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Research Article

Pharmacognostical, Phytochemical and Total Phenolic Content Study of *Lagerstroemia Floribunda* Jack (Kedah Bungor)

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Abstract

No Scientific data of *Lagerstroemia floribunda* Jack leaves and fruits pharmacognostical, phytochemical and total phenolic content was available to date, so we investigated these in this study.

Macroscopy, physicochemical, microscopy, phytochemical analysis and thin layer chromatography of *Lagerstroemia floribunda* Jack was studied using various methods in this research. Total phenolic content was determined using Folin-Coicalteau method.

Results showed that leaves are petiolated, Ovate shaped, with average width of 5.9 - 6.7 cm and length of 11.6 - 13.2 cm. The dicotyledon leaves were pinnate-type reticulate venation with narrow lobes. The leaves have bitter taste without distinct odor, possessing dark green color on dorsal side, while light green on ventral side. Microscopy on leaves and fruit powder showed the presence of anisocytic stomata, xylem, phloem, lignified pericyclic fibers, lignified sclerenchymatous and arc shaped vascular bundle, oil glands, lignified xylem vessels, calcium oxalate crystals and phloem parenchyma. Phytochemical test showed the presence of carbohydrates, reducing sugar, flavonoids and tannins. In physicochemical analysis, loss on drying was found to be 10% for leaves and 12% for fruits, total ash value 7% for leaves and 5.69% for fruits; and water soluble extractive value of 13.6% in leaves and 8% in fruits as highest. Fruits powder showed yellow fluorescence with 1N Hydrochloric acid. Total phenolic content was found to be 7.57 GAE/g in leaves and 3.7 GAE/g in fruits hence it can be concluded content was higher in leaves than fruits.

Keywords: *Lagerstroemia floribunda Jack; Pharmacognostical; Phytochemical; Total Phenolic Content*

Introduction

Lagerstroemia floribunda is a species that belongs to the Lythraceae family (Figure 1). This delightful ornamental plant is also known as kedah bungor and Thai crape myrtle and 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 [1].

Figure 1: *Lagerstroemia floribunda.*

In recent past, there has been a tremendous interest in exportation of plants for its medicinal properties. Lagerstroemia genus is not much explored for pharmacognostical and phytochemical screening except its few species. On contrary, *Lagerstroemia speciosa* is much studied and explored for antidiabetic, antioxidant, antibacterial, antiviral and anti-inflammatory activity [2-7].

Lagerstroemia floribunda found to have traditional use in stomach problems, weight loss and lower blood sugar [8] which needs to be further validated scientifically. In order to do that plant must be screened for its pharmacognostic and phytochemical profile. To our understanding, there is no such data published so far for this plant hence we have carried out this study on *Lagerstroemia floribunda* plant to explore it for pharmacognostic and phytochemical profile. Our research may work as guidance for the researcher who will take this plant for further research and can also support in developing plant scientific profile.

Material and Method

Collection 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. Both the leaves and fruits also dried in the hot air oven to increase the speed of drying process. 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.

Macroscopy Study

Macroscopical examination of different parameters like shape, size, surface, characteristic, colour, odour, taste and texture were done on the plant.

Microscopy Study

Chlorophyll content was removed from the leaves by warming with chloral hydrate. The transverse section of the leaves from the apex and base of the leaves was taken. Coverslips were used to cover the traverse section of the leaves that were mounted on the clean glass slides. Iodine, concentrated hydrochloric acid, phloroglucinol, and 60% sulphuric acid were used to stain the traverse sections of the leaves on different glass slides and they were observed under light microscope. For fruits, the same procedure was followed. Epidermal

cells, trichomes, xylem, stomata, phloem, the characteristic microscopic of the leaves were observed and identified. The results obtained were noted.

Powder Microscopy

A small amount of leaves was stained with phloroglucinol solution and concentrated hydrochloric acid in the ratio of 1:1 in the watch glass, mixed well and allowed to stand for 3 minutes. Then it was mounted on slide and covered with a cover slip. Light microscope was used to observe it. Also, the powder was stained with iodine solution to identify the starch grains. Calcium oxalate crystals were identified by treating the powder with concentrated sulphuric acid (60% v/v) and dilute acetic acid. The same procedures were followed for the fruits powder. All the powder microscopic characteristic of leaves and fruits were observed, identified and results were noted.

Physicochemical evaluation of crude drug

All the crude drugs need to undergo the physical and chemical evaluation in various parameters. The physical evaluation of the crude drugs was carried out by the determination of different physical characteristics like extractive values with different solvents, loss on drying, determination of total Ash values and fluorescence analysis, thin layer chromatography by adopting the methods described in reference textbooks [10].

Extraction

Soxhlet apparatus was used for the extraction. It is the hot continuous 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. The 'thimble' was closed with the filter paper. 3 to 4 boiling chips were placed into the solvent vessels, such as round bottom flask or cylindrical flask. The Soxhlet extractor connection was placed on the top of the flasks. The 'thimble' was inserted into the Soxhlet extractor fitting into the thimble compartment. The condenser was connected on the top of the Soxhlet extractor and the water flow was turned on. 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 Soxhlet apparatus was switched on and maintained at the suitable temperature for about 2 to 3 days continuously. After the continuous extraction, the Soxhlet apparatus was switched off and the content in the round bottom flask was weighed. The extract obtained was concentrated by using rotary evaporator [9].

Phytochemical Screening

As per the methods described in reference textbooks, phytochemical screening was carried out [10].

Total Phenolic Content

Gallic acid stock solution was made by dissolving 1 mg of gallic acid in 100 ml of methanol. The concentration of the stock solution is 10 µg/ml. Different dilutions of standard gallic acid were prepared from the stock solution such as 0.1, 0.2, 0.3, 0.4 and 0.5 µg/ml. Folin-Ciocalteau reagent was prepared by mixing Folin's reagent with phenol reagent in the ratio 1:1 and diluted with the distilled water in the ratio 1:1 before use. 2.5% sodium carbonate was prepared by dissolving 2.5g sodium carbonate in 100 ml of distilled water. Then 100 µl of Folin-Ciocalteau reagent and 2 ml of 2.5% sodium carbonate solution were added to 100 µl of gallic acid. The mixtures were allowed to stand for 2 hours. The absorbance was measured at 750 nm after 2 hours. The stock solution of extracts for both the leaves and fruits were prepared by dissolving 10 mg of extract in 10 ml of methanol. The concentration of the stock solution was 1000 µg/ml. Different dilutions of extract were made from the stock solution such as 50, 100 and 200 µg/ml. The same procedures as the standard gallic acid were followed by the extracts. Calibration curve was plotted for the gallic acid as the standard and the calibration curve for extracts were also prepared. Total phenolic content of the extracts was calculated by using the following formula [11,12].

Total Phenolic Content, C= A/B

A: Equivalent concentration of gallic acid established from calibration curve (mg)

B: Dry weight of extract

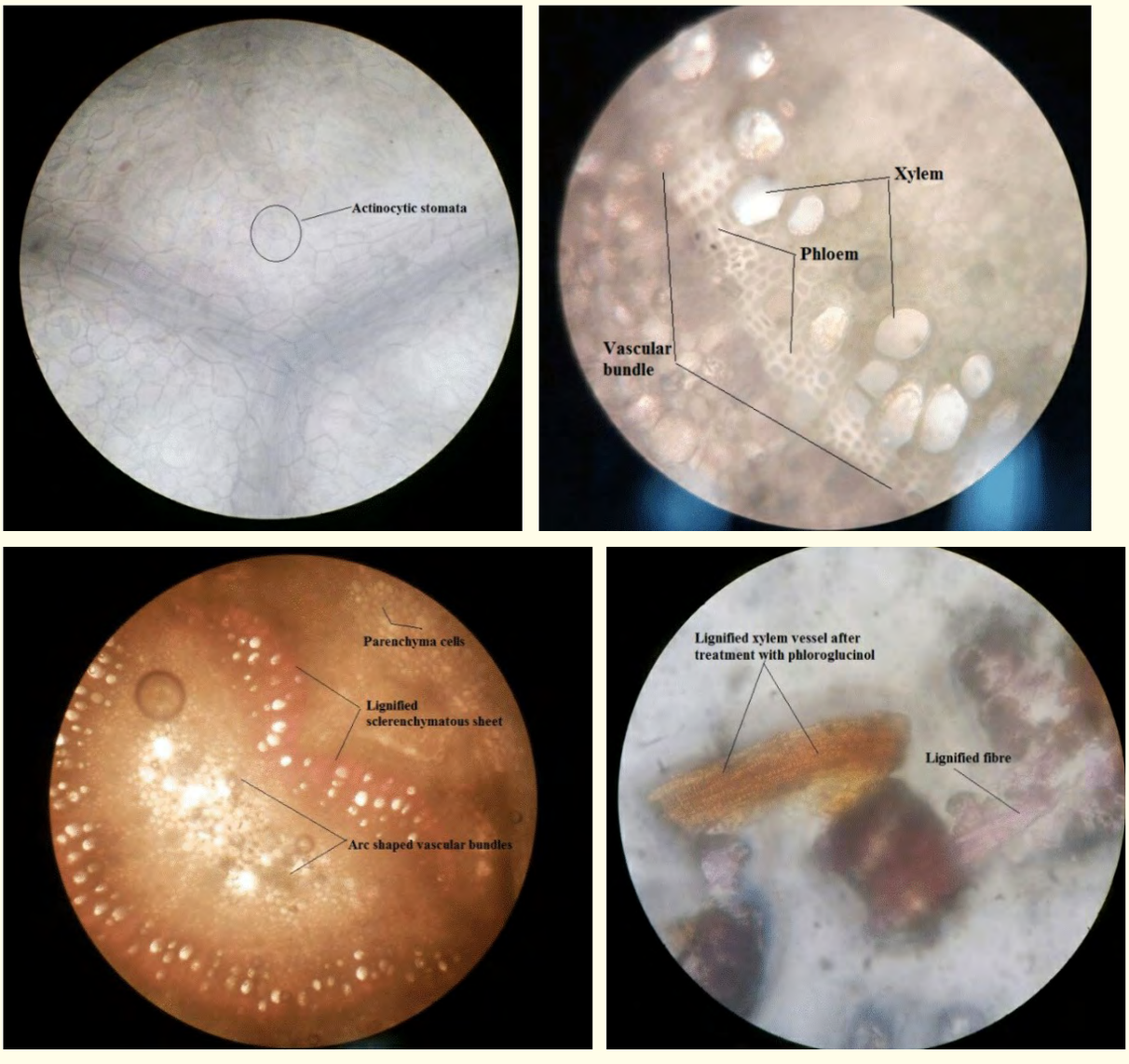
C: Expressed as mg GAE/g dry weight of extract

Results

Macroscopy Study

Organoleptic features of the leaves and the macroscopic characteristics were observed. The leaves are petiolated, arranged in alternate or subalternate manner, irregularly. Ovate shaped, with average width of 5.9 - 6.7 cm and length of 11.6 - 13.2 cm. The dicotyledon leaves are having pinnate-type reticulate venation with narrow lobes. Its prominent veins are devoid of hairs superficially, while its midrib has a rough texture. With cuspidate apexes, the leaves also exhibit thick petioles. The leaves have bitter taste without distinct odor, and possessing dark green color on dorsal side, while light green on ventral side.

Microscopy Study



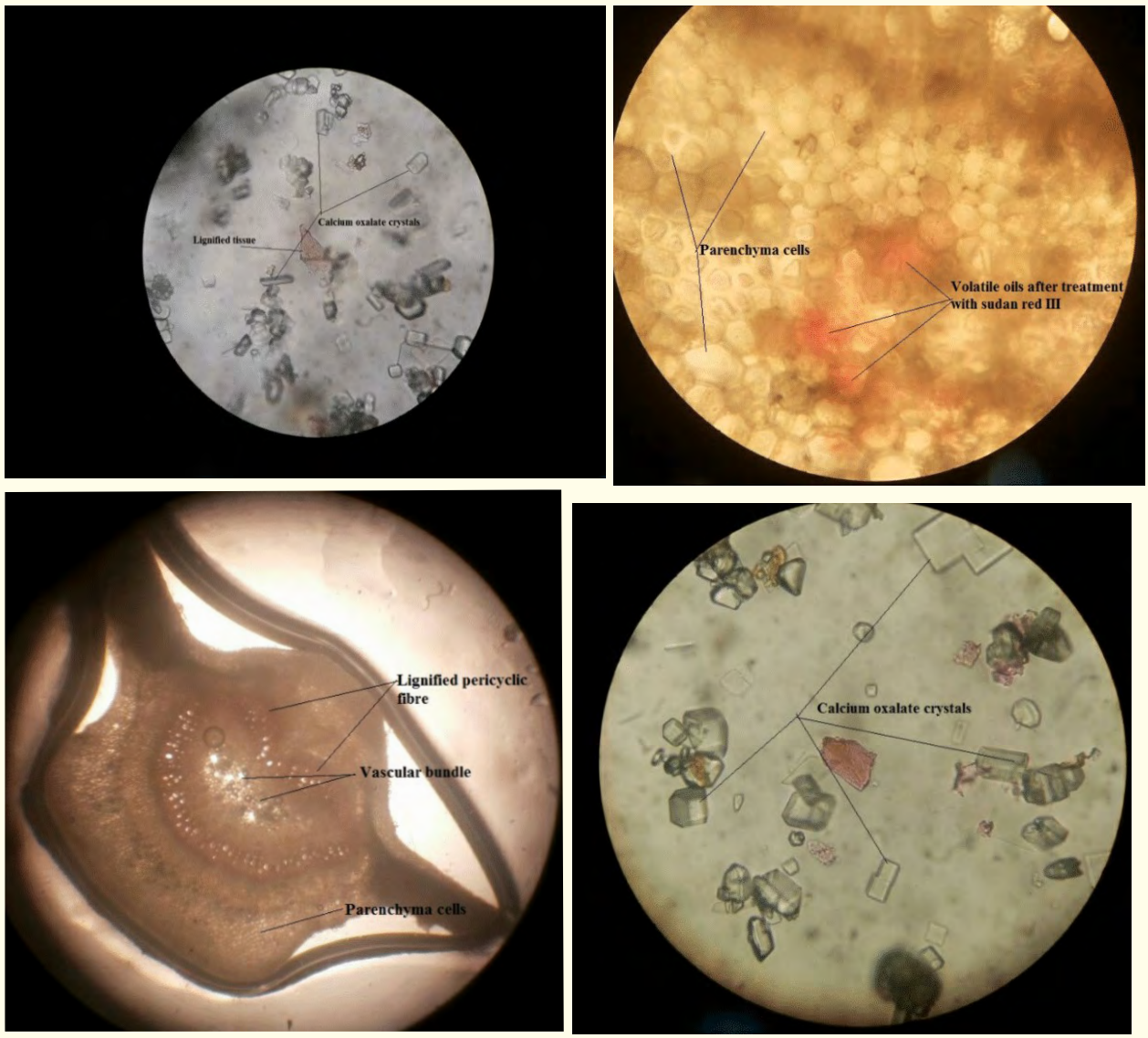


Figure 2: *TS of Lagerstroemia floribunda Jack. leaves.*

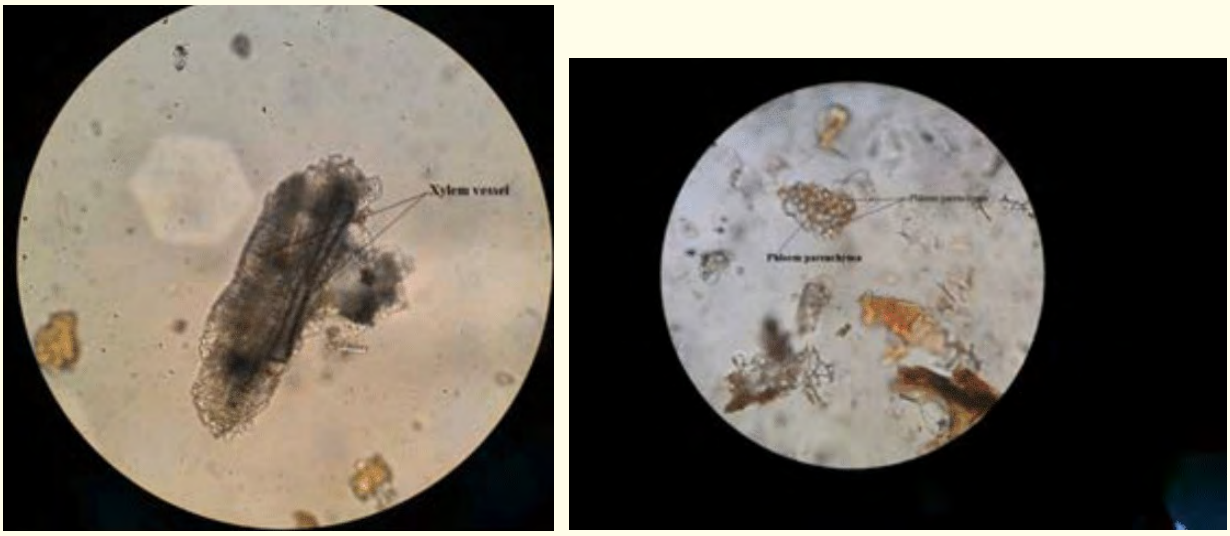


Figure 3: *TS of Lagerstroemia floribunda Jack. fruits.*

Physiochemical Characterization

S No.	Parameters determined	Value in (%) w/w	
		Leaves	Fruits
1	Alcohol-soluble extractive	12.8	3.2
2	Water-soluble extractive	13.6	8.0
3	Chloroform-soluble extractive	5.6	3.2
4	Petroleum ether-soluble extractive	1.6	–

Table 1: *Extractive value.*

S No.	Parameters determined	Values in (%) w/w	
		Leaves	Fruits
1	Loss on drying	10.0	12.0

Table 2: *Loss on drying.*

Weight of empty crucible (g)	Weight of leaves Powder	Weight of crucible + leaves powder (g)	Weight of crucible + ash (g)	Total ash value %
22.78	3.0	25.78	22.99	7.00

Table 3: *Total ash value of leaves.*

Weight of empty crucible (g)	Weight of fruits powder	Weight of crucible + fruits powder (g)	Weight of crucible + ash (g)	Total ash value %
23.16	2.99	26.15	23.33	5.69

Table 4: *Total ash value of fruits.*

Fluorescence Analysis

S. No	Reagents	Leaves Part			Fruits Part		
		Daylight Color	254 µm	365 µm	Daylight Color	254 µm	365 µm
01	Concentrated Sulfuric acid	Light Brown	No	No	Black	No	No
02	Glacial Acetic Acid	Green	No	No	Brown	No	No
03	5 % Iron (III) chloride	Yellow	No	No	Yellow	No	No
04	Ammonia Solution	Brown	No	No	Brown	No	No
05	Acetic Acid + Iron (III) chloride + Sulfuric acid	Yellow	No	No	Yellow	No	No
06	Iodine	Reddish Brown	No	No	Reddish	No	No
07	Acetic Acid + Sulfuric acid	Greenish Brown	No	No	Brown	No	No
08	Picric Acid	Greenish Yellow	No	No	Yellow	No	No
09	1N Hydrochloric Acid	White	No	No	White	Yellow	Yellow
10	10 % Sodium Hydroxide + Few Drops of 10 % Lead Acetate	Milky White	No	No	Milky White	No	No
11	Nitric Acid + Ammonia Solution	Brown	No	No	Brown	No	No
12	10 % Sodium Hydroxide + Copper (II) sulfate	Yellow Brown	No	No	Blue Brown	No	No
13	1N Sodium Hydroxide	Brown	No	No	Brown	No	No

Table 5: *The fluorescence characteristic of leaf and fruit powder under UV after treating with different chemical reagents.*

S No.	Test	Name of Plant (Lagerstroemia floribunda)			
		Fruit		Leaf	
	Extracts	Alcohol	Aqueous	Alcohol	Aqueous
1.	**Test For Carbohydrates**				
a)	Molisch's test	+	+	+	+
b)	Barfoed's test	–	–	–	–
c)	Fehling solution test	+	+	+	+
2.	**Test for reducing sugar**				
a)	Benedicts test	+	+	+	+
3.	**Test for alkaloids**				
a)	Mayer's reagent test	–	–	–	–
b)	Dragendorff's reagent test	–	–	–	–
c)	Hager's reagent test	–	–	–	–
d)	Wagner reagent test	–	–	–	–
4.	**Test for cardiac glycosides**				
a)	Legal test	–	–	–	–
B	Keller-kiliani test	–	–	–	–
5.	**Test for antraquinone glycosides**				
a)	Borntrager test	–	–	–	–
b)	Modified Borntrager test	–	–	–	–
6.	**Test for saponins**				
a)	Foam test	–	–	–	–
7.	**Test for steroids**				
a)	Salkowski test	–	–	–	–
8.	**Test for flavonoids**				
a)	Shinoda test	+	+	+	+
9.	**Test for tannins**				
a)	5% ferric chloride test	+	+	+	+
b)	Lead acetate test	+	+	+	+
c)	Potassium dichromate test	–	–	–	–
d)	Gelatin test	–	–	–	–
10.	**Test for amino acid**				
a)	Ninhydrin test	–	–	–	–

Table 6: *Phytochemical Analysis.*

Thin Layer Chromatography

Spot	R_f Value
1	0.15
2	0.30
3	0.42
4	0.52
5	0.74
6	0.91

Table 7: *R_f values of aqueous extract of Lagerstroemia floribunda fruit using butanol: acetic acid: water (4: 1: 5) as solvent system.*

Spot	R_f Value
1	0.574
2	0.959

Table 8: *R_f values of methanolic extract of Lagerstroemia floribunda leaf using toluene: ethyl acetate: glacial acetic acid (55: 45: 0.5) as solvent system.*

Spot	R_f Value
1	0.899

Table 9: *R_f values of methanolic extract of Lagerstroemia floribunda fruit using toluene: ethyl acetate: glacial acetic acid (55: 45: 0.5) as solvent system.*

Total Phenolic Content

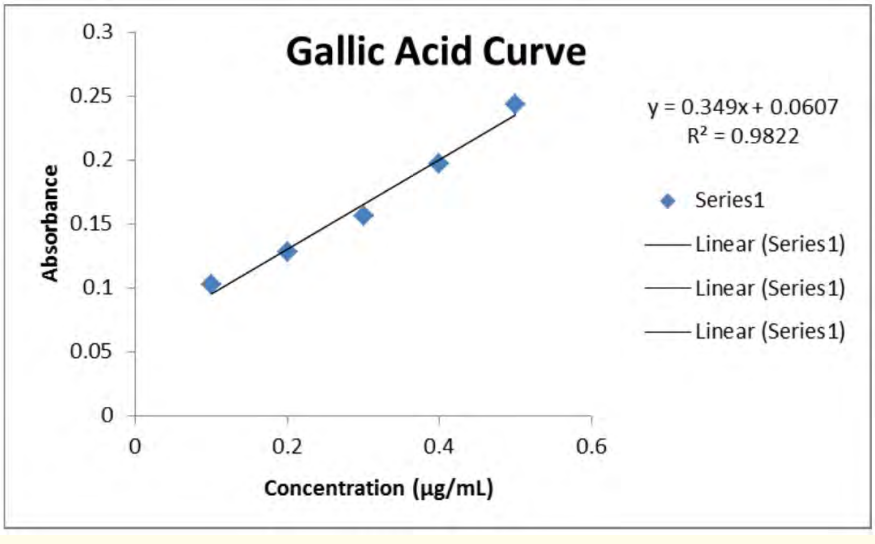


Figure 4: *Graph of gallic acid.*

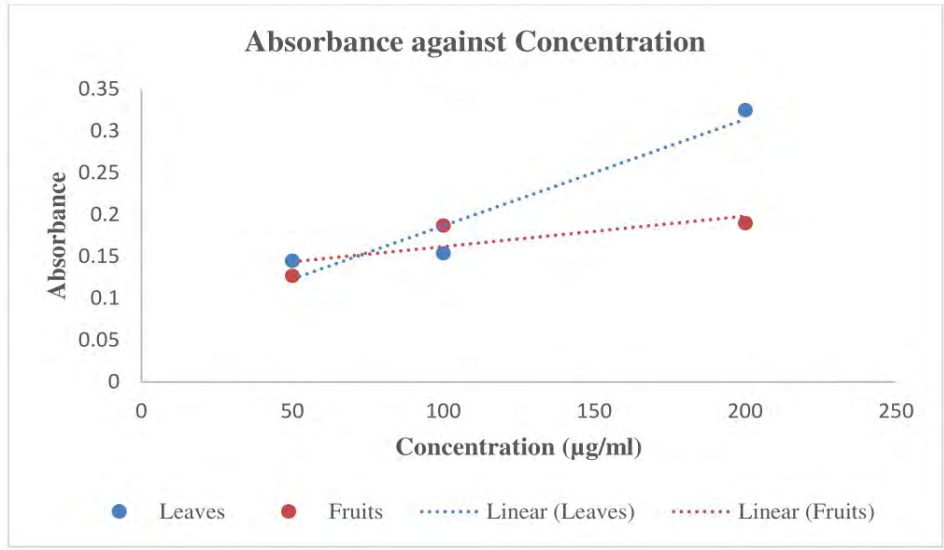


Figure 5: *Graph of absorbance against concentration for leaves and fruits extract of Lagerstroemia floribunda.*

Discussion

In macroscopic study, the morphological features of the leaves and fruits of *Lagerstroemia floribunda* were found to be in agreement with standards cited in official literature.

In microscopic study, transverse section of leaves and fruits of *Lagerstroemia floribunda* plant were being studied by using binocular compound microscope. On treatment of Phloroglucinol, pinkish color suggested the existence of xylem vessels within the leaves and fruits, signifying the presence of lignin within the xylem vessels. On treatment of Sudan Red, several reddish-dyed globules were observed amidst the cell of both transverse section of leaves and fruits featuring occurrence of volatile oil globules. On treatment of Ruthenium Red, mucopolysaccharide were observed as it was tinted with darker red color. Anomocytic stomata were also seen in microscopic study, showed the distinctive features of *Lagerstroemia floribunda*.

In the physicochemical analysis, water soluble extractive value for leaves and fruits were found to be 13.6% and 8% respectively which is the highest value among alcohol soluble, chloroform soluble and petroleum ether soluble extractive values. *Lagerstroemia floribunda* leaves and fruits has a high polar components presence. The values of loss on drying for leaves and fruits were 10% and 12% respectively. The weight of both the powders was high initially because of the presence of the moisture in the powders. The moisture in both the powders gets evaporated when the powders were placed in the hot air oven. Therefore, the final weight of both the powders was reduced. Total ash value for leaves and fruits powder 7% and 5.69% respectively. Apart from quality and purity of the crude drug, total ash value serves as an important index for inorganic compound presence in the crude drug.

Different coloration under visible light and UV light were observed in *Lagerstroemia floribunda* (Kedah bungor) leaves and fruits powder during fluorescent analysis with different chemical reagents. UV light is known to induce a fluorescent nature which is one of the significant features of fluorescence. Among various solvents tested, only 1N hydrochloric acid showed fluorescence in fruit powder. The

fluorescent study helps to identify the class of phytochemical compound (e.g. alkaloids) present in plant. Besides, some of substances may be often converted into fluorescent derivatives by using different chemical reagents though they are not fluorescent, hence we can often assess qualitatively some crude drugs using fluorescence as it is the most important parameter of pharmacognostical evaluation.

Thin Layer Chromatography (TLC) consists of two phase, solid stationary phase and liquid mobile phase. Butanol: acetic acid: water (4: 1: 5) was used as a developing solvent in the TLC analysis of aqueous fruit extract which led to detection of 6 different spots in the TLC plate. TLC analysis of methanolic leaf resulted in two spots while analysis for methanolic fruit showed one spots in in the solvent toluene: ethyl acetate: glacial acetic acid (55: 45: 0.5). Above analysis indicates the phytochemical presence in the respective extracts in varying quantities. Each spot has a different R_f value. The R_f value for any given compound vary depending on the stationary phase and mobile phase used.

Soxhlet or Hot Continuous Extraction involves heating of the extracting solvent in the flask followed by condensation of vapors in the condenser. Vapors condensing drips in to the thimble with crude drug below and extraction takes place by contact. Liquid contents of the chamber containing thimble siphon in to the flask when the chamber is filled with liquid to the top of the siphon tube. This is a continuous process which occurs till a single drop of solvent from the siphon tube does not leave residue when evaporated. Solvent dissolves a portion of the non-volatile compounds of the plant during each cycle. After many cycles of extraction, the extracts became concentrated in the distillation flask. A much smaller quantity of solvent can be used to extract copious amounts of the drug in this method which is advantageous compared to other extraction methods. The disadvantages of this method are the extraction time is long and the agitation can't be provided to accelerate the extraction. Clogging into the extraction apparatus was prevented using a thimble which acts as a filter and is also used to hold the powder. Filter paper was used to cover the thimble to ensure uniform spreading of the powder by the solvent and allowed the maximum extraction of the desired constituents. Methanol was used as a solvent for extraction because it has low density and boiling points as compared to ethanol. Besides that, it is more polar and those compounds that are not extracted with ethanol can be easily extracted by methanol [13-15].

The result of phytochemical screening of the *Lagerstroemia floribunda* suggests the presence of carbohydrates, flavonoids and tannins.

Absorbance of sample and Folin-Ciocalteau Reagent mixture at 760 nm was used for the estimation of the total phenolic content in the sample. Units of total phenolic content were µg/mL and gallic acid was used as standard compound. Gallic acid equivalent was obtained by using the standard curve equation: $y = 0.349x + 0.0607$, $R^2 = 0.9822$, where y is the absorbance at 760 nm, while x is the total phenolic content expressed in µg/mL. The result of phenolic content in methanolic leaves extract was given as 7.57 GAE/g, while the methanolic fruits extract was given as 3.7 GAE/g. Antioxidant activity was reported for plant's phenolic content which acts as hydrogen donating sources, singlet oxygen quenchers, free radical scavengers, and metal ion chelators. *Lagerstroemia floribunda* leaves extract showed higher antioxidant activity than fruits extract, which is directly related to its high existence of phenolic constituents.

Thambi T Priya reported that in *Lagerstroemia speciosa* (L) leaves total polyphenol content were found to be 76.5 mg/g. The fingerprinting techniques helped to identify the polyphenols and tannins varieties present in the plant parts. HPTLC study further Tannins from *L. speciosa* gave peaks at R_f 0.05, 0.18, 0.38 and 0.57 which shows at a wavelength of 380 nm four different constituents were present which answered ferric chloride test.

In our study we found significant amount of total phenolic content in leaves and fruits extract of *Lagerstroemia floribunda*. Since both the species are different and contain different chemical constituents hence there is need for comparative study to further explore this difference by using sophisticated techniques of HPLC and HPTLC profiling [16].

Conclusion

Lagerstroemia floribunda plant was selected for this study and Soxhlet extraction method was used for extraction. All the standardization parameters for the leaves and fruits of selected plant were done according to pharmacopoeia standards. The results of all these

parameters were reported to be found within limits. According to the phytochemical screening studies, we can conclude that the plant contains flavonoid and glycoside. Fruits powder showed yellow fluorescence. Total phenolic content was found to be higher in leaves than fruits.

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