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Inhibition of calcium oxalate crystallization *in vitro* by methanolic leaf extract of *Murraya koenigii* (L.) Spreng

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Abstract
Medicinal plants are old as human society for treatment, prevention or mitigation of diseases. *Murraya koenigii* (*M. koenigii*) is commonly known as curry plant, used in treatment or prevention of many diseases including kidney stone. The objective of this research was *in vitro* study on inhibition of calcium oxalate crystallization through titrimetric method. The results showed that greater inhibitory activity of *M. Koenigii* was found at 48 mg/ml (61.26%) while cystone showed maximum activity at 446 mg/ml (70.18%). Inhibitory activity of *M. koenigii* was concentration dependent manner which indicates the decrease of stone forming constituents. This activity of *M. koenigii* might be due to presence of secondary metabolites such as saponins and other phytoconstituents. Thus, it supports the knowledge of ethnomedicine in some tribes for prevention of kidney stone. And, *in vivo* study and chemical characterization of *M. koenigii* could be new candidate for treatment of kidney stone diseases in future.

Keywords: Calcium oxalate, Kidney stone, *Murraya koenigii*, Saponins, Titrimetric method

1. Introduction
Medicinal plants have been used for treatment, prevention or mitigation of diseases from antiquity [1]. *Murraya koenigii* (L.) Spreng is commonly, known as *karipatta* or *kadipatta* in Nepali as well as in Hindi and curry plant in English [1, 2]. It belongs to Rutaceae family which is widely distributed in Nepal, India, Srilanka, Thailand, Pakistan, Bhutan etc [1, 3]. It is most often used in Nepal and India for its medicinal and aromatic properties [2]. Traditionally, it is used as antidiarrheal, blood purifier, hair tonic, Antinociceptive, antidysentric, antiamnesic, antifungal, memory enhancer, antianemic, antiemetic and antiperiodic [4, 5]. Similarly, it is traditionally used to cure kidney pain, vomiting, itching, dysentery, blood disorder, diabetes mellitus, leucoderma, hypercholesterolemia lightening [2, 5-8]. In addition to this, it has various biological activities such as antioxidant, antibacterial, antidiabetic, antiprotozoal, hepatoprotective, antitumor, antiviral, antileukemial etc. [2, 4, 9, 10].
Kidney stone is oldest painful urological disorder among all age groups [11, 12]. It mainly occurs due to dis-function of promoters and inhibitors which is more common in male as compared to female [12, 13]. Incidence of kidney stone disease is found to be more common in western region rather than eastern region of the earth [11, 14]. Genetic factor, dietary factor and modification of life style could be promising risk factors of kidney stone disease [15, 16]. The objective of this research was to determine the inhibition of calcium oxalate crystallization by *M. koenigii* for the prevention of kidney stone disease, used by some tribes like tharu which was left unnoticed.

2. Materials and methods
2.1 Collection of Plant Materials
Leaves of *M. koenigii* was collected from Shree Shahid Sanghari Bhimdatta Pant Community Forest, Godawari-05, Kailali, Nepal and confirmed from literature reviews [3, 4, 7]. Voucher specimen of *M. Koengii* was deposited in Pharmacognosy Laboratory of School of Health and Allied Sciences, Pokhara University, Kaski, Nepal for its future reference.

2.2 Extraction
Shaded dried sample of *M. koenigii* was pulverized into small pieces. Then, 100 gm of sample was macerated in 700 ml Methanol (crude drug: Methanol = 1:7) for 24 hour. Then, filtration was carried out using whatman filter paper. Afterward, solvent from filtrates was evaporated by using rotatory vacuum evaporator and kept in desiccator till complete removable of solvent. Then, obtained extract was collected into vial and preserved in refrigerator before its use.

2.3 Phytochemical Screening of *M. koenigii*

Phytochemical screening of *M. koenigii* was performed for the detection of secondary metabolites through standard methods [17, 18].

2.4 Inhibition of Calcium Oxalate Crystallization by *M. koenigii*

Inhibition of calcium oxalate crystallization by methanolic leaf extract of *M. koenigii* was carried out by titrimetric method at room temperature [19, 20]. During experiment, stock solution of cystone (Himalaya Herbal Drug) was prepared on keeping its powder in 100 ml distilled water for 2 hour followed by its centrifugation at 1000 rpm. Similarly, stock solution of *M. koenigii* was prepared from dried extract and allowed for its centrifugation at 1000 rpm. Obtained supernatant solution of plant extract and cystone was used for experimentation. Supernatant of plant extract was diluted to different concentrations (16 mg/ml, 20 mg/ml, 24 mg/ml, 28 mg/ml, 32 mg/ml, 36 mg/ml, 40mg/ml, 44 mg/ml and 48 mg/ml). Briefly, Calcium chloride and sodium oxalate i.e. salt forming agents were allowed to fall with equal speed into 250 ml of beaker containing 25 ml of sample. Afterwards, obtained mixture was digested at heating mantle for 10 min, and followed by its cooling at room temperature. Subsequently, it was collected in pre-weighed centrifuge test tube. Then, precipitation of calcium oxalate was obtained after its centrifugation and dried weight of calcium oxalate was calculated. For blank, water was used in place of inhibitor. Then, percentage of inhibition was calculated through Eq. (1).

$$\text{Percentage of Inhibition} = \frac{(\text{Wt. of precipitate in blank set} - \text{Wt. of precipitate in extract or standard set}) \times 100\%}{\text{Wt. of precipitate in blank set}} \quad (1)$$

3. Results & Discussion

3.1 Phytochemical Screening of *M. koenigii*

Phytochemical screening of *M. koenigii* showed the presence of various metabolites such as steroids, terpenoids, saponins, tannins, alkaloids and flavonoids (Table 1). They might have role in inhibition of calcium oxalate crystallization.

Table 1: Phytochemical Screening of *M. koenigii*

	Phytochemical Screening of Methanolic Extracts					
	Alkaloids	**Flavonoids**	**Saponins**	**Steroids**	**Tannins**	**Terpenoids**
M. koenigii	+	+	+	+	+	+

Note: (+) indicates presence of compound and (-) indicates absence of compound

3.2 Inhibition of Calcium Oxalate Crystallization by *M. koenigii*

In the present study, inhibition of calcium oxalate crystallization by *M. koenigii* was dose dependent in manner which indicates the decrease of stone forming constituents (Table 2 and 3). *M. koenigii* showed greater inhibition of calcium oxalate crystallization at 48 mg/ml (61.26%) which was comparable to cystone as standard was 70.18% at 446 mg/ml.

Table 2: Weight of Calcium Oxalate Crystallization at Different Concentrations (Gram)

	Weight of calcium oxalate crystallization at different concentration (gram)									
	16 mg/ml	20mg/ml	24 mg/ml	28 mg/ml	32 mg/ml	36 mg/ml	40 mg/ml	44 mg/ml	48 mg/ml	446 mg/ml
M. koenigii	0.296±0.017	0.303±0.003	0.293±0.003	0.283±0.009	0.263±0.003	0.253±0.008	0.218±0.008	0.209±0.005	0.19±0.003	
Cystone										0.146±0.014
Negative-control				0.493±0.020						

Note: Data were expressed as mean ± standard deviation (n=3)

Table 3: Percentage Inhibition of Calcium Oxalate Crystallization at Different Concentrations (%)

	Percentage inhibition of calcium oxalate crystallization at different concentrations (%)									
	16 mg/ml	20mg/ml	24 mg/ml	28 mg/ml	32 mg/ml	36 mg/ml	40 mg/ml	44 mg/ml	48 mg/ml	446 mg/ml
M. koenigii	39.95	38.53	40.57	42.59	46.65	48.68	55.78	57.61	61.26	
Cystone										70.18

According to Gurocak *et al*. (2006), saponins have well anti-crystallization property by disaggregating the suspension of mucoproteins, the promoters of crystallization [21]. Similarly, saponins rich fraction of Herniaria was found to be potent inhibitor of calcium oxalate crystallization *in vitro* as well as *in vivo* model [22]. And we concluded that inhibitory activity

of *M. koenigii* might be due to presence of saponins and other phytoconstituents.

4. Conclusion

Thus, this study provides the utility of *M. koenigii* (L.) Spreng in kidney stone and also supports the knowledge of ethnomedicine in some tribes for prevention of kidney stone. Further study on *M. koenigii* could lead to develop new drug candidate for treatment of kidney stone disease in future.

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