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In vitro Antioxidant Activities of Methanol and Aqueous Fractions of *Coccinia grandis* L. Voigt Stem Extract

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The present study reports the antioxidant activities of the methanol and aqueous fractions of the stem extract of *Coccinia grandis* L. Voigt (Cucurbitaceae). The antioxidant activities of the fractions have been evaluated by using four *in vitro* assays and were compared to standard antioxidant such as butylated hydroxy anisole (BHA). Both the fractions showed effective H-donor activity, reducing power, free radical scavenging activity. The antioxidant property depends upon concentration and it is increased with increasing amount of the fractions. The free radical scavenging and antioxidant activities may be attributed to the presence of phenolic and flavonoid compounds present in the fractions. The results obtained in the present study indicate that the stem of *C. grandis* is a potential source of natural antioxidant.

Key Words: *Coccinia grandis* (Cucurbitaceae), Free radicals, Antioxidant.

INTRODUCTION

Free radicals are highly reactive species and their over-production may be the cause of a variety of diseases such as cancer, atherosclerosis, arthritis, neurodegenerative disorders, liver injury and degenerative processes associated with aging. In diabetes mellitus, there is oxidative stress associated with release of free radicals. Oxidative stress is significant in the development of many complications of diabetes mellitus such as retinopathy and nephropathy. Free radicals may be neutralized by the action of free radical scavenger. Free radical scavenger protects cells from damaging oxidation reaction by scavenging free radical and other reactive species within the body. They can greatly reduce the damage due to oxidants by neutralizing the free radicals before they can attack the cells and prevent damage to lipids, proteins, enzymes, carbohydrates and DNA[1-3].

Coccinia grandis L. Voigt (Cucurbitaceae) is a climber herb cultivated throughout India. In folklore medicine, the fruit is used to treat leprosy, fever, asthma, infective hepatitis, jaundice and sore throats. It is also used as expectorant and astringent. The alcoholic extract of the plant is used as hypoglycemic and antioxidant agent[4-7].

The objective of the present study is to investigate the antioxidant activity of the methanol and aqueous fraction of the stem extract of *C. grandis* using four *in vitro* models.

EXPERIMENTAL

The plant material consists of dried powdered stem of *C. grandis* L. Voigt. (Cucurbitaceae); collected from in and around Chikhali, Tal-Haveli, Dist.-Pune, Maharashtra, India during the month of September-2008 and was authenticated by Joint Director, Botanical Survey of India, Western Circle, Pune-4110 01 (Ref No. BSI/WC/Tech./2008/477 dated 3/10/2008).

Preparation of the extract: Air-dried powdered stems (500 g) of *C. grandis* were extracted with 2.0 L methanol by continuous hot extraction method using Soxhlet apparatus. An exhausted marc was collected and further used for preparation of aqueous extract. The solvent was concentrated under reduced pressure at 60 ºC, to obtain the solid residues from methanolic extract 43.2 g (8.64 %) and 19.29 g (3.86 %) of aqueous extract, respectively.

All the drugs and chemicals used in the study were obtained commercially and were of analytical grade. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) was obtained from Sisco Research Laboratories Pvt. Ltd., Mumbai. UV measurements were done on Shimadzu 1700 UV-vis spectrophotometer.

Phytochemical screening: Preliminary phytochemical screening of the methanolic and aqueous extracts of the stem of *C. grandis* was carried out[8].

In vitro antioxidant activity

DPPH radical scavenging assay: The free radical scavenging activity of the fractions was measured *in vitro* by DPPH assay procedure[4]. About 0.3 mM solution of DPPH in 100 % ethanol was prepared and 1 mL of this solution was added to 3 mL of the fraction of the extract under study dissolved in ethanol at different concentrations (50-250 µg/mL). The mixture was shaken and allowed to stand at room temperature for 0.5 h and the absorbance was measured at 517 nm using a spectrophotometer. The percentage of scavenging activity at different concentrations was determined and compared using butylated hydroxy anisole (BHA), as the standard[9-11].

Reducing power ability: The reducing power of the extract under observation was investigated by Fe^{3+}-Fe^{2+} transformation in the presence of the fractions as described by Kaur *et al.*[9]. The Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. One mL of the fraction (50-250 µg/mL), 2.5 mL of phosphate buffer (pH 6.6) and 2.5 mL of 1 % potassium ferricyanide were incubated at 50 ºC for 0.5 h and 2.5 mL of 10 % trichloroacetic acid was added to the mixture and centrifuged for 10 min at 3000 rpm. About 2.5 mL of the supernatant was diluted with 2.5 mL of water and shaken with 0.5 mL of freshly prepared 0.1 % ferric chloride. The absorbance was measured at 700 nm using BHA (50-250 µg/mL) as the standard. All the tests were performed in triplicate and the graph was plotted employing the average absorption value of the three determinations, against the concentration of methanolic and aqueous extract, under study, as reported in Fig. 1.

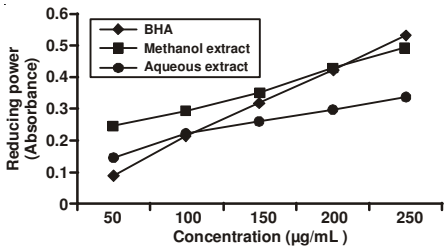

Fig. 1. Reducing power of methanolic and aqueous extract of *C. grandis* stem and butylated hydroxy anisole (BHA). Each value is mean + SE (n = 3)

Super oxide scavenging assay: Scavenging of $O_2^{\cdot-}$ was determined by following the method of Yen and Chen[12]. The reaction mixture comprising of 1 mL of the respective extract solution in distilled water,1 mL of PMS (60 µM) in phosphate buffer (0.1 M, pH 7.4) was incubated at 25 ºC for 5 min and the absorbance was read at 560 nm against blank sample[10] (Table-1).

Hydrogen peroxide scavenging assay: Hydrogen peroxide solution (2 mM/L) was prepared with standard phosphate buffer (pH 7.4). Different concentration of the fractions (50-250 µg/mL) of the extract under study prepared in distilled water was added to 0.6 mL of hydrogen peroxide solution. Absorbance was determined at 230 nm after 10 min against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging activity at different concentrations of the fractions was determined and compared with the standard, BHA[9].

Calculation of 50 % inhibitory concentration (IC_{50}): The concentration (µg/mL) of the fractions required to scavenge 50 % of the radicals was calculated by using the percentage scavenging activities at five different concentrations of the fractions. Percentage inhibition (I %) was calculated using the formula,

$$I\,(\%) = \frac{(Ac - As)}{Ac} \times 100$$

where Ac = absorbance of the control and As = absorbance of the sample.

Statistical analysis: All the experiments were performed in triplicate (n = 3) and results were expressed as mean ± SEM. Statistical analysis was carried out with (INTA package version 10.0) using ANOVA followed by Dunnett test ($p < 0.05$).

RESULTS AND DISCUSSION

Phytochemical screening: Phytochemical screening of the crude methanolic and aqueous extract of the stem of *Coccinia grandis* revealed the presence of flavonoids, saponins, phenols, tannins and terpenoids.

DPPH assay: *Coccinia grandis* stem extract methanolic fraction scavenged 86.97 % and aqueous fraction 89.12 % of

TABLE-1
SCAVENGING OF SUPER OXIDE ($O_2^{\cdot-}$), HYDROGEN PEROXIDE (H_2O_2), REDUCING POWER AND DPPH RADICALS BY METHANOL AND AQUEOUS STEM EXTRACT OF *Coccinia grandis* L. Vigot

Group	O_2^{-}	H_2O_2	Reducing power	DPPH
Methanol extract				
50 (µg/mL)	71.09 + 0.68	94.15 + 0.85	71.09 + 0.68	58.86 + 0.91
100 (µg/mL)	65.21 + 0.40	88.70 + 1.25	65.21 + 0.40	67.27 + 0.90
150 (µg/mL)	58.93 + 0.59	79.46 + 2.27[a]	58.93 + 0.59	72.10 + 0.62
200 (µg/mL)	49.33 + 0.86[a]	71.64 + 3.67[b]	49.33 + 0.86[b]	80.92 + 0.63[a]
250 (µg/mL)	41.68 + 1.32[c]	63.37 + 1.72[c]	41.68 + 1.32[c]	86.97 + 0.44[b]
Aqueous extract				
50 (µg/mL)	82.89 + 1.13	91.01 + 0.55	82.89 + 1.13	58.45 + 0.53
100 (µg/mL)	73.91 + 1.23	80.67 + 1.078	73.91 + 1.23	66.86 + 0.54
150 (µg/mL)	69.64 + 1.20	77.48 + 0.82	69.64 + 1.20[a]	72.20 + 0.71
200 (µg/mL)	65.54 + 1.26[b]	71.12 + 0.46[a]	64.54 + 1.26[c]	79.58 + 0.36[a]
250 (µg/mL)	60.38 + 1.36[b]	65.65 + 1.34[c]	60.38 + 1.36[c]	89.12 + 0.62[b]
Butylated hydroxy anisole				
250 (µg/mL)	37.21 + 0.49	49.89 + 0.29	37.21 + 0.49	67.02 + 0.35

Butylated hydroxy anisole (BHA) was taken as a standard. Results are expressed as percentage of the control. Each value is mean ± SE (n = 3). a: $p < 0.05$, b: $p < 0.01$, c: $p < 0.001$ *vs.* control group.

DPPH radical at a concentration of 250 µg/mL. The IC_{50} value for methanolic fraction was 30 µg/mL and for aqueous fraction 27.5 µg/mL. DPPH scavenging activity of methanolic fraction of *Coccinia grandis* stem is almost equivalent to that of aqueous fraction but fairly higher than that of standard (BHA) as shown in Table-1.

Hydrogen peroxide scavenging assay: Both the fractions of *Coccinia grandis* stem extract were found to scavenge H_2O_2. When compared to the standard (BHA) activity shown by these fraction is quite significant as shown in Table-1 (IC_{50} 19-24 µg/mL).

Super oxide scavenging assay: Both the fractions of the extract significantly and concentration dependently scavenge ($O_2^{\cdot-}$) as well (Table-1). As much as 41.68 % of ($O_2^{\cdot-}$) of methanolic fraction and 60.38 % of aqueous fraction could be scavenged by *Coccinia grandis* stem extract at concentration of 250 µg/mL (IC_{50} 24-30 µg/mL).

Reducing power ability: The reducing capacity of both the fractions, was also found to be greater than that of BHA, used as standard (Fig. 1) (IC_{50} 35-50 µg/mL). It signifies the increase in the absorbance of both fraction which indicates increased reducing power.

Conclusion

The present study demonstrates that the methanolic and aqueous fractions of the stem extract of *Coccinia grandis* possesses significant scavenging activity of superoxide, H_2O_2 and reducing power ability. Its antioxidant activity may possibly attribute due to its polyphenol and flavonoid contents. Further investigations on isolation and characterization of the said active compounds responsible for the antioxidant and other biological activity are under study.

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