

In vitro* Production of Atropine from *Datura metel

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Abstract. The study involved the comprehensive analysis of *Datura metel*, focusing on the extraction and characterization of phytochemical compounds from both the crude plant material and callus extracts. After identification, leaves, internodes, and fruits underwent thorough cleaning and drying before being finely ground into powder. Extraction involved a precise process of mixing the powder with sterile water, gentle heating, filtration, and centrifugation, yielding a clear supernatant for further analysis. Phytochemical screening revealed the presence of various compounds like tannins, alkaloids, saponins, and flavonoids, with variations observed among plant parts and extracts. The rich phytochemical profile of *Datura metel*, suggesting its potential medicinal applications. Tests on crude plant and callus extracts provided insights into their chemical compositions. The callus extracts exhibited higher atropine content than normal plant extracts, hinting at the potential for *in vitro* culture to enhance atropine production. Variations in atropine content across different plant parts, providing valuable insights for pharmaceutical research and medicinal applications. This research highlights on the diverse phytochemical composition of *Datura metel*, warranting further exploration of its medicinal properties and cultivation techniques. The amount of atropine in each source varies: 1.25 mg/g of atropine is found in stem calli, 1.09 mg/g is found in leaf calli, 1.53 mg/g is found in fruit calli, and 1.12 mg/g is found in flower calli. that the amounts of atropine vary according to the tissue from which the calli originate, with the highest concentration found in calli produced from fruit, followed by calli originating from stems, flowers, and leaves. These studies urge for more study into the therapeutic effects of *Datura metel* and advance our awareness of its potential in pharmaceutical applications.

Keywords: *Datura metel*, atropine, phytochemical, tannins, alkaloids, saponins, flavonoids.

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1 Introduction

In the field of pharmaceuticals, the search for innovative and effective ways to produce drugs has prompted researchers to look at natural sources for important molecules. One such substance of importance is atropine, an essential alkaloid with a variety of medicinal uses, including the treatment of certain heart disorders and usage as a mydriatic agent. The in vitro synthesis of atropine, especially from *Datura metel* a plant with a high alkaloid content has garnered a lot of attention in recent years. This project offers a viable path to the sustainable and regulated manufacture of atropine, avoiding the drawbacks of conventional extraction techniques and perhaps offering a more economical and ecologically friendly solution. Atropine, as a naturally occurring compound, is conventionally extracted from various plants belonging to the Solanaceae family, notably *Atropa belladonna* and *Datura* species. These extraction processes often encounter obstacles such as limited availability of plant material, variations in alkaloid content, and environmental concerns regarding the cultivation and harvesting of these plants. In vitro production offers an alternative route to overcome these challenges by employing biotechnological methods to synthesize atropine in controlled laboratory conditions. The use of *Datura metel* as a primary source for in vitro production of atropine presents several advantages. This plant species is known for its high alkaloid content, including hyoscyamine and scopolamine, which are precursors to atropine. By utilizing tissue culture techniques, such as cell suspension culture or organ culture, researchers can stimulate the production of atropine in a controlled environment, independent of seasonal variations and geographical constraints. Moreover, in vitro production eliminates the need for extensive land use and reduces the environmental impact associated with traditional cultivation practices. The scalability of in vitro production allows for consistent and reliable yields of atropine, ensuring a stable supply for pharmaceutical purposes. With advancements in biotechnological methods and genetic engineering, researchers can enhance the biosynthetic pathways involved in atropine production, potentially increasing yield and purity while minimizing production costs. Additionally, in vitro production offers opportunities for the synthesis of novel derivatives and analogs of atropine with tailored pharmacological properties, opening new avenues for drug discovery and development. Atropine, first isolated from *Atropa belladonna* in the early 19th century by Mein and Bell, has a rich history in both traditional and modern medicine. Its pharmacological properties, particularly its ability to block the action of acetylcholine at muscarinic receptors, have made it indispensable in various medical applications. Notably, during World War II, atropine was widely used to treat soldiers affected by nerve agent poisoning (Holstege et al., 1997). Despite its therapeutic benefits, traditional methods of atropine extraction from plants such as *Datura metel* are beset with challenges. The variability in alkaloid content among plant samples, dependence on seasonal factors, and the need for large-scale cultivation pose significant logistical hurdles. Moreover, the extraction process itself can be laborious and environmentally taxing, often involving the use of organic solvents and generating substantial waste (Parry et al., 2019). Biotechnological approaches offer a promising solution to the challenges associated with traditional atropine extraction. By harnessing the inherent metabolic pathways of plants and optimizing growth conditions in vitro, researchers can control and enhance the production of atropine in a more sustainable and efficient manner. In vitro production also enables the cultivation of plant cells in controlled bioreactor systems, allowing for continuous and reproducible production throughout the year (Shi et al., 2022).

2 Materials and Methods

2.1 Plant Collection and Identification

The raw material used for this research was *Datura metel*. The *Datura metel* was collected from nearby village Varapalayam, Tiruchengode at Namakkal District. After the plant components were identified using the protocol, the herbaria was put in Liatris Biosciences LLP's Life Sciences Department in Cochin (Jain et al., 1976).

2.2 Plant Material Preparation

After giving the fruits, leaves, and internode, a good washing with running tap water two or three times, they were allowed to air dry in the shade. The plant components were ground in a mixer once they had completely dried in shade, and that powder stored in little polythene bags with appropriate labelling.

2.3 Plant Material Extraction

5g of powdered plant material, for example, were weighed using an electronic scale and combined with five grams of crushed plant material in twenty-five millilitres of sterile water. Following a 60°C heating step, this mixture was filtered with Whatman no.1 filter paper. Once collected, the filtrate was stored at 5°C in sterile bottles. And centrifugation at a speed of 2500 rpm for a duration of 15-20 minutes (Harborne 1973).

2.4 Preliminary Phytochemical Analysis

The crude powder of *Datura metel L* was subjected to a qualitative phytochemical study using the techniques outlined by (Trease et al., 1989). The results of the numerous phytochemical tests, including those on alkaloids, tannins, saponins, flavonoids, proteins, and so forth (Oguyemi et al., 1979) are displayed below.

2.4.1 Antacid Reagent Test

10% ammonium hydroxide arrangement (NH₄OH) is the sole reagent required for the Soluble Reagent Test, which is utilized to recognize the phenols in plant extricate. To decide whether phenols were display in the plant extricate, a small (2 ml) test of the extricate was treated with a 10% ammonium hydroxide solution.

2.4.2 Benedict's Test

Benedict's Test approves the presence of carbs. After including 0.5 ml of Benedict's reagent to 0.5 ml of extricate, the blend was cooked for two minutes over a bubbling water bath.

2.4.3 Borntrager's Test

The Borntrager's Test may recognize quinones. The essential reagents are fluid alkali (NH₃), sodium hydroxide (NaOH), and diethyl ether ((C₂H₅)₂O). To recognize quinones, blend 1 millilitre of diethyl ether ((C₂H₅)₂O) with 1 millilitre of extricate. At that point, include 2 millilitres of sodium hydroxide (NaOH) and 2 millilitres of watery smelling salts (NH₃).

2.4.4 Ferric Chloride Test

By including a few drops of a unbiased 5% ferric chloride (FeCl_3) arrangement to 5 millilitres of plant extricate, the ferric chloride test may be utilized to recognize tannins.

2.4.5 Flavonoids

It employments concentrated sulfuric corrosive (H_2SO_4) and smelling salts arrangement (NH_3) as reagents. The test included including 1 ml of plant extricate to 5 ml of weakened alkali arrangement (NH_3), at that point 1 ml of concentrated sulfuric corrosive (H_2SO_4) (Evans 1997).

2.4.6 Foam Arrangement Test

The froth era test confirms the nearness of saponin. The extricate was combined with 110 millilitres of refined water in a test tube and well shaken. To confirm the nearness of saponin, steady froth arrangement is utilized.

2.4.7 Iodine test

To confirm that there were carbohydrates (starch) in the plant extricate, a small sum of iodine arrangement (0.1 M potassium iodide) was included. The improvement of a blue-black tint confirms the starch's existence.

2.4.8 Keller-Kilani Test

The nearness of glycosides in the plant extricate was decided utilizing the Keller-Kilani measure. A arrangement of FeCl_3 comprising 1-2% was combined with 2 millilitres of frigid acidic corrosive and unrefined extract. After that, the blend was exchanged to a moment test tube that included two millilitres of concentrated H_2SO_4 . A brown ring shapes in the interphase, demonstrating the nearness of cardiac glycosides.

2.4.9 Libermann-Burchard Test

Libermann-Burchard To find out the nearness of steroids in the plant extricate, a test is conducted utilizing reagents such as acidic corrosive anhydride ($\text{C}_4\text{H}_6\text{O}_3$) and sulfuric corrosive (H_2SO_4). 0.5 ml of extricate was blended with 2 ml of acidic corrosive anhydride ($\text{C}_4\text{H}_6\text{O}_3$) arrangement, and at that point 2 ml of sulphuric corrosive (H_2SO_4) arrangement was presented down the test tube divider (Finar 1986).

2.4.10 Mayer's Test

To decide the alkaloids in the plant extricate, utilize Mayer's Test. The chemicals utilized are Mayer's reagent (potassium mercuric iodide arrangement) and weakened hydrochloric corrosive (HCl). Five millilitres of extricate were blended with a few millilitres of weakened hydrochloric corrosive (HCl) and disturbed. To decide if alkaloids are show in this arrangement, a drop or two of Mayer's reagent is included by the test tube's sides (Evans 1997).

2.4.11 Million's Test

Protein substance in the plant extricate may be decided with the utilize of Million's Test. To confirm that there were proteins in the extricate, 2ml was blended with a few drops of Million's reagent (Rasch and Swift 1960)

2.4.12 Salkowski Test

To test terpenoids, utilize the Salkowski Test. Concentrated sulfuric corrosive (H_2SO_4) and chloroform ($CHCl_3$) were the chemicals utilized. In arrange to distinguish the terpenoids, 1 millilitre of the plant extricate, 2 millilitres of chloroform ($CHCl_3$), and 3 millilitres of absolutely blended concentrated sulfuric corrosive (H_2SO_4) were included to deliver a layer.

2.4.13 Whistler and BeMiller's Test

To decide which gums are display in the plant extricate, utilizing the Whistler and BeMiller's Test. The sole reagent required is outright (99%) ethanol. 5 millilitres of 100% (99%) ethanol (C_2H_5OH) are included to 3 millilitres of plant extricate amid testing, and the blend is ceaselessly whirled (Whistler 1993).

2.5 Aseptic Cultures Establishment

Explants taken from plants grown in the field typically include a variety of microbes. They were repeatedly rinsed in sterile water after being cleaned for 25 minutes using popular sterilizing agents such 3% NaClO, 90% ethanol, and 1% HgCl₂. This was done to remove any surface impurities. Surface sterilizing agents were also used, such as detergent, 0.3% carbendazim , 0.5% streptomycin and 1% benomyl.

2.6 Basic Culture Medium

All of the major and minor plant nutrients that are necessary, vitamins, plant growth regulators, and carbohydrates as a source of carbon are included in nutrition media, along with other organic materials that are helpful additions. The most often used ingredient in the preparation of solid and semi-solid culture medium is agar powder. Throughout, in vitro propagation was conducted using the MS basal medium formulation described by Murashige and Skoog in 1962, which included 30 g/L commercial white table sugar, 0.1 g/L α -cysteine, 10 mg/L calcium pantothenic acid, 2.0 mg/L asparagine, 2.0 mg/L arginine, and 6 g/L agar. After adjusting the medium's pH to 5.8, it was autoclaved for 20 minutes at 1.1 kg/cm² (12° C).

2.7 Growth Agent

Plant growth regulators affect callus development, differentiation, and the beginning of roots and shoots, all of which are critical to the success of tissue culture. Gibberellins promote the production of calluses, although auxins and cytokinin's' relative concentrations in the culture media tightly control these activities (Forsyth and Van 1982)

2.8 Conditions of Incubation

Incubating cultures requires a certain amount of light and warmth. It is often advised to spend 11–15 hours a day under cool, white fluorescent lamps that emit $35\text{--}50 \mu\text{mol/m}^2\text{s}^1$ of light. The temperature of the incubation environment is kept constant at $25^\circ\text{C}\pm 1$.

2.9 Preparation of Explants

8 to 10 week old *Datura metel* plants were gathered from Namakkal district in order to prepare explants. The explants were well cleaned with tap water, then soaked for 10 to 15 minutes in a 0.1% (v/v) fungicide solution, and finally rinsed three to four times with sterile distilled water. They were then chopped into single node stem pieces that were around 11–15 mm long and surface sterilized for 20–25 minutes using a commercial chlorine bleach solution containing 4.5% chlorine. This was followed by four rinses with sterile distilled water.

2.10 Stimulation of Callus from Vegetative Tissues

In order to stimulate callus from vegetative tissues, 8-10 week old plantlets from cultivation fields were evaluated for their stem (10 mm long), leaf (6 mm x 6 mm), fruit, and flower (5 mm long). These fragments were cultivated on MS medium treated with 1.0mgL^{-1} of IBA, NAA, and 2,4-D separately. The control was the medium devoid of plant growth regulators. The combination of plant growth regulators and explant type with the highest callus development percentage was shown to be the most appropriate. After two weeks, the callus quality was evaluated. The explants were injected into glass vials (20 mm × 80 mm) holding 15 ml of treatment material.

2.11 Preparation of Callus extract

All four types of explants (stem, leaf, seed, and flower) had their calli aseptically cut. 10g of each callus were then carefully weighed and finely minced on aluminum foil with a razor blade. Following the transfer of the minced callus tissues to a mortar, 30ml of sterile water were added, and the combination was pounded with a pestle to a fine slurry. This slurry was filtered through Whatman No. 1 filter paper after being heated to 65°C . After the filtrate was produced, it was centrifuged for 15 to 20 minutes at 2500 rpm. The supernatant was then collected in sterile bottles and kept chilled at 5 to 10°C until it was needed (Harborne 1973).

2.12 Extraction of Atropine

Some tropical *Datura* species include fruits that are the primary source of glycol alkaloids for atropine production, which has led to attempts to grow these plants as industrial crops. Recovering glycol alkaloids from *Datura metel* plant material may be done in two ways:

1. The sequential extraction of dried plant material using methanol and petroleum ether, followed by crystallization.
2. Direct extraction of fresh materials using acetic acid (2–5%).

Using a Waring blender, 500 g of fresh plant tissue (leaves) and 750 ml of 3% aqueous acetic acid were homogenized for the latter technique. A filter cloth was used to filter the thick slurry that was produced. After adding equal parts of ethanol to the filter and washings, stirring for two to three hours, and centrifuging the mixture, the filtrate was discarded. A second time, the brown residue was suspended in 250 millilitres of ethanol and extracted for ninety minutes. After adding ammonia to make the alcoholic concentration alkaline (pH 9.5),

it was cooled. A precipitate occurred after two days, and it was centrifuged. To remove the bulk of the glycol alkaloids from the precipitates generated from the combined extracts, hot 50 ml ethanol was used for three to four subsequent extractions. A Buckner funnel was used to filter the ethanol extracts after they had been refluxed with one to two grams of activated charcoal. The filtrate was made 3N by adding concentrated HCl. It was then refluxed for 45–1 hour to extract the alcohol by distillation. The alkaloid crystallized as a light brown solid after cooling (Telek 1997).

2.13 Preparation of Standard Solution of Atropine

A precise dosage of 10–15 mg of pure atropine was dissolved in 30 millilitres of 20% A.R. acetic acid. The solution was then further diluted 10–12 times with 20% acetic acid after an aliquot was taken. This led to the creation of a solution with a 45 mg/ml concentration. 6.55 g of sodium acetate A.R. and 2.40 ml of acetic acid were dissolved in water to create Acetate Buffer pH 4.7. After that, the volume was adjusted with water to 100 ml. Methyl Orange (0.05%) was made by diluting it with water.

2.14 Standard Curve Preparation

The 40 mg/ml standard solution was pipetted into 0, 1.5, 2.5, and 3.5 ml of four appropriate separators. After that, 20% acetic acid was added to each separator to bring its volume to 10 ml. Each separator was then filled with 1.5 ml of methyl orange and 10 ml of acetate buffer. Ten millilitres of chloroform were added after 20 seconds of shaking. After stopping, the separators were shaken for three to five minutes. The chloroform layers were allowed to rest for a few minutes before being removed into dry test tubes, dried with a tiny quantity of anhydrous Na_2SO_4 , and the absorbance was measured using 10 mm cells at 540 nm on a spectrophotometer. On the basis of these results, a standard curve was created.

2.15 Estimation of Atropine

In order to determine the amount of atropine, 200 grams of fresh plant tissue (either callus or leaves) were blended into a fine pulp using a suitable blender and 200 ml of 2% acetic acid. After adding 600 ml of 2% acetic acid to the pulp, it was put into two 500ml conical flasks and agitated for three to four hours. The alkaloid was precipitated by adding 1:2 ammonia to water until the pH reached 9–9.5 after the volume was measured and placed into a 250 ml beaker and heated to boiling. Next, the material was put into conical centrifuge tubes holding 100 ml, and the tubes were spun at 2000 rpm for 20 minutes. 1N HCl was used to dissolve the precipitate after the supernatant was extracted using a vacuum or decanter. After that, 1N HCl was used to adjust the solution to the correct level in a 100 ml volumetric flask. After filtering through filter paper into a dry vessel, 10 ml was pipetted into a tiny flask and refluxed for two to three hours on a water bath heated to 100°C for hydrolysis. After adding 10 millilitres of 1N NaOH and 25 millilitres of concentrated acetic acid to the flask, the contents were poured into a 100 millilitre volumetric flask and levelled with water. This solution equated to 10 mg of fresh plant tissue per millilitre. The process for the standard curve was followed after 3 to 5 ml of this solution were put into a separator.

3 Results and Discussion

3.1 Plant Collection and Identification

The methodology employed for collecting and preparing samples of *Datura metel* involved several meticulous steps. Fresh plant materials were carefully sourced from dry and shaded regions within Namakkal district and identified using a standardized protocol. Subsequently, leaves, internodes, and fruits underwent thorough cleaning with running tap water followed by air drying in the shade.

3.2 Preparation and Extraction of plant material

Once dried, the plant materials were finely ground into powder using a mixer and stored in appropriately labelled plastic bags. For extraction, a measured quantity of the powdered material was mixed with sterile water and gently heated before filtration through filter paper. The filtrate was centrifuged, producing a clear supernatant which was collected into sterile bottles and refrigerated for further use.

3.3 Preliminary Phytochemical Analysis

Following the analytical study, the diverse phytochemical constituents analyzed from *Datura metel* are presented in Table 1. The findings indicate an abundance of Tannins in the extract of *Datura metel*, while alkaloids, saponins, tannins, and flavonoids are moderately present. Moreover, the results suggest a high protein content in the leaf extract of *Datura metel*. The screening of phytochemical constituent in the leaf extract of *Datura metel* demonstrates the presence of alkaloids, saponins, tannins, flavonoids, proteins, among others. The preliminary phytochemical analysis of the crude powder of *Datura metel L* suggests the presence of various phytochemical compounds.

1. Alkaloids were detected using Mayer's test, showing positive results in the plant extract.
2. Flavonoids were identified through the ammonia and concentrated sulphuric acid test, with positive outcomes observed in both the plant extract and callus extract.
3. Terpenoids, detected via the Salkowski test, were absent in both extracts. Quinones, assessed using Borntrager's test, were not detected in either extract.
4. Phenols, determined by the alkaline reagent test, were present in the plant extract but absent in the callus extract.
5. Resins, identified using Whistler and BeMiller's test, were found in both extracts.
6. Steroids, examined through the Liberman-Burchard test, were absent in both extracts.
7. Tannins, analysed using the Ferric chloride test, showed positive results in the plant extract only.
8. Saponins, confirmed by the foam formation test, were present in both extracts.
9. Protein content, as indicated by Million's test, was higher in the plant extract compared to the callus extract.
10. Carbohydrates were detected in both extracts through Benedict's test and the iodine test, with positive results obtained.
11. Glycosides, identified using the Keller-Kilani test, were present in both extracts, showing positive reactions.

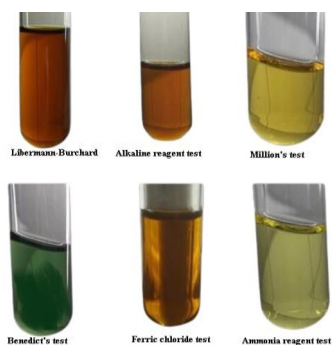


Fig. 1. Phytochemical Analysis of Plant Extract

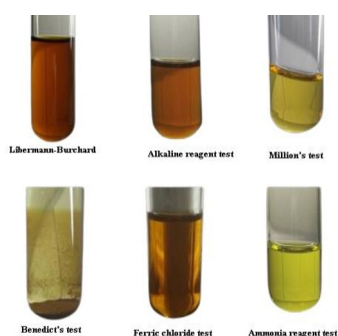


Fig. 2. Phytochemical Analysis of Callus Extract

The analgesic, antispasmodic, and antibacterial properties of basic therapeutic substances, such as pure isolated alkaloids and their synthetic derivatives, are well known (Okwu and Okwu 2004). These substances have noteworthy physiological action when given to animals. The new study suggests that *Datura metel's* widely recognized therapeutic qualities may be due to its reported alkaloid content, while the exact mode of action is yet unknown. A subclass of glycosides known as saponins is distinguished by its soapy properties (Fluck 1973). Red blood cells can precipitate and coagulate due to their aptitude to do so. According to (Abazar et al., 2022) notable characteristics of saponins are their bitterness, haemolytic activity, ability to bind cholesterol, and ability to create foams in aqueous solutions. These characteristics provide *Datura metel* leaf extract a considerable amount of therapeutic efficacy (Diallo et al., 2022).

Table 1. Qualitative Phytochemical Screening of *Datura metel* and its callus

Sl. No	Tests	Compounds for detection	Crude plant extract	Callus extract
1.	Alkaline Reagent Test	Phenols	Moderate	Moderate
2.	Ammonia reagent test	Flavonoids	Moderate	Present
3.	Benedict's test	Carbohydrate	Present	Present
4.	Borntrager's Test	Quinones	Nil	Nil
5.	Ferric chloride test	Tannins	Abundant	Nil
6.	Foam formation Test	Saponins	Present	Present
7.	Iodine test	Carbohydrate	Present	Present
8.	Keller-Kilani test	Glycosides	Present	Present
9.	Liebermann-Burchard Test	Steroids	Moderate	Moderate
10.	Mayer's Test	Alkaloids	Abundant	Abundant
11.	Million's test	Protein	Abundant	Present
12.	Salkowski Test	Terpenoids	Nil	Nil
13.	Whistler & BeMiller's Test	Resins	Present	Present

The table outlines various biochemical tests conducted on crude plant extract and callus extract to detect different compounds present in them. In the alkaline reagent test, both the crude plant and callus extracts show moderate presence of phenols. Flavonoids are detected in moderate amounts in the crude plant extract but are present in the callus extract. Carbohydrates are present in both extracts as indicated by Benedict's test and iodine test. Quinones are absent in both extracts according to the Borntrager's test. Tannins are abundantly present in the crude plant extract but absent in the callus extract according to the ferric chloride test. Saponins are present in both extracts based on the foam formation test. Glycosides are detected in both extracts as per the Keller-Kilani test. Steroids show a moderate presence in both extracts according to the Liebermann-Burchard test. Alkaloids are abundant in both extracts as revealed by Mayer's test. Protein is abundant in the crude plant extract but only present in the callus extract according to the Million's test. Terpenoids are absent in both extracts based on the Salkowski test. Resins are present in both extracts according to Whistler & BeMiller's test. It indicates variations in the presence of different compounds between the crude plant extract and callus extract, highlighting potential differences in their biochemical composition.

Tannins often called tannic acid have antibacterial qualities. Proteins can precipitate as a result of these water-soluble polyphenols, which can be present in many plant diets. They precipitate microbial proteins, making them inaccessible to nutrients, which is known to impede microbial growth (Sodipo et al., 1991). According to observations, tannins prevent the development of bacteria, viruses, yeasts, and fungus (Chung et al., 1998). In phytotherapy, nonspecific diarrhea, mouth and throat inflammations, and mild skin lesions are treated using plants that contain tannins (Westendary 2006). The presence of tannins may help heal wounds and soothe irritated mucous membranes.

According to (Salah et al., 1995) flavonoids show solid anticancer activity and anticipate cell harm caused by oxidants by acting as effective water-soluble cancer prevention agents and

foragers of free radicals. The stomach related tract contains flavonoids that lower the hazard of heart malady. These plants' flavonoids, which work as cancer prevention agents, have anti-inflammatory qualities (Okwu and Okwu 2004). This may offer assistance to clarify why *Datura metel* has long been utilized to recuperate burns, ulcers, and wounds in home grown treatment. The tall protein substance of *Datura metel* takes off is connected to numerous of the plant's helpful qualities in expansion to these auxiliary metabolites. It has been found that a assortment of bioactive proteins extricated from therapeutic plants are useful against specific illnesses (Tsao 1990). According to a few analysts (Tandon and Sharma 2010), the nearness of these phytochemical constituents likely clarifies the wide extend of restorative properties credited to both species in treating ailments and afflictions like hack, liver issues, stomach aches, skin illnesses, aggravation, jaundice, and toothaches.

3.4 Callus Stimulation

After three weeks of culture, 2,4-D, NAA, and IBA at 1.0 mg/l caused callus production from *Datura metel's* leaf, flower, seed, and stem tissues (Figure 3). On the other hand, in the medium lacking PGR (control), these explants did not develop callus. In comparison to 1.0 mg/l IBA or 2,4-D, a larger percentage of explants developed callus in the media supplemented with 1.0 mg/l NAA. Around the borders of the sliced explant tissue region, callus began to develop.

Table 2. *Datura metel* Callus Growth (g) on Various PGR

Plant Growth Regulator	Stem	Fruit	Leaf	Flower
IBA	69	65	41	43
NAA	38	36	18	21
2,4-D	63	61	42	57

The table 2 illustrates the callus growth (measured in grams) of *Datura metel* tissues cultivated with different plant growth regulators (PGRs) - IBA, NAA, and 2,4-D. The data shows the varied response of different tissue types including stem, fruit, leaf, and flower to these growth regulators. For instance, under the influence of IBA, the highest callus growth was observed in stem tissue (69g) followed closely by fruit tissue (65g), while leaf and flower tissues showed relatively lower callus growth (41g and 43g respectively). NAA, on the other hand, resulted in comparatively lower callus growth across all tissue types, with the highest observed in stem tissue (38g) and the lowest in leaf tissue (18g). In contrast, 2,4-D induced robust callus growth in all tissue types, with the highest values observed in flower tissue (57g) and stem tissue (63g), indicating its effectiveness in promoting callus formation across *Datura metel* tissues.

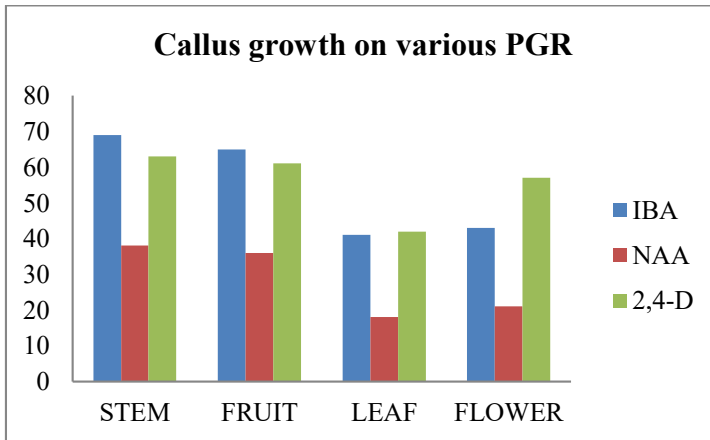


Fig. 3. *Datura metel* Callus Growth (g) on Various PGR



Fig. 4. Callus from *Datura metel*'s Leaf



Fig. 5. Callus from *Datura metel*'s Fruit



Fig. 6. Callus from *Datura metel*'s Flower



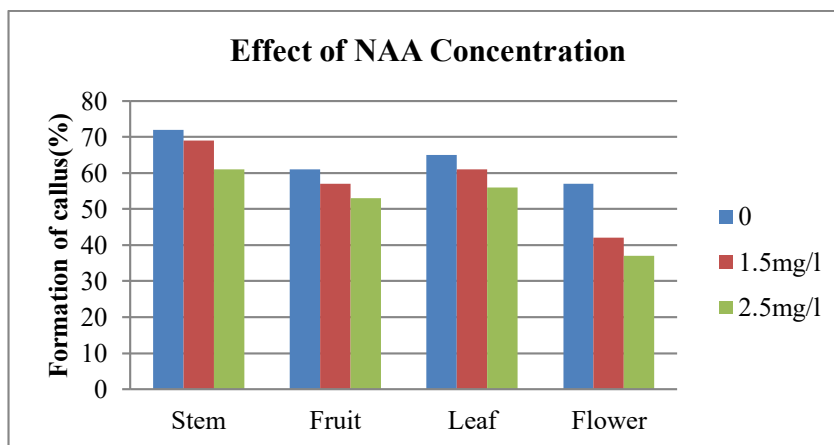
Fig. 7. Callus from *Datura metel*'s Stem

The stem explant yielded the highest percentage of white compact callus, as illustrated in above figures. In contrast, the petiole produced brownish friable callus, while the other two explants produced greenish friable calli with high vacuolation. A small concentration of the growth regulator NAA had a significant effect on the callus mass of *Datura metel*. Among the three concentrations tested (0.5, 1, and 2 mg/L), the smallest concentration (0.5 mg/L) demonstrated a greater ability to induce callus mass in *Datura metel* compared to the higher concentrations across all plant parts studied, as shown in above Figures.

Table 3. Percentage of callus formation on the effect of NAA Concentration on *Datura metel*

NAA Concentration	0 mg/l	1.5mg/l	2.5mg/l
Stem	72	69	61
Fruit	61	57	53
Leaf	65	61	56
Flower	57	42	37

Table illustrates the percentage of callus formation in response to varying concentrations of NAA on different tissues of *Datura metel*. The concentrations tested include 0 mg/L (no NAA), 1.5 mg/L, and 2.5 mg/L. Across all tissue types, including stem, fruit, leaf, and flower, there is a general trend of decreasing callus formation percentage with increasing NAA concentration. For instance, in stem tissue, callus formation decreased from 72% at 0 mg/L NAA concentration to 61% at 2.5 mg/L. Similarly, in fruit tissue, callus formation decreased from 61% to 53% over the same concentration range. Leaf and flower tissues also exhibited a similar pattern of decreasing callus formation with increasing NAA concentration, indicating the inhibitory effect of higher NAA concentrations on callus induction in *Datura metel* tissues.

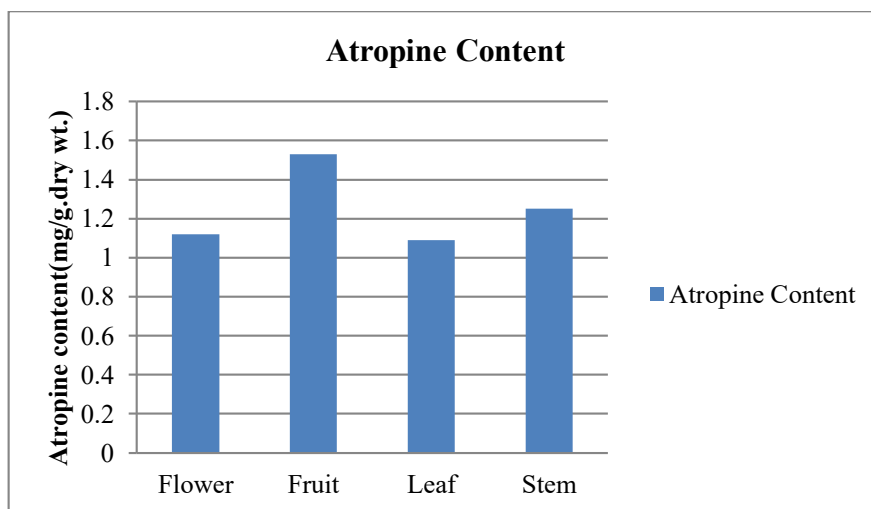
**Fig. 8.** Effect of NAA Concentration on *Datura metel* Callus Growth

3.5 Atropine contents

At 540 nm, the spectrophotometric strategy was utilized to degree the atropine concentration of the rough extricate that was extricated from callus tissues. As appeared in Fig. 8, the discoveries appeared that in spite of the fact that stems had the biggest extent of callus, the rough extricate taken from callus developed from takes off had the most elevated Atropine concentration. It was clear that the calli's add up to calline substance was much bigger than the plant extract's normal sum. It is apparent from this that in vitro culture has the capacity to increment the blend of atropine.

Table 4. Atropine Content in Various Sources of Calli (mg/g Dry Weight)

Sl No.	Source of Calli	Atropine Content
1.	Stem	1.25
2.	Leaf	1.09
3.	Fruit	1.53
4.	Flower	1.12

**Fig. 8.** Atropine from various *Datura metel* Callus

In the table 4 the atropine content in different sources of calli obtained from *Datura metel*, measured in milligrams per gram of dry weight. The atropine content varies among the sources: stem calli contain 1.25 mg/g, leaf calli contain 1.09 mg/g, fruit calli have the highest atropine content at 1.53 mg/g, and flower calli possess an atropine content of 1.12 mg/g as shown in figure 9. That the atropine levels differ depending on the tissue source of the calli, with fruit-derived calli exhibiting the highest concentration, followed by stem, flower, and leaf-derived calli.

4 Conclusion

The comprehensive analysis conducted on *Datura metel* extracts and callus cultures provides significant insights into their phytochemical composition and potential medicinal applications. Thorough collection and preparation of plant samples ensured the integrity of the experimental process, facilitating accurate analysis. The phytochemical screening revealed varying levels of alkaloids, saponins, flavonoids, tannins, and proteins in both the crude plant extract and callus extract, shedding light on the diverse bioactive compounds present in *Datura metel*. Tannins were abundant in the plant extract, while alkaloids exhibited a higher concentration in the crude extract compared to the callus. These researches underscore the pharmacological potential of *Datura metel*, particularly in traditional herbal medicine. The successful induction of callus from various plant tissues demonstrated the feasibility of in vitro culture techniques for mass production of bioactive compounds. Callus

derived from leaf tissue exhibited the highest atropine content, indicating the efficacy of callus cultures as a potential source of medicinal alkaloids. The spectrophotometric analysis provided quantitative data on atropine content, highlighting the potential for optimizing in vitro culture conditions to enhance alkaloid yield. The alkaloid content in the crude plant extract was notably higher than compared to the callus extract, indicating the importance of optimizing culture conditions to maximize alkaloid production. The presence of tannins, flavonoids, and saponins in both extracts suggests their potential pharmacological significance in *Datura metel*. The results of the callus induction experiments further emphasize the feasibility of using tissue culture techniques to manipulate and enhance the production of bioactive compounds in *Datura metel*. The quantitative estimation of atropine content in callus tissues provides valuable data for future optimization studies aiming to upscale alkaloid production through in vitro culture methods.

Declarations

I hereby declared that this manuscript was my original work and had not been previously submitted or published elsewhere. All sources and references used in the preparation of this manuscript were duly acknowledged. I confirmed that I had complied with all ethical standards related to research, and that there were no conflicts of interest related to the content of this manuscript. I further confirmed that the data presented in this manuscript were accurate and had not been manipulated or fabricated in any way. Each author contributed to the study design, data collection, analysis, and manuscript writing. The authors are thankful to the Management and Principal of K.S.Rangasamy College of Technology, Tiruchengode, Namakkal District, Tamil Nadu, India for the support given to carry out this study

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