

## Antimicrobial and antioxidant activities of *Lagerstroemia tomentosa*

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**Abstract: Background:** Some Pathogens are resistance to various antimicrobial agents. Many plants parts contain primarily polyphenols and terpenes which can combat with the problem of resistance bacteria and drug residue hazards. **Materials and Methods:** Methanol (70%) extract of *Lagerstroemia tomentosa* aerial parts was tested for antimicrobial activity against bacterial and fungal strains and for antioxidant activity using oxygen radical absorbance capacity and the trolox equivalent antioxidant capacity assays and also total content of polyphenols with phytochemical analysis of the extract was determined. **Results and Discussions:** The results showed that the extract has a significant antimicrobial activity, it inhibited the growth of *Bacillus subtilis* and also it was highly active against *Candida albicans* suggesting that it can be used in the treatment of fungal infections, and it showed a moderate antimicrobial activity against *Klebsiella pneumoniae*, it has shown high values of oxygen radical absorbance capacity and polyphenol content while it has shown a lower value of the trolox equivalent antioxidant capacity. Phytochemical analysis of the extract showed the presence of carbohydrates, flavonoids, tannins, coumarins and this clarify the high content of total polyphenol in the extract.

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**Key words:** *Lagerstroemia tomentosa*, aerial parts, antimicrobial activity, antioxidant activity, total polyphenol content, phytochemical analysis.

### Introduction

Plants remain the most common source of antimicrobial agents. Their usage as traditional health remedies is the most popular for 80% of world population in Asia, Africa and Latin America and it is reported to have minimal side effects (Bibitha et al., 2002). The development of microbial resistance towards antibiotics makes it necessary to search for new potential effective plant extracts and compounds against pathogenic bacteria and fungi. There have been numerous broad based screening programmes initiated over the past years, in which large numbers of plant species have been evaluated for their antimicrobial activities (Farnsworth, 1966, Afolayan and Meyer, 1997). Free radicals contribute to more than one hundred disorders in humans including atherosclerosis, arthritis, ischemia, injury of many tissues, central nervous system injury, gastritis, cancer and AIDS. Antioxidants or molecules with radical scavenging capacity are thought to exert a potential effect against free radical damage. It has been mentioned the antioxidant activity of plants might be due to their phenolic or flavonoid compounds (Scholar, 1990). *Lagerstroemia* is an important member of Lythraceae consisting of 31 genera. This genus contains more than 56 species of trees or shrubs with colorful flowers distributed from southeastern Asia to Australia (Graham et al., 1993). *Lagerstroemia tomentosa* is a medicinal and ornamental, handsome

deciduous, small tree native of China. The bark of the plant is considered stimulant and febrifuge, leaves and flowers are used as purgative (Chopra et al., 1958), the roots are astringent (Kirtikar and basu, 1935). No previous biological and phytochemical examinations of *Lagerstroemia tomentosa* have been undertaken. In this present study, we investigated the antimicrobial and antioxidant activities of plant and total polyphenol content was determined with phytochemical analysis.

### Material and methods

#### Plant material

Aerial parts of *Lagerstroemia tomentosa* were collected from Al-Zohiriya garden, Giza, Egypt in April 2011. The plant was identified by Dr. Mohammed El-Gebaly, Department of Botany, National Research Centre (NRC) and by Mrs. Tereez Labib Consultant of Plant Taxonomy at the Ministry of Agriculture and director of Orman botanical garden, Giza, Egypt. A voucher specimen No.11232 is deposited in the herbarium of Al-Zohiriya garden, Giza, Egypt.

#### Antimicrobial Assays

The quantitative assay of the antimicrobial activity was performed by broth microdilution method (Petra et al., 2003) in 96-well microplates in order to establish the minimal inhibitory concentration (MIC). The antimicrobial activity was tested against Gram-positive strains (*Staphylococcus aureus*, *Bacillus*

*subtilis*), Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*) and fungal strain (*Candida albicans*). The methanol extract of *L. tomentosa* was tested for its antimicrobial activity using a qualitative screening assay of the antimicrobial properties by the adapted disk diffusion method (Kirby-Bauer method), (Das et al., 2010). The quantitative assay of the antimicrobial activity was performed by binary microdilution method (Petra et al., 2003), in order to establish the minimal inhibitory concentration (MIC). The antimicrobial activity of the investigated extract was tested against bacterial and fungal strains: Gram positive (*Staphylococcus aureus*, *Bacillus subtilis*), Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*) and fungal strains (*Candida albicans*). The microbial strains were identified using a VITEK I automatic system. VITEK cards for the identification and the susceptibility testing (GNS-522) were inoculated and incubated according to the manufacturer's recommendations. In our experiments there were used bacterial suspensions of  $1.5 \times 10^8$  UFC/ mL or 0.5 McFarland density obtained from 15–18 h bacterial cultures developed on solid media. The antimicrobial activity was tested on Mueller-Hinton medium recommended for the bacterial strains and Yeast Peptone Glucose (YPG) medium for *Candida albicans*. Solutions of the extract in DMSO (dimethyl sulfoxide) having 2048  $\mu\text{g}/\text{mL}$  concentration were used.

#### Qualitative screening of the antimicrobial properties of the extract

The antimicrobial activity of the extract was investigated by qualitative screening of the susceptibility spectrum of different microbial strains to the tested extract solubilised in DMSO (1 mg/mL) using adapted variants of the diffusion method. In the 1st variant, 10  $\mu\text{L}$  of the extract solution were equally distributed on the paper filter disks placed on Petri dishes previously seeded "in layer" with the tested bacterial strain inoculums. In the 2<sup>nd</sup> variant, 10  $\mu\text{L}$  of the tested extract solutions were placed in the agar wells cut in the solid culture medium seeded with the microbial inoculum. In the 3<sup>rd</sup> variant of the qualitative antimicrobial activity assay, 10  $\mu\text{L}$  of the extract solutions were spotted on Petri dishes seeded with bacterial/yeast inoculum. In all the three variants, the Petri dishes were left at room temperature to ensure the equal diffusion of the compound in the medium or to allow the drop of solution to be adsorbed in the medium and afterwards the dishes were incubated at 37°C for 24 hours. The solvent used was also tested in order to evaluate a potential antimicrobial activity.

Quantitative assay of the antimicrobial activity

For the quantitative assay of the antimicrobial activity of the extract by the microdilution method

(Petra et al., 2003) in liquid medium distributed in 96-well plates, binary serial dilutions of the tested extract solutions were performed. There were obtained concentrations from 1000  $\mu\text{g}/\text{mL}$  to 0.97  $\mu\text{g}/\text{mL}$  in a 200  $\mu\text{L}$  culture medium final volume, afterwards each well was seeded with a 50  $\mu\text{L}$  microbial suspension of 0.5 MacFarland density. In each test a microbial culture control (a series of wells containing exclusively culture medium with the microbial suspension) and a sterility control (a series of wells containing exclusively culture medium) were performed. The plates were incubated for 24 hours at 37°C.

#### Antioxidant assays

Extraction: 0.2 g of extract with 10 mL Millipore water boiled were sonic, centrifuged and filtered. Evaluation of antioxidant activity of the extract, using methods Oxygen Radical Absorbance Capacity (ORAC), Trolox equivalent antioxidant capacity (TEAC) and determination of total polyphenols content:

Oxygen Radical Absorbance Capacity (ORAC)

This method determines peroxyl radical inhibition capacity, inducing oxidation highlighting the classical radical release, H atom transfer ORAC values were reported as Trolox equivalents, is expressed as micromol TE/ DW. The intensity was monitored at 485 nm and 525 nm for 35 min.

Trolox equivalent antioxidant capacity method (TEAC)

This method is based the neutralizing capacity the radical anion  $\text{ABTS}^+$  by antioxidants. ABTS is oxidized by radicals peroxyl or other oxidants to its radical cation  $\text{ABTS}^+$ , intensely colored ( $\lambda_{\text{max}} = 734$  nm). Antioxidant capacity is expressed compounds tested as potential, to discoloration by direct reaction with it radical  $\text{ABTS}^+$ .

#### The total content of polyphenols

The blue compounds formed between phenols and Folin-Ciocalteu reagent phenolic compounds are independent of structure, thus developing complex between metal center and phenolic compounds. Absorption was recorded at a wavelength of 765 nm. Total phenol content was expressed as gallic acid equivalents.

#### Qualitative Phytochemical Analysis

The extract was tested for the presence of bioactive compounds by using following standard tests (Molisch 's test for carbohydrates, Shinoda test for flavonoids, forth test for saponins, Salkowski 's for terpenes and sterols,  $\text{FeCl}_3$  and Mayer 's reagents for detecting of tannins and alkaloids, respectively (Sofowra, 1993, Trease and Evans, 1989, Harborne, 1973).

## Results

The results of antimicrobial, Antioxidant, total polyphenol content and phytochemical analysis of *L. tomentosa* extract are shown in tables (1, 2 and 3).

**Table 1:** Results of Antimicrobial activity of *L. tomentosa* extract expressed in  $\mu\text{g}/\text{mL}$  (MIC)\*

Material tested	<i>K. pneumoniae</i> IC 13420	<i>E. coli</i> IC 13529	<i>S. aureus</i> IC 13204	<i>P. aeruginosa</i> ATCC 27853	<i>B. subtilis</i> ATC C 6633	<i>C. albicans</i> IC 249
<i>Lagerstroemia tomentosa</i> extract	62.5	125	250	125	62.5	7.8
Blank DMSO	125	125	250	125	125	125

\* MIC =minimal inhibitory concentration

**Table 2:** Results of Antioxidant capacity of the extract tested was expressed as Trolox equivalents and total polyphenol content

Material tested	Total polyphenol content (gallic acid mg/g DW)	Oxygen radical absorbance capacity (ORAC assay value)	Trolox equivalent antioxidant capacity (TEAC assay value)
<i>Lagerstroemia tomentosa</i> extract	677.5 $\pm$ 8.63 mg	5143 mM TE/g	71 $\pm$ 1.08 mM TE

**Table 3:** Phytochemical analysis of *L. tomentosa* extract

Constituents	<i>L. tomentosa</i> extract
Triterpenes and/or Sterols (Quassinoids)	+
Carbohydrates and/or glycosides	+
Flavonoids	+
Coumarins	+
Alkaloids and/or nitrogenous compounds	+
Tannins	+
Saponins	-

+ presence of constituents

- absence of constituents

## Discussion

Antimicrobial activity of *L. tomentosa* extract

For the antimicrobial qualitative methods, i.e. paper filter disks impregnated with the tested extract solution and disposal of the respective solutions in agar wells, the reading of the results was performed by measuring the microbial growth inhibition zones around the filter disks impregnated with the testing extract and around the wells, respectively. The most efficient qualitative method proved to be the direct spotting of the tested solutions on the seeded medium, the results being very well correlated with the results of the MIC quantitative assay. For the quantitative methods of the antimicrobial activity of the tested extract by the microdilution method in liquid medium, the MIC was

read by wells observation: in the first wells containing high concentrations of extract, the culture growth was not visible, the microbial cells being killed or inhibited by the tested extract. At lower concentrations of the tested extract, the microbial culture becomes visible. The lowest concentration which inhibited the visible microbial growth was considered the MIC ( $\mu\text{g}/\text{mL}$ ) value for the extract. In the next wells, including the standard culture growth control wells, the medium become muddy as a result of the microbial growth. In the sterility control wells series, the medium had to remain clear. From the last well without any visible microbial growth and from the first one that presented microbial growth, Gram stained smears were performed for the results confirmation. In table 1 there are the results of the quantitative assay of the antimicrobial activity of the *L. tomentosa* extract. Our results have shown that the extract was highly active against *C. albicans*, suggesting its possible use in the treatment of fungal infections, also it exhibited antimicrobial activity on *B. subtilis* and it has shown a moderate antimicrobial activity against *K. pneumoniae* but it was not active on other bacterial strains.

Antioxidant activity of *L. tomentosa* extract and Total polyphenol content

Antioxidant activity was determined by Oxygen Radical Absorbance Capacity (ORAC) and Trolox equivalent antioxidant capacity method (TEAC) assays. In ORAC assay, *L. tomentosa* extract has shown high ORAC value (5143 mM TE/g) (table 2). In (TEAC) assays, *L. tomentosa* extract showed TEAC value (71  $\pm$  1.08 mM TE/g) (table 2). Total polyphenol content was expressed as gallic acid equivalents and the extract has shown a value of 677.5 $\pm$ 8.63 mg/g (table 2) and this suggest the extract has a high content of polyphenols and this result is in agreement with phytochemical analysis of the extract which has shown the presence of flavonoids, tannins, coumarins (polyphenolic components), alkaloids and carbohydrates (table 3). Phenolic compounds form one of the main classes of secondary metabolites. They display a large range of chemical structures and are responsible for the major bioactivity of plants. Flavonoids have shown a significant antimicrobial activity (Lamb and Cushnie, 2005, Tapas et al., 2008), tannins have shown a significant antimicrobial and antioxidant activities (Reddy et al., 2007) as well as coumarins have shown a good antimicrobial and antioxidant effects (Adriana et al., 2009). On the basis from above, it can be concluded that *L. tomentosa* methanol extract has significant antimicrobial activity and possess better antioxidant activity and these activities are due to the high polyphenol content and the interesting bioactive compounds include flavonoids, tannins and coumarins.

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