Datura stramonium leaves: A Potential Element of Anti –Microbial Activity

Aniket Walia and *Neetu Pandey

Department of Applied Chemistry and Basic Sciences

Sardar Bhagwan Singh University Balawala, Dehradun Uttarakhand, India

*Email: neetu_bhtt@yahoo.co.in

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Abstract- Plant have benefited as an alternative medicine in treatment and prevention of diseases. Medicinal plants like *Datura stramonium* are assessed for phytochemical components and anti-microbial activity. Plants have important medicinal components like tropane, alkaloids, amino acids, tannins, carbohydrate. The components phyto-chemicals are used to cure different human diseases like skin disorder, ear pain, cough, fever, burns and asthma. In the present study the experiments were performed on phytochemicals and antimicrobial activity on leaves of *Datura stramonium* using various specific extracts like petroleum ether, chloroform, ethanol and water, crude extract to indicate the presence of flavonoids, terpenoids, glycosides, etc. Mostly antimicrobial activity was studied by Disc diffusion method. In antimicrobial activity, extracts were found active against pathogens like Bacillus azotoformans, Staphylococcus aureus, Bacillus pasteurii and Pseudomonas aeruginosa

Keywords: *Datura stramonium*, Phyto-constituents, Phytochemicals, antimicrobial activity

Introduction

Plants have always played a major role in the treatment of human traumas and diseases worldwide. The demand for medicinal plant is increasing in both developed and developing countries due to growing recognition of natural product. Herbal medicine is an important part of both traditional and modern system of medicines[1]. *Datura stramonium* is a widespread annual plant from the Solanaceae family. It is one of the widely well-known folklore medicinal herbs. It is a

wild growing flowering plant and was investigated as a local source for tropane alkaloids which contain a methylated nitrogen atom (N-CH3) and include the anti-cholinergic drugs atropine, and scopolamine. From ancient civilization it was traditionally used for religious visionary purposes throughout the world and used by witchcraft in medieval Europe. The God lord Shiva was known to smoke Cannabis and Datura. People still provide the small thorn apple during festivals and special days as offerings in Shiva icons at temples. An extract made from the leaves is taken orally for the treatment of asthma and sinus infections, and stripped hark are applied externally to treat swellings, burns and ulcers. The incidence of *D. stramonium* poisoning is sporadic with a cluster of poisoning cases in the 1990s and 2000s, the United States media reported some cases occurring mostly among adolescents and young adults dying or becoming seriously ill from ingesting. Some medicinal uses of the plant are its anti-inflammatory property, stimulation of the central nervous system, respiratory decongestion and treatment of dental and skin infections, alopecia and in the treatment of toothache. It is a hallucinogenic plant that causes serious poisoning. Consumption of any part of the plant may result in a severe anti cholinergic reaction that may lead to toxicity and occasionally cause diagnostic difficulties. Cases of poisoning have been reported after eating the berries. Death may occur from heart failure after ingesting its seeds, because the seeds contain the highest concentration and has a rapid onset of action, thus may be potentially useful as an alternative to atropine for the treatment of the muscarinic symptoms of organo-plasphate toxicity and some of central anti cholinergic effects. The wide distribution, the strong toxicity and the potential for occurrence in foodstuffs are responsible for the numerous incidents in humans[2]. Datura genus distributes over tropical and warm temperate regions of the world. About ten species of Datura are found, of which Datura anoxia and *D. stramonium* are most important drug plants. Datura has long been known as a medicinal plant and as a plant hallucinogen all over the world. Pre-historic use of Datura in medicinal and ceremonial rituals could be observed in aboriginal in Indian sub-continent[3]. The therapeutic activities of most plants are due to the presence of one or more of such components like alkaloids, tannins, saponins and cardiac glycosides. The phytochemical screening revealed the presence of saponins, tannins, steroids, alkaloids, flavonoids, phenols and glycosides[4]. Atropine and scopolamine are competitive antagonists of muscarinic cholinergic receptors and are central nervous system depressants. All parts of the

plant are toxic, but the highest amount of alkaloids is contained in the ripe seeds[5]. Many cases of accidental poisoning by *D. stramonium* have been reported when these plants were eaten accidentally[6]. Medicinal plants have no doubt remained the major sources of traditional medicine worldwide. The main objective of this research work is to analyze the various solvent extracts obtained from the leaves samples of *Datura stramonium* and to qualitatively screen them for phytochemicals using standard tests. Successful extraction, determination and isolation of biologically active components from plant material are largely dependent on the type of solvent[7] used.

Material and Methods

Collection

Datura stramonium plant samples were collected from Shamsergadh, Balawala, Dehradun, Uttarakhand, India.

Authentication

Plant has been authenticated from Botanical Research Institute, Dehradun, India.

Preparation of Plant Extracts

The fresh plant leaves samples were collected, washed individually under running tap water and dried in an oven at 50 °C for 3 days. The dried leaves material was ground into powder using an electrical blender. About 100 grams of dry powdered plant leaves material was taken and subjected to extraction by soxhlation method using various solvents like petroleum ether, chloroform, ethanol and water. Extracts were then concentrated by air distillation and the concentrated residual extracts were stored at 4 °C in a dry airtight container until further use[8].

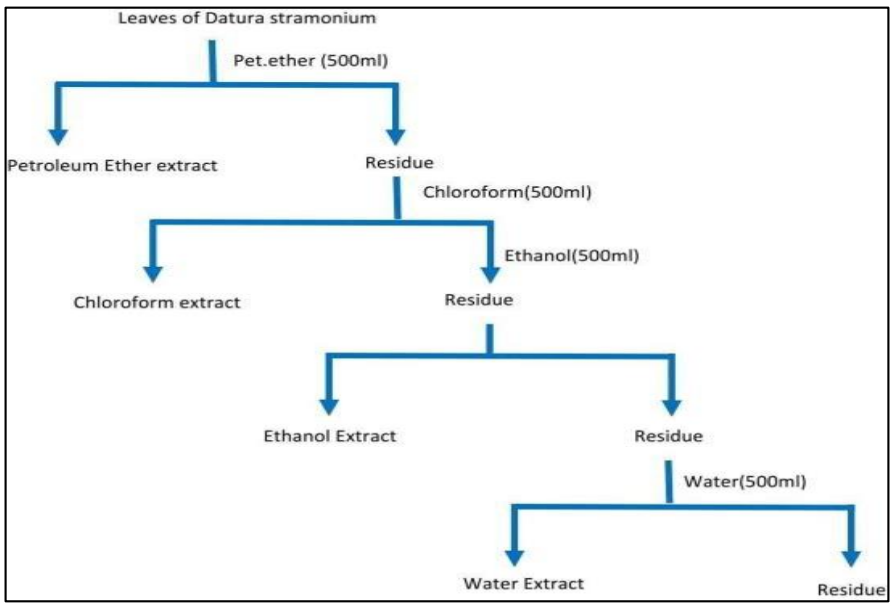

Figure- 1 Extraction Flow Chart

Figure- 2 Soxhlet apparatus

Figure-3 Air distillation assembly

Phytochemical Screening[9-18]

Crude plant leaves extracts were subjected to preliminary screening for the presence of active secondary metabolites. Each plant extract was tested individually with specific chemical reagents according to standard procedures. Visible color change or precipitate formation was taken into consideration for the presence (+) or absence (–) of particular active constituents.

The following tests were carried out to identify the various phytochemical constituents:

The petroleum ether, chloroform extract, ethanol extract and water extract were subjected to preliminary phytochemical screening for the detection of various phytochemical constituents such as alkaloids, steroids, terpenoids, flavonoids, carbohydrates proteins, and amino acids.

Determination of Saponins

Each of the plant leaves extracts (0.4g) was separately stirred in a test tube, foaming which persisted on warming was taken as an evidence for the presence of saponins.

Determination of Tannins

Extract of each sample (0.4g) was separately stirred with 10ml of distilled water and then filtered. To the filtrate was added two drops of 4% Iron (III) Chloride ($FeCl_3$) reagent. Blue – black or blue – green coloration or precipitate was taken as an indication of the presence of tannins.

Determination of alkaloids

Each sample (0.4g) was separately stirred with 1% hydrochloric acid (HCl) on a steam bath. The solution obtained was filtered and 1ml of the filtrate was treated with two drop of Mayer's reagent. The two solutions were mixed and made up to 100ml with distilled water. Turbidity of the extract filtrate on addition of Mayer's reagent was regarded as evidence for the presence of alkaloids in the extracts.

Determination of glycosides

Coarsely powered plant leaf material (1g) was introduced into two different beakers. To one of the beakers was added sulphuric acid (4ml) while water (4ml) was added to the other beaker. The two beakers were heated for 3 minutes and the contents filtered into labelled test tubes. The filtrate was made alkaline with sodium hydroxide (0.4ml) and allowed to stand for three minutes. The presence of reddish brown precipitate in the filtrate was taken as positive for glycosides.

Determination of flavonoids

To the extract of each piece of test plant leaf extract was added a small piece of magnesium ribbon, this was followed by drop wise addition of concentrated hydrochloric acid. Colours ranging from orange to red indicated flavones, red to crimson indicated flavonols, crimson to magenta indicated flavonones.

Carbohydrates

To 1 ml of the filtrate, 4 ml of Benedict's reagent were added. The mixture was heated. Appearance of red precipitate indicated the presence of reducing sugar.

Amino acids

Added 4 drops of millon's reagent to I ml of test solution and heated on a water bath for 10 min, cooled and added 1% sodium nitrite solution. Appearance of red colour confirmed the test.

Proteins

To 2 ml of the test solution added 4 drops of 1% copper sulphate solution and 2 ml of 10% NaOH. Mixed thoroughly. Formation

of purple or violet colour confirmed proteins.

Phytosterols

Extract (2 mg) was dissolved in 2 ml of acetic anhydride, heated to boiling, cooled and then 1 ml of concentrated sulphuric acid as added. A brown ring formation at the junction and the turning of the upper layer to dark green colour confirmed the test for the presence of phytosterols.

Triterpenoids

Approximately 2 mg of dry extract was taken in a test tube and shaken with 1 ml of chloroform and a few drops of concentrated sulphuric acid were added. A red brown color formed at the interface indicated the test as positive for triterpenoids.

Antibacterial Study of different extracts of *Datura stramonium* leaf

The following bacterial cultures were used for antimicrobial activity.

a) *Bacillus azotoformans*
b) *Bacillus pasteurii*
c) *Pseudomonas aeruginosa*
d) *Staphylococcus aureus*

The cultures were obtained from the standard cultures maintained in the microbiology department of Sardar Bhagwan Singh University. These cultures were maintained on nutrient agar slants at first being incubated at 37° C for about 18-24 hrs and thenstored at 4 °C as stock cultures for further antibacterial activity, Fresh cultures were obtained by transferring loop-full cultures into nutrient broth and then incubated at 37°C, over night. To test antibacterial activity, the disc diffusion method was used. After confirming the identity of the isolated bacteria by biochemical tests, tested for susceptibility resistant to number of extracts by disc diffusion method using Muller Hinton Agar assay medium. The organisms were inoculated into Saline water. Broth culture was then spread on Petri plate on Muller Hinton Agar and after 10-14 min discs of different extract to be tested were placed on the surface of seeded Petri plates. Plates were observed for zone inhibition. After 24 hrs of theincubation at 37°C, Zones of inhibition were measured in terms of Inhibition Zone Diameter Scale in mm.

Preparation of culture media: The medium used for anti-bacterial was nutrient agar that was prepared and sterilizedat 121 °C and 14 lbs for 14-30 min.

Nutrient Agar Media

Ingredients	Composition (g/ml)
Beef Extract	3.0 gm
Peptone	4.0 gm
Sodium Chloride (NaCl))	4.0 gm
Distilled Water	1000ml
Agar	18 gm
pH	7.2± 0.229

Figure 3.1 Nutrient Agar Media

Figure 3.2 Different slants of Nutrient Agar Media

Nutrient Broth

Ingredients	Composition (g/ml)
Beef Extract	3.0 gm
Peptone	4.0 gm
Sodium Chloride (NaCl)	4.0 gm
Distilled Water	1000ml
pH	7.2 ± 0.2

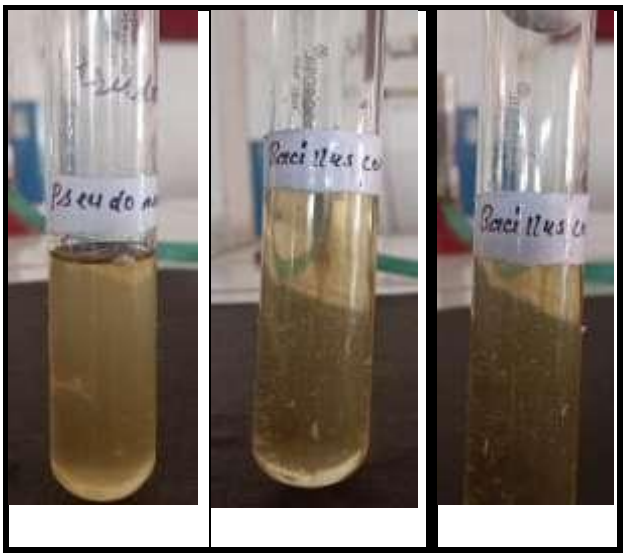

Figure 3.3 Nutrient Broth

Plate preparation

20-24 ml of autoclaved nutrient agar medium was poured into 90 mm diameter pre sterilized petriplates under aseptic conditions and was allowed to solidify in the presence of UV light. Then the prepared plates are pre incubated for 24 hr.

Preparation of dilution

Four different dilution of all the four extract were being prepared of 50mg/ml, 100mg/ml, 150mg/ml and 200mg/ml these dilution had been used for anti-microbial activity along with the standard antibiotic (ciprofloxacin) 30mg/ml solubilised in distilled water.

Figure 3.4 Dilution of petroleum ether extract

Figure 3.5 Dilution of chloroform extract

Figure 3.6 Dilution of water extract

Figure 3.7 Dilution of ethanol extract

Figure 3.8 Dilution of standard (ciprofloxacin) in water

Determination of antimicrobial activity

Determination of antimicrobial activity was done according to the standards recommended by CLSI (Clinical and Laboratory Standards Institute), namely at first Agar well diffusion method. The pre incubated plates were taken and bacteria were spreaded over those plates with the

help of a spreader. After the bacteria had dried the well were bored into agar plates with help of metallic hollow road. Then 100µl of extracts and 30µl of standard antibiotic (ciprofloxacine) were dropped onto discs understerile conditions and were incubated at +3710.1°C for 24 hr. After incubation, the diameters of inhibition zones were measured in millimeters on all plates. All experiments were repeated three times.

Measurements of zone of inhibition

After incubation of the plates, the antibacterial spectrum of the extracts was determined in the terms of zones of inhibition around the wells. The diameters of zone of inhibition produced by the plant extract were compared with those produced by antibiotics. The experiments were performed and average zone diameter was recorded.

The results of cache plate were observed and recorded after 24-26 hrs of incubation at 37˚C by measuring zone diameter (mm), caused by *Datura stramonium* leaves extracts of different solvents.

Result and Discussion

Phytochemical screening[19, 20]

The phytochemical screening test was conducted using four different solvents such as petroleum ether, chloroform, ethanol and water, crude extract of *D. stramonium* leaves were summarized in Table-1. The results obtained from this study pointed that the presences of tannins, alkaloids, triterpenodis, flavonoids, saponins, carbohydrates, proteins, amino acids and phytosterols in the plant extract. According to the previous study, a qualitative phytochemical screening test of water and ethanol extract of *D. stramonium* extract also showed the presence of different class of chemical constituents such as tannins, alkaloids, triterpenodis, flavonoids, saponins, carbohydrates, proteins, amino acids and phytosterols.

Table-1 Phytochemical screenings of crude extracts of *D. stramonium* leaves

Solvents	Petroleum ether	Chloroform	Ethanol	Water
Tannins	-	+	++	--
Alkaloids	-	+	++++	++++
Triterpenodis	-	+	+	-
Flavonoids	+	+	+	-
Saponins	+	+	+	+
Carbohydrates	+	+	+	+
Proteins	+	+	+	-
Amino acids	-	-	-	-
Phytosterols	+	+	+	-

+ = the presence and - = the absence of chemical constituents


Figure 3.9


Figure 3.10


Figure 3.11


Figure 3.12


Figure 3.13


Figure 3.14


Figure 3.15


Figure 3.16


Figure 3.17


Figure 3.18


Figure 3.19


Figure 3.20


Figure 3.21


Figure 3.22


Figure 3.23


Figure 3.24


Figure 3.25


Figure 3.26

Figure 3.27

Figure- Results of phytochemical test performed

Antibacterial

The antibacterial activity of crude extracts of *D. stramonium* was tested by disc diffusion method. The extract of plant leaves has been found to be potent against Pseudomonas, B. cereus and S. aureus. The antibacterial activity of *D. stramonium* leaves extract of different solvents are summarised in Table 2. Chloroforme extract shown the maximum inhibition zone against S.aureus (9.4mm) in 200mg/ml concentration and minimum against B. azotoformans (4.4mm) in 50mg/ml and Ethanol extract shown the maximum inhibition zone against Pseudomonas (10.4mm) in 200mg/ml concentration and minimum against B. azotoformans (4mm) in 50mg/ml. Petroleum ether and water extract has not shown any anti-microbial activity in all the four concentration against all the four bacteria[21, 22].

Table- 2 Antibacterial activities of *D. stramonium* leave crude extracts

Bacteria	Concentration	Petroleumether	Chloroform	Ethanol	Water
Pseudomonas aeruginosa	50mg	-	7.4mm	4.4 mm	-
	100mg	-	7mm	4.4 mm	-
	150mg	-	7mm	4 mm	-
	200mg	-	7.4 mm	10.4 mm	-
	+ve	20 mm	21 mm	19 mm	18 mm
Staphylococcus aureus	50mg	-	7 mm	4.4 mm	-
	100mg	-	8 mm	6 mm	-
	150mg	-	8.4 mm	7.4 mm	-
	200mg	-	9.4 mm	8 mm	-
	+ve	22 mm	20 mm	21 mm	19 mm
Bacillus azotoformans	50mg	-	4.4mm	4 mm	-
	100mg	-	6mm	6 mm	-
	150mg	-	6mm	6.4 mm	-
	200mg	-	7.4mm	8.4 mm	-
	+ve	22.4 mm	20 mm	19 mm	20.4 mm
Bacilluspasteurii	50mg	-	7mm	9 mm	-
	100mg	-	7.4mm	9.24 mm	-
	150mg	-	7.4mm	9.4 mm	-
	200mg	-	8.4mm	10 mm	-
	+ve	20 mm	20.4 mm	22 mm	21 mm

+ve = Cprofloxcin, - = no result

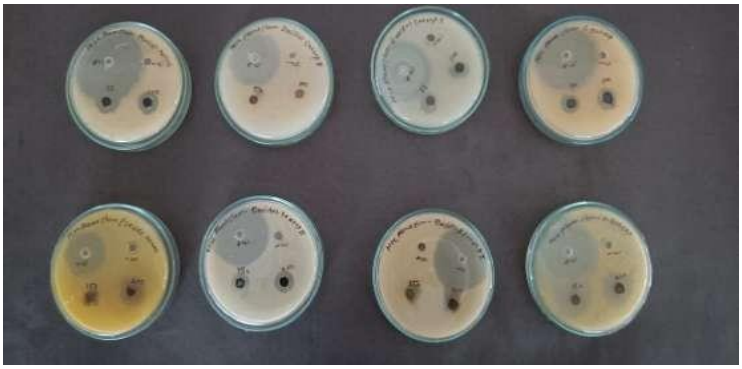

Figure 3.28 Inhibition of zones of chloroform extract on different bacteria.

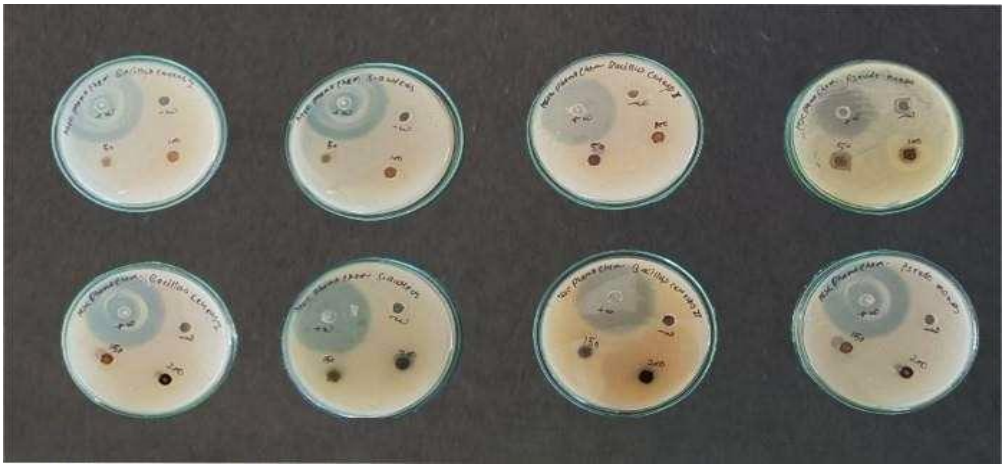

Figure 3.29 Inhibition of zones of petroleum ether extract on different bacteria.

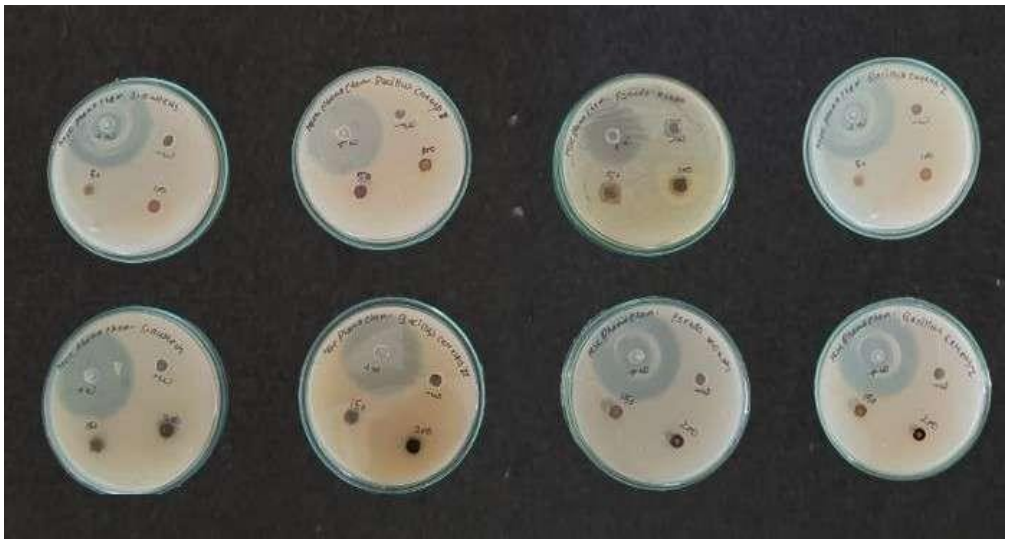

Figure 3.30 Inhibition of zones of ethanol extract on different bacteria.

Figure 3.31 Inhibition of zones of water extract on different bacteria.

Conclusion

From the above study, it can be concluded that the leaf extract of *D. stramonium* have phytochemicals that could contribute in antibacterial activities. Possible antibacterial substance in extract of different solvent includes as tannins, alkaloids, triterpenodis, flavonoids, saponins, carbohydrates, proteins, amino acids and phytosterols. The antibacterial characteristics of the plant can be further tested to use in treatment of bacterial infection. The crude extract of *D. stramonium* can be used against some selective microorganisms. Crude extract of *D. stramonium* can be better alternative to the conventional antibacterial additives in food industry. Also traditionally the anti-bacterial potency of crude extract of *D. stramonium* leaves has been justified.

Disclaimer Statement

Authors declare that no competing interest exists. The products used for this research are commonly used products in research. There is no conflict of interest between authors and producers of the product.

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