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Flavonoid Concentration and Tyrosinase Inhibition Activity of Ethanol Extract of *Piper crocatum* (*Piper crocatum* var. Ruiz & Pav) from Various Regions in Indonesia and Their Correlations

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Abstract

Hyperpigmentation is a condition of darkening of the skin which is generally caused by an increase in melanin production. Melanin is produced in melanocytes by the enzyme tyrosinase. *Piper crocatum* contains flavonoid compounds that are known from previous research to inhibit tyrosinase. The goals of this study were to determine the tyrosinase inhibitory activity and total flavonoid content of seven accessions, as well as look at the Pearson's correlation and clustering PCA (principal component analysis). The method used was water content analysis, extraction yield measurement, total flavonoids analysis, and *in vitro* tyrosinase inhibition. Based on the results, *P. crocatum* from Kendari had the best yield and total flavonoid content of 24.07% and 5.10 mg QE g-1, while *P. crocatum* from Bogor had the lowest water content with a value of 6.21% and the best in tyrosinase inhibition of 13.77. The correlation between total flavonoid content and percent inhibition showed a very weak correlation. The results of clustering formed four clusters of seven accessions based on total flavonoids and percent inhibition. The cluster was divided into Malang (506mDPL) and Jayapura (287mDPL), Banda Aceh (0.80mDPL) and Bandung (670mDPL), Samarinda (8mDPL) and Bogor (190-350m DPL), and Kendari (14mDPL). In conclusion, the correlation between total flavonoid levels and percent inhibition is very weak and regional diversity had a significant effect on total flavonoids and total inhibition.

***Keywords**:* Hyperpigmentation; tyrosinase; *Piper crocatum*; flavonoid; melanin

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1. INTRODUCTION

Hyperpigmentation is a condition of darkening of the skin which is generally caused by an increase in melanin production due to melanocyte activation or hemosiderin buildup in the skin (Bossart et al., 2021). Based on the Department of Economic and Social Affairs, (2021), there has been an increase in cases of hyperpigmentation from 8% in 1950 to 11% in 2009 and has the potential to touch 20% in 2050. Indonesia as a tropical country is exposed to sun's ultraviolet rays throughout the year, so residents will be more susceptible to hyperpigmentation (Ahmad and Damayanti, 2018). This will increase cases of hyperpigmentation. Ultraviolet light contributes to the formation of free radicals and Reactive Oxygen Species (ROS). This stimulates skin inflammation which triggers a series of biochemical reactions in the skin and causes damage to the dermal collagen tissue resulting in premature skin aging (photoaging/premature skin aging or hyperpigmentation) (Charissa et al., 2016).

Piper crocatum is a well-known plant in the medical field and contains secondary metabolites such as alkaloids, flavonoids, tannins, and others (Safithri et al., 2022; Safihtri et al., 2016). According to Allgisna et al., (2021), flavonoids act as anti-hyperpigmentation. Mustopa, (2022) states that *P. crocatum* ethanol extract from Bogor can inhibit 12% tyrosinase *in vitro* and catechin compounds in *P. crocatum* can inhibit tyrosinase *in sillico*. The results of research by Safithri et al., (2015) demonstrated the presence of tyrosinase inhibitory activity from extracts and fractions of *P. crocatum* leaves from Bogor.

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These results indicated that 200 ppm ethanol extract and 200 ppm *n*-hexane of *P. crocatum* leaves inhibited tyrosinase with inhibition values of 52.13% and 40.13%, respectively. Ethanol extract is the highest. Therefore, ethanol is more potent than *n*-hexane.

Putri, (2022) examined the antioxidant test of the DPPH method of *P. crocatum* ethanol extract in various regions including Banda Aceh, Bandung, Bogor, Jayapura, Samarinda, Kendari, Malang and Yogyakarta. The results showed that *P. crocatum* from Kendari had the highest antioxidant activity with an IC_{50} value of 479.29 µg/mL, while Yogyakarta had the lowest with a value of 55.10 µg/mL. This shows that the diversity of locations has a significant effect on the parameters of *P. crocatum* antioxidant activity. In addition, the ethanol extract of *P. crocatum* and the *n*-hexane fraction, the ethyl acetate fraction, and the water fraction were studied on MDA inhibition by Chairunissa, (2022). The results showed that the ethanol extract of *P. crocatum* from Bogor had the best IC*50* and the ethanol extract from Bandung had the lowest, respectively 0.43 µg/mL and 8.49 µg/mL. The difference in strength when testing for antioxidants using the TBA method for each region was carried out with the result that no clusters were formed, meaning that *P. crocatum* leaves from Bogor, Bandung, DI Yogyakarta, and Malang are related (Chairunissa, 2022). To validate that argument, this study aims to determine the tyrosinase inhibitory activity and total flavonoid content of *P. crocatum* leaves from seven regions, as well as to look at the correlation and clustering. This research is useful to find *P. crocatum* which has the best inhibitory activity and total levels of flavonoids.

2. MATERIALS AND METHODS

Materials and Tools

The materials used in the *in vitro* study were 80 mesh size of *P. crocatum* leaf powder from Banda Aceh (05°16'15"–05°36'16" North Latitude, 95°16'15 "–95°22'35" E with an altitude of 0.80 meters above sea level), Bogor (106°43'30" E-106°51'00" E, 30'30" S–6° 41'00" S with an altitude of 190m-350m), Bandung (107° 36' E, 6° 55' S with an altitude of 670m above sea level), Malang (7.06°-8.02° S, 112, 06°-112.07° East Longitude with an altitude of 506 meters above sea level), Samarinda (0°21'81"-1°09'16" South Latitude, 116°15'16"-117°24'16 " East Longitude with a height of 8 meters above sea level), Kendari (3°54'40"-4°5'5" South Latitude, 122°26'33"- 122°39'14" East Longitude with a height of 14 meters above sea level sea), and Jayapura (137°27'-141°41' E and 1°27'-3°49' S with an altitude of 287 meters above sea level). The material needed for powder extraction is ethanol 70%. In addition, to test the levels of total flavonoids and inhibition tests using CH_3COONa 1M, 10% $AlCl_3$, water, ethanol 70%, quercetin, DMSO, and Tirosinase (Sigma, 333 unit/mL).

The tools used in *in vitro* research were oven, porcelain cup and analytical balance, separating funnel, rotary evaporator (Buchi Labortechnik AG type R-11), shaker (WiseBath Daihan Scientific Co), measuring flask, Erlenmeyer, Genesys 10 UV spectrophotometer (190-1100 nm), measuring cylinders, and 96 multi-well plates.

Piper crocatum Leaf Sample Preparation

Preparation of *P. crocatum*'s samples begins with wet sorting to separate dirt or foreign material from the leaves. Leaf parts that are not needed such as leaf bones are removed. Then the leaves are washed under running water to remove dirt. After washing, the leaves are dried in the sun for 48 hours. The dried *P. crocatum* leaves are then mashed with a blender, then sieved through a 80 mesh sieve (Weni, 2014).

Powder Moisture Measurement

The porcelain dish was washed in running water and dried, then the dish was placed in an oven at 105 ºC for 30 minutes. The porcelain cup was then cooled in a desiccator for 30 minutes and its empty weight was weighed. A total of 2 g of powder samples were put into a cup and then baked in an oven at 105ºC for 3 hours. The cup is cooled in a desiccator and weighed. The cup is then in the oven again at a temperature of 105ºC until a constant weight is obtained (Chairunissa, 2022).

Making *Piper crocatum* Ethanol Extract

Sample extraction was carried out by dissolving 20 g of *Piper crocatum* leaf powder from each area in 80 mL of 70% ethanol. The sample was re-extracted by maceration for 24 hours in the dark at room temperature. The sample was filtered using filter paper. The resulting filtrate is a sample extract with a concentration of 0.20 g/mL and will be used for testing (Safithri et al., 2022).

Measurement of Total Flavonoid Content

A total of 0.5 mL of sample with a concentration of 1000 ppm was added with 0.1 mL of 1M $CH_3COOHNa$, 0.1 mL of 10% $AlCl_3$, 1.28 mL of distilled water, and 1.5 mL of ethanol pa. The mixture was incubated for 30 minutes. The absorbance was measured at a wavelength of 430 nm. Quercetin standard curves were made with various concentrations of 0, 25, 50, 100, 125, 150, 200, and 250 ppm. Total flavonoids were calculated in milligrams of quercetin equivalent/gram sample (mg QE/g) (Sari et al., 2021).

Tyrosinase Inhibition Test

The sample from extraction was weighed as much as 50 mg, then dissolved in DMSO to a concentration of 5 g/mL. The sample was diluted using 50 mM phosphate buffer (pH 6.5) to obtain a concentration of extract solution of 1000 and 10000 ppm. Kojic acid as a positive control was tested from the concentrations of: 7.8125; 15,625; 31.25; 62.5; and 125 µg/mL in a 96-well drip plate. A total of 70 µL of each dilution sample was added with 30 µL Tyrosinase (Sigma, 333 units/mL in phosphate buffer). Plates were incubated at room temperature for 5 minutes then added 110µL L-Tyrosine 2 mM and incubated again for 30 minutes at room temperature. The absorbance absorption of the solution was measured using a multiwell plate reader at a wavelength of 490 nm to determine the percent inhibition and IC_{50} (Safithri et al., 2018).

3. RESULTS AND DISCUSSION

Water Content and Yield of *Piper crocatum* Ethanol Extract

The water content of *Piper crocatum* ethanol extract from seven regions showed a good range (below 10%), namely 6.21-9.00%. The water content of the powder obtained has different values for each region (**Table 1**). The *P. crocatum* powder used comes from seven regions in Indonesia, namely Banda Aceh, Jayapura, Kendari, Malang, Bogor, Bandung, and Samarinda. *P. crocatum* from Bogor has the best water content value of 6.21%. Meanwhile, *P. crocatum* from Malang is the lowest with a value of 9.00%. The good quality of powder is shown by the value of the water content which is below 10% (BPOM, 2014). Therefore, it can be concluded that the *P. crocatum* used is good quality. Powder which has been tested for water content is followed by an extraction process to separate the active compounds. The yield obtained from the extraction process has a range of 14.16-24.7%. The yield of Kendari *P. crocatum* extract was the highest compared to the seven regions. The highest yield was obtained by the Kendari area at 24.07% and the lowest by the Malang area at 14.16%. Extraction results of Bogor *P. crocatum* 70% ethanol extract produced from research by Puspita et al. (2018) showed a value of 20.8% with one-time remaceration. The results of Putri's research (2022) showed *P. crocatum* extract of 70% ethanol from Banda Aceh, Bandung, Bogor, Malang, Samarinda, Kendari, and Jayapura with remaceration 3 times each of 11.1%; 14.7%; 12.6%; 11.9%; 12.5%; 14.5%; and 12.3%. The results of the study show greater results than Puspita et al. (2018) and Putri (2022) because the ethanol used is from 100% ethanol or technically mixed with water up to 70% and five times remaceration. A higher concentration of solvent can dissolve more compounds. The longer the extraction time, the yield produced will increase, because the opportunity for contact between the material and the solvent is greater so that the results will increase until the solution saturation point. The longer the extraction time, the solubility of the substance will continue to increase until saturation occurs in the solvent (Pratama et al., 2017).

Table 1. Water content and yield of *Piper crocatum* ethanol extract

Region	Water content (%)	Yield (%)
Banda Aceh	8.17 ± 0.34	20.53 ± 0.19^{b}
Bogor	6.21 ± 0.52	18.48 ± 0.25^{c}
Bandung	7.33 ± 0.72	23.40 ± 0.85^{a}
Malang	9.00 ± 1.00	14.16 ± 0.33^{d}
Samarinda	8.15 ± 0.80	22.58 ± 0.18^{a}
Kendari	7.71 ± 1.16	24.07 ± 0.4^{a}
Jayapura	7.81 ± 0.69	15.14 ± 0.37^{d}

Note: Different letters (a, b, c) in the same column indicate significant differences at the 5% test level (Tukey's advanced test)

Powder is a natural substance in the form of powder. *P. crocatum* drying process aims to reduce the water content, facilitate the manufacture of powder from samples, and prevent the growth and enzymatic activity of microorganisms. Powder that still contains a certain amount of water will induce an enzyme to carry out the process of breaking down the active compounds present in the cells of a material even though the cells are dead (Daud et al., 2020). The extraction process is carried out by maceration. The advantage of this method is not heating, so there is no damage to the bioactive compounds in the powder. Extract yield can be affected by the length of maceration, pH, temperature, light, and the type of solvent used (Wahyuni and Widjanarko, 2015). In addition, the extraction of this study used 70% ethanol. The volatile nature of ethanol facilitates the withdrawal of secondary metabolites from the powder. This solvent also dissolves almost all organic compounds present in the sample, both polar compounds and nonpolar compounds (Noviyanti, 2016).

Total Flavonoid Content

The determination of total flavonoid content was calculated using the standard quercetin curve equation y = 0.0041x + 0.1169 with an R^2 value of 0.9943. The levels of total flavonoids obtained from the seven regions ranged from 2.03 mg QE g^{-1} to 5.13 mg QE g^{-1}. The highest results were obtained from Kendari and the lowest from Malang, respectively 5.13 mg QE g^{-1} and 2.03 mg QE g^{-1} with significantly different results between regions ($p<0.05$). *P. crocatum* from 7 regions showed different responses to total flavonoid levels (**Table 2**). Differences in the total content of flavonoids can occur due to external influences in the form of the environment (eg water, soil, and temperature) which significantly affect several processes related to plant growth and development, even their ability to synthesize secondary metabolites and ultimately cause changes in the whole (Musilova et al., 2016). In addition, specific secondary metabolites are synthesized only under certain circumstances so that high-quality medicinal plants are produced in a controlled environment (Yang et al., 2018).

Several other studies, such as Iqbal et al., (2016) measured the total flavonoid content of *P. crocatum* Palu 96% ethanol extract with a value of 0.11 mg QE g^{-1}. Krisdianty, (2020) reported the results of measuring the quercetin content of *P. crocatum* 70% ethanol extract from Bogor of 1.47 mg/g. Based on previous studies, on average, 70% ethanol extract of *P. crocatum* has a greater value (2.77 mg QE g^{-1}) than 96% ethanol extract (0.11 mg QE g^{-1}).

Temperature is one of the important abiotic factors because it is useful for the biosynthetic processes of growing plants. Variations in high and low temperatures in an environment are influenced by the height of the planting site and are formed simultaneously with changes in the atmospheric climate, one of which is caused by UV-B waves. The high levels of total *P. crocatum* flavonoids from Kendari can occur due to the height of the planting site where Malang is 500 meters above sea level. Temperature as one of the main weather variables can significantly affect the composition of secondary metabolites and in general an increase in temperature can increase almost all secondary metabolites of plant species (Virjamo et al., 2014).

Table 2. Total flavonoid content of *Piper crocatum* from seven regions

Region	Total flavonoid content (mg QE g^{-1} dry weight)
Banda Aceh	2.18 ± 0.09^{d}
Bogor	2.17 ± 0.06^{d}
Bandung	2.81 ± 0.01^{b}
Malang	2.03 ± 0.07^{e}
Samarinda	2.53 ± 0.08^{c}
Kendari	5.10 ± 0.01^{a}
Jayapura	2.60 ± 0.07^{c}

Note: Different letters (a, b, c) in the same column indicate significant differences at the 5% test level (Tukey's advanced test). QE, quercetin equivalent. Different letter notations show that there are significant differences in the results of Duncan's test analysis at the 95% level of confidence.

***Piper crocatum* Inhibition of Tyrosinase**

The results of *P. crocatum* from each region showed the inhibitory activity of the tyrosinase enzyme. The range of tyrosinase inhibitory activity from the seven regions was 2.36% to 13.77%. Negative control showed no inhibitory activity against tyrosinase. Kojic acid gave the best inhibitory effect at 84.85%, and the three areas with the highest inhibition percentage were Bogor, Samarinda and Kendari respectively at 13.77%, 10.72% and 8.90%. *P. crocatum* from Jayapura is the lowest with a value of 2.36%. The numbers in the column followed by the same letter were not significantly different on the Tukey test (α = 0.05) (**Figure 1**). Mustopa, (2022) also used Bogor *P. crocatum* 10,000 ppm ethanol extract, resulting in an inhibition percentage of 12.47%. However, the results of the ethanol extract fractionation showed a large percentage of inhibition, the water fraction was the best among the ethyl acetate and *n*-hexane fractions, namely 84.84%, 82.21% and 34.57%, respectively.

The value of tyrosinase inhibition was shown from the percent inhibition value of the ethanol extract of *P. crocatum* from seven regions in Indonesia. Kojic acid was used as a positive control and the sample without the addition of *P. crocatum* leaf extract and fraction was used as a negative control. The principle in this test is based on the reaction between L-Tyrosine substrate and tyrosinase which produces dopachrome and is followed by the formation of melanin. The formation of dopachrome is indicated by the formation of a brown color. The presence of tyrosinase inhibitors can inhibit the reaction of the substrate with tyrosinase so that the product of dopachrome decreases. This is marked by a decrease in the intensity of the resulting brown color (Charissa et al., 2016).

Correlation of Total Flavonoid Levels with Tyrosinase Inhibitory Activity of Ethanol Extracts of Seven *Piper crocatum* Accessions

The results of the Pearson's correlation test showed a significant (p) relationship between total flavonoid levels and tyrosinase inhibitory activity in the seven *P. crocatum* regions with a p>0.05 value, which was 0.64 and r = 0.108 (**Figure 2**). Based on the p-value it can be determined that the level of total flavonoids does not have a correlation with the activity of tyrosinase inhibition in this study. The value of r 0.108 indicates that the correlation is very weak, which has a range of 0.00 - 0.199. The same result was shown by Morais et al., (2018), Oskoueian et al., (2011), Wang et al., (2011), and Owen and Johns, (2011) who stated that there was no correlation between total flavonoid levels and tyrosinase inhibitory activity.

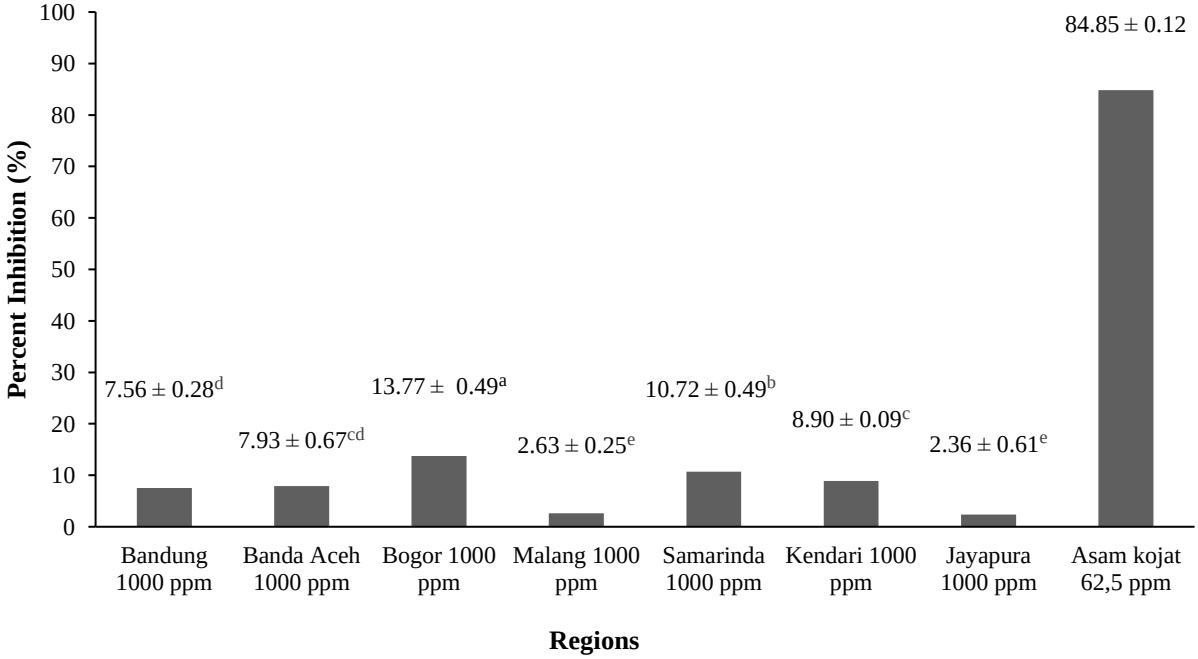

Figure 1. *Piper crocatum* inhibition of the tyrosinase enzyme

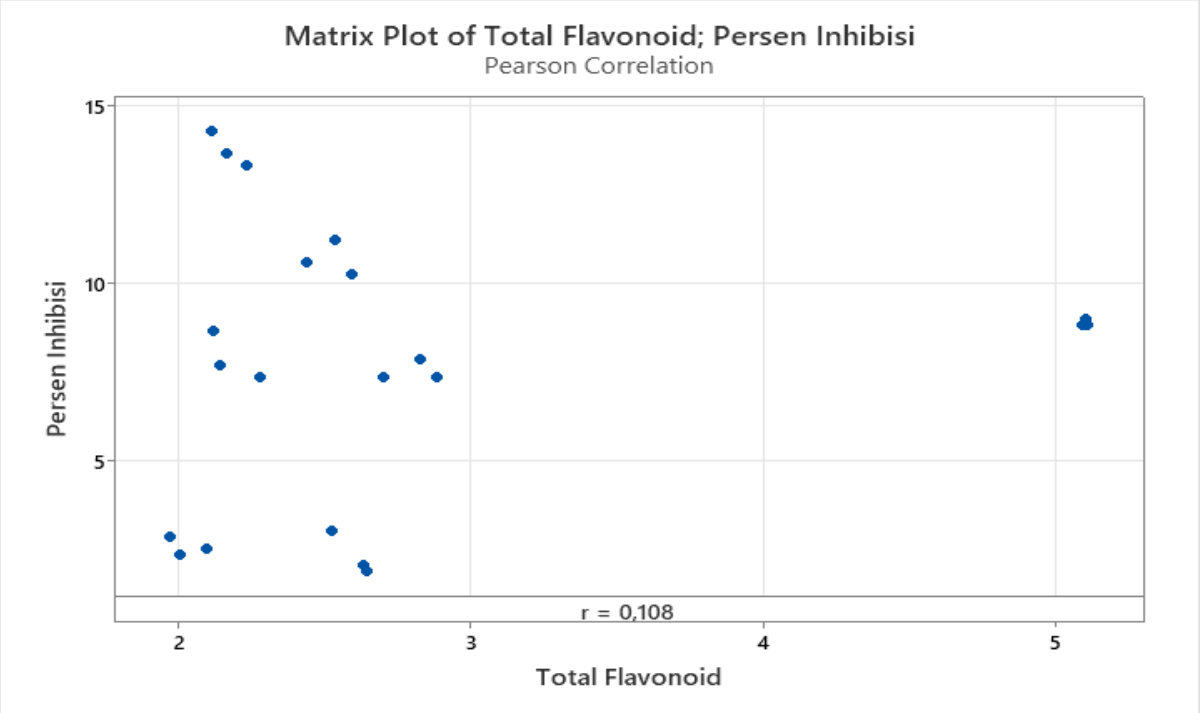

Figure 2. Correlation between levels of flavonoids and percent inhibition

Ndruru et al., (2014) states that the strength of the relationship between one factor and another in Pearson's correlation is classified into several levels based on the Pearson's correlation coefficient (r), which is very weak (0.00-0.199), weak (0.20-0.399), moderately strong (0.40-0.599), strong (0.60-0.799), and very strong (0.80-1.000). The r value obtained from the analysis of the relationship between flavonoids and percent inhibition in this study was 0.108. Therefore, based on the classification, it can be determined that the total levels of flavonoids in the ethanol extract of the seven betel accessions in this study had a very weak correlation with the percentage of inhibition.

Tyrosinase inhibitory activity is inseparable from the content of secondary metabolites of *P. crocatum* extract. *P. crocatum* contains alkaloids, flavonoids, tannins, and others (Safithri et al., 2022). Flavonoids (polyphenols) can act as competitive inhibitors of the tyrosinase enzyme (Fan et al., 2019). This is due to the ability of flavonoids to chelate metals due to their polyhydroxyphenol structure which can interact with copper ions on the active site of tyrosinase. The inhibition mechanism that occurs is competitive inhibition of L-tyrosine oxidation by tyrosinase and the 3-hydroxy-4-keto part of the flavonoid structure which acts as a chelating metal copper (Cu) from the tyrosinase enzyme structure. One molecule of the tyrosinase enzyme contains two Cu atoms, namely CuA and CuB, which are bound to the amino acid histidine. Cu metal acts as a cofactor in the activity of the tyrosinase enzyme. The catalytic ability of the tyrosinase enzyme is reduced by the loss of Cu from the active site of the enzyme, so that dopachrome is not formed (Sagala and Telaumbanua, 2020). The presence of antioxidant mechanisms and tyrosinase inhibition from serum formulations is expected to reduce the formation of melanin and free radicals produced during these reactions so that they can brighten the skin (Dolorosa et al., 2017).

The absence of a correlation between total flavonoid levels and the percentage of inhibition may be due to the possibility that other groups of compounds contained in the ethanol extract of seven *P. crocatum* accessions are responsible for anti-tyrosinase, for example alkaloids and tripernoids. Flavonoids have been shown to inhibit the tyrosinase enzyme (Allgisna et al., 2021; Mustopa, 2022). However, there have been several studies that have shown that other groups of compounds can act as tyrosinase inhibitors derived from plant secondary metabolites, such as flavonoids, phenyls, tannins, triterpenes, or alkaloids (Chen et al., 2014).

Condensed tannins or proanthocyanidins are oligomers or polymers of flavonoids containing flavan-3-ol units. Because of the

tannins is a polymer of flavonoids, thought to have a mechanism of action that is almost like flavonoids (Sholikha et al., 2021). Based on Chang, (2009), triterpenes have copper chelating inability, lack of free radical scavenging, and kinetically uncompetitive inhibitory inhibition. Regarding difficult skin permeability, these hydrophobic steroids or long lipid chain inhibitors appear to have superior potential in development as skin whitening agents because of their easier cellular permeability. The alkaloids inhibit the diphenolase enzyme which belongs to the type-3 copper protein group. It binds to both copper ions at the active site of the enzyme and blocks the enzyme activity. In addition, it was found that N-substituted N-nitrosohydroxylamines inhibit fungal tyrosinase by interacting with copper ions at the active site of the enzyme. Phenyl was found to suppress several key cellular parameters in the melanogenic pathway by downregulating the cAMP-dependent protein kinase K signaling pathway and decreasing gene expression of the transcription factor microphthalmia, which in turn repressed tyrosinase expression (Chang, 2009).

Piper crocatum Clusterization Based on Total Flavonoids and Tyrosinase Inhibition

The results of measurements of total flavonoids and percent inhibition of *P. crocatum* on tyrosinase can be grouped based on the strength of inhibition using PCA analysis. This study uses PCA analysis (principle component analysis) to extract data simpler. The PCA results of total flavonoids and percent inhibition of *P. crocatum* managed to group the data into four clusters, each cluster consisting of several regions (**Figure 3**). The cluster is divided into Malang (506mDPL) and Jayapura (287mDPL), Banda Aceh (0.80mDPL) and Bandung (670mDPL), Samarinda (8mDPL) and Bogor (190-350mDPL), and Kendari (14mDPL) (**Figure 4**).

The PCA method is used to simplify data to make it easier to read, by transforming the data linearly to form a new coordinate with maximum variance. The score plot also shows variations from the data analyzed (Ghani et al., 2016). The score plot describes the grouping of each sample based on the measurement variable. PCA analysis combined data on total flavonoids with the percentage inhibition value of *P. crocatum* ethanol extract.

Each cluster has something in common, this can be caused by geographical location and soil type in each area or agro-biophysic conditions. *P. crocatum* from Malang has the same soil type as red betel from Jayapura, which is of the entisols type. Banda Aceh and Bandung have the same soil type inceptisols. Samarinda and Bogor have spodosols soil types. Meanwhile, *P. crocatum* from Kendari has nothing in common with other *P. crocatum* due to its own different soil type, namely ultisols (Ritung et al., 2000).

Soils owned by Malang and Jayapura, entisols are soils that are still young or do not yet have a pedogenic horizon. Entisols have a high clay content (54%) with a slightly acidic pH (4.7-6.6). Meanwhile, the inceptisols owned by Banda Aceh and Bandung are mineral soils that have begun to develop through the development of a pedogenic horizon, the formation of an ochric epipedon and an albic horizon such as entisols. Entisol soil types are younger than inceptisols. This soil has a high clay content (35%) with a slightly acidic pH (4.6-5.5). Spodosols are soils formed from coarse and acidic sand or clay. This type of soil contains high sand (65%) with a very acidic pH (3.3-4.9). Ultisols are soils that are old or have gone through a process of climate destruction so that the cross section is more than 2 m. Ultisols contain low clay (17%) with a low pH (4.1-4.8) (Ritung *et al*., 2000). Soil types and conditions can affect the content of secondary metabolites and pharmacological activity (Salim et al., 2016). The formation of secondary metabolites is a plant response to environmental stress as plant protection. During stress, the activity of the enzyme phenylalanine ammonia lyase (PAL) will increase the content of flavonoids because it is influenced by phenylalanine as a precursor to the formation of flavonoids (Hawari et al., 2022).

Altitude affects the flavonoid content, where the flavonoid content in the lowlands is higher than the highlands. The degree of soil acidity also affects the content of secondary metabolites. Soil in the lowlands is included in the normal category compared to the highlands (Hawari et al., 2022). In addition, the alkaloid content was higher in the medium and high plains when compared to the lowlands. The increase in levels of alkaloid compounds with an increase in altitude is one of the possible causes of temperature stress between altitudes (Lage and Cantrell, 2009).

The results of the 4 *P. crocatum* clusters may also have occurred due to differences in climate and soil topography, as in the study of Andrianto et al., (2020) Green betel from Sumber Baba and Demta are included in the same cluster because based on climate and soil topography, Sumber Baba and Demta have the same climate and soil. Miswarti et al., (2014) stated that geographical proximity is one of the factors that causes plants to have similar secondary metabolites. This is also in accordance with the research of Nurcholis et al., (2016) who found that agro-biophysics conditions greatly influenced the growth and diversity of secondary metabolite profiles.

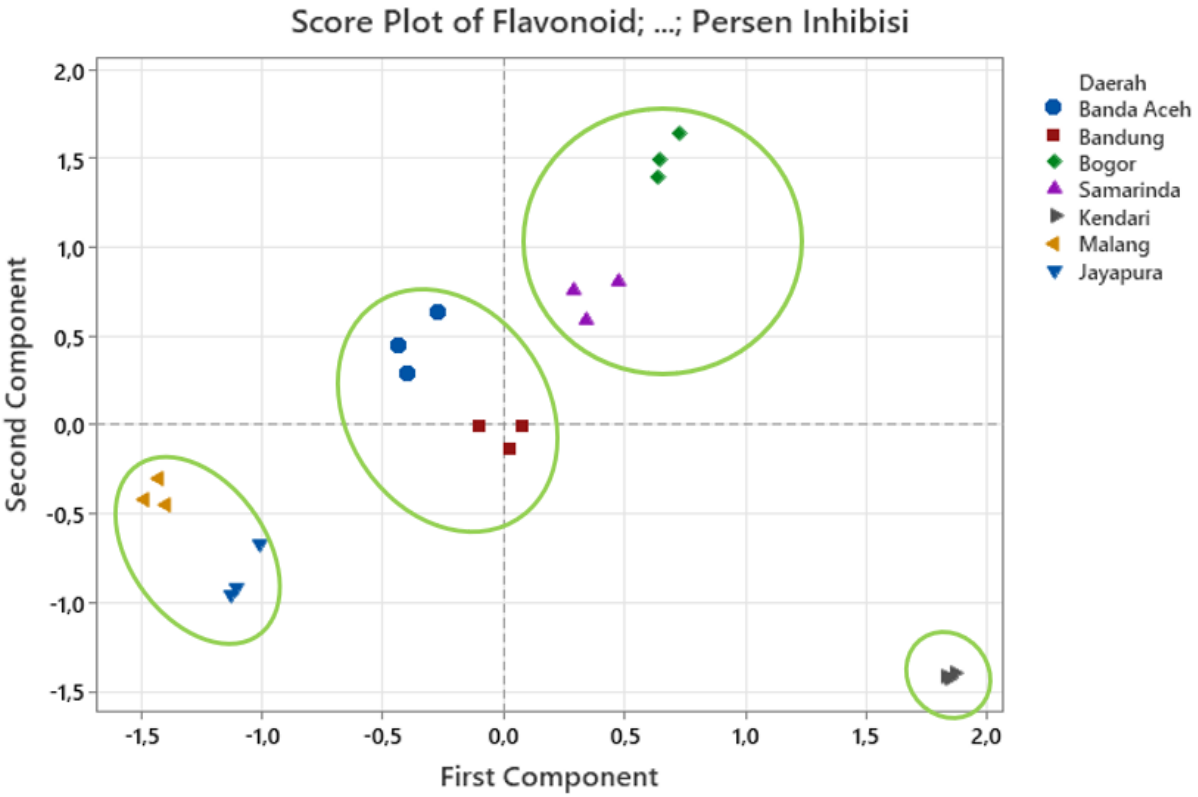

Figure 3. Score plot of total flavonoids and percent inhibition of *Piper crocatum* from seven regions in Indonesia

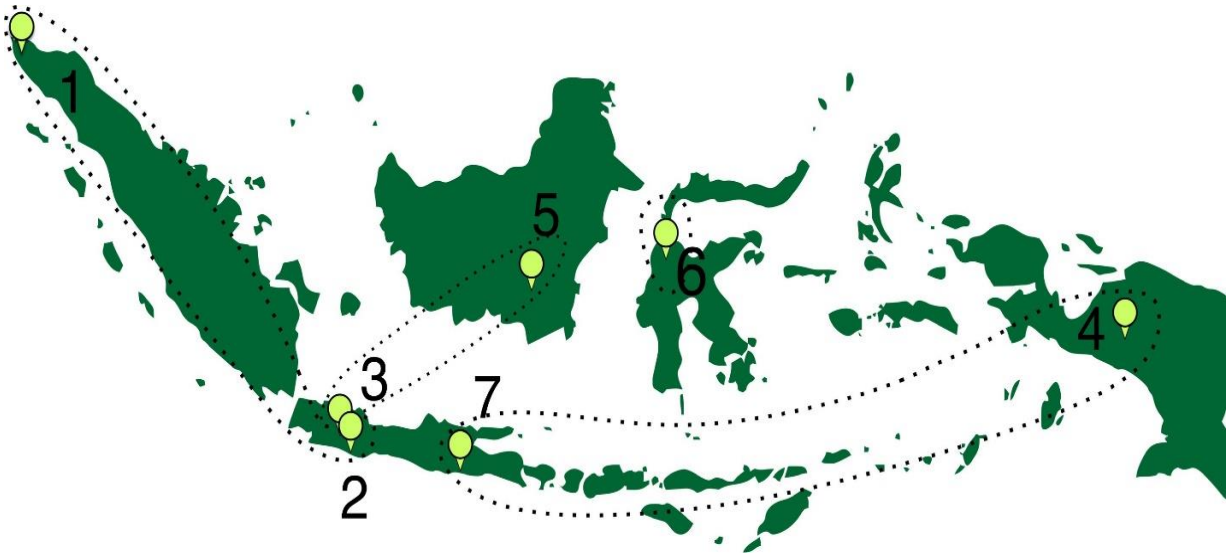

Figure 4. Map of *Piper crocatum* clusters in Indonesia: (1) Banda Aceh, Aceh; (2) Bandung West Java; (3) Bogor, West Java; (4) Jayapura, Papua; (5) Samarinda, East Kalimantan; (6) Kendari, Southeast Sulawesi; (7) Malang, East Java

4. CONCLUSION

Piper crocatum from seven regions was divided into four clusters based on total flavonoid content and total inhibition into Malang and Jayapura, Banda Aceh, and Bandung, Samarinda and Bogor, and Kendari. *P. crocatum* Bogor is the best of them. The correlation between total levels of flavonoids and percent inhibition has a very weak correlation with an r value of 0.108. The total levels of flavonoids in the ethanol extract of the seven *P. crocatum* accessions in this study had a very weak correlation with the percentage of inhibition There is any correlation between the type of soil to the total flavonoid.

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