ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 12, Issue 1, 2019

Online - 2455-3891
Print - 0974-2441

Research Article

# COMPARATIVE ANALYSIS OF MORPHOLOGY AND PHYTOCHEMICAL CONSTITUENTS IN DIFFERENT POPULATIONS AND MORPHOTYPES OF *DATURA INNOXIA* MILL. AND *DATURA METEL* L. FROM PUNJAB PLAINS

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***Received: 11 June 2018, Revised and Accepted: 14 September 2018***

**ABSTRACT**

**Objective:** Comparative analysis of morphology and phytochemical constituents in different populations and morphotypes of *Datura innoxia* Mill. and *Datura metel* L. from Punjab plains.

**Methods:** Morphological analyses of different populations have been done. Methanol extracts of seeds and roots of different populations have been prepared and total phenols, flavonoid contents were measured through spectrophotometry. Antioxidant activity was studied by 2, 2-diphenyl-1-picrylhydrazyl radical scavenging activity and total antioxidant capacity. Two major compounds, caffeic acid and chlorogenic acid were quantified by high-performance thin-layer chromatography (HPTLC) analyses.

**Results:** Two morphotypes of *D. metel* were reported in the present study. Out of five different populations of *D. metel* and *D. innoxia,* the wild populations have more bioactive compound as compared to the cultivated ones from the detailed phytochemical investigation. Pharmacologically important two marker compounds chlorogenic acid and caffeic acid has been identified and quantified by HPTLC technique.

**Conclusion:** Variation in terms of morphology and secondary metabolites exists among the different populations of *Datura* spp. Among the two plant parts studied, seeds have the maximum amount of bioactive metabolites and antioxidant activity. This study revealed that chlorogenic acid and caffeic acid are the potential polyphenolic compounds in *Datura* spp. It has been found that the antioxidant activity of plant is due to its polyphenol contents, which provides insight to various researchers to work on it as it imparts health benefit.

**Keywords:** *Datura metel, Datura innoxia,* Morphotype, Phytochemical analysis, High-performance thin-layer chromatography.

 DOI: http://dx.doi.org/10.22159/ajpcr.2019.v12i1.27720

## INTRODUCTION

The word *Datura* comes from Sanskrit *Dustura* or *Dahatura* [1]. *Datura* is a genus of nine species, poisonous vespertine flowering plants belonging to the family Solanaceae [2]. They are also known as devil's trumpets. Three invasive species grows naturally in Punjab: *Datura metel, Datura Innoxia,* and *Datura stramonium* [3]. There is a lot of variability in the morphology of *Datura* species, i.e., leaf shape, flower and stem color, and seed morphology, which forms the basis of the determination of various morphotypes. There is a lot of variability in the morphological characters of many medicinal plants, and these morphotypes have also variation in their bioactive compounds. Plant morphology is useful in the visual identification of plants [4]. The phenotypic and genetic variation in plants leads to the variation of bioactive components both within the species as well as between the species. The typical bioactive compounds in plants are produced as secondary metabolites. All *Datura* plants contain tropane alkaloids (scopolamine, hyoscyamine, and atropine), primarily in their seeds and flowers. Due to the presence of these substances, *Datura* has been used for centuries in some cultures as a poison. *D. metel* remains a hallucinogenic plant of great enthanopharmacological significance especially in India, Southeast Asia, and Africa. In traditional medicine, it is used to treat chronic bronchitis, seizures, asthma, coma, etc.

The phytochemical techniques are mostly used for the quality control of herbal/Ayurvedic formulations, mainly composed of various chemical components, such as alkaloids, volatiles, saponins, flavonoids, anthraquinones, phenols, tannins, and terpenoids [5,6]. Caffeic acid is a phenolic organic compound that is classified as a hydroxycinnamic acid. It's found in all plants as a result of it acts as a key intermediate in the biosynthesis of lignin, one of the principal components of plant biomass and its residues [7]. Caffeic acid has been reported as antioxidant *in vitro* and also *in vivo* studies [8]. Chlorogenic acid has been studied as a possible chemical sensitizer involved in respiratory allergy to certain plant materials. As there is scanty data available on the comparative analysis of morphology and phytochemistry of different morphotypes of *Datura* spp.; therefore, the present study has been undertaken.

## METHODS

### Plant material

The fresh plant materials were collected from a different area of Punjab during August 2016 (Table 1). The botanical identity of the plant was confirmed by comparing with the herbarium specimens. A voucher specimen with accession number has been deposited at the Herbarium of Botany Department, Punjabi University, Patiala. All the reagent and chemicals were procured from HiMedia of analytic grade.

### Studies of morphological features

Morphological features cover the plant height, leaf color and texture, flower color, shape of flower, leaf margin, flower length, spines, stem color and texture, etc. These parameters were studied to gain data regarding morphological features.

### Plant material and preparation of extracts

In the present study, the seeds and roots of different populations of *Datura* spp. were collected from different parts of the study area for the phytochemical analysis. The collection details of plant materials are mentioned in Table 1. The collected plants were air dried, powdered in grinder, and stored at room temperature. The powdered seeds and roots of each sample 1 g dissolved in 100 mL of aqueous, butanol, chloroform, ethyl acetate, hexane, and methanol for overnight. The extract was concentrated and dried using rotary evaporator under

reduced pressure. The dried extract was re-dissolved in 5 ml of methanol and stored at 4°C until further analysis.

**Preliminary phytochemical screening**

Each extract was subjected to various phytochemical screening to detect the presence or absence of different types of constituents present in it. Different tests were performed for various constituents, namely alkaloids (Mayer's test), flavonoids (lead acetate test), phenols (acetic acid), and steroids and triterpenoids (Liebermann–Burchard test).

**Total phenols content**

Total phenol content in the leaf, stem, and root extracts was determined using Folin–Ciocalteu's reagent [9]. 300 µL of extract solution was mixed with 1.5 ml of Folin–Ciocalteu reagent (1 FC: 9 Water). After 10 min, 1.2 ml of 2.5% $Na_2CO_3$ solution was added, and the mixture was allowed to stand for 1 h. The absorbance was taken at 765 nm using Shimadzu ultraviolet (UV)-visible spectrophotometer. Total phenolic content was expressed as mg/g chlorogenic acid equivalents using the linear regression equation of calibration curve.

**Total flavonoids content**

The total flavonoid content of leaf, stem, and root extracts was determined by the method of with slight modifications [10]. 300 µL of the extract was mixed with 150 µL of $NaNO_2$ 0.5 M and $AlCl_3$ (0.3M). After 10 min, 1.5 ml of NaOH (1 M) was added. The mixture was mixed thoroughly, and absorbance was taken at 506 nm. The total flavonoid content was expressed as mg/g quercetin equivalents using the linear regression equation of the calibration curve.

**Total antioxidant capacity (TAC)**

The TAC of stem and root extracts was determined by the phosphomolybdenum assay [11]. 300 µl of the extract was mixed with 2.7 ml phospho molybdenum reagent (28 mM sodium phosphate and 4 mM ammonium molybdate in 0.6 M sulfuric acid). The reaction mixture was incubated in a water bath at 95°C for 90 min. After cooling of the mixture to room temperature, the absorbance was taken at 695 nm. TAC results were expressed as ascorbic acid (AA) equivalents (mg AA/g of dry sample).

**2, 2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity**

DPPH radical scavenging activity was studied by the method of Otang *et al*. [12]. A stock solution of DPPH was prepared by dissolving 20 mg in 500 ml of methanol and then stored at 10°C until required. Different concentrations (10–100 µg/ml) of each extract were prepared and to this equal volume of a methanolic solution of DPPH were added. The mixture was allowed to stand idle in the dark for 30 min. The absorbance of the mixture was taken at 518 nm. AA was used as a standard control. $IC_{50}$ value of each extract and standard was calculated, which shows the concentration of sample required to scavenge 50% of DPPH free radicals.

**High-performance thin-layer chromatography (HPTLC) instrumentation and conditions**

Concentrations range from 200 to 600 ng of standard solutions was spotted on silica gel 60 $F_{254}$ HPTLC plate (Merck, India) in triplicate using CAMAG Linomat V automatic spotter (Dosage speed: 150 nL/s, Syringe size: 100 µL, Band length: 6.0 mm, and Distance between tracks 10.0 mm). Plates were developed in a twin-through chamber (20 cm×10 cm) to a distance of up to 8 cm using mobile phase as previously reported by Pradhan *et al*. [13]. After development, plates were air dried and scanned densitometric at 366 nm using CAMAG TLC Scanner IV. Peak areas were recorded, and calibration curve of standards was obtained. 8 µL of methanol extracts were applied on HPTLC plates using the above-said conditions with both standard solutions. The plates were scanned at 366 nm and peak area, peak height, and absorption spectra were recorded.

**Statistical analysis**

All the data presented were performed in triplicates. The values are mean with standard error.

## RESULTS

The present study is divided into two main categories, i.e. morphological studies and phytoconstituent analysis of different populations of *D. metel* and *D. innoxia*.

**Morphological analysis**

Two morphotypes of *D. metel* on the basis of morphological character such as number of whorls of corolla, flower color, and leaf margins are compared in Fig. 1. The data regarding morphological characters of different populations of *Datura* spp. are given in Table 2.

**Preliminary phytochemical screening**

Phytochemical evaluation is one of the important tools for quality assessment which includes preliminary phytochemical screening, chemo profiling, and marker compound analysis. In the present study,

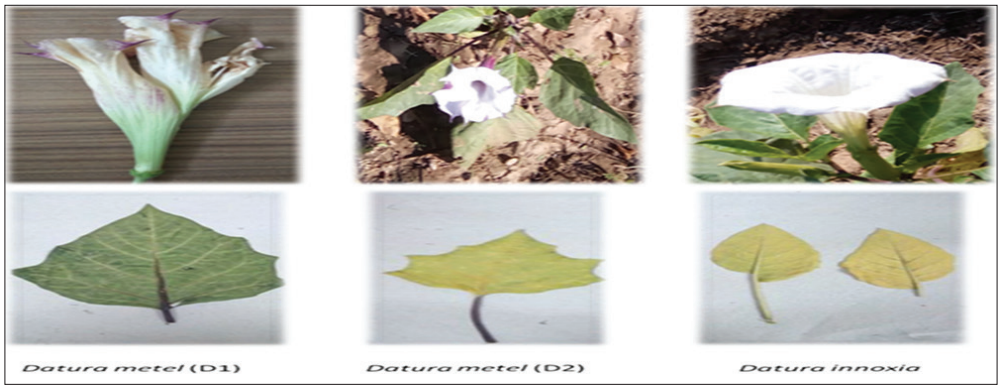



**Fig. 1: The morphology of flower and leaf of *Datura* spp.**

**Table 1: Plant materials collected from different localities, sample codes, accession number, and altitude**

| S. No. | Species | Sample code | Localities | Accession number | Altitude (m) |
|---|---|---|---|---|---|
| 1. | *D. metel* | D1 | Punjabi Bagh, Patiala | 61077 | 255 |
| | | D2 | Plant Conservatory (PUP) | 61034 | 255 |
| 2. | *D. innoxia* | D3 | Plant Conservatory (PUP) | 61033 | 255 |
| | | D4 | Water supply, Abohar | 61032 | 180 |
| | | D5 | Sekhupur, Patiala | 61031 | 255 |

*D. metel: Datura metel, D. innoxia: Datura innoxia*

**Table 2: The comparative morphological characters of different populations of *Datura* spp.**

| S. No. | Plant characters | *D. metel* | | *D. innoxia* | | |
|---|---|---|---|---|---|---|
| | | D1 | D2 | D3 | D4 | D5 |
| 1. | Average plant height (cm) | 128 | 92 | 65 | 95 | 89 |
| 2. | Leaf color | | | | | |
| | Upper surface | Dark green | Dark green | Dark green | Dark green | Dark green |
| | Lower surface | Purple greenish | Purple greenish | Light green | Light green | Light green |
| 3. | Average number of leaves/branch | 24 | 20 | 16 | 23 | 21 |
| 4. | Leaf margins | Serrate | Serrate | Elliptical entire margin | Elliptical entire margin | Elliptical entire margin |
| 5. | Stem (color and texture) | Violet non-hairy | Violet non-hairy | Creamish green, hairy | Creamish green, hairy | Creamish green, hairy |
| 6. | Flower color | Yellowish with purplish margin | White inside and purple outside | White | White | White |
| 7. | Average flower length | 18 | 17 | 15 | 16 | 16 |
| 8. | Number of whorls of corolla | Three | One | One | One | One |
| 9. | Average size of fruit (capsule) with calyx cap (cm) | 3 | 2.8 | 2.6 | 2.9 | 2.8 |
| 10. | Average spine length (cm) | 0.7 | 0.6 | 0.5 | 0.6 | 0.7 |
| 11. | Average number of seed/ capsule (mature) | 49 | 43 | 36 | 48 | 45 |
| 12. | Color of seed (young capsule) | White to pale yellow | White to pale yellow | White | White | White |
| 13. | Color of seed (mature capsule) | Yellowish brown | Yellowish brown | Brownish | Brownish | Brownish |

*D. metel: Datura metel, D. innoxia: Datura innoxia*

**Table 3: Preliminary phytochemical screening of root and seed extracts of *Datura* spp.**

| Test compounds | Seeds | | | | | | Roots | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | A | B | C | E | H | M | A | B | C | E | H | M |
| Alkaloids | + | + | + | ++ | + | +++ | + | + | + | + | + | ++ |
| Phenols | ++ | + | + | ++ | + | +++ | + | + | + | + | + | ++ |
| Steroids | – | ++ | ++ | ++ | + | + | – | ++ | ++ | + | + | + |
| Flavonoids | + | + | + | ++ | + | +++ | + | + | + | + | + | ++ |
| Triterpenoids | + | + | + | ++ | + | +++ | + | + | + | + | + | ++ |

+: Traces, ++: Present, +++: Strongly present, –: Absence. A: Aqueous, B: Butanol, C: Chloroform, E: Ethyl acetate, H: Hexane, M: Methanol

the data regarding preliminary phytochemical screening of seed and root samples in the different solvent extract are given in Table 3. At present, the preliminary phytochemical screening of different extracts revealed that steroids were absent in the aqueous extracts of seeds and root samples. All the other compounds, namely alkaloids, phenols, steroids, flavonoids, and triterpenoids were present in all the root and seed samples screened but roots have a low level of these pharmaceutical compounds as compared to seeds.

**Total phenolic and flavonoids contents**

Polyphenols are one of the major groups of compounds that act as a primary antioxidant scavenger. The medicinal properties of the plant are due to the presence of polyphenols having various biological effects, so it is important to estimate the total phenolic content in the extracts of the plant. The data regarding total phenolic and flavonoids content in the seeds and roots in the different solvent extract were given in Table 4. The total phenolic content in the different solvent extracts of roots and seeds of *D. metel* (D1) ranges from 1.303±0.139 to 6.533±0.135 mg/g dry weight (DW). The effect of different solvents on the extraction of phenolic content is clearly evident in the present study. In case of seed part, the order is M>E>A>C>B>H and from root part, it is M>B>E>A>C>H. As maximum content is recorded in the methanolic extracts of seed parts, so methanol extracts of seeds of the different populations have been further analyzed. Among the different populations of *Datura* spp., maximum content was recorded in D4 and D5 samples (10.39±0.174 and 8.531±0.746 mg/g DW) Table 5.

The total flavonoid content in the different solvent extracts of seeds and roots of *D. metel* (D1) ranges from 2.289±0.118 to 0.071±0.002 mg/g DW. The concentration trend of flavonoids content in different solvents of seed extracts with a decreasing order is M>E>B>H>C>A, while in roots is E>M>H>A>C>B. Interestingly from the root parts, flavonoid content is recorded in ethyl acetate solvent (0.998±0.914 mg/g DW). The total flavonoids content is significantly high in seeds as compared to the roots (Table 4). Among the all samples studied of *Datura* flavonoid content with decreasing order are D1>D2>D3>D5>D4 Table 5.

**Antioxidant assay**

Antioxidant activity was evaluated in terms of DPPH radical scavenging activity and TAC.

**TAC**

This assay is based on the formation of phosphate/Mo (V) complex in acidic medium by the reductive power of analyte. The method is quantitative, as the TAC is expressed as AA equivalents. TAC of seeds is more than roots as clearly seen in the given Table 6. Aqueous and methanolic extracts have the maximum total antioxidant as compared with other solvents in seeds, while ethyl acetate and methanolic extracts in case of roots. In seeds of different accessions of *Datura* spp. D1 has the highest TAC (10.35±0.217 mg/g DW) and D4 with the lowest (7.041±0.091 mg/g DW) as shown in Table 7.

**DPPH radical scavenging activity**

Different series of concentrations are tested to determine the concentration required to attain 50% radical scavenging effect ($IC_{50}$). Larger scavenging activity is indicated by the lower IC50 values, as lesser amount of extract is needed to decrease the concentration of DPPH to 50%. The $IC_{50}$ values of the different solvent extract are given in Tables 6 and 7. Methanol extract has higher DPPH radical scavenging activity as compared with the other solvents extracts in the present study. The decreasing order of activity in the seeds is M>E>H>A>C>B and in the roots is M>E>A>H>B>C (Table 6). Between the various accessions of *Datura,* D1 has the highest value of activity and the lowest in D2 and D3 Table 7.

**HPTLC analysis of chlorogenic acid and caffeic acid**

HPTLC has emerged as an important tool for quantitative and qualitative analysis of different phytochemicals from herbal drug formulations. Analysis of different marker compounds has been done by various workers in different plant species by this technique which is best suitable for identification, visualization, and quantification

**Table 4: The content of total phenol and flavonoids in the different solvent extracts of seeds and roots of D1**

| S. No. | Extracts | TPC(mg chlorogenic acid equivalent/g dry weight) | | TFC (mg quercetin equivalent/g dry weight) | |
|---|---|---|---|---|---|
| | Solvents | Seeds | Roots | Seeds | Roots |
| 1. | Aqueous | 3.756±0.073 | 1.983±0.126 | 0.914±0.046 | 0.090±0.002 |
| 2. | Butanol | 2.578±0.149 | 2.308±0.056 | 1.182±0.021 | 0.071±0.002 |
| 3. | Chloroform | 3.111±0.073 | 1.581±0.169 | 0.926±0.047 | 0.080±0.003 |
| 4. | Ethyl acetate | 4.156±0.713 | 2.039±0.146 | 1.824±0.042 | 0.998±0.041 |
| 5. | Hexane | 2.189±0.179 | 1.303±0.139 | 1.152±0.037 | 0.424±0.024 |
| 6. | Methanol | 6.533±0.135 | 6.511±0.106 | 2.289±0.117 | 0.906±0.021 |

Values are mean±standard error of three replicates. TPC: Total phenolic content, TFC: Total flavonoid content

**Table 5: The content of total phenol and flavonoids in the methanolic extracts of seeds of different accessions of *Datura* spp**

| S. No. | Samples | TPC (mg chlorogenic acid equivalent/g dry weight) | TFC (mg quercetin equivalent/g dry weight) |
|---|---|---|---|
| 1. | D1 | 6.533±0.135 | 2.289±0.118 |
| 2. | D2 | 3.983±0.236 | 2.046±0.101 |
| 3. | D3 | 3.894±0.159 | 1.722±0.055 |
| 4. | D4 | 10.39±0.174 | 1.534±0.013 |
| 5. | D5 | 8.531±0.746 | 1.668±0.046 |

Values are Mean±Standard error of three replicates. TPC: Total phenolic content, TFC: Total flavonoid content

**Table 6: Total antioxidant capacity and DPPH radical scavenging activity of different solvent extracts of seeds and roots of D1**

| S. No. | Solvents | TAC (mg AA equivalent/gm dry wt) | | DPPH ( $IC_{50}$ µg/ml) | |
|---|---|---|---|---|---|
| | | Seeds (D1) | Roots (D1) | Seeds (D1) | Roots (D1) |
| 1. | Aqueous | 10.21±0.217 | 3.928±0.033 | 16.075 | 39.487 |
| 2. | Butanol | 7.235±0.040 | 3.554±0.032 | 38.222 | 90.588 |
| 3. | Chloroform | 6.528±0.035 | 3.272±0.021 | 21.5 | 110 |
| 4. | Ethyl acetate | 7.82±0.091 | 4.316±0.028 | 12.740 | 32.083 |
| 5. | Hexane | 8.655±0.165 | 3.704±0.023 | 13.651 | 59.231 |
| 6. | Methanol | 10.35±0.217 | 4.759±0.089 | 10.886 | 16.559 |

Values are mean±standard error of three replicates. DPPH: 2, 2-Diphenyl-1-picrylhydrazyl, TAC: Total antioxidant capacity

**Table 7: Total antioxidant capacity and DPPH radical scavenging activity of seeds of different accessions of *Datura* spp.**

| S. No. | Samples | TAC (mg AA equivalent/gm dry wt) | DPPH ( $IC_{50}$ µg/ml) |
|---|---|---|---|
| 1. | D1 | 10.35±0.217 | 10.886 |
| 2. | D2 | 7.757±0.113 | 21.536 |
| 3. | D3 | 8.441±0.088 | 20.601 |
| 4. | D4 | 7.041±0.091 | 12.286 |
| 5. | D5 | 9.293±0.126 | 15.177 |

Values are Mean±Standard error of three replicates. DPPH: 2, 2-Diphenyl-1-picrylhydrazyl, TAC: Total antioxidant capacity

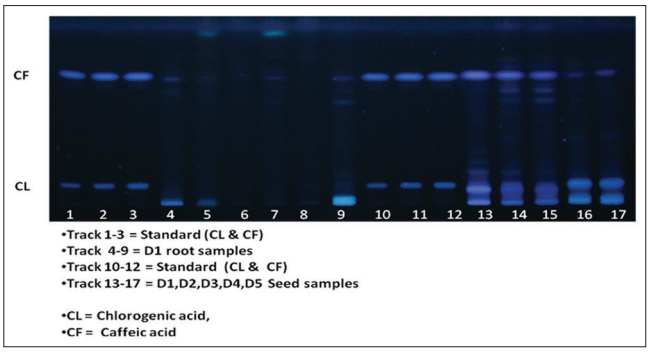



**Fig. 2: High-performance thin-layer chromatography chromatogram at 366 nm**

of biologically active compounds. *Datura* is rich in biologically active compounds; hence, pharmacologically important two marker compounds chlorogenic acid and caffeic acid have been identified and quantified by HPTLC technique.

The bands are visualized at 366 nm under UV light (Fig. 2). The confirmation of the $R_f$ values of chlorogenic acid and caffeic acid is found to be 0.15 and 0.80, respectively (Table 8). HPTLC densitometric chromatogram of standard tracks and sample tracks is given in Fig. 3. Both standard compounds are found to be linear over the range of 200–600 ng/spot**.** The linear regression equations with correlation coefficient values for both compounds are given in Table 8. The content of phenolic compounds in plant samples has been measured using a calibration curve of the standard compounds.

Out of six solvents used for extraction in roots, the maximum amount of caffeic acid was observed in butanol crude extracts, while in case of seeds of all studied populations of *Datura*, maximum content was observed in D1. Chlorogenic acid was not detected from the root parts in the present study shown in Fig. 2. Maximum amount was detected in the D5 and D4 population of *D. innoxia.* Between the two species studied, *D. metel* is rich in caffeic acid and *D. innoxia* is rich in chlorogenic acid. This is the first report of the detection and quantification of these two hydroxycinnamic acids from the root and seed parts of *Datura* spp. shown in Graph 1.

## DISCUSSION

Since the Vedic period there were descriptions on medicinally important plants and their identification, distribution and therapeutical properties for various ailments [14]. Nowadays, people are enthusiastically interested in the study of medicinal plants for different biologically

**Table 8: Different parameters of the HPTLC method**

| S. No. | Marker compounds | Linearity range (ng) | Linear equation | Retention factor (Rf) | Correlation coefficient |
|---|---|---|---|---|---|
| 1. | Caffeic acid | 200–600 | y=18.9x–1503 | 0.15 | >0.990 |
| 2. | Chlorogenic acid | 200–600 | y=7.21x+430 | 0.80 | >0.997 |

HPTLC: High-performance thin-layer chromatography

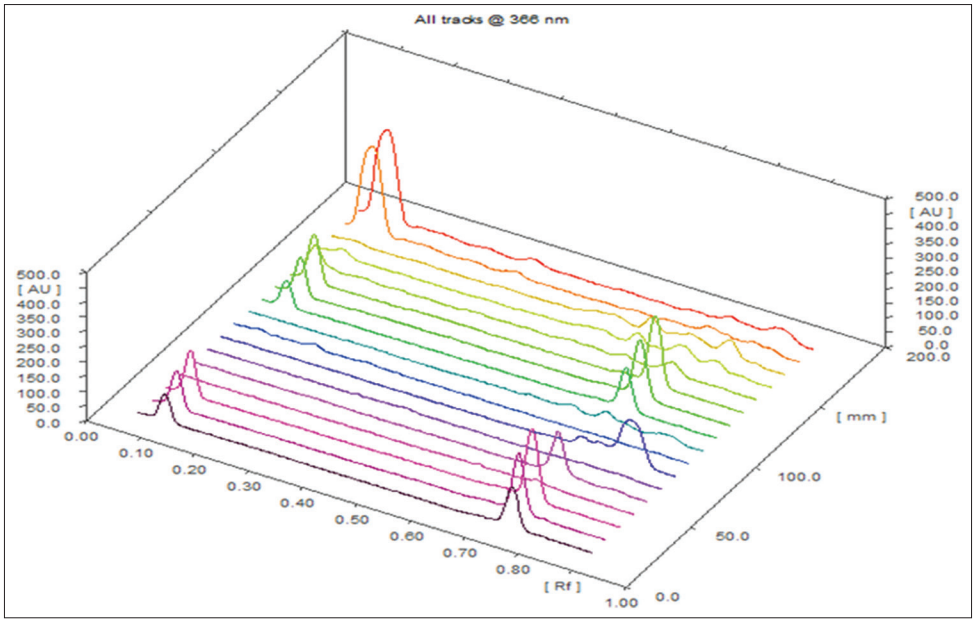
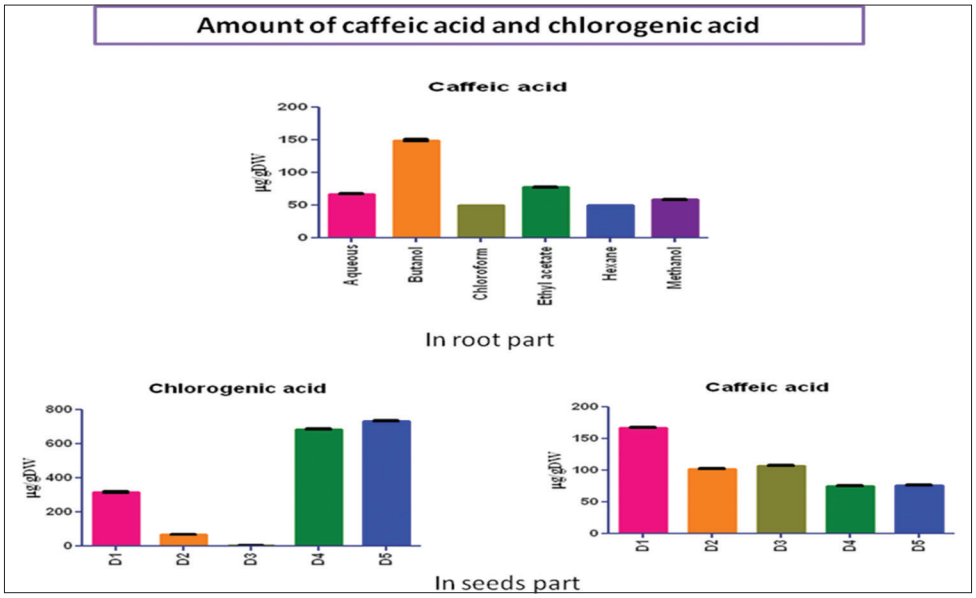



**Fig. 3: Three-dimensional chromatogram of tracks**




**Graph 1: (a-c) Content of caffeic acid and chlorogenic acid in the roots and seeds (µg/g DWE) of different populations of *Datura metel* and *Datura innoxia***

active phytoconstituents which exhibit important therapeutic effects. During the present study, the collections of *Datura* species are made from Punjab for evaluation of phytochemical diversity. In the results, two morphotypes (D1 and D2) have been recorded within the same species of *D. metel.* On morphological comparison, a lot of variation has been recorded between two morphotypes of *D. metel* and in between species. On the basis of morphological comparisons, morphotype D1 had triple whorled corolla; yellowish with purplish margin flower, and broad leaves, while in case of morphotype (D2) has a single whorled corolla, white inside and purple outside flower and comparatively small leaves.

Similarly, morphological diversity and hyoscyamine/scopolamine contents in 12 Algerian samples of *D. stramonium* of different origin were previously reported [15]. Phytochemical constituents in the plant samples are known to be biologically active compounds, and they are responsible for different activities such as antioxidant, antimicrobial, antifungal, and anticancer [16,17]. All secondary metabolite components displayed antioxidant and antimicrobial properties through different biological mechanisms. Most of the secondary metabolite components were isolated and identified in the polar plant crude extracts [18]. In the present study, most of the bioactive

compounds were found in polar methanol and ethyl acetate crude extracts of seeds and roots. Further, during preliminary screening, it was observed that seeds have good amount of phytoconstituents as compared to roots. Jamdhade *et al*. had done a qualitative analysis of the extracts from the root, stem, leaf, seed, and fruit coat sample of *D. metel* and showed that leaf and roots contain more alkaloids, tannins, saponins, and iridoids [19]. The content of phenols and flavonoids is greatly influenced by the type of extraction solvent and parts of the plant, as is very much clear in the present results. Phenols and flavonoids are highly beneficial for human health for combating several diseases, as these have a high effect on the scavenging of singlet oxygen and free radicals [20-23]. The present observations are in accordance with the findings of different workers and our results also suggest that these polyphenols profoundly contribute to the antioxidant potential of *Datura* spp. [24].

Antioxidant activity was evaluated in terms of DPPH radical scavenging activity and TAC. DPPH radical is widely used to check the antioxidant potential of medicinal plants due to its high sensibility and easily availability. The crude extract of *D. metel* and *D. innoxia* contain flavonoid, steroids, alkaloids, phenolic, and triterpenoids. All these bioactive compounds are able to discolor DPPH solution by their hydrogen donating ability [25,26]. *D. stramonium* seeds possess alkaloids and flavonoids and have potent antioxidant and antibacterial activities [27]. The antioxidant activity through free radical scavenging activity (DPPH) method showed that seeds have maximum activity as compared to root parts. So far, between the seed samples of different *Datura* spp. studied, D1 has maximum activity of radical scavenging and antioxidant capacity, due to the presence of more flavonoids. Several reports are available on flavonoid groups which exhibited high potential biological activities such as antioxidant, anti-inflammatory, antimicrobial, anti-angiogenic, anticancer, and anti-allergic reactions [28-32].

HPTLC is a unique technique which can be applied for simultaneous identification of different compounds in the same plant or the specific compounds in different plants in a single attempt. This method is highly economic time saving, needs less expertise, needs a small amount of solvent, and screens a huge number of samples simultaneously. This method can be applied for the qualitative and quantitative determination of various phytoconstituents in medicinal plants. The specific fingerprints of species are developed through HPTLC and can be used to check adulteration at any stage.

In the present study, HPTLC densitogram analysis has been used for quantification of specific pharmacologically important compounds in the seed and root part of *D. metel* and *D. innoxia.* As the morphological study reveals two morphotypes of *D. metel,* i.e., D1 and D2 have been subjected to the qualitative as well as quantitative determination of phytoconstituents. An HPTLC densitometric method for the quantification of these biomarker compounds in various plant species has been developed by many workers [33-38]. Rahmoune *et al*. have investigated phenylpropanoids and fatty acids composition in leaves and roots extracted from *D. innoxia* and *D. stramonium* using gas chromatography-electron impact/time of flight mass spectrometry (GC-EI/TOF-MS) chromatography techniques. They revealed that in both the *Datura* species, phenylpropanoids composition in leaves was remarkably higher than in the roots. However; fatty acids (hexadecanoic acid and octadecanoic acid) are observed in almost equal rate between leaves and roots [39]. To the best of our knowledge, the present investigation is the first report of the detection and quantification of these two hydroxycinnamic acids (chlorogenic and caffeic acid) from the root and seed parts of *Datura* spp.

## CONCLUSION

The present study depicts that there is a lot of variability in terms of morphology and phytoconstituents among the different species and populations of *Datura.* The presence of such diversity in the quantities of active compounds in different morphotypes or populations, establish the necessities of phytochemical screening covering various ecotypes/morpho/cytotypes. Further, these marked chemotypes should be standardized for medicinal dose and authentication of products for the international market.

## ACKNOWLEDGMENT

The authors are grateful to the Department of Botany and Sophisticated Instrumentation Centre, Punjabi University, Patiala, Punjab, India, for providing necessary facilities to carry out HPTLC analysis. The corresponding author is highly thankful to the DBT-IPLS (Ref. No.BT/PR 4548/NF/22/146/2012) for providing Senior Research Fellowship.

## AUTHORS' CONTRIBUTION

The concept and design of the study were done by Dr. RC Gupta and Mr. Saroj Kumar Pradhan. Experimental work was done by Mr. Mahinder Partap. Interpretation of data and draft of manuscript were prepared by Mr. Mahinder Partap and Mr. Saroj Kumar Pradhan. Revision of article and proofreading was done by Dr. RC Gupta.

## CONFLICTS OF INTEREST

The authors declared no conflicts of interest.

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