Investigation of the Resistance and Sensitivity of Infectious Bacteria to *Dendrostellera lessertii* and Chemical Composition Analysis

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Abstract

Introduction: Herbal plants are important sources for finding new and rare products of medicinal value for drug development. The present research aimed to investigate the antibacterial properties of *Dendrostellera lessertii* against infectious bacteria and analyze its chemical composition.

Methods: The different organs comprising root, stem, and leaf of *D. lessertii* from Lorestan province, Iran, were tested. Antibacterial activity was assessed using the agar well-diffusion assay. The total phenolic content (TPC) and total flavonoid content (TFC) were assessed by the Folin Ciocalteu and aluminum chloride methods, respectively. Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were tested by the serial dilution method, and chemical compositions were analyzed by gas chromatography-mass spectrometry (GC/MS).

Results: The chemical composition analysis showed the dominance of bergamotol (10.62%) and bis (2-ethylhexyl) phthalate (7.49%) in the stem extract. However, phytol (12.64%) and E-11-hexadecenal (12.53%) were major constituents in the root extract. Furthermore, major constituents in the leaf extract were phytol (19.658%) and hexadecanoic acid (7.151%). The methanolic extract of root exhibited the highest TPC as 109.1±2.2 mgGA/g and TFC as 2.1±0.33 mgQ/g. The root methanolic extract demonstrated a MIC of 3.125 mg/mL against *Enterococcus faecalis*. Accordingly, the highest sensitivity and resistance were observed on *E. faecalis* and *Pseudomonas aeruginosa*, respectively.

Conclusion: *Dendrostellera lessertii* extract is suggested as a source for antimicrobial drugs, especially to treat bacterial infections.

Keywords: *Dendrostellera lessertii*, GC/MS, Infectious bacteria, Secondary metabolites.

Introduction

Secondary metabolites of plants have been used to treat various infection diseases. Secondary metabolites are widely used for pharmaceutical, microbiology, and agricultural purposes, including as natural antibiotics against microbial growth and development. Children's mortality rate from 12.5 million in 1990 to 8.8 million in 2008 decreased due to the control of infectious diseases. Bacterial strains resistance against herbal drugs are slower compared to chemical drugs. The synthesis of chemical drugs and their widespread usage for treating diseases have been associated with side effects, including resistance against these drugs. Scientists have been attention to research on natural and herbal plants with medicinal properties. Antimicrobial, antioxidant, and anticancer properties of secondary metabolites including as phenol, flavonoid and tannin have been proven.

*Dendrostellera lessertii* is the only species of the genus *Denderostellera* (*Thymeleaceae*) with woody stems and 20-60 cm height, has been distributed in north and northwest of Iran. The species of this genus have been used for the treatment of diseases including as diabetes, skin diseases, rheumatism, and antihistamine. Antibacterial and antiradical properties of flower and root alcoholic extracts by Alamhulu and Nazeri, and the root, leaf and stem hydroalcoholic extracts of *D. lessertii* against some human pathogenic bacteria by Alamhulu and Nazeri have been reported in vitro. The important contributors have been attention to research on natural and herbal plants with medicinal properties.
compound as 3-hydrogenkwadaphnin from *D. lessertii* leaf inhibited the proliferation of acute myeloid leukemia KG1 cells at 5–30 nM concentrations after 24–96 hours treatment.  

In accordance, the flavonoids of *Phaleria macrocarpa* exhibited antimicrobial activity and inhibited DNA synthesis, plasmalemma function, and energy transport. Secondary metabolites including alkaloids, anthraquinones, triterpenoids, and tannins have been reported from *Aquilaria agallocha* Roxb. extract. Phytochemical analysis by GC/MS in herbal plants represents a precise method for the identification and quantification of their chemical components. In this regard, chemical composition with therapeutic properties, including oleodaphnal, oleodaphnone, genkwadaphnin-20-palmitate, and gnidicin-20-palmitate, were reported from the stem methanolic extract of *Daphne oleoides*. Therefore, this research assessed the antibacterial properties of *D. lessertii* extracts against human infectious bacteria and divulged their chemical compositions for the first time.

**Materials and Methods**

**Chemical Materials**

Nutrient broth (NB), Mueller-Hinton agar (MHA), quercetin (Q), and gallic acid (GA) were prepared from Merck Company (Germany), and ciprofloxacin and gentamicin antibiotic discs were obtained from Paten Tab Company (Iran).

**Plant Samples**

The different organs of *D. lessertii* including root, stem, and leaf were collected from Lorestan province, Iran, in 2014. Organs were transferred to Bu-Ali Sina University, then dried and broken by a cylindrical crusher. A volume of 250 mL of methanol, ethanol, and aqueous solvents were added to 25 g of dried powder and shaken at 110 rpm for 48 hours, then filtered and centrifuged. Eventually, the final product was placed at 37°C for drying. The methanolic extract was used for chemical composition analysis.

**Bacterial Strains**

Bacteria were obtained from Tehran University of Medical Science, Tehran, Iran. Antibacterial activities of samples were checked against *Enterococcus faecalis* (PTCC-1385), *Proteus mirabilis* (PTCC-1287) *Neisseria meningitides* (PTCC-4578), *Acinetobacter baumannii* (PTCC-4413), *Staphylococcus aureus* (PTCC-1389), *Pseudomonas aeruginosa* (PTCC-1171), and *Klebsiella pneumoniae* (PTCC-2139) *in vitro*. Next, a bacterial colony was cultured on MHA and incubated at 37°C. Finally, to prepare 0.5 McFarland standard (1.5 × 10⁸ CFU/mL), a bacterial colony was mixed with 1 mL of the nutrient broth and then incubated.

**Agar Well-Diffusion Assay**

In this research, to determine antibacterial properties, the agar well-diffusion method was used. Next, different extracts including distilled water, ethanol, and methanol extracts (i.e., 50, 100 and 200 mg/mL) from the root, stem, and leaf of *D. lessertii* were created. Afterwards, a volume of 200 mL of the bacterial suspension (1.5 × 10⁹ CFU/mL) was spread on MHA broth. In addition, 50 μL of extract solution was transferred into wells and incubated at 37°C for 24 hours. Positive controls included gentamicin (10 μg) and ciprofloxacin (5 μg). Finally, the agar well-diffusion assay was performed by SAS software in three replications.

**Total Phenolic and Total Flavonoid Content**

The total phenolic content (TPC) and total flavonoid content (TFC) were calculated for the methanolic extract. The TPC was calculated by the Folin–Ciocalteu assay at 765 nm using a spectrophotometer (mgGA/g). Next, TFC was determined by the aluminum chloride assay at 415 nm by a spectrophotometer (mgQ/g).

**Minimum Inhibitory Concentration and Minimum Bactericidal Concentration**

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were measured for different extracts by the serial dilution method. Different dilution series, including 3.125, 6.25, 12.5, 25, 50, and 100 mg/mL were used for calculating MIC. First, the nutrient broth (185 μL) was mixed with 200 μL of the extract, and then 200 μL of the solution was transferred to a second tube. Afterwards, 15 μL of the bacterial suspension (1.5 × 10⁹ CFU/mL) was added and incubated. For calculating MIC, the lowest dilution with no bacterial growth was determined. To determine MBC, 5 μL of the samples with no bacterial growth was cultured on MHA. All procedures were conducted in triplicate.

**Gas Chromatography-Mass Spectrometry**

Gas chromatography-mass spectrometry (GC/MS) was used to analyze the chemical composition of the methanolic extract (Razi University of Kermanshah, Iran). The GC/MS analysis was carried out using an Agilent 6890N coupled to Agilent 5973 mass detector, with HP-5, 30 m (length) × 0.25 mm (ID) × 0.25 µm column. The instrument was set to an initial temperature of 55°C, and maintained at this temperature for 2 minutes. Temperature was rose up to 120°C, and then to 200°C at the rate of 3.5°C/min. Injection port temperature was set as 350°C, and Helium flow rate as 0.9 mL/min. The samples were injected in the split/splitless mode. Solvent delay adjusted for 5 minutes with 0.5 µL volume injected.

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Statistical Analysis
The data was analyzed by SAS software with Duncan test at the significance level of P<0.05.

Results
Antibacterial Activity
The inhibitory effect of *D. lessertii* extracts were tested against infectious bacteria (Table 1). The diameter of bacterial growth inhibition zone (as mm) was determined after incubation (Figure 1). In addition, the highest sensitivity was recorded for *E. faecalis* exposed to the root methanolic extract of the plant at the dose of 200 mg/mL. The methanolic extract exhibited more potent inhibitory effects than other extracts. Moreover, the highest resistance and the lowest inhibitory activity were observed for *K. pneumoniae* against the aqueous extract, respectively. Accordingly, the inhibitory effects of the root and stem methanolic extracts at 200 mg/mL were higher than that of gentamicin against *E. faecalis*. Finally, *E. faecalis* and *S. aureus* showed more sensitivity compared to other bacteria.

Determination of TPC and TFC

![Figure 1](image-url). The inhibitory effect of A: the stem methanolic extract against *P. aeruginosa*; B: the root ethanolic extract against *E. faecalis*; C: the leaf ethanolic extract against *S. aureus*.

| Table 1. The Antibacterial Activity of Methanolic, Ethanolic, and Aqueous Extracts of Different Organs of *D. lessertii* Against Pathogenic Bacteria |
|---|---|---|---|---|---|---|---|
| Organ | Ex | Con | *E. faecalis* | *P. mirabilis* | *N. meningitides* | *A. baumannii* | *K. pneumoniae* | *S. aureus* | *P. aeruginosa* |
|---|---|---|---|---|---|---|---|---|---|
| Root | M | 50 | 12±0.33 | 7.5±0.33 | 14±0.55 | 13±0.88 | - | 13±0.66 | 12±0.33 |
| | | 100 | 16±0.33 | 7.5±0.33 | 15±0.33 | 15±0.33 | - | 14.5±0.33 | 12.5±0.66 |
| | | 200 | 18.5±0.57 | 9±0.57 | 16±0.33 | 15.5±0.88 | 9±0.88 | 15±0.88 | 12±0.33 |
| | E | 50 | 13.5±0.88 | 8.5±0.88 | 12.5±0.57 | 11±0 | - | 12±0.66 | 10±0.66 |
| | | 100 | 15±0.57 | 9±0.12 | 15±0.88 | 12.5±0.33 | - | 13±1.2 | 11±0.88 |
| | | 200 | 16.5±0 | 12±0 | 16±0.33 | 13±0.88 | - | 13.5±0.33 | 13±0.33 |
| | A | 50 | 8±0.57 | 7.5±0.88 | - | - | - | 8±0.33 | - |
| | | 100 | 10±0.57 | 9±0.33 | - | 8±0.33 | - | 9±0.66 | - |
| | | 200 | 11±0.33 | 12±0.88 | 8±0.57 | 9.5±0.33 | - | 9±0.66 | 8±0.66 |
| | M | 50 | 14±0.33 | 9.3±0.33 | 12.2±0.88 | - | - | 12±0.66 | 8±0.88 |
| | | 100 | 15±0.88 | 10±0.33 | 13.5±1.2 | - | - | 14.5±0.33 | 9±0.33 |
| | | 200 | 17±0.33 | 12±0.57 | 15±0.57 | 12±0.57 | 13±0.33 | 16±0.66 | 10.3±0.88 |
| | | 50 | 10±1.2 | - | - | - | - | 10±0.66 | - |
| Stem | E | 100 | 11±0.57 | - | - | - | 12.5±0.33 | 11±0.88 | - |
| | | 200 | 13±0.57 | - | 11±0.33 | - | 14±0.57 | 12.5±0.88 | 10.5±0.33 |
| | A | 50 | - | - | - | - | - | - | - |
| | | 100 | - | - | - | - | - | - | - |
| | | 200 | 9±0.33 | - | - | - | - | 8±0.66 | - |
| | M | 50 | 10±0.33 | - | - | - | - | 11.5±0.33 | 13±0.66 |
| | | 100 | 14±0.57 | - | 12.5±0.33 | - | 10±0.88 | 12.5±0.66 | 13.5±0.33 |
| | | 200 | 17±0.88 | 12±0.88 | 13.6±0.88 | 9±0.33 | 12±0.33 | 13±0.88 | 14±0.33 |
| | | 50 | 14±0.33 | - | - | - | 7.5±0.88 | 11±0.0 | - |
| Leaf | E | 100 | 15.5±0.33 | - | 10±0.88 | - | 9±0.33 | 12±0.33 | - |
| | | 200 | 16±0.88 | 9.5±0.57 | 11±0.57 | 10±0.57 | 13.5±0.12 | 13.5±0.88 | 11.5±0.33 |
| | A | 50 | - | - | - | - | - | - | - |
| | | 100 | 7.5±0.88 | - | - | - | 9±0.33 | - | - |
| Gentamicin | | 200 | 9±0.33 | - | 8.6±0.33 | - | 11.5±0.88 | - | - |
| Ciprofloxacin | 16 | 15 | 19 | 17 | 18 | 19 | 21 |
| | 17 | 19 | 20 | 20 | 22 | 21 | 25 |

Note. Co: Concentration; Ex: Extract; M: Methanol; E: Ethanol; A: Aqueous
The results of TPC and TFC of *D. lessertii* extracts are represented in Table 2. The TPC of root, leaf, and stem was calculated as 109.1±2.2, 55.8±1.2, and 68.7±0.88 mgGA/g, respectively. Next, the TFC was calculated as 2.1±0.3, 1.9±0.2 and 1.3±0.88 (mgQ/g), respectively. Based on the findings, the methanolic extract of root contains more secondary metabolites compared to other parts of the plant.

**Determination of MIC and MBC**

MIC and MBC values of the extracts of *D. lessertii* against pathogenic bacteria have been noted in Table 3. The stem ethanolic extract against *E. faecalis* and the leaf methanolic extract against *P. mirabilis* showed the same MIC of 6.25 mg/mL. The root methanolic extract demonstrated the MIC of 3.125 mg/mL against *E. faecalis*. In addition, aqueous extracts demonstrated the MBC of 100 mg/mL against *S. aureus* and *E. faecalis*. Moreover, the most potent antibacterial activity was related to the methanolic extract. Accordingly, the highest sensitivity and resistance were observed against *E. faecalis* and *P. aeruginosa*, respectively.

**GC-MS Analysis**

The chemical compositions of different extracts (i.e., stem, leaf, and root) of *D. lessertii* were analyzed by GC-MS. Overall, 35, 24, and 35 compounds were identified in stem, root, and leaf, respectively. The major constituents including phytol (12.64%), e-11-hexadecenal (12.53%), hexadecanoic acid (10.9%), and n-heneicosane (9.7%) in root methanolic extract. Finally, the most frequent compounds included phytol (19.658%) and hexadecanoic acid (7.151%) in leaf methanolic extract (Table 4).

**Discussion**

Plants are the richest sources of secondary metabolites with varying biological activity. Herbal plants with pharmaceutical properties, especially antimicrobial effects, are collected from the natural flora for the synthesis of rare and natural drugs. An increase in pathogenic microorganism resistance against chemical drugs has led researchers to seek for herbal plants with antimicrobial and antioxidant properties to produce natural drugs with less side effects and more medicinal properties. Multidrug-resistant pathogens, especially bacteria, pose a serious threat for human health.

### Table 2. TPC and TFC of the Root, Stem and Leaf Methanolic Extracts of *D. lessertii*

| Organ | Root | Leaf | Stem |
|-------|------|------|------|
| Phenol (mgGA/g) | 109.1±2.2<sup>a</sup> | 55.8±1.2<sup>b</sup> | 68.7±0.88<sup>c</sup> |
| Flavonoid (mgQ/g) | 2.1±0.33<sup>a</sup> | 1.9±0.2<sup>a</sup> | 1.3±0.88<sup>b</sup> |

Note: The similar letters are not significantly different.

### Table 3. MIC and MBC (mg/mL) of Different Extracts of *D. lessertii* Against Pathogenic Bacteria

| Bacteria | *E. faecalis* | *P. mirabilis* | *N. meningitides* | *A. baumannii* | *K. pneumoniae* | *S. aureus* | *P. aeruginosa* |
|----------|--------------|----------------|-------------------|----------------|-----------------|-------------|-----------------|
| Root M | 3.125 | 12.5 | 100 | 3.125 | 100 | - | - |
| MBC | 6.25 | 50 | - | 6.25 | - | - | - |
| E | MIC | 25 | 50 | 100 | 50 | - | 25 |
| MBC | 100 | 100 | 100 | 100 | - | 100 | - |
| A | MIC | 100 | 100 | - | 100 | - | 100 |
| MBC | - | - | - | - | - | - | - |
| Stem M | 25 | 25 | 50 | 6.25 | 100 | 100 | - |
| MBC | 25 | 50 | 100 | 25 | 100 | - | - |
| E | MIC | 6.25 | 100 | 50 | 25 | - | - |
| MBC | 12.5 | 100 | 50 | 50 | - | - | - |
| A | MIC | 100 | - | 100 | 50 | - | 100 |
| MBC | - | - | - | 100 | - | - | - |
| Leaf M | 50 | 6.25 | 50 | 3.125 | - | - | - |
| MBC | - | 12.5 | 100 | 25 | - | - | - |
| E | MIC | 100 | 100 | 100 | - | 50 | - |
| MBC | 100 | 100 | - | - | 100 | - | - |
| A | MIC | 50 | - | 100 | - | 100 | - |
| MBC | 100 | - | - | - | 100 | - | - |

Note: M: Methanol; E: Ethanol; A: Aqueous; -: Lack of bacteria growth.
In the present study, the chemical compositions of *D. lessertii* were reported for the first time by GC/MS. The antimicrobial, anticancer, anti-inflammatory, antifungal, and antiradical properties of other genera of the family Thymelaeaceae and their constituents (phytol, thymol, and eugenol) have been reported. Secondary metabolites including flavonoid, tannin, and phenolic compounds have been shown to inhibit infectious bacteria growth.
and reduce the risk of cancer to potent antioxidant properties. The antioxidant activity of polyphenols is related to their redox properties that act as reducing agents. Based on the findings, GC-MS results exhibited various antibacterial compounds from leaf, root, and stem extracts of D. lessertii.

In one study, the highest sensitivity (21.33±0.66 mm) was reported for S. typhi against the root methanolic extract of D. lessertii in vitro. Accordingly, TPC and TFC of the root methanolic extract were measured as 111.8±2.69 mgGAE/g and 2.25±0.35 mgQ/g, respectively, which were different from those of the present study. These differences probably could be related to some factors including the type of the extract and bacteria, extract concentration, and the samples collected. Moreover, antibacterial and antioxidant properties of the leaf and stem extracts of D. lessertii against pathogenic bacteria have been reported.

In the recent study, the stem ethanolic extract showed the highest inhibition zone as 27.3±0.6 mm diameter against Micrococcus luteus. The TPC of the leaf and stem methanolic extracts were 69.1±3.2 and 79.4±0.5 5 mgGAE/g, and respective TFC were reported as 2.1±0.1 and 1.5±0.1 mgQ/g. The values of the recent study were slightly higher than ours.

Moreover, the antibacterial activity of the root, leaf, and stem hydroalcoholic extracts of D. lessertii against some human pathogenic bacteria have been reported. In the recent report, the highest sensitivity was related to Bacillus subtilis against the root extract with the TPC of 111.8±1±2.69 mgGAE/g. The difference compared to our study can be due to factors such as differences in species and genus, extract and solvent types, extraction methods, and the time and geographical location of collecting the samples, affecting the chemical composition and antimicrobial properties of plant extracts.

**Conclusion**

Overall, D. lessertii extracts due to the existence of drug metabolites including phenol, flavonoid, and chemical compositions and other antimicrobial activities exhibited antimicrobial properties. Considering the chemical compositions including anticancer and antimicrobial agents such as phytol and eugenol, D. lessertii extracts could be suggested to develop drugs with antimicrobial properties treat bacterial infections.

**Authors’ Contribution**

This research was performed by the corresponding author.

**Ethical Approval**

Not applicable

**Competing Interests**

None.

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