Primary study on the components and physico chemical as well as biological properties of the essential oil from *zingiber montanum* growing in Lai Chau, Vietnam

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Abstract

The essential oil of *Zingiber montanum* (J.Koenig) Link ex A.Dietr. growing wild in the province Lai Chau of Vietnam was obtained by steam distillation and dried with Na_2SO_4. By GC-MS, 31 components in the essential oil were predicted by comparing their retention times and molecular weights mass spectra with those of the standards. In particular, there were 15 hydrocarbons (37.76% monoterpenes and 11.63% sesquiterpenes) and 16 oxygenated components (16.11% aldehydes, 19.99% alcohols, and 13.27% esters). The density (at 20ºC), acid index, and ester index of the essential oil were 0.814 g/ml; 0.651 mgKOH/g, and 0.736 mgKOH/g, respectively. The antioxidant activity was determined by using 1,1-diphenyl-2-picrylhydrazol (DPPH) radical percentage inhibition and it was 48.07± 0.32%. Antimicrobial activity against *Salmonella typhi*, *Bacillus cereus*, *Staphylococcus aureus* and *Escherichia coli* of the essential oil was identified by agar diffusion method.

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Keyword

Essential oil, *Zingiber montanum* growing in Lai Chau, components, physico chemical properties, biological activity

1 Introduction

The plant *Zingiber montanum* (J.Koenig) Link ex A.Dietr., is planted in mountainous areas of the Tam Duong, Muong Te, Nam Nhun, Phong Tho, Than Uyen district of the Lai Chau province. *Z. montanum* shows effects in medical field. It has been used to increase digestion, reduce swelling pain and fever. In particular, in South East Asia, Willd *Z. montanum* is used to treat skin diseases, dyspepsia, some symptoms of digestive tract, flu, malaria, rheumatoid arthritis and some other kinds of infections. *Z. montanum* is also used to produce medicines to treat stomach cancer and throat cancer[1, 2]. The components of different varieties of this plant have shown variability[2]. The components and their bioactivities of some Willd *Z. Montanum* have been reported[1, 2]. However, the components as well as their main physico chemical and biological properties of *Z. Montanum* growing in Lai Chau, Vietnam have not been evaluated yet. Therefore, the aim of this study is to primarily analyze the components, physico chemical indexes and antibacterial activity of the essential oil of *Z. montanum* in Lai Chau.

2 Materials and methods

2.1 Materials

The samples of *Zingiber montanum* (J.Koenig) Link ex A.Dietr. were collected from Tam Duong district, Lai Chau Province, Vietnam in June 2017. The species of sample were identified by MSc Vu Kieu Sam, Bac Giang Agriculture and Forestry University. The essential oil was obtained by steam distillation after drying with Na_2SO_4. After drying with Na_2SO_4, the sample was stored in the Department of Biotechnology and Food Processing, Hanoi University of Industry, Vietnam. The sample has been stored at the Department of Biotechnology and Food, Hanoi University of Industry.

The tested bacterial strains (*Staphylococcus aureus*, *Escherichia coli*, *Salmonella typhi* and *Bacillus cereus*) were obtained from School of Biotechnology and Food Technology, Hanoi University of Science and Technology. All chemicals and culture media were purchased from Sigma.

2.2 Methods

2.2.1. Essential oil extraction

The essential oil was extracted from the rhizome of the plant by hydro distillation using a Clevenger distillation

equipment (Germany) for 180 minutes. The rhizome to water ratio was 1:4 (w/v).

2.2.2. Gas chromatography mass spectrometry (GC-MS)

The sample and standards were run parallelly in the *GC-MS* experiment. Gas chromatography (GC) analysis was performed by using Agilent Technologies HP 6890 Plus Gas chromatograph system equipped with Flame Ionization Dectector (FID) and fitted with HP-5MS columns (30m x 0.25mm, film thickness 0.25µm). The temperature was programmed as follows: The column temperature was programmed from 80 to 150°C in 23.3 min at a rate of 3°C/min and then from 150 to 220°C in 8.75min at a rate of 8°C/min. The used injector temperature was 230°C. The *MS* conditions were as follows: ionization voltage was 70eV, transfer temperature was 250°C, the carrier gas was helium used at a flow rate of 0.5ml/min, and the split ratio of the injector was 1:5[3,4,5]. The *MS* fragmentation patterns were compared with known patterns of other essential oils and with those in the literature by using Wiley (Wiley 9th Version), NIST 08 Libraries (on ChemStation HP). The percentage of each component was calculated by the percentage of its peak area.

2.2.3. Determination of physico chemical properties of essential oil

The density, angle of rotation, refraction index, acid index and ester index of the essential oil were determined by using ISO 8444: 2010[6], ISO 8445: 2010[7], ISO 8446: 2010[8], ISO 8450: 2010[9] and ISO 8451: 2010[10], respectively.

2.2.4. Determination of antioxidant activity using free radical scavenging activity

The free radical scavenging activity of the essential oil was measured by using 1,1-diphenyl-2-picrylhydrazol (DPPH).A 0.5mM solution of DPPH in methanol and 0.005M acetate buffer (pH 5.5) were prepared. An aliquot of 0.1ml of the sample solution was added to the tube containing 2ml of acetate buffer, 1.9ml of methanol and 1 ml of DPPH solution. In the blank tube DPPH was removed; in the control tube, 1ml of DPPH was added to the tube containing2 ml acetate buffer and 2 ml methanol. The mixture was shaken immediately after adding DPPH and allowed to stand at room temperature in the dark. The decrease in absorbance at 517nm was measured after 30 min until the reaction reached plateau. Vitamin C with the concentration of 0.5 mM was used as a positive control and its free radical scavenging activity was performed in parallel in the same experiment. These experiments were run in duplicate [11-13].

The inhibitory percentage of DPPH was calculated as follows:

Scavenging effect (%) = $[(A_o – (A – A_b)) / A_o] \times 100\%$

Wherein A_o is the value of absorbance of the control at the wavelength of 517 nm; A is the value of absorbance of the sample at the wavelength of 517nm; and A_b is the value of absorbance of the blank at the wavelength of 517nm.

2.2.4. Determination of antibacterial activity using agar diffusion method

Antibacterial activity was roughly determined by agar diffusion method. 50µl of the essential oil was put into wellson the plates containing tested bacterial strains. The activity was roughly estimated by the diameter of the antibacterial round(mm), which was calculated by the formula D- d (mm), wherein D was the diameter of the antibacterial round circle (mm) and d was the hole diameter (cm) [14].

3 Results and discussion

3.1 The components of the essential oil

GC-MS of the sample was performed in order to roughly determine the components of the essential oil. Based on the retention times and molecular weights of the sample and the standards (the GC profile was not shown here), 31 components and their percentages in the essential oil were evaluated and shown in the Table 1.

Table 1 The components of the essential oil of *Zingiber montanum* growing in Lai Chau, Vietnam

No.	Components	Retention time (min)	Proportion (%)
1	*α*-pinene	2.269	2.86
2	camphene	2.492	1.98
3	sabinene	6.453	1.15
4	octanal	11.394	6.69
5	*β*-pinene	11.810	10.06
6	myrcene	12.042	1.17
7	*α*-phellandrene	14.082	2.04
8	*δ*-3-carene	20.943	1.43
9	linalool	21.197	1.32
10	octanol	21.691	1.23
11	linalyl acetate	22.632	1.37
12	limonene	23.294	1.58
13	citral	23.380	2.71
14	*p*-cymene	23.640	1.93
15	geranyl acetate	24.171	1.69
16	*α*-citronellol	24.817	1.07
17	*α*-terpineol	25.083	2.76
18	neryl acetate	25.606	7.78
19	*β*-citronellol	25.710	6.93
20	*γ*-terpinene	26.007	4.05
21	*α*-terpinene	26.528	3.62
22	terpinolene	27.422	5.89
23	*β*-cubebene	27.818	4.58
24	decanal	27.966	3.96

25	dodecanal	28.257	2.75
26	terpinen-4-ol	28.790	2.59
27	*trans*-nerolidol	29.655	2.92
28	γ-elemene	36.001	4.07
29	α-humulene	37.422	2.98
30	ledol	39.632	1.17
31	perillyl acetate	42.171	2.43

	Monoterpenes		***37.76***
	Sesquiterpenes		***11.63***
	Aldehydes		***16.11***
	Alcohols		***19.99***
	Esters		***13.27***
	Total		**98.76**

Noted: (%) was calculated by the percentage of chromatographic peak area

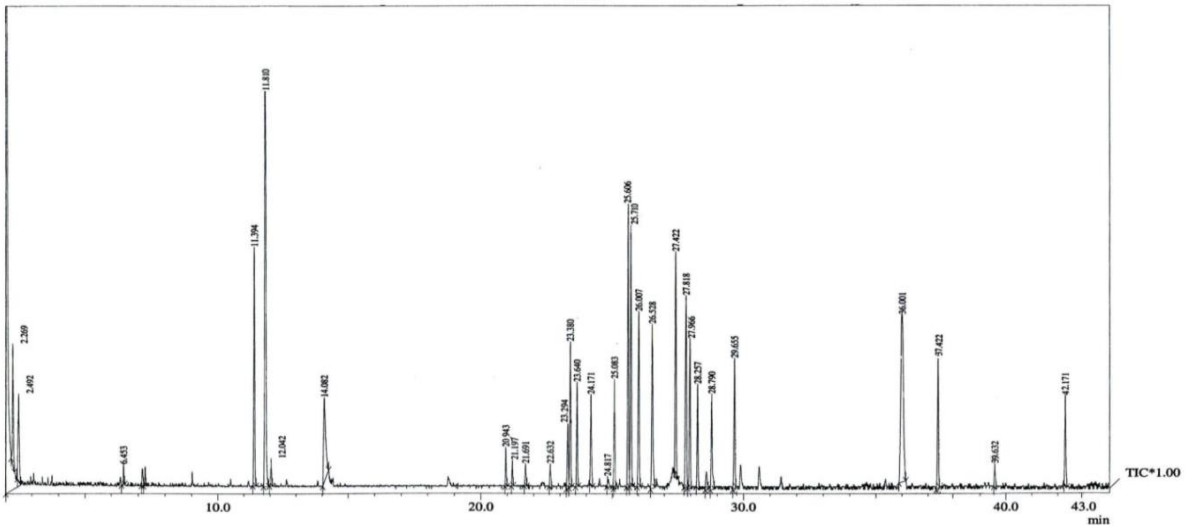

Fig 1 Gas chromatogram of the essential oil of *Zingiber montanum* growing in Lai Chau, Vietnam

The table showed that 31 components were predicted in the essential oil of *Zingiber montanum* growing in Lai Chau, Vietnam. Fifteen out of them were hydrocarbons (37.76% monoterpenes and 11.63% sesquiterpenes) and 16 were oxygenated components (16.11% aldehydes, 19.99% alcohols, and 13.27% esters). The results provided additional evidence to show varied percentages of the components of the essential oils of *Zingiber montanum* growing in Lai Chau, Vietnam. Notably, the amounts of aldehydes and alcohols in the essential oil were higher than those of the essential oil in Indonesia[2] and Malaysian[15]. Probably, the differences were due to the geographical conditions such as the soil factors, weather, climate, growing conditions and harvesting time[2,15].

3.2 The physical chemical indexes of the essential oil of *Zingiber montanum* growing in Lai Chau, Vietnam

The density, angle of rotation, refraction index, acid index and ester index of the essential oil were presented in Table 2.

Table 2 Physico chemical indexes of the essential oil of *Zingiber montanum* growing in Lai Chau, Vietnam

No.	Physical chemical	Result
1	Density at 20°C	0.814
2	Specific rotation α^{20}_D	83°75’
3	Refractive index n^{20}_D	1.457
4	Acid index (mg KOH/g)	0.651
5	Ester index (mg	0.736

In particular, the essential oil had a density (0.821), which was smaller than0.9 and refractive index (1.415), which was smaller than 1.47. However, no significant differences were observed in these values. The value of the angle rotation of the sample showed that the essential oil was capable of being dissolved in both polar organic and nonpolar organic solvents. The acid index of the sample showed that the essential oil could be less of an oxidation. This result was coincident with the percentage of the components of the essential oil. In particular, the total oxygenated components determined in this research were less than 50% (Table 1).

3.3 The biological activities of the essential oil of *Zingiber montanum* growing in Lai Chau, Vietnam

3.3.1 The free radical scavenging activity DPPH of the essential oil

The DPPH free radical scavenging activity of the essential oil of *Zingiber montanum* growing in Lai Chau, Vietnam was 48.07 ± 0.32% and this value was a bit higher than that of 0.5 mM vitamin C (39.65 ± 0.42%). These activities of the essential oils of *Alpinia galangal* (L.) in Phu Tho, Vietnam[4] and *Zingiber purpureum* Roxbwere found to be 47.13 ± 0.34% and 45.32 ± 0.18%, respectively [16]. Therefore, we could say that the DPPH free radical

scavenging activity of the essential oil of *Zingiber montanum* (J.Koenig) Link ex A.Dietris higher than that of *Alpinia galangal* (L.) in PhuTho, Vietnam and *Zingiber purpureum* Roxb.

3.3.2 The antibacterial activity of the essential oil

In order to estimate the antibacterial potentials of the essential oil of *Zingiber montanum* growing in Lai Chau, Vietnam, agar diffusion method was used in this experiment. Tested microorganisms used in this experiment were *Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Bacillus cereus.* The diameters of antibacterial activity rounds of the essential oil against these bacteria were shown in Table 3.

Table 3 The diameters of antibacterial activity rounds of the essential oil of *Zingiber montanum* growing in Lai Chau, Vietnam

No.	Tested microorganisms	Diameter of antibacterial round (mm)
1	*Salmonella typhi*	38.67
2	*Bacillus cereus*	41.34
3	*Staphylococcus aureus*	32.26
4	*Escherichia coli*	37.58

The results showed that the essential oil of *Zingiber montanum* growing in Lai Chau, Vietnam possessed antibacterial activity against all of the four microorganisms tested. Among them, the antibacterial activity against *Bacillus cereus* was the highest one. The activity of the essential oil of *Zingiber montanum* (J.Koenig) Link ex A.Dietr in this research is similar to that of the essential oils of *Alpinia galangal* (L.) in Phu Tho, Vietnam as these essential oils were found to possess antibacterial activities against all of the four tested microorganisms.

4 Conclusions

By GC-MS, 31 components in the essential oil were predicted by comparing their retention times and molecular weights mass spectra with those of the standards. In particular, there were 15 hydrocarbons (37.76% monoterpenes and 11.63% sesquiterpenes) and 16 oxygenated components (16.11% aldehydes, 19.99% alcohols, and 13.27% esters). Physico chemical properties, antioxidant activities as well as antimicrobial activities of the essential oils were also analyzed. The density (at 20^{o}C), acid index and ester index of the essential oil were 0.814; 0.651 mgKOH/g and 0.736 mgKOH/g, respectively. The antioxidant activity was determined by using 1,1-diphenyl-2-picrylhydrazol (DPPH) radical percentage inhibition and it was 48.07 ± 0.32%. Antimicrobial activity against *Salmonella typhi, Bacillus cereus, Staphylococcus aureus* and *Escherichia coli* of the essential oil was identified by agar diffusion method.

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Nghiên cứu sơ bộ thành phần, đặc tính hóa lí và sinh học của tinh dầu chiết xuất từ Gừng gió mọc tại Lai Châu, Việt Nam

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Tóm tắt Gừng gió (*Zingiber montanum* (J.Koenig) Link ex A.Dietr) mọc tự nhiên tại tỉnh Lai Châu, Việt Nam, được chưng cất và làm khô bằng Na_2SO_4 để thu tinh dầu. Sau khi phân tích bằng phương pháp GC-MS, 31 thành phần trong tinh dầu được xác định bằng cách so sánh thời gian lưu và phổ khối lượng với các chất chuẩn. Đặc biệt, kết quả nghiên cứu cho thấy sự có mặt của 15 hydrocacbon (37,76% monoterpen và 11,63% sesquiterpen) và 16 hợp chất oxy hóa (16,11% aldehyd, 19,99% alcol và 13,27% este). Tỉ trọng (ở $20^{O}C$), chỉ số axit và chỉ số este của tinh dầu lần lượt là 0,814g/ml; 0,651mg KOH/g và 0,736mg KOH/g. Hoạt tính chống oxy hóa được xác định thông qua phản ứng bao vây gốc tự do sử dụng 1,1-diphenyl-2-picrylhydrazyl (DPPH) và phần trăm kháng gốc tự do thu được là 48,07 ± 0,32%. Hoạt tính kháng khuẩn chống lại *Salmonella typhi*, *Bacillus cereus*, *Staphylococcus aureus* và *Escherichia coli* của tinh dầu được xác định bằng phương pháp khuếch tán trên thạch.

Từ khóa Tinh dầu, Gừng gió mọc tại Lai Châu, thành phần, đặc tính hóa lí, hoạt tính sinh học