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Chemometric Analysis of Ethanol Extract of Breadfruit Leaves (*Artocarpus altilis*) from Various Regions in Central Java

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ABSTRACT

Breadfruit leaves (*Artocarpus altilis*) contain chemical compounds, including flavonoids, phenolics, tannins, alkaloids, and saponins, which have antioxidant activity. The planting location causes differences in the composition of active compounds, causing their biological activity to change. This research aimed to determine the highest antioxidant activity, flavonoid, phenolic, and tannin content of 96% ethanol extract of breadfruit leaves from 6 sub-districts in Central Java and to group them using the principal component analysis (PCA) and cluster analysis (CA) chemometric methods. Breadfruit leaves were extracted using the maceration method using 96% ethanol solvent. The antioxidant activity test was carried out using the DPPH method using vitamin C as a comparison. Total flavonoid levels were determined using an $AlCl_3$ reagent. Determination of total phenolic and tannin content levels using Folin-Ciocalteu. Then, chemometric analysis was carried out using Principal Component Analysis (PCA) and Cluster Analysis (CA). The results showed that 96% ethanol extract of breadfruit leaves has the highest IC_{50}, flavonoid, phenolic, and tannin levels, respectively, namely 82.79 ± 0.20 µg/ml; 9,96 ± 0,17 mgQE/g; 603.75 ± 2.6; 123.66 ± 0.76 which came from the Tengaran area. The samples were divided into three groups using PCA and CA methods based on the variables used. The first group was Gajahmungkur, Mranggen, and Pamotan. The second group was Gunungpati and Bandungan. The third group was Tengaran.

***Keywords*:** *Breadfruit; Chemometric analysis; Antioxidant; Bioactive molecules*

INTRODUCTION

The breadfruit plant is one of the Indonesian plants that have the potential to be developed as a source of bioactive compounds with several pharmacological activities, including anti-inflammatory, antidiabetic, UV radiation protection, anticancer, antiatherosclerosis, antihypertensive, antibacterial, antifungal, antimalarial, improving kidney function, antioxidant, antihyperlipidemic, and cardioprotective. One part of the breadfruit plant that the Indonesian people utilize as a traditional medicine to keep the body healthy and treat diseases is breadfruit leaves. The potential of breadfruit leaves as conventional medicine can change the paradigm from only waste, and animal feed can be developed into high-value economic products. Breadfruit leaves can be processed into a healthy tea to help cure kidney and heart disease[1]. In addition, natural ingredients are the treatment of choice because they are considered safer, with relatively minimal side effects compared to synthetic drugs. The

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utilization of medicinal plants is also more affordable, especially for rural communities. The compounds that play a role are phenolics, flavonoids, and tannins.[1] The ethanol extract of breadfruit leaves has potent antioxidant activity of 60.13 ± 0.54 µg/mL using the DPPH method.[2]

Breadfruit plants can grow in lowlands to highlands. In addition, breadfruit plants have a relatively high tolerance to climate ranges. Breadfruit plants can grow well in wet and dry climates.[3] The content of compounds in each breadfruit can vary due to several factors, one of which is the plant's geographical location, where soil and environmental conditions such as nutrients, weather, and temperature can affect the formation of compounds and plant productivity.[4] Determining the differences between samples with similar physicochemical properties between variables is often subjective and difficult to do with general statistical methods. Therefore, there is a need for an analytical method that can manage multivariate data effectively and accurately. Chemometrics is used to manage and handle multivariate data and experimental design.[5]

METHODS

Tools and Materials

The materials used were breadfruit leaf samples obtained from several sub-districts in Central Java, namely Gajahmungkur, Mranggen, Pamotan, Gunungpati, Bandungan, and Tengaran. Other materials used are ethanol 96% (Bratachem), ethanol p.a (Merck), DPPH (Sigma Aldrich), vitamin C (Merck), Mg powder (Merck), amyl alcohol (Merck), concentrated HCl (Merck), $AlCl_3$ (Merck), CH_3COOK (Merck), quercetin (Merck), NaCl 1% (Merck), Gelatin 5% (Merck), $FeCl_3$ (Merck), gallic acid (Merck), tannic acid (Merck), and Na_2CO_3 (Merck).

The tools used are a set of glassware (Iwaki Pyrex), a set of maceration tools, pollinating machines (Fomac), electric scales (Ohaus), moisture balance (Ohaus), vacuum (Rocker 600), oven, Buchner funnel, rotary evaporator (Heidolph), spectrophotometer UV-Vis (Shimadzu), and micropipette (Socorex).

Determination

Determination of breadfruit plants was carried out at the Ecology and Biosystematics Laboratory, Biology Department, Faculty of Mathematics and Natural Sciences, Diponegoro University, Semarang.

The results of the determination are 1b-2b-3b-4b-12b-13b-17b-18b-18b-19b-20b-21b-22b-23b-24b-25b-26b-27b-799b-800a (Faimili 117. Moraceae) 1b-2b-4b-6b-8b-9b-15b (Genus. 9. Artocarpus)1a-2a-3b-4b (Species *Artocarpus altilis*).

Research Methods

Preparation of 96% Ethanol Extract of Breadfruit Leaf (EEDS)

Fresh breadfruit leaves are washed with running water, and then wet sorting and chopping are carried out to separate the leaves from impurities. The following process is drying using an oven at 50^{o}C until the water content of the simplisia is obtained at <10%. Then macerated using ethanol 96%.

Phytochemical Screening

a. Phenolic Compounds

EEDS 50 mg was dissolved in 5 mL of ethanol p.a and filtered. The solution was divided into 2 test tubes; tube one was used as a blank, and tube 2 added three drops of 5% $FeCl_3$ solution. The extract is positive for phenol when it produces green, red, purple, blue, or black color[6].

b. Flavonoid Compounds

EEDS weighed 50 mg, dissolved in 10 ml ethanol p.a., and then filtered. The mixture was put into two different test tubes. One of the tubes was added with mg powder, amyl alcohol, and three drops of concentrated HCl through the tube wall. The solution was allowed to stand for a while and observed for color change.[6]

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c. Tannin Compounds

EEDS 50 mg was dissolved in 10 mL of hot distilled water and then filtered. The solution was divided into 4 test tubes: tube 1 was blank, tube 2 added three drops of 1% NaCl solution, tube 3 added three drops of 1% NaCl and three drops of 5% gelatin solution, and tube 4 added $FeCl_3$ solution. In the 3rd tube, a white precipitate indicates a positive extract containing tannin compounds, and tube four shows color changes to corroborate the results.[6]

Antioxidant Activity Test

Antioxidant activity testing starts by determining the maximum wavelength and operating time of DPPH. Determination of the antioxidant activity of DPPH (1,2,3,4 and 5 µg/mL) and EEDS solution (40, 80, 120, 160, and 200 µg/mL), pipetted each 1 ml and added DPPH solution as much as 4 ml, then allowed to stand in a place that is protected from light for 30 minutes and measured the absorption with a Uv-Vis spectrophotometer at a wavelength of 516 nm.[7]

Determination of Phenolic Content

Determining total phenolic content starts with deciding the gallic acid's maximum wavelength and operating time. The following process is determining the standard curve of gallic acid and the total phenolic content of EEDS. Gallic acid solution (50, 100, 150, 200, 250, and 300) and EEDS solution were taken as much as 200 mL each, then 400 mL of *Folin-Ciocalteu* and 4 mL of 7% Na_2CO_3 were added. The mixture was allowed to stand for 130 minutes and read at a wavelength of 754 nm.[8]

Determination of Flavonoid Content

Determining total flavonoid content starts with determining quercetin's maximum wavelength and operating time. The following process is determining the standard curve of quercetin and the total flavonoid content of EEDS. Quercetin solution (2,4,6,8,10, and 12 µg/mL) and EEDS solution were taken 1 ml each reacted with 200 µL of 10% $AlCl_3$ and 200 µL of 1M CH_3COOK. The mixture was allowed to stand for 30 minutes and read at a wavelength of 432 nm.[6]

Determination of Tannin Content

Determining tannin content starts with determining tannic acid's maximum wavelength and operating time. The following process determines the standard curve of tannic acid and the total EEDS tannin content. Tannic acid solutions (40, 80, 120, 160, and 200 µg/mL) were each pipetted with as much as 0.5 ml added with 7.5 ml of water and 0.5 ml of *Folin-Ciocalteu* reagent allowed to stand for 5 minutes. The solution was added 1.5 mL each of 20% Na_2CO_3 solution allowed to stand for 70 minutes in the dark and read at a wavelength of 745 nm. EEDS solution was taken 200 µL each, added *Folin-Ciocalteu* reagent as much as 200 µL, allowed to stand for 5 minutes, then added 100 µL of saturated Na_2CO_3 solution and added distilled water to 5 mL, the solution was allowed to stand for 70 minutes and read at a wavelength of 745 nm.[9]

Data Analysis

The data obtained were IC_{50}, phenolic, flavonoid, and tannin. Data were expressed as mean ± standard deviation and processed using Microsoft Excel (Microsoft Inc., USA). Furthermore, PCA (Principal Component Analysis) and CA (Cluster Analysis) chemometric analysis was performed using Minitab version 19 (Minitab Inc., USA) to group the samples using the variables of antioxidant activity, total flavonoid content, and tannin content.

RESULTS AND DISCUSSION

Preparation of 96% Ethanol Extract of Breadfruit Leaf (EEDS)

Determination of water content using a moisture balance tool and the water content of breadfruit leaves has met the quality requirements of <10%.[10] The extraction of breadfruit leaves used in this study is the maceration extraction method

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because the equipment and method are simple and do not use heating to avoid the decomposition of active substances contained in the sample due to the influence of temperature that is not resistant. Fresh breadfruit leaves of as much as 1.500 grams were dried, and the results obtained are shown in Table 1.

Phytochemical Screening

a. Phenolic Compounds

This study is positive EEDS containing phenolic compounds marked changes in green color to yellow-black. The formation of this color is caused by phenol compounds in the extracted sample forming a complex with Fe^{+3} ions.[6] Phytochemical screening of phenolic compounds can be seen in Table 2.

b. Flavonoid Compounds

This study is positive EEDS containing flavonoid compounds characterized by a change in the color of the solution from green to orange after adding mg and concentrated HCl. Amyl alcohol was used to form two layers. Adding metal Mg and concentrated HCl in the identification reaction reduces the benzopyrone core in the flavonoid structure, creating a red or orange flavylium salt in the amyl layer.[11] Phytochemical screening of flavonoid compounds can be seen in Table 2.

c. Tannin Compounds

Tannin compounds will form copolymers with a greater specific gravity so that they are insoluble in water, thus creating a white residue. The fourth tube containing the sample and $FeCl_3$ can show the presence of phenol groups when there is a change in color to blackish. It happens because tannin is a polyphenol compound. The color change to blackish occurs due to the formation of complex compounds between tannins and $FeCl_3$.[6] Phytochemical screening of tannin compounds can be seen in Table 2.

Antioxidant Activity Test

Antioxidant activity test using the DPPH method. DPPH is an antioxidant activity test that can capture free radicals. When the purple DPPH solution meets with electron donor material, DPPH will be reduced, causing the purple color to fade and be replaced by the yellow color derived from the picryl group.

Table 1. Extraction results of 96% ethanol extract of breadfruit leaves

Place	Dry Leave Powder (g)	Extract (g)	Moisture content (%)	Yield (%)
Tengaran	575	97	4,8	16,86
Bandungan	735	116,4	7,0	15,83
Gunungpati	575	72,4	6,3	12,59
Gajahmungkur	690	91,8	8,6	13,30
Mranggen	595	82,8	7,7	13,91
Pamotan	550	97	6,4	13,96

Table 2. Phytochemical screening results for phenolic, flavonoids, and tannins

Place	Phytochemical Screening of Phenolic	Phytochemical Screening of Flavonoid	Phytochemical Screening of Tannin
Pamotan	+	+	+
Mranggen	+	+	+
Gajahmungkur	+	+	+
Gunungpati	+	+	+
Bandungan	+	+	+
Tengaran	+	+	+

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The intensity of the color depends on the antioxidant ability. EEDS was made in 5 different concentration series to find the IC_{50} value using a mathematical equation obtained through the correlation between inhibition and extract concentration. DPPH reagent that reacts with antioxidants will change color from purple to yellow, where the intensity of the change in DPPH color is directly proportional to the activity of antioxidants to reduce free radicals. The higher the concentration, the more the color fades[12]. The results of antioxidant activity from EEDS can be seen in Table 3. Antioxidant activity in each sub-district has different values because various factors can affect the content of secondary metabolites in a plant, including the temperature of the growing region, the atmospheric environment (CO_2, O_2, and humidity), harvest age, soil nutrients, and fertilization.[5]

Determination of Phenolic Content

This study's phenolic content was determined using the *Folin-Ciocalteu* method with gallic acid as a comparator. Gallic acid is used as a comparator because gallic acid is included in phenolic compounds derived from hydroxyl benzoic acid, which is classified as a simple phenolic acid and has the availability of stable and pure substances. Gallic acid has three hydroxyl groups.[13,14] Gallic acid reacted with *Folin-Ciocalteu* reagent produces a yellow color indicating phenolic compounds, then added with 7% Na_2CO_3 as a base atmosphere giver will change color from yellow to blue. During the reaction, hydroxyl groups on phenolic compounds react with the *Folin-Ciocalteu* reagent, forming a blue *molybdenum-tungsten* complex. The blue color formed will be more intense, meaning that the greater the concentration of phenolic compounds, the more phenolic ions will reduce heteropolyacids (*phosphomolybdate-phosphotungstic*) into *molybdenum-tungsten* complexes so that the resulting color is more intense.[13] The results of EEDS phenolic compound levels can be seen in Table 3.

The table above shows that the highest phenolic content of EEDS is Tengaran District at 603.75 mgGAE/g, while the smallest is Mranggen District at 199.5 mgGAE/g. Several factors can affect the composition of active compounds in plants, including light, temperature, humidity, pH, soil nutrient content, and altitude. Differences in the composition of active compounds in a plant also cause its biological activity to change.[15]

Determination of Flavonoid Content

Determination of flavonoid levels using spectrophotometric methods because flavonoids contain conjugated aromatic systems that show strong absorption bands in the ultraviolet and visible light spectra. Quercetin is used as a comparator because it is a flavonoid from the flavonol group with a ketone group at C-4 and a hydroxy group at C-3 or C-5 atoms so that it can form a color complex with $AlCl_3$[16]. In addition, quercetin is also a flavonoid compound in breadfruit leaves[17]. $AlCl_3$ reagent causes a complex reaction characterized by a change in color to yellow. It is caused by a shift in wavelength towards the visible.[16] CH_3COOK maintains and stabilizes the complex formation between AlC_{l3} and flavonoids.[18] The results of EEDS flavonoid compound levels can be seen in Table 3. Based on Table 3, the highest EEDS flavonoid content is Bandungan District at 13.93 mgQE/g, while the smallest is Mranggen District at 4.75 mgQE/g. Several internal and external factors influence the content of secondary metabolites in plants. Internal factors include genes, and external factors include light, temperature, humidity, pH, soil nutrient content, and the growing place's height. The altitude of a place is one of the factors that affect the growth of a plant. Plant metabolic processes will be disrupted, so the compounds produced from these processes will vary at each altitude.

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Determination of Tannin Content

Determination of tannin content using UV-Vis spectrophotometry at a wavelength of 745 nm read on a UV-Vis spectrophotometer must be reacted with color-forming reagents, namely *Folin-Ciocalteu* and Na_2CO_3. The formation of color is based on oxidation-reduction reactions, where tannin is a reductant. *Folin-Ciocalteu*, as an oxidizer, oxidized tannins will convert *phosphmolybdate* in *Folin-Ciocalteu* into blue *phosphmolybdenim*, which can absorb light in the ultraviolet, visible wavelength region. The more tannins contained, the more *phosphmolybdate* will be reduced to *phosphmolybdenim*. Na_2CO_3 aims to create an alkaline atmosphere so that the *Folin-Ciocalteu* reduction reaction occurs by the hydroxyl group of polyphenols in the sample and will form a blue *molybdenum-tungsten* complex.[13] Tannic acid is used as a comparator in determining the levels of tannin compounds because tannic acid is included in the hydrolyzed tannin group.[19] The results of EEDS tannin compound levels can be seen in Table 3.

Based on Table 3 above, the highest EEDS tannin content is Tengaran District at 123.66 mgTAE/g, while the smallest is Mranggen District at 23.33 mgTAE/g. Several factors can affect the composition of active compounds in plants, including light, temperature, humidity, pH, soil nutrient content, and altitude. Differences in the composition of active compounds in a plant also cause its biological activity to change.[15]

Data Analysis

The results of antioxidant activity (IC_{50}), total phenolic content (TPC), total flavonoid content (TFC), and total tannin content (TTC) obtained were analyzed using principal component analysis (PCA) and cluster analysis (CA) chemometrics techniques with Minitab software version 19.1.

Eigenanalysis of the Correlation Matrix

Eigenvalue	3,6211	0,3368	0,0351	0,0070
Proportion	0,905	0,084	0,009	0,002
Cumulative	0,905	0,989	0,998	1,000

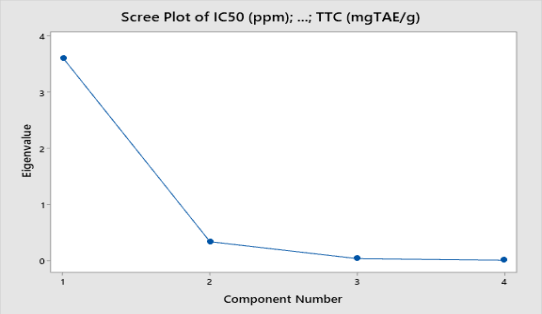

Figure 1. Eigenanalysis and Scree Plot graph of TPC, TFC, TCT dan IC_{50}

The eigenvalue obtained shows that PC1, PC2, and PC3 contribute to the variance of 90.5%, 8.4%, and 0.9%, respectively. The relationship between each PC and eigenvalue is depicted in Figure 1.

Table 3. Results of IC_{50} values, total phenolic, tannin, and flavonoid levels

Village	Value IC_{50} (µg/ml) ± SD	Phenolic Content (mgGAE/g) ± SD	Flavonoid Content (mgQE/g) ± SD	Tannin Content (mgTAE/g) ± SD
Pamotan	158,77 ± 0,54	235 ± 0,28	5,80 ± 0,05	44,41 ± 0,52
Mranggen	170,24 ± 0,1	199,5 ± 0,28	4,75 ± 0,13	23,33 ± 0,52
Gajahmungkur	131,92 ± 0,06	266,5 ± 1,15	5,28 ± 0,1	54,91 ± 0,38
Gungpati	96,31 ± 0,21	412,5 ± 2,5	12,55 ± 0,08	83,58 ± 0,62
Bandungan	90,41 ± 0,05	466,25 ± 1,19	13,93 ± 0,64	84,16 ± 0,76
Tengaran	82,79 ± 0,20	603,75 ± 2,6	9,96 ± 0,17	123,66 ± 0,76

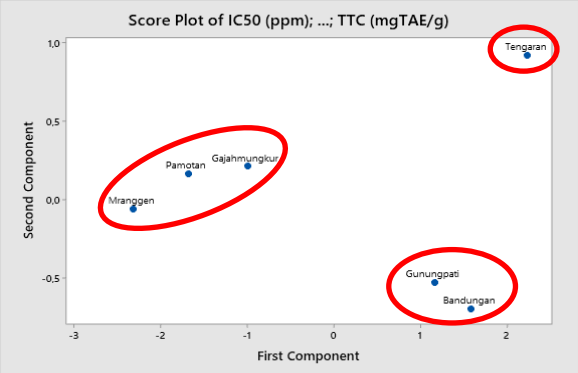

Figure 2. Score Plot

A score plot analyzes samples with adjacent values with similar characteristics and properties. The score plot graph (Figure 2) explains the similarity of a sample with other samples based on PC1, PC2, and PC3, which are characterized by points that are close to each other. The closer the two points are, the more similar the two samples are. Conversely, the farther the two points are, the less similar the samples are based on IC_{50}, TPC, TFC, and TTC values.

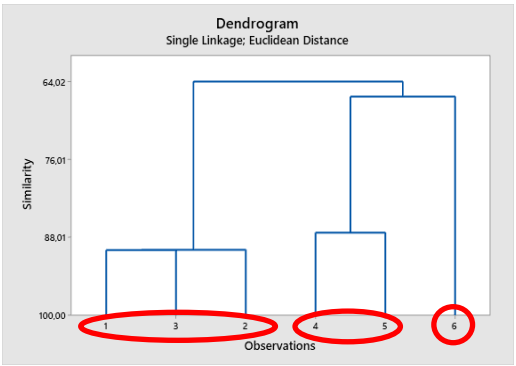

Figure 3. Dendrogram Graph

1 = Gajahmungkur; 2= Mranggen; 3 = Pamotan; 4= Gunungpati; 5= Bandungan; 6= Tengaran

The score plot graph and dendrogram show that the six breadfruit leaf samples produce three groups. The first group is Gajahmungkur, Mranggen, and Pamotan. The second group is Gunungpati and Bandungan. The third group is Tengaran.

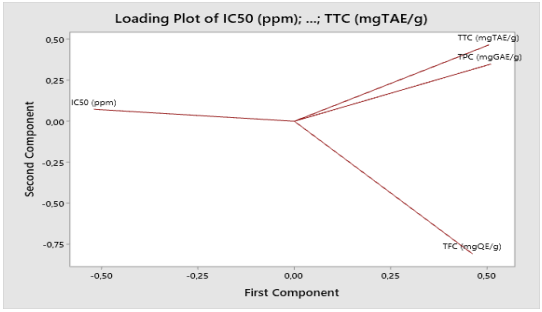

Figure 4. Loading Plot Graph

A loading plot graph (Figure 4) was used to see the correlation between IC_{50}, TPC, TFC, and TTC variables. The loading plot shows how strongly each variable affects the PC by describing it as a vector. The two variables are positively correlated if the two vectors form an angle of less than 90°. The two variables are not correlated if they form an angle around 90°. Meanwhile, the two variables show a negative correlation if they form a wider angle (more than 90°) or around 180°.[4]

The correlation between TPC and IC_{50}, TTC and IC_{50}, and TFC and IC_{50}, which forms a wide angle close to 180°, shows a negative correlation coefficient value and indicates a negative correlation relationship. A negative correlation shows that the higher the value of variable 1, the lower the variable 2.[4] The higher the phenolic, flavonoid, and tannin values will reduce the IC_{50} value. This is because phenolic, flavonoid, and tannin have the potential as antioxidants.[17]

The correlation between the TTC and TPC and TPC and TFC, which forms a slight angle of less than 90°, shows a positive correlation, which means that the higher the variable 1, the higher the variable 2[20]. This is because phenolics, flavonoids, and tannins are in the same secondary metabolic group. The Research conducted by Widyastuti et al[4] shows that flavonoid content negatively correlates with IC_{50}, which means that high phenolic and flavonoid content in Temulawak will affect the lower IC_{50} value. Following this study, it was found that the total phenolic and flavonoid content in breadfruit leaves had a negative correlation with IC_{50}, which

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means that the high phenolic and flavonoid content in breadfruit leaves will affect the lower IC_{50} value. Tannin compounds that correlate with IC_{50} show that tannins affect the antioxidant activity of the sample. It can be caused by tannin, which is a phenolic group compound. Phenolic compounds and flavonoids are compounds that have the potential to counteract free radicals.[21]

CONCLUSION

The ethanol extract of breadfruit leaves has the highest antioxidant activity, flavonoid, phenolic, and tannin content, and it comes from the landmark area. Based on chemometrics, samples can be divided into three groups.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors declare that the work presented in this article is original and that they will bear any liability for claims relating to the content of this article.

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REFERENCES

1. Yumni GG, Widyarini S, Fakhrudin N. Kajian Etnobotani, Fitokimia, Farmakologi dan Toksikologi Sukun (Artocarpus altilis (Park.) Fosberg). Jurnal Tumbuhan Obat Indonesia. 2021 Jul 1;14(1):55–70. DOI: 10.22435/jtoi.v14i1.3944
2. Putra EDL, Cintya H, Satria D. Antibacterial and antioxidant activities of ethanol extract of sukun (Artocarpus altilis.) leaves against Pseudomonas aeruginosa. Bhandari B, Santoso U, Ardiansyah, Julianti E, Yusraini E, Romauli NDM, et al., editors. E3S Web of Conferences. 2021 Dec 13;332:08006. DOI: 10.1051/e3sconf/202133208006
3. Raharjo A, Elida T, Prajitno D. Studi Kesesuaian Lahan Terhadap Sukun (Artocarpus sp.) di Kota Tarakan, Kalimantan Utara. Jurnal Agribisnis Terpadu. 2020 Jun 1;13(1):120. DOI: 10.33512/jat.v13i1.7350
4. Widyastuti I, Luthfah HZ, Hartono YI, Islamadina R, Can AT, Rohman A. Antioxidant Activity of Temulawak (Curcuma xanthorrhiza Roxb.) and its Classification with Chemometrics. Indonesian Journal of Chemometrics and Pharmaceutical Analysis. 2020 Jul 19;29. DOI: 10.22146/ijcpa.507
5. Rohman A, Can AT, Irnawati, Rafi M, Lukitaningsih E, Fadzilah NA. Principal Component Analysis of Antioxidant Activities, Total Phenolic contents, and Total Flavonoid contents of Turmeric (Curcuma longa L.). International Journal of Pharmaceutical Research. 2020 Nov 2;12(sp2). DOI: 10.31838/ijpr/2020.SP2.354
6. Hidayati DN, Maghfiroh HR, Safitri A. Antibacterial activity of ethanol extracts of hibiscus tiliaceus l. leaves from different extraction methods against Escherichia coli and Staphylococcus aureus. Pharmaciana. 2023 Apr 3;13(1):137. DOI: 10.12928/pharmaciana.v13i1.24671
7. Fajarwati I, Solihin DD, Wresdiyati T. Gambier for Diabetes: Comparison of the Antidiabetic Potency between Two Types of Extracts from Uncaria gambir (W. Hunter) Roxb. Jurnal Kefarmasian Indonesia. 2024;14(1):51–62. DOI: 10.22435/jki.v14i1.6621
8. Gultom DK, Saraswati I, Sasikirana W. Penetapan Kandungan Fenolik Total dan Uji Aktivitas Antioksidan dengan Metode DPPH (2,2-difenil-1-pikrilhidrazil) Fraksi Etil Asetat Ekstrak Etanolik Kubis Ungu (Brassica oleraceae var.capitata L). Generics: Journal of Research in Pharmacy. 2021 Oct 31;1(2):79–87. DOI: 10.14710/

ISSN: 2085-675X (Print)
ISSN: 2354-8770 (Electronic)

genres.v1i2.11226
9. Gurning K, Simanjuntak HA, Purba H, Situmorang RFR, Barus L, Silaban S. Determination of Total Tannins and Antibacterial Activities Ethanol Extraction Seri (Muntingia calabura L.) Leaves. Journal of Physics: Conference Series. 2021 Mar 1;1811(1):012121. DOI: 10.1088/1742-6596/1811/1/012121
10. Depkes RI. Farmakope Herbal Indonesia. II. Jakarta: Kementerian Kesehatan Republik Indonesia; 2017.
11. Megawati E, Bangun H, Putra I, Rusda M, Syahrizal D, Jusuf N, et al. Phytochemical Analysis by FTIR of Zanthoxylum Acanthopodium, DC Fruit Ethanol Extract, N-hexan, Ethyl Acetate and Water Fraction. Medical Archives. 2023;77(3):183. DOI: 10.5455/medarh.2023.77.183-188
12. Fatmawati IS, Haeruddin, Mulyana WO. Uji Aktivitas Antioksidan Ekstrak Etil Asetat Daun Belimbing Wuluh (Aveerrhoa bilimbi L.) dengan Metode DPPH. SAINS: Jurnal Kimia dan Pendidikan Kimia. 2023;12(1):41–9.
13. Pérez M, Dominguez-López I, Lamuela-Raventós RM. The Chemistry Behind the Folin–Ciocalteu Method for the Estimation of (Poly)phenol Content in Food: Total Phenolic Intake in a Mediterranean Dietary Pattern. Journal of Agricultural and Food Chemistry. 2023 Nov 22;71(46):17543–53. DOI: 10.1021/acs.jafc.3c04022
14. Hasnaeni H, Usman S, Wisdawati W. Pengaruh Metode Ekstraksi Terhadap Rendemen Dan Kadar Fenolik Ekstrak Tanaman Kayu Beta-Beta (Lunasia amara Blanco). Jurnal Farmasi Galenika (Galenika Journal of Pharmacy) (e-Journal). 2019 Oct 9;5(2):175–82. DOI: 10.22487/j24428744.2019.v5.i2.13599
15. Katuuk RHH, Wanget SA, Tumewu P. Pengaruh perbedaan ketinggian tempat terhadap kandungan metabolit sekunder pada gulma babadotan (Ageratum conyzoides L.). Jurnal COCOS. 2019;1(4):6. DOI: 10.35791/cocos.v1i4.24162
16. A. Makuasa DA, Ningsih P. The Analysis of Total Flavonoid Levels In Young Leaves and Old Soursop Leaves (Annona muricata L.) Using UV-Vis Sepctrofotometry Methods. Journal of Applied Science, Engineering, Technology, and Education. 2020 May 13;2(1):11–7. DOI: 10.35877/454RI.asci2133
17. Kurniawati IF, Sutoyo S. Review Artikel: Potensi Bunga Tanaman Sukun (Artocarpus altilis [Park. I] Fosberg) Sebagai Bahan Antioksidan Alami. Unesa Journal of Chemistry. 2021 Jan 25;10(1):1–11. DOI: 10.26740/ujc.v10n1.p1-11
18. Lindawati NY, Ma'ruf SH. Penetapan Kadar Total Flavonoid Ekstrak Etanol Kacang Merah (Phaseolus vulgaris L.) Secara Spektrofotometri Visibel. Jurnal Ilmiah Manuntung. 2020 Jun 30;6(1):83–91. DOI: 10.51352/jim.v6i1.312
19. Das AK, Islam MN, Faruk MO, Ashaduzzaman M, Dungani R. Review on tannins: Extraction processes, applications and possibilities. South African Journal of Botany. 2020 Dec;135:58–70. DOI: 10.1016/j.sajb.2020.08.008
20. Shiyan S, Nathasia J, Pratiwi G. Evaluation Of Response Corellation Using Chemometrics Analysis For Pre-Optimization Quercetin – Self Emulsion Formulation. Jurnal Farmasi Sains dan Praktis. 2022 Jun 30;9(3):213–24. DOI: 10.31603/pharmacy.v8i2.6660
21. Hikmah N, Arung ET, Sukemi S. Senyawa fenolik dan flavonoid, dan aktivitas antioksidan ekstrak metanol kulit buah ihau (Dimocarpus longan Lour. var. malesianus Leenh.). Bivalen: Chemical Studies Journal. 2020 Sep 30;3(2):39–42. DOI: 10.30872/bcsj.v3i2.447