IN VITRO EVALUATION OF ANTIMICROBIAL ACTIVITIES OF *ALOE VERA* (*L.*) LEAF EXTRACT ON SELECTED HUMAN SKIN PATHOGENS

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In vitro Evaluation of Antimicrobial Activities of *Aloe vera* (L.) Leaf Extract on Selected Human Skin Pathogens

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As thesis research advisors, we hereby certify that we have read and evaluated this thesis prepared under our guidance, by Jalane Gelan entitled: ***"IN VITRO* EVALUATION of ANTIMICROBIAL ACTIVITIES of *ALOE VERA* (L.) LEAF EXTRACT on SELECTED SKIN PATHOGENES"**. We recommended that it can be submitted as the fulfillment of the thesis requirement.

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The author was born on June 1, 1993, in Oromia Region, Adama town. She first started her education at Wonji Community Primary School in 2000/2001 and continued her secondary education at Dembela Secondary School, Adama. She then attended her preparatory education at Hawas Preparatory School, Adama. Then after, she joined Adigrat University in 2012/13 and graduated with B.Sc. degree in Biology in 2014/15. Soon after graduation, the author was recruited by MoE (Ministry of Education) in 2015/16 and joined Haramaya University, College of Natural and Computational Sciences, School of Biological Sciences and Biotechnology in the same year to pursue her postgraduate studies in Microbiology.

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ABBREVIATIONS/ACRONYMS

ANOVA	Analysis of variance
BA	Blood Agar
DMSO	Dimethyl sulphoxide
EPHI	Ethiopian public health institution
GAS	Group A *Streptococcus*
GBS	Group B *streptococcus*
MBC	Minimum bactericidal concentration
MFC	Minimum fungicidal concentration
MIC	Minimum inhibitory concentration
MP	Medicinal plants
NB	Nutrient broth
PDA	Potato dextrose agar
PDB	Potato dextrose broth
SI	Skin infection
TM	Traditional Medicine
TMP	Traditional medicinal plants

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ABSTRACT

*Aloe vera is one of a medicinal plant that used traditionally to treat a number of diseases. Hence, this study was conducted to evaluate antimicrobial activities of Aloe vera (*L.*) leaf and gel extract in different solvents like Ethanol, Petroleum ether and Distilled water (aqueous) against selected human skin pathogens namely; Streptococcus pyogenes, Streptococcus agalactiae and Aspergillus flavus. Disc diffusion method was used to achieve antimicrobial assay. Minimum inhibitory, bactericidal and fungicidal concentration of the extracts were determined by using broth dilution method. The current result showed the inhibitory activity of the gel extracts and the leaf did not inhibit the microbes. The best antimicrobial effect was recorded for petroleum ether gel extracts against Aspergillus flavus (19.7mm), Streptococcus pyogenes (16.2mm) and Streptococcus agalactiae (14 mm). Ethanol extracts showed moderate inhibition against Aspergillus flavus (15mm), Streptococcus pyogenes (13mm) and Streptococcus agalactiae (10.5mm). Aqueous extract showed lowest inhibitory activities with zone diameter of 13, 11 and 7.16mm against Aspergillus flavus, Streptococcus pyogenes and Streptococcus agalactiae respectively. The Minimum inhibitory concentration of both ethanol and petroleum ether extracts were 25mg/ml and 50mg/ml for aqueous extract against Aspergillus flavus and Streptococcus pyogenes. Streptococcus agalactiae was inhibited at 25, 50 and 75mg/ml of petroleum ether, ethanol and aqueous extracts respectively. The Minimum bactericidal concentration value of petroleum ether, ethanol and aqueous extracts for Streptococcus pyogenes were 25, 50, and 75mg/ml respectively. Streptococcus agalactiae was killed at 50, 75 and 100mg/ml of petroleum ether, ethanol, and aqueous extracts respectively. Aspergillus flavus showed Minimum fungicidal concentration at a concentration of 25mg/ml for both ethanol and petroleum ether and 75mg/ml for aqueous extracts. Over-all the results of this study showed the antimicrobial effectiveness of Aloe vera gel extract in different solvents against tested pathogens and the gel part is recommended in order to treat skin diseases caused by Aspergillus flavus, Streptococcus pyogenes and Streptococcus agalactiae rather than the fiber part of Aloe vera.*

Keywords: Broth Dilution, Disc Diffusion, Inhibition, Microbes

1. INTRODUCTION

Aloe vera L.is flowering succulent monocotyledonous plant, which belongs to family Aloeaceae and genus Aloe. It occurs naturally on mainland Africa, in tropical and subtropical latitudes and mainly distributed in Northern Africa. It is characterized by thick fleshy, strangely circularized spiny or thorny leaves (Bozzi *et al*., 2007). The leaf of *A. vera* can be divided into the outer green mesophyll, including the vascular bundles and the inner mucilaginous jelly from the parenchyma cells which is colorless and referred as Aloe gel. When the leaves are cut, exudates are arising from cells adjacent to the vascular bundles. It is used as a bittering agent or as a somewhat violent purgative (Reynolds, 2004).

The central parenchymatous tissues of *A. vera* leaf consists of various components such as polysaccharides, which give glutinous nature, glycoproteins which is biologically active, a nitrogenous compound such as proteins, and different amino acids (Discala *et al.*, 2013). Study of Kedarnath *et al.* (2012) indicated that leaf extract of *A. vera* consists of tannin, saponin, flavonoids and terpenoid compounds that increase antimicrobial effectiveness of the gel. Tannin is a yellowish or brownish bitter-tasting organic substance. Saponin is a toxic compound which foams when shaken with water. Flavonoids are colorless crystalline organic compounds, and terpenoids are any of a large group of volatile unsaturated hydrocarbons with cyclic molecules. Anthraquinones and anthrones are not major components of leaf exudates as they are found in greater variety in the roots and subterranean stems but they have been observed in some leaves of Aloe species (Reynolds, 2004).

Due to its chemical composition, *A. vera* is one of the plants that is being used in traditional medicine. It is one of those medicinal plants that are used to treat different kinds of diseases and infections. Traditionally, it is used for stomach and intestinal disorders including hemorrhoids, and colitis and colon problems. It has great power of penetrating tissues, relieving pain associated with joints and muscles. *Aloe vera* has bactericidal, fungicidal, antiviral and anti-inflammatory activity. Also, it is used as an antiseptic and it has the ability to stimulate macrophages. It breaks and digests dead tissue of the infected skin and moisturizing tissues easily. Additionally, numerous chemical constituents of *A. vera* have demonstrated enhancement of immune system functioning within the body (Davis, 1997).

The leaves of *A. vera* have been used widely in commercial therapeutic and cosmetic preparations, but not accepted medically to prepare drugs because of the difficulty in establishing the dosage (Reynolds, 2004). Furthermore, it has been used externally to treat various skin conditions such as cuts, burns, and eczema. Due to its high moisturizing ability, the skin absorbs extracts of *A. vera* up to four times faster than water, it appears to help pores of the skin open and receive moisture and nutrients of the plants. It also treats infections caused by microbes because of its antiseptic and antibiotic properties of its leaf (Rajeswari *et al.*, 2012).

Different findings have shown the antimicrobial activity of *A. vera* leaf and gel extracts against human, animal and plant pathogens. Study done by Cock (2013) showed the effective antimicrobial activity of the gel against gram-positive and negative bacterial strains such as *Aeromonas hydrophilia, Alcaligenes feacalis, Bacillus cereus, Bacillus subtilis, Citrobacter freundii, Enterobacter aerogenes, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeuroginosa, Pseudomonas fluorescens, Salmonella salford, Serratia marcescens, Staphylococcus aureus* and *Yersinia enterocolitia*, and fungal strain like *Aspergillus niger, Candida albicans and Saccharomyces cerevisiae*. The methanol extract of aloe gel inhibits the growth of *Staphylococcus aureus* and *S. epidermidis* with inhibition zone of 11 and 10 mm, respectively followed by 9mm zone of inhibition against *Escherichia coli. Proteus vulgaris, Pseudomonas aeruginosa, K. pneumonia* and *Proteus mirabilis* were less inhibited with a zone of inhibition (8mm in diameter). Also, ethanol extract of *A. vera* gel established antibacterial activity with the diameter of the zone of inhibition 9 mm against *S. aureus*, and *S. epidermidis*, 8mm against *Proteus vulgaris* and 7mm against *K. pneumoniae, Pseudomonas aeruginosa* and *Proteus mirabilis* and 4mm diameter against *E. coli*. (Udgire and Pathade, 2014). Inhibitory activities of this plat also confirmed against *Bacillus cereus*, *Bacillus subtilis* and *Salmonella typhi* (Lawrence, 2009) *Candida albicans* with a maximum zone of inhibition of 11 mm and *Penicillium* species whit 9 mm zone of inhibition (Malar *et al.*, 2012). *Aspergillus niger, A. fumigates* and *Neurospora crassa* are inhibited with a zone of inhibition measured to about 12mm, 18mm and 22mm, respectively (Kedarnath *et al.*, 2012). The effective antimicrobial activities of fiber or outer layer of the leaves were also reported by Agarry *et al*. (2005). Another study (Reider *et al.*, 2005) reported that extraction from *A. vera* gel was topically effective for

the healing of burns, sunburns, inflammatory skin disorders and wounds that caused by common skin pathogens. Similarly, another study by Asma *et al.* (2011) indicated growth inhibitory activity of *A*loe *vera* leaf and gel extracts against skin infectious pathogens namely *Staphylococcus aureus, Streptococcus pyogenes, S. epidermidis* and *P. aeruginosa* in the percentage of 47.8, 30.4, 8.6, and 13.0%, respectively.

The different solvent extracts of *A. vera* showed a different zone of inhibition against different microbial species. Overall, the petroleum ether extract has shown a high zone of inhibition in *E. coli, K. pnemoniae, A. niger* and moderate zone for *S. aureus*, and *C. albicans*. Chloroform extract has shown a high zone of inhibition in *K. pneumonia, E. coli* and *A. niger*, but the moderate zone of inhibition in *S. aureus*, and *C. albicans*. Methanol extracts have shown a high zone of inhibition in *S. aureus*, *K. pnemoniae* and *C. albicans*, but moderate zone in *E. coli* and *A. niger* (Kaveri *et al.*, 2013). Stanley *et al.* (2014) showed that ethanol extract of *A. vera* gives a better minimum inhibitory concentration than aqueous and methanol extracts on *E. coli, S. aureus* and *C. albicans*.

Traditional medicine (TM) has maintained its popularity in all regions of the developing world including Ethiopia and its use is rapidly spreading in the industrialized countries as well. Low cost, readily availability and easy application of TM gives economic benefits for developing countries (Bannerman, 1993). Over one-third of the population in developing countries lack access to essential medicines or modern medicine. Therefore, provision of safe and effective TM therapies could, thus, become a critical tool to increase access to health care (WHO, 2003).

In Ethiopia, up to 80% of the population uses traditional medicine due to the cultural acceptability of healers and local pharmacopoeia. Healing in Ethiopian traditional medicine is not only concerned with curing of diseases, but also with the protection and promotion of human physical, spiritual, social, mental and material well-being. Traditional medicinal practices can be conducted by professional healers or self-care at home. People exercise traditional medication in different ways such as preventative, curative, and surgical practices (Kebede *et al.*, 2006). There are many conditions such as lifestyle, traditional aspect, low access to obtain modern health care that are the force for Ethiopian population to be more dependent on TM. The majority of

Ethiopia's population lives in rural areas where the health care coverage is low and where existing public sector resources are being stretched to the limits. The traditional aspect of the population is strong on TM or they have strong believes in the curative capacity of those medicinal plants. (CSA, 2001).

The occurrence of antimicrobial drug-resistant bacteria is increasing in developing countries when compared with that of developed countries. The evidence is accumulating to suggest that the emergence and spread of resistance are strongly influenced by socioeconomic factors at the individual and national levels. Also, different patterns of antibiotic drug use, climatic condition and geographic location can influence the occurrence of these microbes. People living in poverty are least able to buffer themselves from the consequences of resistance, even though they may contribute less to the problem in terms of selective pressure. The burden of resistant infection is disproportionately borne by the less privileged. Poor people are typically at a greater risk of becoming infected, but they are also less likely to be able to access the healthcare, including antibiotic drugs (Sosa *et al.*, 2010). In general, the relatively low cost of traditional medicine (Bannerman, 1993), the difficulty of access to modern health facilities and occurrence of antimicrobial drug resistance in developing countries urge the societies to rely on TM to treat any diseases (WHO, 2003).

Skin diseases are pre-dominantly occurring in developing tropical countries including Ethiopia (Roth and James, 1989). Emerging from multiple drug-resistant microorganisms is the cause that pushes the scientists to find another option to treat a number of skin infection and diseases that cause by pathogenic microbes. So that development of new antimicrobial drugs is required to control and treat these diseases. In this regard, plant products provide a good alternative in search for new chemical agents with a wide-ranging antimicrobial activity (Alavijeh and Sharma, 2012). Different plant species including *A. vera* are preferable to apply on skin and do not need any complex medicinal administration and readily available (Asma *et al*., 2011). In addition, most skin diseases are superficial and easy to treat by applying different plant extracts on the skin (Hailu *et al*., 2005). Therefore, this study is designed with the aim of evaluating the *in-vitro* antimicrobial activity of *A. vera* leaf extracts against selected skin pathogens namely *Streptococcus pyogenes, Streptococcus agalactiae* and *Aspergillus flavus*.

General Objective

- The main objectives of this study was to evaluate *in vitro* antimicrobial activity of *Aloe vera* extract against *Streptococcus pyogenes, Streptococcus agalactiae and Aspergillus flavus*

Specific objective

1. To evaluate antibacterial and antifungal activity of ethanol, petroleum and aqueous extracts of *A. vera* leaf and gel extract separately against the pathogens;
2. To compare the efficacy of different solvent extracts of *A. vera* on test pathogens;
3. To determine the minimum inhibitory concentration (MIC) of the extracts; and
4. To determine the minimum bactericidal and fungicidal concentration (MBC and MFC) of the plant extracts

2. LITERATURE REVIEW

2.1. Historical Background of Medicinal Plants

Medicinal plants (MP) have been used as sources of medicine starting from the time when the early man became aware of his environment and in almost all cultures. Traditional medicines have been expanded globally and used widely in both developing and developed countries. Different records indicate that people have used herbal medicinal plants to treat different diseases. Traditional medicinal plants serve the health needs of about 80% of the world's population, especially for millions of people in the vast rural areas of developing countries. In developed countries, the practice of TM is widespread throughout China, India, Japan, Pakistan, Srilanka, and Thailand. China is the leading country for incorporating TM into a modern health care system (40% of all healthcare) and used to treat roughly 200 million patients annually (SATCM, 2011). The population of Singapor, Republic of Korea, Australia, Lao, Spain and Canada, use TM about 76%, 86%, 82%, 80%, 41% and 70% respectively. The majority of African population relies on TM for the treatment of both human and animal diseases. For instance in Ghana, Mali, Nigeria, and Zambia, more than 60% of children with high fever are treated at home with herbal medicine (DW, 2014; WHO, 2013).

Ethiopia has a long history of use of MP and huge biotic riches that can be of paramount importance in future research and drug discovery. But it received very little attention in modern research, development, and less effort has been paid to upgrade the traditional health practices in the country. However, the people came with traditional remedies through the struggle to fulfill their essential drug needs, cultural beliefs and attitudes. Many species of higher plants, shrubs and different spice are commonly used as TM in Ethiopia. Fresh and dried leaves, flowers, roots, bark, seeds, etc. of those plants are used to treat different diseases including skin infection due to skin pathogens (Farr, 2002). Overall more than 95% of traditional preparations in the country are plant origin. In addition to this, many conventional drugs which used in modern medicine are originated from plant sources (Gidey, 2001).

2.2. Some Common Medicinal Plants

A number of plants species are identified as a medicinal plant to treat microbial diseases. *Acokanthera schimperi (Apocynaceae), Calpurnia aurea (Leguminosae), Kalanchoe petitiana (Crassulaceae), Lippia adoensis (Verbenaceae), Malva parviflora (Malvaceae), Olivia rochetiana (Oliniaceae), Phytolacca dodecandra (Phytolaccaceae*) and *Verbascum sinaiticum(Scrophulariaceae)* were investigated as antibacterial and antifungal agents (Hailu *et al.*, 2005). Leaf extracts of *Azadiracta indica, Acacia nilotica* and *Witania somnifera* show significant antibacterial activity against *Bacillus subtilis, Escherchia coli, Staphylococcus aureus* and *Pseudomonas fluorescence* (Alavijeh and Sharma, 2012). Other studies show the use of *Cephaelisi pecacuanha* to treat *Entamoeba histolytic* infections. Lichens which occur naturally on the bark of trees are used as bacteriostatic and fungicidal agents. *Allium sativum, Hydrastis canadensis*as, *Garicinia kola, Aframomum melegueta, Xylopia aethiopica, Cryptolepis sanguinolent* and *Chasmanthera dependens* are among the most widely used species in Africa as TM (Simenew *et al.*, 2014).

Aloe species are predominantly using as antimicrobial agents worldwide to treat common human pathogens that are listed in Table 1. For instance, *Aloe barbadensis or A. vera, Aloe rupestris, Aloe juvenna and Aloe maculata var. pulchra* (Gasteriapulchra) leaf extracts were tested against human pathogens (Sonam and Tiwari, 2015). Especially *Aloe barbadensis* (*A. vera)* is best to treat various kinds of skin disorders. Aloe species used for treating wounds, boils, sores, rashes, and burns. Mostly leaf of this species use as antimicrobial agent against gram-positive bacteria including S. *aureus and S. epidermidis*, *Bacillus species*, and *Salmonella typhi*, gram-negative bacteria including *E. coli, K. pneumoniae, P. aeruginosa*, *P. vulgaris* and *P. mirabilis* (Udgire, 2014) and fungal species including *A. fumigatus*, *C. albicans and Penicillium* species (Malar *et al*., 2012; Stanley *et al.*, 2014).

Table1. Microbes and skin diseases treated by different Aloe extracts (Sanama and Tiwari, 2015; Udgire, 2014)

Species name	plant Part	Inhibited microbes	Skin diseases can be treated
A. barbadensis	Leaves	*B. cereus, B. subtilis, S. aureus, S. epidermidis, E.coli, K. pneumoniae, P. aeruginosa, P. vulgaris, P. mirabilis, A. fumigatus, C. albicans, Corynebacterium, Salmonella typhi,* etc.	Blistering distal dactylitis, Cellulitis, Diphtheria, Folliculitis, Ecthyma, Impetigo, Perianal dermatitis, Intertrigo, etc.
A. rupestris	Leaves	*B. cereus*	Folliculitis, Ecthyma
A. juvenna	Leaves	*B. cereus*	Folliculitis, Ecthyma
*A. maculata*var. *Pulchra*	All part	*B. cereus*	Folliculitis, Ecthyma

2.3. Aloe vera

*A*loe *vera* is an herbal medicinal plant with biological activities, such as antimicrobial, anticancer, anti-inflammatory, anti-diabetic, and immune-modulatory properties (Goudarzi*et al*., 2015). It has been used as an ornamental and medical remedy since ancient times and has gained increasing popularity in recent years. *A. vera* has a great role in therapeutic use (Sahu *et al*., 2013). It is marketed as a remedy for atherosclerosis, allergies, AIDS, prevention of radiation-induced dermatitis, wound healing, psoriasis, insomnia, cancer, pulpitis and several other diseases. Scientific studies investigating these claims are few in number, and the majority of them have been unable to diminish the intuitive skepticism against miracle cures. Despite its widespread use, reports of allergic reactions are rare. The plant is used in cosmetics, drinks, detergents, as well as in stockings, diet foods, toothpaste and clothing (Reider *et al*., 2005).

2.3.1. Physical Structure and Chemical Composition

Aloe vera is a flowering succulent plant, characterized by thick fleshy, strangely circularized spiny or tuberculate leaves and have large, colorful, tubular flowers. The leaf is divided into the outer most layer and inner parenchyma cell. The outer most layer is green mesophyll, including the vascular bundles. It consists 15 - 20 cells thick protective layer synthesizing from carbohydrates and proteins. This part contains derivatives of hydroxyanthracene, anthraquinone and glycosides aloin A and B from 15% - 40% (Saccu *et al.*, 2001). The outer layer (fibers) of *A. vera* leaves are rich in carbohydrates (about 70%) which are a major source of energy and have great contribution in determining bulk, viscosity, stability to emulsions and foams, water-holding capacity, the freeze-thaw stability of the food item (Muaz and Fatma, 2013). The inner part is colorless fleshy parenchyma cell (inner pulp) which containing the Aloe gel. This cell consists three components which are distinctive from each other namely cell wall that is rich in galacturonic acid, degenerated cellular organelles that rich in galactose and mucilage or gel that rich in mannose (Sahu *et al.*, 2013). *The main chemical composition of A*loe *vera* leaf is mention in Table 2. The leaf has high water content approximately 97.42% moisture contents. Ash contents of the leaf are determined as 16.88 %. Crude proteins, ascorbic acid and lipids content of this plant tissue are reported as 7.56-15.4%, 0.004 and 2.91 respectively (Muaz and Fatma, 2013). *A. vera* gel contains a large range of vitamins such as B1, B2, B6, C, β-carotene, choline, α-tocopherol, (Dagne *et al.,* 2000) vitamin B12, Vitamin A, B-Group vitamins, Vitamin E and folic acid. It contains important ingredients including 19 of the 20 amino acids needed by the human body and seven of the eight essential ones that just cannot be made within the human body (Rajeswari *et al.*, 2012).

In other studies, different compounds with their derivatives are identified as the chemical composition of *A. vera*. They are Anthraquinones/ anthrones such as Aloe-emodin, aloetic-acid, anthranol, barbaloin, isobarbaloin, emodin, and an ester of cinnamic acid. Carbohydrates including pure mannan, acetylated mannan, acetylated glucomannan, glucogalactomannan, galactan, galactogalacturan, arabinogalactan, galactoglucoarabinomannan, pectin substance, xylan, and cellulose. Chromones are 8-C-glucosyl-(2'-O-cinnamoyl)-7-O-methylaloediol A, 8-C-glucosyl-(S)- aloesol, 8-C-glucosyl-7-O-methyl-(S)-aloesol, 8-C-glucosyl-7-O-methylaloediol,

8-C-glucosyl-noreugenin, isoaloeresin D, isorabaichromone, and neoaloesin A. The leave of this plant is consisted inorganic compounds or minerals. For example, Calcium, chlorine, chromium, copper, iron, magnesium, manganese, potassium, phosphorous, sodium and zinc are presented in it. Miscellaneous including organic compounds and lipids such as arachidonic acid, γ-linolenic acid, steroids (campestrol, cholesterol, β- sitosterol), triglicerides, triterpenoid, gibberillin, lignins, sorbate, salicylic acid, and uric acid. Alanine, arginine, aspartic acid, glutamic acid, glycine, histidine, hydroxyproline, isoleucine, leucine, lysine, methionine, phenylalanine, proline, threonine, tyrosine, and valine are amino acids in *A. vera* leave. Proteins including Lectins, and lectin-like substance and saccharides like Mannose, glucose, L-rhamnose, and aldopentose are also components of the leaf. There is a number of enzymes in this plant extract such as carboxypeptidase, cyclooxidase, alkaline phosphatase, cyclooxygenase, lipase, oxidase, phosphoenolpyruvate carboxylase, (Dagne *et al*., 2000) catalase, Superoxide dismutase, Peroxidase, amylase, (Muaz and Fatma, 2013).

Table 2.Chemical composition and properties of Aloe vera (Rodríguez *et al.*, 2005)

Constituents	Number and identification	Properties and activity
Amino acids	Provides 20 required amino acids and 7 of the 8 essential ones	Basic building blocks of proteins in the body
Anthraquinones	Aloe-emodin, Aloetic acid, alovin, anthracine	Analgesic, antibacterial
Enzymes	Anthranol, barbaloin, chrysophanic acid, smodin, ethereal oil, ester of cinnamonic acid, isobarbaloin, resistannol	Antifungal and antiviral activity but toxic at high concentrations
Hormones	Auxins and gibberellins	Wound anti-inflammatory
Minerals	Ca, Cu, Fe, Mn, K, Na, Zn	Essential for good health
Salicylic acid	Aspirin like compounds	Analgesic
Saponin	Glycosides	Cleansing and antiseptic
Steroids	Cholesterol, campesterol, lupeol, sistosterol	Anti-inflammatory, Antiseptic and analgesic
Sugars	Glucose, FructoseGlucomannans/polymannose	Anti-viral, immune modulating activity
Vitamins	A, B, C, E, choline, B12, folic acid	Antioxidant (A, C, E), neutralizes free radicals

2.3.2. Therapeutic and Medicinal Uses

Aloe vera has been used for therapeutic and medicinal purposes in different countries. The leaf has contained a mixture of glucosides collectively called aloin which is the active constituent of various drugs (Joseph and Raj, 2010). *Aloe vera* gel is used for the preparation of confection, lotion and useful remedies for curing various diseases. The plant is extensively used topically in treating of wounds, minor burns, skin irritations, pimples, ulcers, gerontology, moisturizing and upgrading of aging skin or skin protection. Internally it can use for treatment of constipation, coughs, intestinal ulcers, diabetes, headaches, arthritis, tuberculosis, stomachic tonic, purgative

(laxative) drug, urine related problems, anti-inflammatory action, antitumor activity, and built immune-system, (Choi *et al.*,2001; Yagi *et al.,* 2003; Reddy *et al*., 2011). Wound healing is a dynamic process, occurring in 3 phases. The first phase is inflammation, hyperaemia and leukocyte infiltration. The second phase consists of removal of dead tissue. The third phase of proliferation consisting of epithelial regeneration and formation of fibrous tissue (Reddy *et al*., 2011). *A*loe *vera* use for healing the first to second-degree burns. Specifically used in collagen synthesis, increased collagen cross-linking for wound contraction and improving breaking strength. It also increases synthesis of hyaluronic acid and dermatan sulfate in the granulation tissue of a healing wound. The wound healing property of *A. vera* gel has been attributed to Mannose-6-phosphate glucomannan, complex carbohydrate and plant growth hormone gibberellins. Glucomannan and plant growth hormone gibberellins interact with growth factor receptors of fibroblast (a cell in connective tissue which produces collagen and other fibers)and stimulate its activity and proliferation for increases collagen synthesis in the topical and oral administration of the gel (Maenthaisong, 2007). The Aloe gel has been used for the treatment of radiation burns and radiation ulcers (Bosley *et al*., 2003). Acemannan is complex carbohydrate of *A. vera*, which is composed of a long chain of acetylated mannose. It accelerates wound healing and reduces radiation-induced skin reactions. This compound may have Macrophage-activating potential and stimulate the release of fibrogenic cytokines. Direct binding of acemannan to growth factors and their stabilization may lead to the promotion of prolonged stimulation of granulation tissue. It stimulates the synthesis and release of interleukin-1 (IL-1) and tumor necrosis factor from macrophages, which in turn initiated an immune attack that resulted in necrosis and regression of the cancerous cells (Reddy *et al*., 2011).

Muco-polysaccharides help in binding moisture into the skin. The amino acids also soften hardened skin cells and zinc acts as an astringent to tighten pores. Its moisturizing effects have also been studied in the treatment of dry skin associated with occupational exposure where *A. vera* gel gloves improved the skin integrity, decrease the appearance of spots wrinkle and decrease erythema (West and Zhu, 2003). The Aloe gel gives cooling effect and has a role in gerontology and rejuvenation of aging skin. This property of Aloe is because it’s biogenic material. (Sahu *et al*., 2013).

The gel is used for soothing the skin and keeping the skin moist to help avoid flaky scalp and skin in harsh and dry weather. Due to its soothing property Aloe gel is recommended to treat minor skin infections, such as boils and benign skin cysts and have been shown to inhibit the growth of fungi that cause tinea (Sumbul *et al.,* 2004).

Anthraquinones present in the latex of the plant is a potent laxative; it's stimulating mucus secretion, increase intestinal water content and intestinal peristalsis. Aloe is primarily contained 1, 8-dihydroxyanthracene glycosides, aloin A and B (which called barbaloin). After oral administration of aloe gel aloin A and B, which are not absorbed in the upper intestine, are hydrolysed in the colon by intestinal bacteria and then reduced to the active metabolites (the main active metabolite is aloe-emodin- 9-anthrone) which like senna (laxative prepared from the dried pods of Cassia tree) acts as a stimulant and irritant to the gastrointestinal tract. Aloe latex is known for its laxative properties. The laxative effect of Aloe is not generally observed before 6 hours and sometimes not until 24 or more hours after oral administration, (Langmead *et al.,* 2004).

*A*loe *vera* gel inhibits the cyclo-oxygenase pathway and reduces prostaglandin E2 production from arachidonic acid to process anti-inflammatory activity. Campesterol, β-sitosterol, and lupeol are the most active anti-inflammatory sterols of this plant that helps in reducing the inflammation by up to 37% and act as a natural analgesic. Another bradykinase that break down the bradykinin (an inflammatory substance that induces pain) and aspirin-like compound are present in Aloe is responsible for anti-inflammatory and antimicrobial properties. Alprogen inhibits calcium influx into mast cells, thereby inhibiting the antigen-antibody-mediated release of histamine and leukotriene from mast cells. Several low-molecular-weight compounds are also capable of inhibiting the release of reactive oxygen free radicals from activated human neutrophils. This helps the plant to stimulate human immune system (www.healingaloe.com, 2008).

Aloe vera has antiseptic property due to the presence of six antiseptic agents namely lupeol, salicylic acid, urea nitrogen, cinnamonic acid, phenols and sulphur. These compounds have an inhibitory action on fungi, bacteria and viruses. Though most of these uses are interesting

controlled trials are essential to determine its effectiveness in all diseases (Langmead *et al.*, 2004). The leaf is containing polysaccharides which increase the insulin level and show hypoglycemic properties. Additionally, five phytosterols of *A. vera,* lophenol, 24-methyllophenol, 24-ethyl-lophenol, cycloartanol and 24-methylenecycloartanol are recognized as an anti-diabetic agent in type-2 diabetic (Tanaka, 2006; Noor *et al.,* 2008).The plant juice enables the body to heal itself from cancer and also from the damage caused by radio and chemotherapy that destroys healthy immune cells crucial for the recovery. *A. vera*emodin, an anthraquinone, has the ability to suppress or inhibit the growth of malignant cancer cells making it have antineoplastic properties (Fenig *et al.,* 2004).

2.3.3. Antimicrobial Activities

Aloe vera leaf has bactericidal, fungicidal and viricidal property. It has antibacterial effect against both gram-positive and gram-negative pathogenic bacteria (Cock, 2013; Agarry *et al*., 2005). The saponins have an antimicrobial effect on both pathogenic yeast and mold forms of fungi (Malar *et al*., 2012). The gel also shows effective antiviral agents such as Acemannan which reduced herpes simplex infection in two cultured target cell lines, Lectins that directly inhibited the cytomegalovirus proliferation in cell culture, perhaps by interfering with protein synthesis. Aloe-emodin is effective against infectivity of herpes simplex virus Type I and Type II and it is capable of inactivating all of the viruses, including varicella zoster, influenza, and pseudorabies. Anthraquinones extract from the gel is directly virucidal to enveloped viruses such as herpes simplex, varicella zoster and influenza. These actions may be due to indirect effect due to stimulation of the immune system (Mariita *et al*., 2011).

2.4. Skin and Association with Micro-Flora

Skin is one of sense organ that has a primary protective function. It provides a permeability barrier, which prevents both the loss of water and electrolytes and the invasion of pathogens (Chiller *et al.*, 2001). The pathogenic organisms have limited time to invade further into the epidermis due to the stratum corneum (outermost layer of the epidermis) which consists of corneocytes (a nucleated keratinocytes or skin cells), and an outer lipid matrix. Corneocytes fit together in an overlapping fashion, making penetration by organisms difficult. It is acidic and

participates in innate immunity via secretion of antimicrobial peptides (eg. human β-defensins, canthelicidin, psoriasin, and dermicidin) that has antimicrobial activity against pathogenic microbes (Schroder, 2010). There are also resident micro floras on the skin that provide additional protection against infection by preventing colonization with pathogenic organisms via competitive bidding to cell surface receptors of skin, and by the production of toxic substances called bacteriocins that inhibit the growth of similar bacteria. Pathogens or transient flora invade the skin from the environment when the skin is disrupted to cause bacterial and fungal diseases (Braff *et al.*, 2005)

2.5. Common Skin Infection and Causative Organisms

Most bacterial skin infections are caused by gram-positive organisms, including species of *Staphylococcus, Streptococcus, Enterobacter, Bacillus* and D*ermatophilus*. Less common causes of infection include gram-negative organisms: *Vibrio*, *Mycobacterium*, *Pseudomonas*, *Aeromonas*, *Proteus*, *Clostridium*, *Corynebacterium, Escherichia coli*., *Klebsiella* and other anaerobes are common bacterial group to cause bacterial skin diseases which are listed in Table 2 (Chiller *et al*., 2001; Steven *et al*., 2006).

Fungal infections of the skin can be superficial (dermatophytoses), intermediate or deep which involve the epidermis, dermis and subcutaneous. Species including *Microsporum, Epidermophyton*, and *Trychophyton*, which are invading in most forms of tinea (superficial skin infection), *Malassezia species*, *Aspergills species*and *C. albicans* (cause both superficial and deeper tissues infection) are determined as a causative agent for fungal skin infection or diseases. Some fungal species are opportunistic that affect a susceptible host while some are truly pathogenic that can infect a healthy person (Malar *et al.*, 2012). Some of skin infections with their causative microorganisms are mentioned in table 3.

a. *Streptococcus pyogenes*

Streptococcus pyogenes are also called group A *streptococcus* (GAS). They are gram-positive extracellular, a non-sporing coccoid-shaped bacterium that grows in chains with large colonies greater than 0.5 mm in size. GASs are small in size which is less than 2 µm in length. They are

facultative anaerobe, non-motile, negative for catalase, salt tolerance and oxidase and susceptible to bacteriocines. Erythrogenic, toxin Streptolysin O, Streptolysin S, Hyaluronidase, DNase, NADase, Streptokinase and M Protein endotoxin are used as virulence factors for *S. pyogenes* (Shet and Kaplan, 2002).

Streptococcus pyogenes are identified as a causative agent for skin diseases such as Impetigo, cellulitis and erysipelas (Cogen *et al., 2008*). Impetigo is a contagious infection which forming pustules and yellow crusty sores. It usually occurs on the face, neck, and hands of young children and infants. Impetigo is more rarely in adults. There is a different type of impetigo named as nonbullous impetigo (Impetigo contagiosa), Bullous impetigo and Ecthyma. Nonbullous usually begin with red sores around the nose and mouth. These blisters burst, leaving a weeping, red rash that becomes crusted. This rash may be itchy but is not painful. Bullous impetigo is most common in children under age two. Blisters usually appear first on the torso, arms, and legs. These blisters may initially appear clear and then turn cloudy and can last longer than blisters caused by another type of impetigo. The areas around the blisters may be red and itchy. Ecthyma is the most serious form of impetigo because it affects the second layer of the skin, rather than just the top layer. Blisters tend to be painful and may turn into ulcers, or aggravated, open sores. The symptoms are red sores that pop easily and leave yellow crust fluid-filled blisters itchy rash skin lesions swollen lymph nodes (Silverberg and Block, 2008).

Cellulitis is inflammation of subcutaneous connective tissue. It is acute skin infection which involving the dermis and subcutaneous tissues. The lower legs are affected most commonly, but infection can occur at any site and it is manifested by edema, erythema, redness, warmth, swelling, tenderness, and pain. Erysipelas is bacterial skin infection involving the upper dermis that characteristically extends into the superficial cutaneous lymphatic. It is a tender, intensely erythematous, an indurated plaque with a sharply demarcated border and characterized by large raised red patches on the face and legs (Stevens *et al., 2005*). Infection of GAS may spread through direct contact with mucus or sores on the skin (WHO, 2003)

Many plants are used as TM to treat infection caused by *S. pyogenes*. Leaves, stems and flowers of *Acacia aroma, Azadirachta indica,* the 3, 4-dihydroxycinnamic acid isolated from leaves of

*Cassia alata (*Paul *et.al*., 2013), ethanol extracts of ginger (*Zingiber officinale*) (Sebiomo *et al.*, 2010), leaf and root of *Lantana indica*, methanol crude extracts of *Scaevola spinescens*, have antibacterial activity against *S. pyogenes* (Mejin, 2009). Leaf and gel of *Aloe vera* also have antimicrobial activity against *S. pyogenes* (Asma *et al.*, 2011).

In Ethiopia, numerous plant species are used to treat different diseases caused by *S. pyogenes*. The study was conducted by Aberra *et al*. (2005) to determine the presence of anti-microbial activity in the crude extracts of 67 commonly used Ethiopian medicinal plants against some common human pathogens including *S. pyogenes* and revealed that *Laggera tomentosa*, *Syzygeum guineense*, *Ageratum conizoides*, *Cordia Africana*, *Ferula communis*, *Olea europea* subsp. *Cuspidate*, *Discopodium peninervum* and *Oleaceae* have been highly inhibiting the growth of *S. pyogenes.* Four Ethiopian medicinal plants, namely *Clerodendrum myricoides* (Lamiaceae), *Ficus plamata* (Moraceae), *Grewia ferruginea* (Tiliaceae) and *Periploca linearifolia* (Asclepediaceae) are screened for antibacterial and antifungal activities. All plants highly affect the growth of *S. pyogenes* with inhibition zone of 16-39mmin diameter (Asmamaw *et al*., 2014). Research is done in Gondar, Ethiopia explains antimicrobial activity effect of *Hibiscus micranthus* leaves against gram-positive bacterial strain like *Staphylococcus aureus*, *Streptococcus pneumoniae*, and *S. pyogenes* while gram-negative bacterial strains like *E. coli*, *P. aeruginosa*, *K. pneumoniae* and *P. mirabilis* (Begashaw *et al*., 2017). Leaves of *Jasminum abyssinicum* (Oleaceae), and *Solanecio gigas* (Asteraceae); the leaves, seeds, and fruit-flesh of *Lagenaria siceraria* (Cucurbitaceae), plants that are widely used in Ethiopian as a traditional medicine for treating skin disorders, have been assessed in vitro on selected species of bacteria and fungi. All plant extracts have an effect on the growth of *S. aureus*, *P. aeruginosa* and *S. pyogenes* with the exception of the fruit-flesh of *L. siceraria* which showed no activity against clinical isolate *S. aureus* (Mesfin *et al*., 2006).

b. *Streptococcus agalactiae*

Streptococcus agalactiae is a gram-positive coccus and known as Group B Streptococcus. It forms glistening gray-white colonies with a narrow zone of beta hemolysis when cultured on sheep blood agar. This bacterium is mainly colonized skin, genital tract, urinary tract, and

respiratory tract. *Streptococcus agalactiae* is an invasive encapsulated organism capable of producing severe disease (Doare and Heath, 2013). Since the 1970s, group B Streptococcus (GBS; *Streptococcus agalactiae*) has been recognized as a pathogen, mainly in neonates and peripartum women (Farley et al., 1993). It is the most clinical importance pathogen to multiple hosts, as it can cause infections especially in humans, fish and cattle. Group B streptococcus is typically related to skin disease and soft tissue infection such as erysipelas/cellulitis, wound infections, arthritis, genital and oral ulcers. They are expressing a capsular polysaccharide (CPS), a major virulence factor that helps the microorganism evade host defense mechanisms (Doare and Heath, 2013; Frey *et al., 2011*).

Traditionally herbal medicinal plants are mostly used to treat infections caused by *S. agalactiae*. Cardoso *et al., (2016*) show inhibitory activities of avocado extracts (*Persea Americana*) against the pathogens *Streptococcus agalactiae* is also inhibited by methanol extracts of *Croton floribundus, Cupania vernalis* and other Brazilian plants (Castro *et al*., 2008). Extracts from leaf, flower and root part of *Catharanthus roseus* show high inhibition zone for this bacteria (Raza *et al*., 2009). The crude extract of leaves, stems and flowers of *Acacia aroma* had also antimicrobial activities against this pathogen (Arias *et al*., 2004). According to a study conducted by Turker *et al*. (2009) about 22 plant species from Bolu (Turkey) are screened for antibacterial activity against *Aeromonas hydrophila, Yersinia ruckeri, Lactococcus garvieae, Streptococcus agalactiae* and *Enterococcus faecalis*. The result indicates that among the tested plants 11 species are capable to inhibit the growth of *S. agalactiae* with high zone diameter. A study done in Bishoftu, Ethiopia, describe antimicrobial effectiveness ant wound healing capacity of steam bark of *Combretum molle,* the leaves of *Xanthium strumarium* against *Staphylococcus aureus* and *Streptococcus agalactiae* (Habtamu *et al*., 2015). *Cenchrus ciliaris*, *Brachiaria sp*, *Abutilon indicum*, *Coccinia grandis*, *Asteracantha longifolia*, *Trichodesma indicum*, *Dactyloctenium indicum*, *Spermacoce hispida* have an inhibitory effect on this pathogen (Doss *et al*., 2012).

c. *Aspergillus flavus*

Aspergillus flavus (*A. flavus*) are widely distributed fungal molds found in soil, air and other organic matter. The species are members of family Trichocomaceae and genus *Aspergillus*

(Krishnan, 2009). Its morphological characters are different from other species of *Aspergillus* when it is growing on appropriate fungal growth medium. Colonies are granular, smooth, or unclear and yellow or yellow-brown to lime green, consisted of a dense felt of conidiophores or mature vesicles bearing phyalides over their entire surface. *Aspergillus flavus* hyphae were septate and showed dichotomous branching. Conidiophores were coarsely roughened, uncolored, with vesicles spherical, covering nearly the entire vesicle. Conidial heads were radiate, uni-seriate, and bi-seriate. Conidia are pale green and conspicuously echinulate, smooth to very finely roughened, spherical (Manisha and Panwar, 2012). *Aspergillus flavus* seems to be more virulent and more resistant to antifungal drugs than most other *Aspergillus* species and has high invasive, allergic, immunosuppressive, toxic, teratogen and carcinogen potential (Hedayati, 2007).

Typically, inhalation of the fungal spores allows entry of the pathogen into the body and the organism has a predilection for the respiratory tract. The spores can also directly inoculated or contaminate at sites of skin injury. Also, they can cause diseases by hematogenous spread from pulmonary sites or by contiguous extension from neighboring sinus. *Aspergillus flavus* causes chronic granulomatous, sinusitis, keratitis, cutaneous aspergillosis, wound infections, onychomycosis (fungal nail infection) and osteomyelitis (fungal infection of the external auditory canal) following trauma and inoculation (Manisha and Panwar, 2012; Krishnan, 2009).

There are a number of plant species that are used as TMP to treat infections caused by *A. flavus*. Different solvents extract of *Sapium sebiferum* leaves were investigated for their antifungal activity against *A. niger* and Aflatoxigenic *A. flavus* (Kumar, *et al*., 2011). A study by Behbahani *et al*. (2014) shows the antimicrobial effect of *Avicennia marina* against this pathogen. *Melia azedarach* leaf extract (Sen and Batra, 2012), various parts of *Datura* species such as *D. ferox, D. inoxia, D. metal* and *D. stramonium* (Gachande and Khillare, 2013), *Ricinus communis* leaf extracts (Naz and Bano, 2012) was recognizes as good inhibitory activities against different fungal pathogens including *A. flavus*. In Ethiopia, several plant species are used to treat different fungal diseases. For instance, Aberra *et al*. 2005 verify the antifungal effect of *Dovyalis abysssinica, Trichila emetic* on this fungal strain. A study was done on antimicrobial activities of Leaves of *Jasminum abyssinicum*, and *Solanecio gigas*; leaves, seeds and fruit-flesh of

Lagenaria siceraria is indicated that antifungal effect all listed plant on this fungus (Mesfin *et al*., 2006). *Lippia adoensis* is locally known as koseret which is endemic in Ethiopia and has been traditionally used to treat different infectious diseases and also in food preparation as a condiment. The crude extract of this plant has been tested for antimicrobial activity against *A. flavus* and inhibition zones were measured as 10.33 and 17.00 mm for its ethanol and methanol extracts respectively (Gemechu *et al*., 2015).

Table 3. Some common skin diseases and causative organism (Chiller *et al*., 2001; Cogen *et al*., 2013)

Infections	**Causative organisms**	**Affected skin area**	**Symptoms**
Impetigo	*B* hemolytic Strep and coagulase positive staph	Whole skin part	pustules, honey-colored crust, erythema, minimal pain, Pruritus, regional adenopathy, and leukocytosis
Anthrax	*Bacillus anthracis*	cut (abrasion) site	edema, bulla ruptures, ulcer, Malaise,
Blistering distal dactylitis	*S. pyogenes, S. agalactiae, Staphylococcus aureus*	nail fold, toes, and palm	non-tender bulla with an erythematous base involving the distal volar fat pad of the phalanges, Dark discoloration
Cellulitis	*Streptococcus agalactiae S. pyogenes, S. aureus*	any site mostly affect lower legs	Edema, warmth, erythema, tenderness, vesicles, bullae, lymphangitis, fever,
Ecthyma	*S. pyogenes*	deep ulcerative infection on legs	erythema; over time the lesion evolves into a crusted ulcer with an elevated rim
Erysipelas	*S. pyogenes*, groups B, C, G S*treptococci*	moist intertriginous zones of skin	well-demarcated reddish-brown patches and plaques located
Bacterial folliculitis	*S. aureus,*	hair follicle	2-5 mm papules and pustules on an erythematous base,
Aspergillosis	*A. Flavus*	Skin, lung,	swelling, skin induration, blisters, fever, coughing, headache, weight loss
Intertrigo	*Staphylococcus aureus, Streptococcus pyogenes C. albicans,*	neck, axillae, fossa, inguinal area	satellite lesions, Bright red, well-demarcated weeping patches and plaques
Perianal dermatitis	*Staphylococcus aureus, Streptococcus pyogenes*	superficial, anus	Erythema, Volvo-vaginitis, vaginal discharge, and vulvar redness, irritability, pruritus, blood-streaked stools, fissures,

3. MATERIALS AND METHODS

3.1. Description of Study Area

The study was conducted from December 2016-June 2017 at Haramaya University, in microbiology laboratory of School of Biological Sciences and Biotechnology and plant pathology laboratory of School of Plant Science. Haramaya University is located at 42^{o} 30' East longitude and 9^{o} 26' North latitude and at an altitude of 1980 m.a.s.l. It has a short rainy season, Belg that stretches from March to May, while the main rain season Kiremt extends from July to September. The annual rainfall and mean maximum and minimum temperature is 800.9mm, 24.18^{0}c and 9.9^{0}c respectively.

3.2. Plant material and Test Pathogens

Fresh mature healthy leaves of *A. vera* were collected in plastic bags from Haramaya University campus and identified as *A. vera* at Haramaya University Herbarium. The leaves were then washed repeatedly with tap water, rinsed with sterilized distilled water and disinfected with alcohol. Then, it was sliced longitudinally with a sharp sterile knife. The inner exposed transparent glutinous and colorless parenchymatous tissue called Aloe gel was scraped out from green fibers by using a sterile spoon and collected into the clean sterile beaker. Then the green fibers were allowed to air dry completely for two weeks at room temperature. *Streptococcus pyogenes* (ATCC 19615) was obtained from Ethiopian Public Health Institute (EPHI), Addis Ababa, Ethiopia. *S. agalactiae* and *Aspergillus flavus* were obtained from plant pathology laboratory, Haramaya University, Haramaya.

3.3. Preparation of Plant Extracts

The gel was blended with an electric blender to make aloe juice and 790g of the juice was macerated with 100ml of each solvent (Ethanol, Petroleum ether and Distilled water) in 250ml conical flak and left for 24h in the laboratory at room temperature. The solution was then filtered through Whatmann No 1 filter paper into a sterile beaker. The filtrates of ethanol and petroleum ether were transferred to round bottom flask and subjected to a rotary evaporator to remove the solvents. Then the concentrates were poured into beaker separately and put in an oven for

complete drying at 40°c for 4 hours. For aqueous extraction, the filtrate was evaporated by using a water bath. The remaining dried green fibers or outer layers of the leaf were powdered by using an electric grinder. Fifty gram of powdered leaf was then mixed with 250ml of solvents (Ethanol, Petroleum, and Distilled water) in a conical flask. The mixture was agitated using an electric blender to enhance proper mixing of the solvent with the powder and it was left for 24 hours at room temperature in the laboratory. The mixture was filtered with Whatman No 1 filter paper. The filtrates of petroleum ether and ethanol were transferred into round bottom flak and subjected to rotary evaporator separately. The obtained gel and fiber crude extracts were sealed with aluminum foil, sterilized and stored at 4°C until use (Malini *et al*. 2013).

3.4. Preparation of Inoculum Suspension

Blood agar (BA) growth media for bacterial strains and potato dextrose agar (PDA) for fungus were prepared according to their manufacture's instruction into conical flasks by using sterile distilled water. Then it was boiled on a hot plate, sterilized by autoclaving at 121^0c for 15mn and poured into sterilized plates. The media were cooled and solidified after 15mn. Test bacterial pathogens (*S. pyogenes and S. agalactiae*) were taken from the stock culture by using a sterile cotton swab and spread on freshly prepared media to acquire bacterial sub-culture. Then, the plates with test microbes were incubated at 37^0c for 24h. Spores of *A. flavus* were sub-cultured on PDA by incubating at 35^0c for 5 days (Mostafa *et al.,* 2011). The bacterial suspension was prepared in sterile saline solution by adjusting the density or turbidity of the organism suspension with turbidity of 0.5 McFarland standard. McFarland Standard is a chemical solution that was formed by mixing 0.5ml of 1% $BaCl_2$.$2H_2O$ (barium chloride hydrated) and 99.5ml of 1% H_2SO_4 (sulfuric acid) in which the reaction produce a fine precipitate and turbidity. Prepared 0.5 McFarland standard was stored in an upright position at 25°C (room temperature) and protected from light until it was used (Andrews, 2009).

About four morphologically similar colonies of *S. pyogenes* and *S. agalactiae* were taken from sub-cultured bacterial strains with a sterile loop and inoculated into labeled test tubes with 10ml saline solution separately. Ten ml of 0.5 McFarland standard was also added to another test tube of the same size. The solutions were mixed on a vortex mixer and the turbidity was adjusted to

0.5 McFarland standard. These suspensions are adjusted to contain 1×10^8 CFU/ml. (Andrews, 2009). Inoculum suspension of *A. flavus* was prepared from fresh, mature and well-sporulated (5-day-old) culture and a number of spores/ml was adjusted by using cell counting hemocytometer. The spores on the surface of the medium were covered with 20ml of distilled water and detached by rubbing with a sterile loop. The solution was filtered with a muslin cloth to remove the majority of the hyphae and to produce an inoculum mainly composed of only spores and transferred into the sterile flask. One ml of spore suspension was dropped on the engraved grid of hemocytometer and covered by coverslip to count the number of spores/ml. A number of spores/ml was calculated by using the following formula.

$$\text{Spores/ml}=\frac{\text{n}\quad\text{o c}\quad\text{s}\quad\text{ii 1m}}{\text{v(}\quad\text{o e}\quad\text{v}\quad\text{g}}$$

The counted spores were 437 in 1ml and the volume of the engraved grid is 10^{-4}. The result was 437×10^4 spores/ml and the suspension was further diluted by twofold in saline solution to obtain 4.37×10^6 spores/ml (Amare, 2017). All suspensions were spread on the newly prepared fresh medium by using a cotton loop to achieve antibacterial assay within 15 min after suspension preparation.

3.5. Disc Preparation

Six mm diameter discs were prepared from Whatman No. 1 filter Paper and were put in drying oven at 100^0c for 6hr. Then the discs were soaked into prepared extracts for 30min and placed on a sterilized slide to prepare experimental discs. Negative control discs were prepared by soaking the discs into beakers containing pure solvents (ethanol, petroleum ether and distilled water). Broad-spectrum antibiotics such as Cephalosporin (30µg/ml) and antifungal Ketoconazole (30µg/ml) were used as positive control for bacterial and fungal strains, respectively (Kedarnath *et al*., 2012: Kaveri *et al.*, 2013). Cephalosporin is beta-lactam type drug that is recommended for beta-hemolytic bacteria (Stevens *et al*., 2014; Jeng *et al*., 2010).

3.6. Evaluating Antibacterial and Antifungal activity of the Extract

A stock solution of each crude extract was prepared by diluting 200 mg of each crude extract in 1ml of sterile distilled water. The antimicrobial activity of *A. vera* leaf and gel extracts were evaluated by disc diffusion technique against the selected pathogens (Parekh and Chanda, 2007). A loop full of the test organisms from prepared bacterial and fungal suspensions were spread on solidified sterile BA and PDA medium respectively. The experimental, positive and negative control discs were placed on the plates that were inoculated with test organisms and the plates were labeled. Both BA and PDA growth medium were poured into other sterile plates separately without extract and microbial inoculation and used as control plates. All plates were incubated at 37°C for 24hrs and 72hrs for bacterial and fungal strains respective. The effectiveness of each extract was assessed by the presence or absence of inhibition zones and zone diameter compared with those antibiotics (Shahzad *et al.,* 2009).

3.7. Determination of minimum inhibitory concentration (MIC)

The MIC is defined as the lowest concentration that inhibits the bacterial growth. The green fiber extracts of *A. vera* leaf were lacked antimicrobial activities. The MIC value of *A. vera* gel extracts was determined for *Aspergillus flavus*, *S. pyogenes*, and *S. agalactiae* by using broth dilution method in test tubes with the same volume. The tubes were sterilized and labeled with different pathogens and crude concentrations. Serial dilutions of the *Aloe vera* gel extracts were prepared in a concentration of 25, 50, 75 and 100mg/ml. Nutrient broth (NB) and potato dextrose broth (PDB) liquid media were prepared according to the instruction of their manufacturer. Two ml of the medium was dispensed into each sterilized and labeled test tubes. The 200 µl of each crude dilution of different extracts and 50µl of each suspension were inoculated to all tubes that contained media. Control tubes were prepared by inoculating only 2ml of sterile medium without microbial suspension and gel extracts. All tubes were incubated at 37^0C for 24hr for bacteria and 72hr for fungal growth. Then the tubes were checked for bacterial growth and the lowest concentration without visible growth was defined as MIC of the corresponding one (Stanley *et al*., 2014).

3.8. Determining minimum bactericidal and minimum fungicidal concentration (MBC and MFC)

MBC is defined as lowest concentration which has bactericidal activity and MFC is the lowest concentration that has fungicidal activity. The MBC for bacterial strains and MFC for fungus were determined by sub-culturing of tested dilution on fresh NB and PDB respectively. New fresh broth media was prepared for both fungal and bacterial strains and 2ml of each medium was transferred into another sterilized and labeled tubes. Then 0.1ml of the solution was transferred from previous non-turbid tubes into tubes containing a fresh medium. The tubes were incubated at an appropriate temperature and checked for the presence and absence of turbidity or bacterial and fungal growth. The lowest concentration with no visible bacterial and fungal growth on fresh medium was regarded as MBC and MFC (Cataldi *et al*., 2015).

3.9. Data Analysis

Zone of inhibition produced by each solvent extracts on bacteria and fungus were measured and recorded. The data were subjected to one-way analysis of variance (ANOVA) to calculate the mean of inhibition zone. The experimental results were expressed as mean and +/- standard deviation. P-value < 0.05 were regarded as significant.

4. RESULTS AND DISCUSSION

4.1. Antimicrobial Activities of *A. vera* Leaf and Gel

The present study carried out on antimicrobial activities of *A. vera* leaf and gel extracts in different solvents (petroleum ether, ethanol and aqueous) against three selected human skin pathogens namely *S. pyogenes, S. aglactiae* and *Aspergillus flavus*. The results showed that *Aloe vera* gel extracts of all solvents had antimicrobial activity against tested pathogens. *Aspergillus flavus* was measured with zone diameter of 19.7, 15 and 13mm for petroleum ether, ethanol and aqueous extracts respectively. *Streptococcus pyogenes* was inhibited in diameter of 16.2mm for petroleum ether, 13mm for ethanol and 11mmaqueous gel extracts. Inhibition zone of *S. agalactiae* was measured as 14, 10.5 and 7.16mm for petroleum ether, ethanol and aqueous extracts (Table 4). The negative control disks that were impregnated with pure petroleum ether, ethanol and distilled water showed no inhibitory effect in all tested pathogens, suggesting that the observed antimicrobial property is attributed to the extract contents only. Different solvents' extracts of *Aloe vera* gel showed different antimicrobial efficiency. The maximum antibacterial activities were observed in petroleum ether extract. Ethanol gel extract was efficacious next to petroleum ether gel extract, whereas aqueous extract performed least antimicrobial activities. This result shows that different solvents have a different capacity of extracting active principles to inhibit microbial growth. Measured inhibition zones of petroleum ether gel extracts were approximate with zone diameter of standard drugs (Cephalosporin and ketoconazole) for bacterial and fungal strains, respectively. This may be due to the gel has different bioactive compounds that particularly enhance antimicrobial activities of plant extracts (Kedarnath *et al*., 2012). These support the strong scientific basis for the use of these plants in the traditional treatment of skin infectious diseases.

Several studies exhibited a higher level of antibacterial activity of *A. vera* gel against another fungus and bacterial strains which are considered as human pathogens. The DMSO gel extracts of *A. vera* has shown antimicrobial activity against *Bacillus subtilis*, *Salmonella typhi*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Aspergillus fumigatus*, *Candida albicans* and *Penicillium* (Malar *et al*., 2012; Goudarzi *et al.*, 2015).The current study indicated that among tested pathogens, *Aspergillus*

flavus was more susceptible to gel extracts as it showed the highest zone of inhibition for all solvent extracts and followed by *Streptococcus pyogenes.* Similar results were reported by Sitara *et al*. (2011).They used acetone extract of *A*loe *vera* gel against *Aspergillus niger, Aspergillus flavus, Alternaria alternata, Drechslera hawaiensis* and *Penicillium digitatum*. The result has been clearly reflecting that the gel has a higher antifungal effect on *Aspergillus flavus* and *Aspergillus niger* among tested microbes. Arunkumar and Muthuselvam (2009) studied the antimicrobial effect of homogenized *Aloe vera* leaf in three solvent namely aqueous ethanol and acetone solvent against *Staphylococcus aureus, Streptococcus pyogens, Pseudomonas aeruginosa, Escherichia coli, Aspergillus flavus* and *Aspergillus niger*. The report showed that *Aspergillus flavus* and *Streptococcus pyogenes* have a higher zone of inhibition. The least inhibition zones of the gel extracts in all solvents were measured for *Streptococcus agalactiea* in the current study. The result is more or less similar with that of Yisehak *et al*. (2014) in which the inhibition zone of *Aloe vera* against *Streptococcus agalactiea* (methanol extract) was 8.9±1.5mm in diameter at a concentration of 100mg/ml. Subramanian *et al.* (2006) have conducted antimicrobial activity of *Aloe vera* gel extract by using ethanol as extracting solvent against different fungal and bacterial species and the result was showed that the gel inhibits *Aspergillus flavus* and *Streptococcus pyogenes* with high zone diameter.

There are contradictory studies about the inhibitory effect of gel and leaf of *Aloe vera*. A study by Agarry *et al*. (2005) showed that both gel and leaf (ethanol extract) inhibited the growth of *Staphylococcus aureus* in 18 and 4mm zone diameter while the gel has no effect on *Pseudomonas aeruginosa, Trichophyton schoeleini,* Microsporum *canins* and *Candida albicans*. The result also explained that *Candida albicans* was inhibited by leaf extract with zone diameter of 3mm diameter. A similar result was reported that leaf shows antimicrobial activity against *Staphylococcus aureus* isolated from wound infection while the gel has no effect on *Pseudomonas aeruginosa* isolates (Ander *et al*., 2010). The observed different result from current study may be due to using different solvents for extraction, material and methods that were used during the extraction process, the strain of pathogens that were tested, bacterial morphology and structures and environmental condition. The difference of agro-climatic zones

has a significant effect on quantities and types of obtained chemical composition in *Aloe vera* gel (Kumar *et al.*, 2015).

Regarding the efficiency of extracting solvents, the current result is in conformity with earlier findings that reported that petroleum ether gel extract had highest antimicrobial activity than ethanol and chloroform extracts against *Escherichia coli* and *P*soedomonas *aeruginosa*. Also, both petroleum ether and ethanol have been showed equal zone diameter against *Klebsilla pnemoniae*, *Neisseria crassa* and *Aspergillus niger* (Kedarnath et al., 2012). Kaveri *et al*. (2013) also reported *Staphylococcus aureus* and *Klebsiella* species were highly inhibited by petroleum ether gel extract than chloroform and methanol extracts. Lawrence (2009) found the varied antibacterial activity of acetone, ethanol and methanol extract of *Aloe vera* gel against *Staphylococcus aureus*, *Streptococcus pyogenes*, *Bacillus subtilis*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella typhi* and *Klebsilla pneumoniae*. The results showed ethanol and methanol extracts had higher activity while acetone extract showed least or no activity against most of the tested pathogens. The aqueous extract had no antimicrobial effect against *Aspergillus flavus*, *Pseudomonas aeruginosa* and *Escherichia coli* (Arunkumar, 2009). Petroleum ether was also showed the best efficiency on the extraction of other plants. For instance, petroleum ether extract of *Clerodendrum myricoides* plant had shown highest inhibition zone than the extract of methanol, acetone and chloroform against *Trichophyton mentagrophytes* (Asmamaw, *et al*., 2007). Similarly, Bruck (2004) was reported that *Inula confertiflora* of petroleum ether extract was found to be higher in antimicrobial activities than the 80 % methanol extract of the same plant against *C*andida *albicans*, *Trichophyton mentagrophytes*, and *Staphylococcus aureus*. According to Linthoingambi and Singh(2013) antimicrobial activities of *Tithonia diversifolia* against fungal pathogens namely *Alternaria alternata*, *Alternaria solani*, *Aspergillus flavus*, *A. niger*, *Cuvularia lunata*, *Drechslera oryzae*, *Fusarium oxysporum*, *Penicillium expansum* and *Penicillium italicum*and bacterial strains such as *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were done in petroleum ether, chloroform and methanol solvents. Among the extracts, petroleum ether extract showed the highest antifungal and antibacterial activity.

Table 4. Growth inhibition zone (mm) of *A. vera* gel extracts in terms of mean ±SD

Solvents		**Microbial strains with zone of inhibition**			
	Number of trailes	Concentration	*A. flavus*	*S. pyogenes*	*S. agalactiae*
Ethanol	3	200mg/ml	15±1	13±1	10.5±0.5
Petroleum ether	3	200mg/ml	19.7±0.57	16.2±0.76	14±1
Aqueous	3	200mg/ml	13±1	11±1	7.16±1.26
Cephalosporin	3	30 µg/ml	-	18.17±1.04	18.16±0.76
Ketoconazole	3	30 µg/ml	22±1	-	-

4.2. Determination Minimum Inhibitory Concentration

Broth microdilution method was carried out to determine MIC values of ethanol, petroleum ether and aqueous extracts of *Aloe vera* gel against selected microbes. Both ethanol and petroleum ether extracts of *Aloe vera* gel were showed MIC value at a concentration of 25mg/ml *against S. pyogenes* and *Aspergillus flavus*. Their growth was also inhibited by aqueous gel extract at MIC value of 50mg/ml. MIC value of *S. agalactiea* was observed at a concentration of 50, 25 and 75 mg/ml of ethanol, petroleum ether and aqueous extracts, respectively (Fig, 1). Among the extracting solvents aqueous extract was needed highest concentration against all tested pathogens and the highest concentration of all type of gel extracts were needed to inhibit the growth of *Streptococcus agalactiea* while the lower concentration of gel extracts were used to inhibit *Aspergillus flavus* and *Streptococcus pyogenes*. These indicated that *Aspergillus flavus* and *Streptococcus pyogenes* are highly susceptible to gel extract than *Streptococcus agalactiea.* In a similar study, *Streptococcus agalactiea* had shown highest MIC value than *Salmonella typhimurium*, *Escherichia coli* and *Staphylococcus aureu* (Yisehak *et al*., 2014). Different

studies reported the lowest MIC value of *Aloe vera* gel extracts against different skin fungal and bacterial pathogens. Kumar *et al*. (2015) were revealed that the gel can inhibit the growth of *Candida albicans, Salmonella typhi, Escherichia coli, Serratia marcescens* and *Staphylococcus aureus* at low concentration. Gel extract in DMSO solvent had MIC value 50mg/ml for *Candida albicans* and *Helicobacter pylori* (Cataldi *et al*., 2015). *Streptococcus mutans* was inhibited by aqueous and DMSO extract at a concentration of 50mg/ml and 25mg/ml respectively (Jain *et al*., 2015). In another hand, higher MIC value of aloe gel extracts were reported to inhibit the visible growth of some other skin pathogens. For instance *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa* and *Escherichia coli* were inhibited at a concentration of 400-800mg/ml (Cataldi *et al*., 2015).

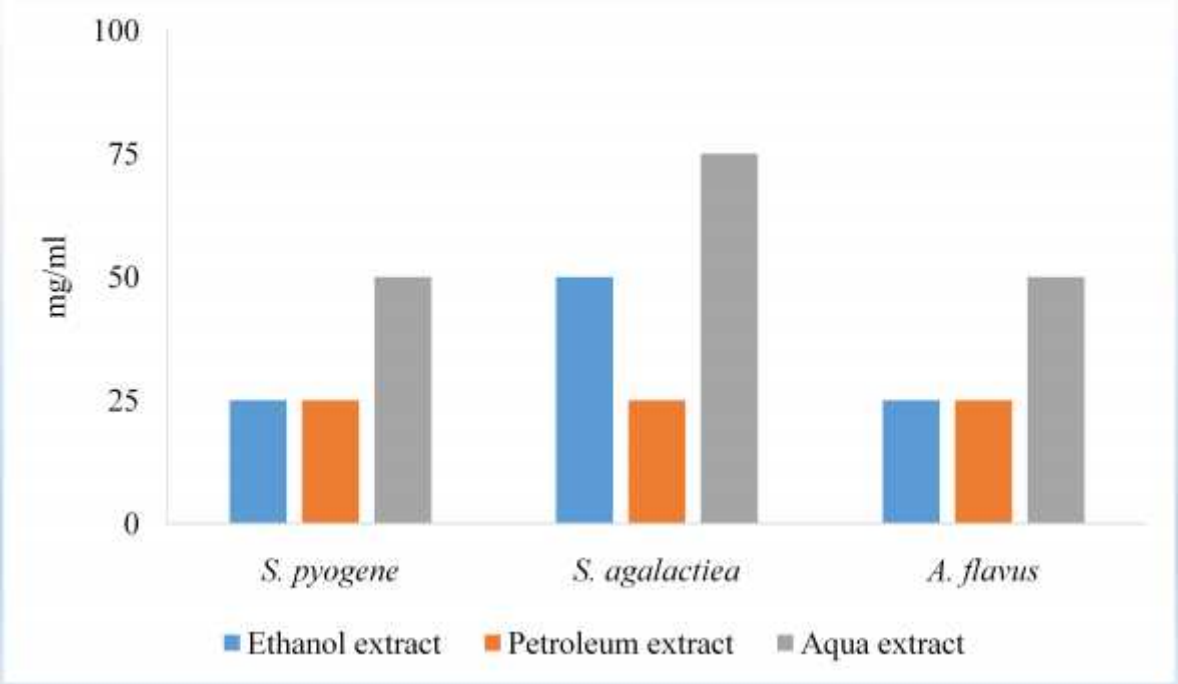

Figure 1. MIC of different extracts of *A. vera* gel against the tested pathogen

4.3. Determination of MBC and MFC

Figure 2 shows the MBC and MFC values of *Aloe vera* gel extracts against all 3 tested pathogens. The MBC result of *Aloe vera* gel extracts was 50 mg/ml of ethanol, 25 mg/ml of petroleum ether, and 75mg/ml for aqueous against *Streptococcus pyogenes. Streptococcus*

agalactiea was totally killed at concentration of 75, 50, 100mg/ml of ethanol, petroleum ether and aqueous extracts respectively. Minimum fungicidal concentration for *Aspergillus flavus* was 25mg/ml for both ethanol and petroleum ether and 75mg/ml for aqueous extracts. Aloe gel extract of petroleum ether showed highest bactericidal and fungicidal activity by inhibiting the growth of the pathogens at the lowest concentration, but aqueous extract killed the pathogens at the highest concentration (Yisehak *et al.*, 2014). Different findings were reported the lowest MBC value of *Aloe vera* extracts in different solvent against different pathogenic micro-organisms. The minimum bactericidal concentration of *Aloe vera* (ethanolic extracts) was 2mg/ml for *Streptococcus salivarius* and *Actinomyces viscosus* while 16 and 4mg/ml for *Streptococcus mutans* and *Streptococcus sanguinis* respectively (Brojeni *et al.*, 2016). Furthermore, the results of another study by Ehsani *et al*. (2013) reported that the MBC value of the ethanol extract as 4.5 mg/ml against *Enterococcus faecalis* and *Streptococcus mutans*.

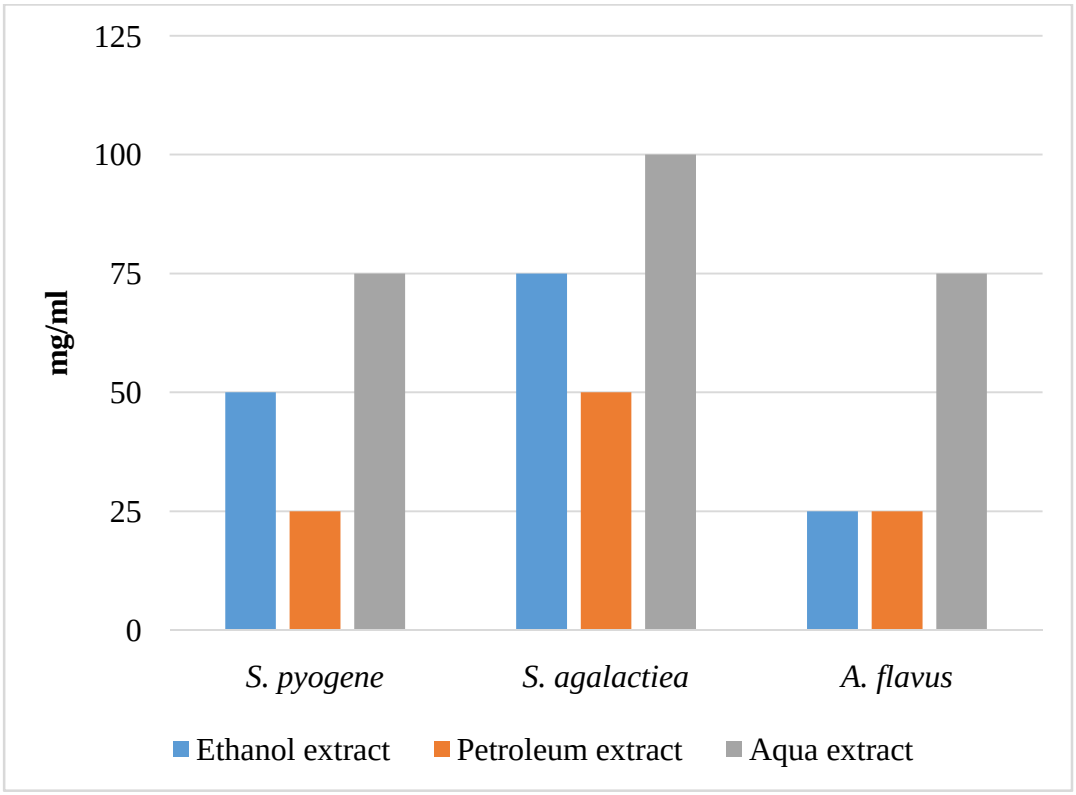

Figure 2. MBC of different extracts of *A. vera* gel against the tested pathogens

5. SUMMARY, CONCLUSIONS, AND RECOMMENDATIONS

5.1. Summary and conclusions

Aloe vera is flowering succulent monocotyledonous plant which belongs to family Aloeaceae and genus Aloe. It is used as TMP in different countries to treat several human skin diseases and wound infection. The present study was aimed to evaluate antimicrobial activities of *A. vera* leaf and gel extract separately in different solvent (petroleum, ethanol and aqueous) against some human skin pathogens namely *A. flavus, S. pyogenes* and *S. agalactiae*. Disk diffusion method was used to test antimicrobial activity of the gel extracts and the MIC, MBC and MFC concentration were determined by using broth dilution method. The result showed that only the gel extracts negatively affected the growth of those microbes. Petroleum ether extract gave highest inhibition zone with a diameter of 15, 13 and 10.5mm against *A. flavus*, *S. pyogenes* and *S. agalactiae* respectively. Relatively aqueous extracts performed least inhibition zone against *A. flavus, S. pyogenes* and *S.agalactiae* with zone diameter of 13, 11 and 7.16mm respectively. Among tested pathogens, *A. flavus* is the most susceptible to all extracts of the gel and followed by *S. pyogenes*. They were inhibited at low concentration (25mg/ml for petroleum) of the extracts while *S. agalactiae* needed the highest concentration with 50, 75 and 100mg/ml for petroleum ether, ethanol and aqueous extracts respectively. In general, the result of the current study showed that antimicrobial effect of *A. vera* gel extract in a different solvent on tested microorganisms and confirmed that the gel can inhibit the growth of tested pathogens at lowest concentration except for *S. agalactiae*. Also, it approved that among the extracting solvent petroleum ether had high extracting capacity than ethanol and aqueous solution.

5.2. Recommendation

- ✓ The current study is recommended that using the gel parts of *A. vera* is preferable in treating different skin infections and diseases that caused by *A. flavus, S. pyogenes* and *S. agalactiae*.
- ✓ Petroleum ether is the best extracting solvent in extracting antimicrobial components of *A. vera* gel than ethanol and distilled water

- ✓ Phytochemical screening should be carried out chromatographically for both leaf and gel separately to know their chemical composition and to find out bioactive elements that have antimicrobial effects;
- ✓ Using different extraction techniques and solvents that were not including in this study may improve the current result. Because all solvents do not have the same dissolving capacity;
- ✓ Further studies are needed to convert the traditional use of this plant into the modern technology.

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7. APPENDICES

Appendix table 1. Mean of inhibition zone of the extracts against the pathogens

	Ethanol extract	**Petroleum extract**	**Aqua extract**	**Control**
S. pyogene	13	16.16	11	18.17
S. agalactiea	10.5	14	7.17	18.16
A. flavus	15	19.7	13	22

Appendix table 2. Analysis of variance for A. flavus

	N	Mean	SD	Std. Error	95% Confidence Interval		Min	Max
					LB	UB		
Ethanol	3	15.000	1.0000	.5774	12.516	17.484	14.0	16.0
Petroleum	3	19.667	.5774	.3333	18.232	21.101	19.0	20.0
Aqua	3	13.000	1.0000	.5774	10.516	15.484	12.0	14.0
Control	3	22.000	1.0000	.5774	19.516	24.484	21.0	23.0
Total	12	17.417	3.8248	1.1041	14.987	19.847	12.0	23.0

LB: lower boundary, UB: upper boundary, Max: maximum, Min: minimum

Appendix table 3. Multiple comparisons depending on variable for*A. Flavus*

(I) solv	(J) solv	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					LB	UB
	Petroleum	-4.6667*	.7454	.000	-6.385	-2.948
Ethanol	Aqua	2.0000*	.7454	.028	.281	3.719
	control	-7.0000*	.7454	.000	-8.719	-5.281
	ethanol	4.6667*	.7454	.000	2.948	6.385
Petroleum Ether	Aqua	6.6667*	.7454	.000	4.948	8.385
	control	-2.3333*	.7454	.014	-4.052	-.615
	ethanol	-2.0000*	.7454	.028	-3.719	-.281
Aqua	Petroleum	-6.6667*	.7454	.000	-8.385	-4.948
	control	-9.0000*	.7454	.000	-10.719	-7.281
	ethanol	7.0000*	.7454	.000	5.281	8.719
Control	Petroleum	2.3333*	.7454	.014	.615	4.052
	Aqua	9.0000*	.7454	.000	7.281	10.719

*. The mean difference is significant at the 0.05 level.

Appendix table 4. Analysis of variance for *S. pyogenes*

	N	Mean	SD	Std. Error	95% Confidence Interval		Min	Max
					LB	UB		
Ethanol	3	13.0000	1.00000	.57735	10.5159	15.4841	12.00	14.00
petroleum ether	3	16.1667	.76376	.44096	14.2694	18.0640	15.50	17.00
Water	3	11.0000	1.00000	.57735	8.5159	13.4841	10.00	12.00
Control	3	18.1667	1.04083	.60093	15.5811	20.7522	17.00	19.00
Total	12	14.5833	3.00631	.86785	12.6732	16.4934	10.00	19.00

Appendix table 5. Multiple Comparisons depending on variable for S. pyogenes

(I) solv	(J) solv	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ethanol	petroleum ether	-3.16667*	.78174	.004	-4.9694	-1.3640
	water	2.00000*	.78174	.034	.1973	3.8027
	control	-5.16667*	.78174	.000	-6.9694	-3.3640
petroleum ether	ethanol	3.16667*	.78174	.004	1.3640	4.9694
	water	5.16667*	.78174	.000	3.3640	6.9694
	control	-2.00000*	.78174	.034	-3.8027	-.1973
Water	ethanol	-2.00000*	.78174	.034	-3.8027	-.1973
	petroleum ether	-5.16667*	.78174	.000	-6.9694	-3.3640
	control	-7.16667*	.78174	.000	-8.9694	-5.3640
Control	ethanol	5.16667*	.78174	.000	3.3640	6.9694
	petroleum ether	2.00000*	.78174	.034	.1973	3.8027
	water	7.16667*	.78174	.000	5.3640	8.9694

*. The mean difference is significant at the 0.05 level.

Appendix table 6. Analysis of variance for *S. agalactiae*

	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval		Min	Max
					LB	UB		
Ethanol	3	10.5000	.50000	.28868	9.2579	11.7421	10.00	11.00
Petrolium ether	3	14.0000	1.00000	.57735	11.5159	16.4841	13.00	15.00
Aqua	3	7.1667	1.25831	.72648	4.0409	10.2925	6.00	8.50
Contro	3	18.1667	.76376	.44096	16.2694	20.0640	17.50	19.00
Total	12	12.4583	4.34039	1.25296	9.7006	15.2161	6.00	19.00

Appendix table 7. Multiple Comparisons depending on variable for *S. agalactiae*

(I) solv	(J) solv	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval	
					Lower Bound	Upper Bound
Ethanol	Petroleum ether	-3.50000*	.75462	.002	-5.2401	-1.7599
	aqua	3.33333*	.75462	.002	1.5932	5.0735
	control	-7.66667*	.75462	.000	-9.4068	-5.9265
Petroleum ether	Ethanol	3.50000*	.75462	.002	1.7599	5.2401
	aqua	6.83333*	.75462	.000	5.0932	8.5735
	control	-4.16667*	.75462	.001	-5.9068	-2.4265
Aqua	Ethanol	-3.33333*	.75462	.002	-5.0735	-1.5932
	Petroleum ether	-6.83333*	.75462	.000	-8.5735	-5.0932
	control	-11.00000*	.75462	.000	-12.7401	-9.2599
Control	Ethanol	7.66667*	.75462	.000	5.9265	9.4068
	Petroleum ether	4.16667*	.75462	.001	2.4265	5.9068
	aqua	11.00000*	.75462	.000	9.2599	12.7401

*. The mean difference is significant at the 0.05 level.