STUDIES ON ANTIOXIDANT ACTIVITY AND PHENOL AND FLAVONOID CONTENT OF SOLANUM TRILOBATUM L

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Abstract: Antioxidant activity, Total Phenol and Flavonoid content of the leaf of *Solanum trilobatum* collected from five geographically distant regions of Tamil Nadu were examined using extracts of aqueous, ethanol, methanol, ethyl acetate and petroleum ether. Butylated Hydroxy Toluene (BHT), Gallic acid (GA) and Quercetin (Q) were taken as standard in case of antioxidant activity, phenol and flavonoid content respectively. The leaf extracts were evaluated for antioxidant activities by DPPH (1, 1 – diphenyl -2- picryl-hydrazyl) radical scavenging assay. Among five accessions with different solvents used, maximum antioxidant activity was found in ethanolic leaf extract (72.66 %) from Chengalpet followed by others. Total phenol and flavonoid contents were quantitatively estimated. Total phenolic content measured by Folin-Ciocalteau method varied from 29.45 to 53.7 mg Gallic Acid Equivalents (GAE)/g and the total flavonoid contents as measured by aluminium chloride method varied from 21.42 to 64.3 mg Quercetin Equivalents (QE)/g. The ethanolic leaf extract of *Solanum trilobatum* (Chengalpet) was found maximum in total phenol and flavonoid contents were 53.7 mg GAE /g and 64.3 mg QE /g respectively.

Keywords: Solanum trilobatum, Antioxidant, Total Phenol, Flavonoid.

Introduction: Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years. Medicinal plants continue to be an important therapeutic aid for alleviating the ailments of humankind (1) .Therapeutic benefits can be traced to specific plant compounds; many herbs contain dozens of active constituents that, together, combine to give the plant its therapeutic value (2). A growing body of evidence indicates that secondary plant metabolites play important roles in human health and may be nutritionally important (3). Phytochemical screening of various plants has been reported by many workers (4, 5). These studies have revealed the presence of numerous chemicals including alkaloids, flavonoids, steroids, phenols, glycosides and saponins. The phenolic compounds are one of the largest and most ubiquitous groups of plant metabolites (6). A number of studies have focused on the biological activities of phenolic compounds which are antioxidants and free radical scavenger (7, 8 and 9).

Free radicals (superoxide, hydroxyl radicals and nitric oxide) and other reactive species (hydrogen peroxide, hypochloric acid and peroxynitrite) produced during aerobic metabolism in the body, can cause oxidative damage of amino acids, lipids, proteins and DNA (10, 11). It has been established that oxidative stress is among the major causative factors in the induction of many chronic and degenerative diseases including atherosclerosis, ischemic heart disease, ageing, diabetes mellitus, cancer, immunosuppression, neurodegenerative diseases and others (12, 13). The most effective way to eliminate free radicals which cause the oxidative stress is with the help of antioxidants. Antioxidants, both exogenous and endogenous, whether synthetic or natural, can be effective in preventing free radical formation by scavenging them or promoting their decomposition and suppressing such disorders (14, 15 and 16).

In addition, phenolic compounds and flavonoids are also widely distributed in plants which have been reported to exert multiple biological effects, including antioxidant, free radical scavenging abilities, anti-inflammatory, anti-carcinogenic etc. (17). The crude extracts of herbs, spices and other plant materials, rich in phenolics and flavonoids are of increasing interest in the food industry because they retard oxidative degradation of lipids and thereby improve the quality and nutritional value of food (18).

Solanum trilobatum L. is an important medicinal plant belonging to the family

Solanaceae, widely used in cough, chronic bronchitis and the leaves are cooked as Vegetable (19). This herbal plant is used as medicine for asthma, vomiting of blood and bilious matter, phlegmatic rheumatism, several kinds of leprosy. It is also antibacterial, antifungal, antimitotic and antitumourous (20, 21).The decoction of entire *Solanum trilobatum* plant is used to treat acute and chronic bronchitis. It has been widely used to treat respiratory disorders (22). The constituents of this plant include sobatum, -solamarine, solanine, solasodine, glycoalkaloid, diosogenin and tomatidine (23).

Materials and Methods:

Collection of Solanum trilobatum *:* The healthy plants of *Solanum trilobatum* were collected from five different regions of Tamil Nadu namely Vellingiri Hills, Arani, Padavedu, Chengalpet and Gummidipoondi. The collected plants were brought to the laboratory and maintained at Poonga Biotech

Research Centre, Plant biotechnology division, Chennai - 600 113, Tamil Nadu, India.

Preparation of the plant extract: Preparation of the extracts was done according to a combination of the methods used by (24) and (25). About 15g of dried leaf fine powder of *Solanum trilobatum* plant materials were extracted with 150 mL methanol (80%), ethanol (75%), ethyl acetate, petroleum ether and aqueous extract for 1 min using an Ultra Turax mixer (13, 000 rpm) and soaked overnight at room temperature. The sample was then filtered through Whatman No. 1 paper in a Buchner funnel. The filtered solution was evaporated under vacuum in a rota-evator at 40° C to a constant weight and then dissolved in respective solvents. The concentrated extracts were stored in airtight container in refrigerator below 10º C.

Qualitative analysis of Antioxidant activity of Solanum trilobatum: The antioxidant activity of leaf extracts of *Solanum trilobatum* was determined by following the method as described by (26).

50µL of leaf extracts of *Solanum trilobatum* were taken in the microtiter plate. 100µL of 0.1% methanolic DPPH was added over the samples and incubated for 30 minutes in dark condition. The samples were then observed for discoloration; from purple to yellow and pale pink were considered as strong and weak positive respectively. The antioxidant positive samples were subjected for further quantitative analysis.

Quantitative analysis of Free radical scavenging activity of Solanum trilobatum: The antioxidant activities were determined using DPPH, (Sigma-Aldrich) as a free radical. Leaf extract of 100µl were mixed with 2.7ml of methanol and then 200µl of 0.1 % methanolic DPPH was added. The suspension was incubated for 30 minutes in dark condition. Initially, absorption of blank sample containing the same amount of methanol and DPPH solution was prepared and measured as a control (27). Subsequently, at every 5 min interval, the absorption maxima of the solution were measured using a UV double beam spectra scan (Chemito, India) at 517nm. The antioxidant activity of the sample was compared with known synthetic standard of (0.16%) of Butylated Hydroxy Toluene (BHT). The experiment was carried out in triplicate. Free radical scavenging activity was calculated by the following formula:

% DPPH radical-scavenging = [(Absorbance of control - Absorbance of test Sample)/(Absorbance Of control)] x 100

Estimation of Total phenol content in Solanum trilobatum*:*Total phenolic content in the ethanolic leaf extracts was determined by the Folin Ciocalteau colorimetric method (28). For the analysis, 0.5 ml of dry powdered ethanolic leaf extracts were added to 0.1 ml of Folin-Ciocalteau reagent (0.5N) and the contents of the flask were mixed thoroughly. Later 2.5 ml of Sodium carbonate (Na_2CO_3) was added and the mixture was allowed to stand for 30 min after mixing. The absorbance was measured at 760 nm in a UV-Visible Spectrophotometer. The total phenolic contents were expressed as mg gallic acid equivalents (GAE)/g extract.

Estimation of Total Flavonoid Content in Solanum trilobatum: Total flavonoids content in the ethanolic leaf extracts was determined by the aluminium chloride colorimetric method (29). 0.5 ml of leaf extracts of *Solanum trilobatum* at a concentration of 1mg/ml were taken and the volume was made up to 3ml with methanol. Then 0.1ml $AlCl_3$ (10%), 0.1ml of potassium acetate and 2.8 ml distilled water were added sequentially. The test solution was vigorously shaken. Absorbance was recorded at 415 nm after 30 minutes of incubation.

A standard calibration plot was generated at 415nm using known concentrations of quercetin. The concentrations of flavonoid in the test samples were calculated from the calibration plot and expressed as mg quercetin equivalent /g of sample.

Results and Discussion: Scavenging activity for free radicals of DPPH (1,1-Diphenyl-2-picryl hydrazyl) has widely used to evaluate the antioxidant activity of natural products from plant and natural sources. Free radicals have aroused significant interest among scientists in the past decade. Their broad range of effects in biological systems has drawn the attention of many experimental works. It has been proved that these mechanisms may be important in the pathogenesis of certain diseases and ageing. Many synthetic antioxidant components have shown toxic and/or mutagenic effects, which have shifted the attention towards the naturally occurring antioxidants (30, 31 and 32). Wild accessions of *Solanum trilobatum* leaf samples were used for antioxidant studies. Analysis on different extraction of methanol (80%), ethanol (75%), petroleum ether, ethyl acetate and aqueous extract showed the presence of antioxidants. 50µl of leaf extracts (methanol, ethanol, petroleum ether, ethyl acetate and aqueous extracts of *Solanum trilobatum* were estimated for free radical scavenging activity using Diphenyl-2-picryl hydrazyl (DPPH) assay. The samples observed for its bleaching from purple to yellow and pale pink were considered as strong positive and weak positive respectively (Table 1; Figure 1). Among the five wild accessions and five different solvent extracts of Solanum trilobatum, the ethanolic leaf extract collected from Chengalpet recorded the most effective DPPH radical scavenging activity (72.66%) followed by, Vellingiri Hills (66.4%), Arani (60.6 %), Padavedu (54.8 %) and Gummidipoondi (49.3%) accessions (Figure 2).

Chengalpet accession values being close to synthetic antioxidant (BHT) as positive control (74.18%). In each case, ethanolic leaf extracts recorded higher percentage of free radical scavenging activity than methanolic extractions followed by aqueous, petroleum ether and ethyl acetate.

Phenolics are the most widespread secondary metabolite in plant kingdom. These diverse groups of compounds have received much attention as potential natural antioxidant in terms of their ability to act as radical scavengers. Phenolic compounds are a class of antioxidant agents which act as free radical terminators (33). In our study, total phenol content (TPC) of *Solanum trilobatum* leaf extract was estimated by using Folin-Ciocalteau colorimetric method and represented in terms of gallic acid equivalent (GAE). The result of the present study showed that the phenol contents of the ethanolic leaf extracts in terms of Gallic acid equivalent were between 29.14 mg GAE/ g to 53.7 mg GAE/g. Total phenol content of *Solanum trilobatum* ethanolic leaf extract was found to be maximum in Chengalpet (53.7 mg GAE/g) followed by Vellingiri Hills (37.4 mg GAE/g), Arani (32.9 mg GAE/g), Padavedu (31.5 mg GAE/g) and Gummidipoondi (29.14 mg GAE /g) Table.2. It has been reported that the antioxidant activity of phenol is mainly due to their redox properties, hydrogen donors and singlet oxygen quenchers (34). Phenolic compounds are important plant antioxidants which exhibited considerable scavenging activity against radicals. Thus, antioxidant capacity of a sample can be attributed mainly to its phenolic compounds (35, 36 and 37). Phenolic compounds are effective hydrogen donors, making them good antioxidants. Similarly, Shahidi and Naczk reported that naturally occurring phenolics exhibit antioxidant activity (38).

Flavonoids are regarded as one of the most widespread groups of natural constituents found in plants. The values of flavonoid content varied from plants. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process (39, 40). The result of the present study showed that the flavonoid contents of the ethanolic leaf extracts in terms of quercetin equivalent were between 36.32 to 64.3 mg QE/g. The maximum amount of flavonoid content *Solanum trilobatum* was found to be in Chengalpet (64.3mg QE/g) followed by Vellingiri Hills (49.53 mg QE/g), Arani (37.82mg QE/g), Padavedu (37.16mg QE/g) and Gummidipoondi (36.32mg QE/g) (Table 3).

In the conclusions, antioxidant activity, total phenol and total flavonoid content of medicinal plants is very important in identifying new sources of therapeutically and industrially important compounds. It is imperative to initiate urgent steps for screening of plants for secondary metabolites. The present research work attempts to assess the importance of antioxidant activity, total phenol and total flavonoid properties in leaves of *Solanum trilobatum* to improve the health status of people and also to use in nutraceutical products of commercial importance. The results indicate that the plant material may become an important source of compounds with health protective potential.

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Table.1 Qualitative analysis of antioxidant activity from leaf extract of Solanum trilobatum (Chengalpet)

S.No	Solanum trilobatum - Chengalpet	Response
	BHT (standard)	+++
S1	Methanol	+
S2	Ethanol	+++
S3	Aqueous extracts	+
S4	Ethylacetate	-
S5	Petroleum ether	-

+++ = Strong positive, + = positive, - = Negative

Table.2 Estimation of Total phenol content from ethanolic leaf extract of Solanum trilobatum

S.No	Plant sample	Total phenol content (mg GAE/g)
S1	*Solanum trilobatum* – Chengalpet	53.7
S2	*Solanum trilobatum* - Vellingiri Hills	37.4
S3	*Solanum trilobatum* – Arni	32.9
S4	*Solanum trilobatum* – Padavedu	31.5
S5	*Solanum trilobatum* - Gummidipoondi	29.14

Table.3 Estimation of Flavonoid content from ethanolic leaf extract of Solanum trilobatum

S.No	Plant sample	Total flavonoid content (mg QE/g)
S1	*Solanum trilobatum* – Chengalpet	64.30
S2	*Solanum trilobatum* - Vellingiri Hills	49.53
S3	*Solanum trilobatum* – Arni	37.82
S4	*Solanum trilobatum* – Padavedu	37.16
S5	*Solanum trilobatum* - Gummidipoondi	36.32

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Figure 1. Antioxidant activity from leaf of Solanum trilobatum (Chengalpet)

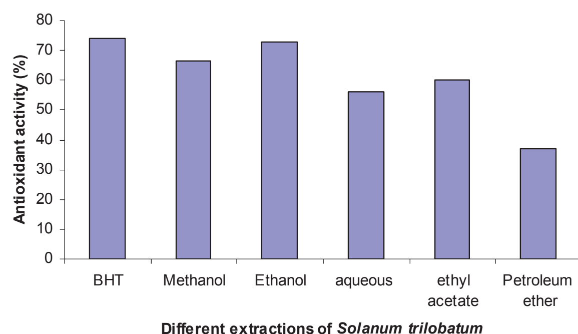
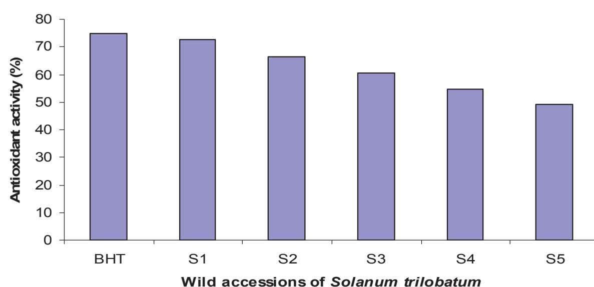


Figure 2. Antioxidant activity from ethanolic leaf extract of *Solanum trilobatum* - different wild accessions



BHT: Standard, S1: Solanum trilobatum (Chengalpet), S2, Vellingiri Hills; S3, Arni; S4, Padavedu and S5, Gummidipoondi.

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