

Biochemical Constituents of *Datura metel*: A Comprehensive HPLC Study

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Abstract : This study investigated the diversity of 25 *Daturametel* accessions by using 7 biochemical parameters. During the present study a high-performance liquid chromatography (HPLC) used to analyse key compounds including Scopolamine, Hyoscyamine, Atropine, Withanolide, Withanolide, Luteolin, and Apigenin in the seeds and leaves of *D. metel*. The HPLC results demonstrate notable discrepancies in the levels of these biochemicals among the selected *D. metel* L. accessions. Within the spectrum of biochemical and morphological attributes, Apigenin (66.28%) and Atropine (44.19%) exhibit the highest coefficients of variance (CV%), while scopolamine (7.38%) display the lowest CV values. In culmination, this study not only provides valuable insights into the wide-ranging physiological effects and properties of *D. metel* but also enhances our comprehension of its intricate diversity by integrating morphological and biochemical characterization.

(Keywords : *Daturametel* L.; diversity; morphological traits; biochemical parameters; HPLC)

Introduction

D. metel L. boasts an array of phytochemicals, notably tropane alkaloids contributing to its toxicity and steroids responsible for its hallucinogenic effects. Notably, other constituents such as scopolamine and atropine alkaloids have also been identified¹. For instance, Scopolamine has garnered international recognition for its effectiveness in addressing motion sickness and serving as a premedication for anesthesia. Conversely,

Hyoscyamine and Atropine have demonstrated versatile utility, ranging from offering symptomatic relief in gastrointestinal ailments to acting as antidotes against particular cases of poisoning. Apart from their pharmaceutical implications, the quantification of these compounds in *D. metel*. holds potential significance in agricultural contexts. For instance, fluctuations in their concentrations could provide indications of the plant's health, its strategies for pest resistance, and its adaptability to specific environmental circumstances². Consequently, a precise and reliable quantification methodology assumes paramount importance for advancing therapeutic applications and driving agricultural advancements within this realm. The inherent diversity inherent in the *D. metel* L. accessions within these regions presents a captivating narrative of the species' broad intra-population diversity. Delving into this diversity has the potential to unveil the genetic richness and adaptive characteristics of these plants, likely influenced by diverse environmental factors and human interactions.

In the quest to explore the intricate characteristics of *D. metel* L. and its diverse compounds, High-Performance Liquid Chromatography (HPLC) emerges as a guiding light in the realm of phytochemical analysis³. Esteemed for its exceptional precision, accuracy, and proficiency in dissecting intricate mixtures, HPLC empowers researchers to delve into the intricate chemical compositions within plant

species. This investigation not only uncovers the inherent variability of *D. metel* but also highlights its potential to make valuable contributions to the realms of medicine and beyond.

Experimental

In the present study, a total of twenty-five distinct datura accessions were used for observing biochemical traits. The accessions, encompassing diversity within the population, were collectively grown in uniform environmental conditions using a randomized block design. Furthermore, the accessions were grown for two seasons one in 2022 and another crop season in 2023. Standard agronomic practices were implemented during the crop growth period. In every replicate of each accession, five robust and typical plants were chosen and labelled through random selection.

The leaf and seed samples had been collected from 25 different datura accessions that were cultivated under uniform environmental conditions. Five typical plants were selected in a random manner from each accession to serve as samples. The plant components were collected during the peak vegetative phase in the case of leaves and at the point of physiological maturity for seeds. The collected samples were subjected to a controlled drying process within a sheltered environment that facilitated passive air circulation, lasting for a period of 7-10 days. Subsequently, the dried samples were preserved in containers that sealed out air before undergoing the extraction process. The process of pulverization was executed using a commercial blender from Sujata, India. Following this, the material was sifted through a stainless-steel sieve with a mesh size of 20, sourced from Prayag, India.

After finely grinding the dried leaf samples, 0.5 g was accurately weighed and placed in centrifuge tubes. These specimens underwent extraction using 20 mL of 0.05 M HCl while

being agitated for a duration of 3 hours at ambient temperature using an orbital shaker. Subsequently, the extracted materials were subjected to centrifugation at 4000 rpm for 10 minutes, leading to the collection of the resultant supernatants. Residues were washed twice with 5 mL 0.05 M HCl, then centrifuged. The accumulated supernatants were combined and brought to a final volume of 50 mL using 0.05 M HCl within 50 mL volumetric flasks. Before being injected into the HPLC system, the extracts underwent filtration through 0.45 µm syringe filters.

The measurement of scopolamine was conducted using an Agilent 1200 series HPLC setup, featuring a C18 column (Zorbax Eclipse XDB, Agilent, 250x4.6mm, 5µm), for accurate quantification. The eluent consisted of a progressive mixture of acetonitrile and a 0.05 M phosphate buffer with a pH of 7.0, flowing at a rate of 1 mL/min. Detection occurred at 215 nm utilizing the diode array detector integrated into the HPLC system, while quantification was accomplished using external standards (scopolamine hydrobromide). Hyoscyamine quantification utilized the Prominence-i HPLC system with a C18 column (Kinetex EVO, Phenomenex, USA). Acetonitrile and phosphate buffer formed the mobile phase. Detection at 215 nm employed the diode array detector, and quantification utilized external standards (hyoscyamine sulfate).

Atropine quantification used an Alliance 2695 HPLC system, with a C18 column (Symmetry Shield, Waters, USA). The composition of the mobile phase involved a mixture of acetonitrile and 0.05M phosphate buffer with a pH of 7. Detection occurred at 215 nm employing the diode array detector, while quantification was dependent on external standard atropine sulfate. Dried seeds had been powdered and exactly weighed samples of 0.1 g were taken into centrifuge tubes. Each sample was extracted with 10 mL of HPLC grade methanol by vortexing for 5 mins using a vortex mixer. The centrifugation

Table 1. The values of biochemical traits identified in the 25 Datura accession.

Germplasm	Scopolamine	Hyoscyamine	Atropine	Withanolide_A	Withanolide_B	Luteolin	Apigenin
D1	0.52de*	0.295jk	0.02f	0.115l	0.095l	46d	26.049d
D2	0.485ghij	0.325gh	0.045c	0.135j	0.115j	37h	17.44h
D3	0.465kl	0.285kl	0.035d	0.155h	0.135h	31i	11.39ij
D4	0.53cd	0.345f	0.02f	0.175f	0.155f	50.5c	30.19c
D5	0.495fgh	0.315hi	0.01h	0.145i	0.125i	25.5j	10.52j
D6	0.475ijkl	0.335fg	0.035d	0.165g	0.145g	18.5k	4.47m
D7	0.55b	0.365d	0.045c	0.195d	0.175d	57.5b	37.57b
D8	0.425n	0.245n	0.02f	0.135j	0.115j	12.5l	5.49l
D9	0.565a	0.375c	0.055b	0.215c	0.195c	47d	26.75d
D10	0.52de	0.295jk	0.01h	0.125k	0.105k	38.5g	18.67g
D11	0.5fg	0.315hi	0.035d	0.145i	0.125i	31.5i	11.49i
D12	0.48hijk	0.285kl	0.025e	0.115l	0.095l	24.5j	8.06k
D13	0.54bc	0.355e	0.045c	0.185e	0.165e	41.5f	21.51f
D14	0.42n	0.235o	0.015g	0.125k	0.105k	18.5k	5.03lm
D15	0.57a	0.39b	0.065a	0.225b	0.205b	50.5c	30.35c
D16	0.51ef	0.305ij	0.025e	0.135j	0.115j	31.5i	11.52i
D17	0.49ghi	0.335fg	0.045c	0.155h	0.135h	37.5gh	17.50h
D18	0.46l	0.28l	0.035d	0.145i	0.125i	18.5k	4.50m
D19	0.58a	0.4a	0.055b	0.235a	0.215a	63.5a	44.16a
D20	0.44m	0.255m	0.015g	0.145i	0.125i	12.5l	5.55l
D21	0.53cd	0.345f	0.025e	0.165g	0.145g	44.5e	24.57e
D22	0.51ef	0.325gh	0.045c	0.185e	0.165e	37.5gh	17.53h
D23	0.5fg	0.305ij	0.035d	0.175f	0.155f	24.5j	8.00k
D24	0.52de	0.335fg	0.025e	0.195d	0.175d	31.5i	11.58i
D25	0.47jkl	0.285kl	0.015g	0.135j	0.115j	18.5k	4.44m

*Values flagged by different alphabets show significant differences at 0.005.

of the extracts was carried out at 3000 rpm for a duration of 10 minutes.

Subsequently, the resulting supernatants underwent filtration using 0.22 µm syringe filters prior to being injected into the HPLC system. The quantification of Withanolide A was accomplished through Agilent 1200 High-Performance Liquid Chromatography (HPLC), employing a C18 reverse-phase column and a mobile phase of methanol-water. Detection was

conducted at a wavelength of 227 nm. Dried seeds were powdered, extracted with methanol as described earlier, and then filtered through Axiva syringe filters before HPLC injection. Quantification employed a C18 column alongside a mobile phase composed of methanol and water, coupled with detection at 227 nm. This process relied on an external standard, specifically withanolide B. The powdered leaves were extracted with 80% methanol using ultrasonication. Extracts were then centrifuged,

filtered, and subjected to UV-Vis spectrophotometry. Absorbance at 272 nm was measured, and quantification utilized external standards: luteolin and apigenin.

Analysis of variance (ANOVA) employed the R program's Variability package (Team RC, 2016), treating accessions as fixed effects and environments as random effects. Statistical significance was set at $P < 0.001$, with result assessment using the least significant difference (LSD) method at $p < 0.05$ through the R program's doebiosearch package. Descriptive statistics including mean, range, and standard error were derived from the same R program package⁴.

The ANOVA outcomes for all assessed traits are detailed in Table 2. Evidently, variations attributed to accessions, environments, and the interaction between the two ($A \times E$) were all notably significant (Table 2). This is in line with

the anticipated complexity of phenotyping. The phenotypic coefficient of variation (PCV) estimates were slightly higher than the corresponding genotypic coefficient of variation (GCV), underscoring the environmental influence (Table 2). Table 3 presents descriptive statistics for all accession traits. The coefficient of variation (CV) ranged from 7.380% for FL to 66.29% for Apigenin. Notably, five traits, mainly biochemical ones, exhibited CV values above 20%, while six traits had CVs between 10% and 20% (Table 3). Traits linked to biochemical tests demonstrated the highest CVs, whereas typically morphological traits (excluding Scopolamine) exhibited lower values. It's noteworthy that traits with substantial quality significance, such as apigenin (66.29%), atropine (48.90%), luteolin (40.60%), withanolide B (23.81%), and withanolide A (20.85%), showed remarkable variability (Table 3).

Table - 2
Analysis of Variance (ANOVA) for 19 different morpho-biochemical traits in 25 accessions tested in two different environments.

Sources of variations	Sum of Square				
	Environments/D F=1	Replication (Within Env)/ DF=2	Genotypes /DF=24	Env: Genotypes /DF=24	Residuals/ DF=98
Scopolamine	0.013***	0.0006	0.011***	0.0	0.0002
Hyoscyamine	0.005***	0.0001*	0.011***	0.0	0.0001
Atropine	0.002***	0.0	0.0014***	0.0	0.0
Withanolide_A	0.004***	0.0	0.007***	0.0***	0.0
Withanolide_B	0.004***	0.0	0.007***	0.0	0.0
Luteolin	17.3***	0.7*	1166.6***	1.6	0.9
Apigenin	8.6***	0.1	736.3***	1.4***	0.3

***0.1% level of significance; **1% level of significance; *5% level of significance. PH: plant height; NoL: No. of leaves; LL: Leaf length; LW: Leaf width; NoF: No. of flowers; FL: Flower length; FW: Flower width; FrL: Fruit length; FrW: Fruit width; SPF: Seeds per fruit; SL: SW: Seed weight.

Table 3.
Statistical analysis of all the traits used for characterization of Datura accession.

Traits	Max*	Min	Mean	SD	SE	CV (%)	GCV	PCV	GA
Scopolamine	0.59	0.41	0.502	0.043	0.006	8.603	8.452	8.452	17.410
Hyoscyamine	0.41	0.23	0.317	0.043	0.006	13.406	13.375	13.375	27.554
Atropine	0.07	0.01	0.032	0.016	0.002	48.904	44.194	44.194	90.938
Withanolide_A	0.24	0.11	0.161	0.034	0.005	20.857	20.600	20.600	42.422
Withanolide_B	0.22	0.09	0.141	0.034	0.005	23.815	23.522	23.522	48.440
Luteolin	64.00	12.00	34.020	13.814	1.954	40.607	40.959	41.015	84.260
Apigenin	44.00	4.00	16.560	10.977	1.552	66.287	66.831	66.957	137.412

Max: maximum; Min: minimum; SD: standard deviation; SE: standard error; CV: coefficient of variation; GCV: genotypic coefficient of variation; PCV: phenotypic coefficient of variation; GA: genetic advance; PH: plant height; NoL: No. of leaves; LL: Leaf length; LW: Leaf width; NoF: No. of flowers; FL: Flower length; FW: Flower width; FrL: Fruit length; FrW: Fruit width; SPF: Seeds per fruit; SL; SW: Seed weight.

Among approximately ten species, *D. anoxia*, *D. stramonium*, and *D. metel* L. are pivotal drug plants. Significantly, key tropane alkaloids hyoscyamine and scopolamine, along with other minor tropane alkaloids, have been identified in *Datura* species⁵. In addition to these, various other alkaloids including tigloidin, aposcopolamine, apoatropin, hyoscyamine N-oxide, and scopolamine N-oxide have also been detected. Furthermore, compounds such as 6 α -ditigloyloxytropane and 7-hydroxyhyoscyamine act as lesser alkaloids in various *Datura* species, such as *D. stramonium*. It is worth emphasizing that the plant extract also contains harmful

tropane alkaloids, namely atropine, scopolamine, and hyoscyamine. Whereas, it was explored the synthesis of hyoscyamine and scopolamine across different plant parts and developmental stages of *D. stramonium* in their research. They discovered the highest concentrations in the stems and leaves of young plants, with hyoscyamine being the prevalent component⁶. The ethanol extracts derived from *D. stramonium* leaves and seeds have exhibited acaricidal, repellent, and oviposition deterrent effects against spider mites (*T. urticae*), as observed in several studies⁷⁻⁹.

References

1. N. Firdaus, U. Viqar, & M.H. Kazmi, *International Journal of Pharmaceutical Sciences and Research*. **11**, 3123 (2020).
2. N. Jabeen, I.H. Khan, & A. Javaid, *Allelopathy J.* **56**, 59 (2022).
3. M. Guo, M. He, J. Zhong, Q. He, B.B. Ismail, G. Chen, & D. Liu, *Science of the Total Environment*. **710**, 134975 (2020).
4. G. Alvarado, F.M. Rodriguez, A. Pacheco, J. Burgueño, J. Crossa, M. Vargas, P. Pérez-Rodríguez, & M.A. Lopez-Cruz, *The Crop Journal*. **8**, 745 (2020).
5. M. Parashuram, *Asian journal of biochemical and pharmaceutical research*. **3**, 501 (2011).
6. M.H. Shagal, U.U. Modibbo, & A.B. Liman, *International Journal of Pharmacy and Pharmacology*. **2**, 16 (2012).

7. S.A. Taha & A.H. Mahdi, *Transactions of the Royal Society of Tropical Medicine and Hygiene*. **78**, 134 (1984).
8. D. Diker, D. Markovitz, M. Rothman, & U. Sendovski, *European journal of internal Medicine*. **18**, 336 (2007).
9. V.A. Boumba, A. Mitselou, & T. Vougiouklakis, *Veterinary and human toxicology*. **46**, 81 (2004).ç