Research Article

Antioxidant potential of *Solanum torvum* (L.) seeds extract using *in-vitro* models

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Abstract

Objective: In the present study, the ability of scavenging free radicals of the Methanol, Chloroform and Ethyl acetate of *Solanum torvum* seed . **Materials and Methods:** *S. torvum* seed was determined by using 1,1-diphenyl-2-picrylhydrazyl (DPPH), 2, 2'-Azinobis-(3-Ethylbenzothiazoline-6-Sulfonic Acid) ($ABTS^{+}$), Ferric reducing antioxidant power (FRAP), Nitric oxide scavenging assay (NO), Superoxide anion radical scavenging (SOD), Hydroxyl radical scavenging assay (HRSA), Hydrogen Peroxide radical assay (HPRA). **Results:** The results indicate that the Ethyl acetate seed extract of *Solanum torvum* has good antioxidant potential and it can be regarded as promising candidates for natural plant sources of antioxidants. **Conclusion:** *S. torvum* seed can be further studied to isolate the phyto compounds responsible for the antioxidant activity.

Keywords: *Solanum torvum L,* DPPH, Nitric oxide radical scavenging, Antioxidant, Oxidative stress

Introduction

Antioxidant research is an important topic in the medical field as well as in the food industry. Recent research with important bioactive compounds in many plant and food materials has received much attention. The oxidation induced by ROS can result in cell membrane disintegration, membrane protein damage and DNA mutation, which can further initiate or propagate the development of many diseases, such as cancer, liver injury and cardiovascular disease (Liao and Yin, 2000). Generally free radicals attack the nearest stable molecules, `stealing' its electrons. When the molecule that has been attacked and loses its electron it becomes a free radical itself, beginning a chain reaction. Once the process is started, it can cascade, initiating lipid peroxidation which results in destabilization and disintegration of the cell membranes or oxidation of other cellular components like proteins and DNA, finally resulting in the disruption of cells (Hertog et al., 1993). The use of traditional medicine is widespread in Africa and medicinal plants are still a large source of natural antioxidants that might serve as leads for the development of novel drug against free radical induced diseases. Medicinal plants are commonly used in treating and preventing specific ailments and diseases and are considered to play a beneficial role in health care (Karandikar, 1997; Vidya, 1997).

Solanum torvum (Solanaceae), commonly known as Turkey berry is native and cultivated in Africa and West Indies (Adjanohoun et al., 1996). The fruits and leaves are widely used in *Camerooninan* folk medicine. It also occurs commonly in the moist farms of India. The fruits of *S. torvum* are edible and commonly available in the markets. They are utilized as a vegetable and regarded as an essential ingredient in the South Indian population's diet. A decoction of fruits is given for cough ailments and is considered useful in cases of liver and spleen enlargement (Siemonsma and Piluek, 1994). The plant is sedative and diuretic and the leaves are used as a haemostatic. The ripened fruits are used in the preparation of tonic and haemopoietic agents and also for the treatment for pain (Kala, 2008). It has antioxidant properties (Ndebia et al., 2007). It is intensively used worldwide in the traditional medicine as poison anti-dote and for the treatment of fever, wounds, tooth decay, reproductive problems and arterial hypertension (Ajaiyeoba, 1999). *S. torvum* also possesses Immuno-secretory (Israf et al., 2000) Antioxidant (Sivapriya and Srinivas, 2007). Analgesic and Anti-

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inflammatory, Anti-ulcerogenic activities (Nguelefack et al., 2008), Cardiovascular (Mohan et al., 2009), Nephroprotective (Mohan et al., 2010), Antidiabetic (Gandhi et al., 2011), Angiotensin and Serotonin receptor blocking activities (Jaiswal and Mohan, 2012).

Material and method

Chemicals

1,1- diphenyl-2-picryl hydrazyl (DPPH), potassium ferricyanide were purchased from Sigma Chemicals Co. USA). butylated hydroxytoluene (BHT) sulfanilamide, N-(1-naphthyl) ethylenediamindihydrochloride, EDTA and ferric chloride were purchased from Merck (Germany). All other chemicals were of analytical grade or purer.

Plant material

S. torvum seed were collected in and around Chidambaram, Cuddalore District in the month of January-February. The herbarium of the plant was identified and authenticated by the botanist Dr.V.Venkatesalu and the voucher specimen was deposited to the Department of Botany, Annamalai University, Tamil Nadu, India.

Preparation of plant extract

S. torvum Seed was shade dried at room temperature (32 ± 2 ºC) and the dried seed was ground into fine powder using a pulverizer. The powder was sieved and kept in deep freezer until use. 100 g of dry fine seed- powder was taken and mixed with 300 ml of three different organic solvents (methanol, chloroform and ethyl acetate) and magnetically stirred in a container over night at room temperature. The extract was filtered using a muslin cloth and concentrated at 40 ± 5 ºC.

DPPH radical scavenging activity

Various concentrations of *S. torvum* of the sample (4.0 mL) were mixed with 1.0 mL of methanol solution containing DPPH radicals, resulting in the final concentration of DPPH being 0.2 mM (Blios, 1958). The mixture were shaken vigorously and left to stand for 30 minutes, and the absorbance was measured at 517 nm. BHT was used as control. The percentage of DPPH decolorization of the sample was calculated according to the equation:

$$\% \text{ decolorization} = [1-(ABS_{sample}/ABS_{control})] \times 100$$

IC_{50} value (mg extract / mL) was the inhibitory concentration at which DPPH radicals were scavenged by 50%. BHT was used for comparison.

$ABTS^+$ scavenging activity

Samples were diluted to produce 5-50 µg/mL. The reaction was initiated by the addition of 1.0 mL of diluted $ABTS^+$ to 10 mL of different concentrations of *S. torvum* of the sample or 10 mL methanol as control (Re et al., 1999). The absorbance was read at 734 nm and the percentage inhibition was calculated. The inhibition was calculated according to the equation:

$$I = A_1/A_0 \times 100$$

Where A_0 is the absorbance of control reaction and A_1 was the absorbance of test compound.

Ferric-reducing antioxidant power assay (FRAP)

A stock solution of 10 mM 2, 4, 6-tripyridyl-s-triazine (TPTZ) in 40mM HCL, 20Mm $FeCl_3.6H_2O$ and 0.3M acetate buffer (pH 3.6) was prepared (Pulido et al., 2000). The FRAP reagent contained 2.5 mL TPTZ solution, 2.5 mL ferric chloride solution, and 25 mL acetate buffer. It was freshly prepared and warmed to 37ºC. FRAP reagent (900 mL) was mixed with 90 mL water and 30 mL *S. torvum* of the sample and standard antioxidant solution. The reaction mixture was then incubated at 37ºC for 30 minutes and the absorbance was recorded at 595 nm. An intense blue color complex was formed when ferric tripyridyltriazine (Fe^{3+} - TPTZ) complex was reduced to ferrous (Fe^{2+}) form. The absorption at 540 nm was recorded.

Nitric oxide radical activity

Nitric oxide radical generated from sodium nitroprusside was measured (Sreejayan and Rao, 1997). Briefly, the reaction mixture (5.0 mL) containing sodium nitroprusside (5mM) in phosphate-buffered saline (pH 7.3), with *S. torvum* sample at different concentration was incubated at 25ºC for 3 hours. The nitric oxide radical thus generated interacted with oxygen to produce the nitrite ion which was assayed at 30 minute intervals by mixing 1.0 mL of incubation mixture with an equal amount of Griess reagent. The absorbance of the chromophore (purple azo dye) formed during the diazotization of nitrite ions with sulfanilamide and subsequent coupling with naphthyl ethylene diamine dihydrochloride was measured at 546 nm.

Superoxide anion radical scavenging activity

This assay was based on the reduction of nitro blue tetrazolium (NBT) in the presence of nicotinamide adenine dinucleotide (NADH) and phenazine methosulfate (PMS) under aerobic condition (Nishikimi and Rao, 1972). The 3 mL reaction mixture contained 50 mL of 1M NBT, 150 mL of 1M NADH with or without sample, and Tris buffer (0.02M, pH 8.0). The reaction was started by adding 15 mL of 1M PMS to the mixture and the absorbance change was recorded at 560 nm after 2 minutes. Percent inhibition was calculated against a control without the extract

Hydroxy radical activity

The reaction mixture 3.0 mL contained 1.0 mL of 1.5mM

$FeSO_4$, 0.7 mL of 6mM hydrogen peroxide, 0.3 mL of 20 mM sodium salicylate, and varying concentrations of *S. torvum* sample (Klein et al., 1991). After incubation for 1 hour at 37ºC, the absence of the hydroxylated salicylate complex was measured at 562 nm. The percentage scavenging effect was calculated as:

Scavenging activity = $[1-(A_1 - A_2)/A_0] \times 100\%$

Where A_0 was the absorbance of the control (without extract), A_1 was the absorbance in the presence of the extract, and A_2 was the absorbance without sodium salicylate.

Hydrogen peroxide radical

S. torvum against H_2O_2 was measured according to the method (Nabavi et al., 2009a). A solution of 40 Mm H_2O_2 was prepared in phosphate buffer (p^H-7.4). Next, 1.4 mL of different concentrations (5-50 µg/mL) of the *S. torvum* was added to 0.6 mL of the H_2O_2 solution. The assay mixture was allowed to stand for 10 minutes at 25ºC and the absorbance measured against a blank solution at ƛ max =230 nm. The *S. torvum* on H_2O_2 scavenging capacity index was calculated as follows:

$$\text{Scavenging capacity index} = \frac{A_{blank} - A_{test}}{A_{blank}}$$

S. torvum was expressed as IC_{50}, which is defined as the concentration (mg/mL) of the *S. torvum* required to scavenge 50 % of H_2O_2. BHT was used as control.

Statistical analysis

The data were subjected to a one-way analysis of variance (ANOVA) and the significance of the difference between means was determined by Duncan's multiple range test ($P < 0.05$) using statistica (Statsoft Inc., Tulsa, USA). Values expressed are means of three replicate determinations ±standard deviation.

Results and discussion

The antioxidant compounds leads to fadedness of deep purple colour by quenching DPPH free radicals (i.e. by providing hydrogen atoms or by electron donation, conceivably via a free-radical attack on the DPPH molecule) and convert them into a colourless- /bleached product (i.e. 2, 2- diphenyl-1-hydrazine, or a substituted analogous hydrazine), which leads to decrease in absorbance and hence provides antioxidant potential (Amarowicz et al., 2003). Figure 1 shows the ethyl acetate of *S. torvum* were exhibited a maximum DPPH scavenging activity of 52.61% at 400 µg/ml whereas for BHT (standard) was found to be 56.09% at 400 µg/ml.

ABTS radical cation scavenging activity also reflects hydrogen-donating ability (Baskaran et al., 2013). Reported that the high molecular weight phenolics (tannins) have more ability to quench free radicals ($ABTS^+$). Since, the extracts from various samples have the ability to scavenge

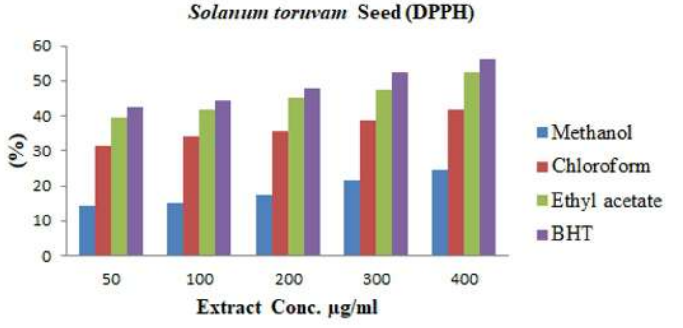

Figure 1. Effect of Methanol, Chloroform and Ethyl acetate seed extracts of *Solanum toruvam* seed on DPPH assay

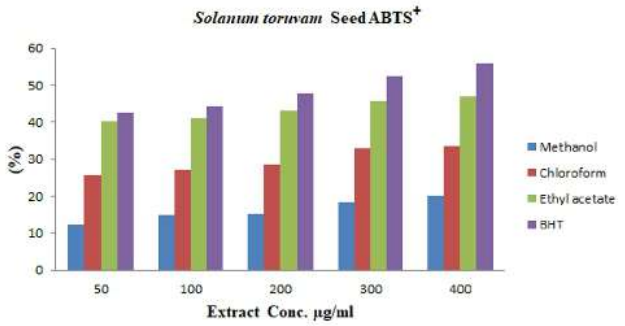

Figure 2. Effect of Methanol, Chloroform and Ethyl acetate extract of *Solanum toruvam* seed on $ABTS^+$ assay

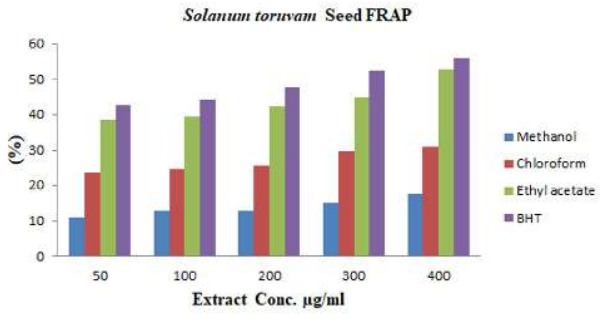

Figure 3. Effect of Methanol, Chloroform and Ethyl acetate seed extract of *Solanum toruvam* Seed on FRAP assay

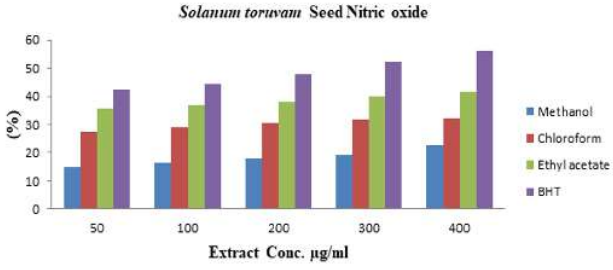

Figure 4. Effect of Methanol, Chloroform and Ethyl acetate seed extracts of *Solanum toruvam* Seed on Nitric oxide assay

free radicals, thereby preventing lipid oxidation via a chain breaking reaction; they could serve as potential nutraceuticals when ingested along with nutrient. Figure 2 shows the Ethyl acetate of *S. torvum* were exhibited a maximum $ABTS^{+}$ scavenging activity of 52.61% at 400 µg/ml whereas for BHT (standard) was found to be 56.09% at 400 µg/ml.

Antioxidants can be explained as reductants, and inactivators of oxidants (Valero and Carmona, 1998). Some previous studies have also reported that the reducing power may serve as a significant indicator of potential antioxidant activity. Antioxidative activity has been proposed to be related to reducing power. FRAP assay was used by several authors for the assessment of antioxidant activity of various food product samples (Siddhuraju and Becker, 2007; Halvorsen et al., 2006; Pellegrini et al., 2003) suggested most of the secondary metabolites are redox-active compounds that will be picked up by the FRAP assay. Figure 3 shows the Ethyl acetate of *S. torvum* were exhibited a maximum FRAP scavenging activity of 52.80% at 400 µg/ml whereas for BHT (standard) was found to be 56.09% at 400 µg/ml.

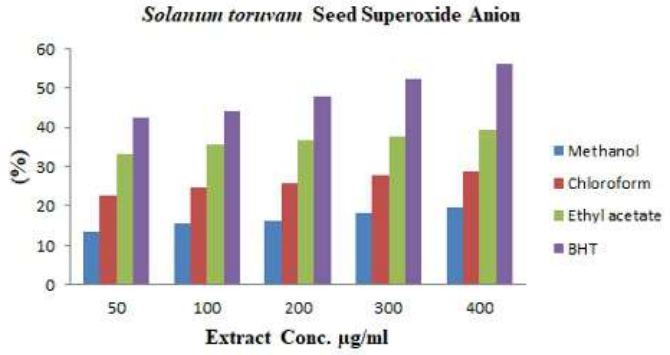

Figure 5. Effect of Methanol, Chloroform and Ethyl acetate seed extract of *Solanum toruvam* Seed on Superoxide Anion assay

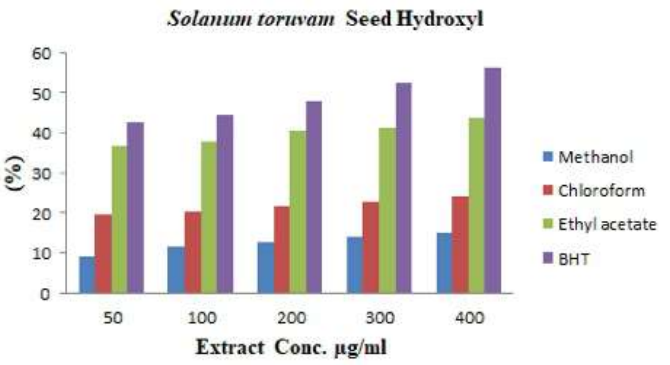

Figure 6. Effect of Methanol, Chloroform and Ethyl acetate seed extracts of *Solanum toruvam seed* on Hydroxyl radical assay

Nitric oxide radical inhibition assay proved that methanolic seed extract *S. torvum* is a potent scavenger of nitric oxide. In this assay sodium nitroprusside generates nitric oxide which form nitrite when reacts with oxygen. The methanol root extract of *Mentha arvensis* L. inhibits nitrite formation by competing with oxygen to react with nitric oxide directly and also to inhibit its synthesis. Scavengers of nitric oxide compete with oxygen leading to reduced production of nitric oxide (Hepsibha et al., 2010). Figure 4 shows the Ethyl acetate of *St* were exhibited a maximum Nitric oxide scavenging activity of 41.74% at 400 µg/ml whereas for BHT (standard) was found to be 56.09% at 400 µg/ml.

The methanolic extract was found to be an effective scavenger of superoxide radical generated by photo reduction of riboflavin. Superoxide anion radical is one of the strongest ROS among the free radicals and get converted to other harmful reactive oxygen species such as hydrogen peroxide and hydroxyl radical, damaging biomolecules which results in chronic diseases (Duan et al., 2007). Figure 5 shows the Ethyl acetate of *S. torvum* were exhibited a maximum Superoxide Anion scavenging activity of 39.49% at 400 µg/ml whereas for BHT (standard) was found to be 56.09% at 400 µg/ml.

Hydroxyl radical can be formed by the Fenton reaction in the presence of reduced transition metals (such as Fe^{2+}) and H_2O_2, which is known to be the most reactive of all the reduced forms of dioxygen and is thought to initiate cell damage in vivo (Battu et al., 2011). Scavenging of hydroxyl radical is an important antioxidant activity because of very high reactivity of the OH radical, enabling it to react with a wide range of molecules found in living cells, such as sugars, amino acids, lipids, and nucleotides (Miller and Rice-Evans, 1997). Figure 6 shows the Ethyl acetate of *S. torvum* were exhibited a maximum hydroxyl radical scavenging activity of 43.73% at 400 µg/ml whereas for BHT (standard) was found to be 56.09% at 400 µg/ml.

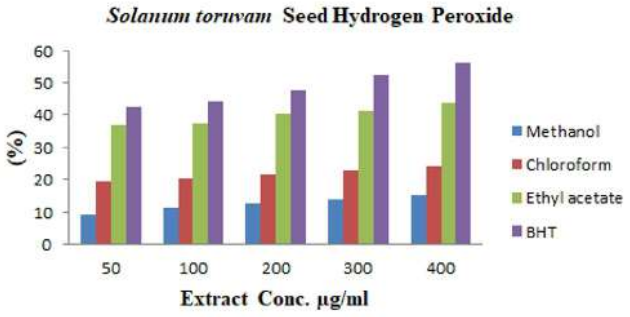

Figure 7. Effect of Methanol, Chloroform and Ethyl acetate seed extracts of *Solanum toruvam* Seed on Hydrogen Peroxide radical assay

The methanol extract was capable of scavenging H_2O_2 in a concentration dependant manner. Hydrogen peroxide is a weak oxidizing agent that inhibits the oxidation of essential thiol (-SH) groups directly by few enzymes. Many of its toxic effects are because H_2O_2 has the ability to rapidly cross the cell membrane and once inside the cell, it can probably react with Fe^{2+} and possibly Cu^{2+} ions to form hydroxyl radicals (Miller and Rice-Evans, 1997). Figure 7 shows the Ethyl acetate of *S. torvum* were exhibited a maximum hydrogen peroxide radical scavenging activity of 40.41% at 400 µg/ml whereas for BHT (standard) was found to be 56.09% at 400 µg/ml.

Conclusions

It is well known that free radicals are one of the causes of several diseases. The result of the present study reveals a strong antioxidant activity of the leaf extract of *S. torvum*. The constituents that are responsible for the antioxidant activity are unclear; hence further studies are required to evaluate the antioxidant activity of the purified fractions.

Conflicts of interest: Not declared.

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