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In Vitro Antioxidant Activity, Total Phenolic and Flavonoid Contents of Hydroalcoholic Extract of Leaves of *Lagerstroemia Parviflora* Roxb

Geeta Parkhe[1]*, Deepak Bharti[1]

[1]Department of Biotechnology, SRK University, Bhopal, MP, India

ABSTRACT

Plants have served human beings as a natural source for treatments and therapies from ancient times, amongst them medicinal herbs have gain attention because of its wide use and less side effects. In the recent years plant research has increased throughout the world and a huge amount of evidences have been collected to show immense potential of medicinal plants used in various traditional systems. *Lagerstroemia parviflora* Roxb (*L. parviflora*, Lythraceae) is a medium-sized deciduous plant indigenous to India and available even up to a height of 900m in the Himalayas. The plant is used for the treatment of syphilis, sores, and carbuncles. The aim of the present study was to determine qualitative and quantitative phytochemical and *in vitro* antioxidant activity of leaf of *L. Parviflora*. Qualitative analysis of various phytochemical constituents and quantitative analysis of total phenol and flavonoids were determined by the well-known test protocol available in the literature. Quantitative analysis of phenolic and flavonoids was carried out by Folins Ciocalteau reagent method and aluminium chloride method respectively. The *in vitro* antioxidant activity of hydroalcoholic extract of the leaf was assessed against DPPH assay method using standard protocols. Phytochemical analysis revealed the presence of phenols, flavonoids, saponins glycosides etc. The total phenolic and flavonoids content of *L. Parviflora* leaves of hydroalcoholic extract was 3.541 and 0.927mg/100mg respectively. The activities of hydroalcoholic leaves extract against DPPH assay method were concentration dependent with IC 50 values of ascorbic acid and extracts 17.681and 62.669µg/ml respectively. These studies provided information for correct identification of this plant material. The diverse array of phytochemicals present in the plant thus suggests its therapeutic potentials which may be explored in drug manufacturing industry as well as in traditional medicine.

Keywords: *Lagerstroemia parviflora* Roxb, Qualitative, Quantitative phytochemical, Antioxidant.

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***Address for Correspondence:**

Geeta Parkhe, Department of Biotechnology, SRK University, Bhopal, MP, India

INTRODUCTION

India is a rich source of medicinal plants and a number of plant derived oils and extracts are used against diseases in various systems of medicine such as Ayurveda, Unani and Siddha. Only a few of them have been scientifically explored. Plant derived natural products such as phenols, flavonoids, terpenes and alkaloids[1, 2] have received considerable attention in recent years due to their diverse pharmacological properties. The qualitative analysis of phytochemicals of a medicinal plant is reported as vital step in any kind of medicinal plant research. Screening of plants constituents accurately can be done by employing chromatographic techniques[3]. Quantification usually employs the use of gravimetric and spectroscopic methods with several advanced approaches now available[4]. Reactive Oxygen Species (ROS), such as hydrogen peroxide, super oxide anion and hydroxyl radical, capable of causing damage to DNA, have been associated with carcinogenesis, coronary heart disease and many other health problems related to advancing age[5]. Antioxidant that scavenges these free radicals proves to be beneficial for these disorders as they prevent damage against cell proteins, lipids and carbohydrates[6]. Erythrocytes, which are the most abundant cells in the human body, possessing desirable physiological and morphological characteristics are exploited extensively in drug delivery[7]. Oxidative damage to the erythrocyte membrane (lipid/ protein) may be implicated in haemolysis associated with some haemoglobinopathies, oxidative drugs, transition metal excess, radiation, and deficiencies in some erythrocyte antioxidant systems[8].This assay is useful either for screening studies on various molecules and their metabolites, especially on one hand, molecule having an oxidizing or antioxidating activity or on the other hand, molecule having a long term action[9]. Several herbal secondary metabolites such as flavonoid have been found to protect cells from oxidative damage[10]. These compounds have been evidenced to stabilize RBC membrane by scavenging free radicals and reducing lipid peroxidation[11,12]. *L. parviflora* (Lythraceae) commonly known as Landia in India and Seja in Bundelkhand; is a species widely distributed in almost all moist and dry deciduous tracts of India. In view of its wide distribution, the tree can withstand great variation in climate. It is often found as a companion to natural sal and teak it occurs as a distinct species in forests of

sub- Himalayan tracts, Assam, Madhya Pradesh, orissa, Maharashtra, Gujarat, Andhra Pradesh, Karnataka and Tamil Nadu (except Nilgiris and arid regions). In Madhya pradesh, it is common in all districts[13]. The tree is primarily use for timber the bark of *L. parviflora* contain tannin (7 % - 10 %) and is used locally for tanning and dyeing lather and for dyeing cotton thread. It also has some medicinal importance[14].Mazumder et al. (2003) reported the antibacterial activities of the leaves of the plant[15] and Bhakuni et al. (1969) reported the antiasthmatic activity of the flowers of *L. parviflora*[16]. Mazumder et al. (2005) reported antipyretic potential of *L. parviflora* leaves in our laboratory[17]. The leaf juice of this plant is used in traditional medicine to treat fever in Jharkhand, India (Jain & Tarafdar, 1970) [18]. The aim of this work was to determine the quality (types), quantity (amount) of bioactive compounds and in vitro antioxidant activity of leaf of *L. parviflora*.

MATERIAL AND METHOD

Plant material

The leaves of *L. parviflora* were collected from Bhimbetka Bhojpur, Bhopal (M.P.) in the month of Feb, 2018. Plant material (leaves) selected for the study were washed thoroughly under running tap water and then were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then the plant material was shade dried without any contamination for about 3 to 4 weeks. Dried plant material was grinded using electronic grinder. Dried plant material was packed in air tight container and stored for phytochemical and biological studies.

Chemical reagents

All the chemicals used in this study were obtained from Hi Media Laboratories Pvt. Ltd. (Mumbai, India), Sigma-Aldrich Chemical Co. (Milwaukee, WI, USA), SD Fine-Chem. Ltd. (Mumbai, India) and SRL Pvt. Ltd. (Mumbai, India).All the chemicals and solvent used in this study were of analytical grade.

Defatting of plant material

Powdered leaves of *L. parviflora* were shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether using maceration method. The extraction was continued till the defatting of the material had taken place.

Extraction by maceration process

75gm of dried plant material were exhaustively extracted with Hydroalcoholic solvent (ethanol: water: 80:20) using maceration method. The extract was evaporated above their boiling points. Finally the percentage yields were calculated of the dried extracts. The extracts were evaporated above their boiling points and stored in an air tight container free from any contamination until it was used. Finally the percentage yields were calculated of the dried extracts.

Phytochemical screening of the extract

The extract of *L. parviflora* was subjected to qualitative analysis for the various phytoconstituents like alkaloids, carbohydrates, glycosides, phytosterols, saponins, proteins, amino acids and flavonoids[19, 20].

Total phenol determination

The total phenolic content was determined using the method of Olufunmiso *et al*[21]. A volume of 2ml of each extracts or standard was mixed with 1 ml of Folin Ciocalteau reagent (previously diluted with distilled water 1:10 v/v) and 1 ml (7.5g/l) of sodium carbonate. The mixture was allowed to stand for 15 min under room temperature. The colour developed was read at 765 nm using UV/visible spectrophotometer. The total phenolic content was calculated from the standard graph of gallic acid and the results were expressed as gallic acid equivalent (mg/100mg).

Total flavonoids determination

The total flavonoid content was determined using the method of Olufunmiso *et al* [21]. 1ml of 2% $AlCl_3$ solution was added to 3 ml of extract or standard and allowed to stand for 15 min at room temperature; the absorbance of the reaction mixture was measured at 420 nm using UV/visible spectrophotometer. The content of flavonoids was calculated using standard graph of quercetin and the results were expressed as quercetin equivalent (mg/100mg).

DPPH free radical scavenging assay

DPPH scavenging activity was measured by modified method[21]. DPPH scavenging activity was measured by the spectrophotometer. Stock solution (6 mg in 100ml methanol) was prepared such that 1.5 ml of it in 1.5 ml of methanol gave an initial absorbance. Decrease in the absorbance in presence of sample extract at different concentration (10-100 µg/ml) was noted after 15 minutes. 1.5 ml of DPPH solution was taken and volume made till 3 ml with methanol, absorbance was taken immediately at 517 nm for control reading. 1.5 ml of DPPH and 1.5 ml of the test sample of different concentration were put in a series of volumetric flasks and final volume was adjusted to 3 ml with methanol. Three test samples were taken and each processed similarly. Finally the mean was taken. Absorbance at zero time was taken for each concentration. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm. The percentage inhibition of free radical DPPH was calculated from the following equation: % inhibition = [(absorbance of control - absorbance of sample)/absorbance of control] × 100%. Though the activity is expressed as 50% inhibitory concentration (IC50), IC50 was calculated based on the percentage of DPPH radicals scavenged. The lower the IC50 value, the higher is the antioxidant activity.

RESULTS AND DISCUSSIONS

The percentage yields of Pet ether and hydroalcoholic extract obtained from *L. parviflora* are depicted in the Table 1. Preliminary phytochemical studies of the extract were done according to the published standard methods. These tests were broad in scope and used to determine the presence of glycosides, flavonoids, phenol, proteins and amino acids, carbohydrates, saponins and diterpins but alkaloids was absents in the extract Table 2. The content of total phenolic compounds (TPC) content was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: **$Y = 0.042X+0.002$, $R^2= 0.999$,** where X is the gallic acid equivalent (GAE) and Y is the absorbance. The content of total flavonoid compounds (TFC) content was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: **$Y = 0.06X+0.019$, $R^2= 0.999$,** where X is the quercetin equivalent (QE) and Y is the absorbance. TPC of hydroalcoholic extract of *L. parviflora* showed the content values of 3.541and fallowed by TFC were 0.927 Table 3. Antioxidant activity of the samples was calculated through DPPH assay. % inhibition was calculated as an indicative of antioxidant potency. The higher the % inhibition the better the activity. Ascorbic acid was taken as standard and the values were comparable with concentration ranging from 10

µg/ml to 100µg/ml. A dose dependent activity with respect to concentration was observed Table 4 & Figure 1.

Table 1 % Yield of leaves of *L. parviflora*

S. No.	Solvents	% Yield
1	Pet ether	3.214
2.	Hydroalcoholic	7.692

Table 2 Phytochemical screening of extract of *L. parviflora*

S. No.	Constituents	Hydroalcoholic extract
1.	**Alkaloids**	
	Mayer's Test	-ve
	Wagner's Test	-ve
	Hager's test	-ve
2.	**Glycosides**	
	Legal's test	+ve
3.	**Flavonoids**	
	Lead acetate	+ve
	Alkaline test	+ve
4.	**Phenol**	
	Ferric Chloride Test	+ve
5.	**Proteins and Amino acids**	
	Xanthoproteic test	+ve
	Ninhydrin Test	+ve
6.	**Carbohydrates**	
	Fehling's test	+ve
7.	**Saponins**	
	Froth Test	+ve
	Foam test	+ve
8.	**Diterpins**	
	Copper acetate test	+ve

Table 3 Total Phenol and total flavonoid content of *L. parviflora* extract

S. No.	Extract	Total Phenol (mg/100mg)	Total flavonoid (mg/100mg)
1.	Hydroalcoholic *extract*	3.541	0.927

Table 4 % Inhibition of ascorbic acid and hydroalcoholic extract using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	10	44.65	31.98
2	20	48.62	38.63
3	40	65.34	46.32
4	60	69.65	51.11
5	80	77.41	55.36
6	100	84.13	58.21
IC 50		17.681	62.669

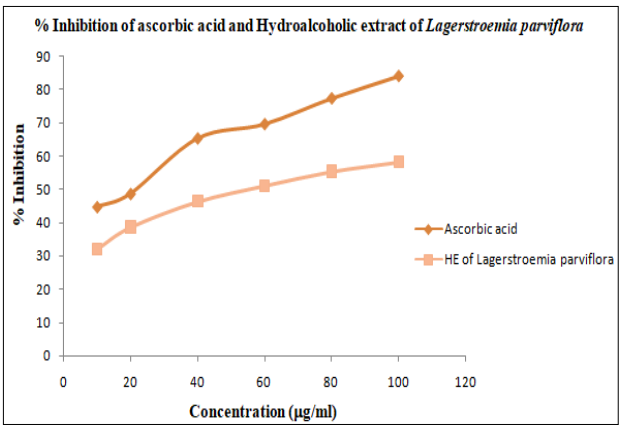

Figure 1 Graph of % Inhibition of ascorbic acid and hydroalcoholic extract using DPPH method

CONCLUSION

It can be concluded that from present investigation the phytochemical investigation gave valuable information about the different phytoconstituents present in the plant, which helps the future investigators concerning the selection of the particular extract for further investigation of isolating the active principle and also gave idea about different phytochemical have been found to possess a wide range of activities. The total phenolic and flavonoid content in hydroalcoholic leaves extract is further proved by in vitro antioxidant studies. Potential antioxidant activity has good correlations with the therapeutic use in the treatment of cardiovascular disorders. Further research to isolate individual compounds, their *in-vivo* antioxidant activities with different mechanism is needed.

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