IDENTIFICATION OF TANNIN COMPOUNDS IN ETHANOL EXTRACT OF CAT'S WHISKERS LEAVES (*Orthosiphon aristatius*) USING THIN LAYER CHROMATOGRAPHY

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A B S T R A C T

Cat's whiskers are one type of medicinal plant that is often used in traditional medicine. Both empirically and clinically, cat's whiskers leaves (Orthosiphon aristatus) are medicinal plants that contain tannin compounds. Tannins are compounds that have a large molecular weight with complex hydroxyl groups related to carboxyl and protein and also several macromolecules. The purpose of this study was to determine the Tannin compounds in cat's whiskers leaves (Orthosiphon aristatus) using the Thin Layer Chromatography method as a method of separating dissolved substances with solvents consisting of 2 12 13 phases or more. The principle of the TLC method is the difference in physical and chemical properties of compounds such as the tendency of molecules to dissolve in liquids, the tendency of molecules to evaporate and the tendency of molecules to adhere to surfaces (adsorption). Thin layer chromatography (TLC) method was used to identify the ethanol extract. This method uses silica gel TLC plate as the stationary phase. According to Hasnaeni and Wisdawati (2019), a yield of 20.8% of the thick extract of cat's whiskers leaves (Orthosiphon aristatus) was obtained to determine the amount of extract obtained during the extraction process. In addition, the yield value correlates with the amount of active compounds present in the sample; a higher yield value indicates that there is a greater possibility that the active compound component is included in the sample. This is in line with the statement made by Harborne (1987), which is quoted in (Wardaningrum, 2019), which states that the high content of active compounds is usually indicated by the high yield produced; the yield is considered good if the value exceeds 10%. with the results found that there are tannin compounds in cat's whiskers leaves.

INTRODUCTION

Indonesia has a lot of natural wealth spread across various regions, with abundant natural wealth showing the potential for utilizing existing plants as modern or traditional medicines. Indonesian society has long utilized medicinal plants as a cure for various symptoms suffered, long before modern health services reached the community (Depkes RI, 2014). Medicinal plants are a series of plants that are spread in nature and can be used as medicine. The usefulness of plants as medicine is none other than hereditary knowledge spread by ancestors in ancient times and has been proven to cure symptoms of disease (Syafriah & Teheni, 2023). Of the many types of medicinal plants that exist, one of them is the cat's whiskers leaf (*Orthosiphon aristatus*), which has antibacterial properties (Gunawan et al., 2014).

In the research, Surahman stated that cat's whiskers leaves showed secondary metabolite compounds that have important biological and pharmacological activities. The existence of bioactive compounds in cat's whiskers was studied using the Gas Chromatography Mass Spectrometry (GCMS) method (Surahmaida et al., 2019).

Tannins are a group of polyphenols with high molecular weights, ranging from 500 to 3000 Da (Dalton). In addition to being soluble in water, tannins can also be soluble in organic solvents such as ethanol, methanol, acetone, and others. The main property of tannins is the ability to precipitate proteins. Tannins have many medical benefits. Tannins in plant extracts can be used as astringents, diarrhea treatments, diuretics, treating duodenal tumors, anti-inflammatory, antiseptic, antibacterial, antiviral, and hemostatic. Research should be conducted to determine the presence of tannin compounds and the amount of tannin contained in cat's whiskers leaf extract, considering its potential (Musman et al., 2015). One of the plants that contains tannin compounds is starfruit (Mustiqawati et al., 2022).

Ismailoff and Schraiber developed thin layer chromatography (TLC) in 1938. Chromatogram is formed when the mobile phase creeps along the stationary phase because the adsorbent is coated on a glass plate that functions as a support for the stationary phase. This method is easy to recover the separated compounds, fast in high separation, and easy to use. Thin layer chromatography (TLC or TLC = Chromatography on Thin Layers) is basically very similar to paper chromatography, especially in the process. Substantially, the separation media is different. A thin layer of fine adsorbent is mounted on a glass, aluminum, or plastic board rather than paper. In the separation process, this thin layer of adsorbent functions as a stationary phase.

By using chromatography, the substance of the mixture can be broken down into certain parts. These include flavonoids and isoflavonoids found in tofu, tempeh, soy powder, and tauco, as well as Scoparia dulcis, Lindernia anagalis, and Torenia violacea. Isoflavones have many benefits for human health. These include being antioxidants, antitumor and anticancer, anticholesterol, antiviral, antiallergic, and can prevent osteoporosis. In addition, the chromatography method is the basis of all chromatography methods. In addition, chromatography involves separating a mixture of chemicals into their pure chemicals and knowing their quantities. In this way, the purity of the material or the composition of the mixture with various contents can be analyzed precisely. not only quality control, food analysis, and environmental analysis, but also the control and optimization of chemical reactions and processes that depend on the measurement of material quantities. The basic principle of chromatography is a preparative analysis and separation technique for a mixture of materials. Several thin layer chromatography techniques are commonly used to separate compounds. In this study, researchers were interested in conducting research on tannin compounds contained in cat's whiskers leaves (Orthosiphon aristatus) using the Thin Layer Chromatography (TLC) method.

METHODOLOGY

Time and Place of Research

This research was conducted in July - August 2024 at the Haluoleo University (UHO) Pharmaceutical Laboratory.

Tools

The tools used in this study were microscope (Yazumi L303), aquarium, dark glass bottle, beaker (Pyrex), Erlenmeyer flask (Pyrex), rack and test tube (Pyrex), dropper pipette, burette. (Pyrex) and dropper plate, and evaporator. Equipment cup (Pyrex), funnel (Pyrex), filter paper, analytical balance (Luck Scale), silica gel plate GF 254, UV lamp 254 nm, chamber.

Materials

The materials used for this study were 70% Ethanol, 1% Chloride (FeCl3), Cat's Whiskers Leaf Extract (Orthosiphon aristatus), Aquadest, Acetic Acid, Sulfuric Acid, Chlorophyll, Magnesium Powder.

Research Prosedures

Sample Preparation

The cat's whiskers leaf sample (*Orthosiphon aristatus*) used came from Baubau. The cat's whiskers leaves were taken and washed clean with running water, then dried by drying them in the sun until dry and have a brownish color until they have a hard and brittle texture. Then they were ground, and the sample was ready to be extracted.

Extract Making

Cat's whiskers leaves (Orthosiphon aristatus) that have been dried and ground, then weighed as much as 510 grams. The leaf powder is put into a clean and closed glass jar, then 2300 ml of 70% ethanol solvent is added. The mixture is stirred periodically for 3x24 hours. After that, the mixture is filtered with filter paper. The liquid extract obtained is then further processed by the concentration method using a water bath until a thick extract is formed according to the specified standard.

Testing Tannin Compounds with Color Reagents

Take 0.5 grams of extract and put it into the prepared test tube. After that, add 2 ml of 70% ethanol to the test tube containing the extract, then stir the mixture until homogeneous. After mixing well, add three drops of FeCl3 (ferric chloride) solution slowly into the mixture. Observations are made after adding FeCl3 to see the color change. If the mixture shows a color change to blue, blue-black, green, or blue-green, and a precipitate forms, this is an indicator of the presence of tannin compounds in the extract.

Tannin Compound Testing with Thin Layer Chromatography (TLC)

Mark the upper and lower limits on the TLC plate with a distance of 1 cm. To make the mobile phase, N-hexane and ethyl acetate are mixed in a ratio of 8:2. Then this mixture is put into a tightly closed chamber and left for 20-30 minutes for saturation, which equalizes the vapor pressure in the chamber. Using a capillary tube, the thick extract of cat's whiskers leaves is spotted on the lower limit line of the TLC plate coated with silica gel. Then, the plate is put into a chamber containing the saturated mobile phase. The eluent rises to the upper limit of the plate before the chromatography process begins. After the eluent reaches this limit, the TLC plate is lifted and allowed to dry in air. Furthermore, the spots that appear on the plate are observed under ultraviolet light at wavelengths of 254 nm and 366 nm to identify compounds in the extract (Kautsari et al., 2021).

After the spots are obtained, the Rf (Retention factor) value is calculated based on the formula below:

$$Rf = \frac{Distance\ traveled\ by\ the\ component}{The\ distance\ traveled\ by\ the\ solvent}$$

RESULTS AND DISCUSSION

In this study, fresh samples of Cat's Whiskers (*Orthosiphon aristatus*) leaves were used, which were taken as much as 4.5 kilograms of leaves. Cat's Whiskers (*Orthosiphon aristatus*) leaves were divided into wet parts and then washed using running water. After being cut, they were dried in an open place without being exposed to direct sunlight. This was done to reduce the risk of damage to the chemical components of Cat's Whiskers leaves caused by high sunlight. The dried Cat's Whiskers (*Orthosiphon aristatus*) leaves were separated and then ground or mixed into Cat's Whiskers (*Orthosiphon aristatus*) leaf powder weighing 510 grams. Furthermore, extraction was carried out by the maceration method using 2300 mL of ethanol solvent for three times 24 hours, with occasional stirring.

Because this method is simple, the maceration method is used for extraction. One way of extraction is maceration, also called cold extraction, where the powdered simplicia is soaked in a solvent liquid without using heating (Pratiwi, 2010 dalam Dewatikasari, 2020). During the solvent maceration process, 70% ethanol is used because of its more selective use, non-toxicity, good absorption, and ability to stop the growth of bacteria and fungi (Suryanto, 2012 dalam Damanis et al., 2020).

The results of compound identification in ethanol extract of cat's whiskers leaves (*Orthosiphon aristatus*) taken from plants in the TOGA garden in Baubau City showed the presence of secondary metabolite compounds, including tannins. Details of the compound identification results can be seen in the following table:

Table 1. Results of Phytochemical Screening Test of Ethanol Extract of Cat's Whiskers Leaves (*Orthosiphon aristatus*)

Compound	Reagent	Result	Information
Polifenol	FeCl3 5%	+	It has a blackish green color
Tannin	Distilled water + FeCl3 10%	+	Blackish in color

Note :
(+) : There are tannin compounds

In the tannin compound test, 2 milliliters of 70% ethanol extract solution from Kumis kucing (*Orthosiphon aristatus*) leaves were boiled in 10 milliliters of distilled water for three minutes. Then cooled and filtered. After the filtrate was put into a test tube, three to five drops of FeCl3 reagent were added. A blackish blue or blackish green color indicates a positive tannin reaction test result (Kusumo et al., 2022). Tannin, a phenolic compound that tends to dissolve in water and polar solvents, is added with FeCl3 to determine whether Cat's Whiskers Leaves (*Orthosiphon aristatus*) contain phenol groups. The presence of phenol groups is indicated by blackish green and blackish blue after FeCl3 is added. Because of the formation of black color, the result is positive (Muthmainnah, 2017).

In addition to phytochemical screening with color reagents, one simple analysis method that can be done to confirm the chemical content in plants is Thin Layer Chromatography (TLC). TLC is a

simple chromatography analysis technique based on the adsorption process. The purpose of this method is to separate compounds based on the differences in affinity between the compounds analyzed (Chandra et al., 2023). Chromatography analysis consists of two phases: the stationary phase (silica) and the mobile phase (eluent). Compounds with low polarity will usually elute faster than compounds with high polarity (Endarini, 2016).

Table 2. Thin Layer Chromatography Test Results

After elution	UV 254nm	UV 366nm	Nilai Rf
			$Rf\ 1 = \frac{7,6}{9} = 0,84$ $Rf\ 2 = \frac{4,6}{9} = 0,51$

Thin layer chromatography (TLC) method was used to identify the ethanol extract. This method uses silica gel TLC plate as the stationary phase. According to Hasnaeni and Wisdawati (2019), a yield of 20.8% of the thick extract of cat's whiskers leaves (*Orthosiphon aristatus*) was obtained to determine the amount of extract obtained during the extraction process. In addition, the yield value correlates with the amount of active compounds present in the sample; a higher yield value indicates that there is a greater possibility that the active compound component is included in the sample. This is in line with the statement made by Harborne (1987), which is quoted in (Wardaningrum, 2019), which states that the high content of active compounds is usually indicated by the high yield produced; the yield is considered good if the value exceeds 10%.

Then, the TLC plate was given an upper and lower boundary line with a distance of 1 cm. With a ratio of 8:2, N-hexane and ethyl acetate solvents were mixed to complete the mobile phase. To carry out the saturation process, the mobile phase mixture was put into the chamber, which was then tightly closed. The purpose of this saturation was to ensure the same vapor pressure throughout the chamber. Then, using a capillary tube, the extract that had been obtained was spotted on the lower boundary line of the TLC plate. Waiting for the eluent to rise until it reached the upper boundary line of the TLC plate was the end point of the process. After the eluent reached the line, the TLC plate was lifted and allowed to dry in the air. Finally, the spots formed on the plate were observed with ultraviolet light at wavelengths of 254 nm and 366 nm (Surahmaida et al., 2019).

CONCLUSION

The results of the study showed that there were tannin compounds in the ethanol extract of Kumis kucing (*Orthosiphon aristatus*) leaves which were positive, as shown by the thin layer chromatography profile, which showed that there were stains on the plate.

This study faced obstacles in obtaining consistent chromatogram patterns, which may be caused by variations in tannin content in cat's whiskers leaves and suboptimal solvent conditions. In addition, the lack of specific references on tannin compounds in these leaves also complicates the identification process.

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REFERENCES

Chandra, M. ., Hidayatullah, M., & Sari, P. E. (2023). Perbandingan Rendemen, Skrining Fitokimia Dan Profil Kromatografi Lapis Tipis Ekstrak Etanol 96% dan Metanol Daun Sungkai (Peronema canescens Jack) M. *Jurnal Kesehatan Islam*, *12*, 48–54.

Damanis, F. V. M., Wewengkang, D. S., & Antasionasti, I. (2020). UJI AKTIVITAS ANTIOKSIDAN EKSTRAK ETANOL ASCIDIAN Herdmania Momus DENGAN METODE DPPH (1,1-difenil-2-pikrilhidrazil). *Pharmacon*, *9*(3), 464. https://doi.org/10.35799/pha.9.2020.30033

Depkes RI. (2014). *Farmakope Indonesia Edisi V*: Jakarta.

Dewatikasari, whika febria. (2020). Perbandingan Pelarut Kloroform dan Etanol terhadap Rendemen Ekstrak Daun Lidah Mertua (Sansevieria trifasciata Prain.) Menggunakan Metode Maserasi. *Journal.Uin-Alauddin*, *5*(September), 125–132. http://journal.uin-alauddin.ac.id/index.php/psb/

Endarini, L. H. (2016). Pusdik SDM Kesehatan Kementrian Kesehatan RI; Jakarta.

Gunawan, D., Sudarsono, Agus P., Subagus W, M Dradjat. Tumbuhan Obat 90,95,106,115 Pusat Penelitian Obat Tradisional UGM Yogyakarta.

Kannan, M., Muthusamy, P., Venkatachalam, U., 2016, Quantification of bioactive components from medicinal herb, Research Journal of Biotechnology, 11(6):49-57

Kautsari, S. N., Purwakusumah, E. D., & Nurcholis, W. (2021). PROFIL KROMATOGRAFI LAPIS TIPIS EKSTRAK KUNYIT (Curcuma longa Linn) SEGAR DAN SIMPLISIA DENGAN VARIASI METODE EKSTRAKSI. *Media Farmasi*, *16*(1), 65. https://doi.org/10.32382/mf.v16i1.1403

Kusumo, F., Mahlia, T. M. I., Pradhan, S., Ong, H. C., Silitonga, A. S., Fattah, I. M. R., Nghiem, L. D., & Mofijur, M. (2022). A framework to assess indicators of the circular economy in biological systems. *Environmental Technology and Innovation*, *28*, 102945. https://doi.org/10.1016/j.eti.2022.102945

Musman, M., Rahmad, A., & Dewiyanti, I. (2015). *AACL Bioflux · February 2015*. *8*(February), 50–56.

Mustiqawati, E., Supardi, S., & Juniadin, J. (2022). UJI AKTIVITAS ANTIOKSIDAN EKSTRAK METANOL DAUN BELIMBING WULUH (Averrhoa bilimbi). *Jurnal Sains Dan Kesehatan*, *1*(1), 11–15. https://doi.org/10.57151/jsika.v1i1.17

Muthmainnah. (2017). Skrinning Fitokimia Senyawa Metabolit Sekunder Dari Ekstrak Etanol Buah Delima (Punica granatum L.) dengan Metode Uji Warna. *Media Farmasi*, *14*(1), 55–64.

Surahmaida, S., Umarudin, U., & Junairiah, J. (2019). Senyawa Bioaktif Daun Kumis Kucing (Orthosiphon stamineus). *Jurnal Kimia Riset*, *4*(1), 81. https://doi.org/10.20473/jkr.v4i1.13176

Syafriah, W. O., & Teheni, M. T. (2023). Uji Aktivitas Antioksidan Ekstrak Metanol Tangkai Daun Kelor (Moringa oleifera Lam) Menggunakan Metode DPPH (1,1-difenil-2-pikrilhidrazil). *Journal of Agricultural Science*, *6*(6), 953–960.

Wardaningrum, R. Y. (2019). PERBANDINGAN AKTIVITAS ANTIOKSIDAN EKSTRAK ETANOL TERPURIFIKASI UBI JALAR UNGU (Ipomoea batatas.L) DENGAN VITAMIN E. *Αγαη*, *8*(5), 55.