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Distribution of Bioactive Compounds and Antioxidant Capacity of Different Parts of *Justicia adhatoda* L. (Acanthaceae)

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Abstract *Justicia adhatoda* L is a well-known medicinal plant used in traditional systems of medicine globally. Different parts of *J. adhatoda* have been used for treatment of various ailments, mainly for the respiratory tract-based ailments. The present study was conducted to quantify the total antioxidant capacity (TAC), total phenolic content (TPC) and total flavonoid content (TFC) of different parts (mature leaves, immature leaves, flowers, bracts, soft stems, bark of mature stem and roots) of *J. adhatoda* using ferric reducing antioxidant power (FRAP) assay, modified Folin-Ciocalteu method and colorimetric method respectively. The results revealed that all tested parts of *J. adhatoda* contained marked amounts of TAC, TPC and TFC. Among tested parts, immature leaves showed a significantly higher TAC (19.28 ± 1.96 mg/TE g DW) followed by flowers (16.97 ± 0.99 mg/TE g DW) and bracts (14.19 ± 1.85 mg/TE g DW). Significantly the highest TPC (11.33 ± 0.14 mg/GAE g DW) and TFC (16.66 ± 3.06 mg/RE g DW) were observed in flowers followed by bracts and immature leaves. Moreover, there were positive correlations of TAC with TPC (R2 = 0.5411) and TFC (R2 = 0.5209). According to the results, it can be concluded that immature leaves, flowers and bracts of *J. adhatoda* contain marked amounts of bioactive compounds and hence could be effectively used for pharmaceutical industries.

Keywords: *Acanthaceae, Justicia adhatoda, bioactive compounds, plant parts, total antioxidant capacity*

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1. Introduction

Justica adathoda L. is a therapeutically important medicinal plant belonging to the family Acanthaceae. This plant is native to Asia and distributed throughout Sri Lanka, India, Pakistan and Myanmar. *J, adathoda* is commonly known in English as Malabar nut, *Pawatta* in Sinhala, *Adadodai* in Tamil and *Vasaka* in Sanskrit [1].

This is an evergreen shrub with average height of three meters. It has opposite and ascending branches. The leaves are broad, leathery and entirely lanceolate in shape with opposite formation. The stem is soft while the flowers are dense, terminal spikes with large, attractive white petals, streaked with purple on the lower lip. Different parts of *J. adathoda* have multiple uses in traditional Unani and Ayurvedic medicines [1,2].

The main phytochemical group present in *J. adathoda* is alkaloids. The prominent compounds present in *J. adhatoda* are vasicine, vasicinone, deoxyvasicine, vasicol, adhatodinine, and vasicinol which are present in different parts of the plant. In addition, vitamin C, saponins, flavonoids, steroids, and fatty acids are also present [2,3] Due to the presence of an array of therapeutically active phytochemicals in different parts of *J. adathoda,* has been extensively used in traditional and Ayurveda systems of medicine to treat respiratory disorders such as cold cough, whooping cough, chronic bronchitis and asthma [1]. The powdered herb is boiled with sesame oil, is used to heal ear infections and arrest bleeding. Boiled leaves are used to treat rheumatic pain, and to relieve the pain of urinary tract infections [4] It was discovered that the main component vasicine present in *J. adathoda* shows oxytocin action helping for speeding up the delivery during child birth [1]. Moreover, this herb is important due to its anti-asthmatic and bronchodilator activity, wound healing activity, anti-ulcer activity, anti-bacterial activity, anti-allergy activity and anti-tubercular activity [4]. Apart from that medicinal uses, the tender leaves and flowers of *J. adathoda* are used as a vegetable in India and Nepal [5] In addition, leaf aqueous extract of *J. adhatoda* and *O. tenuiflorum* exhibited antioxidative effects and impede hyperlipidemia [6].

Generation of reactive oxygen species (ROS) and other free radicals which are normally produced during the

metabolism of the body cells are able to neutralized by natural antioxidant systems. But imbalance of free radicals resulted to oxidative stress which related to cardiovascular disease, cancer and other chronic diseases. Reducing ability of plant extracts are directly related to the phytochemical content of the extract [7]. Though *J. adathoda* is widely used in traditional systems of medicine, information on its bioactive compounds and antioxidant capacity present in different parts of the plant are limited. Therefore, current study was aimed to determine the bioactive compounds and antioxidant capacity of different parts of *J. adathoda*.

2. Materials and Methods

2.1. Sample Collection

Mature leaves, immature leaves (bud with two leaves), flowers, bracts, soft stems, bark of mature stem, and roots of *J. adathoda* plant were collected from Kegalle district in Sri Lanka and brought to the laboratory.

2.2. Chemicals and Reagents

Folin-Ciocalteu reagent, Gallic acid, 2, 4, 6-trypyridyl-2-try-azine (TPTZ), 6-hydroxy-2, 5,7,8-tetramethyl-chroman-2-carboxilic acid (Trolox) and Ferric chloride ($FeCl_3.6H_2O$) were purchased from Sigma Aldrich Chemical Co. (St. Louis, Mo). All other chemicals used were of analytical grade.

2.3. Sample Preparation

The fresh leaves of mature and immature stages, flowers, bracts, soft stems, bark of mature stem and roots were thoroughly washed under running water. Then they were cut into small pieces and air dried at room temperature (28 ± 2°C) for three days.

2.4. Extraction of Phytochemicals

All air-dried samples were powdered using a coffee grinder and sieved with 0.25 mm mesh. Powdered sample (0.1 g) was mixed with 5mL of 80% methanol and vortexed for 15 min. Then it was placed in a water bath at 60°C for 40 min and vortex procedure was repeated in 10 min intervals. After centrifugation at 4,000 rpm for 5 minutes to remove the solid fraction, the supernatant was decanted into a 15 mL centrifuge tube and the pellet was re-extracted with 5 mL of 80% methanol. Supernatants were pooled and stored at -20°C until further usage.

2.5. Determination of Total Antioxidant Capacity (TAC)

Total antioxidant capacity was determined using ferric ion reducing antioxidant power (FRAP) assay described by Benzie and Strain [8], with slight modifications. Briefly, 100 µL of methanolic extracts of samples were mixed with 900 µL of freshly prepared FRAP (Mixing 25 mL of 300 mM Sodium acetate buffer, 2.5 mL of 10 mM TPTZ solution and 2.5 mL of 20 mM ferric chloride solution) reagent of pH 3.6 and incubated for 4 min. Absorbance was measured at 593 nm using spectrophotometer. TAC was calculated using the standard trolox curve and expressed as milligrams of trolox equivalents (TE) per g of DW.

2.6. Determination of Total Phenolic Content (TPC)

Total phenolic content of all samples was determined using a modified Folin-Ciocalteu method [9]. Briefly, 4 mL of distilled water and 0.5 mL of extract were added into a test tube. Then the same amounts of (0.5 mL) 0.2 N Folin-Ciocalteu reagent was added and allowed to react for 3 min. Then 1 mL of a saturated sodium carbonate solution was mixed and incubated in a water bath for 2 hrs. at 30°C. The absorbance was measured at 760 nm using a spectrophotometer. TPC was calculated using the standard gallic acid curve and expressed as milligrams of gallic acid equivalents (GAE) per g of DW.

2.7. Determination of Total Flavonoid Content (TFC)

Total flavonoid content was determined by a colorimetric method as described by Liu *et al*. [10] with slight modifications. Briefly, a volume of 0.5 mLof plant extract was added to centrifuge tube containing 3.5 mL of distilled water. Then the solution was mixed with 0.3 mL of 5% $NaNO_2$. After 6 min 0.3 mL of 10% $Al(NO_3)_3.6H_2O$ solution was added and allowed to stand another 6 min. Then, 2 mL of 2 M NaOH was added. The mixture was diluted with 1.4 mL of distilled water and absorbance was measured immediately using a spectrophotometer at 510 nm wavelength. TFC was calculated using the standard rutin curve and expressed as milligrams of rutin equivalents (RE) per g DW.

2.8. Statistical Analysis

To verify the statistical significance of all parameters, mean values ± SD were calculated. Statistical comparison of mean values was performed by General Linear Model (GLM) of ANOVA followed by Tukey Multiple Range Test using SAS statistical software (Version 9.4).

3. Results and Discussion

Total antioxidant capacity of tested extracts of different parts of *J. adathoda* was ranged from 5.73 ± 0.14 to 19.28 ± 1.96 mg/TE g DW (Figure 1). Significantly the highest TAC was observed in immature leaves (19.28 ± 1.96 mg/TE g DW) followed by flowers (16.97 ± 0.99 mg/TE g DW), bracts (14.19 ± 1.85 mg/TE g DW, bark (13.82 ± 3.5 mg/TE g DW) and roots 10.34 ± 0.65 mg/TE g DW) respectively.

Moreover, order of decrease of TAC of different extracts of *J. adathoda* was as follows; immature leaves > flowers > bracts > bark of mature stem > roots > soft stem > mature leaves. From the results obtained, it is clear that the higher TAC is concentrated around the bud region of *J. adathoda* and decreased with the maturity. This

phenomenon agreed with Izzreen and Fadzelly [11], and Panawala et al [12] , which suggested that antioxidant values are high in immature leaves and decreased with the increment of maturity stage of tea and allspice respectively.. This may be due to the physiological changes of leaf with age and the unique chemical compounds transportation within the plant [13].

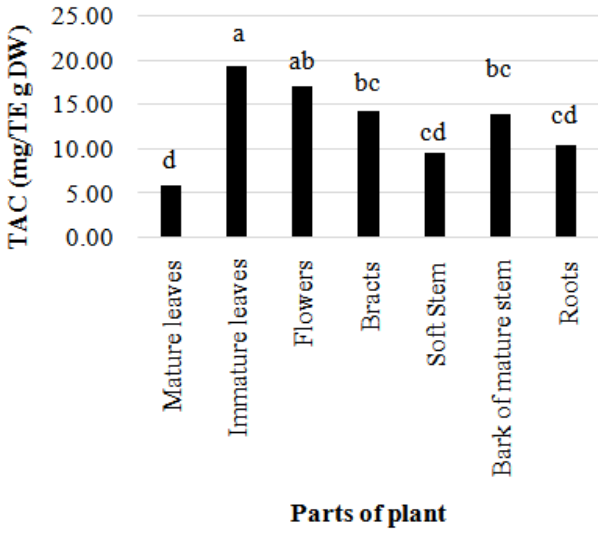

Figure 1. Total antioxidant capacity (TAC) of different parts of *J. adathoda* (Mean with the same letter represent non-significant differences ($p<0.05$)

Results on total phenolic content (TPC) and total flavonoid content (TFC) of different parts of *J. adathoda* are demonstrated in Table 1. TPC and TFC contents of different parts were ranged from 1.94 ± 0.12 to 11.33 ± 0.14 mg/GAE g DW and 3.54. ± 0.46 to 16.66^{a} ± 3.06 respectively. Significantly the higher TPC was observed in flowers (11.33 ± 0.14 mg/GAE g DW) followed by bracts (8.46 ± 0.66 mg/GAE g DW) and immature leaves (7.4 ± 0.32 mg/GAE g DW). The significantly higher TFC was recorded from flowers (16.66 ± 3.06 mg/RE g DW**)** followed by immature leaves, bracts, mature leaves, bark and roots respectively. However, there was no significant difference observed among the results in immature leaves, flowers and bracts parts whereas, least TFC was recorded in soft stem parts (3.54 ± 0.46 mg/RE g DW).

Both TPC and TFC were highly reported in flowers followed by the immature bud region of the plant. Results obtained from this study agreed with the findings of Saha *et al*. [14], who observed the higher content of phenolics in *Saraca asoca* flowers among different parts of the plant.

Table 1. Total phenolic content (TPC) and total flavonoid content (TFC) of different parts of *J. adathoda*

Parts of plant	TPC ± SD (mg/GAE g DW)	TFC ± SD (mg/RE g DW)
Mature leaves	3.35^{e} ± 0.42	6.99^{b} ± 1.84
Immature leaves	7.4^{c} ± 0.32	14.21^{a} ± 2.78
Flowers	11.33^{a} ± 0.14	16.66^{a} ± 3.06
Bracts	8.46^{b} ± 0.66	13.38^{a} ± 1.67
Soft stem	1.94^{f} ± 0.12	3.54^{b} ± 0.46
Bark of mature stem	5.13^{d} ± 0.43	6.63^{b} ± 0.52
Roots	2.16^{f} ± 0.23	5.14^{b} ± 0.22

(Mean denoted by the same letters in a column represent non-significant differences ($p<0.05$); GAE – Gallic acid equivalent; RE – Rutin equivalent; DW – Dry weight; SD- Standard Deviation).

This may be due to the presence of high content of secondary metabolites in flowers and immature parts of *J. adathoda* and also presence of rich in alkaloids, tannins, saponins phenolics and flavonoids [15]. These components are often involved in flower pigmentation and floral fragrances to attract insect pollinators and thus enhance fertilization rates [16]. Moreover, secondary metabolites are important in plant protection against biotic and abiotic stress conditions [17]. According to Duraipandiyan *et al*. [3] *J. adathoda* leaves contain ketone, terpene, and phenols. Presence of phytochemicals in plants provide an important defense strategy to the plants, particularly against herbivorous insect pest and pathogenic fungi [17].

It is a well-known fact that medicinal properties of plants are due to the presence of secondary metabolites in various plant parts [15]. Compounds belonging to alkaloids, terpenoids, and flavonoids are currently used as drugs or as dietary supplements to cure or prevent various diseases [17]. According to Reddy *et al*. [18] flowers of *J. adathoda* are used to cure various ailments like opthalmia, fever, gonorrhoea, to improve blood circulation, jaundice, abdominal tumour and in rheumatism since long time in traditional health practices of India, Pakistan, Sri Lanka and Nepal. Claeson *et al*. [5] reported that there are no records on any serious adverse effects or side effects of *J. adathoda* unless diarrhoea and vomiting may occur if large doses are taken. Findings of the present study showed positive correlations of TAC with TPC (R^2 = 0.5411) and TFC (R^2 = 0.5209). These results are agreed with the findings of Zhang *et al*. [19] also suggested that presence of therapeutically active biomolecules such as phenolic and flavonoid components are significantly enhance antioxidant capacity of different plant parts of *J. adathoda.*

4. Conclusions

According to the results, it could be concluded that all tested parts of *J. adathoda* contain marked number of bioactive compounds and antioxidant capacity. Further, current study validates the traditional claim of obtaining immature leaves, flowers and bracts for drug preparation, since those parts contained the significantly higher amounts of bioactive compounds and antioxidant capacity for the first time in Sri Lanka. Also, immature leaves, flowers and bracts of *J. adathoda* can be used as a good antioxidant supplement for pharmaceutical industries.

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