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STUDY ON THE CHEMISTRY AND BIOLOGICAL ACTIVITIES OF *ELSHOLTZIA WINITIANA* CRAIB. AND ESTABLISHMENT OF CHARACTERISTIC PHYSICOCHEMICAL CRITERIA FOR THE PRODUCT "HA GIANG MINT HONEY"

SUMMARY OF DISSERTATION ON SCIENCES OF MATTER

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The dissertation is examined by Examination Board of Graduate University of Science and Technology, Vietnam Academy of Science and Technology at…….. (time, date……)

The dissertation can be found at:

1. Graduate University of Science and Technology Library
2. National Library of Vietnam

LIST OF THE PUBLICATIONS RELATED TO THE DISSERTATION

INTERNATIONAL JOURNAL ARTICLES:

1. "Optimization of the Essential Oils Extraction Process from Dong Van Marjoram (*E. winitiana* var. *dongvanensis* Phuong.) by Using Microwave Assisted Hydrodistillation and Its Bioactivities against Some Cancer Cell Lines and Bacteria"; *Natural Product Communications*; Volume 16(10): 1–8.
2. "Compositional Comparison of Essential Oils Extracted from Flowers and Aerial Parts of *Elsholtzia winitiana* var. *dongvanensis* Phuong Harvested in Ha Giang Province, Vietnam"; *Asian Journal of Chemistry*; Vol. 32; No. 10(2020); 2438–2442.

DOMESTIC JOURNAL ARTICLES:

3. "Triterpenoid compounds from aerial parts of Dong Van mint (Elsholtzia winitiana var. dongvanensis Phuong) in the Dong Van Karst Plateau, Ha Giang Province"; Proceedings of the 7th Workshop on Research and Development of Natural Products (RDNP 2020); Ho Chi Minh City, Dec. 5–6, 2020; pp. 69–74.
4. "Flavonoid compounds from aerial parts of Dong Van mint (Elsholtzia winitiana var. dongvanensis Phuong)"; Journal of Analytical Chemistry, Physics and Biology, Vol. 26, No. 1/2021, pp. 108–111.
5. "Flavonoid compounds from flowers of Dong Van mint (Elsholtzia winitiana var. dongvanensis Phuong) in Vietnam"; Journal of Analytical Chemistry, Physics and Biology, Vol. 26, No. 3A/2021, pp. 54–57.

UTILITY SOLUTION:

"METHOD FOR HONEY TRACEABILITY BASED ON CHEMICAL MARKERS" – Application accepted as valid in December 2022.

INTRODUCTION

1. Rationale of the dissertation

The Dong Van Karst Plateau – the most majestic karst plateau in Vietnam – possesses multifaceted values in terms of geology, geomorphology, landscape aesthetics, cultural history, and natural resources. As one of the country's most unique limestone regions, situated at an altitude of 1000-1700 meters above sea level, the Dong Van Karst Plateau holds a special appeal for tourists and researchers alike due to its distinctive historical development, unique geological and geomorphological heritage of regional and international significance. Furthermore, nature has bestowed upon the Dong Van Karst Plateau many majestic landscapes, distinctive scenic spots, and a diverse and endemic ecosystem, including many rare and endangered plant and animal groups. More specifically, the natural conditions in the Dong Van Karst Plateau are very favorable for honey production – a famous local product that has been granted a Certificate of Geographical Indication Registration by the Intellectual Property Office.

Honey from the Dong Van Stone Plateau is primarily produced by bees that collect nectar from the flowers of a wild plant that blooms from July to December (commonly known as "stone mint" by the locals) – scientifically known as Elsholtzia winitiana Craib. This plant is found almost exclusively in the Dong Van Stone Plateau, Ha Giang province. We believe this is the source of the precious qualities of Dong Van Stone Plateau honey, a product that is not only beneficial to health but also possesses many valuable medicinal properties. The nectar-producing plant here meets specific conditions to create the sweet, refreshing flavor and color ranging from reddish-yellow to lemon-yellow, which does not change color over time – a unique characteristic found only in Dong Van Stone Plateau honey.

Based on the above, we conducted further research on the chemical composition and biological activity of the nectar-producing plant (Elsholtzia winitiana Craib.), exploring the chemical relationship between honey and this plant, and evaluating the value of this plant and the unique honey product of the Dong Van Karst Plateau region. This is essential and has significant scientific and practical implications. Therefore, we chose Elsholtzia winitiana Craib. as the subject of our research project: ***" Study of the chemistry and biological activity of Elsholtzia winitiana Craib. and development of characteristic physicochemical parameters for 'Ha Giang mint honey' product***" .

2. Objectives of the thesis

- Research on the chemical composition and biological activity of Elsholtzia winitiana Craib. in the Dong Van Karst Plateau, Ha Giang Province.
- A study investigating the correlation between the nectar-producing plant species (Elsholtzia winitiana Craib.) and Ha Giang mint honey products.
- Establishing some characteristic physicochemical parameters for the product "Ha Giang peppermint honey".

3. Content of the thesis

- Chemical composition analysis and evaluation of the biological activity of essential oil from Elsholtzia winitiana Craib.
- Study of the chemical composition of the ethyl acetate residue of peppermint flower.
- Study of the chemical composition of the ethyl acetate residue of the stem and leaves.
- To establish several physicochemical characteristics for Ha Giang peppermint honey, and to determine the chemical marker linkages between Ha Giang peppermint honey and the primary nectar source plant, Elsholtzia winitiana Craib.

4. Scientific and practical basis of the topic

- To evaluate the scientific and practical aspects of the chemical composition and biological activity of the nectar-producing plant (Elsholtzia winitiana Craib.) and the commercially viable mint honey product from Ha Giang through chemical composition analysis and biological screening tests.
- Strengthen international cooperation in research and increase publications in reputable international journals.
- Providing sources of cultivated raw materials, analyzing chemical composition, evaluating biological activity, and guiding the exploitation of these raw materials for use in the pharmaceutical and cosmetic industries.
- To provide a scientific basis for tracing the origin of honey using chemical markers applied to Ha Giang peppermint honey products and to establish some characteristic physicochemical indicators.

5. New contributions of the thesis

- This is the first comprehensive study to investigate the essential oils of the stems, leaves, and flowers

of Elsholtzia winitiana Craib.

- The thesis has developed an integrated analytical framework (HS-SPME/GC-MS, Raman, TLC/HPTLC) for verifying the origin of honey based on chemical markers, which is capable of being implemented at the laboratory level.
- The thesis established a plant ↔ honey marker matrix, linking characteristic compounds of nectar-source plants with the chemical profile of honey (GC-MS peaks, Raman signals, TLC maps) and determining experimental detection thresholds.
- The thesis has standardized several method validation criteria (LOD/LOQ, repeatability, recovery) to ensure reliability, reproducibility, and comparability.
- The initial step involves developing a screening and confirmation SOP, and proposing a set of criteria for managing geographical indications/quality certifications to help prevent fraudulent origin claims for Ha Giang peppermint honey.

6. The structure of the thesis

The dissertation comprises 140 typed pages with 4 diagrams, 33 tables, and 15 figures. The specific distribution is as follows: Introduction (3 pages), Literature Review (41 pages), Research Subjects and Methods (20 pages), Results and Discussion (64 pages), Conclusion and Recommendations (3 pages), List of Published Works (1 page), and References (8 pages).

MAIN CONTENT OF THE THESIS

CHAPTER 1. OVERVIEW

Include six Part 1 : Background scene and meaning belong to research Rescue ; Part 2. Introduction comb Regarding the genus *Elsholtzia* ; Part 3. Composition chemistry learn of the genus *Elsholtzia* ; Part 4. Activity calculate born Learning ; Part 5. Summary mandarin about Vietnamese balm thick (*Elsholtzia) winitiana* Craib.); Part 6. Summary mandarin about honey bee .

CHAPTER 2. RESEARCH SUBJECTS AND METHODS

2.1. Research Subjects

The species *Elsholtzia winitiana* Craib., commonly known as Stone Mint (Thick Mint), was collected in Dong Van district, Ha Giang province in November 2019 and its scientific name was identified by Dr. Nguyen Quoc Binh (Vietnam Natural History Museum - Vietnam Academy of Science and Technology). The specimen is stored at the Institute of Chemistry of Natural Compounds - Vietnam Academy of Science and Technology.

All honey samples were collected from original producers in several localities across the country. Specifically, 9 samples of peppermint honey were collected at honey collection points in Dong Van, Meo Vac, and Quan Ba districts in the Dong Van Karst Plateau region of Ha Giang province at different times throughout 2022.

2.2. Research Methodology.

2.2.1. Sample processing and extraction methods

After harvesting, the plant samples were chopped, dried in the shade, and then dried at 50°C until a constant weight was achieved, after which they were ground into a fine powder. The samples were extracted three times in methanol solvent with ultrasonication and heating at 50°C, with each extraction spaced two days apart. The resulting total extract was distilled to remove the solvent under reduced pressure and at temperatures below 50°C to obtain the total methanol extract. Next, the methanol extract was subjected to fractional extraction with solvents of increasing polarity: n-hexane, ethyl acetate, butanol, and water. Solvent removal under low pressure yielded the n-hexane residue, ethyl acetate residue, butanol residue, and water residue, respectively.

2.2.2. Methods for isolating compounds from plant samples

The analysis and separation of components from plant extracts were performed using various chromatographic methods such as thin-layer chromatography (TLC), conventional column chromatography (CC) with silica gel stationary phase (Merck), reversed-phase column chromatography with YMC RP 18 stationary phase (Merck), diaion, molecular sieve chromatography with sephadex LH-20 stationary phase (Merck), and preparative HPLC liquid chromatography.

2.2.3. Methods for determining chemical structure

Modern spectroscopic methods were used in conjunction with analysis and literature review to determine the chemical structure of the isolated compounds . bag and direction France history use including :

- Point hot Flow (Mp): Point hot run Okay measure above machine Kofer-microhotstage of the Institute of Chemistry learn the Fit matter heaven natural - Korean Institute Forestry Science and Vietnamese technology .

- Prussia block Mass (MS): Spectrum ionization mass Electron dust (ESI-MS) is measure above AGILENT 1100 LC-MSD ion trap spectrometer. Spectrum block quantity fertilizer prize High HR-ESI- MS measure above GILENT 6550 iFunnel Q - TOF LC/MS system

- Prussia add enjoy from seed Nuclear (NMR) spectrum: The NMR spectrum is measure above machine Bruckker advance 500 MHz (substance) internal standard (This is TMS). The techniques technique spectrum add enjoy from seed core Okay history Applications include : General add enjoy from seed core one Dimensions : ^{1H}-NMR, ^{13C}-NMR , and DEPT. Spectra add enjoy from seed core two Directions : HSQC, HMBC, COSY, NOESY, ROESY. Solvent Okay history The applications include : DMSO - *d6* , $_{CD3OD}$, $_{CDCl3}$. The choose select solvent measure extra home enter copy matter belong to each sample , according to original solvent blockage Right dissolve completely whole sample measure .

2.2.4. Method extract cup and fertilizer accumulation wall part chemistry learn in pure oil

2.2.4.1. Steam distillation method using a micro-essential oil distiller.

2.2.4.2. Microwave-assisted steam distillation method for essential oils

2.2.4.3. Methods for analyzing the chemical composition of essential oils

Chemical analysis of essential oils was performed using an Agilent Technologies HP7890A gas chromatograph (GC) coupled with an Agilent Technologies HP5975C mass spectrometer (MS) detector and a flame ionization detector (FID). The equipment is from the Institute of Chemistry of Natural Compounds, VAST.

Gas chromatography coupled with mass spectrometry (GC-MSD) conditions: HP5-MS capillary column (60 m x 0.25 mm x 0.25 µm), carrier gas: He. Inlet: 250 °C, split ratio 100:1, inject volume: 1 µl, temperature program (60 °C – 240 °C, increments of 4 °C/min), ionization energy 70 Ev, scan length 35-450 m/z.

Gas chromatography coupled with flame ionization detector (GC-FID) conditions: HP5-MS capillary column (60 m x 0.25 mm x 0.25 µm), carrier gas: He. Inlet: 250 °C, split 100:1, inject volume: 1 µl, temperature program (60 °C – 240 °C, increments of 4 °C/min), make-up gas N_2 , combustion gases $_{O_2}$ and $_{H_2}$.

Retention Index (RI): Used to convert retention time into a constant independent of chromatographic conditions. The C7-C30 standard mixture sample was analyzed under gas chromatography-mass spectrometry (GC-MSD) conditions, the same analytical conditions as the essential oil sample. The RI of a substance is the retention time interpolated from adjacent alkanes using Massfinder 4.0 software.

Analytical procedure: Essential oils are diluted to 3% in MeOH, then pumped into the inlet where they are vaporized and introduced into the chromatography column. Adsorption and desorption occur on the chromatography column; substances are desorbed based on their evaporation temperature, with substances having lower evaporation temperatures desorbing first, followed by those with higher evaporation temperatures. After exiting the chromatography column, the substances are then sent to a mass spectrometer (MS) or flame ionization detector (FID).

Identification of the components in essential oils: Comparing the mass spectra of the obtained substances using GC-MSD coupled with the HPCH1607, W09N08, and NIST chemistry WebBook spectral libraries, and combining this with the retention index (RI).

Quantitative determination of substances in essential oils: Based on the peak areas of the substances obtained by gas chromatography coupled with flame ionization detector (GC-FID). The percentage content of a substance in an essential oil is the percentage of its peak area relative to the total area of the volatile substances.

2.2.5. Methods for evaluating biological activity

2.2.5.1. Method for evaluating cytotoxic activity using the MTT method

❖ **Ingredient**

Cell line: Hep-G2 (Hepatocellular carcinoma - liver cancer cells) ; A549 (Human lung adenocarcinoma epithelial cells - lung cancer cells) *;* PC-3 (Human Prostate Adenocarnoma - prostate cancer cells) .

Cell culture medium: Cells were cultured at 37 ° C, 5% $CO_{2\ in}$ a suitable medium: DMEM (Dulbecco's Modified Eagle Medium), EMEM (Eagle's Minimum Essential Medium, Sigma-Aldrich, USA) or RPMI 1640 (ThermoFisher, Waltham, Germany) supplemented with 2mM L-glutamine, antibiotics (Penicillin + Streptomycin sulfate) and 5-10% calf serum , *MTT* (3-(4,5-dimethylthiazole-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfo-phenyl)-2H-tetrazolium).

❖ **Method advance onion**

The MTT (3-(4,5-dimethylthiazole-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) colorimetric method was used to evaluate the effect of the substances on the survival of cancer cells including HepG2, PC3, and A549 mentioned above. Cell suspension was then placed on a 96-well microplate (1.5 x 10^5 cells/well) and incubated with the test samples at concentrations ranging from 100 →6.25 µg/ml for the extract or 50 →1 µg/ml (µM) for the purified substance, each concentration replicated three

times. Ellipticine or Paclitaxel (Taxol) in DMSO was used as a positive (+) standard. The crystalline formazan metabolite was dissolved in dimethyl sulfoxide (DMSO, Sigma-Aldrich) and its optical density was measured at λ = 540/720 nm on an Infinite F50 instrument (Tecan, Männedorf, Switzerland).

The ability to inhibit cancer cell proliferation at a given concentration of the test substance, expressed as a percentage compared to the control, is calculated using the following formula.

2.2.5.2. Method for evaluating antimicrobial activity testing

The antimicrobial activity test was conducted to evaluate the antibiotic activity of the extracts using a 96-well microtiter plate according to the modern method of McKane & Kandel (1996). The tests were performed at the Institute of Chemistry of Natural Compounds, Vietnam Academy of Science and Technology.

2.2.6. Methods for analyzing the composition of honey

2.2.6.1. Analysis of some physicochemical parameters to assess the quality of Ha Giang peppermint honey (Pharmacopoeia IV Standard)

Quantitative indicators	Qualitative indicators
Acid value (millie equivalents/kg)	Magazine matter
Road eliminate freedom (%)	Starch and dextrin
Jaw quantity water part (%)	Saccharin
Jaw quantity ash sulfate (%)	Road swap core create
Jaw HMF content (ppm)	Sulfate
	Hydrochloride
	Patch rust iron

2.2.6.2. Method fertilizer accumulation Widespread use of the R&B spectrum corpse pre calculate matter special enemy belong to honey bee silver Ha Giang.

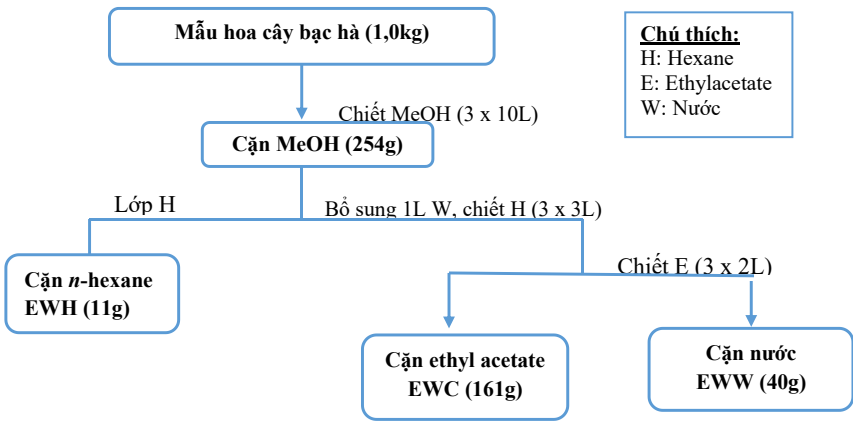
The results fruit fertilizer accumulation Raman spectra were acquired at room temperature using a JASCO NRS-3300 Raman Spectrometer with a CCD sensor and excitation light at a wavelength of 785 nm from a diode laser. To record spectra in the range of 300–1500 cm^{-1}, the instrument used an Olympus UMPLFL 20X lens, a 0.1 × 6 mm aperture, a 600 l/mm dispersion grid, and a central wavenumber at 1150 cm^{-1}. Each spectrum was acquired with an illumination time of 100 seconds and a minimum of three scans; the data were then analyzed using OriginPro 2017 software. Preprocessing procedures were applied to all Raman spectra, including data normalization using OriginPro 2017.

2.3. Experiment

The experimental part was carried out as described in the previous section.

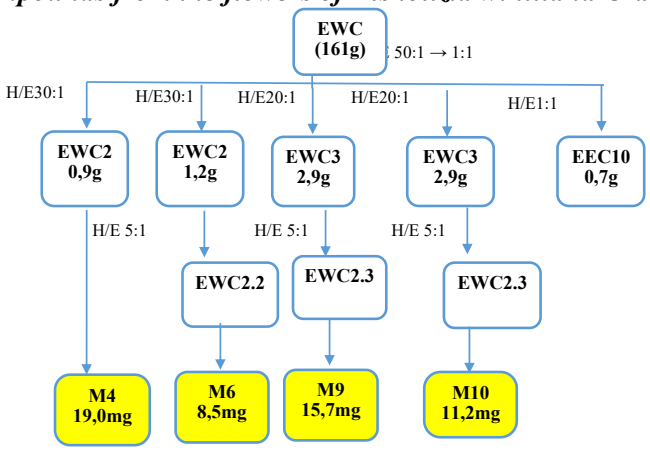
2.3.1. Isolation of compounds from the flower parts of Elsholtzia *winitiana* Craib.

***2.3.1.1. Translation extract from the flowers of Elsholtzia winitiana* Craib.**



Sơ đồ 2.1. Sơ đồ thu nhận dịch chiết từ phần hoa của cây bạc hà đá (Elsholtzia winitiana Craib.)

2.3.1.2. Isolation of compounds from the flowers of Elsholtzia winitiana Craib .

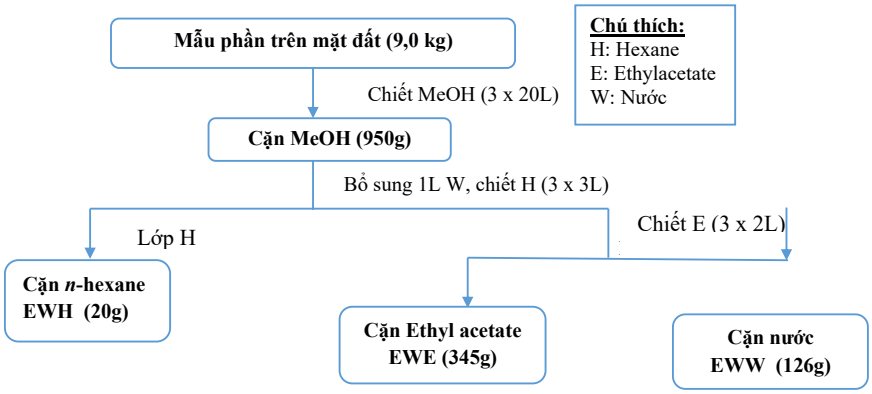


Sơ đồ 2.2. Sơ đồ phân lập cặn ethyl acetate của phần hoa cây bạc hà đá

(Elsholtzia winitiana Craib.)

2.3.2. Classification establish the fit matter from part above face land tree silver river stone (*Elsholtzia winitiana* Craib.)

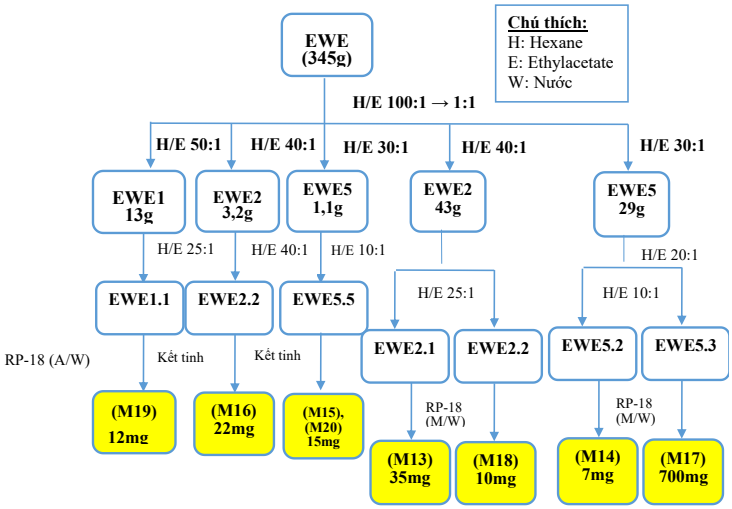
2.3.2.1. Translation extract from part above face land tree silver river stone (Elsholtzia winitiana Craib.*)*



Sơ đồ 2.3. Sơ đồ thu nhận dịch chiết phần trên mặt đất cây bạc hà đá

(Elsholtzia winitiana Craib.)

2.3.2.2. Isolation of compounds from the above-ground parts of Elsholtzia winitiana Craib.



Sơ đồ 2.4. Sơ đồ phân lập cao ethyl acetate phần trên mặt đất cây bạc hà đá

(*Elsholtzia winitiana Craib.*)

2.3.3. Research rescue wall part, jaw quantity and active calculate born learn belong to pure oil species *Elsholtzia winitiana Craib* .

2.3.3.1. Study of the composition and content of the essence oil

Quantitative analysis and identification of the components in the essential oil were carried out using the method described in section 2.2.4. The results are presented in section... 4.1.

2.3.3.2. Isolation of essential oils by column chromatography

In order to obtain the most abundant component in the essential oil of *Elsholtzia winitiana Craib* ., which is Rosefuran epoxide, we performed column chromatography on stem and leaf essential oil samples distilled using conventional methods.

1.1g of essential oil (stem-leaf) was passed onto a standard phase silica gel column with a particle size of 0.040 - 0.063 mm (240-430 mesh) using a hexane-ethyl acetate:9-1 (v/v) solvent system, yielding a pure liquid substance, designated as TD1. The structure of TD1 was elucidated by its physical constants and 1D and 2D spectra.

2.3.3.3. Testing the biological activity of semen oil

The essential oil samples were tested for antioxidant activity, antimicrobial activity, and cytotoxicity. 5 current cell cell MCF7, HepG2, Hela, HGC-27, A549 according to tissue describe in item 2.2.5.

CHAPTER 3. RESULTS AND DISCUSSION

3.1. Results of essential oil extraction, chemical composition analysis, and evaluation of the biological activity of the essential oil of the species. *Elsholtzia winitiana Craib* .

3.1.1. Results of essential oil extraction and some physicochemical indices of the essential oils

All four essential oil samples obtained by the two methods were lighter than water, pale yellow in color, and had a characteristic aroma of the species (similar to rose essential oil). The essential oil obtained from the flower part was lighter in color than the essential oil obtained from the stem and leaves. The essential oil yield and physicochemical indices, including density (d^{25}), refractive index (n^{25}_D), and specific optical rotation (α^{25}_D) of the essential oil samples are presented in Table 3.1.

Table 3.1 . Essential oil extraction efficiency and some physicochemical parameters of essential oils extracted from flower and stem-leaf samples of Elsholtzia winitiana* Craib. *using ppTT and ppVS

No.	Experimental results	Flower **essential** oil		Stem **and leaf essential** oils	
		ppTT	**ppVS**	**ppTT**	**ppVS**
1	Essential oil yield (%, w/w)	1.150	0.910	0.770	0.610
2	Density (d^{25})	0.882	0.879	0.875	0.877
3	Refractive index (n^{25}_D)	1,450	1,451	1,480	1,455
4	Specific rotation angle (α^{25}_D)	85 ° 45 '	85 ° 42 '	85 ° 48 '	85 ° 44 '

3.1.2. Conclusion fruit receive know the wall part chemistry learn in the sample pure oil

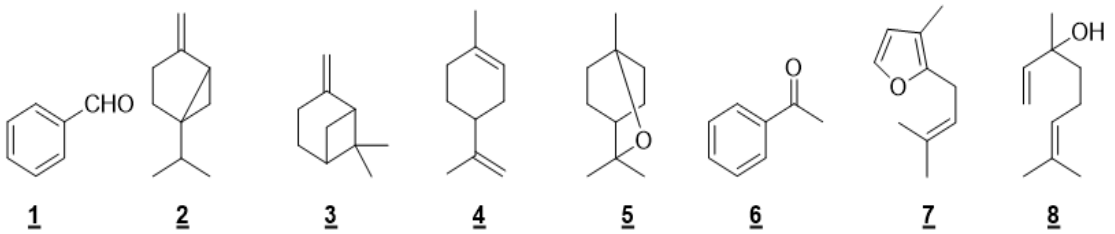
Conclude fruit fertilizer accumulation pure oil from sample flower and sample stem-leaf Okay collect receive equal direction France steam store thunder water (ppTT) and direction France steam store Have included the support assistance of microwaves (ppVS) is take note obtained from Table 3.2 and Table 3.3 (Color) sign thing Have in extra green conclude fruit fertilizer collect 04 samples pure oil). Table 3.2 for know wall part structure death belong to each sample essential oils , only number time space RI , work awake fertilizer molecule (CTPT), mass quantity fertilizer death (KLPT) and distinctive peaks display above spectrum block EI-MS amount of they .

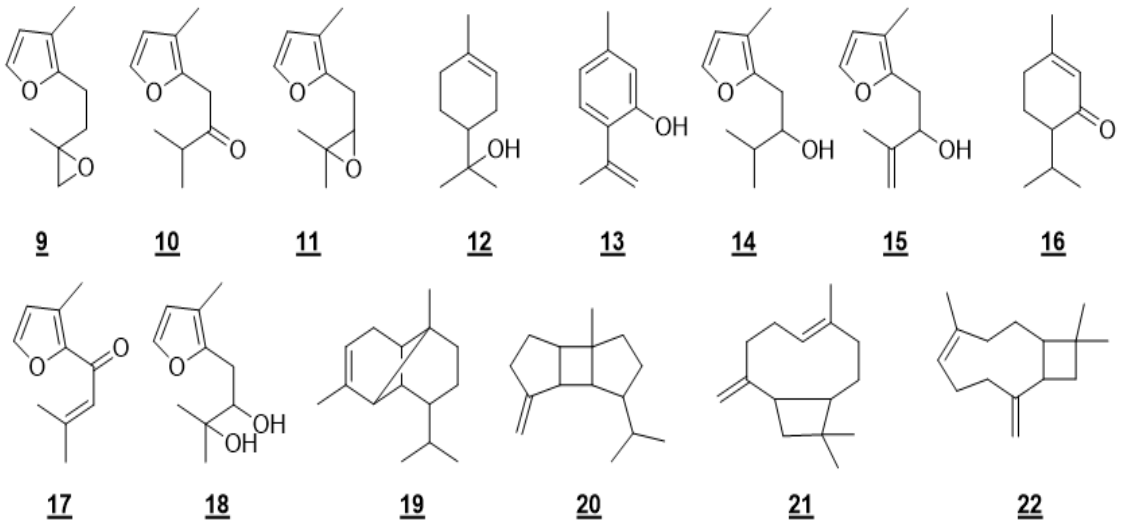
Table 3.2. Components found in the flower essential oil and stem-leaf essential oil of the thick-leaved oregano species *E. winitiana* Craib. obtained by ppTT and ppVS.

TT	Name structure death	RI	CTPT	KLPT	Prussia block quantity
1	Benzaldehyde	967	C_7H_6O	106	
2	Sabinene	979	$C_{10}H_{16}$	136	
3	β -Pinene	985	$C_{10}H_{16}$	136	
4	Limonene	1034	$C_{10}H_{16}$	136	
5	1,8-Cineole	1038	$C_{10}H_{18}O$	154	
6	Acetophenone	1074	C8H8O	120	
7	**Rosefuran**	1100	$C_{10}H_{14}O$	150	150 (100%, M^+), 135 (80), 91 (30)

TT	Name structure death	RI	CTPT	KLPT	Prussia block quantity
8	Linalool	1103	$C_{10}H_{18}O$	154	
9	*Unknown 1 (95, 166, RI 1161)*	1161	$C_{10}H_{14}O_2$	166	166 (10%, M^+), 95 (100), 82 (10)
10	*Unknown 2 (95, 166, RI 1175)*	1175	$C_{10}H_{14}O_2$	166	166 (20%, M^+), 95 (100), 71 (25)
11	**Rosefuran epoxide**	1181	$C_{10}H_{14}O_2$	166	166 (15%, M^+), 151 (5), 95 (100)
12	*α* -Terpineol	1200	$C_{10}H_{18}O$	154	
13	8,9-Dehydrothymol	1206	$C_{10}H_{12}O$	148	
14	*Unknown 3 (96, 168, RI 1213)*	1214	$C_{10}H_{16}O_2$	168	168 (10%, M+), 96 (100), 81 (30)
15	*Unknown 4 (95, 166, RI 1222)*	1222	$C_{10}H_{14}O_2$	166	166 (2%, M+), 95 (100), 81 (15)
16	Piperitone	1265	$C_{10}H_{16}O$	152	
17	**Dehydroelsholtzia ketone**	1310	$C_{10}H_{12}O_2$	164	164 (60%, M+),136 (75), 121(100)
18	*Unknown 5 (95, 184, RI 1336)*	1336	$C_{10}H_{16}O_3$	184	184 (10%, M+), 95 (100), 82 (30)
19	*α* - Copaene	1389	$C_{15}H_{24}$	204	
20	*β* - Bourbonene	1400	$C_{15}H_{24}$	204	
21	(E) -*β* -Caryophyllene	1437	$C_{15}H_{24}$	204	
22	(Z) -*β* -Caryophyllene	1437	$C_{15}H_{24}$	204	
23	*α* -Humulene	1471	$C_{15}H_{24}$	204	
24	γ- Muurolene	1490	$C_{15}H_{24}$	204	
25	Germacrene D	1498	$C_{15}H_{24}$	204	
26	*β* -Selinene	1504	$C_{15}H_{24}$	204	
27	Bicyclogermacrene	1513	$C_{15}H_{24}$	204	
28	γ-Cadiene	1530	$C_{15}H_{24}$	204	
29	*δ* -Cadinene	1536	$C_{15}H_{24}$	204	
30	Scapanol	1594	$C_{15}H_{26}O$	222	
31	Caryophyllene oxide	1605	$C_{15}H_{24}O$	220	
32	Humulene epoxide II	1631	$C_{15}H_{24}O$	220	
33	*α* - Cadiol	1674	$C_{15}H_{26}O$	*222*	

Conclude fruit fertilizer accumulation Satisfied take note receive 33 structures death , in there Satisfied receive know Okay structure bamboo of 28 contracts matter by comparison spectrum block EI-MS levels and only number time space RI of them (with the letters institute spectrum presently there are), years fit matter still again (***unknown 1)*** arrive ***unknown 5***) not yet receive know Okay equal direction comparative method spectrum letters institute and only RI number above machine . Formula structure create belong to the fit matter state above Okay presentation shown in Figure 3.1 below. This .





** (9 , 10 , 14 , 15 , and 18 To be structure bamboo attend guess give the matter* ***Unknown 1-5*** *).*

Figure 3.1. Formula structure create belong to the fit matter Okay receive know Have in pure oil belong to flower and stem-leaf belong to species terrible gender thick *E. winitiana* Craib.

Among the fit matter Satisfied Okay receive know , the fit matter home group furanoid monoterpene is Rosefuran (7), rosefuran epoxide (11) and Dehydro *Elsholtzia* ketone (17) is the wall part main and special display give the sample pure oil this ; in there Rosefuran epoxide (11) is matter Have jaw quantity High best in all sample pure oil (44.86% – 69.56%).

Compare spectrum block quantity belong to the fit matter Not yet known (***unknown 1*** - ***unknown 5***) with spectrum belong to the furanoid monoterpene see in the sample pure oil , especially compared to spectrum belong to Rosefuran epoxide (11), received see they Have the similar copper rather clear distinct at the peaks peak death and peak muscle department) also as in the beginning thing fertilizer piece . On muscle team there Satisfied advance onion fertilizer accumulation spectrum block quantity belong to they and give go out the structure bamboo attend guess belong to ***unknown 1*** – ***unknown 5*** based above the talent the virus survey about spectrum block EI-MS amount of the fit matter Have contain furan kernels **[93, 94, 95, 96, 97, 98, 99]** also like rules fertilizer piece belong to spectrum block EI-MS quantity **[95]** ; because So , the question... export one initial thing fertilizer piece give Rosefuran epoxide (11) as shown in Figure 3.2

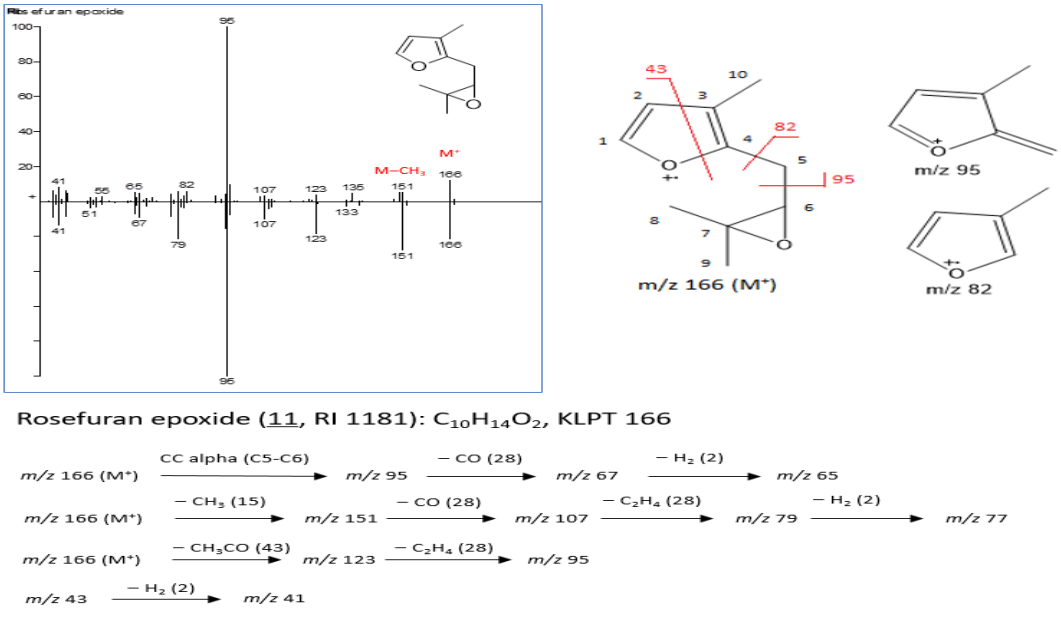
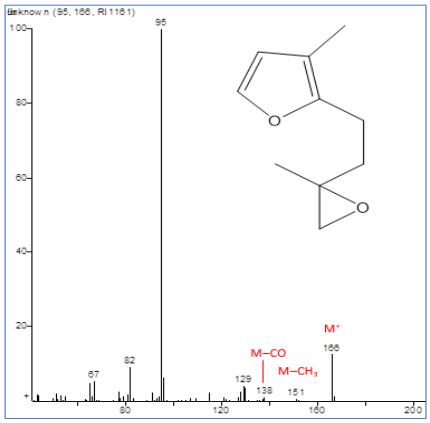
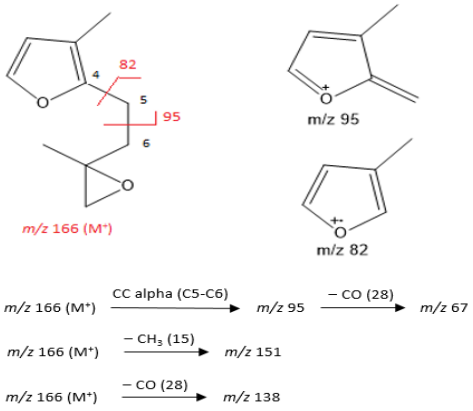


Figure 3.2 . Fragmentation diagram of Rosefuran epoxide.
(*Position of carbon atoms according to the numbering system for the acyclic monoterpene framework*)

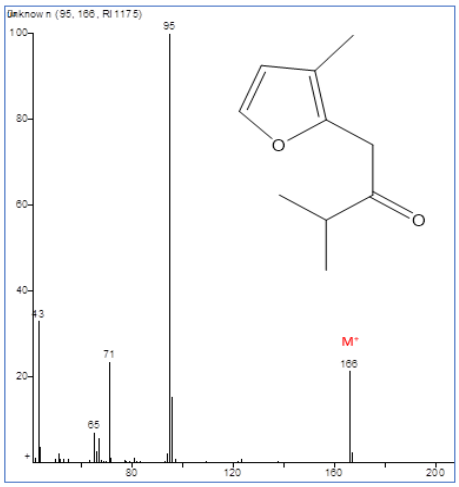




Unknown 1 (RI 1161): $C_{10}H_{14}O_2$, KLPT 166

Figure 3.3. Fragmentation diagram of unknown compound 1

Unknown 2 (RI 1175): $C_{10}H_{14}O_2$, KLPT 166



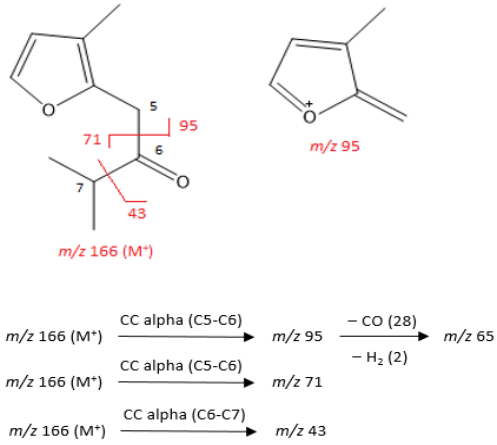
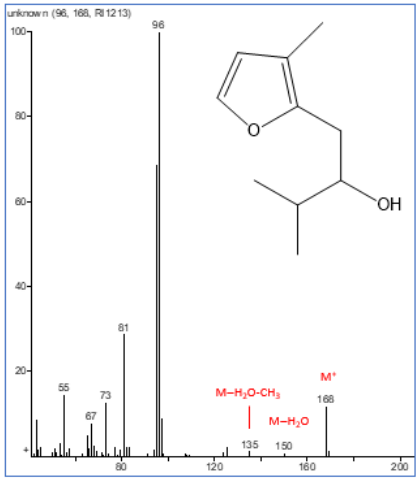
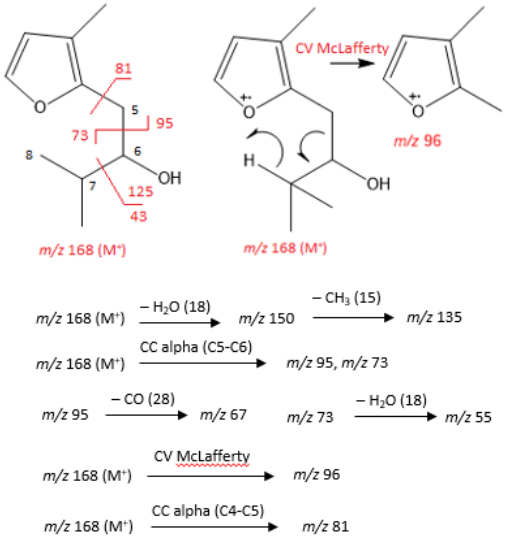


Figure 3.4 . Fragmentation diagram of compound unknown 2





Unknown 3 (RI 1213): $C_{10}H_{16}O_2$, KLPT 168

Figure 3.5. Fragmentation diagram of unknown compound 3

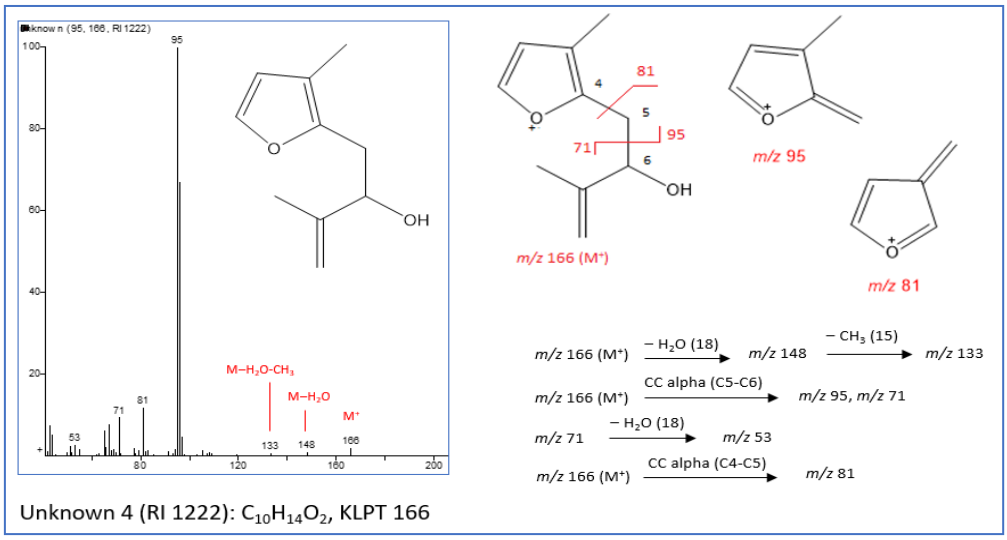


Figure 3.6. Diagram thing fertilizer piece belong to fit unknown substance 4

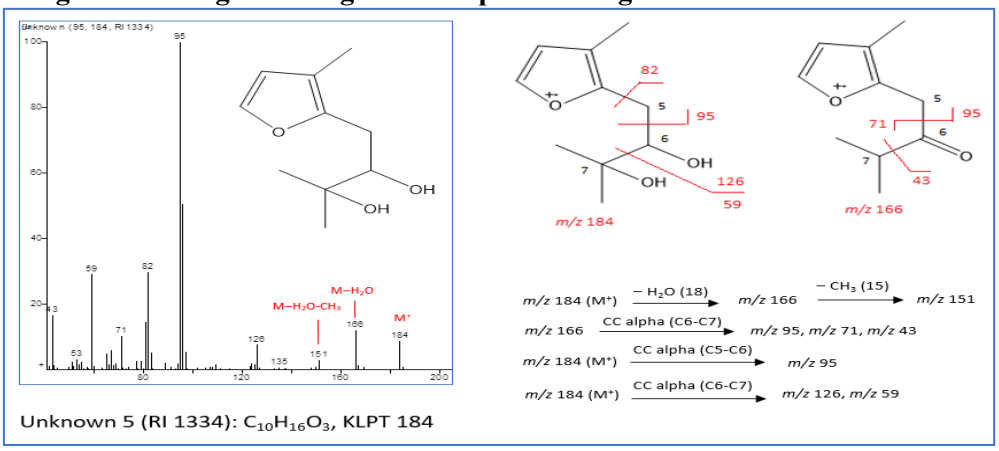


Figure 3.7 . Fragmentation diagram of unknown compound 5

Thus, unknown compounds ***1*** to ***5*** are all predicted to be furanoid monoterpenes with a basic framework of rosefuran epoxide (2-alkyl-3-methyl-furan). ***Unknown compounds 1*** to ***4*** are natural compounds, formed through the biosynthesis of the *E. winitiana plant itself* . Unknown compound ***5*** is only present in essential oils obtained by microwave-assisted distillation, suggesting that a secondary transformation occurred during the microwave-assisted distillation process.

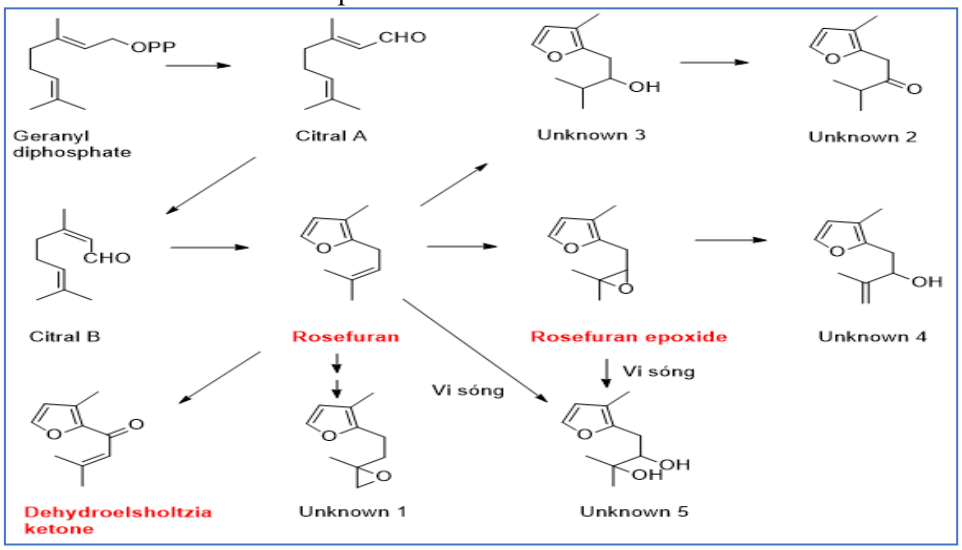


Figure 3.8 . Hypothesis regarding the formation and correlation of furanoid monoterpenes found in the essential oil of rock mint (*E. winitiana* Craib.)

Based on predictions from references **[100, 101, 102, 103]** regarding the biosynthesis of furanoid monoterpene compounds, a schematic hypothesis for the formation and correlation of furanoid monoterpenes in the essential oil of *Elsholtzia winitiana Craib* is hypothesized as shown in Figure 3.8 .

3.1.3. Results of chemical composition analysis of essential oil samples

The composition and relative content (in % FID) of the components in the flower and stem-leaf essential oils *of Elsholtzia winitiana* Craib. collected by traditional (ppTT) and microwave-assisted (ppVS) methods are presented in Table 3.3. In both fractions, oxygen-containing monoterpenes were the dominant group, with furanoid monoterpenes contributing the majority of the peak area. The two dominant components were rosefuran epoxide and rosefuran.

Table 3.3 . Results of GC-MS analysis of the content of components in the essential oil of flowers and stems/leaves of *E. winitiana* Craib. obtained by ppTT and ppVS.

TT	Name structure death	**Flower** Essential Oil		Stem and **Leaf Essential** Oil	
		ppTT	**ppVS**	**ppTT**	**ppVS**
1.	Benzaldehyde	0.97	But	2.10	But
2.	Sabinene	0.11	But	0.16	But
3.	*β* -Pinene	0.20	But	0.24	But
4.	Limonene	0.70	But	0.52	But
5.	Cineole 1,8	0.36	But	0.24	But
6.	Acetophenone	0.36	But	0.40	But
7.	**Rosefuran**	**12.47**	**4.28**	**1.77**	**1.22**
8.	Linalool	0.39	0.20	0.40	0.28
9.	*unknown 1 (95, 166, RI 1161)*	1.10	But	But	But
10.	*unknown 2 (95, 166, RI 1175)*	3.50	2.43	1.75	1.71
11.	**Rosefuran epoxide**	**63.17**	**56.13**	**69.56**	**44.86**
12.	*α* -Terpineol <a->	0.15	0.16	0.22	0.14
13.	8,9-dehydrothymol	0.17	But	But	But
14.	*unknown 3 (96, 168, RI 1213)*	4.61	5.28	4.60	4.29
15.	*Unknown 4 (95, 184, RI 1222)*	2.06	1.19	But	But
16.	Piperitone	0.18	0.23	0.17	0.18
17.	Dehydroelsholtzia ketone	0.26	But	0.21	0.24
18.	*Unknown 5 (95, 184, RI 1336)*	But	June 18th	But	26.65
19.	*α* - Copaene	But	But	0.17	0.37
20.	*β* - Bourbonene	But	But	0.12	0.16
21.	*β* -Caryophyllene <E->	**6.74**	**But**	**11.12**	**But**
22.	Caryophyllene <Z>	**But**	**6.99**	**But**	**10.80**
23.	*α* -Humulene	0.80	0.85	1.29	1.34
24.	γ- Muurolene	But	But	But	0.31
25.	Germacrene D	1.19	1.49	1.74	2.56
26.	*β* -Selinene	But	But	But	0.19
27.	Bicyclogermacrene	0.19	0.19	0.28	0.69
28.	γ-Cadiene	But	But	But	0.16
29.	*δ* -Cadinene	But	But	0.10	0.47
30.	Scapanol	But	But	But	0.20
31.	Caryophyllene oxide	0.31	0.59	1.43	1.21
32.	Humulene Epoxide II	But	But	0.13	But
33.	*α* - Cadiol	But	But	But	0.15
Total number		***99.99***	***98.07***	***98.72***	***98.60***
	Monoterpene hydrocarbon	*1.01*	***But***	*0.92*	***But***
	Monoterpenes contain oxygen.	*88.42*	*87.96*	*78.92*	*79.57*
	Furanoid monoterpene	*87.17*	*87.37*	*77.89*	*78.97*
	Sesquiterpene hydrocarbons	*8.92*	*9.52*	*14.82*	*17.05*
	Sesquiterpenes contain oxygen.	*0.31*	*0.59*	*1.56*	*1.56*

TT	Name structure death	**Flower** Essential Oil		Stem and **Leaf Essential** Oil	
		ppTT	**ppVS**	**ppTT**	**ppVS**
	Simple benzene-containing compounds	*1.33*	*But*	*2.5*	*But*

The results fruit collect Okay give see , group matter owner weak belong to chief pure oil flower and pure oil stem-leaf belong to species *E. winitiana* collect Being in Ha Giang is group the fit monoterpene compounds oxygen accounting for 88.42% (ppTT) and 78.92% (ppVS), in there the wall part main To be the furanoid monoterpenes (87.17% and 77.89%) include Rosefuran epoxide (**11** , 63.17% and 69.56%), Rosefuran (**7** , 12.47% and 1.77%), Dehydro *Elsholtzia* ketone (**17** , 0.26% and 0.21%) and the fit matter ***unknown 1*** (**9** , 1.1% and 0%), ***unknown 2*** (**10** , 3.5% and 1.75%), ***unknown 3*** (**14** , 4.61% and 4.60%), ***unknown 4*** (**15** , 2.06% and 0%).

For the traditional distillation method, comparing the content of flower essential oil and stem/leaf essential oil, there was a superiority in the total content of furanoid monoterpenes (87.17% and 77.89%) and rosefuran content (12.47% and 1.77%); however, the rosefuran epoxide content in the flower essential oil was lower than in the stem/leaf essential oil (63.17% and 69.56%).

In a previous survey conducted in 2015, the composition of essential oils from the flowers and stems/leaves of *Elsholtzia winitiana Craib* . grown in Dong Van district (Ha Giang province) was analyzed. The results regarding the components and their concentrations were quite consistent with the results presented above, especially regarding the content of Rosefuran and Rosefuran epoxide, as well as the differences in their content between the flower and stem/leaves essential oils (Table 3.4) . This suggests the stability of the accumulation and formation of essential oils in the parts of *Elsholtzia winitiana Craib* . grown in Dong Van district, Ha Giang province.

Table 3.4 . Rosefuran and rosefuran epoxide content in flower and stem/leaf essential oil samples collected in 2015 and 2019 .

Sample	Harvest 2019		Harvest 2015	
	Rosefuran	*Rosefuran epoxide*	*Rosefuran*	*Rosefuran epoxide*
Flower	12.47	63.17	15.49	67.34
Stem - leaves	1.77	69.56	3.50	76.31

** The results of GC-MS analysis of essential oil samples harvested in 2015 can be found in the appendix.*

3.1.4. Results of essential oil isolation by column chromatography

To obtain the most abundant component in the essential oil of *E. winitiana* , namely rosefuran epoxide, using the simplest method, column chromatography was performed on stem and leaf essential oil samples distilled using conventional methods. The isolation process is described in Chapter 3, section 3.3.2 . The structure of TD1 was elucidated by physical constants and 1D-, 2D-NMR spectra.

- **Fit Substance 1 (Rosefuran epoxide, TD1)**
- **Fit Substance 1 (Rosefuran epoxide, TD1)**

Form liquid Are not color , [α] $^{25}_{D}-3.8^{0}$ ($CDCl_3$, c 0.021)

spectrum (CDCl3, 500 MHz) shows see two olefinic protons at δH 7.25 (1H, d, J = 1.5 Hz) and δH 6.18 (1H, d, J = 1.5 Hz), specific display give furan ring .

Outside out , trust effect in δH 2.96 (1H, d, J = 1.5 Hz), 2.91 (1H, d, J = 5.5 Hz), and 2.70 (1H, dd, J = 6.0 Hz) for see similar mandarin from methylene group conclude with system circle .

Three methyl groups are shown at δ H 1.96 (3H, s), 1.38 (3H, s), and 1.32 (3H, s).

The 13C-NMR spectrum (CDCl3, 125 MHz), DEPT, and HSQC recorded 10 carbon signals characteristic of the terpenoid structure, including:

- δ C 62.3 (C-3) and 58.6 (C-2): two carbons of the oxirane (epoxide) ring.
- δC 146.9 , 115.3, 112.9, 140.6: carbons of furan ring .
- δC 26.4 (C-4): linked methylene conclude circle .
- δC 24.7, 18.8, 9.8: three methyl carbons at the taste intelligence other each other .

Tuong HMBC officials at δH 1.23/ δC 58.5, δH 1.38/ δC 58.5 ppm pre group dimethyloxirane position at C-2.

Tuong mandarin δH 2.91, 2.71 / δC 146.8 and 115.2 (furan), δC 62.2 and 58.5 (epoxide) confirmed receive conclude The bond is formed via the –CH2– group between furan and epoxide.

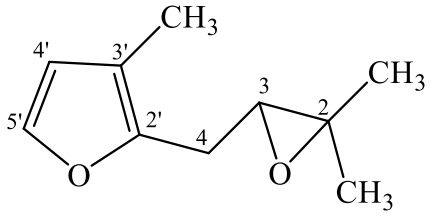
Trust NOESY brand added support assistance do clear structure bamboo Are not space .

Table 3.5. 1H, 13C-NMR spectral data of Rosefuran Epoxide

Taste intelligence	δC (ppm)	δC * (ppm)	δH (ppm, J Hz)	δH * (ppm, J Hz)	Take note uncle
2	58.6	58.49	But	But	Epoxide
3	62.3	62.18	2.96 (1H, m)	2.93 (1H, m)	Epoxide
4	26.4	26.29	2.91 (d, 5.5), 2.70 (dd, 4.0, 14.5)	2.91 (m), 2.66 (m)	CH2
2'	146.9	146.80	But	But	Furan C
3'	115.3	115.21	But	But	Furan C
4'	112.9	112.80	6.18 (d, 1.5)	6.15 (d, 1.4)	Furan H
5'	140.6	140.57	7.25 (d, 1.5)	7.22 (d, 1.4)	Furan H
2-CH3	24.7	24.60	1.32 (s)	1.32 (s)	Me
2-CH3	18.8	18.73	1.38 (s)	1.38 (s)	Me
3'-CH3	9.8	9.71	1.96 (s)	1.96 (s)	Tamarind (furan)

[a] Measure in CDCl 3 , [b] 500MHz, [c] 125 MHz, [d] 300MHz, [f] 75 MHz

From physical constants, spectral data, and comparisons with reference literature, it is confirmed that **TD1** has the structure of rosefuran epoxide.



Rosefuran epoxide

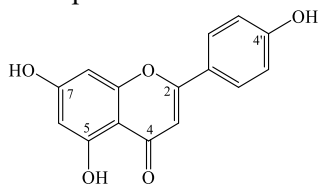
3.1.5. Conclusion fruit fight price active calculate born learn belong to pure oil

Essential oils are obtained by this method. steam store thunder coil Have support Microwave - assisted therapy was evaluated for its antiproliferative activity on two cancer cell lines (PC3 and A549), as well as its antibacterial activity on eight strains of fungi, yeasts, and bacteria. Moderate inhibition was observed in both tested cell lines, with IC50 values of 23.9 µg/mL (for PC3) and 56.2 µg/mL (for A549) (corresponding values for paclitaxel were 3.5 and 3.7 ng/mL, respectively). However, the essential oil showed strong activity against three bacterial strains (*E. coli, B. subtilis, S. aureus*) and one yeast strain (*S. cerevisiae*) with minimum inhibitory concentrations (MICs) ranging from 50 to 100 µg/mL (Table 4.6). Overall, these figures suggest that Dong Van peppermint essential oil extracted by the MAHD method has potential applications in biology.

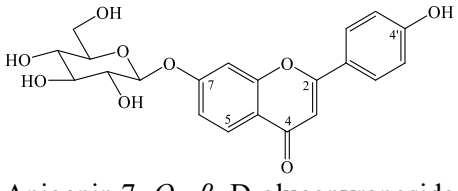
3.2. Results of the study on the chemical composition of the ethyl acetate extracts of the flower parts.

3.2.1. Determination of the structures of compounds isolated from the ethyl acetate extract residue.

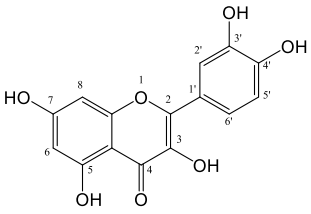
From the methanol extract of the flowers of *Elsholtzia winitiana* Craib. *,* we isolated 11 compounds using extraction and chromatography methods. The structures of these compounds were determined by spectroscopic methods.



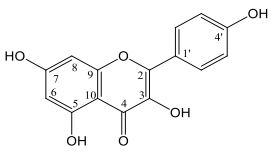
Apigenin [2]



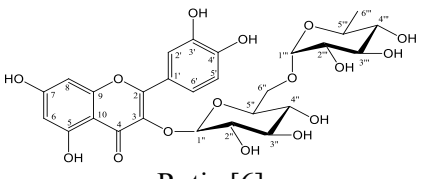
Apigenin 7- *O* - *β* -D-glucopyranoside [3]



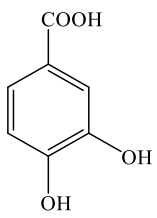
Quercetin [4]



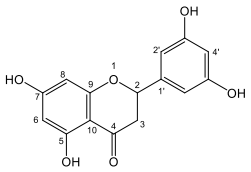
Kaempferol [5]



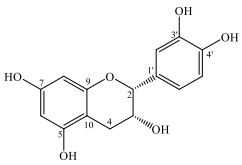
Rutin [6]



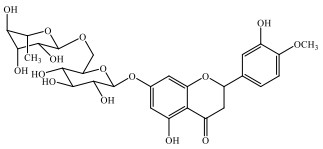
Protocatechuic acid [7]



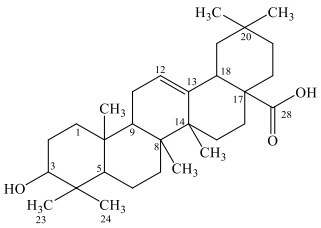
5,7,3',5'-tetrahydroxyflavanone [8]



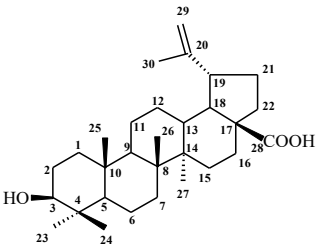
(+)-catechin [9]



Hesperidin [10]



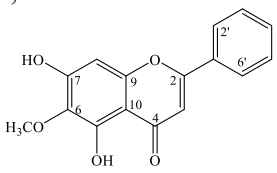
Oleanolic acid [11]



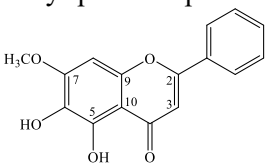
Betulinic acid [12]

3.3. Conclusion fruit research rescue wall part , structure bamboo the fit matter Okay fertilizer establish from scale ethyl acetate extract belong to part stem - leaves

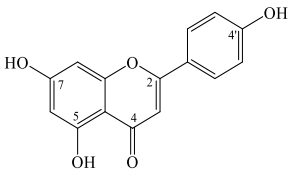
From High met h anol of close Using silica gel column chromatography, we isolated nine compounds from the leaves, and their structures were determined by spectroscopic methods .



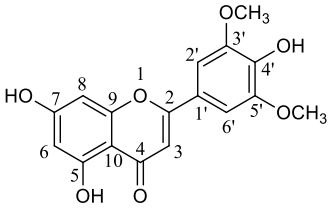
Oroxylin A [13]



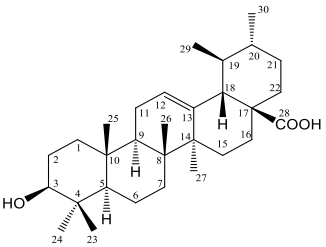
Negletein [14]



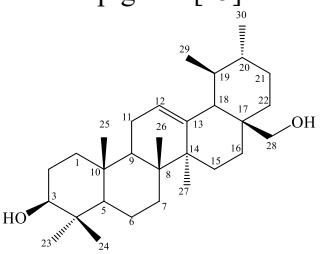
Apigenin [15]



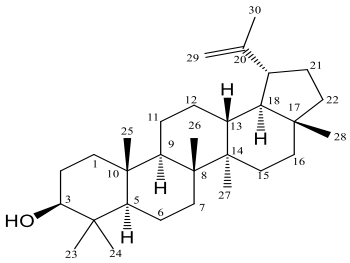
Tricin [16]



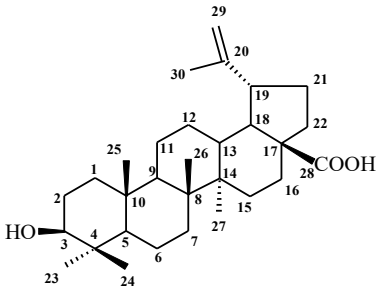
Ursolic acid [17]



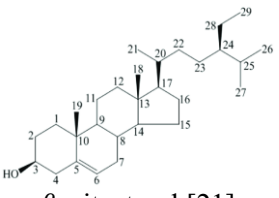
Uvaol [18]



Lupeol [19]



Betulinic acid [20]



β -sitosterol [21]

3.4 . Link only sign chemistry learn between honey bee Mint from Ha Giang and tree source honey main *Elsholtzia winitiana Craib.*

3.4.1. Assessment price matter quantity and wall part belong to honey bee BHHG

3.4.1.1. Conclusion fruit corpse pre one number only pepper matter quantity main belong to Honey bee silver Ha Giang the pepper standard presently onion .

Conclude fruit above Table 3.26 shows see Honey BHHG original water Have matter quantity over dominant about two only pepper mandarin weight To be jaw quantity road reduced (fructose and total glucose) number very tall) and jaw amount of HMF (hydroxymethylfufural very low). However only pepper about water part Have part High than regulated level . Degree acid (meq /kg, milli) right quantity acid over 1 kg)

however Not yet over threshold belong to pepper Vietnamese standards and country cell (Codex Alimentarius), but also need pay attention to cabbage good .

Table 3.26. Results fruit corpse pre one number only pepper matter quantity main belong to Honey bee silver Ha Giang the pepper standard presently onion .

TT	Name only pepper	Unit	Min	Max	Average	TCVN	TCXK
1	Jaw quantity water	%	21.0	23.0	21.8	≤ 20	≤ 20
2	Fructose and total glucose number	%	70.5	75.2	73.4	≥ 60	≥ 65
3	Degree acid	meq /kg	37.6	48.7	40.9	≤ 50	
4	HMF	ppm	4.5	16.2	9.3	≤ 40	< 20

3.4.1.2. Conclusion fruit fertilizer accumulation wall part the fit matter volatile Have in Honey bee silver Ha Giang

Volatile components in the sample honey bee Mint (M1–M9) is corpse pre using GC-MS on evaporation phase give see the presently area fine pre belong to much group fit matter Volatile . Table total fit the fit matter Main components include hydrocarbons (octane, n-undecane, n-tetracosane, α-pinene), alcohols (1-penten-3-ol, 4-hexen-3-ol, nonanol , isoborneol , thymol/carvacrol), aldehydes (n-nonanal, n-decanal, benzeneacetaldehyde), ketones (2-heptanone, damascenone , benzophenone), esters (butyl butyrate, hexyl butyrate, methyl salicylate, methyl dihydrojasmonate), and volatile fatty acids chain short arrive central average (propanoic acid, butyric acid, pentanoic acid, nonanoic acid, benzoic acid, benzeneacetic acid, 2-butenedioic acid, hexanedioic acid).

Among Therefore , α-pinene appears presently with jaw quantity similar opposite to worth tell in socks chief the sample (approximately 2.03–6.08%) price treatment central jar stack approximately 4.02%). This To be a common monoterpene meet in pure oil belong to much species real object fragrant , including chief *E. winitiana* . The presently area fine pre of α-pinene in honey bee Mint also try termite border system between source real object rich in terpenoids and lake initial smell special display belong to honey bee .

However , in thing lawsuit fertilizer accumulation solvent extraction information often pressure use give series Samples M1–M9, recorded receive trust effect belong to Rosefuran and Rosefuran epoxide at levels low . The condition This Have body reaction candidate limit craft direction law : the this furanoid monoterpene very volatile and Are not durable in lip school water - mild acid belong to honey bee ; copper time level exist residual belong to they in honey bee wall product Have body located at the threshold short give direction France Solvent extraction . To chase sign direct next group This is needed . pressure Use headspace HS-SPME/GC–MS now above sample honey bee fresh or sample tell chest dark less , replace because only based enter solvent extraction whole part .

Based above fierce the about the failure Reduce Rosefuran/ Rosefuran epoxide when handle microwave oven and the export presently structure The Unknown-5 group has muscle team attend newspaper that in honey bee Satisfied fine pre love product , mark patch still missing again Are not best design To be Pure rosefuran epoxide form , which Have body To be product product transfer chemistry similar Unknown - 5 (m/z 95/184; RI approx.) (approximately 1330–1340). Because So , the set only sign chemistry learn need calculate chief the guide export transfer chemistry belong to furanoid monoterpenes such as only sign cockroach next give source flower *E. winitiana* .

Table 3.27. Chemical composition of volatile compounds in Ha Giang peppermint honey determined by GC-MS.

TT	Fit matter	Group matter	Min	Max	Average
(1)	(2)	(3)	(4)	(5)	(6)
1	Octane	Hydrocarbon	0.43	1.21	0.84
2	n-Undecane	Hydrocarbon	0.26	0.46	0.36
3	n-Tetracosane	Hydrocarbon	0.32	0.73	0.41
4	Alpha-pinene	Hydrocarbon	2.03	6.08	4.02
5	1-Penten-3-ol	Vodka	0.38	0.5	0.43
6	4-Hexen-3-ol	Vodka	3.01	6.33	4.47
7	Nonanol	Vodka	1.45	10.45	5.87
8	Isoborneol	Vodka	0.12	0.22	0.15
9	Thymol/carvacrol	Vodka	0.27	0.3	0.37

TT	Fit matter	Group matter	Min	Max	Average
10	n-Nonanal	Aldehyde	1.69	10.91	6.19
11	n-Decanal	Aldehyde	0.81	1.96	1.36
12	Benzeneacetaldehyde	Aldehyde	0.26	0.84	0.42
13	2-Heptanone	Ketone	0.22	0.92	0.46
14	Damascenone	Ketone	2.19	4.04	3.37
15	Benzophenone	Ketone	0.33	0.61	0.43
16	Butyl butyrate	Ester	1.41	2.65	2.05
17	Hexyl butyrate	Ester	0.04	2.85	1.38
18	Methyl salicylate	Ester	1.36	1.46	1.41
19	Methyl dihydrojasmonate	Ester	0.64	1.16	0.88
20	Propanoic acid	Acid	0.58	2.31	1.8
21	Butyric acid	Acid	8.19	12.2	6.83
22	Pentanoic acid	Acid	5.06	7.28	6.34
23	Nonanoic acid	Acid	0.47	10.26	4.89
24	Benzoic acid	Acid	0.48	1.33	0.91
25	Benzeneacetic acid	Acid	0.6	0.78	0.72
26	2-Butenedioic acid	Acid	0.27	0.39	0.36
27	Hexanedioic acid	Acid	0.25	0.43	0.33
28	Rosefuran epoxide	Furanoid monoterpene	<0.05	0.80	0.30
29	Rosefuran	Furanoid monoterpene	<0.01	0.20	0.08
30	Unknown 5 (lead) Rosefuran epoxide	Furanoid monoterpene translocation chemistry	<0.10	1.50	0.60

3.4.1.3. Conclusion fruit Broadcast presently the Have face belong to one number fit matter home flavonoid and phenolic acid groups direction France sharp sign copy thin .

Surgery close pre calculate about wall part one number fit matter Have in honey bee Silver Ha Giang direction France fertilizer accumulation sharp sign copy Thin TLC. Six scale Ethyl acetate extract (EM1-EM6) of six sample honey bee collect Received in Ha Giang (M1-M6) run sharp sign class thin together with matter standard Satisfied fertilizer establish Okay or Have readily available , solvent system after : n- Hexane:Ethyl Acetate: Formic acid = 6:3:01 (v/v/v). Result fruit pre calculate Have in board 3.28

Table 3.28. Results of qualitative analysis of some common flavonoids and phenolic acids in Ha Giang peppermint honey samples using TLC.

Matter standard pre calculate	M 1	M2	M3	M4	M5	M6	M7	M8	M9
Gallic Acid	But	But	+	+	But	But	+	+	+
Ferulic Acid	+	But	+	+	But	But	+	But	But
Coumaric Acid	But	But	But	But	+	But	+	+	But
Quercetin	+	+	+	+	+	+	+	+	+
Caffeic Acid	But	+	+	But	But	+	+	But	+
Catechin	+	+	+	+	+	+	+	+	+
Luteolin	+	But	+	But	+	+	+	+	+
Phenylacetic Acid	+	+	+	+	+	+	+	+	+
Kaempferol	+	+	+	But	But	+	+	+	+

Sign Negative (-) No body currently , (+) Body presently

3.4.1.4. Conclusion fruit Broadcast presently the Have face belong to one number fit matter home flavonoid group direction France This is the main street.

Raman spectrum of honey bee Mint spell take note receive quick ' mark Cloud hand total form of sample but Are not need cup Extraction . Raman regions weight mandarin close included : (i) special 1,000–1,200 cm^{-1} region display for C–C and C –O–C oscillations of road reduction (glucose, fructose) – this To be carbohydrate base natural belong to honey bee ; (ii) region approximately 1,440–1,460 cm^{-1} reflection light oscillates variable the $–CH_2/–CH_3$ form of the aldehyde / ketone circuit straight and volatile fatty acids chain short , suitable fit with the presently area of nonanal, 2-heptanone, butyric acid, pentanoic acid… have see in GC-MS; (iii) region approximately 1,600–1,620 cm^{-1} continuous mandarin to C= C oscillation of frame benzenoid aroma , similar prefer with the presently area belong to benzeneacetaldehyde , benzophenone, methyl salicylate – the fit matter decision pre smell sweet flowers special exhibit ; and (iv) region approximately 1,650–1,665 cm^{-1}, attached with C = C oscillation storm peace and frame isopropenyl /terpenoid, is see To be door notebook receive trust effect total body belong to group furanoid monoterpenes have source origin from *E. winitiana* .

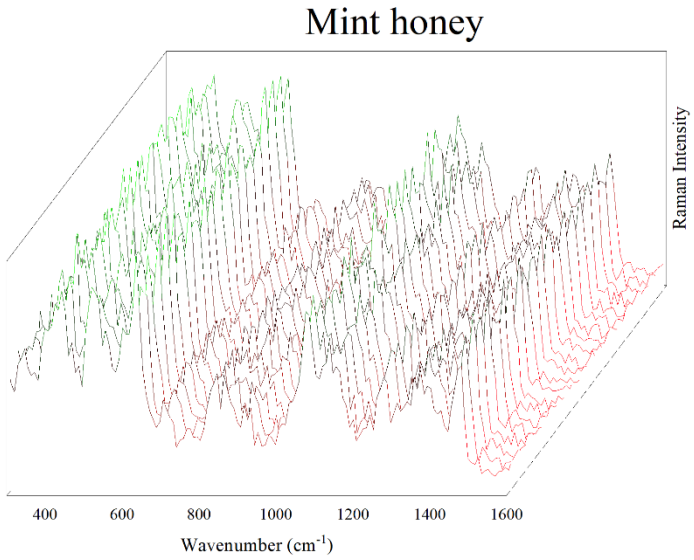


Figure 3.9 . Raman spectrum of Ha Giang peppermint honey

The significance of Raman spectroscopy lies in its ability to quickly screen for potential problems: a commercial honey sample that clearly lacks signals in the aromatic (~1,600 cm^{-1}) and terpenoid (~1,650 cm^{-1}) regions, while still exhibiting a sugar background in the 1,000–1,200 cm^{-1} region, may be suspected of being diluted/adulterated (e.g., sugar syrup) rather than genuine peppermint honey rich in volatile compounds of medicinal plant origin.

3.4.2 . Comparison and identification of chemical marker linkages between volatile components of nectar-producing plants and BHHG honey.

The volatile components of Elsholtzia *winitiana* exhibit a very characteristic chemical profile, in which the furanoid monoterpene group is overwhelmingly dominant. Rosefuran epoxide reaches 63.17% in the flower essential oil and 69.56% in the stem-leaf essential oil (ppTT), while rosefuran accounts for 12.47% in the flower essential oil but only 1.77% in the stem-leaf essential oil. When converted to microwave-assisted distillation (ppVS), the content of both rosefuran and rosefuran epoxide decreases sharply, and compound Unknown-5 (m/z 95/184; RI ~1336) appears in high concentrations (up to 18.06% in the flower essential oil and 26.65% in the stem-leaf essential oil). This demonstrates that the plant's key monoterpene furanoids can be metabolized into secondary products under conditions of water and heat.

The volatile components of Ha Giang Peppermint Honey (BHHG) obtained by GC-MS on the volatile phase of samples M1–M9 show that the main groups of compounds include: hydrocarbons (octane, n-undecane, α -pinene…), alcohols (1-penten-3-ol, 4-hexen-3-ol, nonanol…), aldehydes (n-nonanal, n-decanal, benzeneacetaldehyde), ketones (2-heptanone, damascenone, benzophenone), aromatic esters (butyl butyrate, hexyl butyrate, methyl salicylate, methyl dihydrojasmonate) and volatile short- to medium-chain fatty acids (butyric acid, pentanoic acid, nonanoic acid, benzoic acid, benzeneacetic acid…). Among these, α -pinene is present in relatively high concentrations (approximately 2.03–6.08%, averaging around 4.02%), reflecting the terpene contribution from aromatic plant sources. Methyl salicylate, benzeneacetaldehyde, and other aromatic aldehydes determine the characteristic "floral-honey" aroma of honey from the rocky highlands.

The matching revealed three direct chemical linkages between the nectar source plant and BHHG honey: *(i) Terpene/monoterpene linkage; (ii) Aromatic benzenoid linkage; (iii) Nectar source-specific furanoid monoterpene linkage.*

In other words: with the evaporation phase, the plant-to-honey linkage markers include (a) α -pinene and its monoterpene/terpenoid matrix, (b) aromatic benzenoid clusters (benzeneacetaldehyde, methyl salicylate…), and (c) characteristic furanoid monoterpene markers – which may exist as metabolites (Unknown-5 type) instead of the original epoxide. These three marker clusters are the basis for confirming the biological origin of BHHG honey from *E. winitiana* , and form the basis for establishing GC-MS headspace matching standards for authenticating commercial honey lots.

3.4.3. Comparison and identification of chemical marker linkages between the non-volatile components of nectar-producing plants and BHHG honey.

The floral extract of *Elsholtzia winitiana* revealed a highly identifiable collection of non-volatile secondary compounds, including flavonoid aglycones (Apigenin, Quercetin, Kaempferol), flavonoid glycosides (Apigenin 7-O- β -D-glucopyranoside, Rutin, Hesperidin), polyphenols/flavan-3-ols [(+)-Catechin], phenolic acids (Protocatechuic acid), and natural triterpenoids (Oleanolic acid, Betulinic acid). These components were isolated and their structures described by NMR spectroscopy, meaning they are indeed present in the floral material – the very substance that bees come into direct contact with when collecting nectar/pollen.

These compounds are non-volatile and therefore do not appear in the volatile phase GC-MS analysis of honey. Instead, this group exists in the polyphenol phase of real honey and only becomes apparent when polyphenol/phenolic fractions are extracted and analyzed by LC-MS or HPLC-DAD. This is a crucial difference: this class of compounds does not describe the smell, but rather describes the "deep biomarkers" of the plant source.

Therefore, the non-volatile layer (flavonoids, polyphenols, triterpenoids) serves as "legal evidence" to protect the geographical indication of Ha Giang Peppermint Honey. This layer answers the question: "Did the bees actually collect nectar from the correct native plant species?" while the volatile layer primarily answers the question: "Does the smell and sensory profile of this honey match that of genuine Peppermint Honey?". The two layers complement each other.

3.5. Initial steps in developing a multi-layered chemical marker and a table of characteristic physicochemical parameters for the product "Ha Giang peppermint honey"

3.5.1. Initial steps in developing a multi-layered chemical marker for BHHG honey.

The results of the analysis in Section 4.4 are used to develop a multi-layer chemical indicator system for the authentication of origin and traceability of geographical indications for BHHG honey.

The proposed indicator system comprises three complementary layers, corresponding to three different questions of interest to regulators, scientists, and the market:

- Evaporative layer (GC-MS headspace) : tissue describe lake initial smell and the structure death volatile , including sign special effect source flowers (furanoid monoterpene type) Rosefuran epoxide and product product transfer Unknown-5 chemistry).
- Class Non -volatile (LC-MS/HPLC): tissue describe set transfer chemistry rank grant special Enemies (flavonoids, polyphenols, triterpenoids) from *E. winitiana* , closed shoulder game equal proof born study - law reason belong to source original .
- layer : total fingerprint body to sieve filter fast and broadcast presently no regular / phase Mix

Three classes This attach straight chain : *tree source honey → bees → honey bee wall product* and bow grant one rules presentation check proof according to two Step :

1. Sieve filter fast by Raman + opposite mat lake evaporation special GC–MS headspace .
2. Affirm pre source origin born learn equal chase specific polyphenol/flavonoid/triterpenoid traces (LC –MS/HPLC).

This To be frame skill technique to build Create SOP for auditing definition , proof receive , and tell guard only guide land secret bee " Mint from Ha Giang".

3.5.2. Construction erect only pepper chemistry reason special display give product " Honey " product bee silver Ha Giang

After analyzing the honey samples and comparing them to the Vietnamese Pharmacopoeia IV (DĐVN), TCVN 5267-1:2008; CODEX STAN 12:1981, several basic indicators characteristic of the quality of Ha Giang peppermint honey were established:

Table 3.29. Table of basic quality indicators for Ha Giang peppermint honey.

No.	Name only pepper	Single taste	Level answer application
1	Impurities	Qualitative	Minus calculate
2	Starch and dextrin	Qualitative	Minus calculate
3	Saccharin	Qualitative	Minus calculate
4	Artificial substitution	Qualitative	Minus calculate
5	Sulfate	Qualitative	Minus calculate
6	Chloride	Qualitative	Minus calculate
7	rust	Qualitative	Minus calculate
8	Acid value	(milliequivalents/kg)	35 - 40
9	**Reducing sugars**	**(%)**	**≥ 70**
10	Density	But	≥ 1.38
11	Water content	(%)	< 20
12	**Sulfate ash**	**(%)**	**0.10-0.12**
13	HMF	(ppm)	< 40
14	Total number of microorganisms object	(CFU/mL)	< 1000
15	Have a cold mandarin color sharp	But	Inside, Gold green arrive Yellow
16	Have a cold mandarin smell taste	But	Aromatic , sweet and refreshing , with a distinctive flavor. display
17	Ingredient chemistry learn belong to the fit Volatile matter : Group furanoid monoterpene display and guide output ; monoterpene and sesquiterpene matrix , especially especially α-pinene, limonene, 1,8-cineole, (E)-β-caryophyllene; Aromatic benzenoid group (benzeneacetaldehyde , benzophenone, methyl salicylate...)	%	Have face
18	One number fit matter home Flavonoid and phenolic acid group (Quercetin, Catechin, etc.)	General	Have trust effect distinctive peaks display in region 590 cm^{-1} , 1170 cm^{-1} and 1500 cm^{-1} , region Aromatic zone ~1600 cm^{-1}, terpenoid zone ~1650 cm^{-1}

Samples honey bee Okay fertilizer accumulation If answer application Okay the only pepper above then Have body conclude essay obtain matter quantity belong to the sample honey bee silver Ha Giang.

CONCLUSIONS AND RECOMMENDATIONS

CONCLUDE

From conclude fruit Research rescue chemistry study , activities calculate born learn belong to species *Elsholtzia winitiana Craib* . and build erect only pepper chemistry reason special display give product " Honey " product bee silver Ha Giang " , we I collect Okay one number conclude essay after :

1. This thesis systematically studied the species Elsholtzia winitiana Craib. (rock mint) collected in the Dong Van Karst Plateau, Ha Giang: essential oil was extracted from the flowers and stems/leaves using two methods (conventional steam distillation and microwave-assisted distillation), qualitative and quantitative analysis of components was performed using GC-MS, isolation was carried out by column chromatography, and biological activity was evaluated. The results showed that the essential oil had a high content of furanoid monoterpenes such as rosefuran, rosefuran epoxide, and related derivatives; and also exhibited several noteworthy biological activities (antioxidant, antimicrobial activity). These data contribute to describing the core chemical and biological characteristics of this endemic nectar-producing plant in the Dong Van region.

2. From the highly polar (ethyl acetate) extracts of the flowers and stems/leaves of the mint plant, this thesis isolated and identified the structures of many compounds belonging to the flavonoid, polyphenol, and triterpenoid groups using modern spectroscopic techniques (1H-NMR, 13C-NMR, DEPT, HSQC, HMBC, MS). The obtained compounds include flavonoids (quercetin, kaempferol, apigenin, rutin, (+)-catechin), phenolic acids (protocatechuic acid), and pentacyclic and oleanane/lupane triterpenoids (oleanolic acid, ursolic acid, betulinic acid, lupeol, uvaol, etc.). This set of compounds represents stable "molecular markers" capable of transferring the mark from the nectar source plant to the honey product through the process of bee nectar/pollen collection.

3. The thesis surveyed original Ha Giang peppermint honey (collected in Dong Van). In terms of basic quality, the honey met and/or exceeded some criteria in Vietnamese and international standards (e.g., favorable reducing sugar and HMF indicators), but also revealed a point requiring control: high water content in some field samples. In the volatile compounds layer (GC-MS headspace), peppermint honey showed a characteristic sensory profile of aldehydes, ketones, aromatic esters, short-chain acids, monoterpenes/terpenoids, and hydrocarbons, reflecting the unique flavor profile of the Dong Van Karst Plateau product. This is a sensory indicator – a quick identification layer for the batch.

4. The thesis has demonstrated a direct chemical link between the nectar source plant and Ha Giang mint honey through two layers of indicators :

- (i) Volatile zone: characteristic furanoid monoterpene group of E. winitiana (e.g., rosefuran, rosefuran epoxide and related metabolites) along with aromatic aldehyde/ester/short fatty acid components;
- (ii) Non-volatile zone: The presence (at qualitative detection levels) of characteristic flavonoids and polyphenols such as quercetin, catechin, rutin, etc., in the honey, consistent with compounds isolated from the flowers/stems/leaves of E. winitiana. TLC/HPTLC analysis and Raman spectroscopy allowed the identification of characteristic signals of flavonoid components (e.g., Raman peaks in the ~590 cm^{-1} and 1170 cm^{-1} regions associated with quercetin; the ~1500 cm^{-1} region associated with the aromatic ring vibration of catechin) directly in the mint honey sample.

This match is scientific evidence that the chemical profile of rock mint is transferred into Ha Giang Rock Mint Honey product in both the volatile and non-volatile phases.

5. Based on that, the thesis has developed a "multi-layered chemical marker" framework to serve the authentication of origin and traceability of geographical indications for Ha Giang peppermint honey, including:

The volatile signature class (HS-SPME/GC-MS headspace) describes the region's characteristic aroma profile and volatile compound set.

The non-volatile trace element class (TLC/HPTLC, qualitative LC) is based on the flavonoid/polyphenol/triterpenoid set characteristic of E. winitiana.

Raman "spectroscopic fingerprinting" allows for rapid, non-destructive screening, supporting field testing.

These three layers constitute a two-step process: (i) rapid screening for batch identification and (ii) confirmation of biological origin using markers characteristic of the nectar-producing plant.

From there, the thesis initially proposes some characteristic physicochemical indicators for the "Ha Giang peppermint honey" product, considering it as an initial scientific basis for quality management, geographical indication certification, and combating commercial fraud.

Overall, the thesis not only describes the chemical composition and biological activity of the endemic nectar-producing plant species in the Dong Van Karst Plateau, but also designs a multi-layered chemical marker-based origin verification framework for Ha Giang peppermint honey – a necessary step to protect the economic value and geographical uniqueness of this product.

RECOMMENDATION

1. The study further expanded the sample set spatially (multiple honey collection points in the Dong Van - Meo Vac - Quan Ba region) and temporally/yearly to assess the seasonal and annual stability of the chemical markers, especially the furanoid monoterpene group (rosefuran, rosefuran epoxide, and its metabolites) and the flavonoid-polyphenol group transferred from rock mint to honey.

2. Standardize and refine rapid analytical procedures that can be transferred to local/quality management levels: (i) HS-SPME/GC-MS headspace for reading volatile compound profiles for sensory identification and consignment origin; (ii) TLC/HPTLC and Raman spectroscopy for rapid detection of characteristic flavonoids/phenolics in honey. Standardization includes validation of LOD/LOQ, repeatability, and recovery to ensure legal validity, serving inspection and geographical indication certification.

3. Further research into the transformation mechanism of secondary compounds in spearmint into honey, including the transformation of rosefuran → rosefuran epoxide → other metabolites under

biological conditions (honey gathering, natural fermentation) and during post-harvest processing/storage, is needed. Clarifying this mechanism will strengthen the specificity of the marker and help distinguish genuine spearmint honey from adulterated or artificially flavored products.

Integrating a multi-layered set of chemical markers (volatile – non-volatile – Raman) into the quality management and traceability criteria for "Ha Giang peppermint honey" aims to complete a set of characteristic physicochemical indicators that serve as recommendations for management and as a legal basis for registering, maintaining, and protecting geographical indications at the provincial and national levels.