


# Chemical Composition of Essential Oil of *Amomum xanthioides* Wall. ex Baker from Northern Vietnam

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**Abstract:** The essential oil from leaves, roots, stems, and fruits of *Amomum xanthioides* Wall. ex Baker from Northern Vietnam was obtained by hydrodistillation and analyzed by gas chromatography techniques. The yield of the essential oil obtained from leaves, roots, stems, and fruits of *A. xanthioides* was 0.26%, 0.24%, 0.19%, and 1.45% (w/w) by fresh weight, respectively. The composition of the oil samples was compared; 38, 43, 28, and 22 compounds have been identified in the essential oil of leaves, roots, stems, and fruits, accounting for 96.19%, 96.61%, 98.39%, and 98.12% of the total oil content, respectively. A high variation among organs for the majority of compounds was shown.  $\beta$ -elemene (31.71%),  $\delta$ -cadinene (10.69%), germacrene D (9.55%), bicycloelemene (8.12%), and bicyclogermacrene (7.93%) are the main compounds in leaves, while  $\beta$ -pinene (29.59%), terpinen-4-ol (10.77%), and  $\alpha$ -terpinene (6.96%) are identified as the main compounds in roots. Stems are characterized by high levels of  $\beta$ -elemene (29.58%), spathoulenol (26.89%), and bicycloelemene (6.19%). For fruits, bornyl acetate (37.21%), camphor (19.48%), camphene (14.62%), and limonene (9.64%) are the main compounds. Overall, this study confirms that changes in the yield, composition, and concentration of the essential oil may be caused by several factors such as environmental conditions and climate at the collection site, the time of collection, and the age and properties of plants. This study also provides new data on the chemical composition of the essential oil from *A. xanthioides* collected from Northern Vietnam.

**Keywords:** *Amomum xanthioides*; *Zingiberaceae*; essential oil composition; volatile oil; gas chromatography.

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## 1. Introduction

*Zingiberaceae* is one of the essential oil-bearing plant families. Genus *Amomum* in this family has about 150 species, distributed in tropical Asia, Australia, and islands in the Pacific [1-9]. Vietnam has about 21 species [10]. Species in this genus thrive under the forest canopy, streams, and wet areas. Many species in this genus can be used for medicinal purposes, spices, or to extract essential oils with high economic values [11-14]. They are mainly harvested from natural plants and exported annually to international markets. Some studies on the essential oil of *Amomum* species in the world have been published [15-23]. These species' essential oil has been proved to have antibacterial, antifungal, and antioxidant properties with a high inhibitory effect [17-27]. In addition, it has been shown to inhibit the growth of cancer cells [28,29].

*Amomum xanthioides* Wall. ex Baker is a species in the genus *Amomum*. It is about 1-3 meters tall; Flowers are attached in bunches at plants' foot. Each foot has 3-6 little flower bunches. Each bunch has 4-8 flowers; Fruits are spherical with spines, green and yellow when they are ripe. It lives mainly under the forest canopy and along streams. This species is grown or grows wildy in Northern and Central Vietnam. In addition, it also exists in India, China, Myanmar, Thailand, Laos, and Cambodia [30]. Fruits and seeds of this species are used as medicines, spices, and for the preparation of liqueurs [31-33]. The essential oil composition of *A. xanthioides* fruits from China has been announced [34,35]. However, there are no prior complete reports of the essential oil from other parts of this species analyzed by gas chromatographic techniques in the world. There are several individual studies on *A. xanthioides* essential oil from Central Vietnam [36,37]. Studies also noted the effects of geographic, environmental factors, chemical analysis methods as well as extraction methods on the composition and quality of the essential oil [38-40]. Therefore, this article will provide more new data on the essential oil's chemical composition of *Amomum xanthioides* Wall. ex Baker (leaves, stems, roots, and fruits) from Northern Vietnam was analyzed using gas chromatographic techniques.

## 2. Materials and Methods

### 2.1. Plant material.

*Amomum xanthioides* Wall. ex Baker (leaves, stems, roots, and fruits) was collected at Na Hang Nature Reserve (Tuyen Quang, Vietnam) in July 2018. We based on Ho's (2000) documents to classify flora samples through morphological characteristics [41]. Fresh plant materials were kept at room temperature ( $\approx 25^{\circ}\text{C}$ ). 1 kg of each plant material sample was chopped and hydrodistilled with a Clevenger apparatus for 3 hours to produce the essential oil. The essential oil was dried with anhydrous sodium sulfate and placed in amber-colored vials for storage at  $4^{\circ}\text{C}$  until it was used for further studies.

### 2.2. Gas chromatographic analysis.

Gas Chromatography (GC) Analysis was performed on an Agilent Technologies HP 7890A Plus equipped with a flame ionization detector (FID) of Agilent Technologies, USA and fitted with HP-5MS chromatographic column with the length of 30 m, inner diameter (ID) = 0.25 mm, and film thickness of 0.25  $\mu\text{m}$ .  $\text{H}_2$  carrier gas (1 mL/min). Injector temperature (programmed temperature vaporization-PTV) was  $250^{\circ}\text{C}$ . Detector temperature was  $260^{\circ}\text{C}$ . Programmable thermostat temperature: increasing from  $60^{\circ}\text{C}$  (2 mins hold) to  $220^{\circ}\text{C}$  at  $4^{\circ}\text{C}/\text{min}$ . The temperature of  $220^{\circ}\text{C}$  was held for 10 minutes. Samples were injected by splitting, and the split ratio was 10:1. The volume injected was 1.0  $\mu\text{L}$ . Inlet pressure was 6.1 kPa.

HP 7890A Plus Chromatograph of Agilent Technologies (Santa Clara, California, USA) was equipped with a fused silica capillary HP-5MS (30 m x 0.25 mm, film thickness 0.25  $\mu\text{m}$ ) and interfaced with a mass spectrometer (HP 5973 MSD) used for the GC-MS analysis, under the same conditions as those used for GC-FID analysis. The conditions were the same as described above with He (1 mL/min) as a carrier gas. The MS conditions were as follows – ionization voltage 70 eV; emission current 40 mA; acquisitions scan a mass range of 35-350 amu at a sampling rate of 1.0 scan/s. The chemical compositions of the essential oil were identified based on retention indices (RI) based on a series of n-alkanes, co-injection with

pure compounds when available (Sigma-Aldrich, St. Louis, MO, USA) or identified known essential oil constituents, MS library search (NIST 17 and Wiley Version 10) and by comparing with MS literature data [42]. The relative amounts (%) of components were calculated based on the GC peak area (FID response) without using correction factors.

### 3. Results and Discussion

*Amomum xanthioides* Wall. ex Baker (leaves, stems, roots, and fruits) collected in Tuyen Quang, Vietnam was hydrodistilled to obtain the essential oil. The essential oil with a light-yellow color, lighter weight than water, and a pleasant odor were analyzed by gas chromatography-mass spectrometer (GC-MS) and gas chromatography-flame ionization detector (GC-FID). The *A. xanthioides* fruit in this study achieved the highest yield of the essential oil (1.45% in fresh weight) when compared to other plant parts such as leaves (0.26%), roots (0.24%), and stems (0.19%). However, the *A. xanthioides* fruit's essential oil yield in this study was still lower than previously reported [34,37]. A total of 63 compounds were identified in the essential oil of the parts of *A. xanthioides*, accounting for 96.19-98.39% of the total oil content (Table 1). Monoterpene (67.00-97.21%) is the main group of compounds found in the essential oil of *A. xanthioides* roots and fruits. Sesquiterpene and other groups of compounds account for a low percentage. In contrast, *A. xanthioides* stems and leaves mainly contain sesquiterpene compounds (83.86-93.66%). Other compounds account for a low percentage. The characterized compositions of four essential oil samples are  $\beta$ -pinene (1.23-29.59%) và  $\alpha$ -pinene (1.42-5.56%). The difference in the yield and quality of the essential oil can be explained by the effects of many factors such as chemical analysis method, raw material preparation, collection time, environmental conditions, and climates [38-40]. Along with that, different parts of the same plant species are also factors affecting the yield and quality of the essential oil [43,44].

**Table 1.** Percentage of chemical constituents of *Amomum xanthioides* leaves, roots, stems, and fruits essential oil (%).

No.	Compound name	RI <sup>a</sup>	Percentage composition <sup>b</sup>			
			Leaves	Roots	Stems	Fruits
1	Tricyclene	927	-	0.11	-	0.14
2	$\alpha$ -Thujene	930	0.11	0.42	-	-
3	$\alpha$ -Pinene	939	3.41	5.56	1.48	1.42
4	Camphene	953	0.18	3.16	-	14.62
5	$\beta$ -Pinene	980	2.56	29.59	1.54	1.23
6	$\beta$ -Myrcene	990	0.13	1.10	0.51	3.17
7	$\alpha$ -Phellandrene	1006	0.24	0.13	-	0.24
8	$\alpha$ -Terpinene	1017	0.17	6.96	-	-
9	o-Cymene	1024	0.16	-	-	-
10	Limonene	1032	0.38	1.76	-	9.64
11	1,8-Cineole	1036	-	0.31	0.86	2.43
12	(E)- $\beta$ -Ocimene	1052	-	0.10	-	-
13	$\gamma$ -Terpinene	1061	0.10	1.03	-	0.24
14	$\alpha$ -Terpinolene	1090	0.23	1.10	-	-
15	Linalool	1100	-	-	-	2.93
16	Camphor	1145	-	-	-	19.48
17	2,6-Dimethyl-2,4,6-octatriene	1147	1.44	-	-	-
18	Isoborneol	1156	-	-	-	0.18
19	Borneol	1167	-	-	-	3.79
20	Terpinen-4-ol	1177	0.11	10.77	-	0.14
21	$\alpha$ -Terpineol	1189	0.24	0.36	-	0.13
22	Piperitol	1216	-	0.54	-	-
23	Fenchyl acetate	1222	-	0.48	-	-
24	Bornyl acetate	1289	1.45	2.79	-	37.21
25	1-Terpineol acetate	1294	-	1.27	-	-

No.	Compound name	RI <sup>a</sup>	Percentage composition <sup>b</sup>			
			Leaves	Roots	Stems	Fruits
26	Bicycloelemene	1327	8.12	1.22	6.19	-
27	δ-Elemene	1340	-	1.13	-	-
28	Dodecamethyl-cyclohexasiloxane	1342	0.21	-	-	-
29	α-Cubebene	1351	0.15	0.24	0.63	-
30	α-Copaene	1377	0.55	-	-	-
31	β-Cubebene	1388	1.16	-	-	-
32	β-Elemene	1391	31.71	5.39	29.58	-
33	α-Gurjunene	1412	0.13	-	0.45	-
34	β-Caryophyllene	1419	-	0.77	1.54	-
35	γ-Elemene	1433	-	0.95	-	-
36	β-Gurjunene	1434	0.14	-	2.49	-
37	Aromadendrene	1441	0.75	0.12	0.57	-
38	α-Humulene	1454	0.91	0.26	1.67	-
39	γ-Gurjunene	1477	0.68	-	0.11	-
40	Germacrene D	1485	9.55	2.35	4.51	-
41	α-Amorphene	1485	0.46	-	-	0.12
42	β-Selinene	1486	-	-	1.13	-
43	Epi-bicyclosesquiphellandrene	1489	1.17	-	-	-
44	Cadina-1,4-diene	1496	0.39	-	-	-
45	Bicyclogermacrene	1500	7.93	1.32	4.19	-
46	β-Bisabolene	1506	-	-	1.96	0.24
47	γ-Cadinene	1514	-	0.17	-	-
48	Endo-1-bourbonanol	1522	4.24	1.67	0.12	-
49	β-Maaliene	1522	-	0.10	-	-
50	δ-Cadinene	1525	10.69	0.89	1.37	-
51	Nerolidol	1563	1.12	0.15	4.68	-
52	Spathoulanol	1578	-	-	26.89	-
53	Caryophyllene oxide	1583	-	0.48	0.34	-
54	Guaiol	1601	-	-	1.25	-
55	β-Himachalene	1614	-	0.14	-	-
56	τ-Murolol	1646	1.46	1.76	1.19	0.16
57	β-Eudesmol	1651	-	1.58	0.31	0.29
58	α-Cadinol	1654	2.55	-	2.01	0.10
59	Bulnesol	1672	-	1.19	0.48	-
60	Farnesol	1718	-	2.86	-	-
61	Farnesyl acetat	1726	-	1.17	-	-
62	Benzyl benzoate	1760	1.02	1.91	0.34	0.22
63	Benzyl salicylate	1866	0.19	1.25	-	-
	<b>Total</b>		<b>96.19</b>	<b>96.61</b>	<b>98.39</b>	<b>98.12</b>
	<b>Monoterpene hydrocarbons</b>		<b>9.11</b>	<b>51.02</b>	<b>3.53</b>	<b>30.92</b>
	<b>Oxygenated monoterpenoids</b>		<b>1.80</b>	<b>15.98</b>	<b>0.86</b>	<b>66.29</b>
	<b>Sesquiterpene hydrocarbons</b>		<b>74.49</b>	<b>15.05</b>	<b>56.39</b>	<b>0.36</b>
	<b>Oxygenated sesquiterpenoids</b>		<b>9.37</b>	<b>9.69</b>	<b>37.27</b>	<b>0.55</b>
	<b>Others</b>		<b>1.42</b>	<b>4.87</b>	<b>0.34</b>	<b>0.22</b>

<sup>a</sup> Retention indices on HP-5MS column; <sup>b</sup> Standard deviation was insignificant and excluded from the Table to avoid congestion; (-) Not identified.

In this study, 38 compounds were identified from *A. xanthioides* leaves' essential oil, which accounted for 96.19% of the total amount of the essential oil (Table 1). The main compounds in the essential oil of leaves are β-elemene (31.71%), δ-cadinene (10.69%), germacrene D (9.55%), bicycloelemene (8.12%), and bicyclogermacrene (7.93%). In particular, β-elemene is a compound reported to be able to fight cancer cells [45-47]. The essential oil from *A. xanthioides* roots was the most abundant, with 43 compounds identified as representing 96.61% of the total essential oil content (Table 1). The main compounds of the root essential oil are β-pinene (29.59%), terpinen-4-ol (10.77%), and α-terpinene (6.96%). The β-pinene was reported to have antifungal and antibacterial properties [48]. In addition, it inhibits weed growth through the disruption of membrane integrity as indicated by enhanced peroxidation, electrolyte leakage, and lipoxxygenase activity despite the upregulation of peroxidase [49]. For the essential oil of *A. xanthioides* stems, 28 compounds were identified as

representing 98.39% of the total essential oil content (Table 1). Similar to the leaf essential oil,  $\beta$ -elemene is still the main compound with the highest percentage (29.58%), along with spathulenol (26.89%) and bicycloelemene (6.19%). The spathulenol is sesquiterpene alcohol with the same main frame as azulene. It has antibacterial and antifungal properties along with anti-inflammatory and anti-cancer reactivities [50,51]. It is also considered an inducer of apoptosis. Spathulenol can also be used as a pesticide. In the essential oil of *A. xanthioides* fruits, only 22 compounds were identified, accounting for 98.12% of the total essential oil content (Table 1). Bornyl acetate is the most abundant constituent found in the essential oil of fruits (37.21%). This is a compound with analgesic and anti-inflammatory effects, especially inhibiting cancer cells' proliferation [52,53]. In addition to bornyl acetate, in the essential oil of fruits, there are other main compounds, including camphor (19.48%), camphene (14.62%), and limonene (9.64%). Camphor is widely used in traditional and modern medicine for its antibacterial property and its beneficial effects on the cardiovascular system. Furthermore, it has been used as a topical anti-itch, anti-insect, anti-inflammatory, and analgesic treatment [50]. In addition, its low solubility in aqueous solvents makes it an excellent candidate for distribution in nanocarriers [54]. The research by Feng *et al.* (2019) also showed that the essential oil with two main compounds, bornyl acetate and camphor, were capable of repelling and killing insects [55]. Therefore, the compounds identified in the studied oil samples could play a big role in the essential oil's biological potentials.

There have been some reports on the chemical composition of the essential oil extracted from *Amomum* species globally. For example,  $\beta$ -pinene (9.0%), caryophyllene oxide (6.6%),  $\beta$ -bisabolene (6.4%), and  $\delta$ -cadinene (6.2%) are the main compounds of the essential oil of *A. cannicarpum* leaves [56]. The main compounds identified in the essential oil of *A. subulatum* leaves are 1,8-cineole (39.8%) and  $\alpha$ -terpineol (11.5%) [21]. The research of Singtothong *et al.* (2013) identified that camphor (17.6%),  $\alpha$ -bisabolol (16.0%), camphene (8.2%), and  $\alpha$ -humulene (5.1%) were the main compounds in the essential oil of *A. biflorum* [57]. Those of the essential oil from *A. villosum* fruits are bornyl acetate (51.6%), camphor (19.8%), camphene (8.9%), and limonene (6.2%) [58]. For the essential oil of *A. tsao-ko* fruits, the main compounds are 1,8-cineole (45.24%), *p*-propylbenzaldehyde (6.04%), geraniol (5.11%), geranial (4.52%),  $\alpha$ -terpineol (3.59%), and  $\alpha$ -phellandrene (3.07%) [59]. However, we have not found complete reports on the essential oil's chemical composition of *A. xanthioides* leaves, stems, and roots in the world. We have only recorded a few reports on the essential oil's composition of *A. xanthioides* fruits from China [34,35]. The results obtained in this study, for the essential oil of *A. xanthioides* fruits, were consistent with the study of Ao *et al.* (2019) that bornyl acetate and camphor were two main compounds in the fruit essential oil [34]. Compared with the study of Ao *et al.* (2019), the concentrations of bornyl acetate and camphor in our fruit essential oil were lower. As highlighted previously, this difference can be attributed to growth, genetics, and environmental factors [60].

In particular, we have found some reports on the chemical composition of *A. xanthioides* essential oil in Central Vietnam [36,37]. However, our study samples were collected from Northern Vietnam. This leads to a clear difference between the concentration and composition of the essential oil previously reported and those from the current study. Specifically, the previously reported essential oil of *A. xanthioides* leaves, roots, and stems from Pu Mat National Park (Central Vietnam) was identified as containing 34, 41, and 25 compounds, respectively [36]. There are compounds found in the essential oil of *A. xanthioides* from Pu Mat National Park but not found in our essential oil sample and vice versa. For



example, our study sample's essential leaf oil further identified compounds including  $\alpha$ -thujene,  $\alpha$ -terpineol, bornyl acetate,  $\alpha$ -cubebene, benzyl salicylate but no phytol compounds. Along with that, the concentration of compounds in our essential oil samples was different from that of *A. xanthioides* essential oil samples collected from Pu Mat National Park. The essential oil of *A. xanthioides* fruits from A Luoi – Thua Thien Hue (Central Vietnam) was identified as containing 15 compounds, with bornyl acetate (27.26%), camphor (23.73%), endo-borneol (13.78%), camphene (11.62%), D-limonene (10.96%), and  $\beta$ -myrcene (6.17%) as the main ones [37]. Compared with the essential oil of *A. xanthioides* fruits from A Luoi – Thua Thien Hue, our study has identified 9 new compounds (tricyclene, 1,8-cineole,  $\gamma$ -terpinene,  $\alpha$ -amorphene,  $\beta$ -bisabolene,  $\tau$ -muurolol,  $\beta$ -eudesmol,  $\alpha$ -cadinol, and benzyl benzoate) presenting in the essential oil of *A. xanthioides* fruits, but there has been no compound of (+)-3-carene and geraniol. Along with that, bornyl acetate is also the most abundant compound. It has a higher concentration than the fruit essential oil from A Luoi – Thua Thien Hue with 37.21%. Again, changes in habitat and climate can affect the composition of the harvested products. As growth and development stages, farming, and genetic characteristics can change significantly [40,43,60].

#### 4. Conclusions

Our research on *A. xanthioides* essential oil collected from Northern Vietnam showed a high variation in the chemical composition of oils extracted from different organs. The highest essential oil yield was recorded for fruits. The compounds found in the essential oil of leaves and stems are mainly sesquiterpene (83.86-93.66%). Those found in roots and fruits are mainly monoterpene (67.00-97.21%). The composition and the concentration of the essential oil found in this study are different from those of *Amomum* plants collected in Vietnam or elsewhere in the world. This difference may be due to several factors such as environmental conditions and climate at the collection site, time of collection, as well as the age and nature of plants.

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#### Conflicts of Interest

The authors declare no conflict of interest.

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