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Original Article

A Study on the Antioxidant and Antimicrobial Properties of the Aerial Parts Extract of *Lagerstroemia indica* L.

Moujan Noori Naeiji and Seyed Mohammad Vahdat*

Department of Chemistry, Ayatollah Amoli Branch, Islamic Azad University, Amol, Iran

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***Corresponding author**
vahdat_mohammad@yahoo.com

ABSTRACT

This research aimed to investigate the antioxidant and antimicrobial properties of the extract of the medicinal plant *Lagerstroemia indica* L. against bacteria, including *Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi murium*, *Streptococcus mutans*, and fungi, including *Aspergillus flavus* and *Candida albicans*. The study also evaluated the extract's activities in inhibiting the free radical DPPH, total phenols and flavonoids. The *L. indica* L. plant was collected from the medicinal plant garden of the Islamic Azad University, Ayatollah Amoli branch. In this research, two varieties with purple and pink flowers, and their leaves were used for extraction. The extraction process was performed using the maceration method and a rotary evaporator. The extract's antimicrobial and antioxidant activities were found to be higher in the extract of purple flowers compared to pink flowers. *S. aureus* and *Aspergillus flavus* showed the highest sensitivity to the extract of purple flowers compared to other extracts, with a minimum inhibitory concentration of 12.5 and 25 μg/ml for antimicrobial activity and a minimum concentration of 25 and 50 μg/ml for fungicidal activity, respectively. The average total phenol content in the extract of purple flowers was 369.23 mg of gallic acid equivalent per gram of extract. The average flavonoid content in this extract was 24.1 mg of gallic acid equivalent per gram of extract. In the investigation of the percentage of DPPH free radical scavenging activity, concentrations of 400 μg/ml of purple flower extract had the highest average inhibition percentage of 83.97%, while concentrations of 6.25 µg/ml had the lowest average inhibition percentage of 22.481%. Given its high antioxidant activity and rich content of phenolic compounds, it can be said that extracts derived from this plant exhibit inhibitory and bactericidal effects against the studied microorganisms and can be used as an antimicrobial compound.

INTRODUCTION

Primitive humans used plants as medicine to treat diseases. With the expansion of various branches of science, the use of chemical substances in drug production attracted the attention of researchers. However, it did not take long for scientists to realize the side effects and inefficacy of these drugs, forcing them to turn to herbal compounds again in the treatment of diseases.

One of the most significant therapeutic challenges is dealing with infectious diseases due to their high prevalence and spread. Herbal medicine has been increasingly growing in recent years for the treatment of diseases, particularly infectious ones. The use of plants for therapeutic purposes has been in practice for over a century. Nowadays, a wide variety of antifungal and antibacterial drugs are used for the treatment of infectious agents. However, due to the genetic diversity among microbial pathogens, the emergence of resistant strains, and the side effects associated with the use of these drugs, replacing them with plant-based antimicrobial agents is of particular importance. The presence of biologically active compounds in plants such as antioxidants, antimicrobial, and antitumor agents has made it possible to use them as medicinal plants, preservatives, and dietary supplements [1].

The medicinal herb "*Gol Turi*" with the scientific name *Lagerstroemia indica* L. belongs to the genus *Lagerstroemia* and the family *Lythraceae*. It is also known as "*myrtles crape*" and has around 56 species. It is found in India, Southeast Asia,

southern China, Japan, and Korea, as well as northern Australia and Guinea New Guinea [2,3] and has recently been introduced to Iran. This shrub can grow up to 3 meters in height [4]. It contains alkaloids, cardiac glycosides, tannins, saponins, sterols, triterpenes, anthraquinones, rejuvenating compounds, flavonoids (flavanones/dihydroflavonols and chalcones), and phenylpropanoid glycosides (A-C). The protein content of this plant is 22.53%, carbohydrates are 37.25%, and ash is 12.23% based on the dry weight. According to mineral analysis, this plant is rich in potassium, calcium, magnesium, phosphorus, sodium, and sulfur. The main motivation of this research is to investigate the antioxidant and antimicrobial properties of the extract of this plant [5].

Antibiotics are potent drugs that prevent the proliferation of microorganisms and ultimately eradicate them. However, drug resistance to commonly used antibiotics is a longstanding issue that has existed for many years. Usually, antibiotic resistance occurs through mutation, as chromosomal mutations in bacteria are much more common than in other organisms. Microbes undergo frequent changes, and these changes may be due to the emergence and re-emergence of new infectious diseases. As a result, researchers are forced to develop new antibiotics with appropriate antimicrobial activity or resort to empirically prescribing antibiotics without conducting antibiotic resistance testing, which can ultimately lead to the selection of inappropriate or unnecessary antibiotics and render microbes resistant to them. Given that infectious diseases and toxins comprise a wide range of diseases, and the number of antibiotic-resistant microbial strains is increasing every day, there is a critical need for new and low-risk natural antimicrobial agents. Therefore, investigating the antimicrobial effects of natural plants can serve as a gateway to obtaining new antibiotics. Considering the growing trend of medicinal plants, the existence of biologically active compounds in *L. indica* L. plant, and its distribution in Iran, the present research was conducted with the main aim of investigating the antioxidant and antimicrobial properties of the aerial parts extract of *L. indica* L. plant. This study aims to answer the question of whether the aerial parts extract of the *L. indica* L. plant has antioxidant, antimicrobial, and antifungal properties Several studies have been conducted in this regard. The following points are presented to examine the link between this research and previous studies [6].

Wei *et al*. (2020) identified a total of 114 compounds in the medicinal plant *Goltori*, including compounds with antimicrobial activity, flower color, and essential oil components. Of these compounds, 58, 63, 67, and 61 active substances were found in white, pink, red, and carmine *Goltori* flowers, respectively. The essential oil yields of these flowers were 0.92%, 1.15%, 1.12%, and 1.08%, respectively. The major compounds in the flower of *Goltori* were 2-methylcyclopentanone (9.41%) and m-zylene (7.53%), while the pink and carmine flowers contained octacosane (19.81% and 13.91%) and henicosane (18.02% and 7.98%), respectively. The red flowers contained cyclohexanone (8.13%) and 1-octacosanol (7.87%). In general, 23 compounds were present in the extracted essential oils of the studied flowers, which accounted for approximately 16.57% to 32.72% of the total essential oil. Essential oils extracted from pink, orange, and carmine flowers have shown significant antimicrobial effects against *S. aureus*, *P. aeruginosa*, *E. coli*, *B. subtilis*, and *A. niger* fungi at a minimum inhibitory concentration of 78 µg /ml [7].

In 2016, Ajaib *et al*. investigated the antimicrobial activity of extracts of the skin, leaf, and fruit of *L. indica* L. against two gram-positive bacteria, *S. aureus* and *B. subtilis*, and two gram-negative bacteria, *E. coli* and *P. aeruginosa*, as well as two strains of *A. fungi*, *Aspergillus niger* and *A. oryzae*. The extracts were obtained using petroleum ether, chloroform, methanol, and distilled water. The maximum antibacterial effect of the extract obtained from the petroleum ether of the plant's skin was reported to be 41.33±0.88 mm, while the extract from the petroleum ether of the leaves showed the highest effect against *P. aeruginosa* with an average non-growth halo diameter of 49.33±0.66 mm. The chloroform extract of the skin and the methanol extract of the fruit showed the highest effect against *S. aureus* with an average non-growth halo diameter of 31.33±0.88 mm, while the petroleum ether extract of the skin had the highest effect against *B. subtilis* with an average non-growth halo diameter of 58.33±0.88 mm. Significant antifungal activity was observed for all extracts of *L. indica* L. against

both strains of fungi. Also, Ajaib *et al*. evaluated the antioxidant potential of the fruit, skin, and leaves of the this plant through phytochemical screening, antimicrobial, and antioxidant assays. They assessed the antioxidant activity of this plant using ABTS activity and DPPH radical scavenging activity. The results showed that the highest amount of ABTS, equivalent to 7.946 ± 0.04 mM trolox, was found in the aqueous extract of the leaves, while the highest amount of DPPH, with a mean of 92.92 ± 0.08 percent, was related to the aqueous extract of the bark. Furthermore, the antioxidant activity of the aqueous-methanolic extract of polysaccharide-free leaf (extract A) and polysaccharide-containing extract (extract B) of *L. indica* was evaluated. Extract A showed significantly higher antioxidant activity than extract B at a concentration of 100 mg. They emphasized that the strong antioxidant activity of the *L. indica* extract can be attributed to its phenolic compounds [5].

Xiang-mi and colleagues in 2015 investigated the antioxidant activity of the flower of *Lagerstroemia indica* using DPPH radical scavenging, ABTS radical scavenging, and FRAP assay. The results showed that the flowers of *L. indica* have good antioxidant activity under laboratory conditions. The ethyl acetate extract of *L. indica* showed the highest antioxidant activity, with high DPPH radical scavenging activity (IC50 = 7.4 µg/ml), ABTS radical scavenging activity (IC50 = 8.1 µg/ml), and reducing power of iron equivalent to 2664.7µmol/g [8].

Diab *et al*. (2012) conducted an antimicrobial screening of some Egyptian plants and active flavonoids from *L. indica* leaves against *S. aureus* (ATCC 8095), *Salmonella enteritidis* (ATCC 13076), *Escherichia coli* (ATCC 25922), *Listeria monocytogenes* (ATCC 153112), and *Candida albicans* (ATCC 10231) using the disc diffusion method and determination of minimum inhibitory concentration (MIC) by microbroth dilution method (tube dilution method in liquid medium). They found that the methanolic extract of *L. indica* leaves showed antimicrobial activity against all tested microorganisms, and the flavonoid quercetin exhibited the highest activity against *S. aureus* and *C. albicans*. The results suggest that *L. indica* leaves and their flavonoids have potential as natural antimicrobial agents. The methanolic extract of *L. indica* leaves exhibited antimicrobial activity against all tested microorganisms. A pure active compound was obtained by purifying the methanolic extract of the Goltori leaf, called "4-methoxyapigenin-8-D-β-C-glucopyranoside, cynaroside." The minimum inhibitory concentration (MIC) of this active compound, cynaroside, against *Candida albicans* was 32 µg/ml, against *S. aureus* was 16 µg/ml, against *S. enteritidis* was 16 µg/ml, against *E. coli* was 16 µg/ml, and against *L. monocytogenes* was also 16 µg/ml [9].

MATERIALS AND METHODS

Preparation of Samples

In the autumn of 2021, the flowers and leaves of the *L. indica* L. were collected from the herbal plantations of the Islamic Azad University, Ayatollah Amoli Branch, located in Amol city. After cleaning and separating the damaged parts, they were spread on a clean white cloth to dry under shade and away from sunlight in free air conditions. Then, they were packaged and prepared for the extraction process (Fig. 1).

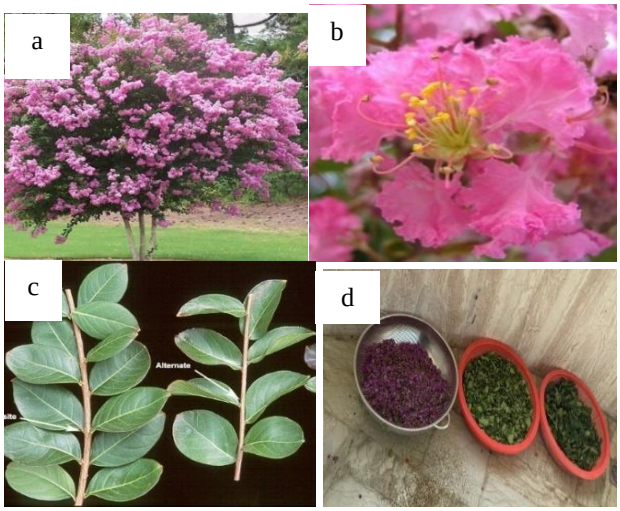

Fig. 1 *L. indica* L. herb: (a) shrub, (b) flower, (c) leaf, (d) dried leaf and flower.

Preparation of Bacterial and Fungal Suspension

Lyophilized vials of *S. aureus* with PTCC=1431, *Escherichia coli* with PTCC=1769, *S. typhimurium* with PTCC=1609, Streptococcus mutans with PTCC=1683, *A. flavus* with PTCC=5006, and *C. albicans* with PTCC=5027 were purchased from the microbial and fungal collection of the Iranian Research Organization for Science & Technology (IROST) under sterile conditions in a laminar hood. The ampoules were shattered from the site of the

cotton ball, and the surrounding area was completely disinfected with 70% alcohol. Then, 2 milliliters of autoclaved culture medium were added to the dry substance inside the ampoule using a syringe, and after uniformity, they were transferred onto the following culture media: Nutrient Broth, Nutrient Agar, Trypticase Soy Agar, Potato Dextrose Agar, and Yeast Mold Agar. The cultures were then placed in an incubator at 37 ^{O}c for 24 hours for bacteria and at 28-25 ^{O}c for 72 hours for fungi. The newly grown microorganisms from the culture medium were streaked linearly onto the aforementioned solid culture media using a sterilized loop and placed inside the incubator to be used as a source of bacteria and fungi [10].

Extraction of Extract by Maceration Method

The extract of *L. indica* L. plant was obtained using the method of immersion in 70% hydroethanolic solvent. 500 mL of the solvent were added to 100 g of the plant's leaves and flowers in a closed Erlenmeyer flask. The resulting mixture was stirred for 72 hours using a magnetic stirrer. After the 72-hour time period, the extracts were separated from the solid portion using regular filter paper. The extract was initially concentrated using a rotary evaporator under vacuum and then stored in amber glass vials in the freezer until use. The extraction efficiency was determined according to the following formula:

$$100\times\frac{Extract\ with\ balloon\ weight-Dry\ extract\ without\ balloon\ weight}{The\ total\ weight\ of\ the\ plant\ has\ been\ used}$$
=Efficiency

To prepare disks containing the extract, blank Padtan Tib disks were used. The blank disks were placed in tubes containing predetermined concentrations of the extract. After 3-5 minutes, when the extract was completely absorbed, the disks were placed at 37 ^{O}c to dry and prepare them for disk diffusion. The necessary tools for performing various stages of antimicrobial effects evaluation were sterilized in an autoclave at a temperature of 121 ^{O}C and a pressure of 15 Ib/In^2 for 15 minutes. All materials used in this study were of high purity and obtained from companies such as Merck, PadtanTeb, and Roushco [11].

Investigating the Diameter of the Lack of Growth Halo by Disk Diffusion Method

To dilute the extract of the medicinal plant *Lagerstroemia indica L.*, sterile distilled water was used. Different concentrations of 400, 200, 100, 50, 25, 12.5, and 6.25 μL/ml of extract were prepared by diluting with distilled water as a solvent. The antibacterial and antifungal properties of the diluted extracts were evaluated separately for each microorganism. A suspension with a concentration of 1.5×10^8 of each bacterium and fungus was prepared in physiological serum. Then, using sterile swabs, the surfaces were cultured on Mueller-Hinton agar for bacteria and Sabouraud dextrose agar for fungi. Blank sterile discs were impregnated with different treatments of the herbal extract of *L. indica* L. and placed on the surface of the culture medium to investigate the zone of inhibition of the studied bacteria and fungi. The plates, along with a control group consisting of one plate containing antibiotic and antifungal discs for bacteria and fungi as positive controls, and a sterile distilled water blank disc as a negative control, were incubated in a 37±2 ^{O}C incubator for 24 hours to examine the growth inhibition zone of each bacterium and fungus [12].

Determining the Minimum Inhibitory Concentration (MIC)

To determine the minimum inhibitory concentration (MIC) of flower and leaf extracts of *L. indica* L., a series of sterile test tubes containing 1 mL of Mueller Hinton agar and Sabouraud dextrose agar were used in a serial dilution method. 1 mL of the Lagerstroemia extract was added to the first tube, and then 1 mL of the content of the first tube was added to 1 mL of the second tube containing the culture medium. This process continued until 2/1 serial dilutions were made in the tubes. At the end of the process, 1 mL of the content of the last tube was discarded. It should be noted that two test tubes were used: one as a positive control (without the extract, which becomes turbid upon the addition of bacteria and fungi and their growth) and the other as a negative control using a mixture of culture medium and plant extract. A total of 9 test tubes were used (7 tubes for different extract treatments and 2 tubes as positive and negative controls). The effect of active ingredients on the growth of *S. aureus*, *E. coli*, *S. typhi*, *S. mutans*, and *A. flavus*, and *C. albicans* fungi, was compared with the cloudiness of the control tube. In the next step, 20 µL of microbial suspension were added to all tubes, and they were incubated at 37 °C for bacteria and 25 °C for the studied fungus for 48 hours and 72 hours, respectively. After incubation, the growth and

turbidity in the tubes were compared with the turbidity observed in the control positive and negative tubes. It should be noted that the seventh tube is examined first, and if bacterial or fungal growth and turbidity are observed, it indicates the correctness of the work. However, if turbidity is not observed in the positive control tube, it indicates a problem in the inoculation of microorganisms, and the process will be repeated. The last tube before the one in which turbidity was observed was considered as the minimum inhibitory concentration (MIC) of the herbal extract of Torilorus plant on the studied bacteria and fungi. To determine the minimum inhibitory concentration (MIC) of the antibacterial agent, 10 µL of the MIC tube and other tubes without turbidity were cultured in Mueller-Hinton agar medium and incubated for 24 hours at 37±2 °C in an incubator. The lowest concentration at which no growth was observed was considered as the minimum bactericidal concentration (MBC) [13].

Determination of Minimum Fungicidal Concentration (MFC)

To determine the minimum fungicidal concentration (MFC), 10 µL of the microbial suspension from the MIC tube and other tubes without turbidity were added to Sabouraud dextrose agar culture medium and incubated at 28±2 °C for 48 hours. The lowest concentration at which no growth was observed was considered as the MFC [13].

Measurement of Total Phenolic Content

In this method, the total phenolic compounds were measured using the Folin-Ciocalteu method. The Folin reagent is a mixture of phosphomolybdic/phosphotungstic acid that is reduced to molybdenum oxide and tungsten by phenolic compounds at basic pH, leading to the formation of blue-colored products with maximum absorption at 720 nm. Gallic acid was used as a standard in this measurement, and its standard curve was plotted. Then, the phenolic content of the extracts was determined using the obtained line equation [14].

Measurement of Total Flavonoid Content

In this method, the total flavonoid content of the extracts was determined based on the Chang method using aluminum chloride as the reagent. Aluminum chloride forms stable acidic complexes with the ketone group at position 4 and the hydroxyl group at position 3 or carbon 5 in flavones and flavonols, as well as unchangeable acidic complexes with ortho-hydroxyl groups in the A or B rings of flavonoids, which have maximum absorption at a wavelength of 415 nm. The amount of flavonoids present in each sample was determined using the standard curve [15].

DPPH Test

The electron-donating ability of hydrogen atoms was assessed based on their capacity to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals. In this method, the change in color of the DPPH free radical was measured using a spectrophotometer. DPPH is a stable radical that acts as an oxidizing agent in redox reactions. The oxidized form of this reagent is purple and absorbs at around 515-520 nm, while the reduced form is yellow. Substances with a higher reducing potential than DPPH can reduce it, causing a change in color from purple to yellow [16].

Statistical Analysis

In this study, we evaluated the antioxidant and antimicrobial properties of the aerial parts extract of the *L. indica* L. plant using a completely randomized design at different concentrations of 400, 200, 100, 50, 25, 12.5, and 6.25 µL/ml with three replicates of each treatment. We analyzed the obtained results using one-way ANOVA, and means were compared using the Duncan multiple range test at a significance level of ($P< 0.05$). We performed the statistical analyses using SPSS v.25 software, and we drew the graphs using Microsoft Excel software.

Table 1 Extraction Efficiency in Investigated Parts of Goltori Medicinal Plant.

Organ under investigation	The color of tree flowers	Extract efficiency (%)
Flowers	purple	6.50
	Pink	6.37
Leaves	Purple	7.24
	Pink	7.30

RESULTS

Extraction Efficiency

Table 1 shows the extraction efficiency of the Goltori medicinal plant in the investigated parts.

Antibacterial and Antifungal Effects of the Extract of the *L. indica* L. by Disk Diffusion Method

The results of the statistical analysis of the comparative study of the effect of *L. indica* L. extracts on the bacteria *S. aureus*, *E. coli*, *S. typhi*, *S. mutans*, and the fungi *A. flavus* and *C. albicans* are presented in Table 2,3. As can be seen in Table 2, the extracts of this plant had significant antibacterial effects on *S. aureus*. Among the different extracts, the extracts obtained from purple flowers and pink leaves had the highest and lowest diameter of growth inhibition zones, respectively. In the extracts of purple and pink flowers and purple leaves, concentrations of 25 and 12.5 µg/ml were used, while in the extract of pink leaves, a concentration of 100 µg/ml was used. Furthermore, concentrations of 50 and 200 µg/ml were used, in addition to concentrations of 6.25 and 12.5 µg/ml, which did not show a statistically significant difference from each other. Additionally, the comparative study results showed that the extract of purple flowers had a greater effect on the bacterium *Escherichia coli* at concentrations higher than 500 µg/ml compared to other extracts. The diameter of the growth inhibition zone in the extracts obtained from the flowers had a greater effect compared to the extracts obtained from the leaves. The maximum diameter of the growth inhibition zone was observed in the extracts of purple flowers, while the minimum diameter was observed in the extract of pink leaves. It has been determined that the extract of this medicinal plant has an effect on *S. typhi* bacteria, and the extract of purple flowers has shown greater effects compared to other extracts. Some concentrations in each extract have not shown statistically significant differences with each other. The maximum diameter of the zone of inhibition was observed in the extract obtained from purple flowers, while the minimum diameter was observed in the extract obtained from pink leaves. Furthermore, the analysis of variance of the comparative study of the effect of extracts of the medicinal plant on *Streptococcus mutans* bacteria demonstrated significant differences between some concentrations of the extracts. The examined extracts have shown suitable antibacterial effects.

The extracts of purple flowers have been more effective at concentrations greater than 50 µg/ml, while the extracts from leaves of the purple flower have been more effective at concentrations less than 50 µg/ml. In addition, the data presented in Table 3 show the results of the investigation of the effect of extracts from this plant on *Aspergillus flavus* fungus. The data indicate that the examined extracts have suitable antifungal effects. Among the different extracts, those extracted from purple flower and pink leaf had the largest and smallest diameter of growth inhibition zone, respectively. In each extract, some concentrations had no statistically significant difference with each other. It has also been determined that this plant has an effect on the *Candida albicans* fungus. The extracts from the purple flowers and leaves have had greater antifungal effects compared to the pink flowers and leaves. Among the various extracts, the ones extracted from purple and pink flowers have had the highest and lowest diameter of inhibition zone, respectively. In each extract, some concentrations have not had a statistically significant difference with each other.

Comparison of the Effect of Different Concentrations of *L. indica* L. Extract on Determining the MIC, MBC, and MFC of the Studied Microorganisms.

Based on the results presented in figure 2, the hydroalcoholic extracts of *L. indica* L. showed an inhibitory effect on the growth of the studied bacteria, The extract of purple flower showed the highest inhibitory effects on Staphylococcus aureus at a minimum inhibitory concentration (MIC) of 12.5 µg/ml, and had greater bactericidal effects on *S. aureus* at a concentration of 25 µg/ml and on *A. flavus* at a concentration of 50 µg/ml compared to the other extracts studied.

Measurement of Total Phenol and Flavonoid Content

As shown in Table 4, the mean total phenol content in the extract of purple flower was 369.230 mg of gallic acid equivalent per gram of extract, which was the highest value, while the extract of purple leafwith a mean of 310.600 mg gallic acid equivalent per gram of extract had the lowest total phenol content among the studied extracts.

Table 2 Results of the effect of different treatments of tagetes extract on bacteria.

Bacteria																Treat ments
Streptococcus mutans				*S. typhi*				*E. coli*				*S. aureus*				
leaf		Flower		leaf		Flower		Leaf		Flower		leaf		Flower		
pink	purple	pink	purple	pink	purple	pink	purple	Pink	purple	pink	purple	pink	purple	pink	purple	
19.000±1.000b	19.000±1.000b	19.000±1.000b	19.000±1.000b	24.000±1.000a	24.000±1.000a	24.000±1.000a	24.000±1.000a	11.500±0.500c	11.500±0.500c	11.500±0.500cd	11.500±0.500c	23.330±1.527a	23.330±1.527a	23.330±1.527a	23.330±1.527a	Ciprofloxacin
26.000±1.000a	26.000±1.000a	26.000±1.000a	26.000±1.000a	20.667±1.154b	20.667±1.154b	20.667±1.154b	20.667±1.154b	15.667±1.154a	15.667±1.154a	15.667±1.154a	15.667±1.154a	27.330±1.516b	27.330±1.516b	27.330±1.516b	27.330±1.516b	Gentamicin
14.500±0.763c	15.167±0.763c	15.667±0.500c	16.500±1.041c	13.500±0.500c	12.167±0.214c	12.320±0.500c	13.940±0.500c	12.500±0.500b	13.500±0.500b	13.500±0.500b	13.833±0.288b	17.167±0.763c	18.500±0.500c	19.167±0.768c	23.000±0.577c	400
13.833±0.276c	13.176±0.276d	14.833±0.275c	14.500±1.063d	12.500±0.500cd	11.160±0.288c	11.680±0.288d	12.950±0.500d	10.833±0.288c	11.833±0.288c	12.333±0.288c	12.620±0.500c	15.000±0.500d	17.167±0.288d	16.500±0.500cd	20.833±1.000d	200
12.750±0.500d	12.500±0.500de	13.500±0.763d	14.167±0.763de	10.330±0.500de	10.960±0.500d	11.500±0.321e	11.650±0.288e	9.667±0.197d	10.333±0.288d	10.667±0.763d	11.000±0.288d	13.750±0.500de	16.167±0.288e	14.500±0.500d	18.500±0.500	100
11.930±0.500e	11.620±0.500e	11.500±0.288d	13.000±0.735ef	10.000±0.500e	9.167±0.301de	8.330±0.292f	10.650±0.265f	8.333±0.265d	9.000±0.500e	9.167±0.763e	9.333±0.288d	12.500±0.500ef	13.833±0.763e	12.833±0.763e	16.500±0.500f	50
9.500±0.866f	12.333±0.866f	9.333±0.263e	7.500±0.500f	8.333±0.763f	8.167±0.210e	7.167±0.262g	8.333±0.273f	7.430±0.241e	7.833±0.288f	7.500±0.500f	8.000±0.500e	10.167±0.288f	11.500±0.500f	9.833±0.288f	14.167±0.763g	25
8.500±0.500f	10.333±0.500f	8.167±0.245f	6.8333±0.288g	6.667±0.265g	6.500±0.500f	6.330±0.211gh	6.667±0.288g	7.000±0.235ef	7.167±0.288fg	7.000±0.500fg	7.000±0.500f	8.000±0.500g	10.500±0.500f	8.833±0.288f	12.500±0.500g	12.5
6.833±0.288g	7.667±0.288f	6.500±0.500g	6.167±0.325h	6.000±0.000g	6.167±0.235f	6.000±0.000h	6.000±0.000g	6.150±0.221f	6.333±0.288g	6.000±0.000g	6.333±0.288f	6.667±0.288g	8.000±0.500g	8.167±0.288g	10.167±0.288h	6.25

Table 3 Results of the effect of different treatments of tagetes extract on fungus

Fungus								
C. albicans				*A. flavus*				Treatments
leaf		Flower		Leaf		Flower		
pink	purple	pink	purple	pink	Purple	pink	purple	
24.000 ± 1.000 b	24.000 ± 1.000 b	24.000 ± 1.000 b	24.000 ± 1.000 b	22.667 ± 1.527 a	22.667 ± 1.527 a	22.667 ± 1.527 a	22.667 ± 1.527 a	Clotrimazole
37.333 ± 3.055 a	37.333 ± 3.055 a	37.333 ± 3.055 a	37.333 ± 3.055 a	16.497 ± 1.154 b	16.497 ± 1.154 b	16.497 ± 1.154 b	16.497 ± 1.154 b	Miconazole
18.000 ± 1.000 c	18.333 ± 0.763 c	17.500 ± 0.500 c	18.833 ± 0.763 c	14.333 ± 0.612 c	14.596 ± 0.574 c	16.000 ± 1.000 b	17.600 ± 0.564 b	400
16.333 ± 0.577 cd	16.000 ± 0.500 d	15.000 ± 0.500 d	16.667 ± 0.557 d	13.000 ± 0.500 d	13.330 ± 0.63 2d	13.833 ± 0.763 c	15.795 ± 0.577 c	200
14.667 ± 0.597 d	14.833 ± 0.288 de	13.500 ± 0.500 d	15.500 ± 0.500 de	12.210 ± 0.288 d	12.167 ± 0.288 de	12.930 ± 0.288 d	13.333 ± 0.549 d	100
11.500 ± 0.500 e	13.500 ± 0.500 e	11.500 ± 0.500 e	13.500 ± 0.500 ef	10.150 ± 0.577 e	11.000 ± 0.500 ef	11.890 ± 0.030 d	12.050 ± 0.288 d	50
8.333 ± 0.279 f	10.833 ± 0.265 f	8.833 ± 0.288 f	12.500 ± 0.500 f	8.350 ± 0.500 f	9.833 ± 0.292 f	9.360 ± 0.265 e	10.594 ± 0.326 e	25
7.500 ± 0.500 f	9.167 ± 0.274 fg	7.000 ± 0.500 fg	9.667 ± 7.000 g	7.167 ± 0.288 fg	7.333 ± 0.201 g	7.333 ± 0.288 ef	8.500 ± 0.500 f	12.5
6.333 ± 0.249 f	6.667 ± 0.269 g	6.167 ± 0.288 g	7.333 ±0.288 h	6.000 ± 0.000 g	6.667 ± 0.288 g	6.000 ± 0.000 f	7.000 ± 0.500 g	6.25

*Numbers with common letters in each column do not have statistically significant differences with each other (P<0.05).

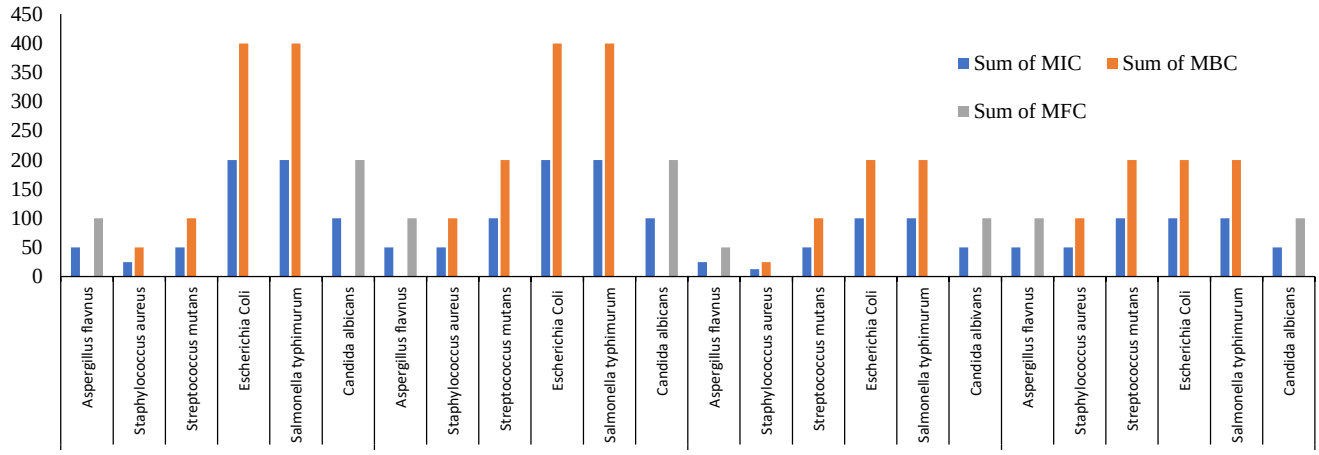

Fig. 2 Comparison of the effects of different concentrations of *L. indica* L. extract on determining the minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC), and minimum fungicidal concentration (MFC) of the studied microorganisms.

Table 4 Mean values of total phenols and flavonoids in *L. indica* L. extract

Flavonoid (mg QE/g EXT)	Total Phenol (mg GAE/g EXT)	*L. indica* L. extract
24.100 ± 0.480 a	369.230 ± 3.257 a	purpel flower
21.720 ± 0.819 b	365.606 ± 5.845 a	pink flower
19.146 ± 0.436 c	310.600 ± 2.724 b	purple leaf

In addition, the mean flavonoid content in the extract of purple flower was 24.100 mg/g equivalent to gallic acid, which was the highest among the studied extracts, while the extract of purple leaf had the lowest amount with a mean of 19.146 mg/g equivalent to gallic acid.

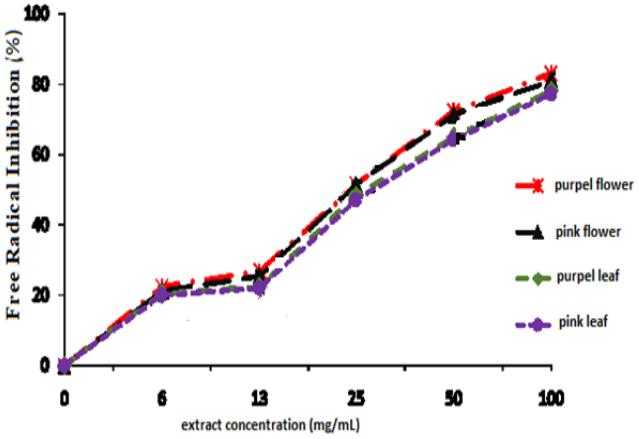

Fig. 3 Comparison of mean DPPH free radical scavenging percentage at different concentrations of *Lagerstroemia* extracts.

Percentage of Free Radical Inhibition

The statistical analysis of the percentage of DPPH free radical scavenging activity at concentrations ranging from 6.25 to 100 µg/ml of different *Lagerstroemia* extracts is presented in Figure 3. Some of the treatments did not show significant differences from each other. With the increase of extract concentration, the percentage of DPPH free radical scavenging activity also increased, such that at concentrations of 100 µg/ml, the extracts from purple flowers and pink leaves had the highest and lowest percentages of free radical scavenging activity, respectively.

DISCUSSION

The results of the statistical analysis of the comparative study of the effects of extracts of *L. indica* L. on *S. aureus* showed that the examined extracts had significant antibacterial effects. Among the extracts, those extracted from purple flowers and pink leaves had the highest and lowest zone of inhibition diameter, respectively. In the extracts of purple and pink flowers and purple leaves, the concentrations of 25 and 12.5 µg/ml, and in the extract of pink leaves, the concentration of 100 µg/ml, showed no significant statistical difference with concentrations of 50, 200, and 6.25 and 12.5 µg/ml.

In this regard, Ajaib *et al*. investigated the antimicrobial activity of the extract of *L. indica* L. skin, leaf, and fruit extracted by petroleum ether, chloroform, methanol, and distilled water on *S. aureus*. They demonstrated that the extracts obtained from the *Lagerstroemia* plant had

significant effects on *S. aureus*. The chloroform extract of skin and methanol extract of fruit had the highest effect on *S. aureus* with an average zone of inhibition diameter of 31.33 ± 0.88 mm. The results of the investigation of the antibacterial effect of *Lagerstroemia* extract on *E. coli* showed that at concentrations higher than 500 µg/ml, the extract of *Lagerstroemia* purple flowers had a greater effect compared to other extracts, and the zone of inhibition diameter in the extracts obtained from the flowers was greater than that of the leaves. The maximum diameter of the growth inhibition zone was observed in the extract of purple flowers, while the minimum diameter was observed in the extract of pink leaves [5].

Diab *et al*. showed that the methanolic extract of *L. indica* L. leaf had significant antimicrobial effects against *E. coli*, with a minimum lethal concentration of 16 µg/ml. In each extract, some concentrations did not show statistically significant differences with each other. The largest zone of inhibition diameter was observed in the extract of purple flowers, while the smallest zone of inhibition diameter was observed in the extract of pink leaves [9].

In 2013, Chandra investigated the antimicrobial effects of methanolic and aqueous extracts of *L. indica* L. leaf against 5 human pathogenic bacteria using the disk diffusion method. The mean diameter of the inhibition zone of the methanolic extract of *Lagerstroemia* leaf against *S. aureus, S. typhi, K. pneumoniae, P. vulgaris*, and *P. aeruginosa* was 12, 12, 13, 20, and 16 mm, respectively. In contrast, the mean diameter of the inhibition zone of the aqueous extract was 8, 6, 9, 5, and 7 mm against the same microorganisms. The results of research on the antibacterial effects of the extract of *L. indica* L. on *Streptococcus mutans* showed that the tested extracts had significant antibacterial effects. The extracts from purple flowers at concentrations greater than 50 µg/ml exhibited stronger effects, while the leaf extract of the purple flower plant had stronger effects at concentrations less than 50 µg/ml. The results of the study on the antifungal effect of the extract of *L. indica* L. flowers on *Aspergillus flavus* showed that the extracts had suitable antifungal effects. Among different extracts, those extracted from purple flowers and pink leaves had the highest and lowest diameters of inhibition zones, respectively. In each extract, some concentrations did not show a statistically significant difference with each other [17].

In this regard, Ajaib *et al*. (2016) investigated the antimicrobial activity of the extract of skin, leaf, and fruit of *Lagerstroemia* extracted against the studied bacteria and fungi. The maximum diameter of growth inhibition zone with an average of 36 ± 3.21 mm against *Aspergillus oryzae* was observed by the extract of tree bark in distilled water. However, the highest antifungal activity against *Aspergillus flavus* with an average diameter of 40.33 ± 0.88 mm was observed by the extract of bark in chloroform. The results of the analysis of variance of the comparative study of the effects of herbal extracts of the *Lagerstroemia* plant on *Candida albicans* showed that the purple flower and leaf extracts had stronger antifungal effects compared to the pink flower and leaf extracts. Among the various extracts, those extracted from purple and pink flowers had the highest and lowest diameters of the inhibition zone, respectively. In each extract, some concentrations did not show a statistically significant difference with each other [5].

In this regard, Diab *et al*. (2012) demonstrated in a study that the methanolic extract of *Lagerstroemia* plant had significant antimicrobial effects on *Candida albicans* fungus at a concentration of 32 µg/ml, which is relevant to the results of this study on *Candida albicans* yeast. The results of the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) tests indicated that the extract of purple flowers had the lowest MIC and MBC values of 12.5 and 25 µg/ml, respectively. This extract showed stronger inhibitory and bactericidal effects on *S. aureus* compared to other bacterial strains studied. Additionally, the examination of the minimum fungicidal concentration (MFC) revealed that the purple flower extract had the lowest fungicidal concentration of 50 µg/ml and demonstrated greater fungicidal effects on *A. flavus* compared to other studied extracts. The investigation on the antioxidant activity of the extracts revealed that the average total phenol content in the purple flower extract was 369.230 mg GAE/g extract, which was the highest among all extracts. In contrast, the leaf extract from the pink flower plant had the lowest amount of total phenols, with an average of 300.247 mg GAE/g extract. In terms of the mean total phenol content, total flavonoids, and DPPH free radical scavenging

percentage, the extract of purple flowers had the highest amount of total phenol, with an average of 369.230 mg GAE/g extract, while the extract of pink flowers had the lowest amount, with an average of 300.247 mg GAE/g extract. The mean amount of flavonoids in the extract of purple flowers was the highest, with 24.100 mg CE/g extract, while the extract of pink flower plant had the lowest amount, with an average of 18.180 mg CE/g extract. The DPPH free radical scavenging percentage in the concentrations of 100 µg/ml of purple flower and pink leaf extracts had the highest and lowest radical scavenging percentage, respectively [9].

In 2015, Xiang-mi *et al*. showed that *Lagerstroemia* flowers have good antioxidant activity under laboratory conditions. The ethyl acetate extract of *Lagerstroemia* showed the highest antioxidant activity and had high DPPH radical scavenging activity (IC50 = 0.4 µg/ml) and ABTS radical scavenging activity (IC50 = 8.1 µg/ml). Additionally, Wei *et al*. (2020) identified a total of 114 compounds in *Lagerstroemia* plant and demonstrated that the extracted essential oil from pink, orange, and carmine flowers showed significant antimicrobial effects against *S. aureus*, *P. aeruginosa*, *E. coli*, *B. subtilis*, and *A. niger fungi* with a minimum inhibitory concentration of 0.078 mg/ml [8].

Overall, the results of this study indicate the presence of antimicrobial and antioxidant effects in the varieties of *Lagerstroemia* flowers under investigation. Flowers exhibited higher antimicrobial and antioxidant effects compared to leaves, and purple flowers had greater effects than pink flowers. The extracts prepared from *Lagerstroemia* flowers had a significantly greater impact on gram-positive bacteria compared to gram-negative bacteria.

Given that drug resistance is considered a serious threat to human health, and individuals with weakened immune systems are more vulnerable, based on the results of this study and similar studies, it can be acknowledged that extracts obtained from traditional medicinal plants have inhibitory and sometimes complete bactericidal effects on various microorganisms. Therefore, the use of these extracts, including *Lagerstroemia*, as an antimicrobial combination, is recommended.

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