EVALUATION OF ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES OF *Citrullus colocynthis* and *Coccinia grandis* UNRIPE FRUITS EXTRACT

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Abstract

The aim of the study to investigate the in vitro antioxidant and anti-inflammatory activities of Citrullus colocynthis and Coccinia grandis unripe fruits extract. Antioxidant activity was done through DPPH, Hydrogen peroxide scavenging activity and reducing power assay. Antioxidant and anti-inflammatory activities were observed that Citrullus colocynthis and Coccinia grandis unripe fruits increased with increasing concentration. Anti-inflammatory activity evaluated by protein denaturation method. Among the two plants, Citrullus colocynthis extract has potential antioxidant and anti-inflammatory activities than Coccinia grandis unripe fruit extract was observed.

Keywords: *Citrullus colocynthis and Coccinia grandis, antioxidant and anti-inflammatory activities*

INTRODUCTION

Oxidative stress is an important risk factor in the pathogenesis of numerous chronic diseases. Free radicals and other reactive oxygen species are recognized as agents involved in the pathogenesis of sicknesses such as asthma, inflammatory arthropathies, diabetes, Parkinson's and Alzheimer's diseases, cancers as well as atherosclerosis. Reactive oxygen species are also said to be responsible for the human aging (Kanwar *et al*., 2009; Chiavaroli *et al*., 2011). An antioxidant can be broadly defined as any substance that delays or inhibits oxidative damage to a target molecule (Yamagishi and Matsui, 2011). The main characteristic of an antioxidant is its ability to trap free radicals. Antioxidant compounds like phenolic acids, polyphenols and flavonoids scavenge free radicals such as peroxide, hydroperoxide or lipid peroxyl and thus inhibit the oxidative mechanisms that lead to degenerative diseases (Wu *et al*., 2011). Herbal plants considered as good antioxidant since ancient times. The aim of the study to investigate the i*n vitro* antioxidant and anti-inflammatory activities of *Citrullus colocynthis* and *Coccinia grandis* unripe fruits extract.

MATERIALS AND METHODS

Collection and authentication of plant materials

The unripe fruits of *Citrullus colocynthis* and *Coccinia grandis* were collected from Pullavarayankudikkadu Village, Mannarkudi (Tk) Thiruvarur (Dt) Tamilnadu, India (Camble, 1935, Mathew 1983), during the month of April 2016. The unripe fruits were air dried at room temperature for one month and the plant authenticated and the specimen was kept in Pushpam Herbarium, Dept of Botany and Microbiology A.V.V.M. Sri Pushpam College, Poondi, Thanjavur Dt, Tamilnadu for further reference..

Extract preparation

Ten grams of unripe fruits of *Citrullus colocynthis* and *Coccinia grandis* powder extracted with 100 ml of methanol using extraction of unripe fruits dried powder was grained with a mixture and added to 10 ml solvent. After 8-10 hours of duration with continuous strirring at 200rpm/min. The mixture was filtered using the filter paper (What man No 1). This operation is repeated four times after each filtration with renewal of the solvent in order to exhaust the match and increase the yield. At the end of extraction and filtration obtained were collected and then were evaporated by rotra vapor at a specific temperature with the solvent.

Determination of *in-vitro* antioxidant activity

Assay of DPPH radical-scavenging activity followed by the method of Shimada, *et al.,* (1992). Determination of Hydrogen peroxide scavenging activity of the extract was estimated by method of Zhang (2000). The reducing power activity was examined by Oyaizu (1986) method.

Determination of *in-vitro* anti-inflammatory activity

Anti-inflammatory activity evaluated by protein denaturation (Egg albumin) method as described by Padmanabhan and Jangle (2012). Anti-inflammatory activity evaluated by protein denaturation (Bovine serum albumin) method as described by Mizushima and Kobayashi, 1968.

RESULTS AND DISCUSSION

Free radicals are responsible for causing a large number of diseases including cancer, cardiovascular disease, neural disorders, Alzheimer's disease, mild cognitive impairment, Parkinson's disease, alcohol induced liver disease, ulcerative colitis, aging and atherosclerosis (Velavan, 2011; Smith *et al*., 2000). Protection against free radicals can be enhanced by ample intake of dietary antioxidants. Substantial evidence indicates that foods containing antioxidants and possibly in particular the antioxidant nutrients may be of major importance in free radical mediated diseases (Blokhina *et al*., 2003).

The human body has a complex system of natural enzymatic and non-enzymatic antioxidant defenses which counteract the harmful effects of free radicals and other oxidants (Badarinath et al., 2010). Antioxidants may be of great benefit in improving the quality of life by preventing or postponing the onset of degenerative diseases. In addition, they have a potential for substantial savings in the cost of health care delivery. Various methods are used to investigate the antioxidant property of samples (diets, plant extracts, commercial antioxidants etc.) (Nur Alam *et al*., 2013).

DPPH radical scavenging activity

1,1- Diphenyl-2-picrylhydrazyl radical is a commonly used method to assess the free radical scavenging ability of various extracts and compounds. DPPH is a nitrogen centered radical and the changes of colour from violet to yellow upon reduction is observed by the process of hydrogen or electron donation. If test extract could perform this reaction antioxidant potential can be measured of the same free radical scavenging activity of the test extract is assessed. It was observed that the free radical scavenging activities of *Citrullus colocynthis* and *Coccinia grandis* unripe fruits increased with increasing concentration. The antioxidant substance present in the extract counters with DPPH free radical solution and converts them into its reduced form either by transferring electron or donating hydrogen atom followed by proton (Nuutila *et al*., 2003).

Table. 1 : DPPH radical scavenging activity of *Citrullus colocynthis and Coccinia grandis* unripe fruits at different concentrations

Concentrations (µg/ml)	% of inhibitions		
	Citrullus colocynthis	***Coccinia grandis***	**Standard as Ascorbic acid**
20	23.87 ± 1.67	19.98 ± 1.39	25.71 ± 1.79
40	42.73 ± 2.99	35.35 ± 2.47	47.21 ± 3.30
60	63.98 ± 4.47	59.24 ± 4.14	73.98 ± 5.17
80	84.64 ± 5.92	80.76 ± 5.65	95.15 ± 6.66
IC_{50} Value	46.29	51.12	41.05

Values are expressed as Mean± SD for triplicates

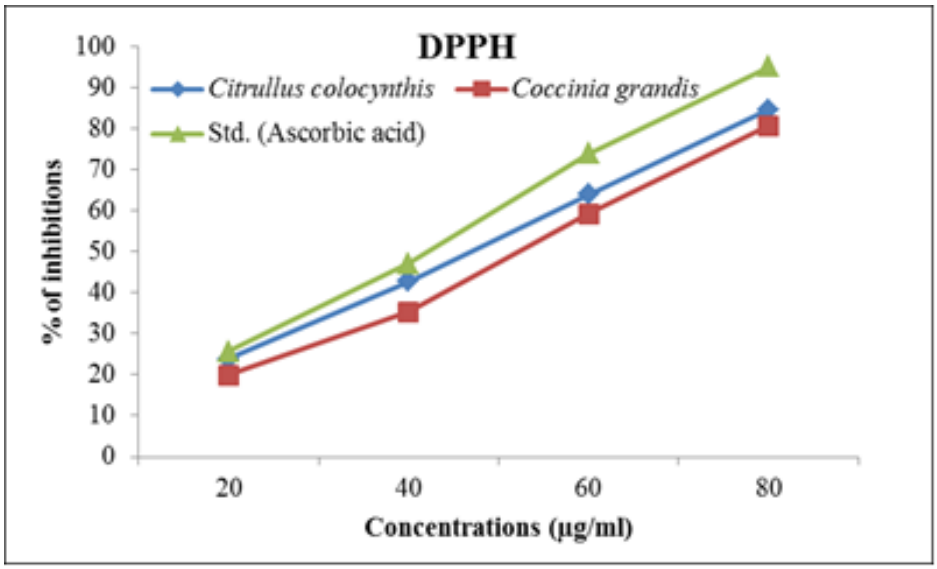

Fig.1: DPPH radical scavenging activity of *Citrullus colocynthis* and *Coccinia grandis* unripe fruits at different concentrations

DPPH radical scavenging activity of unripe fruits extract and standard as ascorbic acid are tested. The half inhibition concentration (IC_{50}) of *Citrullus colocynthis* and *Coccinia grandis* unripe fruits extract and ascorbic acid were 46.29µg/ml, 51.12µg/ml and 41.05µg/ml respectively**.** The unripe fruits extract exhibited a significant dose dependent inhibition of DPPH activity. The potential of L-ascorbic acid to scavenge DPPH radical is directly proportional to the concentrations. The DPPH assay activity of *Citrullus colocynthis* unripe fruit extract is near to standard as ascorbic acid (Table 1 and fig 1).

Hydrogen Peroxide scavenging activity

Fig. 2 depicts the Hydrogen peroxide scavenging effect of *Citrullus colocynthis* and *Coccinia grandis* unripe fruits extract. The hydrogen peroxide scavenging activity of *Citrullus colocynthis* and *Coccinia grandis* unripe fruits extract increased with increasing concentrations in table 2. The half inhibition concentration (IC_{50}) of *Citrullus colocynthis* and *Coccinia grandis* unripe fruits extract and ascorbic acid were 47.31, 51.43µg/ml and 42.74µg/ml respectively**.**

Table.2: Hydrogen peroxide scavenging activity of *Citrullus colocynthis and Coccinia grandis* unripe fruits at different concentrations

Concentrations (µg/ml)	% of inhibitions		
	Citrullus colocynthis	***Coccinia grandis***	**Standard as Ascorbic acid**
20	22.68 ± 1.58	18.31 ± 1.28	24.81 ± 1.73
40	40.81 ± 2.85	38.76 ± 2.71	45.79 ± 3.20
60	65.21 ± 4.56	57.83 ± 4.04	71.36 ± 4.99
80	82.17 ± 5.75	79.31 ± 5.55	90.23 ± 6.31
IC_{50} Value (µg/ml)	47.31	51.43	42.74

Values are expressed as Mean± SD for triplicates

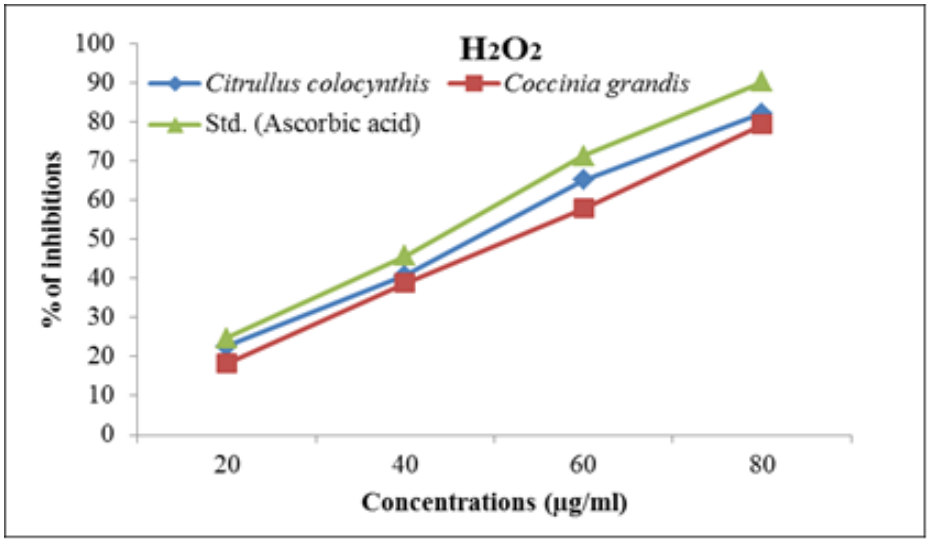

Fig.2: Hydrogen peroxide scavenging activity of *Citrullus colocynthis* and *Coccinia grandis* unripe fruits at different concentrations

Hydrogen peroxide is one of the most important reactive oxygen species formed from superoxide. It could be transformed to the hydroxyl radical via the Fenton reaction where transition metals ions (such as Fe^{2+}) reduce hydrogen peroxide to the hydroxyl radical thus the chelation of Fe^{2+} ions and or the reduction of Fe^{3+} ions is an important event in the prevention or reduction of oxidative stress. The hydroxyl radical reacts indiscriminately with any macromolecule it touches, thereby instigating cellular stress. Hydrogen peroxide also damages cells through direct oxidation of lipid, proteins, DNA, and subsequently necrotic cell death via mitochondrial-driven apoptosis (Nagababu *et al*., 2003; Youdim *et al*., 2000). Thus the scavenging of hydrogen peroxide could reduce these cellular effects and contribute significantly to the improvement of health and wellbeing. The hydrogen peroxide scavenging activity of *Evolvulus alsinoides* increased with increasing concentrations.

Reducing power activity

For measuring the reducing ability, the Fe^{3+} - Fe^{2+} transformation was investigated in the presence of *Citrullus colocynthis* and *Coccinia grandis* unripe fruits extracts. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. However, the activity of antioxidants has been assigned to various mechanisms such as prevention of chain initiation, binding of transition metal ion catalysts, decomposition of peroxides, prevention of continued hydrogen abstraction, reductive capacity and radical scavenging activity (Diplock, 1997; Yildirim *et al.*, 2000). The reducing power of *Citrullus colocynthis* and *Coccinia grandis* unripe fruits extracts increased with increasing dosage. All the doses showed significantly higher activities than the control exhibited greater reducing power, indicating that antioxidant activity *Citrullus colocynthis* and *Coccinia grandis* unripe fruits extract (Table 3 and fig 3).

Table.3: Reducing power activity of *Citrullus colocynthis* and *Coccinia grandis* unripe fruits at different concentrations

Concentrations (µg/ml)	Optical (O.D.)		
	Citrullus colocynthis	***Coccinia grandis***	**Standard as Ascorbic acid**
20	0.19 ± 0.01	0.15 ± 0.01	0.23 ± 0.01
40	0.37 ± 0.02	0.32 ± 0.02	0.47 ± 0.03
60	0.65 ± 0.04	0.56 ± 0.03	0.73 ± 0.05
80	0.81 ± 0.05	0.78 ± 0.05	0.92 ± 0.06

Values are expressed as Mean± SD for triplicates

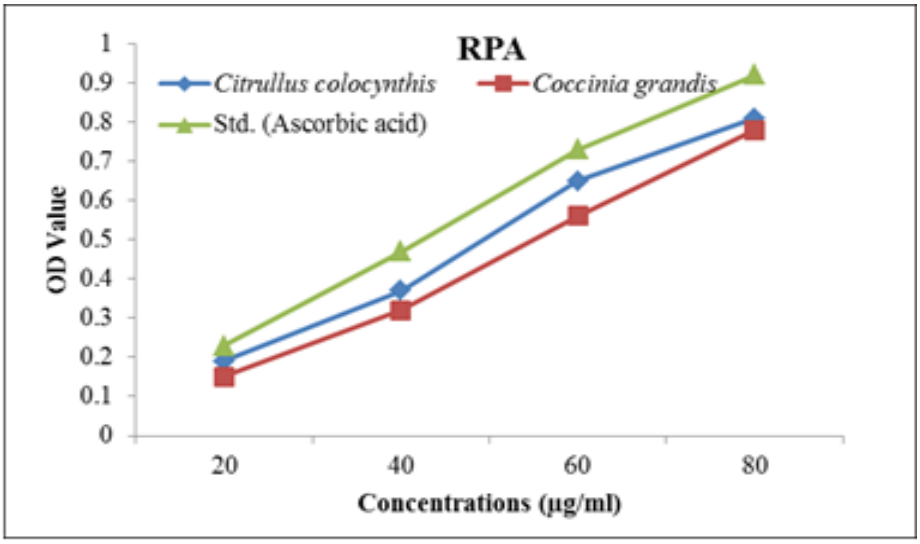

Fig 3: Reducing power activity of *Citrullus colocynthis* and *Coccinia grandis* unripe fruits at different concentrations

The present study has shown that the methanol extract of *Citrullus colocynthis* and *Coccinia grandis* unripe fruits contains significant amount of phenolics and thus, can be inferred that these phenolics are responsible for their noticeable antioxidant activity as assayed through various *in vitro* models used in the study. Among the two plants, *Citrullus colocynthis* extract has potential biological activities than *Coccinia grandis* unripe fruit extract was observed. This is the agreement with several reports that have shown close relationship between total phenolic contents and antioxidative activity (Sofiane *et al*., 2018; Ravishankar *et al*., 2018). Methanolic extract of *Citrullus colocynthis and Coccinia grandis* unripe fruits have considerable antioxidant properties and the consumption of this under-exploited plant may play a role in preventing human diseases in which free radicals are involved, such as cardiovascular disease, cancer and premature aging.

***In-vitro* anti-inflammatory activity**

There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available or could be investigated. During inflammation, lysosomal hydrolytic enzymes are released into the site which causes damages of the surrounding organelles and tissues with observed variety of disorders (Vduvu and lakshmi, 2008). Various methods were employed to screen and study drugs, chemical, herbal preparation that exhibit anti-inflammatory properties or potentials. These techniques include uncoupling of oxidative phospharylation (ATP biogenesis linked to respiration), inhibition of denaturation protein, erthrocyte membrane stabilization, lysosomal membrane stabilization, fibrinolytic assays and platelet aggregation (Gambhire *et al.*, 2009). Hence, in the present study the protein denaturation bioassay was used to confirm *in-vitro* anti-inflammatory property of *Citrullus colocynthis and Coccinia grandis* unripe fruits extract.

The anti-inflammatory activity of *Citrullus colocynthis* and *Coccinia grandis* unripe fruits extract were investigated using protein denaturation (Egg albumin and Bovine serum albumin) method. The present findings exhibited a concentrations dependent inhibition of egg albumin denaturation by the *Citrullus colocynthis* and *Coccinia grandis* and Diclofenac

sodium was used as the reference drug. The highest dose of *Citrullus colocynthis* and *Coccinia grandis* (500 µg/mL) was found to be near to the diclofenac sodium. The IC_{50} value *Citrullus colocynthis* and *Coccinia grandis* was 285.37, 314.33µg/mL and standard was 246.49µg/mL. From the study it can be concluded that *Citrullus colocynthis and Coccinia grandis* showed marked *in-vitro* anti-inflammatory effect against the denaturation of protein (Table 4 and fig.4). Among the two plants, *Citrullus colocynthis* unripe fruit extract has potential activity than *Coccinia grandis* unripe fruit extract.

Table.4: *In-vitro* anti-inflammatory activity of *Citrullus colocynthis* and *Coccinia grandis* unripe fruits extract (Egg albumin)

Concentrations (µg/mL)	***Citrullus colocynthis* (%)**	***Coccinia grandis* (%)**	**Standard (Diclofenac sodium) (%)**
100	18.32 ± 1.28	15.71 ± 1.09	23.63 ± 1.65
200	36.43 ± 2.55	31.87 ± 2.23	45.41 ± 3.17
300	51.06 ± 3.57	48.75 ± 3.41	59.87 ± 4.19
400	69.87 ± 4.89	61.98 ± 4.33	73.49 ± 5.14
500	86.74 ± 6.07	80.32 ± 5.62	91.28 ± 6.38
IC_{50} (µg/ml)	285.37	314.33	246.49

Values are expressed as Mean± SD for triplicates

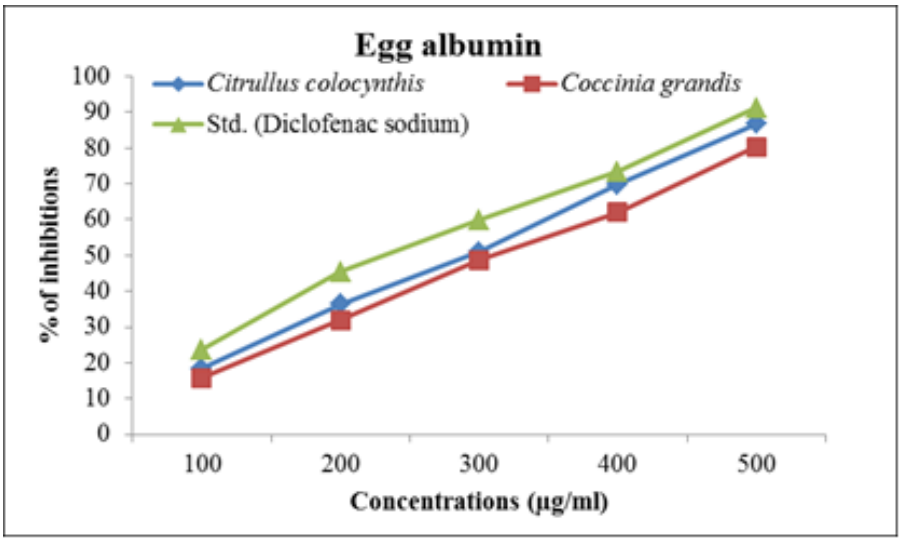

Fig.4: *In-vitro* anti-inflammatory activity of *Citrullus colocynthis* and *Coccinia grandis* unripe fruits extract (Egg albumin)

. The present findings exhibited a concentrations dependent inhibition of Bovine serum albumin denaturation by the *Citrullus colocynthis* and *Coccinia grandis* and Diclofenac sodium was used as the reference drug. The highest dose of *Citrullus colocynthis* and *Coccinia grandis* (500 µg/mL) was found to be near to the diclofenac sodium. The IC_{50} value *Citrullus colocynthis* and *Coccinia grandis* was 291.66, 320.42µg/mL and standard was 246.31µg/mL. From the study it can be concluded that *Citrullus colocynthis and Coccinia grandis* showed marked *in-vitro* anti-inflammatory effect against the denaturation of protein (Table 5 and Fig.5). Among the two plants, *Citrullus colocynthis* unripe fruit extract has potential activity than *Coccinia grandis* unripe fruit extract

Table.5: *In vitro* anti-inflammatory activity of *Citrullus colocynthis* and *Coccinia grandis* unripe fruits extract (Bovine serum albumin)

Concentrations (µg/mL)	*Citrullus colocynthis* (%)	*Coccinia grandis* (%)	Standard (Diclofenac sodium) (%)
100	17.56 ± 1.22	16.95 ± 1.18	21.08 ± 1.47
200	33.29 ± 2.33	30.63 ± 2.14	43.21 ± 3.02
300	52.87 ± 3.70	46.87 ± 3.28	60.75 ± 4.25
400	68.74 ± 4.81	60.79 ± 4.25	79.43 ± 5.56
500	84.65 ± 5.92	79.03 ± 5.53	94.87 ± 6.64
IC_{50} (µg/ml)	291.66	320.42	246.31

Values are expressed as Mean± SD for triplicates

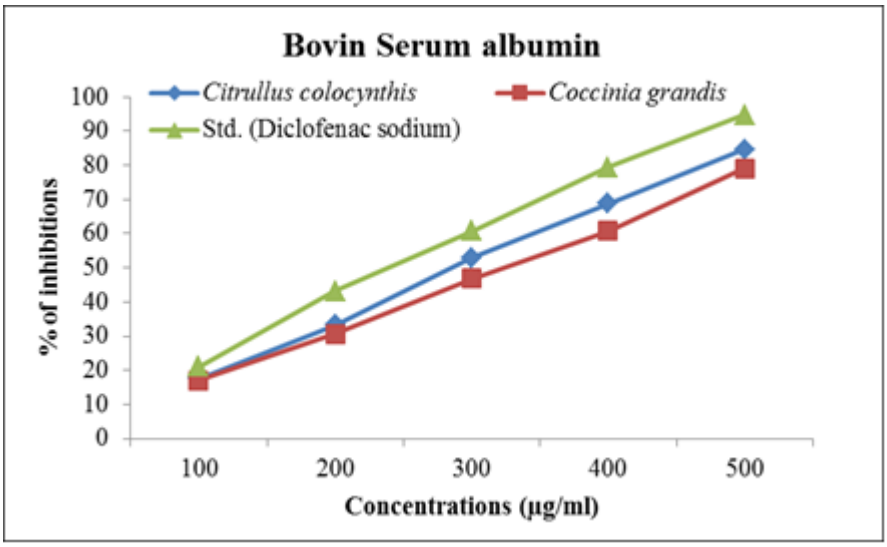

Fig.5: *In-vitro* anti-inflammatory activity of *Citrullus colocynthis* and *Coccinia grandis* unripe fruits extract (Bovine serum albumin)

Denaturation of tissue proteins one of the well-documented causes of inflammatory and arthritic diseases. Production of auto antigens in certain of inflammatory diseases may be due to *in-vivo* denaturation of proteins. The mechanism of denaturation probably involves alteration in electrostatic, hydrogen, hydrophobic and disulphide bonding (Grant *et al.*, 1970). Agents that can prevent protein denaturation therefore, would be worthwhile for anti-inflammatory drug development. The increments in absorbance of *Citrullus colocynthis* and *Coccinia grandis* extract with respect to control indicated stabilization of protein denaturation by and reference diclofenac sodium (Jagtap *et al.*, 2011).

Conclusion

Overall, it can be concluded from the present study demonstrated that methanol extract of *Citrullus colocynthis and Coccinia grandis* unripe fruits has significant *in vitro* antioxidant and anti-inflammatory activities were proved.

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