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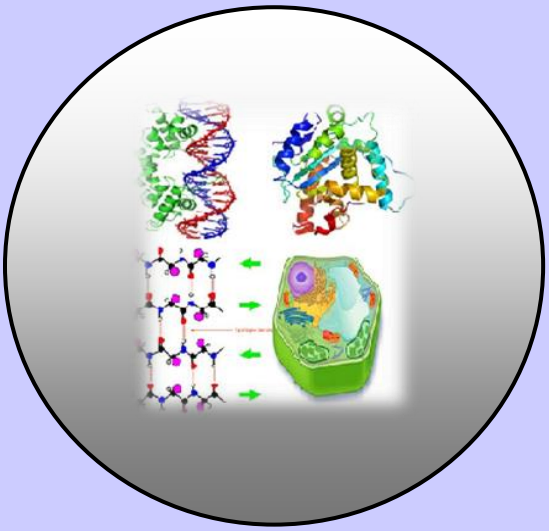

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RESEARCH PAPER

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Extraction and Bioactivity Study of Seeds and Roots Extract of *Datura stramonium*

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ABSTRACT

The main objective of this study is to undertake antibacterial evaluation of root and seed extract of Datura stramonium. Datura stramonium is a wide spread annual plant from the solanaceae family. It is well known folklore medicinal plant. It is used for different ailments such as anti- dandruff, antiasthmatic, swellings, toothache, stomach-ache, burns, skin disease, and diarrhea by traditional methods. The dried and powdered root and seed of Datura stramonium were extracted with increasing gradient of solvents (petroleum ether, chloroform, ethyl acetate and methanol) and screened for antibacterial activity on four pathogenic bacterial strains: Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Busilussubtilis. The antibacterial activity was evaluated by measuring the zone of growth inhibition surrounding the discs in millimeter with the ruler and the results of antibacterial activity were recorded. When antibacterial activities of crude extracts observed, Ethyl acetate extract of seed parts of Datura stramonium was found to have better activity against all the four test strains. On the other hand, the petroleum ether and chloroform extract of root of Datura stramonium were less active against the test strains.
Key words: Antibiotic Resistance, Antimicrobial, Practice and Treatment Strategies.

INTRODUCTION

Plants produce an enormous variety of natural products with highly diverse structures. These products are commonly termed as primary metabolites and secondary metabolites. Primary metabolites are responsible for the growth and development of plant for example; sugars, protein, lipids, starch. Secondary metabolites appear to function primarily in defense against predators and pathogens and in providing reproductive advantage as intra-specific and inter-specific attractants. They may also act to create competitive advantage as poisons of rival species. Some of the most important secondary metabolites (bioactive phytochemical) constituents are alkaloids, essential oils, flavonoids, tannins, terpenoid, saponins, and phenolic compounds [Goh et al., 1955, Getahun et al, 2012 and Kirtikar, 1955]. These secondary metabolites are plant-derived substances that have a great interest

owing to their versatile applications. Medicinal plants are the richest bio-resource on drugs of traditional systems of medicine, modern medicines, folk medicines, and chemical entities for synthetic drugs. A number of interesting outcomes have been found with the use of a mixture of natural products to treat diseases, most notably the synergistic effects and polypharmacological application of the plant extracts [Baris et al., 2006, Hammer et al., 1999 and Gibbons, 2003]. *Datura stramonium* is common weed in disturbed areas, waste ground, infertile soils in field's road sides of at attitude of 600-2800 m. This herb is originated in tropical North American now it is cosmopolitan weed. It occurs in most Ethiopian regions and also in Eritrea, Sudan, and Somalia and throughout tropical Africa, Europe and part of Asia [Ermias, 2011]. It is a widely growing plant and well known to have potent pharmacological activity with great utility and usage in folklore medicine. Water and ethanol extract of *Datura stramonium* contains saponins, tannins, carbohydrates, proteins, steroids, flavonoids, alkaloids, phenols, and glycosides and use in medicine due to its analgesic and anti-asthmatic activities [Sonip et al., 2012 Reddy, 2009]. Leaves extract of the plant contains different types of secondary metabolites such as glycosides, phenols, lignins, saponins, sterols, and tannins [Nain, 2013]. The alkaloids atropine and scopolamine are the primary bioactive substances reported in *Datura stramonium* extracts. Atropine has been used in treating Parkinson's disease, peptic ulcers; diarrhea, and bronchial asthma. Scopolamine is used to treat Parkinson's disease and pain full visceral spasms through injection. The leaves extract of *Datura stramonium* is used for the treatment of baldness , management of pains, anti-inflammatory, and anti-spasmodic, skin disease , ant cholinergic and sedative [Ivan et al., 2006, Van Wyk and Wink, 2004, Khan and Khatoon, 2008, Nijorege, 2012, Dwivedi et al., 2008, Rahmatullah et al., 2010 Wazir et al., 2004]. *Datura stramonium* seed are used for ache and bronchitis in sakaya province of North West Turkey and locally called "Totala", while the petroleum ether extract is also found to possess antimicrobial activities against *Esheria coli* and trachy stemon orientational .It is also used commonly in ethno veterinary practices in Nepal and Gujjar community in India [Uzun et al., 2004, Raut, 2012 and Gour et al., 2010]. *Datura stramonium* is found in most part of Ethiopia. It is known as *"astenagir*" (*ate faris*) and it is used as traditional medicinal plant. *Astenagir,* the name implies it is true drug" during *afersata*" hearing held by elder to solve criminal activities [**Molvaer, 2010**]. Another uses of D. stramonium are as anti-dandruff, wound healing for asthma, toothache. Even though its bioactivity and phytochemicals investigation was not studied by many researchers in Ethiopia. Therefore this research will provide promising information of the presence of chemicals that can be used as a medicinal value and motivates for further advanced researchers.

BOTANICAL INFORMATION

Datura stramonium L, known by the common names Jimson weed or devil's snare. It is a plant in the night shade family .Other common names for *Datura stramonium* includes thorn apple and moon flower [Jimson Weed, 2013] and it has the Spanish name Toloache. Other names for the plant include hell's bell's devil's, trumpet, devil's weed, tolguacha Jimson Town weed, stink weed, locoweed, prickly burr, and devil's cucumber [Thorn Apple].

Distribution

Datura stramonium is native to deserts of the North American south west, central and South American, Europe, Asia, and Africa. It is mainly distributed in the Himalaya region from the Kashmir up to 2700m, in the hilly district of central and south India [Khare, 2007].

Ethno medicinal use

In western Nepal, leaves of *Datura* along with the leaves of cannabis sativa and stem of Neopicrorhiza scrofulariflora, are pounded with water and applied to treat headache. Datura seeds are crushed with grains of rice and taken orally to relieve ingestion. In parts of central Nepal, fresh leaves are warmed and placed on a sprained body part repeatedly, before going to bed, for the alleged analgesic effect. Juice from the leaves is given with warm milk to expel intestinal worms, specifically tape worm [Pressiel and Pressiel, 2002]. In Nigeria, the seeds are mixed with palm oil and applied to severe cases of insect bites and stings [Das et al, 2012]. In India the seeds are used as tonic and febrifuges; the leaves are roasted and applied locally to relieve pain [Joshi et al., 2000].Women in Pakistan warm up 5 to 8 leaves in low fire, and then tie on to sagging breast to bust them up. Two to five seed are added to a cup of green tea to relieve headache [Rajb, 2011]. Native American used Datura seedfor many years as a euphoric agent. Since the 1800s, it was used as a therapeutic agent in Great Britain [Eg Harevba and Hatua, 2008].

Figure 1. Different Parts of *Datura stramonium.*

Statements of the problems

Ethiopia has rich and diverse flora. It is therefore not surprising that some of these plants have chemical compounds of therapeutic value that may be used in the treatment of many diseases.

Nevertheless, most of these medicinal plants within Ethiopian flora have not been phytochemically investigated [Dessanges, 2000, Jakabova et al., 2012]. *Datura stramonium* is among them with no detail phytochemical information and yet it is one of the medicinal plants traditionally used by Oromo people. Thus, the present study is intended to extract the chemical constituents of *Datura stramonium* and screen them on selected bacterial strains. This will give a scientific input of the local use of the plant material.

Objectives of the study

General objective

The main objective of this study is to undertake antibacterial evaluation of root and seed extract of *Datura stramonium.*

Specific objectives

- To extract the roots and seed of *Datura stramonium* using gradient increasing polarity of organic solvents (petroleum ether, chloroform, and Ethyl acetate).
- To examine the antimicrobial activity of the crude extracts from the root and seed of *Datura stramonium* against four bacterial species namely *Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa* and *bacillus subtilis* using *in vitro* tests.

Significance of the study

Datura stramonium is one of the traditional medicinal plants that are used in most parts of Ethiopian regions .The plant is used to treat different types of disease by traditional method. Thus the finding of this research will provide important information on bioactivity, and gives a hint for more advanced research in order to identify important chemical components that are active on pathogens.

MATERIAL AND METHODS

General

Rotary Evaporator (Heidolph Laborata 4000) was used for solvent evaporation from extracts. Chemicals used during this work were products of Sigma-Aldrich of Analytical Reagent (AR) grade. The solvents used were petroleum ether, chloroform, Methanol and Ethyl acetate for extraction. Standard antibiotic drug (Gentamicin 5 µg), Mueller Hinton agar, nutrient agar and saline solution were used as a culture medium during antibacterial test.

Plant material Collection and preparation

The plant materials including; the roots and seeds of *Datura stramonium* were collected from their natural habitat, in Oromia region Jimma zone, around Jimma town in south west of Ethiopia which is 346 km. from Addis Ababa. The seeds and the roots of the plant were air dried under shade separately and powdered to suitable size to improve the subsequent extraction by rendering the sample more homogenous, increasing the surface area, facilitating the penetration of solvent into the cells by using mechanical grinder.

Extraction and antibacterial assay

Extraction

The air dried and powdered plant materials were extracted using petroleum ether, Chloroform and ethyl acetate, methanol using maceration by each solvents for one day (24 hrs). The solvent was removed using a rotary evaporator to afford crude extract. The crude extract was stored at room temperature until use for the next antimicrobial test.

The test solution was prepared by dissolving 100mg of crude extract in 1mL of dimethyl sulfoxide (DMSO) to achieve final stock concentration of 100mg/mL solution of the test sample. Microorganisms used for evaluation of antibacterial activities of the crude extracts were Gram-positive (*Staphylococcus aureus ATCC25903)*, Gram-negative *(Escherichia coli ATCC 25722)*, Gram-negative (*Pseudomonas aeruginosa DSMZ 1117)*, and Gram-negative (*bacillus subtilis).* These standard bacterial strains were obtained from the Department of Biology, Jimma University.

Preparation of fresh inoculums for bioactivity test of crude extracts

The antibacterial activity test was done using disc diffusion method standard procedures [Mesfin, 2004]. Muller Hinton Agar culture media were used for growing of organisms. The culture media was boiled in distilled water to dissolve the media and autoclaved at 121^{o}C for 15min and poured into sterile Petri dishes refrigerator until use.

Antibacterial assay

Preparation of test solutions and bacterial strains for preliminary activity

After the culture media had solidified, organisms were uniformly seeded with it. Four well-isolated colonies of the same morphological type were selected from an agar plate culture and the top of each colony was touched with a loop, and the growth was transferred into a tube containing 4.5ml of a suitable nutrient broth medium. The broth culture was incubated at 37^{o}C until it achieves or exceeds the turbidity of the 0.5 McFarland standards for 8 hours. The turbidity of the actively growing broth culture was adjusted with sterile saline solution to obtain turbidity optically comparable to that of the 0.5 McFarland standards which was resulted in a suspension containing approximately 1 to 2x10^{8}CFU/mL for different strains. Inoculums containing 1 to 2 x 10^{8}CFU/mL of bacteria were spread on the solid plates with a sterile swab moistened with the bacterial suspension. 50mg/mL concentration of 20µL of the working suspension of the same concentration of the sample plant gradient extracts and the same volume of extraction solvent and DMSO for negative control was impregnated using No.1 What man filter paper disc (diameter 6mm) with the help of micropipette. Positive control using Gentamicin was assayed simultaneously. Plates were left for 10 minutes till the extract diffuse in the medium with the lid closed and incubated at 37°C for 24 hours. After overnight incubation, the plates were observed for the zone of inhibition (ZI) and the diameter of the inhibition zone was measured using ruler and mean was recorded.

RESULTS AND DISCUSION

RESULTS

Antibacterial activity test of crude extracts from root and seed of *Datura stramonium*

The air dried and powdered root and seed of *Datura stramonium* were extracted with increasing gradient of solvents. The solvents used were petroleum ether, chloroform, and methanol. 3000g of the powdered plant material for each plant part were used for extraction. The amounts of the crude extracts and their percent yields are summarized here below (Table1).

The crude extracts from seeds and root parts of *Datura stramonium* were subjected to four bacterial strains (*Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa and Bacillus subtilis)*.

The antibacterial activity was evaluated by measuring the zone of growth inhibition surrounding the discs in millimeter with the ruler and the results of antibacterial activity were recorded. Ethyl acetate crude extracts have shown more antibacterial activities, but less active than the observed activity of the reference drug (Gentamicin) as demonstrated by the observed inhibition zone values (Table 2). On the other hand, the petroleum ether and chloroform extract of root of *Datura stramonium* were less active against the test strains (Table 3). When antibacterial activities of crude extracts observed, Ethyl acetate extractof seed parts of *Datura stramonium* was found to have better activity against all the four test strains.

Table 1. Summary of the yield of various root and seed extracts from *Datura stramonium.*

Solvent	**Root** of *Datura stramonium*		*Seed of Datura stramonium*	
	Weight of crude extract (g)	% yield	Weight of crude extract (g)	% yield
Petroleum ether	1.13	4.37 %	5.96	0.2%
Chloroform	1.25	0.004 %	11.74	0.93%
Ethyl acetate	-	-	2.43	0.81%
Methanol	5.0	1.66%	24.02	1.98%

Table 2. Antibacterial inhibition zones of crude extracts of seeds of *Datura stramonium*

Strain	Petroleum ether extract	Chloroform extract	Ethyl acetate extract	Gentamicin	DMSO
E. coli	7.5	7	8.5	24	-
P. aeruginosa	-	7.5	9.5	25	-
B. subtilis	9	7.5	10	23	-
S. aureus	-	-	7.5	32	-

Table 3. Antibacterial inhibition zones of crude extracts of root of *Datura stramonium.*

Strain	Petroleum ether extract	Chloroform extract	Gentamicin	DMSO
E. coli	-	8	24	-
P. aeruginosa	11	7.6	25	-
B. subtilis	-	-	23	-
S. aureus	7	7	32	-

DISCUSSIONS

The antibacterial activity test of seeds and root extracts from *Datura stramonium* that were extracted bysolvents like: petroleum ether, ethyl acetate and chloroform showed varying degree of response towards selected bacterial strains or pathogenic bacteria. As this study indicates, the crude extracts were showed wide range zones of inhibition against the four test bacterial strains.

Among the tested bacteria the crude extract of seed, petroleum extract was found to be the most sensitive against bacillus and escherichia coli. The crude extract found by ethyl acetate has high sensitivity on *bacillus* and less sensitive on *Staphylococcus aureus*. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were less sensitive to a chloroform extracts. The crude extracts from root extracts of *Datura stramonium* has less sensitivity on four bacterial strains. Petroleum ether extract showed wide range zone of inhibition against *Pseudomonas aeruginosa*.

CONCLUSION

Plants played an important role in traditional medicine of Ethiopia and *Datura stramonium*is one of the potential plant used for the treatment of various diseases caused by microorganisms. Though the plant has a variety of medicinal properties there are limited information about the active compounds and their biological activity. The scientific findings of the present study proved that the ethyl acetate and petroleum ether seed crude extract as well of some of the support the traditional use of the plant against various infectious diseases as evidenced by the promising *in vitro* assay result. However, as evidenced from the activity of the crude extract compared to individual isolated compounds there are still more active and unidentified secondary metabolites still present in the plant which initiates further phytochemical analysis of the plant.

This study concludes that the extraction of *Datura stramonium* seeds extract by ethyl acetate are active on four bacterial strains where as roots extract of *Datura stramonium* by chloroform has no activity for all bacterial strains.

RECOMMANDATION

- More biological assay on other strains needs to be conducted on various extracts of the plant so as to establish the traditional use of the plant.
- For further isolation of chemical components of *Datura stramonium* advanced research will be needed.
- Qualitative analysis of the chemical constituents of various extracts has not been done so far and future qualitative determination of the presence of flavanoids, tannins, anthraquinones, terpenoids and alkaloids needs to be conducted so as to assist other researchers who are interested to work on the plant.

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