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ANTI-OXIDANT POTENTIALS OF THE FRUIT OF *COCCINIA GRANDIS*

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ABSTRACT

The existing study was designed at exploring the antioxidant activities of extract of the fruits of *Coccinia grandis* L. (Cucurbitaceae).The antioxidant activities evaluated by means of *in vitro* assays and were linked to standard antioxidants such asascorbic acid and butylated hydroxytoluene. All the extracts showed real antioxidant activity. The antioxidant property rests upon solvent used for extraction as different solvent may extract different components which may have higher phenolic and flavonoid compounds which are accountable for the free radical scavenging. Chloroform extract have high anti-oxidant potential. The results found in the existing study show that the fruits of *C.grandis* are a probable source of good natural anti-oxidants.

Keywords: *Coccinia grandis,* antioxidant, fruits extract, DPPH assay and Reducing power method

INTRODUCTION

The chronic and acute conditions like cardiovascular diseases, diabetes mellitus and many more are leading cause of morbidity and mortality. These conditions are believed to be due to the participation of free radicals. Free radicals can be

defined as atoms or molecules containing one or more unpaired electrons in their orbitals. Their formation occurs continuously in the cells by endogenous and exogenous sources as a consequence of both enzymatic and nonenzymatic reactions. Free radicals can cause a wide range of toxic oxidative reactions which ultimately leads to cell death and progression of chronic and acute disease conditions **[1]**.

Reactive oxygen species are involved in the cell growth, differentiation, progression, and death. Low concentrations of Reactive oxygen species may be beneficial in aging process as well as in a number of human disease states, including cancer, ischemia, and failures in immunity and endocrine functions **[2]**.

C.grandis L. normally identified as "Ivygourd" is a humid plant fit into the family Cucurbitaceae. The roots, stems, leaves and whole plant of *C. grandis* are used in the treatment of jaundice, bronchitis, skin eruptions, burns, insect bites, fever, indigestion, nausea, eye infections, allergy, syphilis, gonorrhoea etc. It has been found in many countries in Asia and Africa. The leaves of this species are widely used in Indian folk medicine for healing the mouth ulcer **[3-17]**.

The objective of the current study was to examine the antioxidant activity of the extract of the fruits of *C. grandis* using *in vitro* methods.

MATERIALS AND METHODS

C. grandis fruits

The fruits were collected in and around Satara district, Maharashtra, India during the month of April and was authenticated by Y.C.I.S. Satara, Maharashtra, India

Preparation of the fruit extract

The fruits of *Coccinia grandis* were collected from Satara district, Maharashtra. The fruits were taxonomically identified and authenticated from Department of Botany, YC College Satara. The fruits of *Coccinia grandis* were air-dried in the shade for 120 hours. Dried fruits were grounded into a fine powder, and extraction was carried out in solvents of different polarities at a ratio of 1:4 (w/v) by using soxhlet extraction apparatus. After 24 hrs the mixture was filtered; filtrate was collected and the residue was again extracted with respective solvents at a ratio of 1:3 (w/v) for 24 hrs. Filtrates was combined and evaporated to dryness **[18]**.

Drugs and chemicals

DPPH was obtained from Hi Media Laboratories Pvt. Ltd., Mumbai and all other drugs and chemicals used in the study were obtained commercially and were of analytical grade.

In-Vitro evaluation of Antioxidant activity [19-26]

DPPH scavenging activity

The sample extract (0.2mL) was diluted

with methanol and 2 mL of DPPH solution (0.5 mM) was added. After 30 min, the absorbance was measured at 517 nm. The percentage of the DPPH radical scavenging was calculated using the equation as given below:

% inhibition of DPPH radical = $([A_{br}-A_{ar}]/A_{br})\times100$

Where A_{br} is the absorbance before reaction and A_{ar} is the absorbance after reaction has taken place.

Hydrogen peroxide scavenging (H_2O_2) assay

A solution of hydrogen peroxide (40mM) was prepared in phosphate buffer (50mM pH7.4). The concentration of hydrogen peroxide was determined by absorption at 230nm using a spectrophotometer. Extract (20–60 µg/mL) in distilled water was added to hydrogen peroxide and absorbance at 230 nm was determined after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage of hydrogen peroxide scavenging was calculated as follows

% scavenged (H_2O_2)=$[(A_i-A_t)/A_i]\times100$

Where A_i is the absorbance of control and A_t is the absorbance of test.

Reducing power method(RP)

2.5 mL of 0.2 M phosphate buffer (pH 6.6) and 2.5 mL of Potassium ferricyanide ($K_3Fe(CN)_6$) (1% w/v) was added to 1.0 mL of sample dissolved in distilled water. The resulting mixture was incubated at 50 °C for 20 min, followed by the additionof2.5 mL of Trichloro acetic acid (10%w/v). The mixture was centrifuged at 3000 rpm for 10 min to collect the upper layer of the solution (2.5 mL), mixed with distilled water (2.5 mL) and 0.5 mL of $FeCl_3$ (0.1%, w/v). The absorbance was then measured at 700 nm against blank sample. Butylated hydroxy toluene (50-800 µg/ml) was used as the standard.

Phosphomolybdenum method

0.1mLofsample(100 µg)solution was combined with 1 mL of reagent (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tube was capped and incubated in a boiling water bath at 95 °C for 90min. After cooling the sample to room temperature, the absorbance of the aqueous solution was measured at 695 nm against blank in UV spectrophotometer. A typical blank solution contained 1 mL of reagent solution and the appropriate volume of the same solvent used for the sample and it was incubated under same conditions as rest of the sample. For samples of unknown composition, antioxidant capacity can be expressed as equivalents of a- tocopherol.

RESULTS

DPPH assay

All the extracts of *C. grandis* shows

DPPH radical scavenging activity. The highest DPPH radical scavenging activity was detected in petroleum ether extract followed by ethyl acetate, and chloroform. These activities are less than that of ascorbic acid **(Table 1)**.

Hydrogen peroxide scavenging assay

All the extracts of *C. grandis* scavenged hydrogen peroxide. The chloroform extract of *C. grandis* showed strong H_2O_2 scavenging activity similar to that of the standard, butylated hydroxytoluene. The petroleum ether and ethyl acetate extract also showed significant scavenging activities but less when compared to the standard. *C. grandis* scavenged hydrogenperoxide may have phenolic groups that could donate electrons to hydrogen peroxide, thereby neutralizing it into water **(Table 2)**.

Reducing power ability

The reductive abilities of diverse extracts of *C. grandis* when compared to the standard, butylated hydroxytoluene. *C. grandis* chloroform extract showed the highest reducing ability than all the other extracts tested. However, the activity was less than the standard, butylated hydroxytoluene. The petroleum ether and ethyl acetate extract also showed significant activity indicating its reductive ability. This indicates presence of hydrogen donating ability with extract that can convert ferricyanide complex to the ferrous form **(Table 3)**.

Phosphomolybdate method

The phosphomolybdate method is quantitative, since the total antioxidant capacity is expressed as α-tocopherol equivalents. Among the extracts tested, the chloroform extract contains highest 21.66µg vitamin E equivalent/ 100 µg. It proves that extract have components that converts the Mo(VI) to Mo(V) and forms a green colored phosphomolybdenum V complex **(Table 4)**.

Table 1: Antioxidant activities of the extracts of *Coccinia grandis* fruits by DPPH assay method

Extracts	% Inhibition of DPPH radical
Petroleum ether	48±0.81
Chloroform	46±0.22
Ethyl acetate	48 ± 0.26
Ascorbic acid	49±0.09

Table 2: Antioxidant activities of the extracts of *Coccinia grandis* fruits by hydrogen peroxide scavenging assay

Extracts	% scavenged (H_2O_2)
Petroleum ether	19 ±0.81
Chloroform	14±1.00
Ethyl acetate	22 ±0.21
BHT	13±0.89

Table 3: Reducing power ability of different extracts of *Coccinia grandis*

Extracts	Absorbance at 700nm
Petroleum ether	0.057±0.013
Chloroform	0.089±0.022
Ethylacetate	0.042±0.012
BHT	0.092±0.012

Table 4: Antioxidant activity of different extracts of *Coccinia grandis* by Phosphomolybdate method

Extracts	Antioxidant activity(µg vitamin Eequivalent/100µg)
Petroleum ether	06.46±0.13
Chloroform	21.66±0.30
Ethylacetate	07.23±0.10

CONCLUSION

Based on the results obtained, it may be concluded that all the extracts of fruits of *C. grandis* showed strong antioxidant activity, reducing power ability, free radical scavenging activity when compared to standards such as ascorbic acid and butylated hydroxytoluene. As the various extracts of *C. grandis* exhibited different reactive oxygen species scavenging activities, there may be different percentages of phytochemical constituents present in the extracts. Further studies to evaluate the *in vivo* potential of the extracts in various animal models and the isolation and identification of the antioxidant principles in the fruits of *Coccinia grandis* are being carried out.

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