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Antioxidant Activities of the Chloroform Extract of *Solanum trilobatum*

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Abstract

A chloroform extract from the anticancer herb *Solanum trilobatum* (CST) (Nadkarni, 1979) was analyzed and compared with reference antioxidants for its *in vitro* antioxidative properties such as scavenging of α,α-diphenyl-β-picryl hydrazyl (DPPH) and superoxide radicals, protection to deoxyribose degradation, reducing power, as well as inhibition of lipid peroxidation. Preliminary phytochemical analysis of CST by silica gel thin-layer chromatography showed the presence of simple phenols, phenolic acids, isoflavones, xanthones, and lignans. The antioxidative effect of CST was found to be concentration dependent to a certain extent and then leveled off with further increase in concentration. The IC_{50} for each antioxidative reaction studied was calculated. When compared to the reference antioxidant butylated hydroxytoluene (BHT), CST exhibited less scavenging effect on DPPH radicals and reducing power but a better superoxide radical scavenging effect. From a comparison of the hydroxyl radical scavenging effect of CST with catechin, it seemed that CST was four-times more effective than catechin. CST was also able to prevent the formation of ·OH-induced malondialdehyde (MDA) in rat liver homogenate.

Keywords: Antioxidant, degradation, deoxyribose, DPPH, lipid peroxidation, malondialdehyde, *Solanum trilobatum*, superoxide radicals.

Introduction

Solanum trilobatum (Solanaceae) is a climbing shrub of Southern India, and Siddha saints have considered it as one of the important rejuvenator drugs. The decoction of various parts of the plant is used in chronic bronchitis. Roots are used for consumption (pulmonary tuberculosis), berries and flowers are used for cough, and leaves are cooked and eaten as a vegetable (Anonymous, 1972).

Cytotoxic potential of different solvent extracts from *S. trilobatum* has been reported. "Sobatum," the partially purified component of the plant obtained from the petroleum ether extract, contained betasitosterol as the active principle and exhibited anti-tumor activity (Mohanan & Devi, 1996; Mohanan & Devi, 1997). Presence of a steroid, solasodine (glycoalkaloid), is also reported (Krishnamurthy & Reddy, 1996).

During the course of normal metabolism, reactive oxygen species (ROS) and free radicals are produced, which induce oxidative damage to biomolecules and play an important role in pathological conditions such as atherosclerosis, aging, cancer, inflammatory diseases, and a variety of other disorders (Halliwell et al., 1992; Kamat & Devasagayam, 2000; Finkel & Holbrook, 2002). Antioxidants with free-radical scavenging activities could have great importance as prophylactic and therapeutic agents in diseases in which oxidants or free radicals are implicated (Halliwell et al., 1995). Phenolic compounds are widely distributed in plants and have been found to possess antioxidative properties and an inhibitory role in the processes of carcinogenesis (Rice-Evans et al., 1995; Vinson et al., 1995; Middleton et al., 2000; Pulido et al., 2000). Tea polyphenols have been reported to exhibit anticarcinogenic, antimutagenic, and cardioprotective effects that are generally associated with their antioxidant (free-radical scavenging and metal chelation) properties (Khokhar & Magnusdottir, 2002).

Based on these observations, the chloroform extractable portion of the shade-dried whole plant excluding roots of

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Solanum trilobatum was tested for its *in vitro* antioxidative activities.

Materials and Methods

Biochemicals and chemicals

Petroleum ether and chloroform (analytical grade), nitroblue tetrazolium (NBT), NADH, phenazoniun methosulfate (PMS), and deoxyribose were purchased from Sisco Research Laboratories Pvt Ltd (Bombay, India). Quercetin, catechin and α,α-diphenyl-β-picryl hydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sprague-Dawley rats from our department animal house facility were used for the study.

Plant material

The plant *S. trilobatum* was collected from the Nagarcoil forest and authenticated by a chief botanist. The whole plant, excluding roots, was cut into pieces, dried in shade, and powdered. The powder (100 g) was extracted exhaustively in a Soxhlet apparatus first with petroleum ether [BP (60–80 °C)] and then using chloroform [BP (60–62 °C)].The extracts were then concentrated *in vacuo* until the solvent was completely removed. The yield of petroleum ether and chloroform extract was found to be 3.33 g and 1.66 g, respectively.

Phytochemical analysis

Estimation of total polyphenol content

The total polyphenol content (g/100 g chloroform extract) present in the chloroform extract of *S. trilobatum* (CST) was analyzed using the Folin-Ciocalteu reagent method (Singleton et al., 1999). Extract solution (0.1 ml) was transferred to a 100-ml Erlenmeyer flask, and then the final volume was adjusted to 46 ml by the addition of distilled water. Afterward, 1 ml of Folin-Ciocalteu Reagent (FCR) was added into this mixture, and after 3 min, 3 ml of Na_2CO_3 (2%) was added. Subsequently, the mixture was shaken on a shaker for 2 h at room temperature, and then absorbance was measured at 760 nm. Catechin was used as the standard for calculation.

Chromatographic analysis

Thin-layer chromatography (Silica gel G) of CST using different solvent systems characteristic for different plant polyphenols was done (Harborne, 1984).

In vitro antioxidant studies

DPPH radical scavenging effect

The DPPH radical scavenging activity was estimated (Yamaguchi et al., 1998) as follows: 200 µl of CST (containing 0.05–1 mg in methanol) was mixed with 100 mM Tris-HCl buffer (800 µl, pH 7.4) and then added to 1 ml of 500 µM DPPH in ethanol (final concentration of 250 µM). The mixture was shaken vigorously and left to stand for 20 min at room temperature in the dark. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. Butylated hydroxytoluene (BHT) was used as a positive scavenger, and its effect on DPPH radical scavenging was also determined. The capability to scavenge DPPH radical was calculated using the following equation:

$$\text{Scavenging effect (\%)} = [(\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}})/\text{Absorbance}_{\text{Control}}] \times 100$$

Measurement of reducing power

The reducing power of CST and the reference antioxidants BHT and quercetin was determined (Yen & Chen, 1995) as follows: 1 ml of sample containing different concentrations (0.05–1 mg in 5% DMSO) was mixed with an equal volume of 0.2 M phosphate buffer, pH 6.6, and 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min. Then, an equal volume of 1% trichloroacetic acid was added to the mixture, which was then centrifuged at 6000 rpm for 10 min. The upper layer of solution was mixed with distilled water and 0.1% $FeCl_3$ at a ratio of 1 : 1 : 2, and the absorbance was measured at 700 nm. Increase in absorbance of the mixture indicated increased reducing power.

Superoxide anion scavenging effect

The superoxide radical scavenging capacity was determined by the PMS-NADH superoxide generating system (Robak & Gryglewsk, 1988). The reaction mixture contained 1 ml (0.05–1 mg in 10% DMSO) of test compound, 1 ml of 936 µM NADH, and 1 ml of 300 µM NBT. After incubation at room temperature for 10 min, the reaction was started by adding 1 ml of 120 µM PMS. The reaction mixture was incubated at ambient temperature for 5 min. The results were determined by reading the absorbance at 560 nm against blank samples. The effect of BHT and quercetin on scavenging of superoxide anion radical was also determined. The capability to scavenge superoxide radical was calculated using the following equation:

$$\text{Scavenging effect (\%)} = [(\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}})/\text{Absorbance}_{\text{Control}}] \times 100$$

Deoxyribose assay for hydroxyl radical scavenging activity

The hydroxyl radical scavenging activity of CST and the water-soluble reference pure compound catechin was determined by the method of Aruoma et al. (1994). The assay mixture, in a total volume of 1.2 ml, contained deoxyribose

(2.8 mM), $FeCl_3$ (25 mM), EDTA (100 µM) [EDTA and iron (III) ions are premixed at the ratio given prior to the addition of deoxyribose], H_2O_2 (2.8 mM), KH_2PO_4/KOH buffer at pH 7.4 (10 mM), various concentrations of CST (0.05–1 mg in alkali and the pH was then readjusted to 7.4) and ascorbate (100 µM). The ascorbate was added to start the reaction. After incubation at 37 °C for 1 h, 1 ml of 1% (w/v) thiobarbituric acid (TBA) in 50 mM NaOH and 1 ml of 2.8% (w/v) trichloroacetic acid (TCA) were added to the reaction mixture and placed in a hot water bath maintained at 80 °C for up to 20 min. The contents were cooled, and absorbance was read at 532 nm. The ability to scavenge hydroxyl radical was calculated using the following expression:

$$\text{Scavenging effect (\%)} = [(\text{Absorbance}_{\text{Control}} - \text{Absorbance}_{\text{Sample}})/\text{Absorbance}_{\text{Control}}] \times 100$$

Lipid peroxidation inhibition effect

The effect of CST on $FeSO_4$-H_2O_2 stimulated lipid peroxidation in rat liver homogenate was determined by malondialdehyde (MDA)-TBA adduct formation (Yang et al., 1997). Liver (0.3 g) from Sprague-Dawley rats (200–250 g) was homogenized with 20 ml 50 mM sodium phosphate buffer (pH 7.4) using a motor-driven Potter Elvehjem homogenizer equipped with a Teflon pestle. A mixture containing 0.5 ml of liver homogenate, 0.05 ml of potassium phosphate buffer (pH 7.4), 0.025 ml of 5 nM $FeSO_4$, 0.025 ml of 0.3% H_2O_2 and various concentrations of CST (0.05–1 mg) was incubated for 10 min at 37 °C. Then, TBA (0.4% in 0.2 M HCl) and BHT (0.2% in 95% ethanol) at a ratio of 1 : 2 : 0.3 were added, and the mixture was heated at 90 °C for 45 min. After cooling, 5 ml of *n*-butanol was added, and the mixture was again shaken vigorously. Then, the *n*-butanol layer was separated by centrifugation at 1000× g for 10 min, and MDA production was measured at 532 nm. Using the external standard tetramethoxy propane, the ability to inhibit MDA formation by CST was calculated using the following equation:

$$\text{Inhibition effect (\%)} = \frac{\text{MDA in liver homogenate wihtout CST - MDA in liver homogenate with CST}}{\text{MDA in liver homogenate without CST}} \times 100$$

Statistical analysis

Statistical analysis was done using SPSS 10.0 for Windows using one sample *t*-test.

Results and Discussion

Preliminary phytochemical analysis of CST revealed that it contained 69.8 ± 0.799 g/100 g of polyphenols, which includes simple phenols, phenolic acids, isoflavones, xanthones, and lignan classes of compounds as identified by silica gel thin-layer chromatography.

Proton radical scavenging action is considered to be one among the various mechanisms for antioxidation. DPPH, which possesses a proton free radical, was found to be scavenged by CST showing 11.6–54.9% scavenging effect at a concentration of 0.05–0.4 mg/ml (Table 1). When compared with BHT, the concentration required for CST was found to be ~4.7-fold of BHT to exhibit 50% radical scavenging effect.

Studies on reducing power of CST exhibited a concentration-dependent increase to a certain extent and then almost remained steady with further increase in concentration. Approximately 3.6- and 11.6-fold concentration of CST was required to exhibit a similar effect in comparison with BHT and quercetin (Table 1), which indicates the presence of antioxidants and reveals that CST is an electron donor and could react with free radicals, convert them to more stable products, and terminate radical chain reaction (Yen & Chen, 1995).

Table 1. The effect of CST and reference antioxidants on DPPH radical and reducing power.

DPPH radical scavenging effect (%)			Reducing power[a]			
Conc.[b]	CST	BHT	Conc.[b]	CST	BHT	Quercetin
0.05	11.6 ± 0.33	33.3 ± 0.95	0.10	0.10 ± 0.003	0.35 ± 0.010	2.20 ± 0.083
0.10	26.2 ± 1.81	60.9 ± 1.89	0.20	0.25 ± 0.008	0.90 ± 0.002	3.95 ± 0.158
0.15	35.0 ± 1.17	70.7 ± 2.36	0.30	0.50 ± 0.017	2.90 ± 0.118	
0.20	38.0 ± 1.31	77.5 ± 2.68	0.50	1.23 ± 0.043		
0.25	42.8 ± 1.55	83.7 ± 3.04	0.75	2.11 ± 0.078		
0.30	45.2 ± 1.72	86.4 ± 3.29	1.00	2.42 ± 0.095		
0.40	54.9 ± 2.21	87.2 ± 3.52				

All values expressed in mean ± SEM.
[a] Absorbance at 700 nm.
[b] Expressed as mg/ml.

Table 2. Effect of CST and reference antioxidants on superoxide and hydroxyl radical scavenging effect.

Superoxide radical scavenging effect (%)			Hydroxyl radical scavenging effect (%)		
Conc.[a]	CST	Quercetin	Conc.[a]	CST	Catechin
0.05	–45.5 ± 1.31	70 ± 2.42	0.0375	9 ± 0.30	0.2 ± 0.005
0.10	–14.6 ± 0.42	84 ± 3.05	0.075	18 ± 0.64	0.8 ± 0.002
0.15	20.7 ± 0.62	97 ± 3.74	0.150	37 ± 1.43	6.0 ± 0.202
0.20	53.5 ± 1.69	99 ± 3.94	0.300	74 ± 2.90	19.0 ± 0.675
0.25	80.9 ± 2.93	99 ± 4.00	0.375	80 ± 3.18	25.0 ± 0.935
0.50	97.7 ± 3.78		0.750	100 ± 4.04	47.0 ± 1.818
			1.000		59.0 ± 2.315

Negative values indicate no scavenging effect. All values expressed in mean ± SEM.
[a] Expressed as mg/ml.

Table 3. Inhibition of CST on $FeSO_4$-H_2O_2–induced lipid peroxidation (MDA production) in rat liver homogenate *in vitro*.

Conc.[a]	% inhibition effect
0.05	6.5 ± 0.184
0.10	20.7 ± 0.655
0.20	41.8 ± 1.443
0.30	67.3 ± 2.514
0.40	90.9 ± 3.672

All values expressed in mean ± SEM.
[a] Expressed as mg/ml.

Table 4. Antioxidative activities of CST, BHT, quercetin, and catechin as expressed by half-inhibition concentration (IC_{50}).

Antioxidative reaction	Antioxidant	IC_{50}[a]
DPPH radical	CST	0.352 ± 0.200
	BHT	0.080 ± 0.001
Superoxide radical	CST	0.196 ± 0.004
	BHT	NA
	Quercetin	0.033 ± 0.003
	CST	0.216 ± 0.017
Hydroxyl radical	Catechin	0.825 ± 0.043

NA, not applicable.
[a] Expressed as mg/ml.

The superoxide radical scavenging effect of CST increased with increase in concentration greater than 0.1 mg/ml. A marked 97.7% scavenging effect was exhibited at a concentration of 0.5 mg/ml CST. One of the two reference antioxidants, BHT, showed no detectable superoxide radical scavenging effect, whereas the other compound quercetin showed significantly high scavenging effect (Table 2). The capacity of CST to scavenge superoxide ($\cdot O_2^-$) radical, which has the potential of reacting with biomolecules and thereby causing tissue damage, reveals that it possesses superoxide dismotase–like activity.

CST has ~4-fold the ability to protect deoxyribose against hydroxyl radicals than catechin (Table 2). This ability of CST is very important in the sense that hydroxyl radical is the most toxic and reactive free radical formed in biological systems and has been implicated as a highly damaging species in free-radical pathology, capable of damaging almost every molecule found in living cells (Hochestein & Atallah, 1988; Gordon, 1991). In addition, this species is considered to be one of the quick initiators of lipid peroxidation process, abstracting hydrogen atom from unsaturated fatty acids (Kappus, 1991).

CST inhibited the OH radical–mediated lipid peroxidation by the $FeSO_4$-H_2O_2 system in a concentration-dependent manner that was determined by the amount of MDA in liver homogenate as given in Table 3. MDA level in normal liver homogenate was 0.062 ± 0.01 µmol/g wet weight. After induction by $FeSO_4$-H_2O_2, the MDA level in liver significantly increased to 0.23 ± 0.01 µmol/g wet weight. However, the addition of 0.05–0.4 mg/ml of CST to rat liver homogenate significantly reduced MDA formation. The results revealed that CST has the capacity to prevent the oxidative deterioration of polyunsaturated lipids.

The half-inhibition concentration (IC_{50}) of CST and reference antioxidants for each antioxidative reaction is summarized in Table 4.

This study demonstrates the capacity of *Solanum trilobatum* to interact with a wide range of species directly responsible for oxidative damage. CST possess proton-donating ability and superoxide dismutase (SOD)-like activity as evidenced through DPPH and superoxide radical–scavenging results. In addition, CST can also be viewed as an electron donor that could react with free radicals, convert them to more stable products, and terminate radical chain reactions. Further studies to purify and structurally identify the active antioxidant principle/principles of medicinal importance from CST is in progress.

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