Comparative free radical scavenging potentials of different parts of *Solanum torvum*

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ABSTRACT

Aim: An attempt has been made to evaluate phytochemicals and free radical scavenging efficacy of different parts of *Solanum torvum*. **Methods:** The herbal powder obtained from various plant parts i.e.dry leaves, dry fruits and fresh fruits were extracted with various solvents. The extracts were analysed for phytochemicals and antioxidants, carotenoids, ascorbic acid, tocopherol, total phenol, proteins, reducing sugars and sterols. Free radical scavenging capacity was analysed in terms of superoxide radial scavenging assay and reducing power assay. **Result:** Phytochemical characterization of the different extracts revealed the presence of the phytochemicals-alkaloids, phenols, flavonoids, sterol, saponin glycosides, reducing sugars, proteins, cardio active aglycones and cardinolides. **Discussion:** Excellent superoxide radical scavenging ability found in almost all extracts of *S. torvum.* In the present study superoxide radical reduces nitroblue tetrazolium (NBT) to a blue colored formazan that is measured at 560 nm. Antioxidant activity has been reported to be concomitant with development of reducing power. This shows that extracts might contain reductones like ascorbic acid, reducing sugar, thiol group containing protein which could react with free radicals to stabilize and terminate radical chain reaction. **Conclusion:** These findings suggest that the promising phytonutrients of the plant could be exploited against oxidative stress, cancer, ageing, Ischemic heart disease in dissolving thrombus, microbial infections and hormone replacement therapy (HRT) justifying their use in traditional medicine as nutraceuticals.

Keywords: *Solanum torvum,* saponins, phytosterols, poly phenols, antimutagenic, cardinolides.

INTRODUCTION

The use of medicinal plants as source of the remedies for the treatment of many diseases dates back to prehistory and people of all continents have this old tradition.[1] In developing countries where medicines are quite expensive, it is obvious that these medicinal plants will find their way in the arsenal of antimicrobial drugs.[2] Phytochemical evaluation plays an important role in the standardization of crude herbal drugs.[3] Limited data from animal studies suggests that very high intake of phytosterols, particularly sitosterol, may inhibit the growth of breast[4] and prostate cancer.[5,6] Indole alkaloids exhibit numerous biological activities such as anti-tumor, anti-microbial, anti-hypertensive and central nervous system stimulant.[7] A large body of scientific evidence associating dietary phytochemicals with health and well-being of population has stimulated tremendous activities to develop and commercialize products variously known as nutraceuticals, phytoceuticals, dietary supplements, functional foods, etc.[8] During 1980s and 1990s, numerous laboratories began studying phytochemicals to "mine" plants for bioactive substances that might be used as medicines (nutraceuticals) or for other chemical applications. Many compounds are showing great promise as disease fighters in the body,

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boosting production or activities of enzymes, which then act by blocking carcinogens, suppressing malignant cells, or interfering with the processes that can cause heart disease and stroke.[9]

In the last few decades, several epidemiological studies have shown that a dietary intake of foods rich in natural antioxidants correlates with reduced risk of coronary heart disease particularly; a negative association between consumption of polyphenol-rich foods and cardiovascular diseases has been demonstrated. This association has been partially explained on the basis of the fact that that polyphenols interrupt lipid peroxidation induced by reactive oxygen species (ROS).[10, 11]

Different *stimuli*, leading to an increase of ROS generation inside the cell, activate the phosphorylation of IkB inhibitory protein and the subsequent proteolysis. Thioredoxin may reduce activated NF-kB proteins facilitating nuclear translocation. Once released from IkB, the NF-kB complex translocates into the nucleus and the binding to DNA domain in the promoters and enhancers of genes such as TNF-a, IL-1, proliferation and chemotactic factors, adhesion molecule. Some of these genes, in turn, may further induce NF-kB activation, leading to a vicious circle if the regulatory cellular system escapes from control. Polyphenolic compounds as natural phytochemical antioxidants that possess anti-inflammatory properties by down regulating NF-kB.[12-14]

A dramatic shift has taken place in consumer attitudes toward food. An increasingly active population, the pursuit of healthier lifestyles and the desire to live longer has given rise to a new category of food products referred to as "nutraceuticals." Incorporated in the nutraceuticals category are functional foods. Functional foods can be described as conventional foods containing ingredients that provide additional health or nutritional benefits beyond basic levels, leading to possible risk reduction of contracting chronic disease.[15]

Solanum torvum belongs to Solanaceae family (nightshade family), a spiny herb or shrub 3-4 m tall found throughout the tropical parts of India and in the Andaman. Leaves are ovate, sinate or bilobed, lobes shallow, rarely deep; flowers white, in dense lateral racemes, berries globere, smooth, yellow or orange-red, seeds smooth. Whole plant is traditionally used as a digestive, diuretic and sedative. Fruits are used for liver and spleen enlargement. Decoction is used as antitussive. Leaves are used as haemostatic. Root-paste is used in rhagades.[16] With this background, the present study was designed to screen for the presence of phytochemicals and free radical scavengers.

MATERIALS AND METHODS

Plant materials

The leaves and fruits of *Solanum torvum* were collected in Chennai, Tamil Nadu, India. The identification and nomenclature of the plant was based on The Flora of Presidency of Madras[17] and The Flora of Tamil Nadu Carnatic.[18] They were later verified at Botanical Survey of India, Southern Circle, Coimbatore, India. All the preserved specimens were deposited at the Herbarium of Entomology Research Institute, Loyola College, Chennai.

Chemicals

All the solvents and chemicals were purchased from Sigma Laboratories, Bangalore, India.

Preparation of extracts for phytochemical analysis

The plant material (leaves and fruits) were air dried in the laboratory at room temperature. It was then powdered and was extracted with hot water by boiling for 30 minutes to get the aqueous extract. The extract obtained was concentrated and dried under controlled temperature (60^{o}C). The dried powder was successively extracted with other solvents and kept in an orbital shaker for overnight. Fresh fruit was homogenized with solvent and then extracted. The obtained extract was filtered with Whatman No. 42 filter paper (125 mm) and the filtrate was collected and used for experimental analysis.

Phytochemical analysis

These studies were performed according to the standard methods[19-21] for alkaloids, glycosides-cardio active aglycones, saponin glycosides, flavonoids, phenols, steroids, proteins and reducing sugars.

Tests for free radical scavenging assay

The antioxidant activity was evaluated using ethanol, chloroform and aqueous extracts for non enzymatic antioxidants, vitamin-C, vitamin-A, vitamin-E, phenol, protein with -SH group, reducing carbohydrate and sterol. Reducing power assay and super oxide radical scavenging assay was also performed with the above extracts.

Estimation of vitamin-A was done by the method of **Carr & Price.**[22] Estimation of vitamin-C was done by the method of **Omaye**.[23] Estimation of vitamin-E was done by **Rosenberg** method.[24] The reducing power of the extract was determined according to the method of

Oyaizu.[25] The superoxide radical scavenging ability was assessed by the method of **Nishimik.**[26] Total phenol was determined using *Lowry's* method.[27] Sterol was estimated by *Liebermann Burchard* method.[28]

Statistical analysis

Experimental results are expressed as means ± SD. All measurements were replicated ten times. The data was analyzed by an analysis of variance i.e. one way ANOVA and student 't' test using Graph Pad Quick Calcs.

RESULTS AND DISCUSSION

The results of the phytochemical analysis were reported in Table 1.

Table 1 shows the qualitative analysis results of the phytochemicals of aqueous and organic extracts of *Solanum torvum* which includes the presence of alkaloids, indole alkaloids, lepac alkaloids, cardio active aglycons, saponin glycosides, flavonoids, phenols, sterol, proteins, and carbohydrates. Table 2 reveals that vitamin–(β.Carotene) content is highest in the leaf, high in dry fruit and less in fresh fruit. Chloroform extract has very high vitamin-A than aqueous and ethanolic extract. Vitamin–A as a powerful, free radical scavenger (singlet oxygen) and chain breaking antioxidant. The function of vitamin-A as a radical scavenging antioxidant can protect the cells from oxidative damage. Several clinical trials showed regression in precancerous lesions of the cervix and the lung as well as the oral cavity with the administration of β-carotene.[29,30] In this study it was found that vitamin-C (ascorbic acid) content is in the following order.

Leaf > Fresh Fruit > Dry Fruit; Aqueous Extract > Ethanolic Extract > Chloroform Extract.

Vitamin-C is an excellent hydrophilic antioxidant; it readily scavenges ROS and peroxyl radical and also acts as a co-antioxidant by regenerating the vitamin- A, E and GSH from radicals.[31, 32] Table 2 indicates the high

Table 1 Qualitative analysis of the phytochemicals of aqueous and organic extracts of *Solanum torvum*

Phytochemicals analyzed	*Solanum torvum*			
	Aqueous	Ethanol	Methanol	Chloroform
Alkaloids	+++	++	++	=/=
Indole alkaloids	+++	+++	+++	=/=
Lepac alkaloids	++	+	++	=/=
Cardio active aglycons	++	++	++	=/=
saponin glycosides	++++	+++	+++	=/=
Flavonoids	++	+	+	=/=
Phenols	++++	+++	+++	=/=
Sterol	+++	++	++	+++
Proteins	+++	=/=	=/=	=/=
Carbohydrates	+++	=/=	=/=	=/=

+ Presence of phytochemicals
++ Definite presence
+++ Definite heavy presence
++++ Definite heaviest presence
=/= Not done

Table 2 Level of different antioxidants in aqueous, ethanol and chloroform extracts of *Solanum torvum* per 100g

Parts of the plant	Leaf	Fresh fruit	Dry fruit	Leaf	Fresh fruit	Dry fruit	Leaf	Fresh fruit	Dry Fruit
Vitamin A (mg)	20.3 ± 0.1491	6.12 ± 0.1874	10.62 ± 0.1874	25.51 ± 0.2514	11.33 ± 0.2983	12.76 ± 0.2830	65.99 ± 0.7578	14.62 ± 0.1874	18.99 ± 0.7578
Vitamin C (mg)	140.9 ± 1.197	130.8 ± 1.398	100.8 ± 1.398	180.9 ± 1.197	180.8 ± 1.398	130.8 ± 1.398	130.9 ± 1.197	95.8 ± 1.398	25.8 ± 1.398
Vitamin E (mg)	20.84 ± 0.1293	15.91 ± 0.09816	10.77 ± 0.1679	23.83 ± 0.1366	13.47 ± 0.3438	6.90 ± 0.1035	26.91 ± 0.09816	18.77 ± 0.167	11.33 ± 0.4342
Polyphenol (mg)	301.3 ± 2.908	151.3 ± 2.908	777.7 ± 2.541	651.3 ± 2.908	301.3 ± 2.908	101.7 ± 2.541	76.3 ± 2.908	52.8 ± 2.781	601.7 ± 2.541
Protein (g)	4.04 ± 0.1174	3.54 ± 0.1174	3.04 ± 0.1174	0.64 ± 0.1174	0.054 ± 0.1174	0.44 ± 0.1174	3.04 ± 0.1174	2.54 ± 0.1174	2.04 ± 0.1174
Carbohydrate (g)	2.092 ± 0.0444	2.204 ± 0.0672	1.7 ± 0.1155	2.004 ± 0.3967	2.388 ± 0.0737	1.8 ± 0.1155	1.132 ± 0.0989	1.18 ± 0.1155	1.096 ± 0.07043

Values are mean ± SD of 10 replications.

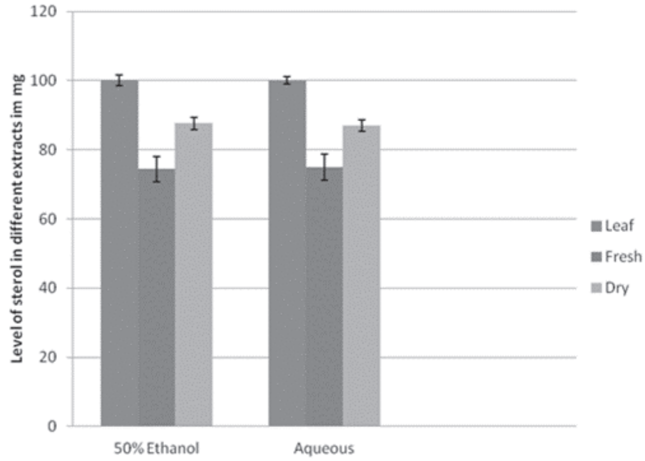

Figure 1. level of sterol in different extracts of S.torvum in mg.

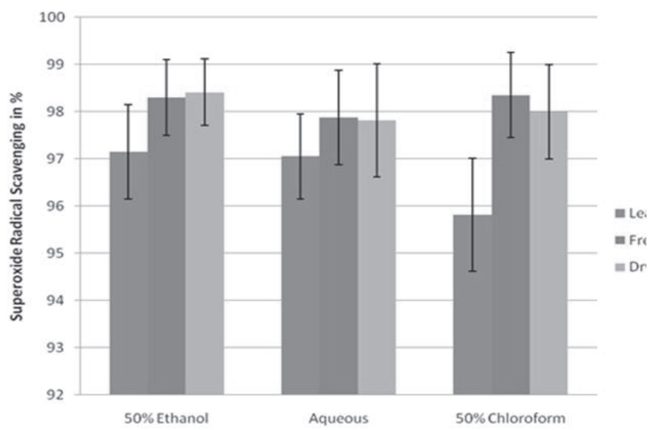

Figure 2. Superoxide Radical Scavenging of Solanum torvum in%.

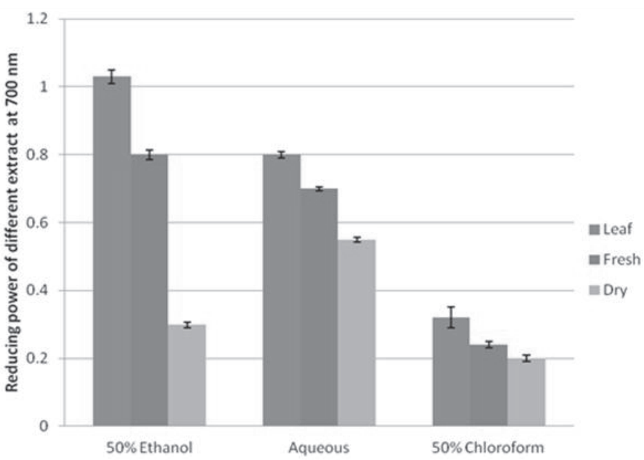

Figure 3. Reducing power of different extract of S.torvun at 700 nm.

vitamin-E content in chloroform extract. Vitamin-E is a fat soluble vitamin which can be extracted effectively by chloroform. *S. torvum* has very high vitamin-C along with vitamin-E content. Vitamin C and E are synergistic antioxidants. Regeneration of vitamin E requires ascorbic acid, an aqueous phase antioxidant.

Table 2 reveals that a high content of phenolic compounds was found in aqueous extract indicating that phenolic compounds in *S. torvum* were mainly soluble in water. Phenolic compounds are likely to contribute to the radical scavenging activity of extract. Polyphenols are a large class of compounds, occur naturally in food plants. The flavonoids are the largest and best studied group of these. Polyphenols are currently sold as dietary supplements and/or herbal remedies. They have antioxidant, antimutagenic, anti-estrogenic, anti-carcinogenic and anti-inflammatory effects that might potentially be beneficial in preventing disease. Epidemiologic findings revealed that polyphenols have cardio protective effects which include inhibition of platelet aggregation and vascular relaxation through the production of nitric oxide which decrease LDL oxidation and prevent atherosclerosis.[33, 34]

Table 2 shows excellent protein content in ethanolic extract of *S. torvum.* It may have more hydrophobic amino acids and lesser hydrophilic amino acids containing proteins. So they are extracted more in ethanol than in aqueous extract. Amino acids, peptides, such as carnosine and anserine, and proteins are common food components. Amino acids were found to be efficient antioxidants in model experiments. Their application is advantageous in mixtures with other inhibitors as they often act as synergists of phenolic antioxidants and as chelating agents. Amino acids convert hydroperoxides into imines, and sulphur containing amino acids reduce hydroperoxides into the respective inactive hydroxylic derivatives. Methionine and selenomethionine were found to be more active than a-tocopherol in olive oil.[35]

Table 2 shows that *S. torvum* has high carbohydrates in aqueous extract indicating that carbohydrates in *S. torvum* were soluble in water. These are likely to contribute to the radical scavenging activity and reducing power of the extract. Simple carbohydrates have antioxidant properties. Hydroxyl radicals generated by a Fenton reaction induce damage on simple carbohydrates with a consequent free radical scavenging activity. Carbohydrate activities were measured by different methods as spin-trapping of hydroxyl radical and electron paramagnetic resonance detection and 1, 1-diphenyl-2-picrylhydrazyl quenching. Carbohydrate damage was evaluated in a Fenton system by measuring the reactive substances to thiobarbituric acid, by their decreased detection with an HPLC test, and by a gas chromatographic determination of formic acid from sugar oxidation. Different intensities of damage and scavenging were found according to molecular structure, and some hyphotheses on the carbohydrate action against free radicals were attempted. The assayed disaccharides were shown to be more active toward and less damaged by hydroxyl radical than monosaccharides.[36]

Table 2 and Figure 3 indicate that *S. torvum* leaf, fresh fruit and dry fruit have excellent sterol content both in organic and aqueous extracts. This may be due the presence of sterol which is extractable with organic and aqueous solvents. *S. torvum* was found to have steroidal saponins and steroidal alkaloid which contributes total sterol content. *S. torvum* has β-sitosterol- an important group of antioxidant. Plant sterols inhibit the intestinal absorption of cholesterol. Functional food ingredient derived from phytosterols has been clinically proven to have significantly lower Low Density Lipoprotein (LDL) or "bad" cholesterol when consumed in different foods. So they have a hypocholesterolemic action. They also inhibit endogenous synthesis of cholesterol by inhibiting and repressing the rate limiting enzyme Hydroxy methyl glutaryl coA (HMG-CoA) reductase in cholesterol synthesis. Steroidal glycosides in plant foods have estrogenic antiestrogenic actions and are known as phytoestrogen. These have antibacterial and antifungal actions. They also produce typical estrogen responses with a biological activity 1/500 to 1/1000 of estradial. Studies proved that phytoestrogens lower the incidence of osteoporosis, breast and uterine cancer.[37]

Saponins are natural surfactants or detergents. It can be used as foaming agents for beverages. Saponins have astypic activity which can be utilized in dissolving thrombus. Recent studies have suggested that the low serum cholesterol levels of Masai tribes in East Africa who consume a diet very high in animal products, cholesterol and saturated fat with saponin richherbs. Saponins act by binding with bile acids and cholesterol. So it cleans or purges fatty compounds from the body, lowering the blood cholesterol level. Digitalis is a saponin used as heart tonic to strengthen contractions of the heart muscle. Saponins are active antifungal agents.[38]

The alkaloid present in *Solanum torvum* is an indole alkaloid. The aromatic nitrogen heterocyclic is a potent cancer fighter/anticancer agent, blocking carcinogenic substances before they reach their cellular targets and eliminating DNA damage in cell nuclei. It may also turn out to be an important chemical tool in fighting breast cancer because it inhibits estrogen induced growth of cancer cells and converts the more dangerous forms of estrogen to safer forms.[39]

Isoflavones have a role similar to hormones, and act as phytoestrogens. They benefit humans in four ways as cancer enzyme inhibitor, as antioxidants and as immune system enhancers or stimulants. Phytoestrogens compete with estrogen for binding to estrogen receptors. Their use could have beneficial effects on preventing osteoporosis and sex hormone-mediated malignancy such as breast and prostate cancer. Clinical trials identified the potential efficacy of isoflavones in the prevention of coronary heart disease, osteoporosis, breast and prostate cancer.[40, 41]

Figure 2 shows the excellent superoxide radical scavenging ability found in almost all extracts of *S. torvum*. Super oxide anions are one of the potent reactive oxygen species (ROS) which are produced from molecular oxygen due to oxidative enzymes[42] of body as well as via non enzymatic reaction such as autoxidation by catecholamine.[43] In the present study superoxide radical reduces NBT to a blue colored formazan that is measured at 560 nm[44] Figure 3 shows the excellent reducing power of *Solanum torvum*. In the reducing power (Fe^{3+}- Fe^{2+} transformation ability) assay, the presence of antioxidants in the extracts would result in the reducing of Fe^{3+} to Fe^{2+} by donating an electron. Amount of Fe^{2+} complex can be then be monitored by measuring the formation of Perl's Prussian blue at 700 nm [45]. Antioxidant activity has been reported to be concomitant with development of reducing power. This shows that extracts might contain reductones, like ascorbic acid, reducing sugar, thiol group containing protein which could react with free radicals to stabilize and terminate radical chain reaction.

CONCLUSION

These findings suggest that the promising antioxidant, cardio active (aglycones), astypic, antimitotic, anti microbial and antitumour properties of the plant could be exploited in herbal preparations against oxidative stress, cancer, ageing, Ischemic heart disease in dissolving thrombus, microbial infections and hormone replacement therapy (HRT) justifying their use in traditional medicine. The plant extracts have assumed an increased importance in medicine and in health care industry and further work on the above suggested aspects may be given prominence. Phytochemicals occur naturally in plant foods which create an entirely new philosophy of "functional foods," eating not just to sustain minimal basic health but also eating to prevent disease. In the future, we may tailor our diets to include the foods that will best address our personal health problems and risks as well as maintain optimal health.

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