Chemical Composition and Cytotoxic Activity of the Essential Oil of *Zingiber ottensii*

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Abstract

The hydrodistilled essential oil from the rhizome of *Zingiber ottensii* collected in Phetchaburi province, Thailand was analyzed by GC and GC/MS. Twenty-eight constituents were identified of which zerumbone (40.1%), terpinen-4-ol (11.2%), p-cymene (6.9%), sabinene (6.5%) and humulene (5.6%) were the major components. Cytotoxic activity using brine shrimp lethality test showed moderate toxicity (LC_{50}= 65.5 μg/ml).

INTRODUCTION

Zingiber ottensii Valeton (Zingiberaceae) is native to South East Asia. It is an interesting ginger differing from the others by the internally violet or ink-coloured rhizomes (Sirat, 1994). In Thai traditional medicine *Z. ottensii* rhizomes are commonly used externally for relieving bruises, sprains and inflammations. Internally, the rhizones are employed as remedies for gastrointestinal disorders and for strengthening purposes (Mahidol University Faculty of Pharmacy, 1995). A few phytochemical studies have been carried on with *Z. ottensii*, which resulted in the isolation of terpenoids including a monoterpene (terpinen-4-ol), sesquiterpenes (humulene, humulene oxide, and zerumbone) and diterpene (E)-labda-8(17), 12-diene-15, 16-dial (Sirat, 1994; Sirat and Nordin, 1994). In this paper we have investigated the composition of the essential oil of the rhizome of *Z. ottensii* from Thailand and its cytotoxic activity using brine shrimp lethality test.

MATERIALS AND METHODS

Plant Material and Isolation of Essential Oil

The plant material was collected in March 2000 from Petchaburi province in the central part of Thailand. The fresh rhizome was hydrodistilled for 5 hours in a Clevenger-type apparatus. The isolated oils were dried over anhydrous sodium sulphate. The oil yield was calculated relative to the dry matter.

Analysis

The oil was analyzed by GC and GC/MS. GC analysis was carried out on a Fisons gas chromatograph model 8000 series equipped with a FID detector and a DB-5 capillary column (30 m × 0.25 μm ; film thickness 0.25 μm). The operating conditions were as follows; carrier gas: helium with a flow rate of 2 ml/min; column temperature: 50-220°C at 4°C/min : injector and detector temperatures: 230°C. GC/MS analysis was performed on a VG Quattro mass spectrometer operating at 70 eV ionization energy, equipped with a DB-wax column (60 m x 0.3 mm x 0.25 μm). The oven temperature was programmed from 35°C (5 min) to 220°C (45 min) at 3°C/min, with helium as carrier gas. The identification of the oil components was accomplished by comparison of their GC retention indices as well as their mass spectra with corresponding data of authentic compounds or published spectra (Heller and Milne, 1978, 1980, 1983; Adams, 2001).

Cytotoxicity Test

Brine shrimp lethality test was modified from the microwell cytotoxicity assay

method. The eggs of brine shrimp (*Artemia* spp., Kuan-Im Brand, USA) were hatched in artificial sea water (Q-SEA®). After 24 hrs, the nauplii of brine shrimp hatched. Stock of the test sample was prepared by dissolving the sample in 10% (v/v) Tween 80 in artificial sea water to the final concentration of 2 mg/ml. Serial dilutions of the sample were made and transferred to six microwells of each concentration. About five nauplii were transferred to each well. Control microwells were prepared by using only 10% (v/v) Tween 80 in artificial sea water and then treated in the same manner as the test sample. The microwells were maintained under illumination. After 24 hrs, the dead and the survivors were counted. The LC_{50} values were calculated by the probit analysis method (Finney, 1964)

RESULTS AND DISCUSSION

The rhizome of *Z. ottensii* yielded 0.38% of a pale yellowish oil with a characteristic camphorous odor. 28 components were identified, representing 84.9% of the total oil. These are listed in Table 1 in order of the elution on the DB-WAX column. The oil contained a mixture of mono and sesquiterpenes with zerumbone (40.1%) being the most abundant component overall. Other important compounds were terpinen-4-ol (11.2%), p-cymene (6.9%), sabinene (6.5%) and humulene (5.6%). These compositions are in accordance with the previously published literature (Sirat, 1994) except for the absence of p-cymene in Malaysian oil and higher zerumbone content in Thai oil. Zerumbone is an oxygenated derivative of humulene which have been reported as a potential fungitoxic agent (Kishore and Dwivedi, 1992) and have been found in the essential oil of *Z. zerumbet* (65%) (Leehat- Vahirua, 1993).

Evaluation of biological activity using brine shrimp lethality test showed that the oil of *Z. ottensii* exhibited moderate cytotoxic activity at LC_{50}= 65.5 μg/ml. According to previous data of antifungal activities of zerumbone, this plant should be the important source of antifungal agent which is required in our country. As a consequence, further study on other biological activities, strain improvement for higher content of zerumbone will be performed.

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Literature Cited

Adams, R.P. 2001. Identification of essential oil components by gas chromatography quadrupole mass spectrometry. Allured publishing Corp. Carol Springs, IL.

Finney, D.J. 1964. Prohibit analysis. 2nd ed. The Syndics of the Cambridge University Press, London. 20-22 p.

Heller, S.R. and Miline, G.W.A. 1978, 1980, 1983. EPA/NIH Mass Spectral Data Base, U.S. Government Printing Office, Washington D.C.

Kishore, N. and Dwivedi, R.S. 1992. Zerumbone: A potential fungitoxic agent isolated from *Zingiber cassumunar* Roxb. Mycopathologia 120(3):155-159.

Leehat-Vahirua, I. 1993. Aromatic plants of Frence polynesia. I. Constituents of the essential oils of rhizomes of three Zingiberaceae: *Zingiber zerumbet* Smith, *Hedychium coronarium* Koenig and *Etlingera cevuga* Smith. J. Essent. Oil Res. 5:55-59.

Mahidol University, Faculty of pharmacy. 1995. Siam Paisat-chaya-pruek (in Thai). Bangkok, Mahidol University Faculty of Pharmacy. 212p.

Sirat, H.M. 1994. Study on the terpenoids of *Zingiber ottensii*. Planta Med. 60:497.

Sirat, H.M. and Nordin, A.B. 1994. Essential oil of *Zingiber ottensii* Valeton. J. Essent. Oil Res. 6:635-636.

Tables

Table 1. Percentage composition of the essential oil of *Zingiber ottensii* from Thailand.

Compound	Percentage
α-pinene	0.92
β-pinene	4.32
sabinene	6.48
myrcene	0.36
α-terpinene	0.11
limonene	0.53
1,8-cineole	1.19
γ-terpinene	0.17
E-β-ocimene	0.08
p-cymene	6.93
terpinolene	0.15
sabinene hydrate	0.29
linalool	0.10
bornyl acetate	0.27
β-elemene	0.12
β-caryophyllene	0.35
terpinen-4-ol	11.17
cis-menth-2-en-1-ol	0.23
humulene	5.64
borneol	0.19
trans-piperitol	0.17
4-phenylbutan-2-one	0.14
p-cymen-8-ol	0.24
caryophyllene oxide	0.77
humulene oxide	2.85
α-eudesimol	0.40
β-eudesimol	0.61
zerumbone	40.14

Figures

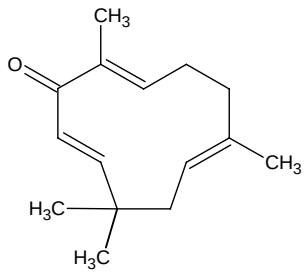

Fig. 1. Structure of zerumbone.