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ANTIOXIDANT CAPACITY OF THE METHANOLIC EXTRACT OF SOLANUM TORVUM LEAVES

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ABSTRACT

Solanum torvum is a pharmacologically important plant which is an important source of many medicinally important chemicals. It is widely studied for their various pharmacological activities like antihypertensive, antioxidant, cardiovascular, antiplatelet aggregation, antimicrobial, antiviral activity etc. The antioxidant activity of the leaves of Solanum torvum was evaluated. The DPPH, FRAP and total Phenolic content of the leaves were 99.71±0.79 mg TE, 42.47±6.73 mg TE and 5.34±0.63 mg GAE/ gm dry leaves respectively. As the antioxidant content of the plant is high, this plant will have many positive effects on the human health such as reducing the hypertension, cardio protective effect, improvement in renal dysfunction etc. by scavenging free radicals and reactive oxygen species which are responsible for number of human disorders.

KEYWORDS: Solanum torvum, antioxidant activity, DPPH, Total phenolic.

INTRODUCTION

Antioxidants can protect the human body from free radicals and retard the progress of many chronic diseases as well as retarding the lipid oxidative rancidity in foods.[1] Recent studies have investigated the potential of plant products as antioxidants against various diseases induced by free radicals. There is an increasing interest in natural antioxidants e.g. polyphenols present in medicinal and dietary plants which might help preventing oxidative

damages.[2] Solanum torvum is a small shrub commonly known as 'Turkey berry'. It is widely distributed in India, Pakistan, Malayan, China, Philippines, Thailand and tropical America.[3,4] Its edible fruits are used as vegetable and are regarded as an essential in Thai cuisine. Its fruit and leaves which are rich in alkaloids can be used for Medicinal or ritual purposes.[4] For many decades, different ethnic groups have used the dried stem and root of this plant for treatment of various ailments. Its Chinese medicinal name is Jinniukou.[5] The fruits are used commonly in traditional medicine as antihypertensive.[6] Phytochemical studies indicated that fruits of this species have as good concentrations of various alkaloids, flavonoids, saponins, tannins and glycosides as sufficient to have pharmacological effects. Therefore, fruit are not only used for nutritive purposes but also fruit decoctions are effective for cough ailments and are considered to be effective medicine in cases of liver and spleen enlargement. The ripened fruits are used in the preparation of tonic and haemopoietic agents and also for treatment of pain.[7,8] It has antioxidant,[9] cardiovascular, anti-platelet aggregation activities,[10] anti-microbial activity against human and clinical isolates[11,12] and sedative, digestive, hemostatics and diuretic activities. [13] This species also exhibited some percentage of antioxidant activity and DNA-repair capability on oxidative DNA damage caused by free radicals.[14] In the present study, the antioxidant activity of the methanolic extract of leaves of Solanum torvum was estimated by three in vitro assay methods namely (1) DPPH free radical scavenging activity, (2) Ferric reducing antioxidant potential (FRAP) assay and (3) Total Phenolic content, spectrophotometerically.

MATERIALS AND METHODS

Plant Material

The plant Solanum torvum was collected from the campus of College of Veterinary Sciences & A.H., Central Agricultural University, Selesih, Aizawl, Mizoram and submitted the herbarium specimens for authentication / identification to the Regional Office, Botanical Survey of India (BSI), Shillong. The BSI, Shillong has authenticated the plants and communicated the identification/ authentication report vides letter reference No.BSI/ERC/Tech/2010/052, dated 27.04.2010.

The fresh leaves of the plant were collected, washed and air dried in shade. On complete drying, the dried plant material was ground to powder with Willey / Laboratory Mill and sifted through sieve number 22. The powdered leaves were then subjected to cold maceration using methanol as solvent following the procedure of Manjunatha et al.[15] and Harborne[16]

with slight modifications. Briefly, five hundred (500g) grams of powder was soaked in 2.5 L of methanol (1:5 w/v) in a conical flask for a period of 3 days with intermittent stirring and at the end of 3rd day the content was filtered with muslin cloth followed by Whatmann filter paper No. 1. For complete extraction of the active principles, this process was repeated three times using fresh solvent on each occasion or until the colour of the methanol becomes light. The filtrate obtained was pooled and further subjected to rotary vacuum evaporator. The material was stored at -40ºC in deep freezer in air tight containers untill further use.

Chemicals and reagents

1,1-Diphenyl-2-picrylhydrazyl (DPPH), 2,4,6-tri(2-pyridyl)-s-triazine (TPTZ), 6-hydroxy-2,5,7,8–tetramethyl chromane-2-carboxylic acid (Trolox), Gallic acid were purchased from Sigma Chemicals Co. (St. Louis, USA); Methanol, Ethanol, Sodium acetate trihydrate, ferric chloride hexahydrate (FeCl3. 6H2O), Folin-Ciocalteu Phenolic reagent, Sodium carbonate were obtained from Merck (Darmstall, Germany). Trichloroacetic acid (TCA) was obtained from Sisco Research Laboratories (SRL), Mumbai. All the chemicals used were of analytical grade.

DPPH free radical scavenging assay

The free radical scavenging activity was measured by the 1,1-Diphenyl-2-picrylhydrazyl (DPPH) method proposed by Leong and Shui with slight modifications.[17] DPPH solution of 0.1 mM was prepared in methanol and the initial absorbance was measured at 517 nm in a UV-Visible Spectrophotometer (Thermo- Evolution 201). An aliquot (20 µl) of extract was added to 3 ml of DPPH solution and the decrease in absorbance was measured at different time intervals at 517 nm until the absorbance remained constant. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity, and vice versa. A standard curve was prepared using Trolox (250 -1250µg/ml) and the free radical scavenging ability of the extracts were calculated from the decreased in the absorbance. The free radical scavenging ability of the extracts were expressed as mg Trolox equivalent (TE) per gram of dry leaves.

Ferric Reducing Antioxidant Potential (FRAP) assay

The ferric reducing antioxidant potential (FRAP) assay was carried out according to the procedure described by Benzie and Strain with slight modifications.[18] Briefly, 30µl of extract was added to 3 ml of FRAP reagents (10 parts of 300 mM sodium acetate buffer of pH 3.6, 1 part of TPTZ and 1 part of 20 mM Ferric chloride solution). The reaction mixture

was incubated at 37ºC for 30 min and the increase in absorbance was measured at 593 nm using a UV-Visible Spectrophotometer (Thermo-Evolution 201). The standard curve was prepared using TROLOX (250 -1000µg/ml) and the value of FRAP was calculated from the standard curve. The results were expressed as mg Trolox equivalent (TE) per gram of dry leaves.

Total phenolic content (TPC)

The total phenolic content of the extracts were estimated by the Folin-Ciocalteau method described by Singleton and Rossi with slight modifications.[19] Briefly, thirty (30) microlitres of the plant extract was added to 1ml of 1:10 Folin-Ciocalteau's reagent and incubated at room temperature for 5 min followed by addition of 970 µl of sodium carbonate (7.5%) solution. After 1 hr incubation at room temperature, the absorbance was measured at 640 nm using a UV/Visible Spectrophotometer (Thermo-Evolution 201). Different volume (20-100µl) of Gallic acid (100µg/ml) was used for calibration of a standard curve. The results were expressed as mg Gallic acid equivalent (GAE) /gm of dry leaves.

RESULT AND DISCUSSION

The antioxidant content of the leaves of Solanum torvum were evaluated by three in vitro assay methods viz. DPPH free radical scavenging, Ferric reducing antioxidant potential assay and total Phenolic content estimation. The DPPH scavenging activity and Ferric reducing antioxidant potential were expressed as mg trolox equivalent (TE) while the total Phenolic content was expressed as gallic acid equivalent (GAE). The antioxidant content observed is given in Table1.

Table 1: Antioxidant content of Solnum torvum

Sl. No.	Methods of estimation	Antioxidant content/ g of dry leaves
01	DPPH free radical scavenging method	99.71±0.79 mg TE
02	FRAP assay	42.47±6.73 mg TE
03	Total phenolic content	5.34±0.63 mg GAE

The substances which are able to donate hydrogen or an electron to DPPH, nitrogen centered free-radical leading to formation of non radical DPPH-H, can be considered as antioxidants and therefore radical scavengers. The degree of discoloration of violet colour of alcoholic DPPH, as it gets reduced, indicates the radical scavenging potential of the antioxidant.[20] The methanolic extract of Solanum torvum was found to reduce the colour of DPPH as soon as it

was added an indicative of a very high ability of the extracts to scavenge the DPPH free radicals. The DPPH free radical scavenging activity of Solanum torvum in the present investigation was 99.71±0.79 mg/ gm of dry leaves. The ability of the extracts to reduce ferric-TPTZ (Fe(III)-TPTZ) to blue ferrous-TPTZ (Fe(II)-TPTZ) was also evaluated. Fe(III) reduction is often used as an indicator of electron-donating activity, which is an important mechanism of phenolic antioxidant reaction.[21] A higher absorbance indicates a higher ferric reducing power. Like the findings of DPPH free radical scavenging assay, the extracts of the leaves exhibited high absorbance. The Ferric Reducing Antioxidant Potential and total Phenolic contents were 99.71±0.79 mg TE and 5.34±0.63 mg GAE / gm dry leaves in present investigation. Many literatures have cited that the phenolics are one of the antioxidants commonly found in most plants. Generally, extracts that contain a high amount of polyphenols also exhibit high antioxidant activity.[22] However, in the present study it was found that total phenols expressed as mg GAE/gm of dry leaves was low in comparison to that of DPPH and FRAP assay. This might be due to the presence of other compounds showing activity indicating that the antioxidant activities of plant extracts are not limited to only phenolics. The activity may also come from the other antioxidant secondary metabolites, such as volatile oils, carotenoids and vitamins.[23] Similar to present finding, many workers have reported high antioxidant activity of Solanum torvum. Winthana and associates[24] reported that Solanum torvum posses significant antioxidant activity in vitro and for this property they are used for reducing oxidative stress in diabetes. Loganayaki and colleagues[25] reported that Phenolic compounds extracted from different parts of S. torvum exhibited anti-oxidant activity. Re and associates[26] reported that chloroform, acetone, and methanol extracts of leaves and fruit were explored for their in-vitro antioxidant activity using ferric reducing antioxidant power, 2,2- diphenyl-1-picryl-hydrazyl (DPPH), ABTS•+, iron chelation, and anti-hemolytic activity. Significantly higher concentrations of phenol were recorded for chloroform extracts. Of note was the fact that the in-vitro antioxidant activity was shown to be highly dependent on total phenolic content ($p<0.01$). The DPPH and 2, 2' azinobis (3- ethyl benzothiozoline -6- sulfonic acid) diammonium salt (ABTS) cation radical scavenging activities were well proved with the ferric reducing antioxidant capacity of the extracts. However, peroxide clearing studies indicated that aqueous extracts of S. torvum fruit had anti-oxidant activities.[26]

The relationship between hypertension, oxidative stress and antioxidant is complex and inadequately understood. Oxidative stress may play a role in the pathophysiology of

hypertension. Human and animal studies have demonstrated that hypertension is accompanied by increase in oxidative stress. Oxidative stress raises blood pressure by promoting functional nitric oxide deficiency (through NO inactivation and tetrahydrobiopterin depletion) and by augmenting arachidonic acid oxidation and formation of vasoconstrictive prostaglandin $F_{2\alpha}$.[27] It is possible that antioxidant might improve some of the deleterious effects of oxidative stress e.g. endothelial function, lipid peroxidation, tissue injury[28] as antioxidant can restore endothelial function. The supplementation with antioxidants, including vitamin C, E or B_6, thiols such as lipoic acid and cysteine and quinine enzyme Q_{10} have been shown to lower blood pressure in animal models and humans with hypertension.[27] As the antioxidant content of Solanum torvum is high as observed in the present investigation, this plant will be a very important medicinal plant besides its importance as vegetable.

CONCLUSION

Solanum torvum is a valuable source for medicinally important compounds besides its edible fruit which is a store house of minerals, vitamins, antioxidants and other nutrients. As the antioxidant content of the plant is high, this plant will have many positive effects on the human health such as reducing the hypertension, cardio protective effect, improvement in renal dysfunction etc. by scavenging free radicals and reactive oxygen species which are responsible for number of human disorders.

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