In Vitro Antioxidant Studies of *Lagerstroemia speciosa* Leaves

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ABSTRACT

The hydro alcoholic extract of leaves of *Lagerstroemia speciosa* (LS) was studied for antioxidant activity on different *in vitro* models namely 1,1-diphenyl, 2-picryl hydrazyl (DPPH) assay, Hydrogen peroxide and Nitric oxide radical scavenging method, and superoxide readical scavenging by alkaline DMSO method. Various standards were also evaluated for comparison. The extract showed dose dependent free radical scavenging property in the tested models. *Lagerstroemia* showed IC_{50} value 4.75±0.25 µg/ml for DPPH method, which was comparable to that of ascorbic acid (IC_{50}=2.75±0.29 µg/ml) and rutin (7.89±0.51µg/ml) . For Hydrogen peroxide method, IC_{50} value was found to be 28.00±0.16 µg/ml, which compares favourable with Ascorbic acid (IC_{50}=187.33±3.45µg/ml) and rutin (35.26±0.166µg/ml). In Nitric oxide model, IC_{50} value was found to be 750±3.66µg/ml, which is very low when compared to Rutin (IC_{50}=68.5±2.27µg/ml). This study demonstrates the antioxidant activity of the *Lagerstroemia speciosa*.

Keywords: Antioxidant, *Lagerstroemia speciosa* (L.), free radicals, DPPH.
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INTRODUCTION

Free radicals oxidatively damage lipids and proteins and compromise genomic DNA integrity. They are widely recognised as the root cause of numerous degenerative diseases, including cardiovascular disease, cancer and aging.[1] Antioxidant compounds scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases.[2]

Lagerstroemia speciosa (Linn) Pers. (Lythraceae), also known as "Queens Crape Myrtle," is a deciduous tree which grow in tropical and subtropical areas. Native of Philippines it is commonly called 'banaba'. The banaba tree grows from 5 to 20 meters in height, and the leaves are smooth, acuminate, oblong, and 12 to 25 centimetres long.[3]

The leaves of Banaba (*Lagerstroemia speciosa* L.) has been used as a traditional oriental medicine to treat diabetes, polyuria and polydipsia.[4] It contains polyphenol compounds and corosolic acid.[5] Tea prepared from banaba leaves possesses antihypertension, diuretic, antiulcer activity.[6] The plant *Lagerstroemia speciosa* L. contains the monomeric and dimeric ellagitannins such as Lagerstoemin, reginin A[7], flosin B, reginine C and D.[8] Most of the reported biological activities and active constituents of this plant may be related to its antioxidant nature. Based on this idea the *in vitro* antioxidant activity of the extracts of leaves of *Lagerstroemia speciosa* has been evaluated and reported hereunder.

MATERIALS AND METHODS

Plant material

The leaves of *Lagerstroemia speciosa* were collected from the local market of Ootacamund, in the month of June 2006 and authenticated by Dr. Suresh Baburaj, Director, Survey of medicinal plants and collection unit, Ootacamund, Tamilnadu, India.

Chemicals

1,1-Diphenyl-2-picryl hydrazyl (DPPH) was obtained from Sigma Aldrich Co., St. Louis, USA. Naphthyl ethylene diamine dihydrochloride (NEDD) was from Roch – Light Ltd., Suffolk, UK. Ascorbic acid was obtained from Merck Ltd., Mumbai. Sodium nitroprusside solution (SNP), Nitro blue tetrazolium (NBT), Dimethyl sulfoxide (DMSO), Sulphanilic acid, Hydrogen peroxide, Glacial acetic acid were from Ranbaxy Laboratories Ltd., Mohali and SD Fine Chem., Mumbai, India. All chemicals and solvents used were of analytical grade.

Plant extract

The collected leaves were shade dried, coarsely powdered and extracted with 50% ethanol by cold maceration process. The extract was filtered and concentrated in vacuum and kept in a vacuum desiccator for complete removal of solvent. Hydro alcoholic extract of leaves of *Lagerstroemia speciosa* was obtained in the yield of 6.8%.

Characterization of plant extract

The identification tests for various phytoconstituents (alkaloids, glycosides, steroids, triterpenoids, tannins etc) of leaves of Lagerstroemia speciosa's hydro alcoholic extract were performed. The hydro alcoholic extract showed presence of alkaloids, glycosides, steroids, tannins, saponins, gums, mucilage, triterpenoids. (Table No. 1)

Preparation of Test and Standard Solutions

Hydro alcoholic extract of leaves of *Lagerstroemia speciosa* and the standard antioxidants (ascorbic acid and rutin) were dissolved in distilled dimethyl sulphoxide (DMSO) separately and used for *in vitro* antioxidant assays using four different methods. As DMSO interfere in the case of hydrogen peroxide method the extracts and the standards were dissolved in distilled methanol and used. The stock solutions were serially diluted with the respective solvents to obtained lower dilutions.

Table 1: Phytochemical Studies on hydro alcoholic extract of *Lagerstroemia speciosa* leaves

Tests	Inference *Lagerstroemia speciosa* Extract
Alkaloid	+
Tannins	+
Carbohydrate	+
Proteins & Amino acids	–
Steroids & Sterol	+
Glycosides	+
Saponins	+
Flavanoes & Flavonoids	+
Fixed oils & Fats	–
Triterpenoids	+
Gums & Mucilages	+

(+) Present , (–) Absent

Total Phenolic Compound Estimation

Antioxidant compounds generally contains phenolic group(s) and hence, the amount of phenolic compounds in the extract of leaves was estimated by using Folin-Ciocalteus reagent[9]. In a series of test tubes, 0.4 ml of the extract in methanol was taken, mixed with 2 ml of Folin-Ciocalteu reagent and 1.6 ml of sodium carbonate. After shaking, it was kept for 2 h reaction time. The absorbance was measured at 750 nm using a Shimadzu UV-160 Spectrophotometer. Using gallic acid monohydrate, standard curve was prepared and linearity was obtained in the range of 2.5 to 25 µg/ml. Using the standard curve the total phenolic compounds content was calculated and expressed as gallic acid equivalent in mg/g or % w/w of the extracts.

Free radical scavenging activity

The extract was tested for its in vitro antioxidant activity using standard methods. In all these methods, a particular concentration of the extract or standard solution was used which gave final concentration of 1000 µg/ml to 0.45 µg/ml after all the reagents were added. Absorbance was measured against a blank solution containing the extract or standards. A control test was performed without the extracts or standard. Percentage scavenging and IC_{50} values ± S.E.M (IC_{50} value is the concentration of the sample required to inhibit 50% of radical) were calculated.

DPPH radical scavenging activity

The assay was carried out in a 96 well micro titre plate. To 200 µl of DPPH solution, 10 ml of each of the test sample or the standard solution was added separately in wells of the micro titre plate. The final concentration of the test and standard solutions used are 1000 to 1.95 µg/ml. The plates were incubated at 37°C for 20 minutes and the absorbance of each solution was measured at 490 nm, using ELISA reader (Bio Rad Laboratories Inc, California, USA, Model 550).[10]

Scavenging of hydrogen peroxide radicals

A solution of hydrogen peroxide (20 mM) was prepared in phosphate buffer saline (PBS at pH 7.4). Various

concentrations of the extracts and standards in methanol (1ml) were added to 2 ml of hydrogen peroxide solutions in PBS. After 10 min, the absorbance was measured at 230 nm against a blank solution that contained extracts in PBS without hydrogen peroxide.[11]

Nitric oxide radical inhibition activity

The reaction mixture (6 ml) containing sodium nitroprusside (10 mM, 4 ml), phosphate buffer saline (PBS, 1 ml) and 1 ml of extract in DMSO were incubated at 25°C for 150 minutes. After incubation, 0.5 ml of the reaction mixture containing nitrate was removed and 1 ml of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) was added, mixed well and allowed to stand for 5 minutes for completion of diazotization reaction, then 1 ml of naphthyl ethylene diamine dihydrochloride (NEDD) was added, mixed and allowed to stand for 30 minutes in diffused light at room temperature. The absorbance of these solutions was measured at 540 nm using ELISA reader against corresponding blank solution in a 96-well micro titre plate (Tarsons Product(P) Ltd., Kolkata, India) using ELISA reader (Bio Rad Laboratories Inc, California, USA, Model 550).[12]

Scavenging of Super oxide radical by alkaline DMSO method

To the reaction mixture containing 0.1 ml of NBT (1mg/ml solution in DMSO) and 0.3 ml of the extracts and standard in DMSO,1ml of alkaline DMSO (1ml DMSO containing, 5 mM NaOH in 0.1 ml water) was added to give a final volume of 1.4 ml. The absorbance was measured at 560 nm.[13]

RESULTS

The hydro alcoholic extract of leaves of *Lagerstroemia speciosa* showed presence of alkaloids, glycosides, carbohydrates, flavonoids, steroids, tannins, saponins, gums, mucilage, triterpenoids. (Table No. 1)

The total phenolic contents of hydro alcoholic extract of leaves of *Lagerstroemia speciosa* was expressed as gallic acid equivalent in mg/g of the extracts. The total phenolic contents of the extract is 20.18%.

Lagerstroemia speciosa hydro alcoholic extract was tested for its antioxidant activity in four different *in vitro* models. The antioxidant activity measured are given in Table No. 2. *Lagerstroemia* showed IC_{50} value 4.75±0.25 µg/ml for DPPH method, which was comparable to that of ascorbic acid (IC_{50}=2.25±0.29). For Hydrogen peroxide method, IC_{50} value was found to be 28.00±0.16 µg/ml, which compares favourable with Ascorbic acid (IC_{50}=187.33±3.45µg/ml). In Nitric oxide model, IC_{50} value was for to be 750.25±3.66 µg/ml, which is very low when compared to Rutin (IC_{50}=68.52±2.27mg/ml). The extract failed to exhibit antioxidant activity in the scavenging of superoxide radical by alkaline DMSO method. The IC_{50} values obtained, however, for the extract in all the methods were found to be higher than the standard used, indicating its low activity compared to the standards.

DISCUSSION

Free radicals have aroused significant interest among scientists in the past decade. Their broad range of effects in biological systems has drawn the attention of many experimental works. It has been proved that these mechanisms may be important in the pathogenesis of certain diseases and ageing. There are many reports that support the use of antioxidant supplementation in reducing the level of oxidative stress and in slowing or preventing the development of complications associated with diseases.[14] Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants. Numerous plant constituents have proven to show free radical scavenging or antioxidants activity.[15]

Table 2: Antioxidant activity of leaves of *Lagerstroemia speciosa* (L) hydro alcoholic extract

Extract/Standard	IC_{50}+ values ± SEM* (µg/ml)			
	DPPH	H_2O_2	Nitric oxide	TBARS
Hydro alcoholic Extract	4.75±0.25	28.00±0.16	750.25±3.66	>1000
Ascorbic acid	2.75±0.29	187.33±3.45	-	>1000
Rutin	7.89±0.51	35.26±0.166	68.52±2.27	–

** Average of three independent determinations, three replicates, values are mean ± SEM.*

+ IC_{50} = Concentration of the sample/standard required to inhibit 50% of free radicals.

SEM = Standard error mean

H_2O_2 = Hydrogen peroxide

DPPH = 1, 1-diphenyl, 2-picryl hydrazyl

TBARS = Thiobarbituric acid reactive species

Flavonoids and other phenolic compounds of plant origin have been reported as scavengers.[16]

The preliminary phytochemical investigation of the extract revealed the presence of steroids, terpenoids, alkaloids, carbohydrate and phenolic compounds such as tannins, flavonoid etc., The phenolic compounds may contribute directly to anti oxidative action.[17] This result indicates that polyphenol present in leaves and its extract could be partly responsible for the beneficial effects.

In our present study demonstrated that, DPPH is a free radical, stable at room temperature, which produces a purple colour solution in methanol. It is reduced in the presence of an antioxidant molecule, giving rise to uncoloured methanol solutions. Hydrogen peroxide radicals Scavenging activity showed that the extract is a potent scavenger of hydrogen peroxide. This hydrogen peroxide is mainly produced by enzymatic reactions. The ability of plant extracts to scavenge $H_2O_2$is followed by decay in H_2O_2concentration. Nitric oxide radical inhibition study proved that the extract is a moderate scavenger of nitric oxide. This nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite. The extract inhibits nitrite formation by competing with oxygen to react with nitric oxide directly and also to inhibit its synthesis. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide[12]. From the nitric oxide test, rutin was used as a standard. In the PMS/NADH –NBT system, superoxide anion derived from dissolved oxygen by PMS/NADH coupling reaction reduces NBT. The decrease of absorbance at 560 nm with antioxidants thus indicates the consumption of superoxide anion in the reaction mixture.

This study suggested that the *Lagerstroemia speciosa* L plant extract possess antioxidant activity, which might be helpful in preventing or slowing the progress of various oxidative stress related diseases. Further investigation on the isolation and identification of antioxidant component(s) in the plant may lead to chemical entities with potential for clinical use.

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