

ESSENTIAL OIL FROM *KAEMPFERIA ANGUSTIFOLIA* RHIZOME: CHEMICAL COMPOSITIONS AND ANTIMICROBIAL ACTIVITIES

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ABSTRACT: Essential oil hydrodistilled (ca. 0.26%, fresh wt.) from the rhizome of *Kaempferia angustifolia* Roscoe (Zingiberaceae) was investigated for its chemical compositions by GC-MS analysis. The oil showed at least 30 components which accounted for monoterpenes (66.73%), oxygenated monoterpenes (27.89%), sesquiterpenes (3.49%), oxygenated sesquiterpenes (1.35%) and others (0.54%). The main constituents in the essential oil from rhizome of *K. angustifolia* were camphene (45.53%), camphor (17.53%), α -pinene (12.22%) and borneol (5.16%). A preliminary antimicrobial activity of the oil was evaluated using agar diffusion method. The oil exhibited moderately active against gram-positive bacterium *Staphylococcus aureus* ATCC 29213 and gram-negative bacterium *Pseudomonas aeruginosa* ATCC 27853 (10.63 ± 0.23 , 10.03 ± 0.22 mm respectively) whilst no activities on *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Streptococcus faecalis* ATCC 29212, *Candida albicans* ATCC 10231 and *Microsporum gypseum*.

Keywords: *Kaempferia angustifolia*, antimicrobial activity, chemical compositions

INTRODUCTION: The *Kaempferia* (Zingiberaceae) is a genus of perennial rhizomatous herbs that consists of economically important species as Prauh-Hom (*Kaempferia galanga* L.) and Krachai-Dam (*Kaempferia parviflora* Wall. ex Baker). *Kaempferia angustifolia*, is known in Thai as Thao-Nhang-Haeng, occurs in monsoon forests as indigenous plant of Thailand and is also used as popular ingredient of charmingly Buddha images. Its rhizome has preferable smell and used traditionally as medicines for the treatment of cold, dysentery and cough¹. Neither phytochemical nor biological studies have been reported on essential oil of this particular species previously. Herein we reported the volatile constituents and its preliminary studied of antimicrobial activity for support the ethnomedical uses.

MATERIALS: The rhizomes of *Kaempferia angustifolia* were purchased from local market in Bangkok during October 2006. Authentication was achieved by comparison with the herbarium specimen at Bangkok Forest Herbarium, Ministry of Agriculture and Cooperative, Thailand. The voucher specimen

has been deposited in Natural Medicine Museum, Faculty of Pharmaceutical Sciences, Chulalongkorn University, Thailand.

METHODS:

Isolation of essential oil

The oil was obtained by hydrodistillation from rhizome using Clevenger apparatus for 4-6 hours until it was no significant increased in the volume of oil collected.

Volatile oil composition analysis

The oil was investigated by capillary column gas chromatography/ mass spectrometry. GC/MS qualitative and quantitative analyses were carried out using a Finnigan Trace GC ultra with Finnigan DSQ Quadrupole detector and BPX5 fused silica column (30 m x 0.25 mm, 0.25 μ m film thickness). The injector temperature was 180°C. Sample, 1 μ l of the oil (1:100 in methanol), was injected by splitter (1:100) into capillary column. The oven temperature was 60°C for 1 min., then ramp to 240°C with the rate of 3°C/ min. Helium was used as carrier gas (flow rate 1 ml/min). MS was performed by EI positive mode at 70 eV ionization voltages. The

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constituents of the oil were identified by matching their mass spectra and retention indices with Adams EO Mass Spectral library and NIST02 MS library and the percentage composition was computed from GC peak areas.

Antimicrobial assay

Test organisms

The bacterial strains used were *Staphylococcus aureus* ATCC 29213, *Pseudomonas aeruginosa* ATCC 27853, *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633 and *Streptococcus faecalis* ATCC 29212. The fungi strains used were *Candida albicans* ATCC 10231 and *Microsporum gypseum*.

Agar well diffusion method

The antimicrobial assay was performed by the agar well diffusion method², a well was prepared in the plates with the help of a cork-borer. The test compound was introduced into the well (50 μ l of 10% oil in tween80 0.05%). The plates were incubated overnight at 37 °C. Microbial growth was determined by measuring the diameter of zone of inhibition. The result was obtained by measuring the zone diameter (Table 2).

RESULTS AND DISCUSSION: The percentage yield of essential oil hydrodistilled from *Kaempferia angustifolia* rhizome was ca. 0.26 % of fresh weight. GC/MS analysis (as shown in Figure 1, 2 and Table 1) revealed the present of monoterpenes (66.73%), oxygenated monoterpenes (27.89%), sesquiterpenes (3.49%), oxygenated sesquiterpenes (1.35%) and others (0.54%). The major constituents were shown as camphene (45.53%), camphor (17.53%), α -pinene (12.22%), borneol (5.16%) and 1,8-cineole (4.38%). The oil exhibited moderately active against gram-positive bacterium *Staphylococcus aureus* ATCC 29213 and gram-negative bacterium *Pseudomonas aeruginosa* ATCC 27853 (10.63 ± 0.23 , 10.03 ± 0.22 mm respectively) whilst no activities were found on *Escherichia coli* ATCC 25922, *Bacillus subtilis* ATCC 6633, *Streptococcus faecalis* ATCC 29212, *Candida albicans* ATCC 10231 and *Microsporum gypseum* (Table 2). The antimicrobial activities might be due to the presence of 1,8-cineole (4.38%) as the reported earlier³. Since this plant species has never been used popularly in Thai traditional medicine, it should be recommended to use as anti-dysentery as described in introduction section.

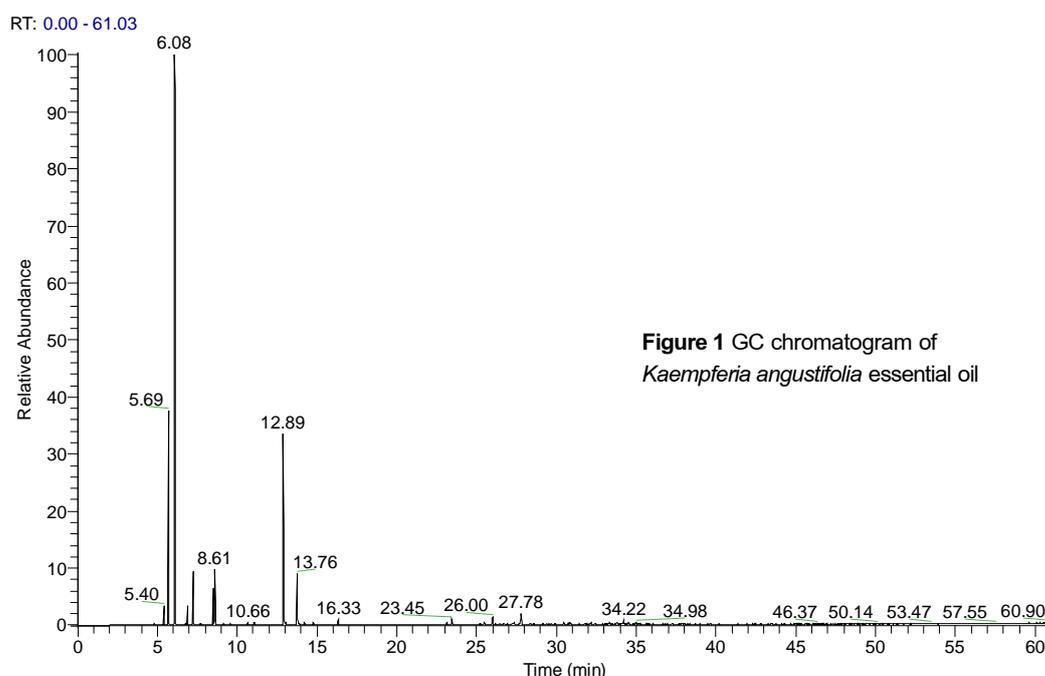


Figure 1 GC chromatogram of *Kaempferia angustifolia* essential oil

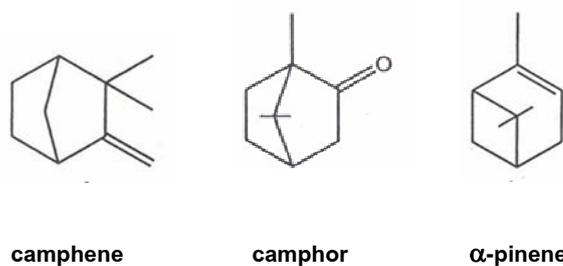


Figure 2 chemical structure of main components of *Kaempferia angustifolia* essential oil

Table 1 Chemical compositions of the essential oil from *Kaempferia angustifolia*

RT	Chemical Composition	Area %	Kovat's Index ³
5.40	Tricyclene	1.22	926
5.69	Pinene<alpha->	12.22	939
6.08	Camphene	45.53	954
6.89	Pinene<beta->	1.52	979
7.24	Myrcene	3.40	990
8.50	Sylvestrene	2.59	1030
8.61	Cineole<1,8->	4.38	1031
10.66	Terpinolene	0.25	1088
11.07	Linalool	0.23	1096
12.89	Camphor	17.53	1146
13.04	Camphene hydrate	0.30	1149
13.76	Borneol	5.16	1169
14.22	Terpinen-4-ol	0.13	1177
14.78	Terpineol<alpha->	0.16	1188
16.33	Isobornyl formate	0.54	1239
23.14	Elemene<beta->	0.16	1390
23.45	Cyperene	0.58	1398
25.50	Ylangene<alpha->	0.28	1375
26.00	Ishwarane	0.81	1466
27.34	Bicyclogermacrene	0.23	1500
27.78	Zonarene	1.18	1529
29.66	Germacrene B	0.11	1561
30.47	Spathulenol	0.14	1578
30.84	Viridiflorol	0.12	1592
32.17	Colocalene<alpha->	0.14	1623
33.35	Selin-11-en-4-alpha-ol	0.13	1659
33.80	Intermedeol	0.19	1666
34.22	Caryophyllene<14-hydroxy-9-epi-(E)->	0.43	1669
34.51	Cadinol<alpha->	0.15	1654
34.98	Cyclocolorenone	0.19	1760

Table 2 Antimicrobial activity of volatile oil from *Kaempferia angustifolia*

Microorganism	Inhibition zone (mm)			Mean ± SD (mm)
<i>E. coli</i>	NA	NA	NA	
<i>S. aureus</i>	10.7	10.6	10.6	10.63 ± 0.23
<i>P. aeruginosa</i>	10.1	10.0	10.0	10.03 ± 0.22
<i>B. subtilis</i>	NA	NA	NA	
<i>S. faecalis</i>	NA	NA	NA	
<i>C. albicans</i>	NA	NA	NA	
<i>M. gypseum</i>	NA	NA	NA	

NA = no activity

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น้ำมันระเหยจากเหง้าเผ่าหนั่งแห้ง: องค์ประกอบทางเคมีและฤทธิ์ต้านจุลชีพ

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บทคัดย่อ: น้ำมันระเหยกลั่นจากเหง้าของเผ่าหนั่งแห้ง (ด้วยผลผลิตประมาณร้อยละ 0.26 จากน้ำหนักสด) ซึ่งได้ทำการศึกษาองค์ประกอบทางเคมีด้วยวิธีแก๊สโครมาโทกราฟี/แมสสเปกโตรเมตรี พบว่ามีองค์ประกอบอย่างน้อย 30 ชนิดซึ่งประกอบด้วย โมโนเทอร์ปีน ออกซีจันเตตเตดโมโนเทอร์ปีน เซทควิเทอร์ปีน ออกซีจันเตตเตดเซทควิเทอร์ปีน และอื่นๆ ร้อยละ 66.73, 27.89, 3.49, 1.35 และ 0.54 ตามลำดับ และมีองค์ประกอบหลักเป็น แคมฟีนิ แคมเฟอร์ แอลฟาไพเนล และบอร์นีออล ร้อยละ 45.53, 17.53, 12.22 และ 5.16 ตามลำดับ ส่วนการศึกษาฤทธิ์ต้านจุลชีพเบื้องต้นของน้ำมันโดยวิธีการแพร่กระจายในวุ้นพบฤทธิ์ต้านแบคทีเรียในระดับปานกลางต่อแบคทีเรียแกรมบวก เช่น *S. aureus* (10.63 ± 0.23 มม.) แบคทีเรียแกรมลบ เช่น *P. aeruginosa* (10.03 ± 0.22 มม.) และไม่มีฤทธิ์ต่อ *E. coli*, *B. subtilis*, *S. faecalis*, *C. albicans* และ *M. gypseum*

คำสำคัญ: เผ่าหนั่งแห้ง ฤทธิ์ต้านจุลชีพ องค์ประกอบทางเคมี

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