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Phytochemical screening and anti-oxidant activity of leaf extracts of *Lagerstroemia lanceolata*(Linn.)

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ABSTRACT

The present study is aimed at investigating the phytochemical screening, fluorescence powder analysis and antioxidant assay of the ethyl acetate and methanolic leaf extracts ofLagerstroemia lanceolata L. (Lythraceae). The antioxidant activities of the extracts have been evaluated by using two in vitro assays and were compared to standard antioxidant butylatedhydroxy anisole (BHA). Both the extracts showed effective H-donor activity, reducing power and free radical scavenging activity. The antioxidant property depends upon concentration of the extracts, which may be due to the presence of phenolic and flavonoid compounds present in these extracts. The results obtained in the present study indicate that the leaves of L. lanceolata may be a potential source of natural antioxidant.

Keywords: *Lagerstroemia*, phenolic compounds, free radical, DPPH, Reducing power assay

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INTRODUCTION

The Lythraceae is a small family of some 22genera, which range in habit from herbs to shrubsand trees[1]. *Lagerstroemia* is an important member of Lythraceae consisting of 31 genera.This genus contains more than 56 species of trees or shrubs with colorful flowersdistributed from southeastern Asia to Australia[2]. *Lagerstroemialanceolata* L. (Lythraceae) is a moderate to large deciduous tree, sometimes attaining 30 metres in height and 2.4 to 3.0 metres in girth with a clean cylindrical bole of 12 to 15 metres[3].

Many plants, particularly medicinal plants, have been extensivelystudied for theirantioxidant activity in recent years[4].Theyare a source for a wide variety of natural products, such as phenolic acids and flavonoids[5] that may lead to the discoveryof new, useful antioxidant sources, providing an incentivefor the preservation of these plants and sustainable developmentwithin this region[6].*In vitro* experiments on antioxidantcompounds in higher plants show how they protectagainst oxidation damage by inhibiting or quenchingfree radicals and reactive oxygen species [7].

The present study aims to investigate the ethyl acetate and methanolic extracts by *in vitro* antioxidant assays, phytochemical screening and fluorescence powder analysis of leaf of*L. lanceolata*.

MATERIALS AND METHODS

Collection of plant material

Plant leaf of *L.lanceolata*was collected from local area in and around from Western Ghat region,Maharashtra.

Chemicals: All the solvents and chemicals used were of analytical grade of Merck Ltd, Mumbai, India.

Preparation of extract:The plant leaves were washed properly with water; then they were air dried under shade and converted into coarse powder.Fifty grams of powdered leaves were extracted with 250 mL of methanol and ethyl acetate separately by maceration for 24 h. The extracts were filteredand the filtrate was evaporated to dryness under reduced pressure at40 °C with a rotary vacuum evaporator.

Preliminary Phytochemical Screening:
Preliminary phytochemical screening of the ethylacetate and methanolic leaf extracts of *L. lanceolata*was carried out by established methods[8, 9,10].

Fluorescence powder analysis:
A small quantity of dried and finely powdered leaf sample was placed on a grease free microscopic slide and 1-2 drops of freshly prepared solution was added, mixed by gentle tilting the slide and waited for 1-2 minutes. Then the slide was placed inside the UV viewer chamber and viewed in day light, short (254 nm) and long (365 nm) ultraviolet radiations. The colors observed by application of different reagents in above mentioned radiations were recorded[11].

DPPH radical scavenging activity:
The free radical scavenging activity of the fractions was measured *in vitro* by DPPHassay procedure. About 0.3 mM solution of DPPH in 100% ethanol was prepared and 1 mLof this solution was added to 3 mL of the ethyl acetate and methanolic extractand dissolved in ethanol at different concentrations (5-80µg/mL). The mixture was shaken and allowed tostand at room temperature for 0.5h and the absorbance was measured at 517 nm using aspectrophotometer. The percentage of scavenging activity at different concentrations wasdetermined and compared using butylatedhydroxy anisole (BHA), as the standard[12 – 14].

Reducing power:
The reducing power of the extract under observation was investigated by Fe^{3+}–Fe^{2+}transformation. The Fe^{2+} can bemonitored by measuring the formation of Perl's Prussian blue at 700 nm. One mL of the extract (5-80 μ g/mL), 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of 1% potassiumferricyanide were incubated at 50°C for 30 min and 2.5 mL of 10% trichloroacetic acid wasadded to the mixture and centrifuged for 10 min at 3000 rpm. About 2.5 mL of thesupernatant was diluted with 2.5 mL of water and shaken with 0.5 mL of freshly prepared0.1% ferric chloride. The absorbance was measured at 700 nm using BHA (50-250 μ g/mL)as the standard. All the tests were performed in triplicate[13,14].

RESULTS

Preliminary Phytochemical Screening:
Phytochemical screening of the crude ethyl acetate and methanolic extracts of leavesof *L. lanceolata*revealed the presence of alkaloids, phenols, resins, flavonoids, anthraquinones and cardiac glycosides(Table 1).

Table1:Preliminary Phytochemical Screening results

Sr. No.	Tests	Ethyl acetate extract	Methanolic extract
1.	Carbohydrates	+	+
2.	Proteins	+	+
3.	Fats & oils	-	-
4.	Steroids	+	+
5.	Glycosides	-	-
6.	Saponins	-	-
7.	Alkaloids	-	+
8.	Flavonoids	++	+++
9.	Phenols	+	++
10.	Tannins	+	+++

+++ strong; ++ medium; + average; - absence

Fluorescence powder analysis:
Fluorescence is the phenomenon shown by various chemical constituents present in the plant material. These constituents undergo fluorescence in ultra violet light, which can be due to formation of fluorescent derivatives or decomposition products in presence of different reagents. This method helps in identifying the crude drugs qualitatively which in turn is an important parameter of pharmacognostic evaluation.
The fluorescence analysis of powdered leaves of *L. lanceolata*is summarized in Table 2.

Table2:Fluorescence powder analysis of leaves of *L. lanceolata*

Powdered drug	Visible/Day light	UV 254 nm(short)	UV 365 nm (long)
Powder drug as such	Light brown	Brown	Yellowish brown
Powder + Methanol	Green	Green	Greenish orange
Powder + 1% glacial acetic acid	Brown	Dark green	Brownish orange
Powder +10% NaOH	Yellowish brown	Reddish black	Blackish brown
Powder + dil. NH_3	Yellowish brown	Green	Brown
Powder + Conc. HNO_3	Brown	Orange	Golden yellow
Powder+ dil.NH_3+Conc.HNO_3	Yellowish brown	Reddish brown	Brownish orange
Powder +1M H_2SO_4	Brown	Faint green	Faint green
Powder +1M HCl	Brownish yellow	Yellowish green	Yellowish green
Powder + 10% $FeCl_3$	Reddish brown	Dark green	Blackish brown
Powder +Acetone + Methanol	Light brown	Dark green	Green
Powder +10% Iodine	Yellowish brown	Dark brown	Blackish brown

Antioxidant assay:

From theTable 3, it is evident that the component from ethyl acetateand methanolic extract of this species can be potent antioxidant in comparison with the widelyused standards and synthetic antioxidants like L-ascorbic acid, BHA and BHT. In DPPH radical scavenging method, increasein concentration follows decrease in absorbance (Fig. 1) where as in reducing power assay, increase in concentration shows anincrease in absorbance (Fig. 2). The above two *in vitro* antioxidant assays indicates that the methanolic extractpossess potent antioxidant activity than ethyl acetate extract and standard (BHA).

Table 3: DPPH and reducing power assay of ethyl acetate and methanolic extract of *L. lanceolata*

Group	DPPH method	Reducing power assay
Control	100±0.0080	100±0.0066
Ethyl acetate extract		
5µg/ml	51.18±1.570[b]	80.186±1.225[b]
10 µg/ml	65.08±2.934[b]	72.413±1.959[b]
20 µg/ml	72.45±2.797[b]	68.168±0.8948[b]
40 µg/ml	81.40±0.9505[b]	60.946±1.440[b]
80 µg/ml	88.86±0.7663[b]	56.203±0.7998[b]
Methanolic extract		
5µg/ml	65.25±0.6909[b]	84.16±0.5759[b]
10 µg/ml	72.84±2.157[b]	80.923±0.6757[b]
20 µg/ml	79.69±2.111[b]	75.1±0.8942[b]
40 µg/ml	84.91±0.8351[b]	69.62±0.5232[b]
80 µg/ml	91.83±0.3694[b]	64.39±2.3000[b]
ButylatedHydroxy Anisole (BHA)		
80 µg/ml	51.33±0.2722	44.206±0.2616

Butylatedhydroxy anisole (BHA) was taken as a standard. Results are expressed as percentage of the control. Each value is mean ±SE (n = 3). a: p< 0.05, b: p < 0.01, c: p < 0.001 vs. control group.

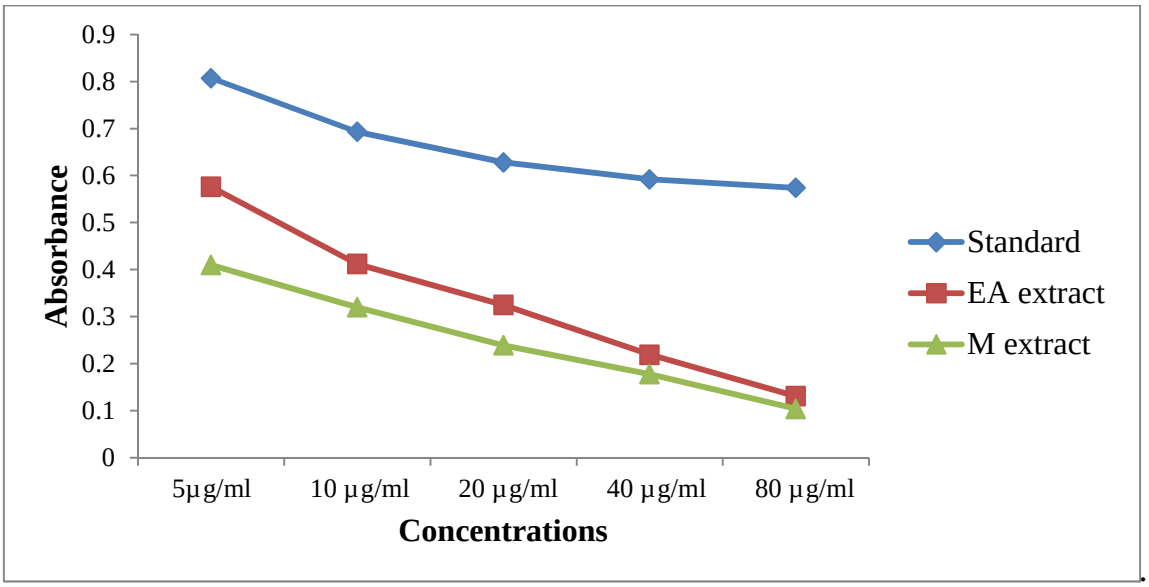

Fig 1: DPPH assay of ethyl acetate and methanolic extract of *L. lanceolata*leaves and butylatedhydroxy anisole (BHA). Each value is mean ± SE (n = 3)

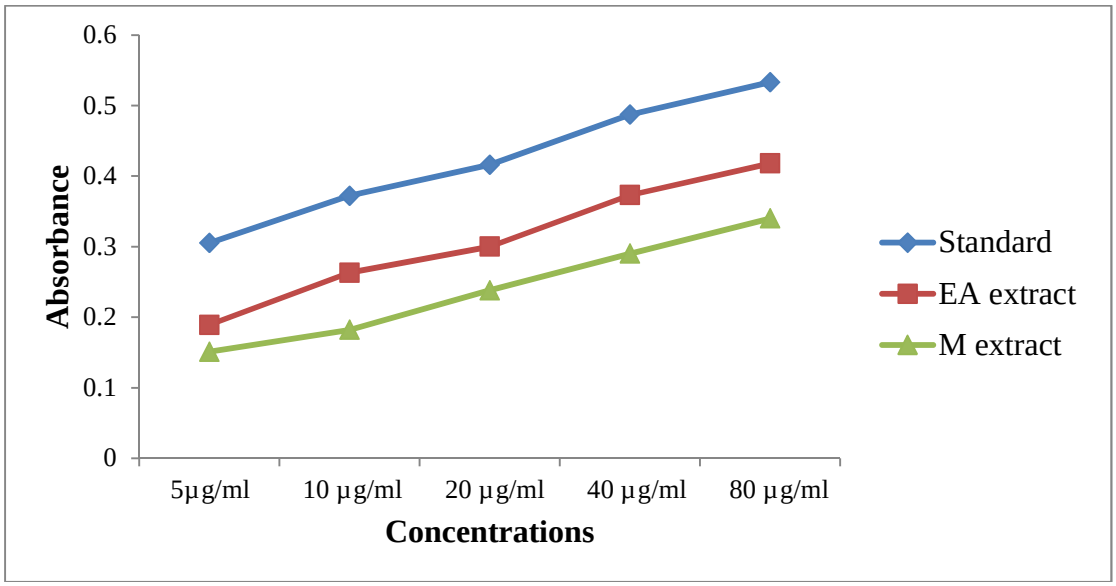

Fig 2: Reducing power assay of ethyl acetate and methanolic extract of *L. lanceolata*leaves and butylatedhydroxy anisole (BHA). Each value is mean ± SE (n = 3)

CONCLUSION

Fluorescence powder analysis is a useful technique which helps in identifying a crude drug qualitatively by understanding the changes that a chemical constituent may undergo when it is exposed to different radiations.The phytochemical screening indicated the presence of different phytochemicals like steroids, flavonoids, phenols, tannins.The antioxidant assay reveals that the methanolicextract of *L. lanceolata*possesses more significant scavenging activity byDPPH and reducing power assay methods. Its antioxidant activity may possiblyattribute due to its polyphenol and flavonoid contents. However, further studies are necessary to elucidate the compound responsible forantioxidant activity of extract.

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