Inter-tree Variation in Chemical Components of North Sumatra Benzoin Gum (*Styrax* sp.)

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Abstract

Benzoin gum, which is known as Sumatra benzoin (*Styrax* sp), is widely used as an ingredient in the incense. Although these resins are widely used as flavours and fragrances, no studies have been made on inter-tree variation of its chemical composition. Therefore, benzoin gum samples were tapped from the 30 individual trees (age 10 years, 9~35 cm in diameter) grown in the community forest of Polung, Humbang Hasundutan Regency, North Sumatra. The gum chemical components were analyzed by GC-MS and identified by comparing the fragmentation pattern with the standard components and literature studies. It was found that the major compounds detected by GC-MS were cinnamic acid (51.48%), cinnamyl cinnamate (62.56%), benzoic acid (1.94%), chavicol (5.18%), benzyl cinnamic acid (7.8%), atropic acid (9.84%), and vanillin (1.47%). Two main constituents that were always detected from 30 benzoin trees were chavicol and cinnamic acid, followed by cinnamyl cinnamate (28 trees) and benzoic acid (14 trees). By cluster analysis, 30 samples of benzoin gum can be classified into clusters I - III based on the average chemical components. Cluster I consisted of 7 individual trees with a higher percentage of benzoic acid and chavicol but lower concentration of cinnamic acid compared to other clusters. Cluster II consisted of 18 individual trees with a high percentage of cinnamyl cinnamate whereas clusters III consisted of 5 individual trees characterized with a high percentage of cinnamic acid. By Pearson correlation, it was observed that no significant correlation between the values of diameter and the amount of chemical components of benzoin gum.

Keywords: benzoin gum, chemical components, tree diameter, cinnamyl cinnamate, cluster analysis.

Introduction

Benzoin gum or 'kemenyan' is one of the potential non-timber forest products that have been related to social, cultural and economic values. In Indonesia, benzoin trees grow in natural forests and mostly found in six regencies of North Sumatra (North Tapanuli, South Tapanuli, Humbang Hasundutan, Papak Bharat, Toba Samosir, and Dairi Regencies). There are 4 types of gum benzoin, i.e. toba (*Styrax paralleloneurum*), durame (*Styrax benzoin*), bulu (*Styrax benzoin* var. hiliferum), and siam (*Styrax tonkinensis*). Sumatra benzoin gum that are commercially available are mostly from *S. paralleloneurum,* P. and *S. benzoin,* Dryand. (Kashio and Johnson 2001). Sumatra benzoin is mainly used for incense purpose. *S. benzoin* has been traditionally utilized for the treatment of skin diseases, arthritis, wounds, muscle pain, anxiety, and nervous disorders.

Previous research confirmed that the main chemical compound found in Sumatra benzoin gum is cinnamic acid (Waluyo *et al.* 2006). The cinnamic acid content indicates the level of purity of the benzoin gum. In addition, benzoin gum contains several other compounds such as styrol, vanillin, styracin, coniferyl benzoate, coniferyl cinnamate, benzoeresinol resin, and suma resinotannol (Waluyo and Setiawan 2007). Furthermore, the volatile content of benzoin gum and research on the chemical composition of secondary products of benzoin gum have been discussed in many studies (Fernandez *et al.* 2003; Hovaneissian *et al.* 2006; Modugno *et al.* 2006; and Filippi *et al.* 2009). However, no studies so far have been conducted to explore the inter-tree variation of gum in any Styrax species.

The objective of this study was to determine and to cluster the chemical composition of benzoin gum from 30 trees grown in Humbang Hasundutan Regency, which has long been one of the centers of benzoin gum production. Another objective was to determine the relationship between tree diameter and the percentage of chemical components of benzoin gum.

Materials and Methods

Sample Preparation

Benzoin gum (*Styrax* sp*)* samples were obtained from 30 individual benzoin trees from the Pollung community forest (*ca.* 4 hectares), Humbang Hasundutan Regency, North Sumatra Province. The selected benzoin tree stands (10 years) were at altitude of 1000~1500 m asl. The diameter selected trees ranged from 9-35 cm and previously has never been tapped. The benzoin gum tapping was done at a diameter at breast height using a straight line wound method (vertically) along 3~4 cm from the bark with a depth of wound toward the sapwood of the benzoin tree. The wounds on the benzoin tree were covered and the tree was left for a month to be harvested for the exudate. The harvested benzoin gum was stored in airtight plastic storage. Due to the small amount and wide variation between trees, the gum production in each individual tree was not measured.

Chemical Component Analysis

Benzoin gum (10 mg) was dissolved in benzene (2.5 mg/ml) for 30 minutes. The dissolved benzoin gum was filtered using filter paper and put into the injection vial. Benzoin gum extraction was analyzed for its extractive components using GC-MS (Shimadzu QP-2010) by direct injection. The gas chromatograph temperature conditions used were initial temperature of 80°C, 2 minutes of isotherm at 10°-200°C, with 4 minutes of isotherm at 6°-280°C, with 40 minutes isotherm. The injector temperature was set at 200°C. Helium (He) was used as the carrier gas at a constant flow of 1.2 ml/min. Chemical component identification was based on comparisons between standard components, references (Faust 1992; Bhone *et al.* 2014), and comparison of the sample mass spectrum with spectrum in the NIST Mass Spectral Database in GC-MS analysis. The standard component used was a synthesized cinnamic acid (Faculty of Math and Natural Sciences, Universitas Gadjah Mada). The quantification of each component was calculated by peak relative method.

Statistical Analysis

SPSS version 22 was used for cluster analysis and Pearson analysis. For cluster analysis, selected agglomerative hierarchical clustering (AHC) using the average relationship with the size of the squared Euclidean distance (the root of the standard deviation of each variable) was applied to examine the relationship between populations and their chemical components.

Results and Discussion

Extractive Component Analysis

Figure 1 shows the GC-MS chromatogram from benzoin gum dissolved using benzene. Nine identified components were cinnamic acid, benzoic acid, chavicol, benzyl cinnamate, cinnamyl cinnamate, atropic acid, vanillin, 4-vinylbenzoic acid, and styrene. The two components with the highest peaks were seen at peak 5 (cinnamic acid) and peak 9 (cinnamyl cinnamate). The fragmentation pattern from mass spectrophotometer is presented in Table 1. The comparison of the fragmentation patterns was conducted by comparing the peak ion fragmentation (Table 1).

The peak number 5 (Figure 1) had an identical peak pattern with standard component of cinnamic acid based on the results of the fragmentation pattern. The identification of other components of benzoin gum was conducted by comparing the fragmentation pattern of the benzoin gum with other studies. The mass spectrum of peak number 1 was compared with the spectrum in the earlier report (Bhone *et al.* 2014) and gave the similar results for styrene compounds, whereas peak number 2 gave the same results for benzoic acid compounds after being compared with a study conducted by Faust (1992). By the same method, the mass spectrum of peak number 4 was vanillin (Srivastava *et al.* 2010). The identification of other components of benzoin gum was done by comparing the fragmentation pattern with the NIST and NCBI libraries. The mass spectra of peaks number 3, 6, 7, 8, and 9 were assigned to chavicol, atropic acid, 4-vinylbenzoic acid, benzyl cinnamate, and for cinnamyl cinnamate compounds, respectively.

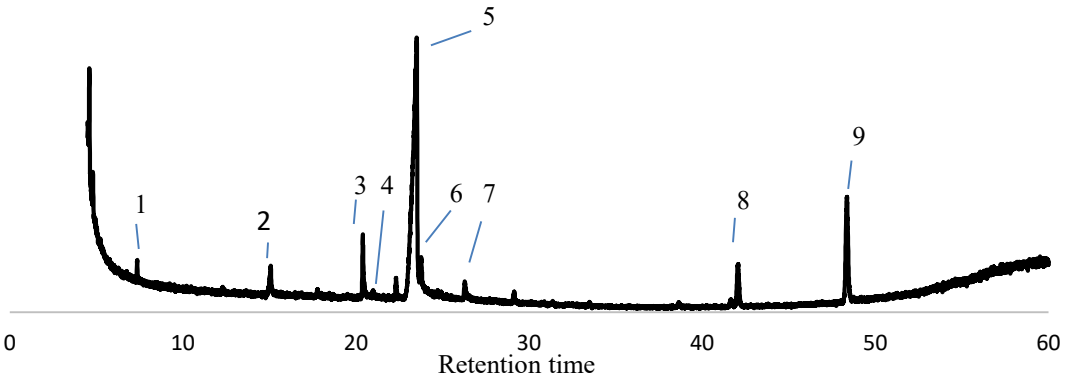

Figure 1. The spectrum of benzoin gum chromatograph; peak 1 (R_t 7.24) = styrene; peak 2 (R_t 15.27) = benzoic acid; peak 3 (R_t 20.77) = chavicol; peak 4 (R_t 22.45) = vanillin; peak 5 (R_t 24.09) = cinnamic acid; peak 6 (R_t 24.10) = atropic acid; peak 7 (R_t 24.65) = 4-vinylbenzoic acid; peak 8 (R_t 43.17) = benzyl cinnamate; peak 9 (R_t 49.41) = cinnamyl cinnamate; and R_t: Retention time (minutes).

Table 1. Peak Ion fragmentation of benzoin gum samples

Peak	Compound	R_T	[M+]	Peak
1	Styrene	7.23	104(100)	**104(100)**. 103(40.2). 92(1). 78(40.3). 63(7). 51(31.8)
2	Benzoic acid	15.27	122(81.8)	122(81.8). **105(100)**. 94(5). 77(48.3) . 65(3.3). 51(19.4)
3	Chavicol	20.80	134(100)	**134(100)**. 115(11.4). 107(26.8). 91(12.10). 77(20.6). 66(8.2). 51(10.2). 39(11)
4	Vanillin	22.45	152(88)	152(88). **151(100)**. 137(12.3). 123(21.8). 109(16.7). 81(38.6). 65(13.3). 53(25.7)
5	Cinnamic acid	24.09	148(71.4)	148(71.4). **147(100)**. 131(19.3). 120(5.1). 103(50.1). 91(19.9). 77(34.3). 63(5.7). 51(26)
6	Atropic acid	24.10	148(51.5)	148(51.5). 131(21.6). 121(17.7). **103(100)**. 91(35.8). 75(52.6). 63(29.9). 51(64.8). 37(12.7)
7	4-Vinylbenzoic acid	24.65	148(100)	**148(100)**. 131(13.6). 118(3.2). 103(38.6). 93(5.8). 78(19.9). 63(17.3). 51(13.7)
8	Benzyl cinnamate	43.17	238(16.4)	238(16.4). 192(62). 178(8.5). 147(7.6). 131(91.9). 115(15.9). 103(41.3). **91(100)**. 77(36.9). 65(16.5). 51(16.2)
9	Cinnamyl cinnamate	49.45	264(3.5)	264(3.5). 219(14.1). **131(100)**. 117(28.7). 115(30.8). 103(23.3). 91(10.8). 77(15.4). 63(1.9). 51(5.9)

Description: R_t = Retention time (minutes); [M+] = Molecular ion; *bold = base peak.

Table 2. The amount of chemical compounds (%) of benzoin gum (*Styrax* sp.)

Compound	Retention ¯ime (minute)	SI (%)	Minimum	Maximum	Mean	Standard Deviation	Tree Diameter [a] (cm)	Number of tree [b]
Styrene	7.24	92	tr	4.29	0.22	0.82	13.69	3
Benzoic acid	15.27	89	tr	1.94	0.39	0.54	18.79	14
Chavicol	20.77	91	1.61	5.18	3.19	0.98	15.61	30
Vanillin	22.45	90	tr	1.47	0.20	0.46	28.66	5
Cinnamic acid	24.09	97	4.46	51.43	21.68	13.15	10.19	30
Atropic acid	24.10	60	tr	9.84	0.45	1.82	15.61	4
4-Vinylbenzoic acid	24.65	62	tr	5.76	0.58	1.42	13.38	6
Benzyl cinnamate	43.17	91	tr	7.80	0.90	1.82	28.34	9
Cinnamyl cinnamate	49.41	94	tr	62.56	26.42	18.83	20.70	28

Note : SI = Similarity Index (%); n = 30 tree ; a = tree diameters with maximum value of the detected compounds, [b] = number of tree the detected compound; *tr= trace (< 0.1 %)

Table 2 presents the summary from the analysis of benzoin gum using GC-MS. Two chemical components of benzoin gum that showed the highest concentration (51.43%) were cinnamic acid (ret. time of 24.09 min.) and cinnamyl cinnamate (62.56%, ret. time of 49.41 min.). Vanillin, styrene, benzoic acid, cinnamic acid, cinnamyl cinnamate, and benzyl cinnamate were previously detected in Sumatra benzoin by HPLC-PAD and GC-MS (Hovaneissian *et al.* 2008) and by HPLC-frit FAB-MS and GC-MS investigations (Pastorova *et al.* 1997). Chavicol, atropic acid, and 4-vinylbenzoic acid were not detected in the earlier reports. The low values of similar index for atropic acid and 4-vinylbenzoic acid should be noticed. Isolation and identification of those peaks in the next works should be conducted to confirm their presences.

No species identification was conducted in this experiment. The number of tree was presented to find out the components distribution regardless the species. Two main components that always detected from 30 benzoin trees are chavicol and cinnamic acid, followed by cinnamyl cinnamate (28 trees) and benzoic acid (14 trees). Chavicol, styrene, and vanillin are from simple neutral aromatic groups, whereas atropic acid, cinnamic acid, benzoic acid, and 4-vinylbenzoic acid are from aromatic carboxylic acids. The esters detected were cinnamyl cinnamate, and benzyl cinnamate.

Cluster and Correlation Analysis

Cluster analysis was applied to all individual data by using a hierarchical method. This method compares the Euclidean distance between individuals as their similarity index. Then, the grouping was performed according to the weighted average linkage method. Cluster analysis was carried out by comparing the 4 main components of benzoin gum with a frequency of more than 10 in each sample of benzoin gum. These components are cinnamic acid, benzoic acid, chavicol and cinnamyl cinnamate. The division of benzoin gum clusters is based on its constituent chemical components. The chemical structures of those components are presented in Figure 2.

Previous report (Waluyo and Setiawan 2007) quantified the cinnamic acid (25~33%) by chemical reaction in Sumatra benzoin from six class qualities. Pastorova et al. (1997) quantified cinnamyl cinnamate (8~14%), methyl cinnamate (10~17%), cinnamic acid (4~7%), and benzyl cinnamate (2~4%) in *S. benzoin* by spectroscopic works. The chemical

components detected in more than 10 tree samples were classified into the major components (cinnamic acid, benzoic acid, chavicol, and cinnamyl cinnamate). The chemical components detected in less than 10 tree samples were classified into minor components (4-vinylbenzoic acid, atropic acid, vanillin, benzyl cinnamate and styrene).

The results of the cluster analysis are in the form of dendrograms or tree diagrams which are shown in Figure 3. Cluster I, II, and III consisted of 7, 18, and 5 individual trees, respectively. Table 3 shows the average percentage of the relative content of chemical components in each cluster. Cluster I had a medium cinnamic acid (23.30%) with a low percentage of cinnamyl cinnamate (9.70%). Cluster II had a low cinnamic acid (14.50%) with a high cinnamyl cinnamate (36.43%). Cluster III had a high percentage of cinnamic acid (45.25%) with a medium percentage cinnamyl cinnamate (17.66%). It was found that there were clear differences between cluster I and cluster II or between cluster II and cluster III. The differences can be seen in the percentage of cinnamic acid and cinnamyl cinnamate. Cluster I and cluster III looks similar in chavicol and benzoic acid percentages. By close examination, it turns that cluster I had a higher percentage of benzoic acid and chavicol, but lower cinnamic acid and cinnamic acid compared to other clusters. Individual trees classified in cluster II had a high percentage of cinnamic acid, whereas cluster III had a high percentage of cinnamic acid compared to other clusters.

Numbers in the vertical direction of the dendrogram indicate the diameter of each individual tree in the cluster. The diameter value was evenly distributed in each cluster, indicating that the diameter value has no effect on the percentage and concentration of the chemical components in benzoin gum. This trend was confirmed by correlation analysis (Table 4). It can be seen that the diameter had no significant effect on the percentage of chemical components.

The relationship between tree diameter and yield has been studied for oleoresin in some pine species (Lekha and Sharma 2010) or sap in rubber species (Woelan 2005). It is assumed that trees with bigger diameters are able to produce benzoin gum with high concentrations of chemical components. From the results of the Pearson correlation, it can be seen that there was no correlation between growth rate (diameter) and the amount of chemical components of benzoin gum. It implies that no special treatment is required to increase the growth rate or diameter of the benzoin tree. Unfortunately, the correlation between benzoin tree diameter and quantity of benzoin gum production was not analyzed in this experiment. Therefore, it is not recommended to use individual trees from this study as benzoin gum-producing trees for production purposes.

Table 3. Mean chemical components of cluster I-III of benzoin gum

Cluster	Benzoic acid	Chavicol	Cinnamic acid	Cinnamyl cinnamate
I	1.19 ± 0.4.9	3.95 ± 0.54	23.30 ± 6.32	9.70 ± 10.85
II	0.43 ± 0.19	2.75 ± 0.97	14.50 ± 7.44	36.43 ± 16.52
III	0.96 ± 0.25	3.74 ± 0.51	45.25 ± 5.30	17.66 ± 6.91

Table 4. Pearson analysis of the amount of benzoin gum components and tree diameter

Compound	Pearson Corelation
Styrene	0.01
Benzoic Acid	0.29
Chavicol	-0.09
Vanillin	0.19
Cinnamic acid	0.29
Atropic acid	-0.07
4-Vinylbenzoic acid	0.06
Benzyl cinnamate	0.28
Cinnamyl cinnamate	0.12

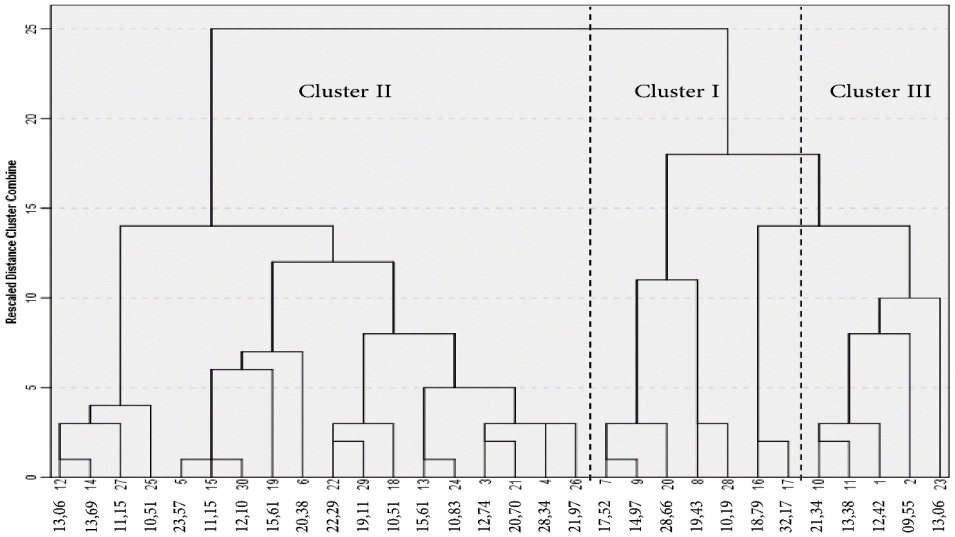

Figure 2. Dendrogram of Benzoin Gum Clusters with X-axis is Tree Diameter (cm)

Conclusion

The results of the GC-MS analysis of benzoin gum on 30 trees (age 10 years) with tree diameters range of 9-35 cm showed cinnamic acid, cinnamyl cinnamate, benzoic acid, chavicol, benzyl cinnamate, atropic acid, vanillin, and 4-vinylbenzoic acid and styrene as the detected components. The major components were cinnamic acid and cinnamyl cinnamate. The results of the cluster analysis of individual trees were grouped into 3 clusters with different percentages of chemical components. Cluster I was classified with average percentage of benzoic acid of 1.19%, chavicol of 3.95%, cinnamic acid of 23.30%, and cinnamyl cinnamic of 6.93%. Cluster II was classified with average percentage of benzoic acid of 0.43%, chavicol (2.75%), cinnamic acid (14.50%) with high cinnamyl cinnamic acid (36.43%). Cluster III was classified with percentage of benzoic acid of 0.96%, chavicol of 3.74%, with high levels of cinnamic acid (45.25%) and cinnamic cinnamic acid (7.66%). There is no significant correlation between the levels of diameter and the amount of chemical components of benzoin gum.

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