



## Preliminary Phytochemical Screening and *in vitro* Antimicrobial Activity of *Datura stramonium* Leaves Extracts Collected from Eastern Ethiopia

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### Abstract

The present study was conducted to identify the preliminary phytochemical identification and antimicrobial investigation of leaves extracts of *Datura Stramonium* Linn collected from eastern Ethiopia. *Datura Stramonium* is traditionally used to cure different human diseases including in skin disorder, ear pain, cough, fever, burns, and asthma in Ethiopia. Phytochemical screening test in five different solvents chloroform, hexane, petroleum ether, ethanol, and acetone crude extracts were indicated the presence of flavonoids, cholesterol, terpenoids, carbohydrates, glycosides, tannins, alkaloids, phenols, proteins, and saponins. However, glycosides, phenols, and cholesterol were not detected in hexane, petroleum ether, and ethanol crude extract, respectively. Antimicrobial activity of crude extract were tested using paper disk diffusion method against four bacteria strains (*S. aureus*, *B. subtilis*, *E. coli* and *S. typhi*) and three fungi strains (*F. solani*, *F. oxysporum* and *A. niger*). *Datura stramonium* petroleum ether extract produced maximum zone of inhibition ( $19.30 \pm 0.18$ mm) against *S. aureus* while minimum zone of inhibition ( $12.30 \pm 0.16$ mm) against *S. typhi*. Hexane crude extract leaves of *Datura stramonium* showed maximum zone of inhibition ( $18.00 \pm 0.27$ mm) against *S. aureus* while minimum zone of inhibition ( $11.05 \pm 0.62$ mm) against *E. coli*. Chloroform extract of the plant also showed maximum zone of inhibition ( $18.43 \pm 0.57$ mm) against *B. subtilis* while minimum zone of inhibition ( $11.51 \pm 0.54$ mm) against *S. typhi*. The various crude extracts of *Datura stramonium* were showed high potential antifungal activity against the tested fungi with maximum zone of inhibition ( $17.07 \pm 0.16$ mm) against *A. niger* was exhibited by petroleum ether extract while minimum zone of inhibition ( $8.05 \pm 0.43$ mm) against *F. oxysporum* was observed by ethanol crude extract. The antimicrobial activities of plant extract were compared with that of chloroamphenicol against bacteria and bavistin against fungi as reference antibiotics.

**Keywords:** *Datura stramonium*, crude extract, phytochemical screening, antimicrobial activity, paper disk diffusion method.

### Introduction

*D. stramonium* is commonly known as Jimson weed or *Datura* belongs to family Solanaceae. It is 60-120 cm or more tall, branched, and pubescent plant. Leaves are 8-17x4-13 cm, ovate, sinuately dentate and minutely puberulose<sup>1</sup>. *D. stramonium* is common weed in disturbed areas, waste ground, in fertile soils in fields, and roadsides at altitudes of 600-2800 m. this herb is originated in Tropical North America, now it is a cosmopolitan weed. It occurs in most Ethiopian regions, and also in Eritrea, Sudan, Somalia, and throughout tropical Africa, Europe and parts of Asia<sup>2</sup>.

*D. stramonium* is widely growing plant and well known to have potent pharmacological activity with a great utility and usage in folklore medicine. Water and ethanol extract of *D. stramonium* contains saponins, tannins, carbohydrates, proteins, steroids, flavonoids, alkaloids, phenol, and glycosides and use in medicine due to its analgesic and antiasthmatic activities<sup>3,4</sup>. Leaves extract of the plant contains different types of secondary metabolites such as glycosides, phenols, lignins, saponins, sterols, and tannins<sup>5</sup>. The alkaloids atropine and scopolamine

are the primary bio-active substances reported in *D. stramonium* extracts. Atropine has been used in treating Parkinson's disease, peptic ulcers, diarrhea, and bronchial asthma<sup>6</sup>. Scopolamine is used to treat Parkinson's disease and painful visceral spasms through injection<sup>7</sup>. The leaves extract of *D. stramonium* is used for the treatment of baldness<sup>8</sup>, management of pains<sup>9</sup>, anti-inflammatory, and antispasmodic<sup>10</sup>, skin diseases<sup>11</sup>, anticholinergic and sedative<sup>12</sup>.

Even though some works on biological activity was done in different area of the world, but the medicinal application of the phytoconstituents of this herb is still insufficient in many of previous reports. The phytochemical screening of leaves crude extract of *D. stramonium* and its antimicrobial activity this herb is not worked up to date in Ethiopia. This is therefore, the main objective of the present study on *D. stramonium* leaves was to estimate the possible antimicrobial activity of using different organic solvent crude extracts against four bacteria strains (*Bacillus subtilis*, *Staphylococcus aureus*, *Salmonella typhi*, and *Escherichia coli*) and three fungi strains (*Aspergillus niger*, *Fusarium oxysporum*, and *Fusarium solani*) and phytochemical

screening of both primary and secondary metabolite present in the crude extracts of *D. stramonium* collected from Eastern Ethiopia were conducted.

## Material and Methods

**Collection and Identification of the Plant Material:** Dry leaves of *D. stramonium* were collected from Haramaya, Eastern Ethiopia on October, 2013. The Botanical specimens of the plant were identified by Mr. Abeduruzak Abdulhahi and the voucher specimen was deposited at the Herbarium of the Department of Plant Science, Haramaya University. After collection, the seeds were washed repetitively and air-dried in the shade to make it easily grindable

**Extraction of leaves of *D. stramonium*:** Air dried leaves of *D. stramonium* were ground by blander and packed in polyethylene bags to avoid entrance of air and any other mixing of surrounding material. Air dried and a powdered leaves of *D. stramonium* (200 g) was extracted by soaking with chloroform, ethanol, hexane, petroleum ether, and acetone separately for 24 hrs at room temperature. Then after, the marc was filtered using Whatman no. 1 filter paper and concentrated by rotary evaporator at 40 °C to yield crude extract of (5.86- 11.50 % w/w) and the various extracts of the plant was kept in refrigerator at 4 °C for further analysis.

**Preliminary Phytochemical Screening of Solvent Crude Extracts:** The crude extracts of the plant were used for screening of phytochemical constituents to identify the presence of primary as well as secondary metabolites such as carbohydrates, proteins, alkaloids, cholesterol, flavonoids, saponins, terpenoids, glycosides, tannins, phenols, according to the standard procedure<sup>13-17</sup>.

**Antimicrobial Activity of Crude Extract of the Leaves of *D. stramonium*:** Different solvent crude extracts leaves of *D. stramonium* was investigated for *in vitro* antibacterial and antifungal assay using paper disc diffusion method against four bacteria strains (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Salmonella typhi*) and three fungi strains (*Fusarium oxysporum*, *Fusarium solani*, and *Aspergillus niger*)<sup>18</sup>. The bacterial cultures were inoculated into the Muller Hinton Agar (MHA) and incubated at 37 °C. Fungal cultures were inoculated into Potato Dextrose Agar (PDA) and incubated at 27 °C. All the microbial were obtained from Plant Pathology laboratory, School of Plant Science, Haramaya University. Bavistin was used against fungi and Chloroamphenicol was used as standard drug against bacteria. Dimethyl sulfoxide (DMSO) was also used as a negative control.

**Preparation of Inoculums:** The test bacterial strains were transferred from the stock cultures and streaked on MHA plates and incubated for 24 hrs at 30 °C oven. Well separated bacterial colonies were then used as inoculums. Then spores of the test

fungi were harvested by washing the surface of the colony using 10 mL sterile distilled water. The mycelial plugs of fungi from stock cultures were transferred to PDA plates and incubated for 7 days at 27 °C oven. The MHA and PDA medias were autoclaved at 121 °C and 1.03 bars for 15 minute in order to sterilized and cooled to about 45 °C in a water bath. The microorganisms were then transferred to their media using sterile loop and mixed by gentle swirling the flasks and then poured to sterile petri plates, allowed to solidify and used for the bioassay test.

**Testing for Antimicrobial Activity:** Filter paper discs of 6 mm diameter placed in a beaker were sterilized in an oven at 180 °C for 1 hrs. Then, 20 and 40 µg/mL of the solutions of the crude extracts as well as the standard drugs was pipetted to the discs in three replications. The paper discs impregnated with the sample were then transferred with sterile forceps to medias seeded with spore suspension of test fungi and bacterial strains as described above. The crude extract was evaluated by measuring the zone of inhibition against the test bacteria and fungi after an incubation period of 24 hrs and compared to that of commercial standard drugs<sup>19</sup>.

## Results and Discussion

**Percent Yield of the Crude Extracts:** The air dried powdered leaves of *D. stramonium* (200 g) were extracted with different solvents chloroform, hexane, petroleum ether, ethanol, and acetone to yield crude extracts of (5.86- 11.50 % w/w).

**Antimicrobial Activity of *D. stramonium* Leaves Crude Extracts:** The antimicrobial activities of the crude extracts of the plant were tested by paper disc diffusion method. The crude extracts of *D. stramonium* leaves has potent antibacterial activity against *S. aureus*, *B. subtilis*, *S. typhi*, and *E. coli* and antifungal activity against *F. oxysporum*, *A. niger*, and *F. solani*, at concentrations of 20 and 40 mg/mL. The antibacterial activity of different solvent leaves extracts of *D. stramonium* were summarized against four bacteria in table -1. Petroleum ether extract was produced maximum zone of inhibition (19.30±0.18 mm) against *S. aureus* while minimum zone of inhibition (12.30±0.16 mm) against *S. typhi*. Hexane extract leaves of the plant revealed that maximum zone of inhibition (18.00±0.27 mm) against *S. aureus* while minimum zone of inhibition (11.05±0.62 mm) against *E. coli*. Chloroform extract of the plant showed maximum zone of inhibition (18.43±0.57 mm) against *B. subtilis* while minimum zone of inhibition (11.51±0.54 mm) against *S. typhi*. Acetone extract of the plant exhibited maximum zone of inhibition (15.60±0.21 mm) against *S. aureus* while minimum zone of inhibition (9.52±0.22 mm) against *S. typhi*. Ethanol extract of the plant also exhibited maximum zone of inhibition (15.50±0.55 mm) against *S. aureus* while minimum zone of inhibition (8.74±0.22 mm) against *E. coli*.

**Table-1**  
**In vitro antimicrobial activities of *D. stramonium* leaves crude extracts**

Cpds	Average inhibition (I)(mm) of Microorganisms													
	Gram(-) Bacteria				Gram(+) Bacteria				Fungi					
	<i>S. typhi</i>		<i>E. coli</i>		<i>B. subtilis</i>		<i>S. aureus</i>		<i>F. solani</i>		<i>F. oxysporum</i>		<i>A. niger</i>	
	20 µg/mL	40 µg/mL	20 µg/mL	40 µg/mL	20 µg/mL	40 µg/mL	20 µg/mL	40 µg/mL	20 µg/mL	40 µg/mL	20 µg/mL	40 µg/mL	20 µg/mL	40 µg/mL
CCE	11.51 ± 0.54	13.14 ± 0.40	12.33 ± 0.22	14.32 ± 0.35	16.94 ± 0.26	18.43 ± 0.57	14.43 ± 0.35	16.27 ± 0.17	12.73 ± 0.35	15.20 ± 0.25	11.53 ± 0.44	14.43 ± 0.85	13.23 ± 0.36	16.08 ± 0.35
ACE	9.52 ± 0.22	11.20 ± 0.32	10.32 ± 0.52	12.15 ± 0.12	12.54 ± 0.32	14.50 ± 0.72	13.30 ± 0.42	15.60 ± 0.21	8.59 ± 0.32	10.15 ± 0.52	9.31 ± 0.42	12.40 ± 0.82	10.09 ± 0.32	13.05 ± 0.22
HCE	12.53 ± 0.42	14.70 ± 0.32	11.05 ± 0.62	14.00 ± 0.22	13.25 ± 0.82	16.08 ± 0.16	15.04 ± 0.47	18.00 ± 0.27	10.24 ± 0.46	13.15 ± 0.19	12.13 ± 0.32	16.35 ± 0.23	11.40 ± 0.20	15.07 ± 0.29
PECE	12.30 ± 0.16	14.25 ± 0.42	13.08 ± 0.14	16.23 ± 0.62	14.06 ± 0.37	18.20 ± 0.26	15.57 ± 0.45	19.30 ± 0.18	13.37 ± 0.65	16.21 ± 0.64	12.50 ± 0.52	15.40 ± 0.38	14.27 ± 0.23	17.07 ± 0.16
EtCE	9.06 ± 0.32	11.20 ± 0.12	8.74 ± 0.22	12.57 ± 0.61	11.54 ± 0.64	13.15 ± 0.35	12.10 ± 0.72	15.50 ± 0.55	9.20 ± 0.67	12.35 ± 0.38	8.05 ± 0.43	12.07 ± 0.54	10.83 ± 0.27	14.03 ± 0.46
CAL	20.40 ± 0.30	23.20 ± 0.25	21.14 ± 0.54	24.43 ± 0.26	22.63 ± 0.20	26.09 ± 0.37	23.28 ± 0.16	27.59 ± 0.17	-	-	-	-	-	-
Bav	-	-	-	-	-	-	-	-	21.07 ± 0.15	24.32 ± 0.45	22.07 ± 0.54	26.03 ± 0.65	19.17 ± 0.20	22.30 ± 0.23
DMSO	-	-	-	-	-	-	-	-	-	-	-	-	-	-

CAL = Chloramphenicol, DMSO = dimethylsulfoxide, Cpds = compounds, CCE = chloroform crude extract, ACE = acetone crude extract, HCE = hexane crude extract, PECE = petroleum ether crude extract, EtCE = Ethanol crude extract, Bav = bavistin, - = No inhibition was observed, Gram (-) = Gram negative, Gram (+) = Gram positive, and the inhibition zone were reported in mean (n=3) ± standard deviation

The various solvent extracts leaves of *D. stramonium* in table-1 were also indicated that significant antifungal activity against the tested fungi strains. Petroleum ether extract were revealed maximum zone of inhibition (17.07±0.16 mm) against *A. niger* while minimum zone of inhibition (12.05±0.52 mm) against *F. oxysporum*. Chloroform extract of the plant exhibited maximum zone of inhibition (16.08±0.35 mm) against *A. niger* while minimum zone of inhibition (11.53±0.44 mm) against *F. oxysporum*. Hexane extract leaves of plant showed maximum zone of inhibition (16.35±0.23 mm) against *A. niger* while minimum zone of inhibition (10.24±0.46 mm) against *F. solani*. Ethanol extract of the plant exhibited maximum zone of inhibition (14.03±0.46 mm) against *A. niger* while minimum zone of inhibition (8.05±0.43 mm) against *F. oxysporum*. Acetone extract of the plant also indicated maximum zone of inhibition (13.05±0.22 mm) of against *A. niger* while minimum zone of inhibition (8.59±0.32 mm) against *F. solani*. The differences the results obtained for antibacterial and antifungal activity of this study are due to the use of various cell culture types and solvents for extraction.

The antimicrobial activities presented in table-1 revealed that petroleum ether, chloroform, and hexane crude extracts of the plant were showed higher inhibition effect than acetone and ethanol extract against the tested bacteria and fungi strains. The crude extracts of the plant were indicated higher antibacterial activity with maximum zone of inhibition (19.30±0.18 mm) against *S. aureus* by petroleum ether extract while minimum zone of inhibition (8.74±0.22 mm) against *E. coli* by ethanol extract in comparison to antifungal activity with maximum zone of inhibition (17.07±0.16 mm) against *A. niger* by petroleum ether extract while minimum zone of inhibition (8.59±0.32 mm)

against *F. solani* by ethanol extract were obtained. The commercial standard drug chloroamphenicol in table-1 showed maximum zone of inhibition (27.59±0.17 mm) against *S. aureus* and minimum zone of inhibition (20.40±0.30 mm) against *S. typhi*. Bavistin also exhibited maximum zone of inhibition (26.03±0.65 mm) against *F. oxysporum* and minimum zone of inhibition (19.17±0.20 mm) against *A. niger*. As a result, the commercial standard drug was revealed higher antimicrobial activity in comparison with the plant crude extracts. DMSO was used as negative control and did not show any inhibition effect against the tested microorganism. The leaves crude extracts of the plant in table-2 revealed that the presence of different types of bioactive secondary metabolites such as glycosides, phenols, saponins, cholesterol, flavonoids, alkaloids, and tannins were likely to be responsible for antifungal and antibacterial activity. As indicated from the results presented in table-1, the antibacterial activity of the plant extract were more pronounced on the Gram-positive bacteria (*S. aureus* and *B. subtilis*) than the Gram-negative bacteria (*E. coli* and *S. typhi*). This might be due to the fact that Gram-negative bacteria have an outer phospholipidic membrane carrying the structural lipopolysaccharide components, which makes their cell wall impermeable to antibacterial chemical substances<sup>20</sup>.

Comparisons of the current finding with the previous study were showed a similar result. *In vitro* agar dilution methods depicted that the chloroform, ethanol and benzene extracts of branches and leaves sample of *D. stramonium* obtained from Pakistan has potent antibacterial activity against Enterobacter *Micrococcus luteus*, *Pseudomonas aeruginosa*, *E. coli*, *Staphylococcus aureus*, and *Klebsiella pneumonia*<sup>21</sup>. Furthermore, the antifungal activities of the methanol extract from different part of the plant

on the vegetative and generative phases of the growth process of four fungi strains (*Fusarium semitectum*, *Fusarium colmorum*, *Ceratocystis ulmi*, and *Rhizoctonia solani*) were reported in Iran showed similar result with the current study<sup>22</sup>.

**Phytochemical Screening:** Phytochemical screening study is intimately related to the needs of finding bio-active chemical constituents from medicinal plant extracts. The phytochemical screening test were conducted using five different solvents such as chloroform, hexane, petroleum ether, ethanol, and acetone crude extract of *D. stramonium* leaves were summarized in table-2. The results obtained from this study pointed that the presences of flavonoids, cholesterol, phenols, alkaloids, tannins, carbohydrates, saponins, proteins, glycosides, and terpenoids in the plant extract. However, glycosides, phenols, and cholesterol were not detected in hexane, petroleum ether, and ethanol, respectively, in crude extract. According to the previous study, a qualitative phytochemical screening test of water and ethanol extract of *D. stramonium* extract showed the presence of different class of chemical constituents such as saponins, flavonoids, alkaloids, phenols, steroids, and glycosides<sup>23</sup>. Both the secondary as well as primary metabolites screened in the leaves of the plant used in this study could be exhibited against the tested microbial.

### Conclusion

From the above study, it concludes that the presence of phytochemical constituents revealed in the solvent crude extract of leaves of *D. stramonium* could contribute for their antimicrobial activities. The various solvent extracts of the plant showed high potential of antibacterial and antifungal activities against the tested microorganisms. The antifungal and antibacterial characteristics of this plant can be further investigated so as to be used in the treatment of fungal and bacterial infections, respectively. Thus, *D. stramonium* crude extract can be used against the selected pathogenic and some microorganisms, and may provide better alternatives or

supplements to the conventional antibacterial and antifungal additives in foods. *D. stramonium* leaves crude extract used for the treatment of various human ailments possess antibacterial and antifungal activity and this also justify its use in the traditional medicine.

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**Table-2**  
**Phytochemical screenings of crude extracts of *D. stramonium* leaves**

S. No	Chemical Constituents	Crude Extracts				
		Chloroform Extract	Acetone extract	Petroleum ether extract	hexane extract	Ethanol extract
1	Flavonoid	+	+	+	+	+
2	Cholesterol	+	+	+	+	-
3	Tannins	+	+	+	+	+
4	Glycosides	+	+	+	-	+
5	Alkaloids	+	+	+	+	+
6	Phenols	+	+	-	+	+
7	Saponins	+	+	+	+	+
8	Proteins	+	+	+	+	+
9	Carbohydrates	+	+	+	+	+
10	Terpenoids	+	+	+	+	+

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

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