INVITRO ANTIOXIDANT AND ANTI – INFLAMMATORY ACTIVITY OF *Coccinia grandis* L.

Amudha M[1], Manikandan R [2], and Sharmila banu A[3]
P.G. and Research Department of Chemistry
A.V.V.M Sri pushpam college (Autonomous) Poondi.
Thanjavur. Tamil nadu.

ABSTRACT

The determination of the antioxidant and anti–inflammatory activity of selected medicinal plant namely *Coccinia grandis* has been studied using different solvents of aqueous, and methanol extracts. *Invitro* antioxidant potential of fruit of *Coccinia grandis* with three different methods like hydrogen peroxide scavenging (H_2O_2) assay, reducing power assay and thiobarbataric acid assay were evaluated. Determination of antioxidant and anti - inflammatory activity of different concentration 0.2, 0.4, 0.6, 0.8 and 1.0 and standard (Diclofenac sodium) of plant extract. Among the three methods, the antioxidant activity for more suitable in Thiobarbataric acid activity than the other methods. The aqueous, and methanol extract of excellent percentage of activity hydrogen peroxide scavenging (H_2O_2) assay when low concentration of aqueous extract also moderate percentage of activity from reducing power assay were observed in the anti – inflammatory activity of *Coccinia grandis* fruit extract of higher concentration was extraordinary performance observed. These observations confirm that methanolic extract of different polyphenolic constituents and its importance in antioxidant and anti - inflammatory activity were performed.

Keywords: Anti oxidant and anti – inflammatory activity, *Coccinia grandis* fruit.

INTRODUCTION

The infectious diseases are troubling the general population on one hand, the pace of modern life is adding to the woes of the middle class. Stress and pollution are known to generate reactive oxygen species termed as free radicals which play havoc with the body. These are found to induce tissue damage resulting in inflammation and physical pain.

Coccinia grandis, the ivy gourd, also called as baby water melon belongs to the family Cucurbitaceae. In traditional medicine fruits have been used to treat leprosy, fever, asthma, bronchitis and jaundice. The fruit

possesses mast cell stabilizing; anti anaphylactic and antihistaminic potential. There is some research to support that compounds in the plant inhibit the enzyme Glucose-6-phosphatase is one of the key liver enzymes involved in regulating sugar metabolism. Therefore, Ivy Gourd is sometimes recommended for diabetic patients Venkateswaran and Pari (2003). Although these claims have not been supported, there currently is a few amount of research focused on the medicinal properties of this plant focusing on its use as an antioxidant, antihypoglycemic agent, immune system modulator. Some countries in Asia like Thailand prepare traditional tonic like drinks for medicinal purposes.

Free radicals play an important role in a number of biological processes including intracellular killing of bacteria and certain cell signalling processes. Free radicals are derived from molecular oxygen under reducing conditions. Excess amount of these free radicals can lead to cell injury which results in many diseases like cancer and diabetes. Free radicals may be involved in Alzheimer's, Schizophrenia, Parkinson's and drug induced deafness. Because free radicals are necessary for life, the body has numerous mechanisms to reduce free radical induced damage and to repair this occurred damage by Rajamani Karthikeyan *et al.,* (2011).

Antioxidants are required. The function of antioxidant is not to remove oxidants entirely, but instead to keep them at an optimum level. In addition to this antioxidants play a key role in defence mechanisms. In recent years, many studies revealed that plants contain high antioxidant and anti-inflammatory activity. Dietary measures and traditional plant therapies as prescribed by ayurvedic and other indigenous systems of medicine are used commonly in India. The world health organization has also recommended the evaluation of plants effective in conditions were safe modern drugs are lacking.

MATERIALS AND METHODS

Collection of plant materials

Healthy plants of *Coccinia grandis* fruits were collected from Kattakudi village, Thiruvarur, Mannargudi. Tamilnadu, India. The fruit materials were cleaned and free from dirt particles and shade dried.

Preparation of plant extracts

Soxhlet method used for extraction of crude materials Ten grams of fruit powder blended with 50 ml of different solvents separately (aqueous and methanol) for different periods with agitation at room temperature. After the fruit extracts were allowed to filtration by using a 0.45 Millipore filter paper. The plant fruit extracts concentrated using a rotary evaporator at 40°C under reduced pressure. Finally the fruit extracts were allowed to weigh and store at -20°C till their usage in the different tests.

ANTIOXIDANT ACTIVITY

Hydrogen Peroxide Scavenging assay (Ruch *et al.*, 1989).

The ability of the fruit extracts to scavenging hydrogen peroxide was determined according the method of solution of hydrogen peroxide (40mm) was prepared in phosphate buffer (pH 7.4). The extracts of 0.2, 0.4, 0.6, 0.8 and 1.0% were added to a hydrogen peroxide solution (0.6 ml, 40ml). Absorbance of hydrogen peroxide scavenging assay at 230 nm was determined 10 minutes later against a blank solution containing the phosphate buffer without hydrogen peroxide. The percentage of antioxidant activity.

$$\% \text{ Scavenged } [H_2O_2] = [(AC - AS)/AC] \times 100$$

Reducing power assay (Oyaizu, 1986)

The reducing power assay with aqueous extract was determined according to this method. One ml of the fruit extract containing of 0.2, 0.4, 0.6, 0.8 and 1.0% were deionized water mixed with 2.5ml of phosphate buffer (0.2M, pH 6.6) and 2.5ml Potassium ferrocyanide (1%). The mixture was incubated at 50°C for 20 minutes. 2.5ml of TCA (10%) and centrifuged at 3000 rpm. The upper layer of the solution was mixed with 2.5ml distilled water and $FeCl_3$ (0.5ml, 0.1%). The absorbance was measured at 700 nm. Higher absorbance of the reaction mixture indicated the higher reducing power. The absorbance compared with the standard ascorbic acid (concentration 20 µg).

The percent increase in reducing power assay was calculated using the following equation

$$\text{Increase in reducing power (\%)} = \frac{\text{A test - A std}}{\text{A}} \times 100$$

Thiobarbitaric Acid (TBA) Method (Sawarka *et al.*, 2009)

TBA method used for evaluating the extent of lipid peroxidation. At low pH and high temperature (100ºC), melonaldehyde binds with TBA to form a red complex that can measured at 532 nm and 2 ml of 20% Trichloroacetic acid and 2 ml of 0.67%TBA solutions were added to 2 ml of the mixtures containing the sample 0.2, 0.4, 0.6, 0.8, and 1.0% were prepared in the FTC (Ferric thiocyanate) method. The percentage of antioxidant activity was calculated by following formula

$$(\%)\ \text{Percentage of activity} = \frac{\text{Absorbance of (Control}-\text{Test)}}{\text{Absorbance Control}} \times 10$$

INVITRO ANTI – INFLAMMATORY ACTIVITY (Mizushima and Kobayashi 1968)

INHIBATION OF ALBUMIN DENATURATION

The reaction mixture consisted of test fruit extracts at different concentration 0.2, 0.4, 0.6, 0.8, and 1.0% aqueous solution of bovine albumin fraction. pH of the reaction mixture was adjusted using small amount of HCL. Diclofenac sodium (10 mg) was used as a standard drug. The sample extracts and standard were incubated at 37˚C for 20 min and then heated to 51˚C for 20 min. After cooling the sample, the turbidity was measured spectrophotometrically at 660 nm. The experiment was performed in triplicate.

Percent inhibition of protein denaturation was calculated as follows:

$$\%\ \text{of inhibition} = \text{control}_{OD} - \text{test}_{OD} / \text{Control}_{OD} \times 100$$

Where, control OD is the absorbance without sample, test OD is the absorbance of sample extract/ standard.

Table 1: Antioxidant activity *Coccinia grandis* extract by various methods

Different concentration (%) of plant extract	Aqueous extract (Percentage of activity %)					
	Standard (ascorbic acid)	Hydrogen peroxide scavenging (H_2O_2) assay	Standard (ascorbic acid)	Reducing power assay	Standard (Ferric thiocyanate)	Thiobarbataric acid
0.2	38.3±3.24	0.18±0.06	17.2±9.06	0.19±0.06	10.1±3.17	0.31±0.10
0.4	28.6±2.10	0.22±0.07	24.5±6.17	0.24±0.03	07.6±2.42	0.29±0.09
0.6	46.3±5.19	0.20±0.07	16.2±0.05	0.27±0.08	12.4±3.38	0.35±0.11
0.8	27.1±2.18	0.35±0.11	15.6±1.72	0.21±0.09	06.7±1.45	0.49±0.16
1.0	65.6±8.43	0.35±0.11	23.3±1.81	0.38±0.12	16.5±1.05	0.28±0.14

Standard deviation ± Standard error

Table 2: Antioxidant activity *Coccinia grandis* extract by various methods

Different concentration (%) of plant extract	Methanol extract (Percentage of activity %)					
	Standard (ascorbic acid)	Hydrogen peroxide scavenging (H_2O_2) assay	Standard (ascorbic acid)	Reducing power assay	Standard (Ferric thiocyanate)	Thiobarbataric acid
0.2	24.3±3.18	0.15±0.05	19.2±9.13	0.29±0.06	13.1±3.27	0.21±0.14
0.4	38.9±2.10	0.23±0.07	38.5±6.17	0.17±0.09	27.6±2.12	0.25±0.14
0.6	26.6±1.19	0.19±0.06	15.3±5.15	0.20±0.18	19.4±3.24	0.42±0.15
0.8	47.1±2.08	0.25±0.10	24.8±1.42	0.16±0.19	15.7±1.05	0.23±0.13
1.0	15.6±3.13	0.15±0.13	16.4±1.31	0.28±0.12	21.5±1.10	0.23±0.12

Standard deviation ± Standard error

Table 3: Effect of Anti- inflammatory activity of *Coccinia grandis* fruit by *invitro* method

Different concentration (%)	(Percentage of activity %)	
	Aqueous extract	Methanol extract
Diclofenac sodium	0.32±0.10	0.30±0.01
0.2	0.38±0.12	0.35±0.11
0.4	0.43±0.14	0.44±0.14
0.6	0.46±0.15	0.54±0.18
0.8	0.64±0.21	0.64±0.21
1.0	0.74±0.24	0.69±0.23

Standard deviation ± Standard error

RESULTS AND DISCUSSION

In the present investigation suggested that the determination of effect of antioxidant activity of medicinal plants like *Coccinia grandis* was carried out by three methods. Among the three methods, the Thiobarbataric acid assay showed excellent antioxidant activity from aqueous extract with other methods of hydrogen peroxide scavenging assay and reducing power assay whereas the aqueous solvent extract of *Coccinia grandis* plant has enomorus free radical scavenging activity from reducing power assay was Standard (10.1±3.17),0.31±0.10, (07.6±2.42),0.29±0.09, (12.4±3.38),0.35±0.11, (06.7±1.45),0.49±0.16 and (16.5±1.05), 0.28±0.14 percentage activity observed with respective plant *Coccinia grandis* concentration of 0.2,0.4,0.6,0.8 and 1.0 percentage treated respectively. The maximum percentage of antioxidant activity was observed due to the properties of photochemical activity (Table – 1).

The total antioxidant capacity is expressed as ascorbic acid equivalents. The percentage of antioxidant activity was in the order of methanol, ethanol, petroleum ether, chloroform and n-hexane were performed. The IC_{50} value of methanol fraction was found to be 140 µg/mL whereas the IC_{50} value of ethanol 200, chloroform and n-hexane and petroleum ether was found to be 400 µg/mL. In reducing power assay, ethanol fraction seemed to have quite high reducing activity when compared to methanol, chloroform, n-hexane and petroleum ether. The reducing power of different extracts. The *invitro* anti-inflammatory activity was estimated by inhibiting protein denaturation by Ashwini *et al.* (2012).

According to the methanolic extract of *Coccinia grandis* fruit with different concentration of 0.2,0.4,0.6,0.8, and 1.0 % has developed ionic stability to promote the activity was Standard (13.1±3.27),0.21±0.14, (27.6±2.12),0.25±0.14, (19.4±3.24),0.42±0.15, (15.7±1.05),0.23±0.13, and (21.5±1.10), 0.23±0.12% observed from Thiobarbataric acid than the other methods whereas *Coccinia grandis* plant extract free radical scavenging activity of methanolic extract has been investigated by reducing power assay recorded respectively (Table - 2).

Preliminary chemical test indicated the presence of flavonoids and terpenoids. DPPH (1, 1-Diphenyl, 2-picryl-hydrazyl) and superoxide free radical was found to be inhibited by *Coccinia grandis*. Among the methanolic and aqueous extracts of fruits evaluated for antioxidant activity, the aqueous extract of fruits showed the maximum activity. A 100µg/ml of methanolic extracts of fruits of *Coccinia grandis* exhibited 43.67 and 52.92 inhibition of DPPH free radical respectively whereas 44.76 and 54.16 inhibition of superoxide radical whereas aqueous extracts of fruits exhibited 52.33 and 61.46 inhibition of DPPH free radical respectively whereas 54.17 and 63.41 inhibition of superoxide radical. The obtained results of absorbance and percentage inhibition showed decrease in the concentration of DPPH radical due to the scavenging ability of extracts. The IC50 values of methanol and water extracts of fruits were 94.33µg/ml, 91.00µg/ml and 79.02µg/ml, 76.36µg/ml respectively which is lower than IC50 values of methanolic extracts by Ashish *et al.* (2011).

Invitro anti-inflammatory activity of methanol extract of *Coccinia grandis.* The revealed that all the concentrations of the extracts showed anti-inflammatory activity. However, the maximum activity was observed at highest concentration (500mg/ml) followed by lower concentrations such as 400 mg/ml, 300mg/ml, 200mg/ml and 100mg/ml respectively. The percentage anti-inflammatory activity of *C. grandis* ranged from 44.46±0.09 % to 84.63±0.04% respectively. Ashwini *et al.* (2012), reported that *Coccinia grandis* exhibited 60-85% anti - inflammatory activity *in vitro*, the report was in agreement with the findings. The obtained were also similar to the reports of stated that the anti-inflammatory and antioxidant potency of the fruit extract highly reduces the risk of getting gastric ulcers.

The effect of anti inflammatory activity of *Coccinia grandis* was higly responsible for reducing activity of inflammation when compared with diclofenac sodium and different concentration of *Coccinia grandis* of 0.2, 0.6, 0.6, 0.8 and 1.0% with anti inflammatory activity Aqueous extract standard 0.32±0.10, 0.38±0.12, 0.43±0.14, 0.46±0.15, 0.64±0.21 and 0.74±0.24% observed from the effect of *Coccinia grandis* plant. As per the methanolic extract of *Coccinia grandis* with different concentration of diclofenac sodium and 0.2, 0.4, 0.6, 0.8 and 1.0 % with anti inflammatory properties of standard 0.30±0.01, 0.35±0.11, 0.44±0.14, 0.54±0.18, 0.64±0.21, and 0.69±0.23% recorded (Table – 3).

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