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Extraction, Optimization and Characterization of Aloe Vera Oil from Aloe vera leaf and Evaluate Antimicrobial Activity

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This is to certify that the thesis prepared by *Nimona Adugna*, entitled: *Extraction, Characterization and Optimization of aloe vera oil from aloe vera leaf and evaluate its microbial activity* and submitted in partial fulfilment of the requirement for the degree of Master of Science Chemical Engineering (Process Engineering) complies with the regulations of the University and meets the accepted standards.

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DECLARATION

I declare that, this thesis entitled "*Extraction ,Optimization and Characterization of aloe vera crude oil from aloe vera leaf and evaluate its antimicrobial activity"* is my original work and has not been done by any other person for an award of a degree anywhere else before or not part of ongoing work in this or any other University.

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We have agreed to supervise the thesis research. We have evaluated the content of the thesis and found to be satisfactory, complete according to the standards and format of the university

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LIST OF ACRONYMS AND ABBREVIATIONS

AAS	Atomic Absorption Spectrophotometers
ANOVA	Analysis of Variance
AOAC	Association of Official Analytical Chemistry
CCD	Central Composite Design
FCCCD	Face Center Central Composite Design
GC- MS	Gas Chromatography Mass Spectrophotometer
GDP	Gross Domestic Product
MAE	Microwave Assisted Extraction
NASA	National Aeronautics and Space Administration
OFAT	One Factor at Time
PFE	Pressurized Fluid Extraction
PMAE	Pressurized Microwave-Assisted Extraction
PLE	Pressurized Liquid Extraction
RNS	Reactive Nitrogen Species
ROS	Reactive Oxygen species
RSM	Response Surface Methodology
FC	Supercritical Fluid Chromatography
SFE	Supercritical Fluid Extraction
SFMAE	Solvent-Free Microwave-Assisted Extraction
SOD	Superoxide Dismutase
TCM	Traditional Chinese Medicine

ABSTRACT

Aloe Barbadensis Miller, commonly known to as aloe Vera, is belonging to family Liliaceae of aloe, one of more than 400 species that originated in South Africa, but have been indigenous to dry subtropical and tropical climates, including the southern USA. The objective of this research work was extraction, optimization and characterization of aloe Vera oil as well as evaluation of antimicrobial effect. The extraction was carried out using Soxhlet extraction method and separated from solvents by using rotary evaporator. The proximate analysis of Aloe vera leaf was revealed that of moisture content 94.72±0.13%, proteins 10.86±0.06%, crude fiber 70.56±0.33% fat content 3.0±0.08%, and ash content 12.88± 0.04%. The minerals analysis were indicated that potassium is the dominant one then the sodium, magnesium, iron and zinc in aloe vera leaves. Response Surface Methodology with composite center design was employed to optimize the extraction temperature (A), aloe vera meal to solvent ratio (B) and extraction time(C) and 20 experimental runs were performed for aloe vera oil extraction. As a result, a polynomial regression model equation was fitted as follows: *Yield = 20.0182 -1.025 * A + 0.85 * B + 0.825 * C -1.53125 * AB + 0.96875 *AC -5.92045 * A^2 -1.04545 * B^2 - 1.42045 * C^2. The optimal extraction temperature, aloe vera powder to solvent ratio and extraction time were found to be 74 ºC, 1:11.6mg/ml and 4.27 hours respectively. The maximum oil yield was found to be 20.413%. Physiochemical property of aloe vera extracted oil at optimum conditions was specific gravity, pH, kinematic viscosity, density, acid value and saponification value were analyzed by ASTM method. FT-IR analysis was used for the identification of the functional group of carbohydrate, carbonyl, alkene, aromatic, alkane, aliphatic amine, carbocyclic, alcohol and methyl ester. Gas chromatography-Mass spectroscopy analysis indicated the presence of major free fatty acids linoleic acid, oleic acid, palmitic acid and stearic acid. The aloe vera oil also have secondary metabolites of alkaloids, anthraquinones, flavonoid, saponins, phenolic compounds, tannins and Terpenoids existence were showed good antimicrobial activity against of gram positive bacteria of Staphylococcus aureus and Bacillus subtiles; gram negative bacteria of Pseudomonades aerginosa and Salmonella typhus; fungus of Aspergillus Niger and Candida albicans.*

Keywords: Aloe Vera; Extraction; Response Surface Method; Antimicrobial.

1. INTRODUCTION

1.1 Background

The number of people using traditional or alternative medicine is increasing rapidly all over the world. According to the World Health Organization, around 65% of the world's population have incorporated plant medicinal agents into their primary aspects of healthcare. Traditional medicine often includes herbal medicines, which consist of biologically active compounds from plant materials, or whole plants. The key reasons behind using plants as sources of therapeutic agents include: isolation of bioactive compounds for direct use as drugs, producing bioactive compounds of new or known structures as references for the synthesis of entities with higher activity and/or lower toxicity, using agents as pharmacological tools and using the whole plant or a certain portion of it as herbal remedy. Consumer interest in herbal and alternative medicines arise from the fact that they consider these products to be both safe and effective, and this has prompted scientists to investigate the numerous bioactive compounds available in plants (Fabricant and Farnsworth, 2001).

However, there is another important reason behind people turning to natural sources in search of compounds with potent antimicrobial activities these days-the alarming rate at which microbial resistance to synthetic antibiotics is growing. Owing to the side effects and resistance that pathogenic microorganisms develop against common, commercially available antibiotics, more attention is being paid to extracts and bioactive components that can be isolated in herbal medicine (Essawi and Srour, 2000). Antibiotic and multi-drug resistance is now a worldwide problem in hospitals, long-stay residential centers and also in the community (Livermore, 2000). A deficiency in new drugs, vaccines and diagnostic aids is also recognized as a major problem in the management of drug resistant infections(Finch and Hunter, 2006). To address all these shortcomings, a significant number of new therapeutics is being derived from natural sources such as plants, as systemic and topical novel drugs and antiseptics to replace or to be used in collaboration with existing products (Woodford, 2005).

Many plant materials used as traditional medicine have been proven to be more effective, and relatively cheaper than their modern counterparts(A *et al.*, 2008). Antimicrobials of plant origin also alleviate many of the side effects that are often associated with synthetic ones(Mukherjee and Wahile, 2006).

Aloe Vera has been used by mankind for thousands of years in folk medicine for healing properties especially on skin. Aloe Vera is one of the oldest known and its first recognized use by humans dates back to an Egyptian papyrus from 3500 BC. Aloe Barbadensis Miller, commonly known to as aloe Vera, is belonging to family Liliaceae of aloe, one of more than 400 species that originated in South Africa, but have been indigenous to dry subtropical and tropical climates, including the southern USA (Reynolds and Dweck, 1999). Aloe Vera is a succulent plant that are xerophytes, Storage tissue has higher water holding capacity ranging from 99-99.5% and 0.5-1.0% solid material has over 75 different potentially active compounds including water and fat-soluble vitamins, minerals, enzymes, simple and complex polysaccharides, phenolic compounds, and many investigators have endeavored to establish the active principles in aloe vera gel (Hamman, 2014).

Today, the aloe Vera industry is rising and the gel is practiced in many products such as fresh juice, gel and other formulations for medicinal, health and cosmetic purpose. The aloe Vera industry claims credible testing regulation to examine the quality and quantity of bioactive chemicals appeared in the most products (Bozzi *et al.*, 2007). The product demands must be tested by exquisite clinical trials, verified and certified by the government regulatory authorities to manufactured consumer confidence and safety of the aloe Vera products. The plant can be produced in drought prone areas and it is a hardy perennials tropical plant whose dynamic is yet to be utilized, despite being known as a fresh crop resource with the foremost promising expectation in the earth.

There are various extraction methods used in the manufacture and extraction of oil and the method used is normally dependent on what type of material is being used. So one of the method is a soxhlet extractions and used for isolation of components in the form of soluble compounds.

This method converts the volatile liquid into a vapor and then condenses the vapor back into a liquid and method of separating mixtures based on differences in their volatilities in a boiling liquid mixture.

Solvent extraction method is used to extract the odoriferous lipophilic material from the plant that use solvents, such as petroleum ether, methanol, ethanol, or hexane. The solvent pull out the chlorophyll and other plant tissue, resulting in a highly colored or thick/viscous extract. The

first product made was concrete that is concentrated extract that contains the waxes and/or fats as well as the odoriferous material from the plant(Joseph and Raj, 2010).

1.2 Statement of the Problem

In Ethiopia, traditional medicines represent not only part of the fight of the people to fulfill the drug essentiality needs but also they are primary components of the cultural beliefs and attitudes. It is customary to find medicinal plants in markets where food items and spices are sold. The dried or fresh leaves, of flowers, roots, bark, seeds of medicinal plants are displayed for sale in most markets in Ethiopia along with spices like pepper, cardamom, ginger but some of the medicinal plants are sold in markets include fumigation, vermifuge, pain relief, treating skin infections were commonly used for antimicrobial and wound healing plants that major medicinal plants that are commonly available in markets (Tadeg *et al.*, 2005).

Infectious disease resulting from the presence of pathogenic microbial agents including bacteria and fungi have become a major healthcare problem an the main reason of deaths in developing countries. Incidence of new and re-emerging infectious diseases and development of resistance to antibiotic is alarmingly increasing. There is need of time to discover new antimicrobial compounds with different chemical structures and novel mechanism of action. Diverse antibiotics of synthetic and microbial origins have been produced. Indiscriminate use of antimicrobial drugs has created very dangerous drug resistance to microbial strains; many bacterial strains have developed resistance against antibiotics, like methicillin resistant Staphylococcus aureus. Due to the development of bacterial super resistant strains currently used antibiotic failed to cure the infectious diseases. The solution of antibiotic resistance is the development of new drugs from synthetic or natural sources. Therefore, discovery of new antibiotic sources that can act either by direct antimicrobial activity or by preventing resistance of microorganism with minimal side effects is emerging and paramount needed (Taukoorah *et al*., 2016; Arya, 2017).

Now a today; rise of new diseases, re-emergence of old, development of resistant strains, side effects of some currently available drugs including toxicity and other undesirable effects in allergic patients are major difficulties which require immediate attention to combat diseases with effective drugs of high therapeutic index. Researchers turned their attention towards herbal products, which is most promising area in research of new biologically activity

compounds with better activity against multi drug resistant strains and reduced antibiotic related side effects.

Thus, this research has been aimed to solve problems associated with pathogenic microorganisms by the application of natural oil extract, optimize and characterize from Aloe vera leaf.

1.3 Objective of the Research

1.3.1 General Objective

The main objective of this study was to extract, optimize and characterize the Aloe vera oil from Aloe Vera leaf and evaluate its microbial activity.

1.3.2 Specific Objectives

- To characterize proximate analysis of Aloe Vera leaf
- To study the effect of extraction parameters in order to optimize oil yield through RSM
- To characterize physiochemical properties and chemical composition analysis of oil extract
- To evaluate activity of crude extract oil of aloe Vera leaf on selected microbial strain.

1.4 The Significance and Scope of the Study

1.4.1 Significance of Study

This presents the existence of huge demand for the product and the burden it is exerting on the country‘s foreign exchange. Moreover, the herbal industry is expected to be the main contributor to the country’s income in the future. The availability of aloe vera in Ethiopia; there is an opportunity to increase importance of oils as pharmaceutical aid besides their traditional role in medicine.

Therefore, the rational of this research work was looking for optimum techniques for the production of aloe vera oil to encourage the farmers to produce the plant of Aloe vera and more beneficiary from the product by substitute the import and also support the establishment of new pharmaceutical industry in Ethiopia which contribute for the development of the country.

The significance of extraction of oil from aloe vera leaf can be Seen from different perspectives to provide optimal extraction method for the production of extract oil from aloe vera; to be responsible for option to produce extract oils locally, which will play a major role to substitute the imported synthetic preservative, antimicrobial which extract from aloe vera and save hard currency and create job opportunity; to assess the potential of new industrial raw material for the extraction of aloe vera oils.

1.4.2 Scope of the Study

This paper focuses on the extraction, characterization and optimization of aloe vera oil and evaluate microbial effect which is used to anti-bacterial and anti-fungal. The methodologies that used in this work were pretreatment, extract aloe by ethanol solvent using soxhlet apparatus and evaluate microbial activity. The statistical data analysis was generated from laboratory experiments and by using analysis of variance (ANOVA) to analyze the effect of process parameters on the yield of crude oil from aloe vera leaf by extraction process and to draw a generalizing conclusion for each parameter on the optimum yield via CCD experimental designs then analysis microbial effect.

2. LITERATURE REVIEW

2.1 Herbal Plants

Now a day the medicinal plants are considerable significance view due to their special attributes as a large source of therapeutic phytochemicals that may lead to the development of novel drugs. Most of the phytochemicals from plant sources such as phenolic and flavonoids have been reported to have positive impact on health and cancer prevention (Venugopal and Liu, 2012).

Modern Mediterranean and DASH (Dietary Approaches to Stop Hypertension) combine a phytochemicals rich diet from fruit and vegetable sources as the plant based diet has shown to extend life span in Okinawan people, that has the highest number of centenarians (Willcox *et al.*, 2007, 2009). Attentiveness in utilizing natural sources in the formulation and development of skin products, as an alternative to conventional drugs and synthetic products, contribute to increase interest in study of research and industrial application of medicinal plants(Mukherjee *et al.*, 2011).

Many plants have shown immense potential as anti-peptic ulcer, antimicrobial and antioxidant properties with common interest in the research of the herbal medicines; these have become an alternate health care system to solve the health problems of world in today's synthetic allopathic era(Jasso De Rodríguez *et al.*, 2005).

With the advancement of research in medicine, it was concluded that plants are biosynthetic laboratories for chemical compounds, which are responsible for curative action of plants. Scientists isolate phytochemical from medicinal plants and many of them are found very active against many diseases. A conitine, atisine, nicotine, atropine,and morphine are some famous examples of such phytochemical (Hori *et al.*, 2003).

2.2 Aloe Vera (Aloe Barbadensis Miller)

2.2.1 Historical Background of Aloe Vera (Aloe Barbadensis Miller)

The name of the plant was derived from the Arabic "alloeh" meaning "shining bitter substance" because of the bitter liquid found in the leaves. The word "Vera" is Latin for "truth". It is also known as "lily of the desert", the "plant of immortality" and the medicinal plant that has the

qualities to serve as alternate medicine(Arunkumar and Muthuselvam, 2009). Al-snafi was stated that distribution of plant is native to Southern and Eastern Africa, it introduced in Northern Africa, Arabian Peninsula, China, Gibraltar, Mediterranean countries and West Indies and it also is cultivated now in many countries(Al-snafi, 2015). Aloe Vera is a typical xerophyte that is a cactus-like plant with thick, fleshy, circularized spiny leaves that grows readily in hot, dry climates(Choi and Chung, 2003; Tan and Vanitha, 2004). Aloe vera plants are stem less or sometimes very short-stemmed plants that grow up to 60-100 cm tall. The thick leaves are green, with some variants that show white flecks on the upper and lower stem surfaces. The serrated margin of the leaves have small, white teeth, and the flowers are produced in summer. Each pendulous flower has a yellow, tubular corolla 2-3 cm long. Aloe vera forms arbuscular mycorrhiza, a symbiotic mechanism that allows the plant better access to mineral nutrients present in soil (Esteban-Carrasco *et al.*, 2002). The fresh leaves of this perennial, drought resistant plant is used to obtain two distinct products: a bitter, yellow latex (exudate) and a mucilaginous gel from the parenchymatous tissues in the leaf pulp. The gel is revealed after removal of the thick out cuticle(Ramachandran and P.Srinivasa , 2008).

Aloe Vera gel is 99.3% water and the remaining 0.7% consists of a range of active compounds including polysaccharides such as glucose, vitamins, amino acids, phenolic compounds and organic acids. These compounds give aloe vera the special property as a skin care product(Borrelli and Izzo, 2000). The gel has the ability to stimulate cell growth and enhance the restoration of damaged skin. Its moisturizing ability arises from its water holding capacity (Eshun and He, 2010)**.**

Products derived from Aloe vera are primarily used in cosmetics, pharmaceuticals, and nutraceuticals and food industries. People use Aloe vera as skin care products and also in the production of cosmetics and medicines (Roussel *et al.*, 1988).

2.2.2 Characteristics of Aloe Vera

2.2.2.1 Physical Properties of Aloe Vera

The leaf is long triangular sheaf with two external membranes which are green and leathery. Inside this tough resistant covering is the gel, which presents itself as a compact, gelatinous mass with a translucent pearly aspect(Iberica *et al.*, 2012). Leaf pulp was used for a

decortications process which having unpleasant sensations was used to extract the juice. It contains some active compounds which detoxified the body stimulating property worthy of highlighting. The nutritional components of Aloe are equally distributed between the pulp and the cortex of the leaf(Bassetti and Saia, 2005).

2.2.2.2 Botanical Properties of Aloe vera

The Liliaceae is botanical genus of Aloe which is germinates from an original bulb in the same way as lilies. In measuring the particular and specifications of characteristics of the Aloe plant, which was categorical new botanical family, that of the Aloaceae. Aloe is a perennial evergreen shrub with succulent leaves having flowers of an elongated tubular form varying in color according to the species, from orange to bright scarlet red, particularly spectacular and reminiscent of an autumn landscape. The Aloaceae family contains approximately three hundred and fifty varieties of the plant throughout the planet(Reynolds and Dweck, 1999).

The total of 132 species Aloe plants were catalogued the range spanned from the miniature type like Aloe aristata and Aloe brevifolia since 1995 studied in South Africa alone, to one which can be defined as the most beautiful in existence in the world. Aloe arborescens Miller, Aloe ferox, Aloe Barbadensis Miller Vera, Aloe chinensis, Aloe saponaria, and Aloe succotrine were the larger-sized Aloes, among a lot and those having a cosmetic, curative value, that can mention Aloe arborescens Miller, Aloe ferox, Aloe Barbadensis Miller Vera, Aloe chinensis, Aloe saponaria, and Aloe succotrine(Kokate et al., 2012).

A more generalized botanical distinction is achieved by observing the trunk and leaves. In this way, we can distinguish large groups of Aloe; acauleas (without a trunk); subcauleas: visible trunk but with a reduced size(Richards *et al.*, 2015; Bayoumi *et al.*, 2016).

Figure 2.1: Aloe vera plant flower

2.2.2.3 Chemical Properties of Aloe vera

Aloe vera is a miracle plant which is a rich source of many chemical compounds and has a great role in the international market. Chemistry of aloe vera plant was discovered the presence of more than 200 different biologically active substances containing minerals ,vitamins ,enzymes, anthraquinones ,sugars, phenolic compounds, lignin, saponins, sterols, amino acids and salicylic acid (Chaturvedi, 2007).

According to Wang and Weller Aloe Vera leaf contained 75 ingredients which have a variety of medical benefits. It is divided into the following categories: Aloe vera latex and gel have physiologically active substances with biological effects, acting alone or indicating a synergistic effect. The identification of these substances is important for the effective use of the plant, (Jasso De Rodríguez et al., 2005; Wang and Weller, 2006). The chemical composition of Aloe vera varies depends on climate, region, growing conditions, the age of the plant or the processing method (Eshun and He, 2010; Boudreau *et al.*, 2017). According to Choi and Chung, 2011 the major bio active substances of Aloe Vera are shown in Table 2.1

Table: 2.1 Major substances of Aloe vera

Anthraquinone	Saccharide	Vitamins	Enzyme	L/M/W./Sub
Aloe-Emodin	Cellulose	B1	Amylase	Arachidonic acid
Aloetic	Glucose	B2	Carboxpeptidas	Cholesterol
Aloin	Mannose	B6	Catalase	Gibberellin
Anthranol	Aldopentose	C	Cyclooxidase	Lectin
Barbaloin	Acetylated mannan	β- Carotene	Lipase	Lignins
Isobarbaloin	Glucomannn	Choline	Oxidase	Salicylic acid
Emodin	Acetylated glycomann	Folic acid		β-Sitosterol
Cinnamic acid	Galactogalacturan	α- tocopherol		Steroids
	Glucoglactomannan			Triglycerides

L/m/w.sub.: low molecular weight substance

Boudreau et al,2017 and Christaki et al,2015 were stated that aloe vera latex is high in anthraquinones, phenolic compounds that have strong laxative effects while they can act also as antibacterial especially against Gram-positive bacteria analgetics and antivirals. In addition, the latex is reported to contain, on a dry weight basis, an acid insoluble resin (16-33%), significant ash content (24.5%) and a small quantity of essential oil that is responsible for the odor of the latex. In spite of these biological activities, anthraquinones may have harmful effects, such as genotoxic, mutagenic and tumor promoting (Brusick and Mengs, 1997). Reynolds and Dweck, 1999; Iuhui, 2003 and Boudreau et al., 2013 stated that potent source of polysaccharides seems to be aloe vera gel that was contained significantly higher levels of Polysaccharides.

2.2.3 Phyto-pharmacological Properties of Aloe vera

Aloes have long been in use for several diseases, predominantly connected with the digestive system; wounds, burns and skin problems. It is the best herbal answer to support the health and healing mechanisms of the body because it does not heal, rather it feeds the body ‘sown systems in order for them to function optimally and be healthy. Rahmani *et al.*, 2015 were stated that pharmacologically of aloe vera was recommended in adjuvant therapy with antibiotics, Non-

steroidal anti-inflammatory drugs and chemotherapy to eradicate drug induced gastritis and other adverse effects.

The phyto-pharmacological properties of aloe vera are such as antioxidant, analgesic, healing, immune-modulating, skin and anti-aging, antiviral and antitumor activity, antifungal and anti - diabetic effect was revealed listed as below:

Antioxidant Properties: Aloe vera exhibited significant inhibition on pan blue cell viability assay, Ehrlich ascites carcinoma cell number dependent on concentration of cytotoxicity against acute lymphocytes leukemia and acute myeloid leukemia cancerous cells. The formation of secondary reactions of monovalent (singlet oxygen) by which were responsible for the destruction of the insurgence of precancerous activity and intercellular tissues are interrupted to some degree. The aloe vera contents acting substances minerals (copper and manganese), vitamins such as B6, B2, C, E etc. and the amino acid. Aloe has many substances that exhibit the antioxidant effects (Okusa *et al.*, 2007; Wojcikowski *et al.*, 2007; Sultana *et al.*, 2009).

Analgesic activity : The Anthracene and anthraquinones of Aloe is dominated molecules, which collaborate with the beneficial action on the cell and enzyme that was cinnamic acid esterified, Isobarbaloin, and salicylic acid that enzyme added is Brady kinase. The bitter compounds of Aloe is Anthracene and acetylsalicylic acid is a natural an aesthetic yielding the well-known. Brady kinase stimulates the immune system, particularly the macrophages, and becomes a part of the pain system on which it has an analgesic action. It inhibits bradykinin, responsible for post-traumatic pain and swelling, called to action by the liposomal enzymes after an elevated loss of granulocytic macrophages that are unable to block the invading foreign bodies that may enter the body. Used topically, Aloe, and the enzyme Brady kinase contained in it, is an effective analgesic and anti-inflammatory remedy(Blitz, Smith and Gerard, 1963)

Healing activity: The aloe vera of a mannose-rich polysaccharide gibberellin and glucomannn; a growth hormone, relates with growth factor receptors on the fibroblast, in that way proliferation and stimulating its activity, which in turn Aloe gel increased collagen content of the wound as well as increased the degree of collagen cross linking and changed collagen composition that was, accelerated wound contraction and increased the breaking strength of resulting scar tissue. The oral or topical treatment are increased synthesis of hyaluronic acid

and derma tan sulfate in the granulation tissue of a healing wound has been reported(Cithara *et al*, 1998).

Immune-modulating activity: The Aloe Vera component Acemannan, Anthraquinones and Glucomannn have immune modulate properties. Glucomannn activity is a neutralizer and powerful anti-inflammatory of the enzymes responsible for damage to the mucosal membranes that acts much like a fire extinguisher, lessening the effects of the harmful enzymes at the intestinal level by reducing of the number of leaks in the intestinal wall and a diminishing of the foreign protein absorption that can stimulate allergic reactions in the body. Acemannan, used directly as, bactericidal, fungicidal, and virucidal activities through which it can assistance the body to control the production and growth of Candida albicans and through which normal gastrointestinal function is re-established. Anthraquinones, also, facilitating to remove allergenic proteins from the small intestine into the colon for elimination in order to stimulate intestinal motility. All these reactions have a normalizing effect on the function and structure of the gastrointestinal walls and, therefore, stop the vicious circle of damage to the immune system (Karaca, *et al*., 1995; Zhang *et al*., 2006).

Antiviral and Antitumor Activity: These actions may be due to indirect or direct effects. Indirect effect is due to stimulation of the immune system and direct effect is due to anthraquinones. The anthraquinones Aloin inactivates various enveloped viruses such as herpes simplex, varicella zoster and influenza. In recent studies, a polysaccharide fraction has shown to inhibit the binding of benzo-pyrene to primary rat hepatocytes, thereby preventing the formation of potentially cancer initiating benzo-pyrene- DNA adducts(Kim and Lee, 1997). Aloe vera inhibits the cyclooxygenase pathway and reduces prostaglandin E2 production from arachidonic acid. Recently, the novel anti-inflammatory compound called C-glucosyl chromone was isolated from gel extracts(Hutter *et al.*, 1996).

Anti-diabetic activity: In a research study Rajasekaran was shown on streptozotocin-induced diabetic rats, oral administration of Aloe vera gel (alcohol insoluble residue extract) significantly reduced the fasting blood glucose, hepatic transaminases, plasma and tissue cholesterol, triglycerides, free fatty acids and phospholipids and in addition also significantly increased plasma insulin levels. The decreased plasma levels of high density lipoprotein

cholesterol and increased levels of low density lipoprotein cholesterol in the streptozotocin-induced rats were restored to normal after treatment with gel extract(Rajasekaran *et al.*, 2006).

Skin and Body Anti-aging activities: The invaluable oligo elements present in Aloe juice, manganese and selenium, constitute the enzymes superoxide dismutase and glutathione peroxidase, recognized as powerful antioxidants and cellular anti-aging agents. Their high antioxidant properties slow down the aging process. This helps cells to become stronger in combating the negative effects caused by oxygen and the broad spectrum radiation that are exposed to daily. The non-essential amino acid proline, is instead a constituent of collagen, whose role is to ensure the perfect holding capacity and elasticity of epithelial tissues. It naturally follows that the intake of the vitamins and minerals present in Aloe stimulates proper blood saturation, thus guaranteeing better oxygenation and faster expulsion of toxins. Skin becomes smoother, hydrated and more elastic, protected from free radicals and their degenerative activity, resulting in substantial ant aging effects(West and Zhu, 2003)

Antifungal activity: Antifungal activity of leaf pulp and liquid fraction of Aloe vera was evaluated on the mycelium development of Rhizoctonia solani, Fusarium oxysporum, and Colletotrichum coccodes, that showed an inhibitory effect of the pulp of A. Vera on F. oxysporum and the liquid fraction reduced the rate of colony growth solani, F. oxysporum, and C. coccodes. From this it is evident that leaf pulp and liquid fraction of Aloe vera act against plant pathogenic fungi (Jasso De Rodríguez *et al.*, 2005).

2.2.4 Antimicrobial Activity of Aloe vera

Antimicrobial potential of some plants had been accepted long before mankind discovered the presence of microbes. The healing power of plants is usually due to presence of secondary metabolites. Plant extracts and large number of phytochemicals exhibited strong inhibiting effect on a broad spectrum of microorganisms (fungi, bacteria). Beside bacterial infections fungal infections are also a big threat to the life of the human beings. Infectious disease resulting from the presence of pathogenic microbial agents including bacteria, fungi and viruses has become a major healthcare problem in current century.

Only few antifungal drugs are available and long use of these drugs caused resistance. The natural anthraquinones effect of aloe vera which have demonstrated in vitro inhibition of

Bacillus subtiles and Mycobacterium tuberculosis. Aloe juice has been found to be bacteriostatic against Streptococcus pyogenes, Staphylococcus aureus, and Salmonella paratyphi (Olaleye *et al*, 2002).

Plants produce a great variety of chemical compound as in their defense system these defense molecules are secondary metabolites. Plants are rich in secondary metabolites such as tannins, alkaloids and flavones, which have been shown antimicrobial properties(Nostro *et al.*, 2000; Sultana *et al*., 2009).

2.2.5 Use and Application of Aloe Vera

Generally, Aloe vera has many uses both for humans and animals. Three distinct preparations of the plant are used: Aloe vera latex, Aloe vera gel and Aloe vera whole leaf extract, whose biological ingredients may act alone or in synergy(Boudreau *et al.*, 2017).

According to Reynolds and Dweck, 1999; Eshun and He, 2010; Franz and Shea, 2013 , Aloe vera used in cosmetics is not new; there are many of them on the market which use in concentrations varying from 1 to 98% .It is well known that Aloe gel enables the plant to hold moisture for extremely long periods of time and has soothing effects so, Aloe vera has found an extensive application in the cosmetic and toiletry industries, such as moisturizers, cleansers, Sun lotions, toothpastes, mouthwashes, shaving creams, deodorants and Shampoos. In Aloe-derived ingredients used in cosmetics anthraquinones levels should not exceed 50 ppm, concentrations too low to induce photo toxicity. In the United States the Food and Drug Administration (FDA) has approved the external use of Aloe vera gel only as cosmetic ingredient (Franz and Shea, 2013).

However, the food and beverage market is a promising arena for Aloe vera that has been used as a resource of functional food such as yogurt or for the preparation of health drinks, including tea(Franz *et al.*, 2005; Eshun and He, 2010). Table grapes coated with Aloe gel significantly delayed the loss of functional compounds such as total phenolic and ascorbic acid. Indeed, Aloe vera inhibits the growth of microorganisms accountable for foodborne illness in humans or animals as well as food spoilage (Klein, 2005;Eshun and He, 2010).

Aloe vera does not appear to affect food taste or appearance, so it seems to be promise as a safe, natural and environmentally friendly alternative solution to conventional synthetic

preservatives FDA, in the United States has approved the internal use of gel as a "dietary supplement". In the European Commission (EC) according to Annex I of Regulation No 1831/2003 Aloe vera can be used by the feed industries as sensory additive functional group "flavoring compounds", to increase smell or palatability of feedings stuff (Esteban-Carrasco *et al.*, 2002).

2.3 Preparation and Extraction Method for medicinal plants

2.3.1 Preparation of Medicinal Plant Samples for Extraction

The initial stage in studying medicinal plants is the preparation of plant samples to preserve the biomolecules in the plants prior to extraction. Plants samples such as leaves, barks, roots, fruits and flowers can be extracted from fresh or dried plants material. Other pre-preparation of plant materials such as grinding and drying also influences the preservation of phytochemicals in the

Fresh vs. dried samples: Both fresh and dried sample is used in medicinal plants studies. In most cases, dried sample is preferred considering the time needed for experimental design. Vongsak *et al.*, 2013 limit the interval between harvest and experimental work at the maximum period of 3 hours to maintain freshness of samples, as fresh samples are fragile and tend to deteriorate faster than dried samples. Comparison between fresh and dried Moringa oliefera leaves showed no significant effect in total phenolics but with higher flavonoids content in dried sample.

Grinded vs. powdered samples: Conventional mortar and pestle or electric blenders and mills are commonly used to reduce particle size of sample. Grinding resulted in coarse smaller samples; meanwhile, powdered samples have a more homogenized and smaller particle, leading to better surface contact with extraction solvents. Lowering particle size increases surface contact between samples and extraction solvents. This particular pre-preparation is important, as for efficient extraction to occur, the solvent must make contact with the target analyses and particle size smaller than 0.5 mm is ideal for efficient extraction(Kharissova *et al* ., 2013). Particle size was a major factor when using enzyme-assisted extraction. Use of pectinolytic and cell wall polysaccharide degrading enzyme in sample preparation was influenced majorly by the particle size as smaller particle enhances enzyme action (Borhan *et al.*, 2013).

Air-drying: Air-drying usually takes from 3-7 days to months and up to a year depending on the types of samples dried (e.g. leaves or seed). Plant samples, usually plant leaves with stem were tied together and hang to expose the plant to air at ambient temperature. This drying method does not force dried plant materials using high temperature; hence, heat-labile compounds is preserved. However, air-drying take longer time in comparison to microwave is drying and freeze drying and may be subjected to contamination at unstable temperature condition(Krokida *et al*., 2015)

Microwave-drying: Microwave-drying uses electromagnetic radiation that possesses both electric and magnetic fields. The electric field causes simultaneous heating through dipolar rotation; alignment on the electric field of the molecules possessing a permanent or induced dipole moment (e.g. solvents or samples), and ionic induction, that produce oscillation of the molecules. Oscillation causes collisions between molecules and resulted in fast heating of the samples simultaneously. This method can shorten drying time but sometimes causes degradation of phytochemicals(Christen and Kaufmann, 2002).

Oven-drying: Oven-drying is another pre-extraction method that uses thermal energy to remove moisture from the samples. This sample preparation is considered as one of the easiest and rapid thermal processing that could be preserved phytochemicals.

Moghadamtousi *et al.*, 2013 stated that oven-drying at 44.5°C for 4 hours using 80% methanol resulted in highest antioxidants activities in Cosmos caudatus extracts and similar result were observed in optimized 80% methanol extracts at 44.12°C for 4.05 hours. Shorter period of extraction time was obtained using this method. However, effect of drying on Orthosiphon stamineus showed no significant effect on the antioxidant activity but the bioactive phytochemicals; such as sinensetin and rosmarinic acid content were affected by the oven- and sunlight-drying, suggesting the sensitivity of the compounds to temperature (Abdullah *et al*., 2012).

Freeze-drying: Freeze-drying is a method base on the principle of sublimation. Sublimation is a process when a solid is changed into gas phase without entering the liquid phase. Sample is frozen at -80°C to -20°C prior to lyophilisation to solidify any liquid (e.g. solvent, moisture) in the samples. After an overnight (12 h) freezing, sample is immediately lyophilized to avoid the frozen liquid in the sample from melting. Mouth of the test tube or any container holding the

sample is wrapped with needle-poked-para film to avoid loss of sample during the process. Most of the time, sample was lost by splattering out into the freeze-flask. Freeze-drying yielded to higher level of phenolic contents compared to air-dying as most of the phytochemicals are preserved using this method. However, freeze-drying is a complex and expensive methods of drying compared to regular air drying and microwave-drying. Thus, the use is restricted to delicate, heat-sensitive materials of high value(Azwanida.Nn, 2015).

2.3.2 Extraction Methods for Medicinal Plants

The study of medicinal plants starts with the pre-extraction and the extraction procedures, which is an important step in the processing of the bioactive constituents from plant materials. The purpose of all extraction is to separate the soluble plant metabolites, leaving behind the insoluble cellular marc (residue).The initial crude extracts using these methods contain complex mixture of many plant metabolites, such as alkaloids, glycosides, phenolics, Terpenoids and flavonoids. Some of the initially obtained extracts may be ready for use as medicinal agents in the form of tinctures and fluid extracts but some need further processing. Several of the commonly used extraction methods are discussed below(Gahlot, Bhatt and Joshi, 2018).

Maceration and Soxhlet extraction are traditional methods commonly used at the small research locale or at Small Manufacturing Enterprise (SME) level. Microwave assisted extraction (MAE), ultrasound-assisted extraction (UAE) and supercritical fluid extraction (SFE), in which these advances are expected to increase yield at lower cost. Moreover, modifications on the methods are continuously developed with such variety of methods present, selection of proper extraction method needs particular evaluation.

Soxhlet extraction or hot continuous extraction: This method was stated by Luque,1998; finely ground sample is placed in a porous bag or “thimble” made from a strong filter paper or cellulose, which is place, is in thimble chamber of the Soxhlet apparatus. Extraction solvents is heated in the bottom flask, vaporizes into the sample thimble, condenses in the condenser and drip back. When the liquid content reaches the siphon arm, the liquid contents emptied into the bottom flask again and the process is continued. Soxhlet, which has been used for a long time, is a standard technique and the main reference for evaluating the performance of other solid–liquid extraction (or leaching) methods. Soxhlet extraction is a general and well-established technique, which surpasses in performance other conventional extraction techniques except for,

in limited field of applications, the extraction of thermo labile compounds. The most widely-used solvent to extract edible oils from plant sources is hexane. Hexane has a fairly narrow boiling point range of approximately 63–69℃ and it is an excellent oil solvent in terms of oil solubility and ease of recovery. The use of alternative solvents such as isopropanol, ethanol, hydrocarbons, and even water, has increased due to environmental, health, and safety concerns(Mamidipally and Liu, 2004).

Soxhlet extraction strongly depends on matrix characteristics and particle size as the internal diffusion may be the limiting step during extraction. For the extraction of total fat from oleaginous seeds, a 2-h extraction obtained 99% extraction efficiency if the particle size was 0.4 mm, while at12-h extraction was necessary to obtain similar efficiency if the particle size was 2.0 mm (Luque-García and Luque De Castro, 2004).

Strength and limitation: According to Luque,1998 study that advantages of conventional Soxhlet extraction include the displacement of transfer equilibrium by repeatedly bringing fresh solvent into contact with the solid matrix, maintaining a relatively high extraction temperature with heat from the distillation flask, and no filtration requirement after leaching. Also, the Soxhlet method is very simple and cheap. The main disadvantages of conventional Soxhlet extraction include the extraction time is long, a large amount of solvent is used, agitation cannot be provided in the Soxhlet device to accelerate the process, the large amount of solvent used requires an evaporation/concentration procedure and the possibility of thermal decomposition of the target compounds cannot be ignored as the extraction usually occurs at the boiling point of the solvent for a long time. The long-time requirement and the requirement of large amounts of solvent lead to wide criticism of the conventional Soxhlet extraction method. The soxhlet extraction another disadvantage comes with such as exposure to hazardous and flammable liquid organic solvents, with potential toxic emissions during extraction. Solvents used in the extraction system need to be of high-purity that might add to cost.

3. METHODOLOGYOF THE RESEARCH

3.1 Chemicals and Equipment

The chemicals that were used for the experiment is chloroform (E. Merk, Stockholm), acetone (Fisher Scientific International Company, United Kingdom), potassium iodide (Hayashi Pure Chemical Industries Ltd.,Japan) solution, petroleum ether (40℃ to 60℃), ethanol absolute(99% ,Avondale Laboratories Ltd., England),diethyl ether, boric acid, sodium thiosulfate solution, starch solution, distilled water, phenolphthalein indicator, sodium hydroxide , Hydro chloric acid , potassium hydroxide, sodium bi-carbonate, sodium carbonate and sulphuric acid, all chemicals were analytically graded reagents.

Experimental Location: The experimental works of the research was conducted mainly in different institutions. Size reduction of the raw material and extraction process were conducted at School of Chemical Engineering, Extraction and Optimization of crude oil from aloe is conducted at College Of Health and Pharmacology in Jimma University and Characterization and physic-chemical testing were carried out in Arbaminch University and Collage of Natural Science department of Chemistry in Jimma University.

Equipment /Apparatus/ used: The equipment used for this experimental study was, Soxhlet apparatus, kjeldahl digester and analyzer apparatus, Gas Chromatograph-Mass Spectrophotometer (GC-MS), Clevenger apparatus, Drying trays, Slicer, Mortar and Pestle, What- man number 1 filter paper ,Centrifuge, 0.2 micro meter nylon membrane filter, Rotary Evaporator ,PH meter, Razor blade, Sieve, Desiccators, Digital weighing balance, Weight bottle, Stopper, Drying oven, Tong , Erlenmeyer flask, Buchan bottle, FTIR, Measuring cylinder, Cotton, Beaker, Scissor, Glove, Mask and Goggles.

3.2 Method of the Research

3.2.1 Preparation of Raw material (Aloe vera leaf) for Extraction

A fresh aloe Vera sample was used in this research study, which was collected from college of agriculture in Jimma university Southern part of Ethiopia. This raw material was washed properly with tap water to remove impurity. Aloe Vera leaves, including the inner gel was then cut into thin slices and dried to remove moisture under shade until the weight became constant.

Air-drying was took a month to dry samples of Aloe Vera leaves. Aloe vera leaves, usually with high contents were tense together and hang to expose the leaf to air at ambient temperature to not be force dried aloe vera leaf using high temperature; hence, heat-labile phytochemicals are unspoiled. The dried aloe vera materials was then pounded manually into fine powder using mortar and pestle to make ready for the next experiment.

3.2.2 Proximate Analysis of Aloe Vera Leaf

The aloe vera leaves surface was cleaned and washed in running tap water to remove adhering debris after which the samples be air dried for a month under shade and be grinded to fine powder. Air dried aloe samples be analyzed for chemical composition and Samples be analyzed chemically according to official analytical chemist (AOAC).

3.2.2.1 Crude Protein Determination of Aloe vera leaf

Crude protein in the residue was determined by the kjeldahl apparatus through the techniques of a digestion, distillation and titration. After 5ml portion of the digest was added the 3ml of 40% (w/v) NaOH was added in the body of apparatus with a 5ml pipette through small funnel aperture. The 50ml conical flask containing 10ml of 2% boric acid was distilled for 2 minutes by steam then mixed indicator solution placed at the receiving tips of the condenser. Besides, 0.50g of ground dried sample was added carefully into the kjeldahl digestion tubes to ensure that all samples put in to the bottom of the tubes then10ml of concentrated H_2SO_4 was added to set in the appropriate hole of the digestion block heater in a fume cupboard.

Consequently, the result obtained from distillation was titrated by 0.01N HCl then green color to be turned to violet/pink color which was indicated that all nitrogen trapped an ammonium borate was removed as ammonium chloride. The total crude protein content was determined by multiplying percentage nitrogen by a constant factor of 6.26%

%N = Titre value x Atomic mass of Nitrogen x molarity of HCl.

$$\text{Crude Protein value} = \%\text{N x } 6.26 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots..3.1$$

3.2.2.2 Fat Content Determination of Aloe vera leaf

The soxhlet flask was then filled to full capacity with petroleum ether 40℃ -60℃ boiling point plus the condenser set was placed on the heating mantel adjusting to boiling temperature of the solvent for condensation of petroleum ether vapor. A gram of dried sample was weighed into the fat free extraction thimble and tightened with cotton wool. The solvent was left to siphon over several times for at least 10-12 times. Distillation was continued until the flask was practically dried. The fat was detached from flask which that exterior cleaned and dried to a constant weight in an oven. Initial weight of dry soxhlet flask W_0 and W_1 is final weight of oven dried flask + fat.

$$\%\text{Fat} = (W_1 - W_0)\ x\ 100\% \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ 3.2$$

3.2.2.3 Crude Fiber Determination of Aloe vera leaf

Two grams of the Aloe Vera leaf was accurately weighed into the fiber flask (W_1) then100ml of 0.255N H_2SO_4 was added. After mixture was heated by heating mantle then hot mixture was filtered through a fiber sieve cloth. The detected residue was mixed with 100ml of 0.313M NaOH then heated by reflux for another one hour. The filtered mixture was added with 10ml of acetone to dissolve any organic constituent. The residue was washed with 50ml hot water twice on the sieve cloth then it was finally transferred into the crucible. The residue and crucible were dried on oven at 150℃ overnight to remove moisture then oven dried crucible containing the residue was cooled in desiccators and later weighed.

$$\%\text{Fiber} = \frac{W_1 - W_2}{\text{Wt ofsample}} * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots .3.3$$

3.2.2.4 Ash Content of Aloe vera leaf

Two grams of the sample of aloe vera leaf powder was weighed in porcelain crucible then transferred into the muffled furnace at 550 degree Celsius and left for about four hours. About this time it has turned to white ash. The crucible containing was cooled to about 100℃ in air then to room temperature in a desiccator and weighed. This was done in duplicate.

$$\%\text{Ash content} = \frac{\text{weight of ash}}{\text{Wt of sample}} * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots .3.4$$

3.2.2.5 Moisture Content of Aloe vera leaf

Two grams of the fresh Aloe vera leaf was weighed into before dried and weighed crucible. The crucible and sample was taken then transferred into the oven set at 100℃ index and allowed to dry overnight. At the end of the 24 hours the crucible plus sample was removed from the oven and transferred to the desiccators and cooled for 10 minutes and weighed.

$$\%\text{Moisture content} = \frac{W_2 - W_0}{W_1 - W_0} * 100 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.5$$

Where: W_0 -the weight of empty crucible; W_1-Weight of crucible +sample; W_2-Weight of crucible +oven dried sample.

3.2.3 Mineral Content Analysis of Aloe vera leaf

The aloe vera leaf dried was analyzed for its minerals profile that wet acid-digested using a mixture per chloric acid and nitric acid (HCSO4; HNO3; 1:2v/v). The amounts of iron, zinc, copper, lead, cobalt and manganese in the digested sample were determined using by Atomic Absorption Spectrophotometer (Varian AA240, Australia). Sodium and potassium were determined by a flame photometer, and magnesium were determined by titration with version 0.0156 N AOAC (1990).

$$\frac{\text{mg}}{\text{L}}\text{of mineral contentnt} = \frac{[(\text{Cs} - \text{Cb})\text{xV}}{[\text{W}]} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.6$$

Cs=concentration of sample in ppm Cb=concentration of blank in ppm

V=Volume (ml) of extract W=Weight (g) of samples

3.3 Extraction Process, Experimental Design and Statistical Analysis

3.3.1 Extraction Process

Experimental work was conducted using soxhlet Apparatus by solvent extraction process via ethanol solvent. The soxhlet extraction parameters of extraction time, extraction temperature and aloe vera leaf powder to solvent ratio procedure extraction method was 20g of the grounded aloe vera samples was extracted in 200ml, 220ml and 240ml of extraction solvents of ethanol at different temperature 70ºC, 75ºC and 80ºC by using a condenser to prevent extraction solvent loss from the rounded flask and also the extraction time for 3hr, 4hr and 5hr respectively. In

such a way, extraction was performed in response surface method with center composite design. Finally, the crude extracted resin was cooled at room temperature, filtered and concentrated from extraction solvent by using an equipment of rotary evaporator and weighed to determine the amount or yield of extract oil and stored in vials at 4°C prior to the analysis(Awang *et al.*, 2014).

Extraction yield for oils was calculated by using the following equation.

$$\text{Oil Yield } (\%) = \frac{(\text{Mass of Oil Extracted}) * 100\%}{\text{Mass of Sample}} \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.7$$

Table: 3. 1 Different level of factors associated with the experiment

Variables	unit	levels		
		-1	0	+1
Solid to solvent ratio	g/ml	1:10	1:11	1:12
Extraction-time	hr.	3	4	5
Extraction-temperature	℃	70	75	80

3.3.2 Experimental Design

Box-Wilson design which is called central composite design (CCD), was experimental used to achieve maximal information about a process at least number of experiments. This research study was used the central composite face-centered (CCFC) experimental design for determine effect of three variables and their ranges were (extraction temperature (70-80℃), extraction time (3-5 hr.), and solid –liquid ratio 1:10-1:12 g/ml) and the optimal process variables conditions on the response of yield of soxhlet extraction in order to produce aloe vera oil(Li *et al.*, 2016).

Then, experiments were established based on a CCFC design with three factors at three levels and each independent variable was coded at three levels between -1 0 ,and +1 that was done by this equation (Maran, 2013).

$$x_i = \frac{X_i - X_z}{\Delta X_i} \quad \mathbf{i}=1, 2, 3 \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots 3.8$$

Where x_i, X_i, X_z, are the dimensionless value; real value; the real value at the center point of independent variable respectively and ΔX_i, step change of real value of the variable i the dimensionless value. In this study, 20 total number of experiments with pure error estimation were calculated from by equation(Azargohar and Dalai, 2005).

$$N = 2^n + 2n + n_c \quad \text{.....3.9}$$

Where is n is the number of factors; and c is the number of center points.

The experimental sequence was randomized in order to minimize the effects of unexpected variability in the responses due to unnecessary factors. An empirical model which correlated with responses to the independent variables were developed through general second-order polynomial equation according to (Maran, 2013).

$$Y = \beta_0 + \sum_{j=1}^{k} \beta_j X_j + \sum_{j=1}^{k} \beta_{jj} X^2{}_j + \sum_{1<1} \sum_{j=2}^{k} \beta X_i X_j + e_i \quad \text{.....3.10}$$

Where Y is the response X_i and X_j are variables (i and j range from 1 to k); β_0 is the model intercept coefficient; β_j, β_{jj} and β_{ij} are interaction coefficient of linear, quadratic and the second-order terms ,respectively ;k is number of independent parameters (k=3 in this study) and e_i is the error.

3.3.3 Statistical Analysis

The multiple regression analysis of experimental data were analyzed through the least square method. The experimental data was tested by model summary statistics and sequential sum of squares in order to observe adequacy of models. The regression coefficients of the linearity, quadratic, and interaction were involved in the model and their effect were analyzed by ANOVA generated were tested which confirmed statistically by F-test at probability levels of ($p \leq 0.05$). Adequacy of the developed models was tested by performing coefficient of determinate; (R^2), adjusted coefficient (R^2adj) and predicted coefficient (R^2pre). The surfaces and contour graphs were plotted to predict the relationship between the independent variables and responses for fitting the models.

3.4 Optimization

A numerical optimization technique was employed for optimizing the yield responses involved in the extraction process. This optimization technique depends on particular response (Y_i) which to be maximized the percentage oil yield, while the independent variables are retained within the range. The general approach is to first convert each response (Y_i) into a dimensionless individual desirability function (d_i) and it was done with the following equation:

$$d_i = h_n(Y_i) \quad \text{.....3.11}$$

The individual desirability (d_i) for each responses i.e., minimize, maximize, in range or target of the response L_i, U_i and T_i be the lower, upper, and target values, respectively, that are desired for response Y_i, with L_i, T_i and Ui(Derringer and Suich, 2018).

The individual desirability's (d_i) for the response was obtained by specifying the goals, which maximize of the response. The individual desirability of maximization of the response depends on which in turn relies on the target value hit by the exponent *s*. For *s*=1, the desirability function increases linearly toward T_i which denotes a large enough value for the response; for *s* < 1, the function is convex ,*s*> 1, the function is concave:

$$d_i(Y_i) = \begin{cases} 0 & Y_i\ x\ < L_i \\ \left(\dfrac{Y_i(x) - L_i}{T_i - L_i}\right)^s & L_i \le Y_i\ x\ \le T_i \\ 1 & Y_i\ x\ > T_i \end{cases} \quad \text{.....3.12}$$

when response was minimized, then its individual desirability function is with the T_i denoting a small enough value for the response:

$$d_i(Y_i) = \begin{cases} 1 & Y_i\ x\ < U_i \\ \left(\dfrac{Y_i(x) - L_i}{T_i - L_i}\right)^s & T_i \le Y_i\ x\ \le U_i \\ 0 & Y_i\ x\ > U_i \end{cases} \quad \text{.....3.13}$$

After desirability values were computed for each response variable, they are combined to obtain a single global desirability index (D), which varies from 0 (completely undesirable response) to 1 (fully desired response)(Maran *et al.*, 2013) and that are equal to their geometric mean of

the individual desired functions. For all the desire functions, a total desired function D ($0 \leq D \leq 1$) Where v_i is a number indicating the relative importance of the i^{th} response.

$$D=\left(\prod_{i=1}^{n} d_i^{vi}\right)1/\sum vi \quad \text{.....3.14}$$

In this research study, desirability functions were developed for specific responses of maximum yield of crude extract. A weight factor, which defines the shape of the desirability function for the response must be between 1 and 10, with larger weights corresponding to more important responses. A weight factor of 1 was chosen for all individual desirability's in this research work. The ''importance'' of a goal can be changed in relation to the other goals. It can range from 1 (least important) to 5 (most important). The default is three representing all goals to be equally important.

3.4.1 Validation of Optimized Conditions and Predictive Models

The suitability of the developed model equations for predicting the optimum response values was verified using the optimal condition. The validity of the model was determined by triplicating experiments were done in the optimal condition and the mean experimental values were compared with the predicted values.

3.5 Characterization of Physicochemical Analysis of Aloe Vera Oil

The physicochemical properties of extracted aloe vera leaves oil were described based on the procedure covered in several standard analyses methods might be analyzed with instruments and titration.

3.5.1 Physical Properties of Aloe vera Oil

Determination of specific gravity: A clean and dry bottle of 25ml capacity of density bottle was weighed (W_0) and then the bottle was filled with the oil, stopper inserted and reweighed to give (W_1). The oil was substituted with water after washing and drying the bottle and weighed to give (W_2).

$$\text{specific gravity} = \frac{W_2 - W_0}{W_1 - W_0} \quad \text{.....3.15}$$

Determination of pH value: 2g of the sample was taken and putted in a clean dry 25ml beaker and 13ml of hot distilled water was added to the sample in the beaker and stirred slowly. And then it was cooled in cold water bath to 25ºC.The pH electrode was standardized with a buffer solution first and then immersed in the sample and the pH was read (AOAC official method analysis 960.19, 2000).

Determination of kinematic viscosity: A15m1 of oil was poured into a test tube and a viscometer was used to measure the viscosity at a temperature of 20℃. A dynamic viscosity of the oil was measured directly using rotary-viscometer which was available in laboratory of school of Chemical Engineering. Then the kinematic viscometer was calculated.

$$\text{Kinematic viscosity} = \frac{\text{Dynamic viscosity of sample}}{\text{Density of the sample}} = V = \frac{\mu}{\rho} \quad \text{.....3.16}$$

3.5.2 Chemical Properties Determination

Acid value determination: 25 ml of toluene and 25ml of ethanol was mixed in a 250ml beaker then mixture was added to 2g of oil in a 250ml conical flask and few drops of phenolphthalein was added to the mixture. The mixture was titrated with 0.1M KOH to the end point with consistent shaking for which a dark pink color was observed and the volume of 0.1M KOH (V_0) was noted ISO 3657 (1988) and AOCS Method.cd 3-25 (1993).

$$\text{Acid value} = \frac{V * C * 56.11}{M} \quad \text{.....3.17}$$

Where, V=volume potassium hydroxide (ml), C=concentration of potassium hydroxide, 56.11=molecular weight of potassium hydroxide M=sample weight.

Saponification value: according to AOCS Method cd 3-25 (1993) Saponification value is an index of average molecular mass of various fatty acids in oil M samples. 2 gm. of the aloe vera sample was weighed into a conical flask; 25ml of 0.5N ethanolic KOH was added that content was frequently stirred and boiled gently for 30min then few drops of phenolphthalein indicator was added and then titrated with 0.5M HCl to the endpoint until the pink color of the indicator just disappeared.

$$\text{Saponification value} = \frac{56.1 * N(V2 - V1)}{W} \quad \text{.....3.18}$$

Where**,** N=normally in ml of HCl used for titration, W=weight of sample (gm.)

V1=volume in ml of potassium hydroxide used for the blank, V2= volume in ml of potassium hydroxide used in determination.

3.6 Chemical Compositional Analysis of Crude Oil from Aloe vera Leaf

3.6.1 Fourier Transform Infrared Ray (FTIR)

Fourier transform infrared (FTIR) spectroscopy was a measurement technique for collecting infrared spectra. Instead of recording the amount of energy absorbed when the frequency of the infra-red light was varied, the IR light was guided through an interferometer. After passing through the sample, the measured signal was the interferogram. FTIR spectra was a useful tool to identify functional groups in a molecule, as each specific chemical bond often has a unique energy absorption band and can obtain structural and bond information on a complex to study compounds. Infra-red (IR) spectrum was recorded on a Perkin-Elmer FT-IR spectrometer. The FT-IR spectrum of the aloe vera oil was obtained using Perkins Elmer Spectrum 65 FT-IR spectrometer in Jimma University and functional groups was determined with the help of IR correlation charts. Liquid oil samples was sandwiched between two plates of a high purity salt of potassium bromide. The plates was transparent to the infrared light onto the spectra. The IR spectrum was reported in % transmittance. The wave number region for the analysis was 4000-400 cm^{-1} in the mid-infrared range(Nurrulhidayah *et al.*, 2011).

3.6.2 Gas Chromatography-Mass Spectroscopy

The extracted crude oil samples was analyzed using gas chromatography mass spectrometry (GC-MS). Agilent6890 gas chromatography instrument coupled to Agilent 5973 mass-spectrometer and Agilent Chem software in order to identify their chemical constituents. This was an essential method to evaluate the quality of the oil samples. The operating parameters of GC-MS was as followed: system operating in EI mode (70 eV), equipped with a split injector (280ºC, split ratio 1:20), using DB -5 column (30 x 0.2 5 mm i.d x 0. 2 5 mm). The temperature program is 50ºC (5 min) rising to 300ºC rate of 5ºC/min. Injector and detector temperature was 280ºC. Helium was used as carrier gas at a flow rate 1 mL /min(Lakshmi and Rajalakshmi, 2011).

3.7 Preliminary Phytochemical Screening of Aloe vera Oil

The presence of phytochemicals like alkaloids, flavonoids, saponins, tannins, Terpenoids and phenol in crude aloe vera oil was evaluated using standard testing the method.

Test for alkaloids: Mayer's test

About 0.25 g of the crude extract aloe vera was stirred with 5 ml of 1% HCl on a steam bath. 1ml of the filtrate was treated with a few drops of Mayer's reagent then with Dragendorff's reagent. Turbidity or precipitation formation was taken as presence of alkaloids(Ayoola *et al.*, 2008).

Test for saponins: Foam test

About 0.25 g of the crude extract aloe vera was dissolved in 5 ml of distilled water into a test tube. Then, the shaken solution was strongly and observed for a stable persistent froth formation that indicate the presence of saponins (Ayoola *et al.*, 2008).

Test for polyphenols

About 0.25 g of crude extract of aloe vera was treated with few drops of 5% neutral ferric chloride solution then appearance of a greenish precipitate indicated the presence of phenol(Shetty *et al.*, 2016).

Test for flavonoid

10 ml of ethyl acetate was dissolved with 0.25 g of crude extracted aloe vera oil into a test tube and heated on a water bath for 3 minutes. Then, about 4 ml of the filtrate was taken and shaken with 1 ml of dilute ammonia 24 solution. The layers were allowed to separate and the yellow color in the ammonia layer indicated the presence of flavonoids.

Test for Terpenoids

About 0.25 gm. of extract oil was liquefied in 2 ml of chloroform into test tube then 3ml concentrated sulfuric acid was wisely added that formation of reddish brown coloration of the interface layer indicates presence of Terpenoids(Shetty *et al.*, 2016)

Test for tannins: Ferric chloride test

About 0.25 g ethanol extract was boiled in 10 ml of water in a test tube and then filtered. Three drops of 0.1% ferric chloride were added to the filtrate. Presence of tannins was confirmed by the formation of brown greenish or blue-black color.

Test for anthraquinones: Borntrager's Test

About 0.5 g of sample of each plant extract was shaken with 5 ml of chloroform and filtered. A 10% ammonium hydroxide solution (5ml) was added to the filtrate, and the mixture was shaken. The presence of a pink, red or violet color in the ammonia phase was taken as an indication of the presence of anthraquinones (Joshi *et al.*, 2011).

Test for Steroids: Salkowski'stest

Joshi *et al.*, 2011 was tested that about 1gm of aloe vera extract was dissolved in 2ml of chloroform. Concentrated sulphuric acids was carefully added on standing yields red color.

3.8 Effect of Extract Crude Aloe Vera Extract on Selected Microbial

Agar well diffusion method was used for the primary screening of the antimicrobial activity of plant extracts against the target pathogens in vitro. The microorganisms were selected for the study Bacillus subtiles and staphylococcus aureus (gram positive bacteria), Pseudomas aerginosa and salmonella typhus (gram negative bacteria), two fungi species namely Candida albicans, Aspergillus Niger.

3.8.1 Preparation of Nutrient Agar (NA) Plates

In order to prepare 1000 ml of NA, 28 grams of the powder was added. Keeping this concentration constant, the required volumes of NA was prepared each time. Using an electronic balance the powder was measured prior adding distilled water. After stirring to break off any lumps, the flask had been heated on a Bunsen burner until the solution turned clear and bubbles appeared. After allowing to cool for a few minutes, the mouth of the conical flask containing the media was covered using aluminum foil and autoclaved. After sterilization, the media was carefully poured on to sterile petri dishes inside a laminar air flow cabinet. For a medium-sized plate, the volume of NA needed is 20 ml.

3.8.2 Preparation of Saline Solution

To make 0.9% saline solution, 0.9 grams of sodium chloride (NaCl) was taken into 100 ml of distilled water. 9 ml of the saline solution was put in each test tube. Several such test tubes were prepared and autoclaved, with the screw cap opened through half turns.

When taken out of the autoclave machine, the screw caps was turned fully to close the mouth of the tube to prevent contamination.

3.8.3 Inoculation and Agar Well Diffusion

Standardized inoculum of 0.5 McFarland (approximate cell count density: $1.5x10^8$) turbidity standard was prepared by taking 1-2 colonies of organisms with loops from 24 hour culture plates and mixing them in sterile saline solutions. The saline containing tubes were vortexed for homogeneous mixture and the turbidity compared to that of 0.5 McFarland standard solution. Using sterile cotton swabs, each of the test bacterial strains were lawn cultured on properly labelled NA plates to achieve even growth. The plates were allowed to dry and then a sterile cork borer was used to bore wells in the agar plates. The extracts (100 µl/well) were then loaded in the wells using a micropipette. The plates was then incubated at 37°C for 24 hours. In the case of fungi test was performed in sterile Petri dishes containing saboraud dextrose agar. The oils was adsorbed on sterile paper disc and placed on the surface of the medium previously inoculated with a suspension of bacteria and fungus. All Petri dishes were sealed with a sterile paraffin film to avoid evaporation of test samples and incubated at 28℃.The zone of inhibition was determined by measuring the diameter of the clear zone around each disc. The standard antibiotics ciprofloxacin was used for bacteria and Ketoconazole used for fungi. The test was conducted in Microbiology Laboratory of Jimma University. Antimicrobial activities of the extracts were determined by measuring the diameters of inhibition zones in millimeters produced against the pathogens. The experiments was repeated three times and the mean values was calculated.

4. RESULT AND DISCUSSION

In this section, the results obtained from solvent extraction of crude aloe vera extract, characterization and preservative effect of the extracted oil were discussed. The effects of main and interaction factors and the optimum levels of the parameters were also investigated.

4.1 Proximate and Mineral analysis of Aloe Vera Leaf

4.1.1 Proximate Analysis of Aloe vera Leaf

Proximate composition is important in determining the quality of raw materials. The results on chemical composition of Aloe Vera leaves was shown in Table 4.1. Aloe vera leaves were analyzed for different quality attributes; contains 94.69 ± 0.02%, 10.86 ± 0.06%, 3.0 ± 0.08%, 70.35 ±0.33% and 12.63 ± 06% of moisture, crude proteins, crude fat, crude fiber, ash contents respectively. The key feature of all the Aloe vera leaf had high water content. The moisture contents is the most fundamental and important analytical procedure since the dry matter that remains after moisture removal is commonly referred to as total solids. Approximately 94.72 ± 0.13% moisture contents were observed. Ash is the primarily step in preparing a biological samples for specific element analysis. Because a certain samples are high in particular minerals, that becomes significant and signifies the total mineral content. Ash content was determined in present study (12.88 ± 04%) were higher than previously estimated **(**7.12-7.30%). Aloe vera leaves are rich sources of fibers as these represented greater than 70% proportions 70.56 ±0**.**33%**)** in this study. These results were partially in accordance with the discoveries of Simal *et al.*, 1999 as they observed 60.34-72.17% crude fibers in Aloe vera tissues.

In proteins most distinguishing element is nitrogen hence nitrogen content in various proteins ranges from 13.4 to 19.1% due to the variation in the specific amino acid composition of proteins. However, a study on compositional features of Aloe vera tissues 7.56-15.4% described by(Ahmed and Hussain, 2013). In this research the protein content of aloe vera leaf found to be , 10.86 ± 0.06%,which comparable to preview works on the aloe vera 7.56-15.4% stated by (Ahmed and Hussain, 2013). Fat was dry matter basis that represented a minor fraction in present analysis 3.0 ± 0.08%).

Table: 4.1 proximate composition analysis of aloe Vera leaves

Constituent's	Results%	(Ahmed and Hussain, 2013) Report
Ash	12.88 ±0. 04	16±0.04
Crude fiber	70.56 ±0.33	73.35±0.30
Fat	3.0±0.08	2.91 ± 0.09
Protein	10.86 ± 0.06	6.86±0.06
Moisture	94.72 ± 0.13	97.42±0.13

Results are expressed as percentages ± standard deviation on dry matter basis

4.2 Mineral Analysis of Aloe vera Leaf

The mineral elemental configuration of Aloe vera leaves samples were shown in Table 4.2 indicates that Aloe vera is rich in essential minerals like: Potassium 3mg/l, Sodium 2mg/l, Magnesium 1.5mg/l, Iron 0.62mg/l, Zinc 0.22 mg/l, Copper 0.19 mg/l and Manganese 0.31 mg/l. Aloe vera also contains heavy metal like: Cobalt 0.52mg/l, Lead 0.53 mg/l and Chromium 0.02mg/l. According to Rajendran *et al*, 2007 result on mineral configuration is covenant with some medicinal plant species analyzed trace elements in Aloe vera leaf by AAS, which showed that concentrations of Potassium, Magnesium, Sodium and Zinc were more than 200 µg and has also been recorded average concentrations of Lead, Manganese, Cobalt, Copper, Cadmium, Nickel, Iron and Aluminum as 0.7 µg, 0.8 µg, 0.1 µg, 1.8 µg, 0.04 µg, 0.3 µg, 20 µg and 11 µg respectively. Minerals like Iron, although extant in threshold level can performance as anti-oxidant and are involved in supporting the immune system while, Zinc are known to prevents cardiomyopathy, muscle degeneration, growth retardation and bleeding disorder. Magnesium element is in large quantities present in the human being, which is dispersed in bones, skeletal muscles, soft tissues and extracellular fluids. Mg plays crucial role in lipid membrane stabilization, replication and metabolic processes Therefore, the presence of these minerals in aloe vera provides bases in pharmaceutical application (Dharajiya *et al.*, 2017).

In general, one more importance of this research study was that Aloe vera demonstrates the biological role and heavy metal absorbance ability. The elements play vital role in formulation

and medicinal properties of any herbal drugs. The Aloe vera leaves shown acceptable amount of elemental concentrations hence evidence for medicinal and nutritional properties.

Table: 4.2 Mineral composition of aloe vera leaves

Elements	Conc.mg/l	Absorbance (µm)	Elements	Conc.mg/l
Copper	0.19	0.019897	Sodium	2
Iron	0.62	0.0169	Potassium	3
Zinc	0.217	0.060033	Magnesium	1.5
Manganese	0.313	0.020467		
Chromium	0.02	0.0036		
Cobalt	0.52	0.024198		
Lead	0.53	0.04262		

4.3 Statistical Analysis and Effect of Process variables of Extraction Oil

4.3.1 Statistical Analysis

In this research work, three process variables had been chosen to be involved in the extraction process which was ratio of aloe vera meal to solvent, time and temperature to assist the extraction process via face-centered composite had designed 20 experimental runs including 6 repeats at the center point on the yield of extract crude aloe vera oil and evaluate the combined effect of the independent variables. The yield of crude aloe vera oil was determined from equation 3.7 below Table 4.3 under yield column was filled that was shown the effect of extraction parameters on crude aloe vera extract yield. The maximum and minimum yield of oil were found to be 20.75 % 8.25 % which obtained at 20gm of meal aloe vera to solvent 220 ml, 240ml; temperature 75, 80 °C; contact time 4, 3 hr. respectively.

Table 4.3 FCCCD the independent variables and their responses in aloe oil

Run	A:Temperature °C	B:Ratio mg/ml.	C:Time hr.	Yield %w/w
1	75(0)	1:10(-1)	4(0)	18.75
2	75(0)	1:11(0)	4(0)	19.25
3	70(-1)	1:10(-1)	3(-1)	10.25
4	70(-1)	1:10(-1)	5(+1)	9.75
5	70(-1)	1:12(+1)	3(-1)	15
6	75(0)	1:11(0)	4(0)	20.75
7	75(0)	1:11(0)	4(0)	20.5
8	75(0)	1:11(0)	4(0)	20.25
9	80(+1)	1:10(-1)	5(+1)	13
10	75(0)	1:11(0)	4(0)	19.75
11	75(0)	1:12(+1)	4(0)	19.5
12	80(+1)	1:12(+1)	5(+1)	12
13	70(-1)	1:12(+1)	5(+1)	15
14	75(0)	1:11(0)	3(-1)	18
15	75(0)	1:11(0)	5(+1)	19.5
16	80(+1)	1:12(+1)	3(-1)	8.25
17	75(0)	1:11(0)	4(0)	19
18	80(+1)	1:10(-1)	3(-1)	9.5
19	80(+1)	1:11(0)	4(0)	12.75
20	70(-1)	1:11(0)	4(0)	15.75

-1: lower, 0: center, +1: higher

4.3.2 Experimental Modeling for Percentage of Oil Yield

The major statistical analysis of the extraction route was model generation; model fitness test and ANOVA analysis are presented and discussed as follows. The different model summary statistics were found in the Table 4.4 that effort on the model maximizing the Adjusted R-

Squared and the Predicted R-Squared values large consequently, the quadratic model is suggested.

Table 4.4 Model Summary Statistics

Source	Sequential p-value	Lack of Fit p-value	Adjusted R^2	Predicted R^2	
Linear	0.7465	0.0001	-0.1023	-0.6896	
2FI	0.7608	< 0.0001	-0.2441	-5.0613	
Quadratic	< 0.0001	0.7335	0.9788	0.9617	Suggested
Cubic	0.6500	0.5399	0.9753	0.2272	Aliased

A second order quadratic regression was performed to estimate the response function is second order polynomial after checking of the model fit summery revealed that quadratic model was statistically major for the response percent yield of oil. Mathematical models was evaluated for response by means of regression analysis. The model statistical significant was checked by F-test and the ANOVA Table 4.5 by utilizing the quadratic model as suggested. The F-value of 98.35 and the P- value of <0.0001 demonstrated the significant of the regression with 99% confidence level. There is only a 0.01% chance that a model F-Value this greater might be occurred due to noise while the value of prob>F less than 0.0500 indicate model term is significant (Muzenda *et al.*, 2012).

The ANOVA result in Table 4.5 was generated that effects of linear, combined interaction and quadratic were resolute the significance of polynomial by calculating the F-value at a probability (P) of 0.001,0.01 or 0.05. In this situation A, B, C, A^2,B^2,C^2, AB, AC are significant model terms. The Lack of Fit F-value of 0.55 implies the Lack of Fit is not significant relative to the pure error.

The response function (y) measured the yield of crude aloe vera oil values by using different independent variables. The values was related to the variables temperature (A), aloe meal to solvent ratio (B) and Time(C) by a second degree polynomial using the Eq. 4.1. An experimental correlation between the response and the significant variables in coded units could be stated by the following equation:

Yield = 20.0182 -1.025 * A + 0.85 * B + 0.825 * C

-1.53125 * AB + 0.96875 * AC

-5.92045 * A^2 -1.04545 * B^2 -1.42045 * C^2……...……..4.1

The coefficient of the polynomial were denoted by a constant term A> B >C (linear effects); AB> AC>BC(combined interaction effects) and A^2> C^2> B^2(Quadratic effect hence,the combined interaction of BC is insignificant effects. However,the quadratic effects A^2 is the most significant effects with compared to linear ,quadratic and combined interaction effect since it has greater F-value which is 252.28.

Negative coefficient values indicate that individual or combined interactions factors negatively affect extraction while positive coefficient values represents that factors increase extraction percentage yield. For instance, among all linear factors temperature; combined interaction of temperature; ratio and all quadratic had a negative effect but linear factors of ratio and time; combined interaction of temperature and time had a positive effect on yield of oil these results were described below graph 4.2.

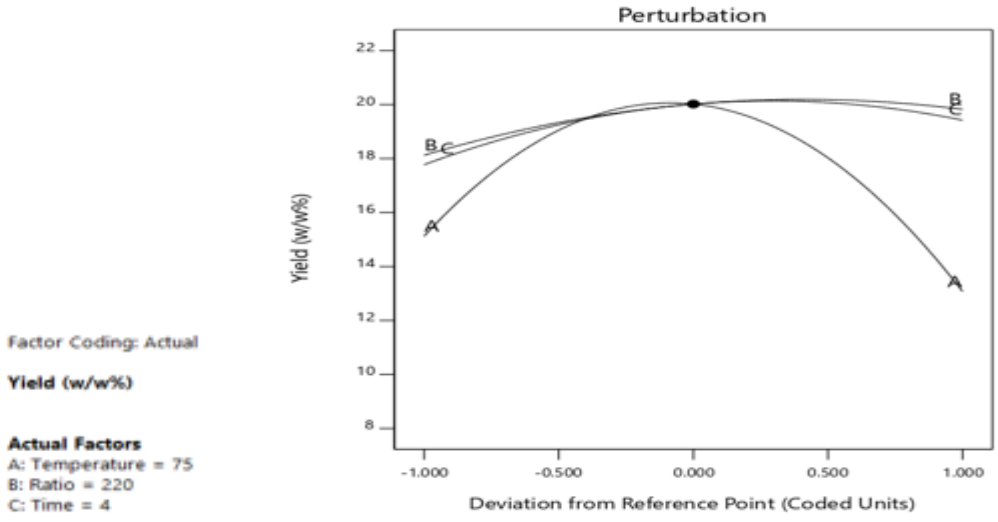

Figure 4.1 Effect of process variable on oil yield

Table: 4.5 ANOVA for the response surface quadratic polynomial model

Source	Sum of Squares	df	Mean of Square	F-value	p-value
Model	338.19	9	37.58	98.35	<0.0001***
A-Temperature	10.51	1	10.51	27.50	0.0004**
B-Ratio	7.23	1	7.23	18.91	0.0014**
C-Time	6.81	1	6.81	17.81	0.0018**
AB	18.76	1	18.76	49.09	<0.0001***
AC	7.51	1	7.51	19.65	0.0013**
BC	0.0703	1	0.0703	0.1840	0.6770*
A^2	96.39	1	96.39	252.28	<0.0001***
B2	3.01	1	3.01	7.87	0.0186**
C2	5.55	1	5.55	14.52	0.0034**
Lack of fit	1.36	5	0.2725	0.5543	0.7335
Pure Error	2.46	5	0.4917		

P<0.05 indicates the model terms are significant while P<0.01 indicates the model term is highly significant
* *Not Significant* **significant *** highly significant

The statistical model was developed by applying multiple regression analysis methods using the experimental data of ethanol extraction of aloe vera leaf which is given in Eq.4 .1. R^2 is a degree of the amount of variation around the mean described by the model. The goodness of fit was evaluated by the coefficients of determination (R^2), which was 0.9888 and this reveals that 98.88 % a, of the data was clarified by the selected models. The adequate precision of 27.4091 for quadratic for extract crude aloe vera extract were greater than 4, which indicates the models could be used to investigate the design space. Characteristic of the constructed model was explained in Table 4.6 as the model adequacies were checked by R-Squared>0.95; Adjusted-R-Squared minus Predicted R-Squared <0.2; CV<10; Adequate Precision >4 with the according to by Myers and Montgomery (2002).

The statistical analysis indicates that the suggested model was adequate with very satisfactory values of the R-Squared; the adjusted-R -Squared ; adequate Precision that compares the ranges of the predicted values at the design points to the average prediction error and ratios greater than 4 indicate adequate model discernment (Sereshti *et al.*, 2009).

Table: 4.6 Fit statistics for the response of crude aloe vera extract yield

Std. Deviation	0.6181	R^2	0.9888
Mean	15.82	Adjusted R^2	0.9788
C.V. %	3.91	Predicted R^2	0.9617
		Adequate. Precision	27.4091

4.3.3 Model Adequacy Checking

Before the model implemented for different applications it should satisfy different criteria such as the normal distribution of the residuals. Unless the model should not satisfy these criteria it is not advisable to use the model for different purpose rather other methods are applied to adjust the model in desirable way. The normal probability plot of the residuals showed that, it follows almost a straight line which implies residuals are approximately normally distributed that satisfy the most important assumption in any model. Normal probability plots of residuals shown Fig.4.3 and Plot of residuals vs. predicted values shown Fig.4.4.

Fig.4.3 shows that the plot of residuals versus predicted value did not follow any pattern, it is random it implies that the model is adequate. As it is seen from the Fig. 4.4 the student zed residuals versus the run numbers were not have a uniform structure which tells us that the experiment was conducted at a randomized design with random runs. So that the experiment conducted was not biased in terms of predicted yield and run number.

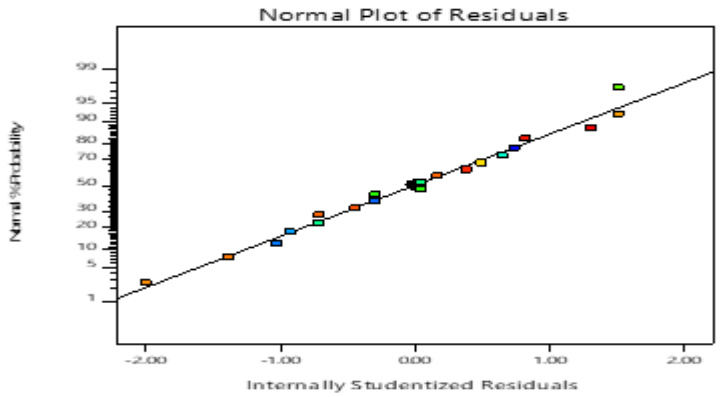

Figure 4.2 Normal probability plots of residuals

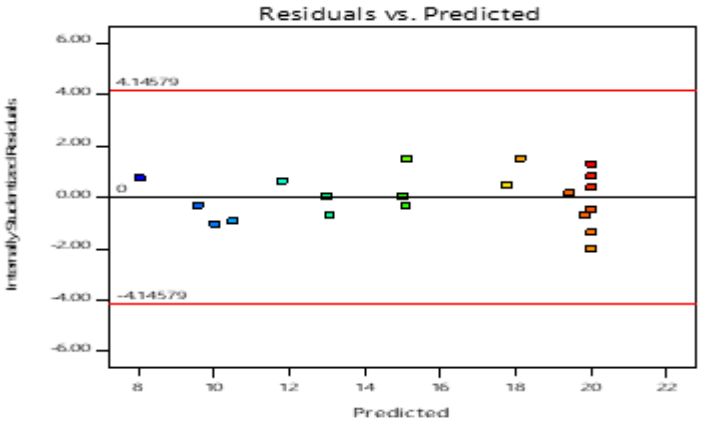

Figure 4.3 Plot of residuals vs. predicted

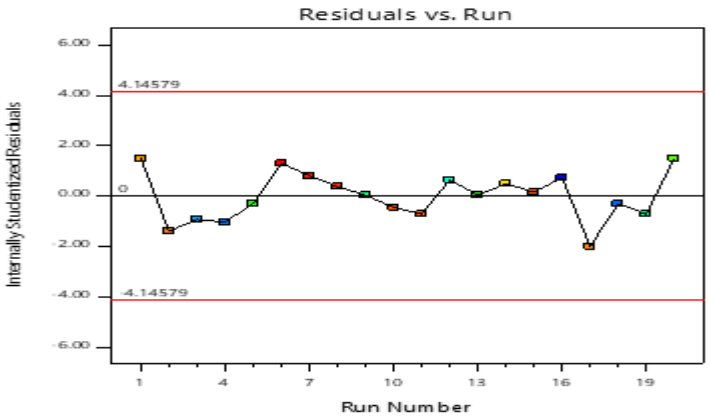

Figure 4. 4 Student zed Residuals vs. Run

4.3.4 Effect of Dependent Variables (Process Variables) on Percentage Yield

In this study work the 3D response surface plot was built from the advanced models that response surface plot is graphical representations of a regression equation that illustrate the main and interactive effects of independent variables on a response variable. These graphs was drawn by maintaining one factors constant (in turn at its central level) and varying the other two factor in order to understand their main and interactive effects on the dependent. These graphs are easy to understand and represent the interactions between pairs of independent variables on the responses and to locate their optimal levels.

4.3.4.1 The Combined Effect of Extraction Temperature and Solid-Liquid Ratio

The effect of temperature on the yield of crude aloe vera oil was revealed that, a positive quadratic effects at limit value. When the temperature was increased from 70 to 75℃, the oil yield of crude aloe vera extracted was increased which is shown below Fig. 4.5. According to (Toma *et al.*, 2001), studied that all over higher temperature used , great number of cavitation nucleus surface was formed hence high cavitation threshold which is responsible for acoustic cavitation. The comparative greater force split the cavitation nucleus and disrupted the cell tissues that improve the mass transfer. However, Chen, 2012 was stated that higher temperature could enhance the solubility of solid meal in solvent and decrease the viscosity and density of the extracts. The reduced density and viscosity that aid the solvent diffusion deeper into the sample surrounding substance which in turn add to the extraction efficiency by revealing more surface area of the sample to the solvent used.

In another factor, crude aloe vera extract was increased gradually when the solid-liquid ratio ranged from 1:10 to 1:12 (g/ml) that higher solvent volume can dissolve phytochemical compounds more effectively and result in a well extraction yield. Instead of, Ying, Han and Li, 2011 study stated that the concentration variance between the exterior plant cell and the interior solvent was affected by the higher solid–liquid ratio, which could dissolve the components more effectively leading to an enhanced mass transfer rate and increase the extraction yield.

4.3.4.2 The Combined Effect of Extraction Time and Temperature

The crude aloe vera extract yield was increased when the duration was maintained from 3 to 5 hours but slowly decreased when the duration continued to be extended which is given away in Fig. 4.6. Most of the bioactive compounds in broken cells are released at the later period of extraction, because heating more enhanced the release of those compounds into the exterior solvent and increased the yield in the first 4 hour. However, Tiwari et al., 2010, stated that longer extraction time might be induced the degradation of bioactive compounds. The number of cavitation micro-bubbles created by siphon increased with the duration extended. The irregular downfall of micro-bubbles near surfaces was also related with oil that could burnish surfaces and damage substance in solution (Vilkhu *et al.*, 2008).The structure of bioactive compounds was damaged and its stability declined because of frequent failure of micro-bubbles. The results was in clearly specified that 4.27 hour of extraction time was sufficient enough to extract bioactive compounds.

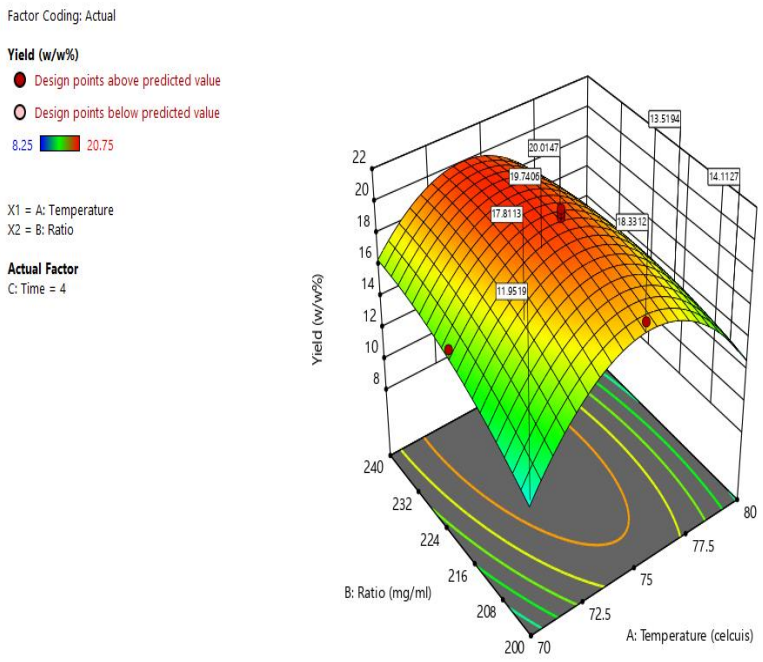

Figure: 4.5 Effect of the interaction between solid to liquid ratio and extraction temperature on oil yield Response surface plot

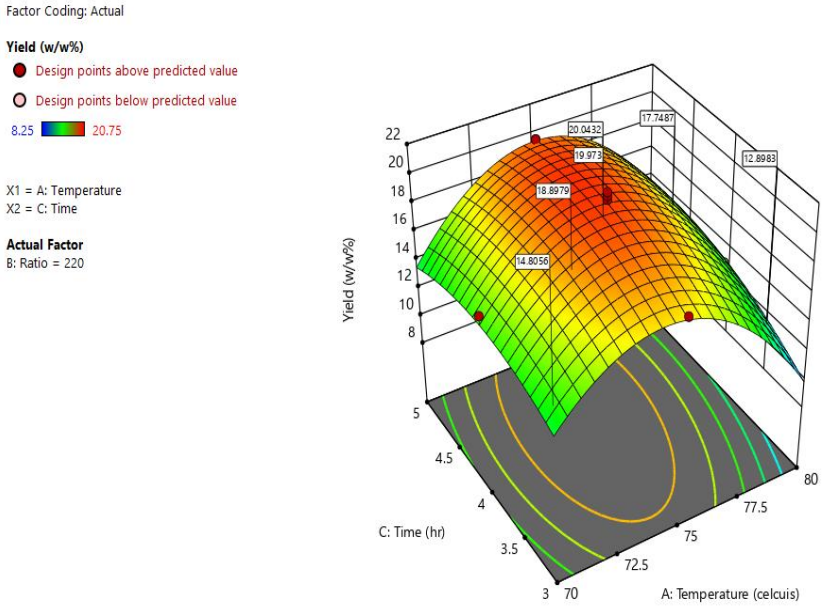

Figure: 4.6 Effect of the interaction between extraction time and extraction temperature on oil yield Response surface plot.

4.3.5 Validation of Optimum Conditions and Verification of Predictive Model

An optimum condition for the extraction of crude aloe vera oil from Aloe vera leaf was determined to obtain maximum yield. In this study second order polynomial model was developed for response in order to achieve specified optimum conditions that numerical optimization estimates a point that maximizes the desirability function.

The optimum operations stated that response and all the factors corresponding with high-limit and low-limit experimental region have to satisfy the criteria in Table 4.7.

Table: 4.7 Settings for criteria optimization

Factor/response	Goal	Lower limit	Upper limit	Importance
A	In range	70	80	3
B	In range	1:10	1:12	3
C	In range	3	5	3
Crude oil yield	Maximize	8.25	20.75	5

Desired function was determined by Derringer's methodology indicated that an extraction temperature of 74.34℃, extraction time of 4.27hr.and solid–liquid ratio of 1:11.6 g/ml were gave 20.431 (w/w %) with an overall desirability value of 0.997. This optimize conditions might be considered as optimum along with possible conditions. The following data took from design expert output and among many possible combinations; five of the highest yield offering combination would take. Possible combination of the variables shown in Table 4.8.

Table: 4.8 Highest yields offering possible combination of the treatments in report form

Number	Temperature	Ratio	Time	Yield	Desirability	
1	74.339	230.379	4.262	20.413	0.997	Selected
2	74.335	230.289	4.259	20.413	0.997	
3	74.332	230.366	4.257	20.413	0.997	
4	74.357	230.185	4.265	20.413	0.997	
5	74.364	230.142	4.264	20.413	0.997	

Experiments were carried out under the optimal conditions in order to compare the experimental results with the predicted values of the responses using the developed empirical model equation Eq. (4.1). The experiments were accompanied in triplicates and the average values are reported in Table 4.9. The mean values of the yield obtained predicted values found to be in covenant

with experimental values that through confirmation experiments indicate the settled quadratic models suitability within optimal values are valid at specified range of process parameters. Upon optimization, a solution combining three factors above suggest at desirability value of one, to obtained maximum Aloe vera oil yield of 20.413%. A confirmation run to validate the model were done at a selected optimal parameters and revealed results of average aloe vera oil yield of 19.94 ± 0.62%.

Table: 4.9 Predicted and experimental values of the responses at optimum conditions

Optimal levels of process parameters	Optimized values (predicted)	Experimental values
A (℃) =74.34	20.413	19.94 ± 0.62
B (gm. /ml) =1:11.6		
C (hr.) =4.27		

4.4 Physiochemical Characterization of Extracted Oil

The process parameters were gave a maximum oil yield at extraction temperature of 74.34℃ ,extraction time of 4.27 hour and solid to solvent ratio of 1:11.6g/ml) extracted was studied for its physical and chemical properties.

4.4.1 Moisture and Volatile matter of Oil

Table: 4.10 Moisture and volatile matters of aloe vera oil

Time in hours	Wt.at time 0 hrs.	Wt.at time=1hrs	Wt.at time 2hrs	Weight in gm. loss by (2-0).hrs.
Weight in gm.	5	4.94	4.94	0.06 ± 0.03

Moisture and volatile matter of oil $= \frac{0.06}{5} * 100\% = 1.2\%$

4.4.2 Specific Gravity

Specific gravity is the ratio of the density of respective substance to the density of water at 4°C. Density bottle method was used to determine the specific gravity of oil as the detail experimental procedure were stated in Eq. 3.15 Thus**,** Specific gravity was 0.9234.Hence the density of oil can be determined using: Density of oil (ρoil) =SG * $\rho water$) = 0.9234*1000kg/m^3 was density of aloe oil.

4.4.3 Kinematic Viscosity

Dynamic viscosity of oil was determined by using rotary-viscometer as 3.45mPa.s at a temperature of 20ºC, substituting the viscosity oil = 3.45mPa.s = 3.45×10^{-3} kg.m-1.s-1 and density of aloe vera leaf oil = 923.4kg/m^3. Therefore, the kinematic viscosity aloe vera leaf oil was considered by Eq.3.16 the result was 3.736m m^2/s .

4.4.4 pH value of Oil

The pH value of aloe vera oil was triplicated and the results obtained are summarized in Table

Table: 4.11 pH value of aloe vera oil

Product	Test-1	Test-2	Test-3	Average pH-value
Aloe vera oil	5.2	4.78	4.82	4.93±.23

The pH value of aloe vera oil was slightly acidic which was 4.93±.23. According to Mueller et al.,2000 that preparation of antimicrobial activity, skin and hair care materials were preferable pH value is in the range of 3.5-6.5.Thus, pH value of aloe vera oil was found in the range that used for antimicrobial activity.

4.4.5 Acid Value

Table: 4.12 Acid value

condition	Volume of KOH for the sample	Mass of sample	Acid value
Dry	7.7	2	20.75

Acid value is the measure of total acidity of the lipid involving contributions from all the constituent fatty acids that make up the glyceride molecule. The acid number measures the amount of acids present in oil. Acid value is an indirect method for determination of free fatty acid of amount in oil samples.

4.4.6 Saponification Values

Table: 4. 13 Saponification values

Condition	Volume of KOH for blank	Volume of KOH for sample	Mass of sample	Sap value
Dry	30.5	16	2	203.36

The result of Table 4.13 shows that the saponification values of aloe vera oil 203.36Gkoh/g oil which had high saponification value involves greater proportion of fatty acids of low molecular weight.

This values obtained was favorably comparable with the saponification value of olive oil (185-196)g KOH/g oil which is a well-known vegetable oil in cosmetics industry. Thus, high saponification value of the aloe vera oil suggests the use of the oil in production of liquid soap, cosmetics, shampoos and creams.

4.5 Chemical Compositional Analysis of Aloe Vera Oil Extracted

4.5.1 Determination of the Functional Groups in Aloe Vera Oil by FT-IR

The functional groups present in the aloe Vera oil was determined by comparing the vibration frequencies in wave numbers of the sample spectrograph was obtained with those of an IR correlation chart. In the FT-IR spectrum of crude extract aloe vera oil the absorption band or frequency 3390.75cm^{-1} shown in Fig.4.7 since Szymczycha-Madeja, A., *et al,* 2013 stated that the presence of medium indicate the region from 3200-3600 cm^{-1} of functional groups such as strong broad stretch free vibration, H-bonded, C-H stretch for presence of alkene, aromatic, alkane and stretching of methyl ester. The stretch band at 2970 cm^{-1} and 2883 cm^{-1} ,C-H revealed the presence of alkane and aromatic since the range of wave number from 2850-3000cm^{-1} indicate the presence stretch vibration C-H alkane and -C-H bending vibration. The range of wave number from 1652.25cm^{-1} illustrate that esterified carboxyl group C=O, from 1700-1600cm^{-1}. From the spectra of selected oils, the bands at 1455.255 and 1044.25 cm^{-1} are evident, which are correlated to stretching vibration of C-O ester, carbocyclic and alcohol groups; a strong absorption at 1016.47cm^{-1} designated the presence of C-N Aliphatic amines, carbohydrate groups whereas; a strong absorption 625cm^{-1} specified the presence of C-H Alkane carbohydrate, aromatic ring but from 1500-400 cm^{-1} finger print (*Nurrulhidayah,* et al., 2011).

Recently, Bran, 2014 stated that oils extracted from various herbs and spices have been a subject of intensive research partially due to the continuous discoveries for multifunctional activities other than classical roles as food additives and/or fragrances. Thus many oil have been investigated and confirmed for antibacterial, antifungal, and antioxidant activities

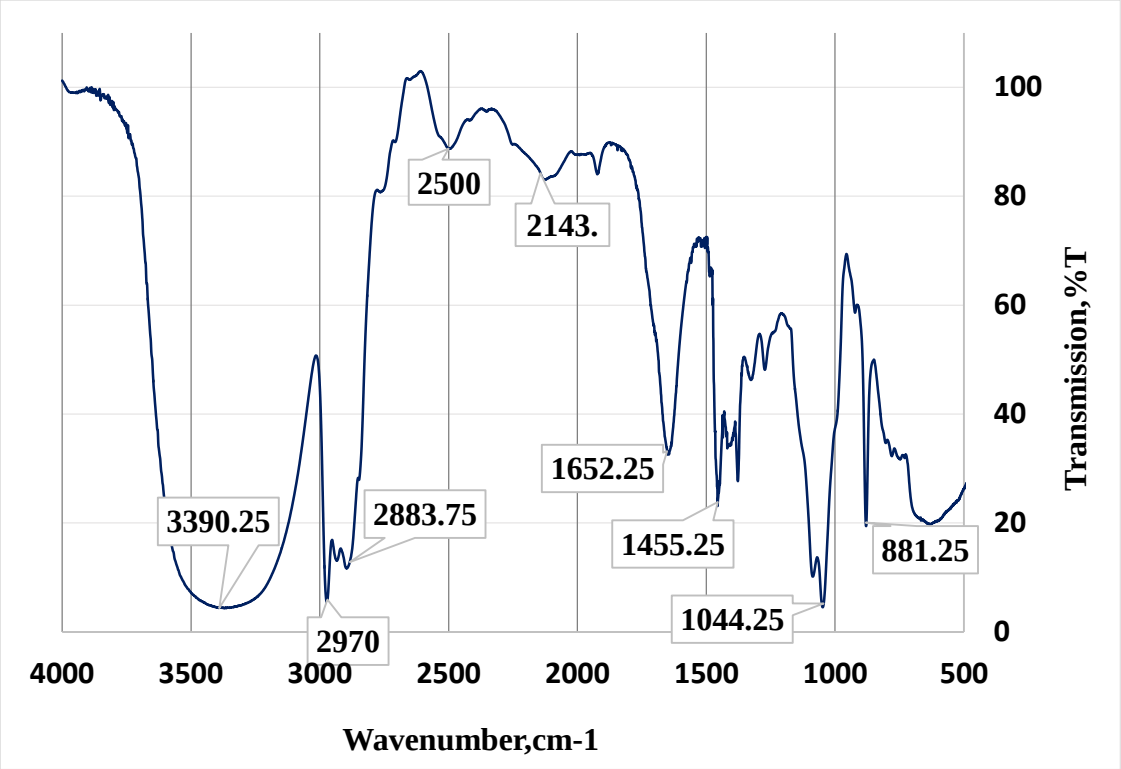

Figure: 4.7 FT-IR analysis of aloe Vera oil.

4.5.2 Determination of Bioactive Compounds in Aloe Vera Oil by GC-MS

In the GC-MS analysis bioactive phytochemical compounds were identified in the ethanolic extract of Aloe Vera. The identification of phytochemical compounds is based on the retention time (RT), molecular formula, molecular weight (WM) and concentration (%) are presented in Table 4.14. Interpretation on mass spectrum GC-MS was conducted using the database of National Institute Standard and Technology (NIST). The spectrum of the unknown component was compared with the spectrum of the known components stored in the NIST library. Essential, major active compounds were found to be 9, 12- octadecadienoic acid (Z, Z), methyl ester ($C_{19}H_{34}O_2$) with RT 47.598 has peak area 30.18% , 9- octadecadienoic acid, methyl ester (E)($C_{20}H_{36}O_4$) with RT 47.731 has peak area 13.14%,Hexadecanoic acid, methyl ester, ($C_{17}H_{34}O_2$) with 42.978 has peak area 8.72% , 9, 12- octadecadienoic acid, ethyl ester ($C_{20}H_{36}O_2$) with RT 49.215 has peak area 6.25%,Methyl strearate ($C_{19}H_{38}O_2$) with RT 48.343 has peak area 5.95%Ethyloleat,9-octadecadienoicacid,ethylester($C_{20}H_{38}O_2$) with RT 49.360 has peak area 3.15%, Oleic Acid, 9- octadecadienoic acid, (E) –($C_{18}H_{34}O_2$) with RT 48.759 has peak area 2.15% were instigated in tested ethanol aloe vera oil.

These findings was confirmed with Arunkumar and Muthuselvam,2009 stated that bioactive phytochemical compounds were identified in ethanolic extraction aloe vera including these were J. Sitosterol (C29 H50O) with RT 38.78 has peak area 13.19% ,Oleic Acid (C18 H 34O2) with RT (21.85) and 9,12,15- Octodecatrienoic acid, methyl ester, (Z,Z,Z). (C19H33O2) with RT 22.06 ranks next having peak area 11.74% and 11.36% respectively.According to the study of Lakshmi and Rajalakshmi, 2011, major compounds in Aloe vera like n-Hexadecanoic acid, Oleic Acid, Squalene, butyl octyl ester ,Tetradecanoic acid were prevailing compounds shown to have the activity as anticancer, antimicrobial ,antioxidant ,antifouling etc.

Table: 4.14 Major Bioactive Compounds Identified In Aloe Vera Oil via GC-MS

RT	Name of compound	MF	MW	PA (%)
30.12	Phenol, 2, 4-bis (1, 1-dimethylethyl)	$C_{17}H_{30}OSi$	278	0.08
37.04	Tetradecanoic acid, methyl ester	$C_{15}H_{30}O_2$	242	0.17
42.99	Hexadecanoic acid, methyl ester,	$C_{17}H_{34}O_2$	270	8.72
	pentadecanoic acid, methyl ester	$C_{16}H_{32}O_2$	256	
47.56	9, 12- octadecadienoic acid (Z, Z), methyl ester	$C_{19}H_{34}O_2$	294	30.18
47.73	9- octadecadienoic acid, methyl ester (E),	$C_{20}H_{36}O_4$	296	13.14
48.34	Methyl stearate	$C_{19}H_{38}O_2$	298	5.95
48.76	Oleic Acid, 9- octadecadienoic acid, (E)	$C_{18}H_{34}O_2$	282	2.15
49.23	9, 12- octadecadienoic acid, ethyl ester	$C_{20}H_{36}O_2$	308	6.25
49.36	Ethyloleat, 9- octadecadienoic acid, ethyl ester	$C_{20}H_{38}O_2$	310	3.15
50.012	octadecadienoic acid, ethyl ester,	$C_{20}H_{38}O_2$	310	1.37
	Heptadecanoic acid, ethyl ester			
51.288	9-octadecadienoicacid, 1 2-hydroxy-	$C_{19}H_{36}O_3$	312	0.25
	Methyl ester,[R(Z)]			
53.223	Methyl18-methanonadecanoate	$C_{21}H_{42}O_2$	326	0.27
	,Eicosanoicacid, methyl ester			

4.6 Phytochemical Constituents of the Crude Extract Aloe Vera Oil

In my research study work was carried out on the aloe vera revealed the presence of medicinal active constituents. The phytochemical active compounds of Aloe Vera were qualitatively analyzed and the results are presented in Table 4.15. Based on the presence or absence of color change indicate positive or negative results indicated .According to the phytochemical screening study, the crude extract oil from Aloe vera leaf was found to be positive for the existence of alkaloids, tannins, flavonoids, saponins, polyphenols and Terpenoids then the negative for absences of steroid all of the tested secondary metabolites.

Neog and Deka, 2013 was stated that groups of phytochemical compounds commonly associated with combating microbial resistance and having antimicrobial activity in medicinal plants are flavonoids, alkaloids, tannins, Terpenoids, and phenols. Even though, at this point in time, it is difficult to judge the mechanism of actions of the bioactivity of the crude of the study plant, that possible to speculate their antimicrobial effect based on the different approach of action of the bioactive phytochemicals detected in phytochemical analysis of this study.

The antimicrobials activities of flavonoids isolated from medicinal plants have long been studied by different studies. For instance, different structural derivatives or congeners of flavonoids like quercetin, naringenin apigenin, luteolin crycristagallin, and orientanol B have been found to have antimicrobial activities against the growth of different bacteria (Kujala *et al.*, 2000). Therefore, the presence of flavonoids in crude extract of the leaf of Aloe vera could contribute their own share for the observed antimicrobial activities, especially against gram positive bacteria. The possible mechanism of action for the antibacterial effects of flavonoids includes the damage or disruption of the cell membranes and inhibition of the synthesis of nucleic acids which can lead to the death of the susceptible bacterium (Dzoyem, Tshikalange and Kuete, 2013).

Polyphenols are the other phytochemicals that might involve in the antimicrobial activities of medicinal plants used for different infectious diseases. The extracts of phenolic compounds (isolated from Carum carvi) had been found to have a growth inhibition effect against different bacteria (E. coli, S. aureus and Salmonella typhimurium). In addition, polyphenols can disturb the metabolic function of microorganisms by forming dense soluble complexes with enzymes. Thus, the antibacterial activity of the crude extract of the present plant could be associated with

the mentioned approach of action of polyphenols and with their possible attack on the cell walls of bacteria (Shivasharan *et al.*, 2013).

Tannins are the other compounds that have been found to have antimicrobial activities against the growth of bacteria. The antibacterial role of tannin constituents in green tea leaf extract has been shown to inhibit and decrease the load and growth of aerobic, mouth cavity colonizing bacteria. In addition, different derivatives of tannins such as catechin, ellagetannin and gallotannin have also been found to be bioactive compounds against the growth of *S. aureus* (Trakranrungsie, 2011). Therefore, the antibacterial activity findings of aloe vera could be due to the presence tannins in the crude extract.

The antibacterial mechanism for tannins might be due to its membranes damaging effects and inhibition of metabolic pathways of bacteria like oxidative phosphorylation which could lead to death of the microorganism(Moghadamtousi *et al.*, 2013).

Terpenoids are the other class of compounds known to have antimicrobial activities. The Terpenoids fractions isolated from Luffa cylindrical (Nagarajan et al., 2010) and Elephantopus scaber (Jasmine et al., 2007) were found to have antibacterial activities against various pathogenic microbes including S. aureus, and P. aerginosa with varying selectivity. Therefore, the antibacterial activities of Terpenoids detected in the crude extract of the study plant might be linked to the disruption of cytoplasmic cell membrane.

Moreover, the antibacterial activities of medicinal plants are associated with its alkaloid components that extracted from the leaf of Prosopis juliflora have been found to be bioactive phytochemicals which inhibited the growth of gram positive and negative test bacterial strains(Ahlawat and Khatkar, 2011). Therefore, the presence of alkaloids in the crude aloe vera oil might be contributed for their respective antibacterial activities to disruption of cell membranes or inhibition of the protein synthesis of bacteria.

Anthraquinones had been detected in the extracts of Aloe Vera leaves. Thus, Yang, Liu and Gao, 2009 were stated that antibacterial activities of the crude of the present study might be related to the presence of anthraquinones.

This antibacterial action of anthraquinones has been strengthened by growth inhibitory effect of different anthraquinones compounds such as Emodin (isolated from Rheum officinale) and 1, 8-dihydroxy-anthraquinone isolated from Porphyra haitanensis.

Finally, saponins have also been considered to have antibacterial activities. Most literatures found that saponins fractions or isolated saponins compounds have antibacterial activities. For instance, the saponins purified fractions isolated from sorghum bicolor (Soetan et al., 2006) and Acacia aroma (Mattana et al., 2010) were found to have antibacterial activity against the growth of S. aureus.

Table: 4.15 phytochemical investigation of Aloe vera oil using chemical test methods

Metabolites tested	Crude extract
Alkaloids	+
Anthraquinones	+
Flavonoids	+
Polyphenols	+
Saponins	+
Steroid	-
Tannins	+
Terpenoids	+

+: present, - : absent

4.7 Effect of Extract Crude Aloe Vera Oil on Selected Microbial

Antimicrobial activity of ethanol extract of aloe vera leaf sample was tested for selected bacterial and fungal strains based on its availability and by considering the likely that can cause bacterial and fungal infections for which the experimental plant is indicated traditionally against of gram positive bacteria (Bacillus subtiles and staphylococcus Aureus) of gram negative bacteria (Pseudomonades aerginosa and salmonella typhus) and of fungal strains (Aspergillus niger and candida albicans). The dimethyl Sulfoxide (DMSO) was used as negative control and Ciprofloxacin was used as positive control and Ketoconazole positive control for bacteria, fungi respectively. The results of the initial antimicrobial screening assay of the crude extracts aloe vera leaf plant on selected microbial strains in different concentration were shown in Table4.16.

Table 4:16 Results for the antimicrobial activity crude extract aloe vera oil

Tested sample	Zone of inhibition (mm) Bacteria Strains				Fungi Strains	
Conc.(%v/v)	Bs	Sa	Pa	St	An	Ca
1/32	14	10	11	9	10	10
1/16	15	15	12	10	12	12
1/8	20	16	11	13	13	13
1/4	22	18	15	12	15	15
1/2	23	21	20	13	20	18
Ciprofloxacin*10	25	30	35	30	NT	NT
Ketoconazole*30	NT	NT	NT	NT	30	35

*Bs=Bacillus subtiles; Sa = Staphylococcus aureus; Pa =pseudomonas aerginosa; salmonella typhus; Ca = Candida albicans; An = Aspergillus niger; NT = not tested, * = positive controls.*

The valuable antimicrobial activities of aloe vera oil showed that it is effective against gram positive bacteria; S. aureus and B. subtiles, gram negative bacteria; P. aerginosa and S. typhus and fungi; A. niger and C. albicans. Aloe vera oil showed a good potential new source of high-value oils of antimicrobial effects. The aloe vera oil was shown good antimicrobial activity against S. aureus B. subtiles, P. aerginosa S .typhus, A. Niger and C. albicans with maximum zone of inhibition 21±0.23, 23±0.25, 20±0.51, 13±0.31, 20±0.15 and 18±0.25mm respectively. The growth inhibition of the crude extract of aloe vera leaf of against tested the gram positive bacteria was greater than gram negative bacteria.

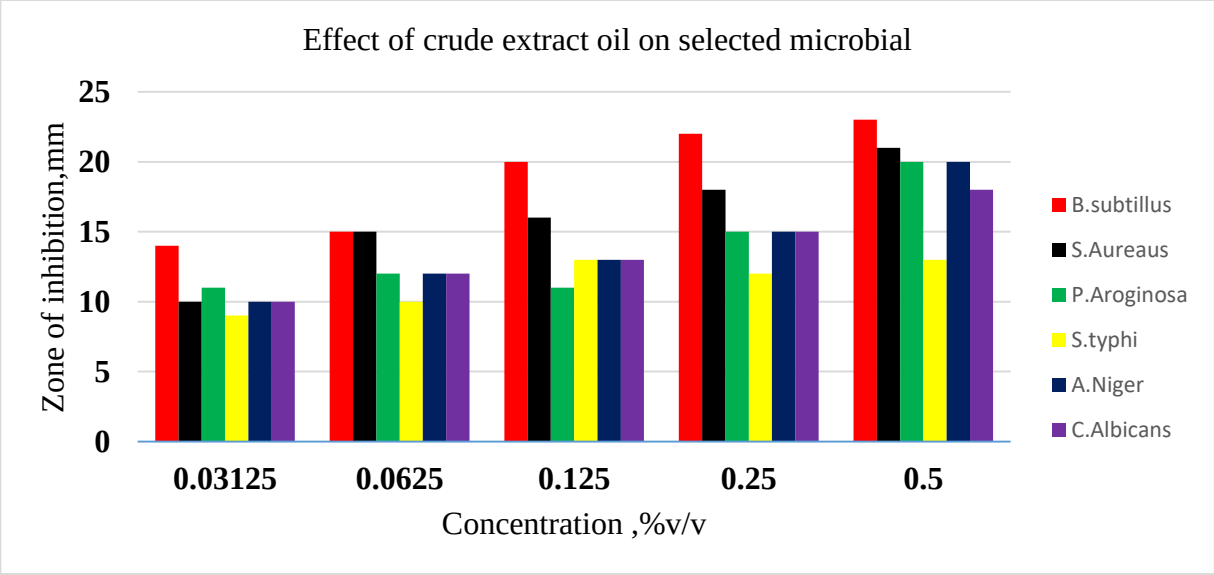

Figure 4.8 Effect of extract oil on selected microbial

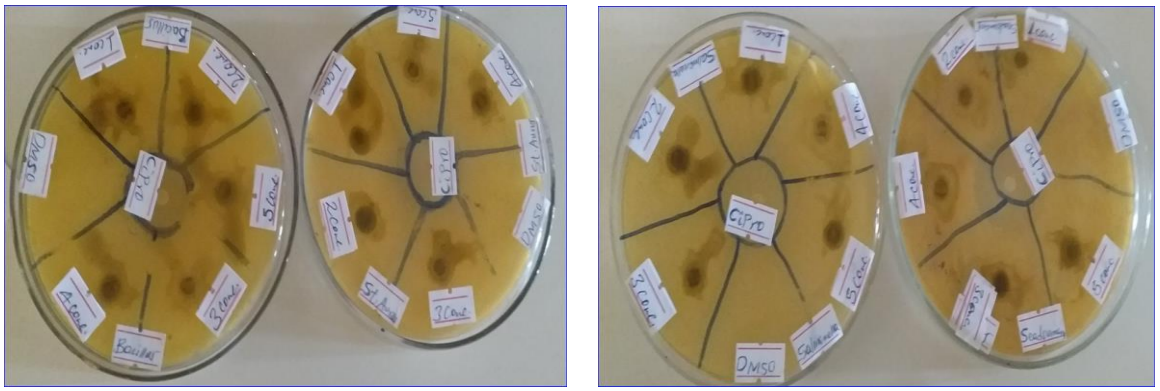

Figure 4.9Antimicrobial effects of aloe vera oil against bacteria isolates of B. subtiles S. aureus, p .aerginosa and S. typhus respectively

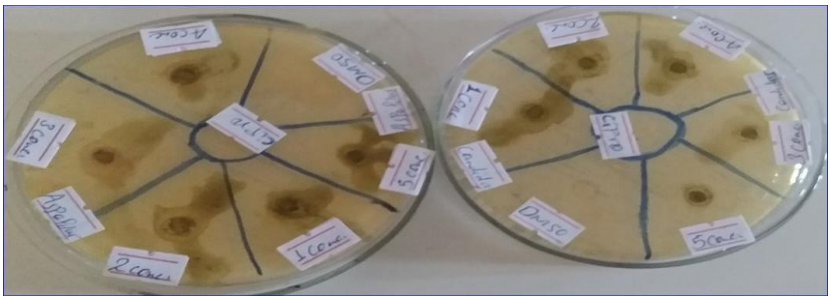

Figure 4.10 Antimicrobial effects of aloe vera oil against fungi isolates of A. Niger and Albicans

5. CONCLUSION AND RECOMMENDATION

5.1 Conclusion

This research was intended to study the influence of different factors (extraction temperature, extraction time and aloe vera powder to solvent ratio) on the quality and quantity of oil extracted from aloe vera using ethanol as solvent and evaluation of aloe vera oil for antimicrobial activity. Variability of these operating conditions is the pre-dominant factors for the quality and quantity of aloe vera oil. There are different methods of oil extraction from aloe vera but in this research, Soxhlet extraction was used. From the experimentation it was found that maximum oil yield of 20.413% was obtained at extraction temperature 74.34ºC, extraction time of 4.26 hour and aloe vera meal to solvent ratio of 1:11.6mg/ml. A minimum oil yield of 8.25 % was obtained at extraction temperature 80ºC, extraction time of 3 hour and Aloe vera meal to solvent ratio 1:11.6mg/ml, the observed quantitative difference in the quantity of the oil was due to effect of extraction parameters. Thus, determination of appropriate extraction temperature, optimal extraction time and aloe vera meal to solvent ratio for the recommended particle size needs to have a consideration to get the maximum amount of the required product. From statistical data analysis of ANOVA P value < 0.0001 for extraction temperature, extraction time and aloe vera meal to solvent ratio indicate that operating parameters have significant effect on oil yield. Physiochemical property of extracted oil were specific gravity, pH, kinematic viscosity, density, moisture and volatile contents, acid value and saponification value were: 0.9234, 4.93 ± 0.23,3.74mm^2/s, 923.4kg/m^3, 1.2, 20.75±0.23mg KOH/g oil and 203.36±0.31mg KOH/g oil respectively. The quality of the oil could be affected due to several reasons like purities with the leaf, genotype of the leaf, operating conditions, maturity stage, drying condition, and type of soil and extraction equipment. From the investigation, extraction temperature, solid to liquid ratio and extraction time was the dominant factor for the change in quality of the oil.

Analysis using Gas Chromatography-Mass Spectrometer was found to be the best method to identify fatty acid composition of particular oil along with major components, and determination of functional group by FTIR. The valuable antimicrobial activities of aloe vera oil showed that it is effective against gram positive bacteria; S. aureus and B. subtiles, gram

negative bacteria; P. aerginosa and S. typhus and fungi; A. niger and C. albicans. Aloe vera oil showed a good potential new source of high-value oils of antimicrobial effects.

In conclusion, this study can be in food, cosmetics and medicinal sectors which have interest to engage in manufacturing natural antioxidant from aloe vera oil

5.2 Recommendation

Recommendation for further work

- Comparison of different extraction technology such as steam distillation, supercritical fluid extraction and cold press with solvent extraction is suggested.
- Detailed feasibility study of aloe vera oil in Ethiopia is recommended
- Effect of particle size and solvent type and other factors on the percentage oil yield is suggested.
- Aloe vera oil for cosmetics, shampoo, and cream and soap application is suggested
- Anti-microbial activity on other micro-organism such as other bacteria, fungus and virus.

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APPENDIX

Appendix: A. Equations and Formulas used for characterization of the oil

Moisture

Crucible 1

(B) Crucible weight =24.60gm

(C) Crucible with sample weight=26.60gm

Result after Cooling

(D) Crucible with sample weight = 24.7056gm

$$\%Moisture(crucible1) = \frac{(C-D)*100\%}{(C-B)}$$

1.8944/2*(100%) = 94.72%

Crucible 2

(B) Crucible weight =26.80gm

crucible with sample weight=28.80gm

Result after Cooling

(D) Crucible with sample weight = 26.91gm

$$\%Moisture(crucible2) = \frac{(C-D)*100\%}{(C-B)}$$

1.8931/2*(100%) =94.66%

Average moisture content =94.69%

Ash Content Determination

Crucible 1

(B) Crucible weight =20.8955gm

(C) Crucible with sample weight=22.8955gm

Result after Cooling

(D) Crucible with sample weight = 22.6379gm

$$\%Ash\ content(crucible1) = \frac{(C-D)*100\%}{(C-B)}$$

$$\%Ash\,content(crucible2) = \frac{(C-D)*100\%}{(C-B)}$$

0.2576/2*(100%) = 12.88%

Crucible 2

(B) Crucible weight =21.9055gm

(C) crucible with sample weight=23.9055gm

Result after Cooling

(D) Crucible with sample weight = 22.6579gm

0.2476/2*(100%) =12.38%

Average ash content = (12.88+11.38)/2=12.63

Appendix B: Laboratory equipment and samples photo.

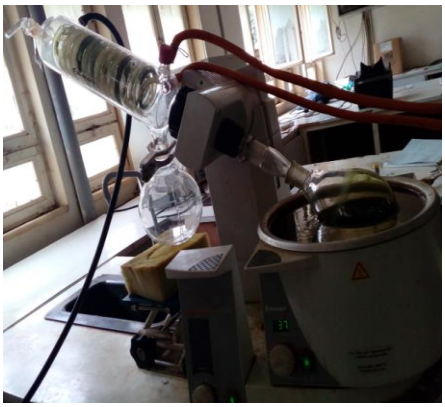
Aloe vera leaf

Aloe vera leaf powder

Aloe vera oil

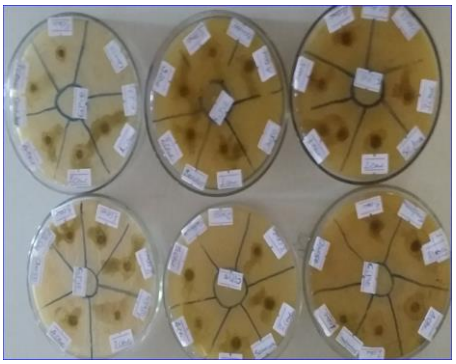
Agar Muller preparation

Soxhlet extraction oil

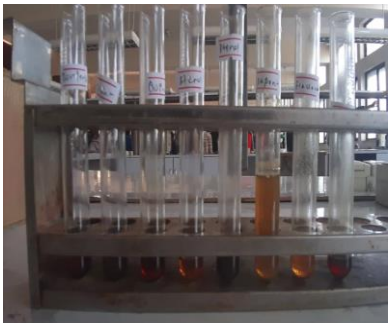


Rotary Evaporator

Laminar air flow



Tests of antimicrobial



Test of phytochemical screen

Atomic Absorption Spectroscopy

GM-MS

Appendix C: Results from the statistical analysis.

Sequential Model Sum of Squares [Type I]

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean vs. Total	5008.61	1	5008.61			
Linear vs. Mean	24.54	3	8.18	0.4122	0.7465	
2FI vs. Linear	26.34	3	8.78	0.3920	0.7608	
Quadratic vs. 2FI	**287.32**	**3**	**95.77**	**250.66**	**0.0001**	**Suggested**
Cubic vs. Quadratic	1.15	4	0.2875	0.6459	0.6500	Aliased
Residual	2.67	6	0.4451			
Total	5350.63	20	267.53			

Select the highest order polynomial where the additional terms are significant and the model is not aliased.

Lack of Fit Tests

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Linear	315.02	11	28.64	58.25	0.0001	
2FI	288.68	8	36.09	73.39	< 0.0001	
Quadratic	**1.36**	**5**	**0.2725**	**0.5543**	**0.7335**	**Suggested**
Cubic	0.2125	1	0.2125	0.4323	0.5399	Aliased
Pure Error	2.46	5	0.4917			

The selected model should have insignificant lack-of-fit.

Appendix D Characteristic IR Absorption of Some Functional Groups

TABLE

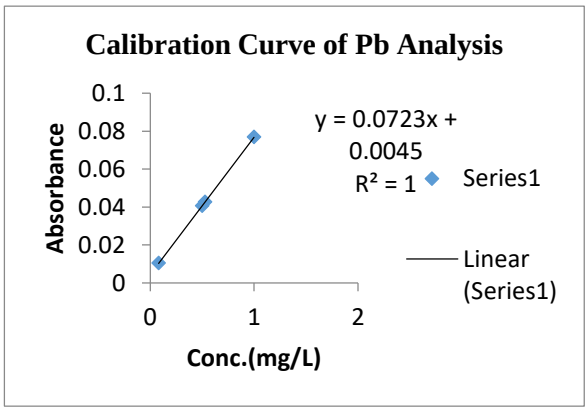
Characteristic IR Absorptions of Some Functional Groups

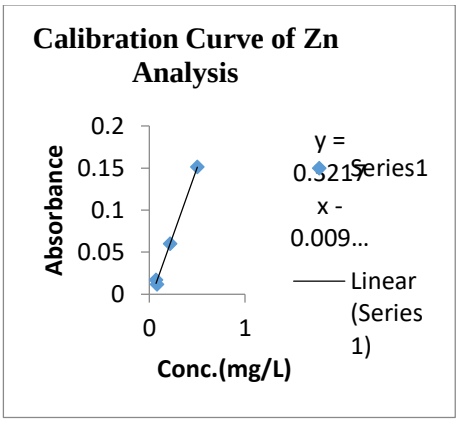
Functional group	Absorption (cm^{-1})	Intensity
Alkane		
C–H	2850–2960	Medium
Alkene		
=C–H	3020–3100	Medium
C=C	1640–1680	Medium
Alkyne		
≡C–H	3300	Strong
C≡C	2100–2260	Medium
Alkyl halide		
C–Cl	600–800	Strong
C–Br	500–600	Strong
Alcohol		
O–H	3400–3650	Strong, broad
C–O	1050–1150	Strong
Arene		
C–H	3030	Weak
Aromatic ring	1660–2000	Weak
	1450–1600	Medium
Amine		
N–H	3300–3500	Medium
C–N	1030–1230	Medium
Carbonyl compound		
C=O	1670–1780	Strong
Carboxylic acid		
O–H	2500–3100	Strong, broad
Nitrile		
C≡N	2210–2260	Medium
Nitro		
NO_2	1540	Strong

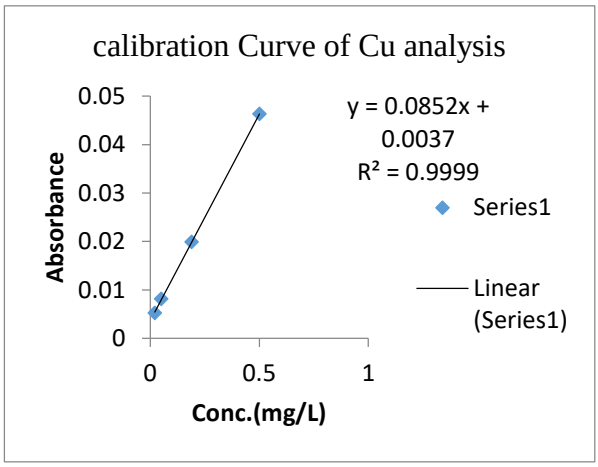
Appendix E Data of calibration mineral element Determination

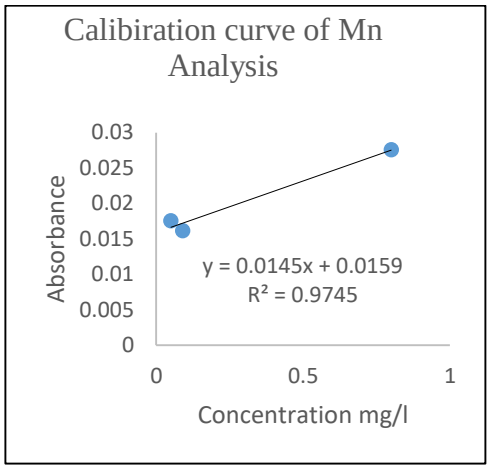
			Concentration(mg/L)	Absorption
1	Auto zero	Pb283		
2	Cal-Std1	Pb283	0.5	0.0406
3	Cal-Std2	Pb283	0.08	0.01036
4	Cal-Std3	Pb283	1	0.0769
5	Compute calibration	Pb283		
		Average	0.526667	0.04262
1	Cal-Zero1	Zn213		
2	Cal-Std1	Zn213	0.08	0.01177
3	Cal-Std2	Zn213	0.07	0.01705
4	Cal-Std3	Zn213	0.5	0.15128
5	Compute calibration	Zn213		
		Average	0.216667	0.060033
1	Auto zero	Cu324		
2	Cal-Std1	Cu324	0.02	0.00522
3	Cal-Std2	Cu324	0.05	0.00816
4	Cal-Std3	Cu324	0.5	0.04631
5	Compute calibration	Cu324		
		Average	0.19	0.019897
1	Auto zero	Mn228		
2	Cal-Std2	Mn228	0.09	0.0162

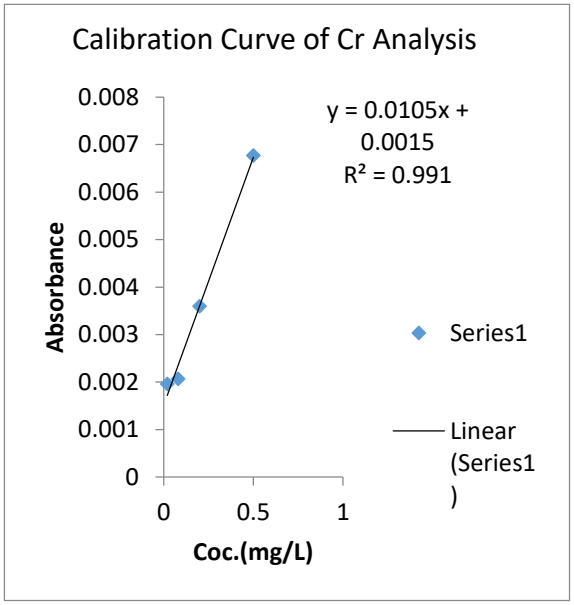
3	Cal-Std2	Mn228	0.05	0.0176
4	Cal-Std3	Mn228	0.8	0.0276
5	Compute calibration	Mn228		
		Average	0.313333	0.020467
1	Auto zero	Co240		
2	Cal-Std2	Co240	0.05	0.00133
3	Cal-Std3	Co240	0.5	0.02707
4	Cal-Std4	Co240	1	0.04419
5	Compute calibration	Co240		
		Average	0.516667	0.024197
1	Auto zero	Cr357		
2	Cal-Std1	Cr357	0.02	0.00196
3	Cal-Std2	Cr357	0.08	0.00207
4	Cal-Std3	Cr357	0.5	0.00677
5	Compute calibration	Cr357		
		Average	0.2	0.0036

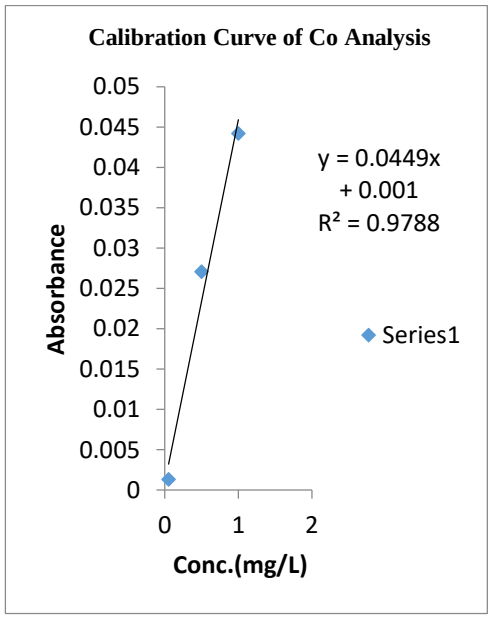

Calibration Curve of Pb Analysis
Absorbance
Conc.(mg/L)
y = 0.0723x + 0.0045
R² = 1
Series1
Linear (Series1)


Calibration Curve of Zn Analysis
Absorbance
Conc.(mg/L)
y = 0.3217x - 0.009...
Series1
Linear (Series 1)


calibration Curve of Cu analysis
Absorbance
Conc.(mg/L)
y = 0.0852x + 0.0037
R² = 0.9999
Series1
Linear (Series1)


Calibration curve of Mn Analysis
Absorbance
Concentration mg/l
y = 0.0145x + 0.0159
R² = 0.9745


Calibration Curve of Cr Analysis
Absorbance
Coc.(mg/L)
y = 0.0105x + 0.0015
R² = 0.991
Series1
Linear (Series1)


Calibration Curve of Co Analysis
Absorbance
Conc.(mg/L)
y = 0.0449x + 0.001
R² = 0.9788
Series1