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EXPLORING THE ANTIBACTERIAL EFFICACY OF DATURA STRAMONIUM L. AGAINST CLINICAL ISOLATES

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Abstract:

Plants produce secondary bioactive compounds that have medicinal use because there are tremendous chances to find novel pharmaceutical compounds. Green pharmaceuticals are becoming a recent topic of research. These plants generally synthesize a diverse range of bioactive compounds, including primary and secondary metabolites, which have been established as potent antimicrobial agents against a wide range of pathogenic organisms. This study explores the antibacterial activity of *D. stramonium* against two bacterial strains: *E. coli* (Gram-negative) and *Bacillus spp.* (Gram-positive). Using three different types of solvents (methanol, petroleum ether, and water), the study evaluates the Zone of inhibition (ZOI). After that, qualitative phytochemical tests were used to investigate the phytochemical compounds. Alkaloids, tannins, flavonoids, anthoquinones, carotenoids, proteins, saponins, lipids, terpenoids, steroids, and other secondary metabolites have been found in it. Using the Bour Kurby test method, all of the extracts showed significant antibacterial activity. The result indicates that *D. stramonium* contains broad spectrum of antibacterial activities. Extract efficacy is higher against Gram-positive *Bacillus spp.* than Gram-negative *E. coli*.

Keywords: Bioactive compounds, antimicrobial activity, Extraction, Natural products

Introduction:

AMR (antibiotic resistance) is one of the major problems in present medicine so science has started looking at ethnobotanical way as nowadays antibiotics doesn't have the same potency against the bacteria. There are many plants that have ethnobotanical uses and one such important alternative choice is *Datura stramonium L.* (also known as Jimson weed or Thorn apple) due to its rich chemical constitution and extensive history in folk medicine (Thakur et al., 2023). A herbaceous annual or subshrub, *Datura stramonium L.* is erect, branching and is characterized by the presence of stout, globous, smooth, dichotomously branching pale green to reddish-purple stems; the plant grows 0.5-1.5 m tall. It contains many bioactive compounds these compounds are divided into three basic categories, alkaloids, phenolic, and terpenoids, which serve as useful source of biologically active compounds due to their diverse mixture (Chassagne F, Samarakoon T, Porras G, et al, 2021). Combining medicinal plants and physiological active compounds derived from plants can lead to more effective antimicrobial resistance towards pathogen diseases. In the process to search for new drugs scientist are increasingly using traditionally known plant sources (Wylie MR, Merrell DS; 2022), where herbal medicine is known to be employed for variety of ailment in certain regions of India and is an important part of primary health care service. It can be used to remedy for fever, cough, skin spots and liver disorder (Chithambharan A, et al., 2021). In different parts of each plant like roots, stem, rhizomes, bulbs, leaves, bark, flowers, fruits and seeds that may contain unique compounds with antibacterial properties. India is a diverse supplier of medicinal plants and the world's largest reservoir of genetic resources of medicinal plants. The diverse geography and climate influence the vegetation and the floristic composition, and thus make it a supplier of medicinal plants and the largest reservoir. Plantae Kingdom contains all kind of vegetation on the earth like mosses, flowering plants, ferns etc. The second largest kingdom after Animalia. It comprises about 250,000 kinds of plants. There are around 90 to 100 genera of varied Solanaceae, a plant family, also known as the nightshade or potato family, with some 2,500-3,000 species.

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Members of this family are found throughout the world on virtually every continent, except Antarctica. Central and South American tropical and subtropical regions have the highest density of species diversity. The important application of herbal medicine Benzie and Wachtel-Galor (2011) mentioned are; (i) the patient's belief is compatible with the use of herbs, (ii) relieved the patient's fear toward the adverse effect of western medicine, (iii) cost-effectiveness of herbs, (iv) increasing demand on personalized medicine and (v) the distribution of health information to the masses.

In order to separate these bioactive compounds a number of methods can be used. Proper selection of extraction method is extremely critical in qualitative as well as quantitative assay of the biologically active constituents of botanical source as stated by Smith (2003) and Sasidharan et al. (2011). The first stage of the research into the medicinal plant is the extraction, and dictates the outcome of the research. The physical parameters that are generally affecting the extraction include physical characteristics of the plant matrix, solvent, temperature, pressure, time (Hernndez et al., 2009).

The primary objective of this research is to evaluate chemical constituents of *Datura stramonium* and its therapeutic potentials using extraction, phytochemical screening and antimicrobial studies.

Materials and methods:

1.1 Collection of plant parts:

Fresh plants of *Datura stramonium* were harvested from Babra-Gir, Gujarat, India. Leaves, stem, flowers and seeds of the plants were collected. Plants were harvested in the early morning time to keep intact the chemical properties of the plant. The plant was physically screened for removal of dead leaf particles or other organic matter from the plant and was washed thoroughly by tap water for removal of dust and dirt, and finally rinsed by distilled water. The tropane alkaloids are susceptible to heat, so to preserve them the plant material was then cut into fine pieces and air dried for 2 weeks at room temperature (25C to 30C approximately) until the material would powder. The dried material was finely grounded in a sterilized mechanical grinder. The grounded material was then sifted and stored in plastic bags at room temperature to avoid damage by moisture and light, before the extraction procedure.

1.2 Extraction of plant material:

Solvent extraction using three different solvents. The three different solvent that have been used for extraction of the bioactive constituents were Petroleum Ether, Methanol and Distilled water. For this 1 gm of the plant powder was put along with 10 ml of solvent in a ratio 1:10 W/V. Normally 48-72 hours are given to the plant material to stand. This procedure is known as maceration. Periodical shaking of the flask was done in order to ensure the maximum efficiency of extraction of the active ingredients with the solvent. Filtered extract was then analysed for phytochemical screening after it was passed through the filter paper. Also, whenever the extract was not in use, the same was stored in the refrigerator.

1.3 Qualitative phytochemical tests:

The following standard procedures were used to test the extract for the presence of bioactive components (Sofowra, A. 1993; Trease, G.E., Evans, W.C. 1989; Harborne, J.B. 1973).

1) Test for proteins

Ninhydrin test:

Crude extract when boiled with 2ml of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.

2) Test for carbohydrates

Benedict's test

When crude extract was mixed with 2ml of Benedict's reagent and boiled, a reddish-brown precipitate formed, indicating the presence of carbohydrates.

3) Test for proteins

Ninhydrin test

Crude extract when boiled with 2ml of 0.2% solution of Ninhydrin, violet colour appeared suggesting the presence of amino acids and proteins.

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4) Test for terpenoids
Crude extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added and heated for about 2 minutes. A greyish colour indicated the presence of terpenoids.

5) Test for alkaloids
Crude extract was mixed with 2 ml of 1% HCl and heated gently. Mayer's And Wagner's reagents were then added to the mixture. Turbidity of the resulting precipitate was taken as evidence for the presence of alkaloids.

6) Test for Cardiac glycosides
Add 2 ml filtrate with 1 ml of glacial acetic acid, 1 ml ferric chloride and 1 ml concentrated sulphuric acid. Green-blue coloration of solution indicated the presence of cardiac glycosides.

7) Test for phenols and tannins
Crude extract was mixed with 2 ml of 2 % solution of $FeCl_3$. A blue-green or black coloration indicated the presence of phenols and tannins.

8) Test for saponins
Crude extract was mixed with 5 ml of distilled water in a test tube and it was shaken vigorously. The formation of stable foam was taken as an indication for the presence of saponins.

9) Test for flavonoids
Alkaline reagent test Crude extract was mixed with 2 ml of 2% solution of NaOH. An intense yellow colour was formed which turned colourless on addition of few drops of diluted acid which indicated the presence of flavonoids.

10) Test for steroid
Crude extract was mixed with 2 ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicated the presence of steroids. Another test was performed by mixing crude extract with 2 ml of chloroform. Then 2 ml of each of concentrated H_2SO_4 and acetic acid were poured into the mixture. The development of a greenish coloration indicated the presence of steroids.

1.4 Assay for Antibacterial activity

Bacterial culture preparation and determination of Zone of inhibition (ZOI):
The test organisms for which the antibacterial activity was tested included species of *Bacillus* and *E. coli.* The plant chosen was tested for its antibacterial activity by Bour Kurby test method. For each of the strains to be tested, 200 ml of nutrient agar was poured and used to seed the sterile petri-dishes. The organisms to be tested were then streaked on each of the petri plates using the sterile streaking loop technique. Using the cork bores of size 8 mm diameter, the circular wells were created in each of the petri dishes and 50 µl of *D. Stramonium* extract dissolved in DMSO were poured into each of them. The petri plates after the inoculation were incubated at 37ºC and the results were recorded for possible antibacterial activity by each of the extracts (determined by the mean value of inhibition diameter (mm)) prepared by different inorganic and organic solvents. The experiment was carried out in 3 replicates.

Results and discussion

Phytochemical analysis of bioactive compounds in different solvent extracts of *Datura stramonium L.*
Different bioactive phytochemicals were screened in the plant extract in different solvents. Alkaloids, tannins, flavonoids, anthoquinones, carotenoids, proteins, saponins, lipids, terpenoids, steroids, etc. are present as secondary metabolites. Higher amount of cardiac glycosides, saponins are present in aqueous and methanolic extracts. These results are summarized in Table 1.

Antibacterial efficacy analysis of different solvent extracts:
Antibacterial efficacy in terms of zone of inhibition (ZOI) of crude plant extracts (10 mg/ml) in different solvents of *D. stramonium* tested against pathogenic organisms (Table 2). The antibacterial potency of *D. stramonium* extract was evaluated by the presence or absence of inhibition zones and zone diameters (mm). From the results, it is evident that the methanolic extract of *D. stramonium* leaves extract showed a maximum inhibitory zone. The bacterial strains *Bacillus spp.* was susceptible to plant extracts with zone of inhibition (≥22 mm) diameter respectively. 15 mm zone of inhibition was shown in aqueous extraction of *D. stramonium* leaves.

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Table 1: Phytochemicals screening of *D. stramonium*

Phytochemical compounds	**Water**				**Methanol**				**Petroleum ether**			
Datura strontium L.	leaves	stem	flower	seed	leaves	stem	flower	seed	leaves	stem	flower	seed
Tannins	-	-	-	-	+	+	++	++	-	-	-	-
Flavonoids	++	-	++	++	+++	+++	+++	+++	-	-	-	-
Phlobatannins	-	-	-	-	-	-	++	-	-	-	-	-
Cardiac glycoside	+++	+++	+++	+++	+++	+++	+++	+++	++	+	+	++
Combined anthoquinones	-	-	-	-	+++	++	+++	+	-	-	-	-
Free anthoquinones	-	-	-	-	+++	+	++	+	-	-	-	-
Carotenoids	-	-	-	-	+	+++	-	-	-	-	-	-
Reducing sugars	+	-	-	+	+	-	++	-	+	-	+	-
Alkaloids	-	-	-	-	++	-	+	-	++	+	++	+
Proteins	-	-	-	-	+++	++	+++	+	-	-	-	-
Amino acids	+++	+++	++	++	-	-	+	-	-	-	-	-
Saponins	+++	+++	+	-	+++	+++	-	-	++	+	+	+
Lipids	-	-	-	-	-	-	-	-	+	+	++	+
Steroids	-	-	-	-	-	-	-	-	++	+	+	++
Terpenoids	++	+	+	+++	-	-	-	-	++	+	+	++
Oils and fats	-	-	-	-	++	+	+	-	++	+	+	+

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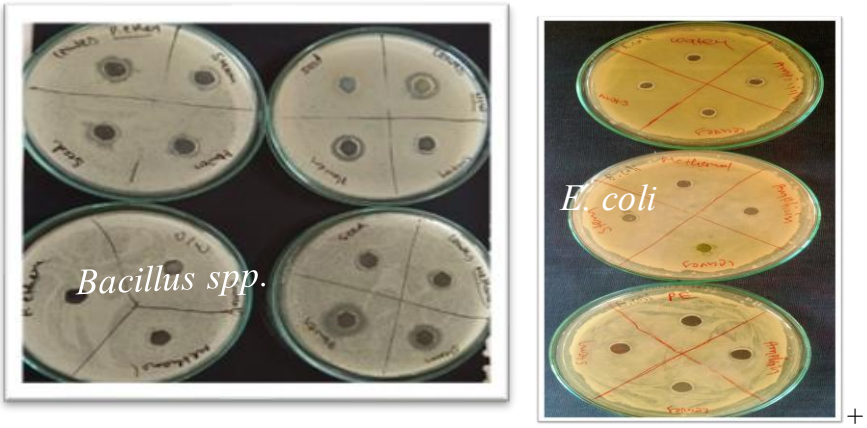


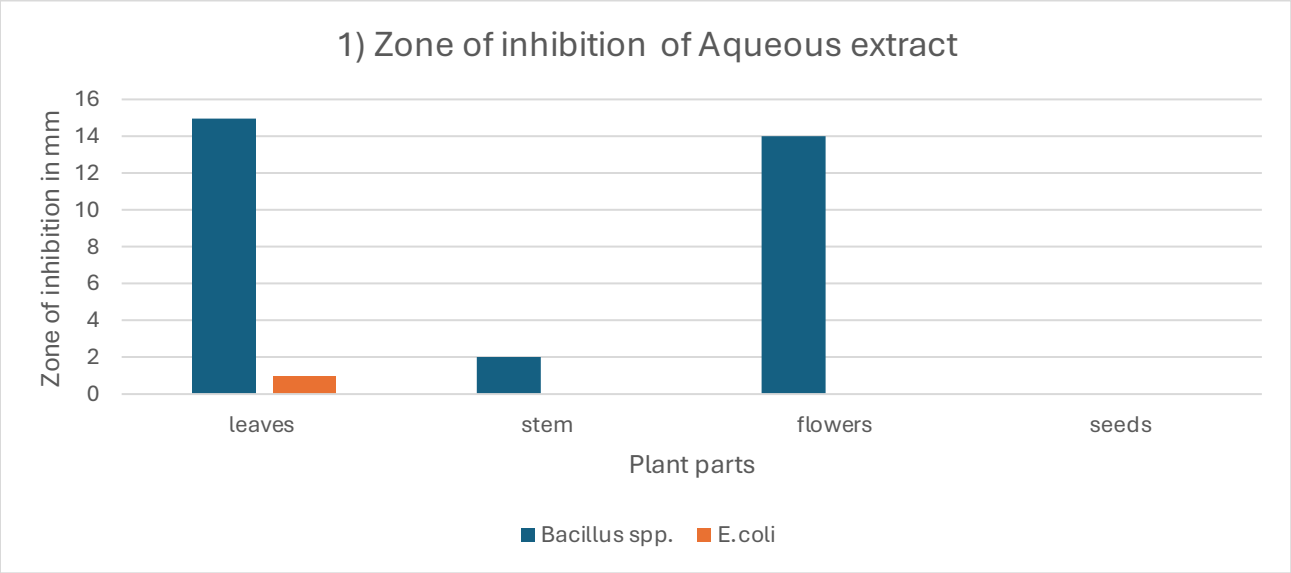
Fig 1 and 2 Zone of inhibition of *Bacillus spp. and E coli*

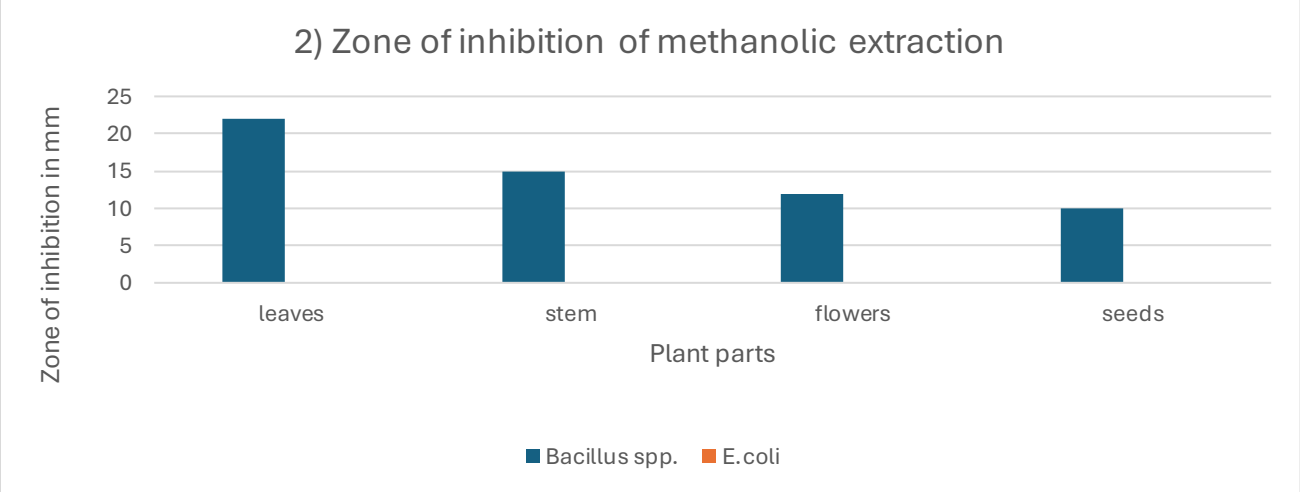
Table 2: Antibacterial efficacy (zone of inhibition) of the extract of *D. stramonium* on test organisms (diameter in mm)

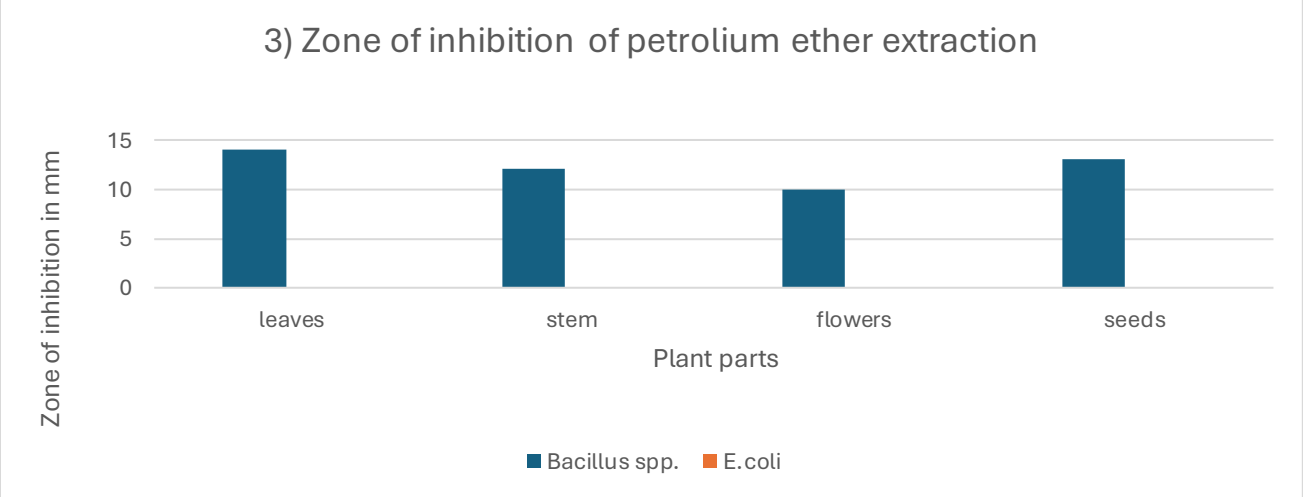
Zone of inhibition in mm				
Solvent	***D. Stramonium***	***Bacillus spp.***	***E. coli***	**Control**
Water	Leaves	15 mm	1 mm	-
	Stem	2 mm	-	-
	Flowers	14 mm	-	-
	Seeds	-	-	-
Methanol	Leaves	22 mm	-	-
	Stem	15 mm	-	-
	Flowers	12 mm	-	-
	Seeds	10 mm	-	-
Petroleum ether	Leaves	14 mm	-	-
	Stem	12 mm	-	-
	Flowers	10 mm	-	-
	Seeds	13 mm	-	-

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Graph 1, 2, 3 indicates Zone of inhibition of Aqueous, methanolic and petroleum ether extraction respectively







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Conclusion:

From the results, the extract of the plant has higher potency against Gram-positive *Bacillus spp*. than Gram-negative *E. Coli*, implying the selectivity of its antibacterial activity. The difference is likely attributed to the difference in structure between cell wall of bacterial species and suggests that the active components might specifically interfere with the cell wall structure or synthesis of peptidoglycans. Furthermore, the result that aqueous and methanolic extracts exhibit better activities compared to less effective petroleum ether fractions implies that the more active antibacterial components are likely to be more polar. These results support that the species has potential in the design of specific phytotherapy (against Gram-positive bacteria).

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