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Recovery Yield and Bioactivities Evaluation on Essential Oil and Ethanolic Extract of Star Anise (*Illicium verum* Hook.f.)

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This study focused on the extraction of the essential oil and extract from star anise (*Illicium verum* Hook.f.) and the evaluation of the physicochemical properties and bioactivities of these products. This study was conducted by one-factor-at-a-time method on the factors affecting on the extraction yield of essential oil and the antioxidant activity of the extract. The optimized conditions for the separating process of the essential oil were raw material size (8 mm), solid/solvent ratio (1/8 w/v), extracting temperature (130 °C) and material non-compression within 180 min. The optimized conditions for the extraction process were two-times extractions at extracting temperature (50 °C), ethanol 60 % and solid/solvent ratio (1/6 w/v) during 75 min. Total phenolic content and antioxidant activity (IC_{50}) of star anise extract were 120.69 ± 0.97 mg GAE/g dried and 36.8 ± 0.4 µg/mL. Star anise essential oil (with 94.7 % of anethole) showed lower antioxidant activity than the extracts. The anti-inflammatory activity of the essential oil and extracts was studied by their ability to inhibit protein denaturation. IC_{20} value of star anise essential oil, extract were 935.5 ± 1.2 µg/mL and 122.4 ± 0.8 µg/mL.

1. Introduction

Illicium verum Hook.f., commonly known as “star anise”, grows from northern Vietnam and southern China’s woods. It is seeded in many regions, mainly Jamaica and some Asian tropical countries (Leandro and Luis, 2016). The fruit is used for flavouring teas and pickles. It is employed for chewing after meals to sweeten the breath (Cleverly et al., 1997). Among other Asian countries, star anise is employed for cooking in Malaysia and Thailand or using as a substitute in mulled wine and particular desserts (Peter, 2004). The star anise fruit contains fixed oil, minerals, catechins, pro-anthocyanidin and 5-9 % essential oil (Gholivand et al., 2009). The principal constituents of essential oil are trans-(E)-anethole (95 %), α-pinene, phellandrene, ρ-cymene, 1,4-cineole, limonene and D-terpineol (Charles, 2013). The oil of star anise is valuable in providing relief from rheumatism and lower back pain (Soher et al., 2016). The star anise fruit has been used in traditional medicine for treatment of stomach aches, vomiting, rheumatic pain, insomnia, skin inflammation (Mosaffa-Jahromi et al., 2017), flatulence, facial paralysis, asthma, and bronchitis (Sung et al., 2012). The compositions of essential oil from star anise were considered by using water distillation method and water distillation combined headspace solvent micro-extraction method (Gholivand et al., 2009). The star anise extract had high value of bioactivities, namely the antioxidant (Wang et al., 2011), anti-bacterial (Zhang et al., 2018) and anti-inflammatory (Deng et al., 2016). Today, many reports confirm the antifungal, antibacterial and anticancer properties of spices. To be specific, star anise has been recognized to have strong antioxidant and antibacterial properties leading in other herbals, as discuses above. This study aims to evaluate the effect of the different extraction conditions on the extraction yield and the antioxidant capacity as well as the potential functional properties of the extract for industrial application.

2. Materials and methods

2.1 Materials

Star anise fruits, collected in Lang Son Province, Vietnam in 2018, were stored in zip bag, placed in dry condition, avoided to moisture. Foline-Ciocalteu phenol reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH),

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quercetin and gallic acid were used in this study. The equipment used in this study were microwave, supersonic path, filtering evaporator, rotating evaporator and ultraviolet-visible spectrophotometer.

2.2 Methods

2.2.1 Distillation process

Dry star anise fruit was entered the distillation system with boiling water (Figure 1), according to Le et al (2018). The Clevenger and condenser system were set up as the various conditions: material size (unblended, 8, 4, 1 and 0.5 mm), bulk density (0, 25 and 50 %), solid/solvent ratio (1/4, 1/6, 1/8 and 1/10 w/v) and extraction temperature (120, 130 and 140 °C). Because density of essential oil was lower than that of water, essential oil was kept in the Clevenger, water was refluxed to continue distillation process. When the process ended, the mixture of essential oil and water was collected and separated to collect the essential oil by anhydrous Na_2SO_4.

2.2.2 Extraction process

50 g of raw material were extracted with different conditions: number of extraction (1 to 3 times: star anise powder was extracted with ethanol - 1 time of extraction. Then, the residue was filtered and reused to extract again with ethanol - 2 times of extraction. Finally, the experiment was repeated one more time with residue from second time extraction and ethanol - 3 times of extraction), ethanol concentration (80, 60, 40, 20 and 0 %), solid/solvent ratio (1/5, 1/6, 1/7 and 1/8 w/v), extraction time (30, 45, 60, 75 and 90 min) and temperature (40, 50, 60, 70 and 80 °C), then this mixture of the solid and solvent was filtrated by vacuum filter to collect filtrate (Figure 1). The filtrates were concentrated to dryness by a vacuum rotary evaporator at 55 °C. The extracts were kept in dark at 4 °C until analysing.

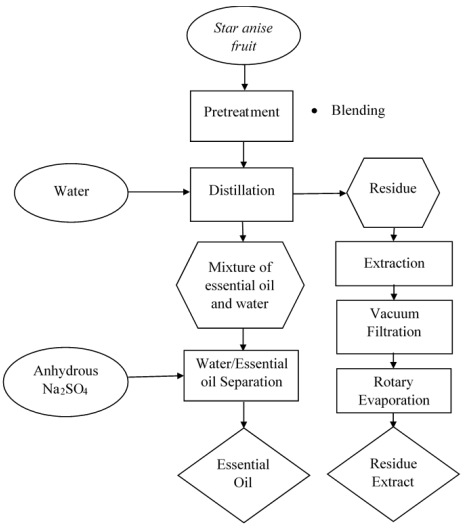

Figure 1: Star anise essential oil distillation and residue extraction procedure.

2.2.3 Analytical methods

Recovery yield of essential oil was evaluated through the quantity of the essential oil composition by weight of absolute dry material. Chemical composition of essential oil was performed on GC–MS machine with column Rxi-5ms, operating program: temperature (50-280 °C) during 37 min with helium as carrier gas, flow rate 1 mL/min, injector temperature at 250 °C. Anti-bacterial activity was controlled by a typing tool based on the resistance phenotype of the microbial strain tested. In this procedure, agar plates were inoculated with a standard inoculum of the test microorganism; then filtered paper discs were placed on the agar surface. Antimicrobial agent diffused into the agar and inhibited germination and growth of the test microorganism and then the diameters of the inhibition growth zones were measured. Antibiogram provides qualitative results by categorizing bacteria as susceptible, intermediate or resistant (Balouiri et al., 2016). Anti-oxidant activity was controlled by the DPPH free radical scavenging method. This method results in the reducing of the absorption at maximum wavelength of solution and the colour of the reaction solution would fade, turning from purple to yellow. The values used to assess strong or weak inhibition of the sample is IC_{50}, which was defined as the concentration of sample at which it could inhibit 50 % of free radicals. In general, the higher the activity

pattern, the lower the IC_{50} value (Le et al., 2018). Anti-inflammatory activity was detected by the method, which investigate the ability to inhibit albumin denaturation, according to Ullah et al. (2014). The reaction mixture consisted of egg albumin, 5 % in phosphate buffered saline and the extract was dissolved in DMSO. Then the mixtures were incubated at 37 °C in a BOD incubator for 15 min and then heated at 70 °C for 5 min; then cooling the reaction mixture and measuring the absorbance at wavelength 660 nm. Diclofenac sodium is used as positive control.

3. Results and discussions

3.1 The effect of different distillation conditions on the recovery yield of the essential oil

It was indicated in Figure 2a that when raw materials were blended at various sizes, the recovery yield of star anise essential oil fluctuated slightly. It can be explained by the fact that the essential oil in the oil bag taking time to be separated by the steam depended critically on the material size. Material (1-4 mm in diameter) has an enormous range of material particle size, the small particles inserted to the space between the more massive particles. This caused starch gelatinization when steam evaporated to moisten small particles, this phenomenon made steam difficult to pass through the material to separate essential oil. The space between particles reduced due to small size which led to result in steam being blocked and unable to escape from the powdered material layer. The diameter of the material (8 mm) can overcome the disadvantages of above particle sizes. The grinding star anise fruit was not extremely small, which can prevent this phenomenon of starch gelatinization. The essential oil was able to escape easily from the fruits, improving the separation rate of essential oil.

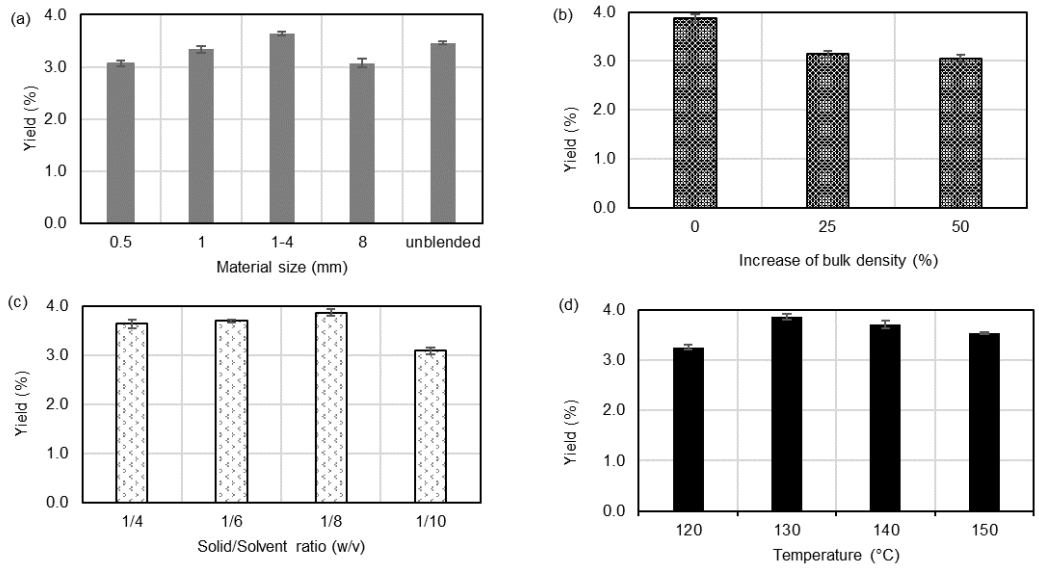

Figure 2: Recovery yields of essential oil at different extraction conditions: (a) material size, (b) increase of bulk density, (c) solid/solvent ratio and (d) temperature.

When the bulk density was unchanged (non-compression), the recovery yield achieved the maximum (3.78 mL/g, Figure 2b). The recovery yield of essential oil decreased when bulk density increased to 50 % and 25 %. The principal reason was due to the uneven resistance in the material block, the steam would follow the way with the least resistance. The resistance was minimal in the case of uncompressed material, resulting in the steam being easily crept through the gaps to separate the essential oil, in this way the amount of essential oil was greatest. The higher the resistance when increasing the bulk density of the material, the more difficult for oil was to recovery. The higher the resistance when increasing the bulk density of the material, the more difficult for oil was to recovery resistance.

According to Figure 2c, the volume of distilled water affected on the recovery yield of essential oil. With intense amount of distilled water, the distance between the water level and the supporter reduced. It was recognizable to produce the bottom layer of raw material transforming to starchy, hindering the separation of essential oil. With moderate amount of distilled water, it was not enough water for separating essential oil, because a part of water was adsorbed. For the system employed in this study, 1/8 of the solid/solvent ratio remain a suitable condition for the highest recovery yield of essential oil.

The extracting temperature affected on the recovery yield of star anise essential oil (Figure 2d). When the temperature was still unstable, the amount of steam evaporating at three levels of the temperatures (130 °C, 140 °C and 150 °C) was relatively equivalent. However, the more heat was transferred to the water at the more elevated temperature, resulting in a steam evaporation rate and the essential oil separation's rate was faster than that at the moderate temperature. In the contract, when temperature was 120 °C, amount of obtained essential oil was low. This could be explained that temperature of 120 °C was not enough to vaporize the essential oil. In addition, temperature was 130 °C, the essential oil produced a burning smell. It was possible at incredibly excessive temperature, because of thermal decomposition, causing a burning smell. Selecting the appropriate heat source's temperature of 130 °C to investigate of other factors.

3.2 The effect of different extraction condition on the antioxidant activity of the star anise extract

In this study, antioxidant activity increased 1.85 times when the number of extractions increased from one-time extraction to two-times extraction (IC_{50} of 154.04 and 83.27 µg/mL). The antioxidant activity of star anise extract decreased with three-times extraction (IC_{50} of 88.92 µg/mL, Figure 3a). The extraction more than two-times was unrecommended in this study because time and solvent, which required for completely extracting, were unjustified by a difference in the yield of extraction. Heating and long extraction times might result in oxidation and decomposition of the desired compounds. In that manner, multiple-stage extraction could remain an effective practice to minimize these problems (Mohamed and Yong., 2008).

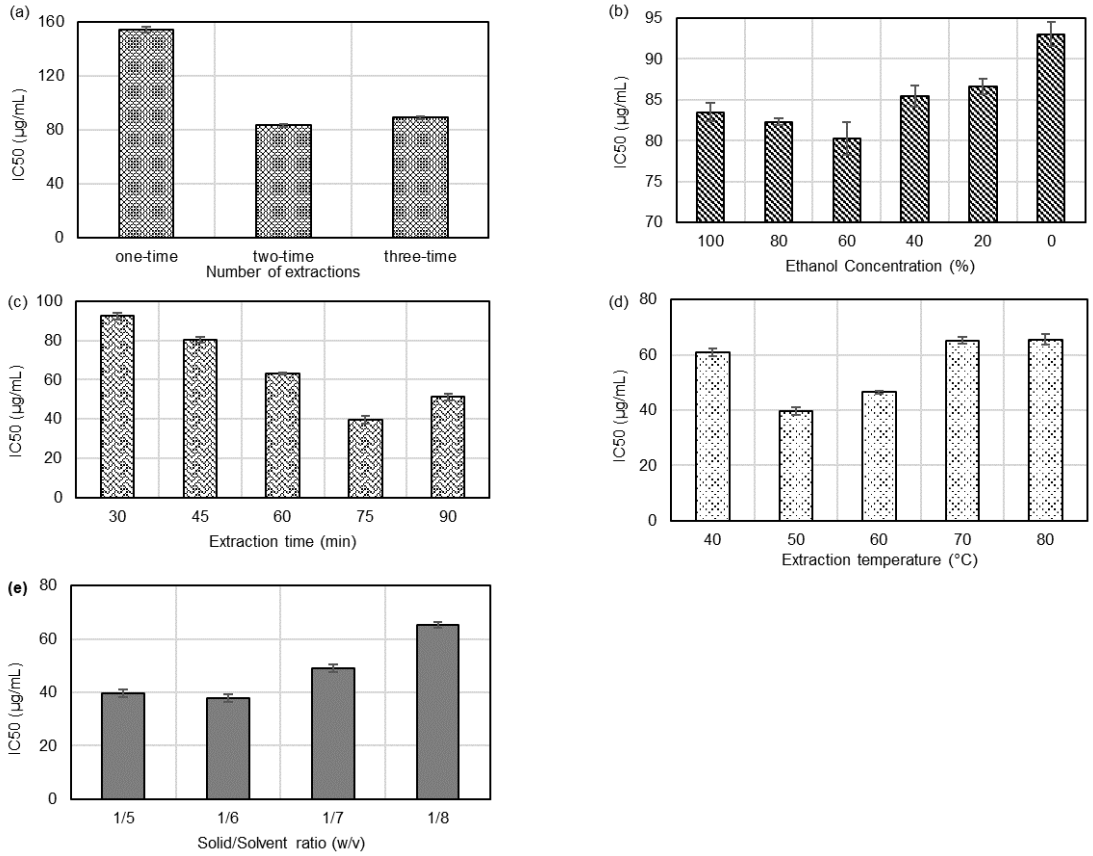

Figure 3: Antioxidant activity of extract from star anise at different conditions: (a) number of extraction, (b) ethanol concentration, (c) extraction time, (d) extraction temperature and (e) solid/solvent ratio.

It can be absolutely sighted in Figure 3b that extract with ethanol 60 % had the most excessive antioxidant activity (IC_{50} of 80.27 µg/mL). Antioxidant activity gradually increased with the concentration of ethanol from 0 % to 60 %, showing that various concentration of ethanol used exhibited different effect on changing the fluid polarity and had effect on the solubility enhancement of the antioxidant compounds. According Figure 3c, extract obtained in 75 min possessed the most significant antioxidant activity (IC_{50} of 39.7 µg/mL) and as twice as the extract in 30 min (IC_{50} of 92.39 µg/mL). The longer the extraction time is, the more antioxidant compounds were obtained. However, if the extraction time was extended to 90 min, there would be a significant decrease in antioxidant activity due to the long extraction time resulting in an increase in impurity, making reducing of antioxidant activity. It can be noted that extraction in 75 min was the most suitable result

for this study. From Figure 3d, antioxidant activity increased from 40 °C to 50 °C and the excellent performance was at 50 °C (IC_{50} of 39.7 µg/mL). This can be explained by the fact that under the influence of temperature, the mobility of molecules increased, the molecules in the mixture would move turbulently due to increasing the mass transfer dynamics, accelerating the diffusive process. The solvent would easily penetrate the material layer and increase the surface contact area between the material and the solvent. Temperature denatured and destroyed cell membranes through the formation of air bubbles, making the extraction process more convenient (Cracolice and Peters, 2009). In this study, the most excessive antioxidant activity was observed at the solid/solvent ratio of 1/6 (IC_{50} of 37.82 µg/mL, Figure 3e). When increasing the solid/solvent ratio from 1/5 to 1/6, antioxidant activity increased due to the mass transfer. The driving force during mass transfer was considered to be concentration gradient, which was greater when a higher solid-to-solvent ratio was used, resulting in an increase of the diffusion rate (Cacace and Mazza, 2003). However, the ratio increased to 1/7 and 1/8, antioxidant activity decreased significantly. It can be explained that the equilibrium achieved, the gradient difference in concentration between solute and solvent was no longer considerably, reducing the amount of extract, leading to reduce of antioxidant activity (Mohamed and Yong, 2008).

3.3 Compositions and bioactivities evaluation of star anise essential oil and extracts

Star anise essential oil after investigation combined 13 components. The fundamental components were anethole (94.75 %) and several other components with low content. Comparing to research of Gurdip et al. (2006), the composition of two essential oils were relatively similar, however, the number of components of India star anise essential oil was higher. This also showed the composition of components in essential oil varied widely due to areas of planting and harvesting.

The antioxidant activity of star anise extract was presented by IC_{50} of 36.75 ± 0.40 µg/mL, which was 2.78 times higher than that of the vitamin C (IC_{50} of 7.75 µg/mL). According Khalaf et al. (2008), IC_{50} values of many kinds of plant's extracts were higher than this value of the vitamin C, for example, IC_{50} values were in descending order of cardamom (681.5 ± 8.4 µg/mL), fenugreek (444.1 ± 5.5 µg/mL), black pepper (144.1 ± 2.2 µg/mL), ginger (65.1 ± 1.7 µg/mL), clove (9.9 ± 0.2 µg/mL). These IC_{50} values of these extracts were 76.57; 49.88; 16.19; 7.30 and 1.11 times higher than IC_{50} of the vitamin C (Khalaf et al., 2008). This evidenced that free radicals scavenging effect of plants were lower than that of vitamin C. The antioxidant of star anise extract was higher than another plant's extracts. IC_{50} value of the residue extract (162.21 ± 0.27 µg/mL) was 20.93 times higher than this value of the vitamin C, it means that antioxidant activity of residue extract was significant. According to results, IC_{50} of essential oil was 4816.17 ± 0.31 µg/mL, higher than steam distillation essential oil of Ming et al. (2013). Besides, essential oil of star anise showed lower antioxidant activity than its extract. It can be explained that bioactivities of star anise were principally in the extract. Based on the preliminary phytochemical screening and GC-MS results, the extract contained many active compounds as polyphenols, flavonoids while essential components of essential oil were anethole.

According to previous research, anethole possess the potential to inhibit protein denaturation and is effective in controlling nonimmune acute inflammation-related disease (Talita et al., 2012). It can be noted that abilities to inhibit of albumin denaturation of extract (IC_{20} of 122.42 ± 1.23 µg/mL) and residue extract (IC_{20} of 134.85 ± 1.27 µg/mL) were higher than its ability of aspirin (IC_{20} of 158.044 ± 1.54 µg/mL). This evidenced that anti-inflammatory of star anise extracts was significant for application. IC_{20} value of essential oil (IC_{20} of 935.45 ± 1.23 µg/mL) was more significant than extracts and 5.92 times higher than that of aspirin. It can be concluded that anti-inflammatory activity of star anise essential oil was unappreciated.

The survey conducted to examine antibacterial ability of essential oil, extract after investigation and residue extract, which was at concentration of 1,000 µg/mL. According to the results, the antibacterial ring of above objects did not appear, which means that essential oil and extract do not perform antibacterial activity at investigating concentration.

4. Conclusions

This critical study reported the results of distillation, extraction of essential oil and extract from star anise and evaluation bioactivities of these products. For the distillation process of essential oil, the optimized conditions to obtain the highest yield of essential oil (3.87 %, main component of anethole – 95 %) were raw material size of 8mm and no compress, solid/solvent ratio of 1/8 (w/v) at 130 °C during 3 to 4 h. For the extraction process, the optimized conditions were two-time extraction with ethanol 60 % and solid/solvent ratio of 1/6 during 75 min at 50 °C. The essential oil (1,000 µg/mL) did not possess anti-bacterial ability with studied bacteria. Star anise essential oil proves weak antioxidant ability, insignificant anti-inflammatory activity. Residue extract has high anti-oxidant activity and anti-inflammatory activity, so it can be applied to produce functional foods of native origins. Bioactivities of extract showed that applicability of star anise extract to antioxidant and anti-inflammatory products was effective. Besides, star anise can also be applied in the pharmaceutical field to

expand the investigations of antioxidant constituents present in star anise which are being not only able to protect the oil against oxidation but also to alleviate diseases by preventing oxidative deterioration.

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