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Chemotaxonomic Studies on the Genus *Amomum* Based on Chemical Components of Volatile Oils

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The study was focused on six species of *Amomum* genus i.e. *A. aculeatum* Roxb., *A. blumeanum* Val., *A. compactum* Soland. ex Maton., *A. longipes* Val., *A. roseum* Benth. et Hook.f., *A. truncatum* Gagn., and one species of *Elettaria cardamomum* Maton. The aims of the study were to find out percentage of volatile oil and the number of chemical components in the volatile oil in the rhizome of plants. In addition, I also determined percentage of volatile oil, the number of chemical components of volatile oil in the rhizome of *A. compactum* (Javanese cardamom); and the phylogenetic relationship of species belonging to genus *Amomum* based on the similarity index of the number of chemical components in the volatile oil. The volatile oil contents in the seven species of rhizome varies from 0.25-2.25% (v/w), and in the seven organs of *A. compactum* from 1.0-3.5%. The total number of volatile oil components of the rhizome (content >1%) was 61 compounds and in several organs of *A. compactum* was 45 compounds. The rhizome showed 10 major components at the RT value of 7.43, 9.04, 11.07, 11.39, 13.57, 14.91, 17.41, 18.30, 18.46, and 18.72. The seven organs of *A. compactum* had seven major components at RT value of 7.34, 7.42, 7.56, 11.08, 13.58, 16.12, and 18.28. The relationships of those seven species were as follows: *A. blumeanum* and *A. longipes* had close relationship on the similarity index of 80%, *A. aculeatum* 75%, and *A. truncatum* 70%. This group joined with *A. roseum, A. compactum* and *E. cardamomum* at the similarity index of 67%. The phylogenetic dendrogram showed that all species had similarity index at least 67%, suggested that taxonomic status of the genus *Elettaria* need to be observed in details. The similarity index of seven organs of *A. compactum* were at least 60%.

INTRODUCTION

Genus *Amomum* (Zingiberaceae) is profitable for flavor spices, perfume, medicinal stuff, garnishing plants, etc (Burkill 1935, Heyne 1950). Their fruits contain high amount of volatile oil that can also be extracted from another organs. Systematic of this genus has been argued among the authors (i.e. Holttum 1950, Backer & Bakhuizen v.d. Brink 1968), because they obtain limited data from the morphological and the anatomical characters. The chemistry of Zingiberaceae volatile oil is one of the most prospective characters for taxonomy (Setyawan 1996). Chemotaxonomic principles are considered and some examples are provided to show the importance of chemical evidence in taxonomic revision (Hegnauer 1986). The advent of chemotaxonomy is closely linked to the introduction of chemical analytical methods, especially that of chromatography (Harborne 1973). There are four prominent groups of compounds that are phenolics, alkaloids, terpenoids and non-protein amino acids, all of which exhibit a wide variation in chemical diversity, distribution and function (Smith 1976). The major secondary metabolite of *Amomum* is volatile oil (terpenoids group). Modern analytical procedures allow extremely rapid and comprehensive determination of volatile oil composition now (Hegarty *et al.* 2001).

The chemotaxonomic characters are better than the morphological and anatomical characters, because the material to be analyzed must not be fresh or complete material, but it uses only dried and crushed material. The character give satisfying result and it can be placed correctly in the taxonomic classification, as long as the microbe or the other materials do not contaminate. A hundred years old herbaria specimen can be examined their secondary metabolites accurately (Harborne 1973).

Volatile oil is a mixture of steam volatile compounds, mainly terpenoids and related substances (Hegarty *et al.* 2001). Terpenes, hydrocarbons built from isoprene units (C_5), are the largest class of plant secondary metabolites (Harborne 1991). Secondary metabolism is generally regarded as a non-essential process that produced by-product or plant wastes. For the majority, no specific function has yet been determined (Liu *et al.* 1998, Hegarty *et al.* 2001). However, volatile oils of terpene group play an important role in chemical ecology, where they act as attractants, repellents, allelopathy, sex pheromones, alerting pheromones or as a part of defense mechanisms from herbivores attack or microorganisms causing diseases (e.g. Luckner 1990, Banthorpe 1994, Agrawal 2000, Olejniczak *et al.* 2000, Grison-Pige *et al.* 2001, Kutchan 2001). They are widely distributed in nature, and over 30 000 different terpenes have been identified (Buckingham 1998).

Volatile oil is one of the principle constituents for providing typical characteristic flavor and medicinal value (Lata *et al.* 2000). The cytotoxic nature of some volatile oil has made them medicinally important (Liu *et al.* 1998). Volatile oil is largely responsible for the flavors and aromas of foods, beverages, perfumes and cosmetics (Keita *et al.* 2000,

Hegarty *et al.* 2001). Secondary metabolites are the most consistently successful source of drug leads (Harvey 2000). Approximately 60% of the world's population relies almost entirely on plants for medication (Farnsworth 1994) and secondary metabolites have long been recognized as an important source of therapeutically effective medicines, i.e. antibacterials and anti-cancer drugs (Cragg 1997).

Volatile oil of Zingiberaceae is deposited in idioblastic secretory cells that coated with suberized walls (Tomlison 1969, Setyawan 1996). Secretory sacs or cavities of vascular plants are relevant taxonomic characters and important anatomical features that have originated many times in distantly and closely related families (Viera *et al.* 2001). Chemical and botanical data are still insufficient to enable the chemical composition of volatile oil to be used for taxonomic purpose (McKern 1965). Volatile oil can be used to investigate taxonomic problems in genera level (Dunlop *et al.* 1997).

Genus *Amomum* belongs to the division of Spermatophyte, the sub-division of Angiosperm, the class of Monocotyledons, the order of Zingiberales, the family of Zingiberaceae, the sub-family of Zingiberoideae, and the tribe of Alpineae. This genus has members of about 90-150 species (Lawrence 1951, Burtt & Smith 1972a, Bhattacharyya & Johri 1998), where 40-50 of them grows in Asia (Burkill 1935, Holttum 1950). We can find *Elettaria* in India and *Aframomum* in Africa, which have a close relationship to *Amomum* (Holttum 1951, Burtt & Smith 1972b). Javanese cardamom (*A. compactum*) is one of the members of genus *Amomum* that is mostly favored in Indonesia. But, true cardamom (*E. cardamomum*) is the most widely used in the world (Naiola 1978). The last species of the genus, which is a native plant in India, has been planted in Indonesia, especially in the highland of West Java (Madjo-Indo 1978). There are more than 20 members of genus *Amomum* in Indonesia (Backer & Bakhuizen v.d. Brink 1968, Danimiharja & Notodiharjo 1978, Kosahara 1986), but the complete chemical study of volatile oil to all members of this genus has never been done before, especially when it is related to the taxonomic status and the relationship.

The study is intend to find out (i) the volatile oil percentage in six species of genus *Amomum* rhizome i.e. *A. aculeatum*, *A. blumeanum*, *A. compactum*, *A. longipes*, *A. roseum*, *A. truncatum*, and one species of *E. cardamomum*, (ii) the number of chemical component of volatile oil in those species, (iii) the volatile oil percentage in seven organs of *A. compactum*, namely rhizome, root, trunk, leaf, fruit, seed and fruit peel), (iv) the number of chemical component of volatile oil in those organs and (v) the phylogenetic relationship of species belonging to genus *Amomum* based on the similarity index of the number of chemical components in their volatile oil.

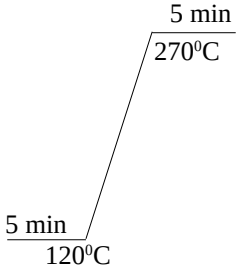
MATERIALS AND METHODS

Plant Material. The specimen of *A. compactum* Soland ex Maton was used to compare the percentage and the components of volatile oil of the rhizome, the root, the trunk (the aerial stem), the leaves, the fruit, the seed and the fruit peel. It was gathered from medicinal plant collection of PT. Jamu Air Mancur Surakarta. While the rhizome of *A. aculeatum* Roxb., *A. blumeanum* Val., *A. compactum* Soland ex Maton, *A. longipes* Val., *A. roseum* Benth. et Hook.f., *A. truncatum* Gagn., and the rhizome of *E. cardamomum* Maton were gathered from Bogor Botanical Garden (Hortus Botanicus Bogoriense). They were used to build dendrogram and to find out the phylogenetic relationship. Each of the specimens was examined in triple.

Distillation Assay. The rhizome or the other organs that was similar in age, size, and form were cleanly washed, then chopped down cross wisely into pieces with 1-2 cm of thickness. The pieces were air dried for 3-4 days. Dried materials were ground and sifted with a sifter (2 mm^2 i.d.). One hundred gram of powder was put into 1 000 ml Stahl-type apparatus flask, and was added with distilled water up to two third of the flask plus 0.2 ml of xylene, and then it was placed on the stove for 4-5 hours to produce volatile oil. The xylene was evaporated in a vacuum or low-pressure rotary evaporator, then the oil was dried with anhydrous Na_2SO_4 and was stored in the cool place (4-5^{0}C) in a dark bottle with the cap put on tightly. The percentage of volatile oil was stated as the amount of distilled volatile oil per 100 gram of the powder (v/w; ml/100 g) as usually used in *Materia Medika Indonesia* (MMI) (Anon 1977, 1978, 1979).

Gas Chromatography (GC) Assay. The volatile oil was analyzed by Gas Chromatography to determine the number and the type of volatile oil components, and the percentage of each component. The types of volatile oil component were identified by the peak enrichment technique. They were leaded by the retention time value of chromatogram (RT). All components that had high enough percentage (> 1%) were analyzed. The value of RT was considered similar at range of 0.05. If, at this point, there were overlapping values, then to find out the value group of RT (the upper value or the bottom value) would solve the problem. The GC condition was:

Merck	: Hewlett-Packard 5890 series II
Sample volume	: 0.1 ml
Column	: DB 5 (5% phenyl methyl siloxane, 30 m, ϕ 0.33 mm i.d.).
Carrier gas	: He
Gas flow	: 40 ml/min
Column pressure	: 60 kpa
Injector temperature	: 260^{0}C
Initial temperature	: 120^{0}C
Initial time	: 5 min
Temperature rate	: 10^{0}C/min
Final temperature	: 270^{0}C
Detector temperature	: 270^{0}C
Detector	: FID (Flame Ionization Detector).



Data Analysis. Phylogenetic dendrogram of seven examined species was numerically made with the method of association coefficient (Sneath & Sokal 1973), where the similarity index of association coefficient was determined through cluster analysis (Pielou 1984). The calculation was covered in unweighted pair group method with arithmetic mean (UPGMA), which was computed in BIOSYS-1 program

(Swofford & Selander 1989). The same way was used to find out the similarity index of volatile components of several organs of *A. compactum*, i.e. rhizome, root, trunk, leaves, seed, fruit, and fruit peel.

RESULTS

More than 20 species of genus *Amomum* and genera allied are known in Indonesia, but only seven species could be prepared in this study. However, all species of *Amomum* that was planted in Bogor Botanical Garden could not be gathered in the same time. They had different age, they bloomed their flowers and matured their fruits at a different time, and finally their fruit could not be gathered at a similar time. In the relationship analysis of the genus *Amomum*, the rhizome is preferable to use than the fruit. On the other hand, *A. compactum* was chosen as representative of genus *Amomum* to compare its volatile oil components of various organs. The material source was taken from medicinal plant collection of PT. Jamu Air Mancur Surakarta. The fruit of Javanese cardamom is well known in our community, especially as ingredient.

Figure 1a showed the percentage of volatile oil of rhizome in six *Amomum* species and *E. cardamomum*. The highest percentage of volatile oil was found in *E. cardamomum* (2.25%). It was followed subsequently by *A. roseum* (2%), *A. blumeanum* (1.75%), *A. compactum* (1.5%), *A. aculeatum* (1.25%), *A. longipes* (0.5%) and *A. truncatum* (0.25%). Figure 1b showed the percentage of volatile oil of various organ of *A. compactum*. The highest percentage of volatile oil was found in fruit (3.5%). It was followed subsequently by leaves (3%), fruit peel (1.75%), rhizome (1.5%), root (1.25%), trunk (1%), and seed (1%).

Table 1 showed number, type, and percentage of each type components which composing volatile oil of the rhizomes in six *Amomum* species and one species of *E. cardamomum*. It was derived from the individual original chromatogram. The seven species that were analyzed result in 61 compounds with the percentage of more than 1%, where 11 of them were the major components with the percentage of more than 10%, and six of those 11 compounds had percentage of more than 20%. Besides, there were many other peaks with the percentage less than 1% and they appear inconsistently in triple assays, so they could be ignored. The volatile oil components in every species varied from 14-24 components. The highest number was found in *A. roseum* (24 components), the next was *A. truncatum* (22 components), *A. longipes* (21 components), *A. aculeatum* (18 components), *A. blumeanum* (15 components), and then *A. compactum* (14 components) and *E. cardamomum* (14 components).

Table 2 showed number, type, and percentage of each type components composing volatile oil of several organ of *A. compactum*. There were components differences in composing the volatile oil in such organs of *A. compactum* as indicated in their peaks. It varied from 4-20 components, and the total number was 45 components, in which seven of them were the major components, and four out of seven components had percentage of more than 20%. The highest number was found in root (20 components), the next was in rhizome (14 components), in trunk (13 components), in leaves (8 components), in fruit peel (7 components), in fruit (5 components), and the smallest was in seed (4 components).

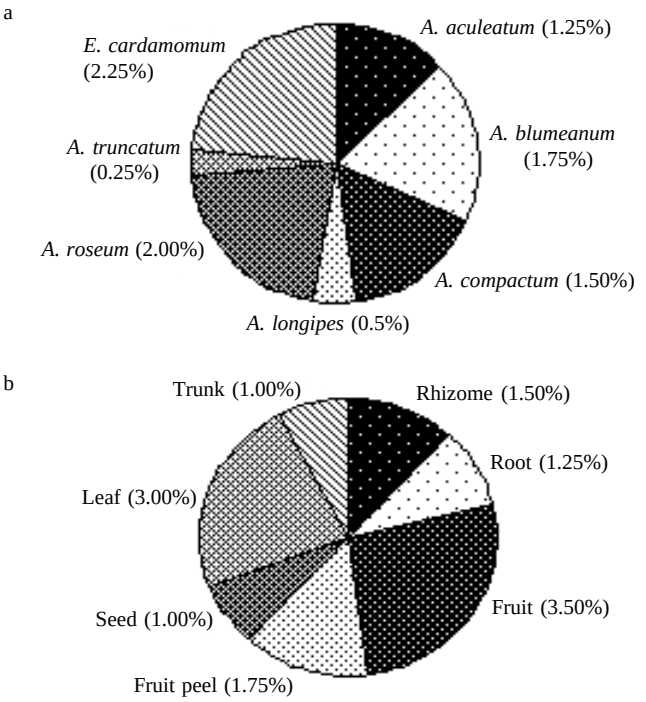


Figure 1. a. percentage of volatile oil of *Amomum* species and *Elettaria cardamomum*, b. various organs of *A. compactum.*

DISCUSSION

Volatile Oil Contents. The volatile oil percentage of the rhizome of six *Amomum* species and *E. cardamomum* tend to be varied (Figure 1a), as well as in several organs of *A. compactum*. It depends on the age and the organ that were analyzed, the soil and the climate that were used to culture, the species and its varieties (Guenther 1948). The percentage of volatile oil could vary from plant to plant of same species, as well as with factors such as maturity, season, flowering cycle, variation in climate, soil, and recent attack by grazing animals or insects (Llusia & Penuelas 2000, Hegarty *et al.* 2001). The genetic factors that present in the form of species variation and its variety were the most influencing factors (Crawford 1990). It answered to question why the volatile oil percentage of those plants are different in some literatures. For example, the seeds of *E. cardamomum* contains 3.5-7% of volatile oil (Guenther 1952), while other literatures say its volatile oil percentage is 2-8% (Hegnauer 1963, Purseglove 1972), 3-7% (Youngken 1948), and 2.8-6.2% (mean of 4%) (Trease & Evans 1978). The rhizome gathering from Bogor Botanical Garden was analyzed to construct phylogenetic dendrogram of seven species, while *A. compactum* gathering from medicinal plant collection of PT. Jamu Air Mancur Surakarta was used to compare the chemical components of several organs.

Table 1. The percentage of chemical components of the volatile oil in rhizome of six species of *Amomum* and *Elettaria cardamomum*

Mean RT values	Mean percentage (%)						
	A. aculeatum	*A. blumeanum*	*A. compactum*	*A. longipes*	*A. roseum*	*A. truncatum*	*E. cardamomum*
5.49	–	–	–	–	–	–	3.15
6.28	–	–	–	1.77	–	–	–
6.31	–	–	–	–	–	–	11.50
7.43	1.53	2.08	2.90	1.45	12.15*	9.13	27.00**
8.98	–	–	–	–	1.19	–	–
9.04	–	–	–	–		–	28.66**
9.16	–	–	2.60	–		–	–
9.52	–	–	–	–	1.64	–	–
9.96	5.31	–	–	3.51	2.05	2.93	–
10.01	–	8.50	1.08	–	–	–	–
10.52	–	–	–	–	2.18	–	–
10.69	1.47	–	9.25	1.13	3.18	–	3.53
11.07	1.56	–	40.21**	–	14.20	–	4.39
11.13	–	–	–	–	–	1.20	–
11.39	13.23*	20.35**	–	8.82	–	10.96*	–
11.82	–	–	2.60	–	–	–	–
12.05	–	–	–	–	1.59	–	–
12.25	–	–	–	–	1.18	–	–
12.37	–	–	–	–	2.45	–	–
12.45	–	–	1.24	–	–	–	–
12.64	6.92	9.43	–	3.22	1.10	5.40	–
13.42	–	–	–	–	2.00	–	–
13.57	–	–	12.96*	–	–	–	–
13.74	–	1.58	–	1.17	–	–	–
14.15	2.67	4.67	–	6.25	–	2.66	–
14.45	–	–	5.48	–	–	–	–
14.91	–	11.18*	–	4.91	–	–	–
15.48	–	1.53	–	–	–	–	–
15.56	–	1.71	–	1.50	–	–	–
15.75	–	–	1.27	–	–	–	1.11
16.00	1.23	–	–	2.73	4.60	–	–
16.08	–	3.93	2.17	–	3.38	2.10	–
16.34	–	–	–	–	1.07	–	–
16.43	–	3.00	–	1.50	–	–	–
16.97	–	–	–	–	2.65	–	–
17.41	27.80**	3.19	1.18	22.13**	3.88	11.12*	–
17.56	–	15.12*	–	–	–	7.78	1.49
17.74	2.27	–	–	2.11	1.02	2.11	–
17.85	–	–	–	–	–	–	1.17
18.09	–	–	–	2.46	–	–	–
18.12	–	–	–	–	–	–	1.30
18.30	–	–	14.51*	1.48	–	–	–
18.46	10.52*	–	–	8.15	4.28	1.03	1.26
18.57	–	–	–	–	–	7.38	–
18.64	4.92	–	–	3.50	–	–	1.55
18.72	–	12.12*	–	2.68	4.82	4.07	–
19.48	–	–	–	–	–	1.07	–
19.65	2.05	–	–	–	–	–	–
19.75	–	–	1.52	–	5.69	2.50	–
20.00	1.41	–	–	–	–	2.21	–
20.25	1.36	1.61	–	3.14	1.16	2.38	1.81
20.83	–	–	–	–	4.05	–	–
20.65	–	–	–	–	–	2.29	–
20.92	–	–	–	–	1.18	–	–
21.26	–	–	–	–	–	2.08	–
21.69	2.00	–	–	–	–	–	–
21.75	–	–	–	–	–	1.74	–
22.07	1.34	–	–	–	–	–	–
22.13	–	–	–	–	–	1.19	–
22.27	3.83	–	–	2.37	–	2.87	–
23.85	–	–	–	–	–	–	4.44
Total percentage (> 1%)	91.42	100	98.97	85.98	69.35	86.20	92.36
Total amount of components	18	15	14	21	24	22	14
Total major components	3	4	3	1	1	2	2

–: not existing or less than 1%, *: major components (> 10%), **: very high percentage of major component (> 20%)

Table 2. The percentage of chemical components of the volatile oil in seven organs of *Amomum compactum*

RT value	Mean percentage (%)						
	Fruit	Fruit pell	Seed	Leaves	Rhizome	Root	Trunk
5.48	–	–	–	1.18	–	–	–
5.57	–	–	–	–	–	1.01	–
5.86	–	–	–	–	–	4.97	–
6.34	2.85	–	3.38	1.16	–	3.22	–
7.25	–	1.31	–	–	–	–	–
7.34	–	–	–	16.45*	–	–	8.80
7.42	–	67.40**	–	–	2.90	10.95*	–
7.56	79.03**	–	80.56**	39.20**	–	28.83**	52.92**
9.07	–	2.22	–	–	–	–	–
9.16	–	–	–	–	2.60	–	–
9.59	–	–	–	–	–	–	1.22
10.00	–	1.31	–	–	1.08	–	–
10.07	–	–	–	–	–	9.35	–
10.19	–	–	–	–	–	1.09	–
10.39	–	–	–	–	–	–	3.08
10.62	–	–	–	–	–	–	1.62
10.71	1.48	5.34	1.99	–	9.25	1.40	2.02
11.08	8.83	18.38*	8.98	–	40.21**	–	4.77
11.16	–	–	–	–	–	1.76	–
11.38	–	1.78	–	–	–	–	–
11.82	–	–	–	–	2.60	–	–
12.08	–	–	–	–	–	–	2.52
12.44	–	–	–	–	1.24	–	–
13.58	–	–	–	–	12.96*	–	–
13.79	–	–	–	–	–	3.03	–
14.45	–	–	–	–	5.48	–	–
15.59	–	–	–	–	–	1.45	–
15.76	–	–	–	–	1.27	–	–
15.89	–	–	–	1.37	–	–	1.36
16.08	1.40	–	–	–	2.17	–	9.36
16.12	–	–	–	22.98**	–	1.87	–
16.16	–	–	–	–	–	2.43	–
16.43	–	–	–	5.66	–	–	2.32
17.38	–	–	–	–	1.18	–	–
17.49	–	–	–	–	–	8.03	–
17.81	–	–	–	–	–	2.10	–
18.15	–	–	–	–	–	1.25	–
18.28	–	–	–	–	14.51*	–	–
18.55	–	–	–	3.48	–	3.26	–
18.63	–	–	–	–	–	–	1.91
18.71	–	–	–	–	–	1.60	–
18.77	–	–	–	–	–	–	1.18
19.80	–	–	–	–	1.52	–	–
20.28	–	–	–	–	–	2.58	–
22.33	–	–	–	–	–	1.01	–
Total percentage (> 1%)	93.59	97.74	94.91	91.48	98.97	91.19	93.08
Total amount of components	5	7	4	8	14	20	13
Total major components	1	2	1	3	3	2	1

–: not existing or less than 1%, *: major components (> 10%), **: very high percentage of major components (> 20%)

The highest percentage of volatile oil of rhizome was found in *E. cardamomum* (2.25%), it was followed subsequently by *A. roseum* (2%), *A. blumeanum* (1.75%), *A. compactum* (1.5%), *A. aculeatum* (1.25%), *A. longipes* (0.5%) and *A. truncatum* (0.25%) (Figure 1a). The aromatic intensity of the crushed plant material could be used to prejudge the percentage of volatile oil, however each species tend to give different aroma. In this study, *E. cardamomum* indicates stronger smell of volatile oil than the other species. It gave the highest percentage of volatile oil of rhizome with 2.25%. It was quite high, but it was lower than the percentage of volatile oil of seed, which had average of 4% (Trease & Evans 1978). The rhizome of *A. compactum* contains 1.5% of volatile oil; it was lower than that in fruit that contains 3.5% of volatile oil (in this research). It was small number, but it was higher than the percentage, which was stated in the MMI, containing 1% of volatile oil (Anon 1979). *A. blumeanum* and *A. roseum* also indicate high percentage of volatile oil that was 2.00% and 1.75% respectively. Their smells were strong enough. While *A. truncatum*, *A.longipes* and *A. aculeatum* had low content of volatile oil, subsequently 1.25%, 0.50%, and 0.25%. Their smells were neutral, as they had no aromatic compounds.

The well known *Amomum* species, *A. compactum* indicates high degree of volatile oil content. The highest percentage of volatile oil was found in the fruit i.e. 3.5%. It was

followed subsequently by volatile oil content in leaves (3%), fruit peel (1.75%), rhizome (1.5%), root (1.25%), trunk (1%), and seed (1%) (Figure 1b). The fruit, which was usually miscalled as seed, was the main source of volatile oil. People use it to flavor their culinary, soft drink, medicinal stuff, etc. However, another part of this plant contains high volatile oil. It was similar or higher than volatile oil percentage of seed in the average 1% (Anon 1979).

The whole fruit contains much more volatile oil than its separation of fruit peel and seed. If the percentage of volatile oil in the fruit peel and seed was accumulated, it resulted only 2.75%. This value was lower than that of the entire fruit (3.5%). There was 0.75% volatile oil loose by evaporation. So, the storage of the entire fruit was better than that of separated parts. The volatile oil extraction of *A. compactum* for the industrial purpose should not use fruit materials. It was too expensive. The leaves materials might be used on this purpose. It contains 3% of volatile oil. However, it needed to be checked for their volatile oil components, because this was the main purpose of the volatile oil (see below).

The aromatic smell of *A. compactum* trunk tends to neutrally; it contains 1% of volatile oil. The aromatic smell of the seed tends to be the strongest one, but actually it contains only 1% of volatile oil in distillation. Probably it is caused by evaporation. The volatile oil of the seed is dominated by low boiling point of components, and it evaporates in the room temperature. This evidence leads us to name it volatile oil or essential oil or ethereal oil (Claus *et al.* 1970).

Volatile Oil Components. Secondary metabolite of Zingiberaceae consists of volatile oil, resinous material, pigment and bitter substance. The most dominant is volatile oil (Hegnauer 1963). Volatile oil component of *Amomum* species consists of cineole, β-pinene, α-pinene, borneol, camphor, terpinene, terpinyl acetate, terpineol, bisabolene, sabinene, linalool, etc (Guenther 1952, Hegnauer 1963). Cineole is the highest percentage of *Amomum* volatile oil. It can reach 65% in *A. subulatum* Roxb. (Hegnauer 1963), and 5-10% (Anon 1979) or 12% (Darwis *et al*. 1991) in *A. compactum*. Percentage of other components is stated rarely in literatures. Sources of chemical variation of natural plants consist of hydroxylation, methylation, glycosides, disaccharide, etc (Denford 1984). They often present in the sesquiterpene compounds, one of the volatile oil components that are useful for taxonomic purposes.

The components mixture of oils from different plants of the same species might be extremely variable, and the results are also influenced by the method of extraction (Hegarty *et al.* 2001). Volatile oils can be produced by distillation or solvent extraction (McHugh & Krukonis 1986). High temperature in distillation process would change some molecular composition of volatile oil. It constructs new compounds that naturally do not present. They are called *artifact* compounds. For example, gingerol, the pungency of ginger (*Zingiber officinale* Rosc.) that is produced in alkali extraction would be dehydrated into shogaol or hydrolyzed into zingeron and n-hexane in distillation procedure (Hegnauer 1963, Trease & Evans 1978). High degree of temperature conducts hydrodiffusion, hydrolysis, polymerization, and resinification process (Guenther 1948). However, it is useful as taxonomic evidences as long as resulting new stabile compounds. On the other hand, non-polar organic solvents isolate volatile oil. Its product is compound that is as natural as the one in the nature, and result compounds depend on the type of solvents. It might need several organic solvents to extract volatile oil. It is a time consuming and expensive process, and the product is not really similar with the volatile oil in the market, that is usually produced by distillation method. Some of volatile oil compounds can only be extracted by distillation, such as caryophyllene of cengkeh (*Syzygium aromaticum* L.) that compose about 10% of clove oil.

Volatile Oil of *Amomum* Species and *E. cardamomum.* In this study, almost one third of the components or compounds or peaks were found only in one species. This distribution was prospective for taxonomic evidences. Some of the peaks were found in a high enough percentage. Though at a small number, the percentage of volatile oil and the components might change during the laboratory assay. Thus, the major component was more valuable for taxonomic evidence than the minor one.

Compound that could be found in every species was the peak with RT value of 7.43. It became major component of *A. roseum* and *E. cardamomum*, subsequently with percentage of 12.15% and 27.00%. This peak could be predicted as chemical marker of the genus *Amomum* and *E. cardamomum*. The major component of RT value of 9.04 was a special characteristic for *E. cardamomum* and would not be found in the other six species, and this would be a useful differentiating character for this species. The RT value of 13.57 could be predicted as chemical marker of *A. compactum* in the similar reason of that *E. cardamomum*. While, the other RT values were found on the other species, and it was common thing because they belong to the same genus of *Amomum*. The RT value of 17.41 that was the major component for *A. aculeatum, A. longipes*, and *A. truncatum* was found at a lower percentage in *A. blumeanum, A. roseum* and *A. compactum*. It would be useful character to differentiate genus *Amomum* with *E. cardamomum.* Some other compounds presented as minor components in one species or more. It was less valuable for taxonomic purpose, because it might be a non-stabile *artifact* compound that could recreate another compound in different conditions. It might be missing disappear caused by evaporation when it was stored.

Each of the seven examined species had 1-4 peaks of major components or main peaks (> 10%), and they had totally 10 peaks; it lied at the RT value of 7.43, 9.04, 11.07, 11.39, 13.57, 14.91, 17.41, 18.30, 18.46, and the RT value of 18.72. The highest major component belonged to *A. blumeanum*, which had four major components. It was followed by *A. aculeatum* and *A. compactum*, which each of species had three major components, and then *A. truncatum* and *E. cardamomum*, which each of them had two major components. At the last was *A. longipes* and *A. roseum*, which each of them had only one major component. The major components of *A. blumeanum* subsequently lied at RT value of 11.39

(20.35%), 17.56 (15.12%), 18.72 (12.12%), and RT value of 14.91 (11.18%). The major components of *A. compactum* subsequently lied at RT value of 11.07 (40.21%), RT value of 18.30 (14.51%), and RT value of 13.57 (12.96%). The major components of *A. truncatum* lied at RT value of 17.41 (11.12%), and RT value of 11.39 (10.96%). The major components of *E. cardamomum* lied at RT value of 9.04 (28.66%), and RT value of 7.43 (27.00%). The major components of *A. longipes* lied at RT value of 17.41 (22.13%). The major components of *A. roseum* lied at RT value of 7.43 (12.15%).

In this research, six major components of five species were presented at very high concentration (> 20%). It was potentially as source of pure compound for practical purposes, such as medical and pharmaceutical. It was presented in *E. cardamomum* at RT value of 7.43 (27%) and RT value of 9.04 (28.66%). It also was presented in *A. compactum* at RT value of 11.07 (40.21%), and in *A. blumeanum* at RT value of 11.39 (20.35%). It was presented together in *A. aculeatum* and *A. longipes* at RT value of 17.41, subsequently with 27.8% and 22.13%.

The total percentage of volatile oil component (> 1%) in the seven species was about 69.35-100%. The highest one was found in *A. blumeanum* (100%). It was subsequently followed by *A. compactum* (98.97%), *E. cardamomum* (92.36%), *A. aculeatum* (91.42%), *A. truncatum* (86.20%), *A. longipes* (85.89%), and *A. roseum* (69.35%). The remainder percentage indicates the volume of volatile oil that could not be used as taxonomic evidences; it consists of many chemical components that each of them less than 1% in volatile oil. The percentage of *A. blumeanum* reached 100%, it means that each component of volatile oil has concentration of 1% or more, and all of them are used as taxonomic evidences. On the contrary, the percentage of *A. roseum* only reach 69.35%, it means that 30.65% of the volatile oil volume consist of components, which their percentage less than 1%. It is useless for taxonomic purpose. The components of *A. roseum*, which percentage less than 1% were about 30-40 compounds (data not show).

Some of the main peaks of the different species or organs lied at similar RT. It was called similar compounds, and it might have similar purpose. Although, the purpose of natural products was usually determined by entire compound rather than one of those chemistry. The main peaks ought to be a marker of the volatile oil purely. It inhibites counterfeiting or contamination of the volatile oil. However, the entire chromatogram must be used to determine it.

Volatile Oil of Various Organs of *A. compactum.* In this study, there was no compound that presents on every organs of the analyzed plant. Each of the seven organs of *A. compactum* had 1-3 peaks of major components (> 10%), and they had totally seven peaks; it lied at the RT value of 7.34, 7.42, 7.56, 11.08, 13.58, 16.12, and the RT value of 18.28. Rhizome and leaves had three major components. Fruit peel and root had two major components. While fruit, seed and trunk had only one major component. The major components of rhizome subsequently lied at RT value of 11.08 (40.21%), 13.58 (12.96%), and RT value of 18.28 (14.51%). The major components of leaves subsequently lied at RT value of 7.34 (16.45%), 7.56 (39.20%), and RT value of 16.12 (22.98%). The major components of root lied at RT value of 7.42 (10.95%) and RT value of 7.56 (28.83%). The major components of fruit peel lied at RT value of 7.42 (67.40%) and RT value of 11.08 (18.38%). The major components of fruit, seed and trunk lied at RT value of 7.56, subsequently with percentage of 79.03%, 80.56%, and 52.92%.

Eight of the peaks of *A. compactum* were found to be having very high enough percentage (> 20%). The RT value of 7.42 presented in fruit peel (67.40%). The RT value of 7.56 presented in fruit (79.03%), in seed (80.56%), in leaves (39.20%), in root (28.83%), and in trunk (52.92%). The RT value of 11.08 presented in rhizome (40.21%). While the RT value of 16.12 presented in leaves (22.98%). The peak of RT value of 7.56 was a characteristic compound. It presented in very high concentration in all organs, but fruit peel and rhizome. It was a valuable source of pure chemical compound for industrial purpose. It must be the main aroma on the culinary spices, because it contains 80% of volatile oil of seed.

The total percentage of volatile oil component (> 1%) in the seven organs of *A. compactum* was about 91.19-98.97%. Its average was higher than the seven species, i.e. 69.35-100%. It indicated that only a few components having percentage less than 1%, thus, the whole of volatile oil was more stabile, and the aroma was not easy to change.

The similarity indexes of various organs were based on the 45 components of volatile oil (see below to know the procedure). The similarity indexes of the components constructing volatile oil of *A. compactum* were as follows: the fruit and seed had the similarity index of 93%, both of them join the fruit peel at the similarity index of 84%, then this group had equal similarity index of 78% with the trunk and the rhizome. Separately, the root and leaves share the similarity index of 77%. All parts of *A. compactum* shared the similarity index of 60% (Figure 2).

Actually, the fruit, the seed and the fruit peel have a similar cell development and in a real life they do not separated in its parts; they have high similarity and tend to be clustered. The trunk and rhizome lied at a same similarity index namely 78%, it caused the trunk (aerial stem) as an elongation of the rhizome (subterranean stem). Still, it was hard to explain why root and leaves lied at a same similarity index namely 77%, though they had different ontogeny and cell developments. All of the organs joined at similarity index 60%; it was a high similarity index. As I suggest before, there is probability to make substitution of volatile oil of fruit with the one of the leaves.

Phylogenetic Relationship and Taxonomic Status. The relationship was stated as similarity index among the seven species. It was based on the 61 components of volatile oil. The present component was scored as 1, while the non-present component was scored as 0. And because the component of the RT value of 7.43 always presented in the seven species, the percentage of component in every species was used. If the percentage was similar or above the mean, it was scored as 1, and if the percentage was less than the mean, it was

scored as 0. While it was possible, I did not use the percentage of components in other RT values, in order to avoid twice consideration and to reject non-comparative characters. In addition, the distance of Nei was stated as similarity index.

The relationships of those seven species were as follows: *A. blumeanum* and *A. longipes* had close relationship by similarity index of 80%, and they had close relationship at the similarity index of 75% with *A. aculeatum*, and then they had close relationship at the similarity index of 70% with *A. truncatum*. On the other hand, *A. roseum, A. compactum* and *E. cardamomum* joined together at the similarity index of 67%, and the group of the three species joined with the first group of four species in this similarity index (Figure 3).

One of the interesting discussions in taxonomy of the genus *Amomum* is the validity of the genus *Elettaria*, as represented by *A. compactum* and *E. cardamomum*. The confusion among the authors is grouping them in a similar genus or to separate it in an independent genus. It is as complicate as the confusion among the ordinary people to differentiate their seed in the market.

In the early modern classification, Linnaeus classified *Amomum, Elettaria*, and several other allied genera in one genus of *Amomum* (Naiola 1978). In the last completely revision of Zingiberaceae, Holttum (1950) separated the two genera, while he stated that they agreed in all essential flower characters, except the form of the inflorescence. Hutchinson (1969) suggested that the inflorescence of *Amomum* had no involucral bracts and more solid cone-like, while the inflorescence of *Elettaria* was simpler and length. However, Backer and Bakhuizen v.d. Brink (1968), and Burtt and Smith (1972b) tend to take no importance on this differentiation, where they gave *E. cardamomum* a basionym, *A. cardamomum* L. In addition, *Elettaria* has amount of member that lower than *Amomum.*

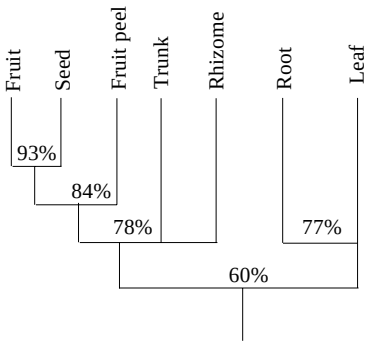


Figure 2. Similarity index of several organs of *Amomum compactum* based on percentage of volatile oil components. Annotation: the percentage indicate the similarity index.

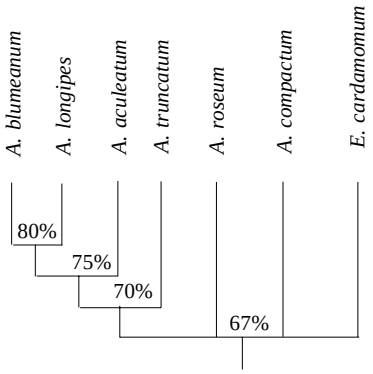


Figure 3. Dendrogram phylogenetic relationship of six *Amomum* species and one species of *Elettaria cardamomum*. Annotation: the percentage indicate the similarity index.

In this study, *E. cardamomum* had lowest similarity index in comparability to six species of *Amomum*, while it had parallel similarity index with *A. roseum* and *A. compactum*, i.e. 67%. It indicated that *E. cardamomum* tend to differ from *Amomum*, but it was high enough to separate it in an independent genus. Thus, it could be concluded that the entire groups had high index of relationship (> 60%), than it must be a genus. As we know in numerical taxonomy, a group of plants that its similarity index is 80% or more is usually grouped into one species. While the similarity index is 60% or more, it is usually grouped into one genus. The similar level on similarity index of *A. compactum* and *E. cardamomum* indicate that they share amount of similar chemical compound, and they have similar uses, especially in traditional medicine and culinary.

The chemotaxonomic studies of genus *Amomum* need to be extended on the other species and the genera allied, which were failed to be available on this study, in order to make the chemotaxonomic structure presented completely. A more detail research must be conducted to determine the taxonomic status of *E. cardamomum.* Further study on the isozymes together with chromosomal data (i.e. karyotype and molecular cytogenetics) of more species should reduce any confusion in the species identification and help to provide a better understanding of evolutionary phylogeny and taxonomic status, especially when combined with study on genomic DNA (Apavatjrut *et al*. 1999).

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