Antioxidant and Free Radical Scavenging Potential of *Justicia gendarussa* Burm. Leaves *in vitro*.

K. Mruthunjaya¹ and V. I. Hukkeri²*

¹Department of Pharmacognosy and Phytochemistry, J.S.S. College of Pharmacy, Vidy nagar, S. S. Nagar, Mysore-570 015 Karnataka, India
²Director, Herball Research Division, Dr Naragund Research Foundation, Dattatreya Nagar, BSK III Stage, 100 feet Ring Road, Bangalore-560 085, Karnataka, India.

Abstract – Antioxidant activity of 70% aqueous ethanolic extract of leaves of *Justicia gendarussa* (EJ) was evaluated. EJ was prepared by cold maceration method. The antioxidant potency of EJ was investigated employing various established *in vitro* systems, such as DPPH radical scavenging, nitric oxide (NO) scavenging, β-carotene linoleic acid module system (β CLAMS), hydroxyl (OH) radical scavenging, anti lipid peroxidation. IC₅₀ values were determined in each experiment. Also, ferric ion reduction capacity of extracts in presence and absence of chelating agent (EDTA) and total antioxidant capacity were determined. Preliminary phytochemical investigation was carried out to know the nature of constituents present in the leaves and correlate it with antioxidant activity. Further total phenolic content was determined in EJ. IC₅₀ values of EJ were 123.09 ± 3.01, 643.0 ± 61.10, 132.3 ± 6.08, 68.5 ± 11.5 and 68.13 ± 11.5 µg/mL in DPPH radical scavenging, NO scavenging, β CLAMS, OH radical scavenging and anti lipid peroxidation activity respectively. In total antioxidant capacity assay, ascorbic acid equivalent value was found to be 205.56 ± 4.69 µg/mg of extract. Total phenolic content was found to be 43.76 ± 4.27 µg equivalent of gallic acid per mg of extract. Phytochemical investigation reveals the presence of flavonoids. The results indicate that EJ possess antioxidant activity and flavonoids are responsible for this activity.

Keywords – *Justicia gendarussa*, antioxidant, DPPH, free radical scavenging, hydroxyl radical scavenging, lignans

Introduction

Reactive oxygen species (ROS) can contribute to the etiology of disorders such as cancer, liver diseases, atherosclerosis, respiratory diseases and inflammatory response syndrome. In recent years there is great deal of interest in developing agents to control damage induced by ROS in biological systems. As plants produce a lot of antioxidants to control the oxidative stress caused by sunbeams and oxygen, they can represent a source of new compounds with antioxidant activity.

*Justicia gendarussa* belonging to family Acanthaceae is known for its medicinal properties in Ayurveda, an Indian system of medicine in inflammations, bronchitis, vaginal discharges, dyspepsia, eye diseases and fever (Kirthikar and Basu, 2001). Decoction of leaves is used to treat chronic rheumatism (Anonymous 1959). *Justicia* species found to contain lignans, naturally occurring phenolic dimers. Lignans reported to have various significant biological activities including antioxidant (Jyotishi and Bagavant, 1992a; 1992b). It is also reported to contain β-sitosterol, friedelin, lupeol and four simple 0-substituted aromatic amines (Chakravarty *et al*., 1982). Its medicinal properties and presence of lignans inspired us to take up the particular study.

Experimental

**Chemicals** – α,α-diphenyl-β-picryl hydrazyl (DPPH), egg phosphatidylcholine, β-carotene and γ-linoleic acid were obtained from Sigma Chemical Co.(St. Louis USA), butylated hydroxy toluene (BHT), ascorbic acid, ethylenediaminotetraacetic acid (EDTA), Tween-40, deoxy-d-ribose, trichloroacetic acid (TCA) and thiobarbituric acid (TBA) were obtained by Hi-Media Labs (Mumbai, India), 1,10-O-phenanthroline, ferric chloride (FeCl₃), hydrogen peroxide, ammonium molybdate, sodium dithionite were obtained from Ranbaxy Fine Chemicals (New Delhi, India), phenyl hydrazine, folin-ciocalteau phenol reagent were obtained from BDH Products (UK). Silymarin was kind gift from Dr. Chidambaramurthy K.N., CFTRI, *Author for correspondence*  
Fax: +91-80-26421903; E-mail: hukkeri_v@rediffmail.com
Mysore, India. All other chemicals used were of analytical grade. The solvents used for extraction were from Ranbaxy Fine Chemicals (New Delhi, India). The UV-visible spectrophotometric values were recorded in JASCO UV-500 spectrophotometer.

**Plant material and extraction** — The leaves of the plant were collected during September 2004 in Wynadu district of Kerala State, India, authenticated, by Dr. B. D. Huddar, Professor and Head, Department of Botany, HSK Science Institute, Hubli, India. The voucher specimen (KMUG01) of the plant is preserved in Department of Pharmacognosy, KLES’ College of Pharmacy, Hubli, India. Leaves dried under shed, powdered and extracted with 70% aqueous ethanol by cold maceration. The plant was preserved in Department of HSK Science Institute, Hubli, India. The voucher specimen B.D.Huddar, Professor and Head, Department of Botany, district of Kerala State, India, authenticated, by Dr.

The plant material was collected during September 2004 in Wynadu district of Kerala State, India, and authenticated by Dr. B. D. Huddar, Professor and Head, Department of Botany, HSK Science Institute, Hubli, India. The voucher specimen (KMUG01) of the plant is preserved in the Department of Pharmacognosy, KLES’ College of Pharmacy, Hubli, India. Leaves dried under shed, powdered and extracted with 70% aqueous ethanol by cold maceration. The voucher specimen was prepared in Department of HSK Science Institute, Hubli, India. The voucher specimen was authenticated by Dr.

**Pharmacognosy, KLES’ College of Pharmacy, Hubli, India.** The voucher specimen was preserved in Department of Pharmacognosy, KLES’ College of Pharmacy, Hubli, India. Leaves dried under shed, powdered and extracted with 70% aqueous ethanol by cold maceration. The voucher specimen was prepared in Department of Pharmacognosy, KLES’ College of Pharmacy, Hubli, India. The voucher specimen was authenticated by Dr.

**HSK Science Institute, Hubli, India.** The voucher specimen was preserved in Department of Pharmacognosy, KLES’ College of Pharmacy, Hubli, India. Leaves dried under shed, powdered and extracted with 70% aqueous ethanol by cold maceration. The voucher specimen was prepared in Department of Pharmacognosy, KLES’ College of Pharmacy, Hubli, India. The voucher specimen was authenticated by Dr.

**Antioxidant assay using β-Carotene Linolate Model System (β CLAMS) (Hidalgo et al., 1994; Singh et al., 2002)** — The antioxidant activity of EJ was evaluated by slightly modified method of Hidalgo et al., 1994. Briefly, 5 mg β-carotene, 40 mg γ-linolenic acid and 400 mg of Tween-40 were mixed in 1 mL chloroform. Chloroform was removed under vacuum using the flash rotary evaporator at 40 °C. The resulting mixture was added with 20 mL water and emulsion was prepared. The emulsion was further diluted with 80 mL of oxygenated water. 100 to 600 μg of extract and BHT were added in separate test tubes and volume was made up to 0.4 mL.