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Comparative study on antioxidant activity of different species of *Solanaceae* family

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ABSTRACT

Antioxidant activity of the ethyl acetate extract of Solanum anguivi Lam., was compared with that of Solanum pubescence Willd, Solanum torvum Swartz, Solanum trilobatum Linn. Solanum nigrum Linn. and Solanum surratense Burm.F., and was determined by use of 2, 2-diphenyl-1-picrylhydryzyl (DPPH) radical scavenging method. The higher the concentration of the extract gave higher free radical scavenging activity. Antioxidant activity of the plant extracts decreased in the order: S. anguivi> S. pubscense> S. torvum> S. trilobatum > S. nigrum> S. surratense. The antioxidant potential of the S. anguivi extract were assessed by employing different in vitro assays such as FTC, TBA, HRSA, PMA, Metal chelating assay and super oxide anion radical scavenging capacity. The present study was undertaken to evaluate and compare the antioxidant properties of six different plant's ethyl acetate extracts of solanaceae family. Strong inhibitions of free radicals were caused by the ethyl acetate extract of S. anguivi. Thus, S. anguivi could be considered as a potential source of natural antioxidants.

Keywords: 2, 2-diphenyl-1-picrylhydryzyl (DPPH), *Solanum anguivi*, Free radical scavenging activity.

INTRODUCTION

Recently, the use of spices and herbs as antioxidants and antimicrobial agents in foods is becoming of increasing importance. Antioxidants have been widely used as food additives to provide protection against oxidative degradation of foods [1]. Free radicals are highly reactive compounds, they are chemical species associated with an odd or unpaired electron and can be formed when oxygen interacts with certain molecules. They are neutral, short lived, unstable and highly reactive to pair with the odd electron and finally achieve stable configuration. Once formed these highly reactive radicals can start a chain reaction they are capable of attacking the healthy cells of the body, causing them to lose their structure and function. Cells may function poorly or die if this occurs [2].

The main characteristic of an antioxidant is its ability to trap free radicals. Highly reactive free radicals and oxygen species are present in biological systems from a wide variety of sources. These free radicals may oxidize nucleic acids, proteins, lipids or DNA and can initiate degenerative disease. Plants are potential sources of natural antioxidants. It produces various antioxidative compounds to counteract reactive oxygen species (ROS) in order to survive [3]. ROS, which include free radicals such as superoxide anion radicals ($O.^{2-}$), hydroxyl radicals (OH.) and non free-radical species such as H_2O_2 and singled oxygen ($1O_2$), are various forms of activated oxygen. These molecules are exacerbating factors in cellular injury and aging process [4]. The majority of the antioxidant activity is due to the flavones, isoflavones, flavonoids, anthocyanin, coumarin lignans, catechins and isocatechins [5]. Antioxidant-based drug formulations are used for the prevention and treatment of complex diseases like atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer [6].

Solanaceae, the nightshade, or potato, family of flowering plants (order Solanales), with 102 genera and nearly 2,500 species, many of considerable economic importance as food and drug plants. Solanaceae are characteristically ethnobotanical, that is, extensively utilized by humans. They are important sources of food, spice and medicine. In this present study we have selected six different plants from solanaceae family to evaluate their antioxidant activity.

MATERIALS AND METHODS

Plants collection:
Fresh plants of *Solanum torvum* and *Solanum trilobatum* were collected from the fields located in Madras University, Maraimalai campus,Guindy, Chennai *Solanum nigrum* and *solanum surratense* were collected from the fields located in Cuddalore district. *Solanum anguivi* and *Solanum pubscense* were collected from the fields located at Tirupathi and Hosur.

Preparation of plant extracts:
The plants were carefully washed with tap water, rinsed with distilled water, and shade dried for one day until it is completely dried. Then it was cut into small pieces & they were ground into powder and stored in room temperature.

Plant extracts preparation:
The ground leaves were extracted with methanol following the method of Eloff [7]. Here, the extraction of the leaf powder was done with solvent in the ratio of 1:10 under shaking condition. The extract was collected in conical flask and the same was repeated thrice to attain maximum extraction. Then the solvent was condensed to concentrate the extract obtained. The concentrated residue was weighed and re-dissolved in different solvents to yield 10mg/ml solutions for further analysis.

Antioxidant activity:
Estimation of Radical Scavenging Activity (RSA) Using DPPH Assay:
The RSA activity of different extracts was determined using DPPH assay according to Nenadis and Tsimidou [8], with small modification. The decrease of the absorption at 517nm of the DPPH solution after addition of the antioxidant (plant extract) was measured in a cuvette containing 2960µl of 0.1 mM ethanolic DPPH solution was mixed with 40 µl of 20 - 200 µg/mL of plant extract. Blank containing 0.1 mM ethanolic DPPH solution without plant extract and vortexed thoroughly, the setup was left at dark at room temperature. The absorption was monitored after 20 min. Ascorbic acid (AA) and Butylated hydroxytoluene (BHT) were used as references. The ability to scavenge DPPH radical was calculated by the following equation.

$$\text{\% of DPPH radical scavenging activity (\% RSA)} = \frac{Abs_{control} - Abs_{sample}}{Abs_{control}} \times 100$$

$Abs_{control}$ is the absorbance of DPPH radical + ethanol; Abs_{sample} is the absorbance of DPPH radical +leaf extract. Measurements were performed in triplicate. Absorbance values were corrected for radical decay using blank solutions. The IC_{50} (concentration providing 50% inhibition) was calculated graphically using a calibration curve vs percentage of inhibition.

Ferric thiocyanate method:
The plant sample of 4mg in 99.5% ethanol were mixed with 2.51% linoleic acid in 99.5% ethanol (4.1mL), 0.05 M phosphate buffer, pH 7 (8mL) and distilled water (3.9mL) and kept in screw cap containers under dark conditions at 40^{o}C. To 0.1mL of this solution, 9.7mL of 75% ethanol and 0.1mL of 30% ammonium thiocyanate were added. After 3 min, 0.1mL of 2M ferrous chloride in 3.5% HCl was added to the reaction mixture and the absorbance of the red color was measured at 500 nm each 24 h until one day after absorbance of the control reached maximum. The control and the standard were subjected to the same procedure as the sample except for the control, where there was no addition of sample, and for the standard 4 mg of sample were replaced with 4 mg of α - tocopherol or Butylated hydroxytoluene [9].

Thiobarbituric acid (TBA) test:
The same samples as prepared for the FTC method were used in TBA test. To 1mL of sample solution, 2mL each of 20% aqueous trichloroacetic acid were added. This mixture was then incubated in a boiling water bath for 10 min.

After cooling, it was centrifuged at 3000 rpm for 20 min and the absorbance of supernatant was measured at 532 nm. Antioxidative activity was recorded based on absorbance on the eighth day [10].

Superoxide Anion Radical Scavenging Assay:
1ml of NBT solution (156µM NBT in 100mM phosphate buffer, pH8) mixed with 1ml of NADH solution (468µM in 100mM phosphate buffer, pH8). Then it mixed with 0.1ml of sample solution (10mg/mL). The reaction was started by adding 100µl of PMS solution (60µM PMS in 10mM, Phosphate buffer, Ph 8). The mixture was incubated at 25ºC for 5minutes. A control was performed with reagent mixture but without the sample. Absorbance was measured spectrophotometrically at 560 nm [11].

Hydroxyl radical scavenging activity:
The scavenging activity of the extract on hydroxyl radical was measured according to the method of Klein *et al* (1991). Various concentrations (50, 100, 150 and 200µg/mL) of extracts were added with 1.0ml of iron-EDTA solution (0.13% ferrous ammonium sulfate and 0.26% EDTA), 0.5ml of EDTA solution (0.018%), and 1.0mL of dimethyl sulphoxide (DMSO) (0.85% v/v in 0.1M phosphate buffer, pH 7.4).The reaction was initiated by adding 0.5ml of ascorbic acid (0.22%) and incubated at 80–90°C for 15 min in a water bath. After incubation the reaction was terminated by the addition of1.0mL of ice-cold TCA (17.5% w/v). Three milliliters of Nash reagent (75.0g of ammonium acetate, 3.0mL of glacial acetic acid, and 2mL of acetyl acetone were mixed and raised to 1L with distilled water) was added and left at room temperature for 15min. The reaction mixture without sample was used as control. The intensity of the color formed was measured spectroscopically at 412 nm against reagent blank. The % hydroxyl radical scavenging activity is calculated by the following formula:

% HRSA = from [(A0 - A1)/A0] X100, where A0 is the absorbance of the control and A1 is the absorbance of the extract/standard.

Metal chelating activity:
The chelation of ferrous ions by the plant extracts and standards was estimated by the method of Dinis *et al*. (1994). Aliquots (1 ml) of the plant extracts dissolved in the same solvents at concentrations of 1, 2.5 and 5 mg/ml were separately added to 2.8 ml of distilled water, followed by mixing with 50 µl of 2 mM $FeCl2.4H_2O$ and 150 µl of 5 mM ferrozine. All the above without plant extract served as control. The mixtures was then shaken vigorously and left standing at room temperature for 10 min. Absorbance levels of the solutions were measured using a spectrophotometer at 562 nm. All tests and analyses were run in triplicate and averaged. The percentage of inhibition of ferrozine– Fe2+ complex formation was calculated using the formula given below:

%Inhibition = [(A0 – A1)/A0] x 100, Where A0 is the absorbance of the control and A1 is the absorbance in the presence of the plant extracts or standards. EDTA was used as a reference compound.

Phosphomolybdenum assay:
The antioxidant activity of samples was evaluated by the green phosphomolybdenum complex formation according to the method of Prieto *et al* [12]. An aliquot of 100µl of sample solution was combined with 1ml of reagent solution (0.6M sulphuric acid, 28mM sodium phosphate and 4mM ammonium molybdate) in a 4ml vial. The vials were capped and incubated in a water bath at 95°C for 90 min. After the samples had cooled to room temperature, the absorbance of the mixture was measured at 695 nm against a blank. The results reported (Ascorbic acid equivalent antioxidant activity) are mean values expressed as g of ascorbic acid equivalents/100g extract.

RESULTS AND DISCUSSION

DPPH radical scavenging activity:
DPPH radical is one of the few stable organic nitrogen free radicals, which has been widely used to determine the free radical scavenging ability of the various samples [13]. The method is based on the reduction of an alcoholic DPPH solution in the presence of a hydrogen donating antioxidant due to the formation of the non-radical form DPPH–H by the reaction [14]. DPPH radical scavenging activity of the tested extracts is concentration dependent and lower IC_{50} value reflects higher scavenging ability. Among the six different plant extracts tested, interestingly, in the DPPH radical scavenging activity of the *S. anguivi* ethyl acetate extract exhibited DPPH radical scavenging potential comparable with that of standard α-tocopherol (Figure 1). The scavenging effect of the six different plant extracts decreased in the order of *S. anguivi*> *S. pubscense*> *S. torvum*> *S. trilobatum* > *S. nigrum*> *S. surratense*.

The data obtained indicate that *S. anguivi* is a powerful free radical scavenger, as well as a primary antioxidant that may prevent free radical damage occurring in the human body. Similarly, Crude extracts of *Leucas linifolia* [15], *Costus pictus* [16], *Samanea saman* (Jacq.) Merr [17], Soy Seed [18] were showed In vitro antioxidant activity in DPPH scavenging activity.

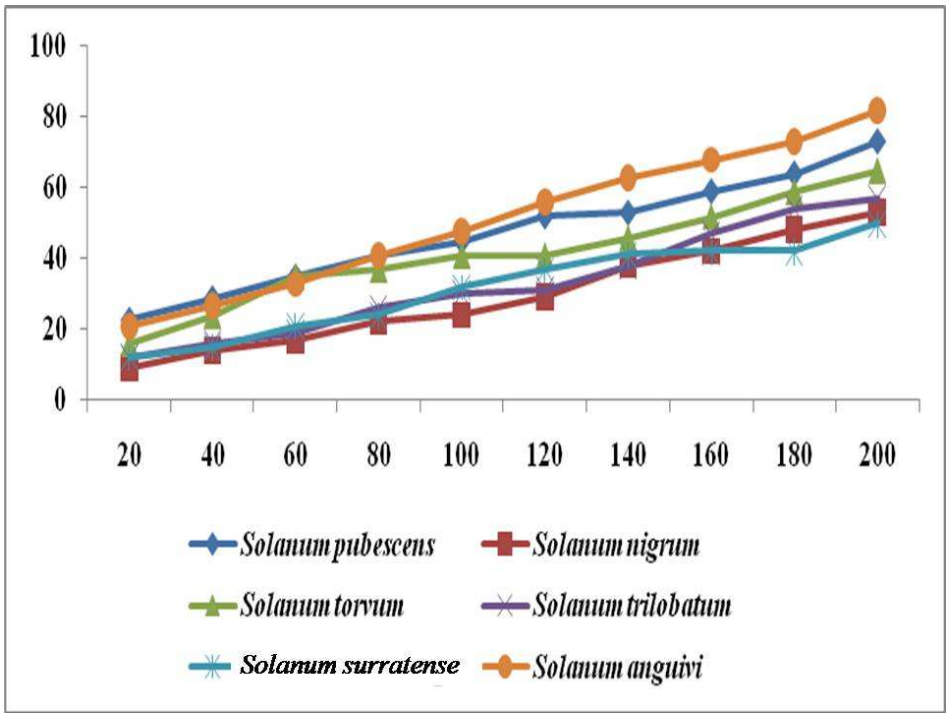

Figure 1. Estimation of Radical Scavenging Activity (RSA) Using DPPH Assay

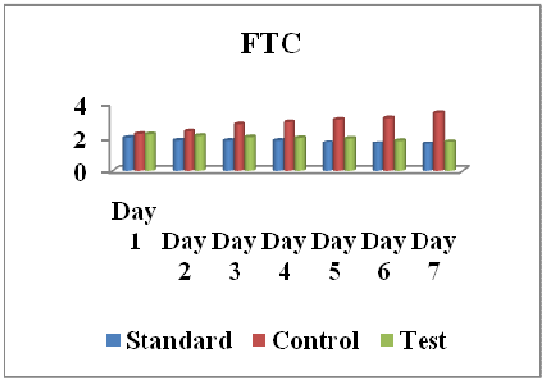

Figure 2. Ferric thiocyanate method

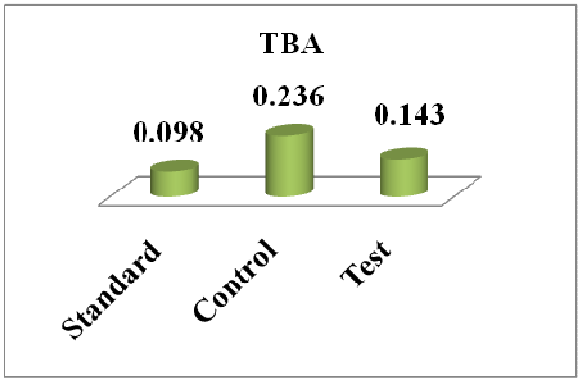

Figure 3. Thiobarbituric acid (TBA) test

Ferric thiocyanate and Thiobarbituric acid (TBA) method:

Antioxidant activity of ethyl acetate extracts is measured to inhibit lipid peroxidation (LPO) by FTC and TBA methods (Fig.2 & 3). The tested plant extract showed strong antioxidant activity or differential capacity to inhibit LPO by FTC and TBA method which is indicated by their low absorbance values. The FTC method measures the amount of peroxide produced during the initial stages of lipid oxidation. Subsequently, at a later stage of lipid oxidation, peroxide decomposes to form carbonyl compounds that are measured by the TBA method [19]. So, at a given concentration, the relatively higher activity was recorded in the extract of *S. anguivi*, surpassing the activity of the standard commercial antioxidants, α- tocopherol. In general, the antioxidant by TBA method is higher than that of FTC method. This might suggest that the amount of peroxide in the initial stage of lipid per oxidation is less than

the amount of peroxide in the secondary stage. Furthermore, the secondary product is much more stable for a period of time [20].

Superoxide radical scavenging activity:
Endogenously, superoxides could be produced in large amounts by various biological processes. It is known to be very harmful to cellular components as a precursor of the most reactive oxygen species (ROS), contributing to tissue damage and various diseases [21]. The ethyl acetate extract of *S. anguivi* exhibited higher ability in scavenging superoxide anion radical, when compared to the standard BHT (Figure 4). The results reveal that the *S. anguivi* seed extracts have superoxide radical scavenging activity which can be of significant interest in health point of view in reducing the level of superoxide radical which is elevated during oxidative stress in the body.

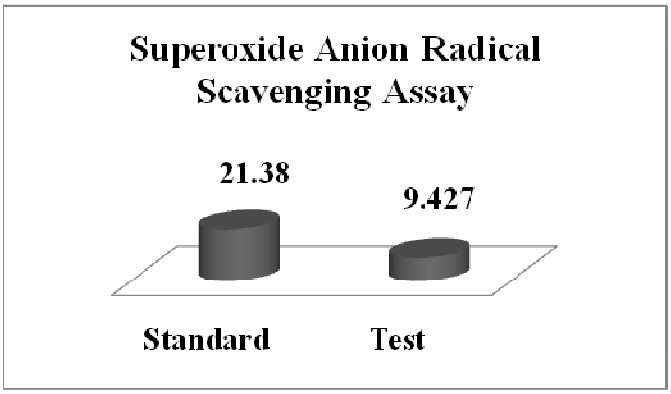

Figure 4. Superoxide Anion Radical Scavenging Assay (%)

Hydroxyl radical scavenging activity:
The hydroxyl radical is one of representative reactive oxygen species generated in the body. These radicals are produced through various biological reactions; one of the common reactions is the Iron (II)-based Fenton reaction. The scavenging capacity of the ethyl acetate extract *S. anguivi* is shown in figure 5. It exhibited the strongest hydroxyl radical scavenging activity. The radical scavenging capacity may be attributed to phenolic compounds in the extract with the ability to accept electrons, which can combine with free radical competitively to decrease hydroxyl radical [22].

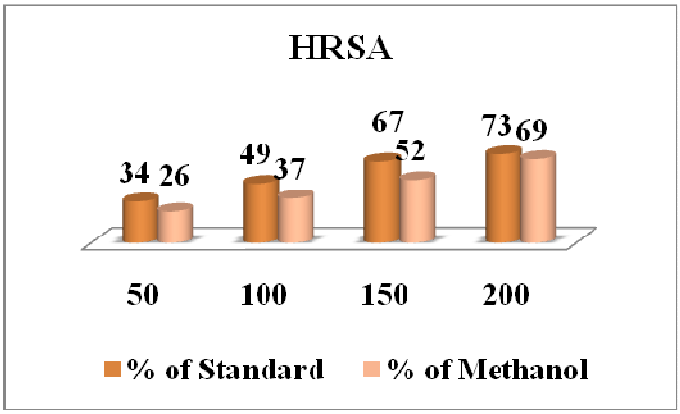

Figure 5. Hydroxyl radical scavenging activity

Metal chelating activity:
Iron is an essential mineral for normal physiology, but an excess of it may result in cellular injury. If they undergo Fenton reaction, these reduced metals may form reactive hydroxyl radicals and thereby contribute to oxidative stress [23]. The chelating ability of ferrous ions by the ethyl acetate extract was estimated by the method of Dinis *et al*. [24]. Ferrozine can quantitively form complexes with Fe2+. In the presence of chelating agents, the complex

formation is disrupted with the result that red color of the complex is decreased. The metal chelating activity of ethyl acetate extract from *S. anguivi* is presented in figure 6. In this assay, ethyl acetate extract of *S. anguivi*, registered higher metal chelating activity. The scavenging potential and metal chelating ability of the antioxidants are dependent upon their unique phenolic structure and the number of hydroxyl groups [25].

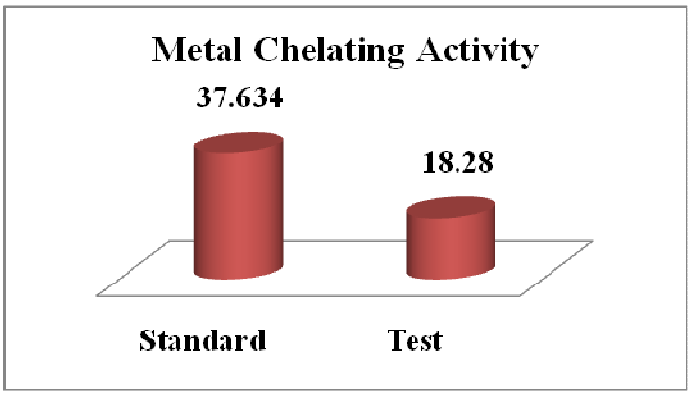

Figure 6. Metal chelating activity (%)

Phosphomolybdenum assay:
The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/Mo (V) complex at acid pH. The phosphomolybdenum method is quantitative since the antioxidant activity is expressed as the number of equivalents of ascorbic acid [12]. The ethyl acetate extract of *S. anguivi* was used to determine their antioxidant capacities by the formation of green phosphompolybdenum complex (Figure 7). The results indicate that the extract is more powerful antioxidant in the reduction of phosphomolybdenum complex.

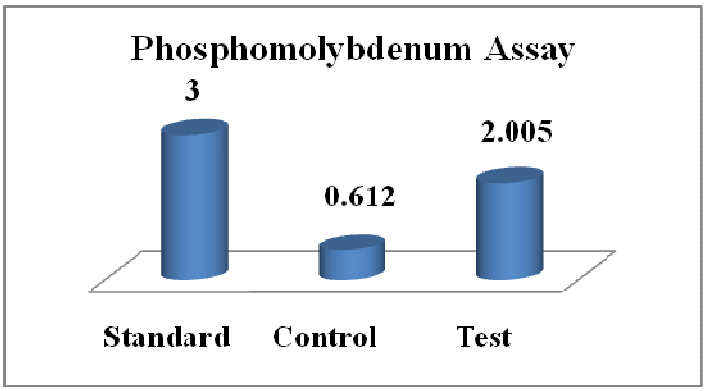

Figure 7. Phosphomolybdenum assay

CONCLUSION

The results of the present study indicate that ethyl acetate extract of *S. anguivi* exhibit strong antioxidant activities. The scavenging activities observed against DPPH, hydroxyl radicals, metal chelating, ferric thio cyanate as well as the thiobarbituric acid assay, lead us to propose *S. anguivi* as promising natural sources of antioxidants suitable for application in nutritional/pharmaceutical fields, in the prevention of free radical- mediated diseases. Further studies are needed to explore the potential phenolics compound(s) from *S. anguivi* and *in vivo* studies are needed for better understanding their mechanism of action.

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