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CHARACTERIZATION OF ESSENTIAL OIL COMPOUNDS ON LEAVES AND RHIZOMES OF TURMERIC (*Curcuma domestica* Val.)

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Abstract: This study aimed to determine the characterization of essential oils in turmeric leaves and rhizomes using Chromatography-Mass Spectrophotometry (GC-MS). In this study, essential oils were obtained by steam distillation on the leaves and rhizomes of turmeric seven months old in the Panjalu area. Based on the research results, there were eight similar compounds, alpha-phellandrene, 1,3-cyclohexadiene 1-methyl-4-(1-methyl ethyl), P-Cymene, D-Lymonene, Eucalyptol, aR-Tumerone, Tumerone, and Curlone.

Keywords: *Turmeric Essential Oil, Steam Distillation, Chromatography-Mass Spectrophotometry (GC-MS).*

INTRODUCTION

Turmeric is a perennial herbaceous aromatic plant from the ginger family (Zingiberaceae). It is assumed that turmeric originated in China, and Buddhist monks or Chinese migration brought it to the Indian subcontinent. Nowadays, it is cultivated in Asian countries (Bangladesh, China, Thailand, Cambodia, Malaysia, Indonesia, and the Philippines) and some parts of South America (Peru and Bolivia) [1-3].

The plant has yellow flowers and reaches a height of about 1 m. The underground rhizome is yellowish, consisting of two main parts: the egg-shaped (mother) rhizome and the long cylindrical, branched primary, secondary, and even tertiary rhizomes. Turmeric rhizome contains two major classes of secondary metabolites: phenolic curcuminoids and essential oil. For the first time, curcumin was isolated by Vogel in 1842. Then, in 1910, its structured manner was described by Lampe and Milobedeska [5].

Curcumin is a liposoluble compound that easily dissolves into an organic solvent such as methanol, ethanol, and acetone. However, poor water solubility often limits biomedical uses using aqueous systems [6]. Curcumin has been studied because of its antibacterial, antifungal, and antiviral activity [5, 7-8].

Members of this genus have a long history for their medicinal purposes [7] and nutritional values in the cosmetics industry. The major pharmacologically active constituents of Curcuma species are curcuminoids and essential oils [9-10]. Curcumin, dimethoxy curcumin, and bisdemethoxycurcumin are present in turmeric [11]. Commonly these three compounds are called curcuminoids [12].

Essential oils are the volatile and fragrant substances of plants. They are obtained from plants through steam distillation or other processes [13, 2].

High yield and high-quality curcumin are very important to choose appropriate and effective methods and operate these under optimal conditions. Various substances co-exist in crude curcumin extracts, including essential oil, sugar, dimethoxy curcumin (DMC) derivatives, bisdemethoxycurcumin (BDMC), and other small molecules. Commercially available crude curcumin usually contains variable amounts of the other two compounds, typically about 16-26 % DMC and 2-8% BDMC [14]. It was found that hydro–distillation of leaves and rhizomes obtained 2.2% oil yield and more than 80 compounds [15].

Chemical constituents of turmeric rhizomes include volatiles and non-volatiles. The chemical constituents of volatile oil were identified using GC and GC-MS. The main components are ar-turmerone, zingiberene, turmerone, and curlonee [2, 16-17]. The non-volatile compounds are coloring agents and rich sources of phenolics. The aroma of turmeric is contributed by its steam volatile essential oils, while the phenolic compounds, curcumin, and its analogs account for its bright yellow color [18].

Essential oils of Curcuma species are relatively complex, with hundreds of components, including terpenes and oxygenated terpenoids [19].

The essential oil could be obtained from fresh and dry leaves, flowers, dry roots, and fresh and dry turmeric rhizomes. Dried rhizomes and leaves are used for essential oil extraction in the industry [4]. Rhizomes (even though they contain a higher amount of active compounds than other plant parts) have a higher oil content than leaves, 5-6% vs. 1-1.5%, respectively. Monoterpenes dominate the essential oils from leaves and flowers. While those from roots and rhizomes primarily contain sesquiterpenes. The major volatile principles of rhizome oil are α- and β-turmerone and ar-turmerone [1, 20].

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RESEARCH METHODS

The research was conducted at the Indoscience Essential Oils Laboratory and the Central Laboratory of Padjadjaran University. The study was conducted in August – November 2021.

This type of research is a descriptive, component analysis of chemical compounds using GC-MS. This research was carried out in several stages: sample determination, essential oil distillation using steam distillation, physical properties test (color, refractive index, specific gravity, and solubility in alcohol), and compound analysis using GC-MS (Gas Chromatography-Mass Spectrometry).

Plants Materials

7-month-old turmeric leaves and rhizomes originating from the Panjalu area that has been scientifically determined for their classification.

Reagents and Chemicals

Methylene-chloride, 70 and 80% ethyl alcohol, and sodium sulfate (Na_2SO_4).
Tools are GC-MS Spectrophotometer and laboratory glass equipment.

Work procedures

1. Sample Preparation
2. Determination of Turmeric Plants
3. Isolation of Essential Oils by Steam Distillation

Processing and data analysis

Processing and analysis of data using the results of the GC-MS analysis process.

RESULT AND DISCUSSION

Research has been conducted at the Indoscience Essential Oils Laboratory and the Central Laboratory of UNPAD on the characterization of turmeric oil compounds using the GC-MS (Gas Chromatography and Mass Spectrometry) method.

Determination of Turmeric Plants

Determination of turmeric plants was carried out in the ITB (Bandung Institute of Technology) laboratory. The results are:

Tribe	: Zingiberaceae
Species Name	: Curcuma longa L.
Synonyms	: Curcuma domestica Valeton
Local Name	: kunyit

The purpose of determination is to classify the turmeric plant in taxonomy correctly. The results of the determination made stated that the type of turmeric plant was Curcuma domestica Valeton.

Turmeric Essential Oil Steam Distillation

The process of making essential oils is carried out using steam distillation. The distillation of turmeric leaves and rhizomes is carried out for ± 7 hours. Before distillation, the leaves and turmeric rhizomes are cut into small pieces and left for 2-3 days in a room without direct sunlight. The cutting process is to speed up the drying of the sample. It expands the material's surface so that water vapor easily penetrates the turmeric tissue and brings the essential oil from the turmeric tissue out with water vapor. During the distillation process, the distillate that comes out of the condenser is in the form of essential oil, which is condensed with water. After completing the distillation process, transfer the essential oil into a measuring cup.

Based on the observations, two layers will be formed, the bottom layer is water, and the top layer is essential oil. The results of the essential oil obtained are then separated by adding anhydrous Na2SO4 little by little, about 5 mg, until the Na2 SO4 does not form lumps as an indication that all the water in the turmeric essential oil has been taken. The purpose of adding Na2SO4 anhydrous is to extract the essential oil water. Then the essential oil obtained is stored in a glass bottle, dry, and tightly closed. The aim is to avoid light oxidation. Furthermore, the oil that can be tested for physical properties and yield calculations is then carried out characterization.

The yield of Turmeric Oil

The yield produced from steam distillation of 4 kilograms of turmeric rhizome was 8 mL, with a yield value of 0.21%, and 1.5 kg of turmeric leaves produced 8 mL of essential oil, with a resulting yield value of 0.44%. The organoleptic test of the turmeric rhizome essential oil was clear yellow, with a distinctive turmeric smell. In contrast, the organoleptic results of the turmeric leaf essential oil were clear white with a distinct minty smell.

Physical Test with Refractive Index Test Parameters, Specific Gravity Test, and Solubility Test in 80% Alcohol

From the results of physical tests carried out on both samples of essential oils on turmeric leaves and rhizomes, the following results were obtained (table 1).

Physical examination of the essential oil on turmeric leaves and rhizomes aims to determine the quality of the essential oil obtained.

Table 1. Physical Test of Turmeric Leaf and Rhizome Essential Oil

No	Test Type	Turmeric Rhizome	Turmeric Leaves
1	Color, smell	Clear yellow, turmeric smell	The clear, distinctive mint smell
2	Specific gravity	0.9430	0.8501
3	Refractive index	1.5063	1.4745
4	Alcohol solubility	1:4	1:4

Characterization Results Using GC-MS

The essential oil obtained from steam distillation was then characterized using the Gas Chromatography-Mass Spectrometry (GC-MS) tool. The chromatogram results from the analysis of essential oils on turmeric leaves showed the presence of 19 peaks of compounds with different retention times, which can be seen in Figure 1.

And which shows the presence of 19 compounds, retention time, peak height, and % area in the turmeric leaf essential oil chromatogram in Figure 1 can be seen in Table 2.

From the results of the analysis of essential oils in turmeric, leaves are compared with the results of the analysis of essential oils in turmeric rhizomes. The analysis results of essential oils in turmeric rhizomes contained 30 compounds, which can be seen in Figure 2.

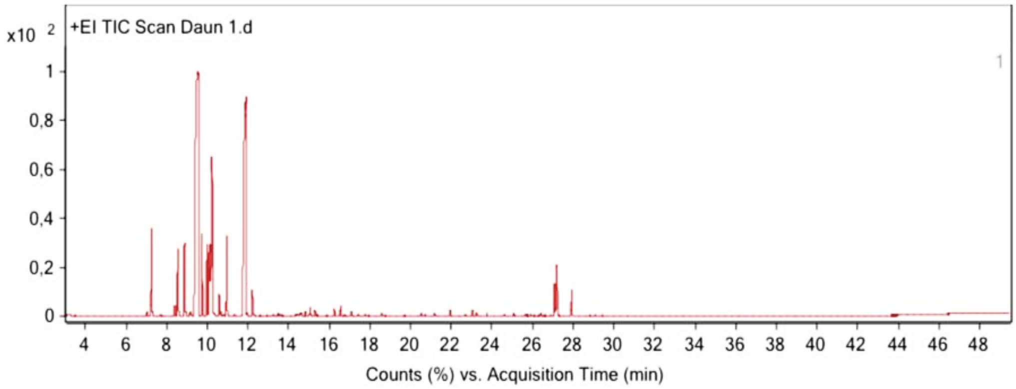

Figure 1. Chromatographic Pattern of GC-MS Analysis of Turmeric Leaf Essential Oil

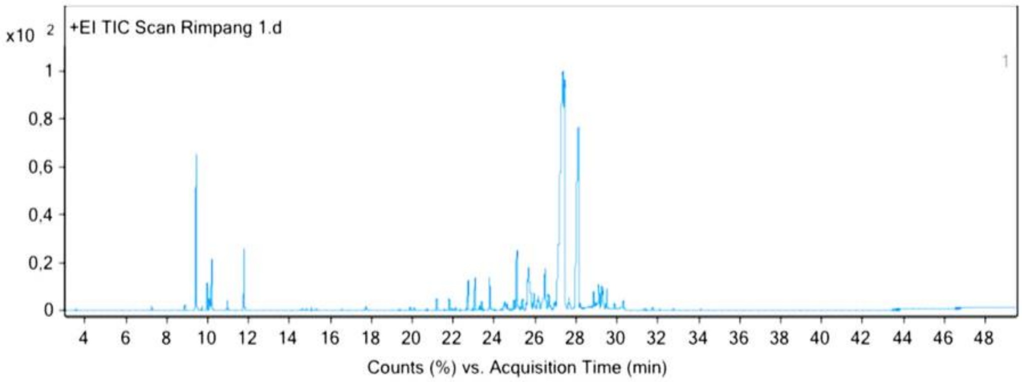

Figure 2. Chromatogram of GC-MS Analysis of Turmeric Rhizome Essential Oil

And which shows the presence of 30 compounds, retention time, peak height, and % area of essential oils in turmeric rhizomes in Figure 2 can be seen in Table 3.

Before characterizing the essential oil on the leaves and rhizomes of turmeric, the sample was prepared using a 10 L pipette and then added with 1 mL of methylene chloride. The aim was to use methylene chloride. It evaporated quickly and did not interfere with the chromatogram of the original compound. It was then homogenized until the oil was homogenized. The volatile matter and methylene chloride were thoroughly mixed before the sample was injected into the GC-MS syringe. It was first rinsed with methylene chloride solution three times. Then 1 L of the sample in the vial was taken using a syringe, injected through the injector at a temperature of 3000C, and evaporated until the sample turned into steam or gas. The sample in the form of gas is carried by a carrier gas, namely Helium gas with a constant flow rate, into a separating column in the form of a capillary column. The sample components will separate when passing through the column because of the difference in the adsorbent power of the stationary phase to the sample components. The mobile phase

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will push the separated components to exit the column.

After the sample is separated into its components, each component will exit the column with the mobile phase. The concentration can be measured with a detector that will generate a signal, the level, and then sent to the reader. Which separated sample components will produce a spectrum or peak because each component is held in the column for different retention times?

The analysis of volatile oil compounds in turmeric leaves and rhizomes using GC-MS obtained 19 compounds in turmeric leaves that were seen from different peak points, with the three highest peak points. It is alpha-phellandrene ($C_{10}H_{16}$), 1,3 – cyclohexadiene, one methyl - 4 - (1 - methyl ethyl) ($C_{10}H_{16}$), and eucalyptol ($C_{10}H_{18}O$).

There are 30 compounds in the turmeric rhizome which have the three highest peak points, namely aR – turmerone ($C_{15}H_{20}O$), curlonee (C15H22O), and turmerone ($C_{15}H_{22}O$).

Table 2. Components of Many Compounds from GC-MS Results of Turmeric Leaf Essential Oil

Peak	Compounds	Start	RT	End	Height	Area	Area %
1	Bicyclo [3.1.1]hept-2-ene, 3,6,6-trimethyl-	7187	7257	7356	135266158	340895273	849
2	Bicyclo[3.1.0]hex-2-ene, 4-methyl-1-(-1-methylethyl)	8327	8386	8470	16238794	45708818	114
3	Bicyclo[3.1.1]heptane, 6,6-dimethylene-	8475	8545	8677	103055622	300628765	749
4	.beta.-Myrcene	8791	8877	9014	112570190	423941795	1056
5	.alpha.-Phellandrene	9316	9548	9628	373908366	4016225588	10000
6	1,3-Cyclohexadiene, 1-methyl-4-(-methylethyl)-	9681	9742	9842	125495860	280320851	698
7	p-Cymene	9904	9979	10042	108946600	312493142	778
8	D-Limonene	10042	10114	10152	109614800	400847089	998
9	Eucalyptol	10152	10233	10317	244907454	919581360	2290
10	.beta.-Ocimene	10521	10578	10621	32703850	76447443	190
11	.gamma.-Terpinene	10893	10961	11074	122375103	318048172	792
12	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)-	11687	11861	11869	321065151	1842318376	4587
13	-	11869	11872	11880	326540991	209404406	521
14	1,3-Cyclohexadiene, 1-methyl-4-(1-methylethyl)	11880	11894	11921	336401840	549745581	1369
15	Linalool	12146	12215	12314	40696375	110856806	276
16	(1R,2S,4S,5R,7R)-5-isopropyl-1-methyl-3,8-dioxatricyclo[5.1.0.02,4]octane	16490	16552	16664	14947254	40598275	101
17	aR-Turmerone	27000	27078	27124	49209962	151642558	378
18	Timezone	27124	27200	27270	78964516	234384277	584
19	Curlone	27852	27922	28000	40982220	109593849	273

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Table 3. Components of Many Compounds from GC-MS Results of Turmeric Rhizomes Essential Oil

Peak	Compounds	Start	RT	End	Height	Area	Area %
1	.alpha.-Phellandrene	9331	9436	9536	199376283	655657545	1895
2	p-Cymene	9901	9962	10032	33997813	89348952	258
3	D-Limonene	10038	10086	10121	13636796	35178808	102
4	Eucalyptol	10121	10194	10269	65612845	180584797	522
5	1,3-Cyclohexadiene, 1-methyl-4-(-methylethyl)-	11707	11777	11893	79392465	202798272	586
6	Caryophyllene	21126	21188	21287	14717264	39851472	115
7	2(1H)-Naphthalenone, 7-ethynyl-4a,5,6,7,8,8a-hexahydro-1,4a-dimethyl-	21730	21798	21892	14588652	39609764	114
8	Benzene,1-(1,5-dimethyl-4-Hexenyl)-4-methyl-	22658	22720	22819	37723670	98248016	284
9	1,3-Cyclohexadiene, 5-(1,5-dimethyl-4-hexenyl)-2-methyl-	22990	23059	23159	42244708	108948807	315
10	.beta.-Bisabolene	23326	23372	23456	11277632	35426747	102
11	.Cyclohexene,3-(1,5-dimetyl-4-hexenyl)-6-methylrne-	23698	23779	23849	41133572	127010135	367
12	(1R,3E,7#,11R)-1,5,5,8-Tetramethyl,12-oxabicyclo[9.1.0]dodeca-3,7-diene	24475	24523	24599	11135182	43621981	126
13	Timezone	24906	24960	25027	12624307	41106893	119
14	2-Methyl-6-(p-tolyl)hept-2-en-4-ol	25027	25100	25195	76921052	267660539	773
15	(Z)-.gamma.-Atlantone	25327	25375	25443	13459692	37503563	108
16	(R-Z)-2Methyl-6-(4 methylcyclohexa-1,4-dien-1-yl)hept-2-en-1-ol	25591	25661	25865	53166222	397269623	1148
17	(1R,4R)-1-methyl-4-(6-Methylhept-5-en-2yl)cyclohexane-2-enol	25876	25939	26055	21820943	72236964	209
18	6-(p-Tolyl)-2-methyl-2-heptenol, trans-	26055	26149	26271	16379960	94791677	274
19	Lanceol,cis	26368	26484	26578	51535901	317733084	918
20	7-Oxabicyclo[4.1.0]heptane, 1-(1,3-dimethyl-	26578	26659	26816	19453333	104570109	302

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	1,3butadienyl)-2,2,6-trimethyl						
21	(Z)-.gamma.-Atlantone	26885	26953	27015	9446287	43452972	126
22	aR-Tuemerone	27015	27361	27382	304971394	3460753613	10000
23	Cameron	27382	27439	27506	293018357	1135061389	3280
24	.alpha.-Bisabolol	27584	27655	27767	15276513	67450843	195
25	Curlone	27841	28105	28153	232288114	1530423302	4422
26	(6R,7R)-Bisabolone	28795	28855	28909	18643113	51203857	148
27	2-Methyl-6-(4-methylenecyckohex-2-en-1-y)hept-2-en-4-one	29034	29119	29147	25834669	66179487	191
28	3-Methyl-2-butenoic acid,tridec-2-ynyl ester	29209	29256	29289	22373172	64270571	186
29	(E)-.gamma.-Atlantone	29289	29313	29337	17584654	36104151	104
30	(E)-Atlantone	29439	29502	29596	27031450	73463532	212

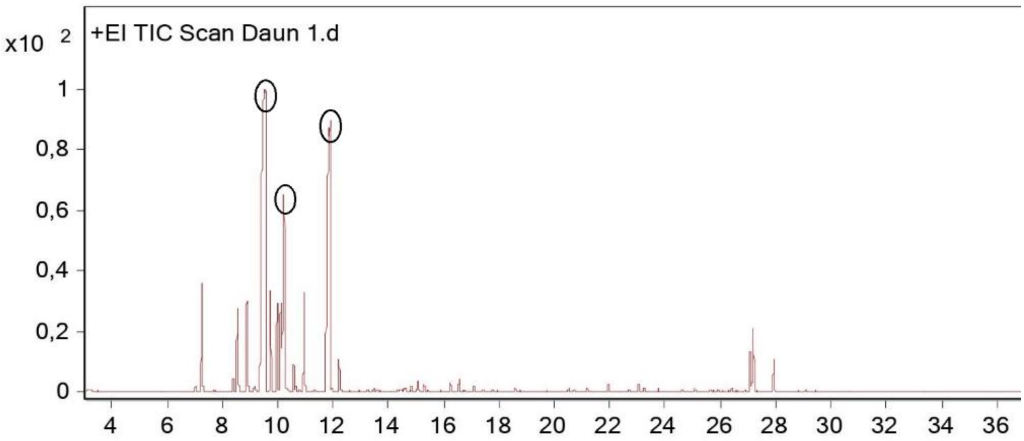

Figure 3. Peak Points in Turmeric Leaf Essential Oil

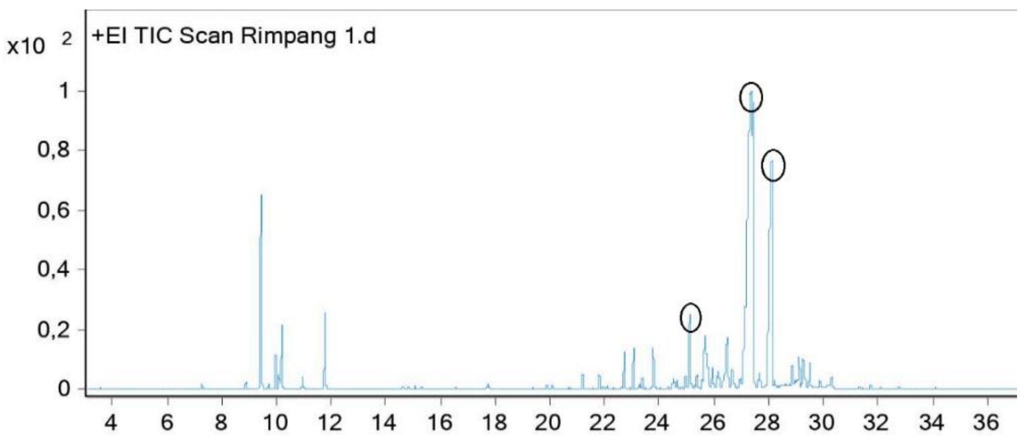

Figure 4. Components of Active Compounds Result of GC-MS Analysis of Turmeric Rhizome Essential Oil

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For the three peak points of essential oils on turmeric leaves, more details can be seen in Table 4 below:

Table 4. Turmeric Leaf Peak

Compounds	Peak Height	% Area
alpha.-Phellandrene	373908366	37.59%
1,3-cyclohexadiene, 1-methyl-4-(1-methylethyl)	321065151	17.24%
Eucalyptol	244907454	8.61%

The three peak points or the most abundant compounds in turmeric leaf essential oil are alpha-Phellandrene, 1,3-Cyclohexadiene 1-methyl-4-(1-methyl ethyl), and Eucalyptol which causes turmeric leaves to function as anti-inflammatory, antimicrobial and has physical properties that are colorless and have a distinctive smell like essential oil-producing plants.

For the three peak points of essential oils on the turmeric rhizome, more details can be seen in Table 5 below:

Table 5. Top Points of Turmeric Rhizome

Compounds	Peak Height	% Area
aR-turmerone	304971394	36.36%
Curlonee	293018357	16.08%
Tumeronee	232288114	11, 93%

From the results of the essential oil in turmeric rhizome, the peak point is aR-Turmerone, Curlonee, and Tumerone. The turmeric rhizome compound has a distinctive odor caused by these three compounds (aR-Turmerone, Curlonee, and Tumeronee) [4].

From the characteristic results in turmeric leaves, 19 compounds were obtained, and 30 compounds were obtained in turmeric rhizome. Turmeric leaves shared eight compounds and turmeric rhizomes. The amount of alpha-phellandrene, 1,3 cyclohexadiene 1-methyl -4-(1-methyl ethyl), P-Cymene, D-Limonene, and Eucalyptol are more abundant in turmeric leaves, while the amount of aR-Turmerone, Tumeronee, and Curlonee are more in turmeric rhizomes for more details can be seen from Table 6.

Table 6. Compounds of Essential Oils That Are Equally Owned by Turmeric Leaves and Rhizomes

No.	Compounds	Amount of Compound		RT	
		Leaves	Rhizomes	Leaves	Rhizomes
1	.alpha.-Phellandrene	37.59%	6.89%	9.354 – 9.583	9.371 – 9.458
2	1,3-cyclohexadiene, 1-methyl-4-(1-methylethyl)-	2.69%	2.13%	11.737 – 11.807	11.732 – 11.869
3	P-Cymene	2.69%	2.13%	11.737 – 11.807	11.732 – 11.869
4	D-Limonene	3.75%	0.37%	10.060 – 10.152	10.050 – 10.121
5	Eucalyptol	8.61%	1.9%	10.152 – 10.257	10.137 – 10.224
6	aR-Tumerone	1.42%	36.36%	27.027 – 27.124	27.069 – 27.382
7	Timezone	2.19%	11.93%	27.138 – 27.224	27.382 – 27.452
8	Curlone	1.03%	16.08%	27.881 – 27.954	27.948 – 28.118

In the physical test results, turmeric leaf essential oil has a clear or colorless color and a distinctive mint smell which is also a physical property of the compound. alpha.-Phelandrene, 1,3-Cyclohexadiene 1-methyl-4-(1-methyl ethyl)-, p-Cymene, D-Limonene, and Eucalyptol. It causes the content of these five compounds to be more abundant in turmeric leaves than in turmeric rhizomes. As for the turmeric rhizome, it has physical properties, namely clear yellow color and a distinctive turmeric smell. It causes the turmeric rhizome to have higher levels of aR-Turmerone, Tumeronee, and Curlonee compounds compared to turmeric leaves, where the three compounds function as turmeric odorants.

In addition to having a peak, the essential oil in turmeric leaves and rhizomes also has the main compounds: aR-Tumeronee, Tumeronee, and Curlonee. Where the levels of the main compounds are mostly found in turmeric rhizomes. For more details, it can be seen in table 7 below:

Table 7. Main Compounds in Turmeric Leaves and Rhizomes

No	Main Compounds	Amount of Compound	
		Leaf	Rhizome
1	aR-Tumerone	1.42%	36.36%
2	Tumeronee	2.19%	0.43%
3	Curlonee	1.03%	16.08%

Based on the observations made on both essential oils of leaves and turmeric rhizome, aR-Turmerone in the leaves

is 1.42%. In turmeric, a rhizome is 36,36%, turmerone in turmeric leaves is 2.19%, and in turmeric, a rhizome is 12 .16%, while curlone in turmeric leaves is 1.03%, and in turmeric, a rhizome is 16.08%.

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CONCLUSION

There are differences in the characteristics of essential oils in turmeric leaves and rhizomes using GC-MS. Namely, the essential oil in turmeric leaves has fewer compounds than in turmeric rhizomes, and turmeric leaves have 19 compounds detected. In comparison, in the turmeric rhizome, there are 30 compounds. Turmeric leaves have three peak points, namely .alpha.-Phellandrene, 1,3-cyclohexadiene, 1-methyl-4-(1-methyl ethyl)-, and Eucalyptus. In contrast, the peak point in the turmeric rhizome is aR-Turmerone, Curlonee, and Tumeronee, the main compounds. The leaves and rhizomes of turmeric contain 8 of the same compounds, namely .alpha.-Phellandrene, 1,3-cyclohexadiene 1-methyl-4-(1-methyl ethyl)-, P-Cymene, D-Limonene, Eucalyptol, aR-Turmerone, Tumerone, and Curlonee.

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