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PHYTOCHEMICAL SCREENING, ANTIMICROBIAL ACTIVITY AND *in vitro* CALLUS INDUCTION IN *Datura metel* L.

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ABSTRACT

***Datura metel*, a well-known medicinal herb, exhibits poisonous and therapeutic attributes, showcasing its significant pharmacological potential. The present study focused on phytochemical screening of *D. metel* through TLC and LC-MS, antimicrobial assay and *in-vitro* callus inuction of *D. metel* through leaf and stem explants. The bioactive compounds from leaf and stem were extracted in ethyl acetate, methanol, and distilled water. Antibacterial activity of *D. metel* against pathogenic bacteria *Escherichia coli* and *Staphylococcus aureus* was performed. The leaf and stem explants were cultured on MS medium supplemented with 30 g L^{-1} sucrose, 8 g L^{-1} agar, and varying combinations of 2,4-D, BAP, kinetin, IAA, and NAA to explore their effects on callus induction. LC-MS analysis of *D. metel* confirmed the presence of three alkaloids *viz*., choline, atropine, and ambelline in its extract. Antimicrobial assay revealed maximum zone of inhibition in ethyl acetate leaf extract (10 mm), with methanolic and aqueous leaf extracts showing minimum zone of inhibition (9 and 8 mm) against *S. aureus.* The MS basal medium supplemented with 2, 4-D + kinetin + IAA (1.5:2:1 mg L^{-1}) combination resulted in early callus initiation on 15th day after inoculation.**

Keywords: Ambelline, atropine, choline, callus, *Datura metel*, phytochemistry

INTRODUCTION

Datura metel L., belonging to family Solanaceae with approximately 25 species, is widely distributed throughout the world (Jamdhade *et al.*, 2010). The plant is an important source of active biomolecules. *D. metel* is a sub-glabrous shrubby herb, frequently used in traditional systems of medicines as narcotic, anodyne and antispasmodic. The leaves of *D. metel* reportedly show anticholinergic activity and are used to relieve the spasm of bronchioles in asthma (Clissold and Heel, 1985). The phytoconstituents of *D. metel* comprise of alkaloids, flavonoids, phenols, tannins, saponins and sterols. The solanaceous alkaloids hyoscyamine, and scopolamines have also been reported (Muthusamy *et al.*, 2014). The phytochemical analysis of *D. metel* extracts have revealed the presence of alkaloids, saponins, flavonoids, tannins, glycosides, amino acids, and phlobatannins, while steroids and terpenoids were not detected (Sundaramoorthy *et al.*, 2014). *D. metel* is long known for its sedative action and as cure for hydrophobia in Ayurveda (Roy *et al*., 2016). The antimicrobial activity of *D. metel* in ethanolic extract are most promising against the pathogens (Krishnan *et al.*, 2017). Pharmaceutical importance of this plant has led the development of efficient tissue culture protocols with leaf explants extensively used for callus production. The media containing 3 mg L^{-1} BAP + 1 mg L^{-1} NAA and 2 mg L^{-1} BAP

+ 1 mg L^{-1} NAA have been reported as the best hormonal treatments for shoot regeneration from callus of leaf and embryo explants (Amiri *et al.,* 2011). Ramachandran *et al.* (2022) studied phytochemical and multi-elemental composition of whole *D. metel* plant. The study involved the analysis of physicochemical parameters, high-performance thin layer chromatographic fingerprint profiling, and high-performance liquid chromatographic profiling at four wavelengths to identify various elements. The present study was aimed to quantify the phytochemicals present in medicinal plant, as well as analyse the secondary metabolites for their pigment patterns using TLC and LC-MS. Additionally, antimicrobial properties and *in-vitro* potential for callus initiation from leaf and stem explants were assessed.

MATERIALS AND METHODS

Collection of plant material and preparation of extracts

The *Datura metel* plants were collected from Shaniwarwada, Pune (India) on October, 2019 in a clean polythene bag and brought to the Department of Botany, Modern College of Arts, Science and Commerce, Shivaji Nagar, Pune (India) for analysis. The plant material was gently dusted, and washed with distilled water. Subsequently, the leaves and stem were dried in shaded. After drying, the plant materials were crushed to fine powder with the help of a mortar and pestle and stored in sterilized bottles. The remaining fresh leaves and stem plant parts were used for tissue culture. The finely powdered dried plant samples were crushed separately in 10 mL ethyl acetate, methanol, and water. The extract was concentrated with the help of a rotary evaporator and dried samples kept at 4°C for further use (Muthusamy *et al.*, 2014).

Phytochemical analysis

The plant extract was tested for its various phytochemical constituents such as bioactive chemical constituents like alkaloids, terpenoids, flavonoids, carbohydrates, tannins, saponins, glycosides and proteins by using the methods of Akharaiyi (2011), Uddin *et al.* (2012) and Muthusamy *et al.* (2014).

Thin layer chromatography (TLC)

Pre-coated TLC plates were used to detect chemical constituents from crude methanol, ethyl acetate and aqueous extracts of *D. metel* leaves. The chromatogram was developed in a mixture of suitable solvent system (hexane: ethyl acetate, 9:1) at room temperature. The spots were visualized by using UV light at 365 and 254 nm (Arjun *et al*., 2012). The relationship between the distance travelled by a solvent front and the substance is usually expressed as R_f value:

$$R_f = \frac{\text{Distance travelled by the solute}}{\text{Distance travelled by the solvent}}$$

Liquid chromatography-mass spectrometry

Chromatographic separations were performed on Waters Alliance 2695 LC-MS system, XTerra MS C18 column (100X 2.1 mm, 3.5 µm), eluted with a gradient delivered at a flow rate of 0.2 mL min^{-1}. The partially purified compound of alkaloid crude extract was dissolved in acetonitrile. For separation of mobile phase, acetonitrile and water were used. An injection volume of 10 µL was used in all cases. A Quattro Micro-triple quadrupole MS (Micromass-Waters) fitted with a Z-spray ion interface was used for analyses. Ionization was achieved using electrospray in positive ionization mode (ES+), the column temperature was 50^0C (Kintz *et al.,* 2006)**.** The aforementioned conditions Are optimal for the analysis of all choline, atropine and ambelline, if any in the test samples.

Antimicrobial activity

The antimicrobial activity of aqueous, methanol and ethyl acetate leaf extract of *D. metel* were evaluated by agar well diffusion method (Vadlapudi and Kaladhar, 2012) against two bacterial pathogens *Escherichia coli* (MCC 3099) and *Staphylococcus aureus* (MCC 2408), procured from

National Centre for Microbial Resource (NCMR), Pune (India). The 100 µL pure bacterial culture was spread on nutrient agar medium. Each well filled with 20 µL methanol, ethyl acetate and aqueous leaf extract (100 µg mL^{-1}) were added aseptically to the wells. The plates were incubated in oven at 37±1°C for 24 h *(*Vadlapudi and Kaladhar, 2012; Ganesh *et al.*, 2015). The experiments were done with six replicates each, and the treatment effects were analysed using analysis of variance (ANOVA) to find significant differences between the means.

Preparation and sterilization of explants

D. metel leaves and stem were washed with liquid detergent under tap water for 30 min and subsequently immersed in 70% ethanol for 45 sec. The leaves were transferred into a sealed bottle containing 1% NaOCl solution for 20 to 30 sec and then it was rinsed thrice with distilled water. The treated plant materials were cut as explants approximate 1 x 1 cm dimensions (Amiri *et al.*, 2011).

Callus induction

The leaf and stem explants were separately transferred to the culture bottles containing 100 mL MS basal medium (Murashige and Skoog, 1962) supplemented with different growth hormones *viz*., BAP @ 4.0 mg L^{-1}, 2,4,-D @ 0.5 mg L^{-1}, kinetin @ 3.0 mg L^{-1}, 2,4-D + kinetin @ 1 mg L^{-1} each, NAA + BAP @ 1 mg L^{-1}each, and 2,4-D + kinetin + IAA @ 1.5: 2.0: 1.0 mg L^{-1}) were evaluated for callus regeneration. Culture bottles were inoculated with explants and incubated under dark (50%) and light (50%) condition at 25 ± 2°C temperature under light intensity of 2000 lux. (Amiri *et al.*, 2011). The number of regenerated calli were observed after two weeks. The experiment was conducted in in six replicates. The significance of treatment effects was evaluated using ANOVA, followed by determining the significant differences between the means (Gomez and Gomez, 1984).

RESULTS AND DISCUSSION

Phytochemical analysis

Phytochemical screening of *D. metel* revealed the presence of proteins, carbohydrates, tannins, flavonoids and glycoside. Similar results were reported by Jamdhade *et al.* (2010). Interestingly, they also detected saponins in the plant, while in present study saponin was not detected in all the three extracts. The ethyl acetate extract showed the presence of glycosides and alkaloids. When methanolic extract was tested for various phytochemical tests, it confirmed the presence of tannins, flavonoids, glycosides, terpenoids and alkaloids, whereas the aqueous extract showed the presence of proteins, carbohydrate, terpenoids and alkaloids.

Thin layer chromatography (TLC) of D. metel leaf extract

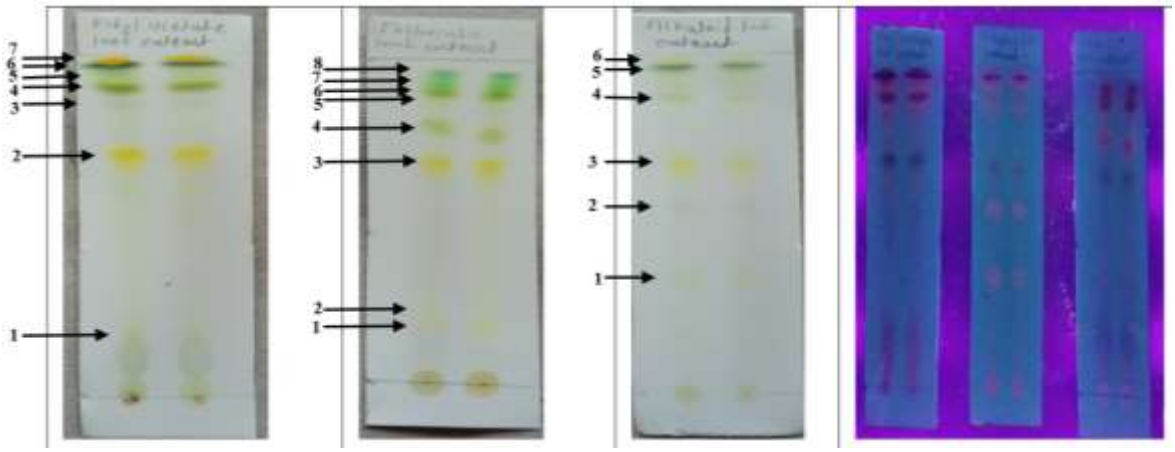
TLC of *D. metel* leaf extracts in methanol, ethyl acetate and ethanol solvents were thoroughly analysed using hexane and ethyl acetate solvents in 9:1 ratio. The spots were visualized under UV light at 254 and 365 nm. The TLC profile of methanolic and ethyl acetate leaf extracts showed discrete bands while only a few bands were observed in the ethanolic leaf extract (Table 1; Fig.1a-c). In all the seven bands, different components were separated in ethyl acetate leaf extracts. Eight bands in methanolic leaf extract and six compounds in ethanolic leaf extract were separated. As many bands were visualized on the TLC plate.

Table 1: R_f values of *D. metel* leaf extract

Band	Ethyl acetate	Methanolic	Ethanol
1	0.125	0.100	0.325
2	0.700	0.187	0.537
3	0.850	0.675	0.662
4	0.900	0.787	0.875
5	0.937	**0.**900	**0.**912
6	0.975	0.925	0.962
7	0.980	0.950	-
8	-	**0.**975	-

LC-MS analysis of ethanolic leaf extract

The LC-MS analysis of ethanol extract of



a) Ethyl acetate leaf extract b) Methanolic leaf extract c) Ethanolic leaf extract d) TLC plate observed under UV light

Fig. 1: Thin layer chromatography of *D. metel* leaf extract

Table 2: Detection of alkaloids in ethanolic leaf extract of *Datura metel*

Parameters	Standards			Plant sample		
	Choline	Atropine	Ambelline	Choline	Atropine	Ambelline
Observed m/z Da/e	-	-	-	104.1072	290.1749	314.1383
Observed RT (min)	0.424	5.034	7.608	0.424	5.034	7.608
Observed mass (Da)	104.1077	289.1675	331.1416	104.1077	289.1675	331.1416
Database formula	-	-	-	C5H14NO	$C_{17}H_{23}NO_3$	$C_{18}H_{21}NO_5$
Database mass (Da)	-	-	-	104.1075	289.1678	331.142
Database mass error (ppm)	-	-	-	1.89	0.95	1.25
Target formula	C_5H_{14} NO	$C_{17}H_{23}NO_3$	$C_{18}H_{21}NO_5$	$C_5H_{14}NO$	$C_{17}H_{23}NO_3$	$C_{18}H_{21}NO_5$
Target name	Choline	Atropine	Ambelline	-	-	-
Manufacturer's formula	$C_5H_{14}NO$	$C_{17}H_{23}NO_3$	$C_{18}H_{21}NO_5$	-	-	-
Final compound's algorithm	-	-	-	Confirm by standard Obs	Confirm by standard Obs	Confirm by standard Obs

Obs: Observation

D. metel confirmed the presence of choline in crude alkaloid extract by noting a clear peak at m/z 104.1071, matching the protonated ion of choline (Fig. 2). The sharp peak and short retention time confirmed its presence in the sample.

The LC-MS analysis strongly indicated the presence of atropine in crude alkaloid extract (Fig. 3). A clear peak at m/z 290.1748 matched the protonated atropine ion, confirming its detection, supported by retention time and fragment peaks. The LC-MS analysis confirmed ambelline in crude alkaloid extract, shown by a dominant peak at m/z 314.1383 matching the protonated ion of ambelline (Fig. 4). The retention time and minor fragment ions confirmed its presence. Studies were conducted to know alkaloids based on standard values (Table 2).

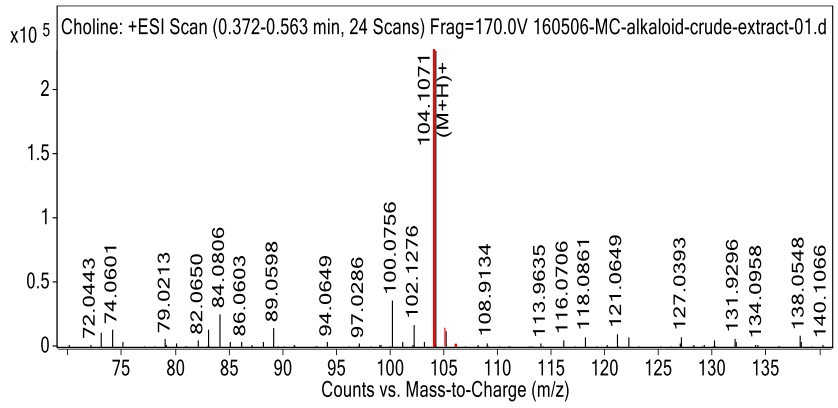


Fig. 2: Detection of alkaloid (choline) through LC-MS

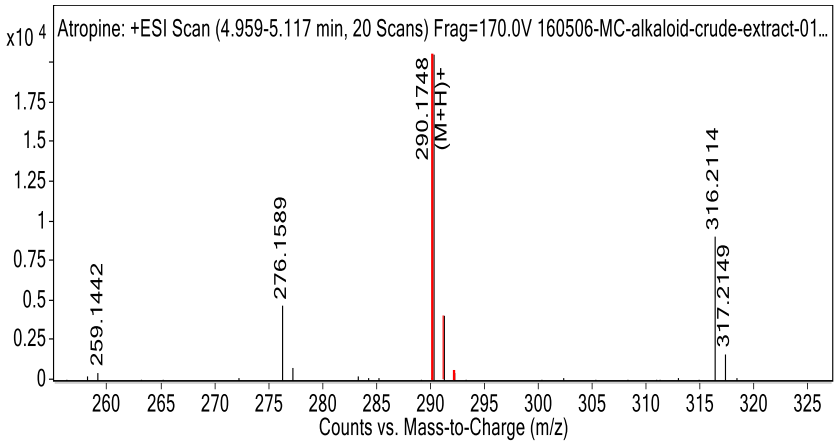


Fig. 3: Detection of alkaloid (Atropine) through LC-MS

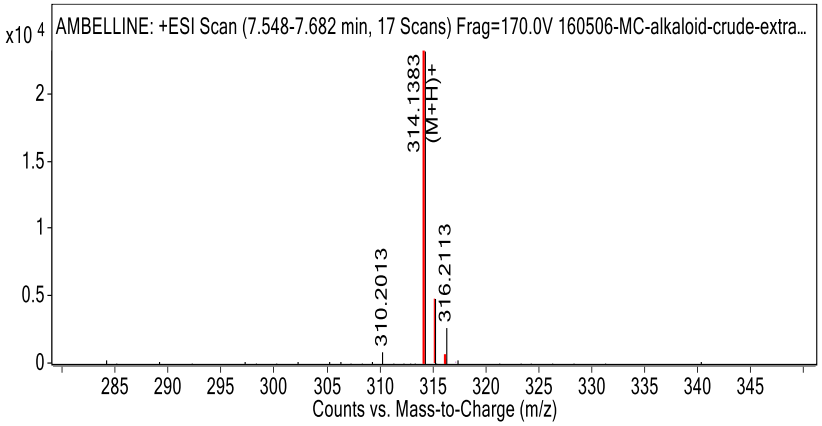


Fig. 4: Detection of alkaloid (Ambelline) through LC-MS

Table 3: Antimicrobial activity of *D. metel* leaf extract

Test samples	Zone of inhibition (mm)	
	S. aureus	*E. coli*
Methanolic extract	9.34 ± 0.34	5.87 ± 0.47
Ethyl acetate leaf extract	10.35 ± 0.82	2.87 ± 0.97
Aqueous leaf extract	6.63 ± 0.84	1.58 ± 0.55

(p-value < 0.05), two-way ANOVA to test for significant differences; F-value: 9.986, p-value: 0.034.

It was observed that *D. metel* plant contains three alkaloids namely choline, atropine, ambelline based on their mass per charge ratio of three compounds i.e. 104.1077, 289.1678, 331.1416, respectively. The observed mass of atropine alkaloid was 289.1678 as well as DB mass was 289.1675 (Table 2) indicate presence of atropine alkaloid, similarly choline (104.1077) observed mass 104.1077 and DB mass (104.1075) it confirms presence of choline alkaloid. Ambelline alkaloid observed mass was 331.1416 (Table 2) DB mass 331.142 whereas the crude ethanol extract show DB mass value 331.142 which confirms presence of ambelline alkaloid.

The F-value of approximately 9.99 suggests more variation between the groups than within the groups. The p-value observed was 0.034, which is less than the common significance level of 0.05, observed between the groups so are statistically significant. Ethyl acetate leaf extract appeared to be most effective against both the test bacteria, mainly against *Staphylococcus aureus*. Methanolic extract showed the second-highest effectiveness, again with a stronger effect against *S. aureus*. Distilled water leaf extract seemed least effective among the three, especially against *E. coli*. All the extracts showed larger zones of inhibition for *S. aureus* as compared to *E. coli*.

D. metel leaf extract was evaluated against two bacterial species *viz.*, *S. aureus* and *E. coli* for its antimicrobial activities. In present study maximum zone of inhibition was observed in ethyl acetate leaf extract, while methanolic and aqueous leaf extracts showed 9 and 8 mm zone of inhibition against *S. aureus*. Methanolic, ethyl acetate and aqueous leaf extracts were less effective against *E. coli* as it showed zone of inhibition values of 6, 4 and 2 mm, respectively (Table 3). The findings are in conformity with Akharaiyi (2011) and Chonde *et al*. (2023), who studied *D. stramonium* species only. Krishnan *et al.* (2017) have reported that *D. metel* leaf extract exhibited higher zone of inhibition in ethanol extract (i.e. 26 mm), methanol extract (21 mm) and acetone extract (20 mm).

A **B** **C** **D** **E** **F**

Fig. 5: ***Datura metel*** **L. leaf explant Callus initiated on MS medium supplemented with various growth hormones; A) Callus formed from leaf and stem explants on MS + 2, 4-D (0.5 mg L^{-1}) medium; B) Callus formed from leaf and stem explants on MS + BAP (4.0 mg L^{-1}) medium; C) Callus formed from leaf and stem explants on MS + kin (3 mg L^{-1}) medium; D) Callus formed from leaf explants on MS + 2,4-D + kin (1 mg L^{-1} each) medium, E) Callus formed from leaf and stem explants on MS + 2,4-D + kin + IAA (1.5:2:1 mg L^{-1}) medium; and F) Callus initiated from leaf and stem explants on MS + NAA + BAP (1 mg L L^{-1}) medium**

Callus induction in Datura metel leaf and stem explants

The present study demonstrated efficient callus induction and regeneration of *D. metel* plant from the leaf and stem explants. The MS basal medium supplemented with 2, 4-D + kinetin + IAA (1.5:2:1 mg L^{-1}) combination resulted in early callus initiation on 15th day after inoculation. The combination of MS + BAP (4.0 mg L^{-1}) showed callus initiation on 28th day (Fig. 5). Kale (2016) reported highest callus formation in MS medium supplemented with 2,4-D and kinetin (2 mg L^{-1} each). Similar results were also achieved by Twaij *et al.* (2019) in *D. innoxia* using stem as explant. The current study confirmed that *D. metel* contains atropine, choline and ambelline alkaloid. The successful initiation of callus confirmed that this alkaloid could be produce using tissue culture technique.

Media used	Explants	Days taken for callus initiation
MS + BAP (4.0 mg L^{-1})	Leaf and stem	27.69
MS + 2,4,-D (0.5 mg L^{-1})	Stem	23.99
MS + kinetin (3.0 mg L^{-1})	Leaf and stem	26.15
MS + 2,4-D + kinetin (1 mg L^{-1} each)	Leaf and stem	17.36
MS + NAA + BAP (1 mg L^{-1} each)	Leaf and stem	16.88
MS + 2,4-D+ kinetin + IAA (in 1.5: 2.0: 1.0 mg L^{-1} ratio)	Leaf and stem	15.18

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