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Research Article

ANTIOXIDANT ACTIVITY OF FRUITS AND LEAVES OF *Lagerstroemia floribunda* Jack (Kedah Bungor)

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ABSTRACT

The purpose of this research project was to study the antioxidant activity of *Lagerstroemia floribunda* fruits and leaves. DPPH assay and FRAP assay were carried out to determine the antioxidant activity of the plant. In DPPH assay, the maximum scavenging activity of the leaves extract was found to be 92.44 % with the concentration of 80 µg/ml and the maximum scavenging activity of the fruits extract was found to be 64.72 % with the concentration of 100 µg/ml whereas in the FRAP assay FRAP (Ferric Reducing Ability of Plasma) the maximum absorbance value for leaves and fruits extract were 0.944 and 0.461 respectively at the concentration of 200 µg/ml. From this study, it can be concluded that the methanolic extract of *Lagerstroemia floribunda* leaves possess excellent antioxidant activity while the fruits extract of the *Lagerstroemia floribunda* possess mild antioxidant activity.

Keywords: Antioxidant activity, DPPH, FRAP, *Lagerstroemia floribunda*

INTRODUCTION

Despite this scientific era, herbal medicine still plays an important role as an alternative medicine, without much scientific method based evidence backed up. Unlike the modern medicine - which stand on a very stiff foundation of evidence-tested pharmaceutical drugs and laboratory-tested data, in utter vice versa, herbal medicines extended its scope including fungal, bee products, minerals, shells and animal parts as well[1].

Antioxidant are of utmost importance in maintenance of normal cell growth and to neutralize the ever hazardous free radicals, which is unstable and so invasive that it could damage cells, tissues or even organ to an enormous extent. So, the naturally occurring antioxidant found within plants, animals and humans, is the "natural buffer" to free radical[2].

Lagerstroemia floribunda, also known as Thai crape myrtle and kedah bungor, is a species of flowering plant in the Lythraceae family. This delightful ornamental plant is native to subtropical and tropical South-East Asia, from southern China to Myanmar, Thailand, Cambodia, Indo-China and Peninsular Malaysia. It's the provincial tree of Saraburi Province in Thailand.

Lagerstroemia floribunda basically consists of carbohydrates and phenolic compounds. The trees bear fruits which prevalently rich in carbohydrate. Since the fruits contain high ratio of seed portion, thus, it does not favoured by animal and human as staple or edible fruit. Yet, the phenolic compounds incorporated within, may contain vast varieties of medical purposes. Besides, antioxidants which serve lots of the fruits and leaves also. [3, 4]

Traditionally Lagerstroemia species used for the treatment of stomach problems, weight loss and lower blood sugar[5].

Figure 1: Parts of *Lagerstroemia floribunda*

To the best of our knowledge, there are no antioxidant studies done so far on this plant. Our research may work as guidance for the researcher who will take this plant for further research and can also support in development of plant scientific profile.

MATERIALS AND METHODS

Collections and Preparation of plant material

Fresh leaves and fruits of *Lagerstroemia floribunda* were collected in and around the campus of AIMST University, Kedah, Malaysia in the month of October 2014. A voucher herbarium specimen was prepared and submitted to Unit of Pharmaceutical

chemistry, Faculty of Pharmacy, AIMST University, Malaysia. The leaves and fruits were separated and shade dried at the room temperature for seven days. Then, the leaves and fruits were homogenized to fine powder by using the electronic blender. Both the fine powder of leaves and fruits were subsequently sieved to get the coarse powder and stored in the air tight container. [6]

Extraction of plant material

Soxhlet apparatus was used for the extraction. In this method, the finely ground crude drug of leaves and fruits were placed in the different porous bags or 'thimble' made of strong filter paper of the Soxhlet apparatus. 250 ml of methanol was added from the top of the Soxhlet apparatus which passed through the thimble. The extracting solvent in the flask was heated and the heat was adjusted to monitor the regular flushing. The extract obtained was concentrated by using rotary evaporator.

DPPH radical scavenging activity assay

0.3 mM DPPH (2, 2 diphenyl-1-picrylhydrazyl) solution was prepared by dissolving 59.1 mg of DPPH in 500 ml of 95 % ethanol. 100 µg/ml stock solution of the extract was prepared by dissolving 10 mg of the extract in 100 ml of methanol. Different diluted working solutions of the test extracts (10, 20, 40, 60, 80 and 100 µg/ml) were also prepared. Positive control or standard was also prepared. Butylated hydroxyl toluene (BHT) was used as standard. Various dilutions of BHT solutions (10, 20, 40, 60, 80 and 100 µg/ml) were prepared. 2 ml of DPPH solution was mixed with 5 ml of different dilutions of extract and BHT solutions separately. The mixture was shaken vigorously and kept in dark for 30 minutes at room temperature. The absorbance was measured at 518 nm using UV-VIS spectrophotometer. Blank solution was also prepared. The blank solution contained only 2 ml of DPPH solution and 2 ml of methanol. No extract or BHT was added into the blank solution. [7-8]

% scavenging activity = [(Ac-As)/ Ac] x 100%
Ac-Absorbance of the control reaction
As- Absorbance of the sample of the extracts

The antioxidant activity of the extract was expressed as IC_{80} and IC_{50}. IC_{80} is the total antioxidant necessary to decrease the initial DPPH radical by 80%. The IC_{50} values were calculated from plotted graph of scavenging activity against the concentrations of the samples. IC_{50} is the total antioxidant necessary to decrease the initial DPPH radical by 50%. The IC_{80} and IC_{50} values were calculated from plotted graph of scavenging activity against the concentrations of the samples.

FRAP assay

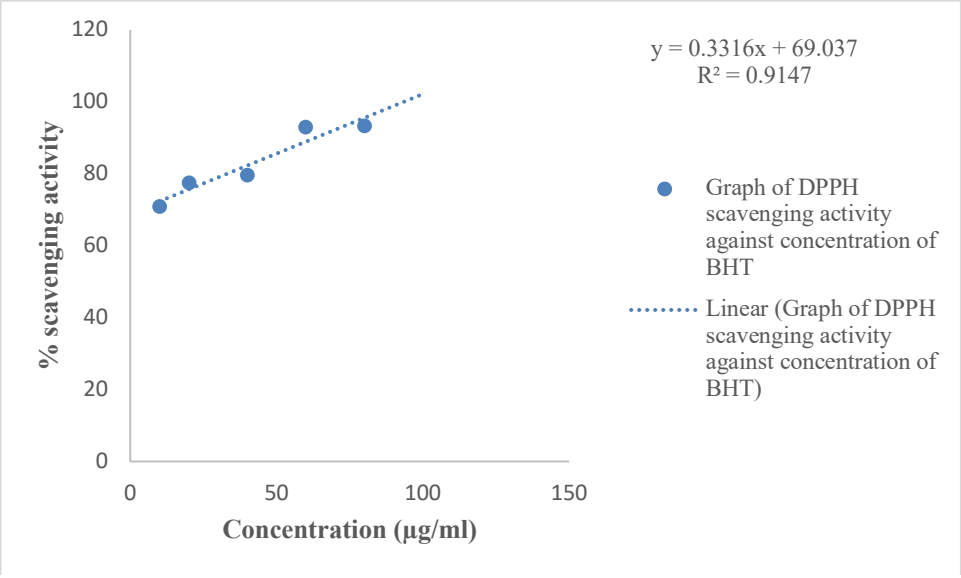
Various dilutions of extract were prepared. 400 µg/ml stock solution was made by dissolving 4 mg of the extract in 10 ml of methanol. 25, 50, 100, and 200 µg/ml of extract were prepared from the stock solution. Ascorbic acid was used as the standard. Different dilutions of standard (25, 50, 100, 200, and 400 µg/ml) were prepared. 1 ml of different concentration of extracts was added to the test tube containing 2.5 ml of phosphate buffer and 2.5 ml of 1 % potassium ferric cyanide. Phosphate buffer with a pH of 6.6 was prepared by mixing 178 ml of 0.2 M NaOH solution with 500 ml of 0.2 M KH_2PO_4 and dilute to 2000 ml with distilled water. To prepare 0.2 M NaOH solution, 1.6 g of NaOH was dissolved in 200 ml of distilled water. For 0.2 M KH_2PO_4, 13.61 g of KH_2PO_4 was dissolved in distilled water and diluted to 500 ml. The reaction mixtures were incubated at 50°c for 30 minutes. Then 2.5 ml of 10 % of TCA solution was added to the mixtures and centrifuged at 3000 rpm for 10 minutes. 2.5 ml of supernatant of the mixture from the test tubes were diluted with 2.5 ml of distilled water. The resulting solutions were shaken with 0.5 ml of 0.1 % of ferric chloride and the absorbance was measured at 700 nm by using UV-VIS spectrophotometer. The same procedure was carried out for the standard ascorbic acid. [9]

RESULTS AND DISCUSSION

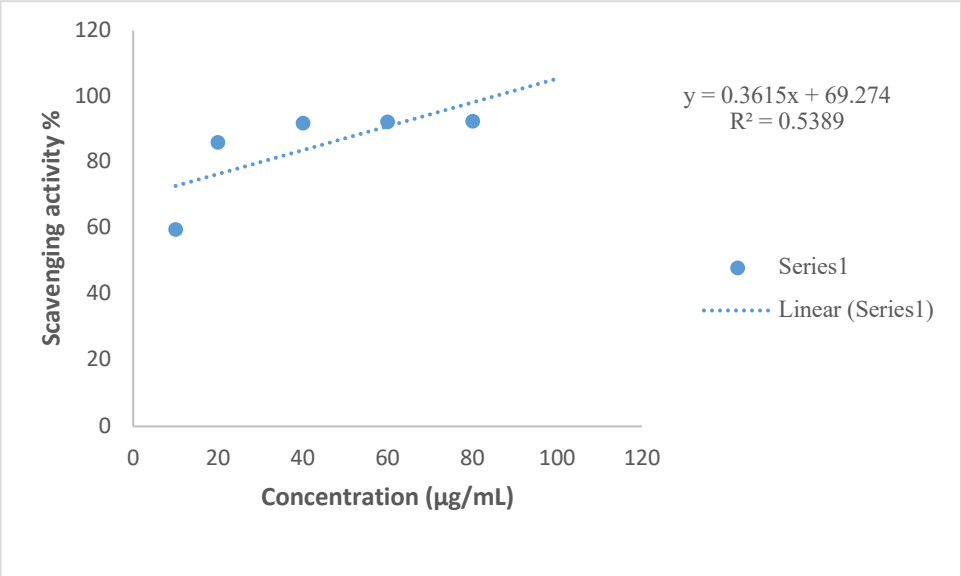
DPPH scavenging activity

Table 1: Sample and corresponding IC_{50} and IC_{80} (µg/mL)

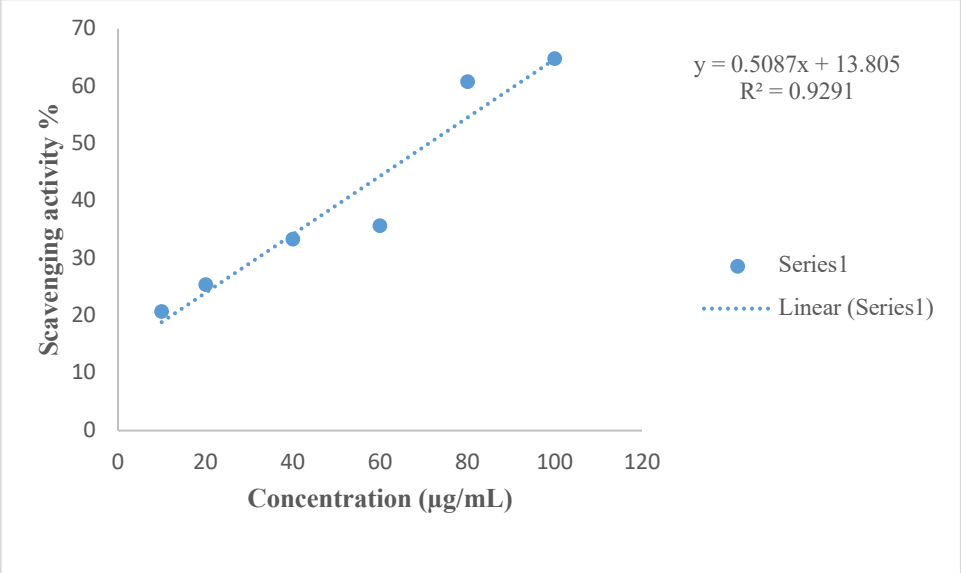
Sample	IC (µg/mL)
BHT (Butylated hydroxytoluene)	IC_{80} = 33.06
Leaf extract of Lagerstroemia floribunda	IC_{80} = 29.67
Fruit extract of Lagerstroemia floribunda	IC_{50} = 71.15



Graph 1: Graph of DPPH scavenging activity against concentration of BHT



Graph 2: Graph of DPPH scavenging activity against concentration of leaves extract of *Lagerstroemia floribunda*



Graph 3: Graph of DPPH scavenging activity against concentration of fruits extract of *Lagerstroemia floribunda*

In DPPH radical scavenging assay, DPPH is a stable free radical and it accepts electron or hydrogen radical to form a stable diamagnetic molecule. The antioxidant reacts with stable free radical (DPPH) which is deep violet in colour and convert it to α, α-diphenyl-β-picryl hydrazine which is of yellow colour. As the DPPH takes up an electron in the presence of the free radical scavenger, the absorption decreases and degree of discolouration shows the radical scavenging potential of the antioxidant. The reaction can be shown as follow:

$$Z\bullet + AH = ZH + A\bullet$$

Where Z• represent DPPH radical, AH represent donor molecules, ZH is the reduced form and A• is the free radical produced. This radical will undergo the further reactions that control the overall stoichiometry which is the number of molecules of reduced form DPPH.

The absorbance values decrease as the concentration increase. The percent scavenging of DPPH was calculated from the absorbance by using the formula as shown below and it shows that the activity had been increased with increased concentration of the extract in the assay. In the other word, as the absorbance values decrease, the antioxidant activity of the extracts increases.

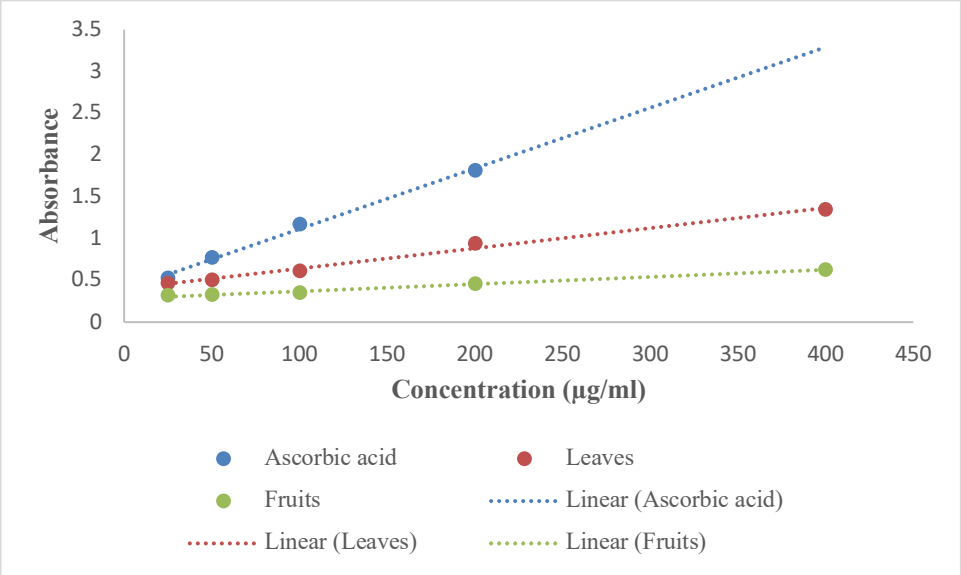
$$\%\ scavenging\ activity = \frac{Ac - As}{Ac} \times 100\%$$

The initial purple colour of DPPH gets reduced by the BHT and extracts and gradually turns into yellow colour. This showed that both the standard and extracts have possessed antioxidant activity. When the concentration is increased, there is more complex nature of the extract that hinders the antioxidant activity. The maximum scavenging activity of the BHT was 93.45 % with the concentration of 80 µg/ml while the maximum scavenging activity of the leaves extract was 92.44 % with the concentration of 80 µg/ml and the maximum scavenging activity of the fruits extract was 64.72 % with the concentration of 100 µg/ml. This shows that the antioxidant activity of the leaves extract is better than the fruits extract. The BHT showed more free radical scavenging potential than leaves extract followed by fruits extract. The leaves of *Lagerstroemia floribunda* have possessed excellent

antioxidant activity, however for the fruits showed only mild antioxidant activity.

IC_{80} of the BHT was 33.06 µg/ml while the IC_{80} of the leaves extract was 29.67 µg/ml. IC_{50} for fruit extract was found to be 71.15 µg/ml. Therefore, the results obtained shows that the leaves extract has more antioxidant activity than fruits extract.

FRAP assay



Graph 4: Graph of absorbance against concentration of standard ascorbic acid, leaves and fruits extract of *Lagerstroemia floribunda*

Antioxidant activity can be indicated by the reducing power. The plants with the high reducing ability are usually having high antioxidant activity. In the FRAP (Ferric Reducing Ability of Plasma) assay, Fe^{3+} was reduced to Fe^{2+} by electron donating activity of the compounds. When the ferric ions were reduced to ferrous ions, the color of the reaction mixture also changes from yellow to bluish green[10-14]. In this assay, ascorbic acid was used as the standard. This assay showed that the higher absorbance value, the higher the reducing power. The reducing power of the ascorbic acid increased as the concentration increased. The ferric reducing power of the ascorbic acid was found to be the highest as compared to leaves and fruits extract. It showed the maximum reducing ability at the concentration of 200 µg/ml with the absorbance value of 1.815. Leaves extract showed the absorbance value of 0.944 at 200 µg/ml whereas fruits extract showed the absorbance value of 0.461 only at the same concentration. Therefore, the leaves extract has showed the better antioxidant activity than the fruits extract. It may duo to the high amount of phenolic compound in the leaves while low level in the fruits of the plant. [15]

CONCLUSION

Lagerstroemia floribunda plant was selected for this study. The antioxidant studies were done by using DPPH and FRAP methods. *Lagerstroemia floribunda* leaves extract showed good antioxidant activity while fruits extract showed low antioxidant activity as compared to BHT (Butylated hydroxytoluene**).** It may duo to the high amount of phenolic compound in the leaves while low level in the fruits of the plant. Hence, leaves extract of *Lagerstroemia floribunda* may be used as antioxidant defense while fruits extract may be used as supportive agent.

Since *Lagerstroemia floribunda* plant is proven to have antioxidant activity, hence further studies on various pharmacological actions supported by antioxidant potential is highly recommended.

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