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ANTIOXIDANT ACTIVITY AND FT-IR ANALYSIS OF *DATURA INNOXIA* AND *DATURA METEL* LEAF AND SEED METHANOLIC EXTRACTS

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Abstract

Background: *Datura* (family- Solanaceae), has a long history of being used as herbal medicine. These medicinal effects have been attributed to the phytochemicals present in the plant leaves and seeds, in particular alkaloids, flavonoids and phenolic compounds. The objective of this study was to investigate the methanolic leaf and seed extracts of *Datura innoxia* (DLP-I & DSP-I) and *Datura metel* (DLP-M & DSP-M) for their total phenolic, flavonoids and *in-vitro* antioxidant properties.
Materials and Methods: Determination of total phenolic content and total flavonoid content and antioxidant activity in terms of total antioxidant assay, ABTS assay, DPPH assay and *in-vitro* lipid peroxidation inhibiting activity were determined along with the FT-IR (Fourier transform infrared spectroscopy) analysis of the extracts.
Results: The highest total phenolic and total flavonoid content were registered by the *D. innoxia* leaf extract (70.26 ± 1.12 mg GAE/g and 34.24 ± 1.28 mg RE/g respectively). Maximum DPPH radical scavenging activity was exerted by the leaf extract of *D. innoxia* (IC_{50} = 146.69 ± 8.46 µg/mL) among the four different methanolic extracts. The highest activity of the ABTS assay was found in *Datura innoxia* leaf extract (IC_{50} value = 149.42 ± 13.43 µg/mL) and the highest total antioxidant capacity was found to be present in *D. innoxia* leaf extract (221.25 ± 1.06 mg AAE/g) whereas *D. metel* seed extract registered the maximum lipid peroxidation inhibition activity (IC_{50} = 112 ± 1.30 µg/mL). The FT-IR data also supported the maximum activity in *D. innoxia* (leaf and seed) extracts.
Conclusion: The results thus obtained suggested that the plant *Datura innoxia* possess considerable antioxidant activity over *Datura metel* and therefore can be established as a potential source of natural antioxidant.

Key words: *Datura* species, Methanolic extract, DPPH, ABTS, FT-IR, Antioxidants.

Introduction

Plants have been important sources of compounds with potential medicinal activity since time immemorial. Indian sub-continent is rich in such medicinal plants and Indian traditional medicinal system (Ayurveda) which is primarily based on plant based medicinals, has survived since thousands of years up to the present times. The genus *Datura* (Solanaceae), commonly known as Jimson weed or Thorn Apple is distributed throughout the world and is comprised of 14 species, of which 10 species are found in India. Among them *D. stramonium, D. innoxia, D. metel* are the most important medicinal plants (Schultes and Hoffman, 1979). *Datura* has been well known for its use in traditional Chinese and Indian systems of medicine for centuries (Rajesh, 2002) and it is frequently used in traditional systems of medicines as narcotic, anodyne, antispasmodic and as a useful remedy for various human ailments including ulcers, wounds, inflammation, rheumatism and gout, sciatica, bruises and swellings, fever, asthma, bronchitis and toothache etc (Kirtikar and Basu, 1999; Gaire and Subedi, 2013). Inflammation has been reported to be accompanied by the formation of reactive oxygen species and free radicals. Excessive ROS production can overwhelm cellular antioxidant defences and can lead to oxidative stress, causing cell injury and cell death. This may lead to the development of many chronic diseases and complications like atherosclerosis, cancer, diabetes, aging and other degenerative disorders in human (Kumpulainen and Salonen, 1999; Cook and Samman, 1996). A wide range of antioxidants from both natural and synthetic origin has been proposed for use in treatment of various human diseases (Cuzzocrea et al., 2001). However, the use of synthetic antioxidants has been questioned due to their potential health risks and toxicity (Gutteridge and Halliwell, 2010). The search for antioxidants from natural sources has received much attention in recent years. Plant secondary metabolites such as flavonoids and other phenolic compounds have been reported as scavengers of free radicals (Rice-Evans et al., 1997).

The objective of the present study was to investigate the antioxidant activity of the methanolic extracts of *Datura innoxia* and *Datura metel* leaves and seeds using different *in-vitro* antioxidant parameters. Total phenolic and flavonoid content were also determined in order to evaluate a relation between the antioxidant activity and phytochemical constituents.

Materials and Methods

Collection of Sample

Fresh leaves and seeds of *Datura innoxia and Datura metel* were collected in the month of January 2013 from Saketri (Latitude 30.7457, Longitude 76.8469) Haryana, India. The plant was authenticated and compared with voucher specimen number 6593 for *D. metel* & 4724 for *D. innoxia* at Department of Botany, Panjab University, Chandigarh, India. The leaves and seeds were thoroughly washed with the tap water, shade dried, grounded to fine powder and stored till further use.

Extraction

10 g each of leaf powder and seed powder were taken in 200 mL of methanol in separate conical flasks and then kept on a rotary shaker for 24 h. The above extracts were filtered through four layers of muslin cloth and then with Whatman No. 1 filter paper. Methanol was evaporated using rota vaporizer (Equitron, Roteva – 8763 RV) at 50 rpm and 70º C. The dried extracts were stored at 4º C in airtight bottles.

Determination of Total Phenolic Content

The concentration of phenolics in plant extracts was determined using spectrophotometric method (Makkar et al., 1997). Methanolic solution of the extract at a concentration of 1 mg/mL was used in the analysis. The reaction mixture was prepared by mixing 0.5 mL of methanolic extract, 1.25 mL of Folin-Ciocalteu's reagent (1:1) dissolved in water and 1 mL of 7.5% $NaHCO_3$. Blank was concomitantly prepared**,** containing 0.5 mL methanol, 1.25 mL Folin-Ciocalteu's reagent (1:1) dissolved in water and 1 mL of 7.5% of $NaHCO_3$. The samples were thereafter incubated in dark for 30 min. The absorbance was determined using spectrophotometer at Λ_{max} of 765 nm. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. The same procedure was repeated for the standard solution of gallic acid and the calibration line was constructed. Based on the measured absorbance, the concentration of phenolics was read (mg/mL) from the calibration curve, then the content of phenolics in extracts was expressed in terms of milligram of gallic acid equivalents per gram of extract (mg of GAE/g).

Determination of Total Flavonoid Content

The content of flavonoids in the examined plant extracts was determined using spectrophotometric method (Chang et al., 2002). Rutin was used to make the calibration curve. The standard solutions or extracts (0.5 mL) were mixed with 1.5 mL of methanol, 0.1 mL of 10% aluminium chloride (w/v), 0.1 mL of 1 mol/L sodium acetate and 2.8 mL water. The volume of 10% aluminium chloride was substituted by the same volume of distilled water in blank. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm. Based on the measured absorbance, the concentration of flavonoid was calculated (mg/mL) from the calibration curve, then the content of flavonoids in the extract was expressed in terms of milligram of rutin equivalent per gram of extract (mg of RE/ g).

Evaluation of Total Antioxidant Capacity

The assay is based on the reduction of Mo (VI) to Mo (V) by the extract and subsequent formation of a green phosphate/ Mo (V) complex at acidic pH (Preito et al., 1999). 0.3 mL extract was mixed with 3 mL of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes containing the reaction solutions were incubated at 95^o C for 90 min. Then the absorbance of the solution was measured at 695 nm using a spectrophotometer against blank after cooling to room temperature. Methanol (0.3 mL) in the place of extract was used as blank. The antioxidant activity was expressed as milligram of ascorbic acid equivalent per gram of extract (mg AAE/ g).

ABTS Radical Cation Decolourization Assay

The method of Re et al. (1999) with slight modifications was adopted for ABTS (2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonate) assay. In this method, the oxidant is generated by persulfate oxidation of 2,2-Azinobis (3-ethylbenzoline-6-sulfonic acid) (ABTS). ABTS radical cation was produced by reacting ABTS solution (7mM) with 2.45 mM ammonium persulfate and the mixture was allowed to stand in the dark at room temperature for 12-16 hours before use. 1 mg/ mL concentration of methanolic extract (20-100µl) was added to 1.9 mL of ABTS and the final volume was made up with methanol to 1 mL and was incubated for 5 min. The absorbance was read at 745 nm and the percentage inhibition was calculated.

DPPH Free Radical Scavenging Activity

The free radical scavenging activity of extracts was measured by 2,2-diphenyl-1-picryl-hydrazyl (DPPH) (Blois, 1958). The reaction mixture consisting of DPPH in methanol (100 µM, 1mL) and different concentrations of solvent extracts (1 mL) was incubated for 30 min in dark, after which the absorbance was measured at 517 nm. BHT was used as a positive control. The percentage inhibition was determined by comparing the result of the test and the control. Percentage inhibition was calculated by the formula:

Inhibition (%) = [1-(A/B)] X 100

Where:

A= absorbance of sample

B= absorbance of control

The inhibiting effects of all the extracts showed varied levels of DPPH radical scavenging activity, expressed as $IC_{50.}$

***In Vitro* Lipid Peroxidation Inhibition Assay**

Freshly excised rat liver was processed to get 10% homogenate in cold phosphate buffered saline pH 7.4 and filtered to get a clear homogenate. The degree of lipid peroxidation was assayed by estimating the TBARS by using the method of Ohkawa et al., 1979 with slight modifications. Different concentrations of extracts in DMSO and water were added to the liver homogenate. Lipid peroxidation was initiated by adding 50 µM ferrous sulphate solution to 0.2 mL of the tissue homogenate. After 30 min, 15% TCA

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and 0.67% TBA was added to the incubated mixture. The mixture was heated for 15 min at 95 ºC. The intensity of pink colour formed was measured at 535 nm. The results were expressed in terms of percentage inhibition.

FT-IR Analysis

The dried methanolic extracts were subjected to FTIR analysis (Perkin Elmer-Model RZX) under IR region in the range of 400-4000 cm^{-1} and the associated functional groups were determined (Coates, 2000).

Statistical Analysis

Assays were performed in triplicate and the results are shown as mean ± standard deviation. Linear regression analysis was used to calculate the IC_{50} values. Pearson's correlation coefficient was calculated using Microsoft excel 2007. Statistical significance was determined among various treatments with one way ANOVA test using SPSS 16.0 for Windows. A statistical significance of $p < 0.05$ was considered to be significant.

Results and Discussion

Phenolic compounds are commonly found in both edible and medicinal plants, and have been reported to have diverse biological effects such as being antioxidant & anti-inflammatory and possessing anti-aggregatory & vasodilating activity (Kahkonen et al., 1999). The Folin-Ciocalteu method was used to determine the total phenolic content. This method measures the reduction of the reagent by phenolic compounds with the formation of a blue complex (Imeh and Khokhar, 2002). The total phenolic content is expressed in terms of milligrams of gallic acid equivalents (GAE) per g of the extract. Total phenolic content obtained from the leaf and seeds methanolic extract of *Datura innoxia* and *Datura metel* are presented in Table 1. The phenolic content was found to be highest in DLP-I extract (70.26 ± 1.12 mg GAE/g) followed by DSP-M (61.93 ± 0.69 mg GAE/g), DSP-I (51.01 ± 0.58 mg GAE/ g) and DLP-M (46.09 ± 0.43 mg GAE/g). Phenolic compounds exhibit their antioxidant activity by several mechanisms such as donating hydrogen atoms to free radicals, scavenging other reactive species such as $OH^{\bullet}$, $NO^{\bullet}_2$, N_2O_3, ONOOH and HOCl (Kumar et al., 2014). Out of the leaf methanolic extracts, the highest phenolic content was found to be present in *Datura innoxia* extract as compared to the *Datura metel* extract and their antioxidant activities were found to be in the same order. Scopoletin, a phenolic coumarin compound, has been reported in the Dr. Duke's phytochemical and ethnobotanical databases. Scopoletin, which is present in the *Datura innoxia* plant may contribute to the high content of antioxidants in the extract (Duke, 1992).

Flavonoids are water soluble polyphenolic compounds which are extremely common and widespread in the plant kingdom as their glycosides. The total flavonoid content is expressed in terms of rutin equivalent (RE) as 34.24 ± 1.28 mg RE/g for DLP-I, 21.71 ± 0.12 mg RE/g for DLP-M, 10.52 ± 0.63 mg RE/g for DSP-M and 6.99 ± 1.11 mg RE/g for DSP-I (Table 1). The antioxidant activity of flavonoids depends on the structure and substitution pattern of hydroxyl groups (Sharififar et al., 2008). They are capable of effectively scavenging the reactive oxygen species because of their phenolic hydroxyl groups (Cao et al., 1997). In view of their wide pharmacological and biological actions, they have a greater therapeutic potential. The flavonoid content is found to be significantly higher ($p < 0.05$) in the leaf innoxia methanolic extract and thereby also contributing to the same antioxidant pattern in the leaf extracts. Luteolin, a flavonoid compound, has been reported to be present in the *Datura innoxia* (Wollenweber et al., 2005). The presence of high phenolic and flavonoid content in the fractions contributes directly to their antioxidant activity.

Table 1: Total Phenolic and Flavonoid content of *Datura innoxia* and *Datura metel* leaf and seed methanolic extracts

Extract	Total phenolic content (mg GAE/g ± SD)	Total flavonoid content (mg RE/g ± SD)
DLP-I	70.26 ± 1.12^{a}	34.24 ± 1.28^{a}
DSP-I	51.01 ± 0.58^{c}	6.99 ± 1.11^{c}
DLP-M	46.09 ± 0.43^{d}	21.71 ± 0.12^{b}
DSP-M	61.93 ± 0.69^{b}	10.52 ± 0.63^{c}

Results are expressed as mean ± SD (n = 3). Gallic acid equivalent (GAE), rutin equivalent (R.E), *Datura innoxia* leaf extract (DLP-I), *Datura innoxia* seeds extract (DSP-I), *Datura metel* leaf extract (DLP-M), *Datura metel* seeds extract (DSP-M). Values in the same column followed by a different letter ($^{a-d}$) are significantly different ($p < 0.05$) and values having same superscript are not statistically significant.

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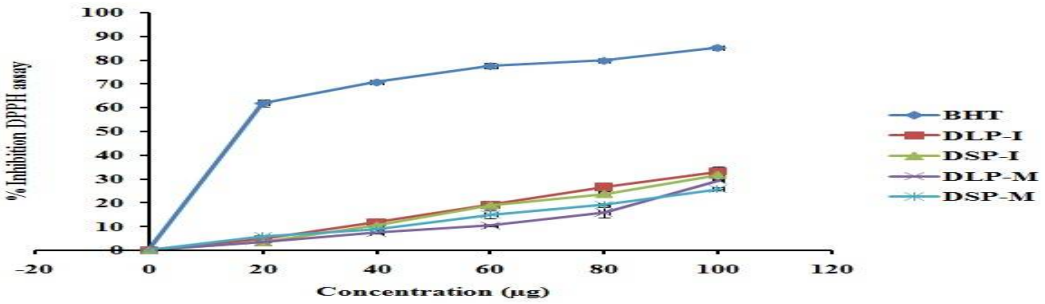

Figure 1: DPPH radical scavenging activity of *Datura innoxia* and *Datura metel* leaf & seed methanolic extracts. Values are represented as mean ± SD (n = 3). DLP-I (*Datura innoxia* leaf extract), DSP-I (*Datura innoxia* seeds extract), DLP-M (*Datura metel* leaf extract), DSP-M (*Datura metel* seeds extract).

The DPPH (2, 2-diphenyl-1-picrylhydrazyl) radical scavenging activity of the extracts is shown in Figure 1. Table 2 shows the IC_{50} values of the extracts, as compared to that of BHT (Butylatedhydroxytoluene) which is a well known antioxidant. The DPPH scavenging assay is based on the ability of 2, 2 diphenyl-1-picryl-hydrazyl, a stable free radical, to decolorize in the presence of the antioxidants. The IC_{50} values obtained from the extracts are 146.69 ± 8.46 µg/mL for DLP-I, 152.40 ± 1.85 µg/mL for DSP-I, 180.97 ± 5.49 µg/mL for DLP-M and 199.34 ± 6.29 µg/mL for DSP-M. The lower IC_{50} value indicates higher antioxidant capacity and hence significantly higher capacity to neutralize the DPPH radical was found in *Datura innoxia* leaf and seed methanolic extracts as compared to *Datura metel* extracts.

Table 2: The IC_{50} values for DPPH, ABTS and *In vitro* Lipid peroxidation inhibition assay of *Datura innoxia* and *Datura metel* leaf and seed methanolic extracts

Extracts	DPPH (µg/mL)	ABTS (µg/mL)	TBARS (µg/mL)
DLP-I	146.69 ± 8.46^{b}	149.42 ± 13.43^{b}	166.98 ± 7.39^{a}
DSP-I	152.40 ± 1.85^{b}	181.10 ± 4.03^{c}	25839.82 ± 195.22^{b}
DLP-M	180.97 ± 5.49^{c}	304.63 ± 25.39^{d}	115.188 ± 1.74^{a}
DSP-M	199.34 ± 6.29^{d}	192.13 ± 4.88^{c}	112.003 ± 1.3^{a}
BHT	16.76 ± 0.41^{a}	5.856 ± 0.27^{a}	----
Rutin	---	----	148.13 ± 4.18^{a}

Results are expressed as mean ± SD (n = 3). *Datura innoxia* leaf extract (DLP-I), *Datura innoxia* seeds extract (DSP-I), *Datura metel* leaf extract (DLP-M), *Datura metel* seeds extract (DSP-M). Values in the same column followed by a different letter ($^{a-e}$) are significantly different ($p < 0.05$) and values having same superscript are not statistically significant.

ABTS radical scavenging assay involves a method that generates a blue/green $ABTS^{+}$ chromophore via the reaction of ABTS and potassium persulfate. The scavenging capacities of the extracts for the ABTS radical were measured & their percent inhibition values are presented in Figure 2. The IC_{50} values for DLP-I (149.42 ± 13.43 µg/mL), DSP-I (181.10 ± 14.03 µg/mL), DSP-M (192.13 ± 4.88 µg/mL) and DLP-M (304.63 ± 25.39 µg/mL) as compared to that of BHT (5.86 ± 0.27 µg/mL), which was used as standard. The scavenging effect of the extracts increased in a concentration dependent manner. *Datura innoxia* leaf extract exhibited a significantly higher scavenging activity as compared to *Datura metel* leaf extract. However, the results obtained from the seed methanolic extracts of *Datura innoxia* and *Datura metel* were not found to be significant with respect to each other.

Table 3: Total antioxidant activity of *Datura innoxia* and *Datura metel* leaf and seed methanolic extracts

Extract	Total antioxidant activity (mg AAE/g) ± SD
DLP-I	221.25 ± 1.06^{a}
DSP-I	130.5 ± 2.12^{b}
DLP-M	115 ± 2.82^{d}
DSP-M	121.5 ± 1.41^{c}

Results are expressed as mean ± SD (n = 3). Ascorbic acid equivalent (AAE), *Datura innoxia* leaf extract (DLP-I), *Datura innoxia* seeds extract (DSP-I), *Datura metel* leaf extract (DLP-M), *Datura metel* seeds extract (DSP-M). Values in the same column followed by a different letter ($^{a-d}$) are significantly different ($p < 0.05$).

The thiobarbituric acid reactive species (TBARS) method has been extensively used for the estimation of peroxidation of lipids in membrane or biological systems. This method measures the malondialdehyde (MDA) formed after lipid hydroperoxide

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decomposition, which forms a pink chromophore with thiobarbituric acid (TBA) (Hodges et al., 1999). The IC_{50} values of the extracts to inhibit the *in vitro* lipid peroxidation is in order of 112.00 ± 1.30 µg/mL for DSP-M, 115.18 ± 1.74 µg/mL for DLP-M, 166.98 ± 7.39 µg/mL for DLP-I and 25839.82 ± 195.22 µg/mL for DSP-I whereas the standard compound rutin has an IC_{50} value of 148.13 ± 4.18 µg/mL. Here, the extracts inhibited the lipid peroxidation in a concentration dependent manner (Figure 3) with DSP-M and DLP-M extracts showing the inhibition activities, although not significantly higher but comparable to the reference compound rutin (Table 2). The antioxidant and lipid peroxidation inhibition activity of *Datura metel* extracts may be attributed to the presence of allantoin (Duke, 1992).

Table 4: Functional group frequencies of *Datura innoxia* and *Datura metel* leaf and seed methanolic extracts

FUNCTIONAL GROUPS	DLP-I	DLP-M	DSP-I	DSP-M
ALKANES				
Methylene C-H asymmetric/symmetric stretch	2925,2854	2926,2854	2925,2854	2925,2854
Methyl C-H asymmetric/symmetric bend, methylene C-H bend	-	-	1455	1457
Cyclohexane ring vibrations	1049,1020	1052	-	1053
Skeletal C-C vibrations	1243,1105, 1049,1020	700,779, 893, 922, 1052	-	720
Methylene (CH2)n rocking	-	-	-	720
ALKENE				
Alkeynyl C=C stretch	1627	1629	1628	1628
Medial cis or trans C-H stretch	3011	-	-	-
Vinyl C-H in plane bend	1411	1414	1415	1416
Vinylidene C-H out of plane bend	-	893	-	-
AROMATIC				
Aromatic C-H out of plane bend	-	893, 779, 700	856,815	-
Aromatic C-H in plane bend	-	-	-	1157,1053
1,4 disubstitution(para)	-	-	-	858
ALKYNE				
Alkyne C-H bend	670, 618	618,669	618	-
Terminal alkyne	-	2111	-	-
HALOGEN				
Fluro compounds (C-F stretching)	1105,1049, 1020	1052,1103	1054	1053
Bromo compounds (C-Br stretching)	670, 618	669	618	618
Iodo compounds (C-I stretching)	534	-	-	-
Chloro compounds (C-Cl stretching)	-	700,779	701	720
HYDROXYL COMPOUNDS				
H-bonded OH stretch	3347	3387	3278	-
Polymeric OH stretch	3347	3387	-	3290
Primary or secondary OH in plane bend	1322	1321	-	-
Phenol or tertiary alcohol, OH bend	-	-	-	1379
Alcohol, OH out of plane bend	670, 618	-	-	720
ETHER				
Alkyl substituted ether C-O stretch (C-O-C)	1105 , 1049	1103	1054	-
Cyclic ethers large rings C-O stretch	1105, 1049	1103	-	-
Aromatic ethers, aryl O stretch	-	1245	-	1239
AMINO COMPOUNDS				
Primary amine C-N stretch	1020	-	1054	1053
Aliphatic primary amine	-	3387	-	-
Primary amine NH stretch	-	1052	-	-
Primary amine NH bend/ secondary amine	-	1629	1628	1628
Aliphatic secondary amine NH stretch	3347	-	-	-
Imino compound	3347	-	-	-
Secondary amine NH bend	1627	-	-	-
Secondary amine, CN stretch	-	-	-	1157
Aromatic primary amine CN stretch	1322	1321	-	-
Aromatic secondary amine	1322, 1243	1321	-	-
Aromatic Tertiary amine	1322	1321	-	-
CARBONYL COMPOUNDS				
Carboxylate ion	1322, 1411	1414	1539	-

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Ester	-	-	1745	-
HETERO-OXY COMPOUNDS				
organic phosphates (P=O stretch)	1322	-	1238	-
Aliphatic phosphates (P-O-C stretch)	1049, 1020	-	-	-
Aromatic Phosphates (P-O-C) stretch	-	-	-	1239
Dialkyl/aryl sulfones	1322	-	-	-
Organic sulphates	1411	-	-	-
Organic siloxane/silicone Si-O-Si	1020	-	1054	1053
Organic silicone Si-O-C	1105	-	-	-
THIOLS				
Open chain azo (-N=N-)	1627		1628	-
Thiol or thioether CH2-S (C-S stretch), disulfides C-S stretch	-		701	-
S-S disulfides	-		618	618
NITROGEN MULTIPLE COMPOUNDS				
Cyanate (-OCN and C-OCN stretch)	-	-	1158	-
INORGANIC IONS				
Carbonate ion	-	-	1455	-
Phosphate ion	-	-	1054	-
Silicate ion	-	-		1053

The total antioxidant capacity of the extracts, as assessed by the phosphomolybdenum method which is based on the reduction of Mo (VI) to Mo (V) by the antioxidant compound and the formation of a green phosphate/Mo (V) complex with a maximal concentration at 695 nm is expressed in terms of ascorbic acid equivalents (Table 3). DLP-I had the highest antioxidant activity (221.25 ± 1.06 mg AAE/g) followed by DSP-I (130.5 ± 2.12 mg AAE/g), DSP-M (121.50 ± 1.41 mg AAE/g), DLP-M (115 ± 2.82 mg AAE/g).

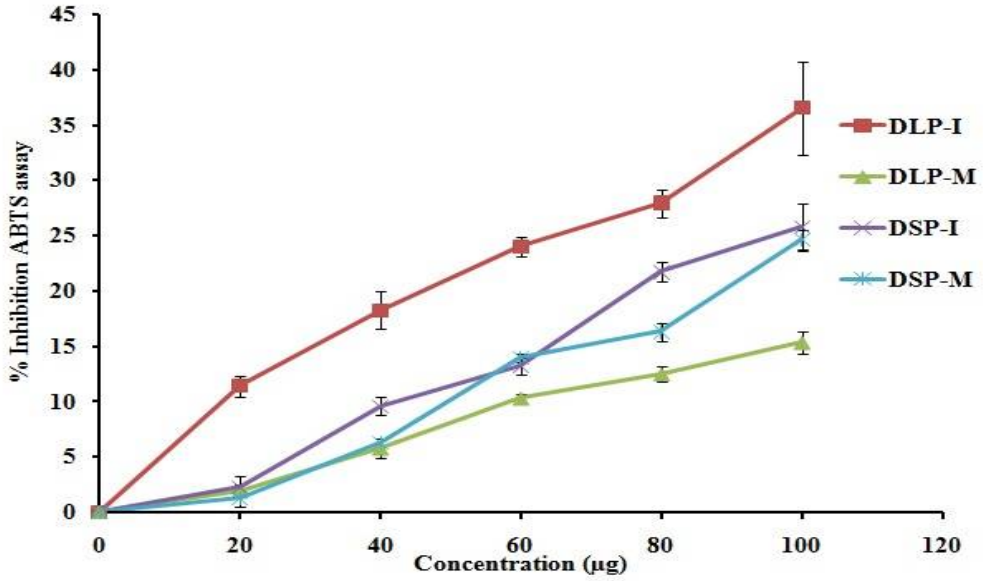

Figure 2: ABTS radical cation scavenging activity of *Datura innoxia* and *Datura metel* leaf & seed methanolic extracts. Values are represented as mean ± SD (n = 3). DLP-I (*Datura innoxia* leaf extract), DSP-I (*Datura innoxia* seeds extract), DLP-M (*Datura metel* leaf extract), DSP-M (*Datura metel* seeds extract).

FTIR has proven to be a valuable tool for the characterization and identification of compounds or functional groups (chemical bonds) present in an unknown mixture of plant extract (Eberhardt et al., 2007; Hazra et al., 2007). The FTIR analysis of the samples was done and the functional groups associated were determined. The IR spectrum of plant samples is shown in the Fig 4-7. The absorption bands and their tentative assignments are given in Table 4. The FTIR analysis confirmed the presence of alkenes, alkanes, alkynes, amides, carboxylic group, aromatic, aliphatic amines and halide groups. The FT-IR data also show the difference in the peaks obtained in the leaf extracts of two *Datura* species. There are 16 peaks observed in the *Datura innoxia* leaf

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extract as compared to 12 peaks in the leaf metel extract. Number of FT-IR peaks in the two extracts may also be associated with their antioxidant activities in the same order.

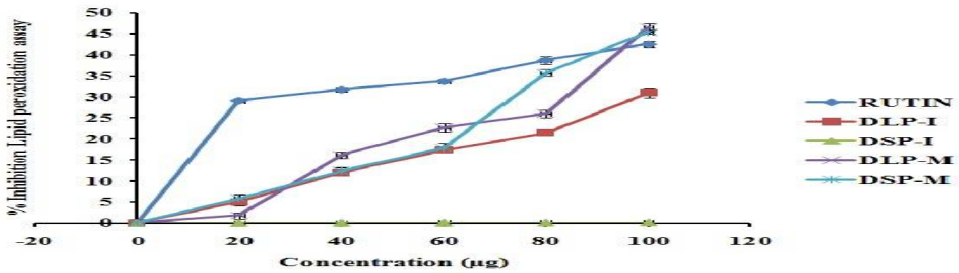

Figure 3: Lipid peroxidation inhibition activity of *Datura innoxia* and *Datura metel* leaf & seed methanolic extracts. Values are represented as mean ± SD (n = 3). DLP-I (*Datura innoxia* leaf extract), DSP-I (*Datura innoxia* seeds extract), DLP-M (*Datura metel* leaf extract), DSP-M (*Datura metel* seeds extract).

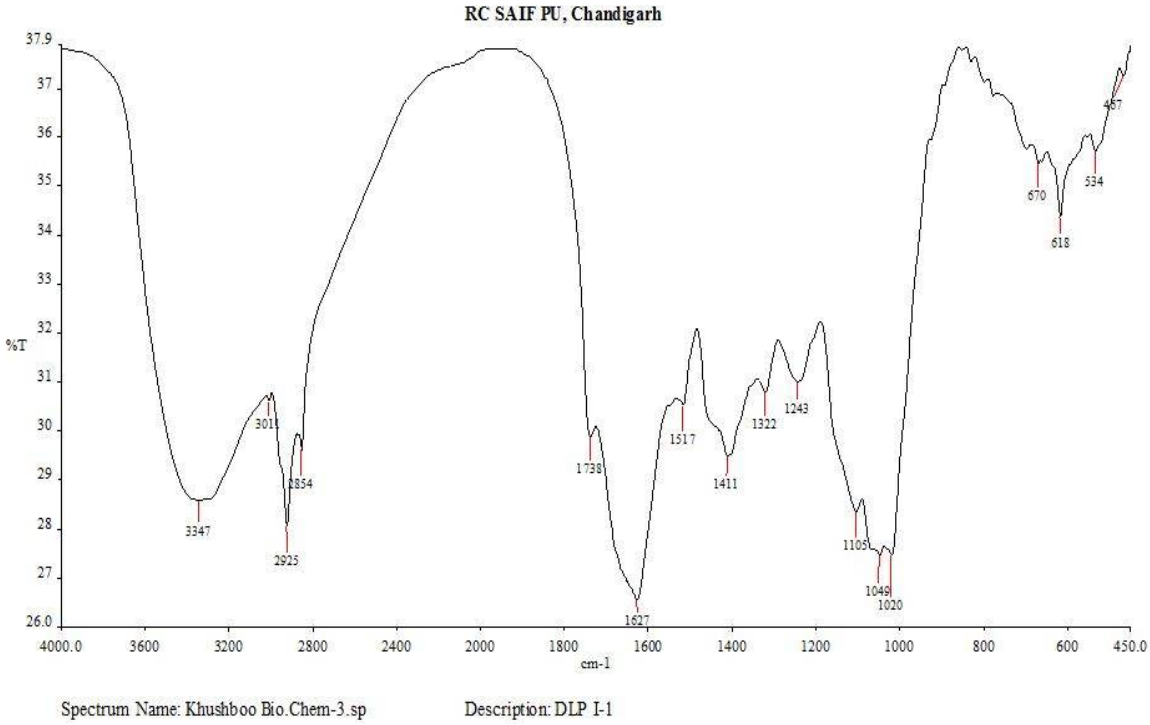

Figure 4: FT-IR spectra of methanolic extract of *Datura innoxia* leaves

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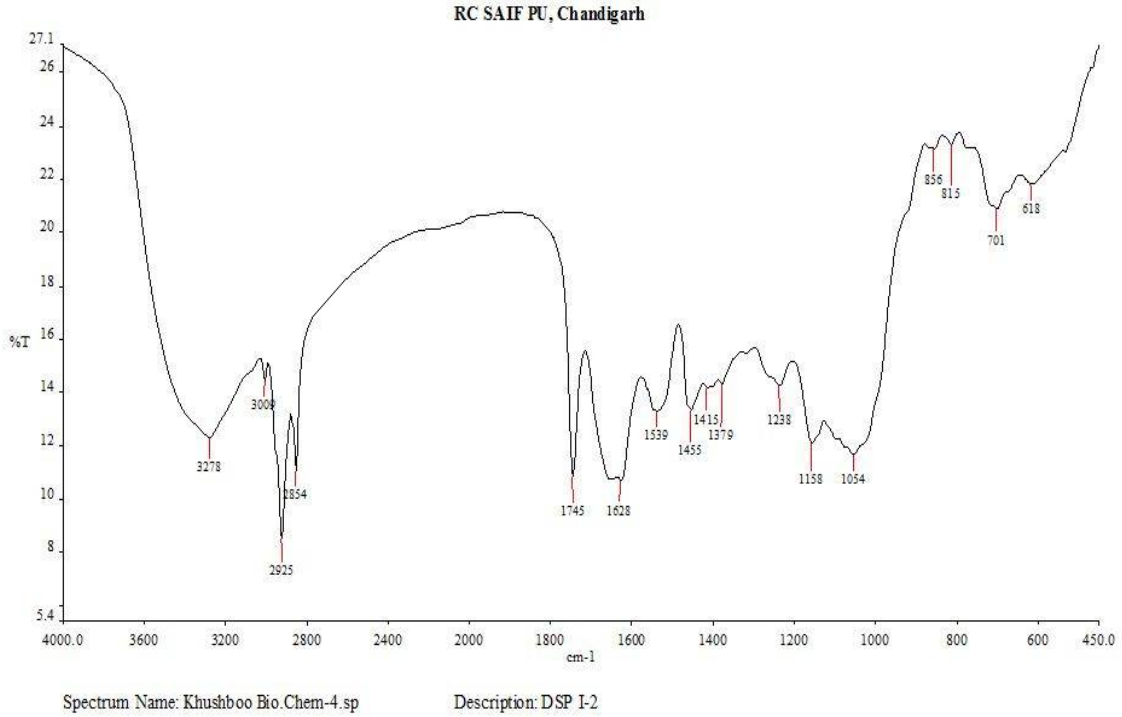

Figure 5: FT-IR spectra of methanolic extract of *Datura innoxia* seeds

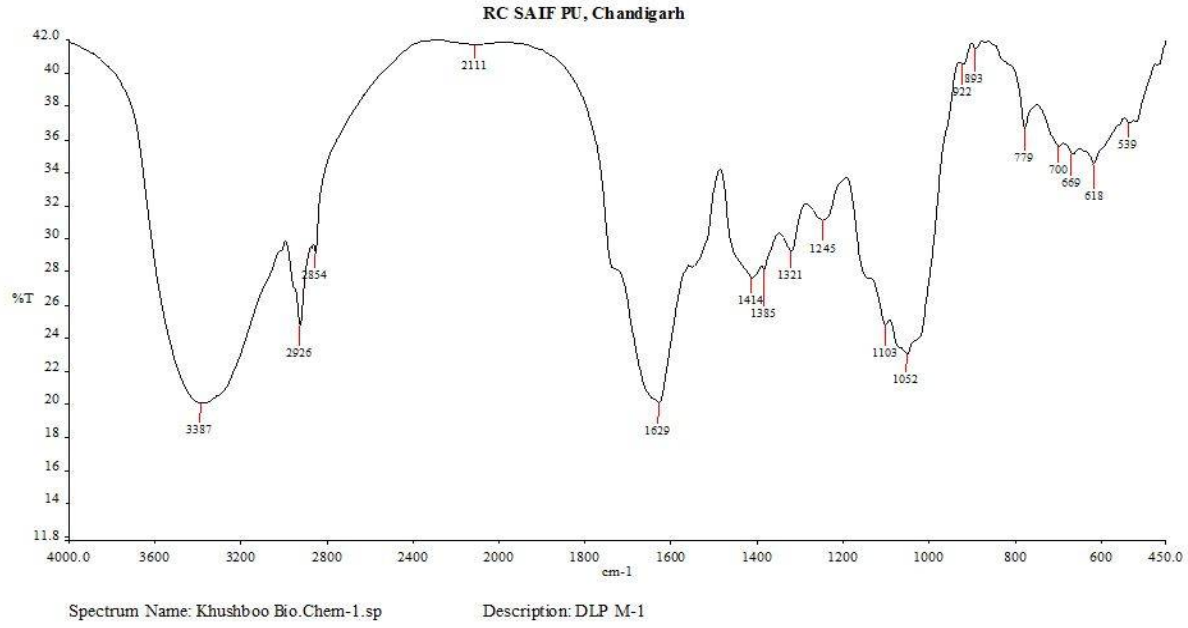

Figure 6: FT-IR spectra of methanolic extract of *Datura metel* leaves

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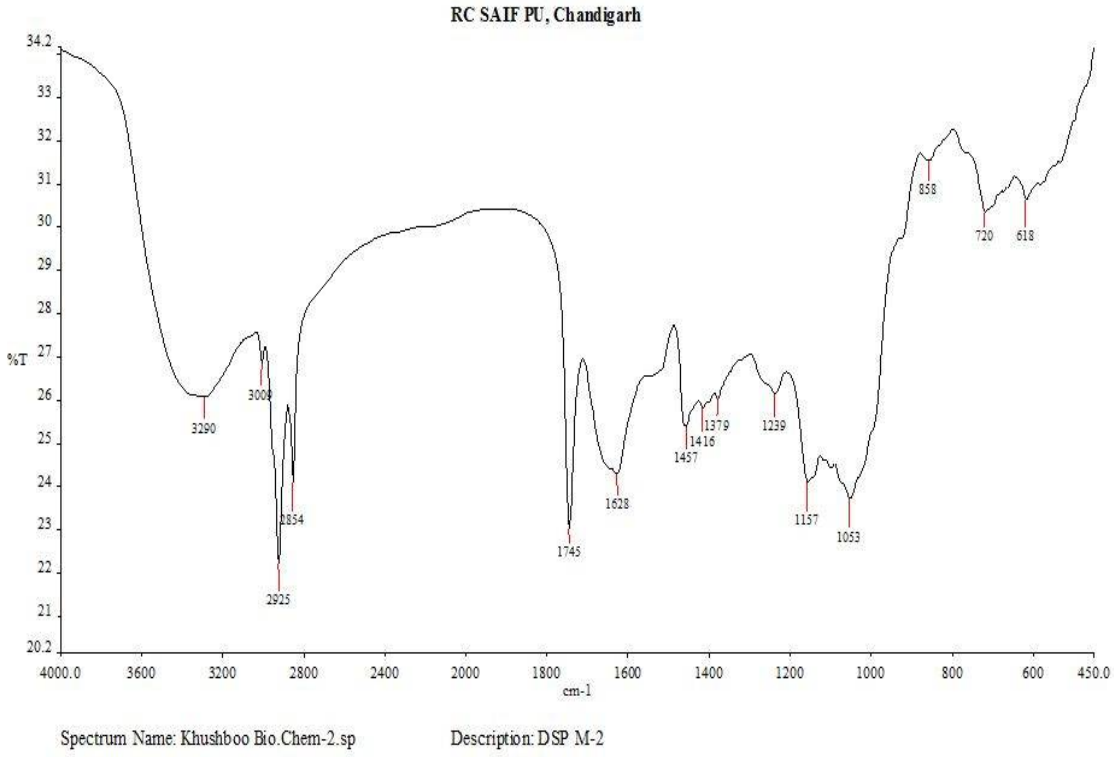

Figure 7: FT-IR spectra of methanolic extract of *Datura metel* seeds

The present study indicates the differences in the levels of antioxidant activity of *Datura innoxia* and *Datura metel* leaf and seed extracts. The differences in their antioxidant activity may be associated with the levels of their phenolic and flavonoid contents. The antioxidant activities of methanolic extracts of four different varieties of *Lantana camara* have been correlated with their phenolic content (Kumar et al., 2014).

Out of the leaf methanolic extracts in the present study, the methanolic extract of *Datura innoxia* leaves (DLP-I) showed the highest total phenolic and flavonoid content. The highest antioxidant activity and free radical scavenging activity as evident from the results of DPPH radical scavenging activity, ABTS cation scavenging assay and total antioxidant activity was observed for the DLP-I methanolic extract. Also, a higher (16) number of FT-IR peaks have been observed for *Datura innoxia* leaf methanolic extract.

In case of seed methanolic extracts, the total antioxidant activity was found to be higher in the methanolic extract of *Datura innoxia* as compared to *Datura metel*. The DPPH radical scavenging activity was found to be higher in the *Datura innoxia* seed extract (DSP-I). In the ABTS radical scavenging assay, *Datura innoxia* showed the significantly higher activity ($p < 0.05$) than *Datura metel* seeds. The FT-IR data also represented the same pattern in the methanolic extract of seeds between these two species. The number of peaks observed in *Datura innoxia* seed extract is 15 whereas in *Datura metel* seed extract the number of peaks is 12. Unlike in leaf extracts, in seed extracts there is an inverse relationship between phenolic & flavonoid contents with their antioxidant activities observed. This may be attributed to the possible differences between the chemical constituents and their nature in the leaves and seeds of *Datura* species. The presence or absence of certain functional groups as evident from the peaks of FT-IR may also contribute to the same.

Conclusion

Datura species had widespread use in phytomedicine and is popular all over the world for its antispasmodic and hallucinogenic properties. The present study indicates a higher antioxidant activity of leaf methanolic extract of *D. innoxia* as compared to *D. metel*. The DPPH free radical scavenging and ABTS cation scavenging activity has been observed to be higher in *D. innoxia* leaf extract again thereby suggesting their probable role towards the antioxidant activity in the same order. This activity has been reported to be associated and dependent upon the levels of phenolic and/ or flavonoids in various extracts by a number of groups of researchers worldwide.

Further work needs to be done for the identification and isolation of safe and natural bioactive molecules from *Datura* species involved in the antioxidant activities.

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Conflict of Interest

The authors declare no conflict of interest.

References

1. Blois, M.S. (1958). Antioxidant determinations by the use of a stable free radical. Nature. 181(4617):1199–1200.
2. Cao, G., Sofic, E. and Prior, R.L. (1997). Antioxidant and Prooxidant Behavior of Flavonoids: Structure-Activity Relationships. Free Radic Biol Med. 22:749-760.
3. Chang, C., Yang, M., Wen, H. and Chern, J. (2002). Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal. 10:178-182.
4. Coates, J. Interpretation of infrared spectra, A practical approach, Encyclopaedia of Analytical biochemistry, R.A. Meyers (Ed.), John Wiley and sons ltd, Chichester. 10815-10837. Notcitedinthetext.
5. Cook, N.C. and Samman, S. (1996). Flavonoids- chemistry, metabolism, cardioprotective effects, and dietary sources. Nutritional Biochemistry.**7**:66-76.
6. Cuzzocrea, S., Riley, D.P., Caputi, A.P. and Salvemini, D. (2001). Antioxidant therapy: A new pharmacological approach in shock, inflammation and ischemia/reperfusion injury. Pharmacol Rev. 53: 135-159.
7. Duke, J.A. (1992). Handbook of phytochemical constituents of GRAS herbs and other economic plants. Boca Raton, FL. CRC Press.
8. Eberhardt, T.L., Li, X., Shupe, T.F. and Hse, C.Y. (2007). Chinese tallow tree (*Sapium Sebiferum*) utilization: Characterization of extractives and cell-wall chemistry. Wood Fiber Sciences. 39: 319–324.
9. Gaire, B.P. and Subedi, L. (2013). A review on the pharmacological and toxicological aspects of *Datura stramonium* L. Journal of Chinese Integrative Medicine. 11: 73–79.
10. Gutteridge, J.M.C. and Halliwell, B. (2010). Antioxidants: Molecules, medicines and myths. Biochem and Biophys Res Commun.393: 561-564.
11. Hazra, K., Roy, M., Sen, S.K. and Laska, S. (2007). Isolation of antibacterial penta hydroxy flavones from the seeds of *Mimusopselengi* Linn. Afr J Biotechnol. 6(12): 1446–1449.
12. Hodges, D.M., Delong, J.M., Forney, C.F. and Prange, R.K. (1999). Improving the thiobarbituric acid reactive- substances assay for estimating lipid peroxidation in plant tissue containing anthocyanin and other interfering compounds. Planta. 207: 604- 611.
13. Imeh, U. and Khokhar, S. (2002). Distribution of conjugated and free phenols in fruits: antioxidant activity and cultivar variations. J Agric Food Chem. 50:6301-6306.
14. Kahkonen, M.P., Hopia, A.I., Vuorela, H.J., Rauha, J.P., Pihlaja, K., Kujala, T.S. and Heinonen, M. (1999). Antioxidant Activity of Plant Extracts Containing Phenolic Compounds. J Agric Food Chem. 47: 3954-3962.
15. Kirtikar, K.R. and Basu, B.D. (1999). Indian medicinal plants. 2nd ed. Volume III. Dehradun: International Book Distributors. 1783-1787.
16. Kumar, S., Sandhir, R., and Ojha, S. (2014). Evaluation of antioxidant activity and total phenol in different varieties of Lantana camara leaves. BMC Research Notes. **7**:560.
17. Kumpulainen, J.T. and Salonen, J. (1999). Natural Antioxidants and Anticarcinogens in Nutrition, Health and Disease. The Royal Society of Chemistry UK.178- 187.
18. Lucy, H. and Edgar, J.D. (1999) Medicinal plants: a re-emerging health aid. Electron J Biotechnol. 2 (2):1-15. notcitedinthetext
19. Makkar, H.P.S., Becker, K., Abel, H. and Pawelzik, E. (1997). Nutrient contents, rumen protein degradability and anti-nutritional factors in some colour and white flowering cultivars of viciafaba beans. J Sci Food Agric. 75:511-520.
20. Ohkawa, H., Ohishi, N. and Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem. 95:351.
21. Preito, P., Pinedo, M. and Aguilar, M. (1999). Spectrophotometric quantification of antioxidant capacity through the formation of the phosphomolybdenum complex: specific application to the determination of vitamin E. Anal Biochem. 269:337-341.
22. Rajesh, S.G.L. (2002). Studies on antimycotic properties of *Datura metel*. J Ethnopharmacol. 80: 193-197.
23. Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M. and Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic Biol Med. 26:1231-1237.
24. Rice-Evans, C.A., Miller, N.J. and Paganga, G. (1997). Antioxidant properties of phenolic compounds. Trends Plant Sci Rev. 2: 152-159.
25. Schultes, R.E. and Hoffman, A. (1979). Plants of the Gods, New York, McGraw-Hill.
26. Sharififar, F., Nudeh-dehghn, G. and Mirtajaldini, M. (2008). Major flavonoids with antioxidant activity from *Teucrium polium*. Food chem. 112: 885-888.
27. Wollenweber, E., Dorsam, M., Dorr, M., Roitman, J.N. and Valant-Vetschera, K.M. (2005). Chemodiversity of Surface Flavanoids in Solanaceae. Z. Naturforsch. 60c: 661-670.