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RESEARCH ARTICLE

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Anti-urolithiatic activity of whole plant aqueous and ethanolic extract of *Coriandrum sativum* L. - an *in vitro* approach

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ABSTRACT

To investigate the anti-urolithiatic activity of aqueous and alcohol extracts of *Coriandrum sativum* L. by *in vitro* turbidity and titrimetric assays. Whole plant powder was extracted with alcohol and water. Turbidity and titrimetry methods were carried out to assess the inhibition of calcium oxalate crystal formation and dissolution of crystals respectively by the extracts. The effects of extracts on calcium oxalate crystal formation were also analyzed by light microscopy. Phytochemical screening was carried out in both extracts. The anti-urolithiatic activity of the extracts was compared with standard drug cystone. Phytochemical screening of the aqueous and alcohol extracts of *Coriandrum sativum* L. revealed the presence of flavonoids, saponins, terpenoids, glycosides and alkaloids. Both the extracts dose dependently (100, 200, 400µg/ml) inhibited the formation of turbidity in nucleation assay. Similarly in a dose dependent manner (10, 50, 100mg/ml) the extracts also dissolved the calcium oxalate crystals. From the results we conclude that the extracts of *Coriandrum sativum* L. exhibit antiurolithiatic activity**.** The anti-urolithiatic activity was found to be more significant in the aqueous extract than alcohol extract of *Coriandrum sativum.*

Keywords: Urolithiasis; Calcium oxalate crystal; *Coriandrum sativum* L.; Cystone

1. INTRODUCTION

Urolithiasis, a multifactorial disorder is one of the oldest and common afflictions of humans and remains a major public health burden. It affects all age groups from less than 1 year old to more than 70, with a male to female ratio of 2:1. A large number of people, nearly 4-15% of the human populations suffer from urinary stone problem all over the globe [1].

The risk of developing urolithiasis in adults appears to be higher in the western hemisphere than in the eastern hemisphere, although the highest risks have been reported in some Asian countries such as Saudi Arabia (20.1%) with lifetime recurrence rates of up to 50% [2]. In India, the "stones belt" occupies parts of Maharashtra, Gujarat, Punjab, Haryana, Delhi and Rajasthan. The formation of urinary calculi is a

serious, debilitating problem in all societies throughout the world.

Medicinal plants are rich source of therapeutic agents for various diseases. They are readily available, cost effective and safe with minimal or no side effects. *Coriandrum sativum* L. belong to the family Apiaceae is an annual, herbaceous plant which originated from the Mediterranean and Middle Eastern regions of the world. All parts of this herb are used as a flavoring agent and/or as traditional remedies for the treatment of different disorders in the folk medicine systems of different civilizations [3]. It has been reported to possess like antioxidant [4], anti-diabetic [5], anti-mutagenic [6], anti-lipidemic [7], anti-spasmodic [8] properties. In this context, the present study is designed to investigate the antiurolithiatic activity of aqueous and alcoholic extracts of *Coriandrum sativum* L. by *in vitro* turbidity and titrimetric assays.

2. MATERIALS AND METHODS

2.1 Collection of plant material

The fresh and fully mature, whole plant of *Coriandrum sativum* L. was collected from local market Annamalainagar, Chidambaram. The plant was thoroughly washed with water and spread in thin layer in trays and finally placed into a dryer having a good air circulating system and temperature controlling thermostat [9]. The plant parts were dried in hot air oven at 60˚C for 3 days. The dried plant parts were ground to coarse powder with a mechanical grinder. The powdered sample was kept in clean closed glass containers till extraction.

2.2 Preparation of Extracts

The plant powder was extracted with ethanol and water using soxhlet apparatus for 24 hours. After completion of the extraction process, the liquid was filtered using a whattman No.42 filter paper. The filtrate was concentrated to dryness under reduced pressure at 40˚C using rotary vacuum evaporator and the powder was preserved in refrigerator.

2.3 Phytochemical screening

The following tests were carried out to screen the presence of various phytochemicals in the extracts.

2.3.1. Test for flavonoids: Sodium hydroxide Test

2 ml of extract was treated with few drops of aqueous NaOH and HCl and the formation of yellow orange color indicates the presence of flavonoids.

2.3.2. Test for saponins: Foam Test

A small amount of extract was shaken vigorously with water and the formation of persistent foam indicates of the presence of saponins.

2.3.3. Test for Quinones: Hydrochloric acid test

A small amount of the extract was treated with Con. HCl and the formation of yellow color precipitate indicates the presence of quinones.

2.3.4. Test for Steroids: Salkowski test

To 2 ml of plant extract, 2ml of chloroform and Con.H_2SO_4 was added and shaken well. Chloroform layer appears red and the acid layer fluoresce greenish yellow. This confirms the presence of steroids.

2.3.5. Test for Tannins: Ferric chloride test

To 3 ml of extract, 3 ml of 5% ferric chloride solution was added. The blue - black color indicates the presence of tannins.

2.3.6. Test for Glycosides: Keller-Kiliani test

To 2 ml plant extract, 1ml glacial acetic acid, three drops of 5% ferric chloride and Con.H_2SO_4 were added. Reddish brown color appears at the junction of the two liquid layers and upper layer appears bluish green, confirming the presence of glycosides.

2.3.7. Test for Terpenoids: Salkowski test

The extract was mixed with 2ml of chloroform and 3ml of Con.H_2SO_4 was carefully added to form a layer. A reddish brown coloration at the interface show positive result for the presence of terpenoids.

2.3.8. Test for Anthocyanins: Sodium hydroxide test

2 ml of extract was treated with 2 M NaOH and the formation of blue green color indicates of the presence of anthocyanins.

2.3.9. Test for Phenol: Ferric chloride test

To 3 ml of extract, 3 ml of 5% ferric chloride solution was added. A dark green color indicates the presence phenol.

2.3.10. Test for Alkaloids: Hager's test

To 3 ml of extract, 1ml of Hager's reagent (saturated picric acid solution) was added. The appearance of yellow precipitate indicates the presence of alkaloids.

2.4. Assessment of Anti-lithiatic activity by turbidity method

In vitro anti-lithiatic activity of the extracts was demonstrated in terms of inhibition of calcium oxalate formation by the extracts in the presence and absence of inhibitors (standard drug and extract). The precipitation of calcium oxalate at 37°C and pH 6.8 has been studied by the measurement of turbidity at 620nm. To 1ml of 0.025M $CaCl_2$, 2ml of Tris buffer (pH 7.4) was added and mixed thoroughly. To this 1ml of 0.025M of sodium oxalate was added and the turbidity formed is measured at 620nm. This serves as negative control. The experiment was repeated with the standard drug cystone and with the extracts (100, 200, 400µg/ml). Standard drug acts as positive control. The percent of inhibition of the drugs and the standard was measured until a period of 10min [10]. The percent of inhibition was then calculated using the formula

$$\text{Inhibition \%} = \{1-[Si / Sc]\} \times 100$$

where;
Si: slope of graph in the presence of inhibitor (drugs/extracts).
Sc: slope of without Inhibitor (Control)

2.4.1. Microscopic Study

With the same experimental protocol described as above the calcium oxalate crystals formed with and without the extracts were observed using microscope which was equipped with digital camera.

2.5. Evaluation for Anti-urolithiatic activity by titrimetric method

2.5.1. Production of kidney stones

Experimental calcium oxalate stones were prepared by homogenous precipitation method described by Byahatti *et al*, 2010 [11]. Equimolar solution of calcium chloride dihydrate in distilled water and sodium oxalate in 2N H_2SO_4 were both allowed to react in saturated amounts of distilled water. The precipitate obtained was calcium oxalate. The precipitate was washed with excess ammonia solution to free them from traces of sulphuric acid. The precipitate obtained were then washed thoroughly with distilled water and dried at 60˚C for about 4-6 h.

2.5.2 Egg membrane preparation

Chicken eggs were used for the experiment. The semi permeable membrane usually lies between the outer calcified shell and the inner albumin and yolk. Shell was removed chemically by soaking the eggs in 2M HCl overnight. After the complete decalcification of the eggs they were further washed with distilled water. The contents were then squeezed out completely by carefully puncturing a hole on the top. Then membrane obtained was washed thoroughly with distilled water, and placed in ammonia solution for deacidification. The membranes were further stored at 4˚C until further use (Figure 1).

Figure 1. Preparation of semi permeable membrane

2.5.3. Estimation of calcium oxalate by titrimetric method

About 10mg of the calcium oxalate and 10, 50,100 mg/ml of the extracts was packed in the semi permeable membrane. The membrane was sutured at one end and placed in a conical flask containing 100ml of 0.1M Tris buffer (Figure.2). Calcium oxalate without the extract serves as negative control. Cystone was used as positive control. The conical flasks were then incubated in a pre-incubated chamber at 37˚C for about 8 hours. Following incubation, the contents from the sac were collected into a test tube. To the contents, 2ml of 1N sulphuric acid was added and titrated against the 0.94N $KMnO_4$ till the appearance of light pink color. 1ml of 0.9N $KMnO_4$ is equivalent to 0.1898mg of calcium. The percent dissolution of the calcium oxalate was calculated using the amount of undissolved calcium oxalate.

Figure 2. Membrane along with the contents suspended in 0.1M Tris buffer

3. RESULTS

3.1. Phytochemical screening of Coriandrum sativum L. extracts

Phytochemical analysis was carried out in the extracts of *Coriandrum sativum* L. They revealed the presence of flavonoids, saponins, terpenoids, glycosides and alkaloids (Table.1). However the presence of anthocyanin, phenols, quinones, steroids and tannins varies between the two extracts.

3.2. Effect of extracts of Coriandrum sativum L. on crystal formation

Turbidity studies done on the aqueous and ethanol extracts of *Coriandrum sativum L.* revealed their inhibitory activity in stone formation. Both the extracts dose dependently inhibited stone formation. Aqueous extracts at 100, 200 and 400µg/ml inhibited the crystallization reaction by 71.64, 76.22 and 81.25% respectively [Figure 3]. The ethanol extract of *Coriandrum sativum* L. at 100,200 and 400µg/ml doses inhibited the crystallization reaction by 33.58, 52.85 and 75.57% respectively (Figure.4). The inhibitory property of the extracts was compared with standard drug cystone, which showed 92% inhibition in crystal formation. The aqueous extract showed more inhibitory property on calcium oxalate crystallization than ethanol extract.

3.3. Effect of extracts of Coriandrum sativum L. on crystal dissolution

The titrimetry values obtained clearly showed the antiurolithiatic activity of the aqueous and ethanol extract of *Coriandrum sativum* L. Both the extracts dissolved calcium oxalate crystals in a dose dependent manner. Aqueous extract at 10, 50 and 100mg/ml showed percentage dissolution of calcium oxalate stones by 44, 47.98 and 60% respectively (Figure.7). The ethanol extract of *Coriandrum sativum L.* at 10, 50, 100mg/ml doses showed percentage dissolution of calcium oxalate stones by 28, 40 and 47.98% respectively (Figure.8). This dissolution property was compared with standard drug cystone, which showed 68% dissolution in calcium oxalate crystals. Thus aqueous extract showed more dissolution property of calcium oxalate stones than ethanol extract.

4. DISCUSSION

Urolithiasis is the formation of calculi anywhere in the urinary system. It is a complex process that results from a succession of several physico-chemical events including supersaturation, nucleation, growth, aggregation and retention within renal tubules [12]. Supersaturation is an initial event in the formation of stones which is characterized by significant turbidity that further develops to urinary calculi. Inhibition of turbidity can retard the subsequent events of urolithiasis. The best way to prevent and treat urolithiasis is to control the process of crystallization events and most important is to control the initial step i.e. nucleation step. If nucleation itself is stopped or controlled by the phytochemical, then the next step which leads to formation, aggregation and retention of crystals do not occur at all. In this context, we measured the inhibition of turbidity by the whole plant extracts of *Coriandrum sativum* L. The aqueous and alcohol extracts of *Coriandrum sativum* L. were prepared and used in a dose depended manner to ascertain its anti urolithiatic activity. The extracts at 100, 200, and 400µg/ml inhibited the formation of turbidity which clearly shows that they prevented supersaturation, an essential step in urolithiasis. This inhibition of turbidity property was significantly higher in aqueous extract at 400µg/ml when compared to alcohol extract at the same dose (Figure 3 & 4).

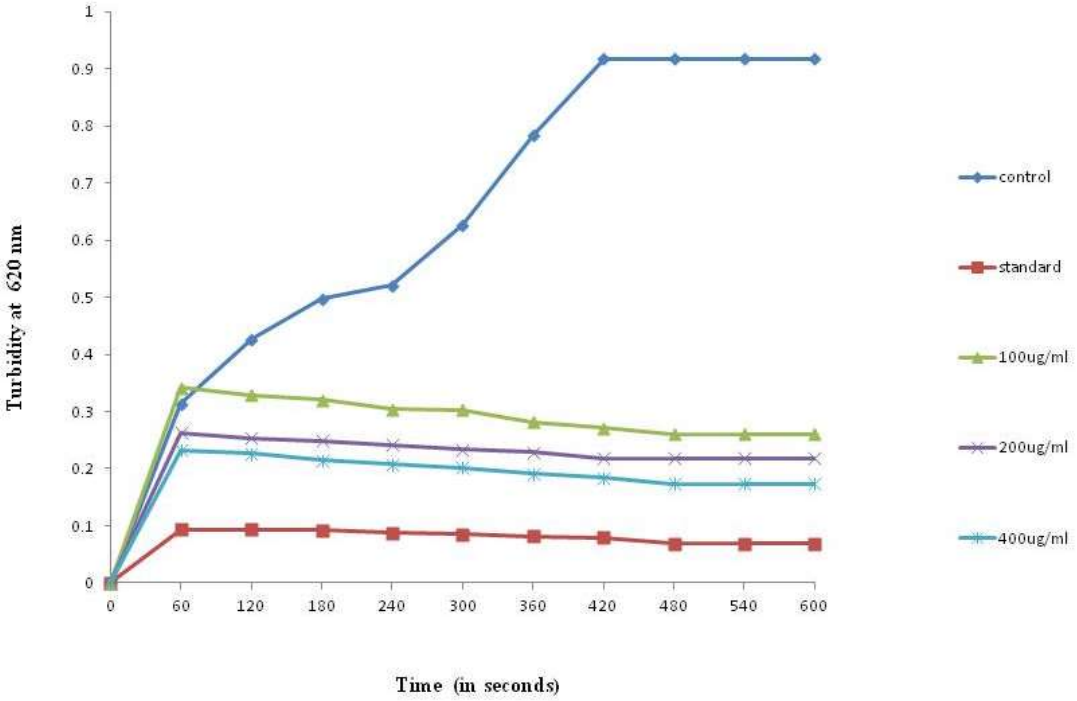

Figure 3. Graphical presentation of percentage inhibition of calcium oxalate crystal formation by aqueous extract of *Coriandrum sativum* L.

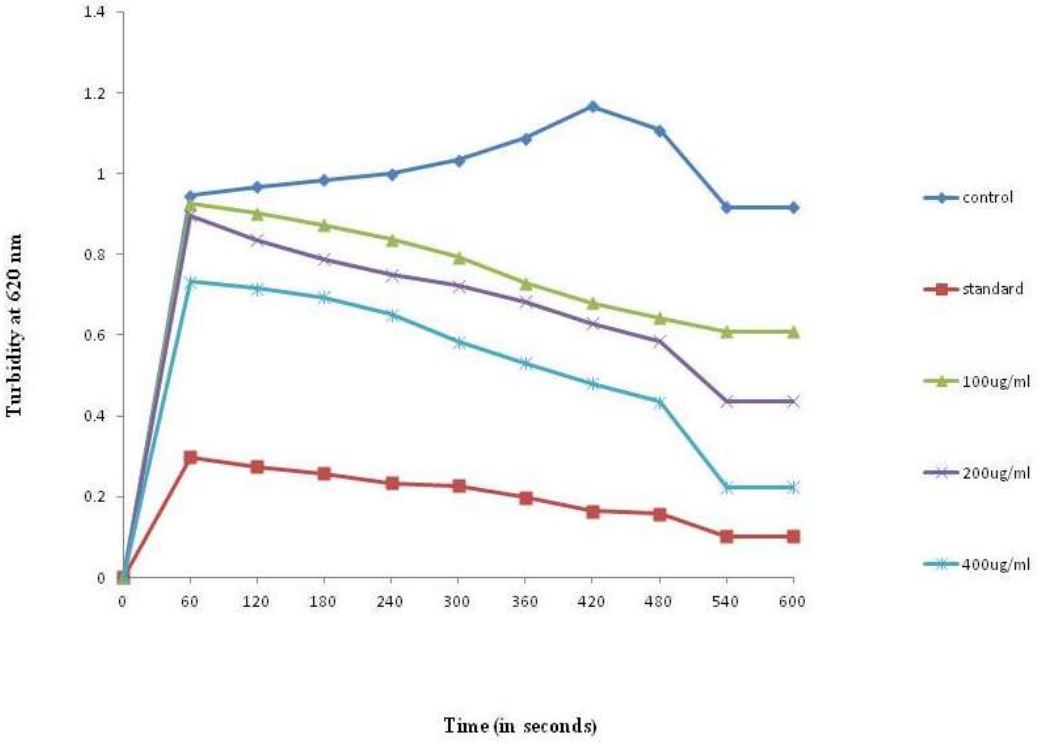

Figure 4. Graphical presentation of percentage inhibition of calcium oxalate crystal formation by ethanol extract of *Coriandrum sativum* L.

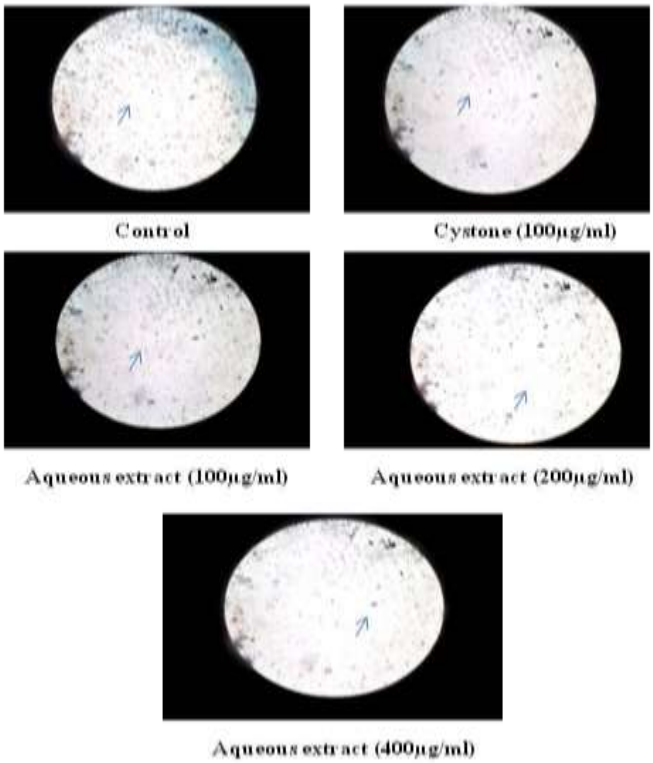

Figure 5. Microscopic examination of CaOx crystal formation in the presence of aqueous extract of *Coriandrum sativum* L.

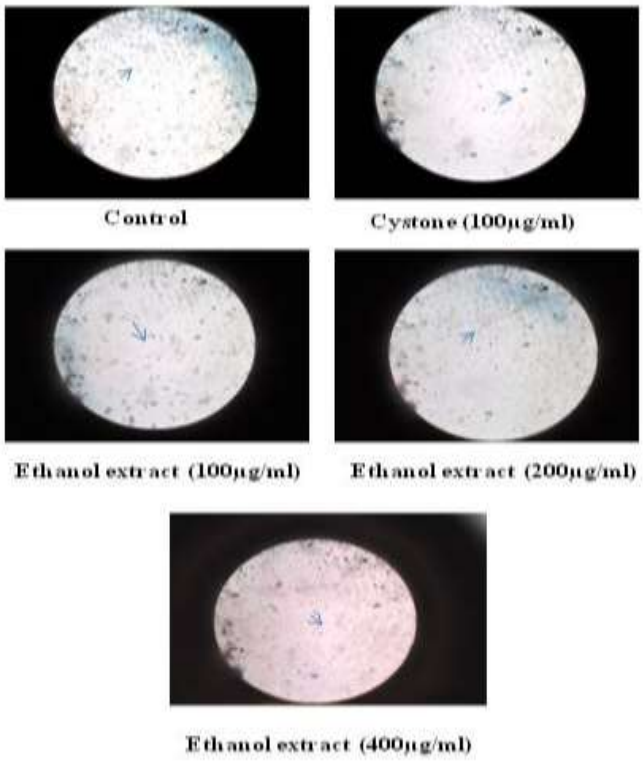

Figure 6. Microscopic examination of CaOx crystal formation in the presence of ethanol extract of *Coriandrum sativum* L.

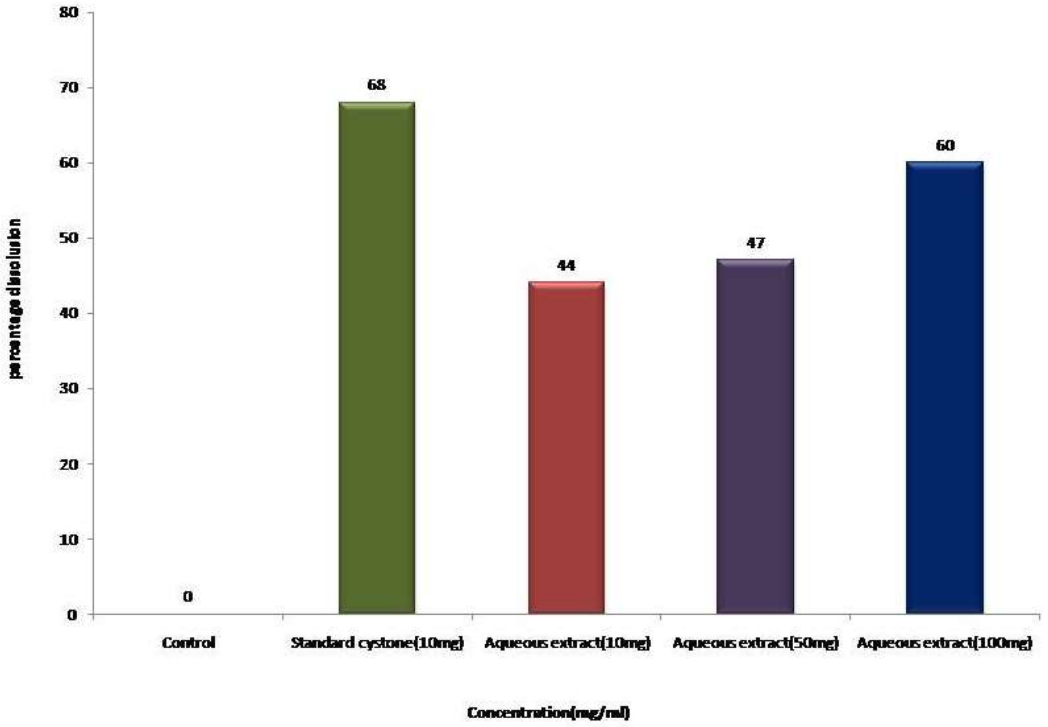

Figure 7. Graphical presentation of percentage dissolution of calcium oxalate crystals by aqueous extract of *Coriandrum sativum* L

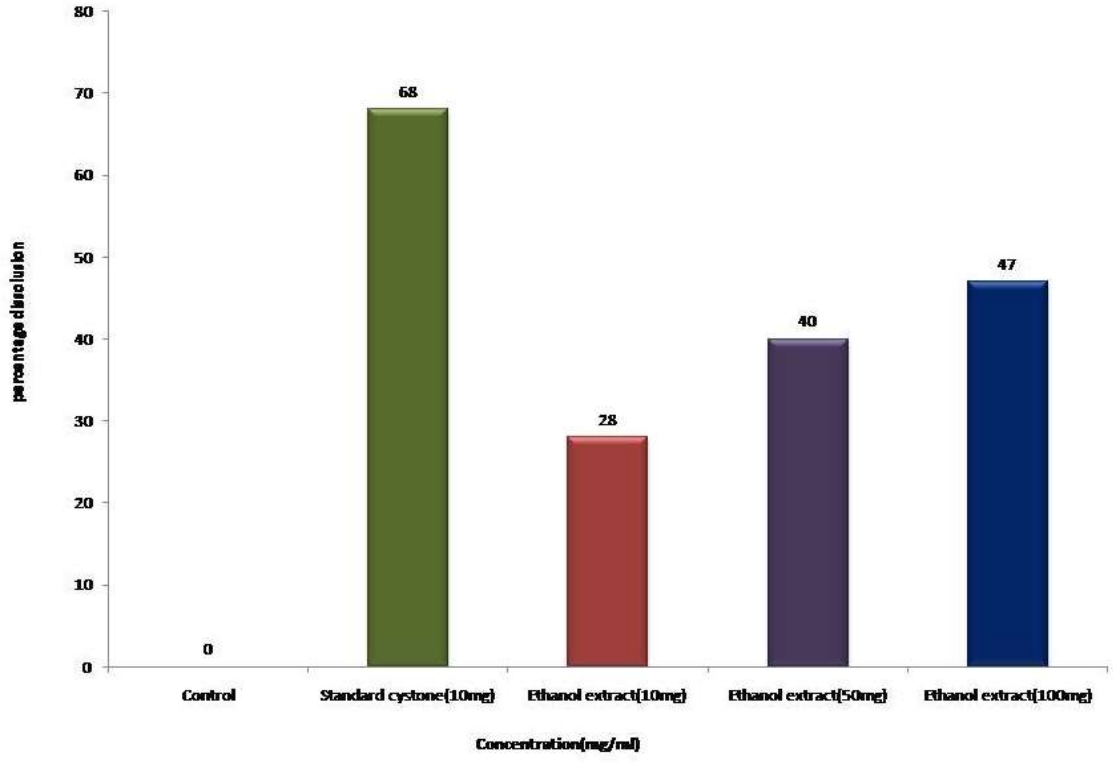

Figure 8. Graphical presentation of percentage dissolution of calcium oxalate crystals by ethanol extract of *Coriandrum sativum* L.

Microscopic studies were further carried out to confirm the inhibition of turbidity by the extracts. The control group showed oval and dump bell shaped calcium oxalate crystals and their aggregates in large numbers. However, the presence of extracts decreased the formation of calcium oxalate crystals in a dose dependent manner (Figure.5 & 6). This also confirmed that the aqueous extract of *Coriandrum sativum* L. significantly prevented supersaturation, an initial step in urolithiasis. Phatak and Hendre confirmed that the aqueous leaf extract of *Kalanchoe pinnata* inhibited turbidity and the formation of urinary calculi *in vitro* [13].

Table 1. Phytochemical analysis of aqueous and ethanol extracts of *Coriandrum sativum* L.

Phytochemical constituents	Chemical tests	Aqueous extract	Ethanol extract
Flavonoids	Sodium hydroxide test	+	+
Saponins	Foam test	+	+
Quinones	Hydrochloric acid test	+	–
Steroids	Salkowski test	+	–
Tannins	Ferric chloride test	+	–
Terpenoids	Salkowski test	+	+
Glycosides	Kaller-kiliani test	+	+
Anthocyanins	Sodium hydroxide test	–	+
Phenols	Ferric chloride test	–	+
Alkaloids	Hager's test	+	+

'+' = Present and '-'= Absent

The final stage in urolithiasis is the formation of solid urinary calculi which gets deposited in different places in the urinary system. This deposition in turn obstructs the urinary flow and output. Gradually patients end up with oliguric and anuric state which leads to azotemia, a life threatening condition. At this stage, the only option available is to lyse the urinary stone and improve urinary output. In this study we examined whether the extracts of *Coriandrum sativum* L. can lyse the most prevalent calcium oxalate stone by adopting titrimetric method. A dose dependent study was carried out (10, 50, 100mg/ml) to test the efficacy of *Coriandrum sativum* L. on stone lysis (Figure 7 & 8). We observed that both the extracts dissolved calculi in a dose dependent manner. Recently Sumayya and Pratima showed *in vitro* antiurolithiatic activity of *Butea monosperma* Lam. and *Nigella Sativa* L. seeds by titrimetric method [14].The antiurolithiatic effects of aqueous and alcoholic extracts of *Coriandrum sativum* L. were compared with the standard drug Cystone, which is a polyherbal tablet marketed by Himalaya Drug Co. Bombay. Each tablet contains various phytochemicals viz., Didymocarpus pedkeflata 65 mg, Saxifraga ligulata 49 mg, Rubia cordifolia 16 mg, Cyperus scariosus 16 mg, Achyranthes aspera 16 mg, Onosma bracteatum 16 mg, Vernonia cinerea 16 mg, Hajrul yahood bhasma 16 mg, Shilajeet 13 mg respectively. It has been extensively reported in the management and treatment of many urinary complaints, urinary tract infections and in urolithiasis. The mode of action of Cystone is linked to calcium metabolism and the proper physicochemical conditions to maintain calcium in solution for normal elimination. It has an effective diuretic activity because of its high content of natural mineral salts and it is known to relax the detrusor muscles and act on the mucin in the calculi that binds the particles together.

Phytochemical analysis was carried out in the extracts of *Coriandrum sativum* L. They revealed the presence of flavonoids, saponins, terpenoids, glycosides and alkaloids (Table 1). However the presence of anthocyanin, phenols, quinones, steroids and tannins varies between the two extracts. Several studies confirmed the beneficial properties of these phytochemicals in the prevention and the treatment of urolithiasis. Recently Noorafshan reported that flavonoids inhibit CaOx crystallization in human urine and in animal models [15]. Similarly saponins are known to possess anti-crystallization property by disaggregating the suspension of mucoprotein the promoters of crystallization reaction [16]. Thus, we conclude that the antiurolithiatic activity observed in the present study was due to the synergetic effect of these phytochemicals present in the extracts of *Coriandrum sativum* L.

5. CONCLUSION

From the results it is evident that alcoholic and aqueous extracts of *Coriandrum sativum* L. dose dependently exhibits antiurolithiatic activity**.** This beneficial property is attributed to the presence of several secondary metabolites present in the extracts. The anti-urolithiatic activity was found to be more

significant in the aqueous extract than in alcoholic extract of *Coriandrum sativum* L. Further *in vivo* studies are required to elucidate the molecular actions of these extracts in preventing urolithiasis.

Conflicts of Interest

There are no conflicts of interest.

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