

EFFECT OF DIFFERENT EXTRACTS FROM *CELOSIA ARGENTEA* ON CALCIUM AND PHOSPHATE INHIBITION IN VITRO

BHAVIK PATEL^{1*}, PARESH PATEL², RAKESH PATEL²

Department of Pharmacology, K. J. College of Pharmacy, Vadasma, Gujarat, India, S.K. Patel College of Pharma. Edu & Res., Ganpat Vidhyanagar, Kherva, Gujarat, India. Email: bhavikpatel1984@yahoo.co.in

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ABSTRACT

Urinary stone disease has afflicted humankind since antiquity and can persist, with serious medical consequences, throughout a patient's lifetime. This study was undertaken to evaluate *In vitro* antilithiatic activity of *Celosia argentea* (L) seeds. *In vitro* activity was determined by inhibition of calcium (titrimetric analysis) and phosphate (colorimetric analysis) precipitation. Cystone (a marketed product) was used as reference drug for compare with ethyl acetate, petroleum ether, ethanol and aqueous extracts of *Celosia argentea* seeds. In which, ethanolic extract of *Celosia argentea* (*C. argentea*) was the most effective in inhibiting of calcium and phosphate precipitate *in vitro*.

Keywords: Antilithiatic, *Celosia argentea*, Cystone, Seeds

INTRODUCTION

A urinary stone disease (urolithiasis) is one of the most common afflictions of modern society that has been described since antiquity¹. Presently, urolithiasis is the third most common disorder of the urinary tract, next too urinary tract infections and benign prostates hyperplasia². It is a consequence of complex physiochemical process and the major contributory factors being urinary super saturation, crystallization, calculogenesis and matrix formation³. The pathogenesis of the lithiasis seems to be multifactor and complex⁴. Surgical management of urolithiasis includes lithotripsy and other surgical that have certain drawback which includes renal injury, decreased renal function and increase incidence in stone recurrence along with possibility of infection⁵. In spite of tremendous advances in the field of medicine, there is no truly satisfactory drug for the treatment of renal calculi². Majority of medicinal plants has potent diuretics activities are used in antilithiatic activity^{6, 7}. A variety of remedies have been used to treat urinary stones throughout history, most of which were taken from plants and have been proven to be useful.

Celosia argentea L (Amaranthaceae) grows as a weed during rainy season throughout India. The leaves are used for the treatment of inflammations, fever and itching. The seeds are bitter, useful in blood diseases, mouth sores. They are efficacious remedy in diarrhoea⁸. Based on ethno botanical practice the plant was investigated for anti inflammatory⁹, anti-pyretic¹⁰ anti diabetic¹¹, anti bacterial and diuretic properties¹². In view of this and on account of alleged usefulness of this plant in the traditional treatment this current study was aimed to evaluate the antilithiatic activity of various extracts from *C. argentea* on calcium and phosphate precipitate *in vitro*.

MATERIALS AND METHODS

Plant Material

Seeds of *C. argentea* were collected from Mehsana district of Gujarat and authenticated by Dr. Y. T. Jasrai, Professor and Head, Department of Botany, Gujarat University, Ahmadabad. A voucher specimen (KJCP, ACC. No. 11) has been preserved in the institute for future reference.

Chemical used

Ethyl acetate, Petroleum ether, Ethanol and Aqueous extracts of seeds of *C. argentea*, aqueous extract of cystone, TRIS buffer pH 7.4, 0.4 M Hcl, 25mm CaCl₂.2H₂O, 25mm Na₂HPO₄.2H₂O, 25mm Na₂C₂O₄.

Preparation of extracts

Celosia argentea

The coarse powder of seed was used for extraction. Continuous hot extraction was carried out by using different solvents of increasing polarity [petroleum ether (60-80°C), ethyl acetate and ethanol] in soxhlet extractor. Finally the marc was allowed for maceration about 24 hours with distilled water to obtain aqueous extract^{13, 14}.

Cystone

Aqueous extract was prepared by grinding a tablet to powder. This powder was mixed with 5 ml of water and kept for 2-3 hrs and then centrifuged at 1000 rpm. The clear supernatant was used for the study¹⁵.

0.1 M TRIS buffer

The buffer composition was: 0.1 M TRIS buffer; Solution A was 0.4 M TRIS [48.4 g of Tris (trihydroxymethyl) amino methane per 1000 ml]; Solution B was 0.4 M hydrochloric acid. [33.6 ml of concentrated hydrochloric acid per 1000ml]; A working solution was made up of 25 ml solution A, 20.7 ml solution B made up to 100 ml, the pH was 7.4¹⁵.

Experimental set-up

Total description of experiments was describe in below figure 1,

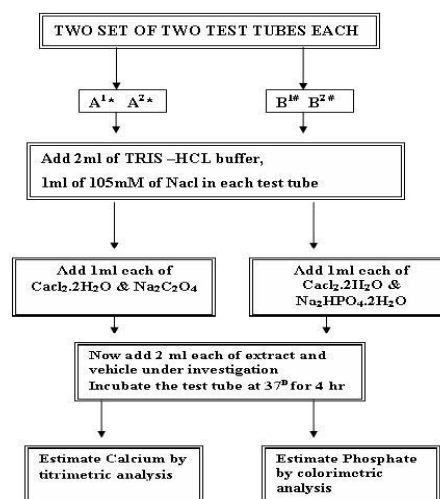


Fig. 1: Total Description of experiments

Calcium was estimated using titrimetry and phosphorus was estimated using colorimetric analysis. Appropriate standard curves were done with each set of experiments. The amounts of precipitate of calcium¹⁶ and phosphate^{17, 18} were determined in each of the sets by above methods respectively. The percent inhibition of the test was calculated in comparison with the control samples.

Statistical analysis

Statistical calculations were carried out with the Graph pad Prism 5.00 for Windows. The data are presented as the mean + SEM of five different sets of experiments. The statistical analysis was performed using the Students t-test with $P < 0.05$ being considered significant. Comparisons were made between the test and control of each group and also between *C. argentea* extracts and cystone groups.

RESULT AND DISCUSSION

A wide range of plants and plant derived products are used in folk medicine for the treatment of urolithiasis as a prophylactic

agent or as curative agent. The result of present study clearly indicate the cystone which is a prescribed treatment for urinary for renal calculi which showed a good inhibitory effect on the formation of the precipitate of calcium and phosphate (Table 1, 2 and 3).

Ethanol extract of *C. argentea* was more active than ethyl acetate, petroleum ether and aqueous extract of some plant. Ethanolic extract of *C. argentea* showed comparable activity to the marketed formulation in terms of inhibiting the formation of phosphate precipitate but showed a significantly better potential in preventing the formation of the calcium precipitate. (Table 4)

Cystone (as positive control) which is a prescribed for treatment for urinary and renal calculi, showed a good inhibitory effect on the formation of the precipitates of calcium and phosphate (Table 5 and Figure 2, 3)

Table 1: Percentage inhibition using ethyl acetate extract of *Celosia argentea*

	Amount of precipitates (μmol)		% of Inhibition	
	Calcium	Phosphate	Calcium	Phosphate
Ethyl acetate extract (0.4mg/ml)				
Control	7.06 \pm 0.01	6.11 \pm 0.02	---	---
Test	6.08 \pm 0.01	5.09 \pm 0.03	13.96 \pm 0.44***	16.70 \pm 0.97***

*** Significant difference when cystone is compared with ethyl acetate extract of *C. argentea* $P < 0.001$.

Table 2: Percentage inhibition using petroleum ether extract of *Celosia argentea*

	Amount of precipitates (μmol)		% of Inhibition	
	Calcium	Phosphate	Calcium	Phosphate
Petroleum ether extract (0.4mg/ml)				
Control	6.69 \pm 0.042	6.5 \pm 0.06	---	---
Test	5.95 \pm 0.05	5.63 \pm 0.06	11.06 \pm 0.51***	13.28 \pm 0.40***

*** Significant difference when cystone is compared with petroleum ether extract of *C. argentea* $P < 0.001$.

Table 3: Percentage inhibition using aqueous extract of *Celosia argentea*

	Amount of precipitates (μmol)		% of Inhibition	
	Calcium	Phosphate	Calcium	Phosphate
Aqueous extract (0.4mg/ml)				
Control	6.68 \pm 0.034	6.5 \pm 0.04	---	---
Test	5.34 \pm 0.044	5.56 \pm 0.06	20.05 \pm 0.88***	14.38 \pm 1.50***

*** Significant difference when cystone is compared with aqueous extract of *C. argentea* $P < 0.001$.

Table 4: Percentage inhibition using ethanol extract of *Celosia argentea*

	Amount of precipitates (μmol)		% of Inhibition	
	Calcium	Phosphate	Calcium	Phosphate
Ethanol extract (0.4mg/ml)				
Control	7.39 \pm 0.03	8.11 \pm 0.03	---	---
Test	4.47 \pm 0.008	3.38 \pm 0.02	39.64 \pm 0.54*	58.34 \pm 1.03***

*** Significant difference when cystone is compared with ethanol extract of *C. argentea* $P < 0.001$.

* Significant difference when cystone is compared with ethanol extract of *C. argentea* $P < 0.05$.

Table 5: Percentage inhibition using aqueous extract of cystone tablets

	Amount of precipitates (μmol)		% of Inhibition	
	Calcium	Phosphate	Calcium	Phosphate
Aqueous extract of cystone tablets (2 ml of 1 tablet/5ml)				
Control	7.15 \pm 0.003	8.03 \pm 0.008	---	---
Test	4.05 \pm 0.037	2.55 \pm 0.012	43.43 \pm 1.75	68.22 \pm 1.32

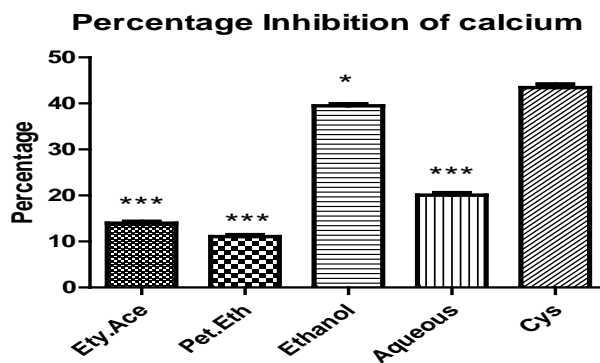


Fig. 2: Percentage inhibition of Calcium precipitation by different extract

*** Significantly different from Cystone $P < 0.001$

* Significantly different from Cystone $P < 0.05$

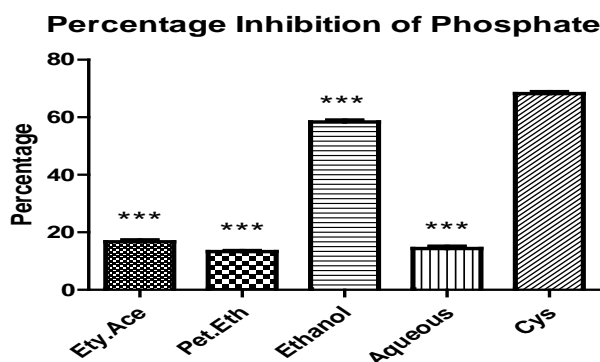


Fig. 3: Percentage inhibition of Phosphate precipitation by different extract

*** Significantly different from Cystone $P < 0.001$

C. argentea seeds extract in Ethanol inhibited the precipitation of calcium and phosphate while the same in ethyl acetate, pet ether and aqueous caused very little inhibition.

CONCLUSION

In conclusion, our study provides a basis for utility of *C. argentea* in the treatment of renal and urinary calculi.

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