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Continuous Essential Oil and Oleoresin Extraction from Star Anise (*Illicium verum*) by Hydrodistillation and Solvent Extraction

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Abstract

Star anise (Illicium verum) is widely used as medicinal herb and spice. The extracts, essential oil and oleoresin, can be produced by hydro-distillation and steam distillation, while solvent and supercritical fluid extraction can be used to extract oleoresin. In this work, the star anise is distilled to obtain the oil, subsequently, its residue is extracted by solvent extraction to get the oleoresin. Whole and grounded star anise fruit is distilled by hydro-distillation for 8, 12, 16, 20, and 24 hours, thus the highest yield is obtained at 20 hours from grounded fruit, and the highest trans-anethole content 68.50% is obtained at 8 hours from grounded fruit. Residue from whole fruit-distillation process is extracted by Soxhlet extraction with three types of solvent (ethanol, diethyl ether, and n-hexane) for 6 hours. The highest yield was obtained from ethanol extraction with 1.16% yield and the highest content of trans-anethol was obtained from n-hexane fraction. One sample with the highest content of anethole from whole and grounded fruit-distilled oil and oleoresin are picked, later they are tested to measure their antioxidant capacity by Ferric Reducing Antioxidant Power Assay (FRAP).

Keywords: *Essential oil, oleoresin, star anise, trans-anethole*

INTRODUCTION

Star anise (*Illicium verum*) is a herb that originated from Southern China and Northern Vietnam, then spread worldwide to all over Asia, the Middle East, and Europe [1]. It is used as a traditional medication and culinary spice. This herb reduces vomiting symptoms, stomach aches, insomnia, skin inflammation, rheumatism, and can be applied as an antiseptic [2]. The famous "Chinese five herbs" are composed of star anise, fennel, cinnamon, and cloves and it is widely used in baked goods, fruit and jam preservatives, and flavour addition in alcoholic beverages. Fragrances companies use its extract as raw material, also pharmaceutical and cosmetical companies use it because it possesses various anti-biological activities, such as antioxidant, antibacterial, antiviral, anticancer, and anti-inflammation [1].

Trans-anethole is the main component of star anise extract which gives a distinct taste and aroma, besides most of the plant's biological activity is provided by this phenolic ether compound which has a double bond and geometrical isomer [3]. The anethole is mainly located in the pericarp surface and lessens its content as it is close to the seed. Anethole is more easily extracted by organic solvent, such as ethanol, than by water [4].

Essential oil and oleoresin are kinds of extract that can be obtained from star anise. The essential oil has a distinctive aroma, clear to yellowish coloured and its content ranged from 2.5-8% [5]. In general, it is composed of *trans*-anethole, limonene, carene, cineole, linalool, terpineol, and others [6]. Star anise oil can be extracted by hydrodistillation and steam distillation [7]. Hydrodistillation is a method in which is the mass soaked into boiling water to evaporate oil in the plant's glands by diffusion through cell walls caused by osmosis. Diffused oil evaporates and is carried away by steam stream [8]. Hydrodistillation can extract volatiles in the plant that has the extreme condition, such as insoluble, very low or high boiling point, and temperature of the system below 100°C [9].

Star anise oleoresin has a thicker texture, a stronger and pungent aroma. It is consisting of essential oil, non-volatile components, pigment, fatty acid, and resin. It is used as an additive in food processing industries [10]. Oleoresin can be extracted by the use of organic solvent and supercritical fluids, then obtained dark green to brownish liquid and slight solvent scented [11]. Soxhlet extraction can be used to produce oleoresin, some conditions such kind of solvent, extraction time, particle size and solvent ration affect the yield and composition of oleoresin [7].

The distillation residue has various biological activity as its fresh. This is caused by remaining of

active component in it. By-product of distilled *Lavandula latifolia* possess antioxidant activity because of remained rosmarinic acid and other phenolic acids. Thus, it is possible to recover the antioxidant components from the waste [12]. Continuous extraction using simple extraction such as solvent extraction from essential oil production can recover the components. It can be done by steam distillation (SD), hydrodistillation (HD), and solvent free microwave extraction (SFME) followed by solvent extraction [13].

Antioxidant activity of star anise is caused by its phenolic compunds, which act as reductor, hydrogen donor, and lowering single oxygen amount [2]. Ferric Reducing Antioxidant Power (FRAP) method measures antioxidant power of a substance by redox reaction. In this method, antioxidants act as reductor, while the FRAP reagent as the oxidating agent will be inactivated by the reductor. Colorimetric titrants whoa acts as reductor reacts with the antioxidants and consumed certain amount of electrical energy which indicating the reduction of the antioxidant [12]. Unlike other method, FRAP directly determine antioxidant power without measuring the inhibition of free radicals produced in the reaction mixture [13].

In this work, whole and grounded star anise will be hydrodistilled for 8, 12, 16, 20 and 24 hours to obtain the highest yield and determined the composition using GCMS. Later, oleoresin in the residue with the lowest yield will be extracted by solvent extraction using Soxhlet apparatus with various solvents (ethanol, diethyl ether, and *n*-hexane) for 6 hours. The oleoresin is purified in the column chromatography by toluene-ethyl acetate as eluent with ratio 9:1 and the product are identified their component by GCMS.

MATERIALS AND METHODS

A. Plant Material

Star anise used for hydrodistillation was obtained from Central Java, Indonesia. The plants kept in the zip lock bag within box in the dry and dark area. Only the fruit was used in this experiment. It is best used before 4 months after the opening of the package.

B. Chemicals

Sodium sulfate anhydrate (Na_2SO_4) was used for drying the essential oils. Ethanol 96%, *n*-hexane and ethyl acetate were obtained from Merck. Diethyl ether was obtained from Mallinckrodt while toluene was supplied from Smart Lab. Aluminium oxide for purification was obtained from Merck.

C. Hydrodistillation

Simple distillation apparatus consisted heating mantle, round bottom flask, connector, Liebig condenser and collecting flask were used in this method. 40 grams of whole or grinded star anise (separately) submerged into the round bottom flask. The process last for 8, 12, 16, 20 and 24 hours. The distillate was collected and separated its oil from hydrosol using separatory funnel. The excess water in the oil was minimized by the use of sodium sulphate anhydrate.

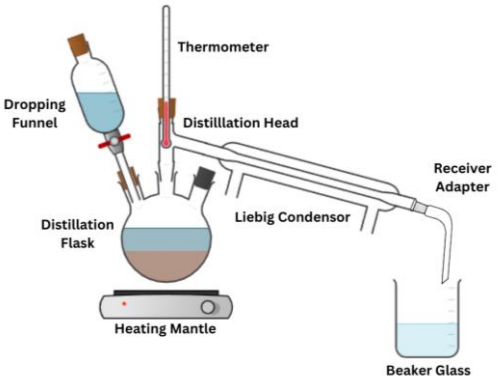

Figure 1. Distillation Equipment Scheme

D. Solvent Extraction

Whole-fruit residues were grinded and used for oleoresin extraction. 10 grams of residues were extracted by Soxhlet technique for 6 hours, followed by solvent evaporation to obtain the oleoresin extract. Ethanol 96%, diethyl ether and *n*-hexane were used as extraction solvent. During the extraction process, the temperature in the solvent chamber was maintained at 79°C for ethanol 96%, 35°C for diethyl ether, and 69°C for *n*-hexane solvent at the atmospheric pressure. This was controlled by thermocouple equipped-heating mantle with magnetic stirrer.

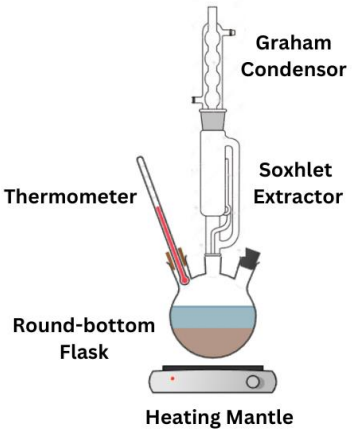

Figure 2. Set of Soxhlet Extraction

E. Purification

Oleoresin from solvent extraction was purified in the column chromatography by toluene-ethyl acetate as eluent with ratio of 9:1. Purified oleoresin was finally obtained by evaporating the eluent.

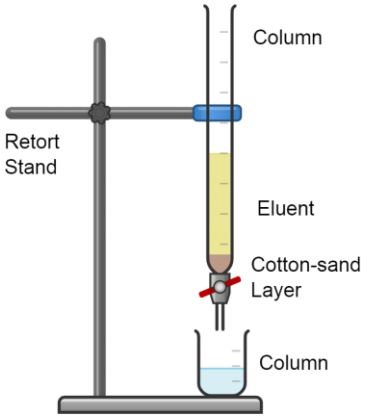

Figure 3. Column Chromatography Scheme for Oleoresin Purification

F. Determination of extract's composition

Chemical components of both essential oil and oleoresin were determined by GCMS using toluene as eluent. The specification of the GCMS (Shimadzu QP2010S) are: column oven temperature 70 C, injection temperature 310 C, sampling time 1 minute, gas pressure 18.8 kPa, and gas low-rate 47.9 L/minute.

G. Determination of antioxidant activity

In Ferric Reducing Antioxidant Power (FRAP) Assay, there are four basic reagents used. They are acetate buffer pH 3.6 from sodium acetat trihydrate, 40 mM HCl solution, 10 mM 2,4,6-tripyridil-striazine (TPTZ) and 20 mM iron(III) chloride hexahydrate ($FeCl_3 \cdot 6H_2O$). A 25 ml of acetate buffer mixed with 2.5 mL TPTZ solution and 2.5 ml $FeCl_3 \cdot 6H_2O$, then added distilled water until the solution reaches 100 ml, this mixture is named FRAP solution. Sample solutions were prepared by diluting the main solution (200 ppm) into various concentration using 96% ethanol. Sample and FRAP solutions were placed into reaction tube with ratio of 1:3, then it was stirred and stand at room temperature for 30 minutes. The absorbance mixture was determined by 96-well microplate reader.

RESULT AND DISCUSSION

A. Essential Oil Extraction

Star anise, the raw material for hydrodistillation, was obtained from Central Java, Indonesia. Only the dried fruit is used in this work. It is stored in the zip lock bag within the box in a dry and dark area. The quality of produced oil is affected by its fresh stock's storage system, such as open-air contact, storage time, temperature, and humidity [14][15]. The essential oils are in the form of light yellow aqueous and oily textured with a strong star anise scent.

Table 1 presents the comparison of the mass and the yield of the oil from **DT** in various particle sizes. Table 1 shows that the grounded-fruit samples (**GF**) produce a higher yield than the whole-fruit samples (**WF**) in every **DT** variation. The residues of WF samples contain adequate trace contents after the distillation process.

GF samples mainly produces a higher amount of oil because the grinding treatment opens the oil glands, thus it is possible to have optimum oil-water diffusion throughout the process and as the **DT** goes longer, so does the mass transfer. Some materials, such as seeds, fruits, and small branches-contained biomass has to be crushed into pieces to achieve complete essential oil recovery, thus shorten the DT and maintaining the quality of the oil [16].

Table 1. Essential Oil yield by hydrodistillation

Distillation Time, h	Particle Size	Mass, gram	Yield, %
8	Grounded (GF)	2.6617	6.65%
12		2.8456	7.11%
16		2.8633	7.16%
20		2.9290	7.32%
24		2.8701	7.17%
8	Whole (WF)	0.7128	1.78%
12		1.6484	4.12%
16		1.8349	4.59%
20		1.8300	4.58%
24		1.4903	3.73%

Moreover, the yields remain increases up to 20 hours of distillation, then it is decreasing afterward. Highest yield was achieved at 20 hours for **GF** and 16 hours for WF. Experiment conducted before [14][17][18] shows that essential oil's yield reached its maximum –based on the resources– and decreased after, even leading to the drop of the yield.

Crushing intensity directly effect on the yield, highly crushed stocks produce higher yield. The comminution of the material can alter the balance of high- and low-boiling point compounds in the oil [19]. Grinding process of the fresh material increase the monoterpenes and drop the sesquiterpene compounds, while non-grounded obtained oil is on the contrary [20].

Meanwhile, compositions shown in Table 2 was obtained by analysing the oils using GCMS and the

amount of each component was determined by its % of peak area. Instead of *trans*-anethole, there are two other compounds identified in the oils, which are 2-butoxyethanole and benzaldehyde. There is a small amount (less than 1%) of limonene found in the GF samples with **DT** 20 hours. Based on Table 2, the *trans*-anethole content declines over time, this is caused by the degradation of the components as **DT** goes longer. There is an increase of 2-butoxyethanole concentration which is two times larger than that of benzaldehyde.

Furthermore, *trans*-anethole can be oxidated into aldehydes and carboxylic acids. Benzaldehyde or anisaldehyde is a common compound found in the star anise oil. It is a trans-anethole derivation by degradation and oxidation reaction [21]. 2-Butoxyethanol or ethylene glycol monobutyl ether is a highly water-soluble ether and has low vapour pressure [22]. This compound mainly does not occur naturally, but produced by oxidation of butanol with ethylene oxide [23]. After distillation process, 2-butoxyethanol is the main component of skunk cabbage oil [24].

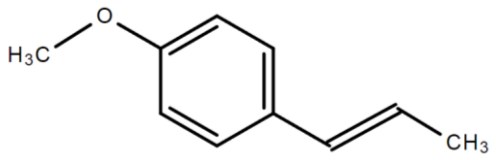

Figure 4. *Trans*-anethole structure

Table 2. Star Anise Essential Oil's Major Composition

Distillation Time, h	Particle Size	2-Butoxyethanol	Benzaldehyde	*trans*-Anethol	Limonene
8	Whole	28.66%	7.56%	63.78%	-
12		64.63%	25.68%	9.69%	-
16		3.90%	45.49%	50.60%	-
20		66.06%	23.70%	10.24%	-
24		66.49%	23.36%	3.12%	-
8	Grounded	22.92%	8.58%	68.50%	-
12		48.43%	16.84%	34.37%	-
16		39.42%	13.82%	46.95%	-
20		34.38%	12.12%	52.99%	0.50%
24		30.63%	10.35%	49.56%	-

B. Oleoresin Extraction

The residues of WF samples were collected and proceeds into the oleoresin extraction process. Utilizing the Soxhlet apparatus, the extractions were done in 6 hours and using three different types of solvent, namely ethanol, diethyl ether, and *n*-hexane. The oleoresin has a thicker consistency than the oil, it has a darker colour and stronger aroma with a slight solvent smell. The highest yield was obtained from ethanol extraction of 11.4%, while diethyl ether produces 6.4%, and *n*-hexane gives the smallest yield of 3%. It is noticeable that the use of different solvent produces a significantly different yield (Table 3).

Table 3. Oleoresins' Mass and Yield from Hydrodistillation Residues

Solvent	Mass, gram	Yield, %
Ethanol	1.16	11.6%
Diethyl ether	0.64	6.4%
n-Hexane	0.30	3.0%

The obtained oleoresins were then purified in a column chromatography using toluene-ethyl acetate (9:1) as eluent. The purification was done to remove non-volatile components for chemical content characterization by GCMS. Sample for the analysis was aqueous purified oleoresin and the amount of each component was determined by its % peak area. The rest purified oleoresin was evaporated by rotary evaporator vacuum and the crude was weighed to determine its final mass.

The main components of the purified oleoresins are *trans*-anethole, 2-butoxyethanol, and benzaldehyde. These major components are identical to that of the essential oils. In the ethanol extract, there was not found any trace of *trans*-anethole, while plenty of it was found in the diethyl ether extract. *N*-hexane gives the highest *trans*-anethole concentrations followed by the diethyl ether. This probably due to the polarity of the solvent used. Ethanol has a higher polarity than *n*-hexane and diethy ether. High polarity solvents are yielded more extract than lower ones, but low phenolic and flavonoid compounds are retrieved larger in non-polar solvents [25]. Star anise oleoresin contain a lot of phenolic and flavonoid components.

Table 4. Oleoresin's Major Components from Hydrodistillation Residues

Components	Solvent		
	Ethanol Extract	Diethyl ether Extract	n-Hexane Extract
2-Butoxyethanol	53.17%	7.91%	8.50%
Benzaldehyde	46.83%	4.56%	5.15%
trans-Anethole	-	34.08%	75.02%
Limonene	-	-	0.75%
Zingiberene	-	2.23%	0.92%
alpha- Bergamotene	-	1.72%	1.48%
trans- Caryophyellene	-	1.32%	0.89%
beta-Bisabolene	-	1.61%	1.02%
Benzopyrene	-	-	6.29%

Some minor components in diethyl ether and *n*-hexane extract were also identified (Table 4), namely zingiberene, alpha-bergamotene, *trans*-caryophyllene and beta-bisabolene. Limonene and benzopyrene were found in the *n*-hexane extracts in a trace amount.

C. Antioxidant Property of Star Anise Essential Oils and Oleoresins

Four samples were selected from essential oil and oleoresin samples with two samples for each category. They were obtained from the 8-hours distillation of WF (EOW) and GF (EOG) and one sample of *n*-hexane extract (OH) residual distillation. In comparison, fresh fruit extracted with *n*-hexane for 6 hours (OHF) was used for antioxidant activity of the residual and fresh fruit's oleoresin.

The antioxidant activity was determined by FRAP Assay, then the IC50 of the samples was measured by calculating the absorbance of the samples and the FRAP solution mixture. The antioxidant activity of the samples is measured by its IC50 value to determine how much antioxidant's concentration needed to reduce the concentration FRAP reagent's into 50% from the initial concentration. Thus, higher antioxidant activity have lower IC50 [27].

Table 5 informs the IC50 of the samples and their AAI Index. The *n*-hexane extract (OH) has the lowest IC50 value, which mean this sample possess the highest antioxidant activity. Both oleoresin samples have significantly lower IC50 then the EO samples. The oleoresin of the fresh fruit (OHF) has a lower IC50 than the residue-based extract (OH). Compared to its composition, OH have the largest *trans*-Anethole content than EOW and EOG. Both EOW and EOG contain 63.78% and 68.50%, while OH have 75.02%. In the essential oil samples, EOW have lower IC50 than that of EOG, but the IC50 of the EOW is higher. The other components, such as 2-butoxyethanol and benzaldehyde can be considered in affecting the IC50 value.

Table 5. IC50 of Essential Oil and Oleoresin Samples

Samples	IC50 (ppm)
EOW	778.2
EOG	941.37
OH	190.22
OHF	220.56

CONCLUSION

The yield of essential oil depends on the particle size of the raw material, while the composition is likely affected by distillation time. The largest oleoresin yield was obtained by ethanol, but the highest *trans*-anethole content was produced by *n*-hexane. Oleoresin has a better antioxidant activity than the essential oils, yet both of them show weak antioxidant properties based on their AAI Index Category.

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