An Assessment of *in vitro* Propagation and Medicinal Properties of *Datura stramonium* (Dhatura)

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ABSTRACT

*Datura stramonium* is a well-known traditional herb with therapeutic properties. It is despically grown wildly and has both noxious as well as medicinal properties. This herb has proven to have very great pharmacological prospects with great utility in old medical practices as well as in the current medical field. It has been scientifically confirmed that *D. stramonium* contains alkaloids, also known as belladonna alkaloids. In the near future, this herb will be used to treat a variety of other human diseases. This herb has contributed to many pharmacological activities in the scientific discipline worldwide and the Indian medical system, including anti-asthmatic action, anti-inflammatory activities, anti-cholinergic activities, etc. *In-vitro* micropropagation is being practiced to grow a large number of datura plants to produce high-quality alkaloids naturally for the pharmaceutical industry which has not been practically realistic in the past.

**Keywords:** *Datura stramonium*, micropropagation, *in vitro* propagation, medicines, anti-asthmatic, virucidal activity.

INTRODUCTION

Treatments for human sickness and trauma caused by diseases, plants, and herbs have played a vital role worldwide from the ancient period and can be traced back at least 60,000 years (Shi et al., 2010). Due to the current appreciation for naturally cultivated products, the demand for medicinal herbs is rising steadily in both developed and emerging nations. Modern herbal medicine is crucial to both traditional and contemporary medical systems (Schultes and Hofmann, 1979).

*Datura stramonium* is an annual plant of the Solanaceae family that may be found all around. It originated in Central America and spread to other parts of the world as an aggressive invasive weed in temperate climate zones. Datura is known by several names in different parts of the world, but it is often known as Thorn apple, Jamestown weed, Jimson weed, stinkweed, Devil’s weed, Devil’s trumpet, Tolguacha, Moonflower, etc.

Plant parts have reportedly been used by some sadhus and Aghoris for entheogen and ordeal poison. It is blended with different hallucinating plants like cannabis and *Aconitum ferox* which is a poisonous plant and creates dysphoric experiences or to attain spiritual liberation (moksha) in the position of extreme discomfort and horror (Rätsch and Christian, 2005). In different cultures, it is used for sacred and visionary purposes, but in between jimson weed has also garnered a reputation for its magical purposes in different regions around the world throughout history. Its uses are also reported in medieval European witchcraft and England and parts of the colonial Northeastern U.S. during the early modern era for creating hallucinogenic effects of magical or lycanthropic salves.
and potions (Schultes and Hofmann, 1979). In some regions, it is also seen as unlucky and improper to grow as it was considered to be an aid to incantations in one’s backyard and lawns (Chopra et al., 1982). In India, it is commonly known as “Datura” and known to be sacred and used for spiritual and occult practices in Hindu culture as it is believed to be the favorite of the Shiva in Shaivism in Hindu mythology.

It is one of the folklore medicinal herbs which is widely well-known for its properties. It is grown in wild and found as a local source having an abundance of various tropane alkaloids which contain a methylated nitrogen atom (N-CH₃) and include the anticholinergic drugs atropine, and scopolamine. For the treatment of asthma and sinus infections, an extract of this plant made from the leaves is taken orally, and for the treatment of burns and ulcers, swellings, the stripped bark is applied externally. It is also practiced for the stimulation of the central nervous system, treatment of skin infections, and treatment of dental and toothache due to its anti-inflammatory property. If mistakenly consumed it is reported as a hallucinogenic plant that causes serious poisoning. A severe anticholinergic reaction can be seen in the individual that may lead to toxicity and poisoning results after consumption of any part of the plant. Many poisoning cases have been reported after eating the berries. Ingestion of approximately 125 seeds may lead to death occur from heart failure, because the plant seeds contain the highest concentration and have a rapid onset of action, thus it may be potentially useful as an alternative to atropine for the treatment of the muscarinic symptoms of organophosphate toxicity and some symptoms of central anticholinergic effects. *Datura stramonium* is an annual, erect plant that can form a bush up to 5-6 ft having stout, erect, smooth, and leafy stems which can be of yellow-green to reddish-purple color. The leaves are soft, smooth, irregularly undulated, and toothed having an underside in light green color, whereas the upper surface of the leaf is dark green. It produces long trumpet-shaped, white, creamy to violet color flowers throughout the summer which are fed upon by nocturnal insects after releasing pleasing fragrance during the night. The pod is 3-8 cm in diameter containing round-shaped seeds covered with thorns. It cracked open into four chambers, each with dozens of small seeds, black-colored at maturity (Chopra et al., 1982).

**PHYTOCHEMICAL COMPOSITION OF DATURA**

Major phytochemicals like carbohydrates, crude fiber, fats, protein, moisture, and ash content (Sharma et al., 2021) along with tannins, cardiac glycoside, alkaloids, phenolic and flavonoids compounds can be found significantly in the datura plants (Bagewadi et al., 2019; Fatima et al., 2015) whereas from the seeds of datura fruit amino acids such as alanine, phenylalanine, glutamate, and tyrosine can be extracted (Al-Snafi, 2017).

Datura plants are rich in tropane alkaloids such as atropine, scopolamine, hyoscyamine, and hyoscine which are found abundantly in every plant part. Its alkaloid content can be varied with the age and growth stage of the plant part at the time. In the case of *D. stramonium*, the plant has a high number of alkaloids after 10-11 weeks of germination of seeds and gradually decreases during the generative stage of plant growth (Al-Snafi, 2017; Al-Snafi, 2021). In general alkaloids concentration differentiate between the plant parts in different aging stages for example leaves contain higher concentrations during the vegetative stage and decreased towards the generative stage (Sharma et al., 2021; Alinejad et al., 2020; Oseni, 2011). Hyoscyamine can be a significant component in the stems and leaves of young plants. However, in young and adult plants the concentrations of atropine and scopolamine differ in different plant parts (Sharma et al., 2021). Chemical structure of the three alkaloid compounds found in the species of datura plant naturally.

**Atropine (C₁₇H₂₃NO₃)**

Atropine is a natural chemical produced by the datura plant which is anticholinergic and lessens the secretions and used for the treatment of different medical cases involved with excessive secretion of mucous, saliva, bladder and intestine problems, ulcers, Parkinson’s disease, heart-related disorders, irritable bowel syndrome (IBS) and patient suffering from brain tumors. Overdose of atropine can show some symptoms like migraine, dizziness, blurry vision, heartburn, nasal congestion, constipation, difficulty in urination, and cause more damage by having high body temperature, irregular heartbeat rate, rashes, and eye pain (ophthalmalgia) (Sharma et al., 2021).

**Hyoscyamine (C₁₇H₂₃NO₃)**

Hyoscyamine is a drug similar to atropine and also produced naturally by the plant which is used for medication problems related to gastrointestinal disorders. It helps to lessen the movement of the stomach and intestine and prevent the excess secretion of acid found in the stomach. Like atropine, it is also prescribed for peptic ulcers, colic, IBS, and diverticulitis-like disorders. Hyoscyamine is used for the treatment of rhinitis, runny nose, or sinus disorder. If it is taken in the excess amount it can cause dizziness and drowsiness which can lead to headaches and blurring of vision, sensitivity to light, dry mouth, and high body temperature, and serious effects.
can be seen as skin rashes, eye pain, rapid heart rate, etc., (Sharma et al., 2021).

Hyoscine or Scopolamine (C17H21NO4)
Hyoscine is the third natural chemical compound found in the parts of the datura plant which is also known as Scopolamine, which is used for the treatment of motion sickness like vomiting, nausea, etc. It is usually used as a patch which can be used for up to 3 days prescribed by the physician. Excess consumption can lead to drowsiness, disorientation, hallucinations, confusion, burring of eyesight, rapid heartbeats, and fever (Sharma et al., 2021).

MEDICINAL PROPERTIES AND POSSIBLE TREATMENTS OF HUMAN DISEASES
Atropine is also known as anti-cholinergic and is found as one of the primary active agents in the D. stramonium with hyoscyamine, scopolamine, and tropine alkaloids which are found in the family Solanaceae and widely used for its medicinal purpose over centuries by physicians in the different parts of the world. In the Datura species, the major tropine alkaloids hyoscyamine and scopolamine with several minor tropine alkaloids have been identified. Tigloidin, aposcopolamine, apoatropin, hyoscyamine N-oxide and scopolamine N-oxide 17-20. 6â-ditigloyloxytropane and 7-hydroxyhyoscyamine are identified for the first time (Das, 2012) and are some examples of minor alkaloids present in D. stramonium.

The young leaves produce mainly scopolamine whereas hyoscyamine becomes the major constituent in mature leaves of the plant which has pharmaceutical effects against cholinergic, asthmatic and spasmolytic diseases. Atropine was mainly used for the treatment of the nervous system and also for the treatment of diseases like Parkinson, peptic ulcers, bronchial asthma, diarrhea, and poisoning due to nerve gas (Abd El-Rahman et al., 2008).

Traditional medicine
In China, doctors used the plant’s leaves as anesthesia during performing surgery as traditional medicine. Dried leaves are widely popularized among the people who generally smoked them using pipes or as cigarettes and especially gain attraction during the 18th century by the General of the East India Company was an English physician, James Anderson who learned about these practices and spread this in the regions of the European continent.

Treatment of Respiratory diseases
Asthma is one of the respiratory diseases having a hard time finding its cure and during the 19th century Henry Hyde Salter suggested that D. stramonium could be used for the treatment of asthma in his work “Asthma: its Pathology and treatment”. In the early 20th century smoking dried leaves of D. stramonium and other herbs established its medicinal use for temporary relief to asthmatics. In the tribe, Datureae (among other plants) Muscarinic antagonists were found, such as atropine, and in some cases of chronic obstructive pulmonary diseases synthetic tropane derivatives selective for muscarinic acetylcholine receptor subtypes such as ipratropium bromide and tiotropium bromide, are prescribed (Chapman, 1991).

Anti-inflammatory activity
In 1597 John Gerard’s Herball states that “The extract of Thornapple pod, cooked with hog’s grease, cure the all inflammations and all manner of burnings and scalding, caused by water, fire, gunpowder, boiling lead as well as the burns which comes by a lightning strike in a very short period.

During the early 19th century William Lewis mentioned, that the fruit juices of the plant could be used as a very powerful remedy in various disorders like convulsive and spasmodic, seizures and fits caused by epilepsy and mania, and was found to give ease in external swellings and piles (hemorrhoids). The bark of the datura stem is used for the treatment of human diseases but it is found to be effective on burns, ulcers, inflammatory wounds, bruises, and swelling.

Anti-cholinergic Activity
In the distinct parts of the plant D. stramonium, the presence of atropine, scopolamine, and tropine alkaloids is used clinically as an anti-cholinergic agent. It is noted as a drug addiction and can lead to poisoning accidentally in human beings and animals if consumed. Symptoms of poisoning by the plant are xerostomia (dryness of the mouth) and dehydration, blurred vision, rapid heartbeat, restlessness, and hallucination, and can result in loss of consciousness in humans and animals (Taha and Mahdi, 1984; Diker et al., 2007; Boumba, 2004; Soni et al., 2012).

Antimicrobial Activity
The extract of D. stramonium in methanol shows the activity opposed to the gram-positive bacteria is concentration-dependent but it shows less or no antimicrobial action against the Escherichia coli and Pseudomonas aeruginosa (Takhi et al., 2011; Rautela et al., 2018).

Vibriocidal activity
Different plant parts of D. stramonium are used for treating cholera and gastrointestinal diseases in traditional medicines. 12 isolates of Vibrio Cholerae non-01 and Vibrio parahaemolyticus were selected from the standard strain of Vibrio Cholerae from an in vitro assay. Aqueous
and organic solvent extracts of different plant parts were tested by using the disk diffusion method; D. stramonium served as broad spectrum virobiocidal agents with other plants Lawsonia inermis, Saraca indica, Syzygium cumini, Terminalia belerica, Allium sativum, and D. stramonium (Sharma et al., 2009).

**Antifungal and biopesticide activity**

D. stramonium leaf extract showed antifungal activities against the Fusarium oxysporum a type of fungal pathogen responsible for the wilt of pigeon pea plant (Cajanus Canjan L.) with other 7 plants during the experiments which can be used as biopesticides. All Leaf extracts having a higher concentration of ethanol of the eight plants showed complete inhibition in linear growth and sporulation in test fungi in vivo and in vitro conditions (Khandare, 2011; Rautela et al., 2018).

**Preventative agent in critical organophosphate toxicity**

Treating patients who had exposure to an organophosphate substance can exhaust the supply of atropine from a hospital. Long-term exposure to organophosphates toxicity can result in headaches, confusion, anxiety, memory loss, loss of appetite, diarrhea, depression, and so on. D. stramonium contains atropine and other alkaloids having anticholinergic compounds which can be easily available as it is common. Extract collected by boiling the crushed seeds of the plant can be used recreationally for the effects of anticholinergic. For the treatment of OP poisoning, this extract can be useful as it has a rapid onset of effects. Pre-treatment of the patient with D. stramonium extracts followed by severe dichlorvos exposure can significantly increase the survival chances of the patient (Bania et al., 2004).

**Mosquito repellent properties**

Extracts of leaves of D. stramonium plant in ethanol solvent results in repellent activity to mosquitoes as well as larvicidal against the Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus species of mosquitoes. It is found that LD$_{50}$ values for larvicidal activity against Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus are 86.25, 16.07 and 6.25 mg/L respectively. A higher concentration (1%) of the leaf extract of D. stramonium in ethanol provided complete protection time (mosquito repellency) against Aedes aegypti, Anopheles stephensi and Culex quinquefasciatus of 2.7, 71.7 and 117.7 min (Swathi et al., 2012; Sharma et al., 2016).

**Toxic concentration in Datura species**

D. stramonium is used for its medicinal purpose by doctors or physicians from ancient periods, but it is still categorized as a poisonous plant that can be harmful to humans after consumption. Every plant part of the datura contains dangerous alkaloids and toxins and can vary between young and older plants. Seeds of the plant are usually containing more toxins that cannot be destroyed even after boiling and trigger severe anticholinergic effects which usually lead to toxicity.

Different plant parts retain some toxicity and can be varied from species to species, parts of the plant consumed, or age and growth stage of the plants as mentioned above and its habitat and weather can also play a part in the concentration of toxicity in the plant.

Many reports were recorded on the toxicity of datura in different regions of the world. Plant leaves were consumed as piped cigarettes and usually used for the cure of respiratory diseases but overdose from the seeds or any other plants can lead to fatal conditions (Pennacchio et al., 2010) where the person suffers from severe headaches, hyperthermia, high and harmful changes in heartbeats, violent behavior, hallucination, pupil dilation, which leads towards the anticholinergic delirium where the patient cannot associate with the reality and can leads to death of the consumer if not getting proper medical attention.

Even if one is consuming datura in regular practice it is reported that it affects the central nervous system, as well as mental health in which one suffer through the anxiety, fatigue, confusion, paranoia, amnesia, delirium, low body energy, hallucinations, time distortion, psychosis, trouble speaking, loss of motor control, irregular heartbeat ratings, high blood pressure, and body temperature, increase in perspiration, can be seen as side effects which generally occur around 30 minutes to 1 hour after the ingestion of the herb and can be generally up to 24 to 48 hours, but there have been some cases to last as long as a fortnight (Pennacchio et al., 2010).

**Anti-cancer activity**

There are still ongoing researches on the plant chemical compounds which shows the potential of the medicinal plant in the upcoming future. One of the studies is going on for its anti-cancer activity. Cancer is a widespread disease that is relentlessly affecting human society for a very long period around the globe. In males lung, prostate, and colon cancer are more frequent while in women breast, ovarian cancer and stomach cancer are more common which can lead to a fatal and painful death. There are different treatments with medicines present in the pharmaceutical industry but it is high priced and time-consuming which cannot ensure the permanent cure of the disease. The anti-leukemic potential of extracts was assessed through different parameters like hematological, histological,
endogenous, biochemical, and antioxidants. Leukemia is a type of blood-forming tissue cancer including bone marrow from which people are suffering all around the world it can be only cured by blood transfusion temporarily and results in the transplant of new bone marrow from the donor which is costly and time-consuming. Further research on the datura plants can become the first step toward the discovery of a new treatment for cancer patients which can be reliable and natural (Abdulla, and Gruber, 2002; Nasir et al., 2020).

**IN VITRO APPLICATION AND METHODOLOGY**

*In-vitro* propagation is opening new doors for scientists to research in variant fields of science in which a large number of plants can be grown by applying this practice anywhere in the world using single plant parts such as tissue, embryo, leaves, stems, and seeds without concerning the meteorological factors of the region and other physical conditions like seed dormancy, plant extinction. The practice of *in-vitro* propagation of the datura plant is applied to producing large-scale plants having desired traits that can be used to harness their medicinal potential for the development of medicines. Which is found to be produced in higher concentration during *in-vitro* culture under controlled culture conditions. Furthermore, the establishment of cell culture of the datura plant holds considerable potential in the upcoming years as an alternative for the production of new secondary metabolites (Cocce et al., 2008; Rautela et al., 2020; Bajpai et al., 2021).

*In vitro* propagation will be giving the opportunities to explore the sustainable way to the production of hyoscyamine and other alkaloids. These techniques help to produce a multiple numbers of plants for green-house propagation in the field and also a sustainable source using *in vitro* callus cultures for producing secondary metabolites (Siddiqui et al., 2017).

In 1973 the first *in vitro* culturing was reported on the different species of the Datura plant, where growth regulators of different chemical composition (2,4-D, kinetin, and Gibberelllic acid (GA₃) were used to assess the potential to promote the growth of regenerated shootlets from callus cultures (Engvild, 1973). Carrying out the *in-vitro* technique especially from the cell or callus culture to process the hyoscyamine and alkaloids can contribute to enhancing their production rate (Abd El-Rahman et al., 2008).

Only limited research was taken placed on the *in vitro* technique of growing callus culture to induce the production of hyoscine or hyoscyamine. Intriguingly the amount of hyoscyamine is varied between the hairy root cultures of different Datura spp. and *D. innoxia* was mentioned to contain a higher number of alkaloids than its other species (Harfi et al., 2016; Rautela et al., 2018).

In a practice, it resulted that plants produced a substantial number of alkaloids and hyoscyamine in comparison to wild-type Datura spp. It is a natural product that is clean and eco-friendly and doesn’t need to depend on meteorological conditions. Thus, higher production can be achieved by the tissue culture forming calli without reducing the quantity. Fresh green calli followed by the leaves and old brown calli produce the highest amounts of alkaloids (Siddiqui et al., 2017).

**Preparation of plant material**

**Collection of ex-plant**

Fresh plants or leaves can be collected locally during the month of April–May when the plant is at the flowering stage. The plant should be identified and described by well-experienced professionals having years of experience in this field or any well-reputed botanical institutes which can assign the voucher number to the specimen and keep it in the herbarium for future preferences.

**Sterilization of ex-plant**

Whole leaves should be washed lightly under running tap water with mild soap/detergent. 0.1% (w/v) HgCl₂ solution work as surface sterilization in which leaves are soaked for 2-3 second and washed with the autoclaved water around 3-5 times and dried on sterile filter paper. This procedure is done under a sterile condition in laminar flow to minimize contamination. Now dried leaves would be cut into small pieces around 1.5x1.5 cm and transferred to callus induction media (Rautela et al., 2018).

**Preparation of culture media**

MS (Murashige and Skoog) media is generally used for callus induction from the leaves of the *D. stramonium* plant with different combinations of plant growth hormones (PGH). For proper media setting, the pH of the media should be 5.8 before autoclaving for 15 minutes at 121 °C. For inducing shoots, MS media containing various combinations of PGH like 6-benzyl amino purine (BAP), kinetin, and Indole-3-butyric acid (IBA), and for inducing rooting the grown shoots around 2-3cm were transferred to another MS media containing various combination of IBA. The cultures should be incubated with a 16/8 h photoperiod at 25 ± 2 °C in a sterile condition. The callus culture is subcultured around every three weeks in the same conditions mentioned above for no contamination and to track down the progress of callus culture (Kinsara, 1994; Rautela et al., 2018; Sundriyal et al., 2021).
Formation of callus
It takes around 15-20 days for the callus to grow around the explant placed in the MS media in a sterile condition.

Shootlet induction
After some time, shootlets start to grow from the callus using combinations of PGH in the MS media which induces the callus culture to grow shootlets, after shootlets reach the height of 2-3cm the plant is ready to transfer to another MS media for rooting.

Root induction
Once the callus culture contains shootlets transfer it to the MS media having a combination of different PGH in media that help to induce the rooting under sterile conditions. It takes about 2 weeks for root formation. After attaining proper growth, the plant is now ready for transplant in the pots in a glasshouse for hardening.

Regeneration and hardening
The grown plant with roots should be removed carefully from the MS media and washed gently with distilled water to discard any solid media stuck around the plant. For hardening prepare a pot containing garden soil, farmyard manure, and vermiculite free from any pest or infectious micro-organism, transfer the regenerated plant to the pot and cover it with plastic film to maintain humidity for the fresh plantlets. Around 10-12 days transfer the plants which are now soil established in the glass house and after that transfer to the larger area or pots filled with mixed soil and sand in equal proportion under greenhouse conditions (Gaurav, et al., 2015, 2016, 2018; Gaurav and Kumar, 2019; Bhoora, et al., 2015; Pant, et al., 2020, 2021, 2022; Sharma et al., 2022; Saini et al., 2021, 2022; Verma et al., 2021; Rawat et al., 2021).

FUTURE PROSPECTUS
D. stramonium has traces as it was used in ancient medical practices widely all around the world and now it is also used in modern medicines to cure a wide range of human diseases (like Ashwagandha, Mentha, Nardostachys jatamansi, Adhatoda vastica, Mucuna puriens, bubble bush, Nyctanthes arbor, and other important medicinal plants) due to the presence of tropane alkaloids which is effective remedies for the diseases. It is still practiced with caution as the plant is toxic and with a little mistake, it can cause severe damage to the nervous system and the physical condition of the body after intoxication. In the past, there are several studies are done on plants which now give us some insight into the chemical compounds which can be now extracted and used in modern medicines with proper precaution. Other studies show the phytochemical analysis with antimicrobial, antifungal properties of the datura plant extracts, which can be further used for the cure of different diseases that arises from the microscopic micro-organisms.

Declaration: We also declare that all ethical guidelines have been followed during this work and there is no conflict of interest among authors.

CONCLUSION
In view of different results from the research on the D. stramonium plant for its medicinal purpose, it is clear now that plant of datura species contains chemical compounds which are naturally produced by the plant parts that can be used as a potential drug for curing the diseases and disorders faced by the humans for a very long period and new and ongoing researches opening the new doors for the treatments of new and profound diseases which is harmful to the human race. This wild weed has shown its potential as a medicinal plant and is now grown by In-vitro process for its better quality of alkaloids, but it is toxic and has taken several lives when it was consumed in excess, and individual experiences severe effects like dizziness, amnesia, high body temperature, rapid and irregular heart rates, eye pain and other symptoms. It should be used as a medicine prescribed by doctors.

REFERENCES
Al-Snafi, A.E. (2021). Medicinal plants alkaloids,


and abuses of plant-derived smoke: Its ethnobotany as hallucinogen, perfume, incense, and medicine. Oxford University Press.


Rautela, I., Parveen, A., Singh, P. and Sharma, M.D. (2018). GC-MS Analyses of Ethanolic Leaf Extract of...


