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Chemical compositions and stability of Vietnamese *Homalomena occulta* essential oil under the influence of storage conditions

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Abstract

The composition of *Homalomena occulta* essential oil in the northern region of Vietnam was analyzed and determined by GC-MS. The main constituents of essential oils are Linalool, Terpinene-4-ol, Geraniol, α-epi-Muurolol, α-Cadino, (-) - Spathulenol, Terpineol, Sabinene, ... Under different light, dark and temperature conditions of 4ºC, 45ºC and the storage time changes in the composition of the essential oils are determined. Under the influence of light and temperature leading to the main Linalool compound, Terpinene-4-ol markedly declined during the period after 6 months of storage. On the other hand, some compounds Hotrienol, Terpineol, (-) - Spathulenol and α-epi-Muurolol tend to increase from 0.057% to 0.459%. Furthermore, the essential oil components showed minimal changes and remained of quality when stored at 4 ° C compared to 45 ° C conditions. Research results have shown that Homalomena occulta essential oil is a promising aroma, it is necessary to conduct many studies to increase the stability and quality of the essential oil when incorporated into the matrix of pharmaceuticals, cosmetics, and food.

Keywords: *Homalomena occulta,* Storage, Essential oïl, Temperature, Light, Oxidation.

1. Introduction

Essential oil is a liquid containing volatile aromatic compounds extracted from plant parts. Almost every part of the plant can extract essential oils, the most common ones are: flowers, leaves, bark, fruit bark, stems, roots ... For centuries, these substances have been widely used in the medical, perfume and cosmetic industries [1-15]. Each essential oil has its own characteristics and effects. Essential oils can be added to foods as part of spices or fragrance ingredients in cosmetics. Essential oils contain very strong natural biological agents with different properties. Essential oils possess a range of antifungal, antibacterial, antiparasitic, and insecticidal properties leading to a wide range of applications [16-27]. Vietnam, with its diverse tropical and ecological climate, containing a rich source of essential oils.

The genus Homalomena (Araceae) is widely distributed in the United States and tropical Asia and includes more than 140 species, *Homalomena occulta* (Lour.) Schott is considered a species of this genus. *Homalomena occulta* is considered to be an herb used to strengthen the body, fight inflammation, cure

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typhoid, rheumatism, tendon aches, or palsy. In folklore, Homalomena occulta is often used to treat rheumatism, stomach pain, as a digestive stimulant, stomach ache. On the other hand, *Homalomena occulta* is often used to treat wounds or mixed with some other herbs to treat fractures, hemorrhages, limb paralysis and stomach pain. Homalomena occulta essential oil has strong antifungal properties, especially fungi such as Curvularia pallesen, Aspergillus niger, Fusarium graminearum ...) [28-31]. Homalomena occulta is a perennial plant, with stout rhizomes, green, 1-2 cm diameter. Staggered leaves, with stalks between 18 and 25 cm long, green, soft, smooth, the blade of an arrowhead shape, 11-15 cm long, 7-11 cm wide, the flowers bloom in April - June, and the tree blooms around April to June, and the fruit is around August to October. Homalomena occulta essential oil is a rich source of Linalool, this is a special aromatic compound with high value in aromatherapy technology [32]. A substance used to produce high-class perfumes. Essential oils are used as aromatherapy and are obtained from different aromatic parts of plants by methods such as steam distillation, cold pressing, leaching, supercritical CO2 extraction, microwave-assisted hydrodistillation [29, 33, 34]. The method of direct distillation with water is one of the most commonly used and easily scaled up to an industrial scale. This method is used to extract the most essential oils. Under extraction by direct distillation with water, the concentration of Homalomena occulta essential oil is usually from 0.8 to 1%, the essential oil is calculated according to the dry content. Essential oil is a clear solution of yellow color, warm aroma, long fragrance time. Homalomena essential oil occulta consists of about 38 compounds, of which Linalool is the main component (accounting for 69.71 - 71.20%). The components with remarkable content are terpinene-4-ol (4%), linalyl acetate (3.3%), α-terpineol (1.2%), and β-caryophyllene (1%) [33]. Essential oils obtained from aromatic plants are a complex mixture of several chemical compounds including terpenes, alcohols, aldehydes, and phenols. The stability of Homalomena occulta essential oil is a factor that needs more research because there is very little chemical information about changes in the content and composition of essential oils under storage conditions. On the other hand, essential oils are also easy to lose smell, reduced activity if exposed to air, sunlight, leading to oxidation occurs... [35]. Therefore, the preservation and storage of essential oils properly is essential. In this study, the essential oil was stored in four different conditions (morning, evening, 4 °C, 45 °C) and lasted for six months to assess changes in content and composition of Homalomena occulta essential oil during storage. The results are analyzed by GC-MS method to identify the modified components. Essential oils will be collated and evaluated based on the results of the controlled essential oil sample. Therefore, the current assessment provides a comprehensive overview of the possible changes in Homalomena essential oil and the factors affecting its stability under various conditions, in order to find out the best storage methods and can be applied to all different essential oils.

2. Materials and methods

2.1. Plant material

Homalomena occulta is collected from natural forests in the mountainous regions of the northern region of Vietnam. The tree prefers wet places and along streams. Plants usually harvest old roots in the spring or autumn. Rhizomes after being collected will be washed, peeled off, and the small roots. Then it will be cut into short pieces of about 10 - 27cm and dried in direct sunlight or dried at a temperature of 50 - 60ºC.

2.2. Extractions methods

8.5kg *Homalomena occulta* dry after processing is pureed, raw materials are into the tank of extraction equipment. The hydrodistillation is performed in a circulating Clevenger type device and the extraction is stopped after ensuring no essential oils are left. Crude essential oil is treated through a filter funnel to remove water and anhydrous with sodium sulfate to obtain the finished essential oil. Essential oils are obtained with an efficiency of 0.718% based on dry material.

2.3. *Storage Condition*

The essential oil was analyzed immediately after extraction and processed as a control, to evaluate and identify changes in quantity and quality of Homalomena mysulta essential oil during storage. The other samples are kept in sealed glass jars, of equal masses. Each set of samples is stored under different light and temperature conditions: in a dark bottle at room temperature, kept in direct daylight at room temperature, in an oven (45°C) and a refrigerator (4°C) for 6 consecutive months. The changes of volatile compounds in essential oils were evaluated based on GC-MS analysis, after storage time was 1 month, 2 months, 4 months, 6 months.

2.4. Gas chromatography-mass spectrometry (GCMS)

GC Agilent 6890 N combined with inert HP5-MS and MS 5973 columns were used to analyze the chemical composition of Homalomena mysulta essential oil. The flow rate constant at 1 mL / min. Injector temperature is 250°C and dispensing rate is 30. Sample heating program: 50°C hold for 2 minutes increase 2°C/min to 80°C, further increase 5°C/min to 150°C, continue to increase 10°C/min to 200°C, increase 20°C/min to 300°C hold for 5 minutes. The pressure of the head column is 9.3 psi. 25µL of essential oil is added to 1.0 mL of n-hexane. The compounds were identified by comparing retention indexes with the Wiley library or with the published mass spectrometry.

3. Results and discussion

For a more accurate comparison and assessment of the quality of the *Homalomena occulta* essential oil product immediately after it has been obtained, conduct analysis by gas chromatography with GC-MS mass spectrometry. The analytical results are presented in Table 1 and Figure 1 shows that there are 35 peaks equivalent to 34 constituents with elution times and different concentrations were quantified, with an unspecified compound. The main constituents of essential oils are Linalool (63,394%), Terpinen-4-ol (7.1%), Geraniol (3,996%), α-epi-Muurolol (2,118%), α-Cadinol (2,581%), (-) - Spathulenol (1,364%), Terpineol (0.723%), Sabinene (0.614%), Caryophyllene (0.449%), Caryophyllene oxide (0.569%),... The chemical composition from essential oils in the research reports will vary. For example, Homalomena occulta was collected from Hubei province, China, studied by Chao Liu et al. Linalool (47.7%) was identified as the main compound of H. constulta essential oil, followed by α-terpineol (11.2%) and 4-terpineol (16.5%) and 87.3% of the total among the components are monoterpenoids [33]. In 2011, Ling-Bin Zeng et al. analyze the chemical composition in the shelf oil essence purchased from Hong Kong. Linalool (11.1%), α-terpineol (13.8%), 4-terpineol (4.92%), δ-cadinene (4.91%), epi-α-cadinol (14.8%) and α-cadinol (14.8%) is the main ingredient, accounting for 64.3% of the total essential oil content [28]. On the other hand, Yang Zaibo and Zhao Chao use SPME (solid-phase microextraction) method to extract Homalomena occulta essential oil and GC-MS analysis. The results indicated 98 components, of which 70 were identified, accounting for about 96.26% of the total number of essential ingredients. The main components are γ-terpinene (1.35%), linalool (29.90%), terpineol-4 (5.73%), linalyl acetate (2.64%), β caryophyllene (2.19%), cad-cadinene (6.09%), α -cedrol (5.24%), tert-cadinol (3.21%), tert-muurolol (4.65%), ... [34]. Linalool (62.5%), terpene-4-ol (7.08%), dcadinene (5.57%), α-selinene (2.19%), t-muurolol (5.32%), ʍ-cymene (2.19%), α-cadinol (3.71%), viridiflorol (3.69%), γ-muurolene (1.81%) and spatulenol (1.81%) are the main compounds in the Homalomena occulta essential oil collected in Sonitpur district of Assam, India [31].

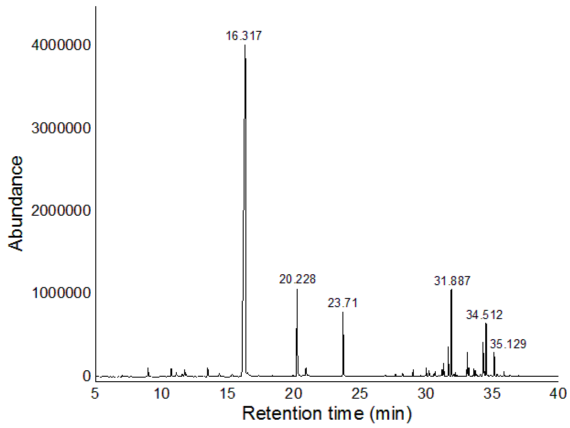


Fig 1. Chromatogram of components identified in the *Homalomena occulta* essential oil

These findings indicate that there is a large difference in the chemical composition of H. constulta essential oils, possibly due to geographical factors, climate, seasonal conditions and delays in harvesting or Shipping to the distillation factory leads to a reduction in the quality of the essential oil components, as well as storage time can contribute to the difference.

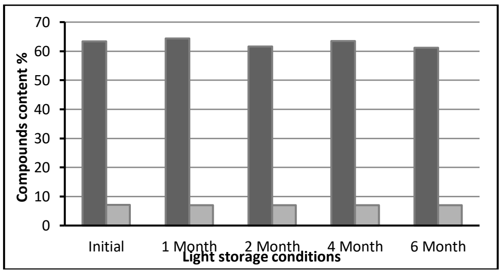
In addition, external factors such as accessibility of oxygen, temperature and light in the atmosphere need to be considered. Moreover, the composition of the essential oil, the compound structure, and the presence of impurities can also govern stability. UV rays in light break down molecules, promoting the formation of free radicals that affect the quality of essential oils [36-38]. When exposed to ultraviolet light, some substances in the essential oil increase, and some substances decrease significantly [39]. The change of the main ingredient in the essential oil is clearly shown in the graphs of Figure 2 and Table 1.

The storage under direct on light, the concentration of Linalool in *Homalomena occulta* essential oil tended to increase slightly after 1 month of storage and instability, phenomenon decreased to 1.798% after 2 months of storage, then increased the level by 1.911% in the 4th month of storage and continue to decrease at the end of the storage period. In contrast, the concentration of Terpinen-4-ol in *Homalomena occulta* essential oil tended to decrease over the time of storage, although Terpinen-4-ol content decreased only by 0.106%. In addition, some other contents in Homalomena occulta essential oils have fluctuations after storage time, specifically the content of Hotrienol, Terpineol, (-) - Spathulenol, and α-epi-Muurolol increased by 0.057%, 0.331%, 0.412% and 0.459% after 6 months of storage.

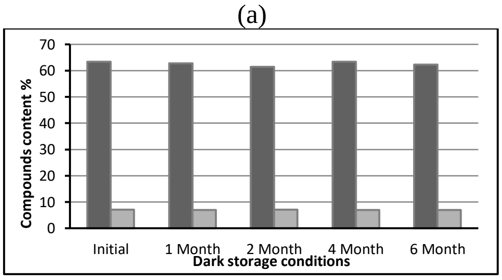
On the other hand, essential oils need to be stored in dark conditions, with a dark glass jar, tightly closed, in a cool place to ensure long-term quality [13]. According to previous studies, significant changes in its composition were observed during storage and its sensory properties remain unaffected by the preservation process. Specifically, the content of Linalool has a change of content after 2 and 4 months, but when stored until the 6th month, the content tends to decrease by 1,116% compared to the original content. At the same time, Terpinen-4-ol also showed a decrease during storage, although the content changed not much compared to the control sample. The difference was shown when α-Cadinol content increased by 0.67% at the end of the storage period.

Temperature is also the main environmental factor that affects the stability of the essential oil, chemical reactions accelerate with the increase in heat due to the temperature dependence of the reaction rate. Essential oils stored under the influence of temperature (4 °C and 45 °C) also occur denaturation in the content and composition of the essential oil is clearly shown in Figure 3 and Table 1. Further, GC-MS analysis results showed that the most prominent change that occurred during storage of Homalomena occulta essential oil at 45 °C compared to 4 °C was the rapid decline of the main ingredient Linalool (1.924%) and Terpinen-4-ol (0.2%) at the end of the storage period. Meanwhile, with a storage temperature of 4ºC the content of the compound was not stable during storage. Specifically, Linalool decreased continuously by 1.452% after 2 months and tended to increase again in the 4th month, but when stored until the 6th month, the overall content decreased by 0.295%. With the increase in storage time, the content of Terpinen-4-ol increased by 0.017% at the end of 6 months of storage.

An important factor that affects the change and damage of essential oils is oxidation under the action of oxygen available in the atmosphere. The storage capacity of various monoterpenes and the changes in composition, as well as the physical and chemical properties of essential oils, are usually more pronounced in bottle when there are few or no there are gaps. The storage of essential oils evaporates compounds of lower boiling point, mainly hydrocarbon monoterpene. From the findings of this study, typically α-Terpinen and Cadina-1 (6), 4-diene evaporated completely during the 4 and 6 month storage periods. Therefore, the quality of essential oils is further reduced during a six-month storage period at room temperature and at 45ºC. A number of reports have been made based on monitoring of volatile plant extracts and essential oil components that have shown that stability is lost with prolonged storage as well as increased temperatures from 23 to 38 ºC (Claudia Turek et. al 2012) and 0 to 28 ºC (Narayanan Gopalakrishnan 1994) [38, 40]. The results indicate that the components of the essential oil easily change when extracted and stored, whereby factors such as light, oxygen and temperature, have an important influence on the change process [41]. This can lead to color changes, increased viscosity or unpleasant aroma formation, often with a pungent odor due to composition changes and the increase of oxidizing compounds.

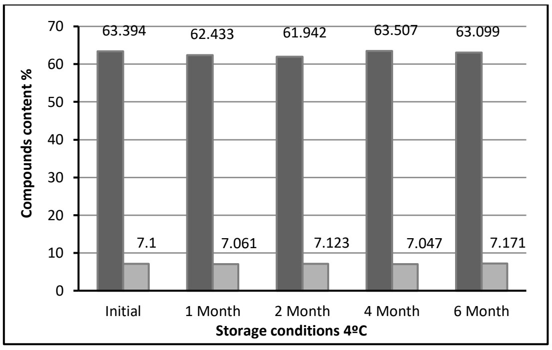


(a)

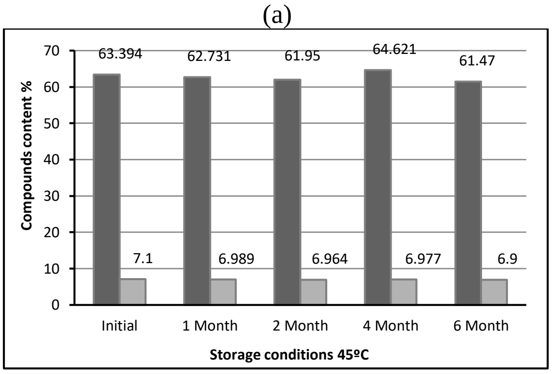


(b)

Fig 2. The influence of light on the storage of *Homalomena occulta* essential oils (a) light, (b) dark



(a)



(b)

Fig 3. The influence of temperature on the storage of *Homalomena occulta* essential oils (a) 4 ºC (b) 45 ºC

Linalool appears to be the main ingredient with different concentrations in essential oils, as indicated in previous analytical reports and included current research. Linalool is related to two optical isomers of a terpene alcohol. An optical isomorphic are mirror images that are not symmetrical and do not overlap. R-linalool produces a scent similar to wood and lavender and S-linalool produces a sweet floral scent [42]. Both linalool optical isomers are found in nature. Linalool functions as a fragrance ingredient in cosmetics and personal care products. In fact, linalool is used to create scents in the range of 60% to 80%. In addition, Linalool exhibits strong antibacterial activity against Escherichia coli, Staphylococcus aureus, Candida albicans, along with periodontal and cariogen bacteria [43]. Linalool is an essential oil compound that is easily oxidized and forms stable hydroperoxide by external factors and in the processing and storage of essential oils. Therefore, the refining process and storage conditions of essential oils are essential, in order to increase the value and increase the efficiency of essential oils.

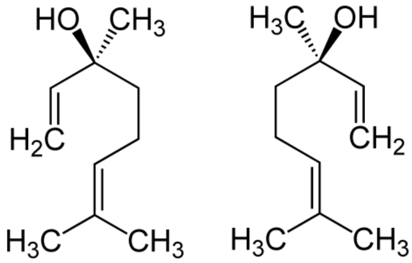


Fig 4. Optical isomers S-linalool và R-linalool of Linalool compound

4. Conclusion

In this study, Homalomena occulta essential oil was obtained through hydrodistillation, with an optimum yield of 0.718%. The 35 compounds of the essential oil are determined through the GC-MS method, with Linalool and Terpinen-4-ol being the main components. The stability of the essential oil was assessed based on the 6-month storage period of the essential oil under conditions of direct sunlight, darkness, temperatures of 4 °C and 45 °C. The results show that storing Homalomena occulta at low temperatures prevents the ability to increase or decrease the concentration of the essential oil components and helps keep the quality of the essential oil less changed. However, the results reported here suggest that storage under light and darkness at room temperature not only does not reduce the quality of essential oils, but also some important index components such as Hotrienol, Terpineol, (-) - Spathulenol and α-epi-Muurolol significantly increased. In a nutshell, the analysis and preservation of secondary plants, especially essential oils, need to be investigated further on oxidation and the importance of light, temperature and the presence of oxygen in promoting recession to increase the value of the compound in today's pharmaceutical and cosmetic industry.

Table 1. Ingredients of *Homalomena occulta* essential oil after for 6 months stored under the conditions of light and temperature

Name	Initial	1 Month				2 Month				4 Month				6 Month			
		Light	Dark	4°C	45°C	Light	Dark	4°C	45°C	Light	Dark	4°C	45°C	Light	Dark	4°C	45°C
Sabinene	0.614	0.554	0.552	0.586	0.523	0.544	0.535	0.596	0.499	0.54	0.554	0.577	0.459	0.478	0.503	0.593	0.388
3-Carene	0.651	0.586	0.567	0.611	0.534	0.547	0.558	0.611	0.514	0.555	0.546	0.588	0.465	0.467	0.52	0.618	0.403
α-Terpinen	0.365	0.263	0.292	0.353	0.205	0.262	0.258	0.332	0.191	0.202	0.187	0.322	-	-	-	0.302	-
o-Cymene	0.313	0.295	0.359	0.318	0.512	0.427	0.4	0.301	0.46	0.527	0.522	0.326	0.481	0.588	0.428	0.258	0.365
Cyclohexen	0.636	0.504	0.564	0.602	0.548	0.546	0.55	0.597	0.5	0.556	0.549	0.585	0.407	0.476	0.493	0.561	0.364
Eucalyptol	0.248	0.181	0.208	0.212	0.216	0.194	0.21	0.228	0.202	0.223	0.227	0.216	0.203	0.226	0.229	0.228	0.205
γ-Terpinen	0.874	0.692	0.742	0.849	0.555	0.657	0.653	0.823	0.537	0.541	0.508	0.805	0.311	0.213	0.388	0.762	0.308
cis-Linaloloxide	0.291	0.258	0.292	0.289	0.33	0.321	0.296	0.269	0.355	0.34	0.339	0.293	0.436	0.397	0.334	0.275	0.381
cis-Linalool Oxide	0.298	0.274	0.297	0.3	0.416	0.317	0.37	0.293	0.423	0.352	0.413	0.301	0.475	0.447	0.342	0.278	0.434
Linalool	63.394	64.377	62.819	62.433	62.731	61.596	61.412	61.942	61.95	63.507	63.391	62.258	64.621	61.189	62.278	63.099	61.47
Hotrienol	0.597	0.545	0.61	0.617	0.58	0.575	0.634	0.622	0.571	0.527	0.576	0.546	0.519	0.654	0.644	0.757	0.633
Terpinen-4-ol	7.1	7.007	7.002	7.061	6.989	7.051	7.07	7.123	6.964	6.993	6.989	7.047	6.977	6.994	7.017	7.171	6.9
Terpineol	0.723	0.687	0.743	0.735	0.747	0.768	0.754	0.978	0.747	0.759	0.759	0.978	0.78	1.054	0.956	0.99	0.756
Geraniol	3.996	3.88	3.817	3.84	3.906	3.949	3.96	4.017	4	3.937	3.938	4.081	4.024	4.085	3.989	3.759	3.997
Copaene	0.239	0.215	0.16	0.234	0.156	0.162	0.164	0.227	0.241	0.156	0.184	0.186	0.144	0.243	0.204	0.205	0.244
β-Elemen	0.19	0.234	0.271	0.262	0.257	0.283	0.27	0.253	0.265	0.218	0.244	0.267	0.246	0.251	0.224	0.257	0.214
Caryophyllene	0.449	0.425	0.434	0.444	0.404	0.426	0.445	0.454	0.405	0.406	0.402	0.442	0.363	0.376	0.397	0.443	0.388
α-Caryophyllene	0.538	0.594	0.521	0.534	0.531	0.516	0.588	0.548	0.529	0.519	0.511	0.599	0.48	0.491	0.512	0.562	0.535
Alloaromadendrene	0.494	0.482	0.486	0.497	0.467	0.397	0.423	0.414	0.418	0.399	0.425	0.403	0.379	0.481	0.47	0.485	0.478
Cadina-1(6),4-diene	0.156	-	0.152	0.167	0.118	0.137	0.146	0.156	0.116	0.113	0.107	0.179	-	-	-	0.144	0.097
Naphthalene	0.353	0.377	0.371	0.374	0.377	0.367	0.384	0.363	0.368	0.363	0.358	0.38	0.362	0.394	0.383	0.35	0.387
Cyclohexane	0.687	0.639	0.648	0.676	0.539	0.6	0.645	0.696	0.543	0.514	0.502	0.751	0.442	0.454	0.501	0.675	0.49
α-Muurolene	0.841	0.855	0.24	0.834	0.832	0.835	0.894	0.872	0.842	0.808	0.803	0.819	0.771	0.876	0.845	0.842	0.841
Naphthalene	1.657	1.678	1.427	1.645	1.635	1.664	1.744	1.703	1.671	1.619	1.611	1.607	1.565	1.783	1.691	1.651	1.721
Cadina-3,9-diene	4.864	4.963	0.582	4.892	4.71	4.856	5.096	5.006	4.803	4.639	4.606	4.786	4.325	4.736	4.751	4.83	4.785
Naphthalene	0.234	0.229	0.432	0.237	0.231	0.24	0.253	0.252	0.237	0.235	0.231	0.229	0.218	0.251	0.241	0.234	0.253
(-)-Spathulenol	1.364	1.389	0.305	1.374	1.509	1.469	1.538	1.384	1.577	1.443	1.463	1.314	1.53	1.776	1.675	1.36	1.703
Caryophyllene oxide	0.569	0.587	0.24	0.551	0.606	0.578	0.619	0.578	0.63	0.581	0.591	0.537	0.62	0.738	0.694	0.582	0.708
Ledo	0.478	0.424	1.427	0.463	0.435	0.437	0.459	0.434	0.452	0.415	0.422	0.446	0.419	0.499	0.542	0.426	0.499
Humulene-1,2-epoxide	0.287	0.292	0.582	0.29	0.316	0.306	0.321	0.296	0.331	0.305	0.311	0.424	0.326	0.402	0.364	0.286	0.381
α-epi-Muurolol	2.118	2.17	2.114	2.462	2.238	2.249	2.301	2.146	2.382	2.077	2.12	2.021	2.12	2.577	2.453	2.25	2.521
1-Naphthalenol	0.353	0.287	0.288	-	0.386	0.313	0.263	0.294	0.26	0.191	0.256	0.27	0.276	0.329	0.306	0.268	0.311
α-Cadinol	2.581	2.655	2.721	2.619	2.852	2.695	2.843	2.677	2.907	2.508	2.713	2.562	2.688	3.387	3.251	2.801	3.279
unknown	1.172	1.138	1.201	1.18	1.214	1.194	1.253	1.19	1.236	1.115	1.167	1.094	1.132	1.396	1.328	1.148	1.37
Oplopanone	0.276	0.262	0.29	0.283	0.313	0.301	0.314	0.228	0.303	0.266	0.288	0.268	0.284	0.34	0.317	0.222	0.37

5. Acknowledgment

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