Essential oil extracted from leaf of *Phoebe bournei* (Hemsl.) Yang: Chemical constituents, antitumor, antibacterial, Hypoglycemic activities

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**ABSTRACT:** The essential oil were extracted from the leaf of *Phoebe bournei* (Hemsl.) Yang by a hydrothermal method and then analyzed by gas chromatography–mass spectrometry. The leaf oil mainly included α-copaene (5.44%), α-muurolene (7.32%), δ-cadinene (11.44%), 1s-calamenene (5.18%). *Phoebe bournei* (Hemsl.) Yang leaf essential oil had significant inhibitory activity against *Epidermophyton floccosum* and *Microsporum gypseum*, the potential antitumor activity towards leukemia, breast, and colon cancer cell lines was good. *Phoebe bournei* (Hemsl.) Yang leaf essential oil had weaker activity on the four tested bacteria, it exhibited a certain role in promoting glucose uptake by adipocytes.

**KEYWORDS:** Essential oil; chemical constituents; antitumor activity; antibacterial activity; hypoglycemic activity
Experimental

Chemical composition analysis

Material

The leaf of *P. bournei* are from Gongdong Township, Rongshui County, Liuzhou City, Guangxi Province. The sample was obtained on January 23, 2018 by the team consisting of researchers from Sichuan Agricultural University and the special committee of China Nanmu Association. The leaf, the bark and tree core was taken. Some of the tree core samples were sliced and observed for microscopic structure, which was consistent with the microstructure of *P. bournei* wood (Chen et al. 2015). The samples of fresh bark and leaves were identified, which were consistent with the shape characteristics of *P. bournei* (*Delecti Flora Repubicae Popularis Sinicae* Agendae Academiae Sinicae. 1982). In summary, the sample species was regarded to be *P. bournei*.

At present, the tree core, bark, leaf samples and tree core wood chips (three slices) are kept in the Wood Engineering Laboratory of Sichuan Agricultural University, and their numbers are 20180123-MNP, 20180123-MNY, 20180123-MNM and 20180123-MNM (01-03). The moisture content of leaf was 12.1%.

Extraction of Essential Oils

First, a crushed sample of *P. bournei* leaf weighing 200±0.01g was accurately measured using an analytical balance and added to a 2000mL round-bottomed flask. Next, distilled water (1200 mL) was introduced, and several glass beads were added to prevent bumping. The extraction apparatus was assembled and heated for 5 h (until the volume of the essential oil remained constant). The volume of the extract was then recorded after settling for 0.5h.

Instrumentation

Agilent 7890A-5975C gas chromatography–mass spectrometry (GC–MS) system (Agilent);
Agilent 7890A gas chromatography (GC) system (Agilent);
Steam distiller (Shuniu Glassware Co., Ltd.);
Sartorius CP224S analytical balance (Sartorius);
Multifunctional enzyme marker (MultiskanFC)

**Experimental Method.**

Gas Chromatography Analysis.

Chromatographic column: Agilent HP-5 column (30m×0.25mm×0.50μm); sample injection volume: 1μL; split ratio: 50:1; Detector temperature: 250°C; carrier gas: ultra-pure helium; oven temperature program: 120°C for 3 min, to 140°C at 5°C/min for 5 min, to 160°C at 2°C/min for 10 min; flow rate: 1 mL/min. equipped with flame ionization detector. The components in the essential oils were identified by retention indices that were determined with C7–C20 alkane standards as the reference and that were confirmed by GC–MS.

Gas Chromatography–Mass Spectrometry Analysis.

*Gas chromatography conditions.* Chromatographic column: Agilent HP-5ms column (30m×0.25mm×0.25μm); sample injection volume: 1μL; split ratio: 50:1; injection port: 250°C; carrier gas: ultra-pure helium; oven temperature program: 120°C for 3 min, to 140°C at 5°C/min for 5 min, to 160°C at 2°C/min for 10 min; flow rate: 1 mL/min.

*MS conditions.* Ion source: electron ionization; ionization energy: 70eV; auxiliary heating zone: 280°C; ion source: 230°C; quadrupole: 150°C; data acquisition mode: full scan; mass scanning range: m/z 50–550; solvent delay: 3 min. The GC–MS spectra were analyzed via a computer-based automatic search, the automated and manual analyses of a mass spectral library (NIST), and a literature search to identify each component. Finally, the relative contents of each constituent were calculated using the peak area normalization method.

Table S1 The chemical composition of *P. bournei* leaf oil
| NO. | Compound               | KI | area | NO. | Compound            | KI | area |
|-----|------------------------|----|------|-----|---------------------|----|------|
| 1   | cyclosativene          | 1508 | 0.43 | 22  | 1s-calamenene       | 1854 | 5.18 |
| 2   | α-copaene              | 1515 | 5.44 | 23  | α-calacorene        | 1897 | 3.12 |
| 3   | bornyl acetate         | 1605 | 0.77 | 24  | tetradecanal        | 1908 | 0.56 |
| 4   | β-elemene              | 1605 | 0.81 | 25  | waitziacuminone     | 1942 | 1.29 |
| 5   | β-caryophyllene        | 1613 | 1.00 | 26  | 11-apollanol        | 2018 | 1.54 |
| 6   | aromadendrene          | 1625 | 0.44 | 27  | globulol            | 2081 | 0.79 |
| 7   | germacrene             | 1672 | 1.35 | 28  | elemol              | 2107 | 1.94 |
| 8   | α-humulene             | 1706 | 1.19 | 29  | spathulenol         | 2143 | 4.57 |
| 9   | γ-selinene             | 1714 | 1.32 | 30  | 1,5,5,8-tetramethylcyclo-3,7-dien-1-ol | 2180 | 1.02 |
| 10  | germacrene-D           | 1720 | 0.63 | 31  | γ-eudesmol          | 2189 | 3.22 |
| 11  | α-amorphene            | 1725 | 2.60 | 32  | T-cadinol           | 2193 | 2.19 |
| 12  | δ-selinene             | 1731 | 3.14 | 33  | torreyol            | 1500 | 2.50 |
| 13  | ledene                 | 1738 | 0.56 | 34  | α-cadinol           | 2206 | 3.32 |
| 14  | epizonaren             | 1742 | 2.18 | 35  | β-eudesmol          | 2243 | 3.82 |
| 15  | β-selinene             | 1746 | 1.61 | 36  | α-eudesmol          | 2246 | 3.95 |
| 16  | α-muurolene            | 1752 | 7.32 | 37  | t-cadinol           | 2252 | 3.17 |
| 17  | valencene              | 1755 | 0.41 | 38  | alloaromadendren    | 2262 | 0.59 |
| 18  | δ-cadinene             | 1779 | 11.4 | 39  | farnesylacetone     | 2471 | 0.41 |
| 19  | β-cadinene             | 1794 | 1.64 | 40  | phytol              | 2533 | 0.40 |
| 20  | selina-3,7(11)-dien e  | 1803 | 2.18 | 41  | myristic acid       | 2646 | 0.46 |
| 21  | α-cadinene             | 1824 | 1.38 | 42  | palmitic acid       | 2737 | 2.02 |

Total 93.90

†the KI values (retention indices) are calculated from the retention times relative to those of the C7–C30

**Antibacterial Activity Measurements of the Extracted Essential Oils**
Materials.

*Escherichia coli ATCC25922*, *Staphylococcus aureus subsp. Aureus ATCC29213*, *Salmonella enterica subsp. enterica ATCC14028*, and *Pseudomonas aeruginosa ATCC27853* were obtained from the China General Microbiological Culture Collection Center (CGMCC). Streptomycin was obtained from HyClone Co., and penicillin G sodium was obtained from Biosharp Co. Terbinafine, amphotericin B, DMSO were obtained from Sigma. Chloramphenicol was purchased from Scientific Research Special.

Experimental Method

Procedure. First, the samples were diluted to specific concentrations and added to the individual wells of a 96-well microplate. Next, the bacterial solutions were added, diluted to obtain a final concentration of $5 \times 10^5$ CFU/mL, and then cultured for 24 h at 37°C. A microplate photometer was used to measure the optical density (OD) below 625 nm, and each treatment was the same as the three controls, and the mean value was taken. Control cultures, blank media, bacterial cultures, and penicillin–streptomycin, and penicillin G sodium-positive controls were prepared. Inhibition rates were calculated based on the following formula. Inhibition rate = $(1 - \text{OD value of sample/OD value of control well}) \times 100\%$

![Figure S1 Inhibition of Four bacteria by different concentrations of the leaf essential oil from *P. bourseni*](image)

Antifungal Experiment. Take 96-well plate, dilute the sample to be tested, and add
fungal liquid to each well. The final concentration of Candida albicans is \(1 \times 10^5\) CFU/mL. Incubate at 37°C for 24h. *Epidermophyton floccosum, Trichophyton rubrum*

The final concentration of Plasmodium sp. was \(5 \times 10^5\) CFU/mL, incubated at 25°C for 5 days, and the OD value at 625 nm was measured with a microplate reader. At the same time, blank control medium, *Candida albicans, Epidermophyton floccosum, Trichophyton rubrum*, and *Pseudomonas aeruginosa* control, and amphotericin B and terbinafine positive drug control were also set. Inhibition rate=\((1-\text{OD value of sample/OD value of control well})\times100\%\)

![Figure S2. Inhibition of Four fungi by different concentrations of the leaf essential oil from *P. bournei*](image)

**Antitumor Activity Measurements of the Extracted Essential Oils**

**Materials**

Leukemia cell line HL-60, lung cancer cell line A549, hepatoma cell line SMMC-7721, breast cancer cell line MCF-7, and colon carcinoma cell line SW480 were used in this study. All cell lines were stored in the Kunming Institute of Botany. Cisplatin(DDP) and Taxol were also provided by the institute. All reagents were of analytical grade.

**Experimental Method**

Dulbecco’s modified Eagle medium(DMEM) culture medium containing 10% fetal bovine serum was used to formulate a single-cell suspension culture.
Approximately 3000–15000 cells per well were seeded into the 96-well plates, with a volume of 100 µL in each well. Adherent cells were cultured 12–24 h in advance. Next, 20 µL of the supernatant from the samples was removed and dissolved in dimethyl sulfoxide. The essential oil samples were rescreened using five concentration gradients: 100, 20, 4, 0.8, and 0.16 µg/mL. Each well had a final volume of 200 µL, each treatment was performed in triplicate, and the mean value was taken. The samples were incubated at 37°C for 48 h. After the incubation period, the media from the adherent cell cultures were discarded, and a solution of 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium(MTS) (20 µL) and culture medium(100 µL) were added to each well. For the cultured suspension cells, 100 µL of the supernatant was discarded from each well; then, MTS solution(20 µL) was added to each well. The cultures were further incubated for another 2–4 h. After the reactions had reached completion, the microplate photometer was employed to measure the OD value below 492 nm. DDP and Taxol were used as positive controls for each measurement. The cell growth curves were plotted with the concentration and cell viability on the x- and y-axes, respectively. The two-point Reed-Muench method was employed to calculate the half-maximal inhibitory concentration(IC$_{50}$) values of the compounds. Inhibition rate=$\left(1-\frac{OD \text{ value of sample}}{OD \text{ value of control well}}\right) \times 100\%$

| Table S2 Effect of _P. bournei_ Leaf essential oil on the IC$_{50}$ of five cancer cells |
|----------------------------------|------------------|------------------|------------------|------------------|------------------|
|                                  | Leukemia cell    | Lung cancer cell | Hepatoma cell    | Breast cancer    | Colon carcinoma |
|                                  | lineHL-60        | line A549        | lineSMMC-7721    | cell line MCF-7  | cell line SW480  |
| IC50±SD(µg/ml)                   | 43.6±1.20        | >100             | 84.8±5.02        | 40.5±0.61        | 41.3±0.58        |
| DDP                             | 2.3±0.81         | 4.7±0.26         | 6.9±0.59         | 8.2±0.57         | 8.1±1.44         |
| Taxol                           | <0.0068          | <0.0068          | <0.0068          | <0.0068          | <0.0068          |

_Hypoglycemic activity Measurements of the Extracted Essential Oils_
Materials

3T3-L1 adipocytes (adipocyte lines were preserved in Kunming Institute of Botany), insulin, berberine, and MTS were provided by Kunming plants Institute.

Experimental Method

After 3T3-L1 cells were induced to differentiate into adipocytes, the cells were digested and seeded in 96-well plates and cultured overnight. After the cells were washed once with a low-glucose medium (glucose concentration 1800 mg/L), 200μl of low-sugar medium containing different drugs was added. The control group was incubated with DMSO and 100nM insulin and berberine, respectively, and the test sample group was incubated at a final concentration of 40μg/ml. Three replicate wells per sample. After incubation for 24 hours, 10μl of cell culture medium was aspirated, and the glucose concentration in the culture fluid was measured by the glucose oxidase-peroxidase method. At the same time, 20μl of MTS was added to the cell rest medium, and incubated at 37°C for 2 hours. The absorbance at 492 nm was measured to judge whether the compound was toxic to adipocytes. Glucose concentration (mmol/L) = (sample absorbance / standard absorbance) × calibrator concentration. Glucose consumption rate (%) = (initial glucose concentration - experimental well glucose concentration) / initial glucose concentration × 100%

Table S3 Effects of *P. bournei* Leaf essential oil on Glucose Consumption Rate

| Sample               | Final concentration | Glucose consumption rate (%) |
|----------------------|---------------------|-----------------------------|
| DMSO (Control)       |                     | 16.23±1.57                  |
| Insulin              | 0.1μM               | 38.22±1.18                  |
| Berberine (235.324)  | 10μM                | 31.23±1.77                  |
| Leaf                 | 40μg/ml             | 20.23±1.45                  |

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