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Original Article

Evaluation of Antiurolithiatic Effects of *Capsicum Annuum* and *Amaranthus Viridis* in Glycol Induced Urolithiasis in Wistar Rats

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HIGHLIGHTS

- Exploration of antiurolithiatic effects of *Amaranthus viridis* and *Capsicum annuum* extracts in rats.
- *In-vitro* assays were performed using aggregation and nucleation assay and *in-vivo* antiurolithiatic activity was performed by ethylene glycol induced urolithiasis model.
- Ethanolic extract demonstrated protective effects against hypertrophy and tissue damage compared to the other extracts.

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ABSTRACT

Introduction
This study investigates the potential antiurolithiatic properties of two nutraceutical herbs, *Amaranthus viridis* and *Capsicum annuum*. Both herbs, known for their medicinal and nutritional benefits, has been explored for their roles in preventing or treating kidney stone formation.
Methods
Ethanolic extracts of *Amaranthus viridis* leaves and *Capsicum annuum* fruits were selected for evaluating anti-urolithiatic activity in Wistar rats due to presence of flavanoids, phenols, saponins and tannins. *In-vitro* assays were performed using aggregation and nucleation assay and *in-vivo* antiurolithiatic activity was performed by ethylene glycol induced urolithiasis model.
Results
In-vitro results showed that both plant extracts and Cystone effectively inhibited nucleation and aggregation steps in kidney stone formation. The plant extracts demonstrated stronger inhibition during the aggregation phase, suggesting they may serve as a potent alternative or complement to cystone in preventing kidney stones. *In-vivo* evaluations using a glycol-induced urolithiasis model indicated that treatment with the extracts inhibited the increment in calcium and uric acid levels, thereby promoting a less acidic urinary environment. Serum analysis revealed reductions in creatinine and urea levels, indicating improved renal function. Kidney weight assessments and histopathological examinations of kidneys showed protective effects against hypertrophy and tissue damage.
Conclusions
Findings suggested that *Amaranthus viridis* and *Capsicum annuum* possess significant antiurolithiatic property, supporting their potential as natural remedies for kidney stone prevention. Further research is recommended to isolate the active compounds and explore their mechanisms of action.

Keywords: Antiurolithiatic Activity; *Amaranthus Viridis*; *Capsicum Annum*; Ethanolic Extracts; Ethylene Glycol; Kidney Stones

Introduction

Urolithiasis is a prevalent kidney stone disease where solid crystals form within the kidneys due to supersaturated urine. The development of these stones is influenced by several factors, including urinary pH, mineral concentration, and the presence of inhibitors or promoters of crystallization (1). When the urine becomes oversaturated, it leads to the formation of crystals, which can grow into stones if they are not flushed out by the body. Kidney stones in urolithiasis are of four types and each type is associated with its own causes and therapy approach (2). Urolithiasis affects around 12% of the global population at some point in their lives, with a higher prevalence in men (approximately 13%) than women

(7%). The recurrence rate is also significant, with up to 50% of patients developing new stones within 5-10 years of their initial episode. Calcium oxalate stones, which are the most prevalent type, represent approximately 50-60% of all kidney stone cases (3). Uric acid stones make up 10-15%, while calcium phosphate stones contribute to around 10-20% of cases (4). Hypercalciuria, a key factor in calcium oxalate stone formation, is present in around 35-65% of individuals who form these stone. Similarly, uric acid stones are more prevalent in individuals with metabolic disorders like diabetes and obesity, accounting for about 10% of all cases. Despite various conventional treatments for urinary stones, there is growing interest in alternative and complementary approaches, such as herbal remedies. This study intends to assess the antiurolithiatic properties of the fruit extract of *Capsicum annuum* and the leaf extract of *Amaranthus viridis*. *Capsicum annum* contains capsaicin, which can increase urine volume, and flavonoids, whichcanprevent calcium oxalate formation. *A. viridisis* also known as Chowlai/ amaranth crop contains flavonoids, steroids, phenols, triterpenoids, tannins and saponin. Due to the presence of saponins this herb can dissolve kidney stone by breaking the crystals of the calcium oxalate into monohydrate. This breakdown process may reduce the size and aggregation of the crystals, thereby promote their dissolution and prevent further stone formation.

Methods

Plant extract

The leaves of *Amaranthus viridis* were harvested in June from the Haldwani region of Uttarakhand, while the fruits of *Capsicum annuum* were collected in September from the Bhowali area of Nainital, Uttarakhand. Both plant samples were authenticated by the Botanical Survey of India (BSI), Dehradun, Uttarakhand, with accession numbers 1227 and 1226, respectively.

Preparation of extract

The leaves of *Amaranthus viridis* and the fruits of *Capsicum annuum* were collected from Haldwani and Bhowali region of Nainital, Uttarakhand respectively. After collection they were washed to remove extra sand and unwanted particles (5). Then they were shade-dried for 15 and 30 days, respectively. Once dried, the plant materials were ground into coarse powder. A total of 100 g of each dried powder was subjected to sequential extraction using 750 mL of petroleum ether, followed by ethanol, a hydroethanol mixture (70:30), and water, using the Soxhlet extraction method to isolate compounds with varying solubilities (6). Extract was filtered (by filter paper), concentrated (by rotary vacuum evaporator) and dried. Percentage yield was calculated of obtained extracts.

Phytochemical screening

Phytochemical screening of powder drug and plant extract was performed using standard procedure (7).

The ethanolic extracts of *Capsicum annuum* and *Amaranthus viridis* were standardized for their phytochemical content. Total phenolic content (TPC) was estimated using the Folin–Ciocalteu method and expressed as milligrams of gallic acid equivalents (mg GAE/g extract). Total flavonoid content (TFC) was determined using the aluminum chloride colorimetric assay and expressed as milligrams of quercetin equivalents (mg QE/g extract).

Total phenolic content estimation: To1mL test sample (1mg/ml), 0.5ml Folin reagent and 3 ml ethanol was added and mixed. After 3 min 2% Na_2CO_3 solution was added and incubated for 90 min at 25 ℃. After incubation absorbance was measured at 430nm and Gallic acid was taken as standard phenolic compound.

Total flavonoid content estimation: To 2% aluminum chloride solution, 1 mL each of methanol and test samples (1mg/ml) was added and mixed. After mixing contents were incubated at 37℃ for 15 min. Absorbance was measured at 415 nm using Shimadzu UV/VIS spectrophotometer. The total flavonoid content was quantitatively estimated by constructing a standard curve using quercetin (8).

In-vitro-antiurolithiatic activity

Nucleation assay: 5mM/L calcium chloride solution and 7.5mM/L sodium oxalate solution was prepared in a Tris-buffered saline (TBS) of pH 6.5. Test samples were diluted in distilled water to concentrations ranging from 100-500 µg/mL.Samples were prepared in concentration ranging from 100-500ug/ml and in to each 1ml samples 3ml of $CaCl_2$ and $Na_2C_2O_4$ solution was added. This reaction mixture was incubated at 37°C for 30 minutes and percentage inhibition of calcium oxalate crystal was measured (620nm) (9).

%Inhibition=(1-treated nucleation/control nucleation)×100

Aggregation assay: 50 mM/L solutions of calcium chloride and sodium oxalate were mixed and heated for 1hr at 60°C and then incubated overnight at room temperature to ensure complete crystal growth. After 24 hours they were centrifuged and supernatant was evaporated to obtain dry calcium oxalate crystals. Then Slurry of calcium oxalate was prepared by mixing 8mg crystals in 10ml TBS (pH 6.5). 3mL of slurry was mixed with 1 ml (100-1000µg dilutions) of test samples. These mixtures were incubated at 37℃ and then after 30 min sample's optical density was measured at 620nm (9).

%Inhibition=(1-treated optical density/control optical density) ×100

Animal Procurement and Housing Conditions

Male Wistar rats, ranging from 100–150 g, were sourced from the animal house of the Pharmacy Department,

Kumaun University, Bhimtal, following the approval of the study protocol by the Institutional Animal Ethical Committee (Protocol No. KUDOPS/167). Upon arrival, the rats were housed under controlled environmental conditions:

Temperature: 22±2°C, Humidity: 35–60%, Light/ Dark Cycle: 12 hours. The animals were provided with a pellet diet and water ad libitum to ensure adequate nutrition and hydration (10).

Acute toxicity studies

Wistar rats were randomly chosen and acclimatized with experimental conditions for one week. After 1-week animals were fasted overnight, with unrestricted access to water, before the dosing procedure. Observations for toxic symptoms and mortality were carried out continuously during the first 4 hours and periodically over the next 24 hours after dosing with AVEE and CAEE. The results indicated no mortality at doses up to 2000 mg/kg, suggesting that the extracts are safe at this dosage (11).

Ethylene glycol-induced urolithiasis model

Seven groups of male Wistar rats, with six animals in each group, were randomly assigned to different treatments: normal control, disease control, standard treatment, and four test groups. The normal control group received normal saline (2.5 mL/kg, i.p., once daily). To induce urolithiasis, the disease control, standard, and test groups were administered 1% w/w ammonium chloride (only for 3 days) along with 0.75% v/v ethylene glycol (continuous for 28 days) in their drinking water for the first three days. This model replicates conditions conducive to calcium oxalate crystal formation, mimicking the pathophysiology of urolithiasis in humans (12, 13).

Urine Acquisition: On 28th day, urine was collected by placing the animals in metabolic cages in groups of three. The collected urine was used for biochemical analysis to evaluate parameters relevant to urolithiasis, such as urinary calcium levels, oxalate, uric acid and pH (14). Calcium level in urine was analyzed by using commercially available colorimetric kits according to the manufacturer's protocols, pH was measured using digital pH meter and was examined under a light microscope at 40× magnification to detect the presence and morphology of calcium oxalate crystals.

Serum Analysis: Blood samples of rats were obtained from retro-orbital plexus under light ether anesthesia on day 28. The serum was separated by centrifugation and analyzed for key biochemical markers, including magnesium, urea, and creatinine, which are indicative of kidney function and metabolic changes associated with urolithiasis.

Kidney Histopathology: After 28 days, the animals were sacrificed under ether anesthesia, and their kidneys were carefully excised. The harvested kidneys placed in vial containing 10% v/v formalin-buffered saline for fixation. The tissues were processed using standard paraffin embedding techniques, sectioned at 5 µm thickness, and stained with hematoxylin and eosin (H&E). Histopathological examination was performed to assess renal structural changes, crystal deposition, and tissue damage associated with urolithiasis. This comprehensive assessment provides insights into the protective or therapeutic effects of the test substances on renal morphology.

Statistical evaluation

The data are expressed as mean standard deviation (SD). To assess the differences between groups, a one-way analysis of variance (ANOVA) was performed, followed by Tukey's multiple comparison test. All statistical analyses were conducted using GraphPad Prism 10 software.

Results

Extractive Yield of *Amaranthu sviridis* leaves and *Capsicum annuum* fruit

In the present study, 100 g dried powder was subjected to sequential extraction using 750 mL of petroleum ether, followed by ethanol, a hydroethanol mixture (70:30) and water, employing the Soxhlet extraction method to obtain compounds with different solubility profiles (Table 1) (6).

Phytochemical Investigation

Preliminary phytochemical screening provides an initial insight into the active principles present in the different extracts *Amaranthus viridis* leaves and *Capsicum annuum* fruit. Preliminary phytochemical investigation was performed with petroleum ether extract, ethanolic, hydroethanolic extracts and aqueous extract. The investigation confirmed the presence of numerous categories of secondary metabolites in the extracts, as depicted in the Table 2.

Total phenolic and flavanoid content

Total phenolic and flavanoid content was estimated by the standard curve of gallic acid and quercetin and total amount of phenol in CAEE (*Capsicum annuum* Ethanolic Extract) and AVEE (*Amaranthus viridis* Ethanolic Extract) was found out to be 1.84 GAE and 0.89 GAE respectively

Table 1. Comparative yield percentages of various extracts

Extract Types	Percentage Yield	
	***Amaranthus viridis* leaves Extracts**	***Capsicum annum* Fruit Extracts**
Petroleum Ether Extract	15.8%	7.44%
Ethanolic Extract	70.2%	60.5%
Hydroethanolic Extract	67.1%	40.7%

Table 2. Phytochemical screening of *Amaranthus viridis* leaves extract and *Capsicum annum* fruit extract

S. No	Phytochemical Class	Test Method Used		Petroleum Ether-Extract		Ethanolic Extract		Hydro-Ethanolic Extract		Aqueous Extract	
				AV	CA	AV	CA	AV	CA	AV	CA
1	Flavonoids	1.	Shinoda Test	+	+	+	+	+	+	-	+
		2.	Sulphuric Acid	+	+	+	+	+	+	+	-
2	Tannins & phenols	1.	Ferric chloride Test	-	-	+	+	+	-	-	-
		2.	Lead Acetate Test	-	-	+	+	+	+	-	-
3	Saponins	1.	Foam Test	+	+	+	+	+	+	+	+
4	Glycosides	1.	Keller-Killiani Test	-	-	+	+	+	+	+	-
		2.	Legal's Test	-	-	+	+	+	+	+	-
5	Alkaloids	1.	Dragendroff's Test	-	-	+	+	+	+	+	+
		2.	Mayer's Test	-	-	+	+	+	+		+
		3.	Hager's Test	-	-	+	+	+	+		+
		4.	Wager's Test	-	-	+	+	+	+		+
6	Carbohydrates	1.	Fehling's Test	-	-	+	+	+	+	-	-
		2.	Benedict's Test	-	_	+	+	+	+	-	-
7	Steroids	1.	Salkowski's Test	+	+	-	-	-	-	-	+
		2.	Liebermann-Burchard's Test	+	+	-	-	-	-	-	+
8	Proteins	1.	Biuret Test	-	-	+	+	+	+	-	+
		2.	Xanthoprotein Test	-	-	+	+	+	-	-	+
		3.	Millon's Test (MercuricNitrate)	-	-	+	+	+	-	-	+
9	Amino acids	1.	Ninhydrin Test	-	-	+	+	+	+	-	+
		2.	Test for cysteine	-		+	+	+	+	-	+
10	Volatile oils		General Test	+	+	-	-	+	+	-	-

"+" indicates Present, "-" indicates Absent, AV indicates *Amaranthus viridis* and CA indicates *Capsicum annum*

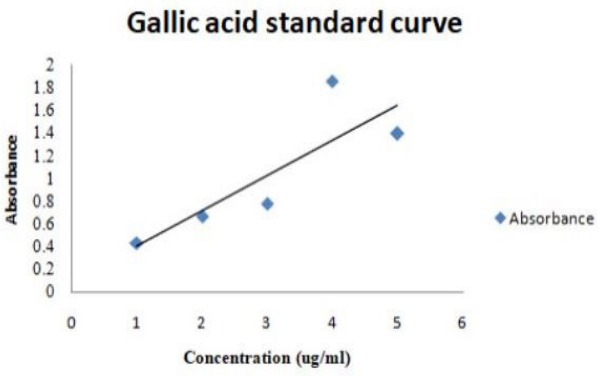

Figure 1. Calibration curve of Gallic acid

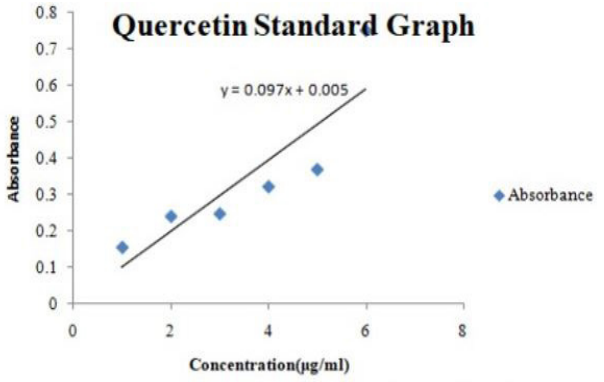

Figure 2. Calibration curve of quercetin

and total amount of flavonoid content in CAEE and AVEE was found out to be 0.24 QE and 0.67 QE (Figures 1& 2).

In-vitro antiurolithiatic Activity

In the *in-vitro* study, well established antiurolithiatic agent Cystone was chosen as standard drug. Cystone a polyherbal formulation by Himalayan Drug Company (India), was selected primarily due to its frequent citation as a reference standard in numerous *in vitro* and *in vivo* anti urolithiatic studies. The effect of various extract of *Amaranthus viridis* leaves and *Capsicum annuum* fruit, on the nucleation of CaOx crystals was evaluated in an *in vitro* experimental setup. Mixing the solution of calcium chloride and sodium oxalate resulted in formation of CaOx crystals. In the nucleation assay (Table 3), AVEE demonstrated a 32% inhibition of calcium oxalate crystal formation at a concentration of 30 µg/mL, while CAEE showed a 42% inhibition at the same concentration.

Table 3. In-vitro antiurolithiatic activity of *Amaranthus viridis* and *Capsicum annum* by nucleation assay

S. No	Concentration (ug/ml)	Cystone	*Capsicum annum*	*Amaranthus viridis*
		%Inhibition	%Inhibition	%Inhibition
1	100	29	17.50	19.65
2	200	38	28.80	30.20
3	300	53.2	31.37	49.22
4	400	61.6	58.61	54.98
5	500	78.04	71.09	65.33

Control absorbance=0.05

Table 4. In-vitro antiurolithiatic activity of *Amaranthus viridis* and *Capsicum annum* by aggregation assay

S. No	Concentration (ug/ml)	Cystone		*Capsicum annum*		*Amaranthus viridis*	
		OD	%Inhibition	OD	%Inhibition	OD	%Inhibition
1	100	0.087	52.97	0.092	50.27	0.118	36.22
2	200	0.085	54.05	0.088	52.43	0.100	45.95
3	400	0.057	69.19	0.079	57.35	0.094	49.19
4	600	0.048	74.05	0.067	63.78	0.082	55.68
5	800	0.046	75.14	0.054	70.81	0.065	64.86
6	1000	0.019	89.73	0.049	73.51	0.054	70.81

Similarly, in the aggregation assay (Table 4), AVEE achieved a 70.81% inhibition of crystal aggregation, whereas CAEE resulted in a 73.58% inhibition, with Cystone showing 89.73% inhibition at their highest concentration.FTIR of calcium oxalate (Figure 3) formed in aggregation assay showed peaks at 1315.21 cm−1^−1 and 780.1 cm−1^−1, which align with known calcium oxalate monohydrate (COM) peaks. Based on these peak positions, it is likely that calcium oxalate crystals were indeed formed in *in-vitro* aggregation assay method). Results indicate that the stronger performance of CAEE implies a higher concentration of effective phytochemicals, highlighting its potential as a more effective natural remedy.

In vivo Antiurolithiatic activity

Based on the finding of the *in-vitro* studies, the extract of *Amaranthus viridis* leaves and *Capsicum annuum* fruit exhibited significant inhibitory activity against all three major phases of calcium oxalate crystallization-nucleation, crystal growth and aggregation. The mechanisms involved are pivotal in the pathogenesis of urolithiasis and effective inhibition at each stage signifies considerable therapeutic potential. So therefore,further the *in vivo* study on wistar rats by ethylene glycol induced urolithiasis was performed.

Ethylene glycol induced urolithiasis in wistar rats

Ethylene glycol-induced model effectively simulates the pathophysiological process of stone formation of humans, making it an ideal choice for investigating the antiurolithiatic potential of EECA and EEAE. Although this model is widely used for its simplicity and reproducibility, it does not fully replicate idiopathic human urolithiasis, which typically occurs under normo- or mildly hyperoxaluric conditions and involves Randall's plaques (15-17). Still, it remains a valuable tool for screening antiurolithiatic agents (18). This study utilized Wistar rats due to their capacity to closely replicate human physiological and pathological conditions, thereby providing a relevant model for research focused on understanding and treating urolithiasis.

Urine parameters and analysis: In *in-vivo* antiurolithiatic study, urine pH was assessed to evaluate the impact of preventive treatment on urinary stone formation, as acidic pH conditions promote the development of stones (calcium oxalate and uric acid). The disease control group exhibited acidic urine due to stone formation, while the normal control group had alkaline urine pH (19) (Figure 4). When comparing the standard and test groups with the disease group there was significant difference (P-value<0.0001) in urine pH and found out to be alkaline in nature The antiurolithiatic treatment significantly influenced calcium metabolism and hyperuricemia in individuals predisposed to kidney stone formation (Figure 5). Observed reductions in urinary calcium and uric acid levels in the treatment groups suggest that EEAV and EECA extracts effectively mitigate the risk of calcium oxalate and uric acid stone development while in disease group there was increased level of calcium and uric acid levels (Figure 6). When urine was observed under microscope at 40×magnification

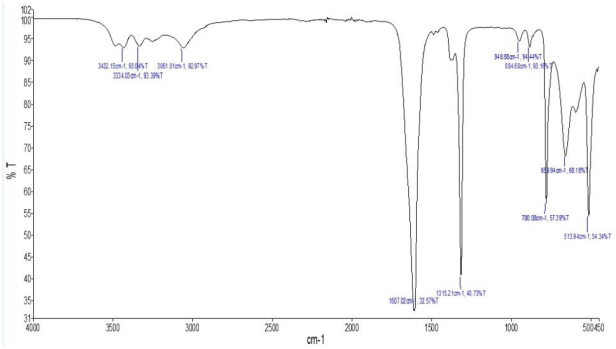

Figure 3. FTIR of calcium oxalate crystal formed in aggregation assay

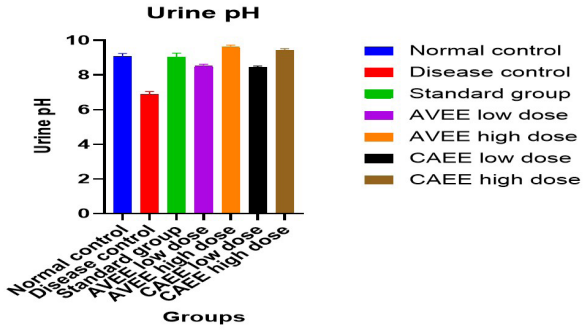

Figure 4. The data represents the urine pH in Wistar rats and results are presented as Mean ± SEM (n=6), CAEE: *Capsicum Annuum* Ethanolic Extract, AVEE: *Amaranthus viridis* Ethanolic Extract

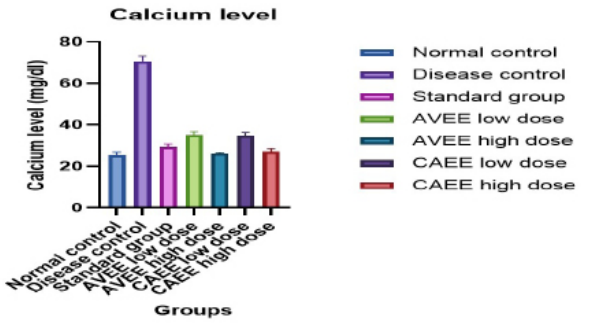

Figure 5. The data represents the calcium level in Wistar rats and results are presented as Mean ± SEM (n=6), CAEE: *Capsicum Annuum* Ethanolic Extract, AVEE: *Amaranthus viridis* Ethanolic Extract

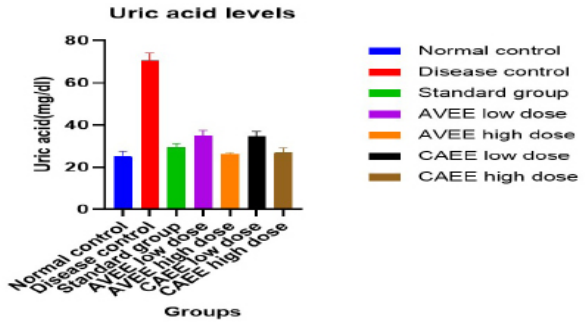

Figure 6. The data represents the uric acid level in Wistar rats and results are presented as Mean ± SEM (n=6), CAEE: *Capsicum Annuum* Ethanolic Extract, AVEE: *Amaranthus viridis* Ethanolic Extract

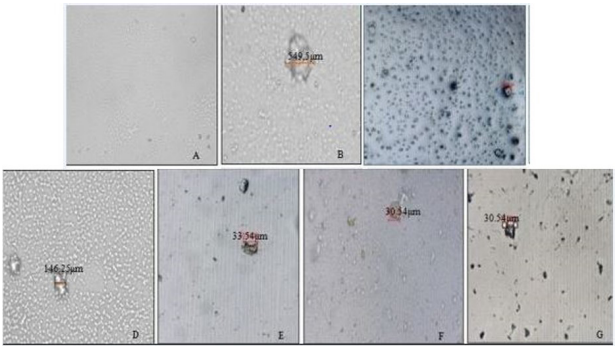

Figure 7. Urine microscopy depicting oxalates, A: Normal Control Group, B: Disease Control Group, C: Standard Group, D: AVEE Low Dose Group, E: AVEE High Dose Group, F: CAEE Low Dose Group, G: CAEE High Dose Group

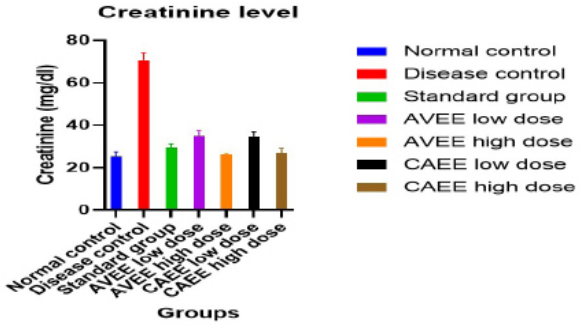

Figure 8. The data represents the creatinine level in Wistar rats and results are presented as Mean ± SEM (n=6),CAEE: *Capsicum Annuum* Ethanolic Extract, AVEE: *Amaranthus viridis* Ethanolic Extract

disease control was seen to develop aggregated forms of calcium oxalate while in treatment groups (standard and test groups) crystals are small, less aggregated and dispersed suggesting EEAV and EECA is effective in preventing stone growth and aggregation (Figure 7).

Serum analysis: Serum investigation indicated reduction in creatinine AVEE and CAEE treated groups, when compared with lithiaticcontrol group (Figure 8). This reduction suggests improved renal function and a lower burden of kidney damage associated with urolithiasis. Elevated creatinine levels are typically indicative of impaired kidney function, and the observed decrease in the treatment group's points to a protective effect from the herbal extracts. Similarly, serum urea levels were also less in treatment group when compared to lithiatic control (Figure 9). Elevated urea levels can signal renal dysfunction, as the kidneys are responsible for filtering urea from the bloodstream. The reduction in urea levels among the treatment groups further supports the notion that *A. viridis* and *C. annuum* exert beneficial effects on kidney health by promoting better filtration and reducing the nephrotoxic effects commonly associated

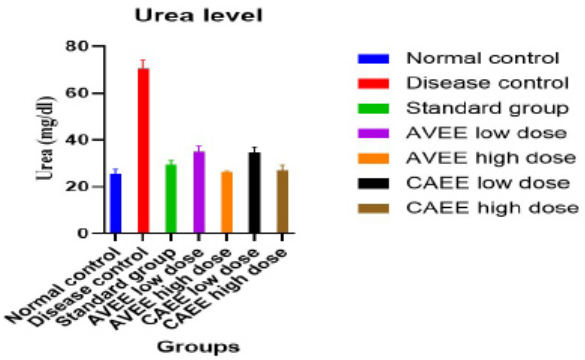

Figure 9. The data represents the Urea level in Wistar rats and results are presented as Mean ± SEM (n=6), CAEE: *Capsicum Annuum* Ethanolic Extract, AVEE: *Amaranthus viridis* Ethanolic Extract

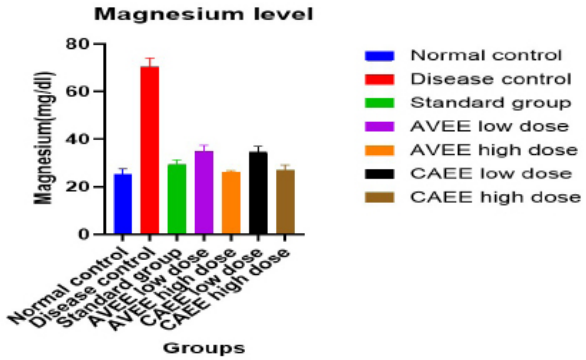

Figure 10. The data represents the magnesium level in Wistar rats and results are presented as Mean± SEM (n=6), CAEE: *Capsicum Annuum* Ethanolic Extract, AVEE: *Amaranthus viridis* Ethanolic Extract

Table 5. Qualitative and quantitative grading of pathological lesions in experimental groups

Group	Lesions	Severity (Qualitative)	Score (0–3)
Normal Control	No visible lesions	None	0
Disease Control	Severe necrosis, crystal deposition	Severe	3
Standard Group	Mild tubular changes	Mild	1
200 mg/kg AVEE	Moderate tubular/structural changes	Moderate	2
400 mg/kg AVEE	Minimal changes	Mild	1
200 mg/kg CAEE	Moderate lesions	Moderate	2
400 mg/kg CAEE	Almost normal (very few/minimal changes)	None–Mild	0–1

CAEE: *Capsicum Annuum* Ethanolic Extract, **AVEE**: *Amaranthus viridis* Ethanolic Extract

with stone formation. When kidney function is impaired, magnesium excretion is reduced, resulting in elevated serum magnesium levels. The analysis revealed that serum magnesium concentrations were found higher (P-value<0.0001) in disease control group compared to the normal group (Figure 10). There was no significant difference between the normal control and standard treatment groups, nor between the high-dose plant extract groups and the Cystone treatment group.

Kidney weight assessment: The assessment of kidney weight offered valuable insights into the effects of *Amaranthus viridis* leaves and *Capsicum annuum* fruits extracts on renal health. The results indicated that kidney weight in the treatment groups was not increased which was opposite to that was observed in lithiatic control group. Notably, *C. annuum* group exhibited a more pronounced protective effect against hypertrophy commonly associated with urolithiasis, as increased kidney weight typically signifies stress or damage related to stone formation and other pathological conditions (Figure 11).

Histopathological Examination: The histopathological examination further clarified the protective effects of both extracts in the context of urolithiasis. Microscopic analysis of kidney sections from the control group revealed significant pathological changes, including tubular degeneration, interstitial fibrosis, and calcified deposits, all indicative of severe damage due to stone formation. In contrast, kidney sections from the extract-treated groups displayed marked improvements in tissue architecture. Specifically, the *Capsicum annuum* group showed minimal tubular damage and a reduction in interstitial fibrosis, indicating effective preservation of renal structure and function (Table 5). In Figure 12 A depicts a normal control group with preserved renal architecture, tubules and glomeruli are healthy and intact, B shows major damage tubular degeneration, fibrosis and crystal deposition, C shows recovery compared to B, D, F and G shows protective effects in E and G depicts better preservation of structure (Figure 12).

Discussion

Urolithiasis is a prevalent kidney stone disease where solid crystals form within the kidneys due to supersaturated urine. The development of these stones is influenced by several factors, including urinary pH, mineral concentration, and the presence of inhibitors or promoters of crystallization (1). This study investigates the potential antiurolithiatic properties of two nutraceutical herbs, *Amaranthus viridis* and *Capsicum annuum*. Both herbs, known for their medicinal and nutritional benefits, has been explored for their role in preventing or treating kidney stone formation. The current study includes *in*

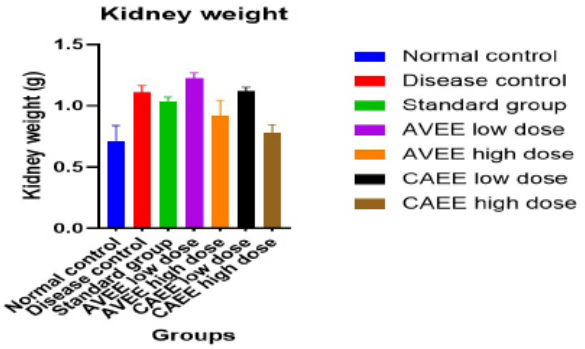

Figure 11. The data represents kidney weight in Wistar rats and results are presented as Mean±SEM (n=6), CAEE: *Capsicum Annuum* Ethanolic Extract, AVEE: *Amaranthus viridis* Ethanolic Extract

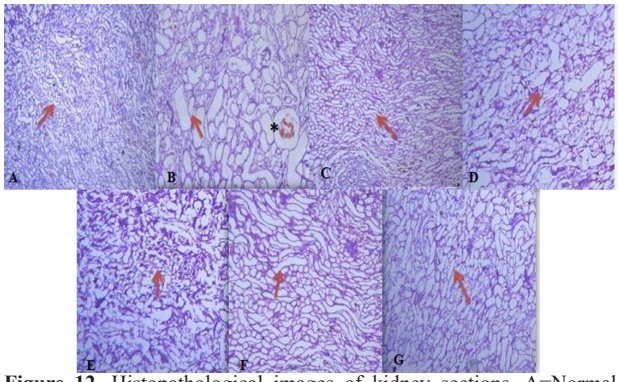

Figure 12. Histopathological images of kidney sections. A=Normal Control; B=Disease Control; C=Standard Group; D=200mg/kg *Amaranthus viridis;* E=400 mg/kg *Amaranthus viridis*; F=200mg/kg *Capsicum annum* G=400 mg/kg *Capsicum annum*. Lesions are marked with arrows (→) and crystal deposits are marked with (*)

vitro and *in vivo* assay, aimed to assess the antiurolithiatic activity of *Amaranthus viridis* leaves and *Capsicum annuum* fruit extracts in Wistar rats, emphasizing their impact on urinary parameters, stone formation, and biochemical markers. Intially the extraction was done using various solvents—petroleum ether, ethanol, hydroethanolic, and distilled water resulted in differing extract yields, reflecting variations in compound solubility and the efficiency of extraction (Table 1). After the extraction phytochemical analysis revealed that both *Amaranthus viridis* leaves and *Capsicum annuum* fruit contain a diverse spectrum of bioactive compounds, which tend to contribute to their antiurolithiatic potential. Phytochemical analysis of *Amaranthus viridis* leaves identified the presence of flavonoids, tannins, phenols, saponins, glycosides, alkaloids, carbohydrates, steroids, and proteins across different extracts, with ethanolic and hydroethanolic extracts showing the most significant activity (20). *Capsicum annuum* fruit extract also showed a wide variety of phytochemicals present across its different solvents. Chemical constituents found in the ethanolic extracts of *Amaranthus viridis* and *Capsicum annum* were more abundant and potent which is why we selected them over extracts from other solvents. Moving ahead in the study *in vitro* studies were carried out to assess the potential of different extract of *Amaranthus viridis* leaves and *Capsicum annuum* fruit to inhibit CaOx crystallization processes such as nucleation and aggregation. The bioactive compounds found in the extracts contribute significantly to their therapeutic properties and interfering with crystallization process by modifying the surface properties of the crystals, thereby preventing nucleation and aggregation. The inhibition of these processes is vital for kidney health, as they are directly linked to the formation of kidney stones. The promising outcomes *in vitro* laid the foundation for its selection in subsequent *in-vivo.* Administration of different extract of *Amaranthus viridis* leaves and *Capsicum annuum* fruit demonstrated a dose-dependent protective effect (21).

The extract of *Amaranthus viridis* leaves and *Capsicum annuum* fruit caused notable pathological alterations such as tubular degeneration, interstitial fibrosis, and calcified deposits, all reflecting severe renal injury from stone formation. However, kidney tissues from animal treated with the extract exhibited considerable restoration of normal architecture. In particular, the *Capsicum annuum* group showed only slight tubular damage and decreased interstitial fibrosis, suggesting strong protection of kidney structure and function. In the rats, the treatment groups receving EEAV and EECA extracts showed reduced urinary calcium and uric acid levels, indicating their potential to lower the risk of calcium oxalate and uric acid stone formation. In contrast, the disease group exhibited elevated levels of both calcium and uric acid. Microscopic examination of urine revealed that the disease control group developed aggregated calcium oxlate crystals, whereas the treatment groups both the standard and test displayed smaller, less aggregated and more dispersed crystals, highlighting the effectiveness of EEAV and EECA in preventing stone growth and aggreagation. Treatment with AVEE and CAEE significantly reduced serum ceratanin and urea levels compared to the lithiatic control, indicating protection against renal dysfunction associated with stone formation (22). Elevated creatinine and urea in the disease group confirmed impaired kidney function, while their reduction oin treated rats highlighted the therapeutic effect of the extracts. These findings indicate that AVEE and CAEE not only prevents stone formation but also offers nephroprotection. *In-vivo* assessments indicated that these extracts positively influenced urinary parameters, reducing the risk factors associated with stone formation, such as calcium and uric acid levels, while promoting a healthier urinary pH (23).

Conclusions

This study demonstrates the promising antiurolithiatic activity of *Amaranthus viridis* and *Capsicum annuum*

extracts in Wistar rats, highlighting their potential as natural therapeutic agents in preventing kidney stone formation. The phytochemical analysis revealed a rich array of bioactive compounds in both plants, particularly in ethanolic extracts, which contributed to significant inhibition of calcium oxalate crystallization *in-vitro*. I*n-vivo* assessments indicated that these extracts positively influenced urinary parameters, reducing the risk factors associated with stone formation, such as calcium and uric acid levels, while promoting a healthier urinary pH20. Additionally, the extracts improved renal function (decreased serum creatinine and urea levels), and showed protective effects on kidney weight and histological integrity. Overall, the findings support the efficacy of *A. viridis* and *C. annuum* in mitigating urolithiasis, underscoring their potential role in dietary and therapeutic strategies for kidney stone prevention. The bioactive constituents present in the extract have interfere with nucleation and aggregation steps of stone formation thereby preventing the enlargement of stones. The extract may have also exerted antiurolithiatic activity through inhibition of key enzyme involved in biosynthesis of oxalate. By inhibiting glycolate oxidase the extract may significantly reduce the intracellular levels of glyoxylate and thereby decrease the formation and excretion of oxalate in urine. Further research is warranted to isolate the active compounds, elucidate their mechanisms of action, and evaluate their effectiveness in clinical settings.

Authors' contributions
K.C: performed experiments, collected and analyzed data related to antiurolithiatic activity; S.K: supervised the work; T.K: co-supervised the work.

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None.

Conflict of interest
All authors declare that there is no conflict of interest.

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Ethics statement
This study was approved by Pharmacy Department, Kumaun University, Bhimtal, by the Institutional Animal Ethical Committee (Protocol No. KUDOPS/167).

Declaration of generative AI
I hereby declare that the information provided above is accurate and complete to the best of my knowledge. I understand that transparency in the use of AI tools is essential for research integrity. During the preparation of this work, the author used [NA] for [NA]. The author subsequently reviewed and edited all content and take full responsibility for the published article's content.

Data availability
Data will be provided on request.

Abbreviations

ANOVA	Analysis of Variance
AVEE	*Amaranthus viridis* Ethanolic Extract
BSI	Botanical Survey of India
CAEE	*Capsicum annuum* Ethanolic Extract
H&E	Hematoxylin and Eosin
SD	Standard Deviation
TFC	Total Flavonoid Content
TPC	Total Phenolic Content

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