CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF THE ESSENTIAL OIL FROM *HERACLEUM SIAMICUM*

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ABSTRACT: *Heracleum siamicum* Craib (Apiaceae) is an important herbal spices having a wide applications in flavoring processed foods. The flat-oval shaped fruit of *H. siamicum* Craib from North Thailand was hydrodistilled and chemical composition of the essential oil was analyzed by GC and GC-MS. The essential oil yield based on dried plant material was 1.25% and twenty-five compounds (corresponding to 97.69% of the total weight) were identified. The main components were: *n*-octyl acetate (65.30%), *o*-cymene (10.35%), limonene (7.52%), δ-2-carene (6.87%), *cis*-thujone (1.92%), isobornyl acetate (0.94%), *n*-octanol (0.73%), 1,8-cineol (0.62%), *n*-tridecanol (0.44%), and safrole (0.37%). *H. siamicum* essential oil demonstrated bactericidal and fungicidal activity against five bacterial strains and two fungal strains, using agar diffusion and minimum inhibitory concentration methods.

Keywords: *Heracleum siamicum*, antimicrobial activity, chemical composition, hydrodistillation, volatile oil analysis, GC-MS

INTRODUCTION: *Heracleum siamicum* Craib (Apiaceae) is a perennial sturdy plant known as "Ma Laep" found in the northern (N) and northeast (NE) parts of Thailand[1)]. The fruits of *H. siamicum* are widely used as spices. In Thai folk medicine, the fruits of *H. siamicum* were used as a carminative herbal drug. Because of wide usage of the fruits of *H. siamicum* as medicinal plant and its use as flavoring agent, it was decided to carry out a phytochemical study on the fruit of this plant.

Many kinds of metabolites including coumarins, furanocoumarins, anthraquinones, stilbenes, furanocoumarin dimers, and flavonoids have been isolated and identified from various species of this genus[2-10)]. Plants belonging to the genus *Heracleum* are aromatic and are excellent sources of essential oils. The essential oil composition of various members of this genus have been reported, *H. persicum*[11,12)], *H. candolleanum* Wight et Arn. Gamble[13)], *H. dissectum* Ledeb.[14)], *H. sphondylium* L. subsp. *ternatum* (Velen.) Brummitt[15)], *H. crenatifolium* Boiss[16, 17)], *H. platytaenium* Boiss.[16)], and *H. candolleanum*[18)] contain monoterpene hydrocarbons (e.g. *p*-cymene; γ–terpene; α- and β–pinene; limonene etc.), oxygenated monoterpenes (e.g. iso-bornyl acetate, linalool, *n*-octanol, terpinene-1-ol-4 etc.), and sesquiterpene (e.g. caryophyllene oxide) in their volatile fractions. The extracts from the fruits of *H. persicum* Desf. ex Fisher showed antibacterial activity that inhibited the growth of *Staphylococcus aureus*, *Bacillus cereus*, and *Bordetella bronchiseptica*[19)]. Different octyl esters, especially *n*-octyl acetate, is reported to be the major constitute in most of the oils investigated[12,16,17,20,21)]. The volatile substances from *H. siamicum* fruits and their antimicrobial activity are reported for the first time in this communication. This study concerns chemical composition and antimicrobial activity of the essential oil from *H. siamicum*.

MATERIALS AND METHODS:

Plant material and Isolation of the Essential Oil

Fruit of *H. siamicum* Craib, was collected in January 2008 from the market of Chiangmai Province in the north of Thailand. A voucher specimen was deposited in the Department of Pharmacognosy and Pharmaceutical Botany, Chulalongkorn University. The dried fruits were hydrodistilled in a Clevenger-type apparatus, according to the literature[22)]. The oil was dried over anhydrous sodium sulfate and stored at 4°C in a vial covered with aluminum foil to prevent the negative effect of light and submitted to chemical and microbiological analysis.

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Analysis of Essential Compound

Analysis was performed with a Varian Star 3400 CX gas chromatograph coupled with a Saturn III mass spectrometer (Varian Inc.) system equipped with a Varian automatic injector and a 30 m long, DB-5 MS (J&W) capillary column (0.25 mm id, 0.25 μm film thickness). The ionization energy was 70 eV. A sample of 1.0 μl of a 4% solution of the fruit oil in hexane was injected with a split ratio of 100:1. The temperature of the injection block was 240°C. The GC oven temperature was programmed as follows: initial temperature 60°C (1 min) followed by a temperature increase of 3°C/min up to 200°C and a second ramp of 5°C/min to the final temperature of 220°C. The carrier gas was helium at 1.0 ml/min at constant volume. Identification of the oil components was established by comparing GC-MS spectra and RI with those of an internal Varian NIST MS 1998 library and those described by Adams[23].

Antimicrobial Activity

The microbial strains used in the antimicrobial assays were: the gram-positive bacteria *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29213), *Streptococcus faecalis* (ATCC 29212) and the gram-negative bacteria *Esherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 27853) and the pathogenic fungi *Candida albicans* (ATCC 10231) and *Microsporum gypseum* (a clinical isolate). Antimicrobial activities of the volatile oil of *H. siamicum* were determined using the agar-disc diffusion method[24-28], as described below. Each bacterial strain was grown on Trypticase Soy Agar plates at 37°C for 24 h. Portions of four discrete colonies were inoculated into 5 ml of Trypticase Soy Broth (TSB) and incubated at 37°C for 2−3 h. The turbidity of each culture was adjusted with sterile saline. For yeast, *C. albicans* was grown on Sabouraud Dextrose Agar (SDA) slant at 30°C for 24 h and some of the growth was transferred to 5 ml of sterile saline. Turbidity of the inoculum suspension was adjusted with sterile saline. *Microsporum gypseum* (of the mold spore) was grown on SDA at 30°C for 96 h, washed from the slant culture and adjusted to the desired turbidity with sterile 0.05% Tween 80. Additionally, the plates with internal diameter of 100 mm containing 25 ml of Muller-Hinton agar and SDA were inoculated with bacterial suspension and fungal suspension by streaking method respectively[25]. The wells (6 mm holes) were produced in the agar with sterile cork borer No.3. The fruit oils were diluted with sterile 0.05% Tween 80 to the final concentration of 1:20. Of this, 50 μL of the diluted samples were pipetted into each well. The plates were left at room temperature for 1 h and then incubated at 37°C for 24 h for bacteria and at 30°C for 96 h for fungi. The tests were carried out in duplicate. The minimum inhibitory concentrations of the oil using the dilution assays were determined as previously described[26]. Reading the results was carried out by measuring the diameters of the zones of inhibition and clear growth (in mm) and the minimum inhibitory concentration (MIC) was defined as the lowest concentration of the volatile oil which prevented growth of the inoculum compared with the growth control plate.

RESULTS AND DISCUSSION: Fruits contained 1.25% (v/w) essential oil (dried weight) was light yellow liquid in color and possessed a distinct sharp odor. Table 1 and Figure 1 showed its chemical composition. Twenty-five compounds were identified by comparison of their retention indexes and the mass spectra of each GC component with those of standards and with reported data. Terpenes and their derivatives predominated, with the most abundant one being *n*-octyl acetate (65.30%), followed by *o*-cymene (10.35%), limonene (7.52%), δ-2-carene (6.87%), *cis*-thujone (1.92%), isobornyl acetate (0.94%), *n*-octanol (0.73%), 1,8-cineol (0.62%), *n*-tridecanol (0.44%), and safrole (0.37%), respectively. These main components comprised more than 95% of the essential oil. We should also note the presence in the essential oil of a total 4.31 % of alcohol hydrocarbons (Table 2). Although most of these compounds are well documented as essential oil components in various plant species[29], to our knowledge this is the first reported of their occurrence in the essential oil of *H. siamicum*.

Table 1 Chemical composition of essential oil from *Heracleum siamicum* determined by GC-MS

No	Compound	RI*	Percent
1	Tricyclene	926	0.22[a, c]
2	α-Thujene	930	0.21[a]
3	δ-2-Carene	1001	6.87[a, c]
4	δ-3-Carene	1011	0.33[a, c]
5	α-Terpinene	1017	0.07[a]
6	*o*-Cymene	1022	10.35[b]
7	Limonene	1030	7.52[b, c]
8	1,8-Cineol	1033	0.62[a, c]
9	*n*-Octanol	1070	0.73[a]
10	Linalool	1097	0.13[b]
11	*cis*-Thujone	1102	1.92[a]
12	*trans*-Pinocarveol	1139	0.10[a, b]
13	Camphor	1143	0.26[b, c]
14	Borneol	1165	0.10[b, c]
15	Terpin-4-ol	1177	0.13[a, b]
16	*n*-Octyl acetate	1194	65.30[b]
17	Isobornyl acetate	1285	0.94[a]
18	Safrole	1285	0.37[a, c]
19	α-Copaene	1376	0.36[a]
20	β-Bourbonene	1384	0.27[a]
21	9-epi-(*E*)-Caryophyllene	1467	0.14[a, c]
22	Citronellyl isobutyrate	1482	0.11[a]
23	Viriflorene	1493	0.39[a]
24	δ-Cadinene	1523	0.24[a]
25	*n*-Tridecanol	1575	0.44[a, c]

*RI determined on a DB-5 column using the homologous series of *n*-hydrocarbons (Kovats index).
[a]Identification was based on RI. [b]Identification was based on comparison of the GC-MS spectra and RI with those of internal (computer) NIST library and those described by Adams. [c]Identification was based on comparison of authentic standards.

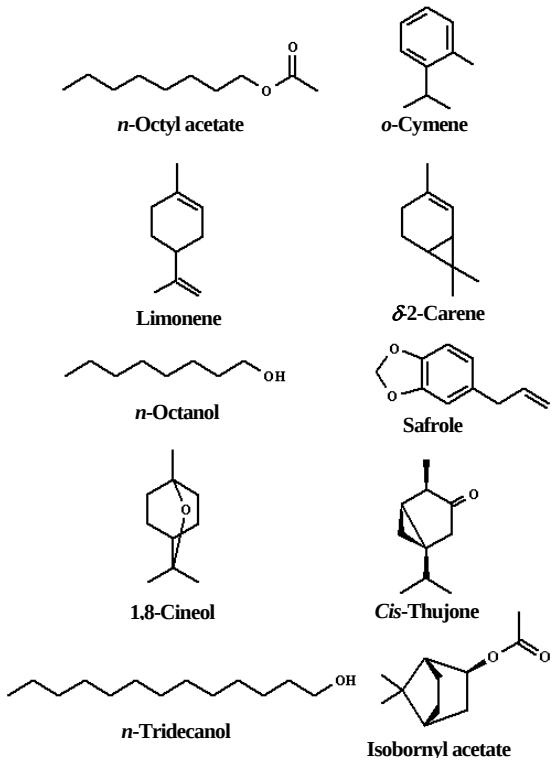

Figure 1 Structure of the major components of essential oil of *H. siamicum*

The dominant compound, *n*-octyl acetate, has been reported as a common component in most fruit oils of *Heracleum* genus and also reported as the constitute in *Boswellia carterii* Birdw[30], *Peucedanum cervaria* (L.) Lapeyr[31], and grapefruit oil[32]. In a recently, the essential oil of *H. sphondylium* subsp. *ternatum* contain the major constitute as *n*-octanol had a high antimicrobial activity against *Candida albicans* with a MIC of 0.5 mg/ml[15]. Limonene has been shown to be biologically active as an antitumour agent[33]. The essential oil of *Grammosciadium platycarpum* Boiss. contained the major constitute as limonene had a high antimicrobial activity against *Staphylococcus epidermidis* with a MIC of 0.6–5 mg/ml[34].

Interestingly, there were significant differences between the main components of the essential oil of *H. siamicum* Craib and those previously determined in *H. crenatifolium* Boiss.[17] which belongs to the same genus. Thus, terpene alcohols such as *n*-octanol, limonene, and linalool are quantitatively abundant in *H. candolleanum* oil, whilst they were only present in much smaller quantities in *H. siamicum* oil (Table 3).

Results of the antimicrobial activity tests of *H. siamicum* essential oil was examined by agar disc diffusion assay and minimum inhibition concentration against an array of five bacteria and two fungi selected on the basis of their relevance to public health (Table 4). The oil demonstrated strong bacteriostatic rather than fungistatic activities. Particularly significant were the inhibition zone diameters observed for dilution essential oil against *Staphylococcus aureus*, *Bacillus subtilis*, and *Candida albicans*, while *Ecsherichia coli*, *Pseudomonas aeruginosa* and *Microsporum gypseum* were less sensitive to the oil.

The oil showed an MIC of 20 μg/ml against *S. aureus*, 25 μg/ml against *B. subtilis*, and 50 μg/ml against *C. albicans*, respectively. Moreover, the paper clearly suggests that the chemical composition (δ-2-carene, *o*-cymene, *cis*-thujone, limonene, isobornyl acetate, *n*-octanol, 1,8-cineol, linalool, and safrole) show synergistic effect which is much more greater than the sum of anti-bacterial effect of each component used alone[35-39].

Table 2 Composition of *H. siamicum* essential oil by substance classes

Compounds	% in essential oil
Monoterpenes	25.14
Sesquiterpenes	1.40
Saturated	66.47
Hydrocarbon total:	***93.01***
Alcohols	4.31
Deoxymethylene	0.37
Oxygenated compounds total:	***4.68***
Total compounds:	**97.69**

Table 3 Main composition of the essential oils from *H. siamicum* and *H. crenatifolium*[17]

H. siamicum **(Relative amount,%)**	***H. crenatifolium*** **(Relative amount, %)**
n-octyl acetate (65.30%)	octyl acetate (88.4%)
o-cymene (10.35%)	octanol (3.10%)
limonene (7.52%)	(*Z*)-4-octenyl acetate (1.0%)
δ-2-carene (6.87%)	octyl 2-methyl butyrate (0.9%)
cis-thujone (1.92%)	octyl hexanoate (0.7%)
isobornyl acetate (0.94%)	hexyl 2-methyl butyrate (0.7%)
n-octanol (0.73%)	α-pinene (0.7%)
1,8-cineol (0.62%)	octanal (0.6%)
n-tridecanol (0.44%)	myristicin (0.4%)
safrole (0.37%)	limonene (0.3%)

Table 4 Antimicrobial activity of essential oil from *H. siamicum*

Tested Microorganism*	Inhibition zone (mm)[a]	MIC (μg/ mL)
Bacillus subtilis ATCC 6633	11.23 ± 0.73	25
Candida albicans ATCC 10231	9.70 ± 0.93	50
Ecsherichia coli ATCC 25922	–	–
Streptococcus faecalis ATCC 29212	–	–
Streptococcus aureus ATCC 29213	11.43 ± 1.01	20
Microsporum gypseum (a clinical isolate)	–	–
Pseudomonas aeruginosa ATCC 27853	–	–

*Tested in 50 μl of 10% oil in Tween 80;
[a] mean values ± SD, The 0.05% Tween 80 did not show any activity, – No inhibition zone.

In addition to the individual monoterpene alcohol of *Salvia fructicosa* Mill. (Lamiaceae), such as 1,8-cineole, and thujone, a major constituents showed relatively high levels of antimicrobial activity against the bacteria, while camphor was inactive against the bacteria tested[33]. However, it is possible that the activity of the main components is modulated by other minor molecules[39-41]. In general, the cytotoxic activity of essential oils is mostly due to the presence of aldehydes, alcohols, methylene dioxy compounds, and phenols[28,42-43].

Our group has previously reported on the antimicrobial activity of *Cinnamomum porrectum* (Roxb.) Kosterm (collected from E. Thailand) essential oil that has safrole (99.8%) as the main component had a very high antimicrobial activity against *Candida albicans* with a MIC of 0.063% by volume[29]. Moreover, *C. porrectum* oil provided a potential for treatment of *Candida* infections and prepared in solution and emulgel topical dosage forms at various concentration (1, 2, and 5% w/w)[44]. *H. siamicum* has moderate oil content and a high proportion of monoterpenes in its profile. The dominance of *o*-cymene, limonene, and the presence of isobornyl acetate in the profile make *H. siamicum* a good candidate for further study in terms of its allelochemical properties, and the agreeable fragrance of the oil makes it of possible interest to the flavoring agent in food and in perfumery.

CONCLUSION: Our GC and GC-MS study of the essential oil of *H. siamicum* from N.Thailand led to the identification of twenty-five compounds, representing 97.69% of the total mass. The main constitutes were terpenes and their derivatives, and the most prominent one was *o*-cymene (10.35%). The antimicrobial activity results presented here demonstrate that this plant essential has a commercial potential.

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