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The combination of ATR-FTIR and chemometrics for discrimination of Red Ginger (*Zingiber Officinale* Var Rubrum) oil in West Sumatra and its antioxidant activity

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ABSTRACT

Their origin influences the chemical components and pharmacological action of essential oils. This study aimed to classify red ginger oil (RGO) based on its FTIR spectra combined with chemometrics and determine its antioxidant activity. Fresh red ginger rhizomes were collected from six West Sumatra regions and extracted using hydrodistillation. ATR-FTIR and Chemometrics were used to classify the oil, and its antioxidant activity was tested using various methods. The study found that RGO was golden yellow with a unique odor, and it had a yield range of 0.52% - 0.86%, a specific gravity of 0.83-0.94 g/ml, a refractive index of 1.4834 – 1.4880, and an optical rotation value of -36.382. However, all RGO samples had low antioxidant activity. Principal Component Analysis (PCA) showed that RGO can be separated based on the altitude of the growing locations at the wavenumber 2000-400 cm^{-1}. Hierarchical Clustering Analysis (HCA) revealed three clusters of RGO: I; Solok, II; Kamang Magek and Batusangkar, III; Dharmasraya, Pesisir Selatan, and Pasaman Barat, with different FTIR fingerprints. In conclusion, red ginger oil from different parts of West Sumatra has unique metabolite profiling and low antioxidant activity levels.

Keywords: Essential Oil, Red Ginger, Antioxidant Activity, Chemometrics, PCA.

INTRODUCTION

In recent years, essential oils have gained popularity in pharmaceuticals due to a growing preference for natural substances. Ginger, a member of the *Zingiberaceae* family, contains essential oils and is extensively utilized as a food preservation, flavor, and traditional medicine in Indonesia and other countries [1]. The primary constituents of red ginger essential oil are *α-zingiberene, β-sesqui phellandrene β-bisabolene, and α-curcumene* [2].

Several factors affect the constituents of red ginger essential oil, such as light, a growing place, rainfall, soil, altitude, etc [3]. Plant's metabolite increases in response to differences in altitude where they grow [3]. For example, phenolic compounds are present in large quantities in plants that grow in the highlands rather than the lowlands [4].

A previous study employed GC-MS to examine white ginger oil from various altitudes in Uttarakhand, India [5], and red ginger oil from Makasar, Indonesia [6]. The study found that there was a significant variation in the chemical composition of white ginger oil from lowland and highland in Uttarakhand and it greatly influenced the antioxidant activity[5]. While, this work proposes an alternative technique using FT-IR fingerprinting with chemometric studies to classify red ginger oil. This method provides speed, cost-effectiveness, sensitivity[7]**.** For instance, FTIR was combined with chemometric analysis to classify *Sida rhombifolia* originating from different locations [8], Similarly, reports the discrimination between *Curcuma xanthorrhiza*, *Curcuma longa*, Jatropha species and Zingiber cassumunar obtained from various places [9],[10].

Red ginger oil has various biological effects, including antibacterial, anti-inflammatory, and antioxidant capabilities [2]. Antioxidants protect the body against the detrimental effects of free radicals, which can cause diseases such as coronary heart disease, stroke, atherosclerosis, kidney failure, cancer, and aging [11,12]. Although the human body generates antioxidants, it frequently requires additional sources, such as essential oils[11]. The chemical composition of essential oils heavily impacts their biological activity [13]. Therefore, this study aimed to identify the fingerprinting pattern to classify the red ginger oil (RGO) based on FTIR spectra combined with chemometrics (PCA and HCA) and determine its antioxidant activity.

Figure 1: The cultivation region of red ginger in West Sumatera, Indonesia

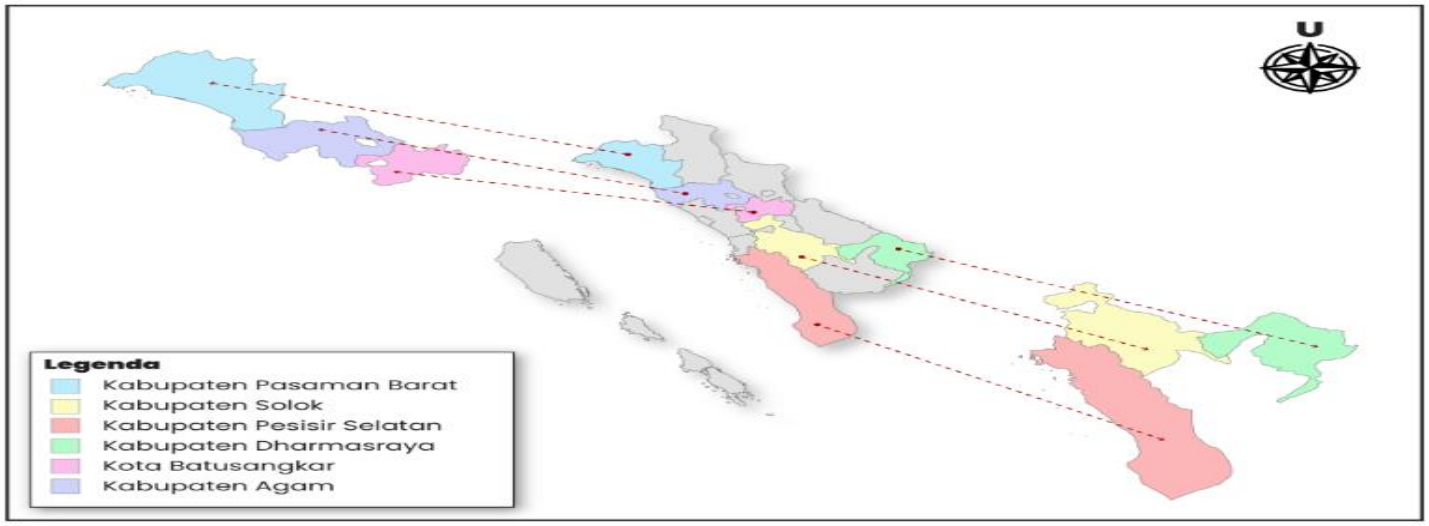


Table 1: Geographical condition

Location	Altitude (m)	Code	Coordinate	Temp. (˚C)	Humidity
Sitiung, Dharmasraya	94 m	DM	$1^0$1'52"S $101^0$37'14"E	26	85%
Lunang. Pesisir selatan	24 m	PS	$2^0$16'25"S $101^0$8'35"E	25	90%
Batusangkar	542	BS	$0^0$56'29"S $100^0$30'21"E	24	89%
Kinali. Pasaman barat	30 m	PB	$0^0$3'17"S $99^0$54'14"E	25	91%
Kamang Magek. Agam	880	AG	0015'30"S 100024'32"E	21	89%
Gunung Talang. Solok	1094 m	SO	$0^0$58'46"S $100^0$37'18"E	21	90%

MATERIALS AND METHODS

The following reagents were used: DPPH reagent, Tris Pyridyl Triazine (TPTZ), ABTS, and trolox, which were supplied by Sigma Aldrich. FeCl3 reagent (30% solution), dimethyl sulfoxide, ascorbic acid, ethanol, and methanol were obtained from Merck. Additionally, PBS tablets (Phosphate Buffered Saline) were obtained from Oxoid.

Plant Collection

Ten kilograms of red ginger rhizome (*Zingiber officinale* var. Rubrum) were gathered from six West Sumatra regions to identify the metabolite fingerprinting of red ginger oil. These regions include Batusangkar, Lunang (Pesisir Selatan), Dharmasraya, Solok, Kinali (Pasaman Barat), and Kamang Magek (Agam) (Figure 1 and Table 1). These areas' selection was random and represented lowland and highland areas. The highlands were at altitudes above 500 m above sea level, while the lowlands were below 500 m above sea level. The samples were identified at the Andalas Herbarium, Department of Biology, Faculty of Mathematics and Natural Sciences, Andalas University, Padang, and deposited with voucher No. **DR-189.**

Extraction of Essential Oil

The fresh red ginger rhizomes were cleaned with running water to remove soil or dirt. Next, the rhizomes were sliced into 4-5 mm thickness using a knife. After that, about 10 kg of pre-cut rhizomes were extracted using a hydro distillation technique for 6 hours. The essential oil was collected in a clean, dark vial. Additionally, anhydrous Na_2SO_4 was added to remove any remaining water, and the vial was stored at 4˚C for future use.

Essential Oil Analysis with FT-IR (Fourier Transform-Infra Red)

Samples of RGO were analyzed using a Shimadzu® FT-IR Spectrophotometer. The RGO was placed on the surface of the ATR prism and run using 32 scans and a resolution of 8 cm^{-1} at wave numbers ranging from 4000 to 400 cm^{-1}. The analysis was carried out at a controlled room temperature of 25°C. The obtained IR spectrum was preprocessed to eliminate noise, including atmospheric correction and smoothing. The peaks visible on the spectra were identified based on the literature to determine the functional groups.

Chemometric Analysis

For chemometric analysis, the absorbance data from the selected peaks of IR spectra were used. This data was then processed using the SIMCA application version 14.1. Red ginger essential oil was classified using PCA (Principal Component Analysis), HCA (Hierarchical Cluster Analysis) and PLS-DA (Partial Least Square-Discriminant Analysis (PLS-DA).

Determination of Antioxidant Activity

Preparation of Test Solution

RGO was mixed with methanol to create a stock solution with a concentration of 10.000 g/mL. The stock solution was then diluted to obtain test solution at the concentrations of 100 g/mL, 10 g/mL, 1 g/mL, and 0.1 g/mL. However, for H_2O_2 scavenging assay, RGO was diluted in DMSO to prepare stock and test solution.

DPPH (2.2–Diphenyl–1–picrylhydrazyl) assay

About 100 µl of test solution was added to the wells of 96-well plates, followed by 100 µl of 0.2 mM DPPH, and incubated at room temperature for 15 minutes. After 15 minutes, the color changes were observed, and then the absorbance was measured using a microplate reader at a wavelength of 517 nm. Methanol was used as a negative control, while Vitamin C was used as a positive control. The test was carried out in triplicate. The percentage of inhibition was calculated using the following formula. and IC_{50} was also calculated [14].

$$\textbf{Inhibition}\ (\%) = \frac{(\textbf{Absorbance of control} - \textbf{Absorbance of sample})}{(\textbf{Absorbance control})} \textbf{x}\ \textbf{100}$$

ABTS (2.20–Azinobis–(3–ethylbenzothiazoline–6–sulphonic acid) assay

The test solution and trolox (positive control) were dispensed in 96-well plates, about 100µl each, followed by 100µL of ABTS solution. After that, the microplate reader was used to measure the absorbance at 645nm. The test was carried out in triplicate, and the % inhibition and IC50 were calculated [15,16].

$$\textbf{Inhibition}\ (\%) = [\mathbf{1} - \left(\frac{\textbf{Absorbance of sample}}{\textbf{Absorbance of control}}\right)\textbf{x100}]$$

FRAP (Ferric Ion Reducing Antioxidant Power) assay

About 20 µL of the test sample was mixed with 180 µL of FRAP reagent in a 96-well microplate. The mixture was allowed to stand for 5 minutes before measuring the absorbance at 595 nm by a microplate reader. The absorbance was compared to a standard curve created using a $FeSO_4$ solution ranging from 1200M to 6.25 uM to calculate the FRAP value. The FRAP value was expressed as uM Fe(II)/mg, calculated using the FeSO4 regression equation. [15,16].

Inhibition of erythrocyte hemolysis (H_2O_2 Scavenging Activity)

The rat blood was collected in an EDTA tube. It was then transferred to a centrifuge tube and centrifuged at 2000 rpm for 5 minutes. The pellets were collected and washed with PBS three times. Finally, they were dissolved in phosphate-buffered saline (PBS) to produce a 5% (v/v) concentration. Next, 50 µl of the test solution was added to each well of a 96-well plate. Then, 100 µl of 100 mM H_2O_2 solution (in PBS pH 7.4) was added and incubated for 1 hour at 37˚C. After that, the absorbance was measured at a wavelength of 540 nm. Vitamin C was used as a positive control. The percentage of inhibition was calculated using the following formula [16,17]

$$\textbf{Inhibition}\ (\%) = [\mathbf{1} - \left(\frac{\textbf{Absorbance of sample}}{\textbf{Absorbance of control}}\right)\textbf{x100}]$$

Statistical Analysis

Statistical Analysis was carried out by Minitab software version 20 using one-way ANOVA followed by Tukey's test. The differences were considered significant at $p < 0.05$.

RESULTSAND DISCUSSION

Physical Characteristics

The physical properties of each red ginger essential oil are shown in Figure 2 and Table 2, including yield, refractive index, optical rotation and specific gravity. RGO was golden yellow in color and has a distinct pungent scent. The extraction yield ranged from more than 0.086 %w/v to 0.52 % w/v. The highest yield obtained was RGO collected from Batusangkar region.

Figure 2: Physical characterization of red ginger essential oil

In this study, the specific gravity for RGO was between 0.83-0.94 g/ml, whereas BS RGO has the highest specific gravity with a value of 0.94 g/mL. RGO has a refractive index of 1.4834 – 1.4880, and optical rotation was -36.382 ˚C. The physicochemical properties of oil indicated an essential oil's quality. The quality should refer to international standards (ISO) or national standards (Indonesian National Standard/SNI) [19]. However, there was no quality standard specifically explained on red ginger oil in both standards. The SNI 06-13-12-1998 only demonstrated the quality of ginger oil (*Zingiber officinale*, Roscoe) (Table 3).

Table 2. Physical Characteristic RGO

Sample	Yield (%)	Color	Odor	Specific Gravity (g/mL)	Refractive index	Optical rotation
DM	0.211	Golden yellow	Distinct scent	0.89	1.4876	-36.382
BS	0.520			0.94	1.4859	-36.382
SO	0.211			0.90	1.4842	-36.382
PB	0.086			0.83	1.4880	-36.382
PS	0.231			0.85	1.4834	-36.382
AG	0.195			0.89	1.4839	-36.382

The physical characteristics of DM RGO fulfilled the SNI standard, but the yield was still less than the yields stated in Indonesian Herbal Pharmacopeia (FHI) 2017, which should not less than 0.8% v/b. But, another study stated the yield from fresh ginger essential oil was 1.02% v/b [20]. The yield varies greatly depending on the species and variety; even within the same species but with different geographic locations could produce a diverse yield of RGO [21,22].

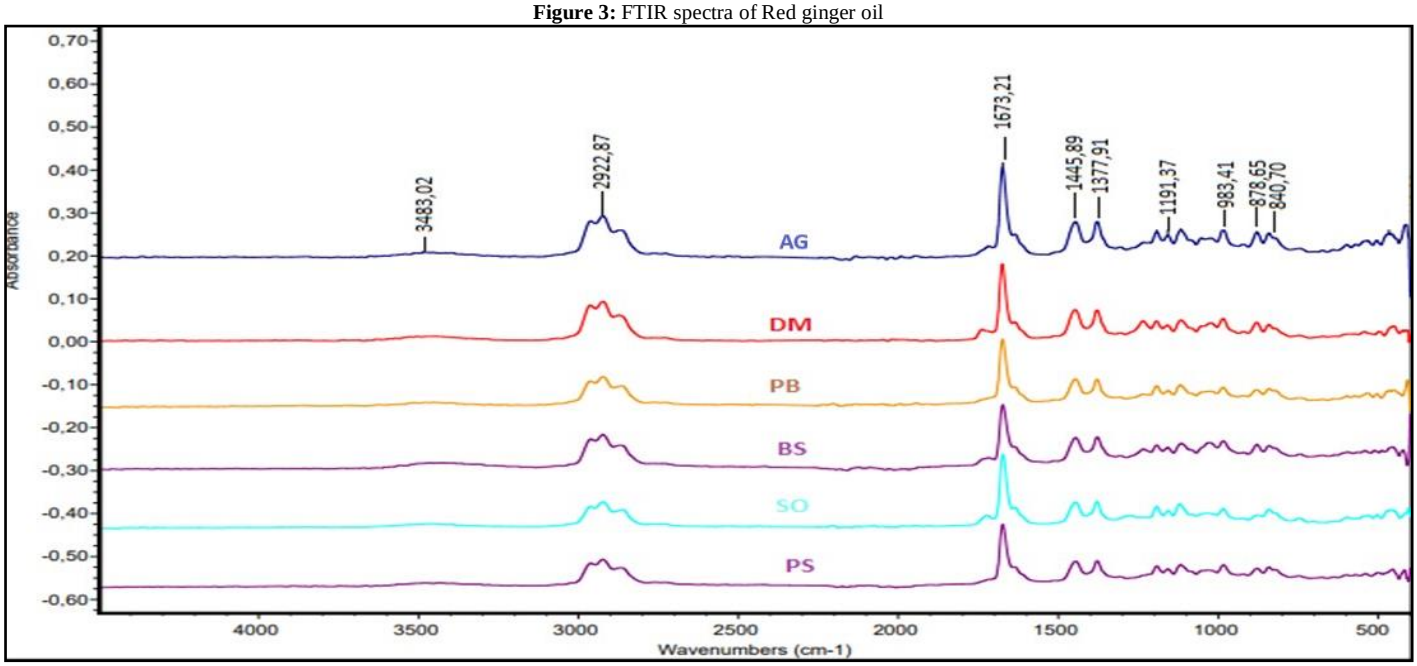
Table 3: SNI (Indonesian National Standard) 06-13-12-1998 for ginger oil [18]

Parameter	Requirement
Specific Gravity (g/mL)	0.8720 – 0.8890
Refractive index	1.4853 – 1.4920
Optical rotation	(-32^0) - (14^0)

FTIR spectra

The FTIR spectra of the six RGO can be seen in Figure 3. Each compound's functional group in RGO absorbs IR radiation, causing a peak to appear at a specific wave number. The functional group was identified according the literature [23,19].

Figure 3: FTIR spectra of Red ginger oil



The peak at 2992 cm-1 is a typical peak in terpene hydrocarbons as results of symmetric and asymmetric stretching of C-H, -CH_2, and –CH_3 which are groups of alkane chains [23]. The peak of 3483 cm-1 resulted from vibrations during O-H stretching. While the peaks of 1673 cm-1 is typical peaks in red ginger essential oil from the results of stretching C = C . The peaks of 1191 cm-1 indicated ether C-O stretching vibrations. The peaks of 878 and 851 cm-1 come from bending of the vibration planes – HC=CH– (trans) and –HC=CH– (cis) [13,21,22].

Chemometric Analysis

The results of the PCA (Principal Component Analysis) chemometric analysis are in the form of a score plot, while the HCA (Hierarchical Cluster Analysis) is in the form of a dendrogram. PCA was performed on the absorbance at wave numbers 2000-400 cm^{-1}, which was then reduced to a principal component (PC) which could represent the structure and variance in the data [24]. The PCA scores plot above shows the values of PC-1 = 85% and PC-2 = 5.42% so that the cumulative results of PCA analysis was 94.20% (Figure 4). This shows that as much as 94.20 % of the data diversity can explain the variable absorbance of functional groups of RGO. In addition, the cumulative R^2 value was 0.991, and the cumulative Q^2 value was 0.982.

The results also show that red ginger oil originated from highland (BS, SO and AG) was located in PC1. In contrast, the red ginger oil originated lowland (DM, PES and PB) was located in PC2. The classification of RGO was further analyzed by HCA analysis to confirm the classification. This algorithm is shown in the form of a dendrogram. Principal Component Analysis (PCA) and Hierarchical Cluster Analysis (HCA) can effectively distinguish and classify RGO collected from different growing locations through identifying similar fingerprint patterns [24]. The PCA score plot is used for grouping based on PC1 and PC2, which have the largest variable values so that they can represent all components [24].

Figure 4: Score plot of PCA based on growing location

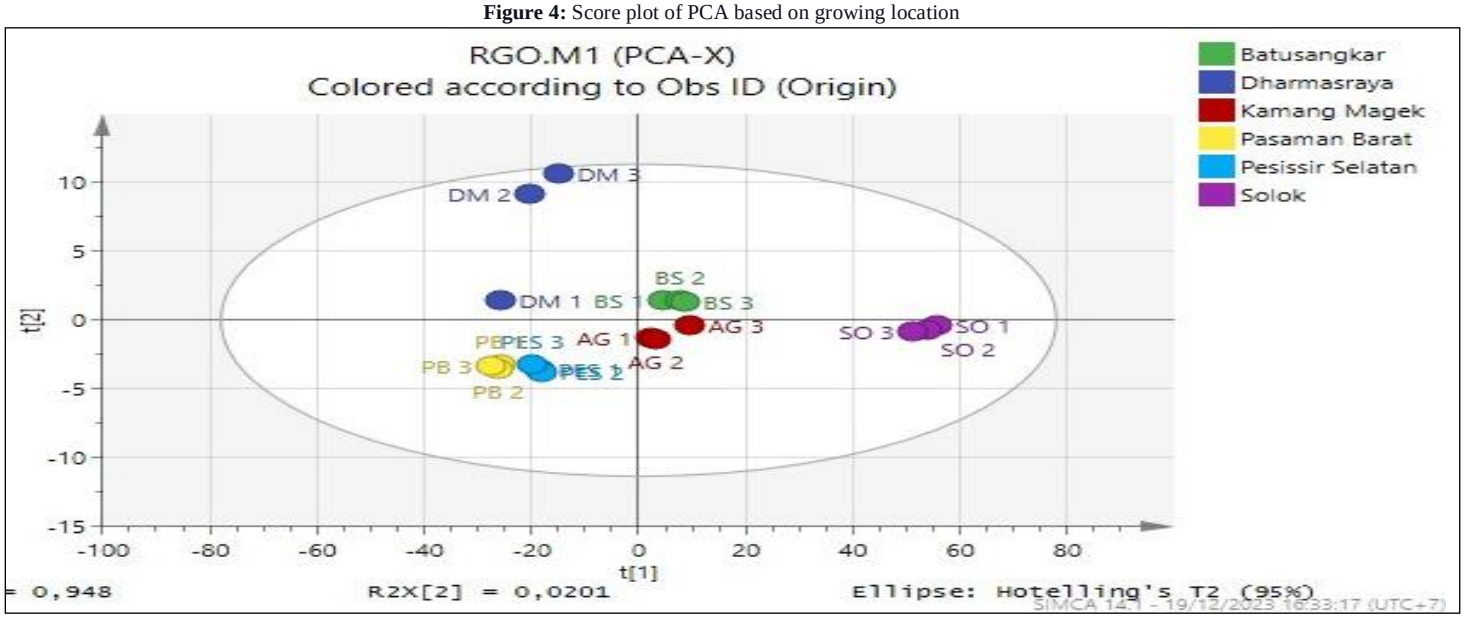


Figure 5: Hierarchical clustering analysis (HCA) Dendogram of RGO

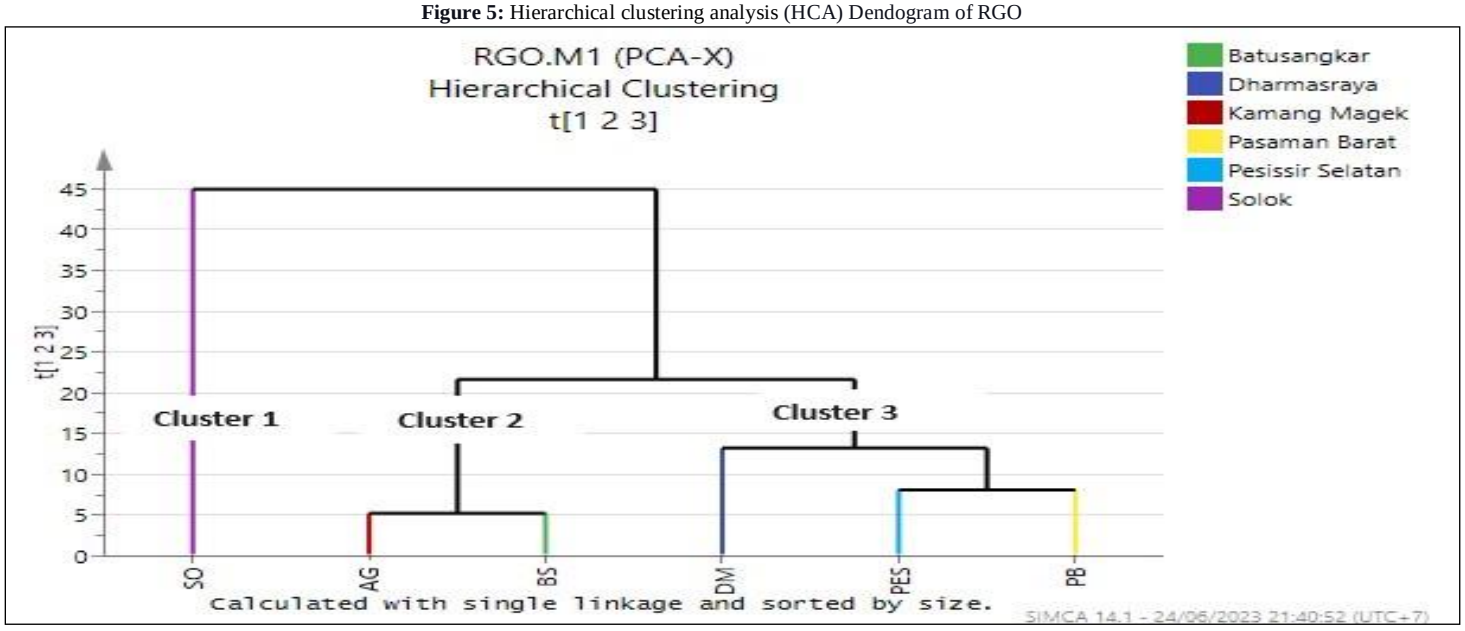
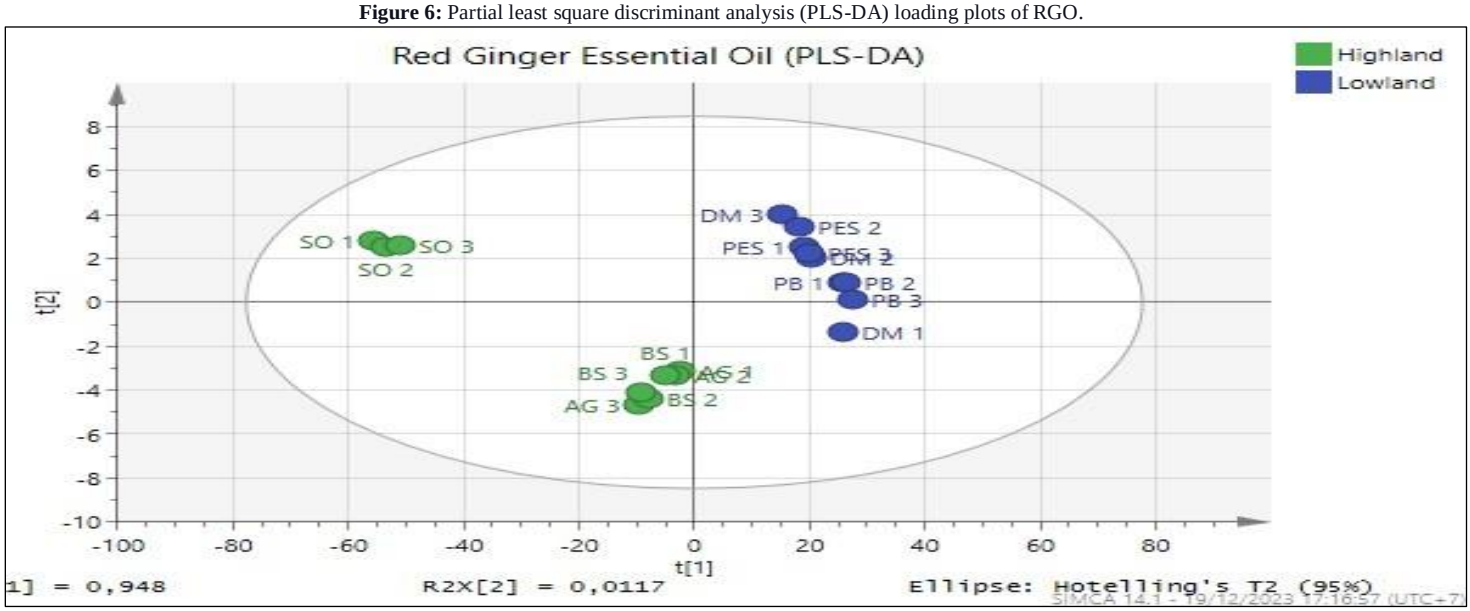


Figure 6: Partial least square discriminant analysis (PLS-DA) loading plots of RGO.



The closer the sample is to other samples the greater the similarity between the samples. Clusters formed with high similarity are also grouped into new parent clusters [25]. This means that although there are structural similarities in RGO, the six RGOs also have differences in chemical composition.

The PLS-DA was used to identify functional groups with the highest ranking using the VIP value parameters (Figure 6). Three functional groups were identified (C-O stretching, C=O stretching, and O-H stretching) based on VIP value greater than 1.0 [26].

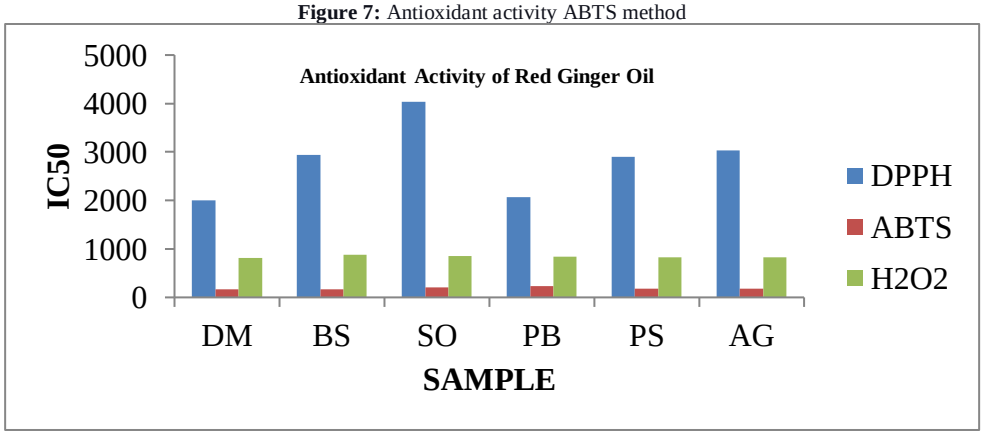
Antioxidant Activity

The antioxidant activity is classified based on the IC_{50} value. It is classified as a very strong antioxidant activity if the IC_{50} value is less than 50µg/mL, weak if the IC_{50} value is between 50-100µg/mL, and very weak if the IC_{50} value is more than 200µg/mL [27]. The results of the antioxidant activity of red ginger essential oil are presented in Table 4 and Figure 7. In DPPH and inhibition of erythrocyte hemolysis assay, all RGO had a high IC_{50} value of more than >200 µg/ml. It means RGO has very weak antioxidant activity. Otherwise, the positive control (ascorbic acid) had very strong activity, with an IC_{50} value of 2.55 µg/mL. All RGO had weak antioxidant activity for the ABTS assay, as indicated by its IC_{50} value of more than 100 µg/mL. In contrast, the positive control (Trolox) had an IC_{50} value of 14.50 µg/mL. The statistical analysis showed no significant differences in the antioxidant activity among the six locations ($p>0.05$).

Table 4. Antioxidant activity

Sample	IC_{50} (µg/mL)			FRAP value (µM Fe^{+} equivalent)
	DPPH	ABTS	H_2O_2	FRAP
DM	1998	161	815	239
BS	2944	170	880	218
SO	4038	211	853	174
PB	2074	229	834	234
PS	2902	174	832	164
AG	3029	181	824.	216
Ascorbic acid	2.55	-	2.09	13821
Trolox	-	14.50	-	-

Figure 7: Antioxidant activity ABTS method



Antioxidant activity was evaluated using DPPH, ABTS, FRAP, and inhibition of erythrocyte hemolysis (H_2O_2 Scavenging Activity) methods. These methods have different mechanisms as antioxidants. The DPPH, ABTS, and inhibition of hemolysis methods are based on ROS scavenging (free radical scavenging). In contrast, the FRAP method does not involve free radicals in the reaction but reduces Fe^{3+} to Fe^{2+}. The results of the antioxidant activity may differ depending on the test method used [28]. Previous study stated ginger rhizome essential oil from the highlands has a better ability to ward off DPPH radicals than ginger essential oil from the lowlands [5]. Where ginger essential oil collected from the highlands of the Uttarakhand region in India has an IC_{50} value of 15.97 µg/mL and from the lowlands of 18.9 µg/mL [8]. Another study found that ginger essential oil has very weak antioxidant activity in counteracting DPPH radicals with an IC_{50} value of more than 1000 µg/ml. The presence of phenolic groups plays an important role in the antioxidant activity of ginger oil [29].

The measurement of antioxidant activity with the ABTS method was more sensitive than the DPPH method [28]. This is due to DPPH can only dissolve in a polar matrix and has a slow reaction in antioxidants with DPPH radicals [30]. A study stated *Zingiber officinale* Roscoe had a weak antioxidant activity using ABTS assay with an IC_{50} value of 110.14 mg/ml [31].

Hemolysis can be used to measure cell damage due to free radicals. H_2O_2 is a non-reactive molecule, but sometimes it can cause cytotoxicity by providing hydroxyl radicals (HO.) in cells. These radicals induce lipid and protein peroxidation at high concentrations, affecting cell integrity. The H_2O_2 method uses mouse erythrocytes as a medium to observe their oxidative reactions to free radicals [32].

CONCLUSION

Red ginger oil was derived from six locations in West Sumatra that can be classified into lowland and highland-originated RGO based on their unique fingerprinting patterns. The oil collected from Dhamasraya meets the SNI quality standards, but all types of red

ginger oil have weak antioxidant activity. It is recommended that the Dhamasraya region be developed for red ginger cultivation, and future works suggest exploring other potential biological activities for RGO.

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Conflict of interests Authors state no conflict of interest.

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