Bacteria are becoming more and more resistant to antibiotics due to the overuse of these agents, turning antibiotic resistance as a global problem. Therefore, novel antimicrobial agents that can effectively and safely eradicate pathogenic bacteria from the body have acquired great attention by researchers around the world. To replace antibiotics, plants are now used as sources of antimicrobials. Plants’ active components, e.g., essential oil, phenols, quinones, alkaloids, terpenoids, flavonoids, tannins, glycosylates, lignans, and some secondary metabolites, have been noted to participate in their activities against bacterial growth. In fact, during ancient times, many people used herbs as natural sources of medicines to treat human ailments. The biological roles identified for herbs’ ingredients have been anti-inflammatory, antiviral, antifungal, antibacterial, anticarcinogenic, antimutagenic, and antiallergic activities. Plant extracts due to effective antimicrobial effects are widely utilized for food preservation, as well as to manage a variety of infectious and non-infectious diseases. The members of the plant family of Verbenaceae have been used to treat ailments since ancient times.

Introduction

**Bacteria** are becoming more and more resistant to antibiotics due to the overuse of these agents, turning antibiotic resistance as a global problem. Therefore, novel antimicrobial agents that can effectively and safely eradicate pathogenic bacteria from the body have acquired great attention by researchers around the world. To replace antibiotics, plants are now used as sources of antimicrobials. Plants’ active components, e.g., essential oil, phenols, quinones, alkaloids, terpenoids, flavonoids, tannins, glycosylates, lignans, and some secondary metabolites, have been noted to participate in their activities against bacterial growth. In fact, during ancient times, many people used herbs as natural sources of medicines to treat human ailments. The biological roles identified for herbs’ ingredients have been anti-inflammatory, antiviral, antifungal, antibacterial, anticarcinogenic, antimutagenic, and antiallergic activities. Plant extracts due to effective antimicrobial effects are widely utilized for food preservation, as well as to manage a variety of infectious and non-infectious diseases.

The members of the plant family of Verbenaceae have been used to treat ailments since ancient times. **Lantana**

**Abstract**

**Introduction:** Today, the resistance of pathogens to antibiotics, as well as the side effects of chemical drugs, have led to our increasing use of medicinal plants. So, our purpose was to investigate the antibacterial and antifungal effects of leaves and flowers’ methanolic extracts of *Lantana camara* L.

**Methods:** Iran’s National Botanical Garden (Tehran, Iran) provided the plant, whose leaves and flowers’ methanolic extracts were prepared by the percolation method. The antimicrobial activity was measured by the disc-diffusion method against some bacteria (gram-negative and gram-positive), as well as fungi. Minimum inhibitory concentration (MIC) was determined by the serial dilution method and determining the diameter of the growth inhibition zone. Data were analyzed by Duncan test, and *P* < 0.05 was designated as the significance level.

**Results:** The results confirmed that the extracts had antimicrobial activities against the microorganisms tested with more potent activity against gram-positive bacteria, gram-negative bacteria, and then fungi, respectively. Antimicrobial activity increased with rising the concentration of the extracts. The extracts of leaves and flowers showed comparable antimicrobial activity (*P* > 0.05). *Staphylococcus aureus* showed the highest sensitivity at the 50% concentration of the leave and flower extracts (29.66 ± 1.15 and 30.33 ± 0.66, respectively), and *Escherichia coli* showed the least sensitivity at the 6.25 % concentration of the leave and flower extracts (2.66 ± 0.88 and 1.00 ± 1.15, respectively). The most potent antifungal activity was seen against *Candida albicans* at the 50% concentration of the leave extract (14.00 ± 0.45), and the lowest antifungal activity was seen against *Aspergillus niger* at the 12.25% concentration of the flower extract (1.33 ± 0.66).

**Conclusion:** *Lantana camara* leaves and flowers’ extracts showed potential to be used as antimicrobial agents against bacterial and fungal infections.

**Keywords:** Antibacterial activity, Antifungal activity, *Lantana camara* L., Methanolic extract

**Investigating of Anti-bacterial and Anti-fungal Activities of Lantana camara L. Against Human Pathogens**

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*L. camara* is a species of this family that primarily grows in America and Africa. It has been shown that the leaf extract of this herb can be effective to alleviate toothache, bellyache, pneumonia, and rheumatism, as well as wound infections and a number of other conditions.6

Phytochemical studies on *L. camara* constituents have successfully identified a wide range of biologically active ingredients, including flavonoids, fatty acids, and many terpenes.24 The main ingredients of this plant are β-caryophyllene (23.3%), α-humulene (11.5%), γ-curcumin (6.3%), germacrene D (10.9%), and davanone (7.3%).9

*Lantana camara* has been reported to have anti-protozoal,4 anti-oxidant,8 insecticidal,19 anti-viral,12 and antimutagenic12 activities, along with allelopathic features. Based on the points noted, we conducted this study to investigate the antibacterial and antifungal effects of the methanolic extract of *L. camara* leaves and flowers on some pathogenic bacterial and fungal strains.

**Materials and Methods**

In this experimental study, the disk diffusion method was used, and the methanolic extracts of the leaves and flowers of the plant were evaluated separately by this method in terms of antimicrobial activity.

**Plant Material**

The leaves and flowers of *L. camara* were prepared from the National Botanical Garden of Iran (Tehran Iran) in 2018.

**Preparation of Extracts**

The leaves and flowers of the plant were dried in shadow and then powdered. Fifty grams of each powder was subjected to extraction by the percolation method and using methanol 80%. The powder was immersed in methanol 80% for one hour. Then it was incubated in the percolator for 48 hours before the liquid extract was harvested. The liquid extract was then concentrated by the use of a rotary system and dried in an oven at 40°C.13 This extract was considered pure (100%) and was used to prepare subsequent concentrations (50%, 25%, 12.5%, and 6.25%) using dimethyl sulfoxide (DMSO).13

**Bacterial Strains**

The microorganisms used in this study included two Gram-positive bacteria, *Staphylococcus aureus* (PTCC1112) and *Bacillus cereus* (PTCC1274), two Gram-negative bacteria, *P. aeruginosa* (PTCC1074) and *Escherichia coli* (PTCC1330), and two fungal strains, *Aspergillus niger* (PTCC16404) and *Candida albicans* (PTCC10231), which were obtained from the Microbiology Laboratory, Department of Biotechnology, Faculty of Pharmacy, University of Tehran, Tehran, Iran. The microorganisms were preserved on nutrient agar at 4°C.

**Preparation of Microbial Suspensions**

The standard McFarland was used as a reference to match the turbidity caused by the bacterial suspension so that a specific concentration of bacteria is obtained. The 0.5 McFarland standard was prepared by mixing 0.05 mL of dehydrated barium chloride (BaCl₂2H₂O) 1.175% and 9.95 mL of sulfuric acid (H₂SO₄) 1%, leading to the formation of barium sulfate precipitate.

To prepare the microbial suspension, microorganisms from fresh cultures were transferred into a tube containing sterile saline using a multi-colony sterile swab. After stirring the solution, the resulting turbidity was compared with the turbidity of the 0.5 McFarland solution. This procedure was performed separately for each microorganism.14

**Antibacterial and Antifungal Activity**

Antimicrobial activity was measured by the disc-diffusion assay using standard discs (6 mm diameter) containing agar-based culture media and by measuring growth inhibition zones.13 For this, sterile blank discs were placed inside plant extracts so that the extracts were completely absorbed by the disc.

Petri plates containing Muller Hilton Agar (MHA) for bacteria and Sabouraud Dextrose Agar (SDA) for fungi were prepared. Then the microbial suspension (0.5 McFarland) of each microorganism was prepared16 and inoculated on the surface of the petri plates by a sterile swab. Then the discs containing the plant extract were placed on the agar surface, and the plates were incubated at 37°C for 24 hours. By measuring the diameter of the growth inhibition zone (in millimeters) around the discs, the susceptibility or resistance of the bacteria and fungi to the extracts was determined. DMSO was used as a negative control,4 and gentamycin and nystatin were used as positive controls.13,15,17 The experiment was conducted in triplicate.

**Minimum Inhibitory Concentration**

Mueller Hinton Broth was used to determine minimum inhibitory concentration (MIC) of the plant extracts (1, 0.5, 0.25, 0.125, 0.0625, and 0.03125 mg/mL) by the twofold serial dilution method after 24 h of incubation at 37°C. The lowest concentration inhibiting the growth of the tested organism was designated as MIC.4,16

**Statistical Analysis**

Each experiment was performed at least three times, and the results were expressed as mean ± SD of three replications. The data were analyzed by the Duncan test at the significance level of P < 0.05.

**Results**

The mean of diameter of the growth inhibition zone was determined for the leaf and flower methanolic extracts of...
Zare

*L. camara* against bacteria and fungi (Figure 1).

The results of the antimicrobial effects of the leaves and flowers' methanolic extracts on four bacterial strains (gram-positive and gram-negative) and two fungal strains based on the measurement of the diameter of the growth inhibition zone have been noted in Tables 1 and 2. The results showed significant differences in the mean diameter of the inhibition zone for all microorganisms. Also, the MICs of the extracts have been shown in Tables 1 and 2.

As the results show, the level of antimicrobial activity increases with increasing the concentration of the extracts (Figures 2 and 3).

The results also showed that among the studied bacteria and fungi, antibacterial activity was higher than antifungal activity for both leaf and flower extracts. Among the bacteria, gram-positive bacteria were more sensitive to the leaf and flower extracts than gram-negative bacteria, and among fungi, *C. albicans* was more sensitive to these extracts than *A. niger*.

In general, the leaf and flower extracts in the studied concentrations showed high inhibitory effects on both bacteria and fungi, and there was no significant difference between the antimicrobial activity of the leaf and the flower extracts (*P* > 0.05).

The most potent antibacterial activity in the 50% concentration of the extracts was observed against *S. aureus* (29.66 ± 1.15, 30.33 ± 0.66), and *E. coli* showed the least sensitivity at the 6.25% concentration of the extract (2.66 ± 0.88, 1.00 ± 1.15). The most potent antifungal activity was seen against *C. albicans* at the 50% concentration of the leaf extract (14.00 ± 0.45), and the least antifungal activity was seen against *A. niger* at the 12.25% concentration of the flower extract (1.33 ± 0.66).

![Figure 1](image1.png) Growth Inhibition Zone Around the Discs Containing the Methanolic Extracts of *L. camara*. (a) Flower methanolic extract against *C. albicans* (1: positive control, 2: 50% concentration, 3: 25% concentration, 4: negative control). (b) Flower methanolic extract against *B. cereus* (1: positive control, 2: 50% concentration, 3: 12.5% concentration, 4: negative control). (c) Leaf methanolic extract against *E. coli* (1: positive control, 2: 25% concentration, 3: 12.5% concentration, 4: negative control).

**Table 1. Antimicrobial Activity of *Lantana camara* Leaf Extract**

<table>
<thead>
<tr>
<th>Bacteria and Fungi</th>
<th>Mean Zone of Inhibition [mm] ± SE</th>
<th>50%</th>
<th>25%</th>
<th>12.50%</th>
<th>6.25%</th>
<th>DMSO</th>
<th>Gentamycin</th>
<th>Nystatin</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>29.66 ± 1.15</td>
<td>24.66 ± 1.33</td>
<td>20.33 ± 1.33</td>
<td>14.33 ± 0.18</td>
<td>-</td>
<td>37</td>
<td>0.0312</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>25.33 ± 1.33</td>
<td>21.33 ± 0.33</td>
<td>18.33 ± 0.15</td>
<td>16.00 ± 1.52</td>
<td>-</td>
<td>26</td>
<td>0.0625</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>19.66 ± 1.66</td>
<td>16.66 ± 0.33</td>
<td>10.00 ± 0.15</td>
<td>6.66 ± 0.33</td>
<td>-</td>
<td>12</td>
<td>0.0312</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>11.66 ± 0.88</td>
<td>8.33 ± 0.88</td>
<td>7.66 ± 1.66</td>
<td>2.66 ± 0.88</td>
<td>-</td>
<td>31</td>
<td>0.0625</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>9.00 ± 0.88</td>
<td>3.66 ± 0.88</td>
<td>2.33 ± 0.66</td>
<td>1.66 ± 0.88</td>
<td>-</td>
<td>30</td>
<td>0.125</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>13.00 ± 1.45</td>
<td>13.00 ± 1.15</td>
<td>7.00 ± 0.57</td>
<td>3.66 ± 0.88</td>
<td>-</td>
<td>28</td>
<td>0.0625</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 2. Antimicrobial Activity of *Lantana camara* Flower Extract**

<table>
<thead>
<tr>
<th>Bacteria and Fungi</th>
<th>Mean zone of inhibition [mm] ± SE</th>
<th>50%</th>
<th>25%</th>
<th>12.50%</th>
<th>6.25%</th>
<th>DMSO</th>
<th>Gentamycin</th>
<th>Nystatin</th>
<th>MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>30.33 ± 0.66</td>
<td>27.33 ± 0.88</td>
<td>22.66 ± 0.88</td>
<td>14.00 ± 2.51</td>
<td>-</td>
<td>37</td>
<td>0.0325</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Bacillus cereus</em></td>
<td>28.33 ± 0.33</td>
<td>26.66 ± 1.85</td>
<td>24.33 ± 0.33</td>
<td>16.00 ± 2.30</td>
<td>-</td>
<td>25</td>
<td>0.0625</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Pseudomonas aeruginosa</em></td>
<td>21.33 ± 1.45</td>
<td>15.66 ± 0.66</td>
<td>11.00 ± 1.45</td>
<td>5.66 ± 1.80</td>
<td>-</td>
<td>11</td>
<td>0.0312</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em></td>
<td>13.33 ± 0.88</td>
<td>9.66 ± 0.66</td>
<td>6.66 ± 0.33</td>
<td>1.00 ± 1.15</td>
<td>-</td>
<td>32</td>
<td>0.0625</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Aspergillus niger</em></td>
<td>7.00 ± 0.57</td>
<td>4.00 ± 0.57</td>
<td>1.33 ± 0.66</td>
<td>0.00 ± 0.00</td>
<td>-</td>
<td>38</td>
<td>0.125</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Candida albicans</em></td>
<td>14.00 ± 0.45</td>
<td>12.00 ± 0.57</td>
<td>5.33 ± 0.17</td>
<td>3.33 ± 0.33</td>
<td>-</td>
<td>35</td>
<td>0.0625</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: MIC, minimum inhibitory concentration; SE, standard error.
Discussion

From ancient times, herbs have been used as natural sources of medications to treat diseases because of their wide range biological activities, including antibacterial and anti-microbial features, secondary to their constituents such as secondary metabolites. Increasing resistance of pathogenic microorganisms to antibiotics has led studies towards producing plants-derived medicinal agents.

Different constituents of *L. camara* plant have been shown to possess biological activities, as well as medicinal and pharmacological properties secondary to the presence of terpenoids, quinones, steroids, flavonoids, alkaloids, and glycosides. The essential oils obtained from the leaves and flowers of this plant have been found to be rich in compounds such as bisabolene, 1,8 cineole, limonene, phellandrene, carvone, germacrene D, bicyclogermacrene, curcumin, humulene, β-caryophyllene sesquiterpenes, and zingiberene.

In this study, the antibacterial and antifungal activities of *L. camara* were evaluated. So far, the antimicrobial properties of this plant have been studied by other researchers, who have reported different activities based on the type of the extract and the plant organ used. In the current study, the methanolic extracts of the leaf and flower of this plant were assessed. The results of this study agreed with those of Satish et al who confirmed the considerable antifungal activity of the plant extract prepared in various solvents.

Saraf et al studied the antibacterial activity of the methanolic and acetone extracts of the leaves and stem of *L. camara* and reported efficient antibacterial activity of stem methanolic extract of the plant against various gram positive and gram-negative bacteria, which was attributed to the high phytochemical capacities of the stem and leaf extracts of this plant, suggesting it as a potential antimicrobial drug.

As well, the antibacterial effects of the methanolic extract of *L. camara* leaves and flowers against the bacteria tested were similar to the results of Saraf et al suggesting that the active ingredients of the plant are dissolved well in methanol. In fact, flavonoids and flavanols, which are aromatic compounds, can be readily dissolved in ethanol and methanol.

Also, our findings are supported by the results of Jhariya and Kakkar, who tested flower alcoholic extracts of *L. camara* against a number of bacteria and reported the potent antibacterial activity of *L. camara* flower extract against *E. coli*, *S. typhi*, *P. aeruginosa*, *S. aureus*, and *B. subtilis*. Agrawal and Varma investigated the antibacterial activity of *L. camara* ethanolic extract against *S. aureus*, *S. typhi*, *P. aeruginosa*, *Klebsiella pneumoniae*, and *E. coli*. They reported that the ethanolic extract of *L. camara* leaves had moderate activity against *E. coli*. Also, in the present study, the methanolic extract showed less...
anti-bacterial activity against *E. coli* than other bacteria, which was similar to the report of Agrawal and Varma. Therefore, it seems that *E. coli* is less sensitive to the alcoholic extract of *L. camara* than other bacteria studied. Kulkarni et al evaluated the antifungal properties of the aqueous and ethanolic leaf extracts of *L. camara* against *A. niger* and *Rhizopus oryzae* and reported that the ethanolic extracts showed marked antifungal properties against these fungi. While the aqueous extract showed anti-fungal activity against *R. oryzae*, it had no effect on *A. niger*. Regarding the antifungal effects of *L. camara* methanolic leaf and flower extracts against *A. niger*, our results were similar to those of Kulkarni et al. Our results also agree with the report of Sailaja who reported that the methanolic extract of *L. camara* showed a high MIC against *A. niger*. Also, we found the studied extracts had antifungal activity against *C. albicans*, which agreed with the report of Das and Godbole who showed that the ethanolic and methanolic extracts of *L. camara* had high antifungal activity against *C. albicans*.

Pattnaik and Pattnaik reported various pharmacological activities for *L. camara*, such as antibacterial and anti-fungal activities and attributed them to the presence of secondary metabolites such as flavonoids, alkaloids, terpenoids, phenolic, palmitic acid, octadecanoic acid, lantanoid, lantanone, and lantanoside. Also, Saraf et al reported that the antimicrobial activity may be due to its triterpene secondary metabolites. Similarly, the antimicrobial activity of this plant against *S. aureus* and *S. typhi* was attributed to a bioactive triterpene- 22 beta acetoxyxylactic acid, as well as a number of other triterpenes. So, the antimicrobial activity of *L. camara* can be due to the presence of secondary metabolites, especially triterpenes and fatty acids in its leaves and flowers, which have good solubility in alcoholic compounds, especially methanol.

**Conclusion**

It can be concluded that the methanolic extracts of the leaves and flowers of *L. camara* were highly active against both Gram-positive and Gram-negative bacteria, as well as some fungi, probably due to the presence of some phytochemicals such as triterpenes, flavonoids, alkaloids, phytosterol, tannins etc. So, *L. camara* has a great potential to be used in the pharmaceutical industry for the production of antibacterial and antifungal herbal drugs against multi-drug resistant microorganisms.

**Acknowledgments**

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**Conflict of Interests**

None to declare.

**Ethical Approval**

No applicable.

**References**


