



## RESEARCH ARTICLE

### PHARMACOLOGICAL EVALUATION OF ANTIUROLITHIATIC POTENTIAL IN DAILY FOODS

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#### ABSTRACT

**Background:** Urolithiasis, commonly known as kidney stone disease, is a common and painful urinary disorder caused by the formation of stones in the kidneys, ureters, bladder, or urethra. It mainly occurs due to the crystallization of substances like calcium oxalate, uric acid, cysteine, and calcium phosphate in urine. The condition has a high recurrence rate and may lead to serious complications if untreated. Conventional treatments include increased water intake, medications, and surgical procedures such as extracorporeal shock wave lithotripsy (ESWL). However, these treatments can be costly, painful, and do not always prevent recurrence. Therefore, there is growing interest in natural and plant-based remedies. **Methods:** This study evaluated the anti-urolithiatic activity of five natural compounds: Ash gourd (*Benincasa hispida*), Bitter gourd (*Momordica charantia*), Coconut water (*Cocos nucifera*), Garlic (*Allium sativum*), and Apple (*Malus domestica*). Preliminary phytochemical screening showed the presence of beneficial compounds such as flavonoids, alkaloids, tannins, and terpenoids. In-vitro studies were conducted using nucleation and aggregation assays to examine their effect on calcium oxalate crystal formation. **Results:** The results showed that all selected compounds significantly reduced crystal formation, especially bitter gourd and garlic, which showed strong inhibitory effects. **Conclusion:** The study concludes that these natural ingredients may help prevent kidney stone formation and could serve as safer, affordable alternatives to conventional treatment. Further in-vivo and clinical studies are needed to confirm these findings.

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## INTRODUCTION

Calculi that arise in the kidneys, ureters, bladder, or urethra are often referred to as urolithiasis. After prostate disorders and urinary tract infections, it is the third most common urological condition (1). Urinary stones afflict about 12% of the world's population, and recurrences are more prevalent in men (70–80%) than in women (47–60%) (2). The emergence of urinary calculi in the renal tubule is the cause of urolithiasis. It is the development of crystals in the urinary tract. The stones fluctuate in size, shape, and crystal formation properties. It can be generated by the precipitation that falls of minerals and urine constituents as struvite, calcium oxalate, uric acid, cysteine, and calcium phosphate (3). The Greek words "Urone" for urine and "Lithos" for stones are the roots of the phrase "Urolithiasis." It has an alarming recurrence rate—14% after a year, 25.5–31.5% after five, 49–52% after ten, and 72% after twenty (4). The five main types of kidney stones are calcium oxalate monohydrate/dehydrate stones, uric acid stones, struvite stones, cysteine stones, and drug-induced stones. Of these, calcium oxalate monohydrate stones are the most widespread, followed by calcium oxalate dehydrate stones, cysteine stones, uric acid stones, struvite stones, and drug-induced stones (5). If not treated promptly, kidney stones can

be potentially hazardous because they obstruct the regular flow of urine and can get lodged anywhere in the urinary system. This can result in a number of secondary infections, which can ultimately cause renal failure, shock, coma, and death. To forestall these issues, kidney stones must be treated (6). Over 85% of human stones comprise a combination of calcium phosphate and CaOX. Formation of CaOx stone is a multi-step technique involving nucleation, crystal growth, crystal aggregation, and crystal retention. Increasing water intake, urine alkalinizing and calcium chelating medications (sodium bicarbonate, potassium) are being used as preventive therapies for eliminating kidney stones. Alkaline citrates lessen the urine's CaOx supersaturation (7). Treatments for urolithiasis rely on the size, location, and composition of the stones in the urinary tract. Alpha-blockers and drinking a lot of water throughout the day are two ways to treat tiny calculi. Extracorporeal shock wave lithotripsy (ESWL) is a therapy option for big stones that fragments them into little pieces. This treatment might damage the urinary system and can be fairly expensive. Furthermore, they are not hindering new stones from emerging (8). Many old traditional kidney stone treatment regimens have made extensive use of medicinal

herbs. These plants are widely accessible, reasonably priced, and thought to be relatively safe with few or no negative effects (9). The etiology is complex and closely linked to eating habits and lifestyle choices. Higher incidences of obesity and hypertension further exacerbate stone formation (10). Urinary pain, excruciating side and back pain, pain in the genitalia, blood in the urine, burning when urinating, increased frequency of urination and cloudy urine, fever with chills (11).

Kidney disease has historically been treated using traditional remedies, the majority of which arise from plants. More than half of patients encounter a stone recurrence within five years of their initial therapy, even though urolithiasis can be managed through surgical removal, which is more costly and has a financial burden (12). It is worthwhile to investigate novel pharmacological therapies for the treatment of kidney stones because both surgical methods and pharmacotherapy alternatives are limited.

## MATERIALS AND METHODS

**Collection of selected medicinal compounds:** Compounds were obtained from an organic store and local market.

**Preliminary phytochemical screening of selected compounds:** To determine the active elements of selected compounds, including tannins, Saponins, flavonoids, phenols, Anthraquinones, alkaloids, Terpenoids, carotenoids, coumarins, etc. A preliminary qualitative screening of phytochemicals has been conducted. The selected compounds are Ash gourd, Apple, Bitter gourd, coconut water, and Garlic.

### Ash gourd:



Figure 1. Ash gourd

*Benincasa hispida* cong. (Thunb.). It is an annual climber plant that is a member of the Cucurbitaceae family. Its fruits are often referred to as winter melon, ash gourd, winter gourd, white pumpkin, etc (13). Volatile oils, flavonoids, glycosides, saccharides, proteins, carotenes, vitamins, minerals, beta-sitosterol, and uronic acid were the main chemical components of *Benincosa hispida* fruits (14). The ash gourd, which is high in water content, effectively lowers body heat and helps maintain the health of the liver and kidneys. It also assists with dysuria, constipation, urinary tract infections, the removal of kidney stones, the regulation of renal processes, and promoting smooth bowel and bladder movements (15).

### Bitter gourd:



Figure 2. Bitter gourd

Bitter gourd (*Momordica charantia* L.) is an important cucurbit vegetable having immense medicinal and nutritional properties (16). The tropical vine known as bitter gourd (*Momordica charantia* L.), often called bitter apple, bitter melon, or balsam pear, is a member of the Cucurbitaceae family. Common sugars, proteins, and chlorophyll are bitter gourd's core metabolites; phenolics, carotenoids, triterpenoids, alkaloids, saponins, etc. are its secondary metabolites (17).

### Coconut water



Figure 3. Coconut Water

Sugar, vitamins, minerals, free amino acids, proteins, and growth-promoting elements are all present in coconut water (*Cocos nucifera* L.), also known as liquid endosperm from coconuts (18). In many cultures throughout the world, particularly in Africa, India, and the Philippines, coconut water is used in traditional medicine or dietary interventions to treat a variety of ailments, such as UTIs, kidney problems including stones, gastroenteritis, and coronary heart disease (19). For example, CW's diuretic qualities facilitate the removal of tiny stones from the urinary system by increasing urine flow (20).

### Garlic:



Figure 4. Garlic

Garlic also known as *Allium*, this consists of bulbs of the plant known as *Allium sativum* Linn. Belonging to family Liliaceae. Garlic contains sulfur compounds (alliin, allicin, ajoene and other), several enzymes (allinase, peroxidases, myrosinase) minerals, vitamins, fiber and water. Amino acids such as lysine, histidine, arginine, teucine etc are present. Oligosaccharides, peptides, steroids, Terpenoids, flavonoids, and phenols carbohydrates are present (21). Allicin possess Antifungal, Anti bacterial activity.

## Apple



Figure 5. Apple

*Malus Domestica*, commonly called Apple belongs to rosaceae family. Different fruit portions contain bioactive substances such as flavonoids, polyphenols, pentacyclic triterpenes, dihydrochalcones, polysaccharides, a good amount of proteins, lipids, and dietary fiber, as well as trace levels of vitamins and minerals (22). Apples have demonstrated positive effects on health against cancer, asthma and pulmonary dysfunction, cardiovascular illnesses, Alzheimer's disease, decrease of natural aging, weight management, and diabetes because of their high nutraceutical qualities and different polyphenols (23).

## MATERIALS AND METHODS

**NUCLEATION ASSAY:** The test was conducted both with and without an inhibitor (control). In a buffer consisting of 50 mM Tris and 150 mM sodium chloride at pH 6.5, solutions containing approximately 5 mM and 7.5 mM calcium chloride and sodium oxalate were prepared. Standard (cystone) and sample stock solutions were made at a concentration of 10 mg/ml. Both the control and sample sets received an addition of approximately 1 milliliter of calcium chloride. In the control set, 1 milliliter of distilled water was also added. Instead of using distilled water, 1 milliliter of different sample dilutions (200,400,600,800, and 1000µg/mL) was added to the sample set. One milliliter of sodium oxalate solution was added to each set to initiate crystallization. After 30 minutes of incubation at 370 nm, a spectrophotometer was used to measure the absorbance at 620 nm. The percentage of nucleation inhibition was computed as follows (24), (25)

$$\text{Percent inhibition} = C-S/C \times 100$$

Where,

C= turbidity of control set

S= turbidity of the sample set

## ASSAY FOR AGGREGATION

Large particle aggregates are created when crystals in liquids adhere to one another. Solutions of  $\text{CaCl}_2$  and  $\text{Na}_2\text{C}_2\text{O}_4$  (50 mmol/l) were combined and heated to 600 degrees Celsius in a water bath for one hour and then prepared seed  $\text{CaOx}$  crystals by incubating at 370°C for the entire night. Following drying, a 0.8 mg/ml  $\text{CaOx}$  crystal solution was made in a pH 6.5 buffer containing 0.05 mol/l Tris-Hcl and 0.15 mol/l NaCl. After adding 1 ml of extract (200, 400, 600, 800, and 1000 µg/mL) to 3 ml of  $\text{CaOx}$  solution, the mixture was vortexed and incubated for 30 minutes at 370 °C. The final mixture's optical density was measured at a wavelength of 620 nm, and the percentage of aggregation inhibition was computed.

$$\% \text{Inhibition} = (1 - \text{OD Test} / \text{OD Control}) \times 100$$

OD Test is optical density of standard drug, OD Control is optical density without sample/ standard drug (26).

## RESULTS AND DISCUSSION

The chosen medicinal substances were examined for their anti-urolithiatic efficacy against chemically generated calcium oxalate crystals due to their well-known use in traditional medicine. Urolithiasis is a common, painful disease that has afflicted the human population since ancient times. Medicinal plants have played a significant role in various ancient traditional systems of medication. Even today, plants provide an affordable source of drugs for majority of world's population. Several pharmacological investigations on the medicinal plants used in traditional anti-urolithic therapy have revealed their therapeutic potential in the in-Vitro models (27). Compared to allopathic medicine which targets one aspect of the pathophysiology of urolithiasis, plant-based drugs have shown to be effective at multiple stages in the prevention of the formation of calculi. Previous studies have reported that the phytoconstituents present in medicinal plants exert their beneficial effects by multiple mechanism, such as changing the ionic concentration of urine, inhibition of nucleation, growth, aggregation during crystal formation, promoting lithotriptic activity by relieving mucin that binds calculi, and regulation of crystalloid colloid imbalance to improve renal function (28). Phytochemical screening of medicinal plants is very important in identifying new sources of therapeutically and industrial importance (29).

In this study, Phytochemical screening showed that coconut water, bitter gourd, garlic powder, ash gourd, and apple contain many bioactive compounds such as alkaloids, flavonoids, steroids, tannins, saponins, terpenoids, carotenoids, phenols, anthraquinones,  $\beta$ -sitosterol, and cardiac glycosides. These samples were tested for the presence other compounds including coumarins, quinones, anthocyanins, and phlobatannins. From the results obtained (Table-1) it was confirmed that Coconut water is missing several compounds like saponins, coumarins, and phenols. Bitter gourd does not contain coumarins, carotenoids, and anthocyanins. Garlic powder lacks compounds such as coumarins, quinones, and anthocyanins. Ash gourd is missing xanthoproteins, quinones, and anthraquinones and Apple lacks many compounds including alkaloids, steroids, tannins, and saponins. The selected medicinal compounds were tested using nucleation and aggregation assays, with Cystone used as the standard drug

Table 1. Summary of phytochemical Analysis of selected compounds

Phytochemicals	Coconut water	Bitter gourd	Garlic powder	Ash gourd	Apple
Alkaloids	+	+	+	+	-
Flavonoids	+	+	+	+	+
Steroids	+	+	+	+	-
Tannins	+	+	+	+	-
Saponins	-	+	+	+	-
Terpenoids	+	+	+	+	+
Coumarins	-	-	-	+	-
Xanthoproteins	-	+	-	-	-
Carotenoids	-	-	+	+	+
Quinones	+	+	-	-	-
Anthraquinones	+	+	+	-	-
Phenols	-	+	+	+	+
Anthocyanins	+	-	-	-	-
Cardiac glycosides	-	+	+	+	-
Phlobatinnins	-	+	-	-	-
$\beta$ -sitosterol	-	-	-	+	-

(+) represents the presence and (-) represents the absence of constituents

Table 2. Nucleation assay of selected compounds

Conc. ( $\mu$ g/ml)	Mean $\pm$ SEM values					
	Cystone(Std)	Apple	Garlic	Coconutwater	Ashgourd	Bittergourd
200	0.13 $\pm$ 0.0115	0.23 $\pm$ 0.0088	0.23 $\pm$ 0.0058	0.27 $\pm$ 0.0153	0.45 $\pm$ 0.0115	0.14 $\pm$ 0.0145
400	0.10 $\pm$ 0.0088	0.21 $\pm$ 0.0058	0.19 $\pm$ 0.0088	0.25 $\pm$ 0.0115	0.41 $\pm$ 0.0115	0.11 $\pm$ 0.0088
600	0.07 $\pm$ 0.0058	0.18 $\pm$ 0.0088	0.17 $\pm$ 0.0067	0.20 $\pm$ 0.012	0.37 $\pm$ 0.0088	0.10 $\pm$ 0.0067
800	0.05 $\pm$ 0.0088	0.15 $\pm$ 0.012	0.16 $\pm$ 0.0088	0.17 $\pm$ 0.0153	0.30 $\pm$ 0.0088	0.07 $\pm$ 0.0088
1000	0.02 $\pm$ 0.012	0.10 $\pm$ 0.0088	0.15 $\pm$ 0.0115	0.10 $\pm$ 0.0145	0.28 $\pm$ 0.0058	0.04 $\pm$ 0.0058
Control	0.21 $\pm$ 0.0088	0.26 $\pm$ 0.0033	0.31 $\pm$ 0.0186	0.31 $\pm$ 0.0088	0.58 $\pm$ 0.0058	0.21 $\pm$ 0.0058

Table 3. Percentage inhibition of Nucleation assay of selected compounds

Conc. ( $\mu$ g/ml)	Cystone (Std)	Apple	Garlic	Coconutwater	Ash gourd	Bittergourd
200	38.09%	11.53%	25.80%	12.90%	22.41%	33.33%
400	52.38%	19.23%	38.70%	19.35%	29.31%	47.61%
600	66.66%	30.76%	45.16%	35.48%	36.20%	52.38%
800	76.19%	42.30%**	48.38%***	45.16%**	48.27%***	66.66%
1000	90.47%	61.53%**	51.61%***	67.74%*	51.72%***	80.95%

\*Statistically significant  $p < 0.01$  \*\*Very statistically significant  $p < 0.001$  \*\*\*Extremely significant  $p < 0.0001$

Table 4. Aggregation assay of selected compounds

Conc. ( $\mu$ g/ml)	Mean $\pm$ SEM values					
	Cystone(Std)	Apple	Garlic	Coconutwater	Ash gourd	Bittergourd
200	0.14 $\pm$ 0.0145	0.17 $\pm$ 0.0145	0.08 $\pm$ 0.012	0.11 $\pm$ 0.0058	0.14 $\pm$ 0.0115	0.09 $\pm$ 0.012
400	0.11 $\pm$ 0.012	0.14 $\pm$ 0.0176	0.06 $\pm$ 0.0088	0.09 $\pm$ 0.0033	0.11 $\pm$ 0.0088	0.07 $\pm$ 0.0088
600	0.09 $\pm$ 0.0088	0.10 $\pm$ 0.0088	0.04 $\pm$ 0.0058	0.07 $\pm$ 0.0115	0.10 $\pm$ 0.0145	0.05 $\pm$ 0.0088
800	0.08 $\pm$ 0.0058	0.08 $\pm$ 0.012	0.03 $\pm$ 0.0058	0.05 $\pm$ 0.0088	0.07 $\pm$ 0.0088	0.04 $\pm$ 0.0088
1000	0.06 $\pm$ 0.0058	0.07 $\pm$ 0.0115	0.02 $\pm$ 0.0058	0.04 $\pm$ 0.0058	0.05 $\pm$ 0.0088	0.03 $\pm$ 0.0088
Control	0.21 $\pm$ 0.0033	0.21 $\pm$ 0.0033	0.22 $\pm$ 0.012	0.23 $\pm$ 0.0088	0.22 $\pm$ 0.012	0.24 $\pm$ 0.0058

Table 5. Percentage inhibition of Aggregation assay of selected compounds

Conc. ( $\mu$ g/ml)	Cystone(Std)	Apple	Garlic	Coconutwater	Ashgourd	Bittergourd
200	33.33%	19.04%	63.63%	52.17%	36.36%	62.5%
400	47.61%	33.33%	72.72%	60.86%	50%	70.83%
600	57.14%	52.38%	81.88%	69.56%	54.54%	79.16%
800	61.90%	61.90%	86.36%**	78.26%**	68.18%	83.33%
1000	71.42%	66.66%	90.90%**	82.60%*	77.27%	87.5%

\*Statistically significant  $p < 0.01$  \*\*Very statistically significant  $p < 0.001$

as these assay methods will indicate the potential activity of the selected medicinal compounds in prevention and control of kidney stone formation.

**Nucleation assay:** The Mean  $\pm$  SEM values of the selected compounds by nucleation assay are mentioned in Table-2. In the nucleation assay, inhibition increased with concentration (200–1000  $\mu$ g/ml) for all samples.(mentioned in Table-3)Cystone showed the highest activity (38.09–90.47%), followed by bitter gourd (33.33–80.95%). Coconut water

(12.99–67.74%) and apple (11.53–61.53%) showed moderate activity, while garlic powder (25.80–51.61%) and ash gourd (22.41–51.72%) showed comparatively lower inhibition.

**Aggregation assay:** The Mean  $\pm$  SEM values of the selected compounds by aggregation assay were mentioned in Table-4. In the aggregation assay, inhibition increased with concentration (200–1000  $\mu$ g/ml) for all samples.(mentioned in Table-5)Garlic powder showed the highest inhibition

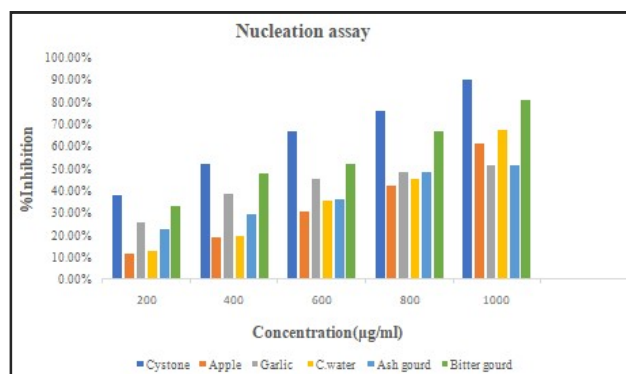


Figure 6. Percentage of Inhibition by Nucleation Assay

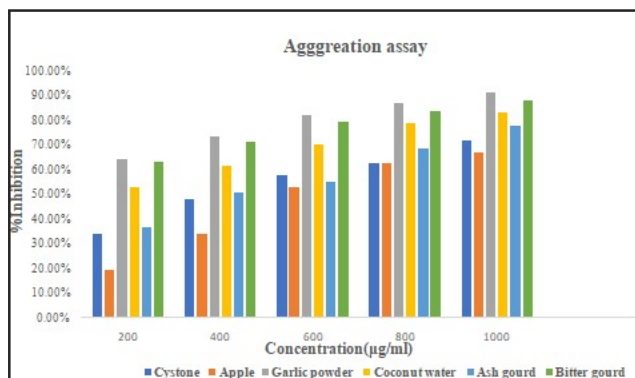


Figure 7. Percentage inhibition of the Aggregation assay

(63.63–90.90%), followed by bitter gourd (62.5–87.5%) and coconut water (52.17–82.60%). Ash gourd (36.36–77.27%) and apple (19.04–66.66%) showed moderate activity, while Cystone showed lower inhibition (33.33–71.42%). All the samples have shown significant inhibition at 800 (µg/ml) and 1000 (µg/ml). Among them Ash gourd and Garlic powder have exhibited extremely significant against Cystone in Nucleation assay and exhibited very statistically significant against Cystone in Aggregation assay.

## CONCLUSION

Urolithiasis is a progressive and recurrent condition that often requires surgical intervention or pharmacological treatment. However, both approaches are frequently associated with significant pain, high treatment costs and an increased financial burden on patients. Furthermore, the recurrence rates remain notably high, with approximately 10% of cases recurring within the first year, 33% by the fifth year and up to 50% by the tenth year, highlighting the urgent need for effective, alternative preventive therapies. Medicinal plants have long served as valuable sources for drug development and continue to play a crucial role in modern therapeutic research. The use of natural foods and plant-based remedies is drawing growing attention because of their easy availability, fewer side effects, and lower cost compared to synthetic medicines. This study adds valuable insight to this area by showing the potential benefits of everyday fruits and vegetables in preventing kidney stones. This research focused on evaluating the anti-urolithiatic activity of Apple, Ash gourd, Bitter gourd, Garlic & Coconut water using In-Vitro methods, nucleation and aggregation assay. The results showed that all selected compounds were effective in reducing calcium oxalate crystal formation, nucleation and aggregation, key processes involved

in kidney stone development. This suggests that these natural ingredients may help in preventing stone formation. All the selected compounds showed varying degrees of activity, indicating the presence of chemical compounds that can either slow down or stop the formation of kidney stones. Although the current investigation was limited to in vitro analysis. The outcomes are encouraging. This suggests that these compounds may be used as an alternative in the treatment of kidney stones.

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## List of Abbreviations

- C – Control (turbidity of control set)
- CaCl<sub>2</sub> – Calcium chloride
- CaOx – Calcium oxalate
- CW – Coconut water
- ESWL – Extracorporeal Shock Wave Lithotripsy
- In-vitro – Laboratory-based experimental study (outside a living organism)
- mM – Millimolar
- mg/ml – Milligrams per milliliter
- NaCl – Sodium chloride
- Na<sub>2</sub>C<sub>2</sub>O<sub>4</sub> – Sodium oxalate
- OD – Optical Density
- pH – Potential of Hydrogen (measure of acidity/alkalinity)
- S – Sample (turbidity of sample set)
- SEM – Standard Error of Mean
- Tris – Tris(hydroxymethyl)aminomethane (buffer)
- UTI – Urinary Tract Infection
- µg/ml – Micrograms per millilitre

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