma, 2018). Free radicals are continuously produced in the human body, as they are essential

46   for energy supply, detoxification, chemical signaling and immune function. Antioxidant

47   compounds from plants, particularly polyphenols, can inhibits the propagation of free radical

48   reactions and protect the human body from different diseases (Perron and Brumaghim, 2009;

49   Lizcano et al., 2010). Increased level of free radical cause oxidative damage to biological

50   macromolecules and disrupt the balance of cellular oxidation-reduction (Dowling and Simmons,

51   2009). The free radicals act as a toxic for cellular system, and it increases the chance of

52   susceptible to disease causing pathogen (Sharma and Sharma, 2017a, b). Antioxidants such as

53   phenolic compounds, can inhibits the propagation of free radical reactions and protect the living

54   being from different diseases (Perron and Brumaghim, 2009; Lizcano et al., 2010). Major

55   sources of natural antioxidant are fruits (Arshiya, 2013), vegetables (Parashar et al., 2014), grains

56   (Karrar, 2014), green (Thasleema, 2013) and black tea (Perron and Brumaghim, 2009), coffee

57   (Nardini et al., 2002) and herbs and spices (Alok et al., 2014). The study done on medicinal

58   plants strongly supports the idea that plant constituents with antioxidant activity are capable of

59   exerting protective effects against oxidative stress in biological systems (Kanta et al. 2018). In

60   this study, *Justicia adhatoda* L. selected for screening of antioxidant that belongs to family

61   acanthaceae and widely spread throughout the tropical region of south-east Asia, a common

62   small evergreen, sub-herbaceous bush plant. In India, it is distributed throughout the country

63   especially in the lower Himalaya up to 1300 meters above sea level. Botanically, leaves are

64   simple, opposite, ovate-lanceolate, acute and shiny; flowers white, capsule shape. In Ayurveda

65 this plant is known as the 'Vasaka' that is well known medicinal plant used by the

66 tribal/indigenous people which is used in treatment of various ailments or disorders including;

67 leprosy, blood disorders, heart troubles, fever, vomiting, jaundice, tumours, leucoderma, mouth

68 troubles, cough, cold, whooping-cough, asthma, bronchitis, tuberculosis, sore eyes and

69 gonorrhea, antispasmodic, expectorant and blood-purifying qualities (Shrivastava et al., 2006;

70 Maurya and Singh, 2010; Bajpai et al., 2015). On the basis of its wide use of medicine our

71 experimental work focused on comparative antioxidant activities of various plant parts such as

72 flower, leaf, stem, bark and root.

73 **Materials and Methods**

74   *Justicia adhatoda* L. was selected on the basis of availability, seasonality, multiple

75 medicinal uses from the campus of BSI (Botanical Survey of India), Northern Regional Centre,

76 Kaulagarh Road Dehradun, Uttarakhand. Plant tissues from different plant parts (flowers, leaves,

77 bark, stem and roots) were homogenized in a chilled pestle and mortar. The homogenate was

78 centrifuged at 12000 g for 20 min at 4 ºC. The resulting supernatant was used as crude enzyme

79 extract. Various antioxidants such as catalase (CAT) (Kato and Shimizu, 1987), peroxidase (PO)

80 (Kar and Mishra, 1975), polyphenol oxidase (PPO) (Soliva et al., 2001), malondialdehyde

81 (MDA) (Heath and Packer, 1968) and ascorbic acid (AA) (Thimmaiah, 1999) were examined by

82 using this enzyme extract. 2, 2'-Diphenyl picryl hydrazyl (DPPH) free radical scavenging

83 activity (Singh et al., 2005) and total phenolic contents (Singleton and Rossi, 1965) were

84 measured on the basis of standard curve with in various concentrations of DDPH methanolic

85 solution and gallic acid equivalent respectively. Whole experiment was done in laboratory conditions

86 with three replicates ($n = 3$) of each treatments statistical analysis of mean values ± SE that were

87 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 pxidase (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 μg 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 ($\mu g/mgGAE$) 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

111   plant physiological functions such as hormone regulation, defence mechanisms, indolacetic

112   degradation and lignin biosynthesis (Serrano-Martínez et al., 2008). These properties of

113   antioxidant found in every parts of plant that make them medicinally important similar to this

114   current study in which each plant parts shows good efficacy of antioxidants (Yen and Duh, 1993;

115   Saran et al., 2019). The medicinal properties of plants generally due to the presence of secondary

116   metabolites as alkaloids, phenols, tannin etc. which are present in various plant parts (Palombo,

117   2006). Phenolic compounds have redox properties that can impart antioxidant properties to the

118   plants where they act as reducing agents, hydrogen donors, singlet oxygen quenchers and metal

119   chelators (Cook and Samman, 1996; Kumar et al., 2013). The phenolic compounds are

120   predominantly associated with antioxidant activities that scavenge free radicals which are

121   generally produced in human body, in this scenario various *in vitro* models have been widely

122   used to investigate antioxidants potential of plant materials (Porto et al., 2000; Rajurkar et al.,

123   2012; Pant et al., 2015). The plant extracts generally rich in antioxidants and other essential

124   phytoconstituents earlier presented in various antioxidant models (Farhan et al., 2012; Amari et

125   al., 2014). The DPPH interactions with antioxidants neutralize the free radicals where

126   antioxidants transfer electron or hydrogen atoms to DPPH (Archana et al., 2005). Many earlier

127   studies on the leaves (Rao et al., 2013; Saran et al., 2019; Bajpai et al., 2015) and flowers (Naqvi

128   et al., 2013) of *Justicia adhatoda* L. were determined that are similar to this study. Nutshell, on

129   the basis of current results each plant part could be medicinally important due to the presence of

130   antioxidant; among all the plant parts leaves and flowers showed maximum natural antioxidants,

131   hence the study could be saying that this plant has good efficacy of antioxidants.

132   **Acknowledgement**

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254   **Table 1.**

Plant Parts	Antioxidant Enzymes				
	Catalase (CAT)	Peroxidase (PO)	Polyphenol oxidase (PPO)	Malondialdehyde (MDA)	Ascorbic Acid (AA)
	(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

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265   **Figures**

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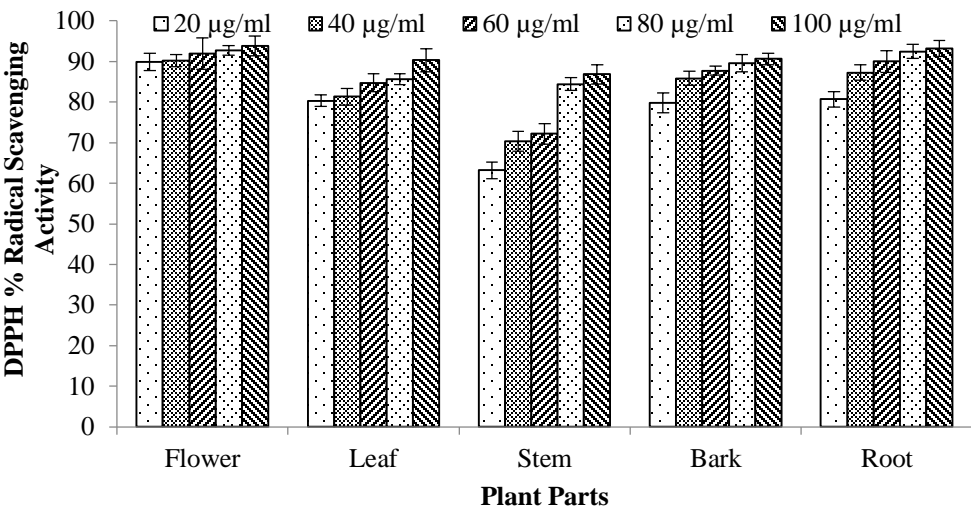
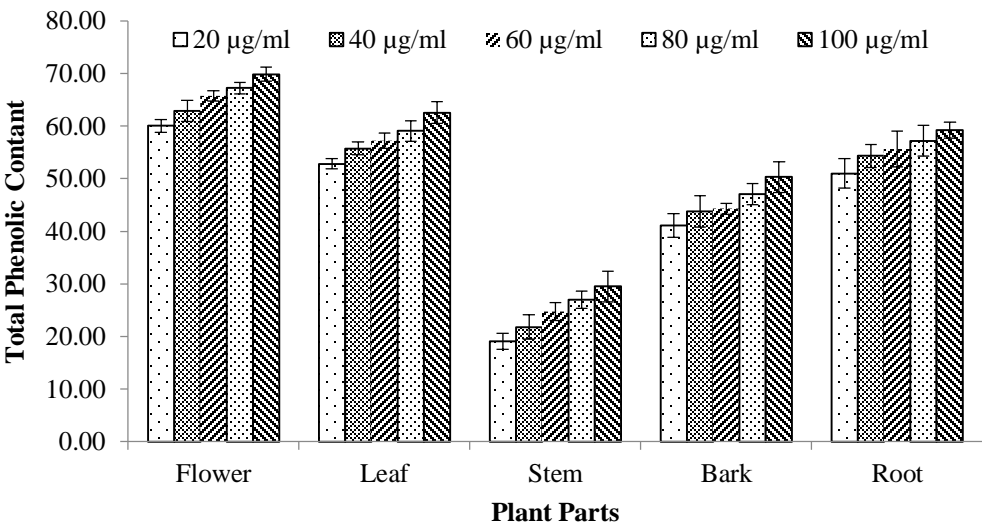

267   **Fig. 1.** DPPH % radical scavenging activity with various concentrations (µg/ml) in various plant

268   parts of *J. adhatoda*

269

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

271   *adhatoda*