ANTIMICROBIAL ACTIVITIES OF *Solanum marginatum* L. AGAINST SOME PATHOGENIC BACTERIA

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FASIL AHMED

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By

Fasil Ahmed

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HARAMAYA UNIVERSITY

Postgraduate Program Directorate

We hereby certify that we have read and evaluated this Thesis entitled '*Antimicrobial Activities of Solanum marginatum L. against Some Pathogenic Bacteria'* prepared under our guidance by Fasil Ahmed. We recommend that it can be submitted as fulfilling the thesis requirement.

Ameha Kebede (PhD)	_____	_____
Major Advisor	Signature	Date
Meseret Chimdessa (PhD)	_____	_____
Co-Advisor	Signature	Date

As members of the Board of Examiners of the MSc Thesis Open Defense Examination, we certify that we have read and evaluated the Thesis prepared by Fasil Ahmed and examined the candidate. We recommend that the thesis can be accepted as fulfilling the Thesis requirement for the *degree of Master of Science in Biotechnology*.

_____	_____	_____
Chairperson	Signature	Date
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_____	_____	_____
External Examiner	Signature	Date

DEDICATION

I dedicate this thesis manuscript to my father Ahmed Mume and my mother Shibre Sintayehu, for nursing me with affection and love and for their dedicated partnership in the success of my life.

STATEMENT OF THE AUTHOR

First, I hereby declare that this thesis is my original work and that all sources and materials used for this thesis have been duly acknowledged. This Thesis has been submitted in partial fulfillment of the requirement for an M.Sc. degree in Biotechnology at Haramaya University and is deposited at the university library to be made available to borrowers under the rules of the library. I solemnly declare that this Thesis has not been submitted to any other institution anywhere for the award of academic degree, diploma or certificate.

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Name: Fasil Ahmed Signature: _____

Place: Haramaya University, Haramaya

Date of Submission: _____

BIOGRAPHICAL SKETCH

The author was born in Afar region, Gewane district, from his father Ahmed Mume and his mother Shibre Sintayehu in April, 1981. He attended his elementary school education at Chiro Number 2 School. He also attended his junior and secondary school education at Chercher Secondary School. After completing his high school education in 1999, he joined Bahir Dar University in September 2000 and graduated with Bachelor of Education Degree in Biology in July 2004.

After graduation, he taught biology in West Hararghe Zone, Gelemso and Chercher Secondary and Preparatory Schools. He joined the Summer Program of the Department of Biology, Haramaya University, in 2012 for his M.Sc. degree in Biotechnology.

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ABBREVIATIONS AND ACRONYMS

ATCC	American Type Culture Collection
CFU	Colony Forming Unit
EPHI	Ethiopian Public Health Institute
GAS	Group A Streptococcus
MHA	Mueller Hinton Agar
MIC	Minimum Inhibitory Concentration
MRSA	Methicillin-Resistant *Staphylococcus aureus*
SD	Standard deviation
SSTI	Skin and soft tissue infection

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ANTIMICROBIAL ACTIVITIES OF *Solanum marginatum* L. AGAINST SOME PATHOGENIC BACTERIA

ABSTRACT

The emergence of antibiotic resistance is a public health problem of increasing magnitude. It is, therefore, important to look for more effective, safer and less toxic options of treatment. The aim of this study was to assess the antibacterial activities of crude extracts of different parts of Solanum marginatum against Escherichia coli, Staphylococcus aureus, Streptococcus pyogenes and Campylobacter jejuni. The stems, fruits, roots and leaves of the plant were shade dried and ground to powder and the bioactive components were extracted using ethanol (99%) and water. The antibacterial activities of the extracts were evaluated using agar-well diffusion method and the inhibitory zones were recorded in millimeters. The minimum inhibitory concentration (MIC) of the plant extracts against E. coli, S. aureus, S. pyogenes and C. jejuni were assessed using the agar dilution method. The antibiotics Tetracycline, Amoxicillin and Penicillin were used as positive controls. The bioassay studies of the crude extracts were undertaken at two different concentrations (20 and 40 mg/ml).The results revealed that the crude extracts of ethanol had antibacterial activities against all bacterial strains. The ethanol extracts had growth inhibitory effect at a concentration of 20 mg/ml and 40 mg/ml with zones of inhibition ranging from 7.10-17.07 mm and 9.40-23.87 mm, respectively. The aqueous extracts showed some antibacterial activity at a concentration of 20 and 40 mg/ml with zones of inhibition ranging from 0.9 – 4.97 mm and 2.87-9.97mm, respectively. However, the two crude extracts had less antibacterial activities than Tetracycline. Campylobacter jejuni was found to be the most susceptible bacterium to crude ethanol extract of fruits and roots with minimum inhibitory concentration of 1.25 mg/ml and 2.5 mg/ml respectively, whereas Streptococcus pyogenes was the least susceptible bacterium to all of the crude extracts. The growth inhibitory activities of the crude extracts were found to be significantly different for the two concentrations (20 and 40 mg/ml) ($p < 0.05$). In conclusion, this study did not only show the antibacterial activities of Solanum marginatum, but also provided a scientific basis for its traditional use against some diseases.

KEYWORDS: Agar dilution, Agar-well diffusion, Antibacterial activities, crude extract, minimum inhibitory concentration

1. INTRODUCTION

Antibiotics have been essential to modern healthcare and their role has extended from treating serious infections to preventing infections in surgical patients, people with compromised immune systems, and promoting growth and preventing disease in livestock. However, once-treatable infections are becoming difficult to cure, raising costs to healthcare facilities, and patient mortality is rising, with costs to both individuals and society. Decreasing antibiotic efficacy has risen from being a minor problem to a broad threat, regardless of a country's income or the sophistication of its healthcare system. Many pathogens are resistant to more than one antibiotics, and new, last-resort antibiotics are expensive and often out of reach for those who need them (CDDEP, 2015).

There are many medicinally important substances in nature which are the basis for the development of significant number of modern medicines. Plants generally produce many secondary metabolites which constitute an important source of microbicides, pesticides and many pharmaceutical drugs (Bobbarala *et al.*, 2009). According to World Health Organization (2001), medicinal plants would be the best source to obtain a variety of drugs. Therefore, such plants should be investigated to better understand their properties, safety and efficacy (Nascimento *et al*., 2000).

In many developing countries, medicinal plants play an important role in meeting the primary health care need of the population and traditional medicine practitioners are the main or sole providers of health care in their communities. Contrary to the synthetic drugs, antimicrobials of plant origin are not associated with many side effects and have an enormous therapeutic potential to heal many infectious diseases (Iwu *et al*., 1999). Medicinal plants represent the oldest and most widespread forms of medication. Despite the cross-cultural and universal nature of herbal medicines, scientific research concerning their effectiveness has been conducted only recently (Halberstein, 2005).

Thousands of plant products with inhibitory effects against microorganisms have shown *in vitro* activities and have been used for centuries by various cultures in the treatment of different diseases. In the early development of modern medicine, biologically active

compounds from plants have played a vital role in providing medicines to combat diseases. Plant-derived medicines continue to occupy an important position in the treatment of diseases worldwide (Prasad and Tyagi, 2015). Therefore, there is currently a growing need to screen medicinal plants for bioactive compounds as a basis for further pharmacological studies.

Many analytical reports showed that Solanum species are important source of a large number of phytochemical compounds with substantial curative applications against human pathogenic diseases (Udayakumar *et al*., 2003). *Solanum marginatum* is one of the important medicinal plants and is also known as the purple African nightshade and white-edged nightshade. The species is native to Ethiopia but naturalized in the Canary Island, the New World and Australia (Symon, 1981).

The plant has some ethnopharmacological basis and members of the genus Solanum are used in ethnomedicine and quite extensively studied for their efficacy. Since taxonomically closely related species may have similar activities, this plant has been selected for investigation of its antimicrobial activities. Though information on the use of *S. marginatum* as traditional medicine to treat different diseases in humans and other animals seems to be plenty in literature, there is little information on the effects of the extracts of different parts of this plant on specific pathogenic bacteria and their minimum inhibitory concentrations (MIC). In addition, the development of resistance to antibiotics by many pathogens has posed an alarming threat on the control of diseases. Therefore, there is a continuous and urgent need to discover new and safer antimicrobial compounds with diverse chemical structures and novel mechanisms of action (Bonjar, 2004). Extracts obtained from this plant species may lead to the discovery of new antibacterial agents and a better understanding of how herbal medicines can be used to treat infections.

In this study, *S. marginatum*, which has traditional claims for the treatment of various skin and enteric infections was investigated *in vitro* for its antimicrobial activities against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Campylobacter jejuni*. *Staphylococcus aureus* is known to cause infections in almost every organ and tissue of the human body. However, the most commonly affected organ is the skin (Lowy, 1998). The other bacterium which causes skin infection is *Streptococcus pyogenes.* This bacterium is an

etiological agent for skin infections like impetigo and necrotizing fascititis (Capoor *et al*., 2006). Even though enteric *E. coli* are part of the normal flora, certain groups can cause severe enteric and extraintestinal diseases in man (Kaper *et al*., 2004). The other bacterium selected for study is *C. jejuni* which is responsible to Campylobacteriosis which produces an inflammatory, sometimes bloody, diarrhea or dysentery syndrome, mostly including cramps, fever and pain in humans (Ryan and Ray, 2004).

General Objective

The general objective of this study was to determine the antimicrobial activities of the crude extracts of white-margined nightshade (*Solanum marginatum*) against some selected human bacterial pathogens.

Specific Objectives

The specific objectives of this research were to:

- determine yield of crude extracts of ethanol and water
- determine the antibacterial activities of the aqueous and ethanolic extracts of the stems, berries, leaves and roots of *Solanum marginatum* against *Staphylococcus aureus, Streptococcus pyogenes, Escherichia coli* and *Campylobacter jejuni*.
- determine the minimum inhibitory concentration (MIC) of each crude extract.

2. LITERATURE REVIEW

2.1. Medicinal Plants

Medicinal plants are plants whose parts (leaves, seeds, stems, roots, fruits etc.), extracts, infusions, decoctions, powders are used in the treatment of different diseases of humans, plants and animals (Nostro *et al*., 2000). Medicinal plants are important sources of traditional medicines for millions of people and additional inputs to modern medicine in terms of exploring and producing new drugs to meet the need for the over grown populations of the planet (Celikel and Kavas, 2008).

Medicinal plants are useful for healing as well as for curing of human diseases because of the presence of phytochemical constituents (Nostro *et al*., 2000). Hundreds of plants are known to have medicinal values and are used to treat infections and ailments in different countries. Many potent and powerful drugs have been produced from these medicinal plants (Gangadevi, 2008).

2.2. History of Use of Traditional Herbal Medicines

Medicinal plants and corresponding preparations have been used for a wide range of purposes and for many centuries people have been trying to treat diseases as well as alleviate symptoms by using different plant extracts and formulations (Cowan, 1999).

Nearly all cultures of the world, both ancient and the recent have heavily depended on plants as a therapeutic agents used in various forms. For centuries, most of the population in Ethiopia, as elsewhere in many other developing countries, has relied on a system of traditional medicine (Dawit *et al*., 2005). In Ethiopia, herbs have traditionally been used in the home to treat family sickness, and occasionally traditional healers may be consulted. Traditional healers may be from the religious traditions of Cushitic Medicine, regional Arabic-Islamic medical system, or the Semitic Coptic medical system practiced by Orthodox Christian traditional healers (Kassaye *et al*., 2006). Due to its long period of practice and existence, traditional medicine has become an integral part of the culture of Ethiopian people (Haile, 2005).

2.3. The Role of Herbal Medicines in Traditional Healing

Natural products perform various functions and many of them have interesting and useful biological activities. There are more than 35,000 plant species being used in various human cultures around the world for medicinal purpose (Philip *et al*., 2009). *Acosmium panamenses* and seeds of *Carica papaya* are used for treating malaria. The bark of *Giiricidia sepium* and fresh leaves of *Ocimum lamiifolium* are used to treat eye infections. Leaves of *Persea americana* are consumed to treat diarrhea. The sap of *Pinus caribea* and the bark of *Bursera simaruba* are used to relief urinary tract infections. *Hamelia patens, Datura stramonium* L. and the leaves of *Neorolaena lobata* L. are used to treat slow healing wounds. *Euclea racemosa* is used in the treatment of Gonorrhoea, diarrhea, eczema and vitiligo. The leaves of *Kalanchoe pinnata* are used to treat Athlete's foot (d'Avigdor *et al*., 2014; Arzu, 2015).

In Ethiopia about 80% of human population and 90% of livestock rely on traditional medicines (Mesfin , 2006) due to the socio cultural appeal, the cultural acceptability of healers and local pharmacopoeias, accessibility and effectiveness of medicinal plant against a number of health problems (Teferi and Hahn, 2002).

In the context of countries like Ethiopia the prohibitively expensive cost of efficacious antibiotics and the emergence of single and multiple antibiotic resistance of bacterial disease call for the search of alternative agents with possible antibacterial effect from natural products (Mesfin, 1986). One possible way this problem can be handled is by searching and screening pharmacologically active agents from plants which are traditionally claimed to treat diseases (Ahmed *et al*., 2007).

2.4. Active Components, Medicinal Applications & Beneficial Effects

Numerous biologically active plants have been discovered by evaluation of ethnopharmacological data and these plants may offer the local population immediate and accessible therapeutic products (Bruck *et al*., 2004). Medicinal plants contain biologically active chemical substances such as saponins, tannins, essential oils, flavonoids, alkaloids and other chemical compounds. These complex chemical substances of different compositions are found as secondary plant metabolites in these plants (Cowan, 1999).

The specific function of many phytochemicals is still unclear; however, a considerable number of studies have shown that they are involved in the interaction of plants, plants and pests and plants and diseases. Antimicrobial screening of plant extracts and phytochemicals, then, represents a starting point for antimicrobial drug discovery. Phytochemical studies have attracted the attention of plant scientists due to the development of new and sophisticated techniques. These techniques played a significant role in the search for additional resources of raw material for pharmaceutical industry (Shakeri *et al*., 2012).

The use of plant extracts for medicinal treatment has become popular when people realized that the effective life span of antibiotic is limited and over prescription and misuse of antibiotics are causing microbial resistance (Alam *et al*., 2009). The antimicrobial effect of medicinal plants is well documented (Valero and Salmeron, 2003). The results of different studies provide evidence that some medicinal plants might indeed be potential sources of new antibacterial agents even against some antibiotic-resistant strains (Kone *et al*., 2004).

Iwu *et al*., (1999) reported that the primary benefits of using plant derived medicines are that they are relatively safer than synthetic alternatives, offering profound therapeutic benefits and more affordable treatment.

2.5. Safety and Efficacy Issues of Medicinal Plants

Traditional medicine, like modern medicine, must be subject to safety and efficacy requirements. For traditional medicine to be more useful it has to meet these requirements. It is the responsibility of practitioners to have a good knowledge of the properties of different products and plants as well as their beneficial and adverse effects on individuals of different ages and sexes in various conditions. Improper use of some herbal materials is a potential source of danger. The common misconception that natural products are not toxic and have no side effects may lead to unrestrained intake resulting in severe poisoning and acute health problems. This misconception also exists in highly developed countries, where the general public often resorts to “natural” products without being properly aware or informed of the associated risks, particularly in the event of excessive use (WHO, 2004a,b). The number of reports of patients experiencing negative health impacts caused by the use of herbal materials has also been rising globally (Banarjee and Sarkar, 2003).

Most reported incidents concerning use of herbal products and medicines are attributable to poor product quality or improper use. Assessment of these products is possible; however, the quality-control methods and specifications for herbal medicines, particularly mixtures, are very complex (WHO, 2004a,b). Generally, plant materials are contaminated with high levels of bacteria, moulds, and yeasts. The microorganisms found in plants are usually native to the soils and surroundings in which the plants are grown. A broad range of microorganisms and microbial loads have been reported in medicinal plants (Banarjee and Sarkar, 2003).

Various forms of traditional medicine are increasingly being practiced outside their original areas and cultures, without adequate knowledge of their use and underlying principles. Thus, traditional products may be used in different doses, obtained by different methods or employed for nontraditional purposes. Use of traditional medicine together with other forms of medicine, which can be commonplace, has become a concern in terms of therapeutic safety (WHO, 2004a,b). The use of traditional medicine has increased in recent years, but questions remain concerning their quality, safety and efficacy (Jung, 2007).

2.6. Current Status and the Future of Herbal Medicines

Bacterial resistance to antibiotics has increased rapidly within recent years which have led to the increase in the incidence of infectious diseases caused by those multi-drug resistant bacteria. Infections caused by multi-drug resistant bacteria involve higher morbidity, mortality, and a burden to health care systems (Sütterlin, 2015). Therefore, there is an interest to search for more effective antimicrobial agents with the aim of discovering potentially useful active ingredients from other sources with proven antimicrobial activity. Medicinal plants have a promising future because the medicinal value of most plants is not investigated yet and their medicinal activities could be decisive in the treatment of existing and emerging diseases. The substantial increase in the popularity of plant-based medicine for treatment of various diseases indicate that medicinal plants are being used extensively as a major source of drugs for treatment of many diseases (Niknan and Farajee, 2015).

Medicinal plants are used in the production of modern drugs, as source of direct therapeutic agents, as raw material for the manufacturing of complex semi synthetic compounds and as taxonomic markers in the search of new compounds (Suppakul *et al*., 2003; Jayalakshmi,

2011). There is a steady rise in utilization of traditional medicinal plants for primary health care (Jung, 2007) and scientists are in search of new phytochemicals that could develop as useful antimicrobial agent for treatment of infectious diseases (WHO, 2010).

The searches for novel antimicrobial agents from medicinal plants are more critical than ever in countries like Ethiopia where maladies caused by bacteria are wild, as well as the causative agents are building up rising resistance against the antibiotics in common use (Abebe *et al*., 2003). Taking into account the non-availability, high cost, limited effective life span and the various side effects of the synthetic drugs, the search for potentially useful plant ingredients used in traditional medicine is further justified (CDDEP, 2015).

Ethiopian plants have shown remarkably effective medicinal values for many human and livestock ailments. Some research results are found on medicinal plants of the south, south west, central, north and north western parts of Ethiopia. However, there is lack of data that quantitatively assesses the resource potential and the indigenous knowledge on use and management of medicinal plants in eastern Ethiopia (Anteneh *et al*., 2012).

A majority of Ethiopians rely on traditional medicine as their primary form of health care, yet they are in danger of losing both their knowledge and the plants they have used as medicines for millennia. Ethnobotanical, ethnomedical and anthropological research must continue in Ethiopia in order to understand the cultural, sociological and practical considerations that inform the wider community at institutional and governmental level. In the future, Ethiopians should be able to take advantage of opportunities to develop the potential of their rich medicinal plant resources via documentation of knowledge of use and pharmacological investigation of medicinal properties of the plants. Integration of traditional herbal medicine with outreach medical services may be a beneficial outcome of supporting further investigations in Ethiopia's medicinal herb knowledge (d'Avigdor *et al*., 2014).

There is urgent need to document the valuable knowledge of medicinal herbs in Ethiopia. Ethnobotanical studies are essential, and related sustainable programmes that support the sustainability of herbal medicine traditions may be considered as a way to collect and disseminate information thereby supporting communities in their efforts to maintain their heritage (d'Avigdor *et al*., 2014).

The future of the plant-based health products and industries is enormously strong as the recent social and cultural tendencies toward natural healing and healthy diets are increasingly growing. Scientists all over the world are therefore trying to explore the precious values of medicinal plants to alleviate human suffering (Hussain *et al*., 2009). Acceptance of traditional medicine and limited access to modern healthcare facilities could be considered as the main factors for the continuation of the practice (Ketema *et al*., 2013).

2.7. Herbal Medicine Research and Trends in Herbal Medicine Use

Despite its significant contribution to society, herbal medicine has received very little attention in modern research and development and less effort has been paid to upgrade the traditional health practices in Ethiopia. But, the long history in the use of medicinal plants in Ethiopia and its huge biotic riches can be of paramount importance in future research and drug discovery (Gidey, 2001). Because of the rise of multi-drug resistant strains of bacteria and an alarming increase in the incidence of new and re-emerging infectious diseases, there is a continuous and urgent need to discover new antimicrobial compounds with different chemical structures and novel mechanisms of action (Semere, 2006).

The potential for developing antimicrobials from higher plants appears rewarding as it leads to the development of new drugs which is needed today. Further research is necessary to find the active compounds within higher plants with their full spectrum of efficacy (Jayalakshmi, 2011**)**.

Even though plants are rich in a wide variety of secondary metabolites with antimicrobial potential, only very few of them are used as antimicrobial agents. It has been indicated that the potential of higher plants as source for new drugs is still largely unexplored. Among the estimated 250,000-500,000 plant species only 35,000 plant species have been investigated and the proportion of phytochemicals that have been subject to biological and pharmacological screening is even less (Ramor and Ponnampulam, 2008; Philip *et al*., 2009). Pharmacological screening of natural compounds or synthetic origin has been the source of enumerable therapeutic agents (Semere, 2006). Random screening as tool in discovering new biologically active molecules has been most productive in the area of antibiotics (Penna *et al*., 2001). At

the present, opposite to common believes medicinal agents from higher plants continue to occupy an important position in modern medicine (Niknan and Farajee, 2015).

Traditional medicines have been used for thousands of years with demonstrated efficacy in treating a wide range of health issues. Many of the medicines in contemporary biomedical treatments are derived from plants and herbs used by indigenous people throughout the world. There has been little research done, however, on the integration of traditional knowledge-based treatments with modern biomedical treatments. The chemistry of plants used by contemporary healers and elders to treat various illnesses has been examined and traditional medicines exhibit chemical properties that can effectively and safely treat illness. The safety of traditional medicines when used in combination with biomedical-based treatments has also been tested (Anonymous, 2012). An integration of traditional medicinal plants with modern medicine has been practiced in countries such as Egypt, Ghana, India, China and Sudan (Penna *et al*., 2001).

2.8. Challenges in Developing Medicinal Plant Potential

According to WHO (2002), the use of medicinal plants in traditional medicine will have to overcome a number of challenges if it is to be recognized and integrated into the health system. Among these challenges the major ones are diversity of practice, inadequate scientific evidence of the efficacy of a large number of traditional therapies, problems protecting traditional knowledge, and lack of resources to ensure that it is used properly.

Plants have traditionally been used for treatment of human and livestock ailments in Ethiopia by different ethnic and social groups. However, this valuable source of knowledge is not adequately documented, which impedes their widespread use, evaluation and validation (Ketema *et al*; 2013). The traditional system and religious beliefs that restrict the manner in which indigenous knowledge should be transferred to others may lead to the declining of information on medicinal plants as time goes on. It is observed that many young people are less knowledgeable about the variety and value of indigenous medicinal plants (Anteneh *et al*., 2012).

Despite significant advances, the regulation of herbal products, practices, and practitioners is not occurring at an equal pace. WHO member states report that faster progress is being made in the regulation of herbal medicines, while that for traditional practices and practitioners is lagging. Of concern is that the safety, quality, and efficacy of traditional medicine services cannot be assured if there is no appropriate regulation of practices and practitioners. This situation presents a serious challenge for many member states, where a lack of knowledge and experience exists regarding the formulation of national policy, leading to weak or absent regulation and a lack of proper integration of traditional medicine services into the health service delivery system. It also reflects the need of all member states to push WHO to update its global strategy on traditional medicine (WHO, 2013).

2.9. Botanical Description and Taxonomy of *Solanum marginatum*

Solanum marginatum is one of the important medicinal plants of the genus *Solanum* in the family *Solanaceae*, which is a large plant family containing two thousand three hundred species with nearly half of which belonging to a single genus, *Solanum* (Sheeba, 2010). It is a much branched hairy shrub growing up to 2 meters tall and densely armed with prickles. It is an easily recognized usually attractive species with a characteristic white to silvery tomentum of dense stellete hairs on the young stems. The leaves are large, broadly ovate with a stout petiole 2-4 cm long. The midribs and primary lateral veins of the leaves are with prickles to over 1 cm long. The upper surfaces of the leaves are green and silvery-grey below. The inflorescence contains several white flowers, hanging or nodding bisexual flowers and erect staminate flowers with large yellow anthers (Figure 1B). The berries of this plant are shiny yellow, up to 5 cm in diameter. The seeds are pale brown and 2.5 x 2 mm in size (Nee, 2013). It is easily recognizable from the more common *Solanum incanum* (*embuay* in Amharic) by its larger leaves that are white beneath and its large fruit and white flowers.

Figure 1A: *S. marginatum* plants
(Source: www.weedbusters.co.nz)

Figure 1B: Aerial parts of *S. marginatum*

2.10. Benefits of *Solanum marginatum*

2.10.1. Antifungal Activity

Plants produce a great deal of secondary metabolites, many of them with antifungal activity. Well-known examples of these compounds include flavonoids, phenols and phenolic glycosides, unsaturated lactones, sulphur compounds, saponins, cyanogenic glycosides and glucosinolates (Bennett and Wallsgrove, 1994).

A number of plants from the Solanaceae family have been reported to possess antifungal activity. Among the plants reported to be used for the treatment of diverse fungal skin infections and plant fungal infections, the genus *Solanum* shows a high index of citation (Shamim *et al*., 2004). Some of the members of the genus *Solanum* which are used as antifungal agents are *Solanum indicum*, *Solanum surattense*, *Solanum trovum, Solanum xanthocarpum, Solanum melongena, Solanum incanum and Solanum marginatum* (Das *et al*., 2010; Roman, 2010; Gavimath *et al*., 2012).

2.10.2. Insecticidal Activity

The application of synthetic insecticides as pest control method has resulted in acute and chronic ecological consequences either by direct injury of non-target organisms like birds and fish or by indirect effects such as elimination of natural enemies (Yadav, 2010).

There are more than 2000 plant species known to have insecticidal properties, where the Euphorbiaceae, Asteraceae, Labiatae, Fabaceae, Meliaceae and Solanaceae families stand out (Castillo-Sanchez *et al.*, 2004). From 60 to 70% of the species in the Solanaceae family produce alkaloids, which play an important role against pathogens and herbivores. They have a toxic and feed deterrent effect on insects like cotton bollworm, armyworm, desert locust and mosquito (Eich, 2008). In particular, *Solanum marginatum* showed inhibitory activity on larval growth of Red flour beetle (*Tribolium casteneum*) and Tobacco hornworm (*Manduca sexta*) (Weissenberg, 1988).

2.10.3. Medicinal Value

According to WHO (2001) plants are well known for their medicinal values. The medicinal value of plants is due to the presence of some chemical substances in the plant tissues which produce a definite physiological action on the human body (Cowan, 1999).

While most medical relevance of genus *Solanum* is due to poisonings which are not common and may be fatal, several species are locally used, particularly by native peoples who have long employed them. A number of research activities have been done on traditional medicinal values of white-margined nightshade in our country. As indicated in Amare (1976), lightly roasted seeds of the plant are used to treat weak heart and stomach complaints. Aerial parts of *S. marginatum* which are prepared by infusion or decoction can be taken orally and have medicinal value for cough, administered as bath for general body joints pain and clean ailments (Manuel *et al.*, 2005). People living in Zegie peninsula, northwestern Ethiopia, used the crushed roots of *Solanum marginatum* to treat wound and swelling (Tilahun and Mirutse, 2007). Similarly, Yigezu *et al*. (2014) reported the roots and leaves of white-margined nightshade (*Solanum marginatum)* are used for treatment of veterinary ailments. The roots and leaves are pounded together by adding water, filtered and given to livestock orally to treat Black leg. In addition, Abraha *et al*. (2013) indicated the highest preference of people for

Solanum marginatum against abdominal pain over the other herbal medicines in Kilte Awulaelo district, Tigray region.

2.10.4. Sanitizer Effect

Solanum marginatum contains products called saponins which have fat emulsifying properties and property of formation of soapy leather when shaken with water similar to soap (Singh & Kaushal, 2007; Colmenares and Corredor, 2011). Saponins have been reported to possess a wide range of biological activities. The toxicity of saponins to insects (insecticidal activity), parasitic worms (antihelmintic activity), molluscs (molluscicidal), and fish (piscidal activity), and their antifungal, antiviral, and antibacterial activities are well documented (Lacaille-Dubois and Wagner, 1996; Milgate and Roberts, 1995).

The presence of saponins in the plant gives it sanitizing power. The alcoholic extract of the fruit is very useful in industries as biodegradable cleaner. The assessment of the sanitizing potential of the cleaner preparation showed that the plant has optimum antimicrobial effectiveness which was comparable with two well-known commercial sanitizers (Colmenares and Corredor, 2011). In Ethiopia, the ripe yellow fruits of this plant are boiled and used as a soap substitute in cleaning clothes (Amare, 1976).

2.10.5. Source of Alkaloids and Tannin

Alkaloids rank among the most efficient and therapeutically significant plant substances. They are chemically very diverse group of organic nitrogen compounds. Generally they are extremely toxic though they do have a marked therapeutic effect in minute quantities. Pure, isolated plant alkaloids and their synthetic derivates are used as basic medicinal agents all over the world for their antispasmodic and bactericidal effects (Ciocan and Bara, 2007).

S. marginatum is one of several *Solanum* species that contain solasodine. It has potential economic importance because its fruits are source of solasodine, a steroid alkaloid used in the commercial production of sex hormones (Dulberger *et al*., 1981). They yield much solasodine which has potential use for corticosteroid production for medicine (Hanlet, 2001).

Tannin is a general descriptive name for a group of polymeric phenolic substances capable of tanning leather (Scalbert, 1991). Since the berries of this plant contain tannin, they are used in

leather tannery (Amare, 1976). Similarly, Jaeger (1985) reported that the fruits of certain African species of *Solanum*, in particular *S. marginatum*, are used by some Africans for tanning leather. Besides its use in tannery, many human physiological activities, such as stimulation of phagocytic cells, host-mediated tumor activity, and a wide range of anti-infective actions have been assigned to tannins (Haslam, 1996). Antimicrobial activity of tannins is expressed due to their ability to link amino acids in proteins, inactivating adhesions, enzymes and transport proteins of cell membranes of microorganisms (Cowan, 1999).

2.11. Inhibition of Bacterial Pathogens Using Plant Extracts

Plants are generally known to possess medicinal agents with microbicidal activities (Bobbarala *et al.*, 2009). A significant proportion of pharmaceutical products in current use are designed from plants. A large number of phytochemicals belonging to several chemical classes have been shown to have inhibitory effect on all types of microorganisms *in vitro* (Cowan, 1999) and some plant extracts have shown activity on both Gram negative and Gram positive organisms (Nascimento *et al.*, 2000) .

The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency and the antimicrobial properties of plants have been investigated by a number of researchers worldwide. Plant extracts have great potential as antimicrobial compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes (Nascimento *et al*., 2000). A number of these agents appear to have structures and modes of action that are distinct from those of the antibiotics in current use, suggesting that cross-resistance with agents already in use may be minimal (Morrison, 2009).

Extracts from medicinal plants are known to have antimicrobial activity against a range of pathogenic bacteria in humans (Palombo and Semple, 2001). There has been growing interest to find new and safer substances from medicinal plant extracts as an alternative approach to discover new antimicrobial compounds. The antimicrobial activities of some herbal medicines in inhibiting and destroying bacterial pathogens have been reported from different countries (Tomoko *et al*., 2002; Rios and Recio, 2005).

Laboratories of the world have literally found thousands of phytochemicals, which have inhibitory effect on all type of microorganisms *in vitro*. However, their effectiveness in whole organisms, toxicity assays and their possible effects on the beneficial micro biota are not yet conducted for many of these compounds (Cowan, 1999).

2.11.1. Inhibition of *Staphylococcus aureus*

Staphylococcus aureus is an important pathogen both in community acquired and healthcare associated infections due to its fast growing resistance to antibiotics. In particular, methicillin-resistant *Staphylococcus aureus* presents major infection control problems and threats globally (Nascimento, *et al.*, 2000).

There has been considerable effort to discover plant-derived antibacterials against methicillin-resistant strains of *Staphylococcus aureus* (MRSA) which have developed resistance to most existing antibiotics, including the last line of defence, Vancomycin. But, Pentacyclic triterpenoid which is a biologically diverse plant-derived natural product has been reported to show anti-staphylococcal activities (Chung *et al*., 2011).

Pharmacological studies done with essential oils from 15 species of aromatic plants obtained in Brazil have shown activity coherent with the use of the medicinal plants in folk medicine. These studies have dealt with the effects of these oils on antibacterial activity (Holetz *et al*., 2002). Essential oils derived from aromatic plants have many biological properties and can be used to prevent infectious diseases. Many of them have high activity against Gram-positive and Gram-negative bacteria. It has been reported that many essential oil producing species of the genus *Pelargonium* have activity against clinical strains of *Staphylococcus aureus* (Bigos, 2012).

McCutcheon *et al.* (1992) tested 100 methanolic extracts of the plants used by British Colombian native people against 11 bacterial isolates. They found that 75% of the extracts were effective against methicillin-resistant *Staphylococcus aureus.*

The guava leaf extract (*Psidium guajava*), crushed garlic extracts (*Allium sativum*), pomegranate extract (*Punica granatum*), stem and leaf extracts of black nightshade (*Solanum nigrum*) botanical extracts of *Salvia officinalis*, *Eucalyptus globulus*, *Coleus forskohlii*, *Coptis*

chinensis, *Turnera diffusa*, and *Larrea tridentate* are some of the common medicinal plants that have been found to possess bactericidal or bacteriostatic effect on *Staphylococcus aureus* (Cutler and Wilson, 2004; Braga *et al*., 2005; Yogananth *et al*., 2005; Snowden *et al*., 2014).

2.11.2. Inhibition of *Streptococcus pyogenes*

Penicillin and erythromycin have been the treatment of choice for *Streptococcus pyogenes* infections. Unfortunately, an increasing incidence of penicillin and erythromycin resistance has been reported (Feng & Shen, 2009). So, the discovery of potential new drugs might be helpful for the treatment of *Streptococcus pyogenes* infections in the near future.

There are several reports on antibacterial activity of plants that inhibit various bacterial pathogens. However, only limited numbers of studies have been published on this important bacterial human pathogen (Limsuwan and Voravuthikunchai, 2013).

Medicinal plants like *Boesenbergia pandurata*, *Cinnamomum bejolghota*, *Cinnamomum porrectum*, *Eleutherine Americana, Gymnopetalum cochinchinensis, Piper betle* L., *Quercus infectoria*, *Quisqualis indica* L, *Rhodomyrtus tomentosa*, *Solanum nigrescens* and *Walsura robusta* have been studied and some have a strong activity and good potential to be developed into an effective drug to treat *Streptococcus pyogenes* infections (Caceres, 1991; Limsuwan and Voravuthikunchai, 2013).

2.11.3. Inhibition of *Escherichia coli*

Escherichia coli (E. *coli*) O157:H7 is of great clinical and epidemiologic importance as the etiologic agent in significant human diseases, including diarrhea, hemorrhagic colitis (HC), and occasionally complications such as hemolytic-uremic syndrome (HUS), and thrombocytopenic purpura (TTP) in developed countries. Complications resulting from the use of antibiotics in the treatment of HUS and TTP encourage researchers to find effective medicinal plants as alternative treatments for *E. coli* O157:H7 infection (Pai, 1988).

There are important medicinal plants known to have antibacterial activity against pathogenic strains of *E coli*. Ushimaru *et al*. (2012) and Voravuthikunchai *et al*. (2004) reported that crude extracts from *Psidium guajava*, *Zingiber officinale*, *Cymbopogon citratus*, *Caryophyllus*

aromaticus, *Mikania glomerata*, *Allium sativum*, *Acacia catechu*, *Holarrhena antidysenterica*, *Peltophorum pterocarpum*, *Psidium guajava*, *Punica granatum*, *Quercus infectoria*, *Uncaria gambir*, and *Walsura robusta* exhibited antibacterial activity against *Escherichia coli* strains.

2.11.4. Inhibition of *Campylobacter jejuni*

Campylobacter spp. is recognized as one of the most common cause of food-borne bacterial gastroenteritis in humans. There are no sustainable strategies for reduction or elimination of this bacterium from the food chain. In addition, antibiotic resistant *Campylobacter* isolates have been reported worldwide (Bester and Essack, 2008).

In their study, Fisher and Phillips (2006) indicated that the extracts of *Acacia farnesiana*, *Artemisia ludoviciana*, *Opuntia ficus-indica*, and *Cynara scolymus* were effective against *Campylobacter jejuni* at minimal bactericidal concentrations. Adherence and cytotoxic activity of the bacteria to host mucosal surfaces which are critical steps in pathogenesis were decreased by these extracts.

2.12. Some Bacterial Diseases

2.12.1. *Staphylococcus aureus*

S. aureus is a bacterium that belongs to of the family *Staphylococcaceae*. *S. aureus* is a Gram-positive facultative anaerobe that exhibits a coccal morphology, and is non-motile and non-spore forming. Its cells are 0.5-1.5µm in diameter and forms grape-like clusters. It is catalase and coagulase positive and some strains produce capsules. On blood agar, colonies of *S. aureus* appear golden (caused by staphyloxanthin, a membrane-bound carotenoid) surrounded by haemolytic zones (Wieland *et al*., 1994).

The bacteria form part of the normal flora of the skin, intestine, upper respiratory tract and vagina. It causes infections in almost every organ and tissue of the human body. The most commonly affected part of the body due to *S. aureus* infection is the skin (Lowy, 1998). *S. aureus* colonizes the skin and nares of 30-50% of the human population. Nasal colonization typically persists for long periods of time and represents a predisposition to future disease, typically skin and soft tissue infections (SSTIs) and it is a common cause of bacteremia and

sepsis. A key feature of *S. aureus* SSTIs is recurrence, which occurs in 20-30 % of all cases even following antibiotic and/or surgical therapy (Peacock, 2001).

The nature of those infections depends on several factors, such as the pathogenic characteristics of the strain, host susceptibility and the route of entry into the host. In addition, *S. aureus* infections vary in seriousness and outcome from minor skin infections such as superficial lesions (furuncles, boils) and wound infections, to life-threatening infections such as septicaemia, ostemyelitis, acute endocarditis and necrotising pneumonia (Lowy, 1998; Keane, 1992).

S. aureus can withstand harsh environments for extended periods allowing susceptible individuals to become infected through contact with contaminated objects, but direct contact with persistently or transiently colonized people is the more important route of transmission (Mulligan *et al*., 1993; Goldmann *et al*., 1996). Such characteristics have made *S. aureus* the most common hospital acquired (nosocomial) pathogen with a formidable array of virulence and resistance strategies (Novick, 1993).

Most *S. aureus* strains produce a capsular polysaccharide that contributes in virulence. The capsule plays vital role in the adhesion of bacterial cells to each other and to host tissues and medical equipment. In addition, the capsule inhibits phagocytosis and restricts the ability of antibiotics to reach the bacterial cell surface. Moreover, peptidoglycan and lipoteichoic acid in the cell wall have a role in pathogenecity. For example, peptidoglycan has endotoxin activity that stimulates macrophages to release cytokines (Lowy, 1998).

Following the initial discovery and use of antibiotics, concern and frequency of *S. aureus* disease decreased significantly. However, the golden age of antibiotic therapy was followed by *S. aureus* acquisition of a wide variety of antibiotic resistance genes that provided escape from the most commonly used therapeutics (Neu, 1992). As a result of emergence of Methicillin-resistant *S. aureus* and acquisition of additional antibiotic resistance including Vancomycin which is the antibiotic of last resort for infections with MRSA, antibiotic resistance has become a major problem globally and it leaves physicians with few options available to treat MRSA infections (Lee *et al*., 2008).

2.12.2. *Streptococcus pyogenes*

Streptococcus pyogenes, also known as Group A Streptococcus (GAS), belongs to the genus *Streptococcus* and it is a spherical, Gram-positive, catalase-negative, facultative anaerobic bacterium that is the cause of Group A streptococcal infections (Ryan and Ray, 2004).

It is one of the most important human pathogens associated with extensive human morbidity worldwide. Diseases caused by *S. pyogenes* are a major public health concern both in developed and developing countries. Carapetis *et al*. (2005) estimated that severe GAS diseases cause over a half million deaths each year.

S. pyogenes can evoke illnesses ranging from mild and quite frequent noninvasive infections as pharyngitis, tonsilitis and impetigo to life threatening infections like necrotizing fascititis and streptococcal toxic shock syndrome. These are often followed by post infective sequel of rheumatic fever, rheumatic heart disease and post streptococcal acute glomerulonephritis (Capoor *et al*., 2006). The ability to produce such a diversity of infections in so many different tissue compartments is evidence to the extensive plasticity of the *S. pyogenes* transcriptome and an abundance of secreted virulence factors (Cole *et al*., 2011).

GAS infection is ordinarily spread by direct person-to-person contact, most likely via drops of saliva, nasal secretions, contaminated fingers, dust or fomites (Arguelles *et al*., 2004). All beta-hemolytic Group A Streptococcus are sensitive to penicillin G, and most are sensitive to erythromycin. A high frequency of resistance to erythromycin in GAS has been reported, particularly in countries where antibiotics are overused (Kim and Lee, 2004).

2.12.3. *Escherichia coli*

Escherichia coli (commonly abbreviated *E. coli*) is a Gram-negative, facultatively anaerobic, rod-shaped bacterium of the genus *Escherichia* that is commonly found in the lower intestine of endotherms. A typical *Escherichia coli* cell is about 1 μm wide and 2 to 3 μm long (Singleton, 1999; Burton and Engelkirk, 2004).

Since its identification in 1885, *Escherichia coli* has become one of the most widely studied bacterial species. *E. coli* strains are comparatively easy to grow and manipulate in the

laboratory, are easy to genetic manipulation, and naturally acquire mobile genetic elements (Clements *et al*., 2012).

Around 95% of coliforms in faeces are *E. coli* and this bacterium is considered to be the best indicator of fecal contamination (Silva *et al.,* 2009). *E. coli* is one of the main inhabitants of the intestinal tract of birds, humans and most mammalian species. Majority of *Escherichia coli* strains are considered as nonpathogen but certain groups of them can cause severe enteric and extraintestinal diseases in man (Kaper *et al*., 2004).

Enteric *E. coli* are part of the natural flora of many animals. Human infections occur through consumption of contaminated food products, drinking water contaminated with animal or human waste, or through direct person-to-person spread from poor hygiene. Infections with Shiga toxin-producing *E. coli* strain have also been linked to consumption of contaminated radish sprouts and pre-packaged spinach (Berger *et al*., 2010).

The constant use of antibiotics in human and animal medicines is causing a rise in *Escherichia coli* resistance to these medicines, interfering with the effective treatment of infections caused by this agent (Carnot *et al*., 2014).

2.12.4. *Campylobacter jejuni*

C. jejuni is a member of the family *Campylobacteraceae*. It is Gram-negative, non-spore forming, S-shaped or spiral shaped bacteria (0.2-0.8µm wide and 0.5-5 µm long), with single polar flagella at one or both ends, conferring a characteristic corkscrew-like motility. It neither ferments nor oxidizes carbohydrates. This bacterium requires microaerobic conditions, but some strains also grow aerobically or anaerobically. *C.jejuni* is thermophilic, growing optimally at 42°C (Garrity, 2005).

It can colonise mucosal surfaces, usually the intestinal tract, of most mammalian and avian species tested (Garrity, 2005). Before 1973, *C. jejuni* was not recognized as one of the most common causes of infectious diarrhea until selective methods for its isolation were developed. *C. jejuni* grows well only on enriched media under microaerophilic conditions. That is, it requires oxygen at reduced tension (5-10%), presumably due to vulnerability of some of its

enzyme systems to superoxides. Growth usually requires 2 to 4 days, sometimes as much as a week (Ryan and Ray, 2004).

It is a widespread and major cause of zoonotic bacterial gastroenteritis carried by animals raised for meat and poultry. Consumption of undercooked meat contaminated with the bacteria causes gastrointestinal diarrhea in humans which produces an inflammatory, sometimes bloody, diarrhea or dysentery syndrome, mostly including cramps, fever and pain (Ryan and Ray, 2004).

3. MATERIALS AND METHODS

3.1. Study Area

The study area is located in Chiro district, which is part of the west Hararghe zone in the Oromia region, Ethiopia. It is about 325 Kilometers away from Addis Ababa towards the east. Geographically, it lies at a latitude and longitude of 8^{O}55'N and 40^{O}15'E, respectively. The daily mean temperature is 14.7-31^{O}C and it receives an annual rainfall of 751.3 mm. The relative humidity of the district is 37.8% and the soil types are chromic cambisol and eutric regosols (BoA, 2001). The experiments were, however, conducted in the laboratory of the School of Plant Sciences and Crop Protection, Haramaya University, which is located at a latitude of 9^{O}26'N, longitude of 42^{O}03'E and an altitude of 1980 m.a.s.l. with a mean annual temperature of 17^{O}C (FAO, 1990).

3.2. Collection of Plant Parts

The leaves, fruits, stems and roots of white-margined nightshade used in this study were collected from Chiro district between the months of September and November 2014. The experiments were carried out between the months of January and March 2015.

3.3. Preparation of Crude Extracts

The collected plant parts (leaves, fruits, stems and roots) were separately washed using tap water followed by sterilized distilled water and cut into smaller sizes of about 1-3 cm long. The washed plant parts were then shade dried at room temperature for three weeks and pounded using electric grinder into fine powder of diameter 40 µm and finally kept in a refrigerator until use (Selvaraj and Narayanasamy, 1993; Singh *et al*., 2007).

3.3.1. Preparation of Aqueous Extracts

Crude plant leaf, fruit, stem and root extracts were obtained by separately suspending 200g of each plant material in 1000 ml distilled water to give 20% (w/v) in a 2000 ml conical flask. The resulting leaf, stem, fruit and root powder suspension were then shaken at 121 rpm for 24 hours using a shaker to produce the required infusion. After filtering the infusion using double layer cheese cloth and Whatman No 1 filter paper, the filtrates were centrifuged for 15 min at

6000 rpm. The supernatants of the extracts were then preserved in air tight bottles for further use in refrigerator adjusted at 4^{O}C (Naduagu *et al*., 2008).

3.3.2. Preparation of Ethanol Extracts

Two hundred (200) grams of air dried powdered plant materials were placed in 1000 ml of ethanol kept in a conical flask each and were shaken in a rotary shaker at 121 rpm for 24 hrs. After 24 hrs, the suspension was filtered with a double layer muslin cloth and Whatman No 1 filter paper. The resulting filtrate was concentrated under reduced pressure in rotary evaporator at 40^{O}C. The gummy residue was further dried in water bath until the solvent was removed. After solvent evaporation, the remaining crude extracts were diluted with 10 ml sterile distilled water and kept in air tight bottle in refrigerator until use at 4^{O}C (Dewanjee *et al.*, 2007; Bhaskarwar *et al.*, 2008; Rajeendran and Ramakrishana, 2009).

3.4. Determination of Percentage Yields of Crude Extracts

Two hundred grams of powdered plant materials were used to obtain crude extracts from each plant part. The percentage yield for each plant part was the amount of crude extract recovered in mass compared with the initial amount of powdered plant materials used. It is presented in percentage (%) and was determined for each extraction solvent used.

3.5. Target Pathogenic Bacteria

Two strains of bacteria that infect the skin (*Staphylococcus aureus* ATCC 25923 and *Streptococcus pyogenes* ATCC 19615) and two enteric bacterial pathogens (*Escherichia coli* ATCC-25922 and *Campylobacter jejuni* ATCC 33560) were obtained from Ethiopian Public Health Institute (EPHI). All bacterial cultures were first grown on 5% sheep red blood agar plates at 37^{O}C for 18-24 h prior to inoculation onto the nutrient agar. Few colonies (4-5) of similar morphology of the respective bacteria were transferred with a sterile inoculating loop to a liquid medium and this liquid culture was then incubated until adequate growth equivalent to McFarland 0.5 turbidity units (1.5×10^{8} CFU/ml) standard was obtained (Hailu *et al*., 2005). The inocula of the respective bacteria were streaked onto Mueller-Hinton agar (MHA) plates using a sterile swab in such a way as to ensure thorough coverage of the plates and a uniform thick lawn of growth was obtained following incubation. The inoculated plates

were left at room temperature for 3-5 minutes to allow for any surface moisture to be absorbed before applying the extract. Wells of 6 mm in diameter were formed onto MHA plates using a sterile cork borer. The wells were filled with the test agents (50 µl each) and the plates were then allowed to stay for 1-2 h at room temperature for proper diffusion. Finally, the plates were incubated at 37^{O}C for 18-24 h and the resulting diameters of zones of inhibition were measured using sliding caliper (Obeidat *et al*., 2012). Amoxicillin, Penicillin and Tetracycline were used as positive controls at concentrations of 0.1 mg/ml and 0.2 mg/ml, with equal amounts as those of the extracts (50 µl), and sterile distilled water (50 µl) was used as a negative control (Hailu *et al*., 2005).

3.6. *In Vitro* Evaluation of the Plant Extracts

The antimicrobial activities of the ethanol and aqueous crude extracts of the stems, fruits, roots and leaves of *S. marginatum* were evaluated using the agar-well diffusion method. The zones of inhibition formed following incubation were measured and the mean diameters were obtained. Overall, cultured bacteria with zones of inhibition equal to or greater than 7 mm were considered susceptible to the tested extract (Nascimento *et al*., 2000).

3.6.1. Aqueous Extracts

The aqueous extracts obtained from different parts (stem, leaf, fruit and root) of the plant were assayed for antimicrobial activities against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Escherichia coli* and *Campylobacter jejuni*.

The four test pathogens were exposed to aqueous extracts of different plant parts by an adaptation of the agar well-diffusion method (Smania *et al*., 1995). A 50 µl of the four types of extracts at a concentration of 20 and 40 mg/ml were placed in 6 mm diameter wells formed in the Mueller-Hinton agar (MHA) plates; the negative controls received the same amount of sterile distilled water. All plates were used with three replications. The inhibition zones were observed after 24 h of growth at 37^{O}C. Amoxicillin, Penicillin and Tetracycline were used as positive controls at concentrations of 0.1 mg/ml and 0.2 mg/ml, with equal amounts as those of the extracts (50 µl), and sterile distilled water (50 µl) was used as a negative control (Hailu *et al*., 2005).

3.6.2. Ethanol Extracts

Four ethanol extracts obtained from the leaf, stem, root and fruit of the plant were used for antimicrobial assay. The growth media were prepared following standard procedures. After complete solidification of the media, separate cultures of each species of bacteria were spread aseptically on to each plate. Immediately following this procedure, small wells (each with 6mm diameter) on each inoculated plate were prepared aseptically using a sterile cork borer and extracts of varying concentrations (20 and 40 mg/ml) were added into the wells. Plates were incubated at 37^{O}C for 24 hours. Amoxicillin, Penicillin and Tetracycline were used as a positive control and sterile distilled water was used as a negative control. All plates were with three replicates. Sizes of colony diameter were measured after 24 h of growth at 37^{O}C.

3.7. Determination of Minimum Inhibitory Concentration (MIC)

The ethanol and aqueous extracts of the different plant parts that showed significant antimicrobial activity in the previous test were selected for determination of MIC. The MIC of the crude extracts of the leaves, the fruits, the stems and the roots of white-margined nightshade were determined by agar dilution method. In agar dilution, the extract solution at 20 mg/ml was serially diluted as 1:2, 1:4, 1:8, 1:16, 1:32 to get 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml and 0.625 mg/ml concentrations respectively. Then, each of the four test pathogens were added to the diluted ethanol extracts of concentrations ranging from 0.625 mg/ml up to 10 mg/ml.

The growth media were first prepared and sterilized by autoclaving. The sterilized media were allowed to cool to 50^{O}C and 18 ml of the molten agar was added to test tubes which contain 2 ml of different concentration of the crude extract and the control. The mixture of the media and the test extracts were thoroughly mixed and poured into pre-labeled sterile petridishes on a level surface. Additional petridishes containing only the growth media were prepared in the same way so as to serve for comparison of growth of the respective organisms. The plates were dried at room temperature. The suspensions of the respective pathogens having density adjusted to McFarland 0.5 turbidity units (1.5×10^{8} CFU/ml) were inoculated onto the series of agar plates using standard loop. Three loopful of the suspension were transferred into each

plate. The plates were then incubated at $37^{O}C$ for 24 h. The lowest concentration which inhibited the growth of the respective organisms was taken as MIC.

3.8. Data Analysis

All the experiments were carried out in triplicates. Zones of inhibition were analyzed using SPSS 20 statistical software package to compare means. The data were subjected to Tukey's HSD analysis. Data were expressed as mean ± standard deviation and statistical significance was calculated. Values corresponding to $p < 0.05$ were considered statistically significant.

4. RESULTS AND DISCUSSION

4.1. Yields of Crude Extracts

As indicated in Table 1, the amount of the extracts ranged from 4.28% to 10.60%. Ethanol extract of the fruits gave the maximum yield (10.60%), followed by ethanol extract of the root (8.72%) and ethanol extract of the leaf (8.54%). The lowest yield was obtained from aqueous extract of the stem (4.28%). The results clearly showed that the percentage yield of the crude extracts of the different plant parts varied significantly ($p<0.05$) from solvent to solvent. This could be attributed to different polarity and extracting potential of ethanol and water. Ethanol can dissolve both polar and non-polar substances. As Cowan (1999) reported, most antimicrobial agents that have been identified from plants are soluble in organic solvents and this reveals the better efficiency of ethanol as extracting solvent than water. Table 1 also shows that the percentage yields of the crude extracts using the same extraction solvent varied significantly ($p<0.05$) from one part of the plant to the other. When different plant parts are compared for their yield, the fruit extracts gave the maximum yield and the stem extracts the least for both extraction solvents. This indicates that the bioactive ingredients are not found uniformly through the plant and some plant parts tend to have more bioactive compounds (Wynn and Fougere, 2006).

Table 1. The percentage yields of the crude extracts of the plant parts

Plant part	Extraction solvent	Crude mass (g)	Percentage yield
Stem	Ethanol	14.60 ± 0.02^{D}	7.30 ± 0.010^{D}
	Water	8.56 ± 0.03^{H}	4.28 ± 0.015^{H}
Leaf	Ethanol	17.08 ± 0.04^{C}	8.54 ± 0.021^{C}
	Water	14.24 ± 0.07^{F}	4.72 ± 0.020^{G}
Root	Ethanol	17.44 ± 0.03^{B}	8.72 ± 0.015^{B}
	Water	9.44 ± 0.04^{G}	7.12 ± 0.034^{F}
Fruit	Ethanol	21.20 ± 0.04^{A}	10.60 ± 0.023^{A}
	Water	14.48 ± 0.03^{E}	7.24 ± 0.015^{E}

4.2. Antimicrobial Activities of the Crude Extracts

A total of 8 crude extracts (ethanol and aqueous) were prepared and screened for antimicrobial activities against the test pathogens. The antimicrobial activities of the different extracts of *S. marginatum* against the four pathogenic bacteria are presented in Tables 2-5.

4.2.1. Antimicrobial activities of crude extracts of the stems of *Solanum marginatum* against test pathogens

In this test, the ethanol extract showed a significant growth inhibition against all the bacterial species. As indicated in Table 2, the zones of inhibition of the ethanol and aqueous stem extracts were in the range of 7.10-15.50 mm and 0.90-5.70 mm respectively.

Table 2. Antibacterial activity of crude extracts of the stem and antibiotics against the test pathogens (mean ± SD, n = 3)

Test pathogen	Zone of Inhibition						
	Conc. of extracts (mg/ml)	Crude extracts obtained using two different solvents		Conc. of antibiotics (mg/ml)	Antibiotics		
		Water	Ethanol		Tetracycline	Penicillin	Amoxicillin
E. coli	20	0.90 ± 0.10^{Dc}	7.10 ± 0.10^{Fb}	0.1	14.27 ± 0.25^{Fa}	0	7.56 ± 0.30^{Hb}
	40	2.87 ± 0.12^{Cd}	9.40 ± 0.10^{Ec}	0.2	17.33 ± 0.15^{Ea}	0	10.73 ± 0.15^{Gb}
S. aureus	20	1.50 ± 0.10^{Dd}	11.40 ± 0.10^{Dc}	0.1	17.80 ± 0.44^{Da}	0	13.57 ± 0.25^{Eb}
	40	4.33 ± 0.06^{Bd}	14.73 ± 0.06^{Bc}	0.2	20.87 ± 0.23^{Ca}	0	16.83 ± 0.15^{Cb}
S. pyogenes	20	1.33 ± 0.15^{De}	7.23 ± 0.15^{Fd}	0.1	17.33 ± 0.25^{Ea}	16.37 ± 0.15^{Bb}	11.57 ± 0.25^{Fc}
	40	3.10 ± 0.10^{Ce}	10.83 ± 0.06^{Dd}	0.2	20.53 ± 0.12^{Ca}	19.67 ± 0.15^{Ab}	15.36 ± 0.21^{Dc}
C. jejuni	20	2.53 ± 0.06^{Ce}	12.30 ± 0.20^{Cc}	0.1	26.90 ± 0.46^{Ba}	9.10 ± 0.20^{Dd}	17.30 ± 0.20^{Bb}
	40	5.70 ± 0.20^{Ae}	15.50 ± 0.20^{Ac}	0.2	29.53 ± 0.38^{Aa}	12.70 ± 0.10^{Cd}	20.40 ± 0.26^{Ab}

n = number of experimental replicates, SD = standard deviation; means with the same letter (lower case) in the same row are not significantly different; means with the same letter (upper case) in the same column are not significantly different

Table 2 also shows that the aqueous stem extracts at both concentrations (20 and 40 mg/ml) didn't show significant antibacterial activities against all tested bacteria. However, the ethanol stem extracts at both concentrations resulted in growth inhibition of all the four bacterial species. The zones of inhibition for *C. jejuni* and *S. aureus* were significantly higher ($p<0.05$) than those observed against *E. coli* and *S. pyogenes*. The antibacterial activities of the ethanol extracts of the stem at both concentrations were significantly higher ($p<0.05$) than Penicillin against *C. jejuni*. In addition, there is no significant difference in activity between the ethanol extract of the stem at 20 mg/ml and the Amoxicillin at 0.1 mg/ml concentration against *E. coli*. However, the zones of inhibition that resulted from Tetracycline were significantly higher than ($p<0.05$) any of the stem extracts. In general, the diameters of zones of inhibition observed due to the antibiotics on the tested bacteria ranged from 14.27-29.53 mm, 0-19.67 and 7.56-20.40 for Tetracycline, Penicillin and Amoxicillin, respectively. The negative control used here, distilled water, showed no inhibition against all the bacterial strains.

4.2.2. Antimicrobial activities of crude extracts of the fruits of *Solanum marginatum* against test pathogens

In a similar manner, the fruit extracts were also tested for their antibacterial properties against the test pathogens. As can be seen in Table 3, the diameters of the zones of inhibition ranged from 3.73-9.97 mm for aqueous fruit extracts and from 9.33-23.87 mm for ethanol fruit extracts.

Table 3. Antibacterial activities of crude extracts of the fruit and antibiotics against the test pathogens (mean ± SD, n = 3)

Test pathogen	Zone of Inhibition						
	Conc. of extracts (mg/ml)	Crude extracts obtained using two different solvents		Conc. of antibiotics (mg/ml)	Antibiotics		
		Water	Ethanol		Tetracycline	Penicillin	Amoxicillin
E. coli	20	4.13 ± 0.06^{Fd}	9.33 ± 0.06^{Eb}	0.1	14.27 ± 0.25^{Ea}	0	7.56 ± 0.30^{Hc}
	40	8.87 ± 0.15^{Bd}	13.6 ± 0.20^{Db}	0.2	17.33 ± 0.15^{Da}	0	10.73 ± 0.15^{Gc}
S. aureus	20	3.73 ± 0.21^{Fc}	13.57 ± 0.25^{Db}	0.1	17.80 ± 0.44^{Da}	0	13.57 ± 0.25^{Eb}
	40	7.60 ± 0.26^{Cd}	17.50 ± 0.30^{Bb}	0.2	20.87 ± 0.23^{Ca}	0	16.83 ± 0.15^{Cc}
S. pyogenes	20	4.07 ± 0.12^{Fe}	9.77 ± 0.06^{Ed}	0.1	17.33 ± 0.25^{Da}	16.37 ± 0.15^{Bb}	11.57 ± 0.25^{Fc}
	40	6.57 ± 0.12^{Dd}	14.83 ± 0.15^{Cc}	0.2	20.53 ± 0.12^{Ca}	19.67 ± 0.15^{Ab}	15.36 ± 0.21^{Dc}
C. jejuni	20	4.97 ± 0.06^{Ed}	17.07 ± 0.15^{Bb}	0.1	26.90 ± 0.46^{Ba}	9.10 ± 0.20^{Dc}	17.30 ± 0.20^{Bb}
	40	9.97 ± 0.15^{Ae}	23.87 ± 0.21^{Ab}	0.2	29.53 ± 0.38^{Aa}	12.70 ± 0.10^{Cd}	20.40 ± 0.26^{Ac}

n = number of experimental replicates; SD = standard deviation; means with the same letter (lower case) in the same row are not significantly different; means with the same letter (upper case) in the same column are not significantly different

The aqueous fruit extracts at a concentration of 20 mg/ml showed no significant antibacterial activity against all the tested bacteria whereas at a concentration of 40 mg/ml there were activities against all tested pathogens except against *S. pyogenes*. The antibacterial activities of the fruit extracts at a concentration of 40 mg/ml against each bacterial species were found to be significantly different form one another ($p<0.05$). When compared with the antibiotics (positive controls), all the fruit extracts showed significantly lower zone of inhibition than Tetracycline against all the test pathogens. However, the ethanol fruit extracts at a concentration of 40 mg/ml showed significantly higher zone of inhibition than Amoxicillin and Penicillin against all tested pathogens except *S. pyogenes*. In addition, the ethanol fruit extracts at 20mg/ml showed significantly higher zone of inhibition than Penicillin against all tested pathogens except *S. pyogenes*. The high antibacterial activities observed in the ethanolic extracts of the fruit is in agreement with the results reported by Beaman-Mbaya and Muhammed (1976) against *S. aureus*, *S. pyogenes* and *E. coli*, and according to this researchers the antimicrobial activities of the White-margined nightshade fruit has been attributed to its high content of solanine and related glycoalkaloids, which are saponins and cytostatic poisons. Moreover, findings from this study are in consonance with findings of other researchers (Alamri and Moustafa, 2012) who reported antimicrobial activity in ethanolic extracts of the fruit against *S. aureus* (18 mm) and *E. coli* (8.46 mm) at a concentration of 25 mg/ml using agar-well diffusion method.

4.2.3. Antimicrobial activities of crude extracts of *Solanum marginatum*'s roots against the test pathogens

The results of the *in vitro* assays of antibacterial activities of the root extracts on the test pathogens are shown in Table 4. The ethanol extracts of the roots had inhibitory activities ranging from 8.03-19.27 mm and the aqueous extracts resulted in the zones of inhibition ranging from 2.03-8.47 mm.

Table 4. Antibacterial activity of crude extracts of the root and antibiotics against the test pathogens (mean ± SD, n = 3)

Test pathogen	Zone of Inhibition						
	Conc. of extracts (mg/ml)	Crude extracts obtained using two different solvents		Conc. of antibiotics (mg/ml)	Antibiotics		
		Water	Ethanol		Tetracycline	Penicillin	Amoxicillin
E. coli	20	3.50 ± 0.10^{EFd}	8.13 ± 0.06^{Fb}	0.1	14.27 ± 0.25^{Ea}	0	7.56 ± 0.30^{Hc}
	40	7.07 ± 0.25^{Bd}	11.57 ± 0.06^{Eb}	0.2	17.33 ± 0.15^{Da}	0	10.73 ± 0.15^{Gc}
S. aureus	20	2.90 ± 0.10^{Fd}	12.33 ± 0.15^{Dc}	0.1	17.80 ± 0.44^{Da}	0	13.57 ± 0.25^{Eb}
	40	5.83 ± 0.15^{Cd}	15.47 ± 0.15^{Bc}	0.2	20.87 ± 0.23^{Ca}	0	16.83 ± 0.15^{Cb}
S. pyogenes	20	2.03 ± 0.06^{Ge}	8.03 ± 0.06^{Fd}	0.1	17.33 ± 0.25^{Da}	16.37 ± 0.15^{Bb}	11.57 ± 0.25^{Fc}
	40	4.30 ± 0.20^{De}	13.03 ± 0.25^{Cd}	0.2	20.53 ± 0.12^{Ca}	19.67 ± 0.15^{Ab}	15.36 ± 0.21^{Dc}
C. jejuni	20	4.13 ± 0.12^{DEe}	13.30 ± 0.20^{Cc}	0.1	26.90 ± 0.46^{Ba}	9.10 ± 0.20^{Dd}	17.30 ± 0.20^{Bb}
	40	8.47 ± 0.06^{Ae}	19.27 ± 0.06^{Ac}	0.2	29.53 ± 0.38^{Aa}	12.70 ± 0.10^{Cd}	20.40 ± 0.26^{Ab}

n = number of experimental replicates; SD = standard deviation; means with the same letter (lower case) in the same row are not significantly different; means with the same letter (upper case) in the same column are not significantly different

The aqueous root extracts at a concentration of 20 mg/ml showed no significant antibacterial activity against all the tested bacteria but they exhibited zone of inhibitions higher than 7 mm at a concentration of 40 mg/ml against *E. coli* and *C. jejuni*. The ethanol root extracts at both concentrations showed significant difference ($p<0.05$) in their activities against the test pathogens as compared with the aqueous root extracts. The antibacterial activity of the ethanol root extract was significantly higher on *C. jejuni* than on the other tested bacteria. Comparisons of the diameters of zones of inhibition of root extracts with the positive controls revealed that Tetracycline was significantly more inhibitory on the tested pathogens than root extracts; and with the exception of *S. pyogenes* the ethanol root extracts showed significantly higher activities than penicillin did. In addition, the ethanol root extracts at both concentrations showed significantly higher inhibitory activities than Amoxicillin did on *E. coli*. The studies conducted by other researchers (Tilahun and Mirutse, 2007; Yigezu *et al*.,2014) on the ethnobotanical usage of the roots of white-margined nightshade support this finding of the present study.

4.2.4. Antimicrobial activities of crude extracts of leaves of *Solanum marginatum* against the test pathogens

The ethanol and aqueous crude extracts of the leaf at concentrations of 20 and 40 mg/ml were evaluated for *in vitro* antibacterial activities against the test pathogens. The zones of inhibition that resulted from these extracts are shown in Table 5.

Table 5. Antibacterial activities of crude extracts of the leaves of *Solanum marginatum* and antibiotics against the test pathogens (mean ± SD, n = 3)

Test pathogen	Zone of Inhibition						
	Conc. of extracts (mg/ml)	Crude extracts obtained using two different solvents		Conc. of antibiotics (mg/ml)	Antibiotics		
		Water	Ethanol		Tetracycline	Penicillin	Amoxicillin
E. coli	20	1.20 ± 0.20^{Ec}	7.63 ± 0.06^{Fb}	0.1	14.27 ± 0.25^{Ea}	0	7.56 ± 0.30^{Gb}
	40	4.53 ± 0.15^{Bd}	10.50 ± 0.20^{Ec}	0.2	17.33 ± 0.15^{Da}	0	10.73 ± 0.15^{Fb}
S. aureus	20	2.53 ± 0.06^{Dd}	11.73 ± 0.12^{Dc}	0.1	17.80 ± 0.44^{Da}	0	13.57 ± 0.25^{Db}
	40	5.17 ± 0.32^{Bd}	15.07 ± 0.31^{Bc}	0.2	20.87 ± 0.23^{Ca}	0	16.83 ± 0.15^{Bb}
S. pyogenes	20	1.43 ± 0.12^{Ee}	7.57 ± 0.15^{Fd}	0.1	17.33 ± 0.25^{Da}	16.37 ± 0.15^{Bb}	11.57 ± 0.25^{Ec}
	40	3.27 ± 0.21^{Ce}	12.53 ± 0.21^{Cd}	0.2	20.53 ± 0.12^{Ca}	19.67 ± 0.15^{Ab}	15.36 ± 0.21^{Cc}
C. jejuni	20	3.53 ± 0.06^{Ce}	12.73 ± 0.25^{Cc}	0.1	26.90 ± 0.46^{Ba}	9.10 ± 0.20^{Dd}	17.30 ± 0.20^{Bb}
	40	8.17 ± 0.15^{Ae}	16.93 ± 0.21^{Ac}	0.2	29.53 ± 0.38^{Aa}	12.70 ± 0.10^{Cd}	20.40 ± 0.26^{Ab}

n = number of experimental replicates; SD = standard deviation; means with the same letter (lower case) in the same row are not significantly different; means with the same letter (upper case) in the same column are not significantly different

The results obtained showed that all the test pathogens were inhibited by the ethanol extracts of the leaf and the zones of inhibition were in the range of 7.57 to 16.93 mm. In the case of the aqueous extracts, the zones of inhibition ranged from 1.20-8.17 mm and these extracts resulted in zones of inhibition which were less than 7 mm on the tested bacteria except on *C. jejuni* (8.17 mm). On the other hand, the inhibition zones produced by Tetracycline, Penicillin and Amoxicillin ranged from 14.27-29.53 mm, 0-19.67 and 7.56-20.40 mm, respectively.

Generally, the results of this study showed differences in activity among plant extracts depending on the nature of the extraction solvent used. Aqueous crude extracts of the four parts of the plant at a concentration of 20 mg/ml were observed to have little antimicrobial activities against the test bacterial species with diameters of zones of inhibition ranging from 0.9 and 4.97 mm. The aqueous crude extracts at a concentration of 40 mg/ml showed zones of inhibition ranging from 2.87-9.97 mm. However, the ethanol extracts of the four plant parts at a concentration of 20 mg/ml and 40 mg/ml showed zones of inhibition ranging from 7.10-17.07 mm and 9.40-23.87 mm, respectively. The aqueous extracts showed some antibacterial activities but their values were significantly lower than standard values set by Nascimento *et al.* (2000), which indicated that these extracts are not having significant effect in the control of the selected bacterial pathogens. This could be explained by the fact that most of the identified components with antimicrobial activities from plants were aromatic or saturated organic compounds and they were soluble in ethanol (Cowan, 1999). As stated above, the activities of the ethanol and aqueous extracts of the different parts of the plant against the test pathogens varied. This findings from the present study revealed that the plant parts extracted by absolute alcohol provided more consistent antibacterial activities compared with those extracted using water. In addition, it has been observed that in all cases the antimicrobial activities of crude extracts increased when used at higher concentrations.

The results also showed that different plant parts assayed here possess different levels of antibacterial activities. Among the extracts of the four plant parts tested against the four bacterial pathogens, the fruit extracts showed the highest range of antibacterial activities (3.73-23.87 mm) followed by the root extracts (2.03-19.27 mm). The stem extracts showed the least zone of inhibition ranging from 0.9-15.50 mm. The standard antibiotics used in this experiment showed varied antibacterial activities and the highest diameter of zone of

inhibition was recorded for tetracycline (29.53 mm) and the least was recorded for Penicillin. The greater activities recorded by fruit and root extracts over the stem and leaf extracts in this study suggest that more of the bioactive ingredients are lodged in these parts (Beaman-Mbaya and Muhammed, 1976). Similarly, active ingredients such as phenols that confer broad spectrum activities in plants were observed by Alamri and Moustafa (2012) in a study of medicinal plants using ethanol extracts of fresh fruits of *S. incanum*. The highest inhibition zone was observed with ethanol fruit extract against *C. jejuni* and the lowest values were recorded for the aqueous extracts of the stem against *E. coli*. Similarly, high resistance to crude extracts of *S. incanum* was reported for *E. coli* by Biruhalem *et al*. (2011).

Among the tested bacteria for susceptibility to various crude extracts of the plant, *C. jejuni* was found to be the most susceptible (2.53-23.87 mm) followed by *S. aureus* (1.50-17.50 mm), *E. coli* (1.20-15.50 mm) and *S. pyogenes* (1.33-14.83 mm). Microorganisms are known to vary widely in their degrees of susceptibility for antimicrobial agents. Nonetheless, the results of the present study indicate that the parts of the plant have promising antimicrobial activities against the selected test pathogens. These results, together with ethnobotanical studies made previously by other investigations (Amare, 1976; Tilahun and Mirutse, 2007; Abraha *et al*., 2013; Yigezu *et al.*, 2014)), suggest that *Solanum marginatum* might have important compounds that can potentially be used for the treatment of skin and enteric diseases.

4.3. Minimum Inhibitory Concentration (MIC) of the Crude Extracts

The minimum inhibitory concentration (MIC) assay was employed to evaluate the effectiveness of the extracts that showed significant antimicrobial activities in the previous tests. MIC was determined for extracts that showed greater than or equal 7 mm diameter of growth inhibition zone at 20 mg/ml. The results are shown on Table 6. The data revealed that the MIC of ethanolic extracts ranged from 1.25 mg/ml for *C. jejuni* to 10 mg/ml for *E. coli* and *S. pyogenes*. Generally, the fruit ethanol extracts had the lowest MIC and the highest was for the leaf and stem ethanol extracts. As reported in Alamri and Moustafa (2012), the MICs of the ethanol fruit extracts for *S. aureus* and *E. coli* were 7.66 mg/ml and 7.56 mg/ml, respectively. The higher MIC observed for *E. coli* in this study could be due to increased resistance to some of the bioactive ingredients in the plant.

Table 6. Minimum inhibitory concentration (MIC) of the extracts of leaves, fruits, stems and roots of *Solanum marginatum* against bacterial test pathogens in mg/ml.

Strain	MIC of the ethanolic extracts (mg/ml)			
	Stem ethanol	Fruit ethanol	Root ethanol	Leaf ethanol
E. coli	10	10	10	10
S. aureus	5	2.5	5	5
S. pyogenes	10	10	10	10
C. jejuni	5	1.25	2.5	5

The relatively low MIC values recorded for the fruit ethanol extracts against the test pathogens confirm the high activity of the extract at low concentrations. Extracts with lower MIC scores are very effective antimicrobial agents. MIC is important because populations of bacteria exposed to an insufficient concentration of the extract can develop resistance to antibacterial agents. The high activity of antimicrobial agents at low concentrations is very essential for chemotherapeutic purposes because of their low toxicity to patients administered with such agents.

5. SUMMARY, CONCLUSION AND RECOMMENDATIONS

5.1. Summary

In the present study, different plant parts of *S. marginatum* were collected from Chiro district and their antimicrobial properties were studied on 4 pathogens. The pathogens were *E. coli*, *S. aureus*, *S. pyogenes* and *C. jejuni*.

For the bioassay study, the bioactive components of the plant parts were extracted with ethanol (99%) and water. The crude extracts of the two solvents were all tested for their antimicrobial activities against the selected pathogens described above at two different concentrations (20 and 40 mg/ml). The bioassay study was undertaken using Agar-well diffusion method and revealed that the crude extracts of water have shown antibacterial activity on one of the bacteria at a concentration of 40 mg/ml. *S. pyogenes* was found to be resistant for the crude extracts. Of the two crude extracts, the ethanolic extract was found to be with the higher growth inhibition effect on all of the bacteria.

The growth inhibition effects of the crude extracts were found to be less than Tetracycline. When the growth inhibition effect of the 8 crude extracts against bacterial pathogens is compared with the antibiotics used as the positive control, the effect observed by the positive controls was very high. However, the distilled water used as a negative control was incapable to show any growth inhibition effect against all the tested pathogens.

5.2. Conclusion

The findings of the present study revealed that *Solanum marginatum* collected from Chiro district exhibited significant antimicrobial effect by the crude extracts against the four bacterial strains (*E. coli*, *S. aureus*, S. pyogenes and *C. jejuni*) which is an indication for the presence of antimicrobial agents in it. The antimicrobial effect of the crude extract of each solvent was found to be concentration dependent against the tested pathogens. The two solvents employed for the extraction process i.e., water and ethanol have showed different power in their extraction efficiency which could be due to their difference in polarity.

The positive findings from this study provide a scientific basis for the traditional use of *Solanum marginatum* and could pave a way for future investigation in this area. In general,

the outcome of this study is that a scientific basis for the use of *Solanum marginatum* as anti microbial agent has been established. This may also provide directives for future studies in the treatment of infections.

5.3. Recommendation

Based on the findings of the experiments conducted in the present study, the following points are recommended.

- The crude extraction process for the different parts of *S. marginatum* showed that high percentage yield was obtained when ethanol was used as the extracting solvent. Therefore, ethanol could be used as the choice of extracting solvent for the extraction of the plant parts for other studies.

- This study has shown that the fruit and root extracts of white-margined nightshade are more effective against the tested bacteria than the stem and the leaves. Therefore, it is recommended that these plant parts should be used for better inhibition.

- Further studies are needed to purify the antibacterial constituents from *S. marginatum* extracts and evaluate their toxicity.

- Studies should also be conducted to examine the synergistic effects of the combination of the white-margined nightshade extracts with antibiotics on bacteria.

- Finally, it is worth-noting that *in vitro* finding is not always dependable because plants which are effective *in vitro* might not work when used *in vivo* orally or as an ointment. Therefore, it is recommended that further identification of the active constituents is needed to evaluate the efficacy and safety *in vivo* against the test pathogens.

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7. APPENDICES

7.1. Appendix Figures

Figure 1. Plant Collection

Figure 2.Shade drying of the plant parts

Figure 3. Laboratory activities (A) Powders dissolved in solvent (B) Filtration of the crude extracts (C) Removal of extracting solvents

7.2. Appendix Tables

Table 1. ANOVA values for the comparison of the antibacterial effect of the crude extract of the stem using two extracting solvents and three antibiotics at different concentrations on the test pathogens

Test Pathogens	Solvent	DF	SS	MS	F-Value	Pr>F	CV
	Water	1	5.80	5.80	497.29	<0.0001	5.73
	Ethanol	1	7.93	7.93	793.50	<0.0001	1.21
E. coli	Tetracycline	1	14.10	14.10	325.54	<0.0001	1.32
	Penicillin	1	-	-	-	-	-
	Amoxicillin	1	15.04	15.04	257.85	<0.0001	2.64
	Water	1	15.04	15.04	694.21	<0.0001	3.57
	Ethanol	1	15.36	15.36	384.00	<0.0001	1.44
C. jejuni	Tetracycline	1	10.40	10.40	58.88	0.0016	1.48
	Penicillin	1	19.44	19.44	777.60	<0.0001	1.45
	Amoxicillin	1	14.42	14.42	262.09	<0.0001	1.24
	Water	1	12.04	12.04	1806.25	<0.0001	2.8
	Ethanol	1	16.66	16.66	2500.00	<0.0001	0.62
S. aureus	Tetracycline	1	14.10	14.10	115.94	<0.004	1.8
	Penicillin	1	-	-	-	-	-
	Amoxicillin	1	16.00	16.00	369.38	<0.0001	1.37
	Water	1	4.68	4.68	280.90	<0.0001	5.82
	Ethanol	1	19.44	19.44	1458.00	<0.0001	1.28
S. pyogenes	Tetracycline	1	15.36	15.36	400.70	<0.0001	1.03
	Penicillin	1	16.33	16.33	700.70	<0.0001	0.84
	Amoxicillin	1	21.66	21.66	406.12	<0.0001	1.71

DF = degree of freedom, MS = mean square, SS = sum of squares, CV = coefficient of variation, level of significance at ($p<0.05$) the error and corrected total are 4 and 5, respectively

Table 2. ANOVA values for the comparison of the antibacterial effect of the crude extract of the fruit using two extracting solvents and three antibiotics at different concentrations on the test pathogens

Test Pathogens	Solvent	DF	SS	MS	F-Value	Pr>F	CV
	Water	1	33.60	33.60	2520.25	<0.0001	1.77
	Ethanol	1	27.30	27.30	1260.31	<0.0001	1.28
E. coli	Tetracycline	1	14.10	14.10	325.54	<0.0001	1.32
	Penicillin	1	-	-	-	-	-
	Amoxicillin	1	15.04	15.04	257.85	<0.0001	2.64
	Water	1	37.50	37.50	2812.50	<0.0001	1.54
	Ethanol	1	69.36	69.36	2080.80	<0.0001	0.89
C. jejuni	Tetracycline	1	10.40	10.40	58.88	0.0016	1.48
	Penicillin	1	19.44	19.44	777.60	<0.0001	1.45
	Amoxicillin	1	14.42	14.42	262.09	<0.0001	1.24
	Water	1	22.42	22.42	395.76	<0.0001	4.20
	Ethanol	1	23.20	23.20	302.69	<0.0001	1.78
S. aureus	Tetracycline	1	14.10	14.10	115.94	0.004	1.80
	Penicillin	1	-	-	-	-	-
	Amoxicillin	1	16.00	16.00	369.38	<0.0001	1.37
	Water	1	9.37	9.37	703.12	<0.0001	2.17
	Ethanol	1	38.50	38.50	2888.00	<0.0001	0.94
S. pyogenes	Tetracycline	1	15.36	15.36	400.70	<0.0001	1.03
	Penicillin	1	16.33	16.33	700.70	<0.0001	0.84
	Amoxicillin	1	21.66	21.66	406.12	<0.0001	1.71

DF = degree of freedom, MS = mean square, SS = sum of squares, CV = coefficient of variation, level of significance at ($p<0.05$) the error and corrected total are 4 and 5, respectively

Table 3. ANOVA values for the comparison of the antibacterial effect of the crude extract of the root using two extracting solvents and three antibiotics at different concentrations on the test pathogens

Test Pathogens	Solvent	DF	SS	MS	F-Value	Pr>F	CV
	Water	1	19.08	19.08	520.41	<0.0001	3.62
	Ethanol	1	17.68	17.68	530.45	<0.0001	1.85
E. coli	Tetracycline	1	14.10	14.10	325.54	<0.0001	1.32
	Penicillin	1	-	-	-	-	-
	Amoxicillin	1	15.04	15.04	257.85	<0.0001	2.64
	Water	1	28.16	28.16	3380.00	<0.0001	1.45
	Ethanol	1	53.40	53.40	2464.69	<0.0001	0.90
C. jejuni	Tetracycline	1	10.40	10.40	58.88	0.0016	1.48
	Penicillin	1	19.44	19.44	777.60	<0.0001	1.45
	Amoxicillin	1	14.42	14.42	262.09	<0.0001	1.24
	Water	1	12.91	12.91	774.40	<0.0001	2.95
	Ethanol	1	14.72	14.72	631.14	<0.0001	1.09
S. aureus	Tetracycline	1	14.10	14.10	115.94	0.004	1.80
	Penicillin	1	-	-	-	-	-
	Amoxicillin	1	16.00	16.00	369.38	<0.0001	1.37
	Water	1	7.71	7.71	355.69	<0.0001	4.65
	Ethanol	1	37.50	37.50	1125.00	<0.0001	1.73
S. pyogenes	Tetracycline	1	15.36	15.36	400.70	<0.0001	1.03
	Penicillin	1	16.33	16.33	700.70	<0.0001	0.84
	Amoxicillin	1	21.66	21.66	406.12	<0.0001	1.71

DF = degree of freedom, MS = mean square, SS = sum of squares, CV = coefficient of variation, level of significance at ($p<0.05$) the error and corrected total are 4 and 5, respectively

Table 4. ANOVA values for the comparison of the antibacterial effect of the crude extract of the leaf using two extracting solvents and three antibiotics at different concentrations on the test pathogens

Test Pathogens	Solvent	DF	SS	MS	F-Value	Pr>F	CV
	Water	1	16.66	16.66	526.31	<0.0001	6.21
	Ethanol	1	12.32	12.32	568.92	<0.0001	1.62
E. coli	Tetracycline	1	14.10	14.10	325.54	<0.0001	1.32
	Penicillin	1	-	-	-	-	-
	Amoxicillin	1	15.04	15.04	257.85	<0.0001	2.64
	Water	1	32.20	32.20	2415.12	<0.0001	1.97
	Ethanol	1	26.46	26.46	496.12	<0.0001	1.55
C. jejuni	Tetracycline	1	10.40	10.40	58.88	0.0016	1.48
	Penicillin	1	19.44	19.44	777.60	<0.0001	1.45
	Amoxicillin	1	14.42	14.42	262.09	<0.0001	1.24
	Water	1	10.40	10.40	195.03	0.0002	5.99
	Ethanol	1	16.66	16.66	312.50	<0.0001	1.72
S. aureus	Tetracycline	1	14.10	14.10	115.94	0.004	1.80
	Penicillin	1	-	-	-	-	-
	Amoxicillin	1	16.00	16.00	369.38	<0.0001	1.37
	Water	1	5.04	5.04	177.94	0.0002	7.16
	Ethanol	1	37.00	37.00	1111.05	<0.0001	1.81
S. pyogenes	Tetracycline	1	15.36	15.36	400.70	<0.0001	1.03
	Penicillin	1	16.33	16.33	700.70	<0.0001	0.84
	Amoxicillin	1	21.66	21.66	406.12	<0.0001	1.71

DF = degree of freedom, MS = mean square, SS = sum of squares, CV = coefficient of variation, level of significance at ($p<0.05$) the error and corrected total are 4 and 5, respectively