Phytochemical screening from leaves of *Datura stramonium* L. and *Justicia schimperiana* and evaluation of their antimicrobial properties against selected human enteric bacteria

M.Sc. THESIS

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DECEMBER 2017
HARAMAYA UNIVERSITY, HARAMAYA

Phytochemical screening from leaves of *Datura stramonium* L. and *Justicia schimperiana* and evaluation of their antimicrobial properties against selected human enteric bacteria

A Thesis Submitted to the School of Biology and Biotechnology,
Directorate for Postgraduate Program
HARAMAYA UNIVERSITY

In Partial Fulfillment of the Requirements for the Degree of
MASTER OF SCIENCE IN BIOTECHNOLOGY

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December 2017
Haramaya University, Haramaya

APPROVAL SHEET
POSTGRADUATE PROGRAMDIRECTORATE
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I hereby certify that I have read andevaluated this Thesis entitled "Phytochemical screening from leaves of *Datura stramonium* L. and *Justicia schimperiana* and evaluation of their antimicrobial properties against selected human enteric bacteria**"**prepared under my guidance by Frehiwot Abebe. I recommend that it be submitted as fulfilling the thesis requirement

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DEDICATION

I dedicate this thesis to my Familyfor their memorable and valuable encouragements in my academic careerand life starting from the day I was born.

STATEMENT OF THE AUTHOR

By my signature below, I declare and affirm that this Thesis is my own work. I have followed all ethical and technical principles of scholarship in the preparation, data collection, data analysis and compilation of this Thesis. Any scholarly matter that is included in the Thesis has been given recognition through citation.

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ACKNOWLEDGEMENTS

First and foremost, I would like to express my heartfelt gratitude to my major advisor Dr. Meseret Chimdessa for his excellent advice, guidance, critical reading, and valuable suggestions during the course of my study. I am very grateful to my co-advisor Dr. Ameha Kebede for providing invaluable guidance and critical supervision while conducting my study.

I would like to extend my gratefulness to Ministry of Education (MOE) for providing me the chance to join School of Graduate Study at Haramaya University.

I would also like to thank the Collage of Natural and computational Science andSchool of Biology and Biotechnology, Haramaya University, for all academic supports I got during the study period.

My heartfelt thanks are also extended to general and microbiology laboratory technicians for providing me all the resources and facilities used in the study. Technical supports of technical assistances of general and microbiology laboratory in particular, Tadele Teklu, Yimeslal Atinafu and Kidist, are gratefully acknowledge.

My special thanks go to my mom, sisters and friends who have been and always are the greatest help in my life.

LIST OF ABBREVIATIONS/ ACRONYM

EPHI	Ethiopian Public Health Institution
ICU	Intensive Care Unit
MDR	Multi Drug Resistance
MDRO	Multi Drug Resistant Organism
MHA	Mueller Hinton Agar
MRSA	Methicillin Resistant *Staphylococcus Aureus*
SE	*Staphylococcus Enterotoxin*
TAE	Tannic Acid Equivalent
TPC	Total Phenolic Content
VRE	Vancomycin Resistant *Enterococci*
WHO	World Health Organization

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Phytochemical screening from leaves of *Datura stramonium* L. and *Justicia schimperiana* and evaluation of their antimicrobial properties against selected human enteric bacteria

Abstract

The growing phenomenon of antibiotic resistance, particularly to pathogenic microorganisms, in current medicine, has directed the concern of scientists for finding novel antimicrobial agents from plant origin with negligible side effect. The aim of this study was to screen the major secondary compounds of leaves of D. stramoinum and J. schimperiana and to evaluate their antimicrobial properties against (S. aureus, S. typhi and S. boydii). Extraction was done by maceration of leaf powders using ethanol as a solvent. Antimicrobial activities of both plant species leaf extracts were determined by disc diffusion and broth dilution methods.The result of phytochemical analysis showed the presence of alkaloid, flavonoids, saponin, steroid, tannin and terpenoidin leaves of both plant species and absence of phylobatannin. All quantified compounds were found in higher amount in J. schimperiana (phen-3.14, alk-410, sap-586.6, and terp-323.3 mg/ml) than in D. stramonium (phen-3.23, alk-42.3, sap-46.3 and terp-39 mg/ml). Results of antibacterial assay revealed that extracts of both planAt species showed inhibitory activity against both the tested bacterial pathogens. Max inhibition was recorded against S. boydii (23.3±3.055) and min inhibition against S.typhi (19±1.000) by leaf extract of D. stramonium. In the other hand, for J. schimperiana max inhibition was recorded against S. boydii (8±1.000) and min inhibition against S. aureus (3±1.000). Lowest MIC in Datura leaf was recorded against S. aureus (2.5 mg/ml) and maximum MIC was recorded against S. boydii (0.625) mg/ml). For J. schimperiana the maximum MIC was recorded against S. boydii (5.0 mg/ml) and minimum MIC against S.aureus and S. Typhi (10 mg/ml). Based on this result it is concluded that leaf extracts of both tested plant species have the major secondary metabolites and antibacterial activities against the tested bacterial isolates.

Key words: Alkaloids, Antibacterial activity, *D. stramonium*, Flavonoids, *J. schimperiana*, Phytochemical analysis, Saponins, Steroids, Tannins and Terpenoids.

1. INTRODUCTION

The growing phenomenon of antibiotic resistance, particularly to pathogenic microorganisms has directed the concern of scientists for finding novel antimicrobial agents from plant origin with negligible side effect (Cowan, 1999). Nature has been a source of medicinal agents for thousands of years and an impressive number of modern drugs have been derived from natural sources, many of these isolations were based on the uses of the agents in traditional medicine (Cragg and Newman, 2001). Medicinal plants have a long history of use in most communities throughout the world. It has been confirmed by WHO that herbal medicines serve the health care needs for about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries (Duru*et al*., 2006). Plants and plant-based medicaments are the basis of many of the modern pharmaceuticals we use todayfor our various ailments. They are best owed with large number of pharmaceutically useful compounds, which can be studied for investigation of new drugs for many serious diseases such as cancer, tumor, AIDS and many human degenerative diseases. Medicinal plants are known to produce certain bioactive molecules which inhibit bacterial or fungal growth (Shanmugam, *et al*., 2002).

In Africa, the use of traditional medicine has persisted over the years and the last few decades have witnessed an upsurge of interest in traditional medicine and other alternative forms of healthcare in the developing and developed countries (Duru*et al*., 2006). In Ethiopia, plants have been used as a source of traditional medicine from time immemorial to combat different ailments and human sufferings (Asfaw*et al.*, 1999). According to Pankhurst (1965), Ethiopia is a country characterized by a wide range of climate and ecological conditions, and possesses enormous diversity of fauna and flora. The country possesses a particularly wide range of potentially useful medicinal plants, more extensive indeed than available in many other parts of the world. Dawit(1986) estimated that 95% of traditional medical preparations in Ethiopia are of plant origin. Due to its long period of practice and existence, traditional medicine has become an integral part of the culture of Ethiopian people (Pankhurst, 1965; Mirgissa, 1996). It is common for people living in rural and urban centers to treat some common ailments using plants available around them.

Datura stramonium (Kingdom: Plantae, Division: Magnoliophyta, Class: Magnoliopsida, Order: Solanales, Family: Solanaceae, Genus: Datura and Species: *Datura stramonium*) belonging to family Solanaceae, is a herb growing to about 60-120 cm or more tall, branched, and pubescent plant. Leaves are 8-17x4-13 cm, ovate, sinuately dentate and minutely puberulose. *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, but 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 (Ermias, 2011).

D. stramonium is well known to have potent pharmacological activity with a great utility and usage in folklore medicine. The presence of different secondary metabolites has been reported previously in its leaf extracts (Preissel and Preissel, 2002). For example, tropane alkaloids such as scopolamine, hyoscyamine, and atropine are found in this plant, primarily in its seeds and flowers (Duke and Ayensu, 1985). Because of the presence of these fundamental biochemials*D. stramonium* is considered as treasured medicine and useful in the treatment of Leucoderma, skin disorders, ulcers, bronchitis, jaundice, hysteria insanity, heart disease, fever and piles (Duke and Ayensu, 1985; Dabur*et al*., 2004).

Justicia schimperiana (Kingdom: Plantae, Division: Magnoliophyta, Class: Magnoliopsida, Order: Lamiales, Family: Acanthaceae, Genus: Justicia and Species: *Justicia schimperiana*) is one of the plants that belong to the family Acanthaceae.It is a very common erect shrub, usually much branched from the base. It is relatively fast growing and prefers altitude of 2438.4 m or above. The shrub is abundant in Ethiopia, Kenya and Tanzania. The plant is used as a live fence. It is an erect shrub up to 4 m high; stem woody and with internodes; leaves decussate, estipulate, simple, ovate-oblong in outline; inflorescence thyroid, with densely flowered spikes; corolla bilabiate white to creamy white; fruit capsule (Abebe and Ayehu, 1993).

Traditionally, the decoction of the leaf of the plant was taken mixed with local beer as a remedy against bronchial asthma. The leaves are used by local people for protection against contagious diseases (Murthy, 1993). Also, different Traditional uses of the plant have been recorded, in Eastern Ethiopia, the plant is used as a laxative (Getahun, 1976). In Northern Ethiopia, the plant alone or in combination with other plants is used for various diseases treatments such as epilepsy, mental illness, eye diseases, jaundice, malaria, leprosy, syphilis, gonorrhea, rabies,

measles, relapsing fever, vitiligo, gout and acute febrile illness **(**Abebe, 1996**)**. In Southwest Ethiopia, it is used for malaria, scabies, where the fresh leaves are crushed and macerated in water and then the affected area is washed with the macerate **(**Teferi, 2003).

The determination of the phytochemical constituents of plant extracts is essential in order to ensure the reliability and repeatability of pharmacological and clinical research, and to understand their bioactivities and possible side effects of the active compounds. Although *D. stramonium* and *J. schimperiana* are widely distributed in Ethiopia and known for their folk medicine. Thus, this study addresses this gap between folk medicine and scientific medical industries by having plant samples collected from East Hararge.

General objective:

- To identify and quantify the major secondary compounds and to evaluate their antibacterial activities of the crude extracts of leaves of *D. stramonium* and *J. schimperiana*

The specific objectives:

- To qualitatively detect the presence of major secondary compounds from leaves of *D. stramonium* and *J. schimperiana*
- To quantify the detected secondary compounds from leaves of *D. stramonium* and *J. schimperiana*
- To evaluate the antibacterial activities of the crudeextracts of leaves against selected bacterial pathogens
- To determine the minimum inhibitory concentration of *D.stramonium and J.schimperiana*

2. LITERATURE REVIEW

2.1. Medicinal plants

Plants are rich in a wide diversity of secondary metabolites which have been found to exhibit antimicrobial, antioxidant, anti-infectious and antitumour activities. The affordability, reliability, availability and low toxicity of bio-chemicals in therapeutic use has made them widespread for implementation in medical health care sector (Akroum*et al*., 2009). An increased number of pathogens have developed resistance to multiple antibiotics (Multiple Drug Resistance), Because of the mutagenic nature of bacterial DNA, the rapid multiplication of bacterial cells, constant transformation of bacterial cells and therefore be untreatable. Thus, countless studies have been directed on medicinal plants for antimicrobial activities and efficacy (Stace, 1997).

Medicinal plants are source of important substance for the study of their traditional use through the verification of pharmacological effects and can be a natural composite source that acts as new anti-infectious agents. A number of medicinal plants have been screened for antimicrobial activity in recent years and efforts have been done to identify their active constituents. In spite of recent development in the synthetic drug, chemistry and production of antibiotics, plants still occupy an important role in the modern and traditional systems in all over the world. Due to the indiscriminate use of antibacterial drugs, the microorganisms have developed resistance to many commercial antibiotics. Therefore, investigation of chemical compounds within medicinal plants has become desirable. Many efforts have been made to discover new antimicrobial compounds from various sources such as microorganisms, animals and medicinal plants. Systematic investigation of folk medicine may result in the discovery of novel effective compounds. Therefore, several medicinal plants have been evaluated for possible antimicrobial activity and get remedy from variety of antimicrobial origin (Shanmuga, *et al*., 2002).

In Africa, traditional medicine plays a central role in health care needs of rural people and urban poor.Here, it is said that, this situation would remain so long as modern medicine continues to be unable to meet the health care of the people of the continent effectively (Jansen, 1981). Their value and role of this health care system will not diminish in the future, because they are both culturally viable and expected to remain affordable, while the modern health care service is both limited and expensive (WHO, 1998).

Plants have been used as a source of traditional medicine in Ethiopia from the time immemorial to combat different ailments and human sufferings (Asfaw,*et al*., 1999). Due to its long period of practice and existence, traditional medicine has become an integral part of the culture of

Ethiopian people (Pankhurst, 1965; Mirgissa, 1998). According to Dawit Abebe (2001), there is a large magnitude of use and interest in medicinal plants in Ethiopia due to acceptability, accessibility and biomedical benefits. In this country, the long history of use of medicinal plants is reflected in various medico- religious manuscripts produced on parchments and believed to have originated several centuries ago (Fassile, 2001).

2.2. Secondary metabolites

Phytochemicals (from the Greek word phyto, meaning plant) are biologically active, naturally occurring chemical compounds found in plants,which provide health benefits for humans further than those attributed to macronutrients and micronutrients (Hasler and Blumberg, 1999). They protect plants from disease and damage and contribute to the plant's color, aroma and flavor. In general, the plant chemicals that protect plant cells from environmental hazards such as pollution, stress, drought, UV exposure and pathogenic attack are called as phytochemicals (Gibson *et al*., 1998). Now a day, it is clearly known that they have roles in the protection of human health, when their dietary intake is significant. More than 4,000 phytochemicals have been cataloged (American cancer society, 2000) and are classified by protective function, physical characteristics and chemical characteristics (Meagher, 1999).

In wide-ranging phytochemicals are found in fruits, vegetables, legumes, whole grains, nuts, seeds, fungi, herbs and spices (Mathai, 2000). Broccoli, cabbage, carrots, onions, garlic, whole wheat bread, tomatoes, grapes, cherries, strawberries, raspberries, beans, legumes, and soy foods are common sources (Moorachian, 2000). Phytochemicals accumulate in different parts of the plants, such as in the roots, stems, leaves, flowers, fruits or seeds (Costa *et al*., 1999). Many phytochemicals, particularly the pigment molecules, are often concentrated in the outer layers of the various plant tissues. Levels vary from plant to plant depending upon the variety, processing, cooking and growing conditions (King and Young, 1999).

These compounds are known as secondary plant metabolites and have biological properties such as antioxidant activity, antimicrobial effect, modulation of detoxification enzymes, stimulation of the immune system, decrease of platelet aggregation and modulation of hormone metabolism and anticancer property. There are more than thousand known and many unknown phytochemicals (Narasinga, 2003).

Some of secondary metabolites are listed below:

Phenolic group

Phenolics are a group of compounds characterized by at least one aromatic ring bearing one or more hydroxyl groups. Most of the thousands of phenolics known to date are of plant origin.These phenolic compounds are biosynthesized through shikimate pathway. Phenolics are of great importance as cell wall components. They form part of cell wall structures such as lignins, cutins and suberins, which provide mechanical support and function as barriers against microbial attack.Phenolics also play a defensive role in plants by protecting against predators.Simple phenolic acids, polyphenolics like tannins and phenolic resins at the plant surfaceare effective feeding deterrents (Carson and Hammer, 2010). Phenolics are accumulated as post-infectional low molecular compounds called phytoalexins as a result of microbial attack.Among the phenolic phytoalexins, hydroxycoumarins and hydroxycinnamate conjugates contribute to disease resistance mechanism in plants. Phenolic compounds also produce allelopathic effect. A well-known compound fromJuglans species is juglone which is highly toxic for a wide range of plants. It occurs in theplant as a non-toxic glucoside and is made active by deglucosylation and oxidation after leaching from the leaves into the soil (Savoia, 2012).

Flavonoids

Flavonoids are phenolic structures found abundantly in photosynthesizing cells. They are usually found in many common edible plant parts such as: Fruits, vegetables, nuts and seeds. Flavonoid compounds have a structural feature of the 2-phenyl-benzopyrane or flavine nucleus, which consists of two benzene rings linked through a heterocyclic pyrane ring. To date, there have been 14 classes of flavonoids identified; they differ based on the chemical nature and position of substituents on the different rings. Many flavonoids have been known to have antioxidant, anti-inflammatory and antitumor activity (Carson and Hammer, 2010).

Alkaloids

Alkaloids are organic heterocyclic nitrogen compounds that are basic-forming water-soluble salts. They contain nitrogen, which is usually derived from an amino acid. Several classes of flavonoids include phenylalkylamines, pyrrolidines, tropane alkaloids, pyrrolizidines and purine

alkaloids (Carson and Hammer, 2010; Savoia, 2012; Ramawat, 2007). These metabolic compounds are grouped into three classes, depending on the precursors and final structure of the molecule. The classes include; (1) True alkaloids, which are basic and contain nitrogen in a heterocyclic ring-includes nicotine, (2) Pseudoalkaloids, which are also basic but they are not derived fromamino acids-includes caffeine and (3) Protoalkaloids, which are amino acid derivatives, basic, but the nitrogen is not in a heterocyclic ring-includes phenylethylamine derived alkaloids including mescaline. Furthermore, alkaloids have been shown to have analgesic effects; morphine alkaloids are pain relievers that are used as narcotics (Agbafor*et al*., 2011).

Terpenoids

Among the terpenes some intoxicating or hallucinogenic compounds can be found; examples are the cannabinoids, which are synthesized predominantly via the methylerythritol–phosphate (MEP) pathway in the plastids. Terpenes can be classified as either essential or nonessential compounds. Essential terpenes include the carotenoids, which play an important role in photosynthesis as components of light-harvesting complexes, and also as protective compounds against high light intensities, but also antioxidative compounds such as tocopherol. Many plant hormones also belong to the terpenoid family, but as these are essential signaling molecules for growth and development and occur in rather small amounts, they are not usually viewed as secondary metabolites. Among nonessential terpenes antimicrobial substances such as the monoterpenes can be found. The term nonessential means that the plant is at an advantage if it can produce these substances under stress conditions; however, under normal conditions nonessential terpenes will not result in a phenotype or prove to be fatal if the biosynthetic pathway is mutated (Savoia, 2012 and Ramawat, 2007).

Saponins

Saponins are steroid and triterpene glycosides, so named because of their soaplike properties. The presence of both lipid-soluble (the steroid or triterpene) and water-soluble (the sugar) elements in one molecule gives saponins detergent properties, and they form a soapy lather when shaken with water. The toxicity of saponins is thought to be a result of their ability to form

complexes with sterols. Saponins may interfere with sterol uptake from the digestive system or disrupt cell membranes after being absorbed into the bloodstream (Savoia, 2012).

2.3. Medicinal properties of *D. stramonium* and *J. schimperiana*

Datura stramonium L. (Solanaceae) is a well-known medicinal plant commonly used in phytomedicine to cure diseases and heal injuries. *D. stramonium* is a wild-growing plant widely distributed and easily accessible. It is traditionally used to cure different human diseases including skin disorder, ear pain, cough, fever, burns, and asthma in Ethiopia. In Morocco, this plant is used traditionally as a healing medicine whereby the leaves and flowers have been used in the treatment of Asthma (El Bazaoui*et al*., 2011). The atropine and related alkaloids have been also used in Japan for the treatment of gastrointestinal diseases, cardiopathy and Parkinson's disease (Takahashi *et al*., 1997). Yet, it has been demonstrated that *D. stramonium* aqueous leaf extract induced oxidative stress in different human cancer cell lines (Iman *et al*., 2011). Another investigation carried out by Hussain *et al*. (2011) verified that the ether extract of *D. stramonium* has antitumor activity and exerts its activity by inhibiting mitosis of cancer cells. One of the toxic components found in *D. stramonium* is tropane belladonna alkaloids (Mahnaz*et al.*, 2012). Tropane alkaloids are allelochemicals found in *D. stramonium* and they are assumed to play resistance role against herbivory (Shonle and Bergelson, 2000). Other research findings have demonstrated that *D. stramonium* contains alkaloids such as atropine and scopolamine, which can cause anticholinergic toxicity (Bontayan, 2010).

In Ethiopia *J. schimperiana* alone or in combination with other plants is used for various diseases such as epilepsy, mental illness, eye diseases, jaundice malaria, leprosy, syphilis, gonorrhea, rabies, measles, relapsing fever, vitiligo, gout and acute febrile illness.

2.4. Distribution of *Datura stramonium* and *Justicia schimperiana*

D. stramonium is among allelopathic plants reproducing by seeds encapsulated in spiny seed pods which are apple-shaped (Wilbur, 1987; Alexander *et al*., 2008). It is an annual poisonous plant that grows to approximately 1.5 m high and it is characterized by solitary white, trumpet-shaped flower (Fatoba*et al*., 2001; Steenkamp *et al*., 2004; Richardson *et al*., 2007). This plant species falls under family Solanaceae (Waseem, 1998; Binev*et al.*, 2006). Furthermore, *D. stramonium* is known with different names across the world such as Angel's trumpet, Jimson

weed, Devil's trumpet, Devil's weed, Thorn apple, Jamestown weed, Stinkweed, Locoweed, Devil's cucumber and Hell's Bells (Mahnaz*et al.*,2012; Oseni*et al*., 2011).*D. stramonium*is naturalized to all four deserts of the American Southwest. Species of Datura can be found throughout the world, except in the colder or Artic regions. The plant lives in sandy flats, plains, arroyos up to 2,500 feet above sea level, and amidst disturbed soils. Jimson weed is commonly seen among roadsides in the Southwest.The origin of *D. stramonium* is not that much clear among conservationists although some botanists refer it to North America or Asia (Richardson *et al*., 2007). According to Mahnaz*et al.*, (2011), the plant is native to Asia, but it is also found in the West Indies, Canada and in USA.

Justicia schimperiana, which belongs to Acanthaceae family is a perennial, evergreen and highly branched shrub (1-2.5m high) with unpleasant smell and bitter taste. It has opposite ascending branches with white, pink or purple flowers. Only a few species are distributed in temperate region. The four main centers of distribution are Indonesia, Malaysia, Africa, Brazil and Central America (Reddy *et al*., 2013).

The plant grows wild in abundance all over Ethiopia, Sudan, Nepal, Sri Lanka, India, and the Pothohar region of Pakistan, particularly in the pharwala area. It is widely used in the treatment of cough, bronchitis, asthma and common cold (Karthikeyan, 2009).

2.5. Selected human enteric bacteria

2.5.1. *Staphylococcus aureus*

Staphylococcus aureus is a bacterium that causes staphylococcal food poisoning, a form of gastroenteritis with rapid onset of symptoms. *S. aureus* is commonly found in the environment (soil, water and air) and is also found in the nose and on the skin of humans.

S. aureus is a Gram-positive, non-spore forming spherical bacterium that belongs to the *Staphylococcus* genus. The *Staphylococcus* genus is subdivided into 32 species and subspecies. *S. aureus* produces staphylococcal enterotoxin (SE) and is responsible for almost all staphylococcal food poisoning (Montville and Matthews 2008; FDA 2012).

The growth and survival of *S. aureus* is dependent on a number of environmental factors such as temperature, water activity (aw), pH, the presence of oxygen and composition of the food. These

physical growth parameters vary for different *S. aureus* strains (Stewart 2003).The temperature range for growth of *S. aureus* is 7–48°C, with an optimum of 37°C. *S. aureus* is resistant to freezing and survives well in food stored below -20°C; however, viability is reduced at temperatures of -10 to 0°C. *S. aureus* is readily killed during pasteurization or cooking. Growth of *S. aureus* occurs over the pH range of 4.0–10.0, with an optimum of 6–7 (ICMSF, 1996; Stewart, 2003).

S. aureus is a poor competitor, but its ability to grow under osmotic and pH stress means that it is capable of thriving in a wide variety of foods, including cured meats that do not support the growth of other foodborne pathogens (Montville and Matthews, 2008). *S. aureus* is a facultative anaerobe so can grow under both aerobic and anaerobic conditions. However, growth occurs at a much slower rate under anaerobic conditions (Stewart, 2003).

Staphylococcus aureus is a major pathogen of increasing importance due to the rise in antibiotic resistance (Lowy, 1998). It is distinct from the CoNS (e.g. *S.epidermidis*), and more virulent despite their phylogenic similarities (Waldvogel, 1990; Projan and Novick, 1997). The species named *aureus*, refers to the fact that colonies (often) have a golden color when grown on solid media, whilst CoNS form pale, translucent, white colonies (Howard and Kloos, 1987).

2.5.2. ***Salmonella typhi***

The genus *Salmonella* was discovered by an American veterinary pathologist, Daniel Elmer Salmon, in 1885. The genus *Salmonella* refers to facultative, anaerobic intracellular bacteria which exhibit predominant peritrichous motility. *Salmonella* are Gram-negative, rod shaped, non-spore forming bacteria, with diameter ranging from 0.7 to 1.5μm and a length of 2 to 5μm belonging to *Enterobacteriaceae* family (Murray *et al.,* 1999; Coburn *et al.,* 2007).

Salmonella is a pathogen of high clinical relevance in both developing and developed nations causing food-borne illness and other diarrheal diseases as well as severe systemic infections and economic losses. Many serovars of *Salmonella* can infect and colonize a wide variety of hosts, with outcomes ranging from sub-clinical infections to life threating systemic fatal disease (Jones *et al.,* 2008a; Lahiri*et al.,* 2010).

Salmonella has been successfully isolated both form warm blooded and cold-blooded animals (Fookes*et al*., 2011; Schikora*et al*., 2011), furthermore, several sub-species of *Salmonella* are

known to infect plants. Hence *Salmonella* is often considered as a 'universal pathogen' (Fedorka*et al.*, 2000).

Salmonella infections in humans are responsible for causing two clinical syndromes mainly typhoid or enteric fever and colitis or diarrheal disease depending upon the serovar that is responsible for infection. Serovars such as *Salmonella typhi, Salmonella paratyphi*A and B cause systemic illness in humans, with clinical manifestations including enteric fever, abdominal pain, headache, transient constipation. Prolonged infections can result in severe hepatic, spleen, respiratory or neurological damage. Untreated, these infections result in high mortality rates of 20-25% (Parry *et al.*, 2002).

2.5.3. ***Shigella boydii***

Shigella are Gram-negative, non-motile bacilli belonging to the family*Enterobacteriaceae*. The genus *Shigella* includes four species: *S. dysenteriae, S. flexneri,S. boydii* and *S. sonnei*, also designated groups A, B, C and D, respectively. The first three species include multiple serotypes. S. *sonnei*and *S. boydii* usually cause relatively mild illness in which diarrhea may be watery or bloody. *S. flexneri*is the chief cause of endemic shigellosis in developing countries. Immunity is serotype specific (Clark *et al*., 2013).

The genus *Shigella* belongs to the family *Enterobacteriaceae*. The *Shigella* are Gram-negative rods, 0.3 to 1 μm in diameter and 1 to 6 μm in length, appearing singly, in pairs and in chains, are non-spore forming, facultatively anaerobic, oxidase negative, and ferment glucose and other carbohydrates without producing gas. By definition, all *Shigella spp.* are nonmotile and lysine decarboxylase negative. Additionally, the *Shigella* are Voges-Proskauer negative and methyl-red positive, do not utilize Simmons citrate, nor produce H2S and are arginine dihydrolase and urease negative. The *shigella*, with the exception of *S. dysenteriae*Type 1, are catalase positive. The optimum temperature of growth is 37ºC. The genus *Shigella* is divided into four species: *Shigella dysenteriae*(Group A), *Shigella flexneri*(Group B), *Shigella boydii* (Group C), and *Shigella sonnei*(Group D) (Collins *et al*., 2004). Each of these species, with the exception of *S. sonnei*, has several serotypes based on the reactivity with hyperimmune serum. The proportion of each species varies from country to country and from region to region. The hallmark of infection with *Shigella* is diarrhea with blood, often termed "dysentery." However, in most cases, *Shigella*

spp causes acute non-bloody diarrhea that cannot be distinguished clinically from diarrhea caused by other enteric pathogens (Dutta, 2001).

2.6. Multidrug resistance

Mutationand selection, together with the mechanism of genetic exchange, enable many bacterial species to adapt quickly to the introduction of antibacterial agents in to their environment. Although a single mutation in a key bacterial gene may only slightly reduce the susceptibility of the host bacteria to that antibacterial agent. It may be just enough to allow its initial survival until it acquires additional mutations or additional genetic information resulting in full-fledged resistance to the antibacterial agent(Keith, 2003). However, in rare cases, a single mutation may be sufficient to confer high level, clinical significant resistance upon an organism(Fred, 2006).

One of the methods used by various authors and authorities to characterize organisms as MDR is based on in vitro antimicrobial susceptibility test results. When they test "resistant to multiple antimicrobial agents, classes or subclasses of antimicrobial agents" (Magiorakos *et al*., 2011).

The definition most frequently used for gram-positive and gram-negative bacteria is "resistant to 3 or more antimicrobial classes". Another method used to characterize bacteria as MDR, is when they are "resistance to one key antimicrobial agent" (Magiorakos *et al*., 2011).

These bacterial isolates may have public health importance due to resistance to only one key antimicrobial agent, but they often demonstrate cross or co-resistance to multiple classes of antimicrobial, which makes them MDR. Creating an acronym for a bacterium based on its resistance to a key antimicrobial agent (e.g. methicillin resistance in *staphylococcus aureus*, i.e. MRSA) immediately highlights its epidemiological significance; the advantage of using this approach for surveillance purposes is that it can be easily applied(Adam *et al.*, 2008).

Multidrug resistant organisms (MDROs) are microorganisms that are resistant to one or more therapeutic classes of antimicrobial agents. The number of MDROs will increase if the selective pressure of antibiotic use continues and the resistant organism is able to spread from one person to another. The MDROs of greatest concern to healthcare facilities include (1) methicillin-resistance *Staphylococcus aureus* (MRSA), (2) vancomycin-resistant *Enterococci* (VRE), (3) multidrug-resistant (MDR) Gram-negative bacilli (such as *Enterobacter*, Klebsiella, *Acinetobacter*, and *Pseudomonas* species and *Escherichia coli*), and (4) vancomycin-resistant

Staphylococcus aureus. For some MDR gram-negative bacilli, such as carbapenem-resistant *Enterobacter* species and extended-spectrum b-lactamase producing *klebsiella* species the specific drug resistance patterns cause concern because of the challenges they present in treatment and infection prevention (Adam *et al*., 2008).

Several factors may lead to the increase in antimicrobial resistance such as hospital stay before ICU admission, hospitalization period before ICU admission, length of ICU stay, surgical ICU stay, the type of operation, previous antibiotic use, inappropriate use of antimicrobial drug, and inadequate adherence to infection control practices. In particular, some patients are more vulnerable to colonization and infections including those with severe disease, those with compromised host defenses because of underlying medical conditions, patients with recent surgery and those with indwelling medical devices. Furthermore, hospitalized patients are likely to have more risk factors and higher infection rates than non-hospitalized patients, especially those who require treatment in the ICU(Shu-hui *et al*., 2011).

It has been shown that antimicrobial drug resistance genes are present in one of the most remote area on Earth, the Arctic. Resistant as well as multi resistant isolates of *E. coli* were detected in the normal flora of Arctic birds. This finding highlights the unique nature of bacterial adaptation and the complexity of dissemination of antimicrobial drug resistance (Fred, 2006).

3. MATERIALS AND METHODS

3.1. Collection of plant material and extraction

Mature leaves of *D. stramonium* and *J. schimperiana* werecollected from around Haramaya University. The collected leaves werewashed through running tap water and subjected to shade drying at room temperature. The dried leave wasground to fine powder by grind mill and stored in refrigerator until use.

The dried powders of the test plants wereextracted by maceration using ethanol as solvent. For This, powder (80 g) and 400 ml of ethanol were mixed in 500 mL Erlenmeyer flasks and mixed by shaking. Then, the flasks werewrapped with aluminum foil to avoid evaporation. Thereafter, the mixture was shaken on a platform shaker for 3 days at room temperature. Some of the obtained extracts were concentrated by heating on a hot plate at about 30 - 40 ºC for 30 min to be used for qualitative analysis. The rest wereevaporated to dryness at room temperature and preserved at 4 ºC until used for qualitative and quantitative analysis, and anti-bacterial activity test (Biswas *et al*., 2011 and Taura *et al*., 2014).

3.2. Analysis of phytochemicals

3.2.1. Qualitative Analysis of Major Secondary Metabolites

Qualitative analysis of major secondary metabolites of both test plants was carried out on the concentrated, solidified ethanolic extract and on the leaf powders of both test plants using standard procedures shown below.

Test for tannins:1 g of each powdered sample was separately added into 20 mL of distilled water in test tubes. Then, the mixtures were boiled in water bath for five minutes and filtered while hot using filter paper into Erlenmeyers flask. After cooling, 1 mL of the filtrate was diluted to 5 mL with distilled water and then 2-3 drops of 10 % ferric chloride wereadded to the mixture so as to get bluish-black or brownish-green precipitateindicating the presence of tannins (Ajayi *et al*., 2011)

Test for phlobatannins:Driedextract **(**0.5 g) was placed into separate test tubes and mixed with 20 ml of distilled water. The mixture was boiled in water bath for 10 min. After cooling, each mixture wasseparately filtered through a Whatman No 1 filter paper. Thereafter, 2 ml of 1%-

aqueous hydrochloric acid was added to each mixture and shaken to develop red precipitate that indicates the presence of phlobatannins (Taura *et al*., 2014).

Test for saponin**:** Powder of the test plant (1g) was put in test tube and mixed with 10 mL of distilled water. Then, the mixture was boiled in a water bath for 10 min and filtered while hot in to Erlenmeyer flask. After cooling, the following tests were done (Ajayi *et al*., 2011).

Foam test: 2.5 mL of filtrate was added to test tube and diluted to 10 mL with distilled water. It was then shaken vigorously for 2 minutes to see formation of froth that confirms the presence of saponin in the filtrate.

Emulsion test:2 drops of olive oil was added to the frothing and the mixture was shaken vigorously for a few minutes in order to seeformation of a fairly stable emulsion that indicates the presence of saponins.

Test for flavonoids:2 ml of the concentrated ethanolic extract was added into test tubes. Then, 4 drops of 10 % NaOH solution was added and the mixture was heated in water bath for 10 min. The intensity of yellow color which becomes colorless on addition of 10 drops of 1 % Hydrochloric acid shows presence of flavonoid (Adachukwu *et al*., 2013).

Test for steroids (Lieberman-Burchard's Test):2 mL of chloroform and 10 drops of acetic acid were placed in test tube. 0.5 mL of the concentrated ethanolic extract was added to the test tube and mixed with the solvents. Then, 2 ml of concentrated sulphuric acid was added from the side of test tube. The change of red color through blue to green indicates the presence of steroids (Gayathri and Kiruba, 2014).

Test for terpenoids (Salkowski test):5 ml of the concentrated ethanolic extract was mixed with 2 mL of chloroform in separate test tubes, andthen 2 mL of concentrated sulfuric acid was added carefully and shaken gently to form a layer. A reddish-brown coloration of the inter-phase confirms positive results for the presence of terpenoids (Biswas *et al*., 2011).

Test for alkaloids**:** 2 mL of 1% HCl was added to 6 mL of the concentrated ethanolic extracts in test tubes. The mixture was heated for 2 min in a water bath while stirring continuously. It was then cooled and filtered. The resulting filtrate were tested with Mayer's Reagent for the presence

of alkaloids as described by Adachukwu *et al*. (2013). 1 mL of the filtrate was added to 0.5 mL of Mayer's reagent. Formation of cream yellow precipitate indicates the presence of alkaloids.

3.2.2. Quantitative Analysis of Major Secondary Metabolites

Leaf powders and preserved solidified extracts of both test plants wereused for standard quantitative estimation of the major secondary metabolites as indicated below.

Total phenol determination**:** Total phenol was determinedspectrophotometrically following the methods of Cavalcanti de Amorim *et al*. (2012). Stock solution of extracts (1 mg/mL, w/v) was prepared by dissolving 10 mg of the solidified extracts in 10 ml of 80% ethanol. 500 µL stock solution of the extract was transferred to test tube. Thereafter, 500 µL of the Folin-Ciocalteu solution and 1 mL of the sodium carbonate solutions were added in the test tube. The final volume was adjusted to 10 ml by adding 8 ml of distilled water. The sample solutions were kept at room temperature for 30 minutes and their absorptions were measured at 760 nm using distilled water as a blank.

Quantification was done based on calibration curve developed using tannic acid standard. For this, stock solution of tannic acid (0.1 mg/mL, w/v) was prepared by dissolving 10 mg of tannic acid in 100 ml of 80% ethanol. Then, 0.10, 0.2, 0.3, 0.4 and 0.5 mL volumes of stock solution was pipetted and transferred into separate pint flasks. 500 µL of 10% Folin-Ciocalteu solution was added to the pint flasks and mixed homogeneously for 10 seconds. Then, they were allowed to stand for 5 minutes. Thereafter, 1 mL of 7.5% Sodium carbonate wasmixed homogeneously for 30 seconds. Then, the final volume was adjusted to 10 mL with distilled water in order to obtain the final standard tannic acid concentration of 1, 2, 3, 4 and 5 µg/ml. These standard reaction mixtures were allowed to stand for 30 minutes after which their absorbance was measured at 760 nm using distilled water as a blank. Calibration curve was then constructed from obtained data.

The total phenolic content (TPC) wasthen calculated as tannic acid equivalent (TAE) by the following equation:

$$TPC = C*V/M$$

Where TPC is the total phenolic content in mg/g of the extracts as Tannic Acid Equivalent (TAE), C is the concentration of tannic acid established from the calibration curve in mg/ml, V is the volume of the extract solution in ml and M is the weight of the extract used in g.

Total alkaloid determination using Harborne (1973) method**:** 3 g of the powder was weighed and added into a 50 mL Erlenmeyer flask. Then, 20 mL of 10% acetic acid in ethanol was added into the flask which was covered and the solution was allowed to stand for 4 hrs. Next, the solution was filtered and concentrated ammonium hydroxide was added drop wise to the filtrate until the formation of precipitate stops. The whole solution wasallowed to settle the precipitate. Then, precipitate wascollected, washed with dilute ammonium hydroxide and then filtered. The obtained residue was dried and weighed. Alkaloid content was calculated as mg per g of the sample powder used.

Saponin determination**:** For this**, t**he method used by Obadoni and Ochuko (2001) was employed. The extract (3 g) was put into a conical flask and 15 mL of 20% aqueous ethanol was added. It was then heated over a hot water bath for 4 hr with continuous stirring at about 55°C. After filtering the mixture, the residue was re-extracted with another 30 ml of 20% ethanol. The resulting filtrates was combined and reduced to 10 mL over water bath at about 90°C. Then, it was transferred into a 250 ml separatory funnel and 5 mL of diethyl ether was added and shaken vigorously. The aqueous layer was then recovered while the diethyl ether layer is discarded. This purification process wasrepeated. Thereafter, 15 ml n-butanol was added to extract saponin. The n-butanol combined extracts were washed twice with 2.5 mL of 5% aqueous sodium chloride. Then, washed n-butanol combined extracts were transferred to pre-weightedPetri plate and heated in a water bath for evaporation. Then, samples were dried in the oven at 60 °C to constant weight and they were measured; the saponin content was calculated as mg per g of the sample powder used.

Total terpenoid determination: two g of powder was soaked in 50 ml of ethanol for 24 hr. The extracts were filtered and the filtrate was extracted with 6 ml of petroleum ether using separating funnel. Then, the ether filter was concentrated to dryness using rotary evaporator at 40 °C. The dried ether extract was considered as total terpenoid (Ferguson, 1956).

3.3. Antibacterial Assay

3.3.1. Collection of Test Organisms

Selected enteric bacterial pathogens of human clinical isolates were obtained from Ethiopian Public Health Institution (EPHI) Addis Ababa, Ethiopia. Thesepathogens were selected for their high prevalence and antibiotic resistance.

3.3.2. Sub- culturing and Standardization of Inoculum

Each of the enteric bacterial pathogen obtained from EPHI *(Staphylococcus aureus, Salmonella typhi murium* and *Shigella boydii*) was cultured on separate nutrient agar plate and incubated for 24 hr at 37 °C to obtain colonies. Two-three formed colonies were picked up with a sterile inoculating loop and transferred into a test tube containing sterile normal saline solution and vortexed thoroughly. This was repeated until the turbidity of each bacterial suspension matched the turbidity of the 0.5 McFarland Standard (Taura *et al*., 2014).The resulting suspension was then used as inoculum for the test pathogen used in the antibacterial susceptibility test.

3.3.3. Antibacterial Activity Test

Preparation of test solution of extracts: The stock solution (200mg/ml) was prepared by reconstituting 0.4 g of the dried extracts in 1 ml of ethanol and 2-fold serial dilutions was made as follows: two sterile test tubes were arranged on a test tube rack and 1 ml of sterile distilled water was dispensed into them (Taura *et al*., 2014).

Preparation of susceptibility discs: Whatman No.1 filter paper discs with 6 mm diameter was punched out with the aid of paper punch and placed in Petri plate. They were then sterilized by autoclaving at 121°C for 15 min. After that, the discs were cooled and impregnated with 0.01 ml of the prepared test solutions of each extract and ethanol (Taura *et al*., 2014).

Inoculation of Mueller Hinton Agar plates: within 15 minutes after adjusting the turbidity of the suspension of inoculums, a sterile cotton swab was dipped into the adjusted suspension, swab was rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This removes excess fluid from the swab. Then, the dried surface of Mueller Hinton Agar plate was inoculated by streaking the swab three times over the entire surface and rotating the MHA plates approximately 60° each time to ensure an even distribution of inoculums. Then, the MHA plates was left open for three to five minutes to allow for any excess surface moisture to be

absorbed (Obadoni and Ochuko, 2001).Following this step, the impregnated discs were dispensed onto the surface of the inoculated agar plate using sterile forceps. Each disc was pressed down to ensure complete contact with the agar surface. The discs were distributed evenly so they were notcloser than 24 mm from center to center (Obadoni and Ochuko, 2001). Commercial ciprofloxacin disc (5 µg) was used as positive control and the pure solvent (ethanol) impregnated disc was used as negative control.The MHA plates was then sealed with parafilm and incubated at 37°C for 24 hrs. After incubation, the diameters of the zone of inhibition around each disc was measured to the nearest millimeter along two axes (i.e. 90° to each other) by using transparent ruler and the mean of the two readings was recorded. For each selected enteric bacterial isolate, the experiment was carried out in parallel and with three replications.

3.4. **Determination of Minimum Inhibitory Concentration**

The stock extract solution (20 mg/ml) was prepared by reconstituting 100 mg of each of the dried extracts in 5 ml of ethanol and 2-fold serial dilutions was made as follows:five sterile test tubes were arranged on a test tube rack and 1 ml of sterile distilled water was dispensed into them. From the stock solutions, 1 ml was transferred into the first test tube and serial dilution of the extract was carried out and the resultant concentrations in the test tubes were then 10, 5, 2.5, 1.25, 0.625 mg/ml. Two mL of nutrient broth was added into six test tubes and 0.1 ml of prepared concentrations of the extracts were mixed with the nutrient broth. Thereafter, standardized inoculums of 0.1 ml of the test pathogen was dispensed into the test tube containing the suspension of nutrient broth and the extract. Then, all test tubes were properly corked and incubated at 37°C for 24 hrs. Then after, they were observed for absence or presence of visible growth. The lowest concentration without visible growth (turbidity) of organism is regarded as the MIC. The experiment was carried out for each organism in duplicates (Taura *et al*., 2014).

3.5. Method of Data Analysis

Statistical package for Social Science (SPSS Version 20; Chicago, IL, USA), was used to analysis the data. The phytochemical contents in leaves of both test plants was analyzed by independent samples T-test. One-way analysis of variance (ANOVA) was used to check significant difference between the effects of extract concentrations on bacterial growth. P-value <0.05 was considered as statistically significant difference between means.

4. RESULTS AND DISCUSSION

4.1. Phytochemical analysis

Presence of major secondary metabolite may be a useful indicator of both the efficacy and potential toxicity of a given plant. In this study analysis of the major secondary compounds from leaves of *D.stramonium* (Local name: atefaris) and *J. schimperiana* (Local name: sensel) was conducted and results showed that leaves of both plant species contain alkaloids, flavonoids, saponins, tannins, steroid and terpenoid, but lack phlobatannin (Table 1).Plants are known to contain a variety of secondary metabolites. The presence or absence of any particular bioactive compound fundamentally depends on the solvent of extraction and the plant part used for the extraction (Dai and Mumper, 2010). The present study agrees with that of Nagesh and Samreen (2016) whoreported the presence of tannins, flavonoids, steroids and alkaloids in the leaf extracts of *Daturastramonium* using ethanol and methanol as solvents. Using maceration method in ethanol and methanol solvents, Ananth and Rajan (2015) also found tannins, steroids, flavonoids and alkaloids in the leaves of *D. stramonium*.However, they did not find terpenoids as opposed to this study.

Preliminary phytochemical screening and *in vitro* antimicrobial activity of *D. stramonium* leaves extractusing maceration method was done by Solomon (2015).He used chloroform, ethanol, hexane, petroleum ether and acetone as solvents and ethanolic leaf extract showed the presence of flavonoids, tannins, saponins which agrees with the present study. However, noterpenoidswere foundas opposed to this study.Ananth (2013) reported the presence of flavonoids, alkaloids, steroids, saponin and absence of tannin and terpenoids from ethanolic extract leaf extract of *D. stramonium*.In another study byChintem and Nzelibe (2015) ethanolic leaf extract of *D. stramonium* found to possess alkaloids, flavonoids, tannins, terpenoids and steroids which agrees with the present study and negative for saponins tests which disagrees with the present study.In agreement with this study, phytochemical screening from ethanolic leaf extract of D. stramonium showed the presence of saponins, tannins and alkaloids while flavonoids were found to be absent from the extract (Aderotimi andSamuel,2006). Habtamu (2014) showed the presence of alkaloids, flavonoids, saponins and steroids in ethanolic leaf extracts of *Justicia schimperiana*. Overall, comparison of the present result with previous

researchers showed some similarities in chemical profile of the same plant and also some differences.

Table 1. Phytochemical analysis of leaf extract of *D. stramonium* and *J. schimperiana*

Plants	secondary metabolites						
	fla	alk	sap	ster	tan	terp	phloba
D. stramonium	+	+	+	+	+	+	-
J. schimperiana	+	+	+	+	+	+	-

Where,(+)= presence of metabolites and (-) = absence of metabolites.

4.2. Quantitative analysis of phytochemicals

There was significant difference between the different secondary compounds class in each of the tested plant extracts. In both plant species, Saponins were the dominant compounds followed by alkaloids, and terpenoids and phenols (Table 2). Likewise, all the tested secondary compound groups showed significant difference between the two-plant species except phenols. *J. schimperiana* found to have large number of alkaloids, saponins and terpenoids (Table 2).

Table 2. Quantitative analysis of phytochemicals form leaves of *D. stramonium* and *J. schimperiana*

Metabolites	Amount of metabolites in mg/g	
	D. stramonium	*J.schimperiana*
Phenols	3.23 ± 0.145^{Ac}	3.14 ± 0.184^{Ad}
Alkaloids	42.3 ± 2.403^{Ba}	410 ± 11.547^{Ab}
Saponins	46.3 ± 2.333^{Ba}	586.6 ± 18.559^{Aa}
Terpenoids	39 ± 3.785^{Bb}	323.3 ± 23.333^{Ac}

The values are Mean ± Standard error of mean (n=3). Capital letter superscripts compare between means in row, whereas small letter superscripts compare between means within a column. Means with the same letter superscripts with row and column are not statistically significant at $P<0.05$, whereas those with different letters are statistically significant.

4.3. Antimicrobial assay

Theantimicrobial activity of a number of plants' extracts for the management and treatment of diseases is attributed to their phytochemical constituents. These phytochemical substances including alkaloids, tannins, flavonoids and phenols have been known for their anti-diabetic, anti-atherosclerotic, anti-inflammatory, anti-carcinogenic andanti-microbial properties (Ananth**,** 2013**).**

In this study, the antimicrobial activities of the crude extracts of*D. stramonium* and *J. schimperiana*were tested by paper disc diffusion method. Compared to the negative control, ethanol, which showed no inhibitory effect, crude extracts of both plant species showed growth inhibitory effect against all the tested bacterial pathogens.

Table 3.The antimicrobial activity of *D. stramonium* and *J. schimperiana* leaf ethanolic extracts and antibiotics against the tested bacterial pathogens.

antibacterial agents	zone of inhibition (in mm)		
	S. a	*S. t*	*S. b*
Ethanol	-	-	-
Ciprofloxacin	17 ± 2.646^{Ab}	14 ± 1.000^{Bb}	14 ± 2.000^{Bb}
D. stramonium	20.67 ± 2.082^{Aa}	19 ± 1.000^{Aa}	23.3 ± 3.055^{Ba}
J. schimperiana	3 ± 1.000^{Bc}	3.3 ± 1.528^{Bc}	8 ± 1.000^{Bac}

The values are Mean ± Standard error of mean (n=3). Capital letter superscripts compare between means in row, whereas small letter superscripts compare between means within a column. Means with the same letter superscripts within row and column are not statistically significant at $P<0.05$, whereas those with different letters are statistically significant.
Where S. a=*Staphylococcus aureus*, S. t=*Salmonella typhi* and S. b=*Shigella boydii.*

Compared to *J. schimperiana* extracts and Ciprofloxacin (positive control), *D. stramonium's* extract showed greater inhibitory effect against all the tested pathogens. There was sensitivity difference between the test pathogens to both plants extractwith *Shigellaboydii* showing higher sensitivity than other bacterial pathogens tested (Table 3).Phytochemicals constituents of plants are known to be biologically active compounds and they are responsible for different activities such as antioxidant, antimicrobial, antifungal and anticancer (Husain and Nagooru, 2011;Suresh & Nagarajan, 2009). Previously many secondarymetabolites have been isolated and identified for their antimicrobial effect (Gonzalez *et al.,* 2004).The presence of secondary compounds such as phenols, saponins, flavonoids, alkaloids, terpenoids, steroids and tannins is most likely to be responsible for the observed antibacterial activity.

Secondary compounds exhibit their antimicrobial effect in different ways including inactivation of enzymes, cell envelope transport proteins and so forth (Sekhar *et al.,* 2012).For instance, plant rich in tannins have antibacterial potential due to their character that allows them to react with proteins to form stable water-soluble compounds thereby killing the bacteria by directly damaging its cell membrane. Flavonoids are a major group of phenolic compounds reported for their antiviral, antimicrobial and spasmolytic properties. Alkaloids isolated from plant are commonly found to have antimicrobial properties. Pure isolated alkaloids and the synthetic derivatives are used as basic medicinal agents because of their analgesic, antispasmodic and bacterial properties as per (Ajayi, 2011).The presence of saponins supports the fact that *Datura*

and *Justicia* leaves have cytotoxic effects such as permealization of the intestine assaponins are cytotoxic.

Preliminary phytochemical screening and *in vitro* antimicrobial activity of *D. stramonium* and *J. schimperiana* leaves extract using maceration method was done by many authors. And the result of the antimicrobial activity of the extracts was put as zone of inhibition in mm. Solomon (2015) worked on preliminary phytochemical screening and invitro antimicrobial activity of *Datura stramonium* leaves extract using chloroform, ethanol, hexane, petroleum ether and acetone by soaking and rotary evaporator for extraction. Paper disc diffusion method and 20 & 40 mg/ml of concentration of crude extract was used for antimicrobial activity and ethanolic leave extract showed 15.5 & 11.2 mm against *Staphylococcus aureus* and *Salmonella typhi*.Nagesh and Samreen (2016) showed their result for antimicrobial activity of *Carica papaya*, *Piper nigrum* and *Datura stramonium* plants on drug resistant pathogens using methanol and ethanol by soaking. Ethanolic leave extract of Datura showed 30 mm zone of inhibition against Staphylococcus aureus.Invitro evaluation of Datura species for potential antimicrobial activity using water and ethanol by soxhelet extraction was done by Gachande and Khillare (2013). Results revealed inhibition zone of 24 & 10 mm against *Staphylococcus aureus* and *Salmonella typhi* by Datura crude leave extract.Hadia, *et al*., 2012 worked on antibacterial and antifungal activity of different extracts of *Datura stramonium* using benzene, chloroform and ethanol by soaking and rotary evaporator. Agar well diffusion method and 200 mg/ml of concentration of crude extract was used for antimicrobial activity. Their result revealed 12 mm zone of inhibition against *Staphylococcus aureus*. Overall, comparison of the present result with previous researchers showed some similarities in zone of inhibition against same bacterial pathogens by the same plant and also some differences.

Minimum inhibitory concentrations were determined by broth dilution method. The MIC in both plant leaves varied against different tested clinical isolates (Table 4). Lowest MIC for *D. stramonium*leaf extract was recorded against *Staphylococcus aureus* (2.5 mg/ml) while maximum MIC was recorded against *Shigela boydii*(0.625 mg/ml). For *J. schimperiana* the maximum MIC was recorded against *Shigella boydii* (5.0 mg/ml) and minimum MIC was against *Staphylococcus aureus* and *Salmonella typhi*(10.0mg/ml). Comparing the two plants *D. stramonium* had maximum MIC.

Minimum inhibitory concentrations of *Datura* and *Justicia* have been done previously by some authorsusing broth dilution method and the result was put in mg/ml. Hadia, *et al*., 2012, who worked on antimicrobial activity of different extracts of *Datura stramonium* using benzene, chloroform and ethanol by soaking and rotary evaporator. Agar well diffusion and 100-0.78 mg/ml concentration of crude extract was used for mic test. And ethanolic extract of *Datura* showed 0.78 mg/ml against *Staphylococcus aureus.* Akharaiyi (2011) reported that the mic of *Datura* against *Staphylococcus aureus* and *Salmonella typhi*was 20 mg/ml. Mic of ethanolic crude extract of *Datura to Staphylococcus aureus* was 24.34 mg/ml as cited by Ram *et al*., 2013, who worked on antimicrobial screening of sequential extracts of *Datura stramonium* using petroleum ether, water, methanol and ethanol by soaking. As reported by Subramanian *et al*., 2012, the mic of *Justicia* against *Staphylococcus aureus* and *Salmonella typhi* was 15 mg/ml and 20 mg/ml.

Table 4. Minimum Inhibitory Concentration

Clinical isolates	MIC in mg/ml	
	Datura	*Justicia*
Staphylococcus aureus	2.5 mg/ml	10.0 mg/ml
Salmonella Typhi	1.25 mg/ml	10.0 mg/ml
Shigella boydii	0.625 mg/ml	5.0 mg/ml

5. SUMMARY, CONCLUSION AND RECOMMENDATION

5.1. Summary and Conclusion

Plants are believed to have active chemical components that help to treat and manage various infectious diseases. This study was conducted with the aim of screening the major secondary compounds known to have anti-bacterial activities from leaves of *D. stramonium* and *J. schimperiana* ethanolic extracts, quantification of the detected secondary compounds and evaluation of anti-bacterial activity of the crude ethanolic extracts.

The results of qualitative phytochemical screeningof leafof both plant species showed the presence alkaloid, saponin, steroid, tannin and terpenoid. Whereas, phylobatannin wasabsent in both extracts.Quantitative analysis showed thatalkaloids are significantly higher than the rest of compounds in both plant species. Compared to *J. schimperiana*, all quantified compounds were found in higher amount in *D.stramonium*.

The result of anti-bacterial activity assay showed that leaf extracts of both plant species showed antimicrobial effect when compared with the negative control (ethanol) against all tested bacterial pathogens. Therefore, it is concluded that both plant species have the major secondary metabolites and antimicrobial property against the tested pathogens.

5.2. Recommendation

Based on the result of the present study the following points are recommended

- The present study was done using one solvent only. Therefore, more studies should be done using other solvents of varying polarity.
- More extraction should be done for finding other phytochemicals.
- The dose for antimicrobial activity should be determined.
- Antimicrobial activity should also be evaluated *in vivo.*
- Active principle *(s)* of the extracts should be identified for drug discovery.

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Appendix Figure

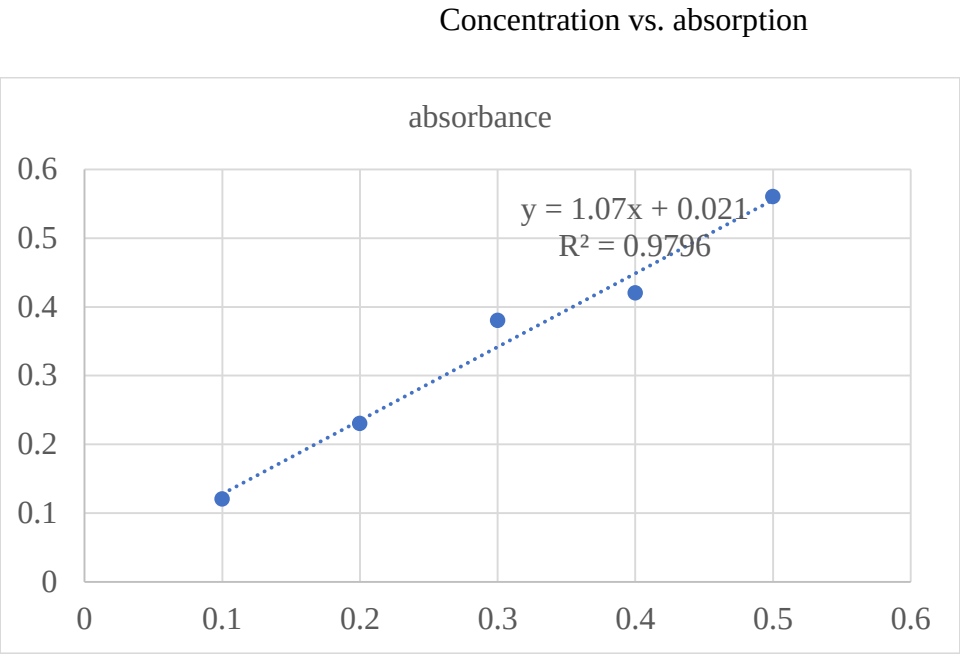
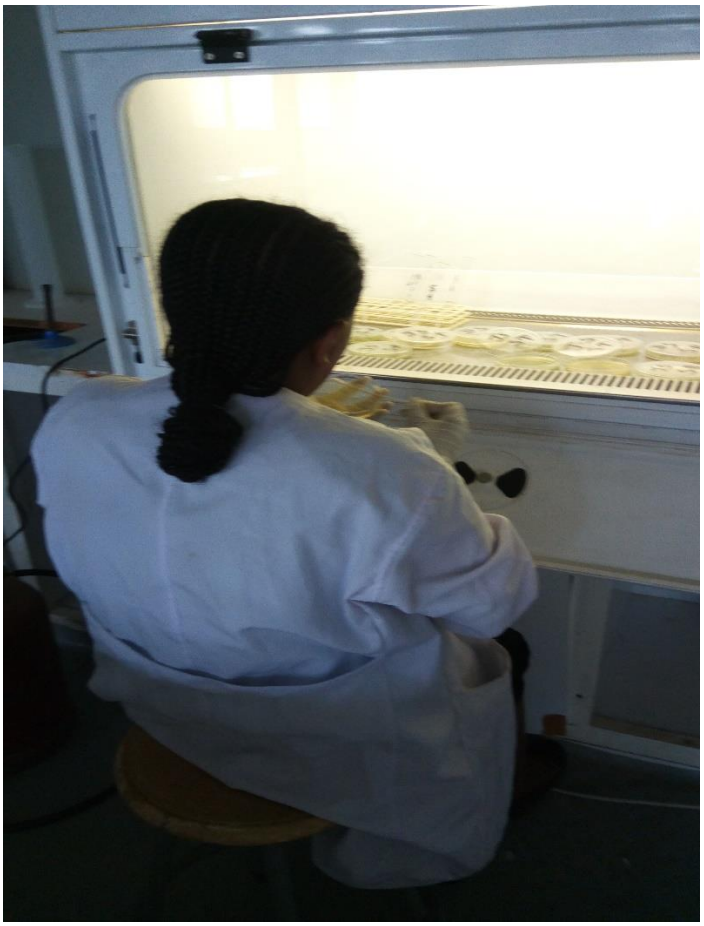


Figure 1 Standard curve of tannic acid

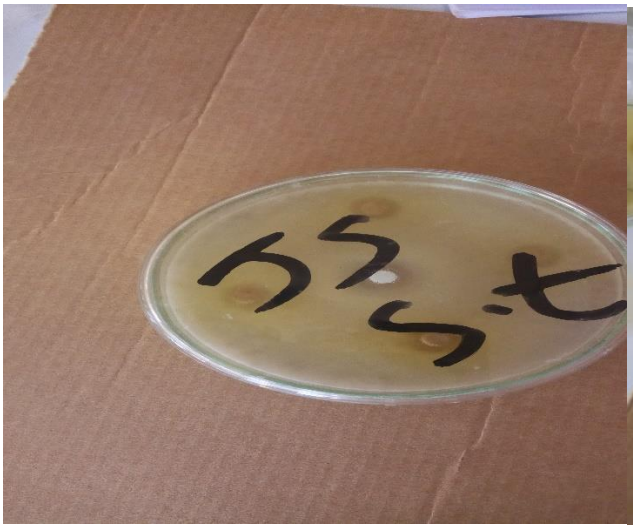
1. Plant collection

3. UV-reading

2. Sample on rotary shaker



4. Placing impregnated disc on agar plates



5. Results of Justicia

6. Results of Datura

7. mic sample in oven