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, cholesterols, terpenoids, carbohydrates, glycosides, tannins, alkaloids, phenols, proteins, and saponins. However, glycosides, phenols, and cholesterols 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±0.18mm) against S. aureus while minimum zone of inhibition (12.30±0.16mm) against S. typhi. Hexane crude extract leaves of Datura stramonium showed maximum zone of inhibition (18.00±0.27mm) against S. aureus while minimum zone of inhibition (11.05±0.62mm) against E. coli. Chloroform extract of the plant also showed maximum zone of inhibition (18.43±0.57mm) against B. subtilis while minimum zone of inhibition (11.51±0.54mm) 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±0.16mm) against A. niger was exhibited by petroleum ether extract while minimum zone of inhibition (8.05±0.43mm) 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, sinutately dentate and minutely puberulous. 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^{2}. 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^{3}. Leaves extract of the plant contains different types of secondary metabolites such as glycosides, phenols, lignins, saponins, sterols, and tannins^{3}. 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^{4}. Scopolamine is used to treat Parkinson’s disease and painful visceral spasms through injection^{5}. The leaves extract of D. stramonium is used for the treatment of baldness^{6}, management of pains^{7}, anti-inflammatory, and antispasmodic^{8}, skin diseases^{9}, anticholinergic and sedative^{10}. 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 (Aspargillus niger, Fusarium oxyspourm, 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.

**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*)

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*.

**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.

**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*.

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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 (7.07±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*. This might be due to the fact that Gram-negative bacteria have an outer lipopolysaccharide membrane carrying the structural lipopolysaccharide components, which makes their cell wall impermeable to antibacterial chemical substances.

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*. Furthermore, the antifungal activities of the methanol extract from different part of the plant

### Table-1

<table>
<thead>
<tr>
<th>Cpd</th>
<th>Gram(-) Bacteria</th>
<th>Gram(+) Bacteria</th>
<th>Fungi</th>
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<td></td>
<td><em>S. typhi</em></td>
<td><em>E. coli</em></td>
<td><em>B. subtilis</em></td>
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<td></td>
<td>20 µg/mL</td>
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<td>Bav</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
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<tr>
<td>DMSO</td>
<td>- - - - - -</td>
<td>- - - - - -</td>
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</table>

CAL = Chloramphenicol, DMSO = dimethylsulfoxide, Cpd = 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 zoon were reported in mean (n=3) ± standard deviation.
on the vegetative and generative phases of the growth process of four fungi strains (Fusarium semitectum, Fusarium colmorum, Ceratocystis ulmi, and Rhizoctina solani) were reported in Iran showed similar result with the current study.22

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 ethan, 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 glycosides23. 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.

References


Table-2

<table>
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<tr>
<th>S. No</th>
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+ = the presence and - = the absence of chemical constituents


