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IN VITRO ANTIOXIDANT STUDY OF PETROLEUM ETHER, CHLOROFORM AND ETHYL ACETATE FRACTIONS OF *COCCINIA GRANDIS* L. VOIGT STEM EXTRACT

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ABSTRACT

The present study aimed at investigating the antioxidant activities of the petroleum ether, chloroform and ethyl acetate 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 as butylated hydroxy anisole (BHA). All the fractions showed effective H-donor activity, reducing power and free radical scavenging activity. The antioxidant property depends upon concentration of the fractions, which 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* may be a potential source of natural antioxidant.

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

INTRODUCTION

The reactive oxygen species (ROS) including superoxide anion radical ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2) and hydroxyl radicals ($^{\bullet}OH$) are implicated in oxidative damage to various cellular macromolecules. Increasing number of evidences suggest that oxidative stress induced biochemical changes are crucial etiological factors in several chronic human diseases such as diabetes mellitus, cancer, atherosclerosis, arthritis, neurodegenerative diseases and also in aging process. Based on growing interest in free radical biology and lack of effective therapies for most chronic diseases, the usefulness of antioxidant in protection against these diseases is warranted. Several synthetic antioxidants such as butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) are available, but

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are quite unsafe and their toxicity is a problem of concern. Therefore, in recent years, considerable attention has been directed towards identification of natural antioxidants that may be used for human consumption[1-4].

Coccinia grandis Voigt. (Cucurbitaceae), commonly known as 'Little gourd' and as 'Kovai' (Hindi), is a climbing perennial herb with a tuberous rootstock producing annual stems up to several meters long, hispid. The plant has been extensively used in Ayurvedic and Unani practice in the Indian subcontinent. The entire plant product has been reported to be useful for the treatment of syphilis, sores and bacterial infections[5]. The fruit is used to treat leprosy, fever, asthma, infective hepatitis, jaundice, and sore throats[6,7]. The plant also possesses potent antidiabetic and anti-dyslipidemic activity[6] and people use various parts of the plant indigenously to get relief from asthma and cough[8]. The objective of the present study was to investigate the antioxidant activity of the petroleum ether, chloroform and ethyl acetate fractions of the stem extract of *C. grandis* using four *in vitro* models.

EXPERIMENTAL

Materials and methods

Plant material

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 petroleum ether by continuous hot extraction method using Soxhlet apparatus. An exhausted marc was collected and used for preparations of chloroform and ethyl acetate extracts. The solvent was concentrated under reduced pressure at 60^{o}C to obtain the solid residues of petroleum ether extract 10.2 g (2.3%), chloroform extract 43.5 g (8.5%) and ethyl acetate extract 35.2 g (7%), respectively.

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.

Phytochemical screening

Preliminary phytochemical screening of the petroleum ether, chloroform and ethyl acetate fractions of the stem extract of *C. grandis* was carried out by established methods[9].

In vitro antioxidant activity

DPPH radical scavenging assay

The free radical scavenging activity of the fractions was measured *in vitro* by DPPH assay procedure[3]. 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 a spectrophotometer. The percentage of scavenging activity at different concentrations was determined and compared using butylated hydroxy anisole (BHA) as the standard[1, 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.[1] 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.

Super oxide scavenging assay

Scavenging of $O_2^{-\bullet}$ was determined by following the method of Ten and Chen. The reaction mixture comprising of 1 mL of the respective extract solution in distilled water and 1 mL of PMS (60 µM) in phosphate buffer (0.1M, pH7.4) was incubated at 25°C for 5 min and the absorbance was read at 560 nm against blank sample[10].

Hydrogen peroxide scavenging assay

Hydrogen peroxide solution (2 mM/L) was prepared with standard phosphate buffer

(pH 7.4). Different concentrations 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 using BHA as standard[1]. 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 AND DISCUSSION

Phytochemical screening

Phytochemical screening of the crude petroleum ether, chloroform and ethyl acetate extracts of the stem of *Coccinia grandis* revealed the presence of flavonoids, saponins, phenols, tannins and phytosterol.

Table 1: Phytochemical constituents present in the stem extracts of *C. grandis*

Test	Petroleum ether extract	Chloroform extract	Ethyl acetate extract
Carbohydrates	+	+	+
Proteins	-	-	-
Glycosides	-	+	-
Alkaloids	-	+	-
Phytosterols	-	-	-
Flavonoids		+	+
Tannins and phenolic compounds	+	+	+

In vitro antioxidant activity

From the Table 2, it is evident that the component from ethyl acetate, chloroform and petroleum ether of this species can be potent antioxidant in comparison with the widely used standards synthetic antioxidants like BHA, BHT and L-ascorbic acid. All the solvent fractions of stem extracts samples had good radical scavenging activity. IC_{50} values obtained for *C. grandis* stem extract for petroleum ether fraction 95-110 µg/mL, for chloroform 50-60 µg/mL and for ethyl acetate 17.5-25 µg/mL. In DPPH radical scavenging method, increase in concentration follows increased % inhibition. It indicates that ethyl acetate fraction possess potent antioxidant activity than remaining two fractions and standard (BHA). However, further studies are necessary to elucidate the compound responsible for antioxidant activity of extracts.

Table 2: Scavenging of Super oxide ($O_2^{-\bullet}$), Hydrogen peroxide (H_2O_2), reducing power and DPPH radicals by methanol and aqueous stem extract of *Coccinia grandis* L. Vigot

Group	$O_2^{-\bullet}$	H_2O_2	Reducing power	DPPH
Control	100 ± 1	100 ± 2.3	100 ± 0.51	100 ± 3.7
Petroleum ether extract				
50 (µg/mL)	92.35 ± 0.20	88.22 ± 0.43	94.48 ± 0.67	53.22 ± 0.35
100 (µg/mL)	90.78 ± 0.23	77.78 ± 0.99	87.65 ± 0.53	61.43 ± 0.71
150 (µg/mL)	88.89 ± 0.17	63.39 ± 0.72[a]	79.88 ± 0.79	68.92 ± 0.53
200 (µg/mL)	86.11 ± 0.82[a]	55.65 ± 0.79[b]	73.27 ± 0.86[b]	77.33 ± 0.35[a]
250 (µg/mL)	83.52 ± 0.20[c]	52.08 ± 0.69[c]	66.25 ± 0.75[c]	82.56 ± 0.27[b]
Chloroform extract				
50 (µg/mL)	92.86 ± 0.27	93.22 ± 0.75	90.34 ± 0.68	44.81 ± 0.80
100 (µg/mL)	85.91 ± 0.60	88.41 ± 1.18	81.24 ± 1.56	58.00 ± 0.95
150 (µg/mL)	78.55 ± 0.90	81.79 ± 1.59	68.64 ± 0.83[a]	64.10 ± 0.44
200 (µg/mL)	71.84 ± 1.21[b]	74.14 ± 1.66[a]	60.89 ± 0.82[c]	77.12 ± 0.45[a]
250 (µg/mL)	64.82 ± 1.532[b]	66.51 ± 0.26[c]	50.89 ± 1.40[c]	82.66 ± 0.27[b]

Cont…

Group	$O_2^{-\bullet}$	H_2O_2	Reducing power	DPPH
Ethyl acetate extract				
50 (µg/mL)	89.96 ± 0.30	95.11 ± 0.14	85.41 ± 0.13	45.02 ± 0.62
100 (µg/mL)	76.19 ± 0.43	90.10 ± 0.15	75. 30 ± 0.15	57.53 ± 0.53
150 (µg/mL)	66.82 ± 0.27	81.65 ± 0.12	67.15 ± 0.13	65.87 ± 0.32
200 (µg/mL)	58.93 ± 0.59[a]	74.07 ± 0.13[b]	56.22 ± 0.10[b]	77.43 ± 0.73[a]
250 (µg/mL)	50.39 ± 0.27[b]	64.68 ± 0.15[c]	48.80 ± 0.17[b]	87.58 ± 0.27[a]
BHA				
	37.21 ± 0.49	49.89 ± 0.29	39.65 ± 0.82	67.02 ± 0.35

Butylated hydroxy anisole (BHA) was taken as a standard. Results are expressed as % of the control. Each value is mean ± S.E. (n = 3).[a]$p < 0.05$, [b]$p < 0.01$, [c]$p < 0.001$ vs. Control group

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