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PHYTOCHEMICAL SCREENING, ANTIOXIDANT ACTIVITY AND ANTIMICROBIAL ACTIVITY OF LAGERSTROEMIA PARVIFLORA

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ABSTRACT

The present study was designed to evaluate the Phytochemical composition, antioxidant activity and antimicrobial activity of *Lagestroemia parviflora* collected from Melghat Forest near Madki Village, Tal- Chikhaldara, Dist- Amravati (M.S) Central region of India. Phytochemical screening was carried out by 'guide to modern techniques of plant analysis". Furthermore antioxidant activity of methanolic extract of *L. parviflora* was investigated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging assay. Antimicrobial activity of *L. parviflora* was investigated by Agar disc diffusion assay. The Phytochemical analysis of *L*. parviflora leaf and seed extracts showed the presence of carbohydrates, alkaloids, flavonoids, cardiac glycosides, phytosterols, steroids, tannins and phenolic compound , and cumarines. The IC_{50} values for the L.parviflora methanolic leaf extract (IC_{50}= 3482 µg / ml) which was said to be far greater than the standard ascorbic acid (IC_{50}= 2.816 µg / ml). The ethyl acetate extract of L. parviflora leaf showed good inhibition against all six Organisms. The highest inhibition was noted in order of S. aureus (19 mm), E. coli (11 mm), P. aeru (11 mm), P. acne (11 mm). The present study describes the phytochemical profile, antioxidant activity and antimicrobial activity of *L. parviflora* which will further used for medicinal applications.

KEYWORDS: Phytochemical, Antioxidant, Antimicrobial, FTIR.

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INTRODUCTION

Lagerstroemia commonly known as crape myrtle or crepe myrtle. The leaves of *L. parviflora* are fed on by the Antheraea_paphia moth which produces the tassar silk (tussah), a form of wild silk of commercial importance in India[1]. L. parviflora roxb is medium sizes deciduous plant cultivated throughout India available even up to height of 900 m in the Maharashtra. It has been found that the local tribes of Chikhaldara region of Maharashtra use the leaves of this plant as an antitussive and astringent.[2] The herbal or traditional medicine involves the use of different types of organic extracts or the bioactive chemical constituents. This type of biochemical investigation provides health care at an affordable cost. Lagerstroemia parviflora is an important medicinal plant with several ethno medicinal properties. The plant is also considered to be used by the tribals of India for treatment of sores, fever strangulation of intestine, syphilis and carduncles.[3]

MATERIALS AND METHODS

Collection of sample

Fresh leaf and seeds of Lagerstroemia parviflora were collected from Melghat Forest Near Madki Village, Tal- Chikhaldara , Dist- Amravati (Central region of India) in the month of November – 2012, were authenticated by a taxonomist from Department of Botany ACS College Amravati. Fresh Fruits of *Lagerstroemia parviflora* were washed well, using tap water and twice using distilled water and it was dried in shade for a period of 20 days, at an ambient temperature of 35˚C. After drying the fruits of *Lagerstroemia parviflora* the leaf and seeds were separated by cutting them into small pieces. The dried samples were grinded properly using a mortar and pestle and later using a grinder, to obtain the powdered and fibrous form.

Preparation of aqueous and ethanolic extract

35 gm of both samples was suspended in 250 ml of distilled water and 95% ethanol. Extraction was done at 70˚C for 45 minutes, followed by filtering of the extracts using Whatman filter paper No.1. Both extracts were then evaporated at 45˚C for 72 hours to form a paste, and further transferred into sterile bottles and refrigerated until use[4,5].

Phytochemical screening

Phytochemical screening was done from the both aqueous and ethanolic extracts. The recommended procedures were followed for determining carbohydrades[6], alkaloid[7], flavonoids[8], phytosterols[9], steroids[10], tannin, phenolic compound[11], saponin[12], and remaining phytochemical analysis as per the methods of Harborne[10].

Table 1
Phytochemical analysis of Lagerstroemia parviflora leaf and seed

SN.	Phytochemical	Test performed	Aqueous extract		Ethanolic extract	
			Leaf	Seed	Leaf	Seed
1	Carbohydrates	Molisch Test	++	-	++	-
2	Sugar	Benedict Test	+	-	+	-
3	Protein	Xanthoproteic Test	-	+	-	-
4	Tannins	Gelatin Test	++	+	++	++
5	Phenolic comp	Lead Acetate Test	++	+	++	+
6	Phytosterols	Libermann Burchard Test	-	-	++	++
7	Steroids	Ring Test	+	++	++	++
8	Amino acids	Ninhydrin Test	-	++	+	+
9	Flavonoids	Ethyl acetate Test	-	-	++	++
10	Terpenoids	Salkowski Test	+	++	+	+
11	Alkaloids	Dragendorff's Test	+	+	+	-
12	Anthraquinone	Borntrager's Test	-	-	-	-
13	Cumarine	Fluorescence test	+	+	+	+
14	Phlobatinins	Spot Test	-	-	-	-
15	Anthrol Glycosides	Borntrager's test	-	+	-	++
16	Cardiac Glycosides	Legal's test	+	+	+	++
17	Saponins	Foam Test	-	+	+	+
18	Fixed oils and lipids	Spot Test	+	+	+	+

++ indicates: strong presence, + indicates: weak presence, - indicates: strong absence

FT-IR Analysis of crude powder of L. Parviflora

Fourier transforms infrared spectrophotometer is perhaps the most powerful tool for identifying types of chemical bonds (functional groups). The wavelength of light absorbed is characteristic of the chemical bond as can be seen in this annotated spectrum. By interpreting the infrared absorption spectrum, the chemical bonds in a molecule can be determined. Dried powder of fruit peel of *L. Parviflora* plant materials was considered for instrumental analysis. The powdered sample plant specimens were treated for FTIR spectroscopy (Shimadzu, IR Affinity 1, Japan). Scan range: from 400 to 4000 cm^{-1} with a resolution of 4 cm^{-1}. [13].

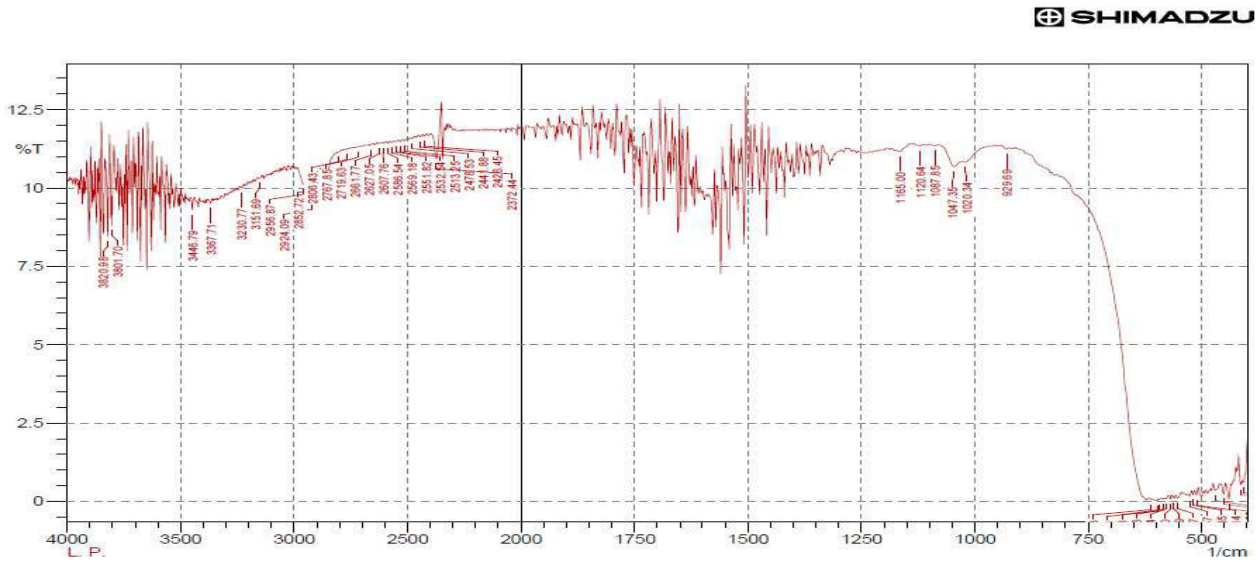


Figure 1
FT-IR for Lagerstroemia parviflora leaf

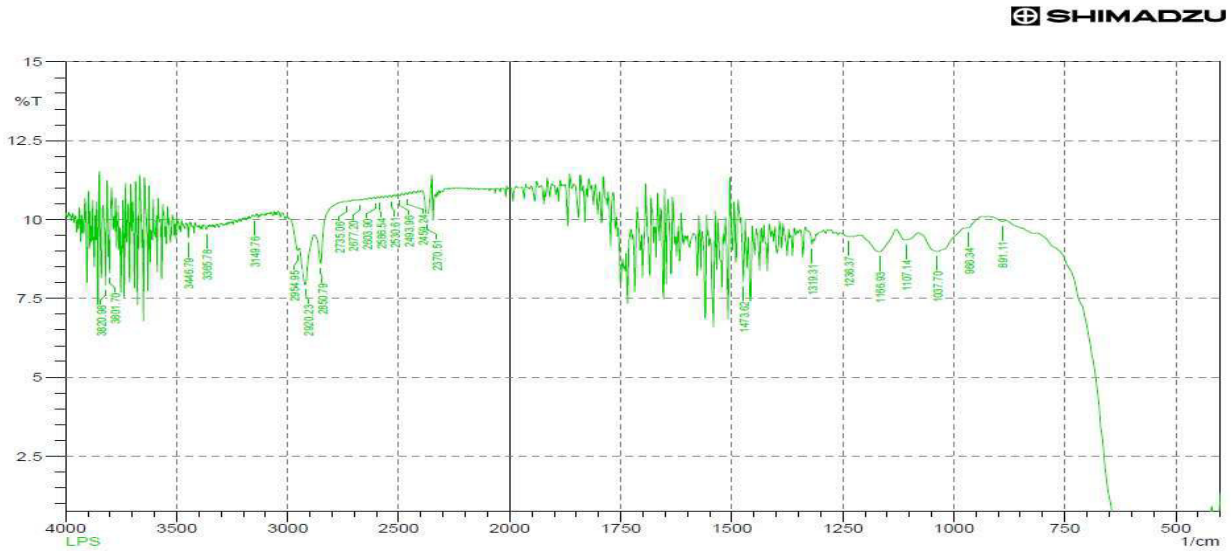


Figure 2
FT-IR for Lagerstroemia parviflora seed

Antioxidant activity

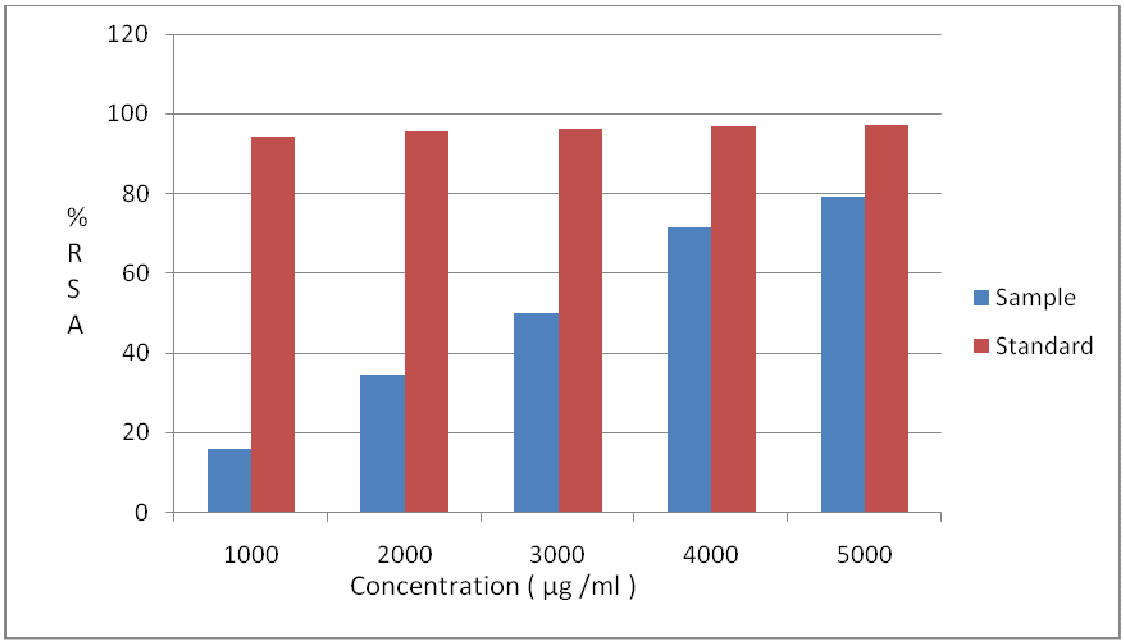
Free Radical Scavenging Activity by 2,2-diphenyl l-1-picrylhydrazyl (DPPH) Assay

The free radical scavenging activity of the extracts, based on the scavenging activity of the stable 2, 2-diphenyl-2- picrylhydrazyl (DPPH) (deep purple color) free radical was determined by the method described by Shen et a [14]. The stock solution of crude extracts was prepared by dissolving a known amount of dry extract in 98% methanol. The working solutions are (1000, 2000,3000,4000,5000 µg/ml) of the extracts were prepared from the stock solution using suitable dilution. The diluted working solutions of the test extracts were prepared in methanol. Ascorbic acid was used as standard in 1000-5000 µg/ml solution. 3.94 mg of DPPH (Sigma Aldrich, USA) was prepared in 100 ml methanol and 2.96 ml of this solution was mixed with 40 µl of sample solution and standard solution separately. These solution mixtures were kept in dark for 20 min and optical density was measured at 517 nm using UV-Vis Spectrophotometer (UV-1700 Shimadzu). DPPH solution was used as blank. The optical density was recorded and % inhibition was calculated using the formula given below

$$\% \text{ of DPPH Radical Scavenging Activity (\%RSA)} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

$Abs_{control\ is}$ the absorbance of DPPH radical + methanol: Abs_{sample} is the absorbance of DPPH radical + Sample extract. The measurements were performed in triplicate. Absorbance values were corrected for free radical decay using blank solutions. The IC_{50} (Concentration providing 50% inhibition) was calculated graphically using calibration curve verses' percentage of inhibition.

Graph 1
DPPH radical scavenging activity of L. parviflora leaf extract



Antibacterial activity

Test organisms

Escherichia coli (ATCC-14948), Staphylococcus aureus (ATCC-33591), Klebsiella pneumoniae (MTCC-4030), Pseudomonas aeruginosa (ATCC-4676), Propioni bacterium acnes (ATCC-1951), Salmonella typhi (ATCC-25812), were purchased from Microbial Type Culture Collection (MTCC), Institute of Microbial Technology, Chandigarh, India and used for assessment of antibacterial activity.

Antibacterial screening

According to the method (Qaralleh et al)[15] Agar disc diffusion assay was used to assay the various antibiotics for bactericidal activity against test strains of E. coli, K. pneumonia, P.aeru, S. aureus, P.acne, S.typhi. The strains of microorganisms obtained were inoculated in conical flask containing 100 ml of nutrient broth. These conical flasks were incubated at 37 ^{0}C for 24 hrs and were referred to as seeded broth. Media were prepared using Muller Hinton Agar (Himedia, Mumbai, India), poured on Petri dishes and inoculated with the test organisms from the seeded broth using cotton swabs. Sterile discs of six millimeter width had been impregnated with 20 µl of test extract and introduced onto the upper layer of the seeded agar plate. The plates were incubated overnight at 37 ^{0}C. Antibacterial activity was assigned by measuring the inhibition zone formed around the discs. The experiment was done three times and the mean values were presented. Rifampicin (10 µg / disc) was used as standards.

Table 2
Antibacterial activity of Ethyl acetate extract of L. parviflora Leaf.

Test Organism used	Extract	Antibiotic (Rifampicin)	Antibiotic + Extract	Ethyl acetate (Control)
K.pneu	10	19	19	0
P.aeru	11	15	15	0
S.aur	19	33	33	0
E.coli	11	18	17	0
P.acne	11	18	18	0
S.Typhi	10	14	14	0
Zone of inhibition in Diameter (mm)				

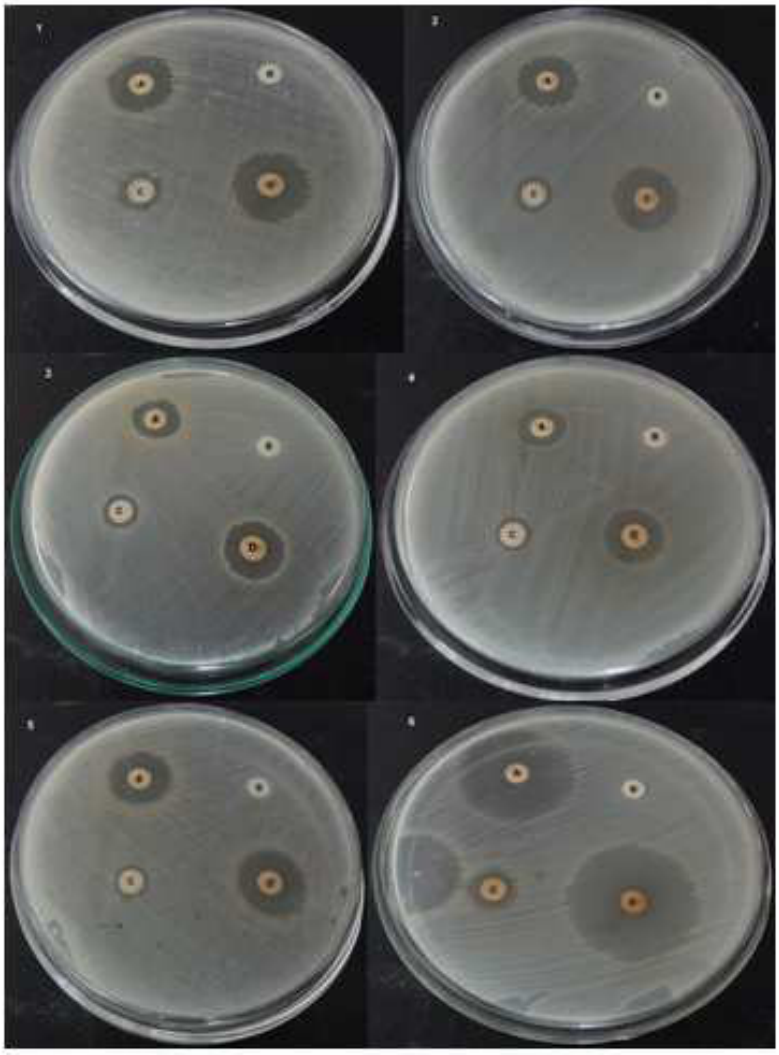


Figure 4
Antibacterial activity of L. parviflora Leaf against (1) Propioni bacterium acnes (2) Escherichia coli (3) Pseudomonas aeruginosa (4) Salmonella typhi (5) Klebsiella pneumoniae (6) Staphylococcus aureus , in each image: (A) Antibiotic disk, (B) Sterile disk(control), (C) Extract disk ,(D) Antibiotic + Extract disk.

RESULTS AND DISCUSSION

The *Lagerstroemia parviflora* leaf and seed extracts was rich in phytochemical activity, as shown in Table 1. The aqueous and ethanol extracts of the powdered leaves and seed of *L. parviflora* showed the presence of carbohydrates , alkaloids, flavonoids, cardiac glycosides, phytosterols, steroids, tannins, phenolic compound , and cumarines. Various herbs and herbal extracts contain different Phytochemical with biological activity that can show valuable therapeutic index. Different active Phytochemical have been found to possess a wide range of activities, which may help in the protection against incurable diseases. Biochemicals and phytochemicals such as saponins, terpenoids, flavonoids, tannins, steroids, and alkaloids have anti inflammatory effects[16,17]. Some cardiac glycosides, flavonoids, tannins, and alkaloids have hypoglycemic activities[18,19]. The mono, di and triterpenoids have also been shown to decrease blood sugar level in animal studies.[20] High molecular weight steroids and triterpenoids showed analgesic properties[21,22]. The steroids and saponins are also responsible for central nervous system activities[23]. FTIR-Spectrum of *L. parviflora* leaf shows strong absorption peaks at 3367.71cm^{-1} which shows strong absorbency N-H Stretch for aliphatic secondary amine, peaks at 3230.77, 3151.69, 2956.87 and 2924.09cm^{-1} represents identical absorbency of stretching frequency for N-H and Ar C-H it indicates aromatic amines. The peaks at 2806.43-2607.76 cm^{-1} stretching frequency and 929.69 cm^{-1} blended frequency shows for aliphatic hydrocarbon C-H group. The peaks at 1087.85 and 1020.34 cm^{-1} represents primary amine C-N stretch and peak at 1165.00 and 1120.64cm^{-1} represents Tertiary amine C-N stretch. The peaks at 2569.18 & 2478.53 cm^{-1} represents Thiols S-H stretch[24].

FTIR- Spectrum of *L. parviflora* seed, shows strong absorption peaks at 3446.79 cm^{-1} which shows strong absorbency N-H Stretch for aromatic primary amine, peaks at 3365.78 cm^{-1} shows strong absorbency N-H Stretch for aliphatic secondary amine. The peaks at 2954.95 cm^{-1}stretching frequency shows Ar C-H stretch, it indicates aromatic hydrocarbon. The peaks at 2920.23, 2850.79 & 2603.90 cm^{-1} stretching frequency and at 891.00 & 966.00 cm^{-1} blended frequency, shows for aliphatic hydrocarbon C-H group in CH_3. The peaks at 1236.37 cm^{-1} represents aromatic primary amine C-N stretch. The peaks at 1166.93 & 1107.14 cm^{-1} represents secondary amine C-N stretch. The peaks at 1037.70 cm^{-1} represents primary amine C-N stretch. The peaks at 2586.54 & 2493.96 cm^{-1} represents Thiols S-H stretch.[24] The radical scavenging activity of the *L.parviflora* leaf extract was tested using stable free radical DPPH (deep purple colour), as DPPH has the advantage of being unaffected by certain side reactions. Figure 3 shows the DPPH radical scavenging activity of *L.parviflora* leaf with ascorbic acid as reference, where the IC_{50} values for the *L.parviflora* methanolic leaf extract (IC_{50}= 3482 µg/ml) which was said to be less than the standard ascorbic acid (IC_{50}= 2.816 µg/ml). The presence of flavonoids and tannins in the plants in phytoconstituent are responsible for the free radical scavenging effects.[25] Flavonoids and tannins are the phenolic compounds and plants phenolics are a major group of compounds that act as primary antioxidants or free radical scavengers.[26] Thus the antioxidants present in the extract quenches the DPPH free radicals (by providing hydrogen atom or by electron transfer, conceivably via a free radical attack on the DPPH molecule) and convert them to a colourless product (2, 2-diphenyl-1-picrylhydrazyl, or a substituted analogous hydrazine) resulting in a decreasing absorbance at the 517 nm[27]. The antimicrobial activities of the plant extracts against the six bacteria strains examined the antibacterial activity are given in Table 2. The *L. parviflora* leaf extract showed good inhibition against all six Organisms. The highest inhibition was

noted in order of S. aureus (19 mm), E. coli (11 mm), P.aeru (11 mm), P.acne (11 mm), K. pneumonia (10mm), S.thyphi (10mm). The ethyl acetate extract of leaves of *L. parviflora*, possessed significant antioxidant activity (IC_{50}= 3482 µg/ml), antimicrobial activity due to the presence of omega-3 fatty acid and various phenolic compounds[28,29].

CONCLUSION

The present study describes the phytochemical profile, antioxidant activity and Antimicrobial activity of *L. parviflora* which will further used for medicinal applications.

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