Research Journal of Botany

ISSN 1816-4919

Academic Journals Inc.

www.academicjournals.com

Research Journal of Botany 10 (1): 30-36, 2015
ISSN 1816-4919 / DOI: 10.3923/rjb.2015.30.36

Studies on Phytochemical Screening and *in vitro* Antioxidant Activity of Ethyl Acetate Leaf Extract of *Justicia gendarussa* Burm. F.

S. Nirmalraj and K. Perinbam
P.G Research Department of Botany, Government Arts College, Nandanam, Chennai, 600035, India

Corresponding Author: K. Perinbam, P.G Research Department of Botany, Government Arts College, Nandanam, Chennai, 600035, India

ABSTRACT

Plant antioxidants have gained significant interest in recent years for their role in preventing the auto corrosion of fats and oils. The present study was aimed to screen the phytochemical constituents such as alkaloids, anthraquinons, cumarins, flavonoids, glycosides, phenols, saponins, steroids and terpenoids from the ethyl acetate leaf extract of *Justicia gendarussa* and also to assess the antioxidant potential of same extract. Result of present investigation clearly revealed that total phenolic content was 34.10±0.79 mg/100 mg GAE and total flavonoid was found to be 15.01±0.30 mg/100 mg QE. *In vitro* antioxidant activity of *J. gendarussa* leaf by DPPH assay showed 74.20±0.44%, FRAP assay showed 72.15±0.44% and nitric acid scavenging assay revealed 65.99±1.93%, remarkably high phenolic content and antioxidant activities were found in the ethyl acetate extract. Further studies, to isolate and identification of bioactive compounds in food and pharmaceutical industries.

Key words: Antioxidants, DPPH, plant extract, phenolic content, flavonoids

INTRODUCTION

Nowadays, plants specific bioactive compounds as sources of natural products, that are screened for their increased various disease like cancer, diabetes, neurological diseases and cardiovascular disease etc. Plants contain naturally occurring nutrients that can prevent or slow that naturally produce free radicals. Exogenous sources of free radicals include tobacco smoke, ionizing radiation, certain pollutants, organic solvents and pesticides (Yildirim *et al*., 2001; Davies, 1995; Robinson *et al*., 1997; Buyukokuroglu *et al*., 2001). The potentially reactive derivatives of oxygen, attributed as Reactive Oxygen Species (ROS), are continuously generated inside the human body. The generated ROS are detoxified by the antioxidants present in the body. However, overproduction of ROS and/or inadequate antioxidant defense can easily affect and cause oxidative damage to various biomolecules including proteins, lipids, lipoprotein and DNA (Farber, 1994). Antioxidant is defined as the agent that neutralizes the effect produced by free radical (Fang *et al*., 2002). The antioxidant can be classified into two categories, namely enzymatic and non-enzymatic. The enzymatic antioxidants are produced itself in our body itself whereas, most of non-enzymatic antioxidants are obtained from either natural plants or synthetics which are used for the treatment for various diseases (Lee *et al*., 2004; Cuzzocrea *et al*., 2001). *Justicia gendarussa* Burm. F. (Family:Acanthaceae) is a shade-loving, quick growing and evergreen

plant mostly found in moist areas. It is believed to be native to China and is distributed widely across India, Sri Lanka and Malaysia. It is commonly called as 'Karunocchi' in Tamil. It is an erect, branched and smooth under shrub. Leaves are linear lanceolate, glabrous; flower small, white with pink or purple spots inside. In Indian and Chinese traditional medicine, the leaf of the plant is recommended to treat ailments such as fever, hemiplegia, rheumatism, arthritis, headache, earache, muscle pain, respiratory disorders and digestive trouble (Periyanayagam *et al*., 2009). However, words from Krishna *et al*. (2010), there are no published scientific studies on the anti-arthritic activities of the leaves of *J. gendarussa* or its potential toxicity. On the basis of the information of the rural and tribal people of the Kolli hills region, in the present study, to investigate phytochemical screening and antioxidant potential of sequential extracts of *Justicia gendarussa* leaf.

MATERIALS AND METHODS

Collection of plant material: Leaf sample of *Justicia gendarussa* were collected from Semmedu Hamlet, Kolli hills, Namakkal District, South India.

Preparation of *Justicia gendarussa* leaf extracts: Shade dried leaf powder measured 200 g were extracted in sequential organic solvents using soxhalet for 7 h 10 cycles for each. The extract was filtered using a Buchner funnel and Whatman No.1 filter paper. The filtrate was evaporated using rotary vacuum evaporator at -37°C and dried for 48 h using a freeze dryer to give a yield of 20 g of dry extract. The resulting extract was reconstituted with respective solvent to get desirable concentration and used throughout the study.

Phytochemical screening of the leaf extract of *Justicia gendarussa*: Ten percent of the dried extracts was used for qualitative screening of phytochemical compounds viz., alkaloids, anthraquinons, cumarins, flavonoids, glycosides, phenols, saponins, steroids, tannins and terpenoids in accordance with the standard methods (Trease and Evans, 1989; Harborne, 1998).

Alkaloids (Mayer's test): The 0.5 g of the extract was stirred with few milliliter of dilute hydrochloric acid and filtered. To a few milliliter of filtrate, one or two drops of Mayer's reagent were added to the sides of the test tube. A white creamy precipitate demonstrated the test as positive.

Anthraquinones (Borntrager's test): About 0.2 g of each portion to be tested was shaken with 10 mL of benzene and then filtered. The 0.5 mL of the 10% ammonia solution was then added to the filtrate. Appearance of a pink, red or violet colour in the ammonical (lower) phase was taken as the evidence for the presence of free anthraquinones.

Steroids (Salkwoski's test): The 0.5 g of the extract was dissolved in 2 mL of chloroform. Sulphuric acid was then carefully added to form a lower layer. A reddish-brown colour at the interface indicated the presence of steroids.

Triterpenoids (Hishorn's test): The 0.5 g of the extract was dissolved in 2 mL of chloroform. The mixture was heated for 10 min, after the addition of 2 mL trichloro acetic acid. The change of yellow colour to red indicates the presence of triterpenoids.

Flavonoids (Ferric chloride test): The 0.5 g of the extract was boiled with distilled water and then filtered. To 2 mL of the filtrate, few drops of 10% ferric chloride solution were then added. A green-blue or violet colouration indicated the presence of flavonoids.

Phenols (Lead acetate test): The 0.5 g of the extract was treated with lead acetate solution. Formation of precipitate indicated the presence of phenols.

Glycosides (Salkwoski's test): The 0.5 g of the extract was dissolved in 2 mL of chloroform. Sulphuric acid was then carefully added to form a lower layer. A reddish-brown colour at the interface demonstrated the presence of glycosides.

Saponin (Frothing test): To 1 g of the extract about 3 mL of distilled water was added and shaken vigorously for about 5 min frothing which persisted on warming was taken as an evidence for the presence of saponins.

Tannins: Exactly 1.0 g of each leaf extract was dissolved in 10 mL of distilled water and filtered using Whatmann No. 1 filter paper. A blue colouration resulting from the addition of ferric chloride reagent to the filtrate indicated the presence of tannins in the extract.

Quantitative assays of *Justicia gendarussa*

Total phenolic assay: Total phenolics were measured following the protocol described by Shetty *et al*. (1995). Phenolics were measured as gallic acid equivalents. One milliliter of leaf extracts were transferred to a test tube along with 1 mL of 95% ethanol, 5 mL of distilled water and 0.5 mL of Folin-Ciocalteu phenol reagent (Sigma Chemical Co., St. Louis, MO). After incubation of about 5 min, 1 mL of 5% Na_2CO_3 was added, mixed well and the solution was kept in the dark for 1 h. Then, the samples were vortexed and absorbance was measured at 725 nm at Elico double beam UV-VIS spectrophotometer.

Determination of total flavonoids: The total flavonoid content was determined using the Dowd method as adopted (Oyaizu, 1986). The 5.0 mL of 2% aluminium trichloride ($AlCl_3$) in methanol was mixed with the same volume of the extract solution (10 mg mL^{-1}). Absorption readings at 415 nm using Elico double beam UV-VIS spectrophotometer were taken after 10 min against a blank consisting of extract solution with 5.0 mL methanol without $AlCl_3$. Total flavonoid content was expressed as mg of quercetin equivalents/100 mg of sample.

***In vitro* antioxidant activity**

DPPH free radical scavenging activity: The antioxidant activity of the samples was determined by the 1, 1- diphenyl-2-picrylhydrazyl (DPPH) inhibition method as described (Garrett *et al*., 2009). To 3 mL of 60 mM DPPH, 100 mL of leaf extract at various concentrations were added, mixed well and incubated at room temperature for 15 min. The absorbance was monitored at 517 nm. Antioxidant activity was reported as percentage inhibition:

$$\text{Inhibition } (\%) = \frac{Ao - As}{Ao} \times 100$$

Ferric Reducing Antioxidant Power (FRAP) assay: The FRAP reagent (1.8 mL) was mixed with 0.2 mL of test sample, then incubated at 37°C for 10 min in a water bath. The FRAP reagent

contains 20 mm TPTZ solution, 20 mm $FeCl_3.6H_2O$ and 0.3 M acetate buffer with pH 3.6. After incubation the absorbance were measured immediately at 593 nm. The above mixture without the leaf extract serves as control (Benzie *et al*., 1996). Antioxidant activity was reported as percentage inhibition:

$$\text{Inhibition } (\%) = \frac{\text{Ao} - \text{As}}{\text{Ao}} \times 100$$

Nitric oxide scavenging activity: Determination of nitric oxide radical scavenging activity of leaf extract was performed by the method described by Garrat (1964). Sodium nitroprusside in aqueous solution at physiological pH spontaneously generated nitric oxide which interacts with oxygen to produce nitrite ions which was determined by the use of Griess reagents. The 2 mL of 10 mM sodium nitroprusside dissolved in 0.5 mL phosphate buffer saline (pH 7.4) was mixed with 0.5 mL of leaf extract at various concentrations. The mixture was incubated at 25°C. After 150 min, 0.5 mL of incubation solution was withdrawn and mixed with 0.5 mL of Griess reagent [1.0 mL sulfanilic acid reagent (0.33% in 20% glacial acetic acid) at room temperature for 5 min with 1 mL of naphthylethylenediamine dichloride (0.1% w/v)]. The mixture was incubated at room temperature for 30 min. The absorbance was measured at 540 nm. The amount of nitric oxide radical was calculated following this equation:

$$\text{Inhibition } (\%) = \frac{\text{Ao} - \text{As}}{\text{Ao}} \times 100$$

where, A_o is the absorbance before reaction and As is the absorbance after reaction has taken place.

Statistical analysis: Experimental results are expressed as Mean±Standard Deviation (SD) of three measurements.

RESULT AND DISCUSSION

The results of present study are encouraging as the tested *Justicia gendarussa* leaf extract revealed the presence of phytoconstituents with remarkable antioxidant potential.

Qualitative analysis of phytochemicals: Table 1 depicts the results for phytochemical screening of six different solvent extracts of the leaves of *Justicia gendarussa* namely hexane, chloroform, ethyl acetate, methanol, ethanol and aqueous extracts. The low polar hexane extract indicated the

Table 1: Qualitative analysis of phytochemical in different solvent of *Justicia gendarussa*

	Justicia gendarussa leaf extract					
Phytochemical screening	Hexane	Chloroform	Ethyl acetate	Methanol	Ethanol	Aqueous
Alkaloids	+	+	+	+	+	-
Anthraquinons	-	-	-	-	-	+
Flavonoids	+	+	+	+	+	-
Glycosides	-	+	+	+	+	+
Phenols	+	+	+	+	+	+
Saponins	-	+	+	-	+	-
Steroids	-	-	-		+	-
Tannins	+	+	+	+	+	+
Terpenoids	-	+	+	-	+	+

+: Presence, -: Absence

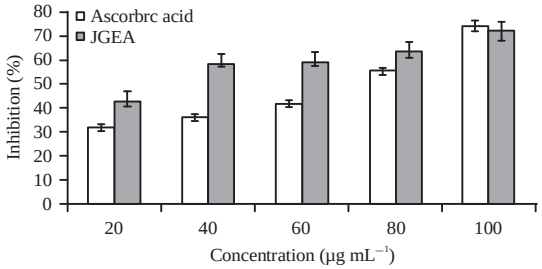

Fig. 1: DPPH assay for ethyl acetate leaf extract of *Justicia gendarussa*. All the values are given means of triplicates determinations. Data presented as the Mean±SD, JGEA: *Justicia gendarussa* ethyl acetate leaf extract

Table 2: Phytochemical compounds of ethyl acetate leaf extract of *Justicia gendarussa* (mg/100 mg)

Bioactive compounds	JG EA
Total phenolic content[a]	34.10±0.79
Flavonoids[b]	15.01±0.30

All the values are given means of triplicates determinations. Data presented as the Mean±SD. a: Gallic acid equivalent, b: Quercetin equivalent d, JGEA: *Justicia gendarussa* ethyl acetate leaf extract

presence of alkaloids, flavonoids and tannins. The high polar aqueous extract revealed the presence of anthraquinons, phenols, glycosides, saponins, tannins and terpenoids. The mid polar ethyl acetate extract showed maximum number of phytochemicals such as tannins, flavonoids, alkaloids, phenols, glycosides, terpenoids and saponins. Presence of tested secondary metabolites in the ethyl acetate leaf extract of *Justicia gendarussa* could be responsible for their antioxidant activity. Taking this into consideration, we choose only the ethyl acetate extract for further studies since, this indicates maximum number of phytoconstituents.

The result of ethyl acetate leaf extracts from *Justicia gendarussa* and its quantitative analysis showed the presence of higher levels of total phenols and flavonoids. The total phenol content was found to be 34.10±0.79 mg/100 mg GAE, flavonoids content was found to be 15.01±0.30 mg/100 mg QE, respectively (Table 2). Phenols are very important plant constituents because of their radical scavenging activity resulting from their hydroxyl group. The phenolic compounds may contribute directly to the antioxidative action (Bidchol *et al*., 2011). The medicinal effects of plants are often attributed to the antioxidant activity of phytochemical constituents mainly phenolics and flavonoids (Miliauskas *et al*., 2004).

***In vitro* antioxidant activity assays:** The measurement of radical scavenging activity of any antioxidant is frequently associated with the usage of DPPH method because it is quick, reliable and reproducible method. It is extensively used to test the ability of compounds as free radical scavengers or hydrogen donors and to evaluate the antioxidant activity of plant extracts (Chanda and Nagani, 2010).

Scavenging activities of *Justicia gendarussa* ethyl acetate leaf extracts against free radicals such as DPPH, FRAP and NO_2 were also investigated. The inhibitory effect on individual radicals was given in Fig. 1-3. Therefore, higher levels of IC_{50} values against studied free radicals. The *Justicia gendarussa* were found to be DPPH is 71.74±0.44%, FRAP was found to be 72.15±0.44% and nitric acid scavenging activity for 65.99±0.10%, with reference to ascorbic acid. Also, strong relationship between total phenolic content and antioxidant activity has been reported (Velioglu *et al*., 1998; Javanmardi *et al*., 2003; Kahkonen *et al*., 1999).

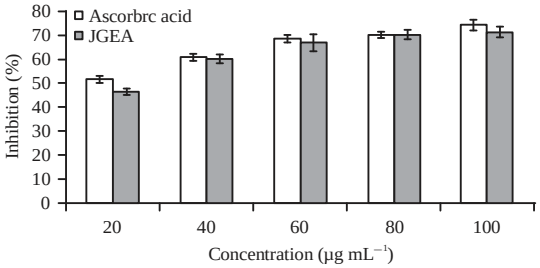

Fig. 2: FRAP assay for ethyl acetate leaf extract of *Justicia gendarussa*. All the values are given means of triplicates determinations. Data presented as the Mean±SD, JGEA: *Justicia gendarussa* ethyl acetate leaf extract

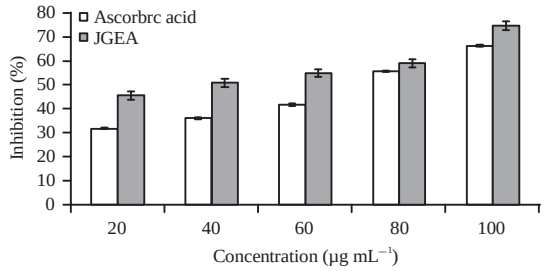

Fig. 3: Nitric oxide scavenging activity of ethyl acetate leaf extract of *Justicia gendarussa*. All the values are given means of triplicates determinations. Data presented as the Mean±SD, JGEA: *Justicia gendarussa* ethyl acetate leaf extract

CONCLUSION

The result of the present investigation suggested that the synthetic antioxidants can be replaced with natural anti-oxidants, our results support the view that medicinal plants are promising sources of natural antioxidants. The results demonstrate that *Justicia gendarussa* ethyl acetate leaf extract with the total phenolic and flavonoid content and antioxidant activity showed that they are potential source of natural antioxidants. Future studies will be performed to find the specific compounds from *J. gendarussa* leaves.

ACKNOWLEDGMENTS

I would like to thank Dr. K. Elumalai and Dr. S. Uma from Center for Entomotoxicity Studies, Department of Zoology, Government Arts College, Nandanam, Chennai, 600035 for their valuable comments and support in this study.

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