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## PHYTOCHEMICAL AND ANTIOXIDANT STUDIES OF *JUSTICIA GENDARUSSA* BURM. F. AN ETHNOMEDICINAL PLANT

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**ABSTRACT:** Present investigation deals with the phytochemical and antioxidant studies of *Justicia gendarussa* Burm.f. (family Acanthaceae), a medicinally important herb. Biochemical studies highlighted that in leaf tissue, quantities of soluble carbohydrate, amino acid and ascorbic acid are of 307 mg/g, 34.93 mg/g and 619µg/g, respectively. In stem bark, quantities of soluble carbohydrate, amino acid and ascorbic acid are of 185 mg/g, 26.86 mg/g and 330.33µg/g, respectively. Pigment content in dried leaf has been estimated as chlorophyll a 46µg/g, chlorophyll b 16.1µg/g and total carotenoids 16.7µg/g. In bark, amounts of chlorophyll a, chlorophyll b and total carotenoids are of 23.9µg/g, 21 µg/g and 14.6µg /g, respectively. Enzyme content in fresh leaf showed that amount of the catalase is 4.89 EU/g fresh tissue and in bark it is 2.39 EU/ g. Peroxide is 3.4 EU/g for fresh leaf and 2.21 EU/g in stem bark DPPH radical scavenging activity was considerably high for methanolic extract of leaf that is 31.36 % and in case of bark the activity was 23.74 %. The IC<sub>50</sub> value of the leaf extract was 75µg/mL and for bark it was 119µg/mL. The total phenolic content in leaf was 33.96 mg/g and in bark it was 31.4 mg/g. The medicinal properties of this plant nicely correlate with its phytochemical content and antioxidant activities. Further scientific investigation of the plant has to be carried out to standardize the bioactive compounds.

**INTRODUCTION:** The use of herbs as medicine is the oldest form of healthcare known in human civilization and has been used in all cultures of human being worldwide.<sup>1</sup> Medicinal plants are the back bone of any traditional or folk medicine. In fact, they are the oldest friend of humans<sup>2</sup>. According to the WHO, more than 80% of the world population, mostly in third world rely on traditional plant based medicine for their primary healthcare needs<sup>3</sup>.

Majority of herbal medicines have now been identified as very effective therapeutic agents. Herbal drugs contain a variety of chemical compounds that are basically responsible for prevention and treatment of diseases or promotion of health and well-being<sup>4</sup>.

Antioxidant means any substance when present at low concentration compared to those of oxidizable substrates, significantly delays or prevents oxidation of those substrates. Oxidative stress occurs due to oxidative biochemical reactions taken place in the cells or organ<sup>5</sup>. During metabolic reactions, aerobic cells produce reactive oxygen species (ROS) such as superoxide anion (O<sub>2</sub><sup>-</sup>), hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), hydroxyl radical (OH•), and organic peroxides as normal products of the biological reduction of molecular oxygen. The

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disparity between the amount of ROS and antioxidant substances leads to damage of important bio-molecules and membrane system of the cells which ultimately creates severe impact on the whole organism. To protect cells and organs from the oxidative stress induced by ROS, living organisms have evolved with very efficient and highly sophisticated protective system, the so called “antioxidant defensive system”.

This defensive system involves a variety of components, both endogenous and exogenous in origin, which function interactively and synergistically to neutralize the free radicals produced through oxidative biochemical reaction<sup>6</sup>. The enzymatic antioxidants [superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase, catalase, Peroxidase, etc.] and non-enzymatic antioxidants [(glutathione (GSH), vitamins C, D, E, carotenoids, xanthophylls, anthocyanins, bilirubin, etc.)] are involved here in protecting the cells or organisms against the harmful free radicals.

There is an interest now growing among the people worldwide in using the herbal products along with the modern medicine for health care. Herbal crude drugs used in many traditional societies in the world are based on the age-old knowledge and superstitions passed from generation to generation<sup>7</sup>. In developed countries, modern medicines are in many cases complemented with plant based drugs for effective health care. For this, research activity on medicinal plants has now got a new momentum throughout the world. The scientific investigation of medicinally important plants is now being carried out covering various aspects of their studies like efficacy, phytochemical evaluation, pharmacognostic characters etc.

Biological assay and chemical evaluation of crude drugs obtained from medicinally important plants are the important aspects for identification of novel bio-active compounds as well as drug discovery. Many members of the family Acanthaceae have so far been studied pharmacognostically<sup>8, 9, 10</sup>. But no work has been done on *Justicia gendarussa* Burm.f. of the family Acanthaceae in respect of its pharmacognostic, phytochemical and antioxidant studies. Therefore, the present investigation has

been under taken to investigate the phytochemical and antioxidant properties of this ethnomedicinal plant to understand the phytochemical groups responsible for its antioxidant activity.

## MATERIAL AND METHODS:

*Justicia gendarussa* Burm.f. (Acanthaceae)

**English name:** Gandarusa

**Common names:** Jagatmadan (Bengali), Kala bashimb (Hindi), Bhutakeshi (Sanskrit)

**Tribal name:** Tao (Sanatli)



FIGURE 1: *JUSTICIA GENDARUSSA* BURM.F. PLANT

*Justicia gendarussa* Burm.f. collected from Santiniketan, West Bengal and voucher specimens have been kept in the Visva-Bharati Herbarium, Department of Botany, Visva-Bharati, Santiniketan. The collected plant parts (leaf and stem bark) were washed, shed dried at room temperature and then ground in a grinder to fine powder for its further study.

### Preliminary microchemical screening:

Freeze dried methanolic extracts of different plant parts was screened for detection of different phytochemical groups by chemical colour reaction tests following different standard methods<sup>11; 12</sup>.

### Estimation of total phenolics:

Total phenolics was estimated by standard methods<sup>13</sup>. The plant sample of 0.5 g was taken and ground with 5 mL of 80% ethanol in a mortar and pestle.

Homogenates was centrifuged at 10,000 rpm for 20 min. Supernatant was collected and the pellet was re-centrifuged. Then both the supernatants were collected and dried. Residues were dissolved in 5 mL of distilled water. 0.5 mL of aliquot was taken in a test tube, the volume was made up to 3 mL with distilled water and 0.5 mL of folin-ciocalteu reagent was added. After 3 minutes, 2mL of 20% Na<sub>2</sub>CO<sub>3</sub> was added to the test tube and mixed it thoroughly. Test tubes were placed on boiling water bath for 1 min and cooled it at room temperature. Then absorbance was measured at 650 nm against a blank.

#### Estimation of ascorbic acid:

1 g of fresh plant samples were homogenized with 25 mL of 4% oxalic acid solution in a mortar and pestle. Homogenates were filtered. 10 mL of the filtrate was transferred to a conical flask and bromine water was added to the flask drop wise until the extract turns orange or yellow. Finally the volume was made to 25 mL with the help of 4% oxalic acid. From this stock solution, 2 mL of sample was pipetted out and volume was made to 3mL with distilled water. 1 mL of DNPH reagent followed by 1-2 drops of thiourea was added to the test tube. Test tube content was mixed thoroughly and incubated at 37<sup>0</sup> C for 3 hours. After incubation, 7 mL of 80% sulphuric acid was added to the test tube and absorbance was measured at 540 nm against a blank <sup>14</sup>.

#### Estimation of total carotenoids:

2g of plant sample was homogenized with 20 mL acetone. Supernatant was filtered and extraction was continued until the tissue became colourless. Pool the filtrates and partitioned thrice with equal volume of peroxide-free ether using a separatory funnel. Combined ether layers were evaporated under reduced pressure at 35<sup>0</sup> C using a rotary evaporator. Residue was dissolved with 5 mL of ethanol. Then 0.5 mL of 60% KOH was added to the ethanol fraction to saponify it.

The mixture was boiled for 5 min and kept in dark over night. Equal volume of water was added and partitioned twice with ether. Combined layer was evaporated as before and residue was dissolved with 2 mL of ethanol. Absorbance was measured at 450nm. <sup>15</sup>

#### Estimation of chlorophyll:

It was done following the standard method <sup>16</sup>. 1 g of finely cut plant sample was homogenized with 20mL of 80% acetone. Pulp was collected, centrifuged it at 5000 rpm for 5 min and then supernatant was transferred to a volumetric flask. Procedure was repeated until the residue becomes colourless. Made the volume to 100mL with 80% acetone. Absorbance of the solution was measured at 645, 663 & 652nm wavelength against the solvent (80% acetone).

Amount of chlorophyll present in the extract was calculated by the following equations-

$$\text{mg Chlorophyll a /g tissue} = 12.7(A_{663}) - 2.69(A_{645}) \times \frac{V}{1000 \times W}$$

$$\text{mg Chlorophyll b /g tissue} = 22.9(A_{645}) - 2.96(A_{663}) \times \frac{V}{1000 \times W}$$

Where, A= Absorbance at specific wavelengths,  
V= Final volume of chlorophyll extract in 80% acetone

W= Fresh weight of the tissue extracted

#### Extraction and estimation of catalase:

For extraction and estimation of Catalase the standard method <sup>17</sup> was followed with some necessary modification. For this 100 mg of plant samples (leaf & stem bark separately) were homogenized with 100 mM sodium- phosphate buffer (pH 7) containing 1% poly vinyl poly pyrrolidone (PVPP). Homogenate was centrifuged at 5000 rpm for 10 min at 4<sup>0</sup> C temperature. For enzyme assay 1 mL of enzyme extract was added to 1 mL of 0.5μM H<sub>2</sub>O<sub>2</sub> and kept it as such for 15 min at 37<sup>0</sup> C temperature. The reaction was stopped by adding 2mL of 1% TiSO<sub>4</sub> (in 25% H<sub>2</sub>SO<sub>4</sub>). The assay mixture was further centrifuged, supernatant was collected and the absorbance was read at 420 nm. The enzyme activity was expressed <sup>18</sup>.

#### Extraction and estimation of peroxidase:

For this, the standard method <sup>19</sup> was followed with some necessary modification. 100mg of tissue samples (leaf and stem bark separately) were homogenized with 5mL of 50mM pre-chilled sodium- phosphate buffer (pH 6.5) at 0<sup>0</sup>c. Homogenates were centrifuged at 1000 rpm for 10 min at 4<sup>0</sup> C. Supernatant was used as enzyme source. The assay mixture was consisted of 2mL of 125mM Na-phosphate buffer (pH 6.5), 1mL

pyrogallol (94.5 mg pyrogallol in 50 mL distilled water), 1 mL of H<sub>2</sub>O<sub>2</sub> (0.56 mL of 30% H<sub>2</sub>O<sub>2</sub> in 100mL distilled water) & 1 mL of enzyme source. The mixture was incubated at 37<sup>0</sup>C for 30 min. The reaction was stopped by adding 0.5 mL of 5% H<sub>2</sub>SO<sub>4</sub>. Finally the absorbance was measured at 420nm. The enzyme activity was expressed<sup>18</sup>.

For the assay of these two enzymes, the blank was taken as zero time control and the activity of the enzyme was expressed as:

$$(\Delta A \times TV)/t \times v$$

Where,  $\Delta A$  is the absorbance of sample after incubation minus the absorbance of zero time control. TV is the total volume of filtrate taken for incubation. 't' is the incubation time in minutes and 'v' is the volume of filtrate taken for incubation<sup>18</sup>.

#### DPPH radical scavenging activity:

DPPH radical scavenging activity was determined following the standard method<sup>20</sup>. The stock solution was prepared by dissolving 24 mg DPPH in 100mL methanol and then stored at -20<sup>0</sup> C until needed. The working solution was obtained by mixing 10mL stock solution with 45mL methanol to obtain an absorbance of 1.1± 0.02 units at 515 nm using the spectrophotometer. Plant extracts (150 $\mu$ L) were allowed to react with 2850 $\mu$ L of DPPH solution for 24 h in the dark.

Then the absorbance was taken at 515nm. The standard curve was prepared with Ascorbic acid. Results were expressed in % of scavenging activity. The experiment was carried out in triplicate. The IC<sub>50</sub> value i.e. the concentration of sample required to scavenge 50% of stable DPPH free radicals was determined from the % inhibition vs. concentration of different plant extract and Ascorbic acid by comparing the absorbance values of control (A<sub>0</sub>) and test compounds (A<sub>t</sub>).

$$\% \text{ Radical scavenging activity} = \left( A_0 - \frac{A_t}{A_0} \right) \times 100$$

**Statistical analysis:** All the data provided in this study represent means  $\pm$  S.E.M. The results were analyzed by ANOVA two factors with replication to establish the significance (p < 0.01). Results of all the experiments were analyzed to establish the correlation among them by MS EXCEL 2007 software.

**RESULTS:** Data on phytochemical screening and antioxidant assay of the investigated plant species are given below (Tables 1, 2, 3 and 4).

#### Microchemical evaluation of powdered plant parts:

Many phytochemical groups have been detected from the methanolic extracts of both leaf and stem bark of the plant. The important phytochemical groups found in both cases are alkaloids, flavonoids, tannins, saponins, reducing sugar, amino acids and anthraquinones which basically confirm the medicinal properties of this plant (Table1; Fig. 2).

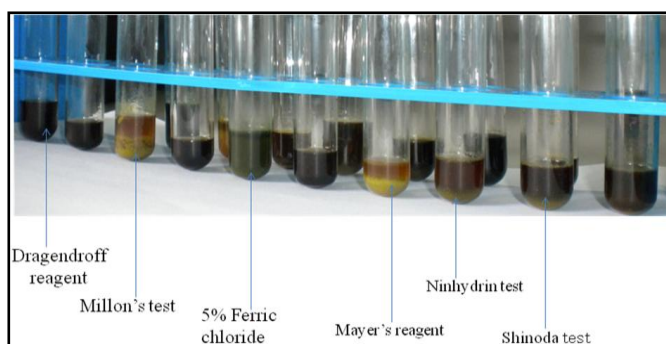


FIGURE 2: MICROCHEMICAL TESTS OF METHANOLIC EXTRACTS OF *J. GENDARUSSA*

#### Soluble carbohydrate content:

Amount of soluble carbohydrate in leaf is 307  $\pm$  2.88 mg/g fresh tissue and it is 185  $\pm$  6.08 mg/g fresh tissue of stem bark (Table 2 Fig. 3). Both leaf and stem bark contained a significant amount of soluble carbohydrate. Leaf extract of the plant showed greater amount of soluble carbohydrate than stem bark.

#### Amino acid content:

The quantity of amino acids in fresh leaf is 34.93  $\pm$  2.16 mg/g tissue and it is 26.86  $\pm$  1.50 mg/g of fresh stem bark (Table 2, Fig. 3). Amount of amino acids is comparatively more in leaf part than stem bark.

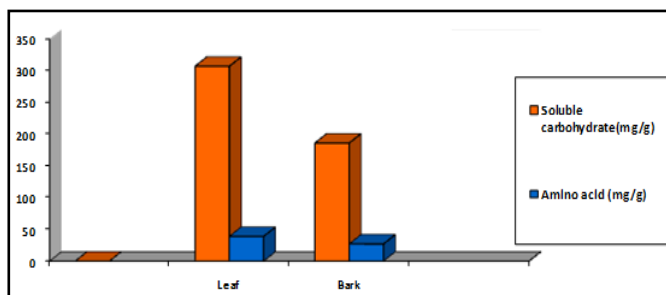


FIG. 3: SOLUBLE CARBOHYDRATE AND AMINO ACID CONTENT OF THE LEAF AND BARK

**Ascorbic acid content:**

Ascorbic acid is a naturally occurring organic compound with antioxidant properties. In leaf, the ascorbic acid content is  $619 \pm 2.30 \mu\text{g/g}$  fresh tissue and it is of  $330.33 \pm 2.96 \mu\text{g/g}$  in fresh stem bark (Table 2). From the findings it is clear that the investigated plant contains greater amount of ascorbic acid in its leaves than stem bark.

**Pigments content:**

Plant pigments play a very crucial role in maintaining the antioxidant properties of the species. In leaf, amount of chlorophyll a is  $46 \pm 0.04 \mu\text{g/g}$  dry tissue, chlorophyll b  $16.1 \pm 0.01 \mu\text{g/g}$  dry tissue and total carotenoids is  $16.7 \pm 0.03 \mu\text{g/g}$  dry tissue. In bark, amount of chlorophyll a is  $23.9 \pm 0.02 \mu\text{g/g}$  dry tissue, chlorophyll b  $21 \pm 0.01 \mu\text{g/g}$  dry tissue and total carotenoids is  $14.6 \pm 0.05 \mu\text{g/g}$  dry tissue (Table 2; Fig. 4). Chlorophyll a and total carotenoids content is more in leaf part than stem bark, whereas Chlorophyll b content is higher in stem bark than the leaf part.

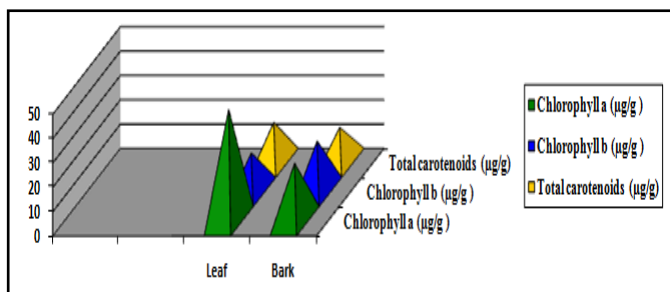


FIG. 4: CHLOROPHYLL A, CHLOROPHYLL B AND TOTAL CAROTENOIDS CONTENT OF THE LEAF AND BARK

**Catalase content:**

The enzyme Catalase catalyzes the decomposition of hydrogen peroxide to water and oxygen in the cells. It is a very important enzyme in protecting the cells from the oxidative damage caused by reactive oxygen species (ROS). Amount of Catalase in leaf is  $4.89 \pm 0.04 \text{ EU/g}$  fresh tissue and in bark it is  $2.39 \pm 0.08 \text{ EU/g}$  fresh tissue (Table 3; Fig. 5). Leaf part possesses greater amount of Catalase enzyme than bark.

**Peroxidase content:**

Peroxidase is involved in many physiological processes in plants which include responses to biotic and abiotic stresses, biosynthesis of lignin and also scavenging of reactive oxygen species (ROS). Peroxidase content is  $3.4 \pm 0.04 \text{ EU/g}$  for

fresh leaf and it is  $2.21 \pm 0.09 \text{ EU/g}$  in stem bark (Table 3, Fig. 5). Peroxidase content is also greater in leaf tissue than the tissue of stem bark.

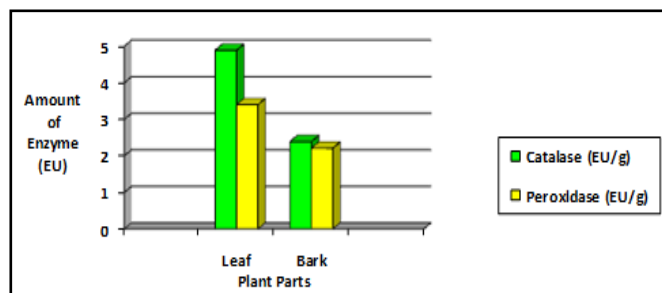


FIG. 5: CATALASE AND PEROXIDASE CONTENT OF THE LEAF AND BARK

**Total phenolic content:**

Phenolics are one of the major groups of antioxidant compounds reported to be involved in free radical scavenging activity. The total phenolic content in leaf is  $33.96 \pm 1.44 \text{ mg/g}$  tissue. Amount of total phenols in stem bark is  $31.4 \pm 5.53 \text{ mg/g}$  (Table 3). Here also amount of total phenolic compounds is significantly higher in leaf tissue than the bark.

**DPPH radical scavenging activity:**

DPPH radical scavenging activity is significantly high for methanolic extract of leaf tissue (at  $10 \mu\text{g/mL}$  concentration) that is  $31.36 \pm 0.05 \%$  ascorbic acid equivalent and in case of bark tissue, the activity is  $23.74 \pm 0.06 \%$  ascorbic acid equivalents. (Table 4; Fig. 6).

$\text{IC}_{50}$  (i.e the concentration of the extracts or compounds that scavenge 50% of a given amount of free radicals) value was determined for both the plant parts.  $\text{IC}_{50}$  value of the leaf extract is  $75 \mu\text{g/mL}$  and for bark it is  $119 \mu\text{g/mL}$ . The  $\text{IC}_{50}$  value for stem bark is higher than leaf of this plant which indicates that greater amount of stem bark tissue is to be needed than leaf to scavenge 50% of a given amount of DPPH free radicals.

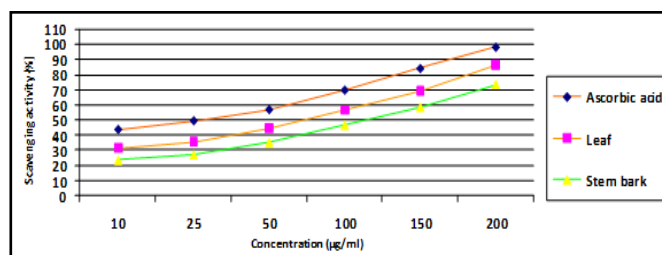


FIGURE 6: DPPH RADICAL SCAVENGING ACTIVITY OF THE LEAF AND BARK

**Statistical analysis:**

All the data provided in the phytochemical and antioxidant studies of *Justicia gendarussa* represent means  $\pm$  S.E.M. The results were analyzed by ANOVA for two factors with replication to understand the degree of significance between different phytochemical constituents and antioxidant activity of two plant parts. ANOVA analysis showed that the results are highly

significant ( $p < 0.01$ ). Here p-value for different parameters is  $2.72 \times 10^{-67}$  and it is  $4.66 \times 10^{-40}$  for different plant parts. There is a positive and strong correlation between different phytochemical groups and antioxidant parameters, except chlorophyll b. Here, chlorophyll b showed negative correlation with other phytochemicals as well as antioxidant activity.

**TABLE 1: MICROCHEMICAL TESTS FOR METHANOLIC EXTRACTS OF DIFFERENT PLANT PARTS**

Test reagent	Test for	Nature of change	Degree of colour change (leaf extract)	Degree of colour change (stem bark extract)
Dragendroff's Reagent	Alkaloids	Orange brown ppt.	++	+
Wagner's reagent	Alkaloids	Orange brown ppt.	++	++
Mayer's reagent	Alkaloids	White/ Cream ppt.	++	++
Shinoda test	Flavonoids	Magenta colour	+	+
10% NaOH	Flavonoids	Yellow colour	++	+
Salkowski test	Steroids and Triterpenoids	Reddish-blue and green fluorescence	-	-
Benedict's reagent	Reducing sugars	Brick red ppt.	++	++
Felling's reagent	Reducing sugars	Brick red ppt.	++	++
Molish's test	Gums	Red-violet ring	-	-
10% potassium dichromate solution	Tannins	Yellowish-brown ppt.	++	+
10% aqueous lead acetate solution	Tannins	Yellow ppt.	+++	++
5% aqueous ferric chloride solution	Tannins	Greenish-black colour	++	++
1% lead acetate	Saponins	White ppt.	-	-
Bontrager's test	Anthraquinones	Pink colour	+	-
Ninhydrin test	Amino acids	Purple colour	+	+
Phloroglucinol test	Lignin	Red colour	-	+
Millon's reagent	Proteins	White ppt.	++	+
Lugol's reagent	Proteins	Faint yellow colour	+++	++

**TABLE 2: BIOCHEMICAL PARAMETERS OF THE PLANT**

Plant parts	Soluble carbohydrate (mg/g dry tissue)	Amino acid (mg/g dry tissue)	Ascorbic acid ( $\mu$ g/g dry tissue)	Chlorophyll a (mg/g dry tissue)	Chlorophyll b (mg/g dry tissue)	Total carotenoids (mg/g dry mass)
Leaf	$307 \pm 2.88$	$34.93 \pm 2.16$	$619 \pm 2.230$	$46 \pm 0.043$	$16.1 \pm 0.018$	$16.7 \pm 0.03$
Stem bark	$185 \pm 6.08$	$26.86 \pm 1.50$	$330.33 \pm 2.96$	$23.9 \pm 0.028$	$21 \pm 0.018$	$14.6 \pm 0.05$

**TABLE 3: CATALASE AND PEROXIDASE AND TOTAL PHENOLIC CONTENT OF THE PLANT**

Plant parts	Catalase (EU/g fresh tissue)	Peroxidase (EU/g fresh tissue)	Total phenolic (mg/g dry tissue)
Leaf	$4.89 \pm 0.04$	$3.40 \pm 0.04$	$33.96 \pm 1.44$
Stem bark	$2.39 \pm 0.08$	$2.21 \pm 0.09$	$31.4 \pm 5.53$

**TABLE 4: ANTIOXIDANT ACTIVITY IN TERMS OF DPPH RADICAL SCAVENGING ACTIVITY**

Concentration ( $\mu\text{g/ml}$ )	Scavenging activity (%) of Ascorbic acid	Scavenging activity (%) of the leaf	Scavenging activity (%) of the stem bark
10	44.05 $\pm$ 0.11	31.36 $\pm$ 0.05	23.74 $\pm$ 0.06
25	49.82 $\pm$ 0.05	35.74 $\pm$ 0.13	27.24 $\pm$ 0.08
50	57.23 $\pm$ 0.02	44.38 $\pm$ 0.10	35.35 $\pm$ 0.07
100	70.14 $\pm$ 0.09	56.54 $\pm$ 0.12	46.7 $\pm$ 0.14
150	84.44 $\pm$ 0.02	69.37 $\pm$ 0.09	58.52 $\pm$ 0.17
200	98.53 $\pm$ 0.03	86.5 $\pm$ 0.12	73.39 $\pm$ 0.12

\*All the results shown above are mean value of three replica  $\pm$  SEM

**DISCUSSION:** The phytochemical groups like alkaloids, flavonoids, reducing sugars, tannins, amino acids and protein are present in both leaf and stem parts of the investigated plant. Anthraquinone group is present in the leaf part only. Among the detected phytochemical groups here, alkaloids, flavonoids, tannins and anthraquinone are known to be therapeutically important which indicate the medicinal value of this herb. It has been reported earlier that methanolic extract of *Carissa spinarum* showed antidiabetic activity due to presence of therapeutically potent phytochemical groups like alkaloids, flavonoids, saponins and tannins<sup>21</sup>. A variety of biological activities like CNS stimulatory, anthelmintic, anti-hypertensive, anti-malarial, anti-diabetic, antirheumatic, anti-cancerous, anti-inflammatory, antiviral etc. of various types of phytochemical groups have been studied by many workers<sup>22, 23, 24, 25, 26</sup>.

Ascorbic acid present in aqueous phase of the cell and is a very good free radical scavenger. It also helps in recycling of Vitamin E, another potent antioxidant. In *Desmodium gangeticum*, a very potent medicinal plant, ascorbic acid content is 213.3  $\mu\text{g/g}$  tissue<sup>27</sup>. Here in this study it has been observed that *J. gendarussa* is a potent source of ascorbic acid where ascorbic acid content is 619  $\mu\text{g/g}$  leaf tissues and it is 330.33  $\mu\text{g/g}$  for stem bark. So, leaf part is again therapeutically more important than bark in respect of its ascorbic acid content.

Excessive generation of reactive oxygen species (ROS) leads to a variety of pathological conditions such as inflammation, diabetes, hepatic damage, cancer and many other physiological disorders<sup>28, 29</sup>. Flavonoids from different plant sources have been reported to have anti-inflammatory, antiarthritic and antioxidant activities. Preliminary phytochemical screening of the plants like *Aegle*

*marmelos*, *Premna optusifolia*, *Oroxylum indicum*, *Desmodium gangeticum* and *Uraria picta* showed that they having high amount of flavonoids and those therapeutic properties<sup>30</sup>. Through phytochemical screening it has been found that *Justicia gendarussa* also contains flavonoids.

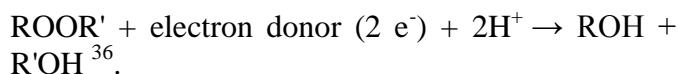
Anthraquinone is a medicinally potent group of phytochemical. Plants rich in anthraquinone are used as cathartic, purgative, laxative, fungal skin infection, antibiotic and also as slimming agents. Different species of the genus *Cassia* used as purgative and antimalarial agent are found rich sources of flavonoids, anthraquinones, mucilage and polysaccharides<sup>31</sup>. Anthraquinone group has also been identified in this investigated plant which highlights the possibility of studies of it for similar kind of remedial potency.

Studies involving animal model have demonstrated that ingestion of phenol rich food led to improvements in blood lipid profile. Low density lipid oxidation (oxLDL) is considered to be a major risk factor for development of atherosclerosis and cardio vascular disorders. Oxidative damage to DNA is a precursor for human carcinogenesis<sup>32</sup>. Phenolic compounds have been identified as very potent antioxidant through various biological activity studies. In *Marrubium peregrinum* L. total phenolic content is 27.26 mg/g which showed very good antioxidant potential<sup>33</sup>. Here in this study, the investigated plant has also been identified as a good source of phenolic compounds where total phenolic content of the leaf part is 33.96 mg/g tissue and for stem bark it is 31.4 mg/g. As leaf part contains larger amount of phenolic compounds than stem bark thus it can be concluded that leaf part is more potent than stem bark. It has clearly been reflected here in case of antioxidant study also where leaf part of the plant shows high activity than the stem part.

Carotenoids have the capacity to trap not only lipid peroxy radicals, but also singlet oxygen species. Carotenoids have extensive applications as antioxidant and are therefore, important for human health. Beet root a potent source of carotenoids where the amount of it is 19 µg/g<sup>34</sup>. It has been observed that carotenoid content in leaf part of *Justicia gendarussa* is 16.7µg/g dry tissue which is very close to the carotenoids content of Beet.

Catalase is a common enzyme found in nearly all living organisms exposed to oxygen<sup>35</sup>. This catalyzes the cellular decomposition of hydrogen peroxide to water and oxygen. Here in *J. gendarussa*, Catalase content is higher in fresh leaf (4.89 EU/ g) than the stem bark (2.39 EU/ g). In respect of Catalase content, leaf part here is also more potent than stem bark.

Peroxidases are a large family of enzymes that typically catalyze a reaction of the form:



For many of these enzymes the optimal substrate is hydrogen peroxide, but others are more active with organic hydroperoxides such as lipid peroxides. Like Catalase, here Peroxidase content is higher in leaf (3.4 EU/g) than the stem bark (2.21 EU/g) which again indicates that leaves are having more therapeutic prospect than stem.

DPPH scavenging activity studies showed that *Justicia gendarussa* is a potent free radical scavenger specially the leaf part of this plant. Here, IC<sub>50</sub> value of leaf extract is 75µg/mL which is lesser than 94.16µg/mL, the IC<sub>50</sub> value of *Hibiscus sabdariffa*, a potent source of antioxidant<sup>37</sup>. Considering both parts of *Justicia gendarussa*, the antioxidant activity is more significant in leaf (75µg/mL) than stem part (119µg/mL). It is interesting to note that DPPH radical scavenging activity is greater in leaf part of the investigated plant where higher content of total phenolics, ascorbic acid, carotenoid, catalase and peroxidase was observed.

**CONCLUSION:** The phytochemical and antioxidant studies of *Justicia gendarussa*

(Acanthaceae) highlight the promising therapeutic potentials of this medicinal plant which need further investigation to explore the novel antioxidant compounds.

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