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Research Article

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Methanol Extract of Seeds of Datura Stramonium L. Show Antioxidant Activities, Inhibit MCF-7 Cell Proliferation

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Abstract

Background: *Datura stramonium besides having several names, is commonly dubbed as thorn apple and jimsonweed weed, belongs to the family of Solanaceae and is found in many regions throughout tropical and subtropical regions. Datura seeds and leaves are used as antiasthmatic, antispasmodic, hypnotic and narcotic. Externally, the jimson seeds are used in treating of fistulas and abscesses. Datura has been used in Ayurveda for asthma symptoms where jimson weed's leaves are smoked in cigarette or pipe.*

Objectives: *Present study investigates the antioxidant and antiproliferative activities of MEDS.*

Materials and Methods: *The 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 Rfvalues of 0.76 and 0.58 in the methanol extract of D. stramonium.The cytotoxic activity for MCF7 cell line was 66.84% at 500 µg/mL concentration of methanol extract.*

Conclusion: *There is a potential of MEDS as a source of Antioxidants and anti-proliferative activities. Further research work required to isolate active compounds for clinical therapeutics.*

Keywords: Antioxidant, cytotoxicity, MCF7, MTT assay.

Introduction

The tumor suppressor BRCA (Breast cancer) gene, implicated in breast and ovarian cancers exerts various properties on the DNA repair system, In addition, mutations of a single allele of the BRCA1 gene are associated with increased genomic instability in breast epithelial cells. Lennart Mucke and his colleagues at the Gladstone institute of Neurological Disease have found that the BRCA1 gene not only affects the way cells can grow by promoting cancer, but can also interfere with nerve cells ability to repair their DNA. Recent investigations as carried out by the E Suberbielle et al. have profoundly explained the direct correlation of the BRCA and Alzheimers, (http://10.0.4.14/ncomms9897). Her team lead to the conclusion that the Amyloid beta oligomers reduced the levels the BRCA 1 levels in the primary neural cultures. The group defined the direct correlation of the alzheimer and the breast cancer where both the proteins are inhibited at the maximal rate, Studies too have investigated that the BRCA cum BRCA2 do have maximal inhibition, ourstudies tried to highlight the antioxidant potential of Methanol Extract of Datura Stramonium (MEDS) cum its potential to target the MCF cell line. 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 biolog-ically 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]. Seeds are used to make somebody unconscious [3]. 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 [4]. 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 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 Pirpanjal Range, being a hilly terrain-it has an average elevation of 2057 metre [5]. 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.

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 MEDS in TLC aluminium sheet (Merck), silica gel 60 F254 (4 x 1 cm), precoated plates [6]. 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 Rf values of the coloured spots were calculated [7].

$$R_f = \frac{\text{Distance traveled by the solute}}{\text{Distance traveled by the solvent}} \times 100$$

Phytochemical screening

The MEDS was subjected to preliminary phytochemical analysis using standard methods [8]. Methanol extract was screened for different classes of phytoconstituents such as alkaloids, steroids, terpenoids, phenolic compounds, flavonoids, saponins and glycosides using specific standard reagents [9].

Antioxidant activities

DPPH radical scavenging assay

The antioxidant activity of MEDS 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. with slight modifications [10]. 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 is based on the capacity of MEDS 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

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

$ABTS^{\bullet +}$ radical cation scavenging assay

Antioxidant capacity was evaluated in terms of the $ABTS^{\bullet +}$ radical cation scavenging activity, by the method of Delgado-Andrade et al [11]. Briefly, $ABTS^{\bullet +}$ was obtained by reacting 7 mM ABTS stock solution with 2.45 m M 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 MEDS were mixed with 1 mL of diluted $ABTS^{\bullet +}$ solution and the absorbance was measured after 10 min.The $ABTS^{\bullet +}$ radical cation scavenging activity was expressed as

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

Hydroxyl ($OH^{\bullet}$) 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 [12]. The percentage of inhibition was calculated using the following formula

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

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

Nitric oxide was generated from sodium nitroprusside and measured by Griess' reaction as described Green et al. Sodium nitroprusside (5 mM) in phosphate buffer saline solution (0.025 M, pH: 7.4) was incubated with different concentrations of MEDS dissolved in phosphate buffer saline (0.025 M, pH: 7.4) and the tubes were incubated at 25ºC for 3 h. Control experiments without the test

compounds but with equivalent amounts of buffer were conducted in an identical manner [13]. 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 diaminedihydrochloride). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthylethylenediamine 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 MEDSwas carried out by the method of Yen and Chen with slight modification [14]. 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. The mixture was incubated at 50°C for 20 min, during which period ferricyanide was reduced to ferrocyanide. Then, 1 mL of 1% trichloroacetic acid was added to the mixture. To this 0.1% $FeCl_3$ and the absorbance at 700 nm was measured to determine the amount of ferric ferrocyanide (prussian blue) formed. Increased absorbance of the reaction mixture indicates the increase in reduction of ferricyanide.

Phosphomolybdenum reduction assay

The antioxidant capacity of the MEDSwas assessed by the method of Sivaraj et al.The 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) [15]. Reaction mixture was incubated in a water bath at 90°C for 90 min. The absorbance of the coloured 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 centre 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 CO2 at 37°C.

Reagents

RPMI-1640 was purchased from GIBCO/BRL Invitrogen (Caithershurg, MD). Fetal bovine serum (FBS) was purchased from Gibco laboratories Trypsin, methylthiazolyldiphenyl- 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 [16]. 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. The 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:

% Cell viability = A_{570} of treated cells / A_{570} of control cells x 100.

Results and discussion

Dose dependence being one of the important criteria of the drug to be effective for treating an particular ailment, Hence any drug metabolism wholly depends upon the concentration of the dose given, Maximally it too can be defined in terms of bioavailability, The given drug in its lower concentration can prove very beneficial, but increasing the concentration of the given drug may prove harmful. Dietary antioxidants are beneficial for brain health in reducing disease-risk and in slowing down disease-progression. Antioxidants are chemicals that interact with and neutralise 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 neutralise 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. Fruits, vegetables and grains are rich sources of dietary antioxidants [17,18]. 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 [19]. Antioxidants are intimately involved in the prevention of cellular damage the common pathway for cancer, aging, and a variety of diseases. BRCA is too unregulated in muscular disorder like Amyotrophic Lateral Sclerosis (ALS), Based on the studies, it can be revealed that the Antioxidant potential of the MEDS, which too can have an therapeutic potential for treating Alzheimer's.

Thin layer chromatography (TLC)

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

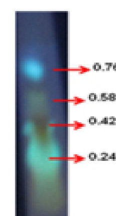

Figure 1b: TLC of methanol extract of seeds of D.stramonium

DPPH radical scavenging assay

Ability of methanol extract of D. stramoniumto scavenge free radicals was assessed using 1,1-diphenyl-2-picrylhydrazyl radical (DPPH). The methanol extract of D. stramoniumhascapacity 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. The capacity of DPPH reduction increases with increasing concentration of the extract as reported earlier by Raa-manet al. The maximum DPPH radical scavenging activity was 59.50% at 120 µg/mL concentration (Table 1a) [20]. It was compared with standard ascorbic acid and the IC_{50} of DPPH radical scavenging activity was 94.87 µg/mL concentration (Figure 2a). The scavenging ability of the petroleum ether fraction of seeds of D. stramoniummay be due to its bio compositions such as phenolic acids and flavonoids. The 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 centred free radical, color of which changes from violet to yellow on reduction by donation of H or e- by the MEDS

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 .Maximum inhibition of superoxide radical was found to be 53.17% at 60 µg/mL concentration of MEDS (Figure 2b). Methanol extract was found to be an effective scavenger of superoxide radical generated by photo reduction of riboflavin. ROS are formed resulting in oxidative DNA damage which is followed by increased DNA repair activity so that initial DNA damage is efficiently repaired.These well-known functions of BRCA show its association with AD, as depicted in (Figure 3b).

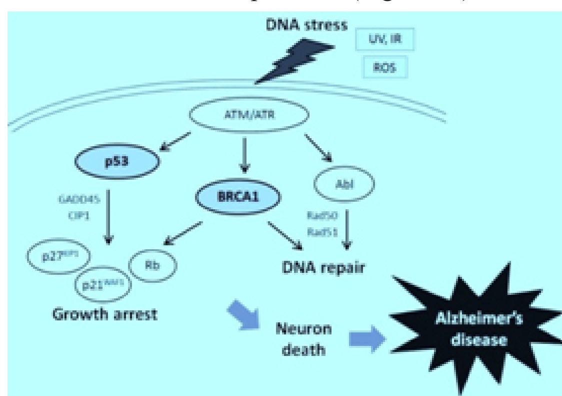

Figure 3b: Schematic illustration of tumor suppressor signaling including BRCA1, p53, Rb, p21WAF1/p27KIP1, and ATM in Alzheimer's disease

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

In the total antioxidant activity, $ABTS^{\bullet+}$ is a blue chromophore produced by the reaction between $ABTS^{\bullet+}$ and potassium persulfate and in the presence of the plant extract orascorbic acid, $ABTS^{\bullet+}$ cation radical gets reduced and the remaining radical cation concentration after reaction with antioxidant compound was then quantified [21]. The maximum $ABTS^{\bullet+}$ radical cation scavenging activity was 63.83% at 60 µg/mL concentration (Table 3a).

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

S. No	Concentration µg/mL	Radical scavenging activity (%)		
		$ABTS^{\bullet+}$	$NO^{\bullet}$	$OH^{\bullet}$
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	66.9z3±4.68	51.65±3.87	55.39±3.61

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. reported the same kind of result in $ABTS^{\bullet+}$ radical cation scavenging activity, which was concentration dependent [22].

Hydroxyl ($OH^{\bullet}$) 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. The 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 [23].The 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 39.59µg/mL (Figure 3c)concentration. The MEDS significantly inhibits generation of NO.radicals in a dose dependent manner.

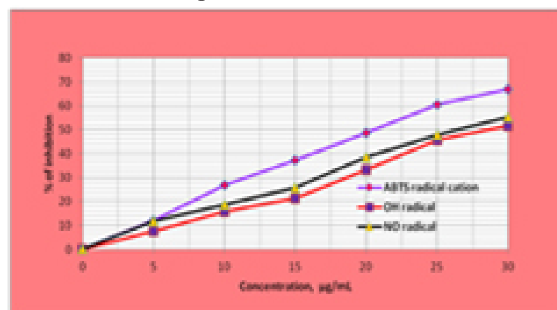

Figure 3c: $ABTS^{\bullet+}$, OH and NO radical scavenging assay

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

A relationship between chronic inflammation and 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. This creates a microenvironment that may enhance cell proliferation, survival, and migration, as well as angiogenesis, thereby promoting tumor [24]. A particularly important role of increased NO generation in this microenvironment is now well recognized as an essential step initiating neoplastic transformation [25]. In this spectrophotometric method, the absorbance of chromophore formed during the diazotization of the nitrile with sulphanilamide and the subsequent coupling with

naphthyethylenediaminedihydrochloride was measured. NO reacts with superoxide anion and form a potentially cytotoxic molecule, the peroxynitrite (ONOO). Its protonated form, peroxynitrousacid (ONOOH), is a very strong oxidant. The main route of damage is the nitration or hydroxylation of aromatic compounds, particularly tyrosine. 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 $NO^{\bullet}$ 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. It was compared with standard ascorbic acid. The IC_{50} of $NO^{\bullet}$ radical scavenging activity was 53.54 µg/mL concentration (Figure 3c) [26].

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 Daturastramoniumand found increasing in showing reduction ability in a dose dependent manner, with increasing concentrations. Since the reducing capacity of the MEDSserve as a significant indicator of its potential antioxidant activity and the reducing ability was 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 [27].

Table 4a: Fe^{3+} reducing power and phosphomolybdenum reduction activity of MEDS

S. No	Concentration µg/mL	Absorbance	
		Fe3+ reducing power assay (700nm)	Phosphomolybdenum reduction assay (695nm)
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

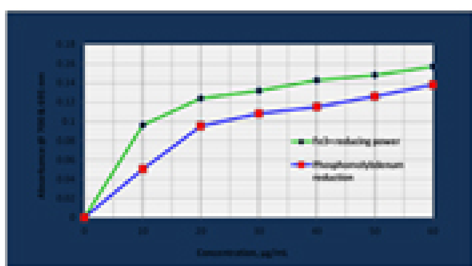

Figure 4c: Fe^{3+} reducing power and phosphomolybdenum reduction activity of methanol extract of seeds of D. stramonium

Phosphomolybdenum reduction assay

The total antioxidant activity of methanol extract of seeds D. stramoniumwas measured spectrophotometrically by the phophomolybdenum 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 [28]. The maximum absorbance was 0.175 at 120 µg/mL concentration (Table 4a) and was compared 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 provides a substantial evidence for this assay [29].

Cytotoxic activity by MTT assay

Experiment on the cytotoxicity of MEDSon human breast adenocarcinoma MCF-7 cells showed increasing cytotoxicity with increasing concentrations of extract and the viable cells detected by MTT assay [30]. The results depicted in Figure 5a &5b, summarise the cytotoxic effects of the extract and the concentration Vs % of cell viability on MCF-7 breast cancer cell lines respectively. Concentration dependent cytotoxic effect on this cell line has too been vouched by the data presented in Table 5c.

Table 5c: Cytotoxic activity of MEDS 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

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 utilising methodology of Spavierj et al. Morphological representation of cytotoxicity of the extract on MCF7 cell line too have been provided inFigure 5a [31].

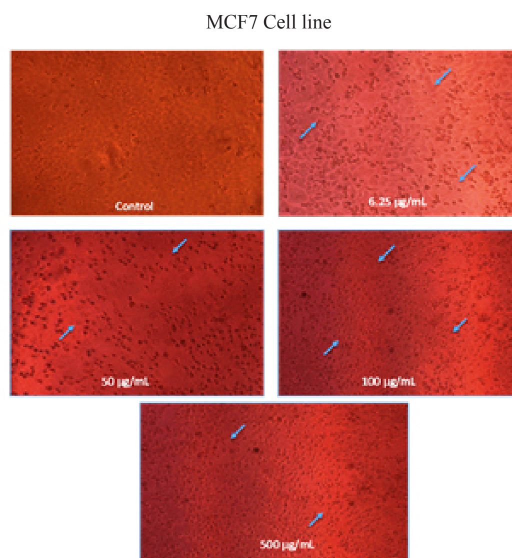

Figure 5a: Cytotoxic effects of the extract on MCF-7 breast cancer cell lines

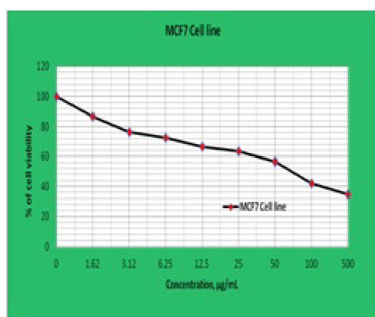

Figure 5b: Cytotoxic effects of the extract on MCF-7 breast cancer lines.

Conclusion

The results of the present work indicated that MEDS is a potential source of natural antioxidants and significantly inhibit free radicals by dose-dependently. Difference in the antioxidant activity may be ascribed to their different group of phenolic and flavonoid compounds. MEDS 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 active antioxidant property, direct correlation between the BRCA and alzheimer' soptimises MEDS, to have therapeutic potential of treating alzheimer's too, keeping dose dependence into consideration. Moreover our studies do confirm that extracts exhibit cytotoxic effect on the breast cancer cell line MCF-7. Further investigations on the isolation of the active component of the extract will throw more information on the mechanism of action.

Further Studies

As we have bridged the gap between the antioxidant potential of MEDS cum MCF, It corroborates its potential for optimising MEDS for its therapeutic potential in treating Alzheimer's. As our recent published findings, Iqbal et al.we have opened new window stating role of Xanthonoid and Flavonoid derivatives having therapeutic potential against Alzheimer's, In vivo studies of mentioned bioactives which have shown Antioxidant activity, are under process in deciphering their role in multi-targeted therapy of Alzheimer's.

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