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Comparative Antimicrobial Activity and Phytochemical Analysis of *Datura stramonium* L. Plant Extracts and Callus *In vitro*

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Authors' contributions

This work was carried out in collaboration between both authors. Author PS designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Author RAS managed the analyses of the study. Both authors read and approved the final manuscript.

Research Article

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ABSTRACT

The phytochemical analysis and antimicrobial activities of *in vitro* grown callus *and in D. stramonium* plant were studied and compared. The crude extracts from *D. stramonium* were analysed for moisture, starch, carbohydrate, ascorbic acid, lipid, proline, crude protein, phenols, DNA, RNA, chlorophyll and carotenoid in plant parts and callus. The phytochemical content of naturally grown plant was comparatively higher than *in vitro* grown callus. The antimicrobial potential of the methanolic extracts of root, stem, leaves, fruits, callus and crude metabolite rich fractions were evaluated against *Escherichia coli* MTCC 1652, *Staphylococus aureus* MTCC 3160, *Pseudomonas aeruginosa* MTCC 847, *Aspergillus flavus* MTCC 2456, *Aspergillus niger* MTCC 282*, Fusarium culmorum* MTCC 349 and *Rhizopus stolonifer* MTCC 2591. The results indicated that methanolic leaf extract exhibited antimicrobial activity against *S. aureus* (IZ=18.2mm) and *E. coli* (IZ=19.8mm), *P. aeruginosa* (IZ=22.2mm), *R. stolonifer* (IZ=21.5mm), and callus exhibited antimicrobial activity against *A. niger* (IZ=12.1mm), *F. culmorum* (IZ=18.9mm) and *A. flavus* (IZ=12.8mm). The present study also revealed that antimicrobial activity was higher in naturally grown plant. In the metabolite rich fraction (flavonoids, phytosterols and alkaloids), greatest bactericidal and fungicidal activity was exhibited by flavonoids against *P. aeruginosa* (IZ=22.4mm) and *A. flavus* (IZ= 20.1mm).

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1. INTRODUCTION

The use of plants by man to treat common ailments is time immemorial and many of the traditional medicines are still included as part of the habitual treatment of various maladies [11]. It is reported that 50% of all modern clinical drugs are of natural product origin and natural products play an important role in drug development programs in the pharmaceutical industry. A revolution came in the medicinal world with the discovery of antibiotics for treatment of various infections. But their indiscriminate use has led to an alarming increase antibiotic resistivity. The intractable problem of antibiotic resistance has led to the resurgence of interest in herbal products as sources of novel compounds to suppress or eradicate the ever increasing problems of reemergence of newer diseases. The need of the hour is to screen a number of new medicinal plants, which are regarded as potentially safe drugs. The selection of crude plant extracts for screening programs is potentially more successful than the pure compounds [18].

Datura stramonium is an Indian medicinal plant and belongs to the family Solanaceae which is widely used in phytomedicine to cure diseases. *Datura* was known to the ancient Hindu Physicians who regarded it as antispasmotic, intoxicant, germicidal, anodyne antipyretic, antiseptic, antiphlogistic, antiproliferative narcotic, sedative, tonic, antidiarrhoeal, antihelmintic, alexiteric and useful in leucoderma, skin disorders, ulcers, bronchitis, jaundice and piles [1].

Antibacterial and antifungal activity have been investigated by various researchers [5,16]. In the present study we compared the phytochemical contents and antimicrobial activities of *in vitro* grown callus and naturally grown plant.

2. MATERIALS AND METHODS

2.1 Plant Material

The plant material was collected from Sodala, Jaipur and voucher specimen no.RUBL15656 was deposited in the Herbarium, Department of Botany, University of Rajasthan, Jaipur used for present investigation.

2.2 Callogenesis of *Datura stramonium*

For callogenesis nodal segments were cultured on Murashige and Skoog's [22] medium contained 0.8% agar including 3% sugar supplemented with 1 mg/l 2-4 Dichlorophenoxyacetic acid (D) and 1.5 mg/l Benzyl amino purine (BAP). Cell cultures were first dried at 100ºC for 15 min to inactivate the enzymatic activity and then at 40ºC until the weight became constant.

2.3 Phytochemical Analysis

Estimation of total soluble carbohydrate was performed by the method of Loomis and Skull [19]; Starch by McCready et al., [21], Proteins by Lowry et al.[20]; Total carbohydrate, fat content and crude proteins by AOAC [4]; Ascorbic acid by Roe and Kuenthar [24]; lipids by

Jayaraman [15]; proline by Bates et al. [6]; phenols by Bray and Thorpe [7]; Nucleic acid by Ogur and Rosen [23]; Pigments by Holden [12] and Moisture by ICMR [13].

2.4 Antimicrobial Analysis

2.4.1 Bacterial and fungal strains

Bacterial strains *Escherichia coli* MTCC, 1652, *Staphylococus aureus* MTCC 3160, *Pseudomonas aeruginosa* MTCC 847 and fungal strains *Aspergillus flavus* MTCC 2456, *Aspergillus niger* MTCC 282*, Fusarium culmorum* MTCC 349 and *Rhizopus stolonifer* MTCC 2591 were procured from the microbial type culture collection MTCC (Institute of Microbial Technology, Chandigarh, India) used for the present study.

2.4.2 Preparation of extracts

20 g of samples (fruit, root, stem, leaf and callus) were homogenized separately with pure methanol and left overnight at the room temperature. Later, each of the homogenates was filtered; the extracts were pooled individually and dried in vacuo. Similarly, various metabolites rich fraction were extracted [flavonoids [27], phytosterols and alkaloids [26] separately and crude extracts were used for further analysis.

2.4.3 Antimicrobial activity

The antibacterial and antifungal activity was evaluated by *in vitro* disc diffusion method [10]. The filter paper disc whatman No. 1 –(6mm in diameter) impregnated with methanol was used as negative control, methanolic extracts (0.4 g/ml) while as reference antibiotics (gentamycin- 10 mg/ml for bacteria and myostatin- 100 units/ml) used as positive control drugs. Such treated discs were air-dried at room temperature, to remove any residual solvent which might interfere with the determination, sterilized in UV light for 24 hrs and inoculated. These plates were initially placed at low temperature for 1 hrs, so as to allow the maximum diffusion of the compounds from the test discs into the plate and later, incubated at 37**º**C for 24 hrs in case of bacteria and 48 hrs at 27**º**C for fungi, after which the zones of inhibition could be easily observed. The media was inoculated with 100ul of the inoculums ($1x10^8$ cfu/ml). Three replicates of each test extract were performed for each microorganism.

3. RESULTS AND DISCUSSION

Various primary metabolites have been summarized. *In vivo* and *in vitro* condition starch concentration was rich in stem (16.80 mg/gdw) as compared to other parts. Maximum concentration of soluble carbohydrates and total carbohydrate was found in fruit (19.4 mg/gdw) and root (69.30 mg/gdw) respectively. Generally fruit are rich sources of soluble carbohydrates (glucose, fructose and sucrose). Ascorbic acid levels were higher in leaves (0.88 mg/gdw) than other plant parts and cell cultures.

Higher levels of lipids were present in plant fruit (31.78 mg/gdw) than callus cultures (12.13mg/gdw). Maximum protein measured as proline and crude protein was found in fruit (proline: 10.90 mg/gdw and crude protein: 24.90 mg/g) than callus culture (proline: 6.12 mg/gdw and crude protein: 20.41 mg/gdw). The total level of phenol was higher in root (10.10 mg/gdw) than callus culture (8.25 mg/gdw). The higher levels of DNA and RNA was

observed in plant leaves (DNA -14.25 mg/gdw: RNA- 7.83mg/gdw) than callus cultures (DNA -3.33 mg/gdw: RNA- 1.01mg/gdw).

In the present study, total levels chlorophylls were found to be maximum in leaf i.e. 0.92 mg/gdw and were not present in root and stem. Similarily carotenoids were measured to be higher in leaf (0.87 mg/gdw) with the minimum levels of carotenoids in root (0.25 mg/gdw) and its level was 0.55 mg/gdw in callus (Table 1). The work on carbohydrates has been reviewed in *D. stramonium* [1]. In *Datura* species considerable amount of fatty acids and oils has been reported by various researchers [1,9].

Table 1. Isolated Primary metabolites contents (mg/gdw) from different plant parts and callus

Primary metabolites	Leaf	Fruit	Stem	Root	callus
Moisture	75.6±0.50	82.10±0.50	74.20±0.40	74.30±0.50	81.97±0.70
Starch	14.8±0.30	13.1±0.32	16.80±0.22	11.50±0.19	9.33±0.27
Soluble carbohydrate	15.4±0.30	19.40±0.50	13.30±0.21	12.40±0.20	14.28±0.28
Total carbohydrate	67.1±0.31	37.18±0.60	60.87±0.37	69.30±0.33	67.27±0.35
Ascorbic acid	0.88±0.18	0.62±0.14	0.76±0.15	0.12±0.01	0.35±0.03
Lipid	21.50±1.50	31.78±0.60	7.41±0.36	8.47±0.39	12.13±0.17
Proline	10.60±0.28	10.90±0.29	14.30±0.1	8.70±0.40	6.12±0.22
Crude protein	24.20±0.20	24.90±0.40	22.30±0.26	17.90±0.25	20.41±0.25
Phenols	9.78±0.39	9.80±0.37	3.54±0.25	10.10±0.28	8.25±0.28
RNA	7.83±0.21	2.45±0.16	3.60±0.27	0.63±0.14	1.01±0.28
DNA	14.25±0.29	6.14±0.19	9.42±0.09	1.09±0.19	3.33±0.21
Chl..-a+b	0.92±0.20	0.28±0.08	0.00	0.00	0.37±0.13
Carotenoid	0.87±0.05	0.34±0.03	0.48±0.07	0.25±0.03	0.55±0.18

mg/gdw : miligram / per gram dry weight
Results are mean value SD from atleast 3 experiments

The antibacterial and antifungal activities of the methanolic extracts both *in vitro* callus and *in vivo Datura stramonium* have been demonstrated in the present study. In plant parts (fruit, stem, root, leaf) and callus, leaf was found to be effective against *S. aureus* (IZ = 18.2 mm) and *E. coli* (IZ = 19.8 mm), *P. aeruginosa* (IZ = 22.2 mm), *R. stolonifer* (IZ = 21.5 mm), callus was found to be effective against *A. niger* (IZ = 12.1 mm), *F. culmorum* (IZ = 18.9 mm) and *A. flavus* (IZ = 12.8 mm).

The flavonoids present in *D. stramonium* (leaf) exhibited maximum antibacterial activity against *P. aeruginosa* (IZ = 22.40 mm), phytosterols against *P. aeruginosa* (IZ = 22.20 mm) and alkaloids against *P. aeruginosa* (IZ = 20.70mm). Maximum antifungal activity among bioactive compounds was recorded against *A. flavus* (IZ = 20.10mm) by flavonoids but minimum antifungal activity was recorded against *F. culmorum* (IZ = 8.50 mm) by alkaloids (Table 2). *Datura* species have been screened for their antifungal activity and antibacterial activity against various microorganisms by number of researchers Kaushik and Goyal [17], Shafique and Shafique [25], Jain et al. [14], Johnson [16], Britto and Gracelin [8].

Table 2. Antimicrobial efficacy of methanolic extracts of *D. stramonium* plant parts, callus and crude extracts of metabolite rich fractions (leaf) on bacteria and fungi

Microorganisms	Standard		Fruit	Stem	Root	Leaf	Callus	Flavonoids	Phytosterols	Alkaloids
S. aureus	**21**	IZ	15.8±0.63	17.6±0.43	13.4±0.56	18.2±0.40	14.78+0.11	14.5 ±0.39	19.5±0.52	18.2 ±0.40
MTCC 3160		AI	0.744	0.829	0.631	0.875	0.696	0.68	0.937	0.875
E. coli	**23**	IZ	17.8±0.71	13.4±0.89	15.1±0.19	19.8±0.63	17.1 ±0.07	16.3 ±0.89	13.2±0.31	14.2 ±0.42
MTCC 1652		AI	0.774	0.582	0.656	0.861	0.743	0.721	0.68	0.731
P. aeruginosa	**23**	IZ	11.9±0.43	13.3±0.56	12.8±0.62	22.2±0.59	21.3 ±0.32	22.4 ±0.86	22.2±0.59	20.7 ±0.52
MTCC 647		AI	0.517	0.578	0.556	0.965	0.968	1.032	0.932	0.869
R. stolonifer	**21**	IZ	11.4±0.05	17.3±0.35	18.2±0.54	21.5±0.41	19.01±0.21	10.8±0.30	10.4 ± 0.56	9.8 ±0.42
MTCC 2591		AI	0.542	0.823	0.87	1.02	0.901	0.843	0.684	0.644
A. niger	**13**	IZ	8.4±0.65	9.1±0.23	7.3±0.63	11.2±0.41	12.1±0.02	10.3±0.45	14.5±0.25	15.2±0.91
MTCC 282		AI	0.656	0.71	0.57	0.875	0.945	0.83	1.169	0.808
F. culmorum	**18**	IZ	9.5±0.80	10.3±0.45	14.5±0.25	17.3±0.35	18.9±0.07	15.0±0.43	10.4±0.21	8.5 ±0.35
MTCC 349		AI	0.532	0.577	0.812	0.831	1.059	0.842	0.584	0.664
A. flavus	14	IZ	10.4±0.56	8.5 ±0.35	10.8±0.30	12.1±0.72	12.8±0.10	20.1±0.62	10.3±0.63	12.6±0.34
MTCC 2456		AI	0.764	0.625	0.794	0.889	0.941	0.975	0.5	0.684

IZ = Inhibition zone (in mm) including the diameter of disc (6 mm)

$$AI = \text{Activity index} = \frac{Inhibition\ area\ of\ the\ test\ sample}{Inhibition\ area\ of\ the\ Standard}$$

Abbreviation: mycostatin = 100 unit per disc; Gentamycin = 10 mg/disc
Results are mean value SD from atleast three experiment

4. CONCLUSION

The phytochemical contents and antimicrobial activity of plant grown naturally was comparatively higher than in vitro grown callus. It is quite possible that due to the optimum in vitro environment conditions callus accumulated lesser amount of active components in comparison to in vivo plant parts and hence shows reduced activity. Different plant parts and metabolite rich fractions showed varying degrees of antimicrobial activities against tested microbes. Further studies on bioactive will unravel its potential as drugs.

CONSENT

Not applicable.

ETHICAL APPROVAL

Not applicable.

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COMPETING INTEREST

Authors have declared that no competing interests exist.

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