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Research Article

Cinnamic Acid in Frankincense Sap as a Criterion for Determining the Best Mother Plant for Vegetative Propagation of *Styrax benzoin* (Sumatra Benzoin) in Sumatra, Indonesia

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Cinnamic acid, contained in frankincense sap produced by *Styrax benzoin* (Sumatra benzoin tree), is an important compound that is used for various purposes, such as preservatives, fragrances, cosmetics, and pharmaceutical products. The production of frankincense sap as a forest product can be increased through the inclusion of *S. benzoin* seeds, which are propagated from the best mother plants. This study aims to use the content of cinnamic acid contained in the sap of *S. benzoin* as a criterion for determining the best mother plant for propagation of seeds. The research was conducted using healthy plants, taking sap samples, identifying and confirmation of cinnamic acid levels, and determining the best mother tree based on the content of cinnamic acid. The results of this study have identified six individual *S. benzoin* trees of very good quality based on their phenotypic advantages and the quantity of sap production. Isolation and identification of cinnamic acid from frankincense samples showed that the composition of cinnamic acid was high (12 to 21%). Three good quality *S. benzoin* trees, with high cinnamic acid composition, were SBN-7 (21%), SBN-3 (18%), and SBN-10 (17%). The SBN-7 tree was then chosen as the best *S. benzoin*, producing 2.70 kg year^{-1} sap, containing *cc.* 21% cinnamic acid. A selected mother plant will be used as a source of plant material for vegetative propagation to produce good quality seeds similar to the properties of the parent plant forforest conservation and to increase the production of nontimber forest raw materials for medicine and other purposes. The finding of this study is the first to use the composition of cinnamic acid as a criterion for determining the best mother plant. The composition of cinnamic acid in the sap is an important parameter in determining the superiority of *S. benzoin* plants.

1. Introduction

Styrax benzoin (Sumatra benzoin), as a woody forest plant, has high economic potential because it produces frankincense sap containing bioactive compounds that have potential as raw materials for medicine. The sap has been used for various purposes, such as religious ceremonies, medicinal ingredients in traditional and modern medicine, raw materials for cosmetics, and industries [1, 2]. Several important chemical compounds contained in frankincense sap include cinnamic acid, benzoic acid, styrene, vanillin, styracin, coniferyl benzoate, benziresinol, resinotannol, flavonoids, and several other bioactive compounds that still need to be elucidated [3, 4]. Cinnamic acid is one of the focal compounds contained in large quantities in the sap. It has biological activities such as being antibacterial, having anesthetic, anti-inflammatory, antispasmodic, antimutagenic, and fungicidal properties, and is a herbicide and tyrosinase enzyme inhibitor [5]. Our focal compound is very important and has high economic value because it is used for various commercial purposes, for example, as preservatives, fragrances in food, cosmetics, and pharmaceutical products.

The economic potential of *S. benzoin* has not been optimized in North Sumatra, Indonesia, because incense gum products are still only sold in the form of raw materials. The economic value of frankincense resin will be higher if the bioactive compounds contained are isolated into pure chemical compounds and used as materials for medicine and industry. Isolating cinnamic acid and other compounds from frankincense sap will increase the selling price of the sap, making Sumatra frankincense a reliable source of income for farmers around forest areas. Thus, the production of frankincense sap needs to be increased through the ecologically sensitive provision of good quality *S. benzoin* seeds that produce very good quality frankincense in large quantities.

Styrax benzoin grows naturally in the forests of North Sumatra. Frankincense sap is produced naturally from the injured bark of the tree, which is a leading commodity from North Sumatra, Indonesia [6, 7]. Frankincense products generate income to farmers living around forest areas in several districts, such as Toba, North Tapanuli, Humbang Hasundutan, and Pakpak Bharat. Some farmers (6.5%) depend on incense products tapped directly from the trees in the forest (Hotdi Berutu 2021: personal communication). The problem faced is that the production of the sap from *S. benzoin* trees varies, and as a result, the trees with low sap production tend to be neglected or cut down. Cutting down the tree reduces the population of *S. benzoin* and threatens forest sustainability [8, 9]. *Styrax benzoin* trees are commonly grown from seeds, which causes the production of frankincense sap to vary, even though they are located in adjacent forest areas. This fact results in low motivation of the community to cultivate *S. benzoin* because there is no guarantee that the tree will produce sap, while a very long time (c. 8 to 10 years) is required from germination of the tree until it can be tapped to produce the gum [10, 11]. To overcome this problem, it is necessary to make efforts to provide good quality *S. benzoin* seeds that produce trees with good quality sap in large quantities, namely through vegetative propagation. Providing superior *S. benzoin* seeds is a good strategy to conserve forests in North Sumatra, through optimizing farmer involvement in planting and caring for trees that are profitable for farmers [12, 13]. The availability of good quality seeds, with the potential for producing trees with high sap production and quality, will motivate farmers to plant and maintain *S. benzoin* in forest areas. This strategy will increase the population of frankincense trees and have prospects for expanding the production of nontimber forest products [14].

Conservation of medicinal forest plants is necessary to preserve forest and at the same time to increase the economic value of nontimber forest products [15, 16]. Various efforts have been made to preserve tropical forests such as reforestation, maintaining forest plant habitats, implementing selective-logging, reducing slash-and-planting, and conservation systems [17, 18]. The above-mentioned strategies require good quality seeds, which are propagated from trees either generatively or vegetatively. Vegetative propagation has the advantage of producing plants with traits similar to the mother plant [19, 20]. The selection of mother plants as a source of plant material in vegetative propagation is pivotal in the success of *S. benzoin* cultivation. The parameters for selecting mother plants that are commonly used are based on phenotypic advantages and plant productivity [21, 22]. Determination of the best mother plant based on phenotypic advantages does not necessarily result in plants with high productivity. It is desirable to obtain *S. benzoin* trees with a superior quantity of sap production and composition of bioactive compounds in the frankincense resin. Achieving these goals can be carried out through the isolation of cinnamic acid compounds contained in the frankincense sap to reveal the superiority of different *S. benzoin* trees. This study aimed to isolate cinnamic acid contained in the frankincense sap and use the composition as a criterion for selecting the best *S. benzoin* trees to be used as mother plants in vegetative propagation. In turn, this is expected to produce good quality seeds that will be planted in the forest as a strategy to conserve forest plants that produce medicinal raw materials.

2. Research Methods

2.1. Chemicals and Materials. The chemicals used in our study included NaCl, HCl, NaOH, HCl, diethyl ether, ammonium hydroxide (NH_4OH), $FeCl_3$, sodium sulfate (Na_2SO_4), and chloroform, all obtained from E-Merck and used without purification. The raw material the chemicals were applied to was frankincense crystals taken directly from selected good quality *S. benzoin* trees from forest areas in Laelange Namuseng village, Sitelu Tali Urang Julu district, Pakpak Bharat Regency, North Sumatra, Indonesia. The sampling location was at Laelange Namuseng village at longitude 98° 22′ 18.171″–98° 27′ 25.764″E and latitude 2° 29′ 54.200″–2° 33′ 14.251″N. Frankincense sap samples were taken during the harvest season and carried out once a year.

2.2. Instrumentation. The instruments used in the isolation, analysis, and identification of cinnamic acid were the Fourier transform infrared (FT-IR) Bruker spectrometer equipped with a Digitech detector (Shimadzu), gas chromatography-mass spectrometry (GC-MS 7890B with MassHunter GC-MS Acquisition Agilent Technologies, Inc.), and analytical balance. Chemical glassware included glass beakers, volumetric pipettes, Erlenmeyer flasks, separating funnels, volumetric flasks, socklets, and pestles and mortars.

2.3. Research Procedure

2.3.1. Selection of Styrax benzoin Mother Plant. Identification and selection of *S. benzoin* trees were carried out in 2020–2021, directly in pristine forest areas with dense tree populations through a field survey, which was directly guided by experienced farmers, and by using a list of good quality mother plant parameters described in the study [6]. The superiority of the mother plant based on the phenotype was recorded by using a checklist algorithm for assessing the quality of woody forest trees. The phenotypic components assessed included plant fertility, leaf density calculated by using the Leaf Area Index (LAI), fruit production was measured based on volume per tree (kg/tree), and plant health determined based on the findings of symptoms on plant parts. Other parameters were also used, such as plant density based on the coverage of the shaded area, estimated tree height using direct girth measurement procedures, measurement of plant stem diameter, and estimated age of mature plants based on experienced informants [23, 24]. All selected *S. benzoin* trees were labelled. Plant productivity was recorded based on the quantity of sap production during the 2020 harvest period.

Identification and labeling of very good quality *S. benzoin* trees were carried out, spreading over the largest forest area that had the largest population of trees in Laelange Namuseng village [6, 25]. All of the selected *S. benzoin* trees were productive, having a high quantity of sap and a superior quality of frankincense sap. A total of six trees of very good quality were selected from 12 trees, which were sorted according to the quantity of the highest sap production per harvest period. All the selected *S. benzoin* trees were used as a source of raw frankincense sap for the purposes of isolation of cinnamic acid. The descriptions of selected mother plants are summarized in Table 1.

The quantity of sap production per harvest period was the main criterion used to select good quality mother plants.

2.3.2. Sample Preparation. The samples used in this study were frankincense saps that were taken from traditional tapping directly from selected good quality *S. benzoin* trees. The frankincense crystal product was cleaned of bark impurities that are still attached to the sap, dried and crushed, then placed in a desiccator to remove moisture, and then used as raw material for the isolation of cinnamic acid.

2.3.3. Isolation and Identification of Cinnamic Acid Compounds. Isolation and identification of cinnamic acid were carried out by an extracting method using the organic solvents mentioned above. Five grams of frankincense powder were placed in an Erlemeyer flask, then 50 mL of solution (KOH, 0.5 M in ethanol) was added, refluxed for one hour, and evaporated to remove the ethanol. Then, 80 ml of distilled water and 75 ml of 3% $MgSO_4$ were added and filtered. The residue was then washed using hot distilled water followed by ethanol. The filtrate was collected and acidified with 4M HCl, and extracted three times using ether (a total of 100 mL), followed by extraction three times using 55% $NaHCO_3$ (a total of 120 mL). The collected extract was then acidified again using 4 M HCl, followed by extraction three times using chloroform (total 120 mL). The chloroform filtrate was then evaporated to produce a balsamic compound. Hot distilled water was added to the compound, which was then filtered while hot, and the filtrate was then cooled on pure ice to produce crystals. The product was then washed with cold distilled water, filtered, and dried in an oven at 50°C to produce the compound cinnamic acid. Cinnamic acid was then analyzed by titration, followed by identification using FT-IR (Fourier-transform infrared spectroscopy) and analyzed by GC-MS using column DB-225, carrier gas helium (4 ml/min.), injector temperature of 220°C, column temperature of 190°C, and detector temperature of 230°C.

3. Results

3.1. Frankincense Sap from Styrax benzoin Mother Plants. The habits of *S. benzoin* and the frankincense sap are shown in Figure 1. A mature, healthy, and tapped *S. benzoin* tree grows in the forest naturally (Figure 1(a)) and was harvested to produce frankincense sap (Figure 1(b)). The sap was then dried and cleaned, producing white frankincense crystals, which is categorised as a "super quality" sap (Figure 1(c)). The sap quality determines the market price for frankincense in the local market of North Sumatra, Indonesia. The quality of frankincense sap, from the highest to the lowest in order, is as follows: (1) super quality sap in the form of large clean white crystals (*Sidungkapi*), (2) medium quality sap in the form of white and yellow medium-sized crystals (*Jurur* or *Barbar*), and (3) low quality sap with small crystal sizes with a dirty texture (*Tahir/Kikisan*). Very good quality frankincense sap from each tree was crushed and used as raw material for the isolation of cinnamic acid (Figure 1(d)).

3.2. Cinnamic Acid Isolation, Identification, and Confirmation. Isolated pure cinnamic acid was produced from raw frankincense sap material by maceration and extraction methods. Qualitative analysis confirmed that the purity level of cinnamic acid was high (86–93% *w*/*w*). The physical and chemical properties of the extracted cinnamic acid compounds are summarized in Table 2.

3.3. Identification and Confirmation of Cinnamic Acid. The presence of cinnamic acid in isolates was confirmed through functional group markers and chemical bonds in the target compound, as shown in Figure 2. These were identified from the analysis results, such as the absorption in the FT-IR spectrum, the main fraction of molecular weight as shown in the MS spectra, and the cinnamic acid peak obtained in the GC chromatogram. The data from the analysis were compared with a standard pure cinnamic acid. The FT-IR spectra and chromatogram GC of the target compound showed the same pattern as standard cinnamic acid in all the sap samples obtained from six *S. benzoin* trees (Figure 2(a)). The data in the FT-IR spectrum confirmed the main functional groups and chemical bonds such as vibrations of O-H groups, aromatic C-H groups, and C=O groups, which were observed in the target samples and in the cinnamic acid standard. The absorbsions in the FT-IR spectrum successively were observed in the wavelength region of 3290.07 cm^{-1} as OH stretching vibrations for the hydroxyl group, 1604.77 cm^{-1} for conjugated C=O (carbonyl), 1511.48 cm^{-1} as the vibration of the C=C alkene group, 2929.60 cm^{-1} as the presence of aromatic CH, 1167.68 cm^{-1} as CO, and the 700–800 cm^{-1} region, indicating that the benzene ring binds one substituent (monosubstituted benzene). Furthermore, the confirmation of cinnamic acid in the sample was identified from the peak

Table 1: Phenotypic characteristics of selected *Styrax benzoin* (Sumatra benzoin) trees used in the isolation of cinnamic acid.

No.	Plants parameters	Mean ± SD ($n = 6$)
1	Phenotypic characteristics through visual assessment parameters (fertility, leaf density, fruit production, and health level)*	4.00 ± 0.00
2	Coverage of shade area (m^2)	117.30 ± 30.32
3	Height of mature trees (m)	12.80 ± 1.92
4	Plant stem diameter measured on circumference of the stem (cm)	26.67 ± 6.06
5	Age of mature plant (years)	11.83 ± 2.04

*The Likert scale criteria are as follows: (4) very good, (3) good, (2) not good, and (1) bad.


(a)

(b)

(c)

(d)

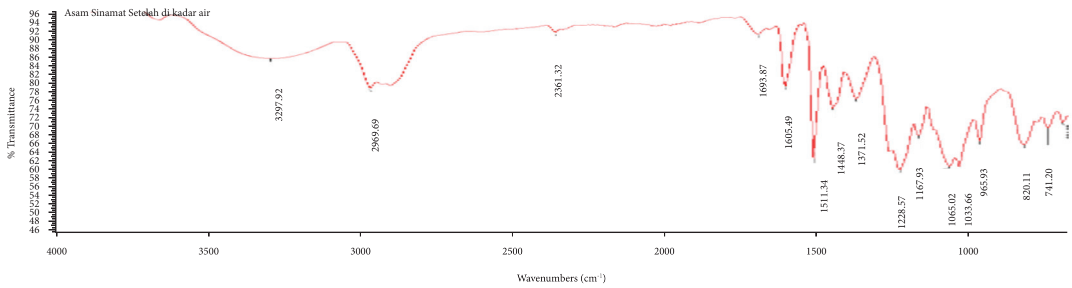
Figure 1: A selected mother tree of *Styrax benzoin* and raw frankincense sap used for the isolation of cinnamic acid: (a) good quality sap producing mother tree, (b) tapped frankincense sap attached to the bark, (c) crystal super-quality frankincense sap, and (d) raw frankincense powder material used for isolation of cinnamic acid.

Table 2: Physical and chemical properties of cinnamic acid isolated from frankincense sap samples.

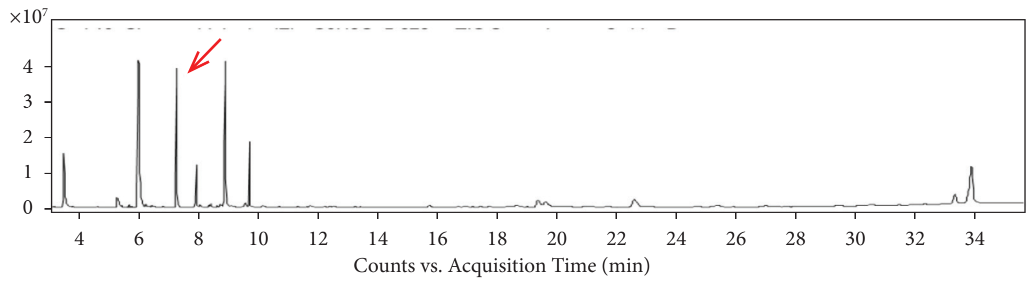
Description of frankincense sap	Purity (%)	Cinnamic acid	
		Physical properties	Chemical properties
Crystal gum, pure white color, has a fragrant smell.	86–93	Amorphous crystal, semisolid soft texture (balsamate), yellowish white color, melting point of 134°C, boiling point of 300°C.	Soluble in organic solvents (chloroform, ether, ethanol/methanol) and hot distilled water. It has a distinctive smell.

indicated by the arrow in the chromatogram GC (Figure 2(b)), and the target analyte of cinnamic acid was confirmed from the fragmentation of the main molecular weight in the MS spectra (Figure 2(c)). All the analytical data confirmed the presence of cinnamic acid as one of the main products in the frankincense gum samples.

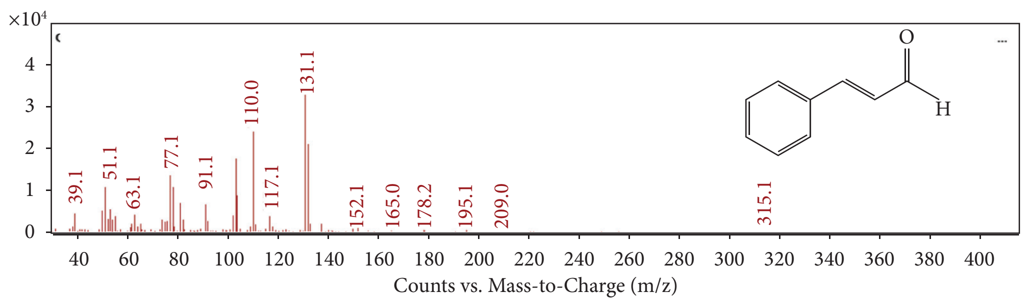
3.4. Selection of Mother Plants Based on Cinnamic Acid Content. The quality of *S. benzoin* trees, based on the production of frankincense sap, was determined. The trees were identified, the quantity of frankincense sap production was recorded, the quality of the sap was assessed, and the content of cinnamic acid in the sap was obtained, as summarized in Table 3.



(a)



(b)



(c)

FIGURE 2: Analysis and characterization of cinnamic acid in the sample: (a) FT-IR spectra, (b) chromatogram GC, with cinnamic acid indicated by the arrow at RT 7.82 minutes, and (c) MS spectra.

TABLE 3: Quantity of frankincense sap produced and composition of cinnamic acid in the sap of selected *Styrax benzoin* trees.

No.	Identity of tree	Production of sap (kg/year)*	Quality of sap**	Cinnamic acid ($n = 3$)	
				Sample (mg/g) (mean ± SD)***	(% w/w)
1	SBN-1	2.70	Super (*Sidungkapi*)	12.20 ± 2.07	12
2	SBN-3	2.70	Super (*Sidungkapi*)	17.63 ± 2.93	18
3	SBN-4	2.60	Super (*Sidungkapi*)	14.61 ± 2.87	15
4	SBN-6	2.65	Super (*Sidungkapi*)	13.42 ± 2.36	13
5	SBN-7	2.70	Super (*Sidungkapi*)	20.70 ± 2.11	21
6	SBN-10	2.60	Super (*Sidungkapi*)	17.47 ± 1.53	17
	Mean value	2.66 ± 0.05	Super quality	16.01 ± 2.31	16

*Production of sap from tapping per tree (kg/year) in each harvest period. **Quality category of frankincense sap is assessed based on the crystal size, shape, color, texture, and the selling price in traditional markets in North Sumatra Indonesia. ***Content of cinnamic acid (mg) contained in a gram of frankincense sap.

A total of six trees were selected as quality *S. benzoin* based on the production quantity of the sap, which had a mean and standard deviation of 2.66 ± 0.05 kg/year per tree. All types of sap products are of "super" quality (*Sidungkapi*), which means they are in the form of large, clean white crystals. The results of the analysis also confirmed the presence of a high composition (16%) of cinnamic acid contained in the sap, with an average yield of 16.01 ± 2.31 mg cinnamic acid per gram of sample. These values will be used as basis to select the best mother plant as a source of plant material for vegetative propagation in further research.

Quantification of compounds isolated from frankincense sap samples was carried out. The composition of cinnamic acid in the sap samples was in the range of 12 to 21% w/w

(Table 3). Three good quality *S. benzoin* trees with high cinnamic acid composition, respectively, were SBN-7 (21%), SBN-3 (18%), and SBN-10 (17%). The composition of cinnamic acid in the sap is an important parameter in determining the superiority of the *S. benzoin* plant. The tree then chosen as the best *S. benzoin*, which produced 2.70 kg/year of sap and contained 21% cinnamic acid, is SBN-7. The tree was then used as a mother plant for vegetative propagation, namely, as a source of tissue explants for micropropagation techniques and plant material for propagation by cuttings. Both propagation techniques (*in vitro* and cuttings) are intended to be applied in the provision of quality *S. benzoin* seeds in this study. This strategy can be implemented to select mother plants for vegetative propagation.

4. Discussion

Styrax benzoin, as a woody forest plant, is proven as a producer of bioactive compounds for medicinal raw materials [26, 27]. The content of bioactive compounds, such as cinnamic acid, ensures that *S. benzoin* has high economic value, which shows the importance of conserving forests [28, 29]. Providing good quality *S. Benzoin* seeds is an obstacle in conserving forests in North Sumatra, Indonesia. Vegetative propagation through *in vitro* techniques and cuttings can be done to provide high volumes of seeds with the same characteristics as the parent plant. Thus, the strategy for selecting mother plants will determine the success of vegetative propagation [30, 31]. The selection of good quality *S. benzoin* trees was carried out based on the production of frankincense sap. However, this strategy was not appropriate to determine the best mother plant because the high quantities of frankincense sap produced often failed to contain high concentrations of cinnamic acid, as shown in Table 3. Thus, determining the mother plant based on the composition of cinnamic acid in the sap becomes an important strategy in the selection of the best mother plant. Solvent extraction was successfully carried out to isolate cinnamic acid directly from the raw material of sap to produce pure cinnamic acid. The composition of cinnamic acid was then able to determine the superiority of individual *S. benzoin* trees. The content of bioactive compounds reflects the importance of medicinal plants, including *S. benzoin* [32, 33]. Our study has succeeded in selecting the best tree, namely, *S. benzoin* SBN-7, with a high quantity of frankincense resin production, accompanied by the highest cinnamic acid composition. The best trees are most suitable to be used as a source of plant material in vegetative propagation, including explant sources for *in vitro* propagation and plant sources for cuttings [34, 35].

This study is the first to use cinnamic acid content to select the best *S. benzoin* mother plant. The success of determining the best parent plant has several advantages, including the availability of sources of plant material to be used in vegetative propagation. This will also enable to increase nontimber forest productivity because the trees produce very good quality frankincense sap. Moreover, the strategy will also help in realizing forest sustainability because plants producing superior bioactive compounds will be well maintained by the community. Planting forests with very good quality saplings in the long term will provide benefits in improving the income of people living around forest areas [36, 37]. Sustainable forest conservation, therefore, will be possible in North Sumatra because farmers in the forest fringes will be motivated to plant *S. benzoin*, take care of the plants in forest areas so that they are free from logging, and make forests the main source of income for the farmers [38, 39]. The findings of this study will also help to develop strategies for preserving medicinal plants that have a high economic impact in forest areas. The model can be used as a reference in selecting the best mother plant to be used as a source of plant material for vegetative propagation. This strategy is appropriate to provide superior planting stock needed for community forests and industrial forests as an effort to increase the production of bioactive compounds as nontimber forest products.

5. Conclusions

Styrax benzoin, as a woody forest plant that produces sap containing bioactive compounds, needs to be preserved because it has high economic value. Preservation is carried out through the provision of very good quality seeds that are propagated from the best mother plants that have the production of large quantities of super quality sap. Identification of *S. benzoin* was carried out in forest areas based on phenotypic advantages and the quantity of frankincense sap production. The sap samples were taken directly from the mother plant tree and were used as raw material for the isolation of cinnamic acid. Analysis results confirmed the composition of cinnamic acid compounds with a high level of purity (86–93%). The content of cinnamic acid in the sap was high (12–21%), which has high potential as a source of raw materials for pharmaceutical and industrial purposes. The composition of cinnamic acid in the sap was used as a criterion to determine the best *S. benzoin* tree. Three of the very good quality Sumatra benzoin trees are SBN-7 (21% cinnamic acid), SBN-3 (18%), and SBN-10 (17%). Sumatra benzoin SBN-7 was the best, with a high productivity of 2.70 kg/year sap and a composition of 21% cinnamic acid. The selected SBN-7 tree was used as the mother plant for vegetative propagation in a follow-up study through in vitro and cutting techniques to provide the very good quality seeds needed to germinate seedlings for planting community forests and industrial forests. The strategy of using the content of bioactive compounds in plant products found in this study is the first in determining the best mother plant. This strategy can be an alternative in increasing the production of bioactive compounds as valuable nontimber forest products.

Data Availability

The data are available in the laboratory at Universitas Sumatera Utara and Universitas Negeri Medan.

Conflicts of Interest

The authors declare no conflicts of interest.

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