

TESTING THE SAPONIN CONTENT OF NUTGRASS (*Cyperus rotundus* L.) USING THIN-LAYER CHROMATOGRAPHY (TLC) METHOD WITH SOLVENT VARIATIONS

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ABSTRACT

Saponins are secondary metabolites widely found in plants and are known to possess various pharmacological activities, including anti-inflammatory, antimicrobial, and anticancer properties. *Cyperus rotundus* L. (nut grass) is one of the plants that contains saponins; therefore, it is important to conduct analysis using Thin Layer Chromatography (TLC) with different solvent systems to specifically identify their presence. The type of research used is a qualitative research type including preliminary tests in the form of foam tests and color tests using Lieberman-Burchard reagent, as well as TLC analysis with three different solvent systems: chloroform:methanol:aquades (5:4:1), (3:5:2) and (4:4:2). The results of the foam test showed the formation of foam as high as 1.5 cm which was stable for 10 minutes, indicating the presence of saponins. The color test produced a brown coloration, which suggested the presence of triterpenoid-type saponins. TLC analysis demonstrated the formation of red spots with R_f values ranging from 0.33 to 0.83. The solvent system with a ratio of 5:4:1 produced the highest number of spots, indicating more effective compound separation compared to the other systems. Based on these findings, it can be concluded that *Cyperus rotundus* L. extract contains triterpenoid-type saponins, and that variations in solvent systems significantly influence the effectiveness of compound separation in Thin Layer Chromatography (TLC) analysis.

INTRODUCTION

Indonesia is recognized as one of the countries with the highest levels of biodiversity in the world. Of the approximately 40,000 medicinal plant species identified globally, around 30,000 are found in Indonesia. This condition underscores Indonesia's significant potential in the development of traditional medicine and phytopharmaceuticals derived from natural resources (Emilda et al., 2017). Such biodiversity must be preserved and utilized sustainably in order to provide meaningful contributions to the health sector. One of the key reasons for utilizing plant diversity lies in the presence of secondary metabolites. These compounds are distributed across various plant parts, including leaves, stems, roots, and tubers, and have been shown to possess a wide range of biological activities. Flavonoids, saponins, alkaloids, steroids, triterpenoids and tannins are among the most commonly utilized secondary metabolites in traditional medicine and the development of modern herbal therapeutics (Khafid et al., 2023).

On the other hand, not all plants have a positive impact on agriculture. Weeds, for example, are widely regarded as undesirable because they can reduce crop productivity when left uncontrolled (S. Ridwan et al., 2022). However, some weed species exhibit significant pharmacological potential due to their secondary metabolite content.

Secondary metabolites themselves are derivatives of primary plant metabolism. Their primary role is to serve as a natural defense mechanism against pathogen attacks, as well as to facilitate plant adaptation and interaction with their environment. Commonly encountered classes of secondary metabolites include flavonoids, alkaloids, saponins, phenols, tannins, steroids and triterpenoids. The presence of these compounds highlights not only their vital role in supporting plant survival but also their potential benefits for humans (Ningsih et al., 2020).

Saponins, in particular, exhibit a wide range of taste profiles, from intensely bitter to sweet, depending on their concentration and structure. These compounds have been applied across multiple

industries, including fisheries, textiles, cosmetics, and pharmaceuticals, owing largely to their surfactant properties and ability to reduce surface tension (Anggraeni Putri et al., 2023).

One plant known to contain saponins is nut grass (*Cyperus rotundus* L.), a weed belonging to the family Cyperaceae. This species grows abundantly, including in industrial plantation forests (Purnama Sari et al., 2020). In agricultural systems, nut grass is considered problematic due to its persistence and difficulty to control. It is categorized as a C4 plant with an efficient photosynthetic pathway, and its tubers can survive for several years, allowing its population to persist even after eradication attempts (Aulia et al., 2022).

To identify saponin content in nut grass, Thin Layer Chromatography (TLC) is among the commonly employed methods. TLC is considered a simple, rapid, and effective technique for separating and identifying secondary metabolites. The choice of mobile phase or solvent system plays a critical role in determining the quality of separation, as differences in solvent polarity influence spot patterns, coloration, and R_f values. Thus, solvent variation becomes a key factor in optimizing the identification of bioactive compounds, including saponins (Mukhriani, et al, 2023).

Based on this background, the present study was conducted to identify the presence of saponins in nut grass (*Cyperus rotundus* L.) using the Thin Layer Chromatography (TLC) method.

Methods

Research Design

This study employed a qualitative research design with an identification method conducted in the laboratory.

Time and Location

The research was carried out on May 28, 2025, at the Pharmacy Laboratory, Baubau Polytechnic, which was selected for its facilities and equipment supporting phytochemical analysis, including preliminary tests and Thin Layer Chromatography (TLC).

Instruments and Materials

The instruments used in this study included a chromatography chamber, Erlenmeyer flask, UV lamp, silica gel F254 plates (Merck), water bath, rotary evaporator, test tubes and micropipettes.

The materials used were chloroform, methanol, 2N hydrochloric acid (HCl), Lieberman–Burchard (LB) reagent for qualitative saponin testing, and distilled water as the supporting solvent.

Sample

The sample used in this study was nut grass (*Cyperus rotundus* L.), which served as the test material for saponin content analysis.

Procedures

Sample Preparation

Nut grass (*Cyperus rotundus* L.) was collected from community farmland. The sample was washed thoroughly with running water, sliced into thin pieces, and dried under shade to avoid direct sunlight. Once dried, the material was ground using a blender to obtain simplicia powder (Elianasari dan Fauziah, 2023).

Extraction

Extraction was performed by the maceration method using methanol as the solvent. A total of 100 g of simplicia powder of nut grass rhizomes was placed in an Erlenmeyer flask and soaked in 600 mL of methanol for three days with occasional stirring to facilitate dissolution of active compounds. The mixture was filtered to obtain the first filtrate. The residue was then re-macerated with 400 mL of methanol for two days under similar conditions to produce the second filtrate. Both filtrates were combined and evaporated using a rotary evaporator until a thick extract was obtained, which was then used for further analysis (Mustiqawati dan Yolandari, 2022)

Initial Test for Rumput Teki (*Cyperus rotundus* L.)

A total of 0.5 g of simplicia powder of nut grass was placed into a test tube containing 10 mL of hot water. After cooling, the solution was shaken vigorously for ± 10 seconds, forming foam with a height of 1–10 cm, which remained stable for 10 minutes, indicating the presence of saponins. Subsequently, 2N HCl was added; if the foam persisted, the sample was confirmed positive for saponins (Sri et al., 2019).

Color Test

The color reaction test was conducted to detect saponins in nut grass extract. Ten milliliters of chloroform was added to a test tube containing 0.5 g of extract, and the mixture was heated in a water bath for approximately five minutes.

After cooling, Lieberman–Burchard (LB) reagent was added and color changes were observed. A brown color indicated triterpenes, while blue or green coloration indicated steroidal saponins (Ngginak et al., 2021).

Thin Layer Chromatography (TLC) Analysis

Silica gel F254 plates were cut into 7 × 3 cm sizes, and a baseline was drawn 1 cm from the bottom edge as a spotting guide. A 5 µL aliquot of sample solution (1 mg/mL) was applied using a micropipette.

The plates were then developed in a chromatography chamber pre-saturated with the mobile phase until the solvent front reached the upper limit. After development, the plates were dried and observed under a UV lamp at specific wavelengths to visualize chromatographic profiles. The following solvent systems were tested:

Solvent A: Chloroform : Methanol : Distilled Water (5:4:1) – nonpolar

Solvent B: Chloroform : Methanol : Distilled Water (3:5:2) – polar

Solvent C: Chloroform : Methanol : Distilled Water (4:4:2) – polar

After development, plates were dried, sprayed with LB reagent and visualized under UV light at 366 nm. Spots were evaluated based on color, number, intensity and Rf values, with interpretation guided by:

$$R_f = \frac{\text{Jarak tempuh noda (Spot)}}{\text{Jarak tempuh pelarut (Front)}}$$

Interpretation based on:

- Spot coloration
- Number of spots
- Rf values

Results and Discussion

This study aimed to identify the presence of saponins in *Cyperus rotundus* L. extract through a series of phytochemical analyses. Three main approaches were employed: the foam test, the color test with Lieberman–Burchard reagent, and Thin Layer Chromatography (TLC) with different solvent systems. The results and discussion for each test are presented below.

Extract Yield

Extraction of 100 g of dried *Cyperus rotundus simplicia* produced 17.6 g of extract, corresponding to a yield of 17%. This yield indicates efficient extraction and reflects a relatively high content of active compounds in the raw material.

Table 4.1. Yield of *Cyperus rotundus* Extract

Weight of Dried Simplicia	Weight of Extract	Yield
100 gram	17,6 gram	17%

Source: Primary Data, 2025

Foam Test

The foam test was conducted as a qualitative method to detect the presence of saponins through their ability to produce stable foam. A total of 0.5 g of extract was dissolved in hot water and shaken vigorously, producing foam. The addition of 2N HCl was used to confirm the observation. The results showed foam with a height of approximately 1.5 cm, which remained stable for 10 minutes, indicating the presence of saponins in the sample.



Figure 4.1. Foam Test

Table 4.2. Foam Test Results of *Cyperus rotundus* Extract

Test Type	Treatment	Result	Interpretation
Foam Test	0.5 g extract + hot water + shaking + 2N HCl	Foam height ±1.5 cm, stable for 10 minutes	Positive indication of saponins

Source: Primary Data, 2025

According to Mustiqawati and Yolandari (2022), the formation of foam ≥ 1 cm that persists for ≥ 10 minutes is considered a positive indication of saponins. This phenomenon is attributed to the surfactant properties of saponins, which reduce water surface tension, thereby enabling foam formation and stability.

Color Test

The color reaction test using Lieberman–Burchard reagent (LB) produced a brown coloration, which is indicative of triterpenoid saponins. As reported by Ngginak et al. (2021), triterpenoid saponins yield brown to brick-red coloration upon reaction with LB reagent. The appearance of brown color in this study suggests the presence of triterpenoid saponins in the extract.



Figure 4.2. Color Test

Table 4.3. Color Test of *Cyperus rotundus* Extract Using Lieberman–Burchard Reagent

Test Type	Reagent Used	Resulting Color	Interpretation
Color Test	Lieberman–Burchard (LB)	Brown	Positive indication of saponins

Source: Primary Data, 2025

The color test using Liebermann–Burchard reagent produced a brown coloration. According to Ngginak et al. (2021), this reagent typically yields a brown to reddish color in the presence of triterpenoid saponins. Thus, the brown color observed in this test indicates the possible presence of triterpenoid-type saponins in the *Cyperus rotundus* extract.

Thin Layer Chromatography (TLC)

TLC analysis was performed to identify compounds based on R_f values and spot coloration. Three solvent systems were tested:

Solvent A: Chloroform:Methanol:Distilled Water (5:4:1)

Solvent B: Chloroform:Methanol:Distilled Water (3:5:2)

Solvent C: Chloroform:Methanol:Distilled Water (4:4:2)

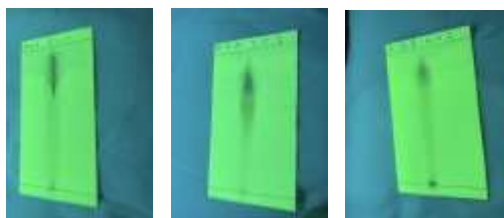


Figure 4.3. TLC Plate under UV 254 nm



Figure 4.4. TLC Plate under UV 366 nm

- Solvent A produced five red spots with varying R_f values, indicating the presence of diverse triterpenoid compounds in the extract.
- Solvent B produced four spots, three of which were red (triterpenoids) and one blue (steroid), suggesting the presence of both triterpenoid and steroidal saponins.
- Solvent C produced only two red spots, suggesting a less complex separation but confirming the presence of triterpenoids.

According to (Sopiah et al., 2019) R_f values are critical indicators of compound identification based on their relative mobility in the mobile phase. High R_f values indicate weak interactions with the stationary phase, whereas low R_f values indicate stronger interactions. Furthermore, (Aritonang,

2022) notes that triterpenoids typically produce red or purple spots, while steroids produce blue or green spots on TLC plates.

Interpretation

The TLC results demonstrated that solvent system variation significantly influenced the separation profile of *Cyperus rotundus* extract. Solvent A (5:4:1) produced the highest number of red spots with different R_f values, suggesting the presence of multiple triterpenoid saponins. Solvent B (3:5:2) produced both triterpenoid and steroidal saponins, while Solvent C (4:4:2) yielded only two triterpenoid spots, indicating limited separation. These findings confirm that the choice of solvent system plays a crucial role in determining the efficiency of saponin detection and separation using TLC.

Conclusion

The preliminary tests confirmed that the extract of *Cyperus rotundus* L. contained saponins, as evidenced by the foam test, which produced foam approximately 1.5 cm in height that remained stable for 10 minutes, consistent with the typical characteristics of saponins. In addition, the color test with Liebermann–Burchard reagent resulted in a brown coloration, further indicating the presence of saponins in the extract.

Further analysis using Thin Layer Chromatography (TLC) with different solvent systems demonstrated variations in separation patterns. Solvent A (chloroform:methanol:distilled water = 5:4:1) produced five red spots, indicating the presence of triterpenoid saponins. Solvent B (3:5:2) yielded four spots, consisting of three red (triterpenoid) and one blue (steroidal), suggesting a mixture of triterpenoid and steroidal saponins. Solvent C (4:4:2) produced two red spots, pointing to the presence of triterpenoid saponins. These findings highlight that solvent system variation significantly affects the number of spots, their color, R_f values, and the types of saponins detected.

Recommendation

It is recommended that future studies on TLC with solvent variations include a positive control (saponin standard) to enhance the accuracy of compound identification and improve the validity of the results.

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