*Egypt. J. Chem.* Vol. 67, SI: M. R. Mahran, pp. **1863 - 1871** (2024)

**Egyptian Journal of Chemistry**

**http://ejchem.journals.ekb.eg/**

# Characterization of Antimicrobial Activity and Fingerprinting for Disorganized and Organized Cultures in *Datura innoxia*

**Mohamed A. M. El-Awady[a], M. S. Abdel-Hady[b], Alia M. Hegazy[c], Dina H. Samy[c], Habibatullah H. Rashad[c], Habiba Shahat[c], Mayar W. Mohamed[c], Minas K. Samha[c], Esmaeel M. Salah[d*]**

[a] *Genetics Department, College of Agriculture, Cairo University, Giza, Egypt.*
[b] *Biotechnology Group, Botany Dept., National Research Centre, Dokki, Giza, Egypt.*
[c] *Biotechnology Program, Faculty of Agriculture, Cairo University, Giza, Egypt.*
[d] *Biotechnology Department, Faculty of Agriculture, Al-Azhar University, Cairo, Egypt.*

**Abstract**

*Datura innoxia* is renowned for its pharmaceutical qualities and traditional medical use. It's crucial for long-term pharmaceutical drug synthesis by regulated micropropagation and tissue culture techniques. This study focused on establishing *Datura innoxia* under aseptic laboratory settings to promote callus formation and comparing its phytochemical profile with leaves using High-Performance Liquid Chromatography (HPLC). The goal was to identify potential changes in phytochemical content between various growing strategies. Antibacterial efficacy of extracts from conventional plants and lab-grown callus were evaluated against *Escherichia coli* and *Staphylococcus aureus* strains. Moreover, aiming to ascertain the genetic stability of *Datura innoxia* cells in different conditions, the fingerprinting profile was performed by Start Codon Targeted (SCoT) and Inter-Simple Sequence Repeat (ISSR) markers. By thoroughly examining these features, the study provides insights into the biochemical, pharmacological, and genetic properties of *Datura innoxia*, which are critical for its use in pharmaceutical research and development. These findings highlight the critical role of regulated cultivation practices in improving the efficacy and sustainability of medicinal plant-based therapies.

Keywords : *Datura innoxia*, Tissue Culture, HPLC, Antimicrobial Activity, SCoT, ISSR, Fingerprinting.

## 1. Introduction

*Datura innoxia*, commonly known as thorn apple or downy thorn-apple, is a member of the Solanaceae family. Datura has diverse pharmacological properties and rich phytochemical profile. Datura explants are considered as traditional medicinal plant for their anti-inflammatory and antimicrobial properties besides being a very effective painkiller. However, scientific investigations into the specific chemical constituents and their bioactivities remain dispersed [1, 2]. The bioactive compounds in *Datura innoxia*, like tannins acted as antioxidants, terpenoids had pain-relieving and anti-cancer properties, saponins aided in lowering cholesterol and managing diabetes, and alkaloids had antibacterial effect [3].

High-Performance Liquid Chromatography (HPLC) is a preferred analytical technique used to identify and compare the chemical components. Due to its precision, sensitivity, and ability to separate complex mixtures into individual components. This analytical technique plays a crucial role in pharmaceutical field, where it is essential for assessing and guaranteeing the purity of products [4]. Chromatographic analysis can be used to establish a comprehensive chemical profile of *Datura innoxia*, enhancing our understanding of its pharmacological properties, and may also lead to the discovery of compounds with significant medicinal value, contributing to the broader field of pharmacology and medicine [5].

Bioactive components of medicinal plants were extracted by using different solvents. Type of explants, bioactive ingredients and availability determined the choice of solvent. Solvents were categorized as polar i.e. (Water, Methanol, Ethanol, Acetone) or nonpolar i.e. (Hexane, Ethyl acetate) [6]. A lot of solvents were used to make an extraction of *Datura innoxia* such as n-hexane (Nh), acetone (A), ethyl acetate + acetone (EthA), ethyl acetate (Eth), ethyl acetate + ethanol (EthE), methanol + ethyl acetate (MEth), methanol (M), ethanol (E) and distilled water (D) [7].

Datura has many biological functions as herbicidal action [8], insecticidal action [2], antifungal activity: *A. flavus*, *A. niger*, *Alternaria solani*, *Fusarium solani*, *and Helianthus sporium* were susceptible to the antifungal effect of extracts of leaves, seeds, stems, and roots of *D. innoxia* [9], and antibacterial activity: The effectiveness of

*Corresponding author e-mail: esmaeel.bio@azhar.edu.eg (Esmaeel M. Salah)
Receive Date: 01 September 2024, Revise Date: 07 November 2024, Accept Date: 11 November 2024
DOI: 10.21608/ejchem.2024.316633.10318

methanolic extracts (80%) of *Datura innoxia* as an antibiotic against *Bacillus subtilis*, *Staphylococcus aureus*, and *E. coli* [10]. Antimicrobial activity of bioactive components is essential for various applications, particularly in medicine, where it underpins the effectiveness of antibiotics, antifungals, and antiviral agents with the increasing resistance of pathogens to current compounds [11].

DNA fingerprinting refers to DNA typing, DNA profiling, genetic fingerprinting, or genotyping. It is a technique that employed to identify individuals based on distinct patterns found in their DNA. This method analyzes specific regions of the genome that exhibit variability among individuals, allowing the differentiation of one person's genetic material from that of another. By examining these unique genetic markers, researchers and forensic scientists can establish individual identities with a high degree of accuracy, making DNA fingerprinting a valuable tool in different fields [12, 13]. DNA fingerprinting was used to differentiate between different species of Datura (*D. stramonium* and *D. ferox*) by using URP Primers [14]. ISSR markers were used to differentiate between nine samples of *Datura stramonium* [15].

Accordingly, the main aim of this study is to investigate the differentiation between callus and leaves of *D. innoxia* through DNA fingerprinting, chemical and antimicrobial analysis. Through this comprehensive analysis, we hope to contribute to the development of sustainable and effective strategies for producing medicinal compounds and enhancing the utility of *Datura innoxia* in modern medicine to the same extent as it was in the old traditional medicinal techniques. This side of the research holds significant promise for several industries. Outcomes of this research will provide insights into the phytochemical composition, antimicrobial potential, and genetic stability of *Datura innoxia*. Our study aims to offer valuable information that could help in some advancements in industrial biotechnological techniques helping in various fields.

## 2. Materials and Methods

### 2.1. Plant Materials

*Datura innoxia* leaves were collected from Faculty of Agriculture, Cairo University, Giza, Egypt. Experiments and analysis were carried out at plant tissue culture lab, Agriculture and biological research institute, National research centre, Giza, Egypt.

### 2.2. Preparations & Sterilization of leaves

Leaves of *Datura innoxia* were placed in a piece of gauze and washed with running water (30 min), then disinfected using 20% Clorox with two drops of commercial detergent (10 min), followed by sterilization using 70% ethyl alcohol (30 seconds), and washing three times by sterile distilled water [16].

### 2.3. Preparation of Callus initiation medium

Murashige and Skoog medium (MS Medium) was used in the experiment [17]. It was supplemented with 30 g/l sucrose.

Benzyl adenine (BA), and α-naphtalene acetic acid (NAA) were used as growth regulators in combination at different concentrations (Table 1). Media were adjusted to pH 5.8 before mixing with 7 g/L agar. Distribute 50 ml of the medium into each jar. Autoclaved at 121°C at a pressure of 1.1 Kpa for 20 min, then left to cool. Plant cultures were maintained at 25±1°C in a constant temperature growth room, under completely dark conditions.

**Table 1. Different concentration of plant growth regulators**

| No. | Code | Plant growth regulators (PGR) mg/L |
|---|---|---|
| 1 | MS1 | Control (Free PGR) |
| 2 | MS2 | 1 NAA + 1 BA |
| 3 | MS3 | 1 NAA + 2 BA |
| 4 | MS4 | 2 NAA + 1 BA |
| 5 | MS5 | 2 NAA + 2 BA |

NAA, α-naphtalene acetic acid; BA, Benzyl adenine.

### 2.4. Callus fresh and weight (g/jar)

Fresh and dry weight of different calli cultures were detected at the end of fifth week of cultivation by using Moisture balance. Three replicates of each treatment were measured.

### 2.5. Extraction of Organized and disorganized Cultures

#### 2.5.1. Extraction of callus

A glass slide cover (instead of liquid nitrogen) was added to 2g of callus and ground. 100 ml ethanol (70%) was used and mixed with them. The whole mixture was filtered in a small flask.

#### 2.5.2. Extraction of leaves

Firstly, leaves were cut using scissors, then a glass slide cover (Instead of liquid nitrogen) was added to 2g of leaves and crushed with the glass. 100 ml ethanol (70%) was used and mixed with them. The whole mixture was filtered in a small flask.

### 2.6. HPLC profiling of *D. innoxia*

HPLC analysis was estimated by using an Agilent 1260 series for 2 Samples (Leaves and Callus). The separation was carried out using Zorbax Eclipse Plus C8 column (4.6 mm x 250 mm i.d., 5 µm). The mobile phase consisted of water (A) and 0.05% trifluoroacetic acid in acetonitrile (B) at a flow rate 0.9 ml/min. The mobile phase was programmed consecutively in a linear gradient as follows: 0 min (82% A), 0–1 min (82% A), 1-11 min (75% A), 11-18 min (60% A), 18-22 min (82% A), and 22-24 min (82% A). Multi-wavelength detector was monitored at 280 nm. The injection volume was 5 µl for each of the sample solutions. The column temperature was maintained at 40 °C.

### 2.7. Antimicrobial Activity

Microbial medium containing 5g/L Peptone, 5g/L Yeast, 20g Agar, and 10g Sucrose was prepared. The pH was 7.0 and the medium was autoclaved at 121 °C for 20 minutes, then poured 25 ml in each sterile petri. *Escherichia coli* and *Staphylococcus aureus* were cultured with disks contained leaves and callus extracts each separately and incubated for 24h at 37°C.

### *2.8.* Fingerprinting of *D. innoxia*

SCoT and ISSR were employed (Table 2) as molecular genetic fingerprinting techniques, where SCoT primer is used for the first time on Datura species. The aim of the work is to analyze genetic stability and diversity. Ensure the authenticity of callus as a disorganized culture compared to the leaves as an organized culture. 1 Kb DNA Ladder (Genedirex) was used. Fingerprinting analysis was performed at biotechnology laboratories, Cairo university research park, Faculty of Agriculture, Cairo university, Giza, Egypt.

**Table 2. Primers, molecular markers, and sequence used for fingerprinting**

| Molecular marker | Primer No. | Name | Sequence | Reference |
|---|---|---|---|---|
| ISSR | ISSR1 | 17898B | $(CA)_6$-GT | [18] |
| | ISSR2 | 17899A | $(CA)_6$-AG | [18] |
| | ISSR3 | HB 10 | $(GA)_6$-CC | [19] |
| SCoT | SCoT1 | SCoT-61 | CAACAATGGCTACCACCG | [20] |
| | SCoT2 | SCoT-14 | ACGACATGGCGACCACGC | [20] |

## 3. Results

### 3.1. Callus initiation and induction

Leaves explants were planted on MS medium with different concentrations of auxins and cytokinins (Table 3). Cultures were incubated at 25ºC ±1 in complete dark. Calli were initiated after 15 days of incubation. Callus grade and color were noticed for different concentrations of plant growth regulators. The best callus grade was obtained as a result of using the concentration (2 mg/L NAA to 2 mg/L BA) (Table 3) and it gives a light green color as shown in figure (1.B).

**Table (3): Concentration of PGR, callus grade and color**

| NO. | Concentration of PGR mg/L | Callus grade | Callus color |
|---|---|---|---|
| MS1 | Control without PGR | - | - |
| MS2 | 1 NAA / 1 BA | ++ | Light green |
| MS3 | 1 NAA / 2 BA | + | Dark green |
| MS4 | 2 NAA / 1 BA | + | Brownish yellow |
| MS5 | 2 NAA / 2 BA | +++ | Light green |

+ Small Callus, ++ Medial Callus, and +++ Bigger Callus

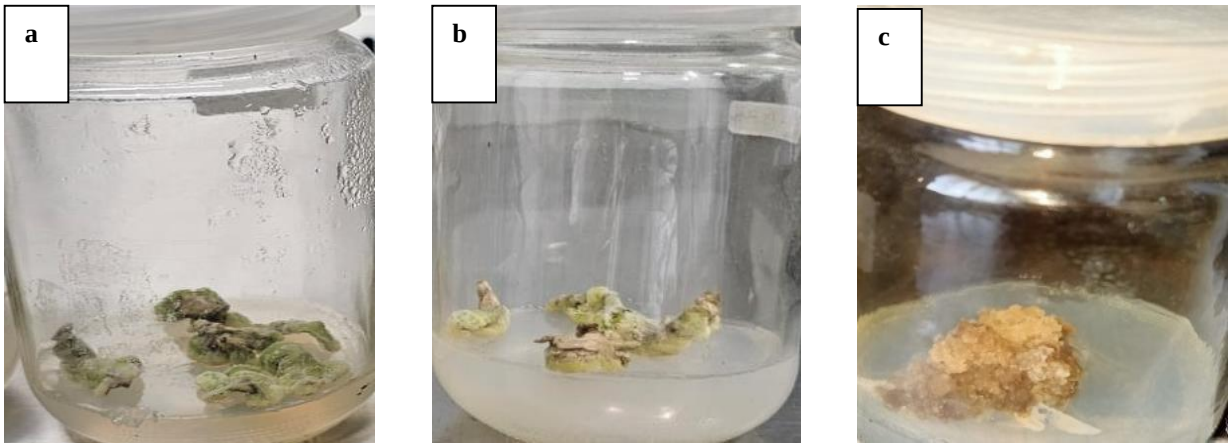


**Figure 1. Callus color for each concentration of plant growth regulator (PGR). (a) Dark green callus: The callus obtained from concentration of (1 mg/L NAA to 2 mg/L BA); (b) Light green callus: The callus obtained from concentration of (1 mg/L NAA to 1 mg/L BA) or (2 mg/L NAA to 2 mg/L BA); (c) Brownish-yellow: The callus obtained from concentration of (2 mg/L NAA to 1 mg/L BA).**

### 3.2. Callus fresh and dry weight

Callus dry weight (DW) was determined to compare the growth rate of callus in different treatments (Table 4). A moisture balance was used to measure fresh weight (FW), moisture percentage (MP), and dry weight percentage (DWP) of callus. FW of callus was measured after 5 weeks of planting the leaves on different treatments, and after 20 days from initiation (20 days old callus).

### 3.3. Biochemical analysis of *Datura innoxia* using HPLC

High-Performance Liquid Chromatography (HPLC) of *Datura innoxia* ethanolic leaves and callus extract revealed the presence of numerous phenolic compounds in the plant (Table 5). These diverse phenolic compounds exhibit various biologically active properties such as antioxidant, anticancer, antibacterial, and anti-inflammatory effect. When comparing the results of the two samples, a significant difference in concentration was observed, with the callus showing higher concentrations than the leaves in

compounds like Gallic acid, Chlorogenic acid, Rutin, Caffeic acid, Methyl gallate, Kaempferol, Quercetin, and Vanillin.

**Table 4. Growth rate of callus formation in different treatments: measuring Fresh weight (FW), Moisture % (MP), Dry Weight % (DWP) and Dry weight (DW) of callus**

| Code | Fresh weight (g) | Moisture % | Dry Weight % | Dry weight (g) |
|---|---|---|---|---|
| MS1 | - | - | - | - |
| MS2 | 1.83 | 84.15 | 15.85 | 0.29 |
| MS3 | 0.78 | 85.77 | 14.23 | 0.11 |
| MS4 | 1.14 | 83.63 | 16.37 | 0.18 |
| MS5 | 2.19 | 86.09 | 13.91 | 0.30 |

**Table (5): Area, Concentration in (µg/ml) and (µg/g), and biological activities of the compounds for leaves and callus of *Datura innoxia* using HPLC**

| Compounds | Classification | Leaves | | | Callus | | | Biological activities |
|---|---|---|---|---|---|---|---|---|
| | | Area | Conc. (µg/ml) | Conc. (µg/g) | Area | Conc. (µg/ml) | Conc. (µg/g) | |
| Gallic acid | Phenolic acid | 41.40 | 3.24 | 323.89 | 296.05 | 23.16 | 3173.01 | Antioxidant, and Anti-inflammatory [21]. |
| Chlorogenic acid | Phenolic acid | 39.70 | 4.90 | 489.52 | 204.02 | 25.16 | 3445.99 | Antioxidant, Anti-inflammatory, Antibacterial, Hypoglycemic, and Anti-cancer [22]. |
| Catechin | Flavonoid | 45.38 | 9.94 | 994.09 | 26.81 | 5.87 | 804.47 | Antioxidant, Anticancer, Anti-inflammatory, and Lower cholesterol [23, 24]. |
| Methyl gallate | Phenolic acid | 8.70 | 0.42 | 42.25 | 15.75 | 0.76 | 104.72 | Antioxidant, Antimicrobial, Anti-inflammatory, Anticancer, and Hepatoprotective [25]. |
| Caffeic acid | Phenolic acid | 1.01 | 0.08 | 7.73 | 1.37 | 0.10 | 14.34 | Antioxidant, Anti-inflammatory, Antimicrobial, and Anticancer [26]. |
| Syringic acid | Phenolic acid | 4.81 | 0.32 | 32.49 | 2.12 | 0.14 | 19.63 | Antioxidant, Anti-inflammatory, Anticancer, Antimicrobial, and Cardioprotective [27]. |
| Rutin | Flavonoid | 1.89 | 0.30 | 30.16 | 3.04 | 0.49 | 66.45 | Antioxidant, Anti-inflammatory, Antidepressant, and Neuroprotective [28]. |
| Ellagic acid | Tannins | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | Antioxidant, Anticancer, Antimutagenic, and Anti-inflammatory [29]. |
| Coumaric acid | Phenolic acid | 0.81 | 0.03 | 2.63 | 0.00 | 0.00 | 0.00 | Antioxidant, and Anticancer [30]. |
| Vanillin | Phenolic aldehyde | 3.90 | 0.14 | 14.02 | 126.49 | 4.55 | 623.42 | Flavoring agent, and Antioxidant [31]. |
| Ferulic acid | Phenolic acid | 38.72 | 2.11 | 211.16 | 4.56 | 0.25 | 34.08 | Antioxidant, Antimicrobial, and Anti-inflammatory [32]. |
| Naringenin | Flavonoid | 11.89 | 1.04 | 103.52 | 0.00 | 0.00 | 0.00 | Antioxidant, Anti-inflammatory, Anticancer, Antimicrobial, and Cardioprotective [33]. |
| Rosmarinic acid | Phenolic acid | 6.19 | 0.61 | 60.78 | 2.20 | 0.22 | 29.66 | Antioxidant, Anti-inflammatory, Antidiabetic, and Anticancer [34]. |
| Daidzein | Flavonoid | 15.15 | 0.93 | 93.14 | 13.69 | 0.84 | 115.29 | Antioxidant, Anticancer- Anti-inflammatory, and Neuron protection [35]. |
| Quercetin | Flavonoid | 5.60 | 0.37 | 36.51 | 6.41 | 0.42 | 57.25 | Antioxidant, Anti-inflammatory, Antihypertensive, Anti-atherosclerotic, and Anti-hypercholesterolemic [36, 37]. |
| Cinnamic acid | Phenolic acid | 7.35 | 0.12 | 12.43 | 0.00 | 0.00 | 0.00 | Antioxidant, Anti-inflammatory, and Antimicrobial [38]. |
| Kaempferol | Flavonoid | 5.97 | 0.35 | 34.82 | 11.30 | 0.66 | 90.31 | Antioxidant, Anticancer, Antimicrobial, Antidiabetic, and Anti-inflammatory [39]. |
| Hesperetin | Flavonoid | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | Antioxidant, Antimicrobial, Antidiabetic, Anti-inflammatory, and Plant metabolite [40, 41]. |

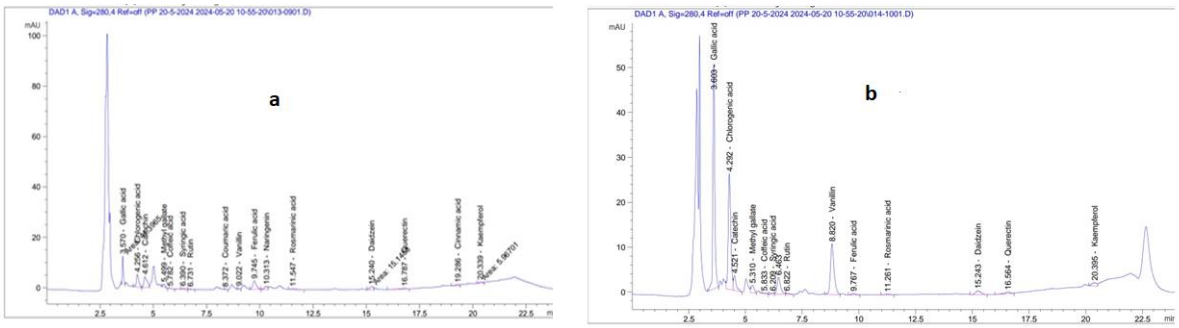


**Figure 2. HPLC Curve for absorption peaks of compounds. (a) Absorption peaks of HPLC analysis for *Datura innoxia* ethanolic leaves extract; (b) Absorption peaks of HPLC analysis for *Datura innoxia* ethanolic callus extract.**

### 3.4. Antibacterial activity of *Datura innoxia*

The inhibitory potential of *D. innoxia* leaves and callus using ethanol as a solvent was observed using an extraction disk diffusion assay against *E. coli and S. aureus.* The results showed zone of inhibition (ZOI) of *E. coli* and *S. aureus* strains to show susceptibility of ethanol extract of *D. innoxia* leaves and callus. Ethanolic leaves extract showed ZOI (12mm ±2) (Figure 3.B) and ethanolic extract of callus showed ZOI (14mm ±2) (Figure 3.C). Ethanolic leaves extract showed ZOI (11mm ±2) (Figure 4.B) and ethanolic extract of callus showed ZOI (13-14mm ±2) (Figure 4.C).

Callus extract give ZOI higher than leaves extract in both strains that prove the results of HPLC as chlorogenic acid compound that has antibacterial properties its concentration in callus higher than leaves.

### *3.5.* Fingerprinting of Datura innoxia

SCoT and ISSR Molecular markers were used to analyze the genetic diversity and compare between the leaves as an organized culture and the callus as a disorganized culture through genetic stability. The ISSR profile resulted from 3 ISSR primers as shown in (Figure 5).

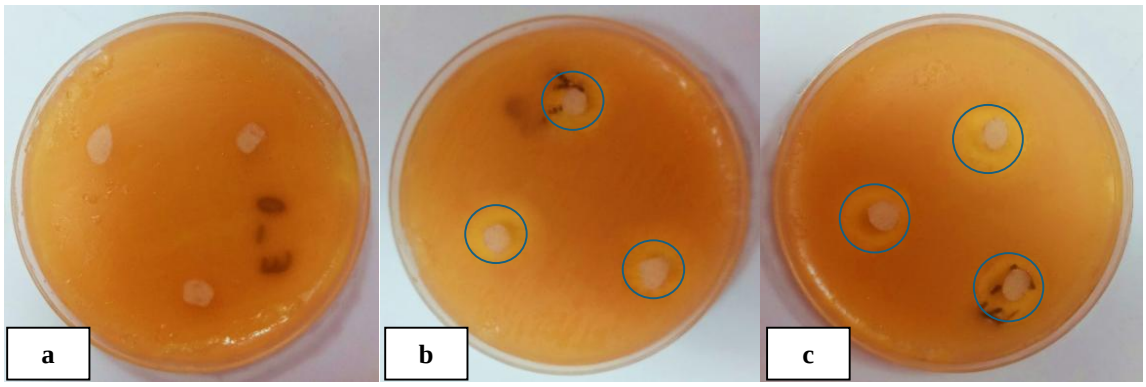


**Figure 3. Zone of Inhibition (mm) of *E. coli* strain to show susceptibility of ethanol extract of *D. innoxia* leaves and callus. (a) Negative control without ZOI; (b) Ethanolic extract of leaves showed ZOI (12mm ±2); (c) Ethanolic extract of callus showed ZOI (14mm ±2).**

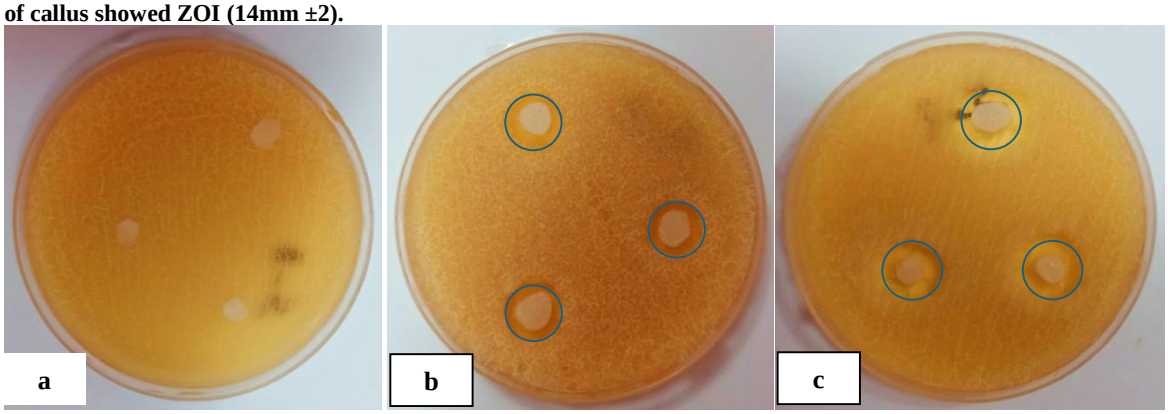


**Figure 4. Zone of Inhibition (mm) of *S. aureus* strain to show susceptibility of ethanol extract of *D. innoxia* leaves and callus. (a) Negative control without ZOI; (b) Ethanolic extract of leaves showed ZOI (11mm ±2); (c) Ethanolic extract of callus showed ZOI (13-14mm ±2).**

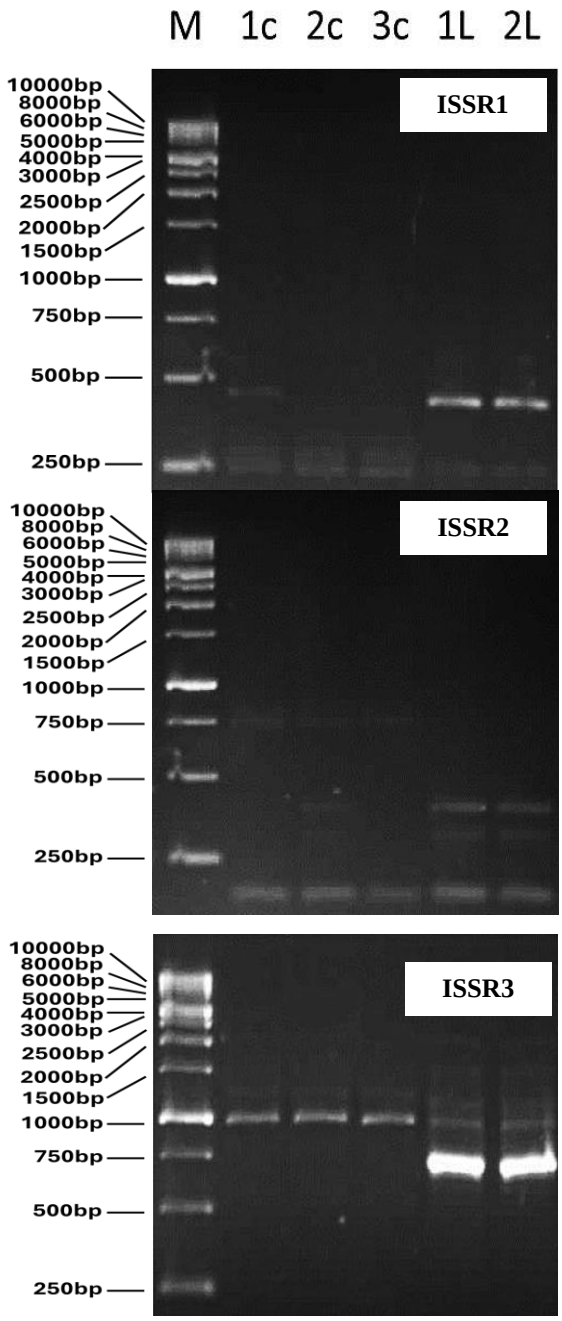


**Figure 5. ISSR profile using ISSR1, ISSR2, and ISSR3 primers. 1c, 2c, and 3c are the callus explant repeats; 1L and 2L are the leaves explant repeats.**

The first leaves biological repeat (1L) of ISSR1 showed no polymorphism with the second leaves biological repeat (2L) and both of them showed low polymorphism with the callus biological repeats. While the primer ISSR2, two leaves biological repeats (1L) and (2L) showed about 75 % polymorphism with the biological repeats of callus, which indicates that this primer can be used to differentiate between leaves and callus of *Datura innoxia*. ISSR3 showed no polymorphism recorded between the biological repeats of leaves (1L and 2L) but both of them showed about 50% polymorphism with the repeats of callus (1c, 2c and 3c).

The SCoT profile resulted from SCoT primers is shown in (Figure 6). After running different biological repeats of the leaves and callus with SCoT1 on the gel, the first leaves biological repeat (1L) showed no polymorphism with the second repeat of leaves.

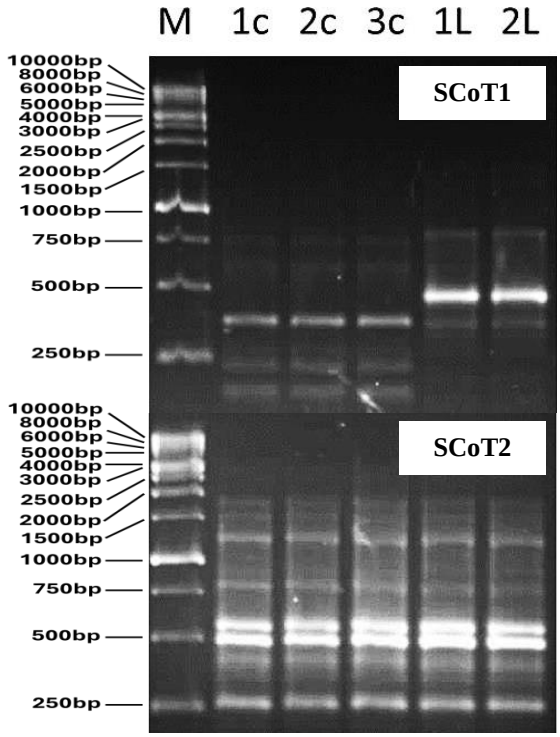


**Figure 6. SCoT amplification profile of primers SCoT1 and SCoT2. 1c, 2c, and 3c are the callus explant repeats; 1L and 2L are the leaves explant repeats.**

The Leaves biological repeats showed about 50% polymorphism with the callus biological repeats. Which indicates that this primer can be used to differentiate between leaves and callus of *Datura innoxia*.

After running different biological repeats with SCoT2 on the gel, the first and second biological repeats (1L & 2L) were identical to the three callus biological repeats (1C, 2C & 3C), Which indicates that this primer can be used to identify the presence of genetic material of *Datura innoxia* but can't be used in differentiation between organized and disorganized cultures.

**4. Discussion**

Initiated callus on 2 mg/L NAA to 2 mg/L BA was the best concentration of Plant Growth Regulators (PGR) and this concentration led to light green. Callus was initiated after 15 days, there is similar to previous studies but with different plant growth regulators and concentrations [42]. Also, Previous studies showed that 0.673g, 0.04g, 0.457g, 0.517g, 0.395g, 0.20g, 0.655g, 0.322g, 0.405g, 0.125g, 0.165g and 0.135g Callus dry weight (30 old days) from (1 mg\L NAA + 1 mg\L BA), (1 mg\L NAA + 3 mg\L BA), (1 mg\L NAA + 5 mg\L BA), (1 mg\L 2.4.D + 1 mg\L BA), (1 mg\L 2.4.D + 5 mg\L BA), (1 mg\L 2.4.D + 1 mg\L kin), (1 mg/L 2.4.D + 3 mg\l kin), (1 mg\L 2.4.D + 5 mg\L kin), (1 mg\L NAA + 1 mg\L kin), (1 mg/L NAA + 3 mg\L kin), (1 mg\L NAA + 5 mg\L kin) respectively. While our study showed that 0.29g, 0.11g, 0.18, and 0.30g Callus dry weight (20 old days) from (1 mg/L NAA + 1 mg/L BA), (1 mg/L NAA + 2 mg/L BA), (2

mg/L NAA + 1 mg/L BA), and (2 mg/L NAA + 2 mg/L BA) respectively [43].

HPLC analysis of Datura innoxia ethanolic leaves and callus extracts revealed the presence of numerous phenolic compounds in the plant. A significant difference in concentration was observed. Callus showed higher concentrations of compounds than the leaves. Concentrations were (489.52 µg/g), (3445.99 µg/g) Chlorogenic acid, (489.52 µg/g), (3445.99 µg/g) Gallic acid and (14.02 µg/g), (623.42 µg/g) Vanillin for leaves and callus respectively. These result differ from a previous study which showed no absorption from the ethanolic leaves extract but indicated results with the Methanolic leaves extract yielding concentrations of Quercetin (0.84 ± 0.02 µg/mg), Catechin (5.41 ± 0.03 µg/mg), and Apigenin (2.11 ± 0.01 µg/mg) [7]. Datura leaves were used as an organized culture where the leaves extract were preferred over stems and fruits extracts because it displayed the best results in terms of the highest extract recovery, phenolic and flavonoid content, total antioxidant capacity, and reducing energy potential [7].

HPLC analysis results also showed various kinds of bioactive compounds that are classified as phenolic acids, flavonoids, tannins, and phenolic aldehyde where *Datura innoxia* has highly amount of bioactive compounds that explained in previous study of phytochemical analysis for methanolic extract of leaves that indicated the concentration of terpenoids (9.0 ± 0.11 mg/g), carbohydrates (34 ± 0.5 mg/g), saponins (14 ± 0.12 mg/g), alkaloids (65 ± 0.35 mg/g), total phenolic content (38 ± 0.27 mg/g), and total flavonoid content (19 ± 0.17 mg/g) [44].

Ethanolic extract of leaves showed Zone of Inhibition (ZOI) on *E. coli* (12mm ±2) and (14mm ±2) with callus extract. Ethanolic extract of leaves showed ZOI on *S. aureus* (11mm ±2) and (13-14mm ±2) with callus extract. These results were different from previous study that reported ZOI (12.64mm) with ethanolic leaves extract on *E. coli* and (23.83mm) on *S. aureus* [45].

DNA fingerprinting was carried out to determine the genetic stability of callus using 3 ISSR markers (ISSR 1: $(CA)_6$-GT, ISSR 2: $(CA)_6$-AG, and ISSR3: $(GA)_6$-CC) and 2 SCoT Primers (SCoT1: CAACAATGGCTACCACCG, and SCoT2: ACGACATGGCGACCACGC) with 3 biological repeats was tested from the callus which gave the best initiation\induction rate (2mg/L NAA + 2mg/L BA). Two biological repeats of leaves were tested and showed high stability for both biological repeats with all primers. While the callus showed somaclonal variation between the biological repeats. All of ISSR primers and SCoT primers showed no stability between leaves and callus genome except primer SCoT2 (ACGACATGGCGACCACGC) that showed high stability between the leaves and callus. From previous studies, Two ISSR markers (ISSR1: GAGAGAGAGAGAGC), and (ISSR2: CACACACACACAGT) have been used to differentiate between nine samples of Datura stramonium [15]. URP Primers were used in another study to differentiate between species of Datura (D. stramonium and D. ferox) [14].

## 5. Conclusions

The study conducted on *Datura innoxia* revealed significant differences between organized and disorganized tissues across multiple research aspects. Optimal callus induction was achieved with a concentration of (2 mg/L NAA to 2 mg/L BA). Datura callus was initiated after 15 days and completed induction by 5 weeks. High-performance liquid chromatography (HPLC) of ethanolic extracts from both leaves and callus identified higher concentrations of phenolic compounds in the callus suggesting potential medicinal and pharmaceutical applications. Inhibitory potential using ethanol extracts against *E. coli* and *S. aureus* showed greater zones of inhibition with callus extracts compared to leaves extracts. Genetic stability assessed through DNA fingerprinting using ISSR and SCoT primers indicated consistent patterns in leaves but somaclonal variations in callus repeats except SCoT2, which demonstrated stability across both tissues. SCoT2 can be used to identify the presence of genetic material of *Datura innoxia* whether leaves or callus. These results findings highlight the distinct biochemical and genetic characteristics between organized and disorganized tissues in *Datura innoxia* emphasizing their relevance in both basic and applied research contexts. Finally, the current results can be used to detect the important bioactive compounds for commercial uses and can be improved by future studies on regulations of genes for these compounds.

## 6. Acknowledgments

The authors appreciate the role of Botany Department, National research centre, Giza, Egypt for allowing them to use the laboratories No. 901, 907.

## 7. References

[1] N. O. Maheshwari, A. Khan, and B. A. Chopade, "Rediscovering the medicinal properties of Datura sp.: A review," *Journal of medicinal plants Research*, vol. 7, no. 39, pp. 2885-2897, 2013.

[2] S. Singh *et al.*, "An update on morphology, mechanism, lethality, and management of dhatura poisoning," *Eur. Chem. Bull*, vol. 12, no. 5, pp. 3418-3426, 2023.

[3] N. Akhtar and B. Mirza, "Phytochemical analysis and comprehensive evaluation of antimicrobial and antioxidant properties of 61 medicinal plant species," *Arabian journal of chemistry*, vol. 11, no. 8, pp. 1223-1235, 2018.

[4] M. Gumustas, S. Kurbanoglu, B. Uslu, and S. A. Ozkan, "UPLC versus HPLC on drug analysis: advantageous, applications and their validation parameters," *Chromatographia*, vol. 76, pp. 1365-1427, 2013.

[5] P. Biswasroy, D. Pradhan, and R. Pradhan, "Quantitative analysis of Hyoscine in different extracts obtained from the seeds of *Datura innoxia* by RP-HPLC," *Journal of Ayurvedic and Herbal Medicine*, vol. 3, no. 4, pp. 192-95, 2017.

[6] A. R. Abubakar and M. Haque, "Preparation of medicinal plants: Basic extraction and fractionation procedures for experimental purposes," *Journal of Pharmacy and Bioallied Sciences*, vol. 12, no. 1, pp. 1-10, 2020.

[7] H. Fatima, K. Khan, M. Zia, T. Ur-Rehman, B. Mirza, and I.-u. Haq, "Extraction optimization of medicinally important metabolites from *Datura innoxia* Mill.: an in vitro biological and phytochemical investigation," *BMC complementary and alternative medicine*, vol. 15, pp. 1-18, 2015.

[8] N. Sakadzo, I. Pahla, S. Muzemu, R. Mandumbu, and K. Makaza, "Herbicidal effects of Datura stramonium (L.) leaf extracts on *Amaranthus hybridus* (L.) and Tagetes minuta (L.)," *African Journal of Agricultural Research*, vol. 13, no. 34, pp. 1754-1760, 2018.

[9] M. Kalim, F. Hussain, H. Ali, I. Ahmad, and M. N. Iqbal, "Antifungal activities of methanolic extracts of *Datura inoxia*," *PSM Biological Research*, vol. 1, no. 2, pp. 70-73, 2016.

[10] F. Eftekhar, M. Yousefzadi, and V. Tafakori, "Antimicrobial activity of *Datura innoxia* and Datura stramonium," *Fitoterapia*, vol. 76, no. 1, pp. 118-120, 2005.

[11] N. Vaou, E. Stavropoulou, C. Voidarou, C. Tsigalou, and E. Bezirtzoglou, "Towards advances in medicinal plant antimicrobial activity: A review study on challenges and future perspectives," *Microorganisms*, vol. 9, no. 10, p. 2041, 2021.

[12] M. Z. Iqbal *et al.*, "DNA fingerprinting of crops and its applications in the field of plant breeding," *Journal of Agricultural Research*, vol. 59, no. 1, 2021.

[13] H. Nybom, K. Weising, and B. Rotter, "DNA fingerprinting in botany: past, present, future," *Investigative genetics*, vol. 5, pp. 1-35, 2014.

[14] I. T. Tsialtas *et al.*, "In the wild hybridization of annual Datura species as unveiled by morphological and molecular comparisons," *Journal of Biological Research-Thessaloniki*, vol. 21, pp. 1-8, 2014.

[15] S. K. Al Taweel and H. A. Al Amrani, "Genetic variability induction, upr, issr and gc-ms identification through ems mutagenesis of *Datura stramonium* l," *Plant Archives (09725210)*, vol. 20, no. 2, 2020.

[16] E. M. Salah, M. A. Eissa, and F. A. El-Feky, "In Vitro Calli Developing and Characterizing from Sweet Potato (*Ipomoea batatas*) to Maximize Secondary Metabolites Production," *Al-Azhar Journal of Agricultural Research*, vol. 49, no. 1, pp. 149-155, 2024.

[17] T. Murashige and F. Skoog, "A revised medium for rapid growth and bio assays with tobacco tissue cultures," *Physiologia plantarum*, vol. 15, no. 3, 1962.

[18] A. D. Wolfe, Q. Y. XIANG, and S. R. Kephart, "Assessing hybridization in natural populations of Penstemon (*Scrophulariaceae*) using hypervariable intersimple sequence repeat (ISSR) bands," *Molecular Ecology*, vol. 7, no. 9, pp. 1107-1125, 1998.

[19] E. Oliveira *et al.*, "Optimizing the efficiency of the touchdown technique for detecting inter-simple sequence repeat markers in corn (*Zea mays*)," *Genet. Mol. Res*, vol. 9, no. 2, pp. 835-842, 2010.

[20] C. Luo, X.-H. He, H. Chen, S.-J. Ou, and M.-P. Gao, "Analysis of diversity and relationships among mango cultivars using Start Codon Targeted (SCoT) markers," *Biochemical Systematics and Ecology*, vol. 38, no. 6, pp. 1176-1184, 2010.

[21] C. Locatelli, F. B. Filippin-Monteiro, A. Centa, and T. B. Creczinsky-Pasa, "Antioxidant, antitumoral and anti-inflammatory activities of gallic acid," *Handbook on Gallic Acid: Natural Occurrences, Antioxidant Properties and Health Implications,(4th Ed.). Nova Publishers*, vol. 1, p. 23, 2013.

[22] L. Wang *et al.*, " The biological activity mechanism of chlorogenic acid," *Frontiers in Nutrition*, vol. 9, 2022.

[23] C.-S. Rha, H. W. Jeong, S. Park, S. Lee, Y. S. Jung, and D.-O. Kim, " Antioxidative, Anti-Inflammatory, and Anticancer Effects of Purified Flavonol Glycosides," *Antioxidants*, vol. 8, no. 8, p. 278, 2019.

[24] V. B. Tatipamula and B. Kukavica, "Phenolic compounds as antidiabetic, anti‐inflammatory, and anticancer agents and improvement of their bioavailability by liposomes," *Cell biochemistry and function*, vol. 39, no. 8, pp. 926-944, 2021.

[25] N. Khan *et al.*, "Nutritional importance and pharmacological activity," *World Journal of Pharmacy and Pharmaceutical Sciences*, vol. 6, pp. 258-273, 2017.

[26] F. Armutcu, S. Akyol, S. Ustunsoy, and F. F. Turan, " Therapeutic potential of caffeic acid phenethyl ester and its anti-inflammatory," *Experimental and Therapeutic Medicine*, vol. 9, no. 5, pp. 1582-1588, 2015.

[27] M. S., A. H. Shaik, M. P. E., S. Y. Al Omar, A. Mohammad, and L. D. Kodidhela, "participate in the enhanced cardiac protection of liraglutide," *Scientific Reports*, vol. 10, no. 1, 2020.

[28] S. Mustapha, R. Magaji, M. Magajic, I. Gayab, Y. Yusha'ub, and S. Chiroma, " Rutin ameliorates lipopolysaccharide-induced depressive-like behaviours in mice," *Journal of African Association of Physiological Sciences*, vol. 10, no. 1, pp. 67-77, 2022.

[29] V. Baradaran Rahimi, M. Ghadiri, M. Ramezani, and V. R. Askari, " Antiinflammatory and anti-cancer activities of pomegranate and its constituent, ellagic acid," *Phytotherapy Research*, vol. 34, no. 4, pp. 685-720, 2020.

[30] W. Tehami, A. Nani, N. A. Khan, and A. Hichami, " New Insights Into the Anticancer Effects of p-Coumaric Acid," *Dose-Response*, vol. 21, no. 1, 2023.

[31] G. Taner, D. Özkan Vardar, S. Aydin, Z. Aytaç, A. Başaran, and N. Başaran, "Use of in vitro assays to assess the potential cytotoxic, genotoxic and antigenotoxic effects of vanillic and cinnamic acid," *Drug and chemical toxicology*, vol. 40, no. 2, pp. 183-190, 2017.

[32] K. Zduńska, A. Dana, A. Kolodziejczak, and H. Rotsztejn, "Antioxidant properties of ferulic acid and its possible application," *Skin Pharmacology and Physiology*, vol. 31, no. 6, pp. 332-336, 2018.

[33] K. Uçar and Z. Göktaş, "Biological activities of naringenin: A narrative review based on in vitro and in vivo studies," *Nutrition Research*, vol. 119, pp. 43-55, 2023.

[34] M. Nadeem *et al.*, " Therapeutic potential of rosmarinic acid: a comprehensive review," *Applied Sciences*, vol. 9, p. 3139, 2019.

[35] F. Zhang *et al.*, " Daidzein ameliorates spinal cord ischemia/reperfusion injury-induced neurological function," *Experimental and Therapeutic Medicine*, vol. 14, pp. 4878-4886, 2017.

[36] Q. Deng, X. X. Li, Y. Fang, X. Chen, and J. Xue, "Therapeutic Potential of Quercetin as an Antiatherosclerotic Agent in Atherosclerotic Cardiovascular Disease: A Review," *Evidence-Based Complementary and Alternative Medicine*, vol. 2020, pp. 1-12, 2020.

[37] F. Elbarbry, K. Abdelkawy, N. Moshirian, and A. M. Abdel-Megied, "The antihypertensive effect of Quercetin," *International Journal of Molecular Sciences*, vol. 21, no. 18, p. 6554, 2020.

[38] N. Ruwizhi and B. A. Aderibigbe, "Cinnamic acid derivatives and their biological efficacy," *International journal of molecular sciences*, vol. 21, no. 16, p. 5712, 2020.

[39] M. Shahbaz *et al.*, " Anticancer, antioxidant, ameliorative and therapeutic properties of kaempferol," *International Journal of Food Properties*, vol. 26, no. 1, pp. 1140-1166, 2023.

[40] H. M. Abdou, F. A. Hamaad, E. Y. Ali, and M. H. Ghoneum, " Antidiabetic efficacy of *Trifolium alexandrinum* extracts hesperetin and quercetin," *Biomedicine & Pharmacotherapy*, vol. 149, p. 112838, 2022.

[41] S.-S. Choi, S.-H. Lee, and K.-A. Lee, "Study of hesperetin, hesperidin and hesperidin glucoside: antioxidant, anti-Inflammatory, and antibacterial activities in vitro," *Antioxidants*, vol. 11, no. 8, p. 1618, 2022.

[42] S. Amiri, S. Kazemitabar, G. Ranjbar, and M. Azadbakht, "In vitro propagation and whole plant regeneration from callus in Datura (*Datura stramonium L.*)," *African Journal of Biotechnology*, vol. 10, no. 3, pp. 442-448, 2011.

[43] R. Abd El-Rahman, A. Gabal, and H. Khlifa, "Production of Scopolamine and Hyoscyamine in Calli and Regenerate Culture of *Datura metel L*," *Journal of Applied Science Research*, vol. 4, pp. 1858-1866, 2008.

[44] Z. K. Bagewadi, U. M. Muddapur, S. S. Madiwal, S. I. Mulla, and A. Khan, "Biochemical and enzyme inhibitory attributes of methanolic leaf extract of *Datura inoxia* Mill," *Environmental Sustainability*, vol. 2, pp. 75-87, 2019.

[45] P. Kaushik and P. Goyal, "In vitro evaluation of *Datura innoxia* (thorn-apple) for potential antibacterial activity," *Indian Journal of Microbiology*, vol. 48, pp. 353-357, 2008.