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Original Article

Antioxidant and Anticancer Activities of Methanol Extract of Seeds of *Datura stramonium* l.

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ABSTRACT

Background: *Datura stramonium* belongs to the family Solanaceae which is commonly dubbed as thorn apple or jimson weed, and is abundant throughout tropical and subtropical regions. *Datura* seeds and leaves are used as anti-asthmatic, antispasmodic, hypnotic and narcotic. Externally, the jimson seeds are used in treating fistulas and abscesses. It has been used by the British soldiers to treat respiratory problems. *Datura* has also finds its utility in Ayurveda for asthma symptoms where jimson weed's leaves are smoked in cigarette or pipe. **Objectives:** Aim of the present study was to investigate the antioxidant and antiproliferative activities of methanol extract of seeds of *D. stramonium*. **Materials and Methods:** Antioxidant assays such as $DPPH^{\cdot}$ radical, superoxide radical, $ABTS^{\cdot+}$ radical cation, $OH^{\cdot}$ radical scavenging assays, Phoshomolybdenum reduction and Fe^{3+} reducing power assays were investigated. Thin layer chromatography was performed to find out compounds present in the methanol extract. *in vitro* cytotoxic activity for MCF7 (breast cancer) cell line was too studied by MTT reagent assay method. **Results:** IC_{50} values of $DPPH^{\cdot}$ radical, superoxide radical, $ABTS^{\cdot+}$ radical cation, $OH^{\cdot}$ radical scavenging assays were 35.26, 10.50, 49.36 µg/mL concentration respectively. TLC analysis showed the presence of compounds with R_f values of 0.76 and 0.58 in the methanol extract of D. *stramonium*. Cytotoxic activity for MCF7 cell line was 66.84% at 500 µg/mL concentration of methanol extract. **Conclusion:** Here Current study corroborates that *D. stramonium* is enriched with fine source of significant natural oxidants with anticancer agents and has therapeutic potential for treatment of diseases, induced by oxidative stress, Further research work is required to isolate active compounds for clinical trials.

Key words: Datura, Antioxidant, Cytotoxicity, MCF7 cell line, MTT assay.

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INTRODUCTION

Datura stramonium is commonly known as Jimson weed or *Datura* belongs to family Solanaceae. Datura refers to species of shrubby herbaceous plants which produces large, white or purple trumpet-shaped flowers and often called angel's trumpet. It is 60-120 cm or more tall, branched and pubescent plant. Seeds are used as purgative, in cough, fever and asthma. Seeds are used for smoking for its narcotic action.[1] The primary biologically active substances in *Datura stramonium* are the alkaloids - atropine and scopolamine. Atropine has been used in treating Parkinson's disease, peptic ulcers, diarrhea and bronchial asthma.[2] Recent Insights have found role of anticancer potential of Nutraceuticals.[3] patients receiving anthracycline based neoadjuvant chemothearpy for breast cancer.[4] optimisation of Antioxidants from Nelumbo seeds,[5] and coumarin derivatives as potential antioxidant agents.[6] manifest the potential of antioxidants having an therapeutic potential in treating various diseases. Seeds and leaves of *D. stromonium* used to treat psychotic patients, insomnia and depression, Relax the smooth muscles of the bronchial tube and asthmatic bronchial spasm. *D. stramonium* is a plant with both poisonous and medicinal properties. Studies too have demonstrated that it has great pharmacological potential with great value and usage in folklore. Seeds of *Datura* are used in the treatment of analgesic, anthelmintic and anti-inflammatory, intestinal pain, infestation, toothache, and fever from inflammation.

Collection and authentication of plant material

To carry out this Research work, plant materials viz., seeds of *D. stramonium* were collected from District Pulwama flanked by District Shopian of Jammu and Kashmir, India, lying at an latitude of 33□ 72 N and a longitude of 74°53 E. It is situated in the laps of foot hills of Pir panjal Range, being a hilly terrain-it has an average elevation of 2057 meter.[7] The plant was authenticated by Prof. Dr. N. Raaman, Director, CAS in Botany, University of Madras, Chennai, India. Seeds were thoroughly washed and dried in shade for 1 day. Dried seeds were made into coarse powder and stored in air tight container till further use

A. Habitat of *Datura stramonium*

B. Seeds of *Datura stramonium*

Preparation of extract

The seed powder (100 g) were soaked in methanol and extracted by maceration method for 72 h. Then, the supernatant was filtered by filter paper. Soaking process was repeated once again in the same powder and the supernatant was filtered. All the supernatant was collected together and concentrated using rotary evaporator which yielded greenish-black coloured sticky residue.

Thin layer chromatography

Thin layer chromatography (TLC) was carried out for methanol extract of seeds of *D. stramonium* in TLC aluminium sheet (Merck), silica gel 60 F_{254} (4 × 1 cm), precoated plates.[8] The methanol extract was spotted at 0.2

mm above from the bottom of the TLC plate. The chromatogram was developed in toluene: chloroform (9:1) solvent system. The spots were visualized under UV light at 254 nm. The R_f values of the coloured spots were calculated.[9]

$$R_f = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent}}$$

Phytochemical screening

The methanol extract of seeds of *D. stramonium* was subjected to preliminary phytochemical analysis using standard methods.[10] Methanol extract was screened for different classes of phytoconstituents such as alkaloids, steroids, terpenoids, phenolic compounds, flavonoids, saponins and glycosides using specific standard reagents.[11]

Antioxidant activities

DPPH radical scavenging assay

The antioxidant activity of methanol extract of seeds of *D. stramonium* was measured on the basis of the radical scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical according to the method of Brand-Williams *et al.*[12] with slight modifications. One mL of 0.1 mM DPPH solution in methanol was mixed with 1 mL of petroleum ether fraction of varying concentrations (10-60 µg/mL). Ascorbic acid was used as reference standard. Mixer of 1 mL methanol and 1 mL DPPH solution was used as control. The decrease in absorbance was measured at 517 nm after 30 min in dark using UV-Vis spectrophotometer. The percentage of DPPH radical inhibition was calculated as

$$\% \text{ of DPPH radical inhibition} = \frac{\text{Control - Sample}}{\text{Control}} \times 100$$

Superoxide radical scavenging assay

Superoxide radicals were generated by a modified method of Beauchamp and Fridovich. The assay was based on the capacity of methanol extract of seeds of *D. stramonium* to inhibit farmazan formation by scavenging the superoxide radicals generated in riboflavin-light-NBT system. Each 3 mL reaction mixture contained 50 mM sodium phosphate buffer (pH 7.6), 20 mg riboflavin, 12 mM EDTA, 0.1 mg of NBT and various concentrations (10-60 µg/mL) of methanol extract. Reaction was started by illuminating the reaction mixture with extract for 90 seconds. Immediately after illumination the absorbance was measured at 590 nm. The percentage inhibition of superoxide radical generation was calculated as

$$\% \text{ of superoxide radical inhibition} = \frac{\text{Control - Sample}}{\text{Control}} \times 100$$

$ABTS^{+}$ radical cation scavenging assay

Antioxidant capacity was evaluated in terms of the $ABTS^{+}$ radical cation scavenging activity, by the method of Delgado-Andrade *et al.*[13] Briefly, $ABTS^{+}$ was obtained by reacting 7 mM ABTS stock solution with 2.45 mM potassium persulfate and the mixture was left to stand in the dark at room temperature for 12-16 h before use. ABTS solution (stable for 2 days) was diluted with 5 mM phosphate-buffered saline (pH 7.4) to an absorbance of 0.70 ± 0.02 at 730 nm. Various concentrations (2 - 12 µg/mL) of 1 mL of methanol extract of seeds of *D. stramonium* were mixed with 1 mL of diluted $ABTS^{+}$ solution and the absorbance was measured after 10 min. The $ABTS^{+}$ radical cation scavenging activity was expressed as

$$\% \text{ of } ABTS^{\bullet+} \text{ radical cation inhibition} = \frac{\text{Control - Sample}}{\text{Control}} \times 100$$

Hydroxyl ($OH^{\cdot}$) radical scavenging assay

Various concentrations (2-12 µg/mL) of methanol extract (1 mL) of seeds of *D. stramonium* were added with 1.0 mL of iron-EDTA solution (0.13% ferrous ammonium sulphate and 0.26% EDTA), 0.5 mL of EDTA solution (0.018%), and 1.0 mL of dimethyl sulphoxide (DMSO) (0.85% v/v in 0.1 M phosphate buffer, pH 7.4). The reaction was initiated by adding 0.5 mL 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 of 1.0 mL of ice-cold TCA (17.5% w/v). An amount of 0.5 mL of Nash reagent (75.0 g of ammonium acetate, 3.0 mL of glacial acetic acid, and 2 mL of acetyl acetone were mixed and made up to 1 L with distilled water) was added and left at room temperature for 15 min. The reaction mixture without sample was used as control. intensity of the colour formed was measured spectroscopically at 412 nm. Ascorbic acid was used as the reference standard. The percentage of inhibition was calculated,[14] using the following formula

$$\% \text{ of } OH^{\bullet} \text{ radical inhibition} = \frac{\text{Control - Sample}}{\text{Control}} \times 100$$

Nitric oxide ($NO^{\cdot}$) radical scavenging assay

Nitric oxide was generated from sodium nitroprusside and measured by Griess' reaction as described Green *et al.*[15] Sodium nitroprusside (5 mM) in phosphate buffer saline solution (0.025 M, pH: 7.4) was incubated with different concentrations of methanol extract of seeds of *D. stramonium* dissolved in phosphate buffer saline (0.025 M, pH: 7.4) and the tubes were incubated at 25°C for 3 h. Control experiments without the test compounds but with equivalent amounts of buffer were conducted in an identical manner. After 3 h, 0.5 mL of incubation solution was removed and diluted with 0.5 mL of Griess' reagent (1% sulphanilamide, 2% o-phosphoric acid and 0.1% napthyl ethylene diamine dihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthyl ethylenediamine was read at 546 nm. The experiment was repeated in triplicate.

$$\% \text{ of } NO^{\bullet} \text{ radical inhibition} = \frac{\text{Control - Sample}}{\text{Control}} \times 100$$

Ferric (Fe^{3+}) reducing power assay

The reducing power assay of methanol extract of seeds of *D. stramonium* was carried out by the method of Yen and Chen with slight modification.[16] One mL each of various concentrations of methanol extract (20 - 120 µg/mL) were mixed with 1 mL of 0.2 M phosphate buffer (pH 6.6) and 1 mL of 1% solution of potassium ferricyanide. Mixture was incubated at 50°C for 20 min, during which period ferricyanide got reduced to ferrocyanide, followed by the addition of 1 mL of 1% trichloroacetic acid to the mixture, followed by the addition of 0.1% $FeCl_3$, and the absorbance at 700 nm was measured to determine the amount of formation of ferric ferrocyanide (prussian blue). Increased absorbance of the reaction mixture signified the increase in reduction of ferricyanide.

Phosphomolybdenum reduction assay

The antioxidant capacity of the methanol extract of seeds of *D. stramonium* was assessed by the method of Sivaraj *et al.*[17] Methanol extract (10-60) µg/mL was combined with 1 mL of reagent solution containing ammonium molybdate (4 mM), sodium phosphate (28 mM) and sulphuric acid (600 mM). Reaction mixture was incubated in a water bath at 90°C for 90 min. Absorbance of the colored complex was measured at 695 nm. Ascorbic acid was used as standard reference. Increased

absorbance of the reaction mixture indicates the increase in phosphomolybdenum reduction.

Cytotoxicity activity
Cell line and culture.

Human breast cancer MCF7 cell lines were obtained from National center for cell sciences Pune (NCCS). The cells were maintained in RPMI-1640 supplemented with 10% FBS, penicillin (100 U/mL), and streptomycin (100 µg/mL) in a humidified atmosphere of 50 µg/mL CO_2 at 37°C.

Reagents

RPMI-1640 was purchased from GIBCO/BRL Invitrogen (Caithershurg, MD). Fetal bovine serum (FBS) was purchased from Gibco laboratories Trypsin, methylthiazolyl diphenyl- tetrazolium bromide (MTT), and dimethyl sulfoxide (DMSO) were purchased from (Sisco research laboratory chemicals Mumbai). All of other chemicals and reagents were obtained from Sigma Aldrich Mumbai.

MTT assay

The Cytotoxicity of extract of seeds of *D. stramonium* on MCF7cells was determined by the MTT assay according to the method of Mosmann.[18] Cells (1 × 105/well) were plated in 100 µL of medium/well in 96-well plates. After 48 h incubation the cell reaches the confluence. Then, cells were incubated in the presence of various concentrations of the extract in 0.1% DMSO for 48 h at 37°C. After removal of the extract solution, cells were washed with phosphate-buffered saline (pH 7.4) and 20µL/well (5mg/mL) of 0.5% 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-tetrazolium bromide (MTT) in phosphate buffered saline solution was added. After 4 h incubation, 0.04 M isopropanol was added. Viable cells were determined by the absorbance at 570 nm with reference at 655 nm. Measurements were performed in 3 times, and the concentration required for a 50% inhibition of viability (IC_{50}) was determined graphically. Absorbance at 570 nm was measured with a microplate reader (Bio-Rad, Richmond, CA), using wells without sample containing cells as blanks. All experiments were performed in triplicate. The effect of the samples on the proliferation of human breast cancer cells was expressed as the % cell viability, using the following formula:

$$\% \text{ Cell viability} = A_{570} \text{ of treated cells} / A_{570} \text{ of control cells} \times 100\%.$$

RESULTS AND DISCUSSION

Antioxidants are chemicals that interact with and neutralizes free radicals, thus preventing damage of cells from radicals. Antioxidants are also known as "free radical scavengers" and body makes some of the antioxidants to neutralize free radicals. These antioxidants are called endogenous antioxidants. However, the body relies on external (exogenous) sources, primarily the diet, to obtain the rest of the antioxidants it needs. These exogenous antioxidants are commonly called dietary antioxidants. Antioxidants benefit the body by neutralizing and removing the free radicals from the blood stream, Fruits, vegetables and grains are rich sources of dietary antioxidants.[19,20] Oxidative stress-the consequence of an imbalance of pro-oxidants and antioxidants in the organism and is a key phenomenon in chronic diseases. Oxidative stress is now recognized to be associated with more than 100 diseases, as well as with the normal aging process, especially people diagnosed with Alzheimer's disease (AD).[21] Antioxidants are intimately involved in the prevention of cellular damage the common pathway for cancer, aging, and a variety of diseases.

Thin layer chromatography (TLC)

TLC analysis was carried out for methanol extract of *seeds* of *D. stramonium* by using toluene: chloroform with the ratio of (2 mL) as the solvent. The separated bands were visualized by UV light at 254 nm. The R_f values of the separated compounds were measured (Figure 1b).

DPPH radical scavenging assay

Ability of methanol extract of *D. stramonium* to scavenge free radicals was assessed using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). Methanol extract of *D. stramonium* has capacity to scavenge free radicals as shown by the data by reducing the stable DPPH (1,1-diphenyl-2-picrylhydrazyl) radical to the yellow coloured 1,1-diphenyl-2-picrylhydrazine. Capacity of DPPH reduction increases with increasing concentration of the extract as reported earlier by Raaman *et al*.[22] Maximum DPPH radical scavenging activity was 59.50% at 120 µg/mL concentration (Table 1a). It was compared with standard ascorbic acid and the IC_{50} of DPPH radical scavenging activity was 94.87 µg/mL concentration (Table 2a). Scavenging ability of the petroleum ether fraction of *seeds* of *D. stramonium* may be due to its bio compositions such as phenolic acids and flavonoids. Radical scavenging activities of the extracts were determined by using DPPH a stable free radical at 517 nm. 1,1-diphenyl-2-picrylhydrazyl is a nitrogen centered free radical, color of which changes from violet to yellow on reduction by donation of H or e^- by the methanol extract of seeds of *D. stramonium*

Superoxide radical scavenging assay

Superoxide anion radical is one of the strongest ROS among the free radicals and get converted to other harmful reactive oxygen species such as hydrogen peroxide and hydroxyl radical which are damaging proteins in the cells which results in chronic diseases .The maximum inhibition of superoxide radical was found to be 53.17% at 60 µg/mL concentration of methanol extract of seeds of *D. stramonium* (Figure 2b). The methanol extract was found to be an effective scavenger of superoxide radical generated by photo reduction of riboflavin.

$ABTS^{\cdot+}$ radical scavenging assay

In the total antioxidant activity, $ABTS^{\cdot+}$ is a blue chromophore produced by the reaction between ABTS and potassium persulfate and in the presence of the plant extract or ascorbic acid, $ABTS^{\cdot+}$ cation radical gets reduced and the remaining radical cation concentration after reaction with antioxidant compound was then quantified.[23] The maximum $ABTS^{\cdot+}$ radical cation scavenging activity was 63.83% at 60 µg/mL concentration (Table 3a). It was compared with standard ascorbic acid. This demonstrates its high anti-oxidative activity with its IC_{50} at 41.10 µg/mL (Figure 3c). The authors viz., Kavimani *et al*.[24] reported the same kind of result in $ABTS^{\cdot+}$ radical cation scavenging activity, which was concentration dependent.

Hydroxyl ($OH^{\cdot}$) radical scavenging assay

Scavenging of hydroxyl radical is an important antioxidant activity because of very high reactivity of the OH radical which enables it to react with a wide range of molecules found in living cells, such as sugars, amino acids, lipids and nucleotides. Hydroxyl radical is an extremely reactive free radical formed in biological systems and has been implicated as a highly damaging species in free radical pathology, capable of damaging almost every molecule found in living cells.[25] Maximum OH radical scavenging activity was 57.88% at 30 µg/mL concentration (Table 3a). It was compared with standard ascorbic acid. This demonstrates its high anti-oxidative activity with its IC_{50} at 39.59 µg/mL (Figure 3c) concentration. The methanol extract of seeds of *D. stramonium* significantly inhibits generation of $NO^{\cdot}$ radicals in a dose dependent manner.

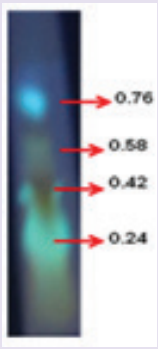

Figure 1b: TLC of methanol extract of seeds of *D.stramonium*

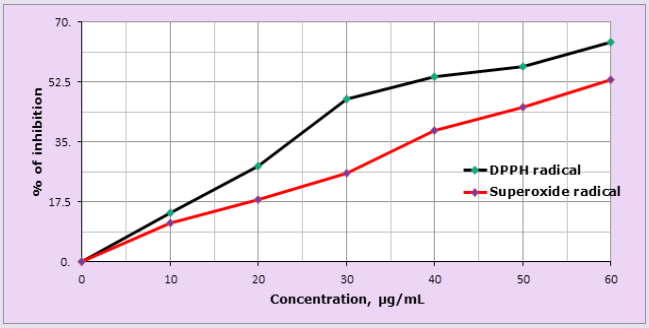

Figure 2b: Superoxide and DPPH radical Scavenging assay

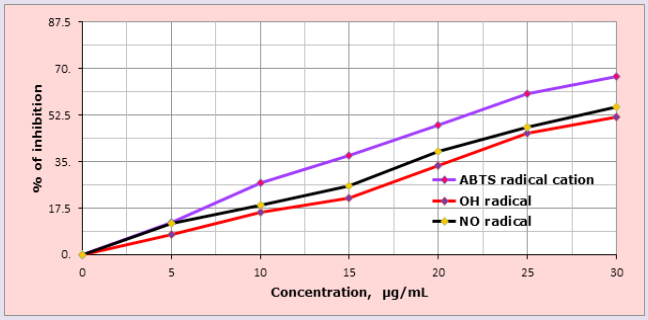

Figure 3c: ABTS.+, OH and NO radical scaverging assay

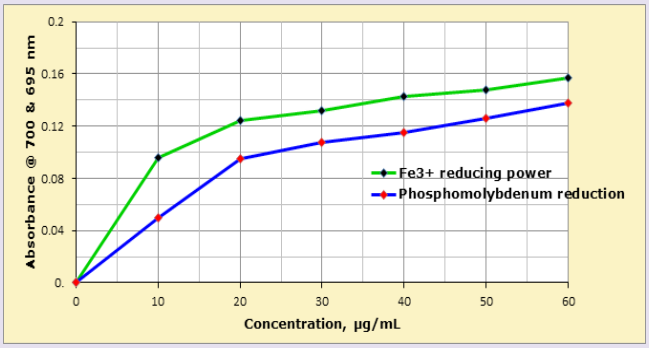

Figure 4a: Fe^{3+} reducing power and phosphomolybdenum reduction activity of methanol extract of seeds of *D.stramonium*

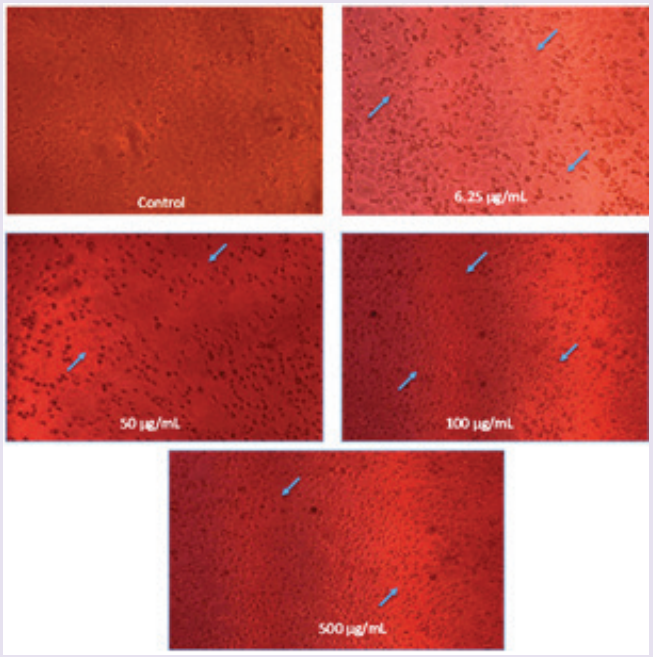

Figure 5a: Cytotoxic effects of the extract on MCF-7 breast cancer cell lines,Treated cells were rounded up and cell to cell adhesion was lost. Arrows show membrane blebbing.

Nitric oxide ($NO^{\cdot}$) radical scavenging assay

Relationship between chronic inflammation and tumorigenesis has long been suspected. It is well known that malignant tissues are infiltrated by leukocytes, which locally secrete cytokines, chemokines, matrix-degrading enzymes, growth factors, free radicals, and oxidants-leading to the creation of microenvironment, thereby enhancing cell proliferation, survival, and migration, as well as angiogenesis, thus promoting tumor.[26] A particularly important role of increased NO generation in this microenvironment is now well recognized as an essential step initiating neoplastic transformation.[27] In this spectrophotometric method, the absorbance of chromophore formed during the diazotization of the nitrile with sulfanilamide and the subsequent coupling with naphthyl ethylenediamine dihydrochloride was measured. NO reacts with superoxide anion and form a potentially cytotoxic molecule, the peroxynitrite (ONOO). Its protonated form, peroxynitrous acid (ONOOH), is a very strong oxidant. In context of post translational modifications, protein oxidative modification-nitration or hydroxylation of aromatic amino acid residue including Tyrosine, do play an pivotal role in redox stress conditions. Under physiological conditions, peroxynitrite also forms an adduct with carbon dioxide dissolved in body fluid and responsible for oxidative damage of proteins in living systems. The maximum $NO^{\cdot}$ radical scavenging activity was 53.03% at 60 µg/mL concentration (Table 3a) and inhibit nitric oxide radical in a dose dependent manner as reported earlier by Rana *et al.*[28] It was compared with standard ascorbic acid. The IC_{50} of $NO^{\cdot}$ radical scavenging activity was 53.54 µg/mL concentration (Figure 3c).

Ferric (Fe^{3+}) reducing power assay

Studies were made on total reduction ability of Fe^{3+} to Fe^{2+} transformation in the presence of petroleum ether fraction of *Datura stramonium* and found increasing in showing reduction ability in a dose dependent manner, with increasing concentrations. Since the reducing capacity of the methanol extract of seeds of *D. stramonium* serve as a significant indicator of its potential antioxidant activity and the reducing ability was

Table 1a. DPPH and superoxide radical scavenging activities of methanol extract of seeds of *D. stramonium*

S. No	Concentration µg/mL	Radical scavenging activity (%)	
		DPPH	Superoxide
1	10	14.28±0.99	11.28±0.78
2	20	27.89±1.95	18.23±1.27
3	30	47.57±3.32	25.86±1.81
4	40	54.08±3.78	38.27±2.67
5	50	57.14±3.99	45.26±3.16
6	60	64.28±4.49	53.17±3.72

Table 2a. DPPH radical scavenging assay of D.S Seeds

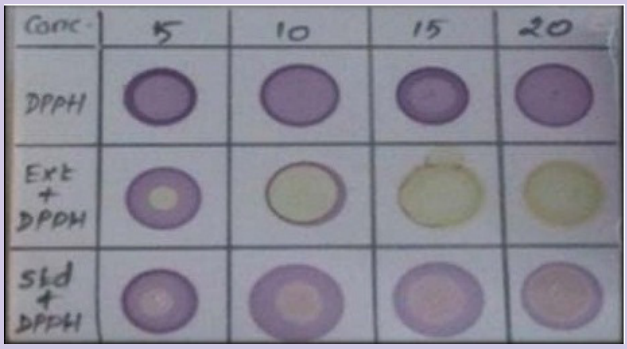

Table 3a: ABTS$^{\cdot+}$, OH and NO radical activities of methanol extract of seeds of *D. stramonium*

S. No	Concentration µg/mL	Radical scavenging activity (%)		
		ABTS$^{\cdot+}$	NO$^{\cdot}$	OH$^{\cdot}$
1	5	12.17±0.85	7.62±0.83	11.87±0.53
2	10	26.82±1.82	15.81±1.30	18.61±1.10
3	15	37.23±2.60	21.27±1.81	25.86±1.48
4	20	48.54±3.39	33.33±2.70	38.65±2.33
5	25	60.63±4.24	45.72±3.35	47.94±3.20
6	30	66.93±4.68	51.65±3.87	55.39±3.61

Table 4a: Fe^{3+} reducing power and phosphomolybdenum reduction activity of methanol extract of seeds of *D. stramonium*

S. No	Concentration µg/mL	Absorbance	
		Fe^{3+} reducing power assay (700nm)	Phosphomolybdenum reduction assay (69 5nm)
1	10	0.096±0.006	0.05±0.003
2	20	0.124±0.008	0.095±0.006
3	30	0.132±0.009	0.108±0.007
4	40	0.143±0.010	0.115±0.008
5	50	0.148±0.010	0.126±0.008
6	60	0.157±0.010	0.138±0.009

Table 5c: Cytotoxic activity of methanol extract of seeds of *D. stramonium* on MCF7 cell line

S. No	Concentration µg/mL	Cell death %
1	1.62	34.61
2	3.12	41.85
3	6.25	56.67
4	12.5	63.38
5	25.0	66.30
6	50.0	72.52
7	100.0	76.30
8	500.0	86.71

0.883 at 120 µg/mL concentration (Table 4a). It was compared with the standard (0.289) ascorbic acid (Figure 4c). The antioxidant activity has been reported to be concomitant with development of reducing power.[29]

Phosphomolybdenum reduction assay

The total antioxidant activity of methanol extracts of D. S seeds was measured spectrophotometrically by the phosphomolybdenum method, which is based on the reduction of Mo (VI) by the extract and the subsequent formation of green phosphate/Mo (V) complex at acidic pH, with a maximum absorption 695 nm. It evaluates both water-soluble and fat-soluble antioxidants with a high absorbance value of the extract indicated its strong antioxidant activity.[30] The maximum absorbance was 0.175 at 120 µg/mL concentration (Table 4a) in comparison with the standard (0.359) ascorbic acid (Figure 4c). A direct correlation between antioxidant activity and reducing power of certain plant extracts reported by the authors,[31] provides a substantial evidence for this assay.

Cytotoxic activity by MTT assay

Experiment on the cytotoxicity of methanol extracts of D. S seeds on human breast adenocarcinoma MCF-7 cells showed increasing cytotoxicity with increasing concentrations of extract and the viable cells detected by MTT assay.[32] Results depicted in Figure 5a &5b, summarize the cytotoxic effects of the extract and the concentration Vs % of cell viability on MCF-7 breast cancer cell lines respectively. The concentration dependent cytotoxic effect on this cell line has too been vouched by the data presented in Table 5c. The IC_{50} of methanol extract of seeds of on cytotoxic activity of breast adenocarcinoma (MCF-7) cell line was 113.05 µg/mL concentration and was defined by utilizing methodology of Spavierj *et al.*[33] The morphological representation of cytotoxicity of the extract on MCF7 cell line too have been provided in Figure 5a.

CONCLUSION

The results of the present work indicated that the methanol extract of D.S seeds is a potential source of natural antioxidants and significantly inhibit free radicals by dose-dependently. The difference in the antioxidant activity may be ascribed to their different group of phenolic and flavonoid compounds. the methanol extract of D.S seeds showed higher phenolic content contributes to the higher antioxidant activity. Based on the results obtained, it can be concluded that the plant contains essential phytochemical constituents and possess antioxidant property, could be beneficial for AD therapy. Moreover our studies do confirm that extracts exhibit cytotoxic effect on the breast cancer cell line MCF-7. Further investigations based on the clinical trial and animal model

will authenticate, the mechanism of action of constituents in disease prevention.

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CONFLICT OF INTEREST

No conflict of interest are declared.

ABBREVIATION USED

DPPH: 2,2-diphenyl-1-picrylhydrazyl; **MCF7:** Michigan Cancer Foundation-7; **DS:** Datura stramonium.

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SUMMARY

- DS extract possesses potential chemopreventive agents.
- Extract of DS has Antioxidant property
- DS extract showed efficacy for the cytotoxicity towards cell line MCF-7 cells, thus suggesting protection against Breast cancer.
- Antioxidant property of DS extract ameliorates its potential in treating AD.

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