Chemical Composition and Antibacterial Activity of the Essential Oil Isolated From Flos Lonicerae (Flower Buds of Lonicera macranthoides Hand.-Mazz.)

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Abstract
Flos Lonicerae (FL, flower buds of Lonicera macranthoides Hand.-Mazz.) is a traditional Chinese medicinal herb that is officially listed in the Chinese Pharmacopoeia. The aim of this study was to screen the chemical composition and to study the antibacterial activity of essential oils of Flos Lonicerae. The chemical composition of the essential oils was investigated using gas chromatography-mass spectrometry (GC-MS). The antibacterial activity was evaluated by the disc diffusion method to determine minimum inhibitory concentration (MIC). The major compounds of Flos Lonicerae essential oils were linalool (10.4%), palmitic acid (8.0%), geraniol (6.9%), hexanal (2.5%), and α-terpineol (2.2%). Flos Lonicerae essential oils demonstrated antibacterial activity against Escherichia coli, Staphylococcus aureus and Pseudomonas aeruginosa. The results of this study suggest that the essential oils of Flos Lonicerae have an interesting antimicrobial effect and may be a new potential source for a natural antimicrobial applied in the pharmaceutical field.

Keywords
flos lonicerae, essential oils, GC-MS, antibacterial activity, natural antimicrobial

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Flos Lonicerae (FL), the dried flower buds of 4 major species of the genus Lonicera (Caprifoliaceae), is a traditional Chinese medicinal herb that is officially listed in the Chinese Pharmacopoeia.¹ The dried buds of Lonicera hypoglauca, L. confusa, L. fulvotomentosa, and L. macranthoides are all referred to as Flos Lonicerae (Shanyinhua in Chinese). It has been used in traditional Chinese medicine for centuries for the treatment of infection by exopathogenic wind-heat or epidemic febrile diseases at the early stage, sores, carbuncles, furuncles, and swelling.²³ These conditions are essentially inflammatory processes involving heat, redness, pain, and swelling, which are often attributed to external pathogenic factors, such as bacteria and viruses. FL is also often used as a raw material for the production of various health care products, such as tea, wine, and cola, which are commonly sold in Asian markets.

A number of phytochemical studies have reported that Flos Lonicerae contains flavonoids, organic acids, iridoids, sapo- nins, and essential oils.⁴⁶ Several studies on essential oils (EOs) from different parts of Lonicera macranthoides Hand.-Mazz., such as leaves and aerial parts collected from various places, have reported the presence of linalool, geraniol, and α-terpineol.⁷⁹ However, there are no earlier studies on the chemical composition and biological activity of the essential oil (EO) of Flos Lonicerae. Furthermore, within scientific literature, essential oils have been reported to show multiple antibacterial activities.¹⁰ They have proven their effectiveness in comparison with conventional antibiotics used to treat several ailments.¹¹ Moreover, essential oils have also demonstrated antimicrobial activity against pathogenic bacteria.¹² In this study, we aimed to investigate the chemical composition of essential oils obtained from Flos Lonicerae collected in China by the use of GC-MS. In addition, the antibacterial activities of the essential oils against pathogenic bacteria, like Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa were tested by the disc diffusion method.

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Results

Essential Oil Yield and Chemical Composition

In this investigation, the essential oil yield of Flos Lonicerae was measured as 1.0% (v/w). Huang reported that the oil yield of ranged from 0.2% to 0.3% on a dry matter basis. The yield of oil in the present study was higher than that reported in the previous study, but there are many factors which can influence the yield. Through GC-MS analysis of the hydrodistilled essential oil of Flos Lonicerae, 66 compounds were successfully identified, representing 67.3% of the total oil. The chemical composition of the essential oil of Flos Lonicerae was mainly alcohols (26.8%), acids (14.0%), aldehydes (8.4%), and esters (5.3%). The major compounds included linalool (10.4%), palmitic acid (8.0%), geraniol (6.9%), hexanal (2.4%), and α-terpineol (2.2%). In Table 1, a comprehensive list of the constituents of the essential oil of Flos Lonicerae is presented.

Earlier studies on the chemical composition of the EOs of Flos Lonicerae pointed out the predominance of alcohols and acids. The essential oil from Flos Lonicerae collected in Guizhou Province has been reported to be mainly composed of linalool, α-terpineol and geraniol, which is in agreement with the findings of our study. Huang et al. have reported on the essential oils of Flos Lonicerae collected at different periods and from different locations of Guizhou Province. Fifty-seven compounds were identified, among which linalool accounted for 21.9% to 30.0%. However, there are 2 biphenyl derivatives, such as peak 43 and 48, which are missing in the previous research. These compounds may come from the drug itself, or from the external environment. In future studies, we will undertake further study on the origin of these compounds.

Disc Diffusion Test

The antibacterial activities of the EOs of Flos Lonicerae were quantitatively assessed by calculations of the inhibition zones. Table 2 shows the antibacterial inhibition zones (including the diameter of the paper disc) of the EOs of Flos Lonicerae, Amoxicillin, and DMSO, against the 3 tested microorganisms (E. coli, S. aureus, and P. aeruginosa). S. aureus was extremely sensitive to FL essential oil (25%, v/v), with an average diameter of 13.5 mm. It was also sensitive to amoxicillin with an average diameter of 24.6 mm. Measured inhibition diameters of essential oils of 25%, 50%, 75%, and 100% (v/v) against E. coli and S. aureus were larger than against P. aeruginosa, with significant differences (P < 0.05). Further, S. aureus was extremely sensitive to 100% of EOs, with an average diameter of 21.0 mm. P. aeruginosa showed some sensitivity to the essential oil at 50% concentration (v/v), but failed to show any sensitivity at 25% (v/v). Inhibition diameters of essential oil of 75% and 100% (v/v) were larger than those of amoxicillin against P. aeruginosa. DMSO did not show any inhibition to growth of any of the 3 bacteria, and its average inhibition diameters were significantly less than those of EOs (P < 0.05). In light of these promising results, it is necessary to undertake further studies on a larger spectrum of antimicrobial strains. The isolation of the essential oil components is in progress.

Determination of MIC

Through the disc diffusion test, we were not able to establish the most inhibited between Gram-negative and Gram-positive bacteria, and in what concentration. Therefore, we decided to calculate the minimal inhibitory concentration (MIC). The results are expressed as means and shown in Table 2. The MIC of the EOs of Flos Lonicerae against S. aureus (20.0 µL/mL) was lower than that of E. coli and P. aeruginosa (37.0 and 71.0 µL/mL, respectively). Furthermore, the results revealed that the essential oils of Flos Lonicerae have a bactericidal effect against Gram positive bacterial strains and a bacteriostatic effect against Gram negative bacteria. In order to determine the mechanism of action of the essential oils against the pathogenic bacteria, several researchers have attempted to correlate antibacterial activity with components of the essential oils. It has been suggested that antibacterial activity is closely associated with high concentrations of linalool and α-terpineol. However, the relationship between most other chemical compounds and bacterial activity is complex and not easily predictable. Some authors believe that essential oils have an effect against the cell membrane of microorganisms. Indeed, linalool exhibits antimicrobial potential. Linalool was active against pathogenic S. aureus and E. coli at very low concentrations (0.3%). Many authors have demonstrated the antimicrobial activity of essential oils, but their mechanism of action has not been studied in great detail. Further research is required to determine the relationship between the antibacterial activity of essential oils and various chemical constituents.

Discussion

Essential oils have shown considerable antimicrobial activity against a wide variety of bacteria, fungi, and viruses. Many claims have been made regarding essential oils and their pharmacological or medicinal properties. This study has highlighted the antibacterial effect of the essential oils of Flos Lonicerae and their chemical composition. The essential oils of Flos Lonicerae contain high amounts of linalool, palmitic acid, geraniol, and α-terpineol. These essential oils demonstrated considerable antibacterial activity and were very effective in inhibiting S. aureus ATCC 25923, with an average minimal inhibitory concentration of 20.0 µL/mL. Flos Lonicerae is traditionally recognized as an antibacterial agent and has been scientifically proven by several studies as having strong antimicrobial potential. We suggest that further research should be undertaken to explore the antibacterial activity of essential oils of Flos Lonicerae against a wider spectrum of bacterial pathogens, and to also evaluate other in vivo pharmacological activities.
| Peak no. | Retention time (min) | Chemical compound | RI   | RI*  | Molecular formula | Area% |
|---------|---------------------|-------------------|------|------|------------------|-------|
| 1       | 2.5                 | Hexanal           | 788  | 788  | C₆H₁₂O            | 2.4   |
| 2       | 2.8                 | 2,4-Dimethylheptane | 823  | 823  | C₇H₁₄O            | 0.1   |
| 3       | 3.3                 | (E)-2-Hexenal     | 840  | 840  | C₇H₁₄O            | 1.0   |
| 4       | 3.3                 | (Z)-3-Hexen-1-ol  | 855  | 855  | C₆H₁₀O            | 1.4   |
| 5       | 3.5                 | 1-Hexanol         | 862  | 863  | C₆H₁₄O            | 1.8   |
| 6       | 4.0                 | 2-Heptanone       | 876  | 875  | C₇H₁₄O            | 0.1   |
| 7       | 4.2                 | Heptanal          | 889  | 889  | C₇H₁₄O            | 0.3   |
| 8       | 4.9                 | 4-Carene          | 952  | 952  | C₁₀H₁₆            | 0.1   |
| 9       | 5.3                 | Camphene          | 954  | 954  | C₁₀H₁₆            | 0.1   |
| 10      | 5.6                 | Benzaldehyde      | 960  | 961  | C₆H₈O             | 1.8   |
| 11      | 6.4                 | 6-Methyl-5-hepten-2-one | 962  | 963  | C₆H₁₂O            | 0.2   |
| 12      | 6.5                 | 2-Pentylfuran     | 977  | 977  | C₆H₁₂O            | 1.1   |
| 13      | 6.8                 | (Z)-2-(2-Pentenyl)furan | 1003 | 1001 | C₆H₁₂O            | 0.2   |
| 14      | 6.9                 | Octanal           | 1004 | 1004 | C₈H₁₆O            | 0.2   |
| 15      | 7.6                 | p-Cymene          | 1020 | 1020 | C₁₀H₁₄            | 0.1   |
| 16      | 7.7                 | Limonene          | 1025 | 1027 | C₁₀H₁₄            | 0.4   |
| 17      | 8.4                 | (Z)-β-Ocimene     | 1029 | 1029 | C₁₀H₁₄            | 0.1   |
| 18      | 10.0                | Tetrahydrodicyclopentadiene | 1027 | 1027 | C₁₀H₁₆            | 0.6   |
| 19      | 10.1                | 3,5-Octadien-2-one | 1048 | 1048 | C₆H₁₄O            | 0.8   |
| 20      | 10.5                | Linalool          | 1085 | 1085 | C₁₀H₁₆            | 10.4  |
| 21      | 12.4                | Lilac aldehyde A  | 1145 | 1145 | C₁₀H₁₄O₂           | 0.3 |
| 22      | 12.8                | (E)-2-Nonenal     | 1149 | 1149 | C₁₀H₁₄O           | 0.5   |
| 23      | 13.0                | Lilac aldehyde D  | 1167 | 1169 | C₁₀H₁₄O₂           | 0.2   |
| 24      | 13.4                | Terpinen-4-ol     | 1170 | 1170 | C₁₀H₁₄O           | 0.5   |
| 25      | 14.0                | α-Terpineol       | 1177 | 1179 | C₁₀H₁₄O           | 2.2   |
| 26      | 14.4                | Estragole         | 1180 | 1180 | C₁₀H₁₄O           | 0.1   |
| 27      | 14.7                | Decanal           | 1185 | 1185 | C₁₀H₁₄O           | 0.3   |
| 28      | 15.7                | Geraniol          | 1255 | 1255 | C₁₀H₁₄O           | 6.9   |
| 29      | 16.6                | Ionone            | 1258 | 1257 | C₁₁H₁₈           | 0.1   |
| 30      | 17.5                | (E)-Citral        | 1272 | 1272 | C₁₀H₁₄O           | 0.3   |
| 31      | 17.6                | 1-Decanal         | 1278 | 1279 | C₁₀H₁₄O           | 1.2   |
| 32      | 18.0                | Anethole          | 1283 | 1283 | C₁₀H₁₂O           | 0.9   |
| 33      | 19.3                | 4-Vinylguaiacol   | 1295 | 1295 | C₁₀H₁₄O₂           | 0.2   |
| 34      | 19.4                | 2E,4E-Decadienal  | 1318 | 1318 | C₁₀H₁₂O           | 0.9   |
| 35      | 21.1                | Eugenol           | 1362 | 1362 | C₁₀H₁₄O₂           | 0.8   |
| 36      | 21.6                | Geranic acid      | 1355 | 1355 | C₁₀H₁₄O₂           | 0.6   |
| 37      | 22.2                | β-damascenone     | 1365 | 1366 | C₁₃H₁₈O           | 2.1   |
| 38      | 23.1                | Methylcinnamaldehyde | 1410 | 1410 | C₁₁H₁₄O₂           | 0.2   |
| 39      | 25.4                | Hexadecane        | 1600 | 1600 | C₁₆H₃₄           | 0.1   |
| 40      | 26.3                | β-Ionone          | 1485 | 1487 | C₁₃H₂₄O           | 0.5   |
| 41      | 26.9                | Pentadecane       | 1500 | 1500 | C₁₅H₃₂           | 0.3   |
| 42      | 27.7                | Myristine         | 1519 | 1517 | C₁₅H₂₄O₃           | 0.4   |
| 43      | 28.2                | 2,2'-Dimethylbiphenyl | 1546 | 1546 | C₁₄H₁₁O₂         | 0.2   |
| 44      | 29.4                | (E)-Nerolidol     | 1557 | 1554 | C₁₃H₂₆O₂           | 0.2   |
| 45      | 29.8                | Dodecanolic acid  | 1567 | 1567 | C₁₂H₂₆O₂           | 1.5   |
| 46      | 30.5                | Cedrol            | 1589 | 1589 | C₁₃H₂₄O₂           | 0.2   |
| 47      | 31.2                | 1,2-Epoxyoctadecane | 1900 | 1900 | C₁₈H₃₀O₂           | 0.2   |
| 48      | 33.4                | 1,1'-Biphenyl, 2,2',5,5'-tetramethyl- | 1663 | 1663 | C₁₈H₃₀O₂           | 0.4   |
| 49      | 34.9                | Tetradecane       | 1614 | 1614 | C₁₄H₂₈O₂           | 0.2   |
| 50      | 35.3                | Tridecanoic acid methyl ester | 1631 | 1631 | C₁₄H₂₈O₂           | 0.2   |
| 51      | 36.5                | Benzyl benzoate   | 1759 | 1759 | C₁₄H₁₄O₂           | 0.4   |
Materials and Methods

Plant Material and Essential Oils Extraction

The plant material was obtained during its flowering period, between March and July 2019, from Longhui Country, Hunan Province, China. The plants were kept in paper bags to protect them from light and moisture. The plant material (Lonicera macranthoides Hand.-Mazz.) was identified by Prof. Dr Jianwei Chen from the School of Pharmacy, Nanjing University of Chinese Medicine, China. A voucher specimen has been deposited at the Herbarium of the Nanjing Haiyuan Prepared Slices of Chinese Crude Drugs Co. Ltd, Nanjing, China. Extraction of essential oil from the flower bud was done by hydrodistillation in a Clevenger-type apparatus for 5 hours. The essential oil was measured directly in the extraction burette, and the extraction yield (%) was calculated as the volume (mL) of essential oil per 100 g of plant material. The oil was then dried over anhydrous sodium sulfate (Na₂SO₄), and stored in a refrigerator until further analysis. Hydrodistillation was performed at least 3 times, and the mean values of the extraction yields were recorded.

Analysis of the Essential Oils

The GC-MS test was conducted using a Shimadzu GC-2010 instrument equipped with an HP-5 capillary column (30 m x 0.25 mm i.d., film thickness 0.25 µm; Agilent, USA). The retention times were converted to RI* values using literature data. The chemical compounds identified are listed in Table 1, along with their RI, RI*, molecular formulae, and area%. The abbreviations used are RI*, retention index from literature.

Table 1. Measured Inhibition Diameters (Mm) of the Disc Diffusion Test and MICs of Flos Lonicerae Essential Oil Against the Test Microorganisms (N = 3).

| Microorganism          | Inhibition diameters (mm) | Flos Lonicerae EOs | Amoxicillin | DMSO | MIC (µL/mL) |
|------------------------|---------------------------|--------------------|-------------|------|-------------|
|                        | 25% 50% 75% 100%          | 25 µg/disc 100%    |             |      |             |
| Escherichia coli ATCC 25922 | 11.5 12.8 14.8 16.5      | 10.5 5.5           | 36.0        |      |
|                        | 11.4 12.8 15.2 16.8      | 11.8 5.4           | 40.0        |      |
|                        | 12.4 13.6 16.0 17.4      | 10.4 5.1           | 35.0        |      |
|                        | 11.8 13.1 15.3 16.9      | 10.9 5.3           | 37.0        |      |
| Staphylococcus aureus (G+) ATCC 25923 | 13.7 19.4 17.7 21.4      | 23.5 5.9           | 20.0        |      |
|                        | 12.8 15.6 19.1 21.1      | 24.7 5.3           | 18.0        |      |
|                        | 14 17.1 18 20.6          | 25.5 5.5           | 22.0        |      |
|                        | 13.5 17.4 18.3 21.0      | 24.6 5.6           | 20.0        |      |
| Pseudomonas aeruginosa (G-) ATCC 27853 | 8.7 10.1 10.8 12.3      | 9.8 5.6            | 65.0        |      |
|                        | 9.5 9.7 10.9 11.5        | 9.5 5.3            | 78.0        |      |
|                        | 8.6 8.8 11.2 11.8        | 9.7 5.7            | 70.0        |      |
|                        | 8.9 9.5 11.0 11.9        | 9.7 5.5            | 71.0        |      |
Bacterial Strains

To test the antibacterial activity of the essential oils of Flos Lonicerae, we used ATCC (American Type Culture Collection) strains of 3 pathogenic bacteria obtained from LMJHP (Laboratory of Microbiology, Jiangsu Haisheng Pharmaceutical Co. Ltd, Nanjing, China.), namely *Escherichia coli* ATCC 25922 (*E. coli*), *Pseudomonas aeruginosa* ATCC 27853 (*P. aeruginosa*) and *Staphylococcus aureus* ATCC 25923 (*S. aureus*).

Disc Diffusion Method

The antibacterial activity was evaluated using the disc diffusion method, with some modifications. Dilutions of the EOs were made in sterile glass using dimethyl sulfoxide (DMSO). The dilutions were: 25%, 50%, 75%, and 100% (v/v) for a final volume of 1 ml. Firstly, the Mueller-Hinton Petri dishes were inoculated with bacterial strains at 0.5 McFarland turbidity, and each essential oil was tested against each bacterial strain. Then, discs of 6 mm diameter were placed in groups of 4 in the Petri dishes and were then soaked with 20 µL of each essential oil dilution. Amoxycillin discs (25 µg/disc) were used as a positive control and discs soaked with DMSO as a negative control. The treated Petri dishes were then incubated at 37 °C for 18-24 h. The diameter of the inhibition zone was then measured in mm. The bacterial sensitivity to each essential oil dilution was classified based on the diameter of the inhibition zone as follows: Diameter < 8 mm: Not sensitive, 9 < Diameter < 14 mm: Sensitive, 15 < Diameter < 19 mm: Very sensitive, and Diameter > 20 mm: Extremely sensitive. Each experiment was carried out in triplicate and the mean diameters of the inhibition zones were recorded.

Determination of Minimum Inhibitory Concentration (MIC)

A broth macrodilution susceptible assay was employed for the determination of the MIC. All tests were performed in a Mueller-Hinton broth (MHB) medium. A total of 11 assay tubes were prepared, and 10 ml of MHB was dispensed in the first tube and 5 ml in the other tubes. The inoculum suspensions of the bacterial strains were prepared from 24 broth cultures and adjusted to obtain a final density of 106 CFU/mL. A solution of the essential oil in DMSO (10%) was prepared and added to the first tube, and then 2-fold serial dilutions were undertaken in the 9 consecutive tubes before adding 20 ml of the inoculum. The last tube, which contained only 5 ml of MHB and 20 ml of the inoculum, was used as a negative control. The assay tubes were then incubated at 37 °C for 24 hours.

Statistical Analysis

Conventional statistical methods were used to calculate the means of 3 performed antibacterial assays. To assess the significant differences between the means, covariance analysis (ANOVA) was applied to the data (P < 0.05). The differences between the sizes of the inhibition zones were determined using the LSD test.

Declaration of Conflicting Interests

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