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In-vitro antioxidant activity of *Datura stramonium* L. leaves

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ABSTRACT

Plants contain various secondary metabolites such as saponin, steroids, flavonoids, and alkaloids, which have shown antioxidant activity that includes scavenging free radical species, inhibiting the production of reactive species, inhibiting the production of reactive species resulting from normal cell metabolism. The present study was undertaken to analyse the antioxidant activity of various extract of Datura stramonium leaves. Standard methods were adopted to assess antioxidant activity and phytochemical nature of the plant materials. The extent of radical scavenging was determined by calculated IC_{50} value. The results revealed that hexane, aqueous, ethyl acetate and ethanol extracts showed good antioxidant activity when compared to Ascorbic acid standard. The ethyl acetate soluble fractions have shown the maximum activity among all.

Key words: *Datura stramonium*, leaves, DPPH assay, secondary metabolites, Antioxidant, IC_{50}.

INTRODUCTION

Oxygen is an essential element for life to perform biological functions such as catabolic and anabolic process of fats, proteins and carbohydrates in order to generate energy for growth and other activities of the cell. Although oxygen is not dangerous by itself, but is involved in the generation of various kinds of "reactive oxygen species" (ROS). ROS can interact with biomolecules and ultimately lead to free radical chain reactions. Free radical chain reactions are produced in the mitochondrial respiratory chain, liver mixed function oxidase, xanthine oxidase activity, atmospheric pollutants and for transition metal catalysts, drugs and xenobiotics [1, 2]. ROS attacks the unsaturated fatty acids present in the biomembranes resulting in membrane lipid peroxidation, a decrease in membrane fluidity, loss of enzymes and receptor activity and damage to membrane protein leading to cell inactivation [3], mutation leading to cancers [4]. ROS also leads to pathological conditions such as ischemia, anemia, asthma, arthritis, inflammation, neurodegenration, Parkinson's diseases, mongolism, ageing process and dementia. Antioxidants are used in the treatment of diseases caused by ROS. Antioxidants are composed of a group of compounds and enzymes potent enough to scavenge free radicals before they cause tissue damage [5]. Vitamin E, vitamin C, carotenoids, natural flavonoids etc., are the natural antioxidants are produced in the body while others must be sequestered from the diet or through supplementation. Most antioxidants were found in citrus and dried fruits, cruciferous vegetables, garlic, onions, carrots, tomatoes, sweet potatoes, sesame and olive oil. There are thousands of naturally occurring and synthetic antioxidants known; these antioxidants belong to different classes of compounds and may cause some side effects [6]. Plant secondary metabolites such as phenolic compounds, carotenoid, ascorbic acid, thiols and tocopherols have shown antioxidant activity that includes scavenging free radical species, inhibiting the production of reactive species, inhibiting the production of reactive species resulting from normal cell metabolism. Thereby prevent the damage to lipids, proteins, nucleic acids and subsequent cellular damage and death [7].

Datura stramonium is a plant belonging to the family Solanaceae and commonly known as Jimson weed, "Haukata-yaro" in Hausa. Medically it has been used in the treatment of madness, epilepsy, burns and rheumatism [8]. *D.stramonium* contains hyoscine, as well as atropine, hyoscyamine, apohyoscine, and meteloidine, thus it is poisonous and hallucinogenic as well as acting as analgesic, and it is also used as mosquito repellent [9]. The

anticholinergic property of the plant results in the inhibition of central and peripheral muscarinic neurotransmission. Leaves of this plant are used as wound healers, anti-inflammatory agent and applied topically [10]. It may have some antioxidant compounds. Hence leaf extract of this plant extract and fractions were subjected for antioxidant screening.

MATERIALS AND METHODS

Plant material

The leaves of *Datura stramonium* were collected from the vegetative land at Ladapuram. Perambalur. The leaves were identified by Prof. John Britto, Department of Botany, St. Joseph's College, Thiruchirapalli, Tamilnadu, India. The leaves of *Datura stramonium* were shade dried at room temperature, coarsely powdered and stored in air tight container till further use.

Preparation of Extracts

Powdered plant material (150gm) was extracted with water, alcohol, ethylacetate and hexane using cold maceration method. All the extracts were filtered with a muslin cloth and the filtrate was concentrated in vacuum evaporator. Dried extracts were used for further studies [11].

Phytochemical analysis

The aqueous and alcoholic extracts of *Datura stramonium* fruit pulp were studied for their phytoconstituents using different phytochemical tests[12].

In-vitro antioxidant assay

A great number of *in vitro* methods have been developed to measure the efficiency of natural antioxidants either as pure compounds or as plant extracts. *α,α-diphenyl-β-picrylhydrazyl* radical scavenging assay (DPPH), Ferric reducing antioxidant power (FRAP), Nitric oxide radical scavenging assay, Superoxide anion radical scavenging assay, ABTS radical scavenging assay, Hydroxyl radical scavenging assay, are the *in vitro* antioxidant assay methods used to assess the antioxidant activity of the leaves extract of *Datura stramonium* [13, 14, 15, 16, 17 ,18].

Statistical analysis

All data were expressed as mean±SD. Statistical analysis was performed by One-way ANOVA using Origin version 6.0 software and $p<0.05$ and p,0.001was considered as statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening of leaf extracts of *Datura stramonium* showed positive results for phenol, tannins, saponins, flavonoids and alkaloids (Table 1). Phenol and phenolic compounds such as flavonoids have been shown to possess significant antioxidant activity [19]. Phenolics are the mostly wide spread secondary metabolite in the plant kingdom. These diverse groups of compounds have potential of natural antioxidant and have ability to act as both efficient radical scavengers. The antioxidant activity of phenols is due to their redox properties, hydrogen donors and singlet oxygen quenchers [20]. The antioxidative characteristics might be attributed to the presence of phytochemical such as flavonoids and other phenolic compounds. Poly phenols have been known to show medicinal activity as well as exhibiting physiological activity. The compounds such as flavonoids; which contain hydroxyls are responsible for the radical scavenging activity in plant [21, 19, 2,].

Table 1 Qualitative Phytochemical analysis *Datura stramonium* leaf extracts

S. No	Phytoconstituents	Aqueous Extract	Alcoholic extract	Ethylacetate Extract	Hexane Extract
1	Alkaloids	Negative	Positive	Negative	Negative
2	Steroids	Positive	Positive	Positive	Positive
3	Terpenoids	Positive	Positive	Positive	Positive
4	Flavonoids	Positive	Positive	Positive	Positive
5	Saponins	Positive	Negative	Positive	Negative
6	Phenolic compounds	Positive	Positive	Positive	Positive
7	Tannins	Positive	Positive	Positive	Positive
8	Lignin	Positive	Positive	Positive	Positive
9	Phlobatannins	Negative	Negative	Negative	Negative
10	Coumarins	Positive	Positive	Negative	Negative
11	Cardiac glycosides	Positive	Negative	Negative	Negative

The reducing capacity of a compound may be used as a significant indicator of its potential antioxidant activity [22]. Reducing power is to the measure of the reductive ability of antioxidant and it is judged by the transformation of Fe^{3+} to Fe^{2+} in the presence of extracts [23]. The reduction power of aqueous and other extracts was summarized in Table 2. The data showed that reducing power of the extracts increased with increased concentration of extracts. The extracts showed potent ferric reducing power. Ethanol & ethyl acetate extract showed 50.33±2.08% and 28.7±12.05% reducing power at 100µg/ml concentrations respectively. However, the activity was found to be less when compared to the standard. IC_{50} value for ethanol extract was found to be 158.99±59.46µg/ml; ethyl acetate extract was 283.06± 135.80µg/ml and 34.62 ± 9.37µg/ml for standard.

Table 2: *In vitro* Free Radical scavenging effect of *Datura stramonium* leaves by reducing power assay

Extracts	Concentration / Percentage of Scavenging					IC50 µg/ml
	20mg/ml	40mg/ml	60mg/ml	80mg/ml	100mg/ml	
Aqueous	07±8.21***	8.73±2.10**	19.11±1.7*	33.21±3.34*	52.42±0.20*	176.17±0.23
Ethanol	5.35±0.66**	8.47±0.81**	17.44±1.36**	31.13±0.80*	50.33±2.08*	158.995±59.463
Ethyl acetate	1.95±1.3***	7.04±2.2***	11.94±1.79***	20.73±8.4***	28.7±12.05***	283.0678±35.801
Hexane	9±8.21***	12.73±2.1**	21.11±1.7*	35.21±3.34*	54.42±0.20*	198.217±4.12
Standard	25±5.26***	32.91±2.0**	37.31±0.97*	48.92±2.15*	60.00±1.60*	34.627±9.377

The aqueous and other extract of leaves of *Datura stramonium* showed significant free radical scavenging action against nitric oxide (NO) induced release of free radicals at the concentration 100µg/ml. Ethanol extract showed 63.74±5.54% inhibition, ethyl acetate extract yielded 52.02±5.37 % inhibition but Ascorbic acid showed 28.64±0.67% of inhibition (Table 3). The available nitric oxide radical is linked with various carcinomas and inflammatory conditions [24]. The nitric oxide generated from sodium nitroprusside reacts with oxygen to form nitrite. The extract directly competes with oxygen to react with nitric oxide and thereby inhibits nitrite formation. The present study proved that the nitric oxide scavenging activity of the extract is better than the standard.

Table 3: *In vitro* Free Radical scavenging effect of *Datura stramonium* leaves by nitric oxide scavenging assay

Extracts	Concentration / Percentage of Scavenging					IC50 µg/ml
	20mg/ml	40mg/ml	60mg/ml	80mg/ml	100mg/ml	
Aqueous	21.11±0.14**	23.06±1.50**	28.12±3.34*	27.33±2.45	65.36±1.81*	96.19±54.26
Ethanol	19.3±0.8*	21.75±1.4**	26.75±1.0*	25.15±2.4**	63.74±5.54**	98.680±40.238
Ethyl acetate	11.02±2.08***	17.11±1.83**	19.33±0.51*	55.35±1.43*	52.02±5.37**	106.243±31.651
Hexane	23.4±1.8*	25.22±0.54**	30.17±1.22*	29.41±3.1**	67.80±4.0**	94.30±44.54
Standard	43.29±2.94**	37.16±2.06**	31.52±1.02*	30.23±1.34*	28.64±0.67*	47.899±30.195

Superoxide anion is harmful reactive oxygen species as it damages cellular components in biological systems [25]. Standard (Ascorbic acid) showed better superoxide radical quenching activity (21.19± 9.36%) at 50µg/ml concentration. The aqueous and other extracts showed potent superoxide radical scavenging activity, 91.41±17.36% for ethanol and 147.89±44.86% for ethyl acetate extract at 100µg/ml concentration. The results suggested that the plant extract is a superoxide radical scavenger but efficiency is low compared to standard. PMS-NADH coupling reaction accelerates the yield of superoxide radicals from dissolved oxygen (Table 4).

Table 4: *In vitro* Free Radical scavenging effect of *Datura stramonium* leaves by superoxide radical scavenging assay method

Extracts	Concentration / Percentage of Scavenging					IC50 µg/ml
	20mg/ml	40mg/ml	60mg/ml	80mg/ml	100mg/ml	
Aqueous	18.14±2.20**	25.77±3.35*	31.42±1.52*	40.22±6.0**	56.06±5.41*	88.53±5.14
Ethanol	15.8±1.05**	22.41±1.0*	28.28±1.1*	37.99±2.6**	53.68±1.7*	91.410±17.365
Ethyl acetate	12.34±2.72***	14.74±1.03**	20.48±1.6**	22.43±2.13**	25.26±1.13*	147.85±44.86
Hexane	21.4±4.16**	28.21±1.22*	34.32±3.31*	43.35±2.55**	59.56±2.31*	85.30±15.05
Standard	52.61±2.63***	64.93±5.77**	72.54±0.80*	73.69±1.85*	75.21±1.43*	21.192±9.36

Effective ABTS radical scavenging process was exhibited by the extracts of *D. stramonium* leaves 94.36±1.42% of radical scavenging activity was exhibited by ethanol extract of *D. stramonium*. Similarly ethyl acetate extract showed 95.12±4.37% inhibitions at 100µg/ml concentrations (Table 5).

Table 5: *In vitro* Free Radical scavenging effect of *Datura stramonium* leaves by ABTS radical scavenging assay method

Extracts	Concentration / Percentage of Scavenging					IC50 µg/ml
	20mg/ml	40mg/ml	60mg/ml	80mg/ml	100mg/ml	
Aqueous	94.32±0.62***	94.52±03.64***	95.21±7.52 ***	96.63±1.93**	95.43±2.93 ***	39.45±2.92
Ethanol	93.01±2.12***	93.54±3.31 ***	93.31±0.73 ***	95.40±1.34***	94.36±1.42 ***	37.11±13.50
Ethyl acetate	85.77±7.11***	90.53±5.97***	91.57±1.40***	71.34±24.10***	95.12±4.37***	35.02±19.16
Hexane	95.52±3.02***	94.27±53.14**	96.81±1.22 ***	98.33±2.63**	96.23±4.03 ***	40.37±3.85
Standard	78.99±6.83***	89.58±2.11***	89.18±4.43***	90.51±3.6***	94.45±3.77***	16.575±8.10

The extracts were also capable of scavenging Hydrogen peroxide in a dose dependent manner & reached 73.77±3.67% for ethanol extract & 69.0±16.40% for ethyl acetate extract at a concentration of 100µg/ml (Table 6). Hydrogen peroxide itself is not very reactive; it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important throughout the systems of human [26]. IC_{50} for scavenging of H_2O_2 were 56.53±12.44 for ethanol extract, 52.19±18.37 for ethyl acetate extract and 26.67±7.51µg/ ml for standard. Scavenging of H_2O_2 by extracts may be attributed to their phenolic compounds, which can donate electrons to H_2O_2, thus neutralizing it to water [27].

Table 6: *In vitro* Free Radical scavenging effect of *Datura stramonium* leaves by H_2O_2

Extracts	Concentration / Percentage of Scavenging					IC50 µg/ml
	20mg/ml	40mg/ml	60mg/ml	80mg/ml	100mg/ml	
Aqueous	41.23±4.26**	53.32±4.811*	54.62±3.04*	67.73±4.46*	75.46±5.29*	58.54±4.62
Ethanol	39.38±2.66**	51.51±1.19*	51.40±1.56*	65.39±3.02*	73.77±3.67*	56.535±12.44
Ethyl acetate	31.70±14.79***	55.76±2.46***	56.39±2.19***	58.93±0.53***	69.0±16.40***	52.190±18.36
Hexane	43.53±6.06**	55.25±5.09*	56.24±4.16*	69.93±0.83*	77.05±4.81*	59.34±2.94
Standard	33.25±2.71**	43.06±2.27*	49.11±2.91**	62.88±2.42*	76.27±7.90**	26.677±7.51

The result of DPPH scavenging activity assay in this study indicates the ethyl acetate extract was potentially active. The aqueous and other extracts produced more or less similar DPPH anion scavenging power of 44.36±2.09% ethyl acetate & 40.12±5.36% ethanol extract at 100µg/ml concentration with 92.648±30.68µg/ml of IC_{50} for ethyl acetate extract & 106.15 ±25.33µg/ml of IC_{50} value for ethanol extract and 63.99 ±25.24µg/ml for Ascorbic acid (Table 7). The scavenging activity of ethyl acetate soluble fractions compared with the standard drug ascorbic acid suggests that the plant phytochemicals are a potent scavenger of free radicals. However further study aimed at characterization of active constituents responsible for antioxidant activity. Overall, the ethyl acetate extract of *Datura stramonium* Linn leaves have most potent antioxidant activity.

Table 7: *In vitro* Free Radical scavenging effect of *Datura stramonium* leaves by DPPH method

Extracts	Concentration / Percentage of Scavenging					IC50 µg/ml
	20mg/ml	40mg/ml	60mg/ml	80mg/ml	100mg/ml	
Aqueous	31.23±4.26**	33.32±4.811*	34.62±3.04*	37.73±4.46*	47.46±5.29*	84.54±4.62
Ethanol	21.33±2.08**	25.06±1.00*	30±4.35**	32.3±3.78**	44.36±2.09*	92.648±30.368
Ethyl acetate	13.40±4.01***	22.48±2.04***	28.35±8.95***	29.25±9.47***	40.12±5.36***	106.158±25.332
Hexane	33.53±6.06**	35.25±5.09*	36.24±4.16*	39.93±0.83*	50.25±4.81*	81.34±2.94
Standard	4.94±6.85***	12.77±0.96**	27.96±1.41*	47.72±5.37**	55.67±2.32*	63.997±25.244

*Significant at **p=<0.05; ***p=<0.001*

CONCLUSION

On the basis of the results it is concluded that the extracts contain higher quantities of phenolic compounds, which exhibit antioxidant and free radical scavenging activity. It also chalets iron and possesses reducing power. *In vitro* assay systems confirm *Datura stramonium* leaves as natural antioxidants. Further *In vivo* assessment also needed to confirm the antioxidant nature of *Datura stramonium* leaves.

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