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CHEMICAL COMPOSITION, ANTIBACTERIAL AND ANTIFUNGAL ACTIVITIES OF ESSENTIAL OIL FROM *HERACLEUM SIAMICUM* CRAIB

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Heracleum siamicum Craib (Apiaceae), is an important herbal species having wide application in food flavoring processes. The flat-oval shaped fruit of *H. siamicum* Craib from North Thailand was hydrodistilled, and the 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, as evaluated using agar diffusion in terms of the minimum inhibitory concentration.

Key words: *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 and northeast 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 material and flavoring agent, it was decided to carry out a phytochemical study on the fruit of this plant.

Many kinds of metabolites including coumarins, furanocoumarins, anthroquinone, stilbene, furanocoumarin dimer, and flavonoids have been isolated and identified from various species of this genus [2 – 10]. Plant belonging to the *Heracleum* genus are aromatic and are excellent sources of essential oils. Essential oil composition of various members of this genus have been reported, including *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]. These oils 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 sesquiterpenes (e.g. caryophyllene oxide) in their volatile fractions. 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, were reported to be the major constitute in most of the oils investigated [12, 16, 20, 21].

The present study addresses the chemical composition and antimicrobial activity of the essential oil from *H. siamicum*.

RESULTS AND DISCUSSION

The fruits of *H. siamicum*. contained 1.25% (w/w) essential oil (DW), which appeared as a liquid of light yellow color and possessed a specific sharp odor. Table 1 shows the established chemical composition. Twenty-five compounds were identified by comparison of their retention indexes and the mass spectra of each component revealed by gas chromatography (GC) to those of standards and reported data. Terpenes and their derivatives predominated, with the most abundant one being *n*-octyl acetate (65.30%), followed by

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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%). These main components (Fig. 1) comprised more than 95% of the essential oil. We should also note the presence of a total 4.31 % of alcohol hydrocarbons in the essential oil (see Table 3). Although most of these compounds are well documented as essential oil components in various plant species [22], to the best of authors' knowledge, this is the first report of their occurrence in the 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 constituent in *Boswellia carterii* Birdw.[23], *Peucedanum cervaria* (L.) Lapeyr. [24], and grapefruit oil [25]. Recently, the essential oil of *H. sphondylium* subsp. *ternatum*, which contains

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

No	Compound	RI[a]	Percent	Identification
1	Tricyclene	926	0.22	a, c
2	a-Thujene	930	0.21	a
3	d-2-Carene	1001	6.87	a, c
4	d-3-Carene	1011	0.33	a, c
5	a-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	a-Copaene	1376	0.36	a
20	b-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	d-Cadinene	1523	0.24	a
25	*n*-Tridecanol	1575	0.44	a, c

[a] RI determined on a DB-5 column using the homologous series of *n*-hydrocarbons (Kovats index).
[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.

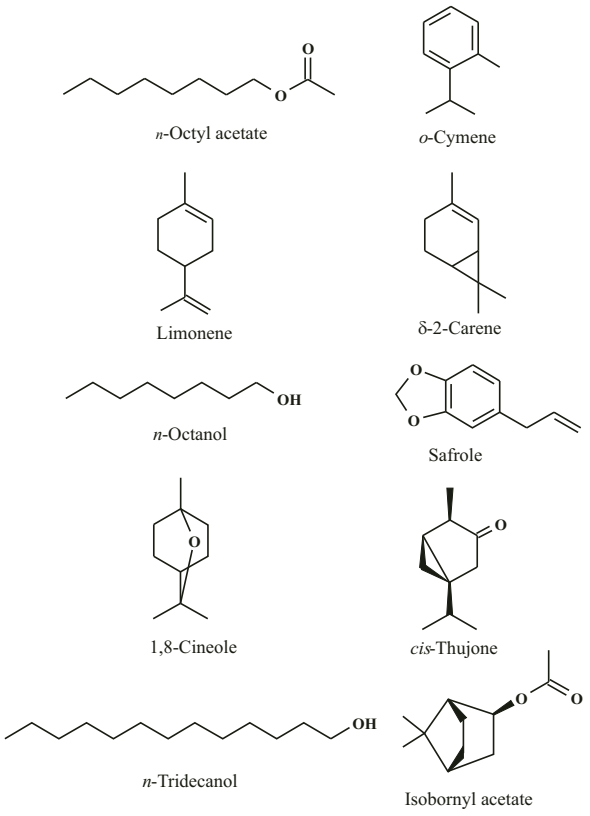

Fig. 1. Structure of the major components of essential oil of *H. siamicum*

n-octanol as the major component, was reported to exhibit d a high antimicrobial against *Candida albicans* [15]. Limonene was shown to be biologically active as an antitumor agent [26]. The essential oil of *Grammosciadium platycarpum* Boiss, which contains limonene as the major constitute, exhibited a high antimicrobial activity [27].

It is interesting to note that 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,

TABLE 2. Composition of *H. siamicum* essential oil by substance class

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**

while being only present in much smaller amounts in *H. siamicum* oil (Table 3).

The antimicrobial activity of *H. siamicum* essential oil was evaluated by the agar disk diffusion assay as minimum inhibition concentration (MIC) against an array of five bacteria and two fungi selected on the basis of their relevance to public health (see Table 4). The oil demonstrated strong bacteriostatic activity (with respect to *Staphylococcus aureus* and *Bacillus subtilis)*, rather than fungistatic activity (which was more or less pronounced only for *Candida albicans*). The species of *Ecsherichia coli*, *Pseudomonas aeruginosa* and *Microsporum gypseum* were much less sensitive to the essential oil.

CONCLUSIONS

Our GC and GC-MS study of the essential oil of *H. siamicum* from Northern Thailand led to the identification of 25 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 essential oil has a commercial potential.

EXPERIMENTAL

Plant Material Preparation and Isolation of Essential Oil

Fruits of *H. siamicum* Craib, were collected in January 2008 from the market of Chiangmai Province, 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 [28]. 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) until chemical analyses and microbiological tests.

Analysis of Essential Oil

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 i.d., 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 the second ramp at 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 [29].

Antimicrobial Activity

The microbial strains used in the antimicrobial assays were the gram-positive bacteria *Bacillus subtilis* (ATCC 6633), *Staphylococcus aureus* (ATCC 29213), and *Streptococcus faecalis* (ATCC 29212), 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 – disk diffusion method [30 – 33], 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

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%)
d-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 *Heracleum siamicum*

Tested Microorganism*	Inhibition zone (mean values ± SD; mm; MIC)
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 ml of 10% oil in Tween 80; number in parentheses refer to MIC values (*m*g/ mL). (The 0.05% Tween 80 did not show any activity)

– No inhibition zone.

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 and fungal suspensions by the streaking method [43]. 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 and 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. All tests were carried out in duplicate. The MIC values of the oil using the dilution assays were determined as described in [32]. The results were evaluated by measuring the diameters of the zones of inhibition and clear growth (in millimeters) 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 in control plates.

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