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Research Article

THERAPEUTIC PROSPECTS OF NEOPHYTADIENE FROM *DATURA STRAMONIUM*: A POTENTIAL BIOACTIVE COMPOUND FOR MEDICINAL INTERVENTIONS

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ABSTRACT

Objective: *Datura stramonium*, traditionally used in medicine, is now scientifically studied for its bioactive properties. This study is to assess the phytochemical composition, antioxidant capacity, and anticancer potential of *D. stramonium* leaf extracts.

Method: The Folin- Ciocalteu method was employed determine the total phenolic content, while the aluminum chloride colorimetric method was used for flavonoid quantification. The antioxidant potential of the extracts was analyzed through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. The cytotoxic effects of the extracts on 3T3-L1, MCF-7, and HepG2 cell lines were evaluated using the MTT assay, which measured cell viability across different concentrations and exposure times. The GC-MS analysis was conducted to identify the chemical constituents present in the extracts.

Result: Methanolic extract exhibited the maximum flavonoid content while ethanolic extract showed maximum phenolic content, whereas the antioxidants present in various extracts exhibited maximum DPPH scavenging at concentrations of 100 µg/mL, and the highest IC_{50} value is obtained for chloroform and acetone extract which contain Neophytadiene as a major compound. The acetone extract shows a strong cytotoxic effect with 50% viability at IC_{50} 165µg/mL and 58µg/mL at 24 h and 48 h, respectively, against the MCF-7 cell line. The GC-MS analysis identified several compounds in which Neophytadiene (R. Time: 24.4) is often found in all *D. stramonium* extracts and may be further investigated for several disorders.

Conclusion: The current study's findings suggest that Neophytadiene (diterpene) in *D. stramonium* leaf extract can found to be a promising anticancerous agent.

Keywords: *Datura stramonium*, DPPH, GC-MS, Phytochemical, Neophytadiene, MCF-7.

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INTRODUCTION

The utilization of therapeutic herbs has increased in recent years, due to their effectiveness as drugs and their considerable worth as natural resources [1]. Many people have been using these plants to treat various ailments throughout history, and even with the availability of today's healthcare services, many people nonetheless rely on medicinal plants for their basic health needs, especially in developing nations [2, 3].

Many medicinal plants contain bioactive compounds with antioxidant [4] and anticancer activity, the genus Datura is one of these plants, characterized as a flowering medicinal herb [5]. As a member of the nightshade (*Solanaceae*) family of plants, *Datura stramonium*, referred to as jimson weed or thornapple, prickly apple, or devil's snare, has hairy leaves, white or purple flowers, and round thorny fruits. The leaves are long, smooth, toothed, sharply margined, and irregularly undulated with a length of about 8–20 cm and they form bushes in height up to 60–150 cm (2–5 ft) [6]. It is native to subtropical and temperate geographical areas such as North America, but it has now been spread to other parts of the world, including Europe, Asia, and Australia [7].

Due to the existence of numerous biologically active compounds such as glycosides, phenols, alkaloids, and flavonoids, *D. stramonium* being utilized to treat many human conditions, including toothaches, bruises, bruising, inflammation, rheumatism, and sciatic nerve irritation [8] and well-known for its natural mosquito-repellent properties and its ability to act as a larvicide and bio-insecticidal capacity against *Anophele* [9,10] and also have antimicrobial properties [11] against variety of microbes.

Free radicals generated in the body during respiration have a beneficial role in cells; however, they become detrimental when produced in large quantities due to external factors such as Ultraviolet radiation, X-rays, gamma rays, pollutants, or when not being eliminated by the endogenous system that induce oxidative stress, result in a number of diseases and conditions [12,13]. Antioxidants as neutralizers of free radical assist in alleviating disorders that release toxic reactive species (ROS) by changing them into less dangerous forms. An imbalance between antioxidant enzymes and oxidative stress increases the risk of developing diseases like cancer, autoimmune disorders, aging, cardiovascular disease, and neurological diseases [14]. Cancer is the unchecked proliferation of aberrant and mutated cells, it is an intricate disease as it can progress and propagate swiftly by several mechanisms, such as cancer resistance to apoptosis, stimulating normal cell signaling, cancer invasion, metastasis, and angiogenesis [15]. Being a leading cause of death and morbidity, cancer is a difficult disease to treat globally. According to some estimates, cancer ranks third in terms of causes of mortality in developing countries and ranks second in affluent countries [16]. Medicinal plants are currently utilized as a natural ingredient in herbal medications due to their antioxidant and anticancer qualities [12,17].

Since technological advancements have made it feasible to investigate natural compounds faster and with greater accuracy than ever before, resulting in the isolation of bioactive compounds with medical advantages [18], this study focuses on phytochemical screening, antioxidant analysis, and metabolite profiling to assess the biologically active compounds in the different solvent extracts of *D. stramonium* leaves using modern analytical methods such as GC-MS and anticancer activity of *D. stramonium* extract.

MATERIALS AND METHODS

Chemicals used

Methanol, Ethanol, Chloroform, Ethyl acetate, Acetone, α-Diphenyl-λ-picrylhydrazyl (2,2-diphenyl-1-picrylhydrazyl [DPPH]), Ascorbic acid,

Aluminum chloride (AlCl3), Sodium Nitrate, Quercetin, Folin-Ciocalteau reagent, Sodium carbonate, Gallic acid, Ethyl alcohol (MP Biomedicals), Dimethyl sulfoxide, 3-(4,5-dimethyl thiazolyl-2)-2,5-diphenyl Tetrazolium Bromide (MTT) (MP Biomedicals), MCF-7, 3T3-L1, and Hep-G2 cell lines, Dulbecco's modified Eagle's medium (DMEM) (Gibco, Thermo Fisher's Scientific, US), Foetal Bovine Serum (FBS) (MP Biomedicals) and 1% penicillin/streptomycin (Gibco, Thermo Fisher's Scientific, US), Doxorubicin Hydrochloride (Thermo Fisher Scientific, United Kingdom).

Collection of plant material

Healthy *D. stramonium leaves* were collected from the Municipal Park in the village of Khedi Manajat, Sonipat, Haryana.

The specimen was identified and Authenticated by ICAR-National Bureau of Plant Genetic Resource, Divison of Plant Exploration and Germplasm collection, Pusa Campus, New Delhi with an assession number AC-253.

Preparation of plant sample

Collected leaves were washed properly and dried under shade and exposure to sunlight for drying was avoided to prevent the loss of bioactive compounds. After properly drying, the leaves were ground into the powdered form using a grinder and were allowed for extraction in aqueous and non-aqueous solvents using maceration.

Preparation of extract

20 g of powdered leaf was emersed in 200 mL of solvent for 7 days for extraction using Methanol, Ethanol, Chloroform, Ethyl acetate, Acetone, and Distilled water as a solvent. The extract was then filtered, and excess solvent was removed using a rotary evaporator to produce a semi-solid, greenish-black extract [19].

Extraction yield: The extraction yields from *D. stramonium* leaves using different solvents can be measured using:

Extraction Yield=(Weight of Extracted Compound/Weight of Plant Material) × 100

Phytochemical screening

Assessment of total flavonoid content

The $AlCl_3$ method was used to measure the extracts' TFC. From a stock solution (1 mg/mL of methanol), various diluted concentrations of the standard (20, 40, 60, 80, and 100 µg/mL) were made. An aliquot of 1 mL of the standard solution was added to 1 mL of 5% sodium nitrate, and after 5 min, 1 mL of 12% $AlCl_3$ was added, and 2 mL of 1M NaOH was added, soon after the amount was then increased to 10 mL by adding methanol and mix thoroughly. The solution was allowed to incubate at ambient temperature for 30 min, and concentration was calculated using a rutin curve as the standard calibrator at a wavelength of 517nm.

The procedure for the extract preparation was the same as that mentioned for the standard rutin was followed, and all the measurements were done in triplicates for each sample.

It was stated as mg of rutin equivalents (RE)/grams of extracts.

$A = (Q_1 \times Q_2)/W$

Where;

A: TFC in mg/g of the extracts as RE

Q_1: Concentration of rutin in mg/ml

Q_2: Volume of the extract in ml, and

W: Weight in grams of the extract [20,21].

Assessment of total phenolic content

The extracts' total phenolic content was quantified using the Folin-Ciocalteau [22] method. From a stock solution (1 mg/mL of Distilled water), various diluted concentrations of the standard (20 µg/mL, 40 µg/mL, 60 µg/mL, 80 µg/mL, and 100 µg/mL) were made. An aliquot of 1 mL of the standard was added to 1 mL Folin-Ciocalteu (FC) reagent (0.5 N), which was then followed by an incubation period of 5-min in the dark at room temperature. After adding 2 mL saturated sodium carbonate, the mixture was left to sit in the dark at ambient temperature for an additional 30 min, with gallic acid as standard at 760 nm wavelength [22].

The procedure for the extract preparation was the same as that mentioned for the standard gallic acid was followed, and all the measurements were done in triplicates for each sample. It was computed as mg gallic acid equivalents (GAE)/grams of the extracts [20,21].

Antioxidant activity

DPPH assay

A stable free radical, α-Diphenyl-λ-picrylhydrazyl (DPPH), is often used to assess an antioxidant compound's ability to neutralize free radicals. According to this method, DPPH is reduced in a methanol solution where hydrogen-donating antioxidants exist, so the non-radical form DPPH-H is formed [23]. This causes a color shift from purple to yellow, which was detected spectrophotometrically at a wavelength of 520 nm.

After mixing 1 mL of DPPH in methanol (0.3 mM) to various diluted concentrations of 1 mg/mL of extract (20 µg, 40 µg, 60 µg, 80 µg, 100 µg/mL), and 1.0 mL of methanol, the mixture was left to sit at ambient temperature for half an hour in the dark conditions before the absorbance were measured at 520 nm.

The positive control used in the assay can be (1:2) DPPH: methanol, the standard used was ascorbic acid. The %RSA can be determined using the formula:

$\%\ RSA=\{(Ab_c - Ab_s)/Ab_c\}\times 100$

Where; Ab_c is the absorbance of the control and Ab_s is the absorbance of the extracts [13,20,23,24].

Gas chromatography-mass spectrometry analysis

GC-MS analysis was employed at Centre Instrumentation Facility, LPU, Phagwara on GCMS-TQ8040 NX system consists of an AOC-20i autosampler and gas chromatograph interfaced to a mass spectrometer instrument uses the following parameters:

The GC-MS program employed helium as the carrier gas with a flow rate of 1 mL/min (split ratio = 10:0), and the oven temperature was programmed between 50°C and 300°C with a hold time of 3 min. The ion source temperature was kept at 240°C, and the interface temperature at 310°C, with a 3.00 min of solvent cut time.

Other conditions were as follows: Detector Gain Mode – In relation to the tuning result with +0.00 kV and 0 threshold, scanning between 3.00 min and 43.00 min, event time- 0.3 s, Q3 scan speed- 2500, wavelength between 45 nm and 700 nm.

Identification of compounds: According to the NSIT database, which holds over 62,000 profiles of known compounds used to examine the mass spectrum and identify the components using their retention indices. The mass spectra for known compounds kept in the NIST mass library (NISTII) were matched with the spectra of the unknown compounds extracted from the test sample. By correlating each compound's mean peak area to the entire area, the relative percentage of each compound was determined [1,19].

Determination of Anticancer activity

Cell culture

MCF-7, 3T3-L1, and Hep-G2 cells were harvested from sub-confluent T-75 flasks using standard trypsinization protocols containing DMEM

(Gibco, Thermo Fisher's Scientific, US) supplemented with 10% Heat-inactivated FBS (MP Biomedicals) and 1% penicillin/streptomycin (Gibco, Thermo Fisher's Scientific, US) antibiotics. Cells were incubated overnight at 37°C with 5% CO_2 for cell attachment.

Cell cytotoxicity assay

Cell cytotoxicity was determined through the 3-(4,5-dimethyl thiazolyl-2)-2,5-diphenyl Tetrazolium Bromide (MTT) test. Cells were planted into 96-well microplates at a number of 1×10^4 cells per well in 100 µL of complete medium specific to each cell line. From the serial dilution tubes, 100 µL of each test concentration was transferred into triplicate wells. Control wells received 100 µL of plain medium. The plates were incubated for 24 h at 37°C with 5% CO_2. A fresh solution of MTT (5 mg/mL in PBS) was prepared and filter sterilized. After 24 h of treatment, 100 µL of MTT solution (typically diluted to 0.5 mg/mL in the medium) was introduced into each well. The plates were then incubated at 37°C for 3 h, permitting MTT to be converted into formazan crystals by viable cells. To dissolve the formazan crystals, 100 µL of DMSO was applied to each well after the MTT solution had been properly removed. The plates were gently shaken for 5 min until the solution became homogenous in each well. Absorbance (OD) was then recorded at 570 nm using a microplate reader. Cell viability percentage was calculated using.

Viability % = $(OD_{control}/OD_{treated}) \times 100$ [25,26]

RESULTS AND DISCUSSION

Extraction yield

The extraction yields obtained from *D. stramonium* leaves using different solvents are summarized in Table 1.

The extraction yield can be measured using:

Extraction Yield=(Weight of Extracted Compound/Weight of Plant Material)×100

Extraction efficiency

Depending on the solvent used, there were considerable differences in the extraction efficiency, as indicated by the % yield of isolated chemicals. Methanol and Chloroform showed greater extraction yields than Acetone, Ethyl acetate, Aqueous, and Ethanol among the studied solvents. The polarity of the solvents and the target component in *D. stramonium* leaves are responsible for this observation.

Phytochemical screening

Total flavonoid content

The $AlCl_3$ method was used to evaluate TFC in various extracts of *D. stramonium* leaves, with rutin serving as a standard. The absorbance values recorded at different rutin concentrations were adopted to construct a calibration curve (Fig. 1).

Using regression equation from the calibration curve (y=0.0028x+0.0742, R^2=0.994), the extracts' TFC was calculated and represented as milligrams of RE/grams of extract.

The findings revealed that the methanolic extract has the highest flavonoid content at 100 µg/mL, i.e. 41.92±30.62 mg RE/g of extract, whereas Chloroform has 23.36±9.25 mg RE/g of extract, and Ethanolic extract has 21.71±10.58 mg RE/g of extract, with Ethyl Acetate being the lowest, 14.46±0.59 mg RE/g of extract (Fig. 2).

Table 1: Extraction yield of different extracts of *Datura stramonium* leaves

Sample	Extraction	Extraction yield (%)
Methanol	3.6875	18.44
Ethanol	3.25	16.25
Chloroform	4.437	22.19
Acetone	1.185	5.93
Ethyl acetate	3.092	15.46
Aqueous	3.58	17.90

Total phenolic content

The FC method was employed to evaluate TPC in various extracts of *D. stramonium* leaves, with gallic acid as a reference compound. All of the absorbance values acquired at the various gallic acid concentrations were utilized to create a calibration curve (Fig. 3).

Based on the calibration curve's regression equation (y=0.012x+0.0912, R^2=0.999), the TPC of the extracts was computed and reported as mg GAE/gram of extract.

The finding revealed that the ethanolic extract has more phenolic content than other solvents at 100 µg/mL, that is, 69.69±1.74 mg GAE/g of extract, whereas Ethyl Acetate has 57.31±2.21 mg GAE/g of extract, and Methanol has 56.76±1.25 mg GAE/g of extract and the lowest was observed in chloroform extract which has 22.23±10.25 mg GAE/g of extract (Fig. 4).

Antioxidant activity

DPPH assay

Several *D. stramonium* leaf extracts were tested for their antioxidant activity using the DPPH assay, and the concentration yielding the IC50 value was used to calculate the extracts' reducing power. It was shown that the concentration of various extracts directly correlated with their

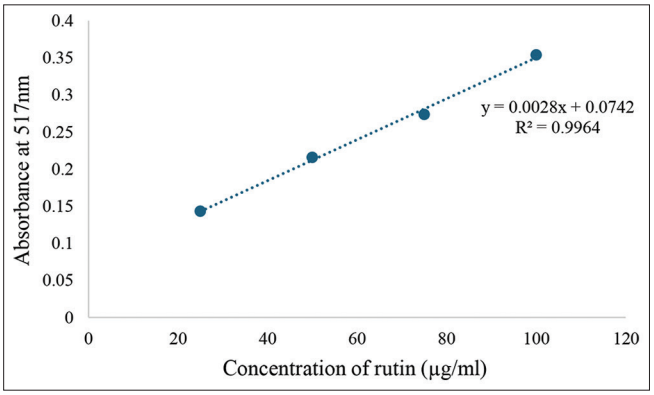

Fig. 1: Calibration curve against the rutin concentration

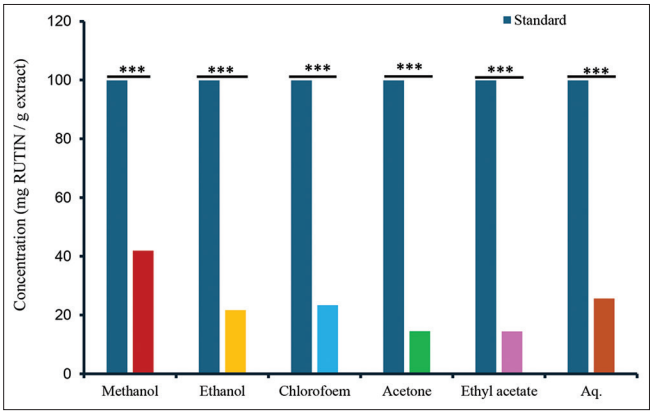

Fig. 2: Total flavonoid content in different extracts of *Datura stramonium*: At a concentration of 100 µg/mL in comparison with positive control (Rutin) and different extracts. The results are shown as the mean±standard deviation of three different experiments. The significant differences were displayed using analysis of variance, with *p<0.05, **p<0.01, and ***p<0.001

capacity to scavenge radicals, that is, the higher IC_{50} value, the lower radical scavenging activity, or the lower antioxidant potential.

Obtained absorbance values of Ascorbic acid were used to produce the calibration curve (Fig. 5) for the determination of %RSA (Fig. 6) and IC_{50} values for standard and extracts of *D. stramonium*.

As a standard against various extracts, ascorbic acid at varying doses exhibited varying antioxidant qualities. Therefore, using the graph of concentration versus percentage inhibition, the IC50 values for each extract at various concentrations were computed. The extracts' corresponding concentration at which their radical scavenging capacity is 50% is indicated by their IC_{50}. Every measurement was taken in triplicates. The findings for IC_{50} values revealed that all the extracts had maximum radical scavenging activity. Methanolic extract was found to have an IC_{50} value that was comparable to the standard, that is, 95.08 and 95.64, respectively. The maximum IC_{50} values were observed at 100 µg/mL in methanol (95.08), at 80 µg/mL in ethanol (86.88), at 60 µg/mL in chloroform (111.3), at 100 µg/mL in acetone (105.8), at 100 µg/mL in ethyl acetate (96.88) and 100 µg/mL in aqueous (105.83).

GC-MS study of *D. stramonium* leaf extracts

A spectrum peak graph was produced by the GC-MS analysis of the samples, as shown in Fig. 7. GC-MS analysis of *D. stramonium* leaves revealed a number of compounds in each extract with hexadecenoic acid, methyl ester; 9,12,15-octadecatrienoic acid, methyl esters; methyl stearate; Neophytadiene; phytol; phenol,2,4-bis(1,1-dimethyl ethyl)-phosphate with 32.53%; 13.5%; 16.95%; 33%; 12.24%; 12.62%, respectively, in abundant amount (Fig. 8).

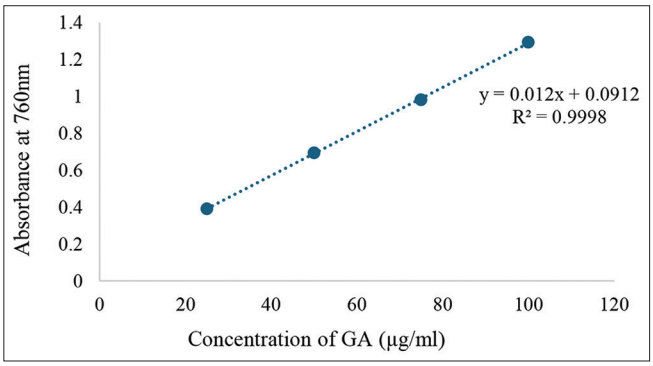

Fig. 3: Calibration curve against the gallic acid concentration

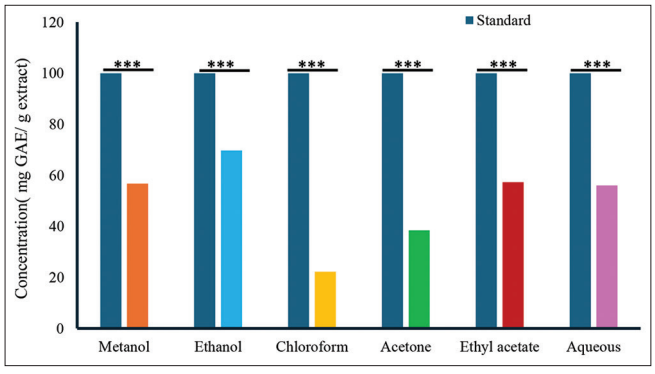

Fig. 4: TPC in different extracts of *Datura stramonium:* At a concentration of 100 µg/mL, in comparison with positive control (Gallic acid) and different extracts. The results are shown as the mean ± standard deviation of three different experiments. Analysis of variance was used to show the significant variations, with *p<0.05, **p<0.01, ***p<0.001

Anticancer activity

The anticancer potential of *D. stramonium* leaf acetone extract was assessed using MTT assay against MCF-7, Hep-G2, and 3T3-L1 and was first assessed as a percent viability at a final dosage of 500 µg/mL, followed by determination of IC_{50} values of the extract. These data reveal that the cytotoxicity of extracts on these cell lines is time-dependent (28 h and 48 h) and dose-dependent (50, 100, 200, 400, 500 µg/mL). Lower concentrations (50 µg/mL) of extract exhibited viability percentages above 70% against the mouse fibroblast cell line (3T3-L1) of the negative control, indicating no cytotoxic effect. Cytotoxicity on MCF-7 and Hep-G2 cells with viability ranges from ~35% at 500 µg/mL to ~60% at 50 µg/mL and ~65% at 500 µg/mL to ~78% at 50 µg/mL at 24 h whereas ~26% at 500 µg/mL to ~51% at 50 µg/mL and 50% at 500 µg/mL and ~63% at 50 µg/mL at 48 h, respectively, with IC_{50} value of 165 µg/mL and 58 µg/mL for MCF-7 at 24 h and 48 h, respectively, and 706 µg/mL and 502 µg/mL for Hep-G2 at 24 h and 48 h, respectively (Figs. 9 and 10) with microscopic analysis of 3T3-L1, MCF-7and HepG2 cell lines (Figs. 11-13).

DISCUSSION

The study provides significant insights into therapeutic potential of Neophytadiene from *D. stramonium* leaf extracts, which demonstrated significant antioxidant and cytotoxic activities, suggesting the bioactive potential of Neophytadiene as a key constituent responsible for these effects.

The phenolic and flavonoid content were determined for each extract of *D. stramonium* leaves, and results showed that the methanolic extract was found to have more flavonoid content (41.93 mg RE/g), whereas ethanolic extract had more phenolic content (69.7 mg GAE/g) compared to other chloroform, acetone, ethyl acetate, and aqueous extracts. Similarly, as reported by Alper and Özay [27],

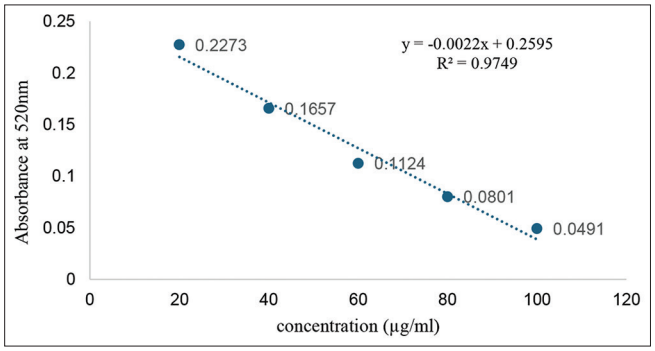

Fig. 5: Calibration curve against the ascorbic acid

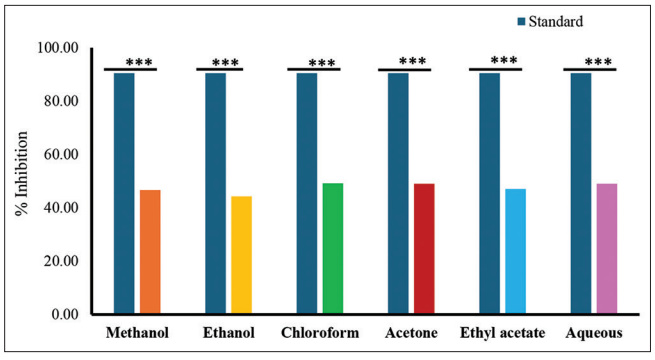

Fig. 6: Plot of the radical scavenging activity by using DPPH Assay between Ascorbic acid and different solvent extracts of *Datura stramonium.* The results are shown as the mean ± standard deviation of the extract's three independent experiments. *p<0.05, **p<0.01, ***p<0.001, and analysis of variance was used to achieve the analysis of variance

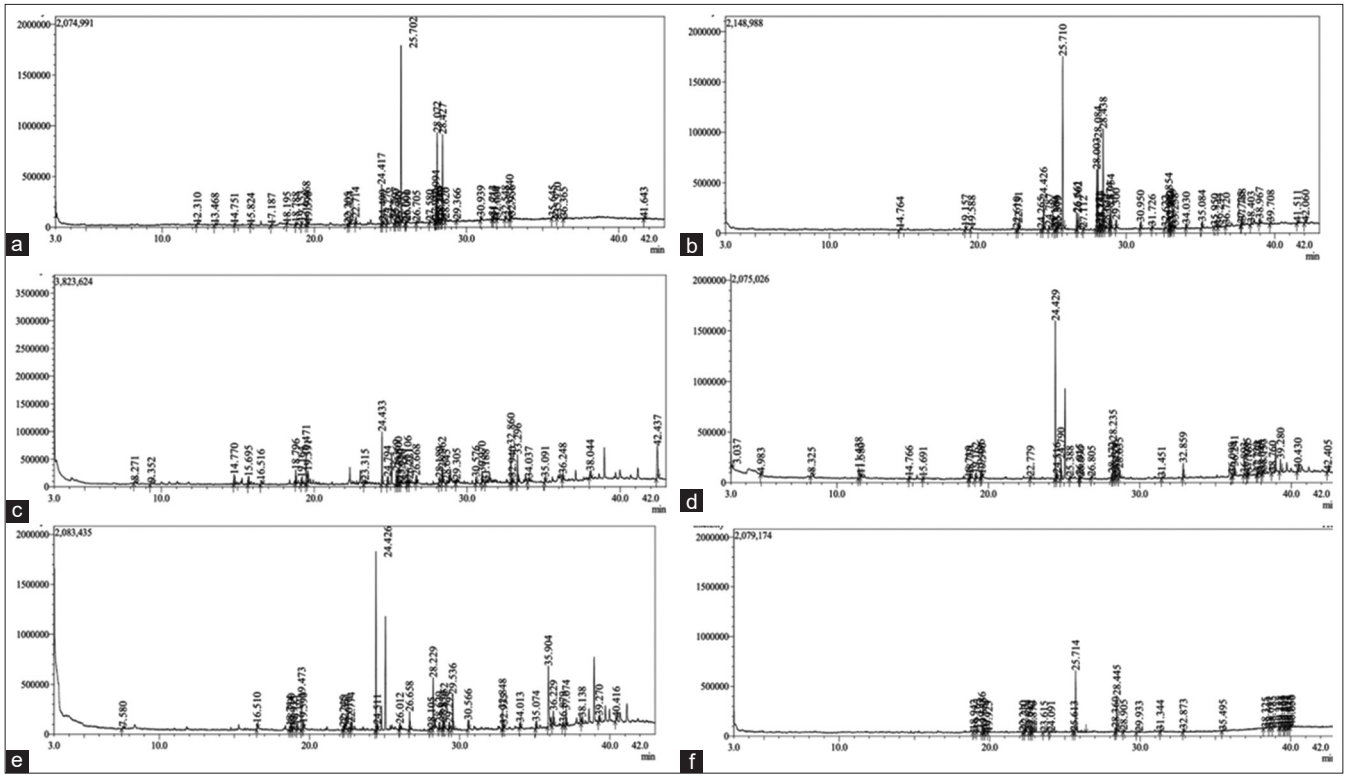

Fig. 7: GC-MS profile of *Datura stramonium* Leaf Extract. (a) Methanol (b) Ethanol (c) Chloroform (d) Acetone (e) Ethyl acetate (f) Aqueous

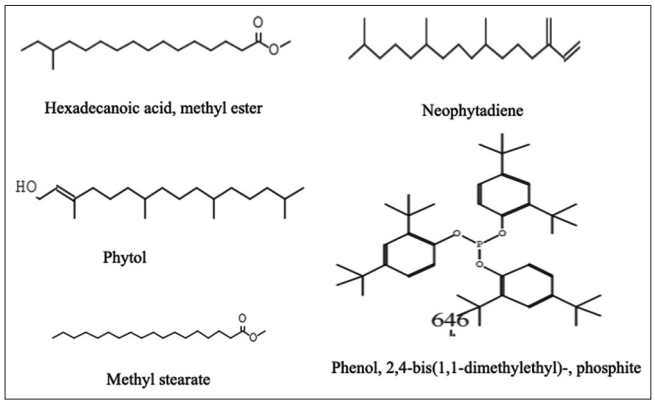

Fig. 8: Structures of bioactive compounds present abundantly in *Datura stramonium* leaf extract, possessing anticancer activity

according to Nohutçu *et al*., total flavonoid content is much less than the total phenolic content [28]. The acetone and chloroform extract exhibit maximum DPPH scavenging activity at concentrations of 100 µg/mL with IC_{50} 92.38±7.77 µg/mL and 102.58±12.06 µg/mL, respectively, which contain Neophytadiene (33%) in acetone extract, as a major compound according to the GC-MS results obtained. According to Chandan *et al*., free radicals scavenging activity was found to be 71.35±1.06 µg/mL, and it could be attributed to the presence of Neophytadiene in the *D. stramonium* essential oil [29]. In the study done by Nohutçu *et al*., the antioxidant activity of *D. stramonium* was 91.74±6.59 µmoL TE/g [28] and 63.19±1.02 mg TE/g in the ethanolic extract resulting from Alper and Özay [27]. The scavenging ability of *D. stramonium* leaf methanol extracts was found by Sreenivasa *et al*., to have an IC50 value of 6.7 µg/mL [30]. Selmy *et al*., Neophytadiene, a diterpene, could be a promising apoptotic inducer, as having a main role in cancer viability by inhibiting the Human A2a receptor at the adenosine binding site, Human LRH1, and hERG K+ channel [31], Neophytadiene demonstrates anxiolytic-like and anticonvulsant effects, likely involving the GABAergic system, and is also recognized for its anti-inflammatory, antioxidant, and cardioprotective properties [32,33]. In this study, *D. stramonium* acetone extract showed the maximum amount of Neophytadiene therefore acetone extract was selected for further anticancerous studies, as Neophytadiene from *D. stramonium* has not yet been studied for its anticancer activity. The results of an anticancer study of acetone extract show minimal toxicity toward normal 3T3-L1 mouse fibroblast cells but exhibited significant cytotoxicity toward MCF-7 and Hep-G2 cancer cell lines. At 48 h, MCF-7 had a notably lower IC_{50} (58 µg/mL) than Hep-G2 (502 µg/mL), implying breast cancer cells may be more responsive to the extract's cytotoxic effects than liver cancer cells. For both MCF-7 and Hep-G2, viability decreased more sharply with longer exposure times (48 h vs. 24 h), indicating the extract's anti-proliferative or cytotoxic mechanisms intensified over time. In the previous study by Pham *et al*., Neophytadiene, a major constituent of *Grewia bulot* leaf extract, shows strong cytotoxic activity against MCF-7, Hep-G2, SK-LU-1, and KB cell lines, with IC_{50} values within 90.60 and 98.27 µg/mL [34]. *D. stramonium* leaf extract shows cytotoxic effects against the MCF-7 cell line (40.56%) [35]. In the study done by El-Naggar *et al*., *D. stramonium* leaf extract shows a potent cytotoxic effect against MCF-7 and Hep-G2 cell lines with IC_{50} values 46.0±2.4 and 30.8±1.1 µg/mL, respectively [36].

This study emphasizes the importance of *D. stramonium* as a natural source for anticancer drug development. The findings suggest that the plant's bioactive constituents, particularly Neophytadiene, could be utilized in the creation of novel cancer therapeutics. The encouraging *in vitro* results underscore the necessity for further investigation, including *in vivo* studies, elucidation of the mechanisms linking specific compounds to anticancer activity, and the identification, separation, and detailed characterization of the compounds causing these properties.

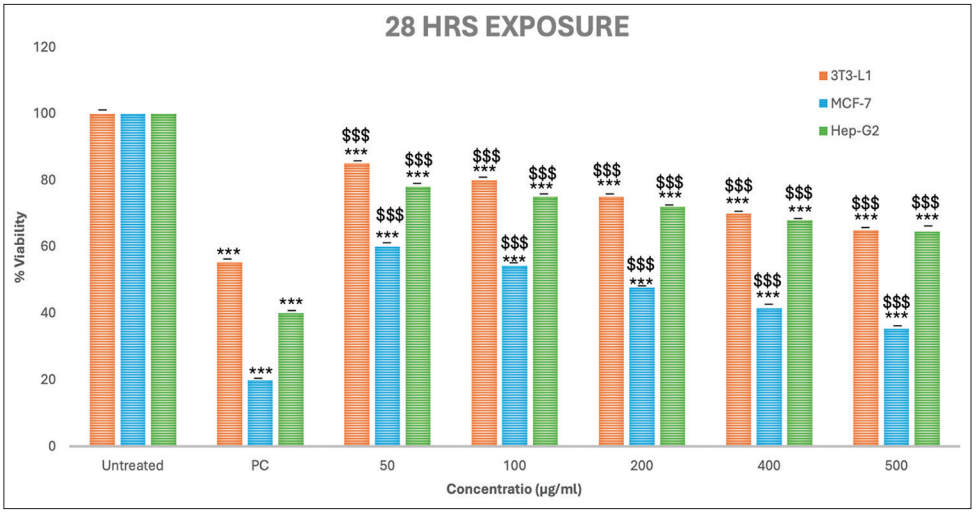

Fig. 9: Cytotoxicity of acetone extracts of *Datura stramonium* on 3T3-L1, MCF-7and HepG2 cell lines. Percent (%) viability of Acetone extract of Datura stramonium at 24 h of incubation in comparison with positive control (Doxorubicin HCL) and untreated cells, where the three independent experiments' means±standard deviations are displayed, p<0.05, p<0.01, and p<0.001 (analysis of variance) for comparisons between the untreated group, the positive control, and the extract. The comparison between 3T3-L1, MCF-7and HepG2 cell lines with varying concentrations is represented by *p<0.05, **p<0.01, ***p<0.001, and $p<0.05, $$p<0.01, $$$p<0.001 represent the comparison between all concentrations of each cell lines

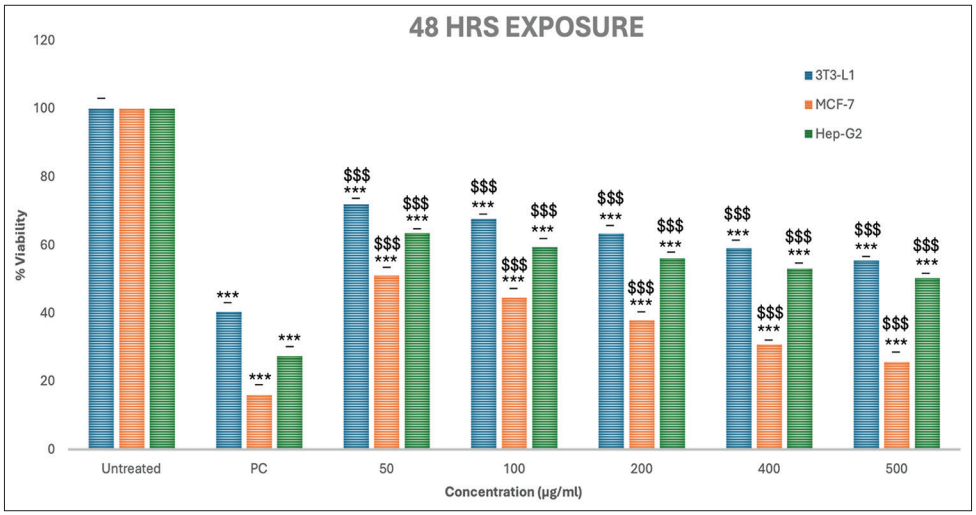

Fig. 10: Cytotoxicity of Acetone extracts of *Datura stramonium* on 3T3-L1, MCF-7and HepG2 cell lines. Percent (%) viability of Acetone extract of *D. stramonium* at 48 h of incubation in comparison with positive control (Doxorubicin HCL) and untreated cells, where the three independent experiments' means±standard deviations are displayed, p<0.05, p<0.01, and p<0.001 (analysis of variance) for comparisons between the untreated group, the positive control, and the extract. The comparison between 3T3-L1, MCF-7and HepG2 cell lines with varying concentrations is represented by *p<0.05, **p<0.01, ***p<0.001 and $p<0.05, $$p<0.01, $$$p<0.001 represent the comparison between all concentrations of each cell lines

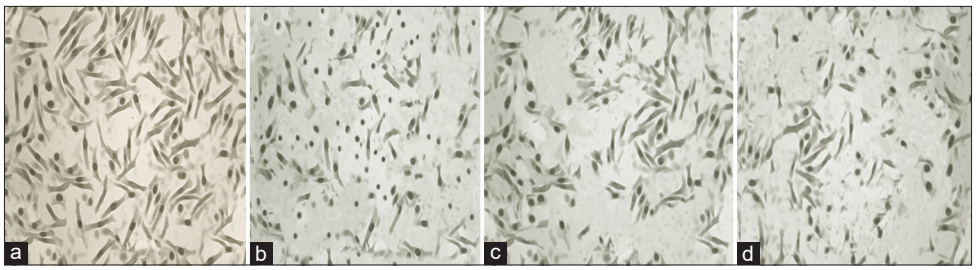

Fig. 11: Microscopic analysis of 3T3-L1 cells treated with (a) 1% DMSO (b) Doxorubicin HCL (5 µg/mL) (c) 500 µg/mL extract at 24 h (d) 500 µg/mL extract at 48 h

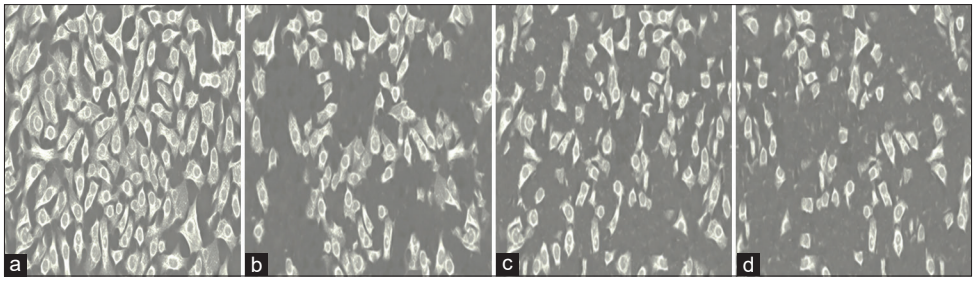

Fig. 12: Microscopic analysis of MCF-7 cells treated with (a) 1% DMSO (b) Doxorubicin HCL (5 µg/mL) (c) 500 µg/mL extract at 24 h (d) 500 µg/mL extract at 48 h

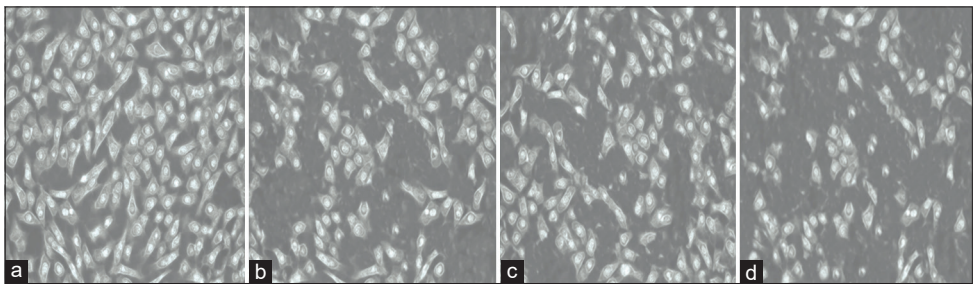

Fig. 13: Microscopic analysis of Hep-G2 cells treated with (a) 1% DMSO (b) Doxorubicin HCL (5 µg/mL) (c) 500 µg/mL extract at 24 h (d) 500 µg/mL extract at 48 h

CONCLUSION

The data produced provide a glimpse into the chemical compounds in *D. stramonium* leaf extracts, indicating the plant's enormous therapeutic potential. The different *D. stramonium* extracts had notably high levels of flavonoids, total phenolics, and antioxidant and anticancer qualities, which make it a powerful natural anticancerous source. Therefore, this plant may be more important in preventing several dangerous human illnesses. In addition, Neophytadiene found in *D. stramonium* leaf extracts can likely be used as a natural anticancer source.

AVAILABILITY OF DATA AND MATERIALS

All data generated or analyzed during this study are included in this published article. Contact the corresponding author to request data from this study.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

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AUTHORS' CONTRIBUTION

Principal author: Mousam Chhillar (lab work and drafting of the manuscript); Corresponding authors: Minhaj Ahmad Khan (research inputs, critical review, and proofreading) (Address for correspondence: minhaj.15324@lpu.co.in)

CONFLICTS OF INTEREST

Authors declare no conflicts of interest.

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