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A STUDY ON ANTIOXIDANT ACTIVITY OF FRUIT EXTRACTS OF *COCCINIA GRANDIS* L.VOIGT.

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ABSTRACT

The aim of the present study was investigating the antioxidant activity of the methanolic extract of the fruit of *Coccinia grandis* L. Voigt. (Cucurbitaceae). The antioxidant activity of the fruit has been evaluated by using three in vitro assays and was compared to standard antioxidant, Butylated hydroxyanisole (BHA). All the fractions showed effective H-donor activity, reducing power, free radical scavenging activity. The antioxidant property depends upon concentration and increased with increasing amount of the fractions. The free radical scavenging and antioxidant activities may be attributed to the presence of flavonoids present in the fractions. The results obtained in the present study indicate that the fruit of *Coccinia grandis* is a potential source of natural antioxidant.

Keywords: *Coccinia grandis*, Free Radicals, Antioxidant and Pro-oxidant.

INTRODUCTION

There are hundreds of medicinal plants that have a long history of curative properties against various diseases and ailments. However, screening of plants for their activity is very essential and needs urgent attention in order to know the value of the plant. The rich knowledge base of countries like India in medicinal plants and healthcare has lead to the keen interest by pharmaceutical companies to use this knowledge as a resource for research and development in the pursuit of discovering novel drugs. However, several plants are used for various aspects in India in the form of crude form without scientific evidence of efficacy. At this juncture it is of interest to determine the scientific basis for the traditional use of these plants. [1]

In recent years, phytochemicals in vegetables have received a great deal of attention mainly because of their role in preventing diseases caused as a result of oxidative stress which releases reactive oxygen species such as singlet oxygen and various radicals as a damaging side-effect of aerobic metabolism that are various tumours, especially epithelial cancers of the respiratory and gastrointestinal tract.[2]

A free radical is defined as any atom or molecule possessing unpaired electrons. There is an increased evidence for the participation of free radicals in the etiology of various diseases like cancer, diabetes, cardiovascular diseases, autoimmune disorders, neurodegenerative diseases, aging, etc. Free radicals can cause a wide range of toxic oxidative reactions like initiation of the peroxidation of the membrane lipids leading to the accumulation of lipid peroxides, direct inhibition of mitochondrial respiratory chain enzymes, fragmentation or random cross linking of molecules like DNA,

enzymes and proteins which ultimately leads to cell death.

Free radicals are highly reactive species and their overproduction may be the cause of a variety of diseases such as cancer, atherosclerosis, arthritis, neurodegenerative disorders, liver injury and degenerative processes associated with aging. [3,4,5,6]

An antioxidant may be defined as any substance that when present at low concentrations, compared with those of the oxidizable substrate significantly delays or inhibits oxidation of that substrate. Oxidation is a chemical reaction that transfers electrons from a substance to an oxidising agent. Antioxidants exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defence mechanisms. They do this by being oxidized themselves, so antioxidants are often reducing agents such as thiols, ascorbic acid or polyphenols. However, it has been suggested that these compounds have shown toxic effects like liver damage and mutagenesis hence, nowadays search for natural antioxidant source is gaining much importance.[7] Radical scavenging action is dependent on both reactivity and concentration of the antioxidant.[8]

MATERIALS AND METHODS

Collection and Authentication

The plant material consists of dried powder of fruits 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 fruit (500 g) of *C. grandis* was 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 42.3 g (8.46 %).

Drugs and Chemicals

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.

DPPH Radical Scavenging Assay

The free radical scavenging activity of the fractions was measured in vitro by DPPH assay procedure. 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 30 min and the absorbance was measured at 517 nm using spectrophotometer. The percentage of scavenging activity at different concentrations was determined and compared using butylated hydroxyanisole (BHA) as the standard. [9-10]

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. 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°C for 30 min 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.

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 minutes against a blank solution containing phosphate buffer without hydrogen peroxide. The percentage scavenging activity at different concentrations of the fractions was determined and compared using BHA as standard. The concentration (µg /ml) of the fractions required to scavenge 50% of the radicals was calculated by using the percentage scavenging activity at five different concentrations of the fractions under investigation.

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

In hydrogen peroxide scavenging assay and reducing power assay methods, there is a direct relation between the sample concentration and the absorbance.

In DPPH Method, there is an inverse relation between the sample concentration and the absorbance. Increase in concentration follows increased % inhibition (table1).

DISCUSSION

DPPH assay is one of the most widely used methods for screening antioxidant activity of plant extracts. DPPH is a stable, nitrogen-centered free radical which produces violet colour in ethanol. In DPPH Method, there is an inverse relation between the sample concentration and the absorbance. Increase in concentration follows increased % inhibition. It indicates that Methanol fraction of fruit extract of *Coccinia grandis* possess potent antioxidant activity than standard BHA. The antioxidant principles present in the *Coccinia grandis* caused the reduction of ferricyanide complex from ferric to the ferrous form, and thus proved to be having reducing power ability.

Scavenging of H_2O_2 is a measure of the antioxidant activity of the methanol fraction of *Coccinia grandis*. Scavenged hydrogen peroxide which may be attributed to the presence of anthraquinone glycosides and flavonoids.

CONCLUSION

On the basis of results obtained, it can be concluded that the methanolic extract of the fruit of *Coccinia grandis* showed significant antioxidant activity. The antioxidant activity of the fruit may be attributed to the presence of flavonoids and anthraquinone glycosides. *Coccinia grandis* fruit also possess reducing power ability, free radical scavenging activity when compared with standard, butylated hydroxyanisole. Further studies on isolation and characterization of the said active compound responsible for antioxidant and other activity are under way.

Table 1: Scavenging of hydrogen peroxide (H2O2), reducing power and DPPH radicals by methanolic fruit extract of *Coccinia Grandis* L. Voigt.

Group	H_2O_2	Reducing Power	DPPH
Control	1.32±0.000	0.928±0.000	1.100±0.000
Standard	0.874±0.00145	0.434±0.0006667	0.693±0.001000
50µg/ml	0.187±0.0006667	0.043±0.002333	0.604±0.001856
100µg/ml	0.214±0.001333	0.055±0.0006667	0.497±0.0005774
150µg/ml	0.234±0.0008819	0.081±0.001202[a]	0.375±0.001453[a]
200µg/ml	0.284±0.0006667[a]	0.104±0.0006667[b]	0.254±0.001155[c]
250µg/ml	0.309±0.0008819[c]	0.132±0.001732[c]	0.129±0.0006667[c]

Butylated hydroxyanisole (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.

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