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Research Article

GC-MS PROFILING AND ANTIDIABETIC EVALUATION OF *DATURA METEL*: AN *IN VITRO* STUDY

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ABSTRACT

Objectives: The aim of this work was to identify different bioactive molecules reported in the leaf and seed extracts of the selected plant *Datura metel* using bioanalytical techniques such as gas chromatography-mass spectrometry (GC-MS) and to assess the efficacy of the extracts for their antidiabetic properties.

Methods: Shimadzu GC-MS QP 2010 was used on plant extracts through a standard protocol for phytochemical analysis. For the *in vitro* antidiabetic analysis, an α-amylase and α-glucosidase inhibitory assay was performed, in addition to total phenol assay, total flavonoid assay, and hydroxyl radical scavenging assay.

Results: A total of 48 compounds in leaves and 46 compounds in seeds were identified through GC-MS analysis. The important bioactive compounds are dibutyl phthalate, neophytadiene, campesterol, 3,4-divanillyltetrahydrofuran, aposcopolamine, (Z)-18-octadec-9-enolide, scopolamine, and 2-methoxy-4-vinylphenol in leaf and seed extracts. The *D. metel* leaves (DM-L) had a high α-amylase inhibition (IC_{50}=53.26 µg/mL), followed by *D. metel* seed (DM-S) (IC_{50}=54.129 µg/mL) with respect to the standard acarbose (IC_{50}=65.46 µg/mL), and this demonstrates its strong antidiabetic properties. In the same manner, the inhibition of α-glucosidase had an IC_{50} of 77.73 µg/mL in DM-L and 115.96 µg/mL in DM-S, as opposed to 42.98 µg/mL in acarbose.

Conclusion: In general, these findings demonstrate that *D. metel* extracts, particularly the leaf extract, have a high antidiabetic potential because it has a great α-amylase inhibitory effect and antioxidant activity, suggesting the use of plant extracts as hypoglycemic agents, and in pharmacological or non-pharmacological approaches for diabetes. The reported phytochemicals are associated with different biological activities, namely, anticancer, antimicrobial, antioxidant, anti-inflammatory, and others.

Keywords: *Datura metel*, Gas chromatography-mass spectrometry, Phenols, Phytochemicals, Antidiabetic activity.

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INTRODUCTION

Diabetes mellitus is a major metabolic and pathophysiological contributor to mortality, illness, and disability worldwide. It is a non-communicable disease correlated to various risk factors that are either modifiable or non-modifiable, such as age, sex, family history, lifestyle, and hypertension [1]. Diabetes is a heterogeneous disease with a common phenotype of impaired glucose tolerance [2]. Other characteristics include persistently increased blood glucose levels, insulin resistance, or reduced insulin production, resulting in various health deterioration issues, namely retinopathy, neuropathy, and nephropathy [3]. The management of this disease involves the use of certain groups of medications with glucose-lowering effects. However, some of these patients have been shown to have associated complications, such as diabetic ketoacidosis, hypoglycemia, cardiovascular disorders, cardiomyopathy, and renal disorders. Therefore, it has become important to look for alternatives that are safe and non-toxic. Traditional medicines have proven their efficacy as a long-standing cure for multiple diseases for ages. These traditional medicines are derived from various parts of the plant and consist of a variety of phytocompounds, namely lipids, steroids, alkaloids, phenols, flavonoids, and reactive oxygen species, responsible for the therapeutic properties of these plants. Yet, in modern times, it is difficult to validate the efficacy, safety, and regulation of such herbal drugs. The techniques using gas phase analytical methods, such as GC-mass spectrometry, structure elucidation through nuclear magnetic resonance spectroscopy, and functional group identification using infrared spectral analysis, have proved to be quite useful in the estimation of such active components. In this study, *Datura metel* (common name: Dhatura) was investigated for the presence of potential metabolites and its efficacy in the cure and mitigation of disease progression.

D. metel, also known as devil's trumpet, shows an annual growth cycle and belongs to the Solanaceae family [4]. *Datura* spp. widely grows in tropical and warm temperate regions of the world [5]. The geographical distribution of the plant *Datura* is widely spread throughout Asia, Europe, and America, and some have also been found in Africa and Australia [6].

Datura, which is widely grown, is an annual herb and a valuable source of pharmacologically active alkaloids used commercially; thus, it is recognized as a medicinally valuable plant. The entire plant, along with its individual components, namely leaf, flower, seeds, and roots, has been employed in the conventional way of treatments with various ranges (Fig. 1).

Daturine, hyoscyamine, atropine, and scopolamine, which have been isolated from the plant Datura and are used as antispasmodic, narcotic, neurosedative, and antasthmatic drugs [7]. A number of nitrogen compounds, including phenylalanine, glutamate, tyrosine, and alanine, depicting various biological functions, are known to be reported in seeds [8]. *D. metel* is rich in a spectrum of active phytoconstituents,

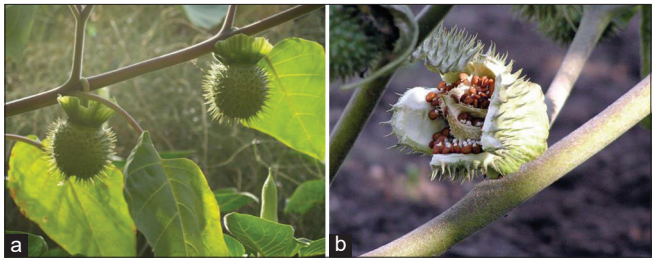

Fig. 1: (a) *Datura metel* plant (b) *D. metel* seed

comprising alkaloids, Polyphenolic compounds, terpenes, tannins, and phytosterols. The solanaceous alkaloids hyoscyamine and scopolamine have been successfully isolated from *D. metel* [9-11]. *D. metel* holds a crucial status in the old conventional system of medicine, similar to that of belladonna and stramonium [12]. This species boasts an enriched history as an herbal medicine. The *D. metel* plant is well acknowledged for its medicinal role in ancient Chinese and long-standing old traditions of Indian medicine [13].

METHODS

Sample collection

The freshly collected plant *D. metel* (leaves and seeds) was collected from Sidhwan Bet, Tehsil Jagraon (Latitude: 30.9115° N, Longitude: 75.45009° E), Punjab, North India. The plant was authenticated at the Department of Botany, Punjabi University, Patiala, India. The collected parts of plants (leaf and seed) were entirely rinsed 3 times with tap water, followed by drying in the shade. The dried leaves and seeds were grounded into fine powder and stored in glass containers for subsequent use.

Preparation of the plant extracts

One hundred grams of finely powdered and shade-dried *D. metel* leaves and seeds were sequentially extracted using solvents of increasing polarity, n-hexane, chloroform ($CHCl_3$), ethyl acetate (C_4HO_2), and methanol. The extraction was done individually on *D. metel* leaves (DM-L) and seeds (DM-S). About 5 g of plant powder was put in a 250 mL Erlenmeyer flask with 50 mL of solvent, then tightly covered with parafilm to reduce evaporation. It was mixed in a rotary orbital shaker (150 rpm) and allowed to mix (72 h) at room temperature (25±2°C) to allow maximum solubilization of phytochemicals. The contents were filtered after every extraction cycle using Whatman No. 1 filter paper to separate the supernatant (filtrate) and the plant residue. To avoid degradation by light, the filtrate was stored temporarily in amber glass bottles, whereas the rest of the residue was dried at room temperature under air to eliminate the remnants of the previous solvent. This residue was then extracted with the next solvent in the order until the last step of methanol extraction was done. Each solvent extraction was filtered and then concentrated under reduced pressure using a rotary evaporator (Buchi R-210), at 45°C to eliminate residual solvents without any loss of bioactive components. The concentrated extracts were dried to a constant weight by air and kept in sterile and airtight glass vials at 4°C to proceed with more gas chromatography-mass spectrometry (GC-MS) profiling and *in vitro* antidiabetic studies (Fig. 2).

Phytochemical screening of DM-L and DM-S extracts

The *Datura metel* leaves (DM-L) and seeds (DM-S) extracts were analyzed with a Shimadzu GC-MS QP2010 Ultra system fitted with an Rtx-5MS capillary column (30 m×0.25 mm i.d., 0.25 mm film thickness) at the central instrumentation facility (CIF), Lovely Professional University, Punjab, India. Helium (99.999% purity) was used as the carrier gas and kept at a steady flow rate of 1.0 mL/min. An auto-injector was used to inject a 1 µL sample of each extract (diluted 1:10 v/v in methanol) in split mode (split ratio=10:1). The injector temperature was adjusted to 250°C, the interface and ion source temperatures were adjusted to 280°C and 200°C, respectively. The instrument was in electron impact (EI) mode with an ionization energy of 70 eV. The following was the program of the oven: the temperature was set at 50°C (held 2 min), then at 10°C/min to 200°C (held 5 min), and at 5°C/min to 280°C (held 10 min). The run time was about 35 min per sample. The scan range of the mass spectrometer was m/z 45 to 800 with a scan rate of 3333 amu/s.

Structural analysis and validation of compounds

The mass spectra obtained from GC-MS analysis were elucidated by matching the spectra with the NIST library database. NIST 2.0 is a reference database of 62,000 mass spectral profiles representing a wide range of chemical compounds [14]. The respective bioactivities of these compounds were analyzed from the literature.

Estimation of total phenolic content (TPC)

To estimate the total amount of phenols present in leaf (DM-L) and seed (DM-S) extracts, a spectrophotometric method was used [15]. The extracts were reconstituted in methanolic solutions with a strength of 1 mg in 1 mL solvent. Then, 500 µL of the above sample was added to 1.25 mL of the FC reagent (1:1), and then the final solution was made in distilled water. 1000 µL of 7.5% sodium bicarbonate was also added to prepare the solution. The blank sample was prepared following the same protocol, except that for the plant extract, 0.5 mL of methanol was added. Both the test and blank samples were put on incubation for 30 min under light-restricted conditions. After half an hour, blank, control, and test samples were read using an ultraviolet (UV)-Vis spectrophotometer at an absorbance range of 765 nm. For each analysis, the test solutions were prepared in three replicates, and the mean calculated optical density values were subsequently calculated. The calibration curve using gallic acid as a standard solution was prepared following the same procedure. The observed different absorbance values were plotted against the calibration curve, and the concentration of phenolics (mg/mL) present was calculated using the following formula and was indicated as milligrams of gallic acid equivalents per gram of extract (mg of GAE/g).

Estimation of total flavonoid content (TFC)

Total flavonoids present in DM-L (leaf) and DM-S (seed) extracts were analysed and calculated by the spectrophotometric method [16]. Rutin is the standard drug used, and a standard curve was established. The plant extracts DM-L and DM-S (500 µL) were dissolved in 1.5 mL of methyl alcohol and added to a 100 µL (w/v) solution of aluminum chloride. A 100 µL of 1 mol/L sodium salt of acetic acid solution and a final volume of 5 mL were made by the addition of distilled water. The blank was prepared similarly, without the extract. The samples were kept for incubation for almost 30 min at 37°C, and the absorbance was recorded; for the same, the absorbance was read at $\wedge_{max}$ 415 nm. The total concentration of flavonoid constituents was measured as per the Rutin standard curve in mg/mL and was indicated as milligrams of rutin equivalent per gram of extract (mg of RE/g).

Determination of hydroxyl radical scavenging activity

The -OH radicals present in the sample were measured by using the deoxyribose method [17]. It is based upon the principle that the hydroxyl radicals produced scavenge the deoxyribose, leading to the formation of products called thiobarbituric acid reactive substances (TBARS), which, when heated with thiobarbituric acid, yielded a reactive pink complex at low pH values. The hydroxyl scavenger drugs and those present in plants, then competes with deoxyribose for hydroxyl radicals, declining the TBARS and hence the pink chromogen [18]. The test mixture was prepared in varying concentrations (100 µg/mL to 500 µg/mL), and to initiate the reaction, 3 mM deoxyribose was mixed with 0.1 mM each of $FeCl_3$, ethylenediaminetetraacetic acid, and ascorbic acid, along with 2 mM hydrogen peroxide, all prepared in 20 mM phosphate buffer at pH 7.4. The solution was left for incubation at room temperature. After 30 min, an equal amount of 5% trichloroacetic acid and 1% TBA (0.5 mL each) was added to make a final volume of 3ml. Again, it was kept for incubation for the next 30 min at boiling temperature. After lowering the temperature of the solution to standard room conditions, the optical density was observed at 532 nm. The scavenging activity of

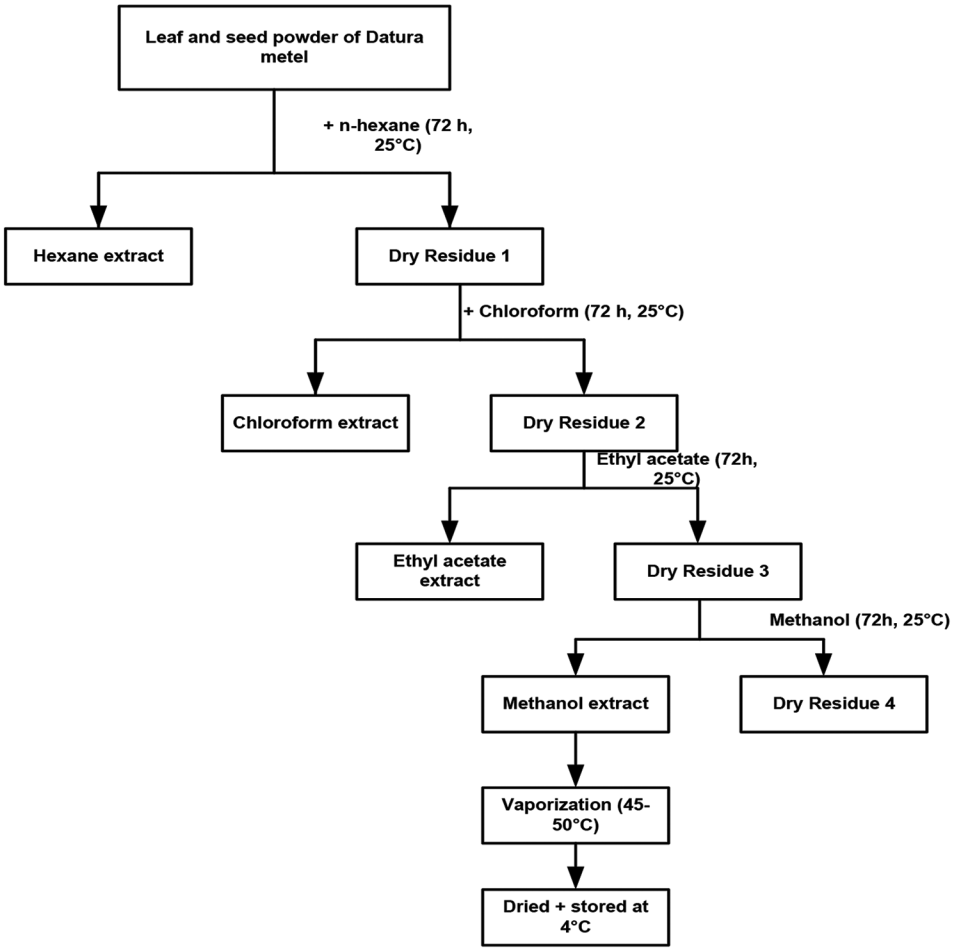

Fig. 2: Flow diagram layout for sequential extraction of *Datura metal*

the samples was expressed in terms of % inhibition value, producing 50% inhibition of the hydroxyl radicals, i.e., IC_{50} values.

Alpha-amylase inhibitory assay

The method of Bernfeld, 1951 [19], was carried out for the determination of enzyme inhibition. 0.02M Na-phosphate buffer adjusted to pH=7.4 was prepared for use. Two hundred fifty milligrams of soluble potato starch were mixed in distilled water to obtain a 50 mL clear solution to be kept on incubation for 15 min. One hundred milliliters of sodium phosphate buffer was added to 0.1 mg of α-amylase to prepare the enzyme mixture. The standard drug, acarbose, was prepared in five varying concentrations from 20 to 100 µg/mL. The plant extracts DM-L and DM-S were also prepared similarly. The 100 mL reaction mixture consisted of 1.0 g of 3,5-dinitrosalicylic acid dissolved in 50 mL of distilled H_2O, 30 g sodium potassium tartrate tetrahydrate, followed by the addition of 2 N sodium hydroxide (20 mL), 30 mL distilled water for the preparation of the DNS reagent. For this assay, the reaction mixture containing 1 mL of both α-amylase and test or standard was left to incubate for half an hour. Later, after the incubation period, 1000 µL of starch mixture was dissolved, and further incubated for another 3 min. The reaction mixture was then incubated at boiling temperature for 15 min. The reaction mixture was brought back to standard temperature, following the addition of 9 mL distilled H_2O, and subjected to UV spectroscopy for the determination of absorbance at 540 nm. A graph was plotted for absorbance against concentration (µg/mL). The IC_{50} values for acarbose, DM-L, and DM-S were calculated from the linear equation of the plotted graph. The inhibitory activity was calculated in percentage following the formula:

$$\text{Inhibition activity (in \%age)} = \frac{\text{Abs.(control)} - \text{Abs. of (test)} \times 100}{\text{Abs. (control)}}$$

Alpha-glucosidase inhibitory assay

A modified method of Kim *et al*. 2005 [20] was used to estimate the inhibitory effect of the enzyme alpha-glucosidase. The alpha-glucosidase was dissolved in 0.1M phosphate buffer, pH 6.9, and was used as an enzyme solution (100 U/mg). 1M p-Nitrophenyl-α-D-glucopyranoside was prepared and used as the substrate. Standard acarbose and plant extracts (DM-L and DM-S) were used in concentrations ranging from 10–100 µg/mL. Different concentrations of the above solutions were premixed with 120 µL of 0.1 M phosphate buffer, pH 6.9 at 37°C for 5 min along with 20 µL of substrate. After pre-incubation, 20 µL of enzyme solution is added and again incubated for 15 min at 37°C. The blank sample contains 40 µL of phosphate buffer, and the control sample contains 20 µL of phosphate buffer. Then, the reaction is terminated by adding 100 µL of 0.1 M Na2CO3. The yellow product can be determined at an absorbance of 405 nm. The percentage of inhibition is calculated as follows:

$$\text{Inhibition activity (in \%age)} = \frac{\text{Abs. (control)} - \text{Abs. of (test)} \times 100}{\text{Abs. (control)}}$$

Statistical analysis

All tests were carried out in triplicate (n=3), and the results were indicated as mean±standard deviation (SD), which included all the experimental assays, such as α-amylase and α-glucosidase inhibitory tests, TPC, and flavonoid (TFC) assays. One-way analysis of variance (ANOVA) was conducted to compare the mean percentage inhibition of

all the concentrations (10–100 µg/mL) of the standard (acarbose) and the two extracts (DM-L and DM-S). In the event where the ANOVA showed significant differences, a Tukey honest significant difference *post hoc* test was used to make pairwise multiple comparisons between the groups to identify which means were significantly different. The independent samples t-test was applied to compare the mean TPC and TFC of the leaf and seed extracts (DM-L vs. DM-S). All analyses were done at a level of significance (p) of ≤0.05. The Data Analysis ToolPak of Microsoft Excel 365 was used to conduct statistical tests, such as p-value calculation, IC_{50} estimation through regression, and *post hoc* analysis.

RESULTS AND DISCUSSION

D. metel is rich in a spectrum of active phytoconstituents, comprising alkaloids, flavonoids, phenols, tannins, saponins, and sterols. As reported by different researchers, it contains tropane alkaloids such as atropine, hyoscyamine, scopolamine (hyoscine), as well as norscopolamine and meteloidine, hydroxylated derivatives such as hydroxy-6-hyoscyamine, tiglic esters of dihydroxytropine, and numerous withanolides [21,22].

The GC-MS spectra for the samples in our study were obtained at varying widths, and the associated libraries of the Wiley 8 and the NIST helped to recognize the phytocomponents based on their retention indices. Thus, the analysed and detected compounds from the computer libraries were produced in tabular form. Tables 1 and 2 list the primary chemical components found in DM-L and DM-S along with their percentage distributions. Figs. 3 and 4 present the GC-MS chromatogram and analysis of both the extracts.

Table 1: Identified metabolites from the methanolic extract of *Datura metel* leaves

S. No	Compound name	Molecular formula	Retention Time (min)	Peak area (%)	Reported biological activity	References
1	2-methoxy-4-vinylphenol	$C_9H_{10}O_2$	15.50	13.8	Anti-inflammatory, antimicrobial, antioxidant	[23,24]
2	Scopolamine	$C_{17}H_{19}NO_3$	31.54	5.83	Anticholinergic, antiemetic, antivertigo	[25]
3	Benzofuran, 2,3-dihydro-	C_8H_8O	13.678	5.74	Pro-oxidant	[9]
4	Dibutyl phthalate	$C_{16}H_{22}O_4$	26.11	3.78	Antibacterial	[26]
5	Neophytadiene	$C_{20}H_{38}$	24.38	3.41	Anti-inflammatory, antioxidant	[27]

Table 2: Identified metabolites from the methanolic extract of *Datura metel* seeds

S. No.	Compound name	Molecular formula	Retention time (min)	Peak area (%)	Reported biological activity	References
1	3,4-Divanillyltetrahydrofuran	$C_{20}H_{24}O_5$	20.41	10.70	Antioxidant; antidiabetic; anticancer	[28]
2	Apoatropine	$C_{17}H_{21}NO_2$	21.7	16.74	Antispasmodic	[9]
3	Scopolamine	$C_{17}H_{21}NO_4$	31.54	6.79	Antispasmodic; anticholinergic	[25]
4	2-Methoxy-4-vinylphenol	$C_9H_{10}O_2$	15.50	4.24	Antioxidant; enzyme inhibitory	[23,24]
5	3-α-phenylacetoxytropane	$C_{16}H_{21}NO_2$	28.62	4.09	Anti-inflammatory; potential antidiabetic	[29]

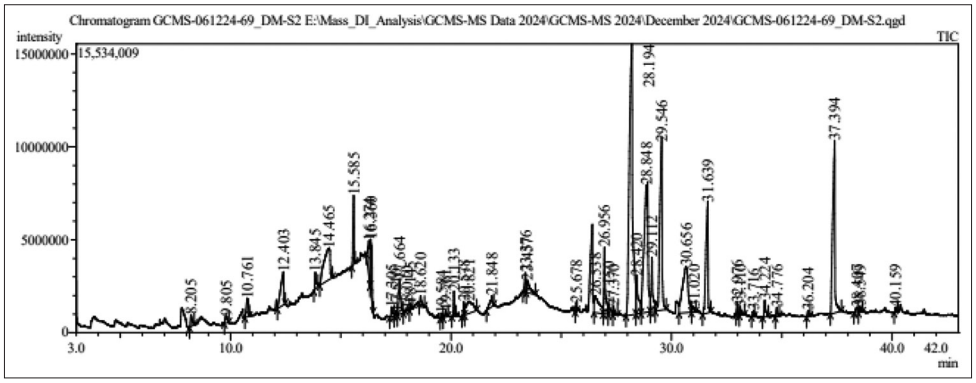

Fig. 3: Gas chromatography-mass spectrometry spectrum (*Datura metel* seed)

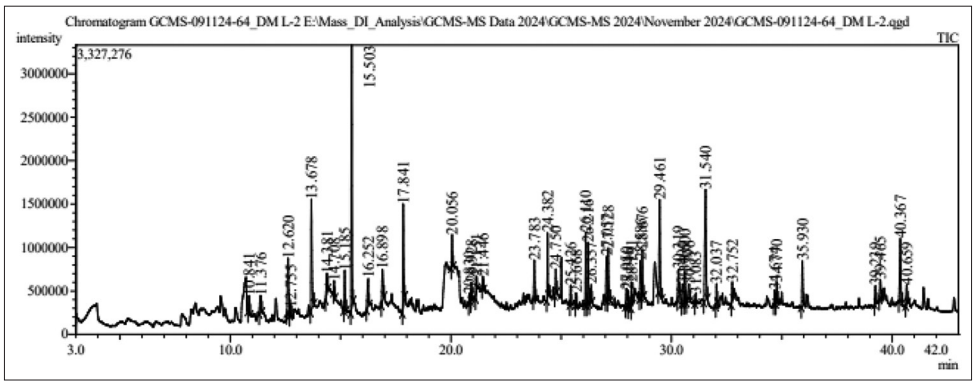

Fig. 4: Gas chromatography-mass spectrometry spectrum (*Datura metel* leaves)

The GC-MS chromatograms of the DM-L and DM-S extracts showed 10 major peaks, confirming the existence of 10 bioactives. The results revealed that apoatropine (21.7%), 3,4-divanillyltetrahydrofuran (9.54%), aposcopolamine (9.21%), (Z)-18-octadec-9-enolide (12.2%), scopolamine (5.7%), and atropine (6.33%) were the major phytoconstituents in DM-S. The major components reported in DM-L include 2-methoxy-4-vinylphenol (13.8%), scopolamine (5.83%), benzofuran, 2,3-dihydro- (5.8%), benzene ethanol, and 4-hydroxy- (5.42%). According to the GC-MS findings, the seed extract contains phytochemicals ranging from alkaloids to their derivatives, benzenoids, phenols, pyranones, and furanones, whereas the leaf extract contains alkaloids, benzenoids, phenols, flavonoids, and phytosterols. Compared with flowers, stems, immature fruits, and leaves, *D. metel* seeds have the highest alkaloid content [30]. The FTIR studies done on the *D. metel* extracts also identified various characteristic functional moieties such as halides, saturated and unsaturated hydrocarbons, carboxylic acids and its derivatives, arenes, planar, aromatic and non-aromatic amino acids [31].

The TPC was determined using the Folin–Ciocalteu method (Standard: Gallic acid). The concentration was indicated in mg gallic acid equivalence (mg GAE/g) (Fig. 5). The investigation for the total phenolic concentration yielded the following values of DM-L=2.921±0.375 (mg GAE/g±SD), whereas for DM-S, it is 3.342±0.315 (mg GAE/g±SD) (Table 3 and Fig. 5). The TFC (standard=Rutin) calculated as 3.101±0.0408 mg RE/g for DM-L, 1.219±0.0396 mg RE/g for DM-S (Table 3 and Fig. 6).

Hydroxyl radicals, known for its the most reactive oxygen, cause critical harm to the neighboring biomolecules. Thus, the radical scavenging was expressed in % activity. Fig. 7 and Table 4 represent the scavenging potential of these extracts for hydroxyl radicals (in percentage); DM-L (56.869±0.00642) and DM-S (34.346±0.011), and with equivalent concentration in microgram/ml of the standard of 0.068 and 0.136 for DM-L and DM-S, respectively. This reflects the presence of the hydrogen-donating ability of the polyphenolic compounds present in the extracts.

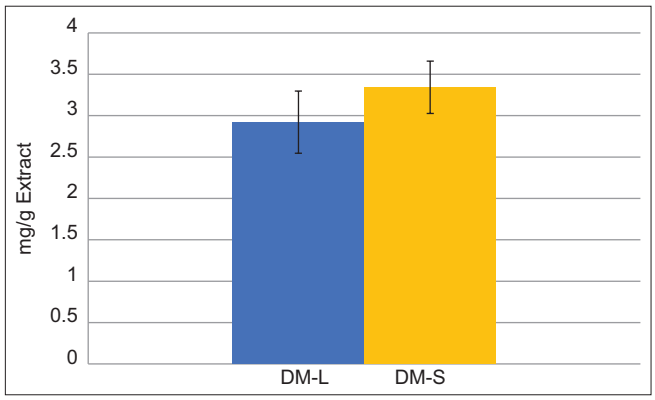

Fig. 5: Total Phenolic Content in DM-L and DM-S

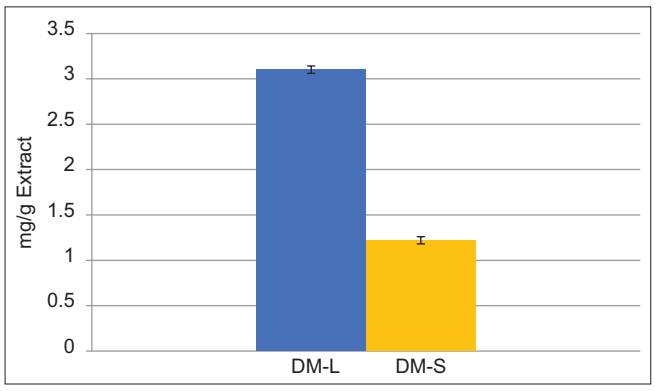

Fig. 6: Total Flavonoid Content in DM-L and DM-S

One of the interesting findings in this research is that the seed extract (DM-S) had a greater TPC (3.342±0.315 mg GAE/g) than the leaf extract (DM-L), but had lower hydroxyl radical scavenging activity (34.35%) than DM-L (56.86%). This paradoxical result indicates that not every phenolic compound has an equal contribution to antioxidant activity. The kind and structural diversity of phenolics in seeds may be very different than those in leaves; the phenolics in seeds may consist of simple phenolic acids with lower redox potential, but the phenolics in leaves may consist of complex polyphenols and flavonoids with higher redox potential.

Furthermore, the seed extract is reported to have a greater percentage of alkaloids and tropane derivatives (including scopolamine and atropine), which can have pro-oxidant effects or affect the deoxyribose assay, thus decreasing the apparent radical scavenging percentage. The same effect has been observed in other *Datura* species in which high levels of alkaloids have moderated the net antioxidant capacity despite high levels of TPC. Such opportunities suggest that the TPC is not necessarily predictive of antioxidant performance, so the quality and composition of phenolics in a matrix should be prioritized over the quantity of phenolics [32].

The high hydroxyl radical scavenging activity of the leaf extract (DM-L) can directly be associated with the fact that it contains more phenolic and flavonoid compounds, which are shown in the GC-MS and TPC/TFC analyses. In particular, phenolic 2-Methoxy-4-vinylphenol, with its high hydrogen-donating ability, and flavonoids such as neophytadiene and campesterol play a significant role in neutralizing reactive oxygen species (ROS). These compounds have conjugated ring structures that have the ability to stabilize free radicals by delocalizing electrons, and hence the higher antioxidant activity of DM-L than DM-S. Such polyphenolic and flavonoid compounds give a mechanistic explanation of the relationship between the phytochemical content of *D. metel* extracts and their hydroxyl radical scavenging and α-amylase inhibitory effects in this paper.

The plant extracts DM-L and DM-S showed significant antidiabetic activity like that of the reference compound used- acarbose, implying a significant role of active phytochemicals contributing toward alpha-amylase inhibition. In our study, the standard showed inhibitory activity ranging from approximately 18% to 62% in a concentration-dependent manner. A study by Kesinee Nanok and Sompong Sansenya [24] also suggested the role of 2-methoxy-4-vinyl phenol, which was identified in both the leaves and seeds of *D. metel* via GC–MS characterization, in the synergistic downregulation of alpha-amylase enzyme activity and significant inhibition of alpha-glucosidase. Moreover, studies of Islam, 2023 [33] reported the presence of nortropane alkaloids and their inhibitory role in glycosidase activity. *D. metel* seed extract has been

Table 3: The total phenols (TPC) and total flavonoids (TFC)

Plant sample	Measured phenol (mg GAE/g)±SD	Measured flavonoids (mg RE/g)±SD
DM-L	2.921±0.375*	3.101±0.0408*
DM-S	3.342±0.315*	1.219±0.0396*

Results are mean±SD (n=3 replicate groups). GAE: Gallic acid equivalent; RE: Rutin equivalent; DM-L: *Datura metel* leaf extract, DM-S: *Datura metel* seed extract; *Significant at p≤0.05

Table 4: The % hydroxyl radical scavenging activity

Plant sample	Hydroxyl radical scavenging potential (%)±SD	Equivalent concentration (µg/ml)
DM-L	56.869±0.00642*	0.068
DM-S	34.346±0.011*	0.136

Results are mean±SD (n=3 replicate groups). GAE: Gallic acid equivalent; RE: Rutin equivalent; DM-L: *Datura metel* Leaf extract, DM-S: *Datura metel* seed extract; *Significant at p<0.05

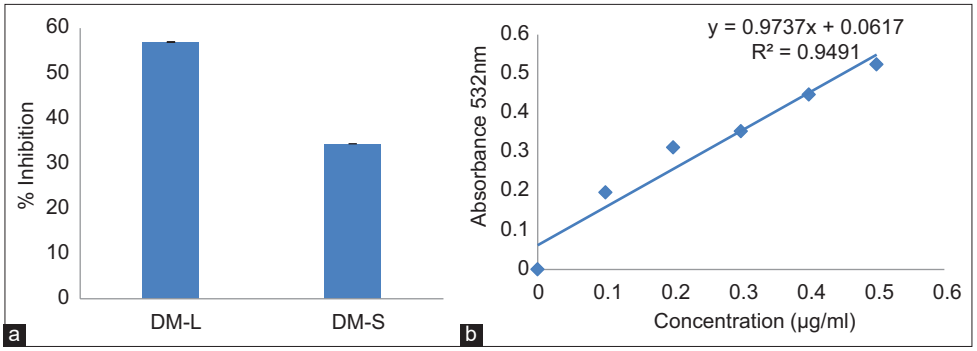

Fig. 7: Hydroxyl radical scavenging activity for (a) *Datura metel* leaves and *D. metel* seed (b) standard curve

shown to have hypoglycemic effects on alloxan-stimulated diabetic rats [34].

The present study suggested that the inhibition of alpha-amylase activity by standard acarbose, DM-L, and DM-S methanolic plant extracts was a dose-dependent pattern for gradual strengths from 0.01 mg/mL to 0.1 mg/mL (Table 5). Thus, the respective calculated IC_{50} values were 65.46, 53.258, and 54.1293 µg/mL. The IC_{50} values were obtained through a linear equation. A maximum of 61.66, 66.166, and 71.071% inhibition of alpha-amylase activity was observed at 1 mg/ml acarbose, DM-L, and DM-S, respectively (Fig. 8).

The pattern of inhibition observed (*D. metel* extracts exhibiting greater α-amylase inhibition than α-glucosidase inhibition than acarbose) is both mechanistically and clinically relevant. α-Amylase catalyses the first step in breaking down starch into oligosaccharides, whereas α-glucosidase acts later in the small intestine to release glucose. Powerful α-amylase inhibition may result in an overabundance of unfermented carbohydrates in the colon, which can be a source of abdominal discomfort or flatulence, an established side effect of potent α-amylase inhibitors. Selective inhibition of α-glucosidase, in contrast, leads to a slower postprandial glucose release, which leads to improved glycemic control with less gastrointestinal side effects. The increased binding of the extract to α-amylase could be due to certain phytochemicals such as 2-methoxy-4-vinylphenol, scopolamine, and 3, 4-divanillyltetrahydrofuran, which have hydrogen-bonding and aromatic ring systems that interact with the catalytic residues of α-amylase more than with the catalytic residues of α-glucosidase. The comparatively low α-glucosidase inhibition could be due to steric or conformational incompatibility between these molecules and the smaller active site of α-glucosidase. This profile indicates that extracts of *D. metel* may have the potential of being mild postprandial glucose modulators, although additional optimization or in combination with selective α-glucosidase inhibitors may enhance their therapeutic utility.

One-way ANOVA results showed an F-statistic value of 12.47 and p=0.0009, which is significant among the groups. Whereas the pairwise t-test suggested the t-statistic value (Acarbose vs. DM-L: 4.12; Acarbose vs DM-S: 3.78) with significance at p<0.001.

For alpha-glucosidase inhibition activity, the plant extracts acted comparatively well as compared to the standard acarbose, with IC_{50} value=42.98 µg/mL, DM-L (77.735 µg/mL), DM-S (115.967 µg/mL), with percentage inhibition ranging from 26% to 73%, 11% to 66%, 22% to 51%, respectively (Fig. 9 and Table 6).

One-way ANOVA for the alpha-glucosidase inhibition assay gave the F-value of 15.83, significant at p=0.0004. The *post hoc* pairwise comparison with t-values of Acarbose versus DM-L: 5.21, p<0.01, and Acarbose vs DM-S: 3.89, p<0.01 was obtained using Analysis Tool Pack in Microsoft Excel 365.

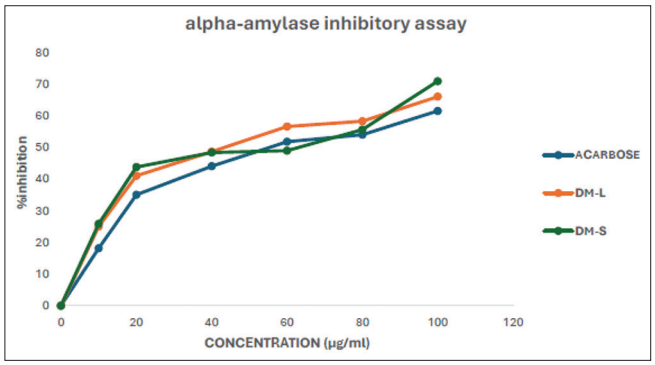

Fig. 8: α-amylase inhibitory activity of *Datura metel* leaves, *D. metel* seed, and the standard drug

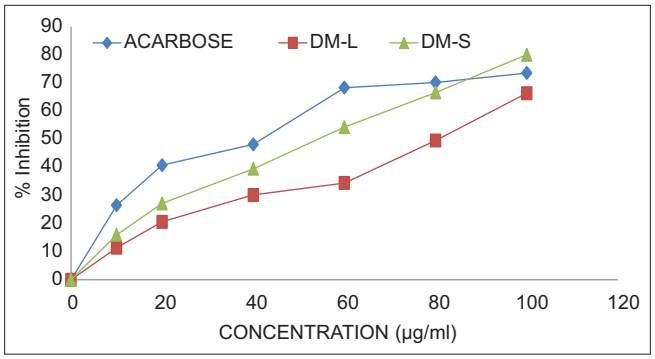

Fig. 9: α-glucosidase inhibitory activity of *Datura metel* leaves, *D. metel* seed, and the standard drug

Table 5: Percentage inhibition values±SDs and IC_{50} (µg/mL) values for standard (Acarbose), DM-L, and DM-S (alpha-amylase inhibitory assay)

Conc. (µg/mL)	STD±SD	DM-L±SD	DM-S±SD
10	18.19±1.533	25.047±0.412	25.909±0.612
20	35.1±1.845	41.124±1.23	43.881±0.911
40	44.181±1.158	48.776±0.626	48.470±1.447
60	51.91±0.511	56.706±1.2	49.099±1.148
80	54.104±0.9905	58.403±0.372	55.752±2.278
100	61.66±2.71	66.166±0.906	71.071±2.073
IC_{50} (µg/ml)	65.46	53.258	54.1293

Results are Mean±SD (standard deviation) n=3 replicates, DM-L: *Datura metel* leaf extract, DM-S: *Datura metel* seed extract

The literature reveals that the majority of bioactive secondary metabolites identified in *D. metel* leaves and seeds (DM-L and DM-S) methanolic extracts have therapeutic potential. A few of them, present

Table 6: Percentage inhibition values±SD and IC_{50} (μg/mL) values for standard (Acarbose), DM-L and DM-S (alpha-glucosidase inhibitory assay)

Conc. (μg/mL)	STD±SD	DM-L±SD	DM-S±SD
10	26.46±0.443	11.402±0.487	22.544±0.362
20	40.69±0.386	20.593±0.252	23.744±0.083
40	48.073±0.567	30.143±0.368	28.45±0.157
60	68.22±0.173	34.391±0.275	33.348±0.462
80	70.083±0.31	49.473±0.368	34.01±0.148
100	73.45±0.359	66.262±0.548	51.038±0.148
IC_{50} (μg/mL)	42.98	77.735	115.967

Results are Mean±SD (Standard Deviation) n=3 replicates, DM-L: *Datura metel* leaf extract, DM-S: *Datura metel* seed extract

in plant extracts, are natural antioxidants (2-methoxy-4-vinyl phenol, hexadecenoic acid), antimicrobial agents (18-octadec-9-enolide, neophytidiene), anti-inflammatory and anticancer agents, and different medicines used in the pharmaceutical industry. Especially because *D. metel* seed extract (DM-S) contains large amounts of phenolics and flavonoids, it is highly effective in antioxidant action and for diabetes. It matters given that diabetes tends to cause a higher level of oxidative stress. In addition, neophytadiene and 3,4-divanillyltetrahydrofuran also help lower the impact of oxidative stress, which plays a role in managing diabetes.

CONCLUSION

Such experimental results supports the therapeutic use of *D. metel* based on the reported biological activities of identified bioactive compounds. This study underscores the potent antidiabetic and antioxidant properties of *D. metel* extracts, particularly the seed extract (DM-S), which exhibited a high concentration of phenols and flavonoids. These phytochemicals have been well-documented for their effectiveness in quenching reactive oxygen species (ROS) and modulating ROS-induced stress, a major contributor to the development and complications of type 2 diabetes mellitus [35]. Given that diabetes is associated with elevated oxidative stress and inflammation, the free radical scavenging activity of these extracts may help mitigate cellular damage and improve metabolic function. Moreover, the strong α-amylase inhibitory activity is particularly significant since this enzyme overactivity contributes to poor glycemic control in diabetic individuals [36].

Beyond enzyme inhibition, bioactive components such as neophytadiene and 3,4-divanillyltetrahydrofuran contribute to reducing oxidative damage and may support glucose homeostasis by modulating oxidative stress pathways [37].

Prospects

While the data strongly support the ethnobotanical use of *D. metel* for diabetes management, caution is warranted due to the presence of toxic alkaloids. In the future, *in vivo* studies, pharmacokinetic profiling, and toxicity assessments are essential to ensure the extracts' safety and efficacy before clinical translation. In essence, with appropriate standardization and rigorous safety validation, *D. metel* holds promise as a plant-based adjunct or alternative to conventional antidiabetic therapies, aligning with a growing interest in integrating traditional medicine into modern health care.

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AUTHOR CONTRIBUTIONS

Khushboo Bhardwaj did the research, execution, and writing of the manuscript. The work plan and review were done by Arvind Kumar and Manoj Jena. The revisions and technical guidance were done by Sudhakar Kancharla, Prachetha Kolli, and Gowtham Mandadapu. All authors have read and consented to publish this study.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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