Calcium Decay Ability of Various Kirinyuh Leaf Extracts (*Chromolaena odorata* L.) on Kidney Stones

Dimas Danang Indriatmoko1, Maulani1, Tarso Rudiana2*

1Department of Pharmacy, Faculty of Science Pharmacy and Health, Universitas Mathla’ul Anwar, Pandeglang, Indonesia

2Department of Chemistry, Faculty of Science Pharmacy and Health, Universitas Mathla’ul Anwar, Pandeglang, Indonesia

*Corresponding author: tarso.rudiana@gmail.com

Submitted: 23 May 2021
Accepted: 18 December 2021
Published: 26 April 2022

**Abstract**

**Background:** Kidney stones are one of the causes of chronic and acute kidney failure symptoms. The flavonoid compounds in *Chromolaena odorata* leaf extract are thought to dissolve calcium in kidney stones. **Objective:** This study aims to determine the activity of *C. odorata* leaves extract as a dissolution agent for calcium kidney stones and to characterize the active extract with a liquid chromatograph mass spectrometry. **Methods:** The leaves of *C. odorata* were extracted by ultrasonication method using 3 solvents in stages, namely n-hexane, ethyl acetate, and methanol. The powder for kidney stones was immersed in an extract solution of n-hexane, ethyl acetate, and methanol for 5 hours at 37°C. The reaction results were analyzed for their absorbance using a UV-Vis spectrophotometer. The fraction with the best activity was analyzed for phytochemical content with various typical reagents and LCMS/MS. **Results:** Methanol extract of *C. odorata* with a concentration of 10,000 µg/mL can reduce calcium in kidney stones by 19.2 µg/mL. Based on phytochemical tests and LCMS/MS analysis, the methanol extract of *C. odorata* leaves contains compounds of the tannins, alkaloids, flavonoids, and steroids. Chromatogram at a retention time of 7.76; 8.96; and 10.01 in methanol extract of *C. odorata* identified 3,5,7,4’-tetrahydroxy-8,3’-dimethoxyflavone; 5,7,4’-trihydroxy-3’,5’-dimethoxyflavone, and 5,6,7,8,4’-pentamethoxyflavone compounds. **Conclusion:** Methanol extract with a concentration of 10,000 µg/mL had the best calcium decay activity in kidney stones of 19.2 µg/mL. 3,5,7,4’-tetrahydroxy-8,3’-dimethoxyflavone; 5,7,4’-trihydroxy-3’,5’-dimethoxyflavone, and 5,6,7,8,4’-pentamethoxyflavone compounds are contained in methanol extract which is thought to play a role in shedding calcium in kidney stones.

**Keywords:** *Chromolaena odorata*, extraction, flavonoids, kidney stones, UV-Vis spectrophotometer

How to cite this article:

P-ISSN: 2406-9388
E-ISSN: 2580-8303
INTRODUCTION

Acute and chronic decline in kidney function is a sign of kidney failure (Rostanti et al., 2016). Kidney failure is characterized by the failure of the kidneys to function optimally. People with kidney disease must undergo hemodialysis throughout their life or get a kidney donor through kidney transplant surgery (Savitri & Parmitasari, 2015). One of the causes of kidney failure is the presence of stones in the urinary tract, and kidney stones, and it is ranked fourth in the occurrence of kidney failure after hypertension, diabetes, and cholesterol (Pranandari & Woro, 2015). According to Purnomo (2003), Kidney stones with a calcium composition are the most common types of kidney stones. Traditional medicine is one of the therapies for kidney stones in addition to surgery, radiation and modern medicine. Treatment of kidney stones with traditional medicine is an alternative option because, besides being cheap, the side effects are also more negligible (Sasmito et al., 2001).

The use of traditional medicine has become the culture of Indonesian society. The use of plants is vital in the development of traditional medicine. Medicinal plants that can be used to treat back pain that leads to urinary tract stones include those from the families of Zingiberaceae, Acanthaceae, Poaceae, Lamiaceae, and Asteraceae (Nisa & Astana, 2018). The content of compounds such as flavonoids and phenolics in plants is used as bioactive compounds. Novalia et al. (2016) reported that the flavonoid content in the ethyl acetate fraction of Clerodendron thomsonae Balf (Lamiaceae) leaves could dissolve calcium in kidney stones. Flavonoids can dissolve calcium in kidney stones because the hydroxy groups on flavonoids can form complexes with calcium (Ratri, 2008), such as luteolin-7-O-glycoside compounds which are capable of preventing and dissolving kidney stones (Dhianawaty et al., 2003).

Kirinyuh (Chromolaena odorata L.) (Asteraceae) is a shrub that contains secondary metabolites such as flavonoids, phenols, tannins, saponins, and steroids. C. odorata extract contains isoflavone, flavone, flavonol, and chalcone group compounds (Saputra et al., 2017). Fitrah et al. (2017) succeeded in isolating a flavonoid derivative, namely methyl ether naringenin. Three chromosome derivatives have been isolated from C. odorata, namely 5,7-dihydroxy-2-(4-methoxyphenyl) chromen-4-on, 3,5-dihydroxy-2-(3-hydroxy-4-methoxyphenyl) compounds 4-on and 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy-chromen-4-on (Jasnie, 2009). The potential content of flavonoid compounds in C. odorata can be used as a dissolution agent of calcium found in kidney stones. In this study, secondary metabolites were extracted from the leaves of C. odorata with n-hexane, ethyl acetate, and methanol as solvents. Each extract was tested for calcium decay in kidney stones. Extracts with the best activity were analyzed for phytoconstituent content by LCMS/MS.

MATERIALS AND METHODS

Sample collection

C. odorata leaves were collected from Serang Regency - Banten (6°05’22.0”S 106°09’25.4”E). Plants were identified in LIPI Research Center for Biology (Identification letter number 2499/IPH.1.01/Ilf.07/XI/2018).

Materials

Technical grade organic solvent such as n-hexane, ethyl acetate, methanol, aquadest, ethanol, anhydrous acetic acid (Merck), 1% iron (III) chloride (Merck), chloroform (Merck), 2 N hydrochloric acid (Merck), metal Mg, Meyer reagent, Wagner reagent, calcium kidney stones, standard calcium chloride (Merck), murexid solution 0.5 N (Merck), silver nitrate (Merck), sulfuric acid (Merck), barium chloride (Merck), 0.1 N sodium hydroxide (Merck), dimethyl sulfoxide (Merck), Harnal D tablets.

Instruments

Various glass and non-glass equipment, analytical scales, waterbath, ultrasonicator (BAKU BK-3A), oven, vacuum rotary evaporator (IKA RB 10 basic), a set of UV-Vis spectrophotometers (Optima SP - 300 Spectrophotometer), a set of LC spectrophotometers MS (Acquaty UPLC®H-Classic System, BEH C18, Xevo G2-S QTof).

Methods

Preparation sample and extraction

The leaves of C. odorata were collected, cleaned, and dried. C. odorata leaves are air-dried at room temperature. C. odorata leaves that have been dried are mashed using a blender. C. odorata leaf powder was extracted using ultrasonication with n-hexane, ethyl acetate, and methanol, alternating from nonpolar, semipolar, and polar. Dried C. odorata leaves powder (282 g) was put into an erlenmeyer flask and added with solvent n-hexane 1.5 (w/v) then extracted using an ultrasonicator for 30 minutes, and the macerate was separated from the residue (Januarti et al., 2017). The residue was extracted again using alternatingly different solvents, namely ethyl acetate and methanol. Each macerate was filtered and concentrated with a vacuum rotary evaporator, resulting in extracts of n-hexane,
ethyl acetate, and methanol from the leaves of *C. odorata*.

**Calcium decay test in kidney stones**

The calcium decay test in kidney stones consists of several stages, including preparation of kidney stones, identification of calcium in kidney stones, and analysis of calcium decay in kidney stones. Kidney stones (3 g) obtained from a patient with kidney stones were cleaned with aquadest and baked in an oven for 10 minutes at 100°C, after being cooled, they were crushed using a mortar.

Kidney stones identified the content of calcium, oxalate, and phosphate. Kidney stone powder is added with a sulfuric acid solution to detect the presence of calcium. Kidney stone powder was added with nitrate treatment to detect oxalate content, and to identify phosphate, kidney stone powder was added with barium chloride.

The standard solution of calcium is made following the research procedure Hayati et al. (2016) includes the making of CaCl₂·2H₂O solutions with concentrations of 4, 6, 8, 10, 20, and 25 µg/mL. Each sample concentration was pipetted 1 mL and put into a 25 mL volumetric flask, added 1 mL of murexide solution, 2 mL of sodium hydroxide and made up to 25 mL with 96% ethanol. The solution was homogenized and the absorbance was measured using UV-Vis spectrophotometer at a wavelength of 500 nm, and the standard curve was determined.

Each extract of *n*-hexane, ethyl acetate, and methanol was made with a concentration series of 100, 500, 1000, 5000, and 10000 µg/mL with 96% ethanol as the solvent. Kidney stone powder was immersed in each extract solution at 37°C for 5 hours and stirred every 15 minutes. The reaction results were filtered using Whatman filter paper, then 1 mL of the filtrate was taken and put into a 25 mL volumetric flask, 1 mL of murexide solution, and 2 mL of 0.1 N sodium hydroxide and 96% ethanol were added to the mark. The solution was homogenized and the absorbance was read at 500 nm (Novalia et al., 2016). The negative control in this study was kidney stone powder at 96% ethanol. The positive control used was Harlan D tablet.

**Phytochemical analysis**

Phytochemical analysis of the extract included qualitative tests for saponins, tannins, alkaloids, flavonoids, triterpenoids and steroids. The extract was dissolved in methanol and put in several tubes, the first tube was added with aqua dest for the detection of saponins, the second tube was added with aqua dest and FeCl₃ for the detection of tannins, the third and fourth tubes were added Mayer and Wager reagents for the identification of alkaloids respectively, the fifth tube was added with aqua dest, Mg and HCl for flavonoid identification. The sixth tube was added with Liebermann Burchard reagent for triterpenoid/steroid identification. The fifth tube was analyzed using LCMS/MS. A total of 0.5 mg of methanol extract of *C. odorata* was dissolved in methanol then pipette 10 µL of the sample and then injected into LCMS/MS with a stationary phase column C-18 (2 x 150 mm), methanol: water (9:1) as the mobile phase with flow rate. 0.3 mL/minute (Rudiana et al., 2019).

**RESULTS AND DISCUSSION**

**Extraction and calcium decay test in kidney stones**

The leaves of *C. odorata* (282 g) were extracted using an ultrasonicator using *n*-hexane, ethyl acetate, and methanol as solvents. The ultrasonication method has advantages, including faster and more efficient extraction times in the use of solvents (Febriyanti et al., 2016). The ultrasonic filtrate is filtered using filter paper. The macerate was concentrated using a vacuum rotary evaporator at 45°C to obtain concentrated extracts of *n*-hexane, ethyl acetate, and methanol.

**Table 1. Yield value data of *C. odorata* extract**

<table>
<thead>
<tr>
<th>Initial powder weight (g)</th>
<th>Extract</th>
<th>Mass (g)</th>
<th>% Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>282</td>
<td>n-Hexane</td>
<td>12.44</td>
<td>4.41</td>
</tr>
<tr>
<td></td>
<td>Ethyl acetate</td>
<td>23.48</td>
<td>8.32</td>
</tr>
<tr>
<td></td>
<td>Methanol</td>
<td>37.97</td>
<td>13.46</td>
</tr>
</tbody>
</table>

Methanol extract had the highest % yield, amounting to 13.46% (Table 1). The high % yield in methanol extract indicated that *C. odorata* leaves contained many polar compounds such as flavonoids, phenolics, and alkaloids. Methanol can dissolve compounds with high polarity due to OH groups such as flavonoids, phenolics, and alkaloids (Rudiana et al., 2018). The terpenoid and steroid compounds are soluble in non-polar to semi-polar solvents (Saidi et al., 2018).

The yield of ethyl acetate solvent is smaller than that of methanol but larger than that of *n*-hexane (Table 1); this is presumably due to the presence of a methoxy group in the chemical structure of ethyl acetate. The presence of the methoxy group causes ethyl acetate to form hydrogen bonds with compounds contained in the
sample. The hydrogen bond formed in the ethyl acetate solvent is weaker than the hydrogen bond formed in the methanol solvent so that it affects the yield of the ethyl acetate solvent, which is less (Romandani et al., 2014).

Analysis of calcium dissolution in kidney stones was carried out using photometry using a UV-Visible spectrophotometer. Each test solution was incubated at 37°C for 5 hours. The incubation temperature of 37°C corresponds to average human body temperature (Kukus et al., 2009). Based on research Hayati et al. (2016) the optimum incubation time is 5 hours. Kidney stones in the body can move due to urine flow, water flow or movement due to the activity of the human body (Efendi et al., 2012), so every 15 minutes during the incubation of the test solution, stirring was carried out (Oktari et al., 2014). The purpose of stirring is to obtain conditions such as those that occur in the body, especially in the kidney organ, which then moves to the urinary tract where kidney stones are usually found (Dewi et al., 2016).

The calcium solubility test of kidney stones was carried out in vitro where the measurement of dissolved calcium levels was carried out using a UV-Visible spectrophotometer at a maximum of 500 nm. The dissolved calcium content was calculated based on the standard curve equation \( y = 0.3627 + 0.004x \) (\( R^2 = 0.9984 \)). The measurement of dissolved calcium levels can be seen in Figure 1.

The bar chart in Figure 1, shows that the methanol extract of C. odorata has a greater effect in dissolving calcium kidney stones than the n-hexane and ethyl acetate solutions.

This study showed that in vitro studies of methanol extract with a 10,000 µg/mL concentration can dissolve calcium kidney stones by 19.2 µg/mL (Figure 1). A positive control using the drug Harnal D was able to dissolve calcium kidney stones by 3.33 µg/mL (Figure 1). The increasing concentration of the extract was followed by the increasing ability to dissolve calcium kidney stones. The high concentration of the extract is directly proportional to the number of flavonoid levels, causing the ability to dissolve kidney stones will also increase. The highest ability to dissolve calcium kidney stones was in 10,000 µg/mL methanol extract. Ethyl acetate is a semipolar solvent. Compounds such as aglycone flavonoids, methylated flavonoids, tannins, and some alkaloid compounds can be extracted by ethyl acetate solvent (Rudiana et al., 2018). The solvent of n-hexane can attract nonpolar compounds such as steroids and terpenoids. Low polarity compounds such as methylated flavonoids, steroids, and triterpenoids have a low ability to dissolve calcium in kidney stones.

Data for the solubility of calcium kidney stones were statistically tested with the normality test (Shapiro-Wilk) and the homogeneity test (Brown-Forsythe and Welch) and followed by a parametric test, namely ANOVA (Analysis of Variance). Based on the statistical analysis results, the percent solubility of calcium for kidney stones is normally distributed with a significance value > 0.05, so the variable data for the percent solubility of calcium for kidney stones is normally distributed. In the homogeneity test with Brown-Forsythe and Welch, it is known that the significance value is 0.000, and the homogeneity test value at the significance level is \( \leq 0.05 \) so that the data on the variance value is homogeneous. The One Way Anova test results showed that the percent solubility of kidney stone calcium had different variant values (\( \alpha = 0.05 \)).

Figure 1. Comparison of the average dissolved kidney stone calcium levels in each extract

P-ISSN: 2406-9388
E-ISSN: 2580-8303
Characterization of kidney stone decreasing active extract

Methanol extract of *C. odorata* leaves had the best activity compared to *n*-hexane and ethyl acetate extracts. The methanol extract of *C. odorata* leaves was characterized and determined its chemical content. The results of phytochemical testing of the methanol extract of the leaves of *C. odorata* are presented in Table 2.

**Table 2.** Phytochemical screening of the methanol extract of *C. odorata*

<table>
<thead>
<tr>
<th>No.</th>
<th>Secondary metabolites</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Saponin</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Tannin</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Alkaloid</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Flavonoid</td>
<td>+</td>
</tr>
<tr>
<td>5</td>
<td>Steroid</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Triterpenoid</td>
<td>-</td>
</tr>
</tbody>
</table>

+ : detected; - : not detected

*C. odorata* leaf methanol extract contains saponins, tannins, alkaloids, flavonoids and steroids (Table 2). In this test, saponins were identified in the methanol extract of *C. odorata* which was characterized by the formation of a stable foam. Tannin compounds were identified in the methanol extract of the leaves of *C. odorata*, the formation of a green-black color in the analysis of tannins was thought to come from a complex compound between Fe metal and tannins contained in the methanol extract of *C. odorata* (Sukarno, 2017). The methanol extract of *C. odorata* contains alkaloids that are characterized by the formation of a white precipitate when reacted with Meyer reagent and an orange residue in Wagner reagent (Sukarno, 2017).

The methanol extract of *C. odorata* contains flavonoids characterized by the occurrence of a color change to orange color when the methanol extract of *C. odorata* is added with Mg metals and HCl. The flavonoids contained in the methanol extract of *C. odorata* are reduced, causing an orange color (Simaremare, 2014). The steroid test was based on the color change of Liebermann Burchard reagent with methanol extract of *C. odorata*. The green ring was identified in this test so that the methanol extract of the leaves of *C. odorata* contains steroid class compounds (Ayoola et al., 2008).

The methanol extract of *C. odorata* was analyzed using LCMS/MS which aims to determine the content of its chemical compounds. The chromatogram of the methanol extract of *C. odorata* is presented in Figure 2. The chromatogram shows 13 peaks at a retention time of 1.23; 5.20; 6.23; 7.32; 7.76; 8.13; 8.96; 9.31; 10.01; 10.68; 11.04; 11.70; and 12.16 minutes.

**Figure 2.** Total Ion Chromatogram of the methanol extract of *C. odorata* leaves
Table 3. Tabulation of interpretation results of LCMS/MS data from methanol extract of *C. odorata* leaves

<table>
<thead>
<tr>
<th>No.</th>
<th>Retention time (minute)</th>
<th>Molecular weight (g/mol)</th>
<th>Molecular Formula</th>
<th>Compound Name</th>
<th>Molecular Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>7.76</td>
<td>346.0689</td>
<td>C&lt;sub&gt;17&lt;/sub&gt;H&lt;sub&gt;14&lt;/sub&gt;O&lt;sub&gt;8&lt;/sub&gt;</td>
<td>3,5,7,4'-tetrahydroxy-8,3'-dimethoxyflavone (1)</td>
<td>![Structure 1]</td>
</tr>
<tr>
<td>2.</td>
<td>8.96</td>
<td>331.0812</td>
<td>C&lt;sub&gt;17&lt;/sub&gt;H&lt;sub&gt;14&lt;/sub&gt;O&lt;sub&gt;7&lt;/sub&gt;</td>
<td>5,7,4'-trihydroxy-3',5'-dimethoxyflavone (2)</td>
<td>![Structure 2]</td>
</tr>
<tr>
<td>3.</td>
<td>10.01</td>
<td>372.1209</td>
<td>C&lt;sub&gt;20&lt;/sub&gt;H&lt;sub&gt;20&lt;/sub&gt;O&lt;sub&gt;7&lt;/sub&gt;</td>
<td>5,6,7,8,4'-pentamethoxyflavone (3)</td>
<td>![Structure 3]</td>
</tr>
</tbody>
</table>

Several flavonoid compounds with a flavonoid basic framework have been isolated from *C. odorata*. Pisutthanan et al. (2005) reported 6 flavonoid compounds with a flavone framework, namely 3,5,3'-trihydroxy-7,4'-dimethoxyflavone (4), 5,3'-dihydroxy-7,4'-dimethoxyflavanone (5), 5,7-dihydroxy-4'-methoxyflavone (6), 3,5,4'-trihydroxy-7-methoxyflavanone (7), 5,7,3'-trihydroxy-5'-methoxyflavanone (8), and 3,5,7-trihydroxy-4'-methoxyflavanone (9), chemical structure can be seen Figure 3. Compounds 1, 2, and 3 (Table 3) identified in this study have a basic framework similar to Pisutthanan et al. (2005) and the number of substituents in rings A and B. The difference in substituents is thought to be due to the difference in the location of different plants so that the enzymatic reactions that occur in plants will produce other chemical structures.

Compounds 1 and 2 are flavonoid derivative compounds with a hydroxyl group (OH). The –OH group in compounds 1 and 2 can react with calcium to form a Ca-flavonoid chelate complex. These complex compounds are more soluble in water, so the water contained in the urine will help remove kidney stones (Nisma, 2011).

CONCLUSION

Extract methanol from the leaves of *C. odorata* with a concentration 10,000 µg/mL can dissolve calcium kidney stones by 19.2 µg/mL. The methanolic extract of *C. odorata* leaves contains 3,5,7,4'-tetrahydroxy-8,3'-dimethoxyflavone; 5,7,4'-trihydroxy-3',5'-dimethoxyflavone, and 5,6,7,8,4'-pentamethoxyflavone compounds.

ACKNOWLEDGMENT

The author would like to thank Mr Nasrullah (Ciwanda - Cilegon), who was willing to voluntarily provide kidney stones after kidney stone removal surgery at Kurnia Hospital, Cilegon. Mr. Azhar at the POLRI Center for Laboratory Affairs and Farhan Riza Afandi at the Chemistry Study Program at UIN Syarif Hidayatullah Jakarta assisted in analysing LCMS/MS. Adawiah at the Center for Integrated Laboratory of FST UIN Syarif Hidayatullah Jakarta and a laboratory
assistant at the Chemical Laboratory of STAK Cilegon who has assisted in the analysis of kidney stone decay.

AUTHOR CONTRIBUTIONS

CONFLICT OF INTEREST
The authors declared no conflict of interest.

REFERENCES


