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Study of Antioxidant Activity of *Datura stramonium* Linn.

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ABSTRACT

The present study aims to investigate the methanolic extract of different plant parts of, *Datura stramonium* Linn (family Solanaceae) for their antioxidant activity. The maximum DPPH scavenging activity of extract and standard was found to be 92.3% at 1000 μg mL^{-1} and 92.8% at 1000 and 512 μg mL^{-1}, respectively. The IC_{50} of the Methanolic extract of *Datura stramonium* and standard was found to be 39.48 and 42.0 μg mL^{-1}, respectively. The maximum superoxide scavenging activity of plant extract and standard was found to be 73.1 and 83.4% at 128 μg mL^{-1}. The IC_{50} value of plant extract and standard was found to be 28.45 and 42.32 μg mL^{-1}, respectively. Maximum chelating of metals ions at 1000 μg mL^{-1} of extract and standard was found to be 63.9 and 89%, respectively. The IC_{50} values of plant extract and standard was recorded as 26.45 and 58.41 μg mL^{-1}, respectively. The maximum ABTS scavenging activity of plant extract and standard was found to be 84.5 and 79.2% at 256 and 128 μg mL^{-1}, respectively. The IC_{50} value of plant extract and standard was recorded as 40.11 and 24.29 μg mL^{-1}, respectively. The maximum nitric oxide radical scavenging activity of extract and standard was found to be 63.1 and 72.3% at 1000 and 512 μg mL^{-1}, respectively. The IC_{50} value of the methanol extract and standard was found to be 35.85 and 29.34 μg mL^{-1}, respectively.

Key words: DPPH, nitric oxide radical scavenging activity, ABTS, superoxide

INTRODUCTION

Datura stramonium Linn. Solanaceae. In most parts of India it grows as a wasteland weed (Oudhia *et al*., 1998, 1999) but is cultivated for its alkaloids in some parts of India and in Europe (Chandra and Pandey, 1989). In India, *D. stramonium* is considered as valuable medicine. Datura was known to the ancient Hindu Physicians who regarded it as antispasmotic, intoxicant, emetic, digestive, acrid, astringent, germicidal, anodyne antipyretic, antiseptic, antiphlogistic, antiproliferative narcotic, sedative, tonic, febrifuge, antidiarrhoeal, antihelminthic, alexiteric and useful in Leucoderma, skin disorders, ulcers, bronchitis, jaundice and piles (Agharkar, 1991). In homoeopathic system of medicine, a widely used drug named stramonium is prepared from mature seeds of *D. stramonium* and considers to act on the human brain (Ghosh, 1988). antioxidants have been reported radical scavenging, to prevent oxidative damage caused by free radical, interfere with the oxidation process by reacting with free radicals, chelating, catalytic metals and also by acting as oxygen scavengers. The potentially reactive derivatives of oxygen, attributed as Reactive Oxygen Species (ROS) are continuously generated inside the human body. The generated ROS are detoxified by the (BHT), antioxidants present in the body.

Ascorbic acid is essential for the normal function of living cells and many enzymatic reactions in humans (Gershoff, 1993; Marcus and Coulston, 2001). Ascorbic acid (Vitamin C) essential for growth, differentiation and metabolism of plants (Chinoy, 1984; Agarwal and Rao, 2000). Ascorbic acid levels were higher in leaves (0.88 mg g^{-1} dw) than other plant parts and cell cultures. Role of ascorbic acid has been reported in tissue culture of *Datura* species (Nag *et al.*, 1974). But no attempts have been made to quantity ascorbic acid in *Datura stramonium*. *Datura* was known to the ancient Hindu Physicians who regarded it as antispasmotic, intoxicant, emetic, digestive, acrid, astringent, germicidal, anodyne antipyretic, antiseptic, antiphlogistic, antiproliferative narcotic, sedative, tonic, febrifuge, antidiarrhoeal, antihelminthic, alexiteric and useful in Leucoderma, skin disorders, ulcers, bronchitis, jaundice and piles (Agharkar, 1991). In Rajasthan the seeds are reported to treat leprosy. In India, the plant is used in the treatment of epilepsy, hysteria insanity, heart disease and for fever and skin diseases (Duke and Ayensu, 1985; Ali and Shuaib, 1996; Dabur *et al.*, 2004a, b).

MATERIALS AND METHODS

Plant extract: About 50 g of the leaves was taken and extracted in a soxhlet extractor with methanol (0.2 Lit.). The crude extract was concentrated to dryness in a rotary flash evaporator under reduced pressure and controlled temperature (40-50°C). The extract was preserved in vacuum desiccators for subsequent use in study.

DPPH radical scavenging assay: To the methanolic solution of DPPH (1 mM) an equal volume of the extract dissolved in alcohol was added at various concentrations from 2 to 1000 μg mL^{-1} in a final volume of 1.0 mL. An equal amount of alcohol was added to the control. After 20 min, absorbance was recorded at 517 nm. Experiment was performed in triplicate (John, 1984; Sreejayan and Rao, 1996). A control reaction was carried out without the test sample linear graph of concentration vs. percentage inhibition was prepared IC_{50} values was calculated:

$$\text{Inhibition or scavenging (\%)} = \frac{\text{Absorbance sample}}{\text{Absorbance control}} \times 100$$

IC_{50} represents the level where 50% of the radicals were scavenged by test samples.

Nitric oxide radical scavenging assay: Sodium nitroprusside 5 mM was prepared in phosphate buffer pH 7.4 to 1 mL of various concentrations of test compound, sodium nitroprusside 0.3 mL was added. The test tubes were incubated at 25°C for 5 h after which, 0.5 mL of Griess reagent was added. The absorbance of the chromophore was read at 546 nm. The experiment was performed in triplicate (Sreejayan and Rao, 1996). A linear graph of concentration vs. percentage inhibition was prepared and IC_{50} value was calculated.

ABTS radical scavenging assay: To the reaction mixture containing 0.3 mL of ABTS radical, 1.7 mL phosphate buffer and 0.5 mL extract was added at various concentrations from 2-500 μg mL^{-1}. Blank was carried out without drug. Absorbance was recorded at 734 nm. Experiment was performed in triplicate (Sreejayan and Rao, 1996; John, 1984). A Linear graph of concentration Vs percentage inhibition was prepared and IC_{50} value was calculated.

Superoxide scavenging assay: Alkaline DMSO was used as a super oxide generating system. To 0.5 mL of different concentrations of the test compound, 1 mL of alkaline DMSO and 0.2 mL of NBT 20 mM in phosphate buffer pH 7.4 was added. The experiment was performed in triplicate (Govindarajan *et al*., 2003). The control tubes were also set up where is DMSO was added instead of sample. The reaction mixture was illuminated for 30 min and absorbance at 560 nm was measured against the control samples. A linear graph of concentration vs. percentage inhibition was prepared and IC_{50} value was calculated.

Iron chelating activity assay: The reaction mixture containing 1 mL O-phenanthroline, 2 mL ferric chloride and 2 mL extract at various concentrations ranging from 2 to 1000 μg mL^{-1} in a final volume of 5 mL was incubated for 10 min at ambient temperature. The absorbance at 510 nm was recorded.

Ascorbic acid was added instead of extract and absorbance obtained was taken as equivalent to 100% reduction of all ferric ions. Blank was carried out without extract. Experiment was performed in triplicate (John, 1984; Sreejayan and Rao, 1996; Benzie and Strain, 1996). A linear graph of concentration vs. percentage inhibition was prepared and IC_{50} value was calculated.

Statistical analysis: Linear regression analysis was used to calculate IC_{50} values.

$$b = \frac{\sum x.y}{\sum x^2}$$

$$a = \bar{y} - b\bar{x}$$

$$IC_{50} = a + b(50)$$

b = Regression coefficient of x on y
a = Intercept of the line
x = Concentration in μg mL^{-1}
y = Percentage scavenging
$\bar{x}$ = Mean of concentration
$\bar{y}$ = Mean of % scavenging

RESULTS AND DISCUSSION

In the present study we have investigated the antioxidant activity of *Datura stramonium* by different assays and found their scavenging activity. In this plant, leaf was found to possessed antioxidant activity. Table 1 shows the % scavenging of methanolic extracts and standards by DPPH, DPPH [1,1-diphenyl, 2-picryl hydrazyl] is a stable free radical with purple colour,. Antioxidants reduces DPPH to 1,1-diphenyl-2- Picryl hydrazine, a colorless compound. Standard shows maximum scavenging 92.8% at 512 μg mL^{-1} concentration while sample shows maximum scavenging 92.3±0.13 at 1000 μg mL^{-1}. Table 2 shows the % scavenging of methanolic extracts and standards by nitric oxide radical scavenging assay. Sodium nitroprusside in aqueous solution at physiological pH spontaneously generates nitric oxide which interacts with oxygen to produce nitrite ions. Standard shows maximum scavenging 72.3% at 512 μg mL^{-1} concentration while sample shows maximum scavenging 63.1±0.51 at 1000 μg mL^{-1}. Table 3 shows the % scavenging

Table 1: Percentage scavenging of methanolic extract and standard by DPPH radical scavenging assay

Concentration (μg mL^{-1})	Scavenging of sample (%)	Scavenging of standard (%)
2	9.2±0.15	9.1
4	9.4±0.19	10.1
8	10.1±0.20	21.1
16	14.1±0.21	59.7
32	18.1±0.22	88.4
64	22.1±0.24	89.0
128	33.0±0.30	91.2
256	68.9±0.06	92.0
512	91.0±0.10	92.8
1000	92.3±0.13	92.0
2000	88.1±0.08	90.3

Each value is expressed as Mean±S.E (n = 3), Standard: Ascorbic acid

Table 2: Percentage scavenging of methanolic extract and standard by nitric oxide radical scavenging assay

Concentration (μg mL^{-1})	Scavenging of standard (%)	Scavenging of sample (%)
2	12.3	34.7±0.25
4	21.1	37.3±0.26
8	24.7	40.8±0.30
16	29.5	47.5±0.32
32	47.9	49.8±0.32
64	53.4	50.1±0.40
128	56.3	52.9±0.41
256	57.1	59.1±0.43
512	72.3	61.3±0.50
1000	61.9	63.1±0.51

Each value is expressed as Mean±S.E (n = 3), Standard: Ascorbic acid

Table 3: Percentage scavenging of methanolic extract and standard by ABTS radical scavenging assay

Concentration (μg mL^{-1})	Scavenging of standard (%)	Scavenging of sample (%)
2	0	29.2±0.30
4	2.1	30.1±0.30
8	4.5	33.5±0.32
16	10.9	49.1±0.33
32	21.2	69.7±0.41
64	66.0	60.4±0.34
128	79.2	62.3±0.40
256	75.0	84.5±0.60
512	74.3	81.2±0.50
1000	68.0	80.7±0.40

Each value is expressed as Mean±S.E (n = 3), Standard: Ascorbic acid, $S.E(\sigma_x) = \frac{\sigma}{\sqrt{n}}$, σ = Standard deviation, n = No. of set

of methanolic extracts and standards by ABTS, ABTS (2,2'-azinobis,3-ethyl-benzothiozline-6-sulphonic acid) assay is based on the scavenging of light by ABTS radicals. The relatively stable ABTS radical has a green color, The decolourization of ABTS cation radical measure the antioxidant activity. Standard shows maximum scavenging 79.2% at 128 μg mL^{-1} concentration while sample shows maximum scavenging 84.5±0.60 at 256 μg mL^{-1}. Table 4 shows the % scavenging of methanolic extracts and standards by superoxide radical scavenging assay,

Table 4: Percentage scavenging of methanolic extract and standard by superoxide anion scavenging activity

Concentration (μg mL^{-1})	Scavenging of standard (%)	Scavenging of sample (%)
2	32.1	18.2±0.23
4	38.3	19.7±0.25
8	44.8	22.3±0.25
16	45.1	42.1±0.50
32	59.3	65.7±0.60
64	79.7	70.3±0.52
128	83.4	73.1±0.54
256	81.1	71.1±0.70
512	80.0	70.9±0.40

Each value is expressed as Mean±S.E (n = 3), Standard: Ascorbic acid, $S.E(\sigma_x) = \frac{\sigma}{\sqrt{n}}$, σ = Standard deviation, n = No. of set

Table 5: Percentage scavenging of methanolic extract and standard by iron chelating activity assay

Concentration (μg mL^{-1})	Scavenging of standard (%)	Scavenging of sample (%)
2	67.2	21.3±0.20
4	68.0	24.7±0.21
8	70.1	29.3±0.20
16	73.6	35.7±0.25
32	75.0	37.3±0.10
64	79.8	38.4±0.17
128	88.1	48.2±0.35
256	85.3	51.7±0.70
512	87.4	56.8±0.72
1000	89.0	63.9±0.40

Each value is expressed as Mean±S.E (n = 3), Standard: Ascorbic acid

Table 6: IC_{50} value for different assays

Assays	IC_{50} value of extract (μg mL^{-1})	IC_{50} value of standard (μg mL^{-1})
DPPH	39.48±0.27	42.00
Nitric oxide	35.85±0.25	29.34
ABTS	40.11±0.30	24.29
Iron chelating	26.45±0.20	58.41
Superoxide scavenging	28.45±0.23	42.32

Each value is expressed as Mean±S.E (n = 3), Standard: Ascorbic acid, $S.E(\sigma_x) = \frac{\sigma}{\sqrt{n}}$, σ = Standard deviation, n = No. of set

Superoxide anion is oxygen centered radical with selective reactivity. It can also reduce certain Iron complexes such as cytochrome (Gulcin *et al*., 2005). Superoxide radical (O^{-}_{2}) was generated from the photoreduction of riboflavin and was deducted by nitroblue tetrazolium dye (NBT) reduction method. Superoxide dismutase enzymes catalyses the breakdown of superoxide radical. Standard shows maximum scavenging 83.4% at 128 μg mL^{-1} concentration while sample shows maximum scavenging 73.1±0.54 at128 μg mL^{-1}. Table 5 shows the % scavenging of methanolic extracts and standards by iron chelating activity assay, iron is essential for life. It caused lipid peroxidation and decomposes the lipid hydroxide into peroxyl and alkoxyl radicals that can perpetuate the chain reaction (Halliwell, 1991). The principle is based on the formation of O-phenanthroline-Fe^{2+} complex and its disruption in the presence of chelating agents. Standard shows maximum scavenging 89% at 1000 μg mL^{-1} concentration while sample shows maximum scavenging 63.9±0.40 at1000 μg mL^{-1}. Table 6 shows the IC_{50} values of all the assays.

Antioxidant activity shown by plant is due to presence of phytochemicals. In our plant this activity was due to presence of flavonoids whose percentage was maximum in leaf as compared to other plant parts. Antioxidant activities of *Datura* has been reviewed and found that leaf exhibit higher antioxidant activity than bark by Kumar *et al.* (2010) and Akharaiyi (2011). It has been shown that the scavenging effects on the DPPH radical increases sharply with the increasing concentration of the samples and standards to a certain extent and hence are said to be strongly dependent on the extract concentration. Also strong relationship between total phenolic content and antioxidant activity has been reported (Sharma *et al.*, 2013a, b; Bhardwaj *et al.*, 2014; Yadav *et al.*, 2013). The scavenging activities of the studied plant could, at least partly, justify the traditional anti-inflammatory use, as phenolics including flavonoids, are known for their antioxidant properties, which could be partially involved in anti-inflammatory mechanisms (Sala *et al.*, 2003). First they act as antioxidants against free radicals which can attract various inflammatory mediators contributing to a general inflammatory response and tissue damage (Nijveldt *et al.*, 2001). Second, the anti-inflammatory activity of flavonoids may be due to a decrease in the activation of the Nuclear Factor jB by ROS, such as HOCl and H_2O_2 which induce the transcription of inflammatory cytokines and cyclo-oxygenase-2 implicated in inflammatory mechanisms *in vivo* (Schinella *et al.*, 2002).

CONCLUSION

Nowadays, there is fairly enough evidence that antioxidants present in foods or plants play a relevant role in the prevention of disease and maintenance of health. With the above study it is proven that *Datura stramonium* shows a good antioxidant activity, with these results it gives new perspectives for the study, The strong antioxidant activity found could lead to the use of this species in the food industry, to prevent rancidity and increase shelf life of the products, or in pharmaceutical industry in anti-ageing formulations. It could also be necessary that full structural identification of the active components of antioxidant compounds of plants is, therefore, required and their toxicological properties be investigated.

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