In – vitro antioxidant activity of leaves on *Datura stramonium*, L.and *Datura metal*, L. Solanaceae – A comparative studies

Anburajan. Veeraragavan ,Hameed Sultan. Sheik Jahabar Ali* & Abdul Hathi. Mohamed Ismail

Herbal Biotechnology Lab, Department of Biotechnology, E.G.S.Pillay Arts & Science College, Nagapattinam, Tamil Nadu, India.

Abstract:
The ethanol extracts of *Datura stramonium* and *Datura metel* (Family: Solanaceae) collected from Pappakovil, Nagapattinam Dist of Tamil Nadu, India. The Ethanol extract obtained by maceration extraction was examined for its antioxidant activities. The antioxidant activity was determined by means of the DPPH radical scavenging test, Total antioxidant (TAA) and superoxide anion scavenging activity assay (SASA).Result from three methods indicate that the antioxidant activity of *D. stramonium* and *D. metel* of ethanol extracts were time and concentration dependent. The antioxidant potential of *D. stramonium* and *D. metel* determined by the DPPH method expressed as IC50 was The half inhibition concentration (IC50) of plant extracts *D. stramonium* , *D. metel* and ascorbic acid were 49.72 µg ml-1, 50.35µg ml-1 and 34.91 µg ml-1 respectively. the antioxidant potential of *D. stramonium* and *D. metel* determined by the Total antioxidant assay (TAA) method expressed as IC50 was The half inhibition concentration (IC50) of *D. stramonium* , *D. metel* and ascorbic acid were 49.59µg ml-1, 49.94µg ml-1 and 42.41 µg ml-1 respectively. the antioxidant potential of *D. stramonium* and *D. metel* from determined by the Superoxide anion scavenging activity assay (SASA) method expressed as IC50 was The half inhibition concentration (IC50) of *D. stramonium* and *D. metel* were, 49.58 and 49.73µg ml-1 andascorbic acid were 31.62µg ml-1 respectively.

Keywords: *Datura stramonium*, *Datura metel*, antioxidant activity, DPPH, Total antioxidant assay, Superoxide anion scavenging activity assay, Nagapattinam.

INTRODUCTION

Datura stramonium and *Datura metel* belongs to the family solanaceae. They are commonly known as seemai oomathai and karu oomathai. This plant can be found on street and Croix growing along roadsides. The disturbed sites, grass-fields and brushwood. The plant was reported to be used in the treatment of wide variety of diseases especially in the treatment of roasted leaves is applied over the area to relieve pain. It is used as herbal medicine, especially in case of ayurveda for asthma and bone setting. Jimson weed is used to treat spasm of bronchitis in asthma. Datura seeds and leaves are used as antiasthmatic, antispasmodic, hypnotic and narcotic.

Antioxidants found in biological system comprise a number of interconnecting and overlapping components, which include both enzymatic and non-enzymatic factors. Antioxidant enzymes primarily account for intracellular defense, while several non-enzyme molecules, small molecule weight antioxidants, protect various components against oxidation (Szaleczky *et al*., 1999).

Superoxide dismutase, catalase, glutathione peroxidase and glutathione *S*-transferase are the most important enzymatic antioxidants. Other antioxidant enzymes include hemeoxygenase-1, thiol-specific antioxidant enzyme and macrophage stress protein. The non-enzymatic antioxidants and other small molecules with antioxidant properties include reduced glutathione, ascorbic acid, α-tocopherol, β-carotene, uric acid and bilirubin. Metal ion chelators that sequestrate metal ions include haptoglobulin, albumin, transferrin, ceruloplasmin and metallothionein (Halliwell and Gutteridge, 1986). Synthetic antioxidants like butylated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) commonly used in processed foods have harmful side effects and are carcinogenic (Hettiarachchy *et al.*, 1996). In recent years, the use of natural antioxidants present in foods and other biological materials has attracted considerable interest due to their presumed safety, nutritional and therapeutic value (Ajila *et al*., 2007).

MATERIALS AND METHODS

Plant Collection

The fresh plant organ (Leaves) of *Datura stramonium*, L. and *Datura metal*, L.. were collected from Pappakovil, Nagapattinam (10.7906°N and 79.8428°E), district of Tamil Nadu, India. The voucher specimen is preserved in the Department of Biotechnology, E. G. S. Pillay Arts and Science college,Nagapattinam.

Preparation of extracts

Solvent extraction by maceration process

25gms of the powder of plant material was transferred into different conical flask (250ml).The conical flask containing 100ml of Ethanol. The conical flask containing plant powder and solvent was shaked it well for 48 hours by mechanical shaker. The extracts were filtered using Whatmann filter paper No.1. The filtrates were evaporated to dryness using water bath. The obtained extracts were stored at 4°c in air tight bottle until further use.

In vitro antioxidant activity

DPPH radical-scavenging activity

DPPH radical-scavenging activity was determined by the method of Shimada, *et al.,* (1992).

Reagents;

1. DPPH : 25 µg/ml in methanol
2. Methanol

Procedure:

Briefly, a 2 ml aliquot of DPPH methanol solution (25µg/ml) was added to 0.5 ml sample solution at different concentrations. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 30 min. Then the absorbance was measured at 517nm in a spectrophotometer. Lower absorbance of the reaction mixture indicated higher free-radical scavenging activity.

$$\text{Radical scavenging activity (\%)} = 100 - \left(\frac{A_C - AS}{A_C} \right) X\ 100$$

Where A_C = control is the absorbance and A_S = sample is the absorbance of reaction mixture (in the presence of sample).

Determination of Total Antioxidant Capacity

The antioxidant activity of the extracts was evaluated by the phosphomolybdenum method according to the procedure of Prieto *et al*., (1999).

Reagents:

1. Sulfuric acid : 0.6M
2. Sodium phosphate : 28mM
3. Ammonium molybdate : 4mM

Procedure:

The assay is based on the reduction of Mo(VI)–Mo(V) by the extract and subsequent formation of a green phosphate/Mo(V) complex at acid pH. 0.3 ml extract was combined with 3ml of reagent solution (0.6M sulfuric acid, 28mM sodium phosphate and 4mM ammonium molybdate). The tubes containing the reaction solution were incubated at 95°C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. Methanol (0.3 ml) in the place of extract is used as the blank. The antioxidant activity is expressed as the number of equivalents of ascorbic acid. The scavenging activity was calculated according to the following equation: % Inhibition

$$\text{\% of Inhibition} = \frac{(A_0 - A_1)}{A_0} \times 100$$

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

Superoxide anion scavenging activity assay

The superoxide anion radicals scavenging activity was measured by the method of Liu *et al*., (1997).

Reagents:

1. Tris-HCl buffer : pH 7.4
2. Nitroblue tetrazolium (NBT) : 300 µM
3. Nicotinamide adenine dinucleotide (NADH) : 936 µM
4. Phenazine methosulfate (PMS) : 120 µM

Procedure

In these experiments the superoxide anion was generated in 3 ml of Tris-HCl buffer (100 mM, pH 7.4) containing 0.75 ml of NBT (300 µM) solution, 0.75 ml of NADH (936 µM) solution and 0.3 ml of different concentrations of the extract. The reaction was initiated by adding 0.75 ml of PMS (120 µM) to the mixture. After 5 min of incubation at room temperature, the absorbance at 560 nm was measured in spectrophotometer.

The superoxide anion scavenging activity was calculated according to the following equation:

$$\% \text{ Inhibition} = ((A_0-A_1) / A_0 \times 100)$$

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

RESULTS

***In vitro* antioxidant activity**

DPPH Assay

Recently, the use of the DPPH• reaction has been widely diffused among food technologists and researchers, for the evaluation of free radical scavenging activity on extracts from plant, food material or on single compounds. In the DPPH assay, the antioxidant was able to reduce the stable radical DPPH to the yellow colored 1, 1-diphenyl-1, 2-picryl hydrazine. The molecule of 2, 2-diphenyl-1-picryl hydrazine is characterised as a stable free radical by virtue of the delocalisation of the spare electron over the molecule as a whole. The proton transfer reaction of the DPPH• free radical by a scavenger causes a decrease in absorbance at 517 nm, which can be followed by a common spectrophotometer set in the visible region. The effect of antioxidants on DPPH• is thought to be due to their hydrogen donating ability (Sindhu and Abraham, 2006). DPPH radical scavenging activity of the ethanol extract of *D. stramonium* and *D. metal* and standard as ascorbic acid are presented in **Fig 1**. The DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants (Nutila *et al.,* 2003). The half inhibition concentration (IC_{50}) of *D. stramonium* and *D. metal* and ascorbic acid were 49.72 µg ml^{-1}, 50.35 µg ml^{-1} and 34.91 µg ml^{-1} respectively **(Table 1).** The plant extract exhibited a significant dose dependent inhibition of DPPH activity. The observed scavenging effect of *D. stramonium* and *D. metal* leaf of ethanol extracts and standard on the DPPH radical decreases in the following order: L- Ascorbic acid > *D. stramonium* > *D. metal* from the experimental data which obtained . It is clear that *D. stramonium* showed maximum percentage of inhibition effect i.e 85.72%at 800µg /mL of concentration.

Total antioxidant activity

The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/ Mo (V) complex with a maximal absorption at 695 nm. The assay is successfully used to quantify vitamin E in seeds and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extract (Prieto *et al*., 1999). Moreover, it is a quantitative one, since the antioxidant activity is expressed as the number of equivalents of ascorbic acid. The yield of the ethanol extract of leaves and its total antioxidant capacity are given in **Fig 2**. Total antioxidant capacity of *D. stramonium* and *D. metal* were expressed as the number of equivalents of ascorbic acid. The study reveals that the antioxidant activity of the extract is in the increasing trend with the increasing concentration of the plant extract. The half inhibition concentration (IC_{50}) of *D. stramonium* and *D. metal* and ascorbic acid were 49.94 µg

ml^{-1}, 49.59 µg ml^{-1} and 42.41 µg ml^{-1} respectively **(Table 2).** The observed scavenging effect of *D. stramonium* and *D. metal* leaves extract and standard on the total antioxidant capacity decreases in the following order: L Ascorbic acid > *D. stramonium* > *D. metal* from the experimental data which obtained. It is clear that *D. stramonium* showed maximum percentage of inhibition effect i.e 83.75% at 800µg /mL of concentration.

Superoxide anion radical scavenging activity

Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, is very harmful to the cellular components in a biological system (Korycka-Dahl & Richardson, 1978). The superoxide anion radical scavenging activities of the ethanol extract from *D. stramonium* and *D. metal* assayed by the PMS-NADH system were shown in **Fig 3**. The superoxide scavenging activity of *D. stramonium* and *D. metal* were increased markedly with the increase of concentrations. The half inhibition concentration (IC_{50}) of *D. stramonium* and *D. metal* were 49.45, 49.58 and 49.73µg ml^{-1} and ascorbic acid were 31.62µg ml^{-1} respectively **(Table 3).** These results suggested that *D. stramonium* and *D. metal* had notably superior superoxide radical scavenging effects. The observed scavenging effect of *D. stramonium* and *D. metal* extracts and standard on the superoxide scavenging activity decreases in the following order: L Ascorbic acid > *D. stramonium* > *D. metal* from the experimental data which obtained. It is clear that *D. stramonium* showed maximum percentage of inhibition effect i.e 85.16%at 800µg /mL of concentration.

On the basis of the results of this study, it clearly indicates *D. stramonium* and *D. metal* possessing antioxidant activity against various antioxidant systems *in vitro*. From our results, the antioxidant capacity of *D. stramonium* and *D. metal* were concentration dependent. Compared between two species viz., *D. stramonium* and *D. metal* extracts and standard tested for the *in vitro* antioxidant activity. The free radical scavenging activity of *D. stramonium* was found to be in close proximity to standard confirmed in the present investigation. From the above assays, the possible mechanism of antioxidant activity of these chemicals includes reductive ability, hydrogen donating ability and scavengers of hydrogen peroxide.

Concentrations	*D. stramonium*	*D. metal*	Standard Ascorbic acid
200µg/ml	24.64±1.65	14.64±0.95	25.6±2.04
400µg/ml	49.73±3.41	24.46±1.78	61.26±4.90
600µg/ml	73.34±5.06	58.28±4.00	88.98±7.11
800µg/ml	85.72±5.93	67.37±4.64	99.34±7.94
IC50	49.72	50.35	34.91

Table 1. DPPH Radical scavenging activity of *Datura stramonium*, L. and *Datura metal*, L.

Values are expressed as Mean ±SD for triplicate

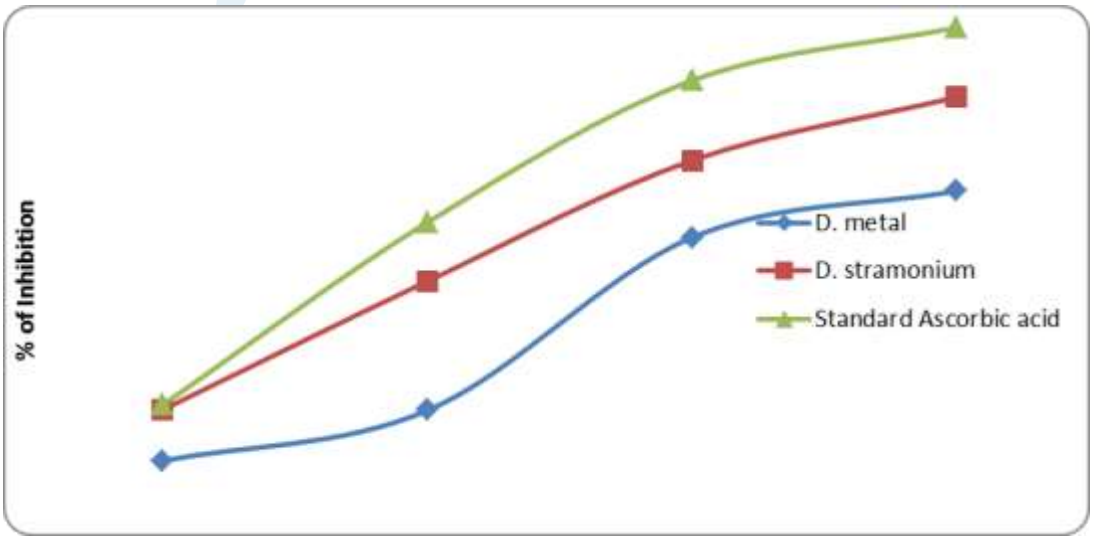

Fig 1 shows % inhibition of DPPH Radical scavenging activity of *Datura stramonium*, L. and *Datura metal*, L.

Table 2: Total antioxidant assay of *Datura stramonium*, L. and *Datura metal*, L.

Concentrations	D. stramonium	D. metal	Standard Ascorbic acid
200µg/ml	28.13±1.96	19.75±1.31	22.35± 1.80
400µg/ml	42.38±2.96	25.00±1.75	51.23± 4.09
600µg/ml	60.63±4.24	51.25±3.58	72.54± 5.80
800µg/ml	83.75±5.86	64.38±4.50	86.35± 6.91
IC50	49.59	49.94	42.41

Values are expressed as Mean ±SD for triplicate

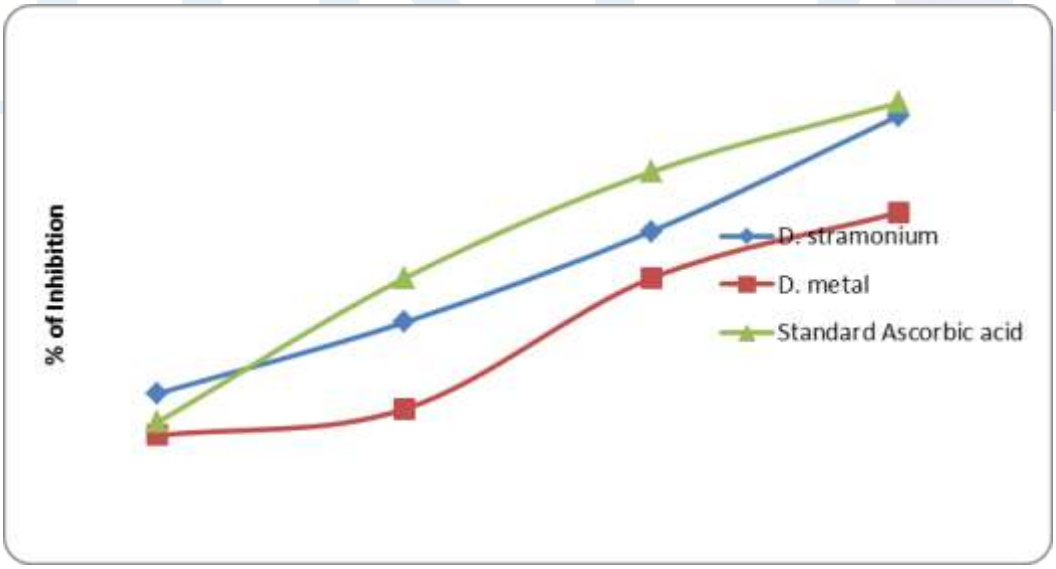

Fig 2 shows % inhibition of Total antioxidant assay of *Datura stramonium*, L. and *Datura metal*, L

Table 3. Superoxide Radical scavenging activity of *Datura stramonium*, L. and *Datura metal*, L.

Concentrations	*D. stramonium*	*D. metal*	Standard Ascorbic acid
200µg/ml	26.14±1.84	20.14±1.40	31.25 ± 2.50
400µg/ml	43.54±3.04	46.41±3.24	64.23 ± 5.13
600µg/ml	71.93±5.03	65.16±4.56	89.54 ± 7.16
800µg/ml	85.16±5.96	72.14±5.04	98.51 ± 7.88
IC50	49.73	49.58	31.62

Values are expressed as Mean ±SD for triplicate

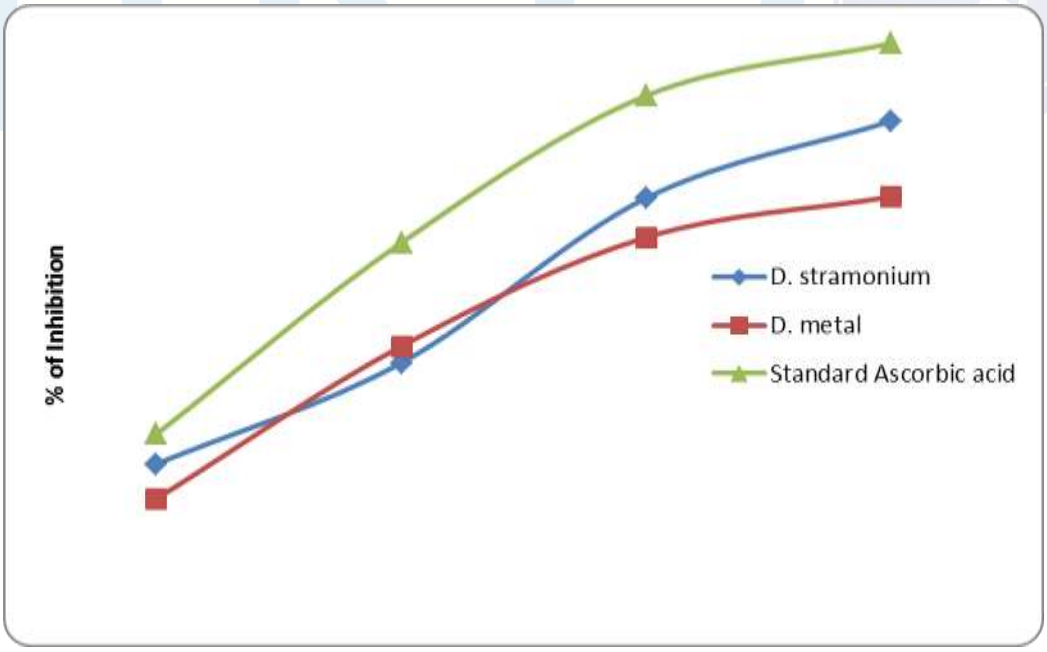

Fig 3 shows % inhibition of Superoxide Radical scavenging activity of *Datura stramonium*, L. and *Datura metal*, L.

DISCUSSION

The proton transfer reaction of the DPPH• free radical by a scavenger causes a decrease in absorbance at 517 nm, which can be followed by a common spectrophotometer set in the visible region. The effect of antioxidants on DPPH• is thought to be due to their hydrogen donating ability (Sindhu and Abraham, 2006).

The DPPH radical was widely used to evaluate the free-radical scavenging capacity of antioxidants (Nutila *et al.*, 2003).

The phosphomolybdenum method was based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/ Mo (V) complex with a maximal absorption at 695 nm. The assay is successfully used to quantify vitamin E in seeds and, being simple and independent of other antioxidant measurements commonly employed, it was decided to extend its application to plant extract (Prieto *et al*., 1999).

Superoxide is biologically important since it can be decomposed to form stronger oxidative species such as singlet oxygen and hydroxyl radicals, is very harmful to the cellular components in a biological system (Korycka-Dahl & Richardson, 1978).

REFERENCES

Ajila, C.M., K.A Naidu, U.J.S Bhat and P. Rao, 2007. Bioactive compounds and antioxidant potential of mango peel extract. Food. Chem., 105: 982-988.

Halliwell, B. and J.M.C. Gutteridge, 1998. Free radicals in biology and medicine, London: Oxford University Press.

Hettiarachchy, N.S., K.C. Glenn, R. Gnanasambandam and M.G. Johnson, 1996. Natural antioxidant extract from fenugreek (Trigonella foenumgraecum) for ground beef patties. J. Food Sci., 61: 516–519.

Korycka-dahl, M.and T.Richardson, 1978 photo generation of superoxide anion in serum of bovine milk and in model systems containing Riboflavin and aminoacids.J.Dairy science, 61: 400-407.

Nutila, A. M., Pimia, R. P., Aarni, M., & Caldenty, K. M. O. (2003). Comparison of antioxidant activities of onion and garlic extracts by inhibition of lipid peroxidation and radical scavenging activity. Food Chemistry, 81, 485–493.

Prieto P., Pineda M., Aguilar M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of a phosphomolybdenum complex: Specific application to the determination of Vitamin E. *Anal Biochem*. **269:** 337-341.

Sindhu M, Abraham TE.(2006) In vitro antioxidant activity and scavenging effects of Cinnamomum verum leaf sample assayed by different methodologies. *Food and Chemical Toxicology* 44 198–206.

Szaleczky, E., J. Prechl, J. Feher and A. Somogyi, 1999. Alterations in enzymatic antioxidants defence in diabetes mellitus [a Rational Approach]. Postgrad Med. J., 75: 13-17.

***Corresponding Author**
H.SHEIK JAHABAR ALI,
Assistant Professor,
Department of Biotechnology,
E. G. S. Pillay Arts & Science College,
Nagapattinam,
Tamil Nadu,
India-611002
Mobile: +91 9791560574
Email: royalherbalist.h42@gmail.com