

The Effect Of Watermelon Seed (*Citrullus Lanatus* (Thunb) Mansf.) Ethanol Extract Concentration And Soaking Duration On The In Vitro Dissolution Of Calcium Kidney Stones

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Abstract

Kidney stones are a collection of mineral salts such as uric acid, phosphate, and calcium oxalate that form from substances that accumulate along the urinary tract. Kidney stones can cause pain in the back, waist, and lower abdomen. Therefore, efforts are needed to dissolve kidney stones, one of which is with watermelon seeds. Watermelon seeds contain flavonoids that are thought to be able to dissolve calcium kidney stones. The aims of this research was to determine whether the concentration of watermelon seed extract and the duration of soaking calcium kidney stones can dissolve calcium kidney stones in vitro. The object of the research was the concentration of calcium kidney stones dissolved after the addition of watermelon seed extract. Watermelon seed powder was extracted using a maceration method with 70% ethanol solvent, then make watermelon seed extract concentrations of 2%, 4%, and 6% and the kidney stones were soaked in the watermelon seed ethanol extract concentration for 3 and 5 hours. Analysis of the dissolved calcium kidney stones was measured used the Atomic Absorption Spectrophotometer method. Identification of the type of kidney stone was carried out used an FTIR spectrophotometer. The result research shows that watermelon seed extract with a concentration of 2%, 4%, and 6% can dissolve calcium kidney stones with a 3-hour soaking duration of 24.34+1.34 ppm, 69.87+1.45 ppm, and 137.06+1.78 ppm on average, while a 5-hour soaking duration of 31.61+1.46 ppm, 82.27+0.84 ppm, and 140.82+1.96 ppm on average. Statistical tests show that there is an effect of extract concentration and soaking time of kidney stones in dissolving calcium kidney stones in vitro and the longer the soaking time the larger the kidney stones that can be dissolved.

Keywords : watermelon seeds, calcium kidney stones, AAS, FTIR, soaking time

1. INTRODUCTION

Kidney stones (nephrolithiasis), medically known as renal calculi, are a condition characterized by the accumulation of salts and minerals in the urinary tract, forming crystals. The process of kidney stone formation involves two stages: supersaturation and nucleation. Supersaturation occurs when urine contains substances such as uric acid, phosphate, and calcium oxalate in amounts exceeding their solubility (saturation). Normally, these substances dissolve in urine and are excreted during urination. However, if the body's natural mechanisms, such as regulating acid-base balance (pH), are disrupted or the immune system is compromised, these substances begin to crystallize. These crystals can then accumulate and form large stones,

a process known as nucleation, which can block urine flow (Alelign & Petros, 2018; Hadibrata & Suharmanto, 2022; Huzen dkk., 2014; Marcellus, 2014; Murphy., 2024; Shastri dkk., 2023).

Kidney stones that accumulate in the urinary tract often cause problems for sufferers. The most common problem is lower back pain. This pain can be sudden and severe (colic) or less sudden (non-colic) (Alelign & Petros, 2018; Hadibrata & Suharmanto, 2022; Margaret et al., 2014; Murphy., 2024; Purnomo, 2008; Rahmawati et al., Erlita dkk., 2018; Shastri et al., 2023).

Many researchers have conducted research on kidney stone dissolution to find ways to prevent and cure this disease. The main goal of this research is to find a simple and effective treatment to make this disease less dangerous. There are many methods for treating kidney stones, such as surgery or radiation. However, these methods are considered impractical and inefficient, so researchers continue to seek alternative treatments (Atmoko et al., 2023; Budiasih, 2017; Fikriani & Wardhana, 2018; Hadibrata & Suharmanto, 2022; Octariani et al., 2021).

Traditional medicine is often considered an alternative, so many plants suspected of containing substances that can help dissolve kidney stones are being studied. The goal of herbal medicine is to restore balance in the body and help the body heal itself. Several studies have been conducted on plants suspected of containing active ingredients for treating kidney stones, such as cat's whiskers (*Orthosiphon grandiflora*), tempuyung leaves (*Sonchus arvensis*), celery leaves, and beluntas leaves. Furthermore, watermelon can also help dissolve kidney stones. Research by Effendi and Sri Wardatun (2012) found that the flavonoids and potassium in watermelon juice can dissolve calcium oxalate kidney stones in vitro. Watermelon is often consumed by the public, but the seeds are usually discarded. Therefore, researchers were interested in examining whether watermelon seeds could also dissolve calcium kidney stones in vitro (Anas et al., 2016; Dewi et al., 2017; Effendi & Wardatun, 2017; Fikriani & Wardhana, 2018; Rani, 2024; Sharma, 2022; Shirani et al., 2020; Sulistiyowati, 2022).

Watermelon seeds are long and flat and come in black, white, yellow, or reddish-brown colors. Watermelon seeds contain citrulline, vitamin B12, the enzyme urease, and an active compound called cucurbitositrin. They also have a sweet, neutral flavor, which can help eliminate urine, improve kidney function, maintain stable blood pressure, and maintain

intestinal moisture. Furthermore, watermelon seeds contain flavonoids. These flavonoid compounds are thought to dissolve calcium kidney stones. Theoretically, the amount and duration of soaking can affect the rate of reaction in dissolving calcium kidney stones (Mukaromah et al., 2022).

2. METHOD

2.1. Object of Research

The object of this research was the calcium content in kidney stones that dissolved after being added with watermelon seed extract (*Citrullus lanatus* (Thunb) Mansf.) in vitro. The independent variables in this study included the concentration of watermelon seed extract, namely 2%, 4%, and 6%, as well as the length of time the kidney stones were soaked, namely 3 hours and 5 hours.

2.2. Tool and Research Materials

The tools used in this research were sieve no. 30/40, grinder, test tube, TLC plate, stirrer, measuring cup, thermometer, water bath, FTIR spectrophotometer (Shimadzu type IR Prestige 21) and Atomic Absorption Spectrophotometer (Perkin Elmer 3110).

The materials used in this research were watermelon seeds, kidney stones, 70% alcohol, distilled water, hydrochloric acid concentrate, amyl alcohol, magnesium powder, 0.1 N sodium hydroxide, sulfuric acid concentrate, n-butanol, glacial acetic acid, potassium bromide, nitric acid p, and lanthanum chloride.

2.3. Reseach Procedure

2.3.1. Making Watermelon Seed Extract

Weighed carefully, 100 grams of dried watermelon seed powder, then soaked at normal temperature using 1000 ml of 70% ethanol solvent for 3 days by changing the liquid every 24 hours. The results of the soaking obtained were collected, then condensed by evaporating the liquid using a rotary evaporator until a thick extract was obtained. The successfully made thick extract was then made in various concentrations, namely 2%, 4%, and 6%.

2.3.2. Qualitative Test of Flavonoid Compounds in Watermelon Seed Extract

The extract was mixed with distilled water, heated for 5 minutes, and then filtered while still hot. The filtered liquid was added with 1 ml of concentrated hydrochloric acid, 2 ml of amyl alcohol, and a small amount of magnesium powder. The mixture was shaken vigorously

and allowed to separate. A red or yellow color change in the amyl alcohol layer indicates the presence of flavonoids. The extract solution is mixed with a few drops of 0.1 N NaOH and then the color is observed. Flavonol and flavone will produce a yellow color (Anas dkk., 2016; Effendi & Wardatun, 2017). The extract solution is mixed with a few drops of concentrated H₂SO₄ and the color is observed. Flavonol will produce an orange color.

Flavonoid identification was also carried out using TLC using n-butanol: glacial acetic acid: water (4:1:5) as the eluent. After the separation process was complete, the plate was dried, then the plate was evaporated with concentrated ammonia (Anas et al., 2016; Effendi & Wardatun, 2017).

2.3.3. Qualitative Analysis of Kidney Stones

Qualitative analysis of kidney stones was performed using an FT-IR spectrophotometer. The analysis process began by weighing 1 to 3 mg of kidney stone powder, then adding 300 mg of KBr and stirring until the mixture was uniform. The mixture was formed into clear pellets by applying 10 tons of pressure for 5 minutes. The resulting spectrum was then compared with standard spectra from the textbook "Analyse des Calculus par Spektrophotometric Infrarouge, Avantages et Limites de la Methode" (Effendi & Wardatun, 2017; Mukaromah et al., 2022)

2.3.4. Measurement of Kidney Stone Calcium Levels Dissolved in Extracts

Watermelon seed extract with a concentration of 2%, 4%, and 6% each as much as 25.0 ml was put into a test tube, then added with 100.0 mg of kidney powder. The kidney powder was soaked at 37°C for 3 and 5 hours, with stirring every 15 minutes, then filtered. The filtered liquid was destroyed using a mixture of HNO₃ p and HCl p in a ratio of 1:3, then the dissolved calcium content was measured using an Atomic Absorption Spectrophotometer (Effendi & Wardatun, 2017; Mukaromah et al., 2022).

2.3.5. Measurement of Calcium Levels in the Extract Solution

Watermelon seed extract with concentrations of 2%, 4%, and 6%, each as much as 25 ml, was put into a test tube, left at a temperature of 37°C for 3 and 5 hours. During the process, the mixture was stirred every 15 minutes, then filtered. The filtered liquid was then destroyed using a mixture of concentrated HNO₃ and concentrated HCl in a ratio of 1:3. After that, the dissolved calcium content was measured using an Atomic Absorption Spectrophotometer (Anas, et al., 2016; Mukaromah et al., 2022).

2.3.6. Measurement of Calcium Levels of Dissolved Kidney Stones in Blanks

25.0 ml of distilled water was placed in a test tube, then 100.0 mg of kidney stone powder was added. The kidney stone powder was soaked at 37°C for 3 and 5 hours, stirring every 15 minutes, then filtered. The filtered liquid was dissolved with a solution of HNO₃ p and HCl p in a ratio of 1:3, after which the concentration of dissolved calcium was measured using an Atomic Absorption Spectrophotometer (Anas et al., 2016; Effendi & Wardatun, 2017; Mukaromah et al., 2022; Permata et al., 2017).

3. RESULTS AND DISCUSSION

The extraction process was conducted using qualitative tests to determine the presence of active flavonoid compounds using chemical reactions and TLC. The results showed that the watermelon seed extract contained flavonoid compounds. This was evident from the yellow color of the amyl alcohol layer. Furthermore, when 0.1 N NaOH was added, the resulting color was also yellow, while with H₂SO₄, the color changed to orange.

Table 1. Phytochemical Screening Results of Watermelon Seed Ethanol Extract

Metabolite Secunder	Result
Alkaloid	+
Flavonoid	+
Saponin	+
Tanin	+
Terpenoid	+
Fenolik	+

Analysis of the kidney stone type using an FTIR spectrophotometer showed that the kidney stone sample used was indeed a calcium stone. This can be seen at the wavelength of 1319.31 cm⁻¹, which corresponds to the Weddellite molecule containing calcium oxalate dihydrate (Ca(COO)₂·2H₂O) (Djamhuri et al., 2016; Hadibrata & Suharmanto, 2022; Suharjo, 2009).

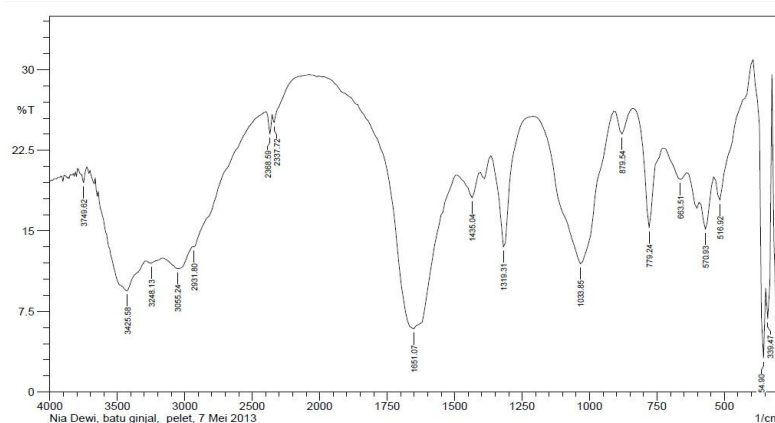


Figure 1. Spectrogram FT-IR of Kidney Stone Sample

The process of soaking kidney stones in a watermelon seed extract solution was carried out for 3 and 5 hours at a temperature of 37°C, with continuous stirring every 15 minutes. The main goal was to make the conditions during the experiment more closely approximate those inside the human body. 37°C was chosen because it is the normal human body temperature. Stirring was carried out every 15 minutes because it is believed that kidney stones in the body can move due to reabsorption processes, both active and passive. Kidney stones can move due to urine flow, water flow, or movement caused by bodily activity. The chosen soaking times, 3 and 5 hours, were based on several studies that state that normal adults excrete approximately 1,200 ml of urine per day, or 50 ml per hour. Meanwhile, other studies indicate that the human bladder capacity is approximately 170 to 230 ml. Therefore, the normal frequency of urination is approximately 3 to 5 hours (Adomako & Moe, 2020; Alelign & Petros, 2018; Anas et al., 2016; Dewi et al., 2017; Djamhuri et al., 2016; Effendi & Wardatun, 2017; Hadibrata & Suharmanto, 2022; Hayati et al., 2016; Shastri et al., 2023).

The filtrate obtained from the soaking was destroyed by adding 10 ml of HNO₃ and HCl (1:3) or until the sample solution became clear. The success of this destruction/destruction process can be seen from the clear solution produced after the process. This destruction process aims to separate the metal elements from other elements. In addition, lanthanum chloride also needs to be added to the sample solution. This is intended to attract phosphate and silicate compounds which, if combined with calcium, will be difficult to decompose and thus can affect the measurement results (Anggriani, 2019; Hayati et al., 2016; Mukaromah et al., 2022).

Analysis of the amount of calcium dissolved in watermelon seed extract is determined by the amount of soluble Ca = the amount of total Ca - the amount of extract Ca - the amount of empty Ca. The amount of total Ca is the amount of calcium measured by an Atomic Absorption Spectrophotometer, the amount of extract Ca is the amount of calcium in the watermelon seed extract, and the amount of empty Ca is the amount of kidney stone calcium

dissolved in distilled water. Determining the amount of kidney stone calcium dissolved in distilled water aims to determine whether the water used to soak kidney stones can dissolve kidney stones or not (Effendi & Wardatun, 2017; Hayati et al., 2016; Permata et al., 2017; Sulistiyowati, 2022).

Table 2. Average Calcium Levels of Kidney Stones Dissolved in Watermelon Seed Extract

Concentration of sample (%)	Dissolved Ca levels during 3 hours (ppm)	Dissolved Ca levels during 5 hours (ppm)
2	24.34±1.34	31.61±1.46
4	69.87±1.45	82.27±0.84
6	137.06±1.78	140.82±1.96

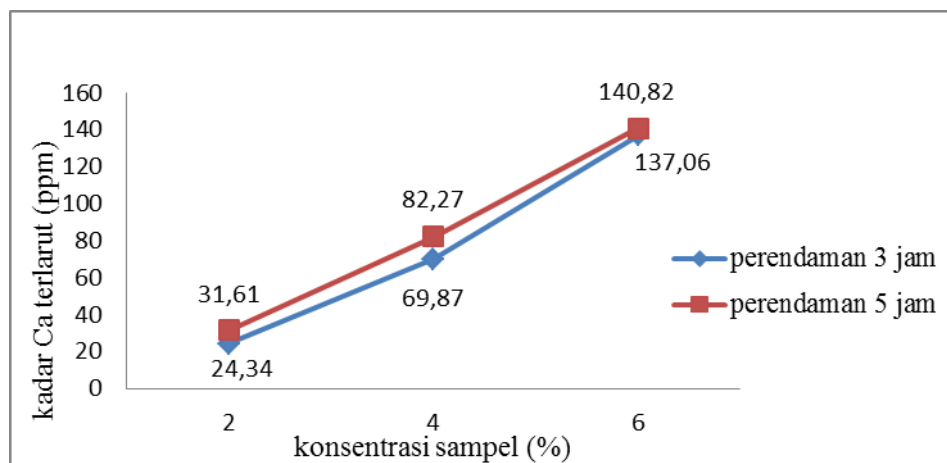


Figure 2. Graph of Dissolved Calcium Levels against Extract Concentration

The table above shows that the amount of calcium dissolved increases with higher extract concentrations and longer soaking times for kidney stones. This occurs because the longer the solution is in contact with the kidney stones, the more calcium is dissolved. The higher the concentration of the solution used, the greater its ability to dissolve calcium from kidney stones.(Effendi & Wardatun, 2017; Sulistiyowati, 2022; Permata et al., 2017)

The results of the ANOVA test showed a significant difference, with a significance value of $\alpha < 0.05$, indicating a difference between each time and concentration group. To determine the significant differences between the groups, a post-ANOVA test was conducted. The results of the post-ANOVA test showed a significant difference between each group. The t-test showed

a significant difference between the 3 and 5 hour soaking times. This indicates that the 3 and 5 hour soaking times affected the in vitro calcium dissolution of kidney stones.

Calcium in kidney stones likely reacts with the –OH group in flavonoids, forming a compound called Ca-flavonoids. This compound is thought to be more soluble in water, so the water in urine can help dissolve kidney stones. Furthermore, the diuretic activity of flavonoids also helps expel stones from the kidneys, typically through the urine (Suharjo, 2009).

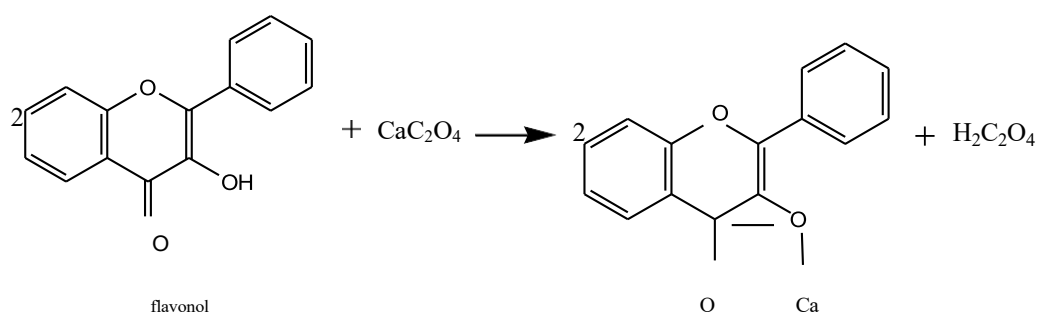


Figure 3. Reaction of flavonoids and calcium stones

4. CONCLUSION

The research results concluded that watermelon seed extract (*Citrullus lanatus* (Thunb) Mansf.) has the ability to dissolve calcium kidney stones in vitro. There were differences in the ability of watermelon seed extract at concentrations of 2%, 4%, and 6% to increase the ability to dissolve calcium kidney stones in vitro. Furthermore, soaking calcium kidney stones in watermelon seed extract for 3 and 5 hours also affected the ability to dissolve calcium kidney stones in vitro.

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