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IN VITRO ANTIOXIDANT ACTIVITY OF METHANOLIC FRUITS EXTRACT OF *COCCINIA GRANDIS*

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ABSTRACT

The study was designed to investigate the antioxidant activities of the methanolic extract of the fruits of *Coccinia grandis*. The antioxidant activity was evaluated by DPPH Free Radical Scavenging, Total Phenolic Content and Total Flavonoids Content which were compared to the standard of Ascorbic acid and Gallic acid. The antioxidative activity was examined by UV Spectrophotometer. The antioxidant activity of methanolic extract of *Coccinia grandis* fruits was showed as Total Phenolic Content (88.81 GAE/g), Total Flavonoid Content (39.08 GAE/g) and DPPH Free Radical Scavenging Activity (219.5 GAE/g). Further research can be performed here to find out the responsible active compounds and to identify the mechanism of actions.

KEYWORDS: Coccinia grandis Fruits; Antioxidant; DPPH Free Radical Scavenging; Total Phenolic Content; Total flavonoids Content.

1. INTRODUCTION

Coccinia grandis is a topical plant which is called "Ivy gourd" and belongs to the family Cucurbitaceae. It grows in many countries in Asia and Africa. Several diseases, such as jaundice, bronchitis, skin eruptions, burns, insect bites, fever, indigestion, nausea, eye infections, allergy, syphilis, gonorrhea, etc, are treated by the roots, fruits, stems, leaves and whole plant of *Coccinia grandis*.[1,2] The plant primarily consists of flavonoid, β-sitosterol, β-carotene and linoleic and palmitic acids, etc.[3,4]

A free radical is an atom or molecule which is possessing unpaired electrons. The primary oxygen deduced free radicals are superoxide anion (O2), hydroxyl (OH·), hydroperoxyl (OOH·), peroxyl (ROO·) and alkoxyl (RO·) radicals and non free radicals are hydrogen peroxide (H2O2), hypochlorous acid (HOCl), ozone (O3) and singlet oxygen (1O2). These reactive intermediates are jointly termed as reactive oxygen species (ROS). Likewise, reactive nitrogen species (RNS) are mainly nitric oxide (NO·), peroxynitrite (ONOO·) and nitrogen dioxide (NO2).[5]

"Free radicals" or highly reactive oxygen assail healthy cells star to loss of their structure and function.[6] Moreover, one hundred disorders in humans including atherosclerosis, arthritis, ischemia and repercussion injury of many tissues, a central nervous system injury, gastritis and cancer are contained by free radicals.[7,8,9,10] As a result of environmental pollutants, radiation, chemicals, toxins, deep fries and spicy foods as well as physical stress, free radicals make exhaustion of the immune system antioxidants, the alter in gene aspect and accelerate unnatural proteins.[11]

An antioxidant blocks oxidation reaction there by reducing cell impairment or death. Anything which deeds against is known as "anti" and "to oxidize" is to unite with oxygen thence antioxidants intends to antagonize oxidation.[12,13] Antioxidants can be divided into two main classes such as, enzymatic and non-enzymatic. The enzymatic antioxidant is an antioxidant which provides endogenously and includes superoxide dismutase, catalase, and glutathione peroxidase. On the other hand, the non-enzymatic antioxidants contain tocopherols, carotenoids, ascorbic acid, flavonoids and tannins which are found from natural plant origin.[14] The molecules of antioxidants have a radical-scavenging capacity that are believe to exercise a protective result against free radical damage. Many chronic diseases, such as cancer, cardiovascular disease, atherosclerosis, diabetes, asthma, hepatitis and arthritis are prevented by these biomolecules.[15,16]

Also, antioxidants act an important function in nutritional by prolongation the shelf life of food and removing nutritional losses as well as shaping of harmful substances.[17] The target of the present study was to inquire the antioxidant activity of the methanolic extract of the fruits of *Coccinia grandis* using three in vitro models.

2. MATERIAL AND METHODS

2.1 Chemicals

DPPH (1,1-diphenyl-2-picrylhydrazyl), methanol, aluminum chloride, sodium carbonate, Folin-Ciocalteu reagent were obtained from Sigma chemical company, USA and Gallic acid is received from Wako pure chemical Ltd, Japan as well as all other reagents were incurred from Merck, Germany.

2.2 Plant Material

Fruits of *Coccinia grandis* were collected from Narsingdi District, Banglaesh, in July 2016, and identified and authenticated by a skillful taxonomist. In the national herbarium of Dhaka, a voucher specimen (DCAB accession no. 44011) has been deposited. The fruits of *Coccinia grandis* were then separated and washed in good order and removed dirty materials as well as shade dried for several days with occasional sun drying. Using a grinding machine to provide coarse powder of the dried fruits.

2.3. Preparation of Plant Extract

About 530 gm of ground powder soaked in 1500 ml (around 80%) of methanol. The whole mixture was continuous shaking and stirring at 15 days and then it was filtered through Whitman filter paper. The filtrate was evaporated by Rotary Evaporator at 4 rpm and at 60ºc temperature. After that, the crude *Coccinia grandis* fruits extract was dried by freeze drier as well as preserved at 4ºc for until further investigation.

2.4. Total Phenolic Content

Total phenolic content in the *Coccinia grandis* fruits extract was determined with the Folin-Ciocalteu reagent according to a procedure described by Singleton and Rossi.[18] Methanolic extract of *Coccinia grandis* fruits in the concentration of 1 mg/ml was applied in the analysis. The reaction mixture was prepared by mixing 0.5 ml of methanolic extract solution, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% NaHCO3 .Blank was concomitantly prepared, containing 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of NaHCO3. The samples were incubated in a thermostat at 25 ºC after 20 min. The absorbance was determined using spectrophotometer at λmax = 760 nm. The same procedure was repeated for the standard solution of gallic acid and the calibration line was construed. Total content of phenolic compounds in plant methanol extracts in gallic acid equivalents (GAE) were calculated by the following formula:

$C = c. V/m´$

where C is the total content of phenolic compounds, mg/g plant extract, in GAE; c is the concentration of gallic acid established from the calibration curve, mg/ml; v is the volume of extract, mL; m is the weight of pure plant methanolic fruits extract.

2.5. Total Flavonoid Content

The total flavonoid content as the methanolic extract of *Coccinia grandis* fruits was determined by the aluminum chloride colorimetric method and gallic acid as standard.[19] There is 0.5 ml of plant extract or standard of 125, 250, 500, 1000 µg/ml concentration solution was mixed with 1.5 ml of methanol, 1 ml of 10% aluminum chloride solution, 0.1 ml of 1 M potassium acetate solution and 2.8 ml of distilled water. Blank was prepared, containing 1.5 ml of methanol, 1 ml of 10% aluminum chloride solution, 0.1 ml of 1 M potassium acetate solution and 2.8 ml of distilled water. The samples were incubated in a thermostat at 25 ºC after 30 min. The absorbance was determined using spectrophotometer at λmax = 420 nm.

2.6. DPPH Free Radical Scavenging

Sample stock solutions (1.0 mg/ml) were diluted to final concentrations of 100, 125, 250, 500, 750 and 1000 µg/mL in methanol. Samples were added to 1.5 ml of DPPH and the mixture was shaken and left to stand at room temperature in the dark. After 30 min absorbance was measured at 517 nm against a blank containing all reagents except the test samples [20]. The percentage of inhibition of DPPH (I%) was calculated using the following equation:

$I\% = (A0 - A/A0) \times 100$

A0 is the absorbance of the blank solution and *A* is the absorbance of the methanolic extract.

The % scavenging activity at different concentrations was determined and the IC_{50} value of the extract was compared with that of ascorbic acid, which was used as the standard.

3. RESULTS

3.1. Determination of Total Phenolic Content

Total Phenolic content of the methanolic extract of *Coccinia grandis* fruits was determined by using Folin-ciocalteu reagent. Phenolic content of the sample was calculated on the basis of the standard curve for Gallic acid as shown in Table.1 and in Fig.1.

Table 1: Absorbance of Gallic acid at different concentrations after reaction with Folin-Ciocalteu reagent.

Concentration (µg/ml)	Absorbance
125	0.025
250	1.115
500	2.173
1000	3.916

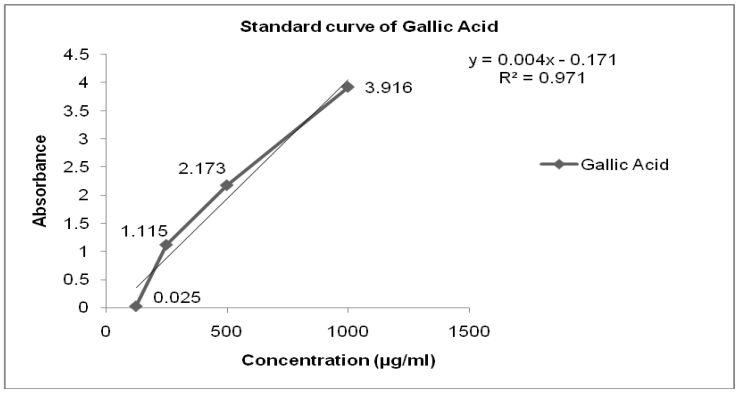

Fig. 1: Standard curve of Gallic acid for the determination of Total Phenolics Content.

Table 2: Determination of Total Phenolic Content of methanolic extract of *Coccinia grandis* fruits.

No of Sample	Concentration (µg/ml)	Absorbance	GAE/mg of dried sample	GAE/mg of dried sample Mean ± STD
1	125	0.103	68.5	
2	250	0.163	83.75	88.81±18.82
3	500	0.201	93.5	
4	1000	0.269	109.5	

As shown in Table.2, the phenolic content was 88.81 mg of GAE/mg of dried *Coccinia grandis* fruits extract. The absorbance values of the extract of *Coccinia grandis* was compared with the standard solution of Gallic Acid equivalents. Total Phenolic Content of the sample was expressed as mg of GAE (Gallic Acid equivalents) gm of extractives.

3.2. Determination of Total Flavonoid Content

Total Flavonoid Content of *Coccinia grandis* fruits extract was determined using much known aluminum chloride colorimetric method. Total Flavonoid content of the sample was calculated on the basis of the standard curve for Gallic Acid as shown in Table.3 and in Fig.2. The results were expressed as µg of Gallic acid equivalent (GAE)/gm of extract.

Table 3: Absorbance of Gallic acid at different concentration for quantitative determination of Total Flavonoid Content.

Concentration (µg/ml)	Absorbance
125	0.347
250	1.003
500	2.114
1000	3.202

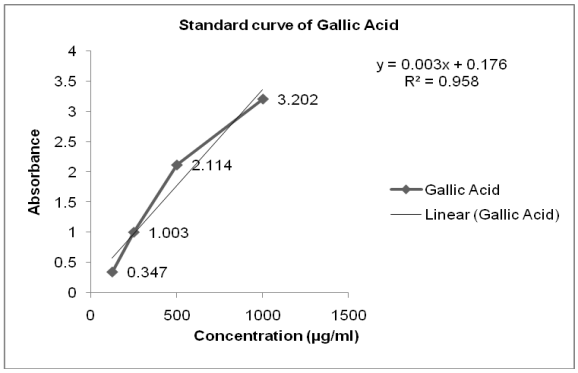

Fig. 2: Standard curve of Gallic acid for the determination of Total Flavonoids Content.

Table 4: Determination of Total Flavonoids Content of the methanolic extract of *Coccinia grandis* fruits.

No of Sample	Concentration (µg/ml)	Absorbance	GAE/mg of dried sample	GAE/mg of dried sample Mean ± STD
1	125	0.186	3.33	
2	250	0.210	11.33	39.08±38.06
3	500	0.353	59	
4	1000	0.424	82.67	

As shown in Table. 4, the Total Flavonoid Content was 39.08 mg of GAE/mg of methanolic fruits extract. The absorbance values of the extract of *Coccinia grandis* was compared with the standard solution of Gallic Acid Equivalents. Total Flavonoid Content of the sample was expressed as mg of GAE (Gallic Acid equivalents) gm of extractives.

3.3. Determination of DPPH Free Radical Scavenging Activity

The antioxidant activity of *Coccinia grandis* fruits was evaluated by DPPH free radical scavenging assay. The results of DPPH free radical scavenging assays of fruits extract and Ascorbic acid (standard) are given in Table.5 and Fig.3.

Table 5: DPPH free radical scavenging activity of methanolic fruits extract and Ascorbic acid (standard) at different concentrations.

Name of Sample	No. of Sample	Concentration (µg/ml)	% of Scavenging	IC_{50} (µg/ml)
Ascorbic acid (standard)	1	100	94.478	6.51
	2	125	94.563	
	3	250	94.854	
	4	500	95.135	
	5	750	95.216	
	6	1000	95.437	
Methanolic extract of *Coccinia grandis* fruits	1	100	44.91	219.5
	2	125	45.39	
	3	250	54.06	
	4	500	60.20	
	5	750	61.54	
	6	1000	63.03	

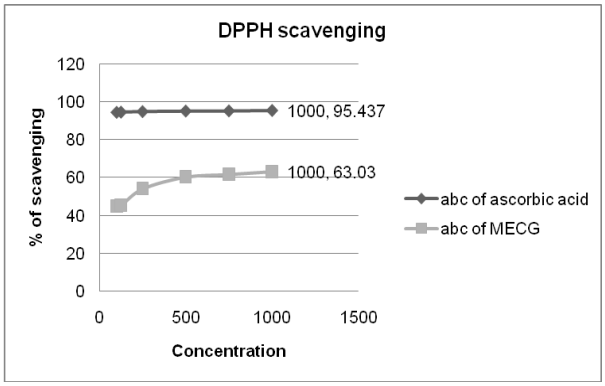

Fig. 3: DPPH free radical scavenging activity of methanolic extract of *Coccinia grandis* (MECG) fruits and Ascorbic acid (standard).

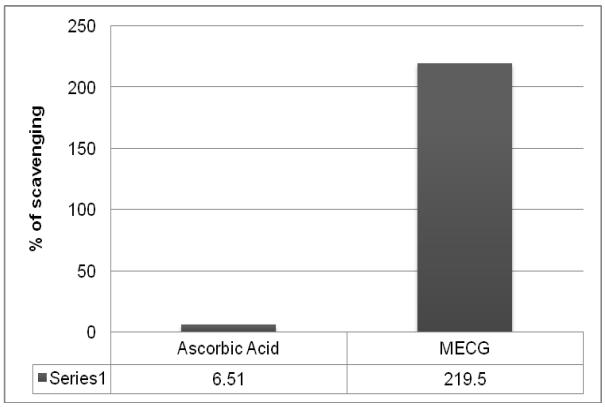

Fig. 4: IC_{50} (µg/ml) values of methanolic extract of *Coccinia grandis* (MECG) fruits and Ascorbic acid (standard).

The scavenging activity of methanolic fruits extract was less than that of Ascorbic acid (standard) as shown in Table.5 and Fig. 3. IC_{50} of Ascorbic acid (standard) and Methanolic fruits extract were 6.51µg/ml and 219.5µg/ml respectively shown in Table.5 and Fig. 4.

4. DISCUSSION

This study was carried on evaluate the antioxidant activity of the methanolic extract of fruits of *Coccinia grandis*. This antioxidant activity was investigated by using three in vitro assays with comparing to standard antioxidant compounds.

Phenolic compounds are high-level antioxidants due to their ability of scavenge free radicals and active oxygen species, for example, singlet oxygen, superoxide free radicals and hydroxyl radicals.[21] The presence of Total Phenolic Content (88.81 GAE/g) and Total Flavonoid Content (39.08 GAE/g) in the methanolic fruits extract of *Coccinia grandis* has imparted directly to the antioxidant activity by negating the free radicals.

DPPH assays is also used for screening antioxidant activity of fruits extract in this study because it is one of the most widely used methods for screening antioxidant activity of medicinal plant extract[22] The DPPH (1,1-diphenyl-2-picrylhydrazyl) assay measures the ability of a substance to scavenge the DPPH radical and produces hydrazine.[23] A substance is added to a solution of DPPH which acts as a donor of hydrogen atoms and provide with a change in color from violet to pale yellow. The IC_{50} value of the methanolic fruits extract was reported to be 219.5 µg/ml in this investigation.

The results of this report forecasts that the fruits of *Coccinia grandis* may be a potential source of natural antioxidant, if further investigation is continued to identify some potential parameters. Different studies have pointed antioxidant property of *Coccinia grandis* leaves, fruits, stem and root extract.[5,24,25,26] The IC_{50} value of *Coccinia grandis* was calculated for antioxidant and was found to be 140 µg/ml (methanolic extract) by Ashwini et al.[27] Further, more elaborated, studies on the

chemical composition of those extracts, and studies with other models, such *in vivo* assays, are indispensable to characterize them as biological antioxidants.

5. CONCLUSION

In conclusion, the present investigation presents that *Coccinia grandis* fruits possess antioxidant activity, which may be assigned to the raising effect on cellular antioxidant defense contribution to the protection against oxidative impairment of assorted cells.

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