ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Research Article

PHYTOCHEMICAL ANALYSIS AND *IN VITRO* ANTIOXIDANT STUDIES OF *LAGERSTROEMIA PARVIFLORA* ROXB BARK

Adesh Anantrao Bawane*, Om Prakash Tiwari, Alok Pal Jain

Sarvepalli Radhakrishnan University, Hoshangabad Rd, Near Nissan Motors, Misrod, Bhopal, Madhya Pradesh, India

**Corresponding author: adeshab@rediffmail.com*

ABSTRACT

The objectives of present studies are to screen the phytochemicals, estimate the content of phenolic and flavonoid compounds and determine the antioxidant capacity of the *Lagerstroemia parviflora* Roxb (*L. parviflora*) barks. 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. The hydro alcoholic extract of bark of *L. parviflora* 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. Phytochemical analysis revealed the presence of phenols and flavonoids. The total phenolic and flavonoids content of *L. parviflora* bark of hydroalcoholic extract was 0.247and 0.687mg/100mg respectively. Ascorbic acid used as standards was also evaluated for comparison. The extract showed dose dependent free radical scavenging property in the tested models. *L. parviflora bark* extract showed IC_{50} value 60.22µg/ml for DPPH method, which was comparable to that of ascorbic acid (IC_{50}=17.68µg/ml). For hydrogen peroxide method, IC_{50} value was found to be 88.60µg/ml, which compares favourable with ascorbic acid (IC_{50}=36.613µg/ml). In nitric oxide model, IC_{50} value was found to be 91.73µg/ml, which was comparable to that of ascorbic acid (IC_{50}=24.63µg/ml). The present study describes the phytochemical profile and antioxidant activity of *L. parviflora* which will further used for medicinal applications.

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

1. INTRODUCTION

Free radicals or highly reactive oxygen species are formed by exogenous chemicals or endogenous metabolic processes in the human body. These are capable of oxidizing bio-molecules viz nucleic acids, proteins, lipids and DNA and can initiate different degenerative diseases like neurological disorders, cancer, emphysema, cirrhosis, atherosclerosis, arthritis etc [1, 2]. Antioxidants are the compounds which terminate the attack of free radicals and thus reduce the risk of these disorders [3]**.** Almost all organisms are protected up to some extent by free radical damage with the help of enzymes such as super-oxide dismutase, catalase and antioxidant compounds viz. ascorbic acid, tocopherol, phenolic acids, polyphenols, flavonoids and glutathione. Prior and Cao [4], reported that antioxidant supplements or dietary antioxidants protect against the damaging effects of free radicals. Presently, much attention has been focused on the use of natural antioxidants to protect the human body especially brain tissues from the oxidative damage caused by free radicals. In last two decades, several medicinal plants have shown such effectiveness through the traditional methods of psychoneuropharmacology [5]. Keeping this in view, the present study has been conducted to evaluate the comparative antioxidant activity of *L. parviflora* which are traditionally well known for their various activities. *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 [6]. 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 [7]. Mazumder et al. (2003) reported the antibacterial activities of the leaves of the plant [8] and Bhakuni et al. (1969) reported the antiasthmatic activity of the flowers of *L.* parviflora [9]. Mazumder et al. (2005) reported antipyretic potential of *L. parviflora* leaves in our laboratory [10]. The leaf juice of this plant is used in traditional medicine to treat fever in Jharkhand, India [11].

2. MATERIAL AND METHODS

2.1. Plant material

The barks of *L. parviflora* were collected from Bhimbetka Bhojpur, Bhopal (M.P.) in the month of Feb, 2018 and authenticated by Dr. Jasvinder Mehta, Department of Botany, Career College, Bhopal (M.P.). Plant material (barks) 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. The bark was cut in suitable size for further evenly drying without any contamination for about 3 to 4 weeks at room temperature. Dried plant material was grinded into coarse size powder using electronic grinder. Powdered plant material was observed for their colour, odour, taste and texture. Dried plant material was packed in air tight container and stored for phytochemical and biological studies.

2.2. Chemical and 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.

2.3. Defatting of plant material

The shade dried bark (110g of air-dried powdered materials) of *Lagerstroemia parviflora* was coarsely powdered and subjected for defatting by hot continuous extraction with 500 ml petroleum ether (60°-80°C) till the defatting of the material had taken place.

2.4. Extraction of defatted bark by soxhlet extraction process

The defatted dried bark were exhaustively extracted with hydroalcoholic solvent (ethanol: water: 70:30) using hot extraction method by soxhlet apparatus. The extract was concentrated in rotary flash evaporator under reduced pressure. The residue was dried in a desiccator over sodium sulphite. Finally the percentage yields were calculated of the dried extracts.

2.5. 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, tannins, proteins, amino acids and flavonoids [12-14].

2.5.1. Total phenol determination

The total phenolic content was determined using the standard method with slight modifications. A volume of 2ml of extracts and each standard (10-50µg/ml) 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) [15].

2.5.2. Total flavonoids determination

The total flavonoid content was determined using the standard method with slight modifications. 1ml of 2% $AlCl_3$ solution was added to 3 ml of extract and each standard (5-25µg/ml) 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) [15].

2.6. Antioxidant activity

2.6.1. DPPH free radical scavenging assay

DPPH scavenging activity was measured by the spectrophotometer with slightly modification of method [15]. Stock solution of DPPH (6 mg in 100ml methanol) was prepared; add 1.5 ml of this DPPH solution in 1.5 ml of methanol gave an initial absorbance (Absorbance of control). Different concentrations (10-100 µg/ml) of standard (Ascorbic acid) and extract (Hydroalcoholic bark extract of *Lagerstroemia parviflora*) prepared and from these solutions 1.5ml of each standard and extracts taken, add 1.5ml of DPPH solution and INCUBATE IN dark at room temperature for 15 min. Decrease in the absorbance was noted 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%.

2.6.2. Free radical scavenging activity (FRSA) using hydrogen peroxide

The hydrogen peroxide FRSA of the methanolic extracts was done as suggested by Czochra and Widensk [16]. 2ml of hydrogen peroxide (43 mM) and 1.0 ml of methanolic sample [20-100 μl of hydroalcoholic extract (4 mg/ml) of plant in methanol] followed by 2.4 ml of 0.1 M phosphate buffer (pH 7.4) were added. The resulting solution was kept for 10 min and the absorbance was recorded at 230 nm. All readings were repeated three times. Blank was prepared without adding hydrogen peroxide and control was prepared without sample. Ascorbic acid was used as a standard compound. Free radical scavenging activity of hydrogen peroxide (%) was calculated as:

$$FRSA\ (\%) = [(V_0\text{-}V_1)/V_0] \times 100$$

Where, V_0 = absorbance of control and V_1 = absorbance of sample.

2.6.3. Nitric oxide scavenging activity

Nitric oxide was produced from sodium nitroprusside and the Griess reagent was measured. Sodium nitroprusside spontaneously produces nitric oxide in aqueous solution at physiological pH, interacting with oxygen to generate nitric ions that can be estimated using Griess reagent. Nitric oxide scavengers compete with oxygen resulting in decreased nitric oxide manufacturing [17]. 0.5 ml of Sodium nitroprusside (10mM) was mixed with 1 ml of various extract concentrations (20 to 100µg/ml) in phosphate buffer saline (PBS) and incubated at 25°C for 150 min. 1 ml of Griess reagent (1% sulphanilamide, 2% H_3PO_4 and 0.1% napthylethylenediamine dihydrochloride) was added to the specimens. The chromophore absorbance created during the diazotization of sulphanilamide nitrite and subsequent coupling with napthylethyleneediamine was read at 546 nm and referred to the absorption of conventional ascorbic acid solutions treated in the same manner with Griess reagent as a standard samples. Control samples without the extracts but with an equal volume of buffer were prepared in a similar manner as was done for the test samples. All triplicate experiments were conducted and the chart was plotted with the mean values. The inhibition proportion was evaluated using the following formula:

$$\text{Radical scavenging activity (\%)} = (A_{control} - A_{test})/A_{control} \times 100$$

Where A control is the absorption (without extract) of the control and where A test is the absorption in the presence of the extract / standard.

3. RESULTS AND DISCUSSION

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. Phytochemical analysis revealed the presence of phenols and flavonoids in the Table 2. The total phenolic content (TPC) was expressed as mg/100mg of gallic acid equivalent of dry extract sample using the equation obtained from the calibration curve: Y = 0.011X+0.011, r^2= 0.998, where X is the gallic acid equivalent ((GAE) and Y is the sample absorbance. Total flavonoids content was calculated as quercetin equivalent (mg/100mg) using the equation based on the calibration curve: Y=0.040X + 0.009, r^2=0.999, where X is the quercetin equivalent (QE) and Y is the sample absorbance. TPC of hydroalcoholic extract of *L. parviflora* showed the content values of 0.247and fallowed by TFC were 0.687 (Table 3). DPPH radical scavenging assay measured hydrogen donating nature of extracts [18]. Under DPPH radical scavenging activity the inhibitory concentration 50% (IC_{50}) value of *L. parviflora* hydroalcoholic bark extract was found to be 60.22µg/ml as compared to that of ascorbic acid (17.68µg/ml). A dose dependent activity with respect to concentration was observed (Table 4). Extracts showed $NO^{\bullet}$ scavenging effects by competing with oxygen to react with NO directly hence inhibited the nitrite ion formation [19]. *L. parviflora* hydroalcoholic bark extract showed nitric oxide (NO^{o}) radical scavenging activity with IC_{50} value of 91.73µg/ml as compared to that of ascorbic acid (IC_{50} 24.63µg/ml) (Table 5) Hydrogen peroxide is generated in vivo by several oxidase enzymes and by activated phagocytes and it is known to play an important role in the killing of several bacterial and fungal strains [20]. There is increasing evidence that, hydrogen peroxide, either directly or indirectly via its reduction product, $OH^{\bullet}$, can act as a messenger molecule in the synthesis and activation of several inflammatory mediators [21]. When a scavenger is incubated with H_2O_2 using a peroxidase assay system, the loss of H_2O_2 can be measured. Table 6 show the scavenging ability of *L. parviflora* hydroalcoholic bark extract and ascorbic acid on hydrogen peroxide at different concentrations.

Extracts were capable of scavenging hydrogen peroxide in an amount dependent manner at all the tested concentrations. Hydrogen peroxide itself is a rather weak oxidant and most organic compounds (except for some sulfur containing molecules) are virtually inert to attack by it at ordinary environmental or cellular concentrations and temperatures. In the presence of reduced transition metal ions, however, hydrogen peroxide is converted to the much more reactive oxidant, hydroxyl radical in the cells by Fenton reaction. Besides this, studies have shown that other transition metals such as copper (I), cobalt (II) and nickel(II) also take part in the process [20]. Thus, the removing is very important for antioxidant defense in cell or food systems.

Table 1: % Yield of barks of *L. parviflora*

S. No.	Solvents	% w/w Yield
1	Pet ether	1.26
2.	Hydroalcoholic	5.69

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

S. No.	Constituents	Hydroalcoholic extract of barks of *L. parviflora*
1.	Alkaloids	
	A) Wagner's Test:	-Ve
	B) Hager's Test:	-Ve
2.	Glycosides	
	A) Legal's Test:	-Ve
3.	Flavonoids	
	A) Lead acetate Test:	+Ve
	B) Alkaline Reagent Test:	-Ve
4.	Saponins	
	A) Froth Test:	-Ve
5.	Phenolics	
	A) Ferric Chloride Test:	+Ve
6.	Proteins and Amino Acids	
	A) Xanthoproteic Test:	-Ve
7.	Carbohydrate	
	A) Fehling's Test:	-Ve
8.	Diterpenes	
	A) Copper acetate Test:	-Ve
9.	Steroid	
	A) Salkowski Tests	-Ve

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

S. No.	Extracts	Total phenolic content (mg /100mg of dried extract)	Total flavonoids content (mg/ 100 mg of dried extract)
1.	Hydroalcoholic	0.247	0.687

Table 4: % Inhibition of ascorbic acid and *L. parviflora* hydroalcoholic extract using DPPH method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Hydroalcoholic extract
1	10	44.65	37.12
2	20	48.62	41.26
3	40	65.34	46.17
4	60	69.65	51.39
5	80	77.41	55.63
6	100	84.13	57.11
	IC 50	17.68	60.22

Table 5: % Inhibition of ascorbic acid and *L. parviflora* hydroalcoholic extract using NO method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ascorbic acid
1	20	47.70	31.11
2	40	52.92	35.98
3	60	67.43	43.21
4	80	68.89	47.36
5	100	74.42	51.36
	IC 50	24.63	91.73

Table 6: Inhibition of ascorbic acid and *L. parviflora* hydroalcoholic extract using H_2O_2 method

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Ascorbic acid
1	20	42.872	32.65
2	40	52.192	38.14
3	60	60.416	43.36
4	80	65.789	49.87
5	100	68.750	51.22
	IC50	34.079	88.60

4. CONCLUSION

The results obtained in the present study clearly demonstrate that the extract, which can effectively scavenge various reactive oxygen species/free radicals under in vitro conditions. This may be due to the number of stable oxidized products that it can form after oxidation or radical scavenging. The broad range of activity of the extracts suggests that multiple mechanisms are responsible for the antioxidant activity. The multiple antioxidant activity of extract demonstrated in this study clearly indicates the potential application value of the both plants. However, the in vivo safety of both plants needs to be thoroughly investigated in experimental rodent models prior to its possible application as an antioxidant ingredient, either in animal feeds or in human health foods. The above results showed that *L. parviflora* hydroalcoholic bark extract could exhibit antioxidant properties. Further studies, on the use of above plants for their antioxidant role in various systems may provide potential natural antioxidants.

5. REFERENCES

1. Halliwell B, Gutteridge JM. *Biochem. J.,* 1984; **219**:1-4.
2. Maxwell SR. *Drugs,* 1995; **49:**45-361.
3. Rice-Evans CA, Miller NJ, Paganga G. *Free Radic. Biol. Med.,* 1996; **20**:933-956.
4. Prior RL, Cao G. Variability in dietary antioxidant related natural product supplements: The need for methods of standardization. *J. Am. Nutraceutical Assoc.,* 1999; **2**:46-56.
5. Dhawan BN. Centrally acting agents from Indian plants. In: Koslow SH, Murthy RS,Coelho GV, editors. Decade of the Brain: India/USA Research in Mental Health and Neurosciences. Rockville: National Institute of Mental Health; 1995, 197-202.
6. Singh S. Safe guard against introduction of against introduction of dangerous pathogens on seed of toxic trees. Proc. Nath. Seminar on tree seeds. Hyderabad, 1986, 147-153.
7. Anonymous. The wealth of India, raw material, Vol. 3. D-E, New Delhi. : Council of Scientific and Industrial Research 1958.
8. Mazumder A, Saha BP, Basu SP, Mazumder R. *Indian J. Nat. Prod.,* 2003; **19**:20-24.
9. Bhakuni DS, Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN. *Ind. J. Exp. Biol.,* 1969; **7**:250-256.
10. Mazumder A, Saha BP, Basu SP, Mazumder R. *Pharm. Biol.,* 2005; **43(1):**64-66,
11. Jain SK, Tarafdar CR. Medicinal plant- Lore of the santals. *Econ. Bot.,* 1970; **24**:241-243.
12. Vikram PK, Malvi R, Jain DK. *Int. J. Curr. Pharm. Res.,* 2012; **4(4)**:47-50.
13. Nayak A, Garg M, Jain S, Khan MA, Jain DK, Khan N. *Sch. Acad. J. Pharm.,* 2019; **8(3)**:94-104.
14. Joshi S, Parkhe G, Aqueel N, Dixit N, Jain DK. *Pharmacologyonline* 2019; **1**:27-33.
15. Jain DK, Nayak A, Patel P, Jain S, Khan MA. *Sch. Acad. J. Pharm.,* 2019; **8 (3)**:86-93
16. Czochra MP, Widensk AJ. *Anal. Chem. Acta.,* 2002; **452:**177-184.
17. Marcocci L, Maguire JJ, Droy MT. *Biochem Biophys Res Commun.,* 1994; **15:**748-755.
18. Hudson BJ. Food antioxidants. In: Gordon MH, editor. The Mechanism of Antioxidant Action *in Vitro*. London: Elsevier Applied Science; 1990.
19. Sunil C, Ignacimuthu S. *Food Chem. Toxicol.,* 2011;**49**:1604-1609.
20. Halliwell B, Aeschbach R, Lolliger J, Aruoma OI. *Food Chem. Toxicol.,* 1995; **33:**601.
21. Sprong RCA, Winkelhuyzen-Jansen C, Aarsman J, et al. *J. Am. J. Crit. Care Med.* 1998; **157:**1283-1293.