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ARTICLE

Investigating *Kalanchoe pinnata* as a potential treatment for acute nephrolithiasis

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Kidneys are vital organs; they are the part of the urinary system that removes waste, extra fluid, and acids that are produced by cells. The kidneys help the body maintain a balance of water, salts, and minerals. Without them, the body may face issues with nerves, muscles, and tissues. Nephrolithiasis, normally known as kidney stones, is a crystal consecration formed from urinal chemicals. The presence of kidney stones may cause the back-up of urine in the kidney and bladder and symptoms such as blood in urine, vomiting, fever and lower back pain. Nephrolithiasis is not rare, as research suggests that one in ten people will face this issue in their lifetime (National Kidney Foundation, 2021). Patients of kidney stones can range from children to adults, although it is more likely to occur in older individuals. Calcium oxalate is the most common type of kidney stone, which is created when calcium and oxalate combine in urine. When kidney stones are small, doctors recommend increasing intake of water, so it naturally passes through the body. For more severe cases of kidney stones, medical treatment has been created where the stones are fragmented in surgery through lasers or shockwaves. However, these treatments are both expensive and invasive, and may not be a necessity for certain patients.

INTRODUCTION

For centuries, herbal medicine has been used for treatment of diseases, including many in the urinary system. *Salvia miltiorrhiza* (red sage) has exhibited properties of aiding patients of chronic kidney disease and is prescribed to treat patients in China. Another herb, *Berberis vulgaris* (barberry) is used for kidney treatment, especially for acute kidney injury and kidney stones. Herbal medicine is a possible avenue of treatment for acute nephrolithiasis, as it is non-invasive, inexpensive, and holds the potential to carry properties that could break or dissolve the stones.

Kalanchoe pinnata is an evergreen succulent herb that is found in the warm climates of Africa, particularly in Madagascar and Mauritius. Research conducted on the plant highlights its medical versatility, as it holds antibacterial and ulcer treating properties. Research suggests that *K. pinnata* contains an abundance of constituents such as organic acids and phytochemicals, but several papers contradict each other on exactly what organic acids and phytochemicals are present. The research will investigate the constituents present in *K. pinnata* and its effect of these extracted constituents on calcium oxalate, while looking into the efficacy of *K. pinnata* tincture in various dose quantities and sizes of kidney stones.

METHODS AND MATERIALS

Four separate tests were conducted; two to test the presence of *Kalanchoe pinnata*'s acidic constituents and two to see its ability to break down calcium oxalate crystals. These tests will indicate its efficacy in clearing the ureter of kidney stones.

Part 1: Organic Acid Tests

Samples of *K. pinnata* were crushed with a mortar and pestle and finally with a coffee grinder, and the extract was later squeezed with a linen cloth. Then, the substance was neutralized with a sodium bicarbonate solution, which was confirmed with a pH test. After the *K. pinnata* extract solution was neutralized, a calcium chloride test was conducted with 5% calcium chloride solution. If the presence of oxalic acid, citric acid and malic acid was found, it would support other studies on the existence of these constituents in *K. pinnata*.

Part 2: Phytochemical Test

1 mL of 95% ethanol was added into *K. pinnata* extract with a pipette into three separate test tubes. In test tube #1, 1 mL of iron (III) chloride was added, with a colour change into red indicating the presence of phenol. Glycosides are compounds that have a sugar molecule linked to a non-sugar molecule, and aid in the breakdown of kidney stones by reducing the concentration of urine. To test glycosides, 1 mL of aqueous NaOH was added

	Potential Results
Oxalic Acid	Immediate formation of white precipitation
Tartaric Acid	Development of precipitation upon shaking
Citric Acid	Formation of precipitation on cooling and boiling
Malic Acid	Development of precipitation after the addition of ethanol

to test tube #2, with a colour change into yellow indicating its presence. Lastly, 3 mL of NaOH was added into test tube #3, with a colour change from light green to colourless indicating the presence of flavonoids. Flavonoids, on the other hand, aid by reducing the formation of calcium oxalate stones and inhibit the growth of existing stones.

Part 3: Herb Percolation

The powder from the previous step underwent percolation to create a tincture for experimentation. During percolation, the powder was damped and tightly packed in a percolation column with a 50% ethanol and 50% water solution. The screw cap was released and drops of the tincture fell into the beaker below. After approximately 24 hours, the process was completed.

Part 4: Artificial Kidney Stone Creation

Sodium oxalate was created with sodium hydroxide and oxalic acid, which was then reacted with calcium chloride to create calcium oxalate.

Part 5: Solubility Test

Equally measured quantities of calcium oxalate crystals were placed into three different Erlenmeyer flasks: beaker #1 as a control, beaker #2 with diluted lemon water and beaker #3 for testing the tincture. Diluted lemon extract was chosen for beaker #2 to confirm that a high pH was not the only reason for crystal breakage. The size of the calcium oxalate crystals was monitored for one week in all three beakers.

Part 6: Kidney Model Test

To model the process of the tincture being used in real applications, a kidney model was created. Simulated blood and the tincture were added to dialysis tubing using a pipette and placed in a plastic cup. Outside the dialysis tubing, measured calcium oxalate crystals were added to deionized water. This model was created thrice, one to act as a control (without the tincture), another with diluted lemon extract and lastly one with the tincture. This was done to see the effect *K. pinnata* has on the calcium oxalate stones in an environment similar to the kidney.

RESULTS

Part 1: Organic Acid Tests

In the organic acid identification test, three organic acids were identified. Clearly visible formation of precipitate in the citric, malic and tartaric acid test tubes indicate the presence of these three organic acids in the extract. Additionally, no precipitation was formed in the oxalic acid test tube.

Part 2: Phytochemical Test

The phytochemical test indicated the presence of phenols, flavonoids, and glycosides.

Part 5: Solubility Test

The tincture was able to break down the kidney stone over a period of seven days by about 49%, while water broke down the stones by 18% and the diluted lemon extract broke down the stones by 27%.

Part 6: Kidney Simulation

In the kidney simulation, the tincture decreased the mass of the stone by 57% compared to only 27% by the control and 25.3% by the diluted lemon extract. Both the solubility and kidney simulation showcased that *K. pinnata* was a more effective treatment than water by around 30% and an average of about 27% more effective than distilled lemon extract.

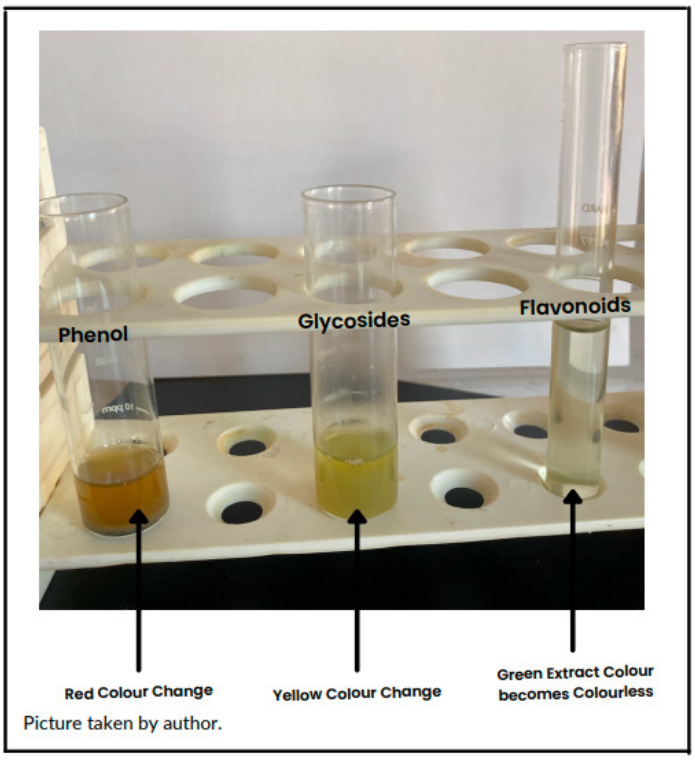

Figure 1. Results for Organic Acid Test

DISCUSSION

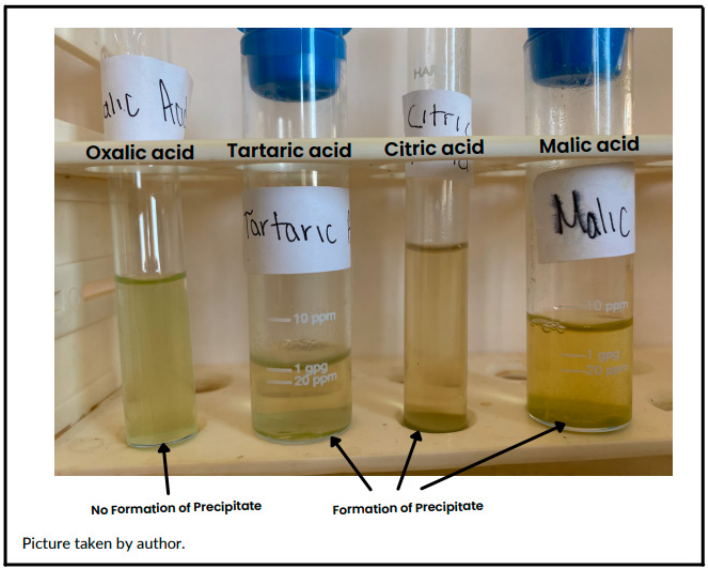

Figure 2. Results for Phytochemical Test

The successful testing of the presence of three separate organic acids in *K. pinnata* suggests that the plant has the constituents to be a very effective nephrolithiasis medicine. The presence of tartaric, malic and citric acid confirms past studies on *K. pinnata*, while a negative result for the presence of oxalic acid contradicts some studies (Pattewar, 2014; Rahman et al., 2019). This can be seen as a positive, as oxalic acid is a binding agent that binds smaller calcium oxalate stones into larger ones. Moreover, finding the presence of the three phytochemicals also shows promise for *K. pinnata*'s abilities to help nephrolithiasis patients. Phenols are

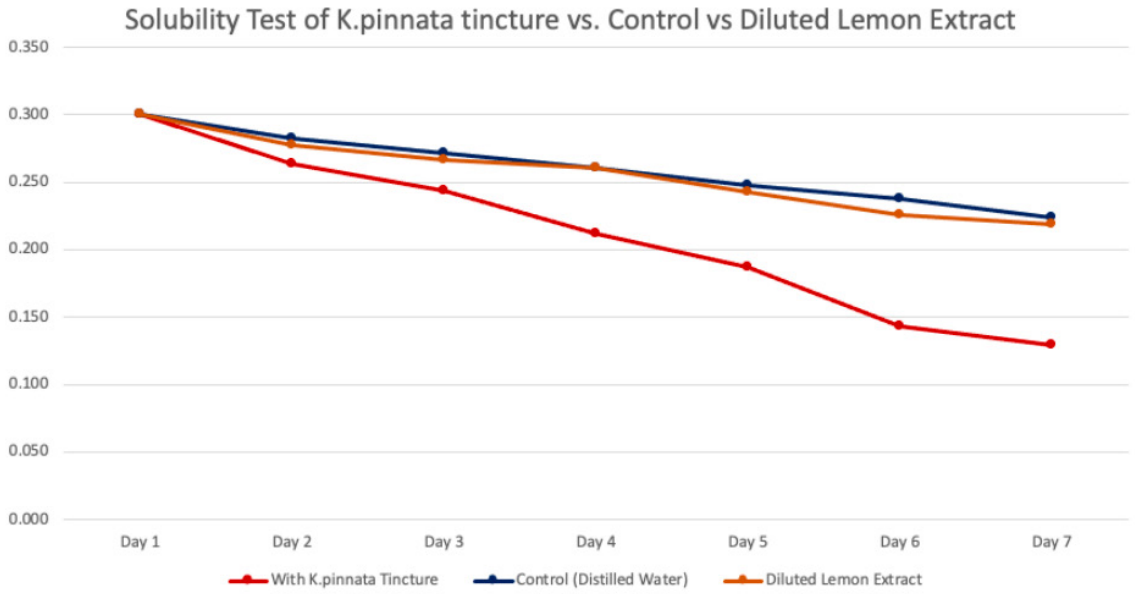

Figure 3. Results for Solubility Test

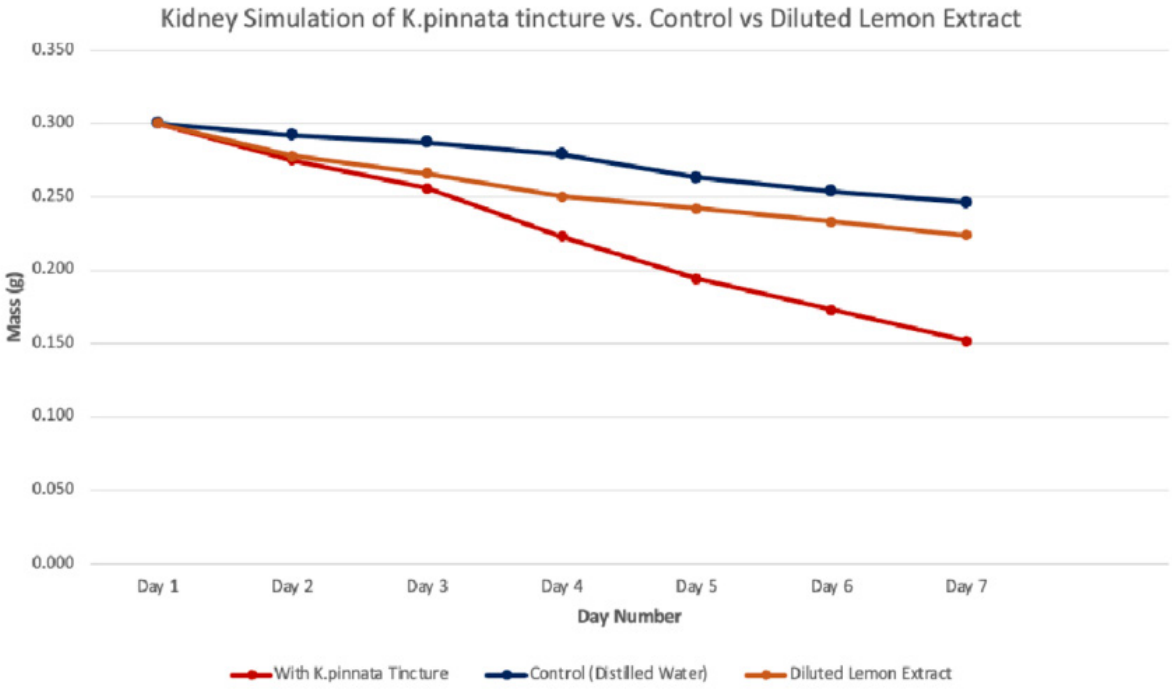

Figure 4. Results for Kidney Simulation

known to be effective in dissolving calcium oxalate and calcium phosphate stones while flavonoids prevent kidney stones from damaging the kidney and lower the likelihood of urinary disorders caused by kidney stones. Glycosides slow down the formation of calcium oxalate stones and are the reason for *K. pinnata*'s diuretic (increasing bodily production of urine), anti-inflammatory, and antibacterial properties.

Furthermore, the solubility test and the kidney simulation test also indicate that *K. pinnata* is an herb that can break down nephrolithiasis at a much quicker rate than water and a substance with a high pH such as lemon extract, once again showing its promise as a potential treatment option.

Although this study does show promise for *K. pinnata*'s nephrolithiasis treating abilities, there are some limitations in the final result. The tests in this experiment were conducted only once and with basic biochemistry tests, but results may vary with more trials and a higher standard of scientific equipment. Also, it is important to note that the rate of breakdown of the stones in both tests is subject to change, as the calcium oxalate stones were artificially made and do not exactly replicate what is formed in the human body. Further testing with real samples of nephrolithiasis could aid in finding a more accurate result of the rate of breakdown.

FUTURE STEPS

In the future with scalability in this research, the existence of even more organic acids, phytochemicals, and compounds that help dissolve calcium oxalate stones can be discovered. Due to the versatility of *K. pinnata*, the plant's impact on other illnesses can be potentially studied, as the organic acids and phytochemicals identified are also known in the scientific community to exhibit positive reactions on other medical issues.

A potential next step is to test *K. pinnata* on rats with kidney stones, to analyze if the plant has any side effects on the animal. In the future, information regarding *K. pinnata*'s ability to break down calcium oxalate stones can be used to create new medicine for acute nephrolithiasis that can be given to patients if basic treatments prescribed by family physicians do not work.

CONCLUSION

In conclusion, *K. pinnata* is a strong treatment against calcium oxalate stones and is more effective than what many doctors currently advise their patients, water. As millions continue to be diagnosed with acute nephrolithiasis, finding novel ways to treat patients in a less expensive and non-invasive method is key. Herbal medicine is a great place to start with this process, as many naturally occurring compounds and acids in herbs can break down kidney stones in the human body. Studies such as this promote scientific research on plants, as there are millions of plantae whose constituents can be used to treat diseases.

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