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Isolation, Identification, and Characteristic of Essential Oil of Iler (*Plectranthus scutellarioides* (L.) R.Br leaves

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Abstract

Objective: This study aims to isolate, identify and characterize determine and verify of essential oil of Iler leaves (Plectranthus scutellarioides (L.) R.Br.

Methods: Iler (Indonesian), P.scutellarioides, obtained from Lembang Plantation, Bandung, West Java. Isolation of essential oils based on methods of sterilization by distillation with Stahl apparatus from Pharmacopoeia Indonesia 3rd edition. Identification of essential oils was carried out by using a refractive index, thin layer chromatography and Gas Chromatography-Mass Spectrometry (GC-MS). Characteristic essential oil was determined based on Pharmacopoeia Indonesia IV edition.

Results: The result of a determination of essential oil content from iler leaf was found to be 0.04% with the refractive index of 1.4835. The organoleptic properties of the volatile oil of the iler leaf obtained in the form of a brownish yellow liquid, the taste of sponge and smells like tea. The identification of chemical compound components with Gas Chromatography-Mass Spectroscopy resulted in phytol of 21.1%, germacrene-d of 9.30%, caryophyllene by 8.84%, β-element as much as 6.84% and neophytadiene as much 6.08% as the main component of volatile oil of iler leaf.

Conclusions: Since the volatile oil content of the iler leaves was not too high, only about 0.4%, although its characteristic was good enough, to be used as a pharmaceutical preparation it was worth considering its use. This research suggests conducting research of anti-inflammatory properties or anti-acne medicines from volatile oil of iler leaf so that can be determined the right dose if it will be used as anti-inflammatory drugs.

Keywords: essential oil, iler leaf, GC-MS, TLC, Stahl distillation, Plectranthus scutellarioides

INTRODUCTION

Iler leaf (Indonesian) with Latin name Plectranthus scutellarioides is traditionally believed to treat hemorrhoids, pelvic menstruation, and appetite enhancers treat constipation, whiteness, hemorrhoid, itching, ear inflammation, stomachache, diabetes, fever, diarrhea, ulcers, dysentery, as a cure for skin diseases, fatigue, atherosclerosis, leucorrhoea and osteoporosis, eye pain, antiviral and acne medications. Depending on the various part of the plant having potential pharmacological values, namely, anti-inflammatory, immune-stimulating, anticancer, hepatoprotective, antioxidant, wound healing, antidiabetic, antinociceptive effect and antibacterial activities [1- 5]. The chemical content of iler plants found in the leaves and roots of saponins, polyphenols, flavonoids and essential oils [6-7]. The essential oil components, among others carvacrol, methyl eugenol, eugenol, ethyl salicylate inhibit irritation. The leaves also contain alkaloids, minerals and a bit of mucus [8]. Amidephrine was reported as part of essential oil of iler leaf [9].

The essential oil is a volatile lipophilic mixture, which is generally obtained by volatile, aromatic, vaporous evaporative distillation, when it is generally freshly colorless or yellowish which turns dark after being let stand, does not moisten water, optically active, has a high refractive index [10]. Whether this essential oil is responsible for its antibacterial properties so that it can be used for anti-inflammatory or for the treatment of acne is unclear. Characterization of essential oils is an important thing done to know the quality of the oil so it is feasible to use and provide optimal benefits. The phytochemical analysis of volatile oil begins with the determination of physical properties including specific gravity, optical rotation, refractive index, solubility in alcohols up to their chemical properties by thin layer chromatography up to gas mass spectrometry chromatography so that the main components can be determined and standardized essential oils are produced. This standardization is useful to obtain a picture of the purity and quality of essential oils so as to know the existence of counterfeiting [11]. This paper report the characterization of essential oils of P.scutellarioides leaf which grows in Lembang, Bandung, West Java which includes the physical and chemical properties and identification of the chemical components of the essential oil so that the components or compounds of the markers can be determined in reference to Pharmacopoeia Indonesia IV edition [12] and Materia Medika Indonesia V [13]

METHODS

Plectranthus scutellarioides leaves obtained from Lembang Plantation, Bandung, West Java. Before dried, chopped made simplicia, plants in determination first in the Department of Biology, Faculty of Natural Sciences, Padjadjaran University. Isolation of essential oils was performed based on the method of determining the levels by distillation from Pharmacopoeia Indonesia III edition [14]. Identification of essential oils using a refractive index, thin layer chromatography and GC-MS [15-16]. Characteristic essential oil was determined on the basis of Pharmacopoeia Indonesia IV edition [13].

RESULTS AND DISCUSSION

Sample collection

The leaves were cut into pieces and then dried in air shielded from direct sunlight for approximately 5 days to obtain a really dry simplicia. It was found that it took 16 kg of fresh iler leaves to produce 1 kg of dried simplicia of iler leaf. This large comparison was because iler was a wet plant with a considerable moisture content.

Result of Isolation and Determination of Essential Oil Content

The result of the determination of the volumes of the volatile oil of the iler leaves carried out by the Stahl distillation device [17], found that for 1 kg of dry simplicia would be produced about 0.38 mL of essential oil, so to obtain 1 mL of essential oil it took about 2.63 kg of dried simplicia which, correlated with the drying ratio then it took about 42.10 kg of fresh iler leaves. The small concentration of essential oil was not explained in the existing articles. The low yield of isolation obtained, the analysis that could be done on iler essential oil was quite limited considering the required amount of leaf was sufficient to perform physicochemical analysis as a whole.

Result of Physicochemical Analysis of Essential Oil Organoleptic Properties

Organoleptic examination obtained brownish yellow oil, smelled sharp like tea smell and specific taste

Refractive Index

With an experimental temperature of 24.0 °C, it was found the average refractive index of 1.4835 was obtained. El-hawary et.al [18] stated that the yield and composition of essential oils produced from Plectranthus amboinicus (Lour.) Spreng and might have an effect on their refractive index values.

Result of Component Analysis of Chemical Essential Oil Compound

A. Thin Layer Chromatography

In measurement with TLC was used clove essential oil as a comparison.

Adsorbent Silica gel GF 254, Eluen: n-hexane-ethyl acetate (9: 1), toluene-ethyl acetate (93:7), toluene - chloroform (1:1). Eluent distance: 8.5 cm

Spotting effect: UV 254, UV 366, Vanilin – sulphate reagent.

The TLC results can be seen in Tables 1, 2, and 3.

Table 1. Results of TLC with n-hexane-ethyl acetate developer (9: 1)

	Essential oil of P.scutellarioides						Clove oil	
Spots	UV 254 nm		UV 366 nm		Vaniline	-H2SO4	Vaniline	-H2SO4
	hR_f	Color	hR_f	Color	hR_f	Color	hR_f	Color
1	-	-	-	-	14	green	-	-
2	27	purple	-	-	24	purple	-	-
3	35	purple	-	-	36	pink	-	-
4	43	purple	-	-	42	blue-black	-	-
5	-	-	-	-	49	blue	-	-
6	55	purple	55	blue	55	yellow	53	yellow
7	62	purple	-	-	60	green-blue	-	-
8	-	-	-	-	64	purple	-	-
9	69	purple	-	-	70	yellow	70	purple
10	76	purple	-	-	76	purple	-	-
11	-	-	-	-	81	blue	-	-
12	85	purple	87	blue	86	pink	-	-
13	-	-	-	-	90	green	-	-
14	94	purple	-	-	95	purple	91	purple

Table 2. Results of TLC with toluene-ethyl acetate developer (93:7)

	Essential oil of P.scutellarioides						Clove oil	
Spots	UV 254 nm		UV 366 nm		Vaniline	-H2SO4	Vaniline	-H2SO4
	hR_f	Color	hR_f	Color	hR_f	Color	hR_f	Color
1	-	-	-	-	6	yellow	-	-
2	-	-	-	-	10	gray	-	-
3	-	-	-	-	23	pink	-	-
4	-	-	-	-	36	dark	-	-
5	-	-	-	-	47	blue	47	purple
6	53	purple	54	blue	52	yellow	-	-
7	-	-	-	-	56	blue	-	-
8	-	-	59	blue	61	pink	-	-
9	-	-	-	-	67	yellow	65	yellow
10	-	-	72	blue	72	pink	-	-
11	-	-	-	-	81	green gray	-	-
12	89	purple	89	blue	89	purple	90	purple

Table 3. Results of TLC with toluene-chloroform (1:1)

Spots	Essential oil of P.scutellarioides						Clove oil	
	UV 254 nm		UV 366 nm		Vaniline	-H2SO4	Vaniline	-H2SO4
	hR_f	Color	hR_f	Color	hR_f	Color	hR_f	Color
1	-	-	-	-	12	yellow	-	-
2	-	-	-	-	41	pink	39	pink
3	-	-	-	-	48	blue	-	-
4	-	-	57	blue	55	yellow	-	-
5	-	-	61	blue	62	purple	-	-
6	-	-	-	-	66	dark blue	66	yellow
7	-	-	-	-	73	light purple	-	-
8	-	-	-	-	79	light pink	-	-
9	-	-	-	-	83	grayish blue	81	blue
10	92	purple	94	blue	92	purple	91	purple

Of the three mixtures of the developers, the n-hexane-ethyl acetate mixture (9:1) gave the best splitting segregation of 14 patches with vanillin-sulfuric acid spotting, 9 spots with 254 nm UV and 2 spots with 366 nm UV. The toluene-ethyl acetate mixture (93:7) gave 12 patches with vanillin-sulfuric acid spotting, 4 spots with 366 nm UV and 2 spots with 254 nm UV. The toluene-chloroform mixture (1:1) gave 10 patches with vanillin-sulfuric acid spotting, 3 spots with 366 nm UV and 1 spot with 254 nm UV.

For qualitative examination, TLC was performed with a comparative mixture. Based on the literature, the volatile oil of iler leaves contained eugenol [7, 15, 19], then clove oil was used as a comparison. The comparative solution was applied to the same plate as the oil tested. The identification of the compound could be seen at the time of its propagation (Rf). Figures Rf futures were between 0.00 to 1.00, whereas hRf is the Rf number multiplied by the factor 100 (100 = h). The reference value of hrf eugenol in clove oil was ~ 50 in brownish yellow [20]. In the n-hexane-ethyl acetate mixture (9:1) the value of the comparative hrf eugenol was 53 in yellow and on the volatile oil, there were also spots on hrf 55 in yellow. With this comparison, it could be concluded that the essential oil of iler contains eugenol. The same was seen in the mixture of toluene-ethyl acetate solvent (93:7) and toluene - chloroform (1:1). Brownish brown spots with hRf between 50-65 were suspected to be eugenol compounds. The value of hRf eugenol on the above two solvent mixtures higher than the reference hRf could be solved by decreasing the solvent polarity. This meant that both toluene-ethyl acetate (93:7) and toluene chloroform (1:1) mixtures are more polar than the n-hexane-ethyl acetate mixture (9:1).

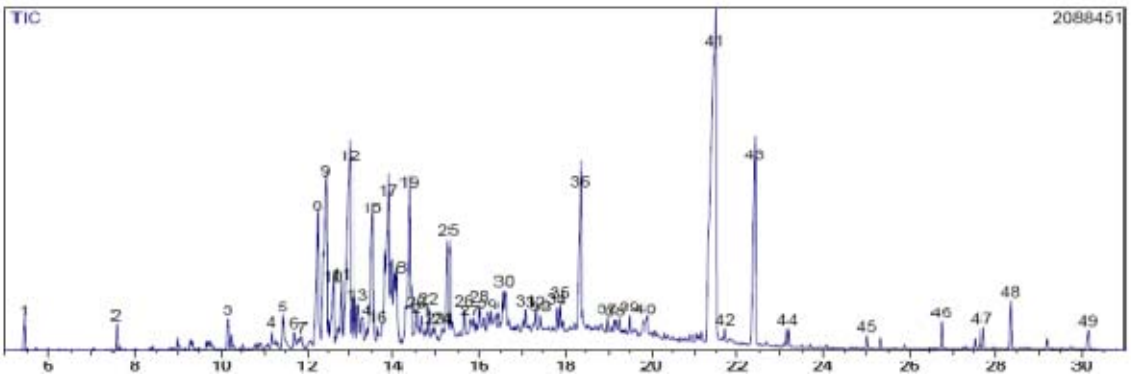
A. Gas Chromatography-Mass Spectrometry (GC-MS)

Essential oil samples were analyzed using the Shimadzu QC-5050 Series GC-MS instrument. From the results of gas chromatography-mass spectroscopy of the volatile oil of iler leaves in the chromatogram obtained as shown in Figure 1.

Each peak on the chromatogram of Fig. 1, then analyzed by using the library. Figure 2 shows the analysis for the phytol compound. From the peak analysis, 43 compounds were obtained as seen in Table 4.

Table 4 Components of essential oil compound of iler leaf

No	Compounds	Concentration (%)
1	Phytol	21.1
2	Germacrene-d	9.67
3	Caryophyllene	8.84
4	Beta-elements	6.84
5	Neofitadien	6.08
6	Delta-cadinene	5.74
7	Alpha-humulene	5.54
8	6, 10, 14-trimethyl-2-pentadecanone	4.27
9	Copaene	3,88
10	(-) cariophyllene oxide	3.05
11	Alpha-selinene	2.57
12	Methyl Eugenol	2.01
13	Oktadecane	1.88
14	Alpha-cadinol	1.74
15	1,2-Dimethyl-3-ethylbenzene	1.66
16	1,3-diisopropenyl-6-methyl-cyclohexene	0.91
17	Nonadecane	0.9
18	Beta-sesquiphellandrene	0.85
19	Dokosan	0.81
20	Vitamin A aldehyde	0,79
21	(+)-Aromadendrene	0.78
22	Nerolidol	0.7
23	Acetamide	0.66
24	Alpha-calacorene	0.62
25	Torreyol	0.6
26	9-Octadecenoic acid	0.59
27	7-Octene-4-ol	0.5
28	1,1'-oxybis- Dodecane	0.56
29	Trimethyl-tetrahydronaphthalene	0.49
30	2-methyl-4-(1-methyl ethyl-2-cyclohexenone	0.48
31	1-(3,7-dimethyl-1-octenyl)- cyclopropanol	0.47
32	5-Benzofuran acetic acid	0.44
33	Caryophyllene oxide	0.43
34	6, 10-dimethyl-2-Undecanone	0.42
35	Alpha-Cubebene	0.38
36	1-(1-oxo-15-tetracocenil)- pyrrolidine	0.37
37	Heneiosen	0.36
38	Linalool	0.31
39	2,6,10,14,18,22-Tetracosahexaene	0.29
40	Neomenthol	0.28
41	Heptacosane	0.61
42	Olealdehyde	0.22
43	Isophytol	0.2



**** Peak Report ****

PKNO	R.Time	I.Time - F.Time	Area	Height	A/H(sec)	MK	%Total	Name
1	5.420	5.325 - 5.517	470976	123037	3.828		0.57	
2	7.565	7.492 - 7.650	255864	74093	3.453		0.31	
3	10.159	10.075 - 10.217	395586	110929	3.566		0.48	
4	11.167	11.117 - 11.383	350377	46924	7.467		0.42	
5	11.446	11.383 - 11.633	753955	144273	5.226	V	0.91	
6	11.713	11.633 - 11.775	313699	69169	4.535		0.38	
7	11.900	11.775 - 11.967	405466	49661	8.165	V	0.49	
8	12.232	12.117 - 12.308	3227158	615180	5.246		3.88	
9	12.431	12.308 - 12.517	5686562	877147	6.483	V	6.84	
10	12.609	12.517 - 12.683	1673809	299421	5.590	V	2.01	
11	12.810	12.683 - 12.867	1376931	243237	5.661	V	1.66	
12	12.998	12.867 - 13.117	7353000	1016819	7.231	V	8.84	
13	13.155	13.117 - 13.208	706830	167280	4.225	V	0.85	
14	13.258	13.208 - 13.333	650611	128891	5.048	V	0.78	
15	13.497	13.333 - 13.600	3835632	648719	5.913	V	4.61	
16	13.633	13.600 - 13.717	304968	50588	6.028	V	0.37	
17	13.895	13.717 - 14.025	7734770	807431	9.579	V	9.30	
18	14.078	14.025 - 14.192	2136784	377324	5.663	V	2.57	
19	14.361	14.192 - 14.475	4770807	714990	6.673		5.74	
20	14.537	14.475 - 14.608	771053	141391	5.453	V	0.93	
21	14.651	14.608 - 14.733	512693	101368	5.058	V	0.62	
22	14.827	14.733 - 14.892	622621	118163	5.269	V	0.75	
23	14.983	14.892 - 15.075	368285	50686	7.266	V	0.44	
24	15.150	15.075 - 15.200	231141	42801	5.400		0.28	
25	15.285	15.200 - 15.458	2539042	479895	5.291	V	3.05	
26	15.642	15.575 - 15.717	356629	93909	3.798		0.43	
27	15.792	15.717 - 15.967	660739	59765	11.056	V	0.79	
28	16.011	15.967 - 16.075	499219	114624	4.355	V	0.60	
29	16.216	16.075 - 16.467	1448244	93006	15.571	V	1.74	
30	16.585	16.467 - 16.758	1565697	200686	7.802	V	1.88	
31	17.081	16.958 - 17.167	461742	79159	5.833		0.56	
32	17.323	17.167 - 17.383	390682	74899	5.216	V	0.47	
33	17.436	17.383 - 17.525	189178	48321	3.915	V	0.23	
34	17.792	17.742 - 17.833	253499	64564	3.926		0.30	
35	17.867	17.833 - 18.025	498264	105020	4.744	V	0.60	
36	18.362	18.250 - 18.500	3548372	709914	4.998		4.27	
37	18.958	18.917 - 19.108	193220	34164	5.656		0.23	
38	19.154	19.108 - 19.317	360439	49953	7.216	V	0.43	
39	19.504	19.450 - 19.567	163673	50514	3.240		0.20	
40	19.867	19.750 - 19.958	488748	88580	5.518		0.59	
41	21.469	21.225 - 21.608	17544020	1798235	9.756		21.10	
42	21.725	21.608 - 21.775	182762	32253	5.666	V	0.22	
43	22.387	22.233 - 22.500	5059301	978225	5.172		6.08	
44	23.164	23.092 - 23.242	173013	49576	3.490		0.21	
45	25.012	24.942 - 25.100	145477	40162	3.622		0.17	
46	26.736	26.658 - 26.817	303169	86649	3.499		0.36	
47	27.677	27.608 - 27.758	238340	67947	3.508		0.29	
48	28.354	28.258 - 28.450	676360	182054	3.715		0.81	
49	30.134	30.050 - 30.233	309993	75707	4.095		0.37	
Total			83159395				100.00	

Figure 1. GC-MS results of the essential oil of iler leaf

The largest component contained in the volatile oil of iler leaf was found phytol with a content of 21.1%. Phytol (C20H40O) was a dihydrogen compound having a C20 carbon skeleton derived from four isoprene units. Denapena was a more volatile compound than monoterpene and sesquiterpene. Most of the spreads were very limited. However, phytol was a widespread acyclic mother compound in the universe [10]. Phytol (Figure 2.) was universally distributed in green plants as a component of chlorophyll molecules in which it was in ester form and as a component part of vitamin E and K [21].

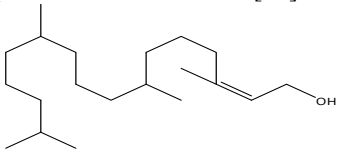


Figure 2 Structure of phytol

Other ingredients in iler essential oils such as germacrene-d, caryophyllene, β-element, delta-cadinene, alpha-

humulene, copaene, alpha-selinene were mostly sesquiterpenes. The composition of hydrocarbon and oxygenated hydrocarbons was almost as much. Oxygenated hydrocarbons were the most important compounds in essential oils because they generally provide a stronger odor [22].

Mariano *et.al* [23] at the DLSU Research Congress 2017 De La Salle University, Manila, Philippines reported their Quantitative analysis of P. scutellarioides and P. amboinicus conducting through GC-MS revealed the major components in each plant were terpenes and phenolic compounds that possessed insecticidal properties. Erny Sabrina *et.al* [24] stated phytochemical analysis using gas chromatography-mass spectrophotometer (GC-MS) of Plectranthus amboinicus suggests that camphor, carvacrol, and 3-carene were the major components that might contribute to the antimicrobial activity. Rodrigues *et.al* [15] mentioned that Plectranthus was one of the most representative genera of Lamiaceae family. In their study, the essential oils of Plectranthus ornatus, Plectranthus ornatus, and Plectranthus barbatus reported that the major components found were carvacrol (54.4% -P. Amboinicus) and eugenol (22.9% -P. Ornatus e 25.1% -P barbatus) . In their reviewed article, Arumugam *et.al* [7] only stated that Plectranthus amboinicus (Lour.) Spreng had 76 volatiles and 30 non-volatile compounds belonging to different classes of phytochemicals such as monoterpenoids, diterpenoids, triterpenoids, sesquiterpenoids, phenolics, flavonoids, esters, alcohols, and aldehydes. Velasco *et.al* [16] reported fifteen components were identified by GC-MS and carvacrol (65.2%) was found to be the major constituent. Manjamalai *et.al* [25] studied Bioactive Evaluation of the essential oil of Plectranthus amboinicus by GC-MS analysis and its role as a drug for carbochol -14 %, Thymol - 18%, Cis-Caryophyllene, t-Caryophyllene, p-cymene -10%. Mwangi et.al [26] reported the composition of essential oil of Plectranthus tenuiflorus (Vatke) Agnew was a total of 17 compounds accounting for 72.3% of the oil were identified. Carvacrol (14.3%) α-terpinene (10.2%) and p-cymene (10.9%) were the major constituents. The oil had low quantities of oxygenates terpenes. Irshad *et.al* [27] used fresh plant material of Angelica glauca, Plectranthus rugosus and Valeriana wallichii, growing wild in the state of Jammu and Kashmir, collected from different locations at different days were subjected to Clevenger-type hydro distillation apparatus. The essential oils were analyzed by GC-MS. P. rugosus yields 0.17% oil. Twenty-three compounds were identified from p. rugosus which yields about 83% of the essential oil. Major constituents were spathulenol (21%), germacrene-d (20%) and β-caryophyllene (10.6%). Mota *et.al* [28] studied volatile-oils composition, and bioactivity of the essential oils of Plectranthus barbatus, P. neochilus, and P. ornatus grew in Portugal. They found monoterpene hydrocarbons (12-74%) and sesquiterpene hydrocarbons (4-45%) constituted the main fractions in all volatiles. α-Pinene (3: 12-67%), oct-1-en-3-ol (6; traces-28%), β-pinene (7; 0.1-22%), and β-caryophyllene (50; 7 -12%) dominated P. barbatus volatiles. P. neochilus major volatile components were α-terphenyl acetate (41, traces-48%), α-thujone (2; 2-28%), β-caryophyllene (50; 2-28%), β-pinene (7; 1-25%), and α-pinene (3, 1-19%). Oct-1-en-3-ol (6; 13-31%), β-pinene (7; 11-24%), α-pinene (3; 11-19%), and β-caryophyllene (50; -11%) were the main constituents from P. ornatus volatiles.Yoshi in 2014 [29] found in twenty-seven compounds was identified, which comprised 98.6% of the total constituents. The main compound was identified as fenchone (32.3%), followed by alpha-humulene (17.3%), piperitenone oxide (8.5%), cis-piperitone oxide (6.0%) and E-beta-farnesene (5.9%). The oil was found rich in oxygenated monoterpenes type constituents (52.0%), followed by sesquiterpene hydrocarbons (40.2%), oxygenated sesquiterpenes (4.9%), and monoterpene hydrocarbons (1.5%). Agnaniet *et.al* [30] in their study of essential oil of Plectranthus tenuicaulis leaves from Gabon, the source of (R), (E) -6,7-epoxyocimene. An unusual chemical composition within the genus Plectranthus was isolated and formally identified as being the (+) - (R) - enantiomer of (E) -6,7-epoxyocimene [(E) -myroxide]. This enantiomer, which represented about 75% of the essential oil, had been previously identified as a pheromone emitted by the male fruit-spotting bug Amblypelta nitida.

As can be seen in these findings, our findings are slightly different from those of other researchers analyzing oil from iler leaves. It should be admitted that the differences in these results are generally due to species, growing spots and different shooting seasons.

CONCLUSIONS AND SUGGESTIONS

From the results of this study, it could be concluded that the volatile oil of iler leaves had a small content of 0.04% v/w so it took the leaf of iler in very large quantities for further analysis. To be used as a pharmaceutical preparation it was necessary to consider its use. The essential oil of the iler leaves was a brownish-yellow liquid, tasteful and smells like tea. Refractive index of 1.4835. The result of identification of iler compound component was phytol 21,1%, germacrene-d 9,30%, caryophyllene 8,84%, β-element 6,84% and neophytadiene 6,08% as main component. This research suggests conducting research of anti-inflammatory properties or anti-acne medicines from an essential oil of iler leaf so that can be determined the right dose if it will be used as anti-inflammatory drugs.

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