Research Article

In-vitro Comparison of Anti-urolithiatic Effect of *Crataeva nurvala*, *Zea mays* (Corn Silk) and Ayurvedic Formulation Neeri Tablet

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ABSTRACT

The main objective of this study was to determine the anti-urolithiatic activities of the *C. nurvala bark, Zea mays (corn* silk), and ayurvedic formulation Neeri tablet taking Cystone as a standard formulation. The *in vitro* anti-urolithiatic activity of the extracts was performed based on their ability to neutralize synthetic calcium oxalate crystals in a semi-permeable egg-shell membrane. The quantity of undissolved calcium oxalate was determined titrimetrically based on the volume of standard potassium permanganate ($KMnO_4$) consumed. Each mL of 0.9494 N $KMnO_4$ is equivalent to 0.1898 mg of calcium, the percentage dissolution of calcium oxalate was calculated. The results showed that the aqueous extract of corn silk dissolved a 60.42 % calcium oxalate crystal, comparable to Ayurvedic formulation, Neeri, 59.52 % and standard Cystone 64.29 %, along with moderate effect of methanolic extract of corn silk, aqueous and methanolic *C. nurvala* extract. The study concluded the significant anti-urolithiatic activity of corn silk aqueous extract.

Keywords: *C. nurvala*, Corn silk, Anti-urolithiatics, Cystone, Neeri tablets.

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INTRODUCTION

Plants have been serving as a significant source of drug discovery since antiquity. Ethnopharmacology, the multidisciplinary approach allows the innovative way of development of anthropological rationale and pharmacological rationale of tremendous medicinal plants.[1] Urolithiasis is the process of formation of stony concretion within the bladder or urinary tract, excruciating pain, and requires prompt medical attention.[2] Approximately 12% of the global population is suffering from this condition where the reoccurrence rate is higher (70-80%) in males than in females (47-60%). Likewise, the highest risk has been reported from Asian countries.[3] Among numerous types of inorganic and organic kidney stones such as calcium oxalate, calcium phosphate, uric acid, struvite, cystine stones, etc., more than 60% of patients with renal stones suffer from calcium oxalate stones.[4] Calcium calculi consisting of oxalate or phosphate (80%) is reported to be preponderance than other (20%).[5,6] Stones incidence and composition are affected by genetic and environmental factors such as hot climate (prone to fluid loss) and sun exposure (vitamin D). Since urolithiasis is a highly recurring disease, the relapse rate is 50% in 5-10 years and 75% in 20 years old peoples. Several factors have influenced its reoccurrence, such as the young age of onset, positive family history, infection stones, and other underlying pathological conditions.[6]

C. *nurvala* belonging to the Capparidaceae family is commonly called as three-leaved capers. It is originated from India. The genus *Crataeva* is represented by eight different species throughout the world. Among them, four are found in India.[7] Each part of *C. nurvala* has its value, like leaves, roots, and, barks and is used both internally and externally. Externally, a paste of leaves or bark is used in cervical adenitis, abscess, edematous wound, and also in rheumatic joints to relieve pain whereas internal decoction of leaves is used as a laxative, cholagogues, appetizer, vermicide and helminthiasis. The decoction of bark is used in gout, internal abscess, adenitis, obesity, urinary disorder, fever and gastric irritation. Leaves decoction along with ghee relieves flatulence and abdominal pain.[8] Furthermore, different pharmacological effects such as analgesic activity, antifertility activity, cardioprotective, antiarthritic, antiurolithiatic activity, antidiabetic activity, etc. have been proven from this plant.[9,10]

Zea mays (Poaceae) is an annual grass, originated from Mexico and Central America, which is the top three cereal crops grown in the world. Corn silk has been ethnomedicinally valued for the treatment of cystitis, edema, kidney stone, diuretic, prostate disorder, and

urinary infection as well as bed-wetting and obesity.[11] Several scientific studies have proven that cornsilk exhibited diverse potent biological effects such as antitumor activity[12], antioxidant activity[13], antibacterial activity[14], neuroprotective activity[15], etc.

Although various treatment modalities for urolithiasis have evolved over the years, discrepancies exist regarding the clinical indications and the efficacy of each of these treatment options. Since there is no absolute effective drug available for the treatment of urolithiasis, the use of herbal formulations has drawn potential interest.[16] Various poly-herbal ayurvedic formulations are used to normalize the deviation regarding kidney functions. The two major polyherbal formulations widely prescribed and used for the treatment of urolithiasis are Cystone tablets (The Himalaya Drug Company)[17] and Neeri tablets (Aimil Pharmaceuticals)[16].

Due to increasing interest in herbal medicine for the treatment of renal calculi, many studies are carried based on ethnomedicinal values, basically in Nepal and India.[18] The entire motive of this study is to compare whether or not the ethnomedicinally used plant products (*Crataeva nurvala* and corn silk) show the anti-urolithiatic effect and their comparison with the marketed polyherbal formulations such as Cystone and Neeri. The selections of species used in this study were mainly based on their ethno medicinal uses.

MATERIALS AND METHODS

Collection, Drying, and Comminution of dried parts

The plants were collected from Hungi village, Palpa district, Western Nepal, during the month of June, 2018. Prepared herbariums were identified by Rashtriya Herbarium and Banaspati Prayogsala, Godwari, Lalitpur, Kathmandu, Nepal. After the preparation of herbarium, voucher specimens were stored in the Pharmacognosy Laboratory of Crimson College of Technology, Pokhara University (Voucher specimen number: CCT-HRB-2018/179). Collected plant materials were cleaned with distilled water. They were shade dried at room temperature in a well-ventilated room. The drying was carried out for 15 days with proper checking at regular intervals. After the plants were dried, corn silk particle size was reduced using the portable grinder while the size of *C. nurvala was reduced* by the cutter.

Preparation of Extract

The double cold maceration method was used for the extraction of both plant samples. Both samples were extracted separately in methanol and water. About 200 g of crude sample was soaked with 1000 mL of menstruum in conical flasks with occasional shaking for 48 hours. Liquids were strained and filtered. The process was repeated up to double maceration and the filtrates were mixed. The filtrates obtained from both processes were evaporated by using a rotary vacuum evaporator. The methanolic extracts were evaporated at 40 ^{0}C and aqueous extracts were evaporated at about 70 ^{o}C. The distilled water extracts and methanolic extracts of *C. nurvala* and corn silk were used without any trituration. The coating of standard (Cystone) was removed by using ethanol and for ayurvedic formulation (Neeri), coating was peeled out by using a blade. Both tablets were triturated and used in powdered form.

Phytochemical screening

Preliminary phytochemical test for all the extract was carried out to test the presence of saponin, alkaloids, phytosterol, flavonoid, phenol, and terpenoids, by using standard procedures.[19-21]

Preparation of semipermeable membrane

Very carefully with the help of a sharp pointer, a hole was made on the eggshell and inner content (yolk and albumin) were removed. The eggshells were decalcified by keeping in 15 % v/v HCl for 7-8 hours. The finally obtained membrane was washed with distilled water and was placed in an ammonia solution to neutralize the traces of acid present on the egg. Subsequently, the membranes were washed with distilled water.[22]

Preparation of calcium oxalate crystals

1.4 g of calcium chloride dihydrate was dissolved in 100 mL distilled water and 1.34 g of sodium oxalate was dissolved in 100 mL of 2N H_2SO_4. So prepared solutions were mixed equally with continuous stirring and calcium oxalate precipitate was obtained and was freed from the traces of sulphuric acids using ammonia solution, washed with distilled water and dried at 60 °C for 2 hours. Thus, calcium oxalate stone was formed.[23]

Pilot Study

In the pilot study, it was found that the inhibition of nucleation and aggregation by the methanolic extract of corn silk was not effective enough at below 50 mg/mL. The methanolic extract exhibited good inhibition activity above at 100 mg/mL in 10 mL of stone-forming solution, and thus, a 100 mg dose was taken in the present study.[23]

Group division

Group I: Blank, 10 mg of calcium oxalate only

Group II: Standard, 10 mg of Calcium oxalate + 100 mg of Cystone

Group III: Test, 10 mg of calcium oxalate + 100 mg of distilled water extract of corn silk Group IV: Test, 10 mg of calcium oxalate + 100 mg of distilled water extract of *C. nurvala*

Group V: Test, 10 mg of calcium oxalate + 100 mg of methanolic extract of corn silk Group VI: Test, 10 mg of calcium oxalate + 100 mg of methanolic extract of *C. nurvala*

Estimation of calcium oxalate by Titrimetry

Exactly 10 mg of calcium oxalate and 100 mg of extract/

compound/ standard were packed together in the semi-permeable membrane by suturing model, which was suspended in a conical flask containing 100 mL of 0.1 M Tris HCl buffer. One group served as a negative control, containing only 10 mg of calcium oxalate. Thus prepared a conical flask of all groups was placed in an incubator, preheated to 37 °C for 2 hours, for about 7 to 8 hours. The content of the semi-permeable membrane from each group was squeezed out into the conical flask, 2 mL of 1 N sulphuric acids was added and finally titrated with 0.9494 N $KMnO_4$ till light pink color end point was obtained. 1 mL of 0.9494 N $KMnO_4$ equivalent to 0.1898 mg of Calcium.[24]

RESULTS

Extractive Yields

The extractive yields of *C. nurvala* bark in the methanol and water was found to be 4.16 % and 9.12 respectively. Similarly it was found to be 10.65 % and 5.97 % for methanolic and aqueous extract of corn silk.

Phytochemical screening

Phytochemical screening of both the distilled water extract and methanolic extract of *C. nurvala* showed the presence of terpenoids, alkaloids, saponins, flavonoids, phenols, tannins and phytosterol. Similarly, both extracts of Corn silk revealed the presence of terpenoids, saponins, flavonoids, phenols, tannins, and phytosterols. However, alkaloid was absent in both extract of corn silk.

Anti-urolithiatics activity

The calculation of the volume of $KMnO_4$ consumed was based on the expression of numerical data as Mean ± SEM and was evaluated by a two-tailed student t-test. The result obtained was compared with the control group. The $p<0.05$ was considered to be statistically significant. As shown in the Table 1 and Figure 1, the dissolution percentage of Calcium oxalate was calculated using the titrimetry method after incubating the groups in an incubator for 7 to 8 hours. Extracts, as well as Ayurvedic formulation (Neeri tablets), showed dissolving ability for oxalate stone at the dose of 100 mg per 10 mg of calcium oxalate stone. However, among the extract distilled water extract of Corn silk (60.42%) showed significant dissolving ability when compared to standard Cystone (64.29%), as shown in figure 1. Nevertheless, another extract as well as Ayurvedic formulation has also shown good anti-urolithiatic activity but less significant as compared to distilled water extract of corn silk.

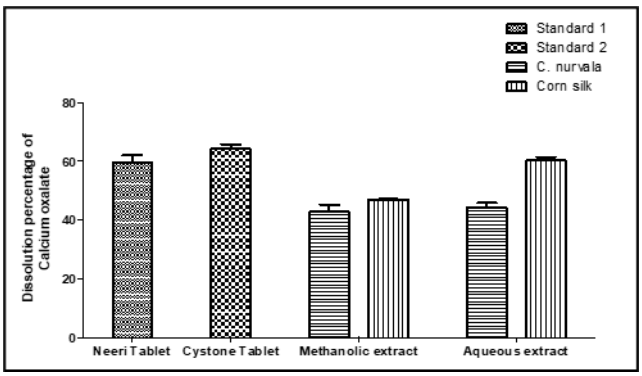

Figure 1: Bar diagram showing the effect of methanolic and distilled water extract of *C. nurvala*, Zea mays (corn silk), Neeri tablets and Cystone in calcium oxalate stone.

Table 1: Effect of methanolic and distilled water extract of *C. nurvala, Zea mays* (corn silk), Neeri tablet and Cystone in calcium oxalate stone.

Groups	Vol. of $KMnO_4$ (mL)	Wt. of calcium estimated (mg)	Wt. of calcium dissolved (mg)	Dissolution percentage (%)
Blank	3.36±0.08	0.63773	-	-
Cystone (standard-1)	1.20±0.05*	0.22776	0.40997	64.29±1.48
Neeri (standard-2)	1.36±0.08*	0.25813	0.37960	59.52±2.53
C. nurvala (methanol)	1.92±0.008*	0.36442	0.27331	42.86±2.32
C. nurvala (aqueous)	1.87±0.005*	0.35493	0.28280	44.34±1.51
Corn silk (methanol)	1.78±0.005*	0.33784	0.29989	47.02±0.14
Corn silk (aqueous)	1.33±0.03*	0.25243	0.3853	60.42±0.97

Where,

- 1mL of 0.9494 N $KMnO_4$ equivalents to 0.1898 mg of calcium.
- The volume of $KMnO_4$ consumed is expressed as Mean ± SEM.
- *Indicate statistical significance as $P<0.001$, compared with control group using two tailed student's t-test (Microsoft Excel)

DISCUSSION

The increasing practices and interest of herbal remedies for the treatment of urolithiasis is due to the limited choice in pharmacotherapy. Numerous medicinal plants have been evaluated continuously and reputed with positive results. However, the rationale behind their use is not well established except for few ones.[18] In our study, the bark of *C. nurvala* and corn silk has also shown a positive effect as anti-urolithiatic agent. The different solvent extract of both plants has shown the ability to dissolve calcium oxalate stone compared to the control group.

In the present study, *in vitro* urolithiasis model was designed by using egg semi-permeable membrane containing chemically synthesized calcium oxalate. In fact, it resembles the urolithiasis because of the fact that the major proportion of kidney stone is calcium oxalate. The ability of the extracts to neutralize the calcium oxalate was determined titrimatrically by calculating the volume of standard $KMnO_4$. Kidney stone-forming parameters, namely urinary calcium, oxalate, magnesium, uric acid, sodium, potassium, and creatinine, characteristically play an important role. Hypercalciuria is caused from primary metabolic alteration, whereas hyperoxaluria is caused due to either enzymatic disturbance in oxalate biosynthesis or increase urinary oxalate level (>45 mg/day), because of increased availability of substrate (as ethylene glycol, ascorbic acid, etc.) or reduced degradation of oxalate. These can play as hallmarks for calcium oxalates stone formation.[25]

The probable mechanism for the anti-urolithiatic effect of *C. nurvala* bark extract might be due to the presence of high lupeol content. Lupeol can exert this activity by potentiating demineralization or dissolution of the preformed mineral phase or its further development in the urinary bladder. Lupeol also help to neutralize the urinary pH and prevent the precipitation of calcium ions.[26] Previous study has reported that, the *C. nurvala* extract induce hypercalciuria and hyperphosphoruria with increased urinary output.[27] Bark and root extract of *C. nurvala* are reported to reduce urinary magnesium excretion along with increase of urinary output, calcium and uric acid excretion.[8]

The husk extracts and fractions of *Zea mays* showed the potential renoprotective and anti-oxidative potential as observed by a remarkable increment of renal protective markers as well as decrease in the toxic markers. Husk extract/fraction administration also exerted a reduction in serum creatinine level and urea. The improvement observed in histological tissues also supported the nephron protective activity.[28] Aqueous infusion of corn silk can help to prevent urolithiasis by diuretics effect.[29]

Phenolic compounds, flavonoids, tannins, and glycosidic flavonoids are responsible for the dissolution of both calcium oxalate and calcium phosphate stones. However, they are found to be more effective in dissolving calcium phosphate stone than oxalate. Betulin, a triterpenoid and quercetin, a flavonoid is found to be responsible for increment in inhibiting oxalate absorption, facilitate urinary excretion and preventing super saturation of urine and thus reducing the risk of calcium oxalate stone formation.[30] Presence of all these bioactive compounds in both plants might produce anti- urolithiatic effect by different mechanism.[8,31]

CONCLUSION

In the inference, we can verify the possibility of anti-urolithiatic activity of bark of *C. nurvala* and corn silk seeing that the facilitating dissolution of calcium oxalates crystals as compared to the negative control. Furthermore, it can be confirmed that the folk knowledge of using distilled water extract of corn silk has greater potential to posse significant anti-urolithiatic activity after Cystone, letting behind Ayurvedic formulation (Neeri). Although the exact chemical compound responsible for the anti-urolithiatic activity of corn silk remains imprecise, the presence of different phytochemicals such as flavonoids, saponins, phenols, glycosides, and triterpenoids may be responsible for the activity. Thus, this study gives the direction for the isolation of the potent active constituent for the drug development in the future.

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Author Contribution

Abaru Panta and Pooja Panth act as First author.

Conflict of interest

We declare that we have no conflict of interest.

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