Chemical Composition and Antimicrobial Activities of the Essential Oil From the Leaves of *Pterocephalus hookeri*

Peng-fei Yang¹, Hui Lu¹, Qiong-bo Wang², Zhi-wei Zhao³, Qiang Liu³, Xu Zhao³, Jing Yang¹, Shen Huang¹, Zhi-fei Chen³, and Duo-bin Mao¹

Abstract

Detailed chemical constituents of essential oil from the *Pterocephalus hookeri* leaves and its antimicrobial activities were investigated in this study. The essential oil, obtained by hydrodistillation, was characterized by gas chromatography-flame ionization detection and gas chromatography-mass spectrometry analyses. Among the 90 identified compounds, hexadecanoic acid (21.27%), phytol (8.03%), furfural (7.08%), oleic acid (5.25%), and phytone (4.56%) were the major components. In the antimicrobial assay, the essential oil showed strong inhibitory activities against *Escherichia coli*, *Candida albicans*, and *Staphylococcus aureus* with minimum inhibitory concentration values of 31.3, 62.5, and 125 µg/mL, respectively. To our knowledge, this is the first report concerning chemical composition and antimicrobial activities of the essential oil from *Pterocephalus hookeri*.

Keywords

essential oil, *Pterocephalus hookeri*, GC-FID, GC-MS, antimicrobial activity

Results and Discussion

Essential Oil Composition

The yield of leaf essential oil obtained by water distillation was 0.33% (w/w relative to dry material weight). Composition analysis by gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS) identified 90 volatile compounds, accounting for 96.16% of the total extracted reported. We here report the chemical composition of the hydrodistilled essential oil from *Pterocephalus hookeri* leaves and evaluate their antimicrobial properties.

Essential Oil Composition

The yield of leaf essential oil obtained by water distillation was 0.33% (w/w relative to dry material weight). Composition analysis by gas chromatography-flame ionization detection (GC-FID) and gas chromatography-mass spectrometry (GC-MS) identified 90 volatile compounds, accounting for 96.16% of the total extracted...
oil, which were identified by matching retention times of available authentic standards, retention indices (RIs), and mass spectra in the NIST 17 database (Table 1). The essential oil was mainly composed of 15 oxygenated monoterpenes (10.62%), 6 sesquiterpenes (0.92%), 5 diterpenes (15.32%), 11 carboxylic acids (34.8%), 16 benzenoid aromatics (5.82%), 11 alkanes (2.29%), 3 aliphatic

Table 1. Chemical Composition of the Essential Oil From *Pterocephalus hookeri*.

| Compounds                          | RI<sup>a</sup> | RI<sup>b</sup> | %      | Compounds                          | RI<sup>a</sup> | RI<sup>b</sup> | %      |
|------------------------------------|----------------|----------------|--------|------------------------------------|----------------|----------------|--------|
| 3-Methyl-2-butenal                 | 784            | 790            | 0.09   | a-Cubebene                         | 1380           | 1378           | 0.25   |
| Hexanal                            | 802            | 800            | 0.16   | β-Damascone                         | 1388           | 1388           | 1.20   |
| Furfural                           | 834            | 835            | 7.08   | (E)-Jasnone                         | 1391           | 1393           | 1.19   |
| Isopropyl butyrate                 | 844            | 843            | 1.60   | a-Terradecane                       | 1400           | 1400           | 0.34   |
| (E)-2-Hexenal                      | 851            | 853            | 0.60   | a-Cedrene                           | 1417           | 1410           | 0.20   |
| (Z)-3-Hexen-1-ol                   | 854            | 857            | 0.82   | a-Santalene                         | 1422           | 1420           | 0.04   |
| Norsabinane                        | 859            | 856            | 0.09   | 4,11-Dimethyl-tetradecane           | 1459           | 1462           | 0.23   |
| Hexyl alcohol                      | 866            | 867            | 0.11   | a-Curcumene                         | 1486           | 1483           | 0.20   |
| α-Angelica lactone                 | 868            | 869            | 0.23   | (E)-β-Ionone                        | 1489           | 1485           | 0.16   |
| 2-Cyclopentene-1,4-dione           | 885            | 880            | 0.10   | n-Pentadecane                       | 1500           | 1500           | 0.33   |
| (2E,4E)-2,4-Hexadienal             | 910            | 911            | 0.17   | n-Heptadecane                       | 1700           | 1700           | 0.37   |
| 2-Acetylfluran                     | 913            | 914            | 0.43   | Cuparene                            | 1513           | 1512           | 0.19   |
| 3-Ethylpyridine                    | 957            | 959            | 0.40   | (2)-Calamene                         | 1531           | 1531           | 0.44   |
| Benzaldehyde                       | 961            | 961            | 1.15   | Dihydroactinidolide                 | 1538           | 1535           | 0.12   |
| 5-Methylfurfural                   | 964            | 966            | 0.71   | Lauric acid                         | 1569           | 1570           | 2.37   |
| 3-Ethenylpyridine                  | 966            | 968            | 1.28   | Carophyllene oxide                  | 1591           | 1589           | 0.17   |
| Hexanoic acid                      | 978            | 981            | 0.26   | n-Hexadecane                        | 1600           | 1600           | 0.23   |
| trans-Dehydroxylinalool oxide      | 995            | 994            | 0.49   | Cedrol                              | 1609           | 1608           | 0.15   |
| (2E,4E)-2,4-Heptadienal            | 1012           | 1007           | 0.17   | Cadalin                             | 1681           | 1674           | 0.23   |
| Benzyl alcohol                     | 1035           | 1033           | 0.15   | n-Heptadecane                       | 1700           | 1700           | 0.37   |
| Benzenecetaldehyde                 | 1045           | 1049           | 0.61   | Myristic acid                       | 1763           | 1765           | 1.91   |
| Acetophenone                       | 1068           | 1065           | 0.29   | Benzyl Benzoate                     | 1769           | 1770           | 0.21   |
| cis-Linalool oxide (furanoid)      | 1076           | 1083           | 1.57   | Phenanthrene                        | 1781           | 1784           | 0.14   |
| trans-Linalool oxide (furanoid)    | 1091           | 1094           | 0.88   | n-Octadecane                        | 1800           | 1800           | 0.10   |
| Linalool                           | 1101           | 1104           | 1.54   | Hexadecanal                         | 1817           | 1818           | 0.16   |
| Phenylethyl alcohol                | 1115           | 1114           | 0.53   | Neophytiadiene                      | 1839           | 1840           | 0.19   |
| Lilacdehyde A                      | 1145           | 1145           | 0.29   | Phytone                             | 1846           | 1845           | 4.56   |
| Camphor                            | 1149           | 1144           | 1.14   | Pentadecylic acid                   | 1861           | 1862           | 0.44   |
| Lilacdehyde C                      | 1155           | 1160           | 0.61   | n-Nonadecane                        | 1900           | 1900           | 0.20   |
| Bornol                             | 1170           | 1172           | 0.54   | (E)-11-Hexadecenoic acid           | 1922           | 1915           | 0.43   |
| 2-Methylecophenone                 | 1187           | 1174           | 0.22   | Methyl palmitate                   | 1927           | 1926           | 1.44   |
| Anethofovan                         | 1189           | 1191           | 0.16   | (E)-9-Hexadecenoic acid            | 1940           | 1942           | 0.27   |
| α-Terpineol                        | 1194           | 1187           | 1.03   | Isophytol                           | 1948           | 1950           | 1.16   |
| n-Dodecane                         | 1200           | 1200           | 0.10   | Palmitic acid                       | 1970           | 1975           | 21.27  |
| Carvomenthene                      | 1219           | 1217           | 1.86   | Manool oxide                        | 1983           | 1982           | 1.38   |
| Citronellol                         | 1222           | 1221           | 1.60   | n-Icosane                           | 2000           | 2000           | 0.13   |
| Nerol                              | 1230           | 1229           | 0.13   | 1-Octadecanol                      | 2084           | 2081           | 1.36   |
| Cuminaldehyde                      | 1247           | 1240           | 0.36   | Linoleic acid                       | 2096           | 2104           | 0.58   |
| Geraniol                           | 1256           | 1258           | 0.25   | Phytol                             | 2115           | 2122           | 8.03   |
| 4-Phenyl-2-butanol                 | 1261           | 1261           | 0.18   | Methyl octadecanoate               | 2123           | 2127           | 0.93   |
| Perillyl alcohol                   | 1293           | 1295           | 0.27   | Elaidic acid                       | 2136           | 2140           | 1.70   |
| n-Tridecane                        | 1300           | 1300           | 0.17   | Oleic Acid                          | 2144           | 2141           | 5.25   |
| 1,1,6-Trimethyl-1,2-dihydronaphthalene | 1357        | 1355           | 0.54   | Oleic anhydride                     | 2152           | 2157           | 1.46   |
| Chavibetol                         | 1359           | 1362           | 0.38   | Ethyl linolenate                    | 2170           | 2169           | 0.29   |
| Capric acid                        | 1364           | 1374           | 0.32   | Phytol acetate                     | 2223           | 2225           | 2.51   |

<sup>a</sup>RI: Retention index determined relative to n-alkanes (C7-C30) on the HP-5ms column.

<sup>b</sup>RI: literature retention indices<sup>12-14</sup>.
aureus and furfural were confirmed against E. coli, C. albicans with MICs of 125 and 250 µg/mL. The antimicrobial activities of phytol, furfural, oleic acid, and phytone, possesses pronounced antimicrobial activity against E. coli, C. albicans, and S. aureus. The results indicate that the essential oil of Pterocephalus hookeri might be suitable for use as a natural antimicrobial agent. However, further studies should focus on the synergism, mechanism of action, and bioavailability of different essential oil components.

### Materials and Methods

#### Plant Material

The fresh leaves of Pterocephalus hookeri in the present study were collected from Shangri-La (27°82′N, 99°71′E), Yunnan Province, China, in October 2019. The plant was authenticated by one of the authors (Shen Huang) and the voucher specimens (No. 0136) were deposited at the Department of Plant Resources, School of Food and Bioengineering, Zhengzhou University of Light Industry, China.

#### Extraction of the Essential Oil

The collected leaves were dried in the shade at room temperature and powdered. The essential oil was extracted by hydrodistillation of powdered leaves (150 g) for 3 hours in a Cleverenger-type apparatus. The essential oil was dried using anhydrous sodium sulfate and stored in a dark glass vial at 4 °C until analysis.

#### Identification of the Chemical Components of the Essential Oil

The components of the essential oil were determined by using an Agilent GC-MS (8890A-5977B, USA) equipped with a fused silica capillary column HP-5ms (60 m × 0.25 mm × 0.25 μm). The injector temperature was 280 °C. The oven temperature was 280 °C. The injector temperature was 280 °C. The oven temperature was 280 °C.

### Conclusion

This study first reports that the essential oil extracted from Pterocephalus hookeri, with main components of hexadecanoic acid, phytol, furfural, oleic acid, and phytone, possesses pronounced antimicrobial activity against E. coli, C. albicans, and S. aureus. The results indicate that the essential oil of Pterocephalus hookeri might be suitable for use as a natural antimicrobial agent. However, further studies should focus on the synergism, mechanism of action, and bioavailability of different essential oil components.

| Microorganisms         | Diameter of zones of inhibition (mm) | MICs (µg/mL) | MBCs (µg/mL) |
|------------------------|--------------------------------------|--------------|--------------|
|                        | Essential oil                        | Antibiotic   |              |
| Staphylococcus aureus  | 19.2 ± 0.6                           | 23.3 ± 0.2   | 125          | 250          |
| Bacillus cereus        | 18.3 ± 0.4                           | 24.5 ± 0.4   | 250          | 500          |
| Escherichia coli       | 20.2 ± 0.6                           | 21.9 ± 0.4   | 31.3         | 62.5         |
| Pseudomonas aeruginosa | 10.9 ± 0.5                           | 24.2 ± 0.7   | NT           | NT          |
| Candida albicans       | 20.7 ± 0.7                           | 23.8 ± 0.2   | 62.5         | 125          |
| Aspergillus fumigatus  | 17.4 ± 0.5                           | 23.2 ± 0.2   | 250          | 250          |

Abbreviations: MBC, minimal bactericidal concentration; MIC, minimum inhibitory concentration.

* Results were mean ± SD of triplicate values.

* Antibiotics used were ampicillin for Gram-positive bacteria, gentamicin for Gram-negative bacteria, amphotericin for fungi.

* NT: not tested.
was initially held at 50 °C for 2 minutes and increased to 140 °C at the rate of 2 °C/min and held for 10 minutes, then increased to 180 °C with a rate of 3 °C/min, held for 2 minutes, finally the temperature was increased to 270 °C with a rate of 1.5 °C/ min, held for 10 minutes. Carrier gas was helium at 1.0 mL/min. One-microliter aliquot of oil was injected into the column using a 20:1 split injection. The mass spectrometer was operated in electron-impact ionization mode with 70 eV energy. The ion source temperature was 230 °C and the scanning range of ion mass was from 35 to 550 amu. The GC analysis was conducted on a 6890N apparatus (Agilent Technologies, Santa Clara, California, USA) equipped with a flame ionization detector (FID) and an electronic pressure control injector. GC-FID analysis was conducted under the same experimental conditions using the same column as described for the GC-MS. Relative percentages of components were calculated based on the peak areas using the normalization method without correction factors. The components of essential oil were identified by matching retention times of available authentic standards, RIs, and mass spectra in the NIST 17 database. RIs were calculated for all components using a homologous series of n-alkane mixtures (C7-C30) injected under conditions similar to those of the samples and computer matched with the NIST libraries.

Antimicrobial Activity Assays
The microbial strains used in this investigation were S. aureus ATCC 25923, B. cereus ATCC 10987, E. coli ATCC 25922, and P. aeruginosa ATCC 15542. The fungal strain used in this study was C. albicans ATCC 10231 and A. fumigatus ATCC 1022. All strains were maintained on an agar slant at 4 °C. The bacterial strains were cultured in a Muller-Hinton broth at 37 °C for 24 hours. The fungal strains were cultured on Sabouraud Dextrose Agar (SDA) at 28 °C for 120 hours before testing. The disc diffusion method was used to determine the antimicrobial activities of the essential oils. Petri plates were prepared by pouring 20 mL Muller Hinton Agar (MHA) or SDA and the solution was allowed to solidify. The plates were then dried, and 0.1 mL of the standardized inoculum containing 10^5-10^6 colony-forming units/mL of the bacterial suspension was poured, uniformly spread, and allowed to dry for 5 minutes. The oil was prepared in sterile dimethyl sulfoxide (DMSO) at a concentration of 1 mg/mL, of which 100 µL was added to the respective wells. The control well received only 100 µL DMSO. The reference antibiotics used were ampicillin for Gram-negative bacteria, gentamicin for Gram-negative bacteria, and amphotericin for fungi. The plates were left at room temperature to allow diffusion and then incubated at 37 °C for 24 hours for bacterial growth or at 28 °C for 48 hours for fungal growth. The antimicrobial activity was evaluated by measuring the diameter of the zones of inhibition against the test organisms. The experiments were repeated in triplicate and the results are expressed as average values. The MIC was determined using the broth microdilution method using 96-well microplates. The inoculum of the microbial strains was prepared from 24 to 48 hours broth cultures and suspensions were adjusted to 0.5 McFarland standard turbidity. Serial concentrations (500, 250, 125, 62.5, 31.3, 15.6, 7.81, 3.9, 1.95, 0.98, and 0.49 µg/mL) of essential oil were prepared. One hundred microliters from culture broth was mixed with 100 µL of different concentration oils in the corresponding well and plates were incubated either at 37 °C for 24 hours for antibacterial activity or 28 °C for antifungal activity. The lowest concentration of the tested oil showing no microbial growth was defined as the MIC. MBC values were determined by taking a part of the liquid from each well that showed no growth and incubating on agar plates at 37 °C for another 24 hours. The lowest concentration that disclosed no visible growth of bacteria or fungi was confirmed as MBC.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by National Natural Science Foundation of China (No. 81903507), and Doctoral Scientific Research Foundation of Zhengzhou University of Light Industry (0123-13501050063).

ORCID ID
Peng-fei Yang https://orcid.org/0000-0003-4180-5000

References
1. Jiang H, Wang J, Song L, et al. GC×GC-TOFMS analysis of essential oils composition from leaves, twigs and seeds of Cinna numomum camphora L. Presl and their insecticidal and repellent activities. Molecules. 2016;21(4):423. doi:10.3390/molecules21040423
2. Seow YX, Yeo CR, Chung HL, Yuk H-G. Plant essential oils as active antimicrobial agents. Crit Rev Food Sci Nutr. 2014;54(5):625-644. doi:10.1080/14786419.2011.599504
3. Boren K, Crown AA, Multidrug CR; Pan-Antibiotic Resistance. The role of antimicrobial and synergistic essential oils: a review. Nat Prod Commun. 2020;15(10):1-19.
4. Hu J, Jia M, Zhu L. Chemical composition and antimicrobial activities of essential oil from Wedelia articulata growing wild in Hunan Province, China. Nat Prod Res. 2019;33(18):2685-2688. doi:10.1080/14786419.2018.1460830
5. Akhgar MR, Safavinia L. Essential oil composition of Pterocla pas gedanica. Chem Nat Compd. 2016;52(3):514-515. doi:10.1007/s10600-016-1693-5
6. Zhang L, Hu J-J, Lin J-W, Fang W-S, Du G-H. Anti-inflammatory and analgesic effects of ethanol and aqueous extracts of Pterocla pas hookeri (C.B. Clarke) Höeck. J Ethnopharmacol. 2009;123(3):510-514. doi:10.1016/j.jep.2009.01.039
7. Wu Y-C, Guo C-X, Zhu Y-Z, Li Y-M, Guo F-J, Zhu G-F. Four new bis-iridoids isolated from the traditional Tibetan herb.
8. Wu Y, Yin Y, Li Y, Guo F, Zhu G. Secoiridoid/iridoid sub-type bis-iridoids from *Pterocephalus hookeri*. Magn Reson Chem. 2014;52(1):734-738. doi:10.1002/mrc.4116

9. Shen X-F, Zeng Y, Li J-C, Tang C, Zhang Y, Meng X-L. The anti-arthritic activity of total glycosides from *Pterocephalus hookeri*, a traditional Tibetan herbal medicine. Pharm Biol. 2017;55(1):560-570. doi:10.1080/13880209.2016.1263869

10. Tang C, Wen J, Wang J, et al. Simultaneous determination of ten major compounds including iridoid glycosides and phenolic acids in *Pterocephalus hookeri* by UPLC-PDA. Chin J Chin Mater Med. 1993;42(7):1234-1237.

11. Guan XL, Yan YN, Wei TM, Ren ZH, Song CS. Anti-inflammatory effects and acute toxicity test of *Pterocephalus hookeri*. J Beijing Univ Trad Chin Med. 2004;27(2):71-73.

12. Adams RP. *Identification of Essential Oil Components by Gas Chromatography/Mass Spectrometry*. Allured Publishing Corporation; 2007.

13. Essien E, Ogunwande IA, Flamini G, Cioni PL, Kinola AO. Analysis of the essential oil of *Dialium guineense* wild. J Essent Oil Res. 2007;19(6):545-547. doi:10.1080/10412905.2007.9699327

14. Afsharpyuor S, Ranjbar M, Mazaheri M, Shakibaei F, Aslani A. Essential oil constituents of seeds and fresh leaves of garden lettuce (*Lactuca sativa* L.) grown in Isfahan, Iran. Res J Pharma. 2018;5(3):1-5.

15. Vahedi H, Nasrabadi M, Lari J, Halimi M. Volatile constituents and antimicrobial activities of *Pterocephalus canus*. J Med Plants Res. 2011;5(23):5646-5648.

16. Abdullah FO, Hussain FHS, Mannucci B, et al. Composition, antifungal and antiproliferative activities of the hydrodistilled oils from leaves and flower heads of *Pterocephalus nestorianus* N abełek. Chem Biodivers. 2017;14(7):e1700009. doi:10.1002/cbdv.201700009

17. Paolini J, Barboni T, Desjohbert J-M, Djabou N, Muselli A, Costa J. Chemical composition, intraspecies variation and seasonal variation in essential oils of *Calendula arvensis* L. Biochem Syst Ecol. 2010;38(5):865-874. doi:10.1016/j.bse.2010.07.009

18. Morelli F, Ferarrese L, Munhoz CL, Alberton O. Antimicrobial activity of essential oil and growth of *Ocimum basilicum* (L.) inoculated with mycorrhiza and humic substances applied to soil. Genet Mol Res. 2017;16(3):1-11. doi:10.4238/gmr16039710

19. Pejin B, Savic A, Sokovic M, et al. Further in vitro evaluation of antiradical and antimicrobial activities of phytol. Nat Prod Res. 2014;28(6):372-376. doi:10.1080/14786419.2013.869692

20. Ghaniean MT, Ehrampoush MH, Jebali A, Hekmatmoghadam S, Mahmoudi M. Antimicrobial activity, toxicity and stability of phytol as a novel surface disinfectant. Environ Heal Eng Manage J. 2015;2(1):13-16.

21. Chai WM, Liu X, Hu YY, et al. Antityrosinase and antimicrobial activities of furfuryl alcohol, furfural and furfuroic acid. Int J Biol Macromol. 2013;57:151-155. doi:10.1016/j.ijbiomac.2013.02.019

22. Ullah I, Khan AL, Ali I, et al. Benzaldehyde as an insecticidal, antimicrobial, and antioxidant compound produced by *Photorhabdus temperata* M1021. J Microbiol. 2015;53(2):127-133. doi:10.1007/s12275-015-4632-4

23. Li L, Shi C, Yin Z, et al. Antibacterial activity of α-terpineol may induce morphostructural alterations in *Escherichia coli*. Braz J Microbiol. 2014;45(4):1409-1413. doi:10.1590/S1517-83822014000400035

24. HA CT'T, Thai TH, Hien NT, et al. Chemical composition and antimicrobial activity of the leaf and twig essential oils of *Magnolia hypolampra* growing in Na Hang nature reserve, Tuyen Quang province of Vietnam. Nat Prod Commun. 2019;14(6):1-9.