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Authentication of Cherry (*Muntingia calabura*) Fruit from different Geographical Locations in Indonesia by FTIR Spectroscopy Combined with Chemometrics and Their Antioxidant and Antiaging Activities

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Abstract

Cherry (*Muntingia calabura* L) is a plant with scientific properties in various treatments. Cherry can overgrow in a variety of environments. This study aims to determine the similarity profile of the compound content of cherries obtained at different geographical locations using the FT-IR-chemometric spectroscopy approach and their bioactivity as antioxidants and anti-aging. Cherry fruit obtained from 8 regions in South Sulawesi Province-Indonesia was dried and extracted using the sonicator method for 30 minutes. The extract obtained was analyzed by FT-IR spectroscopy and authenticated by the chemometric similarity of the compounds (PCA and Cluster analysis). The test results showed that the ethanol extract of cherry fruit from various regions had a similarity of 65.4% and, by dendogram, tended to form 2 clusters based on the profile of chemical content and intensity. Antioxidant activity profiles using DPPH and ABTS methods showed that bioactivity varied from strong to powerful. The same thing was also found in the anti-elastase activity showing variations based on a percentage of elastase inhibition and the IC_{50} value. But overall, the various test results did not significantly affect the bioactivity of cherry fruit as an antioxidant and anti-aging.

Keywords: Authentication, Antioxidant, Anti-aging, Chemometric, Elastase, FT-IR Profile, Muntingia calabura

1. Introduction

Cherry fruit (*Muntingia calabura* L) is a plant from the Muntiangiaceae family which already has some scientific evidence related to its activities, namely antioxidant activity [1,2], a protective effect against UV exposure [3,4], antiaging [5], antibacterial [6], anti-inflammatory [7]. Besides having good activity, cherry fruit also has a variety of nutritional content [8] as well as secondary metabolites i.e., anthocyanins, quercetin, gallic acid, phenolic derivate, catechin derivate, hydroxycinnamates, potassium, fiber, vitamin C, carotenoids, and melatonin [1,7,9] which can support the activities of cherry fruit.

In supporting the successful use of cherry fruit as a natural raw material, it is necessary to monitor and guarantee the quality standards of raw materials. One of the approaches taken is to authenticate the uniformity of content of cherries which can be done through infrared spectroscopy and combined with chemometric analysis. This authentication was carried out because differences in the plant's geographical location will affect the plant's secondary metabolite content [10–12].

FT-IR spectroscopy analysis can provide molecular structure information by looking at the

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specific absorption bands for each compound to distinguish a raw material with similar compounds. Analysis of samples from the same genus or the same species but with different geographical locations have identical spectral patterns, making it difficult to distinguish them. Therefore, combining chemometric methods will make it easier to authenticate similar samples [13,14].

In this study, chemical similarity analysis was carried out using FT-IR spectroscopic approach and chemometric analysis to standardize the similarity of the content of ethanol extract of cherry fruit obtained from different locations in the province of South Sulawesi, Indonesia. In this study, an evaluation of the bioactivity profile as an antioxidant and antiaging agent of the ethanol extract of cherry fruit was also carried out based on differences in the location of its growth.

2. Material and Methods

2.1 Materials

The chemicals used include ABTS (2,2-Azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (Sigma Aldrich, Germany), Aluminium Chloride (AlCl3) (Sigma Aldrich, Germany), ascorbic acid (Sigma Aldrich, Germany), aqua distillate (OneMed, Indonesia), DPPH (2,2-diphenyl-1-picrylhydrazil) (TCI, Japan), ethanol pro analysis (Merck, Germany), $FeCl_3$ (Merck, Germany), N-Succinyl-Ala-Ala-Ala-p-nitroanilide/SANA (Sigma Aldrich, S4760), porcine pancreatic elastase (Sigma Aldrich SLBV 9311), and quercetin (Sigma Aldrich, Germany).

2.2 Plant Collection

Half-ripe cherry (*Muntingia calabura* L) fruit samples were obtained from eight growing locations in South Sulawesi Province, Indonesia. The eight locations are Telkomas (M1, -5º12'71"S, 119º 51'01"E), Tamalanrea (M2, -5º13'92"S, 119º 51'38"E), Biringkanaya (M3, -5º 08'59"S, 119º53'28"E) which were located in Makassar City, Gowa (M3, -5º33'12"S, 119º87'24"E) Takalar (T, -5º31'94"S, 119º34'69"E), Soppeng (S, -4º33'93"S, 119º96'08"E), Barru (B, -4º48'81"S, 119º61'15"E), Tana Toraja (TO, -3º11'02"S, 119º84'59"E) regency. The cherry fruit obtained was then washed and dried using an oven at 40º C for 2 times 24 hours.

2.3 Sample extraction

Each dried cherry fruit powder was weighed 100 g and extracted for 30 minutes using 70% ethanol solvent by ultrasonic method (42 kHz). The dregs and filtrate were separated, and the dregs were re-extracted by the same procedure. The filtrate was collected and then evaporated using a rotary evaporator to obtain a thick extract.

2.4 FT-IR analysis

Cherry fruit samples were mixed with a certain amount of KBr and made in pellet form, and then measured using an FT-IR spectrophotometer (QATR-10, Shimadzu, Japan) in the range of 4000–400 cm–1; the absorption mode (absorbance) was 32 scan minutes^{-1}, and the resolution was 4 cm–1. The spectral data is then stored using the appropriate sample name and code. The measurement data were processed using the Metaboanalyst 5.0 and Origin lab 2022 programs to see the differences or similarities between the cherry fruit samples.

2.5 Antioxidant Activity

2.5.1 Radical DPPH reduction assay

Testing of antioxidant activity using the DPPH method was carried out according to the procedure with minor modifications [15]. Each test sample was made a working solution and varied in concentration (10-1000 µg/mL), then 50 µL of each extract series was added to 100 µl of 0.4 mM DPPH solution was put into a 96 well plate. The volume of the sample mixture was made up to 200 µl using pro-analysis ethanol into the 96-well plate. Then homogenized and incubated for 30 minutes at room temperature. After incubation, the absorbance of the sample solution was measured using a microplate reader at 515 nm (Epoch Biotek, Germany). The potential antioxidant activity of a sample in reducing DPPH radicals using the following formula:

$$\text{Inhibition (\%)} = (\text{Abs. blank} - \text{Abs. sample} / \text{Abs. blank}) \times 100\% \quad (1)$$

2.5.2 Radical ABTS reduction assay

ABTS radical solution was prepared by mixing 7.0 mM ABTS solution (5 mL in aqua distillate) and 2.45 mM $K_2S_2O_8$ solution (5 mL in aqua distillate). The mixture was allowed to stand for 12-16 hours in a dark room. After the incubation period, the ABTS cation radical solution was diluted in a ratio of 1:10 (ABTS: ethanol pro analysis) to obtain a working solution of ABTS cation radical [16]. The antioxidant activity test was carried out by making a series of concentrations of each sample solution (1-1000 µg/ml) by taking a 50 µL of stock solution and putting it into a 96-well plate. Then 100 µl of ABTS solution was added to each sample solution. Then the volume was made up to 200 µl with ethanol p.a. Furthermore, the absorbance at 745 nm (Epoch Biotek, Germany) was measured after an incubation period of 30 minutes. The potential antioxidant activity of a sample in reducing DPPH radicals using the following formula:

Inhibition (%)= (Abs. blank – Abs. sample / Abs. blank) x 100% (2)

2.6 Anti-aging activity by inhibition of elastase

In the anti-elastase assay, a concentration series (10-1000 g/mL) of each working solution sample was made and put into well plate 96 as much as 30 µL. 130 µL of Triz-HCl buffer pH 8 and 20 µL of 0.22 U/mL elastase enzyme solution were added to the sample in well plate 96 and then incubated for 20 minutes at 25º C. After incubation, the mixture was reacted with 30 µL of N-succinyl-(Ala)3-nitroanilide substrate solution. Then the solution was incubated for 50 minutes at 25º C, and the absorbance was measured using a Microplate Reader (Epoch Biotek, Germany) at 405 nm [17].

The inhibitory effect of the elastase activity of the extract sample can be calculated as follows:

% Elastase inhibition =((A-B)-(C-D))/((A-B)) x100% (3)

Information:

A : absorbance of blank solution with enzyme (blank)

B : absorbance of blank solution without enzyme (blank control)

C : absorbance of sample solution with enzyme (sample)

D : absorbance of sample solution without enzyme (sample control)

2.7 Data Statistically

The results of the FTIR spectrum obtained were processed using a chemometric analysis program using the Metaboanalyst 5.0 and Origin Lab 2022 program. Antioxidant and anti-elastase activity data in each sample were interpreted into the mean±SD. Each sample was determined by the value of 50% inhibitory concentration (IC_{50}), which was plotted between the percent inhibition of each sample on the concentration of the sample solution using Microsoft Excel 2011 version.

3. Result and Discussion

3.1 Authentication Profile of Ethanolic Cherry Fruits

The cherry plant is widely distributed in Indonesia and is generally easy to grow in various soil conditions and with a pH of 5-5.5. This cherry plant can grow up to a geographical location of 1000 meters above sea level [18]. The chemical content is widely distributed in cherry fruit, so it has been used empirically and with scientific evidence as herbal medicine. The existence of efficacy and prospects of cherry fruit to be developed as a food nutrition ingredient, medicine, nutraceutical, and even cosmeceutical needs to be characterized and authenticated for its chemical content so that the efficacy of its use can be standardized [2,5].

In this study, authentication was carried out on the similarity of the chemical content of the ethanol extract of cherry fruit obtained from different growing locations in the Province of South Sulawesi-Indonesia. Eight sampling locations for cherry fruit represent the environmental conditions in which they grow. Cherry fruit samples obtained from Makassar City (M1, M2, and M3) are in the city centre with varying geographical environments (0-25 masl). Cherry fruit samples obtained from the districts of Takalar, Gowa, and Barru represent areas with a coastal geographic location (0-1000 masl). Cherry fruit originating from the regencies of Soppeng and Tana Toraja represents an area with mountainous geography (>1000 masl).

Authentication studies carried out by FT-IR combined chemometrics were analyzed at wave numbers 4000-400 cm-1. The IR spectrum profiles produced from each cherry fruit ethanol extract can be seen in Figure 1.

Representative FT-IR spectrum of each cherry fruit sample with the different geographical locations at intervals of 4000-400 cm-1. Overall, there is no significant difference in the spectrum in each sample. However, some vibrations are different from other samples, specifically on the spectrum of cherry fruit samples from Soppeng Regency. The cherry fruit sample from Soppeng (S) only had OH alcohol vibrations in the 722 cm-1 area. Meanwhile, there was no CH-aromatic vibration in the 800-900 cm-1 (Table 1). Only the vibrations in the 800-900 region differ from the cherry fruit samples from Soppeng from other regions. In addition, there is a vibration formed in the 3700-3800 cm-1 area, which is a signal of the -NH (amine) group that appears in samples from the Tamalanrea (M1) and Gowa (G) regions. However, the overall vibrations have a similar profile, so it can be concluded that there is no significant difference in each area based on geographic location. FTIR and chemometric analysis in studying the fingerprint ethanol extract of cherry fruit is instrumental and can be applied to distinguish and study the similarities between each extract [13,14,19].

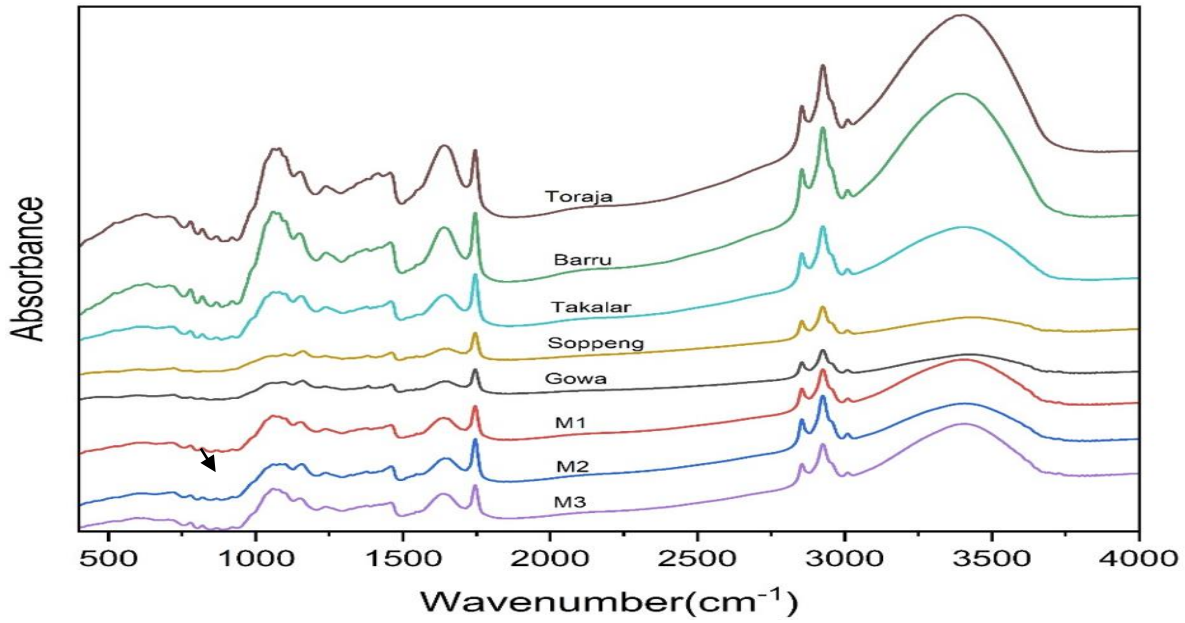


Figure 1. FT-IR spectrum profile of cherry fruit ethanolic extract. The data were analyzed in triplicates (n=3).

Table 1. The wave number (cm^{-1}) of cherry fruit extract is based on the FTIR spectroscopy profile observed at 4000-600 cm^{-1}.

Wave Number (cm^{-1})	**Functional Group**	Structure	**Intensity**	**Assignment**	**Presence in Extract**
3855-3700	Amina	-NH2	W	-NH stretch	M1, G
3400-3450	Alcohol Aromatic	-OH	S	-OH stretch	all extract
3010-3000	Alkene	-CH	S	-CH, vinyl	all extract
2925-2926	Alkane	-CH	S	-CH asymmetries, stretch	all extract
2854	Carboxylic Acid	-COOH	S (broad)	OH-dimer	all extract
1745	Ester	-COOR	S	C=O, stretch	all extract
1638-1650	Amina primer	-NH	M	C=C, stretch	all extract
	alkene	-C=CH2	M	C=C, stretch	all extract
1450-1460	Aromatic	-C=C	M	C=C, stretch	all extract
1378-1400	Phenol/OH tarsier	-OH	M	-OH, bend	all extract
1160-1140	Alcohol/hydroxy	-C-O	M	-C-O, bend	all extract
1059-1100	Alcohol/Eter	-C-O	S	-C-C, bend	all extract
818	Aromatic	-CH	S	-C-H, bend	M1, M2, M3, T, G, B, TO
620-750	Alcohol	-OH	S	-OH	all extract

Note: Data were accumulated from the overall FT-IR profile of eight cherry fruit extracts from different geographies and interpreted and summarized in table 1. The data were analyzed in triplicates (n=3).

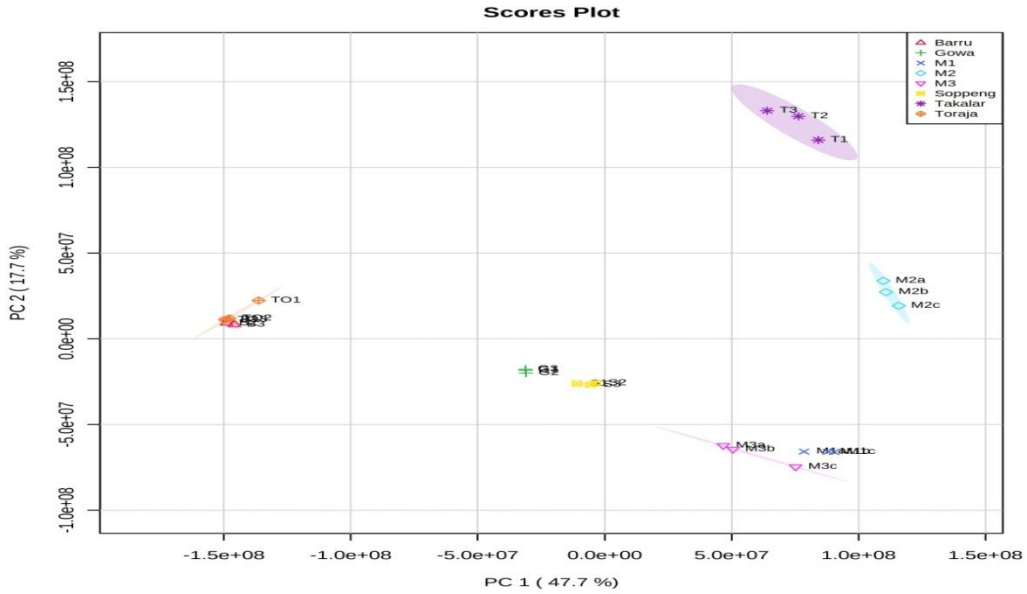


Figure 2. PCA Score plot for main components 1 (PC 1) and 2 (PC2) on ethanol extract of cherry fruit. The data were analyzed in triplicates (n=3).

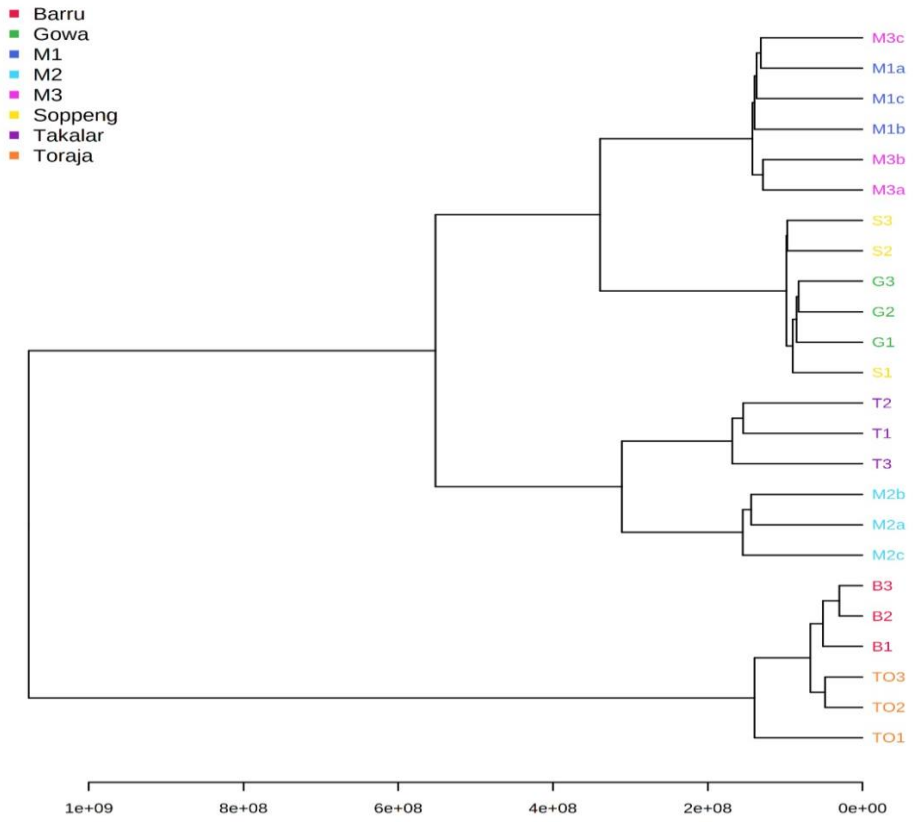


Figure 3. The dendogram of each cherry fruit ethanol extract was repeated 3 times (n=3).

Table 2. Antioxidant activity of ethanolic cherry extract based on geographic location

Samples	Antioxidant Activity (IC_{50})	
	DPPH (µg/mL)	ABTS (µg/mL)
M1	61.06±0.79	46.03±0.09
M2	45.08±0.28	18.67±0.62
M3	43.87±0.81	24.60±0.11
Gowa (G)	27.26±1.02	19.11±0.95
Takalar (T)	62.41±0.17	47.68±0.24
Barru (B)	75.85±0.32	57.18±1.02
Soppeng (S)	30.08±0.22	46.30±0.99
Toraja (TO)	63.46±1.19	54.76±0.28

The data were analyzed in triplicates (n=3).

Table 3. Percentage of inhibition and IC_{50} value of elastase inhibitory activity test of cherry fruit ethanol extract.

Conc. (µg/mL)	Elastase Inhibition (%)							
	M1	**M2**	**M3**	**Gowa**	**Takalar**	**Barru**	**Soppeng**	**Toraja**
4.6875	40.19	44.64	35.74	29.93	32.84	15.77	39.47	23.79
9.375	43.19	45.11	41.26	37.99	39.63	20.78	44.49	34.44
18.75	48.72	51.50	45.94	42.22	44.08	33.79	58.10	37.40
37.5	64.55	65.22	63.87	44.93	54.40	36.11	65.66	57.16
75	75.31	73.11	77.51	72.68	75.10	60.83	72.44	71.87
150	83.88	84.27	83.48	79.45	81.47	64.11	76.02	81.00
IC_{50} (µg/mL)	**18.24**	**7.84**	**15.62**	**32.02**	**39.17**	**84.23**	**11.57**	**25.11**

The data were analyzed in triplicate (n=3).

The similarity of the spectra obtained from the results of the FTIR spectroscopic analysis was then analysed using the PCA (Principal Component Analysis) technique. The results of the PCA analysis are presented in (Figure 2), the principal component values of PC-1 are 47.7%, and PC-2 are 17.7%. So cumulatively, from the results of PCA analysis using spectral data on samples with wavelengths of 4000–400 cm^{-1} can explain the variables of 65.4% of the sample data set, and the variance can be said to be suitable for building a model. If the number of principal components of PC1 and PC2 is more significant than 60%, then the results of the principal component plot show two suitable dimensions. The main groups in each cherry fruit ethanol extract had >60% similarity. These differences can occur based on the content of compounds systematically and clearly. The PCA results confirm that the altitude of the growing place affects the concentrations of different compounds and plays an important role in determining the differences between individual samples [20,21].

PCA is a method often used to make closely related plant discrimination or similarity models, which are

included in the unsupervised pattern recognition technique. PCA is used to reduce data and extract information to find combinations of variables or factors that can explain the main trends in a data set. The PC value provides information about the pattern contained in the sample. Plots for the initial two PC values are usually most helpful in analysis because these two PCs contain the most variation in the data [14,21].

The subsequent authentication analysis performed on the ethanol extract of cherry fruit was cluster analysis. Cluster analysis is a method for dividing a group of objects into classes so that similar ones are in the same class [20,21]. Figure 3 shows the clustering of each cherry fruit extract based on the FT-IR profile. The dendrogram of the FT-IR profile of each extract tends to form two clusters. The first grouping cluster was found in cherry fruit extracts from Barru (B) and Tana Toraja (TO). The second cluster formed clusters from the Biringkanaya (M2) and Takalar (T) areas, and the Tamalanrea (M1), Telkomas (M3), Gowa, and Soppeng subgroups mainly formed clusters together, indicating that the samples had more similar Physico-chemical profiles as reflected in the infrared spectrum. The clustering of each group illustrates the similarity in terms of the profile of the secondary metabolites as well as the intensity or concentration of the compounds.

3.2 Antioxidant Assay

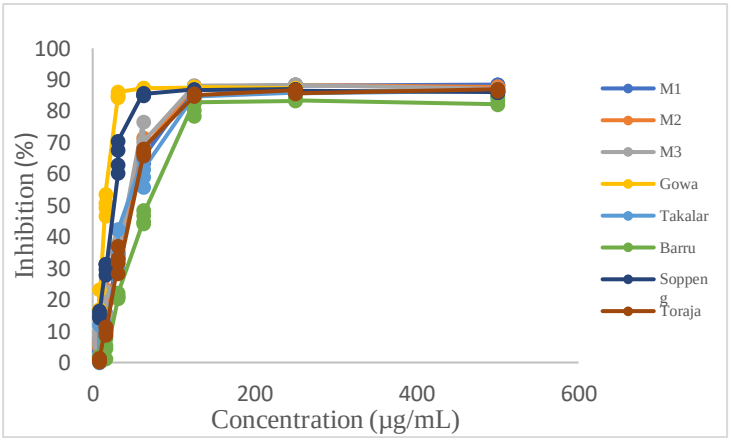
In testing the activity of the ethanol extract of cherry fruit using the DPPH and ABTS methods by determining the IC_{50} value as stated in the study [2]. IC_{50} values were categorized as very strong (<50 µg/mL), strong (>50-100 µg/mL), moderate (>100-150 µg/mL) and weak (>150 µg/mL) to determine the capacity of inhibition against free radicals [5]. The profile of antioxidant activity using the DPPH and ABTS methods from each ethanol extract of cherry fruit can be seen in Figure 4.

Based on the results of testing the antioxidant activity of the ethanol extract of cherry fruit (Figure 4), it shows that along with the increase in the concentration of the sample, it has a correlation with activity in inhibiting free radicals by DPPH (Figure 4a) and ABTS (Figure 4b) methods. The higher the concentration of the sample solution, the greater the inhibitory power of free radicals.

The antioxidant activity test of cherry fruit extract showed that cherry fruit extract provided antioxidant power from strong (IC_{50} >50-100 µg/mL) to very strong (IC_{50}<50 µg/mL), both in the DPPH method and the ABTS method. Extracts M2, M3, G, and S based on geographic location showed very strong antioxidant activity, and extracts M1, T, B, and TO gave strong activity in scavenging DPPH radicals. The same thing also gave very strong (M1, M2, M3, S, T, and G) and strong (B and TO extract) antioxidant activity in the ABTS method.

Previous studies have reported that ethanol extract from cherry fruit has very strong antioxidant activity in reducing DPPH and ABTS radicals with IC_{50} values <50 µg/mL [2,5].

These results have similarities with the study conducted even though the ethanol extract of the cherries analyzed was obtained from different growing locations. Overall, the studies conducted showed antioxidant activity that varied from strong to very strong activity.



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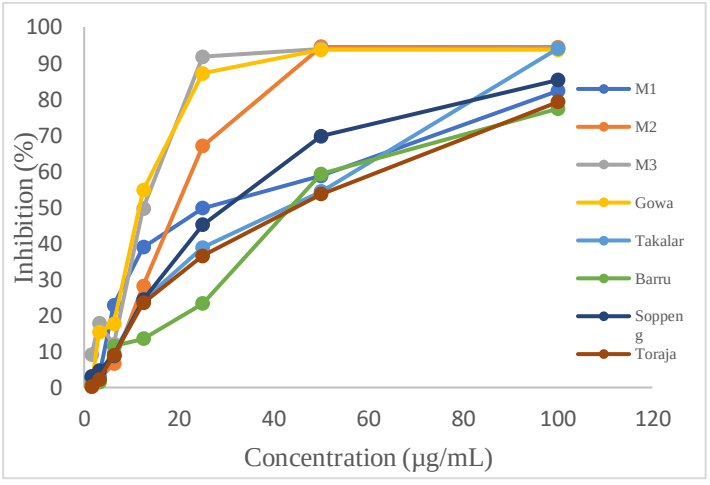

B

Figure 4. The trend of increasing the percentage of inhibition of each cherry fruit ethanol extract along with the increase in concentration using the DPPH (A) and ABTS (B) methods. The data were analyzed in triplicates (n=3).

3.3 Anti Elastase Activity

The activity of the elastase enzyme was carried out colorimetrically on cherry fruit extracts obtained from different growing locations. This test's principle is the interaction between the substrate and the enzyme. The substrate used in this study was N-succinyl-(Ala)3-nitroanilide (SANA). This substrate has three alanine which were then degraded by elastase. Alanine was detected in tropoelastin as elastin-forming in human skin. This model underlies N-succinyl-L-Ala-L-Ala-L-Ala-p-nitroanilide as a substrate in vitro test model. At a wavelength of 401-410 nm, there was an increase in absorbance due to the hydrolysis of N-succinyl L-Ala-L-Ala-L-Ala-p-nitroanilide [17,22]. The formation of yellow-colored nitroaniline indicates the reaction results. The results of the bioactivity of each extract against elastase can be seen in Figure 5 and Table 3.

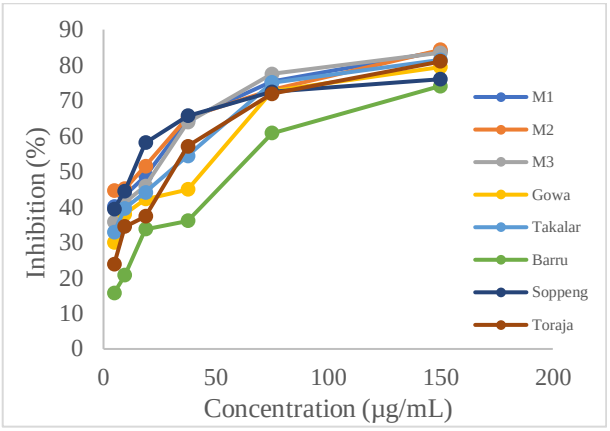

Figure 5. The trend of increasing the percentage of elastase inhibition of each cherry fruit ethanol extract along with the increase in concentration. The data were analyzed in triplicate (n=3).

Based on the results of the elastase enzyme inhibition test (Figure 5), it was shown that there was a correlation with the increase in the sample concentration towards the elastase enzyme inhibition. The ability of ethanol extract from cherry fruit to inhibit elastase was observed at concentrations of 4.68 to 150 µg/mL. At the highest concentration, the average elastase inhibition in each extract was 80.46%. Based on the IC_{50} value of each extract, mixed results were obtained. The lowest IC_{50} value was found in the M2 extract (7.84 µg/mL) and the highest in the Barru (B) extract (84.23 µg/mL). The lower the IC_{50} value from the analysis results, the stronger the bioactivity in inhibiting elastase. Although the IC_{50} value varies, the ability to inhibit the work of elastase is still powerful.

Authentication and bioactivity studies of ethanol extract of cherry fruit obtained from various locations in South Sulawesi-Indonesia, by analysis of their bioactivity as an antioxidant and anti-elastase, found varying results from each extract. Various factors certainly influence variations in the results obtained. Environmental factors, geographical location, climate, and soil conditions where the sample grows can affect the concentration of potential chemical constituents [23,24]. In addition, several treatments in the cherry fruit harvesting process are factors that cause various effects on the bioactivity of each extract. In the analysis of compounds based on the FT-IR spectroscopic approach, the similarity of the chemical content of each extract was 65.4%. Therefore, about > 30% of discrimination or differences in chemical content and the intensity of the secondary metabolites produced. Therefore, this study provides an overview of the geographical differences in the location of the extract growing on its chemical content and bioactivity profile**.**

Conclusion

Authentication study of the similarity of the chemical content of ethanol extract of cherry fruit based on the geographic location where it was grown obtained a picture of the similarity of chemical content from the results of PCA analysis of 65.4%. There is about >30% discrimination between samples and several FT-IR profiles from each sample that tend to group to form clusters (Clusters 1 and 2). From the bioactivity profile of antioxidants and anti-elastase, it was also found that the percentage of inhibition and IC_{50} values varied. However, these variations did not affect the activity of each sample in inhibiting free radicals (DPPH and ABTS) and elastase enzymes. The results showed that the ethanol extract of cherry fruit from various locations where it was grown provided a bioactivity profile from the strong to powerful category.

Conflict of Interest

The Authors declare no conflict of interest in this research

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