

Chemical constituents of the essential oil and antibacterial activity of *Zingiber wrayi* var. *halabala*

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Abstract

Chairgulprasert, V., Prasertsongskun, S. and Wichaporn, W.
Chemical constituents of the essential oil and antibacterial activity of
Zingiber wrayi var. *halabala*

Zingiber wrayi var. *halabala*, a local herb from the Bala Forest in Narathiwat, was investigated for its chemical constituents and antibacterial activity. The essential oil was obtained by steam distillation of fresh rhizomes in 3.6 % yield. The GC-MS data indicated the presence of four compounds including *trans*-anethole, estragol, camphor and *m*-phenylphenol. Further quantitative analysis showed the essential oil to contain 96.8% w/w of *trans*-anethole. The oil, together with petroleum ether, dichloromethane and methanol extracts, were assayed for antibacterial activity. The essential oil, petroleum ether and dichloromethane extracts exhibited antibacterial activity against *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* and *Sarcina* sp. However, none of the extracts was active against *Pseudomonas aeruginosa*.

Key words : *Zingiber wrayi*, essential oil, antibacterial activity, *trans*-anethole, halabala

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บทคัดย่อ

วนิดา เจียรกุลประเสริฐ สมพร ประเสริฐสงสกุล และ วิรัตดา วิชาพร
องค์ประกอบทางเคมีของน้ำมันหอมระเหยและฤทธิ์ต้านแบคทีเรียของปลูดหวาน
(*Zingiber wrayi* var. *halabala*)

การวิเคราะห์องค์ประกอบทางเคมีและฤทธิ์ต้านแบคทีเรียของปลูดหวานซึ่งเป็นพืชสมุนไพรที่พบในป่าบาลา จังหวัดนราธิวาส พบว่าเหง้าปลูดหวานสดเมื่อนำมากลั่นด้วยไอน้ำ ได้น้ำมันหอมระเหย 3.6% จากการตรวจสอบองค์ประกอบทางเคมีของน้ำมันหอมระเหยด้วยเครื่องแก๊สโครมาโทกราฟี-แมสสเปกโตรเมตรี พบสารองค์ประกอบ 4 ชนิด คือ *trans-anethole*, *estragol*, *camphor* และ *m-phenylphenol* และเมื่อวิเคราะห์หาปริมาณของสาร *trans-anethole* พบว่ามีอยู่ในน้ำมันหอมระเหยบริสุทธิ์ 96.8% การทดสอบฤทธิ์ต้านแบคทีเรียของน้ำมันหอมระเหยและสารสกัดหยาบ 3 ส่วนที่ได้จากการนำเหง้าปลูดหวานแห้งมาสกัดด้วยตัวทำละลาย 3 ชนิดตามลำดับคือ ปิโตรเลียมอีเธอร์ ไดคลอโรมีเทน และเมธานอล พบว่าน้ำมันหอมระเหยและส่วนสกัดหยาบ 2 ส่วนคือ ปิโตรเลียมอีเธอร์ และไดคลอโรมีเทน สามารถออกฤทธิ์ต้านแบคทีเรียสายพันธุ์ *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus* และ *Sarcina* sp. แต่ไม่พบสารสกัดได้ออกฤทธิ์ต้านแบคทีเรียสายพันธุ์ *Pseudomonas aeruginosa*

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Zingiber is a genus of plants belonging to the Zingiberaceae, one of the largest and most important families. It is widely distributed throughout tropical and subtropical regions, particularly in Southeast Asia. Ninety species have been identified worldwide and at least 35 of these are found in Thailand (Sirirugsa, 1999). Plants in this genus are rich in volatile oils and are used as sources of foodstuffs, spices and traditional medicines (MedPlant; HerbMed®). Some *Zingiber* species display antioxidant and antimicrobial activities (Habsah *et al.*, 2000; Jitoe *et al.*, 1994; Ficker *et al.*, 2003).

Z. wrayi var. *halabala* is a subspecies native to Bala forest in Narathiwat, a province of Southern Thailand (Kharukanunt and Promchum, 2001). The rhizomes are similar to those of ginger (*Z. officinale* Roscoe), but are slightly smaller. In common with ginger, *Z. wrayi* var. *halabala* prefers a damp, humid and shady habitat. While all parts of this plant are sweet and aromatic, the rhizomes have been used by local people to brew medicinal drinks. They believe that the herb promotes healthy circulation, shiny skin and general well-being.

To date, no study has been made of the

phytochemistry and antimicrobial activity of this plant. Thus, this is the first paper to identify the chemical constituents of the essential oil and antibacterial activity of the extracts from rhizomes of *Z. wrayi* var. *halabala*.

Materials and Methods

Plants, Materials and Chemicals

The plant materials were collected from Bala forest Narathiwat, in 2003. The specimen was identified by Mr. Bamroong Kharukanunt and deposited at Chulaporn Development Project, Number 7, Yala. Rhizomes were separated from loose earth and washed with water. Fresh rhizomes were divided into 2 parts. One part was used for extraction of the essential oil. The other was sliced longitudinally into pieces and dried at 30°C before extraction. The resulting dried rhizomes were used for solvent extraction.

A reference standard of *trans-anethole* was purchased from Sigma Chemicals and used without purification. All solvents for extraction processes were used as received. Hexane for analysis by GC-FID was dried and distilled prior to use.

Extraction of the essential oil

The fresh rhizomes (600 g or 73 g of dry rhizomes) were sliced into small pieces and ground in a blender. The material was subjected to steam distillation for 4 h using a Dean-Stark apparatus. After the essential oil was separated, it was dissolved in dichloromethane. The resulting solution was dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure at room temperature to obtain the essential oil (2.65 g).

Preparation of the crude extracts

The dried rhizomes were ground and extracted successively with petroleum ether, dichloromethane and methanol. Evaporation of the solvent under reduced pressure yielded crude petroleum ether, dichloromethane and methanol extracts, which were used for bioassays.

GC-MS and GC-FID analyses

Constituents of the essential oil were analyzed by GC-MS and the major component found was further characterized by GC. Quantitative determination was carried out using linear calibration graphs obtained from standard solutions of authentic compounds diluted with hexane in the concentration range of 50-150 ppm. Each dilution was carried out in triplicate and the mean value was used.

GC-MS analysis was performed on a HP 5890 GC- HP 5972 Mass Selective Detector. The GC was fitted with HP-INNOWAX column. The inlet temperature was set at 250°C and the oven temperature programmed from 70°C to 220°C (15 minutes) at 10°C / minute. The mass spectrometer was run in electron ionization mode, scanning at 45-550 amu, with a solvent delay time of 3.0 minutes and a transfer line temperature of 300°C. The relative proportion of each individual component of the oil was expressed as a percentage relative to the total peak area.

Quantitative analysis was performed by GC on a Hewlett Packard HP-6890 series analyzer fitted with a flame ionization detector (FID) using a Shimadzu CBP 5: capillary column (25.0 m × 220

µm × 0.25 µm) with 5% methyl siloxane as the stationary phase. The carrier gas was helium at a flow rate of 3.5 mL/min. Injector and detector temperatures were 250 and 300°C, respectively, and the oven temperature was programmed from 70°C to 220°C (15 minutes) at 10°C / minute with a running time of 30 min and an injection volume of 2 µL.

Bacterial Organisms

The bacterial organisms used in this study were obtained from biology laboratory, Department of Science, Faculty of Science and Technology, Prince of Songkla University, Pattani Campus. The bacterial stock cultures were maintained on nutrient agar (NA) following the procedure of Bayder (Bayder *et al.*, 2004). The essential oil and three crude fractions, extracted from petroleum, dichloromethane, and methanol, were individually tested against five strains of bacteria: *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Sarcina* sp. and *Pseudomonas aeruginosa*. Each microorganism was cultured in an appropriate broth at 30°C for 24 h. The concentrations of the cultures were adjusted to 10⁶ - 10⁷ cell / mL in 0.1% peptone, as counted using a haemocytometer.

Antibacterial screening

The agar disc diffusion method was employed for the determination of antibacterial activities following the procedure of Baydar *et al.*, 2004. For initial screening, filter paper discs (6 mm in diameter) were impregnated with 1 g / mL extracts in dichloromethane and mounted on inoculated plates. The discs were allowed to dry in a biological safety cabinet, and then incubated at 30°C for 24 h. Diameters of any clear inhibition zones were measured in millimeters. Extracts that showed positive activities were diluted to concentrations of 0.75, 0.50 and 0.25 g/mL and tested for biological activity as described above. Discs impregnated with dichloromethane and allowed to dry were used as control. All experiments were conducted in triplicate.

Results and Discussion

Determination of the essential oil

The essential oil from *Z. wrayi* var. *halabala* was obtained as a pale yellow oil in 3.6% yield based on dried weight or 0.4% based on wet weight. The rhizomes are rich in oil content compared with other Zingiber species such as the Fijian ginger, green ginger (0.08-0.21% based on wet weight) (Smith and Robinson, 1981) and Sri Lankan gingers, Sidda and Chinese varieties (1.8-4.3% of dried weight) (MacLeod and Pieris, 1984). According to GC-MS analysis under the conditions described above, *trans*-anethole was detected as the main component (96.5%) of the essential oil. The remaining constituents, including estragol, camphor and *m*-phenylphenol, were present at very low concentrations. The structures and relative percentages of these compounds are shown in Table 1.

Further quantitative analysis of *trans*-anethole was carried out by GC comparison with the authentic compound under the conditions described above. The calibration graph of authentic *trans*-anethole was linear with a correlation coefficient of 0.9975. Based upon the graph, 96.8 % w/w of *trans*-anethole was found in the essential oil.

Trans-anethole occurs naturally in the volatile oils of more than 20 spices (Newbrene *et*

al., 1999). It has a sweet and aromatic odor and has been used as a fragrance or flavoring in a variety of foods, alcoholic beverages and cosmetic products. The total annual use of *trans*-anethole in the USA in 1978 was estimated at around 70 tons (FEMA, 1978). *Trans*-anethole has been reported to increase salivary secretion (Fox, 1987) and has carminative and expectorant activity; it is widely used in therapeutics (Gracza, 1981). Thus, the consumption of rhizomes from *Z. wrayi* var. *halabala* by the natives of Bala forest as a herbal medicine is partially explained.

Antibacterial Activities

The essential oil, crude petroleum ether and dichloromethane extracts were active against *B. subtilis*, *E. coli* and *Sarcina* sp. at all concentrations studied (Table 2). The antibacterial activities of petroleum ether and dichloromethane extracts were found to increase with increasing concentration while the essential oil at concentration 0.50 g/mL was more active on *B. subtilis* and *Sarcina* sp. than that of concentration 1.0 g/mL. The dichloromethane extract tended to be the most effective, especially at high concentration (0.75 and 1.0 g/mL). However, the extracts were only active against *S. aureus* at the highest concentration (1.0 g/mL) and none of the extracts inhibited the growth of *P. aeruginosa*. Control discs did not show inhibitory activities on any of the bacteria.

Table 1. Constituents of the essential oil from rhizomes of *Z. wrayi* var. *halabala*.


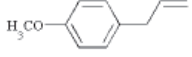
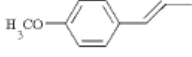
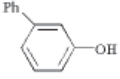
Retention Time	Compound	Structure	%
3.77	Camphor		0.91
5.08	Estragol		1.90
6.90	<i>Trans</i> -anethole		96.51
19.11	<i>m</i> -Phenylphenol		0.42

Table 2. Diameters of clear zones (mm) of the oil and crude extracts from *Z. wrayi* var. *halabala*.

Bacteria	Concentrations of the oil and crude extracts (g/mL)											
	Essential oil				Petroleum extract				Dichloromethane extract			
	1.00	0.75	0.50	0.25	1.00	0.75	0.50	0.25	1.00	0.75	0.50	0.25
<i>B. subtilis</i>	11.9	12.0	12.5	9.7	12.7	11.5	9.1	9.5	14.7	13.0	10.5	9.3
<i>E. coli</i>	11.1	8.7	9.7	7.0	10.2	8.5	8.0	7.7	9.3	9.5	9.3	7.8
<i>Sarcina</i> sp.	11.6	9.3	12.3	8.3	9.8	9.5	9.1	8.3	12.4	10.6	9.8	8.8
<i>S. aureus</i>	13.2	-	-	-	12.3	-	-	-	13.5	-	-	-
<i>P. aeruginosa</i>	-	-	-	-	-	-	-	-	-	-	-	-

(-) No clear zone was present.

The minimum inhibitory concentration values (MIC) of the essential oil, crude petroleum ether and dichloromethane extracts against *B. subtilis*, *E. coli* and *Sarcina* sp. were 0.25 g / mL and against *S. aureus* 1.0 g/mL.

Conclusions

As a result of this study, the essential oil of *Zingiber wrayi* var. *halabala* has been extracted and its components identified. The high concentration of *trans*-anethole in the oil makes it potentially useful in the food, beverage and cosmetic industries. Additionally, the oil and the crude extracts have the possibility to be applied as a constituent of food preservatives and medicines because they exhibit antibacterial activities. However, further study has to be conducted.

Acknowledgements

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