A Study on Factors Influencing the Hydrodistillation of *Triphasia trifolia* Essential Oil

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Abstract: *The present study attempted to evaluate the suitable conditions for essential oil extraction from the fruit of* Triphasia trifolia *using hydrodistillation technique. The effect of various factors such as material size, solvent to solid ratio, heating temperature and distillation time, state of maturation, and fruit preservation on the oil yield was investigated. The physicochemical property, chemical composition, and biological properties were accessed. The highest yield of essential oil (7.9 mL/kg dry matter) from* T. trifolia *was obtained at optimal conditions such as fine particle size, solid to solvent ratio of 1:5, temperature oil bath of 140 °C, and distillation time of 3 h. The state of maturation of the fruits significantly affects the essential oil yield and insignificantly influences chemical composition based on the gas chromatography-mass spectrometry (GC-MS) analysis. It was found that the essential oil yield decreases significantly when ripened fruits are not kept in cool conditions before distillation. It implied that ripened fruits should be immediately processed or kept in cool storage to maintain the essential oil yield. Our findings will help produce high-quality essential oil from* T. trifolia *at a pilot or industrial scale.*

Keywords: Triphasia trifolia*; essential oil; hydrodistillation; GC-MS*

■ INTRODUCTION

Essential oil is a liquid containing volatile compounds extracted from leaves, peels, seeds, or other parts of plants and is widely used for various purposes [1-3]. Nowadays, essential oils are known as basic materials for food and pharmaceuticals worldwide. *Triphasia trifolia* (*T. trifolia*) has grown in many countries, including Vietnam. Its essential oil (EO) is a great source of antioxidants and medicinal properties such as vermicide and expectorant for diabetes [4-5].

T. trifolia fruit EO contains many medicinal compounds reported that immature fruit contains cryptoxanthin, α- and β-carotene that disappear on ripening [6]. In addition, fully ripe fruit contains pigments (e.g., triphasiaxanthin, semi-α-, semi-β- and β-carotenone). Rastogi et al. [7] reported that heraclenol, isomeranzing, and coumarins from *T. trifolia* have antimycobacterial activity against *M. kansaii* and *M. avium*. It was mentioned that essential oils from *T. trifolia* show comparable antioxidant potential as ascorbic acid. This EO has anti-inflammatory and anti-viral effects [8]. Moreover, at 0.2 μL/cm^2, the EO displayed repellency against *Tribolium castaneum* within 2-h exposure [9].

Hydrodistillation is the most common method to extract EO because of its simplicity in equipment and ease of operation [10]. The applicability of hydrodistillation can be done from laboratory to pilot scale and beyond to production scale, so this method is still being evaluated to this day [11-12]. Indeed, this method has been used to extract various aromatic plants such as lemongrass [13], lavender [14], mandarin [15], and grapefruit peels [16]. This method has been used to extract EO from the aerial parts of *T. trifolia* [9]. The results suggested many active compounds such as pinene, hexadecanoic acid, sabine, *p*-cymene, and *D*-limonene. Hydrodistillation was also used by Zoghbi et al. [17] to extract EO from *T. trifolia* stem, leaf, and fruit. Sabiene, β-pinene and γ-terpinene are considered the main components in all essential oils extracted from parts of *T. trifolia* cultivated in the State of Pará, Brazil.

To our best knowledge, the factors which affect the quality and yield of *T. trifolia* EO are not documented in the literature. Unique research related to *T. trifolia* EO from parts such as stem, leaf, and fruit in Brazil was reported [17]. However, the authors only analyzed the chemical composition, and the parameters related to the extraction process have not been mentioned. Therefore, this research aims to investigate these factors and evaluate the chemical composition of *T. trifolia* EO in Vietnam. Our results can help produce high-quality EO from *T. trifolia* fruits that forms the necessary database for extraction processes related to fruit *T. trifolia*.

■ EXPERIMENTAL SECTION

Materials

T. trifolia (Burm. f.) fruits were harvested in August 2018 from Ben Tre, Vietnam. A sample of fruits was selected, washed, and kept in a plastic bag in a cool room (15 °C). Anhydrous sodium sulfate (Na_2SO_4) was used to remove water from essential oils and purchased from Sigma Aldrich (US). Deionized water by Milli-Q purification system (Millipore) (Massachusetts, USA) was used as a solvent in the extraction process.

Instrumentation

Equipment used in the study included a distillation device with a volume of 1 L glass heat by thermal oil (110–160 °C). In addition, the unit was connected to a water-cooled condenser, Clevenger-type apparatus, and temperature sensor. The EOs were analyzed on a Shimadzu-QP2010 Plus system equipped with an Rtx 5MS fused capillary column (30 m × 0.25 mm; 0.25 µm film thickness). The brightness was determined through the Chromacolorimeter (NR60CP model) computer scanner.

Procedure

Essential oil distillation

Firstly, 1 kg of fruit in the ripe state was grounded into a flask with 600 mL of water and hydrodistilled using a Clevenger – type apparatus. The method of using thermal oil to transfer heat to the extraction process can accurately determine the temperature supplied to the system. The hydrodistillation operated at 155 °C until the amount of EO obtained was almost unchanged. The EO was obtained and dehydrated using anhydrous Na_2SO_4 of 5 g and subjected to GC-MS. The EO yield was expressed as mL/kg of the dry matter.

Determination of color change

The color change of the sample was determined using CIE Lab * color space as previously described by Nhi et al. [18]. The brightness was determined through the Chromacolorimeter (NR60CP model) computer scanner. Results are displayed in a numerical form via L* (lightness ranging from 0–100), a* (from green to red) and b* value (from blue to yellow).

Physicochemical properties of essential oils

The physicochemical properties of EOs, such as density, acid value, and saponification value, were carried out in the EO of a plant, which is used to determine the quality of essential oils extracted from plant parts [19].

GC-MS

The EOs were analyzed on a Shimadzu-QP2010 Plus system equipped with an Rtx 5MS fused capillary column. The following conditions were applied: helium as carrier gas with 36.5 cm/s of linear velocity, injector temperature of 250 °C, oven temperature program of 60–240 °C at 3 °C/min, electron energy of 70 eV, interface temperature of 250 °C. Results were compared with MS library system NIST-98 and literature data [20].

RESULTS AND DISCUSSION

Effect of Material Size

The size of the materials is an important factor that affects the yield and quality of EO. In this experiment, *T. trifolia* fruits were grounded by different methods resulting in material sizes: fine, coarse, and raw material, as shown in Fig. 1.

As shown in Fig. 2, the material size influenced the extraction yield, which controls the mass transfer process. The most rapid extraction process was observed as coarse size (raw) materials were used. This may be explained by the difference in mass transfer capability and the possibility that some EO can be adsorbed on the fruit flesh. The smaller fruit size leads to a smaller intraparticle diffusion resistance due to shorter diffusion paths, thus enhancing the extraction rate [21]. However, when the particle size is reduced, the possibility that EO can bind to the fruit flesh increases, thus lowering the extraction rate. The fact that coarse size material is the most appropriate material for distillation results from the competition between two opposite trends as fruit size decreases. Hence, we chose coarse size as the optimized size for EO distillation, and this size is used in other experiments.

Effect of the Amount of Water Added

The amount of water added is considered an important factor in the extraction process. The more water was used for extraction, the greater the diffusivity of *T. trifolia* essential oil in the water achieved. If the amount of water is too large and the efficiency of EO collection increases insignificantly, it is inefficient, time-consuming, and has other energy costs. Therefore, it is necessary to determine the amount of water to be added appropriately for the most economical extraction process.

Various amounts of water were added to the system (0–600 mL/kg of fruit). It was observed from Fig. 3 that the amount of water added significantly affected the time

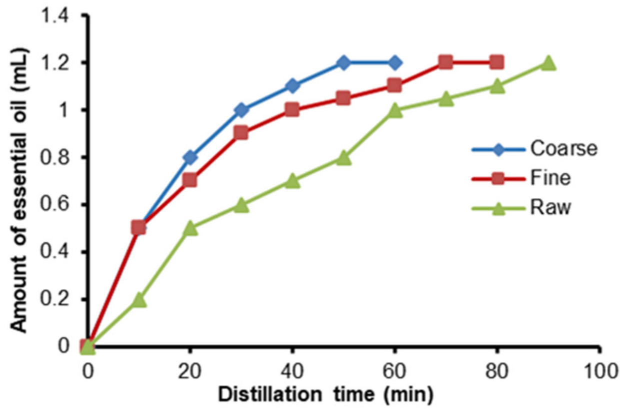

Fig 2. Effect of particle size on the EO yield

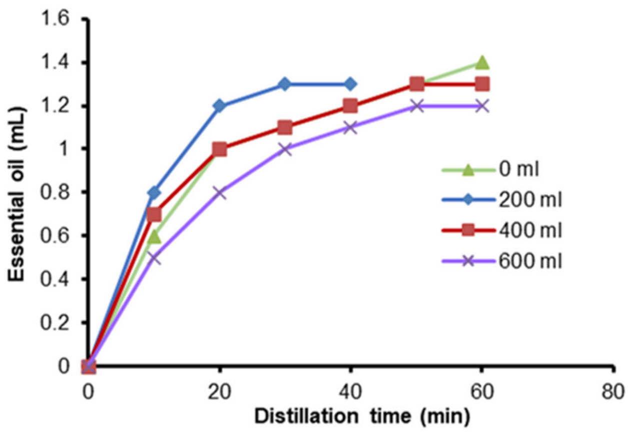

Fig 3. Effect of the amount of water added to the essential oil content

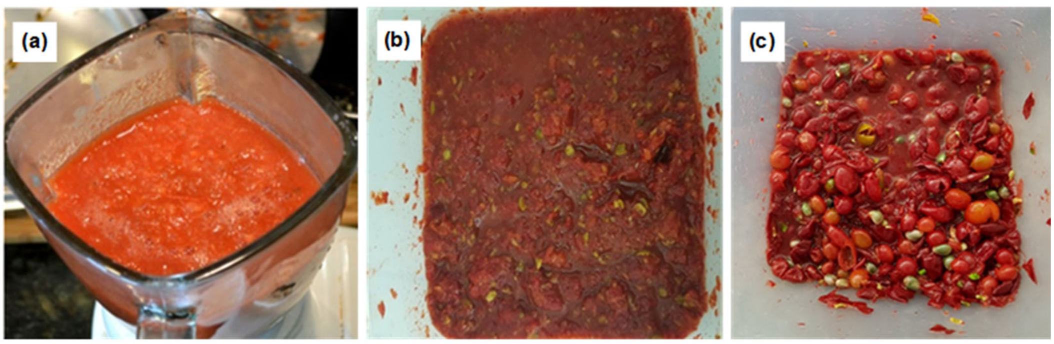

Fig 1. Various sample sizes were used in this experiment; (a) fine size; (b) coarse size; (c) raw material

of EO extraction process yet had little effect on the yield (Fig. 3). The highest amount of EO was obtained (1.4 mL/kg) when no water was added, followed by 1.2 mL/kg when 200 and 400 mL of water was added. In general, the yield of EO tended to decrease with the addition of more water, possibly due to the dissolution of the polar components in the essential oil. Besides, the hydrodistillation extraction process requires a covering of water to protect the raw materials exposed to high temperatures and limit thermal degradation [22]. Therefore, 200 mL of water was kept constant with the earlier problems while reducing extraction time by more than a third, which resulted in the highest energy costs for the extraction process. Based on the obtained results, 200 mL of water per kg of fruit was taken for the next experiment.

Effect of Oil Bath Temperature

Oil bath temperature has a significant effect on the yield. This factor is important since it affects the solubility, mass transfer rate of the solute in the material, and cavitation phenomenon. Fig. 4 demonstrates the influences of oil bath temperature on the EO yield. The amount of EO increased from 100 to 155 °C. At 100 °C, the lowest EO was obtained at 0.6 mL/kg. The temperature of 125 °C reached 1.2 mL/kg, and at the temperature of 140 °C obtained the maximum amount of 1.3 mL/kg. Indeed, low oil bath temperature would result in slight cavitation, thus minimizing the mass transfer. As a consequence, the obtained EO is negligible. As oil bath temperature increases, the effect of cavitation increases, together with the increased solubility of solutes in the solvent, leading to the rise in the amount of EO yield [20-21]. However, the excessive increase in oil bath temperature might enhance EO thermal degradation, which decreases the amount of obtained EO Besides, the short amount of extraction time can ensure the cost of the process and limit the oxidation of essential oils. Hence, 140 °C was chosen as the optimum temperature for hydrodistillation.

Effect of Fruit Ripening States

Various ripening stages of the fruits were considered. Fig. 5 shows the ripening stages of *T. trifolia* fruits. The fruits are characterized by measuring the color of the peels, and the values of L*, a*, and b* are shown in Table 1. We also presented the average weight of 20 species and the corresponding moisture content. The weight of the 20 species increases with the increase of the ripening stage. Therefore, the difference between the samples can be visually observed, and the Lab color value in Table 1 can make sample selection easy.

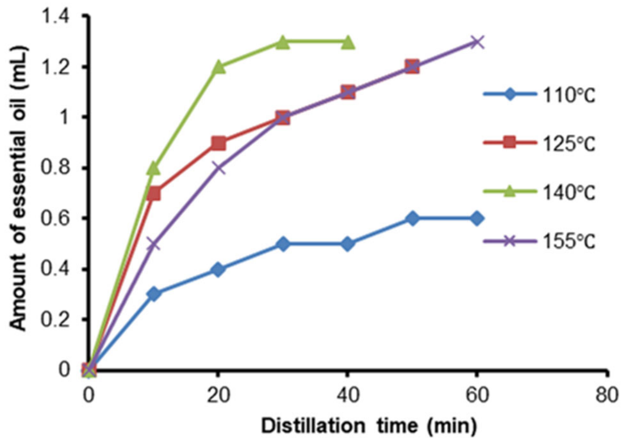

Fig 4. Variation of EO obtained as a function of time at various oil bath temperatures

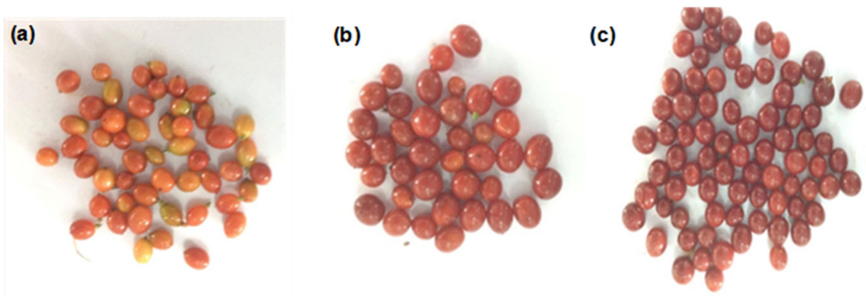

Fig 5. Image of *T. trifolia* fruits at different ripening stages. (a) Green stage, (b) Ripening stage, and (c) Fully ripened stage

Table 1. Color parameters of *T. trifolia* fruits at different ripening stages

	L*	a*	b*	Weight of 20 species (g)	Humidity (%)
Green stage	44.77	31.93	23.33	21.3246	83.63
	45.20	30.48	27.06		
	46.36	23.66	23.90		
	44.99	27.41	23.00		
	47.26	20.98	28.55		
Ripening state	39.48	29.17	18.27	24.3590	81.89
	37.44	26.67	12.33		
	37.31	29.46	14.53		
	37.25	26.49	12.36		
	39.28	29.17	15.22		
Ripe stage	31.24	21.29	7.33	36.3997	80.36
	31.66	22.06	7.41		
	31.02	21.06	6.60		
	32.23	21.49	7.81		
	32.24	21.12	7.92		

Based on Fig. 6, it can be seen that the amount of EO recovered gradually decreased with the maturity of *T. trifolia* fruits. In the samples of green, ripening, ripe fruits, the amount of EO obtained was 1.2, 1.3, and 1.5 mL/kg, respectively, and green fruit showed the highest value of essential oil. On the contrary, samples collected at maturities such as ripening and ripe fruits yielded lower EO This result is also reported in the study of Sedláková et al. [23], choosing the correct ripeness of the raw materials brings maximum economic efficiency.

Effect of Storage Time

Production of EO requires the storage of materials, and hence the condition of storage material plays a critical role in affecting EO's yield. The material was subjected to storage after collecting for 1, 2, and 3 days. As observed in Fig. 7, EO decreased from 1.2 to 0.8 mL/kg after 3 days of storage at 15 °C. It was shown that the EO yield ranked in order yield (1 day) > yield (2 days) > yield (3 days). This can be explained by the oil reservoir in the peels can be degraded and the volatile oil easily when exposed to the environment. Indeed, the fresh sample selected for evaluation was the best at all times. The same change was also found in the study of Zhang et al. [24]; the closest storage and harvesting time has a good effect on both the yield and quality of the EO.

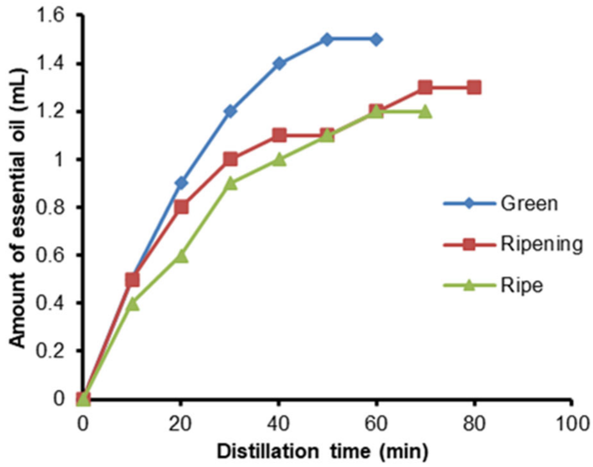

Fig 6. Effect of the ripening stage on the EO yield

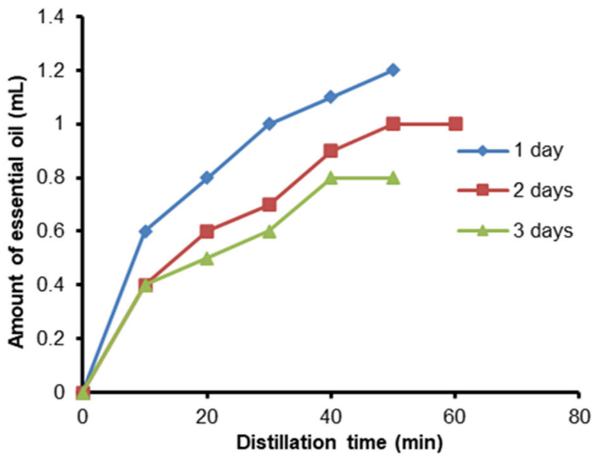

Fig 7. Yield of EO obtained at various storage conditions

Besides, extraction time is also considered an important criterion, and it was evaluated in parallel with the survey factors. Extraction time depends on many factors such as material size, amount of water added, and temperature. The longer the extraction time, the higher the recovered EO content. However, when the time is extended to a certain limit, the amount of EO obtained does not increase anymore and may adversely affect product quality [25]. On the other hand, the long extraction time consumes much energy for the heating process. Therefore, it is necessary to determine the appropriate extraction time.

Physicochemical Property and GC-MS Analysis

The EO sample was obtained at optimal conditions, such as green ripening, coarse size, 200 mL of water per kg of fresh fruit, and 140 °C, which was used to evaluate the physicochemical properties and GC-MS analysis of *T. trifolia* EOs.

The physicochemical characteristics of *T. trifolia* fruits EO such as density, acid value, and saponification value, were measured and presented in Table 2. These indexes depend on the extraction method and the freshness of the raw materials. With long-preserved raw materials, the acid index increases due to oxidation, the esters in EO are oxidized, and the ester in the EO is decomposed. The amount of free acid in the EO can be known from the acid number. A large soap index indicates that EOs have small molecular weight acids and vice versa. These results have not been compared because there are no official and published standardized reports on the characterization of *T. trifolia* fruits EO. For that reason, it is impossible to compare data on the density, acid value, and saponification value evaluated as EOs obtained by hydrodistillation in the southern region of Vietnam.

Eleven components were identified and quantitatively evaluated in the hydrodistillation extract from *T. Trifolia* fruits of different ripening states, representing more than 95% of the total EO (Table 3). Monoterpene hydrocarbons (more than 85%) predominated in all EO samples analyzed and reached maximum levels at full maturity. Oxygenated terpenoids were found to form from 7.166–7.750%, which was prominent in the green fruit stage and decreased equally in the other two states. Sesquiterpenes represent mainly in ripening fruit. The main components of *T. trifolia* fruits EO were found as sabinene (approximately 52.391–54.835%); D-limonene (5.534–8.342%), and γ-Terpinene (17.649–18.417%). The most found in the green fruit as sabinene (52.391%) and D-Limonene (8.342) were found the most.

Table 2. Physicochemical characteristics of *T. trifolia* fruits EO

Physicochemical characteristics	Results
Density at 25 °C (g/mL)	0.864–0.868
Acid value (mg KOH/g)	1.53
Saponification value (mg KOH/g)	30.70

Table 3. Chemical composition of the EO from *T. trifolia* obtained by hydrodistillation

Composition	Retention time (min)	Green (%)	Ripening (%)	Ripened (%)
a-Thujene	5.133	0.424	0.406	0.422
α-Pinene	5.377	2.371	2.463	2.596
Sabinene	6.568	52.391	55.372	54.835
α-Terpinene	7.774	1.992	1.608	1.875
p-Cymene	8.000	0.480	0.190	0.047
D-Limonene	8.197	8.342	7.464	5.534
γ-Terpinene	9.227	17.649	17.396	18.417
1-*p*-Menthene	10.210	1.985	1.870	2.029
Terpinen-4-ol	14.148	7.750	7.166	7.436
trans-α-Bergamotene	24.575	1.494	1.800	1.582
(E)-β-Farnesene	25.459	0.567	0.650	0.560

This result shows a slight similarity with the results reported by dos Santos et al. [26]; the chemical composition assessment was performed on both leaves and fruits of *T. trifolia*. The results showed that sabinene content accounted for the majority (37.2%), followed by β-pinene (23.95%) and γ-terpinene (16.3%) in the EO from the fruit, while the component in the leaves was sabinene (35.4%), and myrcene (34.1%). EOs of *T. trifolia* fruit and leaves have almost the same main composition and moderate antibacterial activity. In Brazil, Zoghbi et al. [17] analyzed volatile components of *T. trifolia* EO from plant parts by hydrodistillation extract. The EOs are characterized by high content of sabine (leaf: 31.1%, stem: 21.1%, fruit: 23.9%), and β-pinene (leaf: 40.8%, stem: 36.2%, fruit: 32.4%). It is worth mentioning that the chemical composition of a substance may vary with harvest time and geographical location [27].

Previously, sabinene exhibited strong to moderate antibacterial activity against gram-positive bacteria and anti-fungal activity against pathogenic fungi [28]. γ-Terpinene exhibits good antibacterial activity against many gram-negative and gram-positive bacteria [29]. Terpinen-4-ol has been previously reported to have potent antibacterial and anti-inflammatory properties. In addition, terpinen-4-ol was developed for the treatment of thrush in animals and for tissue healing applications [30].

■ CONCLUSION

This study considered various factors affecting *T. trifolia* EO obtained by hydrodistillation. It was found that the coarse size material, water to solid ratio of 200 mL/kg fresh fruit, and oil bath temperature at 140 °C were optimal values for EO distillation. The *T. trifolia* fruits should be used for distillation after harvesting. *T. trifolia* EO consists of many biologically active compounds such as sabinene, D-limonene, γ-terpinene, and terpinen-4-ol. Therefore, EOs have great potential to be applied in the food, pharmaceutical, and cosmetic fields because of their antioxidant properties equivalent to ascorbic acid.

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■ AUTHOR CONTRIBUTIONS

Phuoc-Sang Huynh Ngo, Tien-Xuan Le conceived and designed the experiments; Xuan-Cuong Luu contributed reagents, materials, and analysis tools; Minh-Thuan Huynh, Thien Hien Tran, Tan Phat Dao analyzed and interpreted the data; Tan Phat Dao, Tien-Xuan Le revised the manuscript; Tan Phat Dao, Tien-Xuan Le wrote the paper, and Tan Phat Dao, Tien-Xuan Le performed the experiments. All authors agreed to the final version of this manuscript.

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