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RESEARCH ARTICLE

In-vitro Micropropagation and Pharmacognostical Studies in *Datura inoxia* Mill.

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ABSTRACT

Datura inoxia Mill. (Solanaceae) is non-traditional medicinal plant. *Datura inoxia* has many medicinal properties like anodyne, antispasmodic, hallucinogenic, hypnotic and narcotic etc. *Datura inoxia* used in the treatment of insanity, fevers with catarrh, diarrhea, scabies, piles, ulcers, colds, asthma, Cardiac disorders, Impotency, Antispasmodic, Malaria, Baldness and skin diseases. The plant contains several alkaloids, the most active of which is scopolamine. It is also useful in respiratory ailments, rheumatism, elephantiasis, insanity, earache and eye diseases. During the present investigations efforts have been made to establish protocol for in vitro propagation and Pharmacognostic screening of this important medicinal plant.

Keywords: *Datura inoxia Mill*, *In vitro* studies, Phytochemistry.

INTRODUCTION

Ayurveda is an oldest traditional system of medicine which originated and is practised in India from 5000 years ago. (Naik, 1998). The term "non-traditional" medicine may be better known as complementary and alternative medicine which includes "traditional" Chinese medicine, naturopathic medicine, mind-body medicine, osteopathy, Ayurvedic medicine, etc. Despite research supporting many "non-traditional" practices, many people familiar only with Western medicine believe that most, if not all, alternative methods are useless and ineffective in a technologically and scientifically advanced society (Rebecca Nekolaichuk, 2013).

Medicinal plants have gained more importance as possible source of alternative and effective drugs. Around 12,000 plants secondary Metabolites of antimicrobial importance have been isolated. These compounds fall in one of the major groups of compounds like Phenols, Quinones, Flavonoids, Tannins, Terpenoids, Alkaloids and other mixtures. Neeraj *et. al.* (2012) worked on rediscovery the medicinal properties of *Datura inoxia* Mill. All parts used in the medication for leprosy and rabies. Humaid (2003) worked on effects of compound fertilization on growth and alkaloids *Datura inoxia* Mill. has he found leaves and fruit contains high alkaloids. Das *et. al.* (2014) recently worked on *Datura inoxia* Mill. leaf extract mediated one-step green synthesis and characterization of magnetite (Fe304) nanoparticle.

Result shows the phytochemicals present in the *Datura inoxia* leaf extracts shows flavonoids, phenolic compounds, cardiac glycosides and Sugars and the formation of the Fe304 nanoparticles was first monitored using UV-Vis absorption spectroscopy typical surface plasma absorption maxima at 270-290 nm. Zayed *et.al* (2006) worked on *in vitro* organogenesis and alkaloids accumulation in *Datura inoxia* Mill. Taking into consideration the following aspects it was decided to carry out In vitro, and Phytochemical screening of the important medicinal plant *Datura inoxia* Mill

MATERIAL AND METHODS

Data Collection and Identification

Datura inoxia Mill. Plant was collected from University campus, Dr. Babasaheb Ambedkar Marathwada University, Aurangabad. Traditional knowledge about this plant species was collected through informal discussions, interviews and through communication. Identification and authentication of the collected specimens was made with the help of standard floras (Hooker, 1872-1897; Naik, 1998). Herbarium specimens are deposited in the index herbarium "BAMU" in Department of Botany, Babasaheb Ambedkar Marathwada University, Aurangabad. Library and Herbarium of Botanical Survey of India, Pune was consulted for review of literature and also for identification of the specimen.

In-vitro Studies

Seeds of *Datura inoxia* Mill. were collected from the best quality and large size mature fruits. For seed germination, seeds were first washed under running tap water for 3 - 5 min. Floating seeds were considered to be empty and discarded. Later the seeds were dipped in 70% alcohol for 30 sec, followed by washing with distilled water. The seeds were surface sterilized with0.2% (w/v) mercuric chloride for 10 min. with continuous shaking. Finally it was washed four times with sterilized distilled water.

Culture media and Growth Conditions

Full strength M.S medium (Murashige and Skoog, 1962) supplement with 3 %sucrose (Hi-media Mumbai, India) ,0.2% Clerigel (Hi-media, Mumbai India) and different combination of Auxin (2,4- D,NAA,IBA) and Cytokinin (BAP, KIN) at the concentration 0.5,1.0.....3.0 mg/l was used as the callus induction medium. The pH of the medium was adjusted to 5.8 before the addition of Clerigel .Culture bottles were filled with 50 ml of the media. The media was autoclaved at 15 lbs. (121°C) for 15 min.

The surface sterilized seeds were transferred on minimal medium for regeneration of explants. After growth in vitro grown plantlets were used as an explant. The cultures were incubated at 25+2^{0}C with 16 h photoperiod with the light intensity of 3000 lux under cool white fluorescent tubes. All the experiment were conducted in 5 replicates and repeated for 3 times. The Number of days, frequency of multiple shoots formation after 4 weeks of culture.

Phytochemical Screening

All samples were analysed for mineral content at Centre for Analytical Research and Studies (CARS), Maharashtra Institute of Technology (MIT), Aurangabad (MS) India. The mineral from the samples were analysed according to Tandon HLS, (2017) by using various techniques.

RESULTS AND DISCUSSION

Regeneration of Plants *in vitro*

After inoculation of explants like leaf and stem callus was recorded after two weeks, which was compact, yellowish green with nodular structure and slow growing .The callus was transferred to the medium similar to the shoot initiation medium. Meanwhile the plantlet regeneration was observed from embryonic callus which was indicated by the development of morphologically normal plantlets which shoots and roots.

Effects of different Auxins & Cytokinins on the cotyledon explants for callus induction:

Effects of different Auxins & Cytokinins were studied by using cotyledon as explants. The basic culture medium utilized in present piece of work was Murashige and Skoog medium (MS) supplemented with different concentration of Auxins and Cytokinins.

Result revealed that when 2,4-D and NAA tried alone and in combination of BAP and KIN shows induction of callus. Maximum proliferation was achieved on (2 mg/ L) 2, 4 D when it was tried alone and in combination 2 mg/L of BAP and from 0.2 mg/L to 0.4 mg/L of NAA increased the proliferation in cotyledons tissue.

Table 1: Effect of different concentration of PGR's on callus formation and direct somatic embryogenesis from explant of *Datura inoxia* Mill.

Concentrations of Plant Growth Regulator (g/l)				Frequency of Callus formation	Frequency of Direct Somatic embryonic callus	Colour of callus/ somatic embryo
NAA	2,4-D	BAP	KIN			
0.1	-	2.0	-	+	+	Yellowish green
0.2	-	2.0	-	+++	+++	Yellowish light green
0.3	-	2.0	-	++++	++++	Yellowish green
0.4	-	2.0	-	+++++	+++++	Whitish
-	1.0	-	-	+	-	Creamish
-	1.5	-	-	+++	-	Creamish
-	2.0	-	-	++++	-	Creamish
-	2.5	-	-	+	-	Creamish
1.0	0.5	-	0.5	+	+	Yellowish
1.0	0.5	-	1.0	+++	+++	Yellowish
1.0	0.5	-	1.5	+++	++++	Yellowish
1.0	0.5	-	2.0	+++++	+++	Creamish

(+) Very weak ; (+++) Moderate; (++++) Profuse; (+++++) Very profuse

Highest rate of callus indication was found on MS medium supplemented with NAA 1mg/L + 0.5mg/L of 2, 4 D + 2.0 mg/L of KIN and 0.4 mg/L of NAA with 2.0 mg/L of BAP.

Callus formed in present study were showing variability in terms of colour and texture, Yellowish, Green, Light Green, White, cream coloured callus was frequently observed with different type of texture viz. smooth, rough, and crystalline.

Callus develop in present piece of work were show direct somatic embryogenesis with different type of shapes.

Table 2 : Effect of different concentration of PGR's on Shoot induction

Concentrations of Plant Growth Regulator (mg/l)				No of shoot induction	Mean ± SE
NAA	2,4-D	BAP	KIN	-	
0.1	-	2.0	-	10	8 ± 0.577
0.2	-	2.0	-	22	18 ± 1.632
0.3	-	2.0	-	36	28 ± 1.527
0.4	-	2.0	-	52	45 ± 2.848
-	1.0	-	-	-	-
-	1.5	-	-	-	-
-	2.0	-	-	-	-
-	2.5	-	-	-	-
1.0	0.5	-	0.5	07	5 ± 0.577
1.0	0.5	-	1.0	12	9 ± 0.881
1.0	0.5	-	1.5	32	28 ± 1.201
1.0	0.5	-	2.0	25	19 ±1.452

Phytochemical Analysis

Qualitative Analysis of secondary metabolites and phytochemicals in *Datura inoxia* Mill. was carried out and have shown the positive test for the presence of starch, protein, fat, saponins, glycosides and alkaloids in all the plant part i. e. root, stem and leaf. Total amount of ash in the root was 18.5%, water soluble was found to be 0.4% water insoluble ash was18.1%, acid soluble ash was 15.2% acid insoluble ash was found to be 3.3%. Total amount of ash in the stem was 16 %, water soluble was found to be 0.7% water insoluble ash was15.3%, acid soluble ash was 13.8% acid

Table.3: Phytochemical analysis of *Datura inoxia* Mill.

Sr. No.	Part of the plant	ash %	Water SA	Acid SA	Non-Reducing Sugar	Total Sugar	Alkaloids	Crude protein	% Amino Acids
1	Root	18.5	0.4	15.5	0.20%	0.72%	0.01%	11.30%	0.17%
2	Stem	16	0.7	12.3	0.35%	0.57%	01%	11.38%	0.75%
3	Leaf	13	2.5	10.6	0.25%	4.87%	9.5%	26.01%	1.77%

insoluble ash was found to be 2.2%. Total amount of ash in the leaf was 13 %, water soluble was found to be 2.5% water insoluble ash was10.5%, acid soluble ash was 12% acid insoluble ash was found to be 01%. The highest amount of water-soluble ash is noted in leaf ie. 2.5% as compare to root and stem. Results are tabulated in Table.3.

CONCLUSIONS

From the in vitro studies it could be stated that, these studies are fruitful for regeneration of callus as well as multiple shoot formation. Even though plant is abundant in nature but it is restricted for certain season. There were significant differences in regeneration frequencies of callus and multiplication of shoots. As stated by Martin (2002) the high morphogenic efficiency of node segments derived callus may be due to the presence of some internal components from the pre-existing axillary buds that are essential for induction of caulogenesis. Shoot buds were also developed from callus culture elongated. Similar kind of results was reported in *Tylophora indica* by (Kakde and Pandhure 2016). Somatic embryogenesis has been reported from mature leaves of *Tylophora indica* (Das and Rout, 2005, **Choudhary** et.al. 2005). Another similar protocol has been developed for high-frequency shoot regeneration and plant establishment of *Tylophora* from petiole-derived callus (Faisal and Anis, 2005).

Phytochemical studies revelled that plant is rich in Potassium (0.290%), Calcium (0.80%), Phosphorus (1.90%) thus, highest amount of bioactive compounds present in the leaf is confirmed during this study. This also confirms that, the local people using this plant as maximum benefit from plant as medicinal herb.

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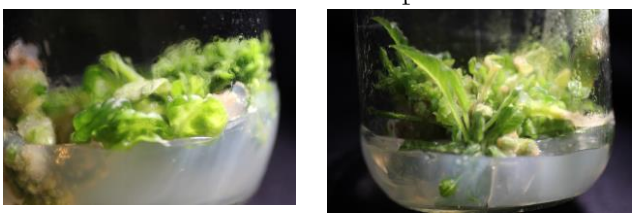
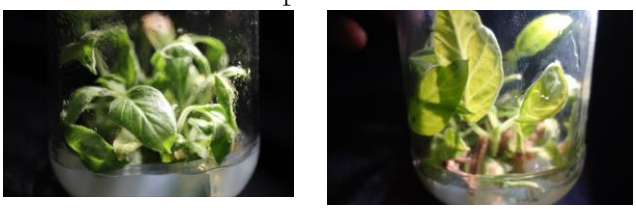
PHOTO PLATE I

a. Callus Development



b. Multiple Shoot Formation



c. Full Grown Plants

Results : MS Medium with NAA 1mg/L + 0.5mg/L of 2, 4 D+ 2.0 mg/L of KIN and 0.4 mg/L of NAA with 2.0 mg/L of BAP.