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## The Potential of Pineapple Crown (*Ananas Comosus*) Extract as Removal of Kidney Stone

Naimatul Khoiroh<sup>a\*</sup>, Fatati Nurmalia Sari<sup>b</sup>, Aldi Gunawan<sup>b</sup>, Anif Mega Hidayati<sup>b</sup>,  
dan Siti Umroka<sup>b</sup>

**Abstract.** Some people in Mojokerto use pineapple crowns as medicine to remove the kidney stone. However, research related to this topic is quite limited, with no specific data regarding the content of active substances in pineapple crowns. Therefore, in this study, kidney stones' decay was tested with pineapple crown extract and analyzed the phytochemicals of pineapple crown. In this research, preliminary testing was carried out through in vitro test with kidney stones obtained from one of the patients with kidney stone's disease. The solubility of calcium oxalate, the majority content of kidney stone, was tested against pineapple crown extract with various solvents. Several phytochemical tests, such as saponins, tannins, flavonoids, triterpenoids, and steroids, were carried out six times on the pineapple crown extract using solvent variations. It was confirmed that the highest phytochemical content in pineapple crown extract with water and ethanol solvents. From the in vitro test results of kidney stones' solubility, it was found that there was an effect of pineapple crown on kidney stones' solubility, although it was not significant. The optimum concentration of pineapple crown extract from several variations of the concentration tested was 0.0625 M. Through this research can provide scientific facts to the public about the use of pineapple crown extract for kidney stones' removal.

**Keywords :** Pineapple crown, kidney stone, calcium oxalate.

<sup>a</sup>UIN Sayyid Ali Ramhatullah Tulungagung, Jl. Mayor Sujadi No.46, Kudus, Plosokandang, Kec. Kedungwaru, Tulungagung and 66221, Indonesia

<sup>b</sup>Institut Teknologi Sepuluh Nopember, Jl. Teknik Kimia, Keputih, Kec. Sukolilo, Kota SBY, Jawa Timur 60111, Indonesia

Correspondence and requests for materials should be addressed to Khoiroh, N. (email: [naimatul.khoiroh@uinsatu.ac.id](mailto:naimatul.khoiroh@uinsatu.ac.id))

## Introduction

Nephrolithiasis is one of the most common urological diseases affecting 1–13% of the world's population. Its prevalence and morbidity are predicted to continue increasing over the coming decades [1]. Nephrolithiasis, also known as kidney stones, renal calculi, or urolithiasis, are irregularly-shaped or crystal stones, made of mineral and salt deposits that form in the kidneys as a solid mass. This disease can cause a significant burden on public health which can result in hydro-nephrosis, impaired renal function, and ultimately renal insufficiency [2] and it is likely to involve many factors, including climate, diet, and genetic background [3].  $\text{CaO}_x$  is reported become the dominant type of kidney stone, the majority 77,5% of all urinary calculi [4], but the mechanism of  $\text{CaO}_x$  stone formation has not been elucidated. Currently, patients with kidney stone in Indonesia have increased, and comprised region with urinary stone dominance over the ten countries with other sufferers in Asia [5]. Many kinds of drugs to treat patients with kidney stone have been found either alternative (herbal) or medical.

First, through medical treatment, such as used medicine (allopurinol, citrate, cystone, and thiazide diuretics) or directly removed through medical means, ESWL, endourology, laparoscopic surgery, or open surgery [6]. Medical treatment is indeed fast healing, but the high cost that must be paid causes people with middle to lower economic levels not to be able to take medical treatment. In addition, the continuous consumption of chemical-based drugs causes the deposition of chemicals in the kidneys [7]. Second, for herbal treatment, there are many plants that used as kidney stone herbal treatment, such as *Elymus repens* (couch grass rhizome), *Solidago* spp. (golden rod), *Eupatorium purpureum* (Gravel root), etc.[8]. Other herbal medicines that have been used by the Mojokerto society who have kidney stone disease was pineapple crown (*Ananas comosus*). However, the use of this pineapple crown is still not widely known by the public and the pineapple crown often just ends up becoming environmental waste. Whereas Indonesia is involved as the fourth major pineapple-producing country with a total production of 2196.46 thousand metric tons of pineapple [9]. Almost 15-20% of pineapple part ends as unused pineapple crowns, its waste production estimated at 1203.5 tons per day.

Pineapple crown has great benefits in medical herbal treatment, especially kidney stone treatment based on the Mojokerto society judgment through consuming pineapple crown continuously then it can exuviate kidney stone gradually. However, in this case, there has never been a study related to the use of pineapple crown as a kidney stone medical treatment. So, there is no scientific evidence to support this opinion. In addition, there is a limited explanation regarding the pineapple crown specifically. Research conducted by Dutta et al (2013) is also limited to the enzymatic, antimicrobial, and toxicity properties of the *Ananas comosus* (pineapple) crown leaf [10]. Therefore, it was worthwhile to characterize pineapple crown extract in terms of its different standard metabolite compounds to evaluate its kidney stone dissolving capability.

## Experimental

### *Raw Material dan Extract Process*

Pineapple crowns obtained from a group of fruit traders at Pucang Traditional Market in Surabaya as much as 1 sack (about 4-5 kg) have been washed, sliced into smaller sizes, and then dried at room temperature. The dried pineapple crown was crushed into pineapple crown powder as much as 540 gr and the extraction process can be carried out. Extraction was carried out using a maceration process through several stages. First, 85 gr pineapple crown powder total powder was macerated for 24 hours with 700 mL solvents. There were obtained 6 extracts from different solvents, consisting of water, methanol, hexane, ethyl acetate, methylene chloride, and ethanol 30%. Furthermore, the extract obtained was concentrated using a rotary evaporator, and the results were used in the next procedure.

### *Phytochemical Analysis*

Phytochemical analysis of pineapple crown in this study was carried out qualitatively. This analysis aims to determine the active compounds in pineapple crown extracted using several variations of solvents. The compounds identified were alkaloids, flavonoids, saponins, steroids and triterpenoids, phenolic hydroquinone, and tannins. The phytochemical test method consists of 6 tests as described below based on Harborne (1987) [11].

**Alkaloids.** 0.5 grams of the extract from each variation of solvents was taken and added with 1 mL of 2N HCl and 9 mL of distillate water. The mixture was heated in a water bath for 2 minutes, cooled, and filtered. Finally, three drops of filtrate from Von Mayer's reagent, Bouchard's reagent, and Dragendor's reagents were added to the solution.

**Flavonoids.** 0.5 grams of sample was taken and then extracted using 10 mL of distilled water and filtered. 5 ml of the liquid was removed and added 0.1 g of Mg powder, 1 mL of concentrate HCl and 2 mL of amyl alcohol. It was necessary to shake to find out the test results.

**Tannins.** 0.5 g of the sample was taken and added to 10 ml of distilled water, filtered, then diluted with distilled water until colorless. Next, 2 mL of filtered results mixed with 1-2 drops of  $\text{FeCl}_3$  1%.

**Saponins.** 0.5 grams of sample was taken in the test tube and added to 10 mL of warm, chilled distilled water, shaken vigorously for 10 seconds. When the foam formed reached a height of 1-10 cm, at least 10 minutes, added 12 N HCl.

**Steroids/terpenoids.** 1 gram of sample was taken and added with 20 mL of n-hexane for 2 hours, then filtered. Filtrate it evaporates on water. 2 drops for the rest added anhydrous acetic acid and 1 drop concentrated sulfuric acid.

### Kidney Stone Solubility Test

Calcium oxalate is the most abundant compound in kidney stone. Therefore, kidney stones' solubility can be identified from the solubility of calcium oxalate. This method has been used as an initial test of the solubility of kidney stone (Andrianto, 2010). The solubility of calcium oxalate in this study can be seen from its turbidity. Solubility testing was carried out using several variations of the pineapple crown extract content. Concentrations to be tested from 1%, 2%, 3%, 4%, to 5% (w/v) were made in the same volume, which was 25 ml. Each solution with a certain concentration added calcium oxalate up to 4,000 ppm. Turbidity was calculated three times, starting before the addition of the extract, directly after the addition of the extract, and 6 hours after the incubation time. Calcium oxalate concentration was calculated using a standard curve that had been made previously.

## Results and Discussion

### Extraction Results

Pineapple crown extracts of as many as six samples have been successfully produced from a series of extraction processes (Figure 1). The highest yield of extraction results was obtained from extraction using water and ethanol 30% as solvent (Table 1). This show that water and ethanol 30% were good solvents for extraction and can dissolve many phytochemical compounds in the pineapple crown.



**Figure 1.** Extraction Process; a. pineapple crown; b. dried powder pineapple crown; c. pineapple crown extract

**Table 1.** The yield of extraction from each solvent

Extract	Yield (%)
Water	8,68
Methylene chloride	1,09
N-hexane	0,47
Ethyl acetate	1,20
Methanol	4,34
Ethanol 30%	8,33

**Phytochemical Test**

Phytochemical test results showed that the pineapple crown contains many phytochemical compounds (Table 2). This compound was extracted more using water and ethanol 30% as solvent. This phytochemical test was an initial test to determine the presence of secondary metabolites in the analyzed sample. From Table 2 it can be seen that the secondary metabolites contained in the aqueous and ethanolic extracts of pineapple crown were saponins, tannins, flavonoids, triterpenoids, and steroids. The methanol extract contains saponins and alkaloids, the ethyl acetate extract contains alkaloids, while the methylene chloride extract and n-hexane extract show negative results in all tests.

The characteristics of the positive results seen from each phytochemical test were different. The sample shows positive results containing saponins if the foam can be formed until 2-3 minutes after shaking it. In the case of kidney stone disease, saponins (yellow highlight in Table 2) were secondary metabolites in plants that can inhibit the growth of calcium oxalate monohydrate crystals and the occurrence of calcium phosphate mineralization [12].

Positive tannins show a blackish green color after  $\text{FeCl}_3$  15% was poured into it. Positive alkaloids show precipitate after the addition of  $\text{H}_2\text{SO}_4$  2N and Wagner reagent. Positive triterpenoids change color to orange/purple after  $\text{H}_2\text{SO}_4$  and  $\text{CH}_3\text{COOH}$  were added, while positive steroids change color to reddish brown after  $\text{H}_2\text{SO}_4$  and chloroform were added to the sample [13]. The

last phytochemical screening test carried out in this study was the flavonoid test (orange highlight in Table 2). A positive result was indicated by a change in the color of the sample to yellow-orange after the addition of concentrated Mg and HCl powder.

Several phytochemical compounds found in plants and have antiurolithiatic activity were phenolic compounds, saponins, flavonoids, alkaloids, and tannins [14]. In patients with kidney stone disease, oxalate can cause lipid peroxidation by reacting with polyunsaturated fatty acids in cell membranes and damaging kidney tissue. This calcium oxalate can be decayed through several mechanisms. Triterpenes will help in the destruction of oxalate crystals and show antioxidant activity. Organic substances were adsorbed on the crystal surface and inhibit the crystallization process. Saponins can help the process of mucoprotein disintegration and cause a reduction in the adhesion of CaOx crystals to renal epithelial cells through pre-coating the crystals. Flavonoids significantly prevent crystallization with antioxidant, anti-inflammatory and antimicrobial properties [15]. Calcium in kidney stone was predicted to be able to form complex compounds with the -OH group of flavonoids to form Ca-flavonoids then kidney stone can dissolve and also dissolve out with urine [16]. Based on these explanations, the kidney stone solubility test was carried out using extract containing the most phytochemical compounds. The extract should be water extract and ethanol 30% extract. The water extract was chosen between the two extracts because water extract has higher yield than ethanol 30% extract.

**Table 2.** Phytochemical test of pineapple crown extract

Test	Extract					
	Water	Ethanol 30%	Methanol	Ethyl acetate	N-hexane	Methylene chloride
Saponins	+	+	+	-	-	-
Tannins	+	+	-	-	-	-
Alkaloids	-	-	+	+	-	-
Flavonoids	+	+	-	-	-	-
Triterpenoids	+	+	-	-	-	-
Steroids	+	+	-	-	-	-



**Table 3.** In vitro test of kidney stones' solubility of pineapple crown extract water solvent

Extract concentration	Blank Absorbance	Sample Absorbance		%Solubility
		Before Incubation	After 6 Hours of Incubation	
0.0078 M	0.029	0.337	0.300	0.120% ± 0.015
0.0156 M	0.061	0.532	0.507	0.053 % ± 0.015
0.0313 M	0.063	0.713	0.678	0.054% ± 0.015
0.0625 M	0.189	0.754	0.618	0.241 % ± 0.015
0.1250 M	0.387	0.967	1.000	-0.139 % ± 0.015

### Kidney Stone Solubility In Vitro Test

The solubility test of kidney stone was carried out with a UV-Vis spectrometer at 315 nm wavelength based on the turbidity of the solution due to the presence of kidney stone. The effect of pineapple crown extract was investigated with variations in the concentration of pineapple crown extract of 0.0078, 0.0156, 0.0313, 0.0625, and 0.1250 M. Due to the influence of kidney stone, the solution will be more turbid and the absorbance from UV-Vis Spectrometer will be greater.

Based on the data obtained (Table 3), it can be confirmed that the absorbance of the solution after incubation was lower than the absorbance of the solution before being added by the extract. This result remained the same until the addition of a concentration of 0.0625 M. It was also seen that the highest percentage of kidney stones' solubility was at an extract concentration of 0.0625 M, which means that the optimum concentration of pineapple crown extract was 0.0625 M. This indicates that there was an effect of pineapple crown extract activity on the decay of kidney stone even though not such a significant value. This was predicted to occur due to the low content of active compounds in the pineapple crown. Further exploration is still needed by looking at the quantity (amount) of the content of each bioactive compound in pineapple crown in further research as evidence.

The ability of pineapple crown extract to dissolve calcium contained in kidney stone was thought to be due to the presence of flavonoids and potassium in it [17]. Flavonoids and potassium can inhibit the formation of calcium kidney stone. Calcium in kidney stone can form complex compounds with the -OH group of flavonoids to

form Ca-flavonoids. This complex compound was more soluble in water, so the water contained in the urine will help dissolve the stone.

Flavonoids were included as a group of phenolic compounds that have a polyphenolic structure with a low molecular weight. Flavonoids that were useful for urolithiasis include rutin, catechins, epicatechins, and diosmin. Flavonoids help diuretic activity, antioxidant activity, anti-apoptosis, anti-infection, anti-inflammatory, and antibacterial. The anti-inflammatory and antioxidant properties of these flavonoids inhibit the formation of urinary tract stone, preventing the destruction of epithelial cells caused by CaOx crystals. Several in vitro and in vivo studies have shown that the antioxidant activity of flavonoids has an important role in preventing CaOx accumulation by reducing ROS (Reactive Oxygen Species) levels and protecting against oxidative kidney damage [18]. The diuretic activity of flavonoids can help expel stones from the kidney, which were excreted with urine, while potassium will compete and separate calcium bonds with oxalate so that calcium kidney stone become dissolved.

### Conclusion

From the research results that have been described previously, it can be concluded that the highest content of phytochemicals was in extracts from aquades and ethanol solvents. From the results of the in vitro test for the solubility of kidney stone, it was found that there was an effect of pineapple crown on the solubility of kidney stone, although not significantly. The optimum concentration of pineapple crown extract from several variations of the concentration tested was 0.0625 M. Therefore, these preliminary research results

show such a good start for further explore about the potential of pineapple crown to be a kidney stones' removal drug with further development in the next research.

### Author Contributions

Naimatul K. wrote the paper, conceived and designed the analysis; Anif M. H. collected the data; Siti U contributed data; Fatati N.S. and Aldi G performed the analysis.

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