

Determination of Total Phenolic, Flavonoid Contents and Antioxidant Potentials of Methanolic Extract Fractions of *Delonix elata* Barks

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ABSTRACT

Plant produces a diverse range of bioactive molecules, making them a rich source of different types of medicines. A regular and widespread use of herbs throughout the world has increased serious concern over this quality, safety and efficacy. Thus a proper scientific evidence or assessment has become the criteria for acceptance of herbal health claims. In the present study, we examined the antioxidant effects of barks of *Delonix elata*. Dried and powdered barks of *Delonix elata* were extracted with methanol and fractionated with Dichloromethane (DM), Ethyl acetate (EA) and the remaining was considered as Mother residue (MR). Total phenolic and flavonoid contents of different fractions were determined. EA fraction had the highest phenol and flavonoid content of 33.5 ± 0.25 and 18.6 ± 0.30 mg/g respectively followed by MR fraction. The antioxidant activities were poor in the dichloromethane fraction. These results suggest that, the active antioxidant compounds are better fractionated in Ethyl acetate and there is a direct correlation between the total flavonoid and its antioxidant activity.

Keywords: *Delonix elata*, Methanol extract, Solvent fractions, Antioxidant activity.

INTRODUCTION

Stress is one of main reasons for various abnormal biochemical changes in the human system. Plant derived antioxidants scavenge free radicals and modulate oxidative stress-related degenerative effects.¹ Free radicals have been implicated in many diseases such as cancer, atherosclerosis, diabetes, neurodegenerative disorders and aging.² Several research reports suggest that higher intake of antioxidant rich food is associated with decreased risk of degenerative diseases particularly cardiovascular diseases and cancer.³

Plants are potential sources of natural antioxidants. In recent years much attention has been devoted to natural antioxidant and their association with health benefits.⁴ Plant phenolics are commonly found in both edible and non-edible plants, and have

been reported to have multiple biological effects, including antioxidant activity. The antioxidant activity of phenolic compound is mainly due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. In addition, they have a metal chelation potential.⁵ The phenolic compounds are increasingly of interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food. The importance of natural phenolic compounds from plants materials is also raising interest among scientists, food manufacturers and consumers due to the specific health effects.⁶ *Delonix elata* is a flowering plant in the pea belonging to the family Caesalpinioideae. This plant was reported to have anti-oxidant,⁷ anti-arthritis

Received Date : 03-04-2013

Revised Date : 26-06-2013

Accepted Date : 01-07-2013

DOI: 10.5530/rjps.2013.2.10

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and anti-inflammatory activities.⁸ In this study we made an attempt to fractionate the methanol extract and studied the various fractions for antioxidant potentials.

MATERIALS AND METHODS

Plant material

Delonix elata barks were collected from the out skirts of Tiruchengode, Tamilnadu. It was authenticated by Dr. Sreenath, Taxonomist, Dept. of Botony, Bangalore University, Bangalore. The barks were dried under shade and size reduced later passed through sieve no. 40.

Extraction and fractionation of the bark

Shade dried powder bark material 400 gm was extracted continuously by methanol until the plant material became colourless. Extract was distilled by rotary flash evaporator. The crude methanol extract obtained was fractionated with dichloromethane (DM), ethyl acetate (EA) and the remained portion was considered as mother residue (MR). These fractions were evaporated to get the concentrates which were stored in desiccators and used for the study.

Total phenolic content

The total phenolic content of methanol extract fractions was determined with Folin-ciocalteu assay. An aliquot 1 ml of extracts or standard solution of Gallic acid (2, 4, 6, 8, 10 µg/ml) was added to a 25 ml volumetric flask, containing 9 ml of distilled water. 1 ml of Folin-ciocalteu reagent was added to the mixture and shaken thoroughly. After 5 min, 10 ml of 7% Na₂CO₃ was added to the mixture. The solution was made up the mark to 25 ml with distilled water and mixed. After incubation for 30 min at room temperature, the absorbance against the prepared reagent blank was determined at 750 nm with an UV-Visible spectrophotometer (Shimadzu). All Samples were analysed in duplicates.⁹

Total flavonoid assay

The total flavonoid content was measured with an AlCl₃ colorimetric assay. Aliquots of 1 ml of fractions extracts and standard solution of Rutin (2, 4, 6, 8, 10 µg/ml) were transferred in to 10 ml volumetric flasks containing 4 ml of distilled water. 0.3 ml of 5% NaNO₂ was added in each flask followed by 0.3% of AlCl₃. At the sixth minute, 2 ml of 1 M NaOH was added and the absorbance was measured against a prepared reagent blank at 410 nm with an UV-Visible spectrophotometer (Shimadzu). All Samples were analysed in duplicates.⁹

Ferric reducing antioxidant power assay (FRAP)/ Total antioxidant activity assay

The stock solutions were prepared by mixing 300 mM acetate buffer (pH 3.6), 10 mM 2, 4, 6-tripyridyl-s-tri-azine solution in 40 mM hydrochloric acid and FeCl₃.6H₂O

solution. The fresh working solution was prepared by mixing 25 ml of acetate buffer, 2.5 ml of FeCl₃.6H₂O solution. The temperature of the solution was kept at 37°C before using. Extract fractions 0.1, 0.3, 0.5, 0.7 and 0.9 ml were mixed with (2850 µl) of the FRAP solution for 30 min in the dark condition. Reading of coloured products (ferrous tripyridyltriazine complex) was recorded at 593 nm. The experiment was repeated triplicates. The samples were compared with standard Butylated Hydroxytoluene (BHT).¹⁰

Hydrogen peroxide assay

A solution of hydrogen peroxide (40 mM) was prepared in phosphate buffer (50 mM pH 7.4). Fractions of methanol extract were taken (0.1, 0.3, 0.5, 0.7 and 0.9 mg/ml) in distilled water hydrogen peroxide was added. The scavenging potentials of fractions were determined by recording the absorption at 230 nm by UV Spectrophotometer after 10 min against a blank solution. The activity was compared with reduction by L-ascorbic acid.¹¹

Superoxide radical scavenging activity

Stock solutions of Nitro blue tetrazolium (NBT), Nicotinamide adenine dinucleotide (NADH) and phenazine methosulphate (PMS) were prepared in 100 mM phosphate buffer (pH 7.4). 1 ml of NBT (156 µM), 1 ml of NADH (468 µM) and different concentrations of fractions were mixed and 100 µl of PMS (60 µM) was added to each flasks and the mixture was incubated at 25°C for 5 min. Superoxide ion scavenging power by the fractions were measured at 560 nm. Quercetin was used as a reference standard.¹²

DPPH Radical scavenging activity

The free radical scavenging activity of the fractions were measured in terms of radical scavenging ability using the stable 1,1-Diphenyl-2-Picrylhydrazyl (DPPH) radical method. A solution of 0.1 mM DPPH in methanol was prepared and 1.0 ml of this solution was mixed with 3.0 ml of fractions containing 10, 20, 30, 40 and 50 µg/ml concentration. The procedure was followed for L-Ascorbic acid which is used as standard. The reaction mixtures were mixed thoroughly. After 30 min incubation at room temperature the absorbance of mixture was measured at 517 nm by UV-Visible Spectrophotometer (Shimadzu). It was compared to control containing same amount of ethanol and DPPH without samples or standard.¹³

Nitric oxide radical scavenging activity assay

2 ml of 10 mM sodiumnitropruside in 0.5 ml of phosphate buffer saline (pH-7.4) was mixed with 0.5 ml of reference compound and fractions of various concentrations (0, 0.3, 0.5, 0.7 and 0.9 mg/ml). All the mixtures

were incubated at 25°C for 90 min. 1.0 ml sulphanilic acid reagent (33% in 20% Glacial acetic acid) was added to all the flasks and incubated at 25°C room temperature for 5 min. Finally 1.0 ml of Naphthyl Ethylene diamine dihydro chloride (0.1% w/v) was mixed and incubated at room temperature for 30 min. The absorbance was measured at 540 nm by UV-Spectrophotometer (Shimadzu). α -Tocopherol was used as a reference standard.¹⁴

RESULTS

Total polyphenol and flavonoid content

Results obtained in the present study revealed that the level of polyphenols in the Ethyl acetate fraction was 33.5 ± 0.25 mg/g which was higher when compared to DM and MR fractions of *Delonix elata* bark. The flavonoid content of EA fraction was 18.6 ± 0.30 while other fractions tested showed less amount (Table 1).

FRAP assay/Total antioxidant activity assay

EA fraction showed 55.44% of ion scavenging effect at 0.9 mg/ml followed by MR 46.56%. While DM fraction showed less effect. All the fractions exhibited the antioxidant potential in a dose dependent manner (Figure 1).

Hydrogen peroxide assay

Results obtained in the method revealed that the EA fraction exhibited 54.42% activity which was higher when compared to DM and MR fractions. (Figure 2)

Table 1: Total Polyphenol and Flavonoid Content of Methanolic Extract Fractions of *Delonix elata* Bark

Fractions	Total phenols (mg/g)	Total flavonoid (mg/g)
Dichloromethane (DM)	8.8 ± 0.15	5.7 ± 0.15
Ethyl acetate (EA)	33.5 ± 0.25	18.6 ± 0.30
Mother residue (MR)	15.8 ± 0.30	9.2 ± 0.25

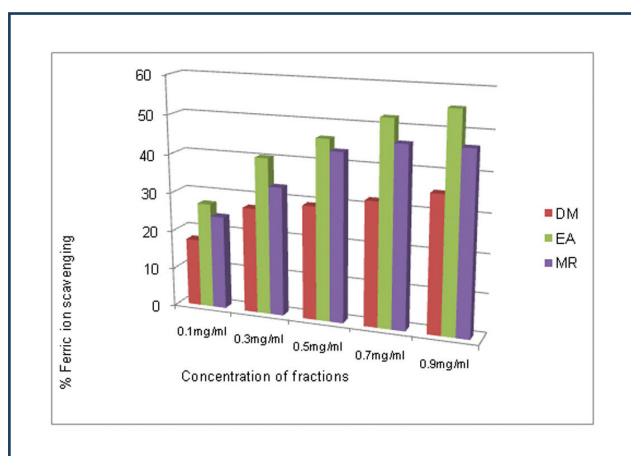


Figure 1: Total antioxidant activities of methanolic extract fractions of *Delonix elata* bark.

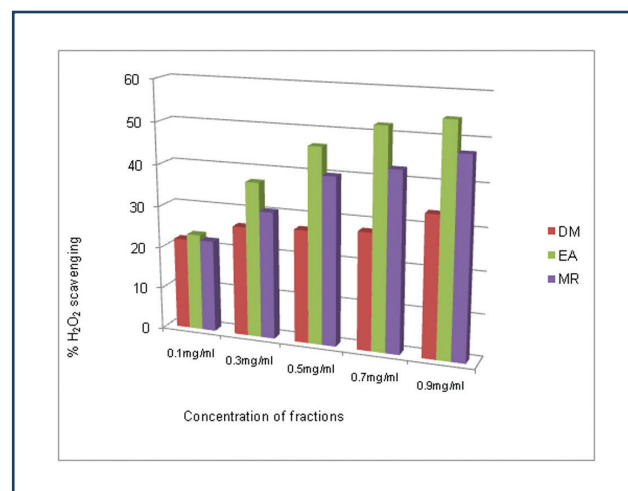


Figure 2: Hydrogen peroxide scavenging activity of methanolic extract fractions of *Delonix elata* bark.

Superoxide radical scavenging activity

EA fraction showed promising free radical scavenging effect of superoxide in a concentration dependent manner 56.03% while DM, MR fractions showed moderate antioxidant potential. (Figure 3)

DPPH radical scavenging activity

Free radical scavenging effect of DPPH was observed in a concentration dependent manner. 67.5% inhibition was shown by EA fraction which was higher than the other two fractions. (Figure 4)

Nitric oxide radical scavenging activity assay

EA fraction showed 60.23% of nitric oxide scavenging effect at 0.9 mg/ml concentration followed by MR 49.21%. While DM fractions showed less effect. (Figure 5)

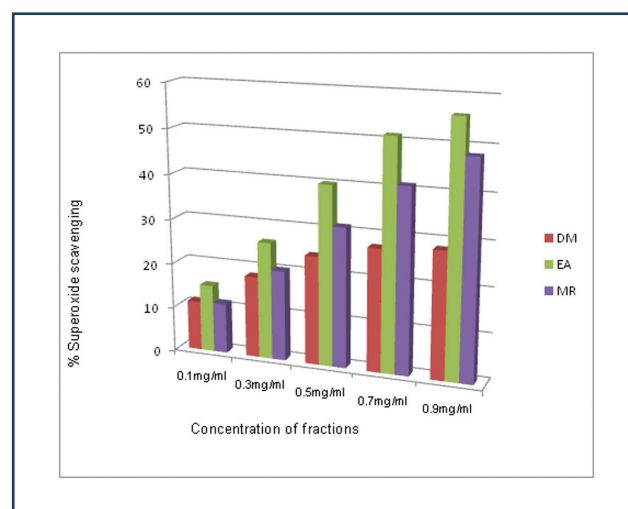


Figure 3: Superoxide radical scavenging activity of Methanolic extract fractions of *Delonix elata* bark.

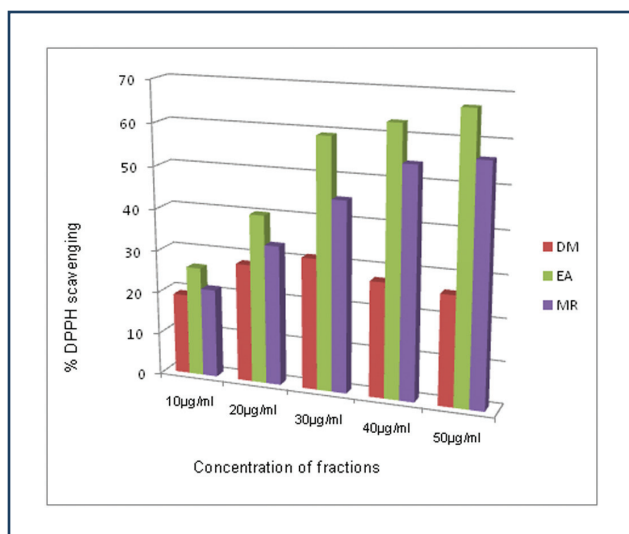


Figure 4: DPPH radical scavenging activity of methanolic extract fractions of *Delonix elata* bark.

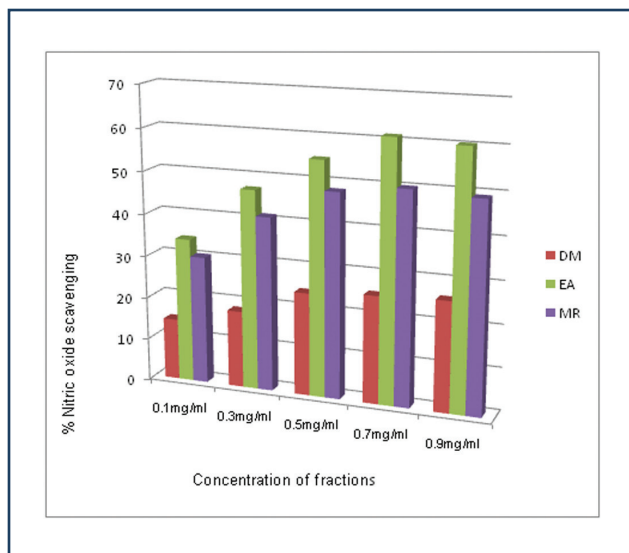


Figure 5: Nitric oxide radical scavenging activity of methanolic extract fractions of *Delonix elata* bark.

DISCUSSION

The present study revealed that the antioxidant effects of barks of *Delonix elata*. Among the DM, EA and the MR fractions, EA fraction had the highest phenolic and flavonoid content of 33.5 ± 0.25 and 18.6 ± 0.30 mg/g respectively. This may be the reason for the potential antioxidant effect of the EA fraction. The antioxidant activities were poor in the DM and MR fractions. These results suggest that, the active antioxidant compounds are better fractionated in Ethyl acetate and there is a direct correlation between the total flavonoid and its antioxidant activity. Hence we have taken up the fraction for further isolation and characterisation of constituents from the active fraction.

CONCLUSION

The EA and MR fractions of *Delonix elata* barks exhibited significant antioxidant activity compare to DM fraction. The activity may be related to the flavonoids and phenolic compounds in fractions of methanolic extract. Since reactive oxygen species are important contributors to several serious ailments. In the present study, it is observed that in all antioxidant models the fractions of *Delonix elata* bark EA fraction exhibited significant effect. Hence further study might be useful for the development of newer and potent natural antioxidants.

ACKNOWLEDGMENT

Authors are thankful to Management PES College of Pharmacy for their support and facilities provided to carry out the study.

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