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Antioxidant and Antimicrobial Activities of Methanolic Leaves Extract of *Lagerstroemia parviflora*

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ABSTRACT

An antioxidant is a molecule capable of terminating the chain reactions that damage cells by removing free radical intermediates and inhibit other oxidation reactions by thereby reducing stress responsible for many degenerative disorders. The present study was concerned with phytochemical screening, antioxidant, antimicrobial activities of methanolic leaves extract of *Lagerstroemia parviflora*. It was observed that the methanol extract showed IC 50 value of 76.05µg/ml as compare to standard ascorbic acid 14.66 µg/ml. It was evident that the extracts showed proton-donating ability and this could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. Thus the methanol extract of this plant possesses the strongest ability to scavenge DPPH radical. The leaves extracts of *Lagerstroemia parviflora* exhibited potential antibacterial activity against *Staphylococcus aureus* (20±0.2, 21±0.1 and 23±0.1), *Salmonella bongori* (9±0.3, 12±0.1and 15±0.5) and *Aspergillus niger* (7±0.1, 9±0.1 and 11±0.2) at the concentration level of 25-100mg/ml. The Results of antimicrobial activity of extract showd more effectiveness against both the selected bacteria (*Staphylococcus aureus* and *Salmonella bongori*) found less effective against fungus (*Aspergillus niger)*. The present study suggests that the use of leaves of this plant may be exploited for health supplements and has potential for topical treatment.

Keywords: *Lagerstroemia parviflora,* Antioxidant, Antimicrobial

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INTRODUCTION

The plants with the antioxidant activity have drawn attention in recent years due to the development of a large number of different diseases[1,2]. It has been hypothesized that these diseases are caused by action of free radicals in most cases[3]. Antioxidants have the ability to inhibit the free radicals generation or to remove the already formed free radicals by their direct action[4]. There are a number of natural antioxidants, e.g. α-tocopherol[5]; ascorbic acid [6,7]; retinol, thiamin and riboflavin, flavonoids[8] and phenolic acids[5,9] as well as a number of synthetic antioxidants[10,11]. Synthetic antioxidants such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) have a potentially harmful effect on human health, leading to various dysfunctions[10]. One of the most important trends in the food and pharmaceutical industry today is searching for natural antioxidants from the plant material. Medicinal plants have been used for centuries as remedies for various diseases because they contain bioactivecomponents of a therapeutic value[11-14]. There is the increasing use of a traditional medicine as an alternative form for treating various diseases due to the resistance of microorganisms to the existing synthetic antibiotics[13,15]. A large number of studies investigate the antimicrobial activity of natural phenolic compounds from plants[1,16-19] in order to find new, natural antimicrobial agents. Flavonoids are synthesized in plants in response to microbial infection. It is therefore not surprising that these compounds have *in vitro* antimicrobial activity against a wide range of microorganisms[20,21]. Some phenolic compounds, such as resveratrol, hydroxytyrosol, quercetin and many phenolic acids may inhibit many pathogenic microorganisms[22-24]. Acne vulgaris is a cutaneous disorder of multifactorial origin which manifests in the pilosebaceous follicle. It is characterized by open and closed comedones and inflammatory lesions like papules, pustules and nodules[25]. Micro-organisms like *Propionibacterium acnes*, *Staphylococcus aureus* and *Staphylococcus epidermidis* proliferate rapidly[26] leading to the development of acne. In clinical management of acne vulgaris, a considerable number of antibiotics and

chemotherapeutic agents are available in the global market as topical or systemic treatment modalities[27]. Topical therapy is preferred as first-line treatment in mild acne whereas for moderate and severe type of acne, systemic therapy is required in addition to topical therapy. Topical therapy has associated side effects and the undesirable physicochemical characteristics of certain important agents like tretinoin and benzoyl peroxide affect their utility and patient compliance[28]. The latest treatment regimen followed is the one-step acne solutions [29], but they too have disadvantages in that, they are 99% oil based creams and contain either (or both) benzoyl peroxide or (and) salicylic acid. Oil based products are counterproductive because they both fight and contribute to acne by clogging pores. Benzoyl peroxide[30] and salicylic acid[31] are generally more irritating than acne itself. So the authors felt a need to develop a formulation that is water based and devoid of harmful chemicals. Herbal therapies on the other hand are gaining attention in comparison to existing formulations which cause enormous side effects like skin dryness, rashes, wrinkling, erythema, pruritis, skin eruption and development of resistance[32]. Thus, the aim of this study is phytochemical analysis, antioxidant and antimicrobial activities of methanolic extract of *Lagerstroemia parviflora* leaves.

MATERIALS AND METHODS

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. 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.

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. The pathogenic microbes used in the current study were obtained from Microbial Culture collection, National Centre Forcell Science, Pune, Maharashtra, India.

Extraction by maceration process

89gm of dried plant material were extracted with methanol using maceration method for 48 hrs. 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, tannins, proteins, amino acids and flavonoids.

Antioxidant activity (DPPH model) of methanolic extract of Lagerstroemia parviflora (Leaves)

DPPH scavenging activity was measured by the spectrophotometer [33]. 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. Final decrease in absorbance was noted of DPPH with the sample at different concentration after 15 minutes at 517 nm.

Calculation of % Reduction

$$= \frac{\text{Control Absorbance} - \text{Test absorbance}}{\text{Control Absorbance}} \text{X } 100$$

Antimicrobial activity of methanolic extract of Lagerstroemia parviflora (Leaves)

Pathogenic Antimicrobial used

The pathogenic microbes used in the current study obtained from microbial culture collection, national centre forcell science, Pune, Maharashtra, India.

Media preparation (broth and agar media)

Composition of nutrient agar media

Agar	1.5 gms.
Beef extract	0.3 gms.
Peptone	0.5 gms.
Sodium chloride	0.55 gms.
Distilled water	to make 100 ml.
pH – 7	

Composition of Potato Dextrose Agar media;

Agar	2.0 gms.
Potato infusion	20 gms.
Dextrose	2.0 gms.
Distilled water	to make 100 ml.
pH – 7	

Method of preparation

This agar medium was dissolved in distilled water and boiled in conical flask of sufficient capacity. Dry ingredients are transferred to flask containing required quantity of distilled water and heat to dissolve the medium completely. The flask containing medium was cotton plugged and was placed in autoclave for sterilization at 15 lbs /inch^2 (121^{o}C) for 15 minutes. After sterilization, the media in flask was immediately poured (20 ml/ plate) into sterile petri dishes on plane surface. The poured plates were left at room temperature to solidify and incubate at 37^{o}C overnight to check the sterility of plates. The plates were dried at 50^{o}C for 30 minutes before use.

Antibiogram Studies

Broth cultures of the pure culture isolates of those test microorganisms which are sensitive towards the phytoextracts used in present study were prepared by transferring a loop of culture into sterile nutrient and potato

dextrose broth and incubated at 37°C for 24-48 hours. A loop full was taken from these broths and seeded onto sterile nutrient and potato dextrose agar plates through sterile cotton swab to develop diffused heavy lawn culture. The well diffusion method was used to determine the antimicrobial activity of methanolic extract prepared from the leaves of *Lagerstroemia parviflora* using standard procedure[34]. There were 3 concentration used which are 25, 50 and 100 mg/ml for extracted phytochemicals in antibiogram studies. It's essential feature is the placing of wells with the antibiotics on the surfaces of agar immediately after inoculation with the organism tested. Undiluted overnight broth cultures should never be used as an inoculums. The plates were incubated at 37°C for 24 hr. and then examined for clear zones of inhibition around the wells impregnated with particular concentration of drug.

RESULTS AND DISCUSSION

Results of antioxidant activity using DPPH method

The effect of antioxidant on DPPH is believed to be due to their hydrogen-donating ability. The DPPH assay measures the antioxidant activity of water soluble phenolics. Table 1 and Figure 1 shows the dose-response curve of DPPH radical scavenging activity of the different methanolic extract of *Lagerstroemia parviflora*. It was observed that the methanol extract showed IC 50 value of 76.05µg/ml as compare to standard ascorbic acid 14.66 µg/ml. It was evident that the extracts showed proton-donating ability and this could serve as free radical inhibitors or scavengers, acting possibly as primary antioxidants. Thus the methanol extract of this plant possesses the strongest ability to scavenge DPPH radical.

Table 1: % Inhibition of ascorbic acid and methanolic extract of *Lagerstroemia parviflora* (Leaves)

S. No.	Concentration (µg/ml)	% Inhibition	
		Ascorbic acid	Methanolic extract
1	10	38.16	30.25
2	20	47.58	35.45
3	40	54.91	41.66
4	60	70.24	48.19
5	80	79.83	50.87
6	100	87.01	54.74
IC 50		14.66	76.05

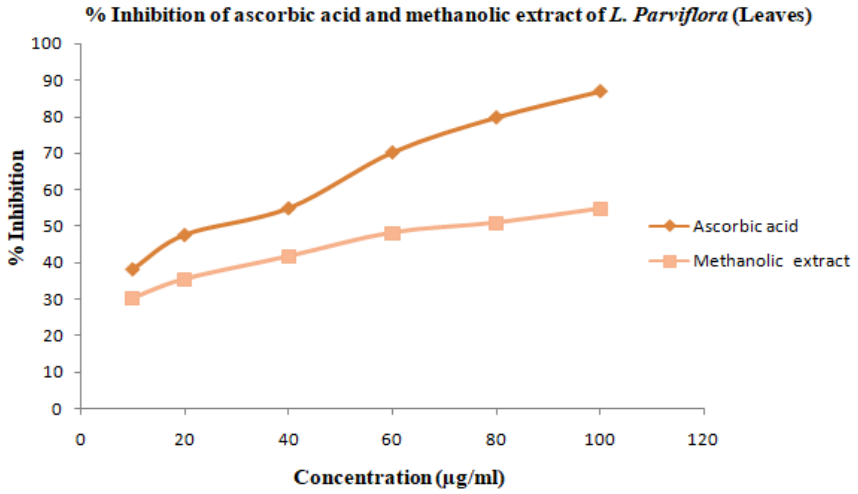

Figure 1: % Inhibition of ascorbic acid and extract of *Lagerstroemia parviflora* using DPPH method

Results of antimicrobial activity of methanolic extract of *Lagerstroemia parviflora*

A study of zone of inhibition against bacterial strains with leaves extracts of *Lagerstroemia parviflora* revealed that all bacteria subjected to cold water extract showed zone of inhibition against *Staphylococcus aureus, Salmonella bongori* and *Aspergillus niger* Table 2, 3 and 4. The results of present study observed variation in antibacterial activities of the leaves extracts of *Lagerstroemia parviflora* to inhibit selected bacteria *in vitro*. However higher antimicrobial activity of *Lagerstroemia parviflora* was seen with increase in concentration (25-100mg/ml). The leaves extracts of *Lagerstroemia parviflora* exhibited potential antibacterial activity against *Staphylococcus aureus* (10±0.47, 14±0.74 and 18±0.74), *Salmonella bongori* (12±0.35, 14±0.6 and 17±0.65) and *Aspergillus niger* (7±0.74, 10±0.5 and 11±0.94) at the concentration level of 25-100mg/ml. The Results of antimicrobial activity of extract showd more effectiveness against both the selected bacteria (*Staphylococcus aureus* and *Salmonella bongori*) found less effective against fungus (*Aspergillus niger).*

Table 2: Results of sensitivity of methanolic extract of *Lagerstroemia parviflora*

S. No.	Microbes Codes	Microbial Strains	Sensitivity
1.	Bact-1	*Staphylococcus aureus*	Yes
2.	Bact-2	*Salmonella bongori*	Yes
3.	Fungus-1	*Aspergillus niger*	Yes

Table 3: Antimicrobial activity of *standard drug* against selected microbes

S. No	Name of drug	Microbes	Zone of Inhibition (mm)		
			10 µg/ml	20 µg/ml	30 µg/ml
1.	Ciprofloxacin	*Staphylococcus aureus*	23±0.47	25±0.5	27±0.94
		Salmonella bongori	16±0.5	22±0.86	24±0.57
2.	Fluconazole	*Aspergillus niger*	7±0.74	10±0.5	11±0.94

Table 4: Antimicrobial activity of methanolic extract of *Lagerstroemia parviflora* against selected microbes

S. No.	Name of microbes	Zone of inhibition		
		Methanolic extract		
		25mg/ml	50 mg/ml	100mg/ml
1.	*Staphylococcus aureus*	20±0.2	21±0.1	23±0.1
2.	*Salmonella bongori*	9±0.3	12±0.1	15±0.5
3.	*Aspergillus niger*	7±0.1	9±0.1	11±0.2

CONCLUSION

The results of the present work indicated that the hydroalcoholic extract of *Lagerstroemia parviflora* leaves is a potential source of natural antioxidants and significantly inhibit free radicals by dose-dependently and also showed better antibacterial activity. Hence, from the overall results, finally it was concluded that the formulated herbal gels have significant antimicrobial properties and hence will be better, safe and effective than allopathic medications.

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