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Original Article

Development of Chromatographic Fingerprint Profile and Multi-elemental Analysis of *Datura metel* L.

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ABSTRACT

Background: The Solanaceae family member *Datura metel* L. has been related to therapeutic benefits. The plant is widely used in Ayurveda and Siddha systems of Indian medicines. **Objectives:** Present study was focused to understand the phytochemical and multi elemental composition of *D. metel* whole plant. **Materials and Methods:** The whole plant of *D. metel* was collected, shade dried, powdered and subjected to analysis of physico-chemical parameters, thin layer chromatography, high performance thin layer chromatographic finger print profilings (HPTLC), high pressure liquid chromatographic profile at four wavelengths, X-ray fluorescence, energy dispersive X-ray analysis, X-ray photoelectron spectrometer, powder X-ray diffractometer and inductively coupled plasma optical emission spectrometer. **Results:** In HPTLC, 7 spots under 254 nm, 10 spots under 366 nm and 11 spots after derivatization with vanillin sulphuric acid reagent appeared. In HPLC, 9 peaks at 230 nm, 11 peaks at 254 nm, 5 peaks at 286 nm and 7 peaks at 366 nm separated. Presence of 17 elements such as copper, cobalt, nickel, manganese, zinc, iron, sodium, potassium, calcium, magnesium, phosphorus, aluminium, chlorine, carbon, oxygen, sulphur and silicon were identified. **Conclusion:** The elements K, Mg, Ca, Si, S and Fe play vital role on human health. Chlorine, silicon, sulfur and silica are first time identified which have significant biological functions.

Key words: Ummattai, Devil's trumpet, Solanaceae, Cardiarhythm, Asthma.

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INTRODUCTION

Datura metel is commonly known as devil's trumpet. It is an annual, perennial shrub. It is wilding growing in warmer parts of the world. It is also cultivated worldwide for its medicinal properties.[1] It belongs to the Plantae Kingdom, Magnoliophyta Division, Angiospermae Subdivision, Magnoliopsida Class, Asterids Subclass, Solanales Order, Solanaceae Family, *Datura* Genus and *D. metel* (syn. *D. fastuosa*) Species.[2] It is also known as Thorn apple in English, Dhattura and Kaladhattura in Hindi, Dhotra, Dhatura and Dhutura in Bengali, Dhatura, Dhaturo and Dhanturo in Gujarati, Unmatta and Unmatte-Gida in Kannada, Ummattu, Unmatta, Rotecubung and Ummam in Malayalam, Dhotra and Dhatura in Marathi, Dhattur and Dhattura in Punjabi, Ummattai and Umate in Tamil, Ummetta and Ummatta in Telugu, and Khunuk in Bihari.[3]

The chemical constituents of *Datura metel* are hysocyamine,[4,5] scopolamine,[4] atropine, hyosocyamine,[5] hyoscine,[6] fastumine, fastudine, fastusuidine,[7] fastuic acids, daturanolone,[8] 31-norlanosterol, 31-norlanost-8-enol, 31-norcycloartanol, 31-norcycloartenol, cycloeucalenol, 31-norlonost-9(11)-enol, 14α-methyl-24-ethylcholesta-7,24-dienol,4α,14α,24-dienol, 4α-methylcholest-8-enol, lephenol, 24-methylophenol, (24R)-24-ethylphenol, gramisterol, citrostadienol,[9] withafastuosins A-C&E, baimantuoluoline A-C, dmetelins A-D, withametelin, withafastuosin D-F,[10-15] oleic, linoleic, palmitic, palmitolic, stearic acids, ethyl palmitate, ethyl linoleate, phytol,[16,17] withanolides, withametelins B,I-P, withametelin, isowithametelin, daturafolisides A-I, daturataturin A&B, baimantuoluoside B, daturametelin J, 12-deoxywithastramonolide,[15,18-22] cis-2-nitro-4-*t*-butylcyclohexanone, trifluoroacetic acid, 2,2-dimethylpropyl ester, 4-trifluoroacetoxyoctane, 1,4-cyclohexadiene,[23] arenarine D, daturadiol, hyoscyamilactol, isofraxidin, scopatone, scopoletin, β-sitosterol, vanillin and N-trans feruloyl tyramine.[24]

Datura is ascribed as a poisonous as well as a medicinal plant. Its traditional claims include diarrhea, epilepsy, fever with catarrh, heart diseases, hysteria, insanity and skin diseases.[25] This plant is used to treat asthma in China; antiasthmatic cigarettes are packed up with cut pieces of dried leaves and flowers in Vietnam; allergy, eczema and scabies are treated with leaves in Bangladesh.[26] Oral consumption of 3 to 5 g of flower extract can cause general anesthetic within 5 min of administration which can long last for 5-6 hr.[27]

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The smoke from the dried whole plant powder cure irregular breathing. External or internal administration of leaf juice reduces pain and swelling. The inflammation in swollen gums and ear base is reduced by the application of leaf juice admixed with few opium. Breast pain is reduced by the application of leaf juice admixed with lime and turmeric.[28] Flowers are used for curing asthma, convulsions, pain and rheumatism.[29]

The pharmacological activities of *D. metel* are antimicrobial activity,[30-35] antispasmodic and spasmogenic activity,[36] toxicity and side effects,[2,25,27,37-41] analgesic activity,[42] neurological activity,[37,41,43,44] antidiabetic activity,[45] xanthine oxidase inhibitory activity,[46] cytotoxic activity,[22,46-47] reproductive activity,[48,49] anti-inflammatory activity,[50,51] wound healing activity,[52] antioxidant activity.[47,53,54]

In Ayurvedic system of medicine, *D. metel* whole plant is one of the ingredients in Kanakāsava, Ekāngavīra Rasa, Puspadhanva Ras, Tribhuvana Kīrti Rasa, Śri Jayamangala Rasa, Laghu Viśagarbha taila, Visatinduka Taila, Dhattura Taila, etc. The therapeutic uses as per Ayurveda includes, Śvasa (dyspnea), Jvara (fever), Krmi (worm infestation), Kandu (itching sensation), Kasa (cough), Kustha (leprosy), Unmāda (psychiatric illness), Mutrakrccha (dysuria), Alarka Visa (rabies), Indralupta (alopecia), Pada dāha (burning feet), etc. and 100 to 200 mg is the prescribed dose of the drug.[55]

According to Siddha literature, *D. metel* whole plant is a remedy for dog bite sore, acute oral ulcer, tumour and toxicity. The daily intake of 32 mg to 100 mg of dry leaf powder cures asthma. Or the smoke from 325 to 1000 mg leaf rolls if inhaled also cures asthma, dismisses sputum, banishes shortness in breathing. Hot fomentation with sauted leaves cures inflammation in bones, pain due to tumour, etc. Its fruit cures eczema, cancer, scabies, etc.[56] Leaf juice is used in the Siddha preparations like Kattuvai māttirai, Kanēca kulikai, Paccaik kalimpu, Milakut thailam.[57] Application of the ash from the leaves of the plant along with fat reduces the facial neuralgic pain.[58]

The extensive use of the plant in Indian systems of medicines and various pharmacological studies envisage the medicinal importance of the plant. A complete review of literature from various sources revealed that not extensive work on elemental composition of *D. metel* has been carried out using XRF, EDAX and ICP-OES. The present study includes TLC photodocumentaion, HPTLC finger print profiles, HPLC analysis, and elemental analysis of *D. metel* by EDAX, XRF, XPS, PXRD and ICP-OES.

MATERIALS AND METHODS

Plant Material

The whole plant of *D. metel* (DM) was collected from natural habitat in Kumarapalayam region of Namakkal District, Tamil Nadu, India. It was authenticated by the Pharmacognosist of this Institute, dried at room temperature and powdered.

Physicochemical Analysis

The plant was analyzed for moisture content in terms of loss on drying, inorganic content in terms of total ash, water soluble ash, silicious matter in terms of acid insoluble ash, extractive values in water and alcohol as per WHO methods.

TLC/HPTLC analysis

A high performance thin layer chromatography (HPTLC) system for detection of separation; Linomat IV applicator for extract application; twin chamber for developing the TLC plate; scanner 030618 attached with WINCATS software for qualitative and quantitative scanning; visualizer for photo documentation at dual wavelengths 254 and 366 nm; in visible lights after derivatization with vanillin sulfuric acid reagent and heating till color development are from CAMAG, Muttenz, Switzerland. Analytical reagent grade solvents toluene, ethyl acetate, methanol and formic acid with 99.8% purity were obtained from E. Merck chemicals. Plant powder (100 mg) was extracted with 100 ml aqueous methanol by sonicating for 5 min, filtered using Whatman no.1 filter paper, concentrated to 1 ml using Rotavapor R-300, and used as test solution for HPTLC analysis. The mobile phases for methanol extract, *ethyl acetate: toluene: formic acid* (2:5:0.5, *v/v/v*) was finalized. Sample solution (10 µl) of was loaded as band having length of 8 mm on 4×10 Silica gel $60F_{254}$ TLC plate using auto sampler. The sample applied plate was positioned in a presaturated twin trough chamber and the plate was developed in the mobile phase up to 90 mm. After evaporation of solvents from plate on air, the plate was viewed under photo documentation chamber at UV 254 and 366 nm and their images were captured. Vanillin sulfuric acid reagent was sprayed over the developed plate and heated at 100°C on a plate heater for developing the colored spots. Scanning of the plate was done in duel at 254 and 366 nm and after derivatization at 520 nm to record densitogram with finger prints and peak tables.

HPLC conditions

Column: Shim pack GIST, C_{18}, 4.6×250 mm, 5 µm; Sample preparation: 1 g in 10 ml methanol; Mobile phase: 100% Methanol; Flow rate: 1 ml/min; Elution: Isocratic elution; Volume injected: 20 µl and Detection wave length: 200 nm; Detector: PDA

Elemental Analysis

SEM - FESEM, TESCAN-MIRA 3XMU, Czech Republic); EDX - EDS, TESCAN-MIRA 3XMU, Czech Republic); XRF - XGT-2700 X-ray analytical microscope, with X-ray tube and an Rh-anode - Horiba, Japan); XPS - Perkin-Elmer PHI 5500 ESCA System, monochromatic Al K radiation (1486.6 eV). PXRD - Aeris PANalytical Diffractometer. ICP-OES - Agilent, Model 720 series, Santa Clara, California, USA. Wet digestion of the sample was performed using a microwave digester with 40 closed vessels (Mars 6, CEM, USA) were used.

RESULTS

The physicochemical results are summarized in Table 1. TLC of methanol extract showed seven bands (all green) under 254 nm; it revealed 10 bands with under 366 nm; post derivatized plate showed eleven bands under 520 nm [Table 2, Figure 1]. In the HPTLC fingerprint profile of methanol extract, peaks 9, 8, 4 and 3 at R_f 0.62 (33.29%), 0.50 (20.16%), 0.13 (13.72%) and 0.26 (10.85%) were major under 254 nm; peak 7 and 3 at R_f 0.59 (66.82%) and 0.27 (10.68%) were major under 366 nm; peak 7, 8, 9, 4 and 5 at R_f 0.50 (19.65%), 0.58 (17.50%), 0.63 (13.82%), 0.13 (12.10%) and 0.26 (10.68%) were major under 520 nm. Two spots at R*f* 0.62 and 0.26 were found in all the three fingerprint profiles [Figure 1]. HPLC results of hexane extract of the plant sample were shown in Table 3 and Figure 2.

The elemental compositions of *D. metel* at different surface locations were tabulated in Table 4. The EDAX results indicated the presence of eleven elements in descending order: potassium, oxygen, chlorine, sodium, carbon, sulphur, calcium, phosphorous, aluminium, silicon and iron. The SEM micrographs of the sample containing various metal elements are given in Figure 3.

XRF data obtained from the sample showed the presence of eight elements in the descending order: potassium (37.75 %), sodium (26.81 %), chlorine (15.27 %), calcium (17 %), iron (2.54 %), zinc (0.25 %), nickel (0.20 %), and manganese (0.14 %). The elemental composition of the *D. metel* sample obtained by XRF is presented in Table 5, Figure 4. The elements C, O, P, K, Mg, Na, Ca, Si and Cl were identified with various

Table 1: Physicochemical Parameters.

S.No.	Physicochemical Parameters	Mean (%, w/w)
1	Loss on Drying	10.85
2	Total Ash	12.54
3	Water Soluble Ash	6.57
4	Acid Insoluble Ash	3.21
5	Water soluble Extractive	14.54
6	Alcohol soluble Extractive	10.11

Table 2: R_f value and color of spots.

At 254 nm		At 366 nm (Post Derivatized)		At 520 nm (Derivatized)	
Color	R_f value (s)	Color	R_f value(s)	Color	R_f value(s)
Green	0.05	Red	0.04	Blue	0.03
Green	0.09	Red	0.09	Blue	0.05
Green	0.16	Red	0.16	Pink	0.08
Green	0.29	Red	0.29	Blue	0.10
Green	0.37	Red	0.32	Blue	0.16
Green	0.52	Red	0.36	Green	0.29
Green	0.63	Blue	0.42	Blue	0.38
		F. green	0.62	Green	0.52
		Red	0.65	Violet	0.60
		Red	0.69	Violet	0.66
				Violet	0.70

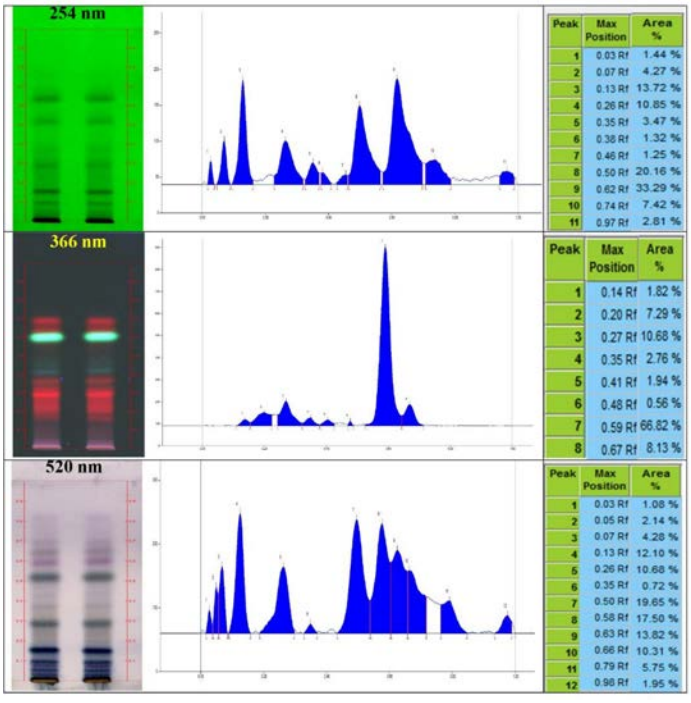

Figure 1: TLC fingerprint profile at 254 nm, 366 nm and 520 nm and peak area.

Table 3: HPLC Peak R_t and peak area at different wavelengths.

Peak	230 nm		254 nm		286 nm		366 nm	
	R_t	Area	R_t	Area	R_t	Area	R_t	Area
	2.418	40377044	2.440	16015455	2.432	13271369	2.415	4321940
	2.713	37172298	2.710	14943538	2.707	14094578	2.714	4515676
	3.149	39908362	3.419	9297192	3.450	1438916	5.088	820793
	3.531	9212346	3.514	2133310	5.298	954323	5.295	2118053
	4.105	13509834	4.104	3005576	6.858	507195	6.134	288532
	4.287	3788960	4.289	1490817	-	-	6.858	1809680
	6.856	970332	6.867	645952	-	-	10.291	87060
	14.183	1443841	8.155	644293	-	-	-	-
	15.791	522408	9.033	924431	-	-	-	-
	-	-	14.814	1079152	-	-	-	-
	-	-	15.792	382586	-	-	-	-

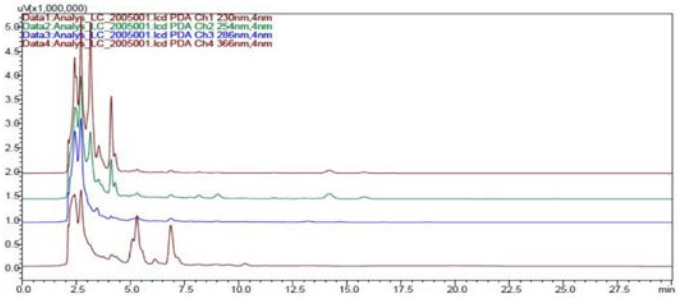

Figure 2: HPLC chromatogram of methanol extract.

Table 4: Composition by EDAX.

Elements	Mass %
Carbon	9.57
Oxygen	27.71
Aluminium	0.47
Silicon	0.35
Phosphorous	2.60
Sulphur	3.41
Chlorine	21.48
Sodium	18.41
Potassium	33.35
Calcium	2.73
Iron	0.26

chemical states (Table 6, Figure 5). The peaks were matched with the standard of the constituents through ICDD (International Centre for Diffraction Data). Strong peaks with high intensities at 2θ angles of 28.33 and 40.50; intermediate and small peaks with low intensities at 2θ angles of 26.61, 29.37, 31.62, 50.16, 58.69, 66.36, 73.76 and 94.68 were recorded. PXRD results (Figure 6) showed the presence of elements such as Ca, K, Cl, C, S and O. In chemical composition or phase identification present in the form of potassium chloride, sodium chloride, silicon dioxide and calcium carbonate and their ICDD reference numbers 00-041-1476, 00-005-0628, 01-075-8320, 04-023-8700 respectively.

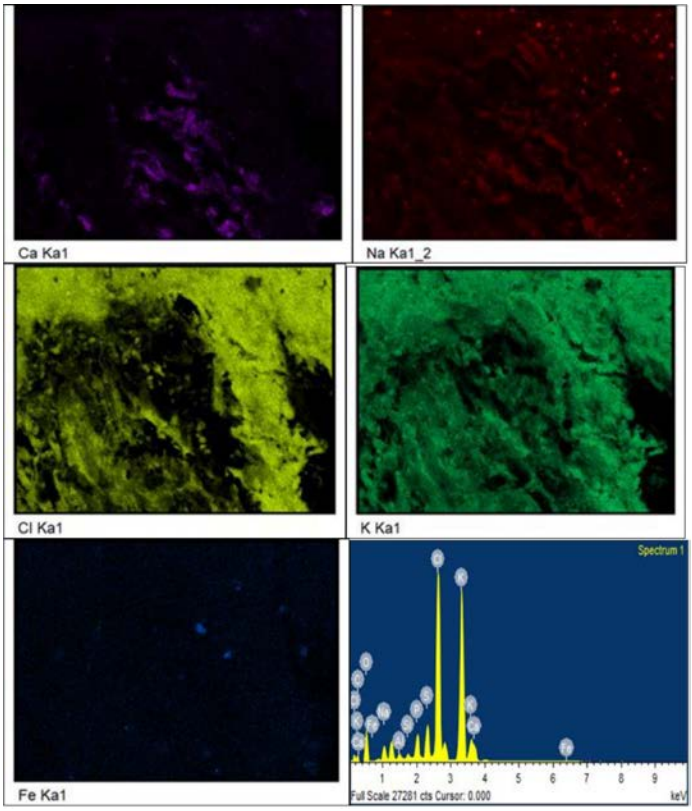

Figure 3: SEM-EDAX Micrographs.

Table 5: **Composition by XRF.**

S.No.	Elements	Mass %
1	Sodium (Na)	26.81
2	Chlorine (Cl)	15.27
3	Potassium (K)	37.75
4	Calcium (Ca)	17.00
5	Manganese (Mn)	0.14
6	Iron (Fe)	2.54
7	Nickel (Ni)	0.20
8	Zinc (Zn)	0.25

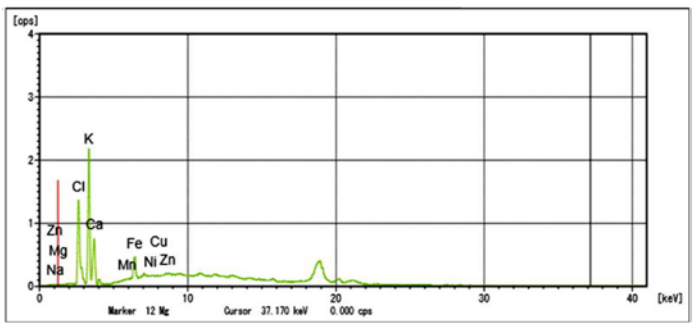

Figure 4: XRF spectra.

Table 6: **Composition by XPS.**

S.No.	Elements	Electronic state	Binding Energy (eV)
1	C	1s	284.0
2	O	1s	530.0
3	P	2p	131.0
4	K	2p	293.0
5	Mg	1s	1302.0
6	Na	1s	1070.0
7	Ca	2p	350.0
8	Si	2p	101.0
9	Cl	2p	198.0

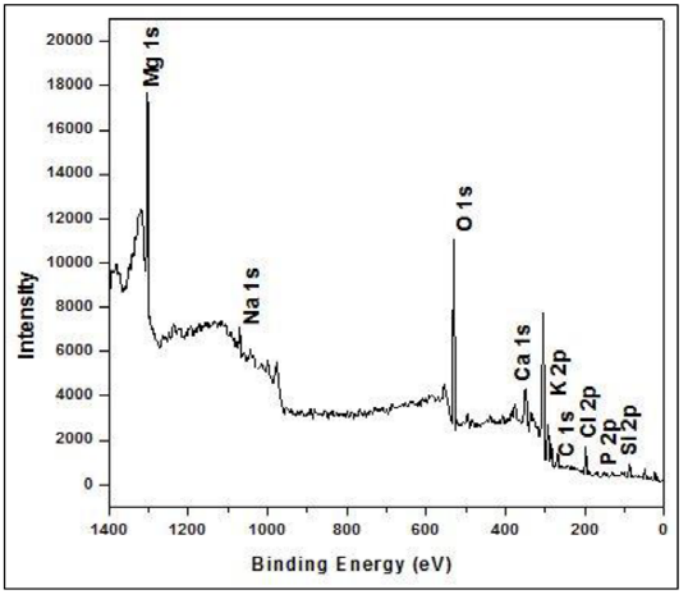

Figure 5: XPS spectra.

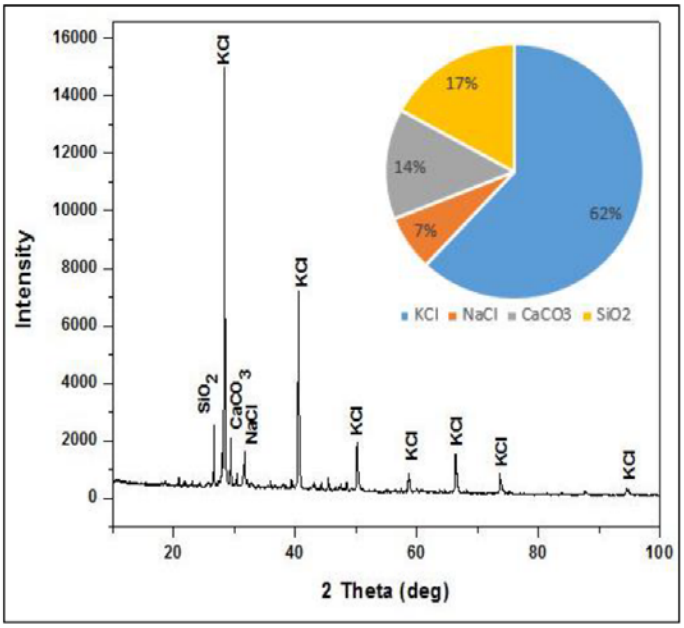

Figure 6: PXRD spectra.

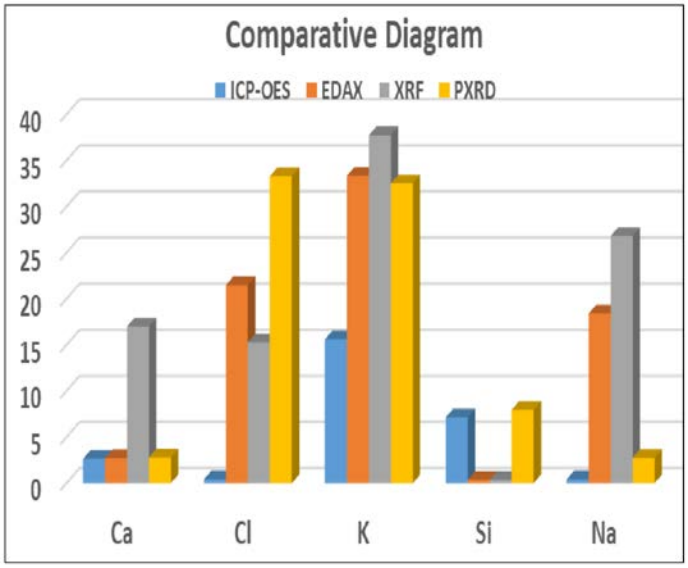

Figure 7: Comparison of ICP-OES, EDAX, XRF & PXRD.

Table 7: Composition by ICPOES.

Sl.No.	Elements	Mass %
1	Al	0.40
2	Ca	2.65
3	Cl	0.41
4	Co	<0.001
5	Cu	0.01
6	Fe	0.74
7	Mg	2.67
8	Mn	0.01
9	Ni	<0.001
10	P	0.015
11	K	15.60
12	SiO_2	15.28
13	Na	0.42
14	S	0.74
15	Zn	0.05

The ICP-OES data indicated the presence of 15 elements in descending order: potassium, silica, magnesium, calcium, sulfur, iron, sodium, chlorine (gravimetry), aluminum, zinc, phosphorus, copper, manganese, nickel and cobalt (Table 7).

DISCUSSION

For the quantification of atropine and scopolamine from *D. metel* by HPLC-MS has been reported[59] but TLC or HPTLC date for the identification of the plant is not available.

The correlation of elemental composition of *D. metel* in ICP-OES and XRF techniques with respect to Mn, Ca, Fe, K and Zn is given in Figure 7. The disparity between XRF, EDAX, PXRD and ICP-OES values is due to the fact that range of elements which are detected by ICP-OES is wide;[60] EDAX, XRF, PXRD are surface elemental analytical technique which give different elemental composition in different locations of the sample; ICP-OES measures the content of elements in the whole sample.[60] The wide variation in elemental composition obtained using XRF, ICP-OES and EDAX has also been reported during examination of geochemical sample,[61] soil sample,[62,63] and municipal landfill.[64]

Despite the above facts, the ICP-OES data and some of other data are common in the detection of elements such as calcium, chlorine (as chloride in PXRD), potassium, silica and sodium. The content of Al was almost same in EDAX, XRF and ICP-OES but not identified in XPS and PXRD. The content of Ca was comparable in EDX, PXRD and ICP-OES but it was very high in XRF other techniques. Fe was not detected in XPS and PXRD. The content of K in almost double time higher in other spectral methods than ICP-OES. But the content of Na and Si, results of EDAX and XRF were comparable and similarly ICP-OES with PXRD. However, Co, Cu and S were not identified in any of the spectral technique except ICP-OES. The lower detection limits and accuracy of ICP-OES is better when compared to the XRF and SEM-EDX,[65] the multi elemental composition of *D. metel* is taken into consideration.

D. metel plant has a high concentration of K which is vital for maintenance of cardiarhythm,[66] Mg which is essential for cardiovascular health,[67] Ca which is an important mineral normal functioning of human body,[68] silica which is indispensable for liver health,[69] permissible level of S which protects the cells in the body from heavy radiation and pollution,[70] and Fe which is important nutrient for impairing immune system.[71] From the literature, the presence of copper, cobalt, nickel, manganese, zinc, iron, sodium, potassium, calcium, magnesium, phosphorous and aluminum have been reported in *D. metel*.[72]

CONCLUSION

In the presence study, in addition to earlier reported elements, chlorine, silicon, sulfur and silica are reported which also find biological applications. In addition, the TLC photo documentations would serve as a new scale for the authentication of the plant along with the pharmacopoeial standards.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

ABBREVIATIONS

HPTLC: High performance thin layer chromatography; **HPLC:** High pressure liquid chromatography; **HPLC-MS:** High pressure liquid chromatography-mass spectrometry; **PDA:** Photo diode array; **XRF:** X-ray fluorescence; **EDAX:** Energy dispersive X-ray analysis; **XPS:** X-ray photoelectron spectrometer; **PXRD:** Powder X-ray diffractometer; **ICP-OES:** Inductively coupled plasma optical emission spectrometer; **WHO:** World Health Organization.

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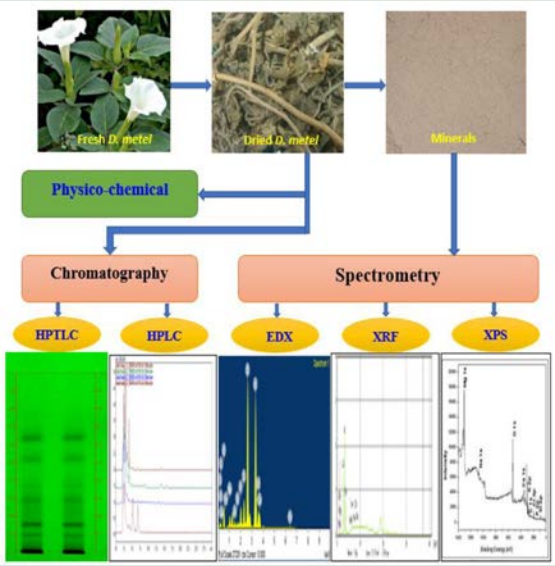
GRAPHICAL ABSTRACT



SUMMARY

The powdered *D. metel* whole plant subjected physicochemical parameters, TLC, HPTLC, HPLC analysis; its ash was subjected to XRF, EDAX, XPS, PXRD and ICP-OES. In HPTLC, revealed the separation of 7, 10 and 11 compounds under 254, 366 nm and white light after derivatization with vanillin sulphuric acid reagent. In HPLC a maximum of 11 peaks were separated at 254 nm. Seventeen elements such as copper, cobalt, nickel, manganese, zinc, iron, sodium, potassium, calcium, magnesium, phosphorus, aluminium, chlorine, carbon, oxygen, sulphur and silicon were identified.

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