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Evaluation of Antimicrobial Activities, Antioxidant Properties and Phytochemical Components of Aloe ankoberensis

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LETTER OF APPROVAL SHEET

Debre Berhan University

College of Graduate studies

Department of Chemistry

This is to certify that the thesis prepared by Minda Getaneh, entitled: Evaluation of Antibacterial Activities, Antioxidant Properties and Phytochemical Components of Aloe ankoberensis, submitted in partial fulfillment of the requirement of the Degree of Masters of Science in Chemistry complies with the regulation of the University and meets the accepted standards with respect to originality and quality.

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Declaration

I declare that this thesis work entitled “Evaluation of Antibacterial Activities, Antioxidant Properties and Phytochemical Components of Aloe ankoberensis” is based on my work done under the esteemed supervisor of Dr. Minbale Gashu and the data presented in this work are original and authentic .They have not been published before in any journal .Beside that, I have properly acknowledged the reference materials I used in this thesis work.

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First and for most I would like to thank the Almighty God and St.Mary who gave me health, moral and strength throughout my life.

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Abstract

In Ethiopia there are many species of medicinal plants which may contain disease-curing bioactive compounds which are distributed all over the country. The traditional medicine still plays an important role in the primary health care in Ethiopia. Aloe ankoberensis is one of the most common endemic plants in Ethiopia used in traditional medicines. In this study the leaves of Aloe ankoberensis were extracted using hexane, chloroform, ethyl acetate and ethanol using maceration. The antibacterial activities of the extracts were investigated against Gram positive, and Gram negative bacteriausing Muller Hinton Agar medium by disc diffusion method. Ethanol extract of aloe ankoberensis indicated a good antibacterial activity against a Staphyloccus aures, Listerial monocytogenes Escherichia coli and, candida albicans with zone of inhibition 11, 11, 13 and 10, respectively. Chloroform extract of Aloe ankoberensis also showed a medium antibacterial activity against a Staphyloccus aureus and listerial monocytogenes and Escherichia coli and, candida albicans with zone of inhibition 9, 7, 8, and 8, respectively. The antioxidant activity of the extracts of Aloe ankobernsis was determined using 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. DPPH radical scavenging activity of hexane, chloroform, ethyl acetate, and ethanol extracts were 83.73%-96.04%, 72.14%- 95.43%, 67.57%-91.64%, 42.61%-90.47%, respectively. Generally ethanol crude extract had good antibacterial and antioxidant activities than ethyl acetate, chloroform and hexane extracts due to the presence of saponins and phenolics compounds.

Keywords: **Aloe ankoberensis, antibacterial activities,** A**ntioxidant activity**

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List of acronyms

%RSA	Percentage scavenging assay
µg/ml	Microgram per milliliter
A	Aloe
AA	Ascorbic acid
Abs	Absorbance
Ac	Absorbance of the control
As	Absorbance of the sample
CA	Chloramphnicol
CH	Chloroform
DMSO	Dimethyl sulfoxide
DPPH	2, 2- diphenyl -1-picrylhyorazyl
EA	Ethyl acetate
ET	Ethanol
HE	Hexane
ME	Methanol
ROS	Reactive Oxygen Spcies
UV	Ultra Violet
WHO	World health organization
APGI V	Angiosperm phylogeny group

1. INTRODUCTION

1.1 Background of the study

The value of medicinal plants in drug discovery is well known and the human being used them for various purposes from the beginning of the human history (Farns worth, 2008). Traditional folk remedies from plants have always guided scientists to search for new medications in order to maintain and promote healthy life for human and animals (Achterberg J.,2013).

Medicinal plants represent a rich source of antimicrobial agents (Khaing TA.,2011). Many of the plant materials used in traditional medicines are readily available in rural areas at relatively cheaper prices than modern medicines (Mann A et al., 2008). Over 80% of the Ethiopian population depends on traditional medicine for their healthcare need (Kassaye KD et al, 2006).

Medicinal properties of plants are normally dependent on the presence of certain phytochemical principles such as alkaloids, anthraquinones, cardiac glycosides, saponins, tannins and polyphenols, which are part of bioactive compounds responsible for the antimicrobial and antioxidant property. (Mann A. et al, 2008).

Oxidation is a chemical reaction that transfers hydrogen or electrons from a substance to an oxidizing agent, and oxidation reactions can generate free radicals. Free radicals are also produced as a consequence of the incomplete reduction of oxygen molecule ((kohen R and Nyaska, 2002). The free radicals formed can cause structural and functional changes. Antioxidants protect biological systems against free radical damage. Insufficient levels of endogenous and exogenous antioxidants can cause oxidative stress, an imbalance of oxidants and antioxidants resulting in cellular damage or death. Oxidative stress plays a significant role in diverse diseases such as cardiovascular conditions, cancer, inflammatory diseases and early ageing (valko M,et al,2007 and ultra B,et al 2009).

Antioxidant components of plants are also effective in preventing many diseases (krishaiah D. et al, 2011).The DPPH assay is easy way to estimate antioxidant

activity by UV-Vis spectrometry, so it can be useful to assess various product at a time (Huang .D et al, 2005).Recently, natural products have received much attention as sources of biological active substances including antioxidants. Numerous studies have been carried out on some plants, vegetables and fruits because they are rich sources of antioxidants, such as vitamin A, vitamin C, Vitamin E, carotenoids, polyphenolic compounds and flavonoids (A.T.Diplock, et al, 1998). Thus, the consumption of dietary antioxidants from these sources is beneficial in preventing cardiovascular diseases (lipidol L.et al, 2000).

Aloe ankoberensis is one of the medical plants used to treat different diseases. Plants remain the most common source of antimicrobial agents. Their usage as traditional health remedies is most popular for 80% of world population in Africa, Asia and all over the world and is reported to have minimal side effects (Bibitha B.et al, 2002). Recently pharmaceutical companies have spent a lot of time and money in developing natural products extracted from plants, to produce more cost effective remedies that are affordable to the population. The rising incidence in multidrug resistance amongst pathogenic microbes has further necessitated the need to search for newer antibiotic sources. Because of its wide usage and availability, this study was set out to investigate the antimicrobial, antioxidant activities and phytochemical components of extracts of the *Aloe anokobernsis*

1.2. Statement of the problem.

Aloe ankobernisis is one of medical plants in Ethiopia found around North Shoa in ankober wereda, is used as medicine where many researchers in Ethiopia and other countries keen to study the antioxidant and antibacterial activity of aloe species. However, the studies were not enough to confidently propose further use of *Aloe ankoberensis* to its potential towards antibacterial and antioxidant activity.

1.3. Significance of the study

Recently, there has been much research emphasis on the antioxidant and antibacterial properties of plants. Plants with these attributes are good resources

for general health maintenance and well-being. Anti-bacterial and antioxidant agents of plant origin have been studied from various plant extracts with the objective of developing antibacterial and antioxidant drugs. This is because plants are richest in bioactive compounds and these bioactive compounds are responsible for the antibacterial and antioxidant effect of plants. Evaluation of plant products for pharmacological and medicinal effects is of growing interest as they contain many bioactive substances which have therapeutics potential. The result of this study will indicate the antioxidant and antimicrobial ability of the leaf extracts of aloe ankobernsis and it shows a kind of phytochemicals present in leaf parts of this plant. Generally this study will strengthen the medicinal use of *Aloe ankobernsis*

1.4. Objectives of the study

1.4.1 General objectives of the study

The main objective of the study was to investigate the antibacterial activity and antioxidant properties, and phytochemical analysis of *Aloe ankoberensis* of leaf extract.

1.4.2. Specific objectives

The specific objectives of this study were:-

- To evaluate the antibacterial activities of the leaf extracts of *Aloe ankoberensis*
- To evaluate the antioxidant properties of the leaf extracts of *Aloe ankoberensis*
- To carry out a qualitative phytochemical test of ethanol, ethyl acetate, chloroform and hexane leaf extracts of *Aloe ankoberensis*

2. LITERATURE REVIEW

2.1. The family Asphodelaceae

Asphodelaceae is the family of flowering plants in the order asparagales such a family has been recognized by most taxonomists, but the circumscripition has widely. In its current circumscription in the APGIV system, it includes about 40 genera and 900 known species (Christenhusz, M.J.M andByng, J.W, 2016). The type genus is Asphoddelus. The family has a wide but scattered distribution throughout the tropics and temperature zones.*Aloe ankobernsis* pendent shrub distribution Ethiopia, Shewa Region (SU), on eastern escarpment; not known elsewhere. Ethiopia. Shewa Region, Mussolini Pass, above Debre Sina, 8 Jan. 1966, W. de Wilde 9623; 34 km from Debre Berhan on road to Ankober

Table 2.1. Classification of Aloe ankobernsis

Kingdom	Plant
Order	Asparagales
Family	Asphdelacea
Genus	Aloe
Species	Aloe ankoberensis

Figure .2.1. Aloe ankoberensis plant

2.2. The genus aloe

The genus Aloe is represented by 600 species and belongs to the family of Asphodelaceae (kawai K.et al, 1993,). Aloe plants are native to sub-Saharan Africa, many islands of western Indian Ocean, including Madagascar and Saudi Arabian Peninsula. In Ethiopia and Eritrea, about 46 species of Aloe have been described so far with a high proportion of endemics adapted to harsh climates (Norda L., 2003). It has been reported that the leaf latex of several Aloe species and their constituents possess wide spectrum of biological activities, such as antimicrobial (Asmamaw W. et al, Minal G., 2011 et al,2014) and antimalarial (Gemechu W. et al, 2014).

Medicinally, the gel and dried leaf exudates of aloe species have been used since ancient civilizations of the Egyptians, Greeks and Mediterranean people (Trease, G. E., Evans, W., 976). Aloe species have enjoyed a very wide folkloric usage and are also now used in modern medicine in many parts of the world. The bitter leaf exudates of some aloe species are commercially important sources of the laxative aloe drugs (Cheney, R. H., 1970) and are also used in the cosmetics industry as additives in shampoos, shaving and skin care creams(Leung, A. Y.,1977) and in the treatment of skin disorders.

The exudates have also been used as a bittering agent in alcoholic beverages (Council of Europe ed., 1981). The leaves and roots of Aloe species elaborate many interesting secondary metabolites belonging to different classes of compounds including, anthrones, chromones, pyrones, coumarins, alkaloids, glycoproteins, naphthalenes, anthraquinones and flavonoids (Dagne E., 1996**).**

2.3 Traditional use of aloe species

Aloe plants have been widely known and used for centuries as topical and oral therapeutic agent due to their health, beauty, medicinal, and skin care properties (Surjushe A.,et al 2008, Boudreau M.D., et al, 2006).

Aloe arborescens, *Aloe barbadensis*, *Aloe ferox*, and *Aloe vera* are among the well-investigated Aloe species. Presently, they account among the most

economically important medicinal plants and are commonly used in primary health treatment, where they play a pivotal role in the treatment of various types of diseases, through biochemical and molecular pathway modulation (Rahmani A.H., et al, 2015).

Indeed, Aloe plants have been reported for multiple biological properties, including antibacterial and antimicrobial, antitumor, anti-inflammatory, anti-arthritic, anti-rheumatoid, anticancer, and antidiabetic activities, detoxification, treating constipation, flushing out toxins and wastes from the body, promoting digestion (on gastrointestinal disorders treatment), and reducing illnesses likelihood, as well as for immune system deficiencies (Boudreau M.D., et al,2006 Rahmani A.H., et al , 2015, Radha M.H., Laxmipriya N.P 2015) . The nutrients and phytochemicals that have been identified in Aloe plants include vitamins, minerals, enzymes, simple and complex polysaccharides, fatty acids, indoles, alkanes, pyrimidines, aldehydes, dicarboxylic acids, ketones, phenolic compounds, phytosterols, and alkaloids with potential biological and toxicological activities (Boudreau M.D., et al, 2006).

On a side note, A. vera gel coating has demonstrated postharvest preservative and stabilizing effects in some foods and beverages, and for instance, table grapes (Serrano M, et al, 2006, Ahlawat K.S., Khatkar B.S, 2015).

2.4. Antioxidant activity

Oxidative damage is a critical etiological factor implicated in several chronic human diseases including cardiovascular dys- function, inflammation, atherosclerosis, carcinogenesis, drug poi- sonousness, reperfusion damage and neurodegenerative diseases. Antioxidants play an important role in the prevention of chronic ailments such as heart diseases, cancer, diabetes, hypertension, stroke and Alzheimer's disease by protecting the cells from damage caused by free radicals, highly reactive oxygen compounds (S.A. Bakasso,et al, 2008). Thus, antioxidant compounds can be used to counteract oxidative damage by reacting with free radicals, chelating free catalytic metals and also by acting as oxygen scavengers (A. Nagarajan, M. Sellamuthu, 201

Anti- oxidants play an important role in inhibiting and scavenging free radicals, thus providing protection against infections and degenerative diseases (F. Annie Felicia, M. Muthulingam, 2012). Plants contain several phytochemicals which possess strong antioxidant activities (A. Senthilkumar, V. Venkatesalu, 2009). The antioxidant activity of natural and synthetic compounds and extracts of various natural sources including medicinal herbs are determined using various antioxidant methodologies (woidylo A et al, 2007, dudonne S et al, 2009, sahin H,2016 and Topal M et al, 2016).

Antioxidant assays may be classified based on the type of antioxidants measured (lipophilic or hydrophilic, enzymatic or nonenzymatic), character of solvent (aqueous or organic), type of reagent (radicalic or non-radicalic), and mechanism of reaction (hydrogen atom transfer, HAT; electron transfer, ET (Huang D et al, 2005). ET-based assays include 2, 2-diphenyl-1-picrylhydrazyl (DPPH–) scavenging (Brand-william W et al, 1995).

DPPH assay is one of the most popular and frequently employed methods among antioxidant assays. The method is simple, efficient, relatively inexpensive, and quick. However, as with most antioxidant assays, it require a UV–Vis spectrophotometer. DPPH_ method is developed by Blois (Blois MS, 1958). DPPH is a stable free radical which possesses a deep purple colour and a strong absorption around 517 nm. The antioxidant compounds present in the medium convert DPPH_ radical to a more stable DPPH molecular product by donating an electron or a hydrogen atom. The colour change from purple of DPPH radical to pale yellow of reduced form of DPPH allows the spectrophotometric determination of the antioxidant activity.

Antioxidants from trees have been also measured. Phenolics from almond hulls (Prunus amygdalL.) and pine sawdust (Pinus pinaster L.) have been extracted employing various methods in order to determine the gram fresh yield of polyphenol compounds andantioxidant activity (Barla et al, 2007). The antioxidant activity is measured by the DPPH radical scavenging method. The results showed that methanol was most appropriate either for phenolics or any bioactive compounds, while methanol was more selective for extracting polyphenolics

2.5. Active phytochemicals in medicinal plants

These are primarily secondary metabolites and include alkaloids, phenolic and polyphenols, steroids, tannins, saponins, terpenoids, and flavonoids

2.5.1 Phenols

Phenolic compounds are important plant secondary products that are non-nutrients, but are useful for the plant's defense against foreign bodies. These phenolic compounds also have antioxidant properties that enable them to quench free radicals in the body. It is probable that herbal remedies contain active flavonoids. Their capability to interact with protein phosphorylation and the antioxidant, iron chelating, and free radical scavenging activity may account for the wide pharmacological profile of flavonoids. These include vasoprotective, anticarcinogenic, antineoplastic, antiviral, anti-inflammatory, antiallergic, antiproliferative activity on cancer cells, antimicrobial activity and hepatoprotective activity (Jain et al., 2006; Li et al., 2007).

2.5.2. Terpenoids

This refers to compounds with basic skeletons derived from mevalonic acid or closely related precursor. The fragrance of plants is carried in the so-called quinta essential, or essential oil fraction. The oils are based on iso-prene structure.

2.5.3 Alkaloids

These are heterocyclic nitrogen compounds. They are basic in nature and contain one or more nitrogen atoms. They have pronounced pharmacological actions in animals and man. Morphine was first use full alkaloid to be isolated in 1805. They are distributed in various parts of plants in different quantities including the leaves, bark, fruits, seeds, aerial parts, roots and rhizomes. They include diterpenoid alkaloid, glycoalkaloid and berberine. Alkaloids have antimicrobial activity and smooth muscle contractility (Rattmann et al., 2005).

2.5.4 Tannins

These are complex substances containing mixture of polyphenols and are widely distributed in plant kingdom. They are protective and most tannins have molecular weight of 1000-5000. They are subdivided into hydrolysable and condensed (proanthocyanidins) tannins. Hydrolyzable tannins (HT) are polymers esterified to a core molecule, commonly glucose or a polyphenol such as catechin. Hydrolyzable tannins are potentially toxic to ruminants. Proanthocyanidins (condensed tannins) are relatively stable in the digestive tract of the animal, and rarely have toxic effects. They have antiparasitic properties (Githiori, 2004).

2.8. Phytochemical screening

The subject of phytochemistry has developed in recent years as a distinct discipline. It is concerned with the enormous variety of organic substances that are elaborated and accumulated by plants and deal with chemical structures of these substances, their biosynthesis, turnover and metabolism, their natural distribution and their biological function. Ideally, fresh plants tissues should be used for phytochemical analysis (Harborne, 1973). However, alternative methods have been developed where the plant material is air dried under a shade, crushed into powder and subjected to solvents extraction. The acquired extract is then subjected to chemical test to identify the constituents using standard procedures (Sofowora, 1993).

2.9. TEST MICROGANISMSFOR ANTIBACTERIAL ACTIVITY

2.9.1. Escherichia coli

Escherichia coli are normal flora in the body of human beings and they can be non-pathogenic, commensal or pathogenic (Kaper et al., 2004). When pathogenic they usually cause uinary tract infections, systematic infections and enteric infections (Mandell et al., 2005). The development of resistance by Escherichia coli due to increasing in the use of antimicrobial agents has led to the use of medicinal plants extracts against it (Akram et al., 2007). Medicinal plant extracts have shown to

have antimicrobial activity against enteropathogenic Escherichia coli found in food material (Fullerton et al., 2011).

2.9.2 Salmonella typhi

Salmonella typhi is a Gram-negative bacteria pathogen that causes gastroenteritis in humans (Ibarra and Steele, 2009). Studies carried out have shown that herbal extracts and dietary spices from medicinal plants have antimicrobial activity against Salmonella typhi (Shan et al., 2007). Other studies have shown that herbal extracts from medicinal plants not only have antimicrobial activity on Salmonella typhi found in vegetables but also against other disease-causing bacteria pathogens such as *enteropathogenic Escherichia coli and Listeria monocytogenes (Cutter, 2000).*

2.9.3 Staphylococcus aureus

Staphylococcus aureus is Gram-positive bacteria that cause diseases such as skin and soft tissues infections as well as food poisoning and toxic shocks (Perez et al., 2009). The increasing use of antimicrobials against Staphylococcus aureus has led to the development of resistance hence needs to develop new antimicrobial agents (Kwon et al., 2007). Medicinal plant extracts have shown a wide range of antimicrobial activity against both bacterial and fungal pathogens (Manvi et al., 2010). Studies carried out have shown that some edible plants extracts also have antimicrobial activity against Staphylococcus aureus (Alzoreky et al., 2003). Other studies carried out have shown a great synergistic activity of plant extracts and spices when used against not only pathogenic, probiotic and food spoilage pathogens such as *Staphylococcus aureus*, *Salmonella typhi, Escherichia coli* and other bacteria organisms, both Gram positive and Gram negative (Das et al., 2012).

2.9.4 Candida albicans

Candida albicans is a normal microbiota mainly found in the mucosal cavity, vagina and gastrointestinal tract of an individual (Shao et al., 2007). They are a yeast-like

fungus that are commensals in healthy humans but can cause systemic infections in immunocompromised individuals (Pfaller et al., 2007). The incidence of fungal infections has led to increased antimicrobial resistance hence making a few antifungal agents active (Arendrup et al., 2005; Espinel - Ingruff et al., 2009). There has been an increase in resistance by Candida albicans to conventionally produced antimicrobials recently, leading to the search of a new antifungal agent (White et al., 1998; Sardi et al., 2011).

2.9. The role of UV-Vis spectrometer in analysis

UV-Vis spectroscopy is a sensitive method in molecular spectroscopy that uses ultraviolet and visible light in the wave length range between 200 and 780nm (Schmid.F, et.al 2001). The Beer–Lambert law describes the correlation between light absorption by the molecule, the light path length of the sample, and the concentration of the absorbing molecules in the liquid medium (Schmid.F, et.al 2001).

3. MATERIALS AND METHODS

3.1. Plant materials

Fresh leaves of *Aloe ankobernsis* were collected from Ankober, which is located in North Shoa Zone, Amhara Regional State, Ethiopia in March, 2020. The plant material was identified and authenticated by a botanist Dr. Abyou Tilahun, Department of Biology, Debre Berhan University.

3.2. Chemicals and reagents

The chemicals and reagents used for this study were distilled water, chloroform, ethanol, hexane, ehyl acetate, 10% ferric chloride, Wagner's reagent (Iodine in potassium iodide), hydrochloric acid (HCl), sulfuric acid (H_2SO_4), 2%sodium hydroxide (NaOH), iodine, 5% iron chloride, Ascorbic acid, DPPH, methanol.

3.3. Instruments and apparatus

The necessary apparatus and instruments used for this study were electronic beam balance, vacuum rotary evaporator, electrical shaker, UV–Visible spectrophotometer, volumetric flask, beaker, round bottom flask, Whatman No.1 filtrate paper, aluminum foil, micropipette, incubated agar, cuvette, Erlenmeyer flask, test tube, measuring cylinder, petridish, micropipette tips, spreader, laminar air flow hood, forceps, autoclave were used for different purposes

3.4. Preparation and Extraction of the plant materials

The collected and identified fresh leaves of *Aloe ankobernsis* were washed and air dried at room temperature for a week under a shad until it became well dried for grinding. The dry plant materials was taken separately and ground to a powdered. One hundred grams of leaf powder were macerated separately in four Erlenmeyer flasks using ethanol, ethyl acetate, chloroform, and hexane as extraction solvents. The flasks were closed with aluminum foil and shaken thoroughly for 72 hour on electrical shaker. Then, the extracts were filtered using Whatman No.1 filter papers, the filtrates evaporated to dryness under pressure in a rotary evaporator

and weighed. The extracts were stored in closed and dark place for the next experiment.

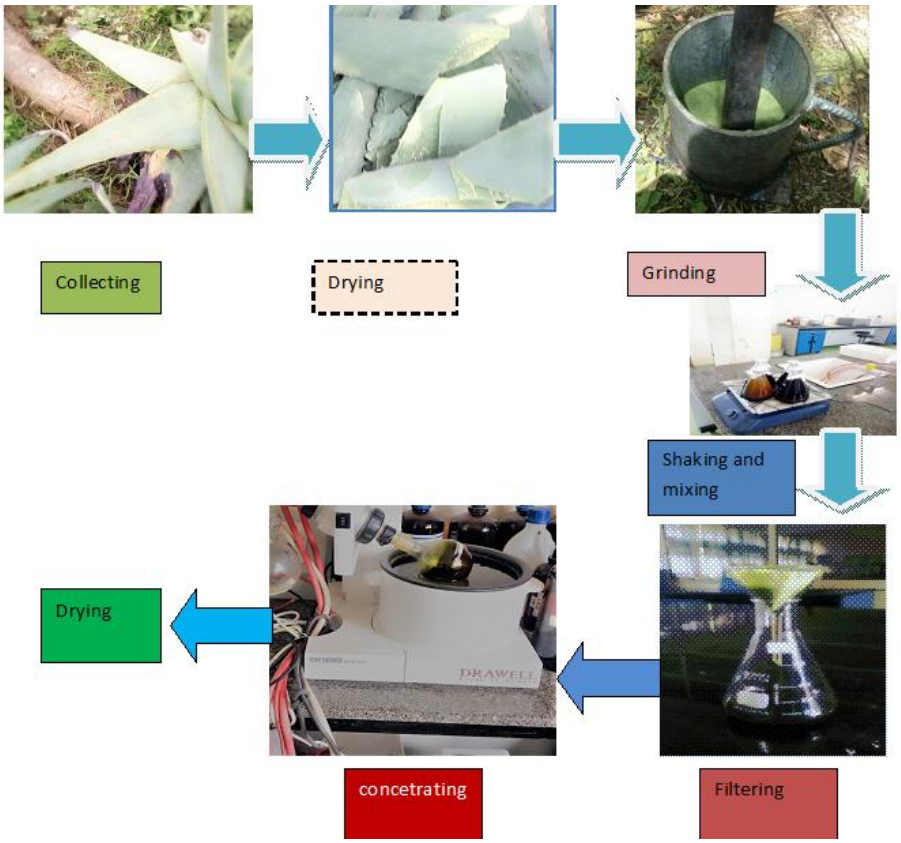


Figure 3.1: Preparation and extraction of *Aloe ankoberensis*

3.5 Anti-microbial activity by Disc Diffusion Method

The antimicrobial test was carried out at Debre Behan University, Microbiology laboratory. The antimicrobial activity for different extracts was determined by the disc diffusion method (Bauer et al., 1966). Both Gram positive (*Staphylococcus aureus* and *Listeria monocytogenes*) and Gram negative bacteria *Escherichia coli* and *Salmonella typhimurium*) and yeast (*Candida albicans*) , were used for the test. All materials used for antimicrobial activities (Petridish, flask, forceps, swaps, beakers, loop) were first sterilized by autoclaves at 121 ^{0}C for 20 minutes. Nutrient agar media was prepared by mixing agar powder with distilled water in 100 ml flask. The growing media Muller Hinton Agar was prepared as follows.

Muller Hinton Agar: This media was prepared by dissolving 3.9 g powder of Muller Hinton agar in 100 ml of volumetric flask and distilled water. Then gently heated and bring to boiled using heating mantle. Finally sterilized using autoclave at 15psi pressure at 121℃ for 15 minutes.Finally activated microbes in nutrient agar were transferred and distributed evenly in to the sterilized Petri dish containing Muller Hinton agar for bacteria by swapping method. In this Petri dish, solutions of plant extracts with concentration (100, 200µg/ml) were introduced by paper disk method (Lalas.S.et al.,2012). The standard antibiotic, chlora amphenicol,was used as a positive control applied to the center of the agar plate and DMSO as negative control. These plates were then incubated at 37ºC for 24 h to allow maximum growth of the organisms. The test materials having antibacterial activity on the growth of the microorganisms showing a clear, distinct zone of inhibition was visualized surrounding the medium.The antimicrobial activity of the test agents was determined by measuring the diameter of zone of inhibition expressed in millimeter. The diameter of inhibited zone formed was compared with the standard drug.

3.6. Preparations of stock solutions, reagents and extracts

Ferric chloride (5%): Dissolved 5 g of ferric chloride solution in 100ml of distilled water.

Lead acetate (10%, w/v): Dissolved 10 g of lead acetate in 100 ml of distilled water.

DPPH reagent: 0.04mg/ml DPPH solution is prepared by adding 4 mg of DPPH in to 100 ml of methanol in volumetric flask.

Wagner’s reagent: 2 g of iodine powder was measured and mixed with 6 g of potassium iodide (KI) salt. Then the mixture was transferred into 100mL volumetric flask and filled with distilled water up to the mark.

Ascorbic acid (800µg/ml): prepared by dissolving 0.01 g of ascorbic acid with methanol in 100 ml of volumetric flask and methanol was added up to the mark. Similarly, extracts of hexane, ethyl acetate, chloroform and ethanol (800µg/ml)

were prepared by dissolving 0.01 g in methanol in a separate 100 ml of volumetric flask.

3.7 Antioxidant capacity assay (DPPH free radical scavenging activity)

The DPPH free radical scavenging activity was assayed according to the method reported (Nagananda, et al., 2013). Diluted solutions of ascorbic acid were taken in the following concentration range (50, 100, 200 and 400, 800µg/ml) in each test tubes and make up the volume up to 2 ml using solvent, methanol. Then 2 ml of 0.04% DPPH was added into each test tube. The mixture was shaken well and incubated at room temperature for 30 min at dark place and absorbance was measured at 517 nm in spectrophotometer against solvent, methanol as blank and solvent with DPPH as control. The experiments were performed for all ethanol, ethyl acetate, chloroform and hexane extracts.

The radical scavenging activity of samples corresponded to the intensity of quenching DPPH. The results were expressed as percentage inhibition (Shekhar,TC. and Goyal, A.2014).

$\%\,\boldsymbol{RSA} = \frac{(\boldsymbol{Abscontrol - Abssample})}{\boldsymbol{Abscontrol}} \times \mathbf{100}$, Where, RSA is the Radical Scavenging Activity

Abs control is the absorbance of DPPH radical + methanol

Abs sample is the absorbance of DPPH radical + plant extract.

3.8. Phytochemical screening test

Phytochemical screening tests for alkaloids, steroids, terpenoids, flavonoids, tannins, and saponins were carried out as described below (Sofowora, 1993; Harborne, 1973; Ogbuewu, 2008) using ethyl acetate,chloroform,hexane and ethanol extracts of Aloe ankoberensis.

3.8.1. Test of saponins

5ml of extract was shaken vigorously with 5ml of distilled water in a test tube and warmed. The formation of sable foam, honey comb in shape, was taken as an indication for presence of saponins.

3.8.2. Test for flavonoids

To 1ml of extract, 1ml of 10% lead acetate solution was added. The formation of a yellow precipitate was taken as a positive test for flavonoids.

3.8.3 Test for terpenoids

2ml of the organic extract was dissolved in 2 ml of chloroform and evaporated to dryness. 2 ml of concentrated sulphuric acid was then added and heated for about 2 min. A greyish colour indicates the presence of terpenoids.

3.8.4. Tests for steroids

A red colour produced in the lower chloroform layer when 2 ml of the extract dissolved in 2 ml of chloroform and 2 ml concentrate sulphuric acid added in test tube indicates the presence of steroids.

3.8.5. Test for alkaloid (Wagner's test)

A few drops of Wagner's reagent were added to few ml of plant extract along the sides of test tube. A reddish- Brown precipitate confirms the test as positive. (wanger.H. 1993).

3.8.6. Test for phenolic compound (ferric chloride test)

Few extract was dissolved in 5 ml of distilled water. To this few drops of neutral 5% ferric chloride solution was added. A dark green colour indicates the presence of phenolic compounds (Mace.M.D.1963).

3.9. Analytical methods

Calibration curves of standards were constructed to analyze antioxidant activity (radical scavenging percentage) of extracts where a linear equation is used to calculate the value using the formula

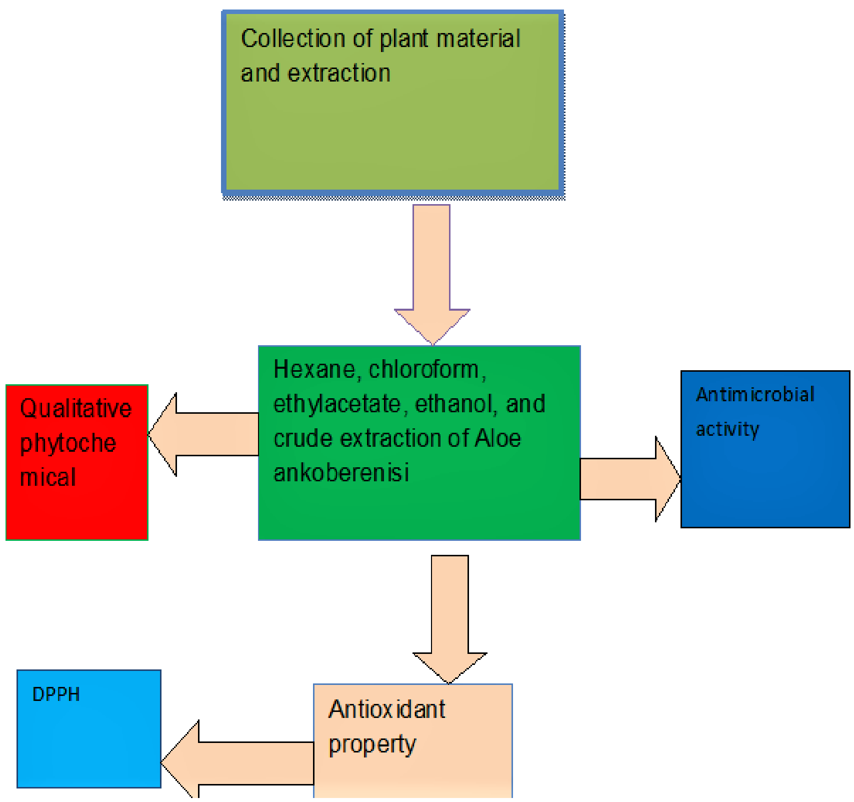
Y=ax+b……………………………………………………………………1

Where, a = slope, b = y-intercept

$$\% \textit{Inhibition} = \frac{(Ac - As)}{Ac} X100 \ldots\ldots\ldots\ldots\ldots\ldots \quad 2$$

Where Ac is the absorbance of the control and As is the absorbance of the sample.

3.10. Over all method of the experiment



Figre 3.2. Over all method of the extraction

4. RESULTS AND DISCUSSION

In this study the antimicrobial and antioxidant activities of the extracts of the leaf of *Aloe ankoberensis* were evaluated and the phytochemical components of each solvent crude extracts were identified

4.1. Percentage yield of the extracts

The percentage yields of hexane, chloroform, ethylacetate, and ethanol extracts of *Aloe ankoberensis* leaves were 0.426 g (0.426%), 0.506 g (0.506%), 0.669 g (0.669%), and 4.022 g (4.022%), respectively (Table:4.1, figure 4.1). Ethanol extract gives more yield than that of hexane, chloroform and ethylacetate. Hexane extract gives lower yields than ethylacetate, chloroform and ethanol extracts. These results showed that more polar solvents extract more polar compounds and gives high yields but non polar solvents cannot extract polar compounds and as the result gives low yields.

Table 4.1 Percentage yield of the extracts

Solvent	Mass of the sample (g)	Extract Mass (g)	Percentage Yield (%)	Color
Hexane	100	0.426	0.426	Dark
Chloroform	100	0.506	0.506	Brown
Ethylacetate	100	0.669	0.669	Brown
Ethanol	100	4.022	4.022	Brown

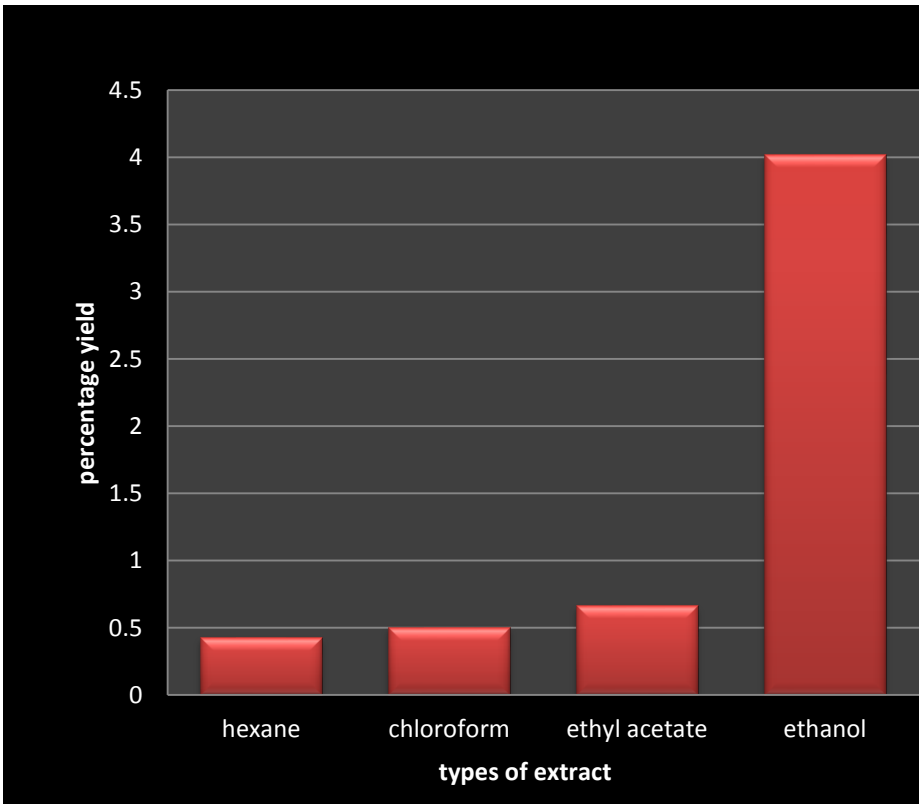


Figure 4.1 Percentage yields of extracts of aloe ankoberensis

4.2. Antimicrobial activity test of leaf extract of Aloe ankoberensis

The present study carried out to evaluate antimicrobial activities of various extracts of Aloe ankoberensis. The successive leaf extracts using hexane, chloroform, ethyl acetate and ethanol of *Aloe anokoberensis* were tested for their antimicrobial efficiency against pathogenic bacteria like (Gram positive *Staphylococcus aureus* and *Listeria monocytogenes* and Gram negative bacteria *Escherichia coli* and *Salmonella typhimurium*) and yeast (*Candida albicans*) at a dose of 200mg/ml and 100mg/ml. The standard drug used for comparison was chloramphenicol against bacteria and yeast**.** Among the samples the leaf extracts showed moderate to high activity against both Gram positive and Gram negative bacteria. The hexane, chloroform, ethyl acetate and ethanol extracts of Aloe ankoberensis showed better antimicrobial activity against *Staphylococcus aureus, Listeria monocytogenes,*

Escherichia coli and Candida albicans (Table 4.2, Fig 4.2).The ethyl acetate and ethanol extracts showed highest inhibition zone at higher concentration (200mg/ml). Overall the ethanol extracts showed greater inhibition of all pathogenic microorganisms used when compared to ethyl acetate, chloroform and hexane extracts.

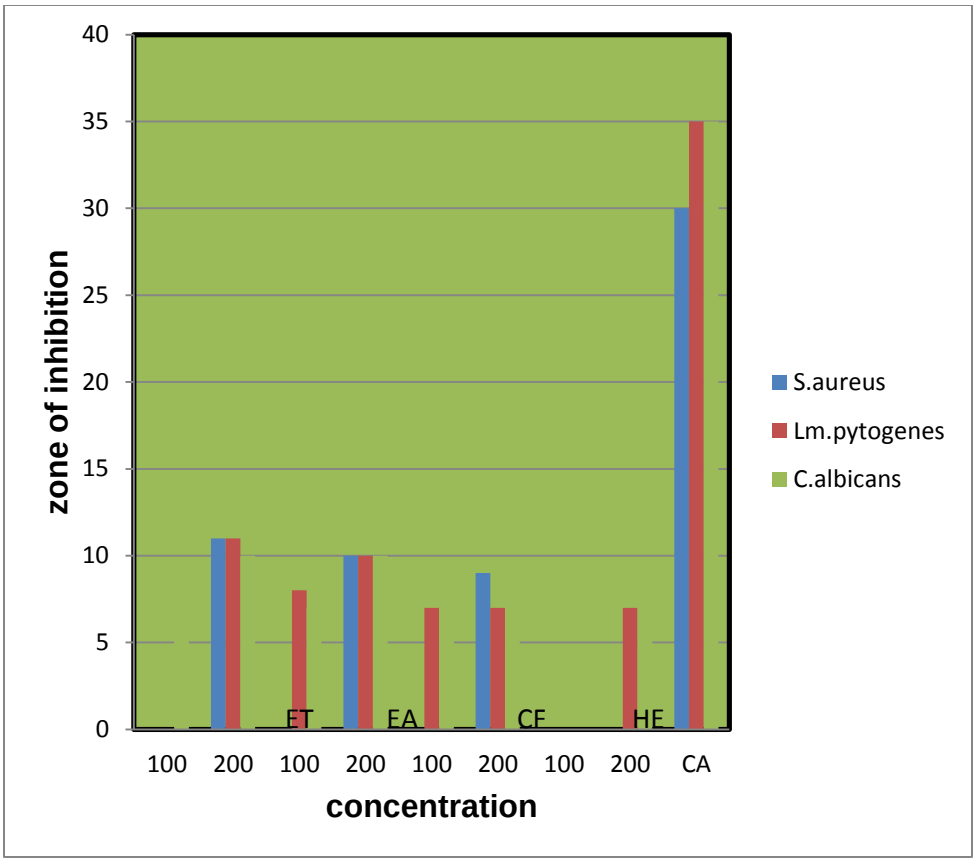
Table 4.2 Comparison of zone of inhibition among leaf extracts of *Aloe ankoberensis* and standards against bacteria and Yeast

Extractive solvent	**extract Concentration (mg/ml)**	**Zone of inhibitition (mm) against bacteria and Yeast**				
		Gram positive		Gram negative		Yeast
		S.aure	L.monocytogenes	E.coli	Sal	C,albicans
EA	200	11	11	13	-	10
	100	-	-	-	-	7
ET	200	10	10	10	-	10
	100	-	8	-	-	7
CF	200	9	7	8	-	8
	100	-	7	7	-	7
HE	200	-	7	-	-	7
	100	-	-	-	-	-
Chloramphenicol	0.1g/ml	30	35	18	10	35

Higher inhibition zone was recorded in ethanol extract than ethyl acetate, chloroform and hexane extracts of Aloe ankoberensis in the control of *Staphyloccus aureus, Listerial monocytogenes* and *Candida albicans* (Table 4.2,

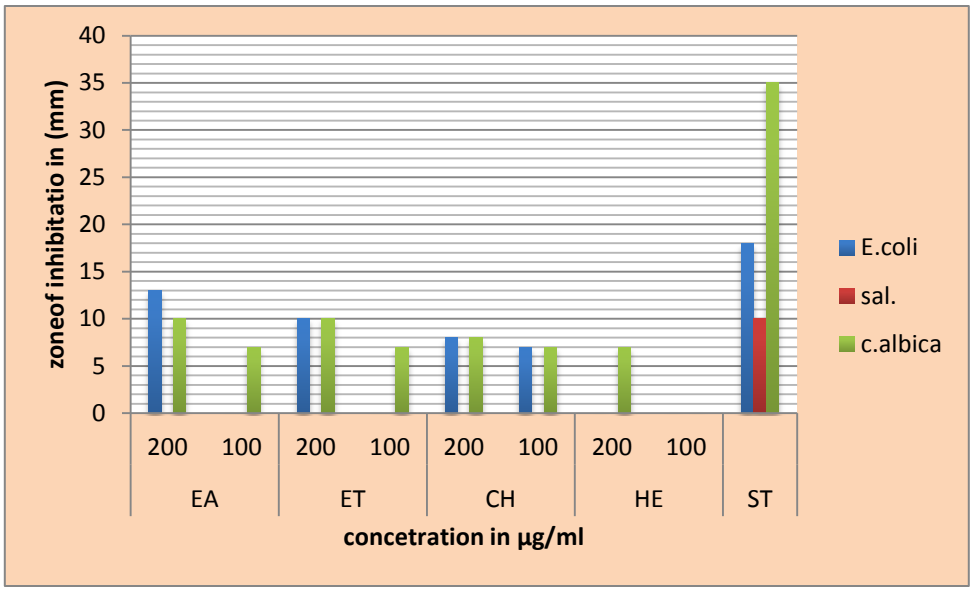
Fig 4.2). Ethanol has the higher inhibition zone recorded than ethyl acetate, chloroform and hexane extracts of *Aloe ankoberensis* against *Escherichia coli*. But, hexane extract has the lower inhibition zone than ethanol, ethyl acetate, and chloroform extracts of the plant. All extracts had lowest antibacterial potential as compared with the standard antibiotic (chloroamphenicol). In this study a good antibacterial results were recorded at higher concentration. All extracts had no inhabitation zone recorded against *Salmonella typhimurium* (table 4.2, Fig 4.3) and *Candida albicans* potential.

Ethanol extract of *Aloe ankoberensis* indicated a good antibacterial activity against. *Staphyloccus aureus* and *Listerial monocytogenes* and *Escherichia coli* and, *Candida albicans* with zone of inhibition 11, 11, 13 and 10, respectively. Ethyl acetate extract of *Aloe ankoberensis* also showed a good antibacterial activity against *Staphyloccus aureus* and *Listerial monocytogenes* and *Escherichia coli* and, *Candida albicans* with zone of inhibition 10, 10, 10, and 10, respectively. Similarly, chloroform extract of *Aloe ankoberensis* also showed a medium antibacterial activity against *Staphyloccus aureus* and *Listerial monocytogenes* and *Escherichia coli* and, *Candida albicans* with zone of inhibition 9, 7, 8, and 8, respectively. But, hexane showed very low zone of inhibition. In general the results of this study indicated that ethanol extract has higher antimicrobial effect than that of ethyl acetate, chloroform and hexane extracts due to the presence of phenolic and steroids.



KEY: S.aureus= staphyloccous aureus, Lmpytogenes= listerial monocytogeges, C.albicans= candida albicans, ET= ethanol, EA= ethyl acetate, CF= chloroform, HE= hexane, CA= chloro amphenicol

Figure 4.2: Zone of inhibition of extracts and standard antibiotics against bacteria and yeast with different concentration**.**



KEY: E.coli = Escherichia coli, Sal. = salmonella typhimurium ,
C.albcans = candida albicans, ST =standard anbiotic

Figure 4.3: Zone of inhibition of extracts and standard antibiotics against the two gram negative bacteria and yeast with different concentration

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4.3. Antioxidant activity test

For the DPPH radical scavenging assay different concentrations of ascorbic acid (50, 100, 200, 400, and 800 µg/ml) were used to construct the calibration curve. The calibration curve was constructed as a function of absorbance versus concentration of ascorbic acid. The absorbance at 517 nm and the percentage inhibition of ascorbic acid was given below in Table 4.3.

Table 4.3: Absorbance and percent inhibition value of ascorbic acid (at 517 nm)

Concertation in µg /mg	Absorbance	%RSA(inhibitin)
50	0.1225	74.53
100	0.1145	76.2
200	0.1007	79.02
400	0.07389	84.64
800	0.02107	95.62

The equation obtained from the calibration curve of ascorbic acid (Figure 4.5) for DPPH radical scavenging assay was y = -0.00016x + 0.128 with linear regression coefficient (R^2) of 0.999.

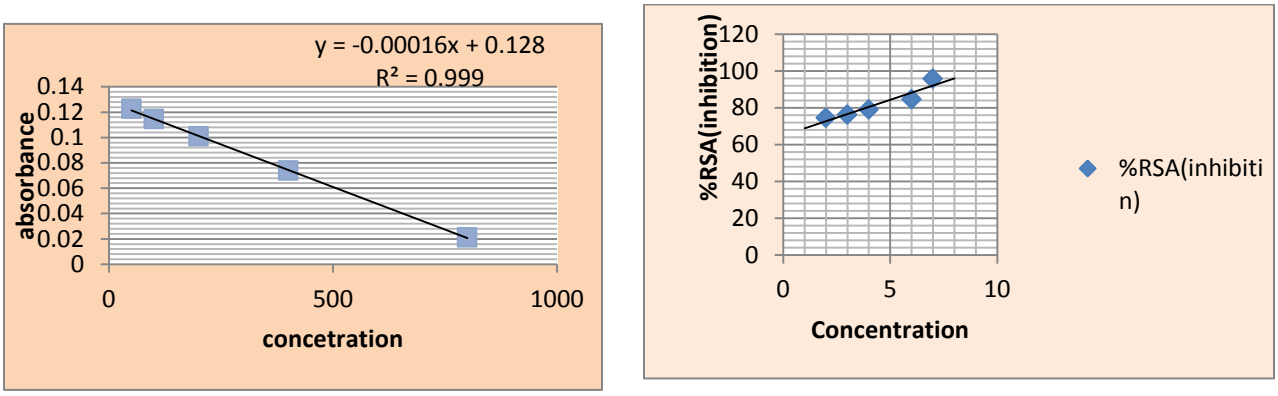

Figure 4.5 calibration curve of ascorbic acid for DPPH scavenging assay**.**

For DPPH antioxidant activity study different concentration of hexane, chloroform, ethyl acetate and ethanol leaf extract of Aloe ankoberensis (50, 100, 200, 400 and 800 µg/ml) were screened, and shows strong radical scavenging activities. The activities of the test sample in DPPH scavenging assay can be expressed as a decrease in absorbance.

Figure 4.5 and Table 4.3 showed that decreased in absorbance of the extract sample as the concentration of the each extract increased from 50µg/ml to800µg/ml respectively.

The change in absorbance of DPPH radicals caused by antioxidants is due to the reaction between the antioxidant molecules of the plant extract and the DPPH radicals. As the absorbance decreases, the free radical scavenging activity becomes high**.** The scavenging activities of the extracts of *Aloe ankoberensis* can be expressed using percentage inhibition of DPPH free radical using equation 2 and it was found that percentage inhibition increases with increasing plant concentration (Table 4.4). The data in the Table describes the percentage inhibition of DPPH free radicals of ethanol, ethyl acetate, chloroform and hexane extracts(50µg/ml to 800µg/ml) and have the value ranges from 83.73%-96.04%, 72.14%- 95.43%, 67.57%-91.64%, 42.61%- 90.47%, respectively. This result showed that ethanol extract has strong free radical scavenging activity than chloroform, ethyl acetate, and hexane extracts.

Table 4.4 Absorbance and percentage inhibitation of Aloe ankoberensis at 517 nm

Conc. (µg/ml)	Absorbance at 517nm				% RSA(inhibition)			
	ET extract	EA Extract	CH extract	HE extract	ET extract	EA extract	CH extract	HE Extract
50	0.059	0.13	0.16	0.28	87	72	68	43
100	0.049	0.12	0.12	0.15	90	74	76	70
200	0.038	0.1	0.11	0.09	92	80	77	82
400	0.021	0.04	0.08	0.06	95	92	84	87
800	0.019	0.02	0.04	0.05	96	95	92	90

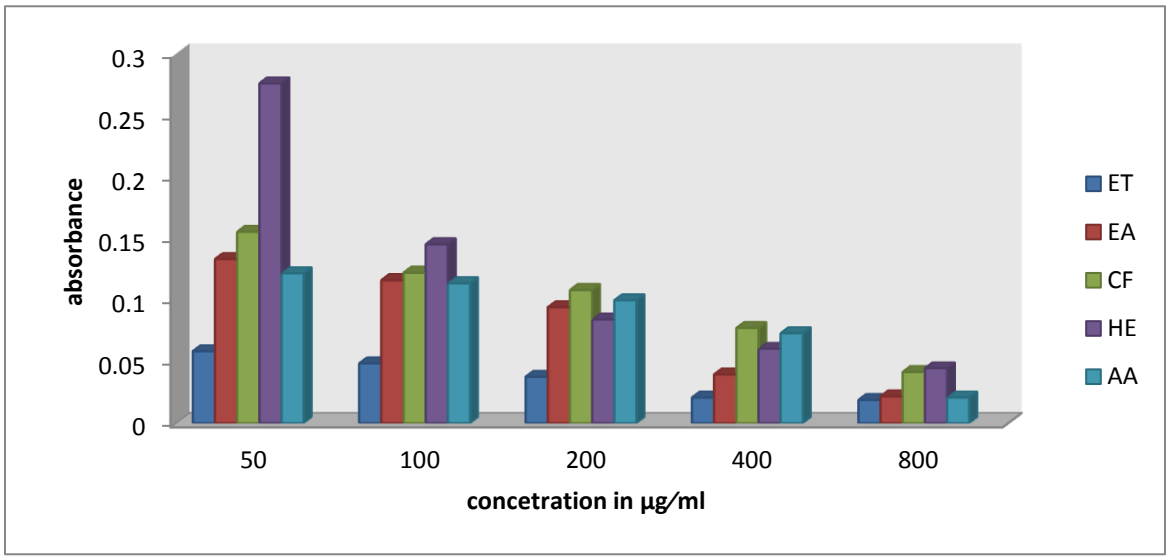

Figure 4.6 graph of DDPH scavenging activity of extracts and standard

4.4. Qualitative phytochemical analysis

The qualitative analyses of presence of bioactive compounds in the four solvent extracts have been done as follows. The presence and absence of useful bioactive substances such as tannins, flavonoid, phenols, terpenoids, steroid, alkaloids, and saponins in the leaf extracts of Aloe ankoberensis were revealed by the confirmatory test, involving color changes (Table. 4.5, Fig 4.7).

Table .4.5. Phytochemical analysis of leaf extracts of Aloe ankoberensis

Phytochemical	Reagent	Hexane	Chloroform	Ethyl acetate	Ethanol
Alkaloids	Wagner's test	+	+	+	+
Phenols	Ferric chloridetest	-	+	+	++
Steriods	Salkowski test	−	−	+	+
Flavonoid	lead acetate test	−	−	−	−
Terpenoids	Lieberman-burchard test	−	−	−	−
saponins	Foam test	−	−	−	++

key:- ++ = highly present, + = present, - = abscent

Most bioactive compounds highly found in ethanol extract and in ethyl acetate and chloroform extract in smaller amount but most bioactive compounds are not found in hexane extract. Because polar solvents like ethanol can extract almost all bioactive compounds and hexane being highly non-polar in natureit was able to extract very limited compounds, thus it showed negative results in most qualitative test of bioactive compounds (Table 4.5). This result indicated that the extraction of bioactive compounds from plant material is largely dependent on the type of solvent used in extraction steps.

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This study showed the importance of Aloe ankoberensis as antioxidant and antibacterial agent. The ethanol extract of Aloe ankoberensis showed higher percentage yield of the extracts than ethyl acetate hexane, and chloroform. The ethanol extract of Aloe ankoberensis showed higher free radical scavenging properties than ethyl acetate; chloroform and hexane extracts. The antimicrobial activity for ethyl acetate and ethanol extract which are comparable to the other two extracts (hexane and chloroform). Generally ethanol was higher in percentage yield, antioxidant, antibacterial activities, and in cotrolling Candida albicans than ethyl acetate hexane, and chloroform due to the presence of saponins and phenolics.

5.2 Recommendation

This study needs further investigations to identify and isolate individual compounds from Aloe ankoberensis which are responsible for the bioactivity studied.

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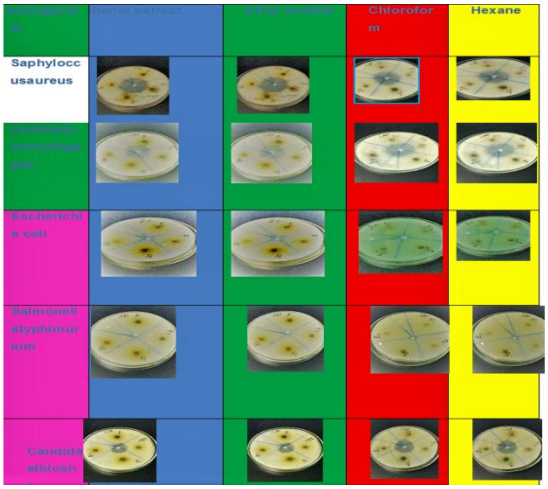
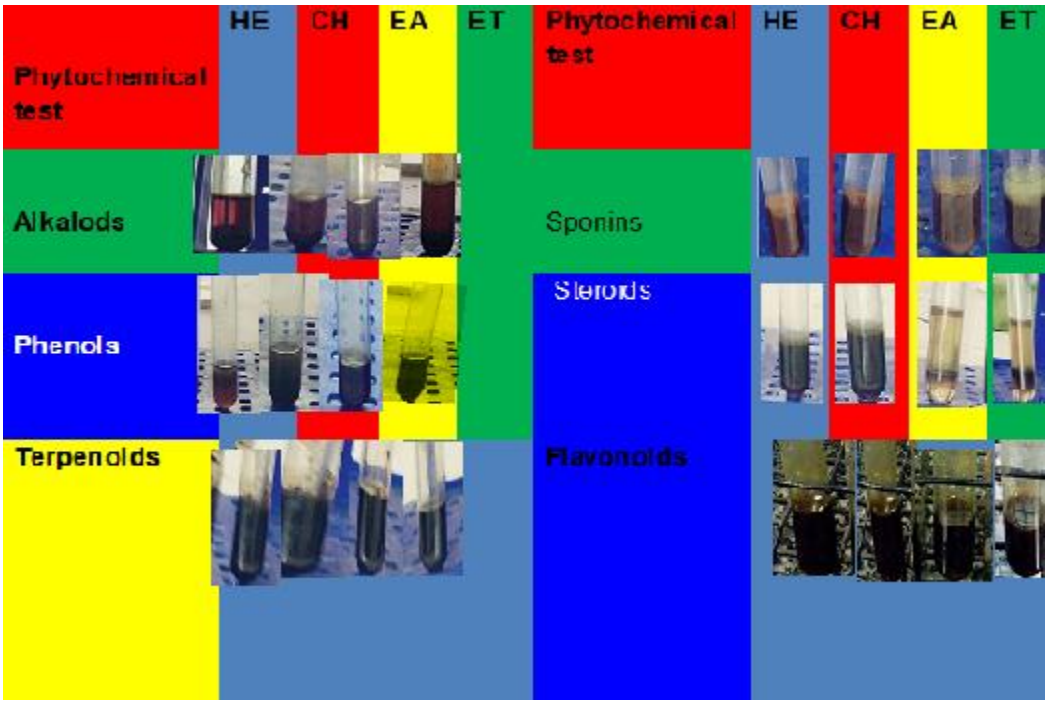
7. APPENDIX



Key: - D1, E1, F1, and G1 = 100µg/ml , D, E, F, and G = 200µg/ml of extracts and white at the center positive control (= chloro amphenicol)

Figure 4.4:- Comparison of zone of inhibition between extracts and standard.



Key: **HE=hexane, CH = chloroform, EA= ethyl acetate, ET= ethanol**

Figure 4.7Colorobserved in some phytochemical analysis tes