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Combination of Extraction and Distillation Red Ginger Rhizome on the Composition of Active Compounds and Tyrosinase Inhibitory Activity

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***Abstract*— In Indonesia, red Ginger is commonly used as traditional medicine and health drink. Its bioactive compounds are generally extracted separately in essential oils or oleoresin only. This study aims to maximize the extraction of red ginger rhizome bioactive compounds in the form of oleoresin and essential oil simultaneously and examine the effect of the different methods of extraction and distillation on the quality. The first method is essential oil extraction by water-steam distillation, continuing with oleoresin extraction of the ginger rhizome, while the second method is oleoresin extraction of fresh rhizome, continuing with distillation. The essential oil quality is determined based on the chemical composition and the tyrosinase enzyme inhibitory activity. The results showed that the essential oil produced by the second method contains higher zingiberene content (28.5%) with clear pale-yellow color, while the first method is orange with 15.4% zingiberene. The 6-gingerol, 8-gingerol, 6-shogaol, and 10-gingerol content of the oleoresin by the second method is higher than the first method. Furthermore, the inhibition test of two tyrosinase enzyme activities showed that the essential oil and extract from the two method is not significantly different. Thus, in addition to obtaining two extracts at once in the form of oleoresin and essential oil, this study also showed that the extraction process carried out before distillation of the rhizome could increase the yield of both extracts and increase the quality of red ginger essential oil.**

***Keywords*— Red Ginger; solid waste; essential oils; oleoresin.**

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I. INTRODUCTION

Red Ginger (*Zingiber officinale* var. *rubrum*) is one of three Ginger cultivated in Indonesia, and it is used as a healthy drink and traditional medicine. Another Ginger is a big white rhizome, also known as Gajah ginger (*Z. officinale* var. *officinale*), and a small white rhizome also known as Emprit Ginger (*Z. officinale* var. *amarum*). This red Ginger is recognized visually by its red skin rhizome color. Several studies have demonstrated that ginger rhizome contains bioactive compounds with pharmacological properties such as anticarcinogenic, antioxidant, anti-inflammatory, antimicrobial, anticancer, neuroprotective, cardiovascular protective, respiratory protective, and antidiabetic activities [1]–[4]. These bioactive compounds are usually extracted as essential oils and oleoresins.

The main components of essential oil from ginger rhizome are monoterpenes and sesquiterpenes, with zingiberene as the main component. In addition, camphene, monoterpenes, and other sesquiterpenes were also detected in essential oils at varying levels [4]–[7]. In addition to essential oils, the ginger rhizome also contains oleoresin. This important non-volatile compound is also responsible for its pharmacological properties and gives a warm sensation due to its sharp and spicy taste, including gingerols, shogaol, paradol, gingerdiol, and zingerone [1], [3], [4], [8], [9].

However, ginger essential oil from Indonesia, including red Ginger, has difficulty meeting international standards. According to ISO 16928:2014(E), ginger essential oil from China contains zingiberene (29.0 – 40.0%) and camphene (4.5 – 10.0%), and from India contains zingiberene (35.0 – 40.0%) and camphene (5.0 – 8.0%) [10]. However, Ginger essential oil produced from Indonesia generally has lower levels of zingiberene and higher camphene than international standards [6], [11]. This is presumably due to the production process that applies the distillation method with high temperatures leading to camphene forming and reducing zingiberene

content. Souza et al. [12] showed that the hydro distillation method, where the rhizomes are cooked in boiling water during the distillation process, will produce essential oils with lower zingiberene levels than the steam distillation or Supercritical Fluid Extraction (SFE) method.

Several studies have shown that zingiberene levels decrease due to high-temperature heating during the ginger essential oil production process, while at the same time, camphene levels increase [12], [13]. The composition of the chemical compounds in it influences the quality and pharmacological properties of ginger essential oil. Purnomo *et al.* [13] study that has been carried out proved that heating ginger rhizome resulted in changes in the composition of essential oils which resulted in a decrease in antioxidant activity. Therefore, the levels of zingiberene and camphene indicate purity and the correct production technique of ginger essential oil.

Several studies on alternative methods to improve the quality of Indonesian ginger essential oil have been reported, including the SFE and Microwave-Assisted Hydro distillation (MAHD) methods [12], [14]. However, applying both methods in Indonesia still faces major challenges. In addition to the distillation method, other factors have also been known to affect the chemical composition of ginger essential oil, including the maturity stage/age of the rhizomes, variety, geographic location of the planting area [15], the drying method of the rhizomes [16], roasting the rhizomes [17]. Thus, efforts are still needed to continue studying the best method to improve the quality of Indonesian ginger essential oil.

In Indonesia, the extraction of ginger rhizome bioactive compounds is generally done to obtain one product, namely in the form of essential oil or oleoresin, so that each process only produces one product and leaves ginger rhizome dregs which still contains several bioactive compounds. Yulianto et al. [18] have demonstrated that ginger rhizome dregs collected from the herbal medicine industry waste still contain several bioactive compounds, including zingiberene, Beta-sesquiphellandrene, and E-citral. Although the waste still had active components, the process order will determine the optimum results. The extraction without heat will produce extracts and residues that are not degraded, while distillation involves high temperatures, so heat degradation is possible. Therefore, getting good quality oleoresin and essential oil from one source is necessary.

This study aims to maximize the extraction of red ginger rhizome bioactive compounds in the form of oleoresin and essential oil simultaneously and examine the effect of the combination of the different methods of extraction and distillation on the quality. The first method is distillation to extract essential oil before extracting the oleoresin of the red ginger rhizome, while the second is a distillation of the rhizome after oleoresin extraction. The quality of the essential oil produced is determined based on the chemical composition, especially the zingiberene content and the tyrosinase enzyme inhibitory activity.

II. Materials and Method

A. Plant Materials

Fresh red Ginger (*Z. officinale* var. *rubrum*) rhizome was collected from Biopharmaca Cultivation and Conservation Unit, IPB University (Dramaga, Bogor–West Java), with a maturity stage of 9-10 months. Then, the fresh rhizomes were washed to remove soil materials and other impurity materials. After cleaning, the rhizomes were air-dried and ready for extraction. The following flowchart can briefly explain the extraction steps (Fig. 1).

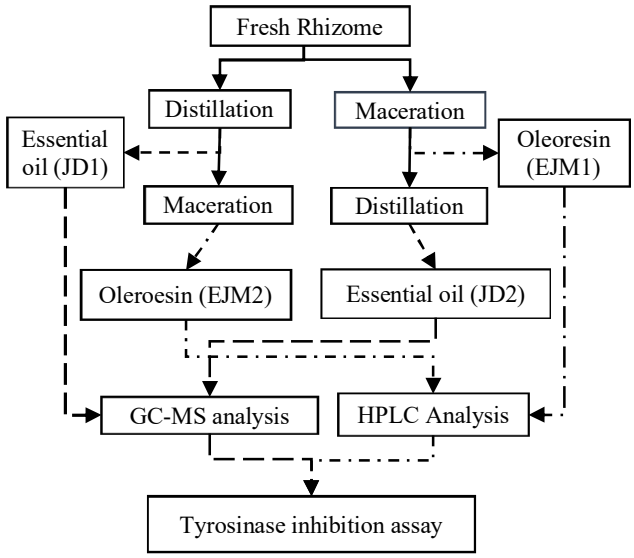

Fig. 1 Extraction combination of red ginger rhizome

B. Chemicals

Chemical reagents such as ethanol, L-tyrosinase, L-DOPA, dimethyl sulphoxide (DMSO), Kojic acid, 6-gingerol, 8-gingerol, 10-gingerol, 6-shogaol and methanol (HPLC grade) were purchased from SIGMA Aldrich and Merck.

C. Extraction

The cleaned fresh red ginger rhizome was sliced with a knife into small pieces, then divided into two for extraction by a combination of two methods, namely distillation and maceration. The difference between the two methods is only in the order of extraction. The first method is to distill fresh ginger rhizomes first. Then the residue is macerated with methanol to obtain oleoresin. The second method is that the fresh ginger rhizome is macerated first, then the residue is distilled to obtain the essential oils. The extraction yields were calculated as the mass of extracted oil/oleoresin divided by mass of fresh dried ginger rhizomes.

1) Essential Oil Distillation Followed by Oleoresin Extraction (1st Method): About 2.6 kg of fresh red ginger rhizomes is cut into small sizes and put into a water-steam distillation device to which water has been added as a steam medium. Distillation was carried out for five hours until the essential oil was obtained. Finally, the essential oil obtained is separated from water based on the principle of difference in density. JD1 names the essential oil from the first method. Oleoresin was extracted by soaking the distilled dregs in ethanol (ratio of distilled dreg to ethanol 1:5) for 3 x 24 hours at room temperature. After that, the rhizome samples and ethanol extract were separated using filter paper. The ethanol extract was then concentrated using a rotary evaporator to obtain a concentrated oleoresin extract. The oleoresin extract of the first method is named EJM2.

2) Oleoresin Extraction Followed by Essential Oil Distillation (2nd Method): The second method has the reverse order of extraction with the first method; maceration is carried out first, then the dregs are distilled. The distillation and maceration methods are the same as the first method; about 2.6 kg of fresh ginger rhizomes cut into small pieces are macerated with ethanol (ratio of distilled dreg to ethanol 1:5) for 3 x 24 hours at room temperature. The oleoresin extract of the second method is named EJM1. After the rhizome dregs were separated from the ethanol extract, it was distilled for a total of five hours to obtain ginger essential oil. JD2 names the essential oil from the second methods.

D. Gas Chromatography-Mass Spectrometry (GC–MS) Analysis of Ginger Essential oil

Gas Chromatography-Mass Spectrometry (GC-MS) is one of the chromatographic techniques used to detect volatile chemical compounds. The GC-MS analysis was carried out with GC-MS instrument Agilent 7890 B using a temperature of 250 °C. Helium gas was used as a mobile phase with a flow rate of 1, 2 $mL.min^{-1}$. The mass analyzer used was MSD. The identification of the compounds was completed by comparing the obtained mass spectra with the mass spectrum database from the NIST Library.

E. Quantitative Analysis of Gingerols and Shogaol

The methods used was modified from Akamine et al. [19]. Gingerols and shogaol content of oleoresin were analyzed by HPLC Shimadzu LC 20A with C18 column (150 mm x 4,6 mm, Shimadzu, Japan) was used as stationary phase, the mobile phase was acetonitrile: water in a gradient mode (1 $mL.min^{-1}$), while detection using PDA at 280 nm. Standard solution of 6-, 8-, 10- gingerol, and 6-shogaol were prepared with an initial concentration of 1000 ppm, then diluted to 50 ppm in methanol. Meanwhile, as much as 100 mg of ginger oleoresin sample was dissolved in 8 mL of methanol. The dissolution was accelerated with ultrasound-assisted for 60 minutes with a break for 15 minutes every 30 minutes to avoid thermal degradation. Then, the volume of the oleoresin solution was adjusted to 10 ml with methanol in a 10 mL volumetric flask. After homogenization, the sample solution was filtered using 0.45 µm filter and was ready to determine the levels of gingerols and shogaol using an HPLC instrument using standard compounds and analytical conditions as described above.

F. Tyrosinase Inhibition Assay

Tyrosinase inhibitory activity was evaluated based on the potency of inhibition to monophenolase and diphenolase. The assay was carried out using L-tyrosine and L-DOPA as the substrates, following the method described by Kartina et al. [20], and kojic acid was used as a positive control. ELISA plate well reader (Merck Biotec Epoc Spectro UV-Vis) was used for tyrosinase inhibition assay.

III. Results and Discussion

The ginger rhizome contains two groups of secondary metabolite bioactive components: volatile and non-volatile. Volatile compounds are extracted as an essential oil by distillation, while non-volatile compounds are extracted as oleoresin by various methods; one of them is maceration extraction with an organic solvent. Bioactive compound composition of Ginger essential oil (and oleoresin), especially sesquiterpenes and monoterpenes groups, could be changed by high temperature during drying rhizome and extraction processes. Zingiberene and camphene, the two compounds that determine the quality of Ginger essential oil, are strongly affected by temperature. Zingiberene is thermolabile and can decrease in concentration due to high-temperature treatment, while camphene has been shown to increase with heating in the rhizome processing [12], [13].

Purnomo et al. [13] reported that the essential oil from boiled and roasted Gajah ginger had lower sesquiterpenes content, especially zingiberene, while monoterpenes, such as camphene, are higher. However, the distillation method commonly applied in Indonesia is hydro distillation and steam distillation by drying the rhizomes using sunlight or an oven. The two heating processes further reduced zingiberene while increasing camphene, oxygenated mono- and sesquiterpenes. Several studies demonstrated the drying ginger rhizome method effect on the volatile components of ginger [16], [21], [22]. In addition, some compounds in ginger oleoresin could also easily be degraded due to high temperatures. 6-gingerol will be converted to 6-shogaol due to pre-treatment and extraction technique that applies high temperature. Changes in the composition of gingerols and shogaols were also shown to affect antioxidant activity [23]–[26].

Therefore, to observe the effect of the combination of extraction and distillation methods on the quality of essential oils and gingerols in the oleoresin produced, this study used fresh red ginger rhizomes and did not go through the drying process under the sun or oven so that it was hoped that they had not changed the composition of volatile compounds before extraction process is carried out. Therefore, this study shows a comparison of the quality of extracts produced from a combination of two extraction methods, namely the distillation and maceration extraction. Combining this method also adds value because two extracts are obtained from ginger rhizomes, namely essential oil and oleoresin. Generally, people only extract oleoresin or essential oil so that the waste rhizome is wasted or is only used as fuel or organic fertilizer additives. The basic difference between the two methods in this study is that the first method of oleoresin components is extracted by maceration at room temperature so that oleoresin components that are soluble in ethanol and susceptible to thermal degradation can be extracted.

A. Extraction

Figure 2 shows four extracts obtained from the combination of the two methods. The extraction yields were calculated as the mass of extracted oil/oleoresin divided by mass of ginger rhizomes. The yield of EJM1 oleoresin extract obtained from the second method was 2.96%, higher than the EJM2 from the first method, which was only 2.13%. This happened because not many components were lost from the ginger rhizome before maceration. After all, the extraction was carried out directly from fresh Ginger, while the EJM2 extract was obtained after going through the distillation extraction stage, which allowed the loss of some oleoresin components during the process. Meanwhile, the yield of essential oil obtained from the first method (JD1) was 0.02% with a transparent orange color, while the essential oil from

the second method (JD2) reached 0.04% with a pale-yellow color. Based on ISO 16928:2014(E) [10], the color of JD2 complies with international standards for ginger essential oil, while JD1 does not meet these standards.

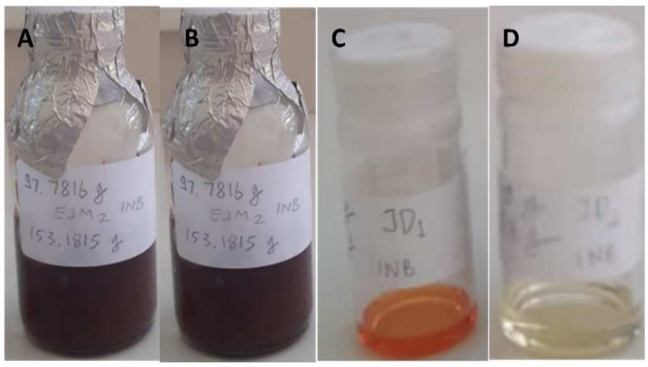

Fig. 2 Red Ginger oleoresin extract EJM1 (A), EJM2 (B), and the essential oil JD1 (C), and JD2 (D).

B. Essential Oil Composition

The GC-MS chromatogram (Fig. 3) shows the results of the chemical composition analysis of the essential oil samples from both method. To make sure about the components, the Chemical Abstracts Service (CAS) number and the Molecular weight of the compound (g.mol^{-1}), as well as the Kovats Retention Index (RI) is determined.

Based on GC-MS analysis data (Table 1), wherein JD1 camphene is higher (27.3%) compared to JD2, which is 23.7%. The decrease in camphene is the expected result because camphene can cause the optical rotation of essential oils to be more positive. Apart from camphene, the oxygenated monoterpene group is the ingredient that distinguishes JD1 and JD2 essential oils. This group of compounds was not detected in JD2 essential oil but found in JD1 about 7%, namely eucalyptol, geraniol, and Neral. Therefore, some mono- or sesquiterpenes oxidation in the JD1 happens, whereas in the JD2, these compounds were extracted during maceration.

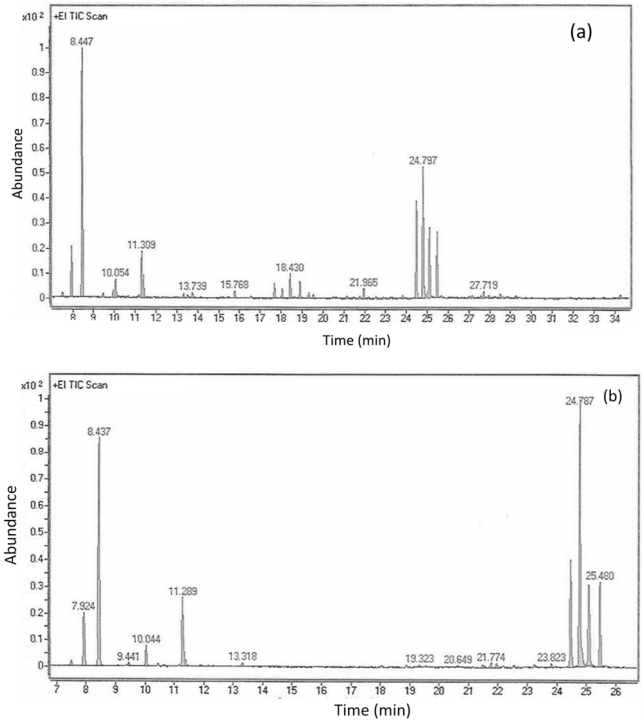

Fig. 3 Chromatogram of essential oil JD1 (a) and JD2 (b)

TABLE I
COMPOUND NAME AND % RELATIVE AREA OF JD1 AND JD2 ESSENTIAL OILS

No	Compound Name	Molecular Formula	MW	CAS No.	RI	Area %	
						JD1	JD2
1	Cyclohexane	$C_{10}H_{16}$	136	110-82-7	663	4.99	6.88
2	α-Pinene	$C_{10}H_{16}$	136	80-56-8	948	5.57	5.57
3	Camphene	$C_{10}H_{16}$	136	79-92-5	943	27.25	23.68
4	β-Myrcene	$C_{10}H_{16}$	136	123-35-3	953	2.00	2.00
5	Eucalyptol	$C_{10}H_{16}O$	154	470-82-6	1059	2.47	-
6	Neral	$C_{10}H_{16}O$	152	96680-15-8	1174	1.75	-
7	Geraniol	$C_{10}H_{18}O$	154	106-24-1	1228	2.72	-
8	α-Curcumene	$C_{15}H_{22}$	202	644-30-4	1464	10.65	11.04
9	Zingiberene	$C_{15}H_{24}$	204	495-60-3	1492	15.36	28.50
10	β-guaiene	$C_{15}H_{24}$	204	88-84-6	1494	0.78	-
11	β-Bisabolene	$C_{15}H_{24}$	204	495-61-4	1509	9.31	10.01
12	β-Sesquiphellandrene	$C_{15}H_{24}$	204	20307-83-9	1543	-	8.4
13	Sesquisabinene	$C_{15}H_{24}$	204	58319-04-3	1565	7.07	-
14	β-Sesquiphellandrene	$C_{15}H_{24}$	204	20307-83-9	1543	-	8.4
15	psi.-carotenoid acid	$C_{35}H_{46}O_2$	498	1109-11-1	-	0.32	-
16	Lycoxanthin	$C_{40}H_{56}O$	552	19891-74-8	-	0.38	-
17	psi.-Carotene	$C_{42}H_{64}O_2$	536	145678-42-8	-	0.41	-

Note: MW is Molecular weight of compound (g.mol^{-1}); CAS No. is Chemical Abstracts Service Number of chemical substance; RI is the Kovats Retention Index

Based on this study, level of oxygenated mono– and sesquiterpenes detected in red ginger essential oil from Indonesia still lower than maximum level of West Africa ginger oil standard [10]. In addition to this study, oxygenated monoterpenes were also detected in Gajah ginger essential oil grown in Indonesia [13]. Therefore, the presence of

oxygenated monoterpenes may also be due to a heat-catalyzed reaction [12], [13]. Purnomo et al. [13] study revealed that the total oxygenated monoterpenes in the Gajah ginger essential oil experienced a drastic increase of more than two times in the heated ginger rhizome. In this study, JD2 essential oil was also produced through distillation involving heat after going through the maceration extraction process; however, the oxygenated monoterpene levels remained undetectable or did not increase compared to JD1. Thus, oxygenated monoterpene group compounds may indeed be contained in the red ginger sample used but then extracted during the maceration process before distillation.

Another difference between the essential oils produced by the first method (JD1) and the second method (JD2) is that the zingiberene content in JD2 essential oil is much higher than that of JD1; the difference between the two is almost doubled, namely 28.5% and 15.4%, respectively. Thus, combining the oleoresin extraction method as an initial step followed by essential oil distillation may increase the zingiberene levels in the resulting essential oil. In addition to zingiberene, the content of β-sesquiphellandrene is also significantly different between the two essential oils, where this compound was not detected in JD1 extract and was only found in JD2. Thus, the combination method may also affect increasing the levels of this compound. In total, the ratio of monoterpene levels in essential oils from the first method to the second method were 39.90% and 38.20%, 7% and 0% oxygenated monoterpenes, while 42.50% and 57.90% sesquiterpenes, respectively.

Based on this comparison, combining the first extraction method may improve the quality of essential oils by increasing zingiberene and decreasing camphene. In addition to monoterpenes and oxygenated sesquiterpenes, the content of total carotenoid derivatives in JD1 were also higher than JD2. JD1 essential oil contains psi.-carotenoid acid; lycoxanthin and psi.-carotene for a total of 1.11%. The presence of these carotenoid derivatives compounds may be the reason why the color of JD1 essential oil is orange. Carotenoids (and its derivative compounds) are natural red-orange pigments produced by plants, fungi and yeasts. More than a thousand carotenoids (including several compounds identified in this study) are produced by various sources, including plant, fungi, and microalgae [27]. Carotenoids content in red ginger essential oil has not been previously reported [2]. This study reports the presence of carotenoid-derived compounds in red ginger essential oil for the first time. These carotenoids may be extracted from the red skin of the rhizome.

C. Gingerols Content of Oleoresin Extracts

The oleoresin extract and ginger rhizome essential oil are responsible for ginger's bioactivity, aroma and warm effect. Essential oils contain volatile compounds, while oleoresins mainly contain non-volatile compounds. Several methods can be used to extract oleoresin from ginger rhizomes, two of which are high-temperature extraction, namely Soxhlet and lower-temperature CO_2 SFE [28], [29]. The SFE method is advantageous because the extract obtained is oleoresin and essential oil [29]. Red ginger extract, collected by infusion process, has the highest phenolic content and antioxidant activity compared to the emprit and gajah ginger [30]. The main phenolic compounds in ginger oleoresin are gingerols and shogaol.

In this study, oleoresin extraction was carried out at room temperature to prevent thermal degradation using the ethanol solvent maceration method. The size of the ginger rhizome extracted by both methods was not in the form of powder because it would go through a water-steam distillation process. Table 2 shows the levels of gingerol and shogaol in the oleoresin extract from both methods. Oleoresin from the first method (EJM2) contains more gingerol and shogaol than the extract from the second method (EJM1). This may be due to no heat treatment and all the compounds directly extracted in the second method.

TABLE II
THE 6-, 8-, 10- GINGEROL AND 6-SHOGAOL CONTENT OF RED GINGER RHIZOME EXTRACT

Compounds	Yield ($mg.g^{-1}$)		Analysis Method
	EJM1	EJM2	
6-Gingerol	21.37	44.37	HPLC
8-Gingerol	4.07	8.99	HPLC
6-Shogaol	4.73	15.91	HPLC
10-Gingerol	9.33	19.31	HPLC

D. Tyrosinase Inhibition Activity

Melanin is the primary pigment responsible for skin, hair, and eye pigmentation in humans and is produced through melanogenesis by melanocytes. Melanogenesis and skin pigmentation are essential in protecting the skin from harmful ultraviolet radiation that can cause cancer and damage. Tyrosinase plays an important role in melanogenesis, and enzymatic browning in fruit is tyrosinase. However, in addition to its photoprotective effect, tyrosinase is also responsible for the unwanted browning of fruits and vegetables and diseases that produce excess melanin. Therefore, the activity of the tyrosinase enzyme needs to be controlled to treat hypopigmentation disorders in mammals and enzymatic browning in fungi and fruits [31].

All oleoresin and essential oil extracts were tested for their bioactivity against two tyrosinase enzyme activities: monophenolase and diphenols. The second enzyme activity test was carried out to determine the components' effect on the two tyrosinase catalytic activities on all oleoresin and essential oil extracts. The two activities involve two different substrates; the first activity of the tyrosinase enzyme is monophenolase, which converts the substrate hydroxylates monophenols (i.e., L-tyrosine/L-tyr) into o-diphenols (L-DOPA), while the second activity is diphenolase, which converts o-diphenols. (L-DOPA) to o-quinones (0-dopaquinone) [31]. The tyrosinase activity test of the extracts of the two methods (Table 3) showed that the order of the extraction methods affected the tyrosinase inhibitory activity of L-Tyr in the oleoresin extract and its essential oil.

TABLE III
TYROSINASE INHIBITION ACTIVITY OF RED GINGER RHIZOME EXTRACT

Sample	Conc. (ppm)	L-Tyr substrate inhibition (%)	L-DOPA substrate inhibition (%)
EJM1	1000	21.99 ± 0.12	29.55 ± 0.19
EJM2	1000	23.71 ± 0.04	29.44 ± 0.22
JD1	1000	15.38 ± 0.17	34.03 ± 0.15
JD2	1000	33.07 ± 0.23	31.80 ± 0.28
Kojic Acid[a]		100.00 ± 0.00	100.00 ± 0.00

All extracts showed moderate monophenolase and diphenolase tyrosinase inhibitory activity, between 15,380 – 34,029 compared to kojic acid. The essential oil obtained from the second method (JD2) showed the highest inhibitory effect on the activity of monophenolase tyrosinase in converting L-tyr substrate to L-DOPA, which was two times higher than the inhibitory activity of essential oil obtained from the first method (JD1). Meanwhile, for oleoresin, the extract from the first method (EJM2) showed slightly higher inhibition of the monophenolase tyrosinase catalytic activity than the oleoresin of the second method (EJM1). However, diphenolase tyrosinase inhibitory activity of both oleoresin and essential oil extracts showed almost the same inhibition. Thus, one of the differences in tyrosinase inhibition ability may be influenced by differences in the content of gingerol and shogaol in EJM2, which is higher than EJM1.

IV. Conclusion

In conclusion, this study reveals for the first time the advantages of the combination of maceration and distillation techniques performed on *Z. officinale* var *rubrum*. This technique is able to produce two forms of extract, namely oleoresin and essential oil with yield and quality supported by chemical composition and tyrosinase enzyme inhibitory activity. Compared to the first method, the second method which combines the maceration process followed by distillation, can improve the quality of red ginger essential oil. In addition, the combination of the second method can prevent wasting red ginger rhizome waste, which still contains essential oils. The second essential oil contains higher (almost doubled) zingiberene compounds and lower camphene contains. The second method also had a higher yield of oleoresin extract, and the tyrosinase inhibitory activity showed a similar level of activity. This study showed that the extraction process carried out before the distillation of the rhizome produced two products (oleoresin extract and essential oil) in the continuation process. This provides a way for future research to optimize the extraction parameters of the combination of maceration and distillation.

Nomenclature

MW	Molecular Weight	g/mol
RT	Retention Time	minute
RI	Retention Index	

Superscripts:

a	Positive control

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