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In-vitro anti-inflammatory, anti-oxidant and *In-vivo* anti-urolithiatic activities of *Coccinia grandis* fruit extract

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Abstract

This research study was performed to evaluate the *in-vitro* anti-oxidant, anti-inflammatory and *in-vivo* activities of ethanolic fruit extract of *Coccinia grandis*. The fruit extract of *Coccinia grandis* at a concentration of 100 µg/ml was tested for its free radical scavenging activity adopting the standard methods like DPPH and Nitric Oxide. *In-vitro* anti-inflammatory activity was determined using Protein Denaturation Method and Human Red Blood Cells Method using *Coccinia grandis*. Further, *In-vivo* anti-urolithiatic activity was carried out by administering 0.75% of Ethylene Glycol solution to male rats for drinking, which caused urolithiasis. *Coccinia grandis* fruit extract exhibited significant anti-oxidant, anti-inflammatory and anti-urolithiatic activities. The results indicate that the extract possessed good therapeutic potential.

Keywords: *Coccinia grandis*; Anti-oxidant; Anti-inflammatory; Anti-urolithiasis; Ethylene glycol; Human Red Blood Cells method

1. Introduction

Coccinia grandis [Ivy gourd] belonging to the family Cucurbitaceae [1-4], a well-known vegetable grown throughout the state of Andhra Pradesh. Local natives use this fruit extract for the treatment of sores of tongue and infection caused by helminthes. Research work has been carried out on the whole plant, leaves and on root, scanty work is reported on the fruit. Sincere initiatives were made by the researchers to investigate the biological activities on *Coccinia grandis* fruit. In Ayurveda, *Coccinia grandis* fruit juice being used for the treatment of urinary stones and calculi. Based on this fact anti-urolithiatic [5, 6] activity is aimed to evaluate the pharmacological investigation.

2. Materials

2.1. Collection of *Coccinia grandis* fruit

Unripe fresh fruits of *Coccinia grandis* were procured from local sellers of Surampalem, Peddapuram, Samarlakot and Jaggampeta are collected for biological studies.

2.2. *Extraction*

100 grams of coarsely ground, air-dried *Coccinia grandis* fruit extract is continuously heated with ethanol in a sox-let extractor for 8-10 hours. After the filtration, the extract was filtered, and the filtrate was dried by evaporating it.

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2.3. Chemicals and Apparatus

All the chemicals used for this research study were of Analytical grade and Glassware used is Corning and Borosil apparatus

3. Results

3.1. *In-vitro* Anti-oxidant activity:

- **DPPH Method-** Ethanolic fruit extract of *Coccinia grandis* was subjected to DPPH radical scavenging assay [5,7] and the extract at a concentration of 100 µg/ml showed maximum inhibition of 68.50% and the results are compared with standard agent Gallic acid 2.5 µg/ml which produced maximum inhibition of 79%. Table 1 and Figure 1 present an overview of the results.

Table 1 DPPH Radical Scavenging Assay of *Coccinia grandis* fruit extract

S.No	Test	Concentration (µg/ml)	Percentage of Inhibition
1	*Coccinia grandis* fruit extract	100	68.50 ±0.30
2	Gallic acid (Standard)	2.5	79.00±0.50

Values are Mean ± SEM, n=3.

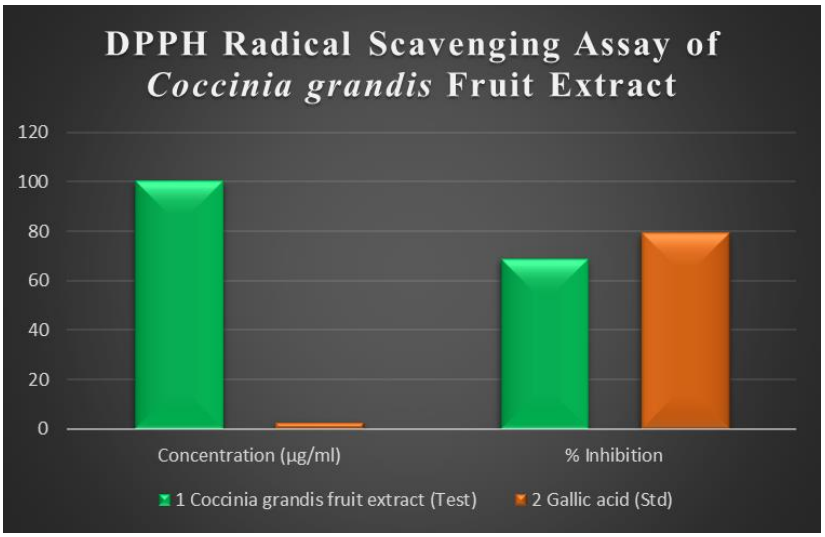

Figure 1 DPPH Radical Scavenging Assay of *Coccinia grandis* fruit extract

- ***Nitric oxide scavenging activity:*** The fruit extract of *Coccinia grandis* is subjected for Nitric oxide scavenging assay [8] and the extract showed significant inhibition at 100 µg/ml as 51.50 and this result is compared with standard reference drug Gallic acid 2.5 µg/ml which showed 65.50%. Table 2 and Figure 2 show tabulated results.

Table 2 Nitric-oxide Scavenging Assay of *Coccinia grandis* fruit extract

S.No	Test	Concentration (µg/ml)	Percentage of Inhibition
1	*Coccinia grandis* fruit extract	100	51.50 ±0.80
2	Gallic acid (Standard)	2.5	65.50 ±0.45

Values are Mean ± SEM, n=3.

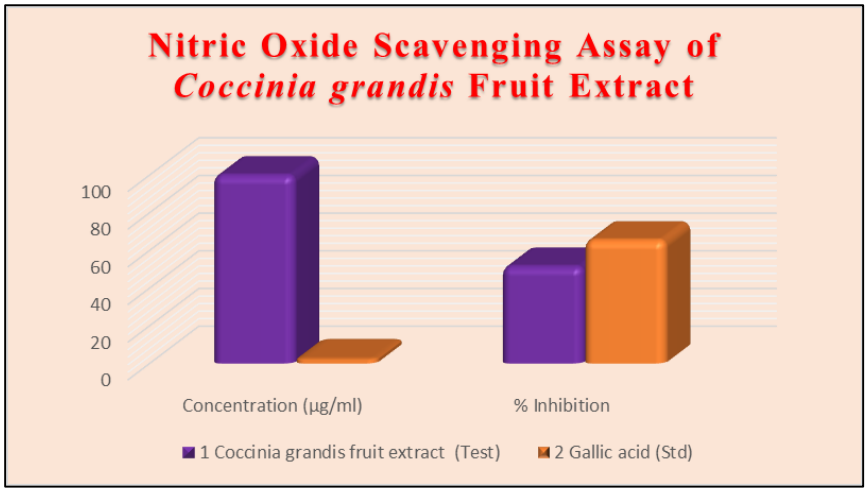

Figure 2 Nitric-oxide Scavenging Assay of Coccinia grandis fruit extract

3.2. *In-vitro* Anti-inflammatory activity:

3.2.1. Protein denaturation method [9,10,11]*:*

One of the most adaptable *in-vitro* anti-inflammatory approach used to assess *Coccinia grandis* anti-inflammatory properties is protein denaturation. The Standard anti-inflammatory drug Diclofenac sodium at 10 µg/ml and 25 µg/ml concentrations produced inhibition of 65.50 and 80.20 respectively, compared to the fruit extract which showed tremendous percentage of inhibition of 30.50 and 50.40 at concentrations of 100 µg/ml and 200 µg/ml. The extract's anti-inflammatory properties depend on concentration. The results were summarized in Table-3 and Figure-3.

Table 3 *In vitro* Anti-inflammatory effect of *Coccinia grandis* fruit extract by Protein denaturation method

S.No	Concentration (µg/ml)	Percentage of Inhibition	
		***Coccinia grandis* fruit extract**	**Diclofenac Sodium (Standard)**
1	10	-	65.50
2	25	-	80.20
3	100	30.50	-
4	200	50.40	-

Values are Mean ± SEM, n=3.

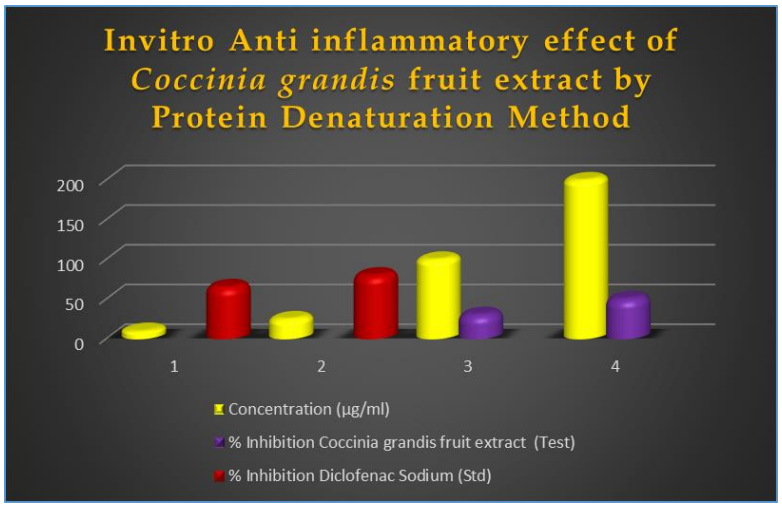

Figure 3 *In-vitro* Anti-inflammatory effect of *Coccinia grandis* fruit extract by Protein denaturation method

3.2.2. Human Red Blood Cell Membrane Stabilization Method

This method is an important method used to demonstrate *Invitro* Anti-inflammatory activity of *Coccinia grandis* fruit extract. In the HRBC Membrane Stabilization Method, the extract inhibited the lysis of membranes caused by hypotonicity to a degree of 35 and 58% at concentration of 100 µg/ml and 200 µg/ml which is compared to that of standard reference drug Diclofenac Sodium 55.50 and 70.20 at concentration of 10 µg/ml and 25 µg/ml respectively. The anti-inflammatory effect varies with the concentration and the results are described in Table-4 and Figure-4.

Table 4 *In vitro* Anti-inflammatory effect of *Coccinia grandis* fruit extract by HRBC method

S.No	Concentration (µg/ml)	Percentage of Membrane Lysis	
		***Coccinia grandis* fruit extract**	**Diclofenac Sodium (Standard)**
1	10	-	55.50
2	25	-	70.20
3	100	35	-
4	200	58	-

Values are Mean ± SEM, n=3.

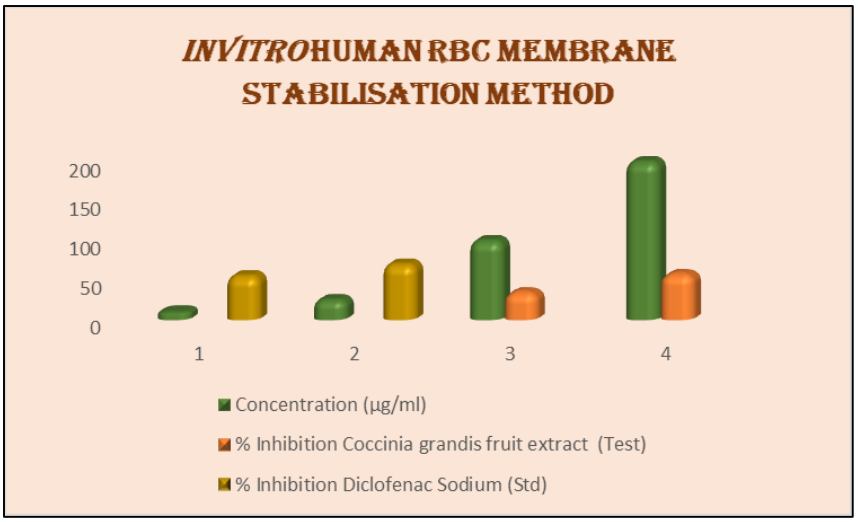

Figure 4 *In vitro* Anti-inflammatory effect of Coccinia grandis fruit extract by HRBC method

3.3. *In-vivo* Anti- urolithiatic activity

Ethylene glycoside induced hyper-oxaluria method was used to access the anti-urolithiatic activity in Albino male rats [12, 13]. Rats were divided into four groups, each group containing three animals. Ethylene glycol 0.7% v/v in drinking water is given to all Groups for the induction of renal calculi for 28 days. The *Coccinia grandis* fruit extract was assessed for its anti-urolithiatic activity and its curative action in urolithiasis. In this experiment the extract is given from 15th day to 28th day

- Group I: Normal control group received regular rat food and drinking water
- Group II: [Calculi induced] the control received ethylene glycol 0.7% v/v in drinking water for induction of renal calculi for 28 days.
- Group III: Received ethylene glycol and standard Urolithiatic drug Cystone 750 mg/ kg body weight from 15 to 28th day [14].
- Group IV: Received 200 mg/kg of *Coccinia grandis* fruit extract from 15th to 28th day.

3.4. Estimation of Biochemical Parameters

Urine was taken at the end of the experiment, on day 28, and its Calcium, Phosphate and Oxalate levels were measured. Tail vein method is utilized for collecting blood in order to measure uric acid and calcium creatinine in the serum.

Table 5 *In vivo* Anti-urolithiatic activity of *Coccinia grandis* fruit extract

S.No	Group	Urine			Serum		
		Calcium	Phosphate	Oxalate	Calcium	Creatinine	Uric acid
1	Control	3.3±1.10	16±1.80	4.0±1.02	7.0±1.40	0.5±0.14	3.0±1.02
2	Calculi	13.5±0.12	36.0±0.15	7.8±1.95	15.0±2.04	36.0±1.48	7.2±1.05
3	Cystone(750mg/kg)	3.65±1.02	16.8±0.20	3.8±1.90	4.10±1.30	0.85±0.12	3.4±0.20
4	*Coccinia grandis* (200mg/kg)	8.0±1.30	22.30±0.12	4.2±1.20	10.50±1.60	0.7±0.10	3.9±1.30

Values are Mean ± SEM, n=3, t<0.001.

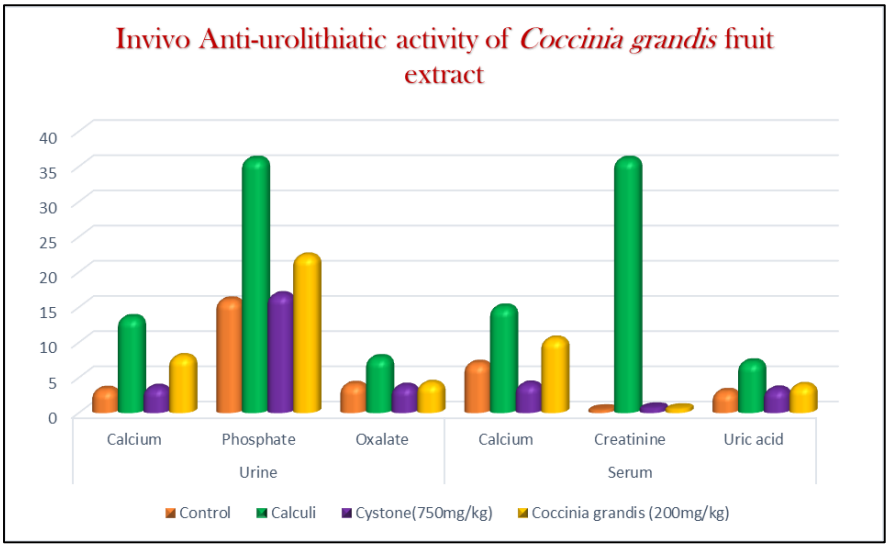

Figure 5 *In-vivo* Anti-urolithiatic activity of Coccinia grandis fruit extract

4. Discussion

No much pharmacological work is carried out on *Coccinia grandis* fruit and based on the folkloric usage of this fruit by the natives of East Godavari residents a sincere attempt was made by the researchers to explore the important biological activities of this medicinal plant.

The ethanolic extract of *Coccinia grandis* fruit is subjected for free radical scavenging activity and most important versatile methods were employed to evaluate the antioxidant activity. *Coccinia grandis* at a concentration of 100 µg /ml produced 68.5 0 percentage of inhibition as compared to the Standard drug Gallic acid 2.5 µg/ml producing 79% of inhibition in DPPH radical scavenging method. Further Nitric oxide scavenging method is also used for the detection of antioxidant activity. *Coccinia grandis* at a concentration of 100 µg/ml exhibited 51.5 percentage of inhibition when compared to Gallic acid 2.5 µg/ml producing 65.50% of inhibition.

In vitro anti-inflammatory activity of *Coccinia grandis* fruit extract is evaluated by implementing two important methods such as Protein denaturation and Human RBC membrane stabilizing methods. The fruit extract tremendously produced significant anti-inflammatory activity at a concentration of 100 and 200 µg/ml producing 30.5 0 and 50.40 of maximum percentage of inhibition and the results were very well compared with standard anti-inflammatory drug Diclofenac sodium which produced significant percentage of inhibition of 65.5 and 80.20 at a concentration of 10 and 25 µg/ml respectively. Similarly another versatile Human RBC method is employed for *Coccinia grandis* fruit extract at a concentration of 100 and 200 µg/ml which produced good percentage of inhibition with 35 and 58 percentage and

Diclofenac sodium was used to compare these results which produced 55.5 and 70.20 percentage of maximum inhibition at a concentration of 10 and 25 µg/ml.

The different phyto-constituents present in the *Coccinia grandis* fruit extract could be responsible for urolithiatic activity. Ethylene glycol in liver is metabolized to Glycolic and Glycoxylic acid which is further oxidized to Oxylate thus promoting hyperoxyluria. The combination of Uric acid, Calcium, Oxalate and Inorganic Phosphate in urine may enhance crystallization. Urolithiasis can be induced by ethylene glycol in rats with 28 days resulting in substantial extraction of oxalate and deposition of crystals in kidney [15]. Apatite (calcium phosphate) or calcium oxalate precipitation and subsequent crystal formation are both favored by elevated urine calcium concentrations. The *Coccinia grandis* fruit extract significantly reduced concentrations of Calcium, Phosphorus and Oxalate in urine when compared to Calculi induced group and these results are very well compared with Group III Reference drug. Similarly the concentration of Calcium, Creatinine and Uric acid in Blood Serum is drastically lowers in Group IV treated with fruit extract of *Coccinia grandis* when compared with Calculi induced group which is compared with cistone the standard drug Cistone of group III. Based on the results obtained in Group IV *Coccinia grandis* fruit extract significantly exhibited anti-urolithiatic activity in experimental animals.

5. Conclusion:

The researches have made a sincere attempt to evaluate the possible pharmacological potential of ethanolic fruit extract of *Coccinia grandis* for various biological activities. Based on the experimental results obtained it is very evident that the medicinal plant possessed and exhibited significant Free radical scavenging, Anti-inflammatory and Anti-urolithiatic activities. Furthermore research work can be attempted to establish and elucidate the responsible phyto-constituents present in this medicinal plant that may be responsible for the biological activities.

Compliance with ethical standards

Acknowledgments

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Disclosure of conflict of interest

Conflicts of interest have not been brought up by the authors.

Statement of ethical approval

The ethical approval for this work was obtained in accordance with the IAEC criteria and the Aditya College of Pharmacy's CPCSEA regulations.

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