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Extraction and Antioxidant Activity Test of Black Sumatran Incense

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Abstract. Benzoin absolute essential oil is a high-value oleoresin derived from the dried sap of the *Styrax benzoin* tree. One type of *Styrax benzoin* traded in Indonesia is black Sumatran incense. Reflux extraction method with ethanol is used to gain the benzoin absolute essential oil. The purpose of this study is to investigate the antioxidant activity of benzoin absolute essential oil produced using ethanol reflux extraction. Antioxidant test showed that black Sumatran Incense has active antioxidant properties with IC_{50} value is 90.03; the antioxidant activity shows potential alternative utilization of this essential oil industry, especially those derived from incense resin and its derivatives.

Keywords: antioxidant, essential oil, reflux, *Styrax benzoin*

INTRODUCTION

Benzoin balsam is a resin derived from the sap of trees in the family of *Styraceae*, this type of plant grows in many Asian countries such as Indonesia, Thailand, Laos and Vietnam [1]. In Indonesia, this plant generally grows endemic to the islands of Sumatra, Java, and Kalimantan. *Styrax benzoin* is a plant native to Indonesia [2]. The tree will not produce resin under normal conditions. The resin is produced and flows out as a response to a pathological incision made on the stem [3]. This sap is obtained from an incision or wound on a tree, which will dry out when exposed to air, the color of the sap can vary from white, yellow to blackish brown. In general, the sap will be taken after one month after the incision in the tree.

Benzoin balsam has been widely used since ancient times [4] by the Romans and Egyptians to treat respiratory infections. This is based on the therapeutic properties and pharmacological effects of the resin and is classified as disinfectant and expectorant [5]. In Asian countries, the smoke generated from burning benzoin gum is used as an antidote to evil spirits and diseases, this explains its use in various religious ritual ceremonies [6].

Today, the use of benzoin gum has been expanded not only to be used as fragrances and incense because of its fixative effects but also used as an antioxidant in the cosmetics industry and flavor enhancers in the food industry [7]. The therapeutic and pharmacological effects of disinfecting (anti-bacterial) and expectorants in benzoin gum are also used in the pharmaceutical industry. In fact, benzoin gum in the Asian region has been used in wound healing, erythema and cough [3].

In 1914, Reinitzer stated that Siam Benzoin contains coniferyl benzoate with a percentage of 75-80% [8]. Fifty-four years later, Schroeder's research stated that the components of Siam benzoin are cinnamyl cinnamate (0.56%), siaresinolik acid (6%), p-coumaryl benzoate (10-15%), acid benzoate (12%), and coniferyl benzoate (65-75%) [9]. Other studies have shown that cinnamic acid and cinnamic acid esters, especially cinnamyl cinnamate, coniferyl cinnamate and pinoresinol are constituent components of Sumatran benzoin [10]. However, traces of vanillin

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commonly found in Siam benzoin are also present in Sumatran benzoin, and hence both resins have a distinctive vanilla aroma [11].

Tchapla et al. (1999) stated that the content of styrene, benzaldehyde, cinnamic acid and cinnamic acid derivatives such as cinnamyl, ethyl, benzyl and phenyl propyl cinnamate in Sumatran Benzoin is greater than Siam benzoin [12]. The Pastorova study, reported that the aromatic composition of the ingredients contained in Siam benzoin and Sumatran benzoin not only depends on the geographical origin and the botanical conditions of the plant but also depends on the extraction process carried out [13].

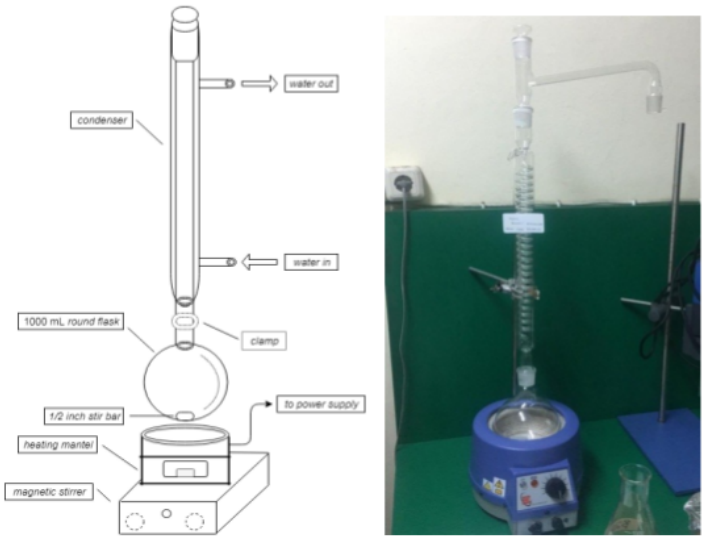
Other research results show the main aromatic compound contained in Siam and Sumatran resins is benzyl benzoate [1]. The main constituent that composes in both benzoin is benzyl benzoate (76.1-80.1%). While the differences in both are the presence of methyl benzoate (1.5%), benzoate acid (12.5%), and allyl benzoate (1.5%) in Siam benzoin. Whereas in Sumatra benzoin contains benzyl cinnamate (3.3%) and cinnamic acid (3.5%) [3,14].

Several studies on the extraction of Siam benzoin and Sumatra benzoin have been carried out by researchers in the world. These studies generally focus on identifying both aromatic and non-aromatic components that make up the two resins. On the other hand, unfortunately, food and beverage producers have been looking for more economical flavor enhancers, in addition to the high consumer demand for non-synthetic natural flavor-enhancing products [14].

The antioxidant activity is a very important characteristic of essential oil [15]. Due to the large need for benzoin absolute essential oil benefits and high economic importance, a special study is needed to calculate the antioxidant activity test of benzoin absolute essential oil which gains from the extraction process. This study examines the process of extracting absolute essential oil with the raw material of benzoin gum derived from *Styrax benzoin* by the ethanol reflux method. Benzoin's absolute essential oil has a higher economic value than benzoin gum which is directly sold in the market.

MATERIALS AND METHOD

The black Sumatran incense samples used in this study was bought from local market in North Sumatra. The solvents used in the optimization test were ethanol p.a (Merck) and methanol p.a (Merck), the material used for the antioxidant test was DPPH (*2,2-diphenyl-1-picrylhydrazyl*, Aldrich) and quercetin p.a (Merck) as a comparison.



(a) (b)

FIGURE 1. (a) Schematic Diagram of Reflux Extraction; (b) reflux equipment

After cleaning, as much as 40 g of dried gum *Styrax benzoin* is carefully weighed, then roughly ground using mortar. As can be seen in Figure 1, benzoin absolute essential oil was extracted using the reflux extraction at a constant temperature determined according to the boiling point of ethanol (solvent), which is 78-79 °C. Furthermore, 40 g of incense and 95% ethanol with mass ratio 1:5 were put into a 1000 mL round flask. After the extraction process, the solution extract was collected and separated from solid residue by vacuum filtration using 42-grade Whatman filter paper with a diameter of 125 mm. The extracted filtrate solution was evaporated using a vacuum rotary evaporator (Rotary Evaporator RV 8, Germany) at a temperature of 79 °C with a speed of 40 rpm to a pressure of 95-100 mbar. Then, the oleoresin obtained is weighed and calculated as a result of absolute essential oil. The obtained oleoresin is stored in an airtight bottle and stored at room temperature for further analysis.

The antioxidant activity test using DPPH was carried out based on the method carried out by Abe N et al [16]. The antioxidant activity test using DPPH was conducted to evaluate the antioxidant activity of benzoin essential oil produced during the experiment. A total of 10 mg of each extract from *Styrax benzoin* was dissolved in methanol p.a. with a concentration of 1000 µg/mL as the mother liquor. From the mother liquor, sample solutions were made in various concentrations, namely 50, 75 and 100 µg/mL. Then 0.2 mL of each sample made, put in a test tube, in each test tube added 3.8 mL DPPH then incubated at 37 °C for 30 minutes, then the absorption was measured at a wavelength of 517 nm. As a comparison used quercetin with concentration 2; 4; 6; 8 and 10 µg/mL. Each IC_{50} value is calculated using a regression equation [17].

RESULTS

Figure 2 showed the raw material and benzoin absolute essential which produced from ethanol reflux extraction. Beside yield, in this experiment also calculated % ethanol recovery and mass of residue from the extraction process, benzoin essential oil produced is 45.5 g with ethanol recovery 81.07% and mass of residue is 7.93 g.

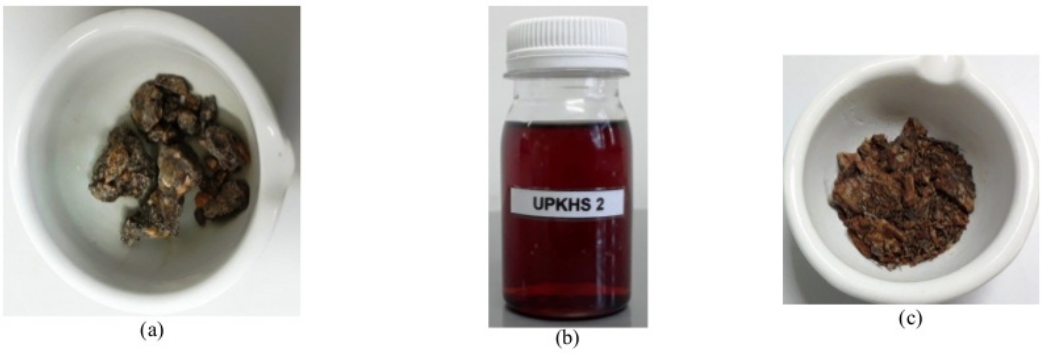


FIGURE 2. (a) Raw Material (black Sumatran incense); (b) benzoin absolute essential oil produced; (c) residue from ethanol reflux extraction

The antioxidant properties of a material can be indicated by the IC_{50} value as shown in Table 1.

TABLE 1. Correlation of IC_{50} values with Antioxidant Properties [18]

IC_{50} values	Intensity
<50 µg/mL	Highly active
50-100 µg/mL	Active
101-250 µg/mL	Moderate
250-500 µg/mL	Weak
>500 µg/mL	Inactive

Table 1 shows the level of antioxidant strength by the DPPH method. In this test method, the antioxidant intensity of *Styrax benzoin* extract was determined based on the calculation of the IC_{50} value. This value can be

defined as the concentration of the sample tested when it can reduce 50% of free radicals contained in DPPH. In this process, a reaction occurs between the samples to be tested for antioxidant activity with free radicals contained in DPPH as shown in Figure 3. Phenolic compounds in antioxidants will attract free radicals in DPPH.

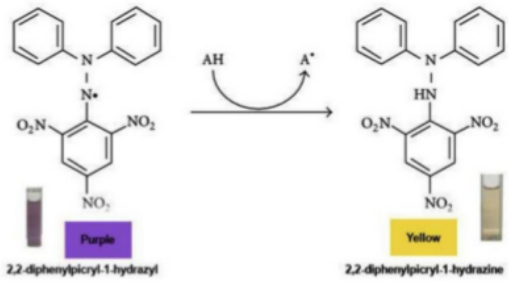


FIGURE 3. Schematic Diagram of the Reaction between Antioxidant Substances and DPPH [19]

An unpaired electron in the outer orbitals of a free radical can trigger a chain reaction. Therefore, free radicals are very reactive to cellular molecules. The binding process of electrons in cellular molecules and free radicals will produce new free radicals. Measurement of the value of the antioxidant activity is done by measuring the absorbance of *Styrax benzoin* extract sample solution using UV-Vis Spectrophotometry. The sample is incubated for 30 minutes before measurement, the aim is to provide sufficient time for the reaction between free radicals in the DPPH and the sample to be tested.

The results of measurements using UV-Vis Spectrophotometry showed a decrease in absorbance of DPPH caused by test samples at various concentrations. DPPH color change from purple to slightly yellowish after incubation for 30 minutes can be seen and observed. This process occurs because the hydrogen atom which is owned by the compound in the sample is donated to free radicals contained in DPPH. In this reaction, DPPH will be reduced to DPPH-H (1,1-diphenyl-2-picrylhydrazine). DPPH-H has more stable properties than DPPH. This measurement, as shown in Table 2 indicates that the absorbance value will be lower when the concentration of the sample used is higher.

Table 2 shown results for the antioxidant test in black Sumatran incense. Trial and error tests to obtain uptake at the maximum wavelength of black Sumatran incense were achieved at concentrations of 50, 75, and 100 ppm. From the results in Table 2, then a linear regression curve is made as shown in Figure 4.

TABLE 2. Antioxidant Activity Test of Benzoin Absolute Essential Oil from Black Sumatran Incense

Concentration (ppm)	Abs Sample	Abs Sample (*Mean*)	Abs Blank	% inhibition (%)
50	0.4455 0.4391 0.4337	0.4394	0.6786	35.24
75	0.3864 0.3728 0.3802	0.3798	0.6786	44.03
100	0.3079 0.3160 0.3146	0.3128	0.6786	53.9

The strong or weakness antioxidant properties of benzoin absolute essential oil are influenced by the molecular composition. The more phenolic compounds contained in essential oils, the stronger the antioxidant properties, phenolic compounds is a compound containing the -OH group which is bound to an aromatic rings with conjugated double bonds. The -OH groups found in phenolic compounds will stabilize unpaired electrons contained in DPPH which act as free radicals.

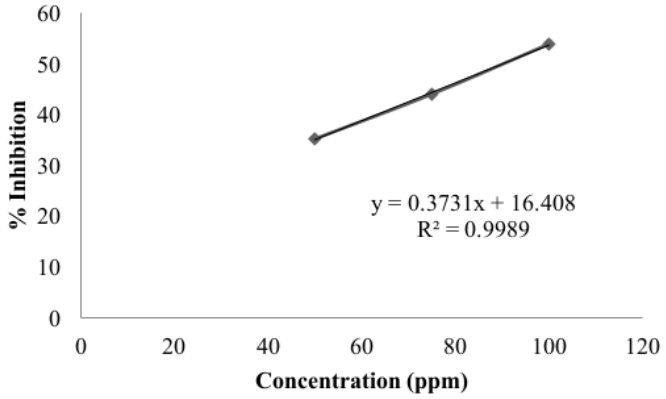


FIGURE 4. Linear Regression Calculation on Antioxidant Activity Test of Black Sumatran Incense

From the calculations made it is known that the linear equation obtained has a significant R square. From the calculations performed, the IC_{50} value obtained is 90.03. Based on this calculation, black Sumatran incense is classified as a compound with active antioxidant properties (IC_{50} value 50-100 µg/mL).

Compared with other studies, the antioxidant activity possessed by the black Sumatran incense is similar to the antioxidant activity possessed by Senggani leaves *(Melastoma candidum* D. Don), with an IC_{50} value of 65.65 µg/mL (IC_{50} value 50-100 µg/mL; Active Antioxidant Properties) [19]. The IC_{50} value possessed by black Sumatran incense is greater than the IC_{50} value possessed by *Sargassum serratifolium* extract with IC_{50} 35.1 ± 0.27 µg/mL [20] and smaller than IC_{50} value that is owned by *Jatropha curcas* L. leaves with IC_{50} value 314 ± 0.74 µg/mL [21]. These results indicate that the antioxidant activity possessed by black Sumatran incense resin is greater than *Jatropha curcas* L. and smaller than *Sargassum serratifolium* extract.

CONCLUSION

The antioxidant test results in the form of IC_{50} value for black Sumatran incense is 90.03; The antioxidant test results showed that black Sumatran incense has active antioxidant properties against DPPH (IC_{50} value 50-100 µg/mL).

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Conflicts of interest: None.

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