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Research Article

Comparıson of Tannın-Compound Levels from Cacao Leaf Ethanol Extract (*Theobroma cacao* L.) by Place of Growıng

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ABSTRACT: Cacao (*Theobroma cacao* L.) is one of the plantations commodities in Indonesia. The most abundant part of the cacao plant are the brown leaves. Cacao leaves contain several secondary metabolite compounds, one of which is tannin. Tannin has several properties, such as being astringent, antidiarrheal, antibacterial, and antioxidant. This research aims to determine the comparison of tannin compound levels from the ethanol extract of cacao leaves (*Theobroma cacao* L.) using the UV-Vis spectrophotometric method. Samples were taken from three areas, namely Jeneponto, Wajo, and Malino. This research method begins with sample preparation by maceration with a 96% ethanol solvent, then a qualitative analysis test is carried out using the Thin Layer Chromatography (TLC) method, and a quantitative analysis test is carried out by standard measurement of tannic acid and determination of tannin content using a UV-Vis spectrophotometer at a maximum wavelength of 756 nm. The results showed that the ethanol extract of cacao leaves (*Theobroma cacao* L.) was positive for containing tannin compounds and had total tannin levels in the Jeneponto area (86.244 mgEAT/g extract), Wajo (79,417 mgEAT/g extract), and Malino (79.365 mgEAT/g extract). From the results above, it shows that the ethanol extract of cacao leaves from the Jeneponto area contains the highest levels of tannin compounds compared to the other two areas.

KEYWORDS: Antibacterial; cacao leaf (*Theobroma cacao* L.); tannin; UV-Vis Spectrophotometry.

1. INTRODUCTION

Indonesia is a country that has abundant flora diversity. Some types of plants are used in many fields, one of which is the pharmaceutical field. Cacao plants are one of the plant commodities that have been cultivated on Indonesian plantations. However, the use of other parts of the plant, such as brown leaves, still needs to be developed so that other parts of the plant can be put to good use to be cultivated into raw materials as other products (Hasanah et al., 2017). The phytochemical screening results of brown leaf extract contain several secondary metabolite compounds, one of which is tannin. Tannins are polyphenolic compounds found in many plants, including cacao leaves (Singh et al., 2015).

Tannins are one of the active compounds of secondary metabolites that have several properties, such as being astringent, antidiarrheal, antibacterial, and antioxidant (Fathurrahman, 2018). However, the tannin content of cacao leaves may vary depending on where they grow. For example, the areas used are Wajo Regency, Malino Regency, and Jeneponto Regency. Plant growth and development are strongly influenced by plant intrinsic and extrinsic factors. Intrinsic factors include genetic and hormonal factors. While extrinsic factors come in the form of environmental factors such as altitude, soil pH, light intensity, temperature, humidity, rainfall, soil texture, and others (Raharjeng ARP, 2015), The temperature difference in each altitude range causes different metabolic processes in a plant, so the production of secondary metabolites is different (Fatchurrozak et al., 2013).

Therefore, a comparison of tannin compounds from cacao leaf ethanol extract based on the growing place has been carried out to determine the difference in tannin levels between cacao leaves growing in various places using the UV-Vis spectrophotometry method.

2. EXPERIMENTAL SECTION

2.1. Sample collection and processing

The samples used in this research are cacao leaves (*Theobroma cacao* L.) taken from three regions, namely Malino, Wajo, and Jeneponto. The samples of fresh cacao leaves that have been collected are each cleaned first of impurities that are still attached to the leaves using clean water and dried. The dried leaves are then mashed by blending, put in a closed container, and stored in a dry, clean place. Simplisia of cacao leaves (*Theobroma cacao* L.) that have been mashed before weighing as much as 300 g and extracted by the maceration method The reason for using the maceration method is to avoid the risk of damage to compounds in plants that are thermolabile (Badaring et al., 2020). This maceration method uses a 96% ethanol solvent, which has volatile polar properties or solubility, so it is good to use as a solvent. In addition, ethanol is also one of the organic solvents that is widely used to dissolve organic compounds (Wiratmaja, 2011).

2.2. Materials and tools

The materials used in this research The ingredients used are aquadest, cacao leaves (*Theobroma cacao* L), tannic acid p.a., ethanol (96%), $FeCl_3$ 0.1 M, potassium ferricyanide, N-hexane, and ethyl acetate. The tools used in this study are a set of extraction tools with maceration methods, blenders, jars, stirring rods, filter paper, ovens, a set of glassware (Pyrex), micropipettes, UV-Vis spectrophotometry (Evolution type 201), analytical balances, rotary evaporators (Ika® RV 10 basic), a water bath (Memmert), and split funnels.

2.3. Solution making

2.3.1. Preparation of a standard curve solution

A total of 10 mg of tannic acid was weighed and then dissolved with ethanol in a beaker. Next, put in a measuring flask of 10 mL and add ethanol until the limit mark. The solution is used as a 1000 ppm mother solution, and then dilution is carried out from the stock solution to 100 ppm. From such solutions are made standard solutions with concentrations of 15, 20, 25, 30, and 35 ppm. Standard solutions of each concentration are taken as much as 0.5 mL, then mixed with 8 mL of aquades, then added with 0.1 mL of $FeCl_3$ as much as 0.5 mL and 8 mM potassium ferricyanide as much as 0.5 mL, then incubated for 10 minutes, then measured at a maximum wavelength of 756 nm. The absorbance readings obtained are used to make a standard calibration curve for the concentration of tannic acid solution (Ojha et al., 2018).

2.4. Qualitative analysis

The tannin qualitative test was carried out using the KLT (Thin Layer Chromatography) method. In the separation of tannin compounds with KLT, GF254 silica plates with a size of 8 cm x 4 cm were used. Tannin extracts are tolerated on plates at a distance of 1 cm from the bottom edge of the plate with capillary pipes, then dried and traced using the ratio of n-hexane mobile phase to ethyl acetate (6:4). The stains formed were examined with UV-Vis lamps at wavelengths of 254 nm and 366 nm (Kusumo et al., 2017) by spraying the appearance of FeCl3 spots, where the positive results of the active tannin compounds are characterized by colored spots ranging from green to blackish (Widyaningrum & Ningrum, 2021).

2.5. Quantitative analysis

2.5.1. Determination of the maximum wavelength of tannic acid

A total of 10 mg of tannic acid was weighed and then dissolved with ethanol in a beaker. Then I put it into a 10 mL volumetric flask and added ethanol until I reached the limit. The solution was used as a 1000-ppm parent solution. The parent solution was pipetted as much as 1 mL to get a 100 ppm stock solution, then pipetted as much as 2.5 mL, and then put into a 10 mL volumetric flask to get a standard solution with a concentration of 25 ppm. The 25 ppm standard solution was taken as much as 0.5 mL, then mixed with 8 mL of distilled water, and then added with $FeCl_3$ 0.1 M as much as 0.5 mL and potassium ferricyanide 8 mM as much as 0.5 mL, and then incubated for 10 minutes, after which the absorption was measured in the wavelength range of 400–800 nm. So that the maximum wavelength of 756 nm was obtained (Ojha et al., 2018).

2.5.2. Measurement of standard solutions of tannic acid

A total of 10 mg of tannic acid was weighed and then dissolved with ethanol in a beaker. Then I put it into a 10 mL volumetric flask and added ethanol until I reached the limit. The solution was used as a 1000 ppm master solution, then diluted from the stock solution to 100 ppm. The solution was made into a into a standard solution with concentrations of 15, 20, 25, 30, and 35 ppm. The standard solution of each concentration was taken as much as 0.5 mL, then mixed with 8 mL of distilled water, then added with $FeCl_3$ 0.1 M as much as 0.5 mL and potassium ferricyanide 8 mM as much as 0.5 mL, then incubated for 10 minutes, then measured at a maximum wavelength of 756 nm. The absorbance readings obtained were used to make a standard calibration curve against the concentration of tannic acid solution (Ojha et al., 2018)

2.5.3. Determination of tannin content of ethanol extract of cacao leaves (Theobroma cacao **L.***)*

The ethanol extract was weighed at 10 mg and dissolved with ethanol up to 10 mL (1000 ppm). Each of the replicates was pipetted into 3 mL and then put into a 10 mL volumetric flask to obtain a sample solution with a concentration of 300 ppm. Pipetted as much as 0.5 mL of sample carefully, mixed with 8 mL of distilled water, added with $FeCl_3$ 0.1 M as much as 0.5 mL and potassium ferricyanide 8 mM as much as 0.5 mL, and then incubated for 10 minutes. Then, the absorbance was measured at a maximum wavelength of 756 nm (Basri et al., 2023).

2.6. Data analysis

Data analysis was first carried out using the standard curve method; linear regression $y = a + b\ x$ was made based on absorbance and concentration data from standard solutions and obtained the average tannin (Wijayanti et al., 2021).

3. RESULTS

The samples used in this research are cacao leaves (*Theobroma cacao* L.) taken from three areas, namely Malino, Wajo, and Jeneponto, which contain tannin compounds. This research began by preparing samples, then extracting them using the maceration method. The maceration results are then collected and evaporated using a rotary vacuum evaporator until a thick extract is obtained. After maceration, calculate the yield value, which can be seen in **Table 1**.

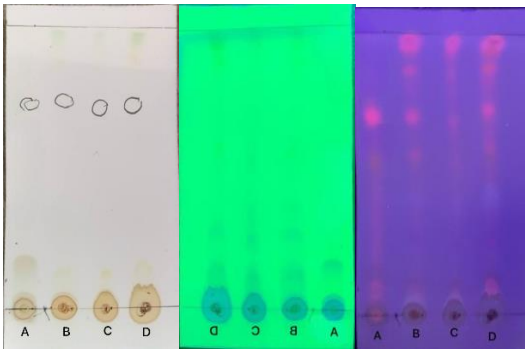
Table 1. Calculation results of percent soakage of ethanol extract of cacao leaves (*Theobroma cacao* L.)

Sample	Volume Ethanol (mL)	Simple weight (g)	Extract weight (g)	Rendement extract (%)
Malino cacao leaf ethanol extract	5000	300	14.72	4.906
Wajo cacao leaf ethanol extract	5000	300	9.97	3.323
Jeneponto cacao leaf ethanol extract	5000	300	17.2	5.733

In this research, thin layer chromatography and qualitative analysis were used to identify tannin compounds contained in cacao leaves. The reason for using the KLT (Thin Layer Chromatography) method is that it is a simple, cost-effective, easy-to-do analysis technique, and only a small sample size is needed for analysis (Husna & Ratnawulan Mita, 2020). As for the results obtained from the qualitative analysis of the KLT method, they can be seen in **Table 2**. obtained the Rf value in the three regions that identify tannin compounds; the Rf value of tannin compounds, according to Harborne (1996), is 0.65.

Table 2. Results of qualitative analysis using thin-layer chromatography method

Sample	Spot movement	*Rf*
Malino cacao leaf ethanol extract	4.4	0.676
Wajo cacao leaf ethanol extract	4.4	0.676
Jeneponto cacao leaf ethanol extract	4.5	0.692
tannic acid comparator	4.4	0.676



Visible light 254 nm UV lamp 366 nm UV lamp

Figure 1. TLC profile from the identification of tannin compounds from the ethanol extract of cacao leaves (*Theobroma cacao* L.) with the mobile phase n-Hexane: Ethyl Acetate (6:4)

Quantitative analysis was carried out to determine tannin levels using the UV-VIS spectrophotometry method (Fawwaz et al., 2023). The UV-Vis spectrophotometer can be used to obtain information for qualitative and quantitative analysis (Putri, 2015). The presence of chromoform and autochrome groups in tannin compounds is the fundamental reason for using spectrophotometric methods. The types of chromoform groups in tannin are C=O, C-H, and benzene

rings, while the type of ausochrome group is -OH (Jafar, 2018). Quantitative testing begins with measuring the maximum wavelength of a standard solution of tannic acid, as shown in **Table 3** and **Figure 2**.

Table 3. Results of absorbance measurements of standard solutions of tannic acid

Concentration (ppm)	Absorbance
15	0.348
20	0.433
25	0.563
30	0.637
35	0.779

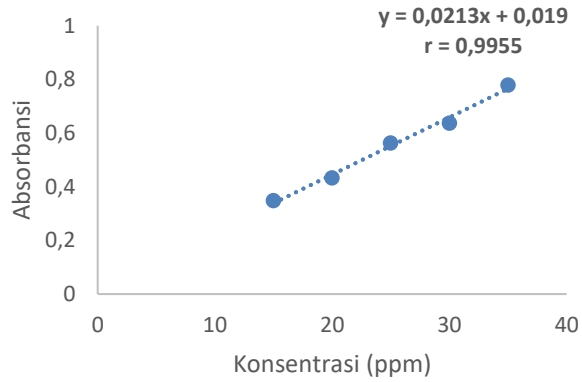


Figure 2. Tannic acid standard curve

After the measurements, absorbance data and a linear curve were obtained, namely, y = 0.0213 + 0.019 with a coefficient of determination (R2) value obtained of 0.9911 and a relationship coefficient value (r) of 0.9955, which meets the requirements for the feasibility of the relationship coefficient in the analytical method, namely r > 0.995 (Mayerhöfer TG & Popp J, 2018).

Next, absorbance measurements were carried out on samples of cacao leaf extract (*Theobroma cacao* L.) from the three regions. The absorbance of these three samples was measured using a UV-Vis spectrophotometer at a wavelength of 756 nm, and each sample was made in three replications. After the measurements, the sample absorbance data was obtained, which is presented in the table as follows:.

Table 4. Results of determining tannin content in the ethanol extract of cacao leaves (*Theobroma cacao* L.) in the Malino area

Replication	Abs (Y)	Initial tannin content	Total tannin content (mgEAT/g extract)	Average total tannin content (mgEAT/g extract)
1	0.535	24.225	80.669	79.365
2	0.525	23.755	79.104	
3	0.520	23.521	78.324	

Table 5. Results of determining tannin content in the ethanol extract of cacao leaves (*Theobroma cacao* L.) in the Wajo area

Replication	Abs (Y)	Initial tannin content	Total tannin content (mgEAT/g extract)	Average total tannin content (mgEAT/g extract)
1	0.515	23.286	77.542	79.417

2	0.530	23.990	79.886	
3	0.536	24.272	80.825	

Table 6. Results of determining tannin content in an ethanol extract of cacao leaves (*Theobroma cacao* L.) in the Jeneponto Region

Replication	Abs (Y)	Initial tannin content	Total tannin content (mgEAT/g extract)	Average total tannin content (mgEAT/g extract)
1	0.598	27.183	90.519	86.244
2	0.590	26.807	89.267	
3	0.524	23.708	78.947	

Data obtained for quantitative analysis included the tannin content of the ethanol extract of cacao leaves (*Theobroma cacao* L.) from Jeneponto, which was 86.244 mgEAT/g extract, which means that each gram of ethanol extract of cacao leaves (*Theobroma cacao* L.) contained 86.244 mg of tannin. The tannin content of Wajo cacao leaves (Theobroma cacao L.) ethanol extract is 79.417 mgEAT/g extract, which means that each gram of brown leaf (*Theobroma cacao* L.) ethanol extract contains 79.417 mg of tannin. The tannin content of the ethanol extract of cacao leaves (*Theobroma cacao* L.) from Malino is 79.365 mgEAT/g extract, which means that each gram of ethanol extract of cacao leaves (*Theobroma cacao* L.) contains 79.365 mg of tannin.

The results obtained show that the Jeneponto area has a high tannin compound content, namely 86.244 mg. There are several factors that can influence the differences in tannin compound content in the three regions, namely the existence of different environmental conditions in the three regions. The Jeneponto area has fertile soil, namely vertisol soil (Badan Pusat Statistik, 2010).

In the Jeneponto area, the air temperature ranges between 21° and 34°C with a relative humidity level of ±76%. According to Andriani and Karmila (2019), plant growth is greatly influenced by temperature; at temperatures around 21 oC, the photosynthesis process will take place optimally. Under such conditions, the glucose formation process will run smoothly. So that the photosynthetes resulting from the photosynthesis process can be distributed properly throughout the plant body, This factor can result in the high content of tannin compounds contained in cacao leaves (Theobroma cacao L.) in the Jeneponto area.

4. CONCLUSION

Based on the research that has been carried out, it can be concluded that cacao leaves (Theobroma cacao L.) in the Jeneponto, Wajo, and Malino areas contain tannin compounds, respectively, namely 86.244 mgEAT/g extract, 79.417 mgEAT/g extract, and 79.365 mgEAT/g extract.

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REFERENCES

Andriani, V., & Karmila, D. R. Pengaruh Temperatur Terhadap Kecepatan Pertumbuhan Kacang Tolo (Vigna sp.). In *Stigma* (Vol. 12, Issue 1). Mei. 2019.

Badan Pusat Statistik. *Kabupaten Jeneponto dalam Angka 2009*. 2010

Badaring, D. R., Puspitha, S., Sari, M., Nurhabiba, S., Wulan, W., Anugrah, S., Lembang, R., & Biologi, J. Uji Ekstrak Daun Maja (Aegle marmelos L.) terhadap Pertumbuhan Bakteri *Escherichia coli* and *Staphylococcus aureus*. *Indonesian Journal of Fundamental Sciences*, 2020: *6*(1).

Basri, R., Abidin, Z., & Pratama, M. Penetapan Kadar Tanın Ekstrak Etanol Bıjı Alpukat (*Persea americana* Mill.) Dengan Menggunakan Metode Spektrofotometrı Uv-Vis. *Makassar Natural Product Journal*, 2023: *1*(3), 2023–2125. https://journal.farmasi.umi.ac.id/index.php/mnpj

Fatchurrozak, Suranto, & Sugiyanto. Pengaruh Ketinggian Tempat Terhadap Kandungan Vitamin C dan Zat Antioksidan Pada Buah Carica pubescens di dataran tinggi dieng. *Jurnal Pascasarjana UNS*, 2013: 25.

Fawwaz M, Pratama M, Musafirah, Wahab I, Iriani R, Aminah, Kusuma AT, Arsul MI. Evaluation of Antioxidant Activity of *Vernonia amygdalina* Leaves and Its Flavonoid-Phenolic Content. Indonesian Journal of Pharmaceutical Science and Technology. 2023; 10(2): 104-110.

Fathurrahman, N. R. Teknık Analısıs Instrumentası Senyawa Tanın. *Farmaka*, 2018: *16*, 450–450.

Gondo Kusumo, G., Hanny Ferry, M. F., Asroriyah, H., Ilmu Farmakognosi, B., Farmasi Surabaya, A., Ilmu Kimia, B., & Program Studi DIII Farmasi, M. Identifikasi Senyawa Tanin Pada Daun Kemuning (Murraya panicullata L. Jack) Dengan Berbagai Jenis Pelarut Pengekstraksi. *Journal of Pharmacy and Science*, 2017: *2*(1). http://pustan.bpkimi.kemenperin.go.id/files/SN

Harborne, J. B. Metode Fitokimia Penuntun Cara Modern Menganalisa Tumbuhan. *Http://Www.Ansci.Cornell.Edu/Plants/Toxicagents/Ta Nnin/ Definition.Html. Cornert University.* 1996: *Diakses Tanggal 3 Januari 2016*.

Hasanah, M., Amaliani, S., Rikmasari Sekolah Tinggi Ilmu Farmasi Bhakti Pertiwi Palembang Jl Ariodillah III No, Y., Ilir Timur Palembang, A. I., & Selatan, S. (2017). Analisis Antioksidan dari Berbagai Fraksi Daun Cokelat (*Theobroma cacao* L.). In *Jurnal Ilmiah Bakti Farmasi: Vol. II* (Issue 1).

Husna, F., & Ratnawulan Mita, S. *Farmaka* Identıfikası Bahan Kımıa Obat Dalam Obat Tradısıonal Stamına Prıa Dengan Metode Kromatografı Lapıs Tıpıs. 2020.

Jafar, R. Analisis Kadar Tanin Total Ekstrak Etanol Daun Kopasanda (*Chromolaena odorata* L.) Menggunakan Metode Spektrofotometri UV-Vis. 2018.

Mayerhöfer TG, & Popp J. Beer's Law-Why Absorbance Depends (Almost) Linearly on Concentration. *Chemphyschem*, 2018: *20*, 511–515.

Ojha, S., Raj, A., Roy, A., & Roy, S. Extraction of total phenolics, flavonoids and tannins from paederia foetida l. Leaves and their relation with antioxidant activity. *Pharmacognosy Journal*, 2018: *10*(3), 541–547. https://doi.org/10.5530/pj.2018.3.88

Raharjeng ARP. Pengaruh Faktor Abiotic Terhadap Hubungan Kekerabatan Tanaman Sansevieria trifasciata L. *Jurnal Biota. UIN Raden Fatah Palembang*. 2015.

Rahma Widyaningrum, N., & Noerlita Ningrum, A. Identıfikası Kromatografı Lapıs Tıpıs dan Aktıvıtas Antıpıretık Ekstrak Etanol Daun *Ipomoea carnea* Jacq Melaluı Induksı Pepton Pada Mencıt Jantan. *Avicenna : Journal of Health Research*, 2021: *4*(2). https://doi.org/10.36419/avicenna.v4i2.534.

Singh, Nidhi, & Datta. Antimicrobial Activity and Cytotoxicity of *Theobroma cacao* extracts. *Der Pharmacia Lettre*, 2015: *7*, 287–294.

Wijayanti, N., Wirasti, W., Waznah, U., & Nur, A. V. (n.d.). Prosiding Seminar Nasional Kesehatan Lembaga Penelitian dan Pengabdian Masyarakat Penetapan Kadar Tanin pada Teh Hitam Kering Produksi Pekalongan dengan Metode Spektrofotometri UV-Vis. In *Seminar Nasional Kesehatan*.

Wiratmaja, I. G. *Pembuatan Etanol Generasi Kedua Dengan Memanfaatkan Limbah Rumput Laut Eucheuma Cottonii Sebagai Bahan Baku I Gede Wiratmaja (1), I Gusti Bagus Wijaya Kusuma (2) dan I Nyoman Suprapta Winaya (2)*. 2011.