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EVALUATION OF ANTI-LITHIATIC POTENTIAL OF AQUEOUS EXTRACT OF TRIANTHEMA DECANDRA BY *IN VITRO* CALCIUM AND PHOSPHATE INHIBITION ASSAY

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ABSTRACT

Kidney stone formation is one of the oldest and most wide spread diseases known to humans. The present study was aimed to investigate invitro Antilithiatic effect of aqueous extract of *Trianthema decantra* (whole plant) with various concentrations (200-1000µg/ml). *Invitro* Calcium and phosphate inhibition assay was adopted, which includes undissolved calcium was determined by titrimetric analysis and phosphate was estimated by colorimetric analysis. Cystone (as marketed product for kidney stones) was used as a standard references drug with same concentration. AETD (Aqueous extract of *Trianthema decandra*) was inhibited 54.87% of calcium precipitation and 55.64% phosphate precipitation at the concentration of 1000µg/ml. At the same concentration Cystone was inhibited 71.83% of calcium and 68.30% of phosphate precipitation. When compare with standard drug AETD is having moderate activity. Further research towards Isolation, purification, characterization and explaining the mechanism of action of phytochemicals responsible for antiurolithiatic activity is in progress.

Key word: Antilithiatic activity, aqueous extract, calcium precipitation inhibition, phosphate precipitation inhibition, kidney stones, *Trianthema decandra*.

INTRODUCTION

In the kidney stone formation several stages are involved. During this condition severe pain can be produce and it can leads to infection.[1] Supersaturation is the first step to promote these undesirable conditions and further steps involved are crystallization, nucleation,

aggregation and growth. Calcium containing stones consist of calcium oxalate monohydrate; calcium oxalate dihydrate and calcium phosphate are present. In most of the cases the commonly occurring stones are calcium oxalate or magnesium, ammonium phosphate type[2,3]. *Trianthema decandra* belongs to family Aizoaceae. It is commonly known as gadabani (Hindi) and vellai sharuni (Tamil). It is a prostrate, glabrous, succulent and annual weed found almost throughout India. *Trianthema decandra* has been used in various parts of Asia, Africa, Australia and South America for curing various diseases. This plant roots are used to cure corneal ulcers and used for itching, dimness of sight and night blindness, curing bacterial infections and it's also given in combination with ginger as a cathartic. The leaves contains huge amount of vitamin C which is used to treat edema.[4] The decoction of the herb is used as a vermifuge and is useful in rheumatisms. A decoction of this herb is used as an antidote to alcohol poison[5], which also reduces blood sugar[6]. It is also having Antioxidant properties[7] such as Nitric Oxide Scavenging Activity [8]

Earlier we did crystallization, nucleation, aggregation and growth inhibition assays by using AETD. The results were suggested that crude extract is having moderate activity[9]. At this time we have made an attempt to prove AETD is having calcium phosphate inhibition assay by using *Invitro* method.

2. MATERIALS AND METHODS

2.1. COLLECTION AND IDENTIFICATION OF PLANT MATERIAL:

The fully mature, whole plant of *Trianthema decandra* Linn. was collected from Midhilanagaram, Mellacheruvu village, Chittoor district, Andhra Pradesh. All the parts were air dried at room temperature (25^0C) for 15 days and converted into fine powder with an auto mix blender. The powder part was kept in a deep freezer until the time of use. The plant was identified and authenticated by Dr.Madhavachetty; Assistant Professor, Department of Botany, S.V.University, Tirupathi.

2.2. PREPARATION OF PLANT EXTRACT:

500 gm of dry fine powder was suspended in 1.5 liters of Double distilled water and then stirred magnetically for 24 hours at room temperature. The extract was double filtered by using muslin cloth and whatmann No. 1 filter paper. The filtrate was concentrated to dryness under reduced pressure at 40^0C using rotary vacuum evaporator (Buchi labortech AG, Switzerland). The percentage of yield was 16.2%. The dried AETD (Aqueous Extract of *Trianthema decandra* (whole plant) was stored in vacuum desiccators under controlled

conditions till it used for experimental purpose.

2.3. AQUEOUS EXTRACT OF CYSTONE

Aqueous extract was prepared by grinding tablets to powder. This powder was mixed with 5 ml of water and kept for 2-3 hrs and then centrifuged at 1000rpm. The clear supernatant was used for this study with different concentrations (200-1000µg/ml)[10].

2.4. PRELIMINARY PHYTOCHEMICAL SCREENING

1 gm of the aqueous extract of *Trianthema decandra* was dissolved in 100 ml of its own mother solvent to obtain a stock of concentration 1% (w/v). The standard methodology of Harborne (1998)[11] and Kokate (2001)[12] were adopted for the Phytochemical screening.

2.5. CHEMICAL USED

0.1M TRIS-buffer pH 7.4, 25mm $CaCl_2.2H_2O$, 25mm $Na_2HPO_4.2H_2O$, 25mm Sodium oxalate.

2.6. 0.1M TRIS BUFFER

The buffer composition was: 0.1M TRIS buffer; solution A was 0.4M TRIS (48.4g of Tris [trihydroxymethyl] amino methane per 1000ml); solution B was 0.4M hydrochloric acid. [33.6ml of concentrated hydrochloric acid per 1000ml]; A working solution was made up of 25ml solution A, 20.7ml solution B made up to 100 ml, the pH was 7.4[13].

2.7. EXPERIMENTAL PROTOCOL

Invitro calcium and phosphate precipitations inhibition assay methodology was adopted [14] with minor modifications. Four experimental setup made which contain a set of six test tubes. In each experiment one test tube used as control and other test tubes are used for different concentrations. Each test tube was filled with 2ml of Tris-Hcl buffer (pH 7.4), 1ml of 105mM of NaCl, 1ml of 25mM $CaCl_2.2H_2O$ and 25mM $Na_2C_2O_4$ add 2ml of AETD with different concentrations (200-1000µg/ml). Second experimental setup was added same composition instead of $Na_2C_2O_4$ add $Na_2HPO_4.2H_2O$. Third and fourth experimental setup was made as above composition, instead of crud extract, Cystone (200-1000µg/ml) may be used. All the test tube was incubated at 37^oC for 4hrs. After that undissolved calcium was estimated by titrimetric analysis[15] and phosphate was determined by colorimetric analysis[16]. A standard curves were draw with each set of experiment. The amount of

precipitate of calcium and phosphate percentage of inhibition of the test was calculated in comparisons with the control samples.

3. RESULT AND DISCUSSION

Traditional medicines are used by 70% of the world population. Traditional herb medicines are an important part of the health care system in India[17]. Preliminary phytochemical analysis showed the presence of Phenols, Terpenoids, Tannins, Saponins, Phytosterols, Carbohydrates, Flavonoids, Amino acids like phytoconstituents which already reported.

Table: 1. Calcium, Phosphate precipitation percentage inhibition of Aqueous Extract of *Trianthema decandra*. (Cystone as Standard Drug) (200-1000µg/ml).

S. No	Conc. of extract (µg/ml)	Amount of precipitate(µmol)				% inhibition			
		Calcium		phosphorus		calcium		Phosphorus	
		AETD	Cystone	AETD	Cystone	AETD	Cystone	AETD	Cystone
1	Control	8.20	8.70	7.82	7.92	_	_	_	_
2	200	7.21	7.44	6.84	6.24	13.17	14.51	12.41	14.75
3	400	6.32	6.26	5.46	4.92	22.92	26.87	30.08	32.78
4	600	5.31	5.04	4.84	3.82	35.24	40.84	38.02	47.81
5	800	4.22	4.00	3.92	3.34	48.53	53.28	49.80	54.37
6	1000	5.70	2.40	3.48	2.51	54.87	71.83	55.64	68.30

(AETD- Aqueous Extract of *Trianthema decandra*.)

The results of present study clearly indicate the AETD was inhibiting 54.87% of calcium precipitation and 55.64% of calcium phosphate precipitation at the concentration of 1000µg/ml. At the same concentration Cystone which is prescribed for treatment of urinary stones showed a good inhibitory effect. Calcium oxalate precipitation was inhibiting 71.83% and calcium phosphate precipitation was inhibited 68.30%. (Table-1)

In the earlier studies we tried to prove AETD is having antiurolithiatic activity which is proved by *Invitro* crystallization, nucleation, aggregation and growth inhibition assays. The results of this research work were presented in the figure 1 and 2. We are assuming that the crude drug may contain some phytochemical which is responsible for inhibiting the

precipitation of calcium and phosphate by donating some ions which may reduce the complex formation the same may be leads to precipitation ultimately stone formation.

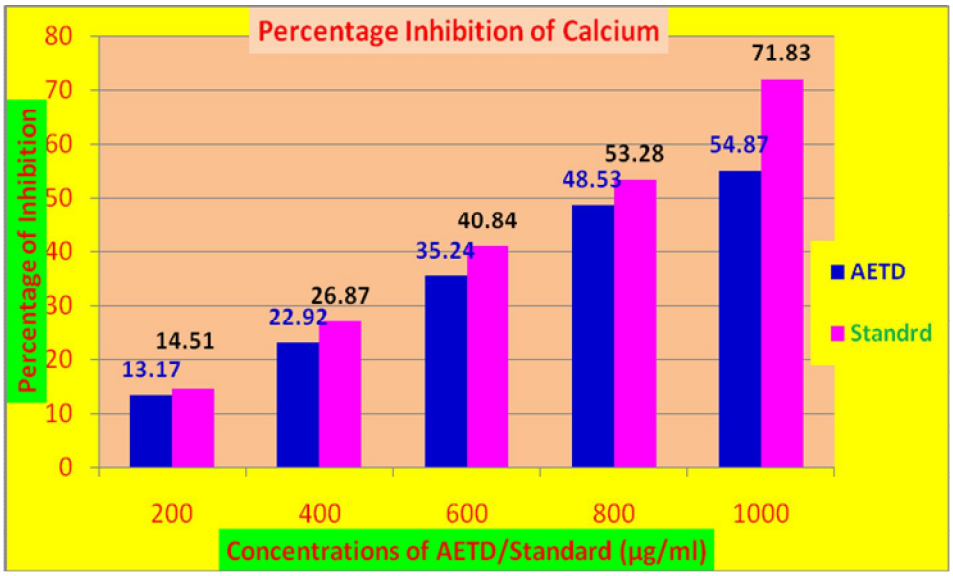

Figure: 1. Percentage Inhibition of Calcium precipitation by different concentrations of AETD and Cystone (Standard drug).

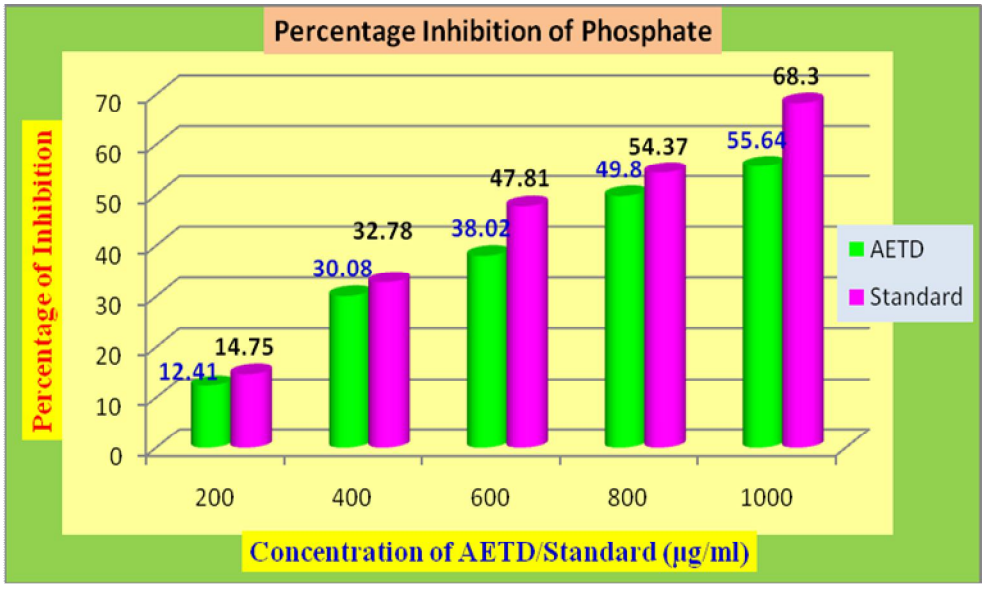

Figure: 1. Percentage Inhibition of Phosphate precipitation by different concentrations of AETD and Cystone (Standard drug).

4. CONCLUSION

Antiurolithiatic activity has been performed by using AETD (whole plant) by Calcium oxalate and Calcium phosphate precipitation method *Invitro.* From above results we conclude that, crud extract is having moderate antiurolithiatic activity when compared to

Cystone which shows good results. The study provides a new source of plant product which can be utilized for renal Calculi if suitable formulation developed. Isolation, purification, characterization and explaining the mechanism of action of phytochemicals responsible for antiurolithiatic activity is in progress.

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