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EVALUATION OF IN-VITRO ANTIOXIDANT ACTIVITY OF METHANOLIC EXTRACT OF *BENINCASA CERIFERA* FRUIT

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ABSTRACT

The methanolic extract of *Benincasa Cerifera* fruit was screened for their antioxidant activity using different in-vitro assay procedures. In this study, Antioxidant activity of methanolic extracts of *Benincasa cerifera* fruit was screened with DPPH free radical scavenging assay, super oxide free radical scavenging activity, hydroxyl radical scavenging activity, H_2O_2 scavenging activity and reducing power ability. In present investigation methanolic extract of *Benincasa cerifera* fruits exhibited significant DPPH scavenging activity with IC50 value 590.59 µg/ml, Significant scavenger of superoxide radical at IC50 value 555.76 µg/ml, significant OH• radicals scavenging activity with IC50 value 555.28 µg/ml, scavenging activity against H_2O_2 with IC50 value 423.5 µg/ml and shows significant reducing activity. All the methods are compared to standard (ascorbic acid). Results suggested that fruit extract of *Benincasa cerifera* possesed strong antioxidant activities. Presence of phytochemicals like flavonoids, phenolic compounds, carbohydrates, proteins and amino acids might contribute to observed antioxidant activity. The tested plant extracts showed promising antioxidant and free radical scavenging activity, therefore justifying their traditional use.

KEYWORDS: Antioxidant activity, free radical scavenging activity, *Benincasa cerifera* fruit, DPPH, H_2O_2.

INTRODUCTION

Reactive oxygen species (ROS) are molecules generated from the metabolism of oxygen. It is a byproduct of metabolic reaction. ROS produced DNA damage is crucial step in development of mutation and other disorders like neurodegenerative disorder, cardiovascular disorders etc.[1-4]

Mammalian cells possess defense mechanisms which detoxify free radical. Antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and nonenzymatic molecules like thioredoxin, thiols and disulfide-bonding play important roles in antioxidant defense systems.[5] Some exogenous antioxidants are obtained from food, like α-tocopherol, β-carotene and ascorbic acid, effectively scavenge free radicals. If defense mechanism do not effectively scavenge free radicals than lead to disease condition as described above.[6]

Antioxidant-based drugs/formulations for the prevention and treatment of complex diseases like atherosclerosis, diabetes, stroke, Alzheimer's disease, and cancer have appeared during the last 3 decades. Among them natural antioxidants are great interest of research and worldwide use of natural phytochemicals has increased.[7-9]

Benincasa cerifera (which commonly called winter melon, ash gourd, ash guard, winter gourd, white pumpkin and wax gourd. white gourd, tallow gourd, gourd melon and Chinese watermelon) belongs to the cucurbitaceae family. The *Benincasa cerifera* has been used as a food and medicine. Ash gourd is ideal for diabetics and patients suffering from obesity. It combats general debility by stabilizing nerve cells. The cooling properties of its juice are helpful in treating peptic ulcers and for relieving acidity. Ash gourd provides relief from constipation and tones the gastrointestinal tract. The seeds of Ash Gourd are anabolic and encourage tissue growth.[10-12] In this report, we investigated the crude methanolic extracts of *Benincasa cerifera* for their potential antioxidant activity with DPPH free radical scavenging assay, super oxide free radical scavenging activity, hydroxyl radical scavenging activity, H_2O_2 scavenging activity, reducing power ability.

MATERIAL AND METHODS

Collection, identification and authentication of plant material

The matured fruits of *Benincasa cerifera* were collected from market. Fruits were identified and authenticated by Dr. Ritesh Vaidya, Senior lecturer, Department of bioscience, Ganpat

university. After removing skin and the seeds, the fruit pulp was dried under shade. The dried fruit pulp was finely powdered and stored in polythene bags at room temperature until needed in order to active components.

Extraction of plant material

The fruit powder of *Benincasa cerifera* was subjected to extraction using soxhlet apparatus. The powdered material was exhaustively extracted with methanol in soxhlet apparatus by continuous hot extraction. After each extraction, the solvent was recovered using distillation assembly, and the extract was concentrated under reduced pressure. The final yield of extract was calculated and stored in air tied container for experiment.

Preliminary phytochemical screening

The methanolic extract of *Benincasa cerifera* was subjected to qualitative phytochemical analysis for presence of various constituents like Alkaloids, Carbohydrate, Glycosides, Terpanoids, Protein and Amino acids, Phenolic and Tannins, Flavanoids, Oils and Fats, Saponins etc.

Evaluation of antioxidant activity of extracts

Antioxidant activity of fruit extracts of *Benincasa cerifera* was evaluated under different conditions.

DPPH free radical scavenging activity[13]

The DPPH free radical scavenging activity of the extract was measured in terms of hydrogen donating or radical scavenging ability using the stable free radical DPPH, according to the method described by Ashvin & Mishra 2007.

Procedure: Accurately weighed 4.3 mg of DPPH was dissolved in 3.3 ml of methanol in a test tube. Solution was protected from light by covering with aluminum foil. 150 µl of above solution was taken and diluted up to 3ml with methanol, the absorbance of this solution was taken immediately at 517 nm on UV spectrophotometer using methanol as blank. This reading was served as control reading. Aliquots of different concentration ranging from 10 µg/ml to 1000 µg/ml for the test and 5 µg/ml to 150 µg/ml for the standard were prepared. For the assay 150 µl of the test or std solution was added to 150 µl of DPPH solution and diluted up to 3ml with methanol, the absorbance of this solution was taken after 15 min at 517 nm on

UV spectrophotometer using methanol as blank. The absorbance was taken in triplicate manner. Ascorbic acid was taken as reference.

Percentage scavenging of DPPH free radical was calculated based on the control reading, which contained DPPH and methanol without any extract using the following equation.

$$\% \text{ scavenging activity} = \frac{A \text{ control} - A \text{ test}}{A \text{ control}} \times 100$$

Where A Control was the absorbance of the control reaction and A Test was the absorbance in the presence of the sample extract.

A low absorbance of the reaction mixture indicated a high free radical scavenging activity. The antioxidant activity of the extract was expressed as IC50. The IC50 value was defined as the concentration (in µg/ml) of extracts that inhibits the formation of DPPH radicals by 50%.

Super oxide free radical scavenging activity[13]

100 µl of riboflavin (20 µg), 200 µl EDTA (12 mM), and 200 µl methanol and 100 µl (0.1 mg) nitro-blue tetrazolium were mixed in a test tube and diluted up to 3 ml with phosphate buffer (50 mM). The reaction mixture was allowed to react for 5 min. Absorbance of the mixture was measured at 590 nm using phosphate buffer as a blank after 5 min. This reading was served as control reading. Aliquots of different concentration ranging from 10 µg/ml to 1000 µg/ml for the test and 5 µg/ml to 150 µg/ml for the standard were prepared. For the assay 150 µl of different concentration of test or standard solution was taken in a test tube and 100 µl of riboflavin, 200 µl of EDTA, 200 µl of methanol and 100 µl of nitro-blue tetrazolium were added. The above reaction mix was diluted up to 3 ml with phosphate buffer. The reaction mixture was allowed to react for 5 min. thereafter absorbance was measured at 590 nm using phosphate buffer as a blank. The absorbance was taken in triplicate manner. Ascorbic acid was taken as reference. Percentage scavenging of super oxide free radical was calculated like DPPH free radical scavenging activity.

Hydroxyl radical scavenging activity[14]

The ability of compound to scavenge $OH^{\cdot}$ was assessed using the classic deoxyribose degradation assay. 2.0 ml of the assay mixture containing EDTA (1 mM), $FeCl_3$ (10 mM), H_2O_2 (10 mM), deoxyribose (10 mM) and sample extract was dissolved in distilled water with ascorbic acid (1 mM) in 50 mM phosphate. The mixture was incubated at 37 °C for 1 h. and 1.0 ml of the incubated mixture was mixed with 1 ml of 10 % TCA and 1 ml of 0.4%

TBA (in glacial acetic acid, pH 3.5 adjusted by NaOH) to develop the pink chromagen. The absorbance was measured at 532nm against corresponding blank solution. Ascorbic acid was taken as reference.

The hydroxyl radical scavenging activity of the extract was reported as % inhibition of deoxyribose degradation and was calculated as

$$\% \text{ inhibition of deoxyrebose degradation} = \frac{A \text{ control} - A \text{ test}}{A \text{ control}} \times 100$$

H_2O_2 scavenging activity[15]

The ability of the compound to scavenge H2O2 was determined according to the method of Suganthy et al., 2008. 40 mM H_2O_2 was prepared in phosphate buffer (pH 7.4) and the H_2O_2 concentration was determined spectrophotometrically by measuring the absorption with the extinction coefficient for H_2O_2 of 81 $M^{-1}cm^{-1}$. Extract at the different concentrations in 3.4 ml phosphate buffer were added to a H_2O_2 solution (0.6ml, 40mM) and the absorbance of H_2O_2 was determined at 230 nm after 10 min incubation against a blank solution containing phosphate buffer without H_2O_2. Ascorbic acid was taken as reference. The percentage of scavenging of H_2O_2 was calculated like DPPH free radical scavenging activity.

Reducing power ability[15]

The reducing power of plant extract was determined according to the method of Suganthy et al., 2008. The capacity of extract to reduce the ferric-ferricyanide complex to the ferrous-ferricyanide complex of Prussian blue was determined by measuring the absorbance at 700 nm after incubation.

Different concentrations of extract in 1 ml of distilled water were mixed with phosphate buffer (2.5ml, 0.2 M, pH 6.6) and potassium ferricyanide (2.5 ml, 1%) and the mixture was incubated at 50°C for 20 min. 2.5 ml of 10% TCA was added to the reaction mixture which was centrifuged at 5000 rpm for 10 min. The upper layer of solution (2.5 ml) was mixed with distilled water (2.5 ml), FeCl3 (0.5 ml, 0.1%) and the absorbance was measure at 700 nm. Ascorbic acid was taken as reference.

The higher the absorbance of the reaction mixture the greater is the reducing power.

RESULTS AND DISCUSSION

Table 1: IC50 value of *Benincasa cerifera* fruit in various invitro antioxidant assay.

Assay	IC50 value (µg/ml)	
	Ascorbic acid	Methanolic extract of *Benincasa cerifera*
DPPH free radical scavenging assay	28.04	590.59
H_2O_2 scavenging assay	24.52	423.53
SO· free radical scavenging assay	30.46	555.76
OH· free radical scavenging assay	25.18	555.28

Antioxidant compounds reduce the level of oxidative stress and prevent the development of complications associated with oxidative stress-related diseases by scavenging free radicals such as peroxide, hydroperoxide or lipid peroxyl. [16] Synthetic antioxidants have shown toxic and mutagenic effects, which have shifted interest towards naturally occurring antioxidants. Numbers of naturally occurring substances have been recognized to have antioxidant abilities.[17]

1,1-Diphenyl-2-picrylhydrazyl (DPPH) is a stable nitrogen centered free radical which can be effectively scavenged by antioxidants. [18] Hence it has been widely used for rapid evaluation of the antioxidant activity of plant and microbial extracts relative to other methods. DPPH is also considered as a good kinetic model for peroxyl radicals. [19] The ability of methanolic extract of *Benincasa cerifera* fruits to scavenge DPPH radicals was determined by the decrease in its absorbance at 517 nm. In present investigation methanolic extract of *Benincasa cerifera* fruits exhibited significant DPPH scavenging activity with IC 50 value 590.59 µg/ml (Table 1, Fig. 1). As DPPH is free radical, the scavenging activity of any antioxidant is thought to be due to their hydrogen donating ability.[20] Hence, the DPPH scavenging activity of *Benincasa cerifera* may be due to its hydrogen donating ability.

The methanolic extract exhibited an IC50 value of 555.76 µg/ml (Table 1, Fig. 2). The methanolic extract was found to be an effective scavenger of superoxide radical generated by photo reduction of riboflavin. Superoxide anion radical is one of the strongest ROS among the free radicals and get converted to other harmful reactive oxygen species such as hydrogen peroxide and hydroxyl radical, damaging biomolecules which results in chronic diseases.[21]
In present investigation methanolic extract of *Benincasa cerifera* fruits exhibited significant super oxaide free radical scavenging activity with IC 50 value 555.76 µg/ml (Table 1, Fig. 2).

OH• has a short half-life and is the most reactive and damaging ROS. It causes oxidative damage to DNA, lipids and proteins. [22] The extract was examined for its ability to scavenge OH• radicals generated by the Fenton reaction. In present investigation methanolic extract of *Benincasa cerifera* fruits exhibited significant OH• radicals scavenging activity with IC 50 value 555.28 µg/ml (Table 1, Fig. 3). According to the method [22]; the scavenging effect of any antioxidant on OH• radical is due to its inhibitory activity on the degradation of 2-deoxyribose-2-ribose. And therefore the scavenging effect of *Benincasa cerifera* fruit extract on OH• radicals may be by preventing degradation of 2-deoxyribose-2-ribose.

Hydrogen peroxide is a weak oxidizing agent and can inactivate a few enzymes directly, usually by oxidation of essential thiol (-SH) groups. H_2O_2 can cross cell membranes rapidly and once inside the cell it can probably react with Fe^{2+} and possibly Cu^{2+} to form hydroxyl radicals and this may be the origin of many of its toxic effects.[23] It is therefore advantageous for cells to control the amount of hydrogen peroxide that is allowed to accumulate. Results shows the strong scavenging activity of *Benincasa cerifera* fruit extract against H_2O_2 with IC50 value 423.5 µg/ml as compared to other anti oxidant method (Table 1, Fig. 4).

The reducing ability of a compound greatly depends on the presence of reductones, which have exhibit antioxidative potential by breaking the free radical chain by donating a hydrogen atom.[24] For the measurement of reductive ability, we investigated the Fe^{3+} to Fe^{2+} transformation in the presence of methanolic extract using the method of Suganthy et al., 2008. Methanolic extract of *Benincasa cerifera* fruit (1000 µg/ml) showed higher absorbance when compared with the control. It indicates reducing capacity of methanolic extract of *Benincasa cerifera* fruit, which is a indicator of its antioxidant activity (Fig. 5).

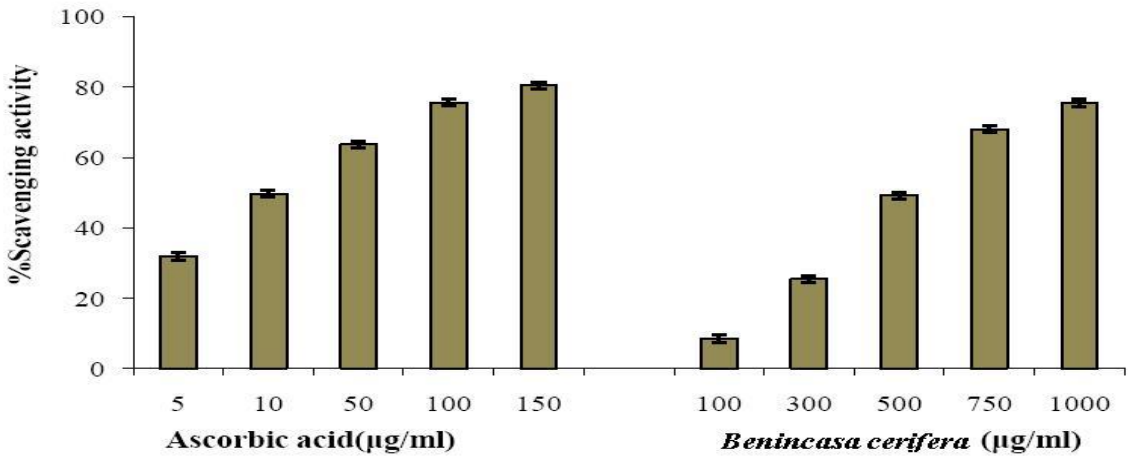

Figure 1: Comparison of % DPPH free radical scavenging activity of methanolic extract of *Benincasa cerifera* fruit and ascorbic acid.

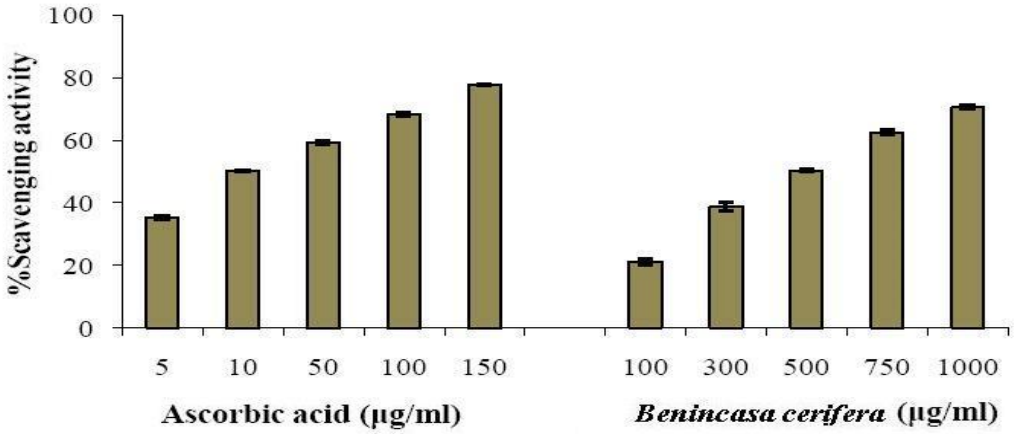

Figure 2: Comparison of % Super oxide free redical Scavenging activity of methanolic extract of *Benincasa cerifera* fruit and ascorbic acid.

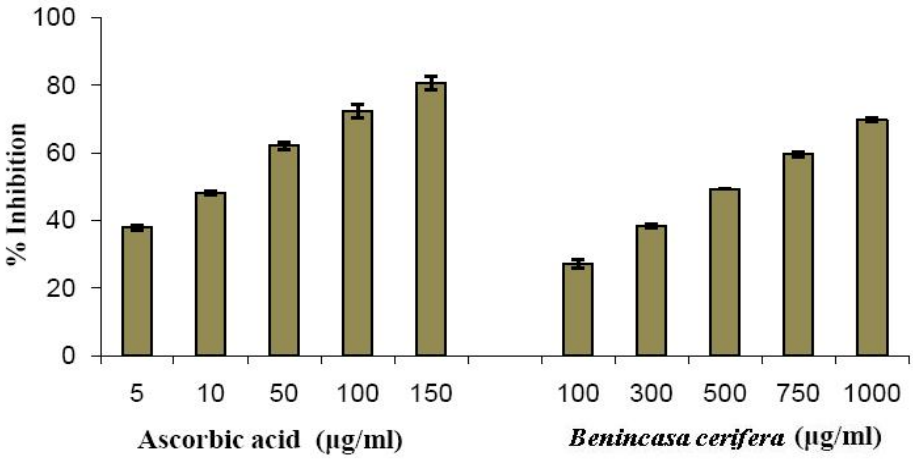

Figure 3: Comparison of % inhibition of deoxyrebose degradation by methanolic extract of *Benincasa cerifera* fruit and ascorbic acid.

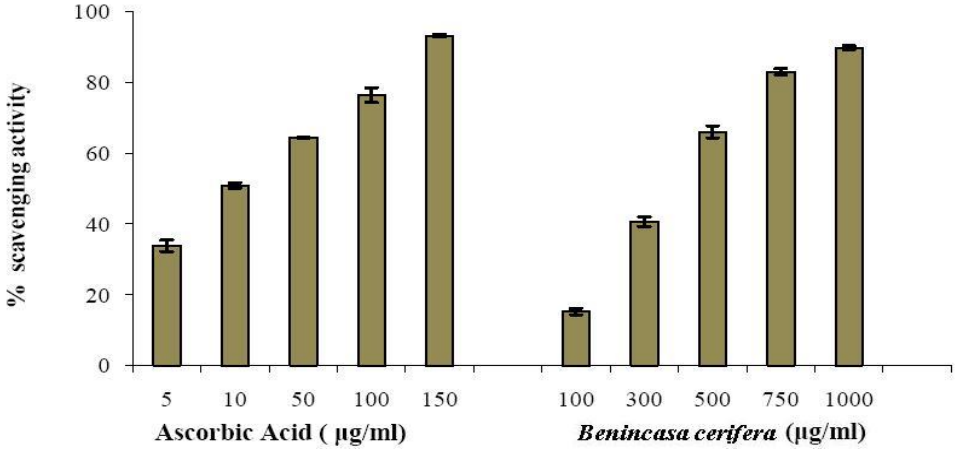

Figure 4: Comparison of % H2O2 scavenging activity of methanolic extract of *Benincasa cerifera* fruit and ascorbic acid.

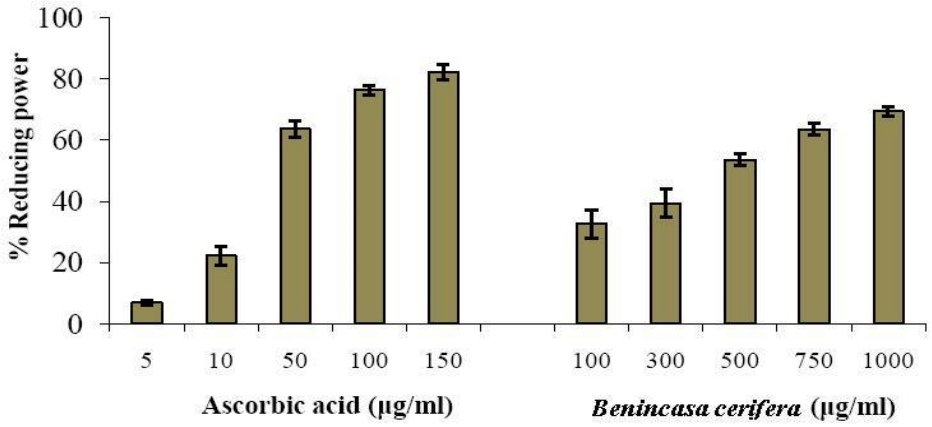

Figure 5: Comparison of reducing power ability of methanolic extract of *Benincasa cerifera* fruit and ascorbic acid.

CONCLUSION

This investigation supports the view that *Benincasa cerifera* fruit extract is promising source of natural antioxidants. Methanolic extract of *Benincasa cerifera* fruit possess significant antioxidant activity, which is confirmed by various in-vitro antioxidant-screening methods.

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