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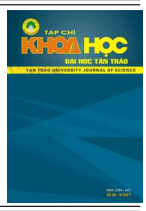

RESEARCH OF CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIVES OF CINNAMOMUM BURMANNII ESSENTIAL OIL IN BAO LAC, CAO BANG PROVINCE

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Cinnamomum Burmannii; essential oil; GC-MS, Cao Bang, IC50

Abstract:

The objectives of the study was to investigate the chemical composition and biological activity of *Cinnamomum burmannii* essential oil in Cao Bang province. Use steam distillation method to extract essential oils. The chemical composition of essential oils was determined by Gas chromatography mass spectrometry (GCMS). Evaluation of the antioxidant capacity of Cinnamomum burmannii essential oil by using DPPH free radical method. The results of this study have determined that *Cinnamomum burmannii* essential oil has 23 components with the main components including: Citronellal (52.82%), Citronellol (25.13%), 1, 8-Cineole (5.04%). *Cinnamomum burmannii* essential oil has antioxidant capacity with IC50 value = 12.03 µg/ml. These results created a base for further research, and development of functional products, care healthy products from the chemical components of this plant.

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NGHIÊN CỨU THÀNH PHẦN HÓA HỌC VÀ HOẠT TÍNH SINH HỌC CỦA DẦU TINH DẦU QUẾ TRÈN TẠI BẢO LẠC, TỈNH CAO BẰNG

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Cinnamomum Burmannii; tinh dầu; GC-MS, Cao Bằng, IC50

Tóm tắt

Mục tiêu của đề tài là khảo sát thành phần hóa học và hoạt tính sinh học của tinh dầu Quế ở tỉnh Cao Bằng. Sử dụng phương pháp chưng cất lôi quốn hơi nước để chiết xuất tinh dầu. Thành phần hóa học của tinh dầu được xác định bằng phương pháp sắc ký khí ghép khối phổ (GC-MS). Đánh giá khả năng chống oxy hóa của tinh dầu *Cinnamomum burmannii* bằng phương pháp gốc tự do DPPH. Kết quả nghiên cứu này đã xác định tinh dầu *Cinnamomum burmannii* có 23 thành phần hợp chất hóa học với các thành phần chính gồm: Citronellal (52,82%), Citronellol (25,13%), 1,8-Cineole (5,04%). Tinh dầu *Cinnamomum burmannii* có khả năng chống oxy hóa với giá trị IC50 = 12,03 µg / ml. Những kết quả này đã tạo cơ sở để tiếp tục nghiên cứu, phát triển các sản phẩm chức năng, chăm sóc sức khỏe từ các thành phần hóa học của loại cây này.

1. Introduction

Cinnamomum burmannii is a species closely related to C. cassia (Cinnamomum aromaticum Nees). They are native to Southeast Asia and Indonesia, also known as Indonesian cinnamon, Padang cassia, Batavia cassia, or Korintje. In addition, it has been used commercial name is a cinnamon stick (Shan, B. et al., 2007). The plant has oblong-elliptical leaves that are 4-14 cm long, glossy green, and oppositely oriented, as well as an ovoid long fruit, and small yellow flowers that bloom in early summer. The plant's dried bark is sold on the market in the shape of rolls and quills, which are used in cooking and flavoring (Tan, 2005). As a traditional plant, *C. burmannii* has been cultivated for everyday requirements (cinnamon spice in food) and illness treatment (Zhang,2008; Al-Dhuhiab, 2012), and logging residues (e.g., berries and leaves) are created as agricultural waste (Wang et al.,2006). *C. burmannii* leaves have high antioxidant and antibacterial characteristics (Chandurkar et al., 2014).

2. Materials and methods

2.1. Materials

The major raw material utilized in the study to extract essential oils is the leaves of the *Cinnamomum Burmannii* tree, which grows in the Bao Lac area of Cao Bang, identified by Mr. Nguyen Quoc Binh, Vietnam Academy of Science and Technology identify the scientific name was *Cinnamomum burmanii* (Nees.) Blume, 1826, Lauraceae family.

The leaves of *Cinnamomum Burmannii* used to extract essential oil must be fresh, not moldy, not damaged, bruised, wilted, not infested by pests and illnesses, mature leaves; do not gather leaves that are too young or too old because the leaves are too old. That means the essential oil content of the leaves is minimal.

Figure 2.1. Cinnamomum Burmannii leaves

2.2. Research scope

Research was carried out in the laboratory scale

2.3. Work place and time to proceed

- Location : Laboratory of Institute of Life Sciences, at Thai Nguyen University of Agriculture and Forestry.

2.4. Equipment and chemicals

Table 2.1: Experiment Chemicals

Numbers	Chemical experiment	Origin
1	Ethanol (96%)	Vietnam
2	Phenolphthalein	China
3	Potassium hydroxide	China
4	Hydrochloric acid	China
5	Ascorbic acid	China
6	Sodium Sulphate	China
7	2,2-diphenyl-1-picrylhydrazyl	America
8	Distilled water	Vietnam

Table 2.2: Experiment Equipment

Numbers	Experiment Equipment	Origin
1	Analytical balance	China
2	UV- Spectrophotometer	Germany
3	Vortex mixer	Germany
4	TDW Muffle furnace	China
5	Oven air dryer	Germany
6	Essential oil distillation set	China
7	Gas chromatography–mass spectrometry	America

Table 2.3: Laboratory instruments

Numbers	Laboratory instruments	Origin
1	Measuring cylinder	Vietnam
2	Filter paper	Vietnam
3	Separating funnel	Vietnam
4	Measuring pipette	Vietnam
5	Desccicator	Vietnam
6	Volumetric flask	Vietnam
7	Erlenmeyer flask	Vietnam

8	Test tube cleaning brush	Vietnam
9	Aluminium foil	Vietnam
10	Crucible	Vietnam
11	Beaker	Vietnam

2.5. Research content

2.5.1. Extraction of *Cinnamomum burmannii* essential oils by direct steam distillation

To extract essential oil from *Cinnamomum Burmannii* leaves, they must be fresh, reach the maturity leaves, and be free of pests and illnesses. The leaves are washed with water after harvest to eliminate pollutants and dirt before being processed [Bổ sung tài liệu tham khảo].

2.5.2. Investigate the factors affecting the content of *Cinnamomum Burmannii* essential oil

- ***Content 1***: Determination the effect of extraction time on essential oil content.

- ***Content 2***: Determination the effect of volume of distilled water on essential oil content.

- ***Content 3***: Determination the effect of raw material withered time on essential oil content.

2.5.3. Determination of the chemical composition of *Cinnamomum Burmannii* essential oil by GC-MS method

In this content, the chemical components of Cinnamomum Burmannii essential oil are determined and identified by GC-MS method, in order to assess the quality of obtained essential oil.

2.5.4. Investigation of antioxidant capacity of essential oils

In this content, investigation of the antioxidant capacity of Cinnamomum Burmannii essential oils was tested using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) technique by Radical Scavenging Activity method [Bổ sung nội dung và các tài liệu tham khảo].

2.6. Statistical analysis methods

Data were analyzed by one-way analysis of variance (ANOVA) and Fisher's PLSD post-test at $P \leq 0.05$ using SPSS software (version 20).

3. Results and discussion

3.1.Result for determining of moisture and ashing content in *Cinnamomum Burmannii* leaves

Table 3.1. Results of determining moisture and ashing content in *Cinnamomum Burmannii* leaves

Component of raw materials	Unit (%)
Moisture	53.17
Ashing	10.61

The moisture content in Cinnamomum Burmannii leaves from the experimental results was 53.17%. This result shows that the moisture content in Cinnamomum Burmannii leaves is not too high, but it also contains a lot of water. With a content value of 53.17%, during the distillation process, it is necessary to add a lot of water to increase the permeability of water into the raw tissues, destroy the colloidal system and attract organic components in the essential oil of the Cinnamomum Burmannii leaves.

The ashing content in *Cinnamomum Burmannii* leaves from the experimental results was 10.61%.

3.2. The result of studying factors affecting the distillation process of essential oils

3.2.1. Effect of extraction time

Conduct a survey on the influence of extraction time on essential oil content gets. Surveying the extraction time on 5 kg of *Cinnamomum Burmannii* samples at 150°C with a solvent volume of 15 liters of distilled water, the result is presented in Table 4.2.

Table 3.2. Results of survey on extraction time of essential oils

Formula	F1	F2	F3	F4	F5
Extraction time (minutes)	20	30	40	50	60
Essential oil content (%)	0.1214^{a}	0.2196^{b}	0.5086^{c}	0.6442^{e}	0.6186^{d}

(Note: Values in the same row with different exponents have significant differences at the level α = 0.05) (Bổ sung thông tin về các chỉ số a, b, c,e,d của các số liệu trong các bảng)

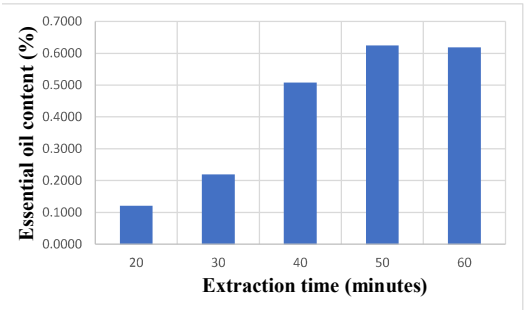


Figure 4.1. Graph showing the effect of time on essential oil content

The process of surveying and analyzing the data obtained from Table 4.2, shows that when the extraction time increases, the amount of essential oil obtained also increases, the highest rate increases in the period from 30 to 50 minutes, then decreases. gradually. The content of essential oil obtained after 50 minutes was 0.6242%, then there was no significant change. Therefore, to save effort and fuel, the optimal distillation time was chosen to be 50 minutes.

3.2.2. Effect of volume of distilled water

Investigation of the effect of the volume of distilled water added was carried out during the distillation process. Conducting a survey on the volume of water used for extraction on 5 kg of *Cinnamomum Burmannii* samples at 150°C for 50 minutes. The correlation between the volume of distilled water and the change in essential oil content is shown in Figure 4.2.

Table 3.3. Result for effecting distilled water volume on essential oil content

Formula	F1	F2	F3	F4	F5
Volume of distilled water (liters)	8	10	12	15	20
Essential oil content (%)	0.4393^{a}	0.5202^{b}	0.5607^{d}	0.6127^{e}	0.5491^{c}

(Note: Values in the same row with different exponents have significant differences at the level α = 0.05)

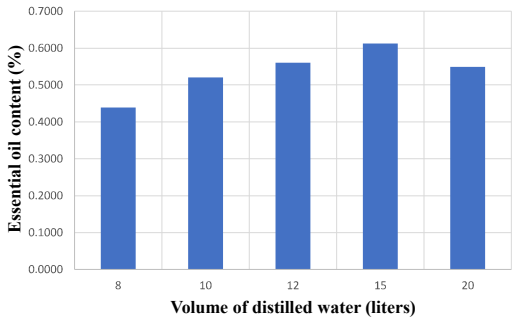


Figure 4.2. The graph shows the effect of the amount of distilled water on the essential oil content

As the volume of distilled water added during the distillation process increases, the amount of essential oil obtained also increases because the larger the volume of water, the more steam will rise, and the more essential oils will be attracted. The maximum oil content obtained was 0.6127% when the volume of water added was 15 liters. But if the water volume is too large (more than 2/3 of the volume), it will make the surface airy, so the amount of water vapor will decrease, leading to a decrease in the amount of essential oil obtained. Therefore, the volume of distilled water added during distillation is chosen is 15 liters.

3.2.3. Effect of raw material wilting time on essential oil content

Conduct a survey on the effect of raw material exposure time on the amount of essential oil obtained with samples exposed after 48 hours, 60 hours, 72 hours, 84 hours, and 96 hours. Surveying the extraction time on 5 kg of *Cinnamomum Burmannii* samples in 50 minutes, the volume of distilled water is 15 liters with different degrees of wilting, the following results were obtained:

Table 3.4. Effect of sample wilting time on essential oil content

Formula	F1	F2	F3	F4	F5
Time after harvest (hours)	48	60	72	84	96
Essential oil content (%)	0.5780^{b}	0.6069^{c}	0.6820^{d}	0.7225^{e}	0.5375^{a}

(Note: Values in the same row with different exponents have significant differences at the level α = 0.05)

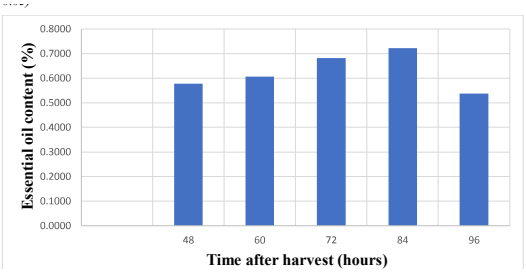


Figure 3.3. Graph showing the effect of wither on essential oil content

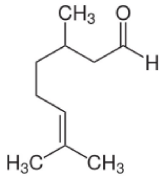
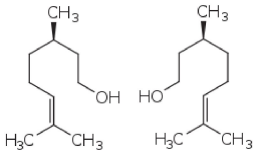
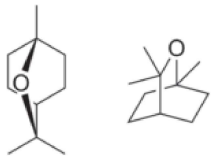
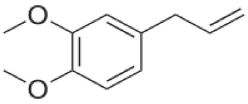
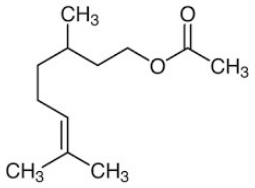
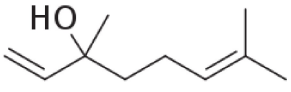
Under the same survey conditions, the longer the exposure time (in the shade), the higher the essential oil content and reached the highest at about 84 hours, then the oil content gradually decreased when exposed for longer than 96 hours. This is explained by the fact that the amount of water in the plant decreases gradually when drying, so when taking the right amount for the survey, more samples are needed. It is possible that the essential oil is also evaporated during the drying process, but this is not significant compared to taking a larger amount of samples.

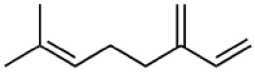
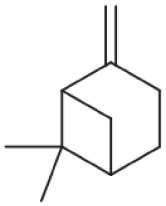
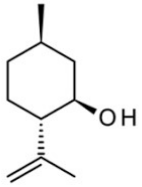
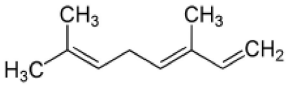
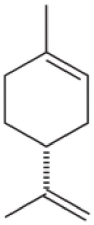
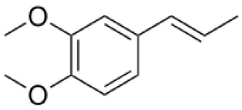
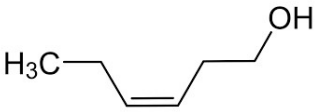
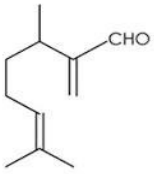
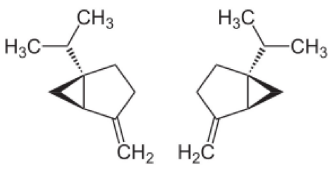
Thus, through surveying the factors affecting the extraction process of essential oils by steam distillation, the optimal extraction conditions were selected when the sample was crushed and wilted for about 84 hours, the volume add distilled water is 15 liters at 150°C, distilled for 50 minutes.

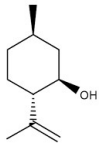
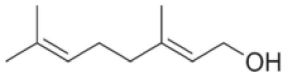
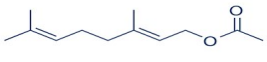
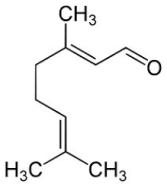
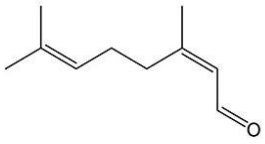
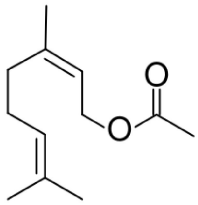
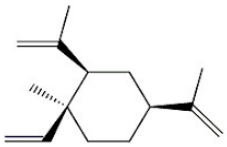
3.4. Result for determining the chemical composition of *Cinnamomum Burmannii* essential oil by GC-MS method

By means of gas chromatography-mass spectrometry (GC-MS) the chemical components in essential oils were determined and recorded in Table 4.8.

Table 3.5. Chemical composition of *Cinnamomum Burmannii* essential oil

No	Substance name	Moleculr formula	Structural formula	Content (%)
1	Citronellal	$C_{10}H_{18}O$		52.82
2	Citronellol	$C_{10}H_{20}O$		25.13
3	1,8-Cineole	$C_{10}H_{18}O$		5.04
4	Methyl eugenol	$C_{11}H_{14}O_2$		3.78
5	Citronellyl acetate	$C_{12}H_{22}O_2$		2.96
6	**α-Pinene**	$C_{10}H_{16}$		2.40
7	Linalool	$C_{10}H_{18}O$		1.96

8	Myrcene	$C_{10}H_{16}$		1.38
9	**β-pinene**	$C_{10}H_{16}$		1.11
10	Isopulegol	$C_{10}H_{18}O$		0.50
11	Trans-β-Ocimene	$C_{10}H_{16}$		0.49
12	Limonene	$C_{10}H_{16}$		0.39
13	Methyl isoeugenol	$C_{11}H_{14}O_2$		0.29
14	Cis-3-Hexen-1-ol	$C_6H_{12}O$		0.25
15	Bergamal	$C_{11}H_{18}O$		0.21
16	Sabinene	$C_{10}H_{16}$		0.20

17	Iso-Isopulegol	$C_{10}H_{18}O$		0.18
18	Geraniol	$C_{10}H_{18}O$		0.18
19	Geranyl acetate	$C_{12}H_{20}O_2$		0.18
20	Geranial	$C_{10}H_{16}O$		0.13
21	Neral	$C_{10}H_{16}O$		0.12
22	Neryl acetate	$C_{12}H_{20}O_2$		0.11
23	Cis-**β-** Elemene	$C_{15}H_{24}$		0.11

From the above results, the chemical composition of Cinnamomum Burmannii essential oil obtained 23 compounds, of which the highest content was Citronellal (52.82%), Citronellol (25.13%), 1,8-Cineole (5.04) %. The results of the study are different from the results of previous studies on the composition of C. Burmannii essential oil. According to Research by Su J et al. (2010), the essential oil of C. burmannii leaves analyzed by GC-MS showed the presence of 40 volatile components, accounting for 99.4% of the total oil. The main components found were D-borneol (78.6%), Bornyl acetate (3.26%), (-)-spathulenol (2.60%) and eucalyptol (1.92%). In another effort, *Deng et al. (2010)* investigated 61 components in C.Burmannii essential oil in Guangxi, the main components were identified as caryophyllene (21.71%), eucalyptol (18.22%), guaiol (7.52%). %), (+)-α-terpineol (7.06%), (-) -0β-pinene (3.57%), γ-eudesmol (3.33%), bulnesol (3.16%). According to research by Nguyen Thi Thu Thao et al.(2021), studying the chemical composition of cinnamon essential oil from leaves and young branches in Phu Tho, the obtained results show that there are 31 compounds identified, of which the main component E-cinnamaldehyde (75.25%), E-o-methoxycinnamaldehyde (9.31%), benzaldehyde (3.54%). The cause of this difference may be due to differences in climate, soil or experimental conditions, so the composition of essential oils is different.

3.5. Result for determining the antioxidant capacity of *Cinnamomum Burmannii* essential oil

Conducted a survey on the antioxidant capacity of *Cinnamomum burmannii* essential oil on DPPH, the results shown in Figure 4.5 were obtained.

Table 3.6: Antioxidant activity of *Cinnamomum Burmannii* with DPPH

Formula	F1	F2	F3	F4	F5
Concentration (µg/ml)	8.68	17.41	26.14	34.81	43.41
DPPH scavenging effect (%)	45.42	55.98	70.87	80.92	91.28

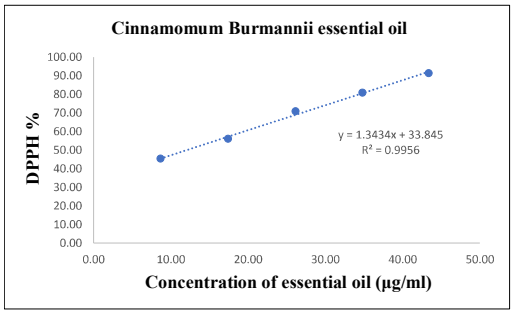


Figure 4.4: Correlation between free radical inhibitory activity and

Figure 4.4: Correlation between free radical inhibitory activity and concentration of Cinnamomum Burmannii essential oil

Table 4.7: Antioxidant activity of Ascorbic acid with DPPH

Formula	F1	F2	F3	F4	F5
Concentration (µg/ml)	10	20	30	40	50
DPPH scavenging effect (%)	39.82	49.05	59.03	74.87	84.48

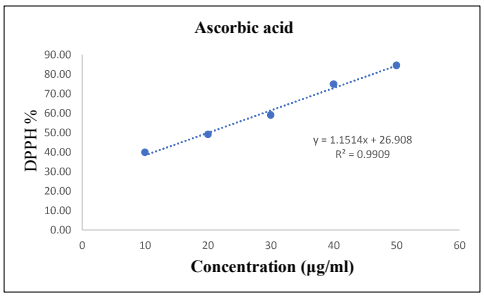


Figure 3.5: Correlation between free radical inhibitory activity and

Figure 3.5: Correlation between free radical inhibitory activity and concentration of Ascorbic acid

From the equation deduced the IC50 value of Ascorbic acid is: IC50 = 20.06 (µg/ml).

From equation (Figure 4.5) deduced that *Cinnamomum Burmannii* essential oil has an IC50 value = 12.03 µg/ml, 1.5 times lower than the IC50 value of ascorbic acid (20.06 µg/ml). Thus, compared with Ascorbic acid, the antioxidant activity of essential oil is 1.5 times higher than that of ascorbic acid. This study has results consistent with the study of Harlinda Kuspradini et al. (2016) the highest rate of DPPH radical scavenging activity (98%) was expressed in the 100 ppm µg/ml essential oil of *Cinnamomum burmannii*. Their values at different concentrations (25–100 ppm) were higher than those of ascorbic acid (97%); *Deng et al. (2010)* investigated the oxidizing activity of essential oils from *Cinnamomum* Burmannii leaves and found that the maximum removal rate on the DPPH radical was 21.71%.

The antioxidant activity of *Cinnamomum Burmannii* essential oil compared with cinnamon is often similar. In the research of Nanasombat, S., Wimuttigosol, P.(2011): ‘’Cinnamon, mace, and prikhom oils had a strong antioxidant activity with 0.29–5.66 mg/mL IC50, 61.46–68.52% antioxidant activity, 0.22–2.19 mM/mg reducing capacity, and 78.28–84.30% inhibition by 2,2-diphenyl-1-picrylhydrazyl (DPPH), β-carotene bleaching, ferric reducing (FRAP), and superoxide anion scavenging activity assays, respectively”.Compared with others essential oils such as ginger, lemongrass or rosmarinus officinalis L, the antioxidant activity of *Cinnamomum Burmannii* essential oil is higher.

In other research by G. S. El-Baroty et al.(2010), in the DPPH assay the ability antioxidant of cinnamon and ginger essential oils showed that cinnamon oil had high potential DPPH radical scavenging activity with IC50 of 13.1 µg/ml while ginger essential oils offered lower antioxidant activity (IC50 = 65.5 µg/ml) compared with the cinnamon. According to research of Nguyen Ngoc Yen et al.(2019), antioxidant activity of rosmarinus officinalis L essential oil is low with IC50 = 75,7 µg/mL. In one other research of Marta O Soares et al.(2020), the lemongrass essential oil was able to reduce the stable free radical 2,2'-diphenyl-1-picrylhydrazyl to diphenylpicrylhydrazine with an IC50 of 41.7µg/ml..

3.6. Result for determining some characteristics of essential oils

Cinnamomum Burmannii essential oil after extraction with the steam distillation method, there are some characteristics of *Cinnamomum Burmannii* essential oils are presented in Table 4.8

Table 3.8: Analysis results of some features of *Cinnamomum burmannii* essential oils

Features	Result
Color	Light yellow
Odor	Specific smell of essential oil
Taste	Bitter, warm nature
Solubility	Insoluble in water, soluble in organic solvents: methanol, diethyl ether, chloroform...
Density	0.867g/ml
Acid index (IA)	4.24
Saponification index (IS)	22.61
Ester index (IE)	18.37

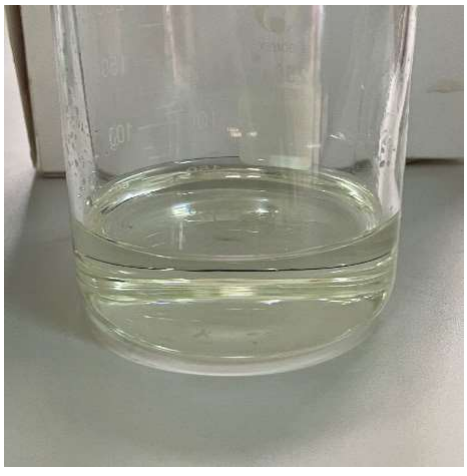


Figure 3.6. *Cinnamomum Burmannii* essential oil

3.7. Completing the extraction process of Cinnamomum burmannii essential oils

- Treatment: Use fresh, green *Cinnamomum Burmannii* leaves that are free of rot and injury. Leaves that are too young or too old should not be used since they contain a little amount of essential oil. After selecting the leaves, they are cleaned and dried. Weigh precisely 5 kg.

- Grind: Bring the leaves out after weighing, chop them into little pieces, and place them in the steam distillation pot.

- Direct steam distillation: After the leaves are placed in the distillation pot, pour water until the leaves are face down. The equipment is sealed, and the cooling water outflow valve is open. The boiling mixture evaporates water, the steam passes through the condenser tube, the steam is cooled by the cooling water outside the tube, and the steam condenses and falls into the 1-liter cylindrical glass jar. The pressure of distillation is equivalent to the pressure of the atmosphere. The distillation procedure is repeated until the amount of essential oil drawn to condense under the glass cylinder stays consistent.

- Condensation: The steam mixture that is attracted to evaporate during distillation is condensed at the condenser, converting from vapor to liquid and dropping into a glass cylinder.

- Separation: The resulting mixture contains water and essential oils. Remove the mixture from the glass cylinder and place it in a 1000 ml separating funnel. If you leave the mixture in the funnel, the essential oil of *Cinnamomum Burmannii* leaves will be lighter than water and will float on top of the water, separating it into two different phases:

the water phase below and the essential oil on top. Open the drain valve below the hopper to drain all the water below, then take the essential oil tank to recover the essential oil. That is crude oil.

- Dehydration: The essential oil obtained after distillation still contains water; if the water is not removed, the essential oil will be harmed since it contains numerous polar chemicals that are quickly oxidized. Because Na_2SO_4 salt is hydrophilic, it may be used to remove water from essential oils. Different quantities of salt are used as anhydrous depending on how much water is left in the essential oil. Place the Na_2SO_4 in the crude oil container and shake vigorously until the Na_2SO_4 crystals begin to separate.

- Collection: Allow the mixture to settle after drying, then transfer the anhydrous essential oil to a dark bottle, securely seal it, and preserve it at 2°C - 4°C.

4. Conclusion

Due to limited research time as well as equipment, the topic has not fully exploited or brought into full play the meaning of essential oil research. Based on the obtained results, the topic should be further studied in the following directions: Investigate the optimal conditions when extracting *Cinnamomum Burmannii* essential oil by microwave-assisted steam distillation and liquid CO2 extraction; Survey and study the process of isolating biologically active substances from *Cinnamomum Burmannii* essential oil; Researching mineral components and chemical substances in essential oils to be able to apply in the pharmaceutical and cosmetic industries. In order to find the most effective method of essential oil extraction while still ensuring the quality of *Cinnamomum Burmannii* essential oil, contributing to a wider application in practice.

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