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Original Research Article

FT-IR Fingerprinting Analysis for Classification of West Sumatra Small Ginger (*Zingiber officinale* Roscoe) Essential Oil and Its Antioxidant Activity

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ABSTRACT

Due to its essential oil content, the community utilises the small ginger rhizome as traditional medicine and spices. This study aims to compare the antioxidant activity of small ginger essential oil (SGEO) from six different altitudes in West Sumatra and then categorise the SGEO using FTIR spectroscopic and chemometric analysis. Fresh rhizome of small ginger was extracted by hydrodistillation method. Antioxidant activity was evaluated using DPPH, ABTS, FRAP, and inhibition of erythrocyte hemo lysis by H_2O_2. The SGEO has a golden yellow colour with a distinctive ginger odour. The yield was between 0.1- 0.2%; the refractive index was 1.4860-1.4880, the optical rotation value was - 30.3207°, and specific gravity was 0.80-0.95 g/mL. The data was analysed using one-way ANOVA at a 95% significance level. The FTIR spectra revealed the typical peak at 2923 $cm^{-1,}$ corresponding to terpene hydrocarbons. The results showed that the six essential oils had weak antioxidant activity with an IC_{50} value >200 µg/mL and a FRAP value < ascorbic acid (positive control). PCA analysis showed that the SGEO from lowland and highland regions could be classified based on the 200-400 cm^{-1} fingerprint area. The hierarchical clustering analysis (HCA) dendrogram showed 3 clusters of SGEO (I; Ampek Angkek, II; Paninjauan, Sitiung, III; Koto laweh, Kinali, Lunang). Altitude plays little or no role in the FTIR fingerprint properties of small ginger essential oils. The essential oils exhibited weak antioxidant activity.

Keywords: *Zingiber officinale*, essential oil, antioxidant, fingerprinting, chemometrics

Introduction

Unhealthy lifestyles such as smoking, poor diet, and lack of exercise increase the risk of developing severe diseases due to the presence of free radicals such as peroxide anion (RO2), hydroxyl (OH), nitric oxide (NO), superoxide anion (O2-) and reactive oxygen compounds. These free radicals have the potential to cause damage to biomolecules, resulting in neurodegenerative diseases, diabetes mellitus, cardiovascular disease, premature ageing, and cancer.[1] Antioxidant agents have been used to scavenge free radicals in the human body.[2,3] Antioxidants are essential in reducing the harmful effects of metals that can trigger oxidative stress to cells in the body.[4] Recently, there has been a growing interest in using natural ingredients such as herbs (rosemary, oregano, marjoram, sage, basil, etc.) and spices (garlic, cloves, cinnamon, ginger, nutmeg, black pepper, etc.) as safer alternatives to synthetic antioxidants.[5]

Generally, ginger can be distinguished based on the rhizome's scent, colour, shape, and size. The ginger varieties are classified into white, red, and small ginger. Small ginger has softer fiber than other types of ginger. Despite the small size of the rhizome, small ginger has a sharp aroma and spicy taste6. Small ginger rhizome also contains high nutrients, including starch, protein, oleoresin, and essential oils.[7,8]

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Ginger essential oil is rich in hydrocarbon compounds, including a high percentage of sesquiterpenes (66.66%) and monoterpenes (17.28%), as reported in a previous study.[9,10] However, the chemical constituents can vary depending on where the ginger was grown. Higher altitudes, for example, can decrease soil pH and micronutrient content, leading to lower mineralisation processes. Some compounds may increase in response to different altitudes, aiding plants in adapting to various environmental conditions. [11] Whereby the chemical composition of essential oils heavily impacts their biological activity.[12] Identifying differences in compound composition among plant species is possible through FTIR fingerprinting experiments. FTIR has several advantages, such as being cost-effective, fast, and requiring minimal sample preparation.[13] However, the fingerprint area has a complex pattern, necessitating chemometric analysis. Chemometrics employs statistical and mathematical methods to extract significant insights from absorbance data from IR spectra efficiently.[14] The classification of herbal components has been made possible through FTIR spectra combined with chemometrics.[15] Currently, no studies in literature determine the fingerprinting pattern of small ginger oil originating from West Sumatra. Therefore, this study aims to identify metabolite profiling of small ginger essential oil (SGEO) from West Sumatera using FTIR spectra in combination with chemometrics (PCA and HCA) and to evaluate its antioxidant activity.

Materials and Methods

Materials

DPPH reagent, Tris Pyridyl Triazine (TPTZ), ABTS, and trolox were supplied by Sigma Aldrich. Additionally, $FeCl_3$ reagent, 30% H_2O_2 solution, DMSO, ascorbic acid, ethanol, and methanol were obtained from Merck, while PBS tablet (Phosphate Buffered Saline) was obtained from Oxoid.

Plant collection
About ten kilograms of small ginger (*Zingiber officinale*) rhizome was obtained from six areas in West Sumatra, Indonesia: IV Angkek, Kinali, Lunang, Sitiung, Paninjauan, and Koto Laweh in May – June 2022. These areas were categorised as highlands (600 meters above sea level) and lowlands (below 600 meters above sea level). The plant identification process was conducted at the ANDA Herbarium Andalas (ANDA), part of the Department of Biology in the Faculty of Mathematics and Natural Sciences at Universitas Andalas in Padang, Indonesia. The voucher specimen was kept with voucher No. DR-188. The characteristics of each location are listed in Table 1.

Extraction of essential oil
Fresh rhizomes of small ginger were sliced and extracted by hydrodistillation methods using the Clevenger apparatus. The distillation process was carried out for 6 hours. The essential oil (SGEO) obtained was collected in dark bottles, and Na_2SO_4 was added to remove the remaining water. After that, the essential oil was stored at 4°C for further use.

Essential Oil Analysis with Fourier Transform-Infra Red (FT-IR)
SGEO were dropped on the ATR (Smart iTR) surface of the FTIR spectrometer (Shimadzu) and scanned in the MIR region with wave numbers of 4000–400 cm^{-1} and at a controlled room temperature of 25 °C. The scan ran for 32 scanners and a resolution of 8 cm^{-1}. The previously measured air served as a background, and the resulting spectrum was automatically modified or corrected. The spectral measurements were made in triplicates. All the spectra obtained were pre-processed, including atmospheric correction and smoothing.

Chemometric analysis
Absorbance data from the peaks of IR spectra at wave numbers 2000-400 cm^{-1} were used for chemometric analysis. Then, this data was processed using the SIMCA application version 14.1. For the classification of the six SGEOs, the data were subjected to PCA (Principal Component Analysis) and HCA (Hierarchical Cluster Analysis).

Ethical clearance
This study was approved by The Research Ethics Committee, Faculty of Medicine, Universitas Andalas N0: 27/UN.16.2/KEP-FK/2023. This certification confirms that the research protocol qualified to conduct a study of this nature.

Determination of Antioxidant Activity
Preparation of Sample Solution
SGEO was diluted with methanol to produce a stock solution with a 10 mg/mL concentration for the DPPH, ABTS, and FRAP assay. The stock solution was then diluted to obtain the test solution at the concentrations of 100 µg/mL, 10 µg/mL, 1 µg/mL, and 0.1 µg/mL. For the H_2O_2 scavenging assay, SGEO was diluted with DMSO to prepare the stock and test solutions.

DPPH assay
The DPPH (2,2–Diphenyl–1–picrylhydrazyl) assay was performed in a 96-well plate. The wells were filled with 100 µL of test solution, followed by 100 µL of 0.2 mM DPPH, and kept at room temperature for 15 minutes. The colour changes were observed after 15 minutes, and the absorbance was measured with a microplate reader (Biocrom Asys UVM 340, Agilent Technologies) at a wavelength of 517 nm. Methanol was the negative control, and ascorbic acid was the positive control. The test was performed in triplicates. The following formula was used to calculate the percentage of inhibition, and the IC_{50} value was also calculated.[16]

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

ABTS (2,2'–Azinobis–(3–ethylbenzothiazoline–6–sulphonic acid) assay
ABTS reagent was prepared by dissolving 38 mg of ABTS powder in 10 mL of purified deionised water to obtain the final concentration of 7.0 mM. Then 6.5 mg of potassium persulphate was added and incubated for 16 hours to form ABTS radicals. The ABTS solution was then diluted with water to obtain a final absorbance value between 2.0 and 2.4 at the wavelength of 645 nm. Positive control (Trolox solution) was dissolved in absolute ethanol to obtain a concentration of 20 mM. The well was filled with 100 µl of SGEO ginger test solution or trolox, and then 100 µL of ABTS solution was added to each well. The absorbance was measured at 645 nm using the Microplate Reader (Biocrom Asys UVM 340, Agilent Technology). The test was performed in triplicates.[17] Inhibitory activity (% inhibition) and IC_{50} were calculated from the formula below.

$$Inhibition\ (\%) = \frac{[\,1 - (Absorbance\ of\ sample)]}{Absorbance\ control}\ x\ 100$$

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, allowed to stand for 5 min, and the absorbance was then measured using a microplate reader (Biocrom Asys UVM 340, Agilent Technologies) at a wavelength of 595 nm. The standard curve was generated using a solution of $FeSO_4$ (12 M - 6.25 M). The $FeSO_4$ regression equation was used to calculate the FRAP value, expressed as µM Fe(II)/mg.

Inhibition of erythrocyte hemolysis (H2O2 Scavenging Activity)
Blood from the experimental animals' tails was collected into EDTA tubes, then transferred to centrifuge tubes and centrifuged at 2000 rpm for 5 minutes. Pellets were collected and washed with PBS thrice, then dissolved in phosphate-buffered saline (PBS) to produce a 5% (v/v) concentration. In each well of the 96 well-plates, 50 uL of test solution was added, followed by 100 µL of 100 mM H_2O_2 solution (in PBS pH 7.4) and incubated for 1 hour at 37˚C. Then, the absorbance was measured at a wavelength of 540 nm. Ascorbic acid was used as the positive control.[18] The percentage of inhibition was calculated using the following formula:

$$Inhibition\ (\%) = \frac{[\,1 - (Absorbance\ of\ sample)]}{Absorbance\ of\ control}\ x\ 100$$

Statistical analysis
The antioxidant activity was analysed using Minitab version 20 software using one-way ANOVA followed by Tukey's test. The differences were considered significant at $p < 0.05$. The analysis results were considered significantly different at a p-value <0.05.

Table 1: Geographical conditions

Location	Altitude (m)	Code	Coordinate	Temp. (C°)	Humidity
IV Angkek	877	AA	$0^0$16'41''S100026'09''E	25	90%
Kinali	30	KN	$0^0$3'17 "S 99^{0}54'14"E	25	91%
Paninjauan	904	PN	$0^0$26'49"S100˚25'22"E	23	90%
Koto Laweh	1245	KL	$0^0$25'10"S100˚21'59"E	22	92%
Lunang	41	LN	$2^0$16'25"S 101^{0}8'35"E	25	90%
Sitiung	94	ST	$1^0$1'52"S 101^{0}37'14"E	26	85%

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Results and Discussion

Physical characteristics

Figure 1 shows the six locations in West Sumatera, Indonesia, where the plant samples were collected. Figure 2 and Table 2 show the physical characteristics of each small ginger essential oil, including specific gravity, optical rotation, refractive index, and yield. The typical oil obtained was a light yellow to golden yellow with a distinct ginger aroma.

The yield of small ginger essential oil was in the range of 0.125 – 0.2 %. According to the Indonesian Herbal Pharmacopoeia, ginger contains essential oils that should exceed 0.8%. However, studies on the composition of ginger essential oil from several areas in India revealed the yield was in the range of 0.11% to 0.45%.[19] Several factors, such as the distillation method, sample preparation, and distillation time, can influence the yield of essential oils.[11,20]

The quality and purity of ginger essential oil could be detected through its specific gravity and refractive index. The specific gravity values of small ginger essential oil range from 0.75 – 0.91 g/mL. A previous study stated the specific gravity value of small ginger oil was 0.929 g/mL.[21] According to the Indonesian National Standard (SNI), the specific gravity of small ginger essential oil should range from 0.8720 - 0.8890 g/mL.[22] It means that only PN SGEO met the requirement. The specific gravity value is often associated with the weight fraction of the components contained therein. Therefore, the greater the weight fraction contained in the oil, the greater the specific gravity value.[23] The refractive index values of ginger essential oil obtained from each region were 1.4851 – 1.4879. A previous study stated that the refractive index value of ginger oil ranges from 1.488 to 1.494.[24] However, according to the Indonesian National Standard (SNI), the refractive index value of ginger essential oil is 1.4853 to 1.4920.[22] Thus, the refractive index of KN SGEO was out of the standard range. The optical rotation value of small ginger essential oil was -30.2°. According to the Indonesian National Standard (SNI), the optical rotation value is -32° to -14°.[22] However, ISO 279-1981 and ISO 280-1976 stated the relative density = 0.870-0.882, the refractive index = 1.488-1.494, and optical rotation = -47° to -28°.[24]

FTIR Spectra

In Figure 3, the FTIR spectra of the six SGEOs are shown. The functional groups in SGEOs absorb IR radiation, causing a peak at a specific wave number (as listed in Table 3). Identification of the functional group was done based on existing literature. Although the peak pattern of the six SGEOs was similar, their absorbance varied, suggesting that all SGEOs have similar chemical components but in different concentrations.

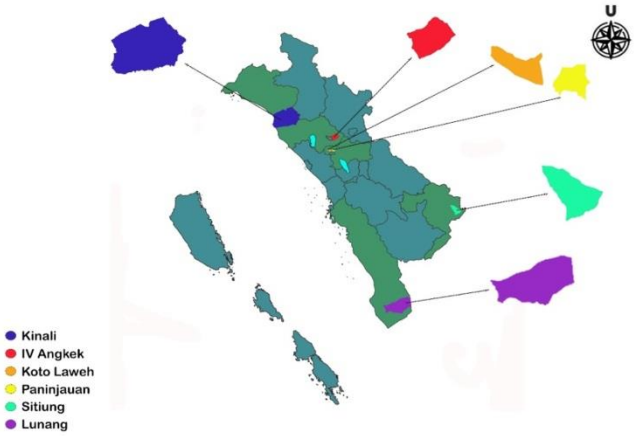

Figure 1. The origin of small ginger essential oil

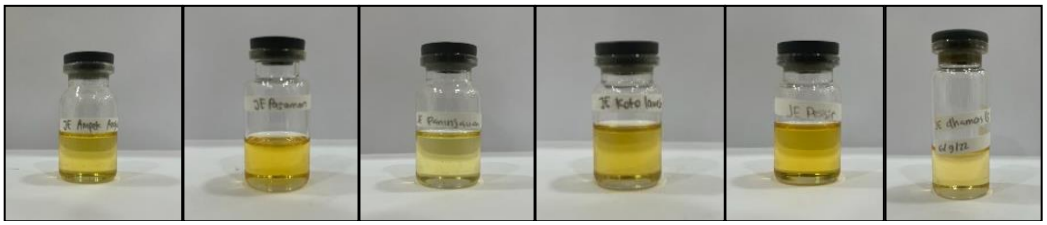

Figure 2: The essential oil of the small ginger

Table 2: Physical characteristics of SGEOs

Sample	Yield (%)	Colour	Odour	Specific Gravity (g/mL)	Refractive index	Optical rotation
AA	0.138	Golden yellow	Distinct scent	0.85	1.4860	-30.2
KN	0.2	Golden yellow	Distinct scent	0.81	1.4851	-30.2
PN	0.19	Golden yellow	Distinct scent	0.88	1.4867	-30.2
KL	0.156	Golden yellow	Distinct scent	0.84	1.4867	-30.2
LN	0.125	Golden yellow	Distinct scent	0.75	1.4879	-30.2
ST	0.129	Dark Golden yellow	Distinct scent	0.91	1.4876	-30.2

Table 3" Functional groups on the SGEOs FTIR spectra

Wave number (v, cm^{-1})	Functional group vibration
2923	Stretching symmetric and asymmetric C-H, -CH_2, –CH_3
1673	Stretching C=C (aromatic and aliphatic))
1445 and 1377	stretching CH_3
1191, 1114, 1021	Stretching C-O
983	Bending C-H aromatic (in of plane)
878	Bending C-H aromatic (out of plane)
840	Stretching C-H aromatic

The peak at 2923 cm^{-1} is typical in terpene hydrocarbons caused by symmetric and asymmetric stretching of C-H, -CH_2, and -CH_3 alkane chain groups.[25] Peaks of 1673 cm^{-1} are typical in SGEO due to stretching C = C.[26] The 1191 cm^{-1} peak indicated ether C-O stretching vibrations. The peaks at 983 and 878 cm^{-1} result from plane bending vibration - HC=CH- (trans) and -HC=CH- (cis).

Chemometric analysis

Principal component analysis (PCA) is a multivariate data reduction technique when each variable correlates. This method can be used if objects (samples) with almost the same main components have the same physicochemical properties. Cluster analysis is a method for dividing a group of objects (sample) into a group (class) so that similar objects will be in the same group.[14,27,28] The score plots of PCA analysis can be seen in Figure 3. The cumulative R^2 value was 0.998, and the cumulative Q^2 value was 0.994. The PCA scores plot shows PC-1 = 19.4% and PC-4 = 4.79%, resulting in a total PC value of 24.19% from PCA analysis using spectrum data at 2000-400 cm^{-1}.

Figure 4A reveals SGEO separation based on altitude. The blue square indicated SGEO from the highland, while the green circle indicated the lowland SGEO. In addition, Figure 4B shows six SGEO groups based on their origin. The PN and KL were located in Quadrant I, while the ST and LN were in Quadrant II. On the other hand, KN was in quadrant III, but AA was in quadrant IV. Although the SGEOs share structural similarities, they may differ in chemical composition. As a result, HCA analysis was performed to confirm the SGEO classification. This algorithm is depicted as a dendrogram, whereby the dendrogram groups the samples into clusters according to the similarity of the sample characteristics. If cut at t=16, the dendrogram divides the SGEO into 4 clusters: I: PN SGEO, II: AA SGEO, III: KL SGEO, and IV: LN, ST, KN SGEO (Figure 5).

Antioxidant activity

The IC_{50} value is used to classify antioxidant activity. If the IC_{50} value is less than 50 µg/mL, the test sample possesses very strong antioxidant activity, weak between 50-100 µg/mL, and very weak if greater than 200 µg/mL.[29] The results of the antioxidant activity of small ginger essential oil are presented in Table 4 and Figure 6. The study shows that SGEO, collected from six locations, had weak antioxidant activity. All SGEO had an IC50 value of more than 200 µg/mL for all antioxidant methods. Ascorbic acid, used as the positive control, possesses very strong antioxidant activity with an IC_{50} of 2.55 µg/mL for the DPPH assay, 2.09 µg/mL for the hemolysis method, and 13821 µM FRAP value. Additionally, the IC_{50} value for Trolox was 17.08 µg/mL. According to the statistical analysis, there were significant differences in the antioxidant activity of SGEO from the six locations compared to the positive control ($p<0.05$). The post hoc analysis (Tukey's test) revealed that the growth location significantly affected the antioxidant activity. For the DPPH, ABTS, and hemolysis assays, all six SGEOs were located in different groups, whereas, in the FRAP assay, some SGEOs were found in the same group. For instance, KN and ST were in the same group, indicating no significant difference in their antioxidant activity. This study also confirmed that ginger essential oil has lower antioxidant activity than turmeric and galangal oil.[30–32] Another study showed that ginger essential oil has weak activity with an IC_{50} value of 110 µg/mL.[33] The previous study concluded that the antioxidant activity may vary due to various factors such as the method of testing employed and the location from which the plant was collected.[34,35]

Conclusion

This study concluded that small ginger essential oils can be classified according to their growing location altitude using FT-IR and chemometrics. However, the antioxidant activity of small ginger essential oil obtained from the six regions in West Sumatra was low. The SGEO from Lunang (LN) showed higher antioxidant activity than others. Further evaluation of essential oils for in vitro and in vivo biological activities is recommended.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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Table 4: Antioxidant activity

Sample	IC_{50} Value(µg/mL)			FRAP Value (µM)
	DPPH	ABTS	H_20_2	
AA	1289.1 ± 15.18^{b}	552.09 ± 1.91^{d}	867.19 ± 1.53^{b}	214.09 ± 13.56^{e}
KN	1236.4 ± 2.08^{c}	568.75 ± 5.29^{c}	893.15 ± 3.51^{a}	$193.76 \pm 2.12^{c,d}$
PN	2155.7 ± 17.6^{a}	1065.23 ± 2.65^{a}	844.07 ± 1.53^{c}	283.47 ± 10.31^{c}
KL	759.62 ± 10.12^{d}	308.61 ± 3.96^{f}	832.91 ± 2.52^{c}	191.64 ± 16.29^{b}
LN	450.97 ± 5.00^{e}	420.55 ± 3.33^{e}	864.94 ± 9.35^{b}	213.20 ± 14.34^{c}
ST	1248.80 ± 4.36^{c}	653.13 ± 9.41^{b}	857.26 ± 4.04^{b}	$168.53 \pm 13.38^{d,e}$
Ascorbic acid	2.55 ± 0.0376^{f}	-	2.09 ± 0.16^{d}	13821 ± 20.3^{a}
Trolox	-	17.08 ± 4.11	-	-

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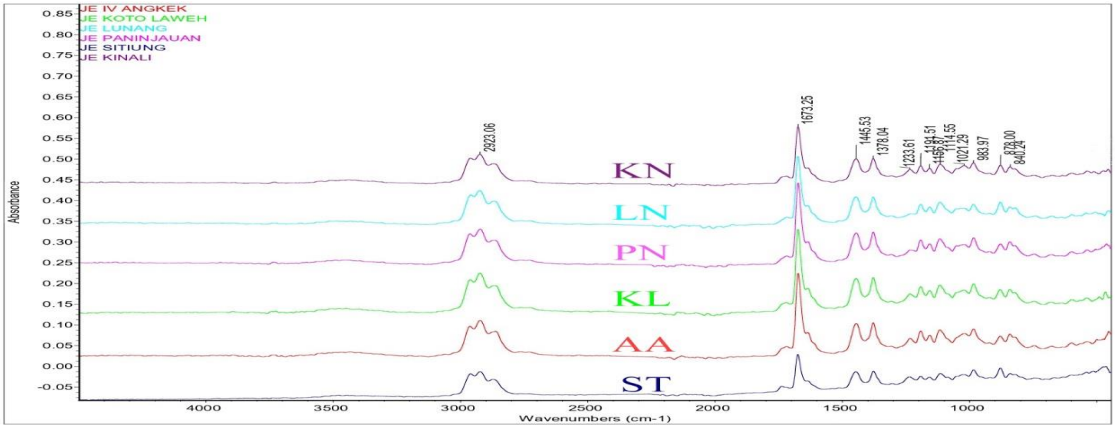

Figure 3: FTIR spectra of the small ginger essential oil

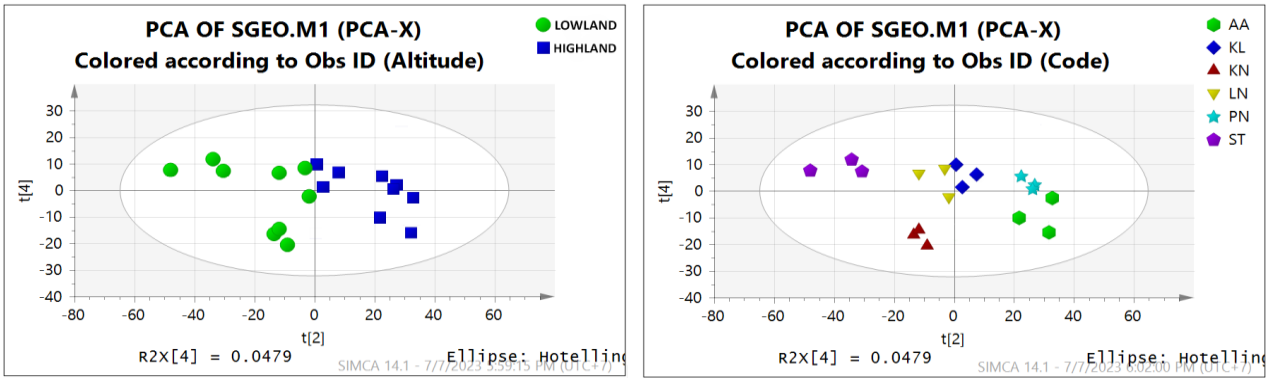

Figure 4: The PCA score plot is based on A) altitude and (B) origin**.**

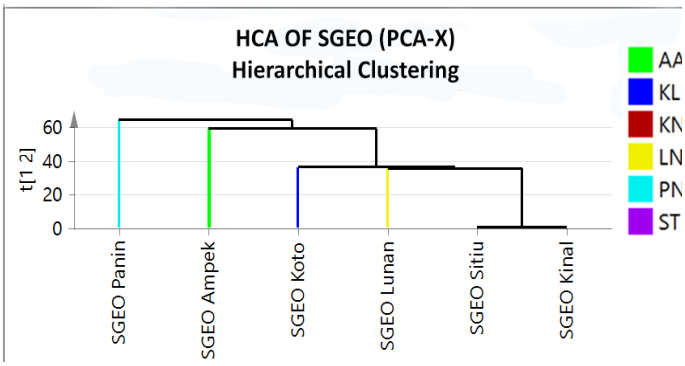

Figure 5. Dendogram of SGEO based on origin

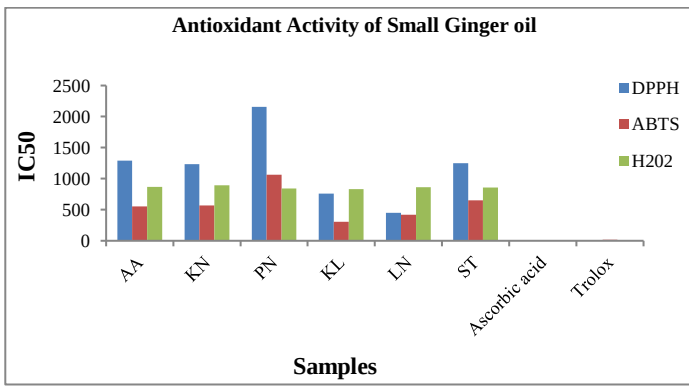

Figure 6: Antioxidant activity

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