UNIVERSITY FOR DEVELOPMENT STUDIES, TAMALE

ISOLATION, CHARACTERIZATION AND ANTI-INFECTIVE STUDY OF COMPOUNDS FROM THE LEAF OF *ALOE BARBADENSIS* MILLER

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BY

KUUBABONGNAA NGMENPONE (BSc. APPLIED CHEMISTRY)

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THESIS SUBMITTED TO THE DEPARTMENT OF APPLIED CHEMISTRY AND BIOCHEMISTRY, FACULTY OF APPLIED SCIENCES, UNIVERSITY FOR DEVELOPMENT STUDIES IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF PHILOSOPHY DEGREE IN APPLIED CHEMISTRY (PHARMACEUTICAL CHEMISTRY OPTION)

JUNE, 2018

DECLARATION

I hereby declare that this thesis is the result of my own original work and that no part of it has been presented for other degree in this university or elsewhere:

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I hereby declare that the preparation and presentation of the thesis was supervised in accordance with guidelines on supervision of thesis laid down by the University for Development Studies.

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ABSTRACT

Extracts from the leaves of *Aloe barbadensis* Miller were prepared and two compounds, namely, 1,8-dihydroxy-3- hydroxymethyl-9- antracenone (Aloin A) and 5, 22-Cholestadien-24-ethyl-3β-ol, (Stigmasterol) were isolated, formulated as ointments and tested for antimicrobial activities against *E. coli, K. pneumoniae, P. aeruginosa, S. aurous, C. albicans* and *T. fluvus*. The structure of the two compounds were characterized on the basis of extensive spectral data (^{1}H-NMR, ^{13}C-NMR, IR and GC-MS). Although not novel compounds, their formulation with polyethylene glycol (PEG) as an ointment against wound pathogenic microorganisms may have been accomplished in this work for the first time. The activity of Aloin A- PEG-ointment against the test microorganisms was stronger and more effective than the pure Aloin A alone. This was observed from the low MIC values recorded; 2.5, 0.63, 0.63, 0.32, 0.32 and 1.25 mg/g for *P. aeruginosa, E. coli, K. pneumoniae, S. aureus, C. albicans* and *T. fluvus* respectively. Similarly, the activity of Stigmasterol-PEG ointment on the test microorganisms resulted in very low MIC values as compared to that of pure stigmasterol only and was statistically significant ($P < 0.05$). The MIC values obtained were as follows: 0.08, 0.63, 1.25, 0.63, 1.25, and 0.16 mg/g for *P. aeruginosa, E. coli, K. pneumoniae, S aureus, C. albicans* and *T. fluvus* respectively. The data of this current research confirms the antibacterial and antifungal actions of *Aloe barbadensis* Miller and presented the biologically active bioactive compounds which include Aloin A and a phytosterol known as stigmasterol. The plant isolate-PEG ointments should be explored further in vivo to determine the effectiveness of these ointments for possible potential use for treating infectious diseases.

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I dedicate this work to my lovely wife, Mad. Louisa Kuubaar and my beautiful daughter, Rynna Kuubabongnaa.

LIST OF ABBREVIATIONS

WHO:	World Health Organization
US:	United State
R_f:	Retention factor
EAC:	Ehrlich ascites carcinoma
SOD:	Super Oxide Dismutase
HSV-2:	Herpes Simplex Virus type-2
TCM:	Traditional Chinese Medicine
HPLC:	High Performance Liquid Chromatography
TLC:	Tin Layer Chromatography
NMR:	Nuclear Magnetic Resonance
^{1}H NMR:	Proton Nuclear Magnetic Resonance
^{13}C NMR:	Carbon-13 Nuclear Magnetic Resonance
FTIR:	Fourier Transform Infrared
GC-MS:	Gas Chromatography-Mass Spectrometry
ETEC:	Enterotoxigenic *E. coli*
A/E:	Attaching and Effacing
UPEC:	Uropathogenic *E. coli*
UTI:	Urinary Truck Infections
AIDS:	Human Immune Deficiency Syndrome
HIV:	Human Immune Virus
CDC:	Center for Disease Control

STD:	Sexually Transmitted Disease
PEG:	Polyethylene Glycol
MHA:	Mueller Hinton agar
NB:	Nutrient Broth
MIC:	Minimum Inhibitory Concentration
MBC:	Minimum Bactericidal Concentration
MFC:	Minimum Fungicidal Concentration
E. coli:	*Escherichia coli*
S. aureus:	*Staphylococcus aureus*
P. aeruginosa:	*Pseudomonas aeruginosa*
K. pneumoniae:	*Klebsiella pneumoniae*
C. albicans:	*Candida albicans*
T. fluvus:	*Talaromyces fluvus*
n:	Number of replications/repetitions
STDEV:	Standard Deviation

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CHAPTER ONE

1.0 INTRODUCTION

1.1 Background of Study

Natural products, such as plants extract, either as pure compounds or as standardized extracts, provide unlimited opportunities for new drug discoveries because of the unmatched availability of chemical diversity (Cosa *et al.*, 2006). According to the World Health Organization (WHO), more than 80% of the world's population relies on traditional medicine for their primary healthcare needs. The use of herbal medicines in Asia, Africa and many other places represent a long history of human interactions with the environment. Plants used for traditional medicine contain a wide range of substances that can be used to treat chronic as well as infectious diseases (Duraipandiyan *et al*., 2006). Due to the development of adverse effects and microbial resistance to the chemically synthesized drugs, men turned to ethnopharmacognosy. They found literally thousands of phytochemicals from plants as safe and broadly effective alternatives with less adverse effect. Many beneficial biological activity such as anticancer, antimicrobial, antioxidant, antidiarrheal, analgesic and wound healing activity have been reported (Duraipandiyan *et al.,* 2006). In many cases, the people claim the good benefit of certain natural or herbal products.

Research on plants with medicinal properties and identification of the chemical components responsible for their activities have justified the ancient traditional healing wisdom and have proven the enduring healing potential of many plant medicines (Babu *et al.*, 2009). However, wild plants have always been the major source of primary health care and other necessities of daily life for local communities throughout the world, an

indication that medicinal plants can provide the best alternative source to obtain a variety of drugs (Barbara, 2014). Drugs of natural origin now play an ever more important role in medical and healthcare services because metabolites produced by plants constitute a major source of bioactive substances which can be used as an alternative for cheap and effective herbal drugs against common infections (Sati and Joshi, 2011).

It has been reported of the use of medicinal plants against bacteria by different ethnic communities throughout the world. These medicinal plants having antagonistic efficacy against bacteria have been evaluated pharmacologically and several active components have been isolated (Sabo, 2015). Pharmacologically, investigated plants using various models and plant parts include; *Allium cepa, Allium sativum, Pimpinella anisum, Sassafras albidum, Morinda citrifolia, Gaultheria procumbens, Zingiberofficinale roscoe*; occasionally *Z. capitatum,* Siegesbeckia orientalis *Linn. Berberistinctoria Lesch. Justiciabetonica Linn*. (Sasikumar *et al*., 2007), *Satureja bakhtiarica* (Ahanjan *et al.,* 2014). *Anaphalis margaritacea* (L.), *Apocynumandrosaemifolium* L., *Arctostaphylosuva-ursi* (L.) Spreng, *Cornuscanadensis* L., *Rauvolfia serpentine, Tageteserecta, Xanthium strumarium* L. and *Aloe barbadensis* Miller (Hassan *et al*., 2014). Aloe is a very important plant that has been used in folklore for different purposes, such as for healing boils, wounds, burns and treatment of different ailments (Bruneton, 1995). The plant is one of the most abundant in Ghana. It is distributed in all parts of the country being abundant in the southern parts. The plant has been used from time in memorial for various purposes such as, treatment of acute wounds and burns including sun-ray burns, and as a major component of hair cream and skin smoothing cream, it is also used for the treatment of constipation problems, skin diseases, urinary tract infections as well as

stomach ulcers and treatment of asthma using steam from exudates (Karkala and Bhushan, 2014). Many researchers have endeavored to establish the active ingredients in *Aloe vera* since it has been used for many centuries for its therapeutic and curative properties. Although over 75 active ingredients from the Aloe gel have been identified, therapeutic effects have not been correlated well with each individual component (Habeeb *et al.,* 2007). This research therefore seeks to validate the activity of *Aloe vera* as a result of some bioactive compounds by isolating and testing some of these compounds.

1.2 Problem statement

Despite the existence of potent antibiotic and antifungal agents, resistant or multi-resistant strains are continuously appearing (Silver, 1993). Widespread antibiotic resistance, the emergence of new pathogens in addition to the resurgence of old ones, and the lack of effective new therapeutics exacerbate the problems therefore imposing the need for a permanent search and development of new drugs (Turkoglu *et al*., 2006). It is therefore very necessary that the search for newer antibiotic sources be a continuous process. Although, there are several researches aimed at evaluating the antimicrobial potential of extracts of *Aloe barbadensis* Miller, the antimicrobial activity of isolated compounds from this plant have not been reported extensively. Hence, the motivation for the current research work.

1.3 Justification

It has been reported that, Plants are the cheapest and safer alternative sources of antimicrobials (Doughari *et al*., 2007). Since the medicinal properties and chemical information of some compounds isolated from *Aloe barbadensis* Miller is not conclusively documented, this research would provide comprehensive chemical information about the leaf of the plant and also provide information about the potent constituents responsible for its antibacterial activity against wound and skin diseases.

1.4 Statement of the Research Hypothesis

The leaf of *Aloe barbadensis* Miller contains bioactive constituents, which have antimicrobial activity.

Chemical constituents of the leaf of *Aloe barbadensis* Miller are not bioactive.

1.5 Research Aim

To isolate and characterize stable bioactive compounds in the leaf of *Aloe barbadensis* Miller which are partly responsible for its medicinal properties.

1.5.1 Specific Objectives

- To isolate stable compounds from the crude extract obtained from the leaf of *Aloe barbadensis* Miller.

- To carry out Spectroscopic analysis on isolated compounds using FT-IR, $^{1}H/^{13}CNMR$, GC-MS in order to determine the structures of the compounds.
- To determine physicochemical parameters of the isolated compounds (log P and pKa) in order to determine the mechanisms of action of the said compounds.
- To formulate the isolated compounds into an ointment using polyethylene glycol (PEG)
- To screen for antibacterial and antifungal properties of the pure compounds and their formulations

1.6 Significance of study

To validate the medicinal use of *Aloe barbadensis* Miller from Ghana

To establish stable active compounds in the leaves of the plant potent against some selected microorganisms.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Description of the aloe plant

Throughout the world today, extensive investments regarding therapeutic applications of herbal plant- resources, which are of unlimited abundance to improve the quality of life, have been done. One of the medicinal plants which is unique in terms of geographic distribution, specie abundance and chemical composition is the aloe plant (Atherton, 1998).

The name aloe is from the Greek word "alsos" meaning "bitter juice" from the leaves of the aloe plant. In Hebrew, it is termed as "allal" and 'alloeh" in Arabic both meaning bitter (Park, 2006).

The aloe plant is known to have several species with some being hybridized. Some of the species include: *Aloe barbadensis* Miller *(Aloe vera), Aloe indica, Aloe chinesis, Aloe affinalis, Aloe aborescens, Aloe litoralis, Aloe perfoliata, Aloe vulgaris* etc. of which only *Aloe barbadensis* Miller (Figure 2.1) and *Aloe aborescens* are grown commercially (Kokate, 2005).

Aloe vera plants are succulent and are xerophytes adapted to living in areas of low water availability and characterized by possessing a large water storage tissue (Maharjan et al., 2015). The principal feature of the *Aloe vera* plant is its high water content, ranging from 99-99.5% with the remaining 0.5-1.0% solid material containing over 75 different potentially active compounds including simple and complex polysaccharides, minerals,

water- and fat-soluble vitamins, enzymes, organic acids and phenolic acids (Hamman, 2008).

Aloe vera (Figure 2.1) is a perennial stemless, short-stemmed succulent plant from the family of Liliaceae, which grows to about 30 cm tall, spreading by offsets. The leaf resembles a 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 (Poor *et al.*, 2002; Ramachandran *et al.*, 2012; Vogler and Ernst, 1999). The leaves are spirally arranged as a clustered rosette, thick and fleshy, green to grey-green, with some varieties showing white flecks on their upper and lower stem surfaces. The margin of the leaf is jagged and has small-hardened pale teeth, 2 mm long, 10-20 mm apart, along the margin. Most mature leaves are present on the outward region of the rosette (Sampathkumar *et al.*, 2010).

The flowers are produced in summer on a spike up to 90 cm tall, each flower being pendulous, with a yellow tubular corolla 2–3 cm long. The fruits are woody capsules with many black seeds (Carter *et al.*, 2011; Holmes and White, 2002; Wood, 1997). Like other Aloe species, *Aloe vera* forms arbuscularmycorrhiza, a symbiosis that allows the plant better access to mineral nutrients in soil (Gong *et al*., 2002). The plant reaches maturity after four years with a complete life cycle of twelve years (Evans, 1996). This however is at variance with another studies which says the plant will have fully mature leaves in 3 years (Karkala and Bhushan, 2014). Each plant usually has 12-16 leaves and may weigh up to 3 pounds when matured. The plant can be separated into two products: aloe latex and aloe gel. Aloe latex (aloe juice) is the bitter yellow exudates from the pericyclic tubules found in the outer skin of the leaves. The major and active constituents of aloe

latex are hydroxyanthracene derivatives (15-40%) such as the anthraquinone glycosides, Aloin A and B (Saccu *et al.,* 2001; Rowe and Parks, 1941). Aloe gel is the colourless gel contained in the inner part of the fresh leaves (Reynolds and Dweck, 1999). The gel consists primarily of water (> 98%) and polysaccharides (pectins, cellulose, hemicelluloses, glucomannan, acemannan and mannose derivatives). Traditionally, *Aloe vera* gel is used both, topically (treatment of wounds, minor burns and skin irritations) and internally to treat constipation, coughs, ulcers, diabetes, headaches, arthritis and immune-system deficiencies (Eshun and He, 2004). Aloe plant can survive for more than 7 years without water. It takes the water it needs for survival and growth from dew collected on the surface of its leaves. It repels attacking insects, rodents, snakes by means of the bitter Aloin (the yellow colored part of the sap) just beneath the rind (Karkala and Bhushan, 2014).

Figure 2.1: Picture of *Aloe barbadensis* Miller. Source: (Spohn, 2013).

2.2 Historical use of *Aloe vera.*

There are many romantic tales about Aloe, suggesting that Egyptian queens Nerfertiti and Cleopatra, used it as part of their regular beauty regimen (Danddof, 1987). The earliest known record of medicinal application of aloe dates to a Sumerian clay tablet from approximately 2200 BC indicating the use of the plant in what is now Iraq over 4000 years ago (Park and Lee, 2006). Similarly, Ancient Egyptians papyrus, Mesopotamian clay tablets contain Aloe as an active ingredient used in curing infections, treating skin problems and as a laxative (Atherton, 1998).

Supposedly, Alexander the Great in 333 BC occupied the Island of Socotra in the Indian Ocean for its famed Aloe supplies needed to treat his wounded soldiers (Atherton, 1998). Aloe is also common in both traditional Chinese and Ayurvedic medicine. The Chinese describe Aloe's skin and the inner lining of its leaves as a cold, bitter remedy which is downward draining and used to cure constipation due to accumulation of heat (Jeffrey, 1985). In Arabian medicine, the fresh gel is used on the forehead as a headache remedy or robbed on the body to cool it in case of fever, as well as being used for wound-healing, conjunctivitis and as a disinfectant and laxative (Robson *et al.,* 1982). As with many other succulent plants noted for their unusual or even bizarre appearance, aloes have been used in various activities relating to superstition (Reynolds, 1966). Aloe products, such as dried leaves, have been found amongst the items used for fetish purposes by traditional priests and witch doctors. As uprooted aloes can survive for years, and even flower in this condition, they are often hung over doors of houses as charms intended to ensure long life for the occupants. In the home of a childless woman in Botswana, it is supposed to indicate whether or not the woman will bear a child; according to whether the plant

flowers or dries up. Several species including *Aloe vera*, are planted on graves as the home of the spirits of their male ancestors (Jackson, 1964; Reynolds, 1950).

According to the Roman scholar, Pliny, the plant was also used for embalming (Sikarwar Mukesh *et al*., 2010). Today, Egyptians still hang an aloe plant over the door of a new house to provide a long and fruitful life for its occupants. In India the whole leaves, exudate, and fresh gel aloe are used as a cathartic, stomachic, emmenagogue, and anthelmintic. Until the 1930s in the U.S., the primary commercial use of aloe was the dried latex as a laxative (Crosswhite and Crosswhite, 1984). No wonder the plant is being referred to as the "plant of sanctuary immortality" and was given as an offering at the funerals of pharaohs by the Egyptians (Schmelzer, 2008).

Since aloe has naturalized throughout the warm regions around the world, it is difficult to correctly establish its origin. It is supposed to be a native of North Africa or the Nile region in Sudan. Presently, it is widely distributed throughout the tropics and subtropics (Schmelzer, 2008).

2.3 Modern Use of *Aloe vera*

The genus Aloe contains over 400 different species with *Aloe barbadensis* Miller (*Aloe vera*), *Aloe arborescence* and *Aloe chinensis* being the most popular. *Aloe barbadensis* Miller is considered to be the most biologically active (Joshi, 1997). It is also reported that, *Aloe vera* is the oldest medicinal plant ever known and the most applied medicinal plant worldwide. (Parthipan *et al*., 2011). Many beneficial effects of this plant are attributed to the polysaccharides present in the gel which are widely used in various

cosmetic, neutraceutical and medical applications (Hamman, 2008). Recently, only a few species of aloe have been considered for commercial importance, of which *Aloe vera* is considered the most potent and thereby the most popular plant in the research field (Eshun and He, 2004). Although *Aloe vera* is effective when taken orally, it is also beneficial when included in topical formulations like ointment, cream or lotion. It mainly protects the wounds due to its moisturizing properties (Karkala and Bhushan, 2014).

Commercially, aloe can be found in pills, sprays, ointments, lotions, liquids, drinks, jellies, and creams. Numerous aloe species around the world are used for conditions ranging from dermatitis to cancer (Kathi and Victoria, 1999). Various researches have revealed that *Aloe vera* leaf possesses many therapeutic activities, including antimicrobial, anticancer, antioxidant, antidiabetic, antiulcer, hepatoprotective, immunomodulatory and many more (Blitz *et al.*, 1963; Borra *et al.*, 2011; Bruneton, 1995). Many of the health benefits associated with *Aloe vera* have been attributed to the polysaccharides contained in the gel of the leaves (Atul et al., 2011; Bashir et al., 2011; Borra et al., 2011; Chandan et al., 2007; Josias, 2008; Miladi et al., 2008; Naveena et al., 2011).

The original commercial use for the Aloe plant was in the production of a late substance called aloin, a yellow sap used for many years as a laxative ingredient. This product became synonymous with the name "Aloe" and recorded in the trade, technical, and government literature during the early 20th century. This terminology created much confusion later when aloe's other main ingredient, aloe gel, beginning in the 50's gained respect as a useful commodity that is used as a base for nutritional drinks, as a moisturizer, a healing agent in cosmetics and drugs (Karkala and Bhushan, 2014).

2.3.1 *Aloe vera* gel

This is a clear, mucilaginous and aqueous extract of the inner central area of the leaf pulp as shown in figure 2.2. *Aloe vera* gel serves as the water and energy storage component of the plant. The mechanical extrusion of the mucilaginous gel from the inner leaf pulp gives a 70% yield with a water content of 99–99.5% (Femenia *et al.,* 1999). The Aloe gel has been extensively used in gastrointestinal disorders, including peptic ulcer and its clinical efficacy has been documented (Blitz *et al.,* 1963).In recent times, *Aloe vera* gel has been used as an active ingredient in hundreds of skin lotions, sun blocks and cosmetics (Crew, 1939). Aloe gained popularity in the United States in the 1930's with reports of its success in treating x-ray burns (Rowe, 1941).The inner gel, or "pure gel", is the more readily known part of the *Aloe vera* plant. This is the section of the plant that is most commonly known to be used for treatments of sunburns. It is reported that if prepared properly, the inner gel can be consumed to help treat certain internal ailments (Karkala and Bhushan, 2014). *Aloe vera* gel has been sold in the health food market as a tonic, as well as for "supporting the immune system" and "supporting healthy breathing" (Bruneton, 1995). Externally, the gel has been used in various ways: in cosmetics, dermabrasion, wound healing, and psoriasis. In cosmetics, the gel is added to moisturizers, cleansers, shampoos, suntan lotions, and sunburn treatments (Bruneton, 1995). Some in vivo studies revealed that, aloe gel has shown significant results in the treatment of asthma, peptic ulcers, and diabetes mellitus (Bruneton, 1995).

Figure 2.2: Nature of *Aloe vera* gel

2.3.2 Aloe latex

The restricted distribution of the bitter latex within the margins of the leaves of the *Aloe vera* plant suggests that this thin layer is the primary site of secondary metabolites biosynthetic compounds that do not function directly in plant growth and development but serve as a plant defense strategy (Boudreau *et al.,* 2013). A wide variety of secondary compounds have been isolated from the *Aloe vera* latex including; phenolic compounds, anthraquinone *C*-glycosides, anthrones, and free anthraquinones (Reynolds, 2004).The drug derived from aloe latex is part of one of the strongest anthraquinone groups that function as stimulant laxatives. The latex is officially approved as a laxative in the U.S., England, and Germany. The German Commission recommends the use of aloe for

occasional constipation and for conditions that require a soft stool, such as anal fissures, hemorrhoids, and after rectal or anal surgery (Blumenthal *et al.*, 1998; Bradley, 1992). Topically, the latex is used like the gel and as a soothing agent in treating burns and mild cuts (Blumenthal *et al.*, 1998; Bradley, 1992).

2.4 Chemistry of Aloe

The chemistry of the aloe plant has been studied for many years from a number of viewpoints. The leaf, the most frequently studied organ, can be divided into the outer green mesophyll, including the vascular bundles and the inner colourless parenchyma containing the well-known aloe gel and the exudates; arising from cells adjacent to the vascular bundles (Beaumont *et al.*, 1985), which is usually yellow-brown. The first real investigation which provided scientific enlightenment concerning Aloe's therapeutic efficacy started in the twentieth century (Rowe and Parks, 1941). That was the age when a medical chemist travelled extensively and brought back plants reputed for medical applications including cathartics, in order to isolate and characterize active substances using the chemistry of the day (Saeed *et al.,* 2004). *Aloe vera* leaves contain phytochemicals, such as acetylated mannans, polymannans, anthraquinone,C-glycosides, anthrones and anthraquinones, such as emodin and various lectins (Parthipan *et al.,* 2011). Numerous scientific studies on *Aloe vera* have demonstrated its analgesic, anti-inflammatory, wound healing, immune modulating and anti-tumor activities as well as antiviral, anti-bacterial, and antifungal properties (Blitz *et el.*, 1963; Borra *et al.*, 2011; Bruneton, 1995). However, Aloes medicinal properties can be attributed to the

synergistic effect of the combined nutritional elements producing a more powerful effect than the individual components (Karkala and Bhushan, 2014).

2.4.1 Chemical Constituents of *Aloe vera*

There are many chemical constituents derived from *Aloe vera* such as; Acidic galactan, Arabinans, Glucogalactomannan, Glucomannan, Polyuronide, Cellulose, 7-Hydroxy aloin, Aloe-emodin, Aloe saponarin I&II, Aloin A and B (barbaloin), Anthranol, Beta barbaloin, Chrysophanol, Chrysophanolglucoside, Isobarbaloin, Capric acid, Hexadecadienoic acid, Palmitleic acid, Stearic acid, β-Carotene, Choline, Folic acid, Vitamin K, Vitamin D, Vitamin E, Arginine, Glutamic acid, Magnesium, Calcium, Zinc, Copper, Amylase, Catalase, Echitamine, Picrinine (Ali, 2009; Dayu, 2012; Ebadi, 2007; Evans, 2008; Kiran *et al.*, 2012; Nandy *et al.*, 2013). Similarly, Table 2.1 indicates a summary of the chemical constituents of *Aloe vera* as reported by Hamman (2008). The main feature of the *Aloe vera* plant is its high water content, ranging from 99% to 99.5%, while the remaining 0.5–1.0% solid material is reported to contain over 200 different potentially active compounds, including vitamins, minerals, enzymes, simple and complex polysaccharides, phenolic compounds, and organic acids (Boudreau *et al.*, 2013; Rodríguez *et al*., 2010). Acetylated mannan is the primary polysaccharide in *Aloe vera* gel (Ni *et al*., 2004). Other chemical constituents of *Aloe vera* include lectins such as aloctins A and B (Kuzuya *et al*., 2004).

In compositional studies on the structural leaf portions of the *Aloe vera* plant, the rind was found to compose 20–30% and the pulp 70–80% of the whole leaf weight. On a dry-weight basis, the rind and pulp contain 2.7% and 4.2% lipids, and 6.3% and 7.3%

proteins, respectively (Femenia *et al.*, 1999). The percentages of soluble sugars (11.2% and 16.5%), primarily as glucose, and the percentages of ash (13.5% and 15.4%), in particular calcium, were relatively high in the rind and pulp, respectively. The physical and chemical constituents of the products derived from *Aloe vera* plants differ depending on the source (e.g. part of the plant), the species of the plant, the climatic conditions, seasonal and grower influences and processing techniques (Waller *et al*., 2004; Boudreau *at al*., 2013).

Table 2.1: Composition and properties of *Aloe vera* gel

Class	Compounds	Properties
nes	Aloe-emodin, aloetic-acid, anthranol, barbaloin (aloin A and aloin B), ester of cinnamic acid	Aloin and emodin act as analgesics, antibacterials, and antivirals
es	Pure mannan, acetylated mannan, acetylated glucomannan, glucolactomannan, galactan, galactogalacturan, arabinogalactan, galactoglucoarabinomannan, pectic substances, xylan, cellulose.	A glycoprotein with antiallergic properties called alprogen and novel anti-inflammatory compound
	8-C-glusoly- (2-O-cinnamoly)-7-O-methylaloediol A, 8-C-glucosyl- (S)-aloesol, 8-C-glucosyl-7-O-methylaloediol A, 8-C-glucosyl-7-O-methylaloediol, 8-C-glucosyl-noreugenin, isoaloeresin D, isorabaichromone, neoalosin A.	The novel anti-infalmmatory commands
	Alkaline phosphates, amylase, brandykinase, carboxygenase, lipase, oxidase, phosphoenolpyruvate, carboxylase, superoxide dismutase	Brandykinase helps to reduce excessive inflammation when applied to the skin topically, while the others help in the breakdown of sugars and fats
	Calcium, chlorine, chromium, copper, iron, manganese, potassium, phosphorous, sodium, zinc.	They are essential for the proper functioning of various enzymes systems in different metabolic pathways and few are antioxidants
ıs ınd	Arachidonic acid, Y-linolanic acid, steroids (campesterol, cholesterol, Bsitosterol), gibberillin, lignins, potassium sorbate, salicylic acid, uric acid	
proteins	lactins, lactin-like subtances	It also contains salicylic acid that possesses anti-inflammatory and antibacterial properties, lignin, an inert substance, when included in topical preparations, enhances penetrative effect of the other ingredients into skin. Saponins that are the soapy

		substances form about 3% of the gel and have cleansing and antiseptic properties
Saccharides	Mannose, glucose, L-rhamnose, aldopentose	
	Vitamin A, B12, C, E, choline and folic acid	Vitamin A, C and E are antioxidants and Antioxidants neutralizes free radicals
	Auxins and gibberallins	They help in wound healing and have anti-inflammatory action

rrif and Sandeep, 2011)

2.4.2 Acemannan

Acemannan (Figure 2.3) is a linear polysaccharide composed of (1,4)-linked mannosyl residues, with C2 or C3 acetylated and some side-chains formed by galactose units attached to C6. It is a β-(1,4)--linked polydispersed, highly acetylated mannan with an average molecular weight of approximately 1000 kDa and the structure as shown in figure 2.2. It is obtained from the inner leaf gel of *Aloe vera* (Tanwi *et al.*, 2014). Acemannan works in part by stimulating the macrophage, a key component of the immune system that is responsible for a wide range of potential health benefits. Acemannan has shown to accelerate wound healing (Erik *et al.*, 2009). Acemannan produces immune agents such as interferon and interleukin which help to destroy viruses, bacteria, and tumor (cancer) cells (Tanwi *et al.*, 2014; Erik *et al.*, 2009).

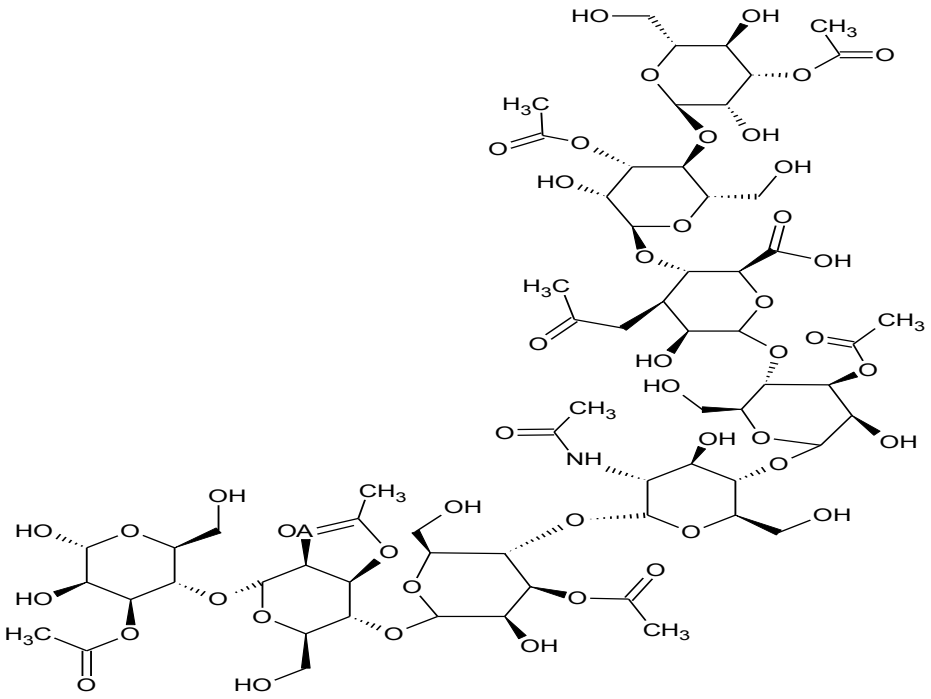


Figure 2.3: Structure of acemannan: Source (www.chemicalbook.com, n.d.-b)

2.4.3 Aloe emodin

Aloe emodin (Figure 2.4) is an anthraquinone present in aloe latex, an exudate from the aloe plant (Mc Analley, 1993). Aloe-Emodin was able to inhibit cell growth in several tumor cells, including human lung carcinoma (Lee *et al.,* 2001), hepatoma (Yeh *et al.*, 2003), and leukemia cell lines (Kuo *et al.*, 2002).

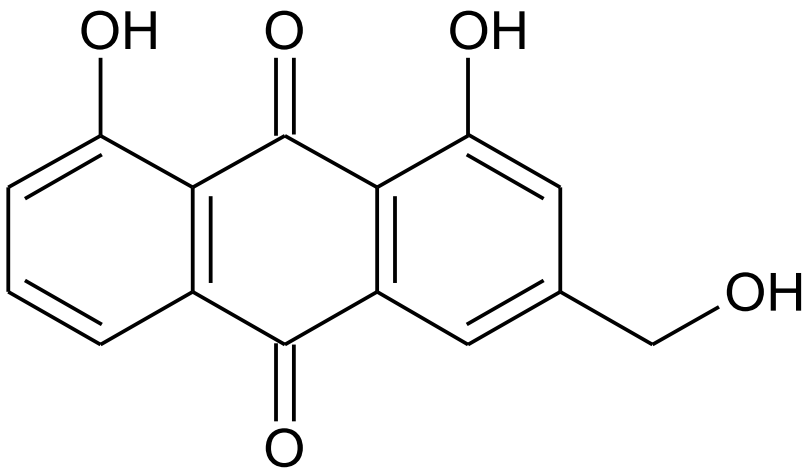
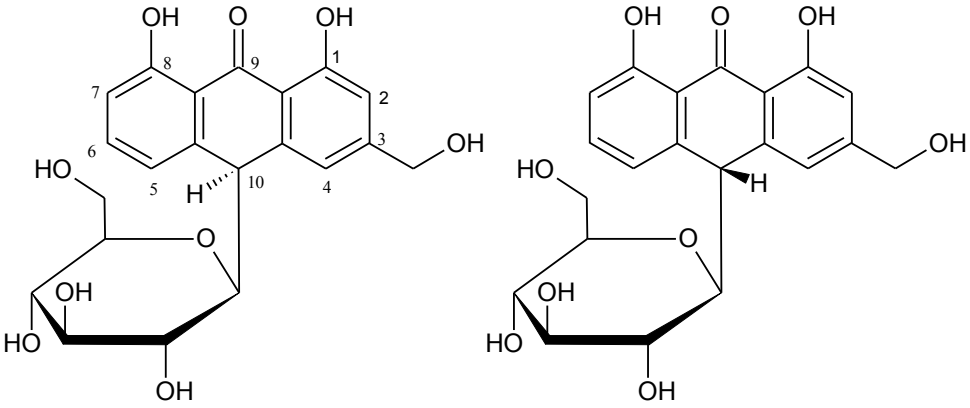


Figure 2.4: Structure of aloe emodin Source: (Jain *et al*., 2011).

2.4.4 Aloin

Aloin (Figure 2.5) is an anthraquinone glycoside. Aloin is also known as barbaloin. It has molecular weight 418, molecular formula $C_{21}H_{22}O_9$ and its chemical structure is shown in figure 2.5. Its IUPAC name is 8-Dihydroxy-10-(-D-glucopyranosyl)-3- hydroxymethyl) -9(10H)-anthracenone (Femenia *et al.,* 1999). It is a yellow-brown compound estimated at levels from 0.1 to 0.66 % of dry leaf present in cells adjacent to the rind of the leaf in gel. It is used as laxative to maintain digestive system and for treating constipation by inducing bowel movements (Grindlay and Reynolds, 1986).When ingested, it increases peristaltic contractions in the colon, and induces bowel movements (Atherton, 1997).

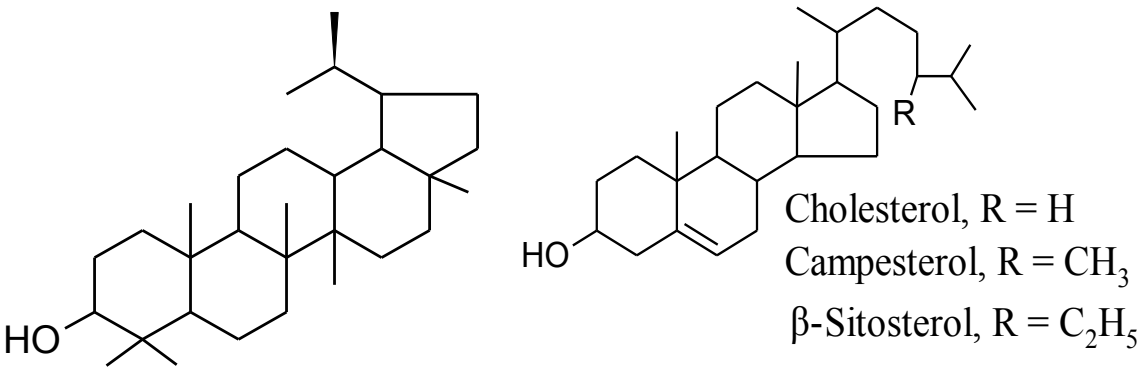


Aloin A (Barbalion) Aloin B (Isobarbaloin)

Figure 2.5: Structure of Aloin: Source (www.chemicalbook.com, n.d.-a)

2.4.5 Steroids

Campesterol is a phytosterol whose chemical structure is similar to that of cholesterol. It is the precursor of anabolic steroid bolden one which is commonly used in veterinary medicines to induce growth in cattle (Gallina *et al.*, 2007). It was found beneficial in lowering LDLs and cholesterol. It is thought that the campesterol molecules compete with cholesterol and thus reduces the absorption of cholesterol in the human intestine (Choudhary, 2011). Cholesterol, campesterol, stigmasterol, β-sitosterol, and lupeol were found in substantial amounts in the lipid fraction of *Aloe vera* leaf extract (Tanwi *et al.,* 2014) with structures shown in figure 2.6.



Lupeol

Figure 2.6: Structure of triterpenoid and sterols in *Aloe vera:* Source (Http://www.chemicalbook.com, n.d.)

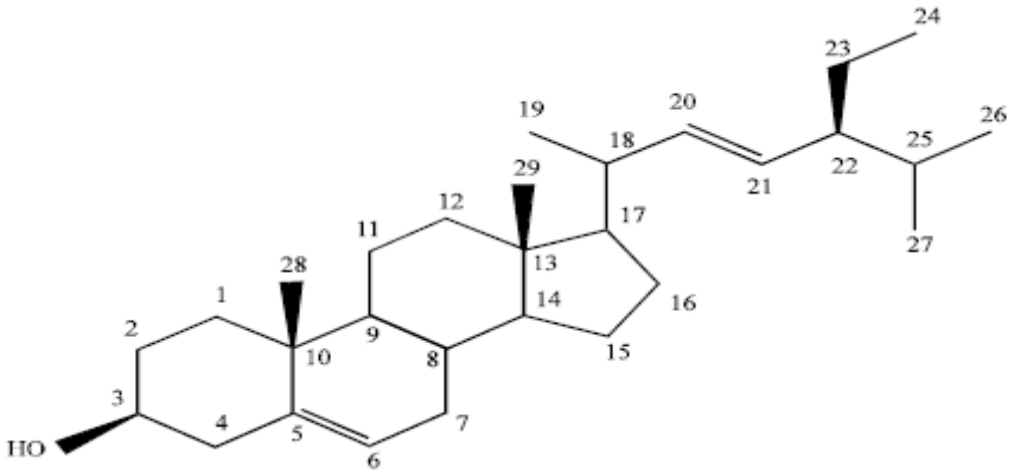


Figure 2. 7: Structure of stigmasterol in *Aloe vera:* Source (Http://www.chemicalbook.com, n.d.)

2.5 Pharmacological Studies of *Aloe vera*

Aloe has varied pharmacological activities; some of the important studies carried out are given below:

2.5.1 Wound Healing Effect of *Aloe vera*

A study was designed to assess the wound healing properties of *Aloe vera (Aloe barbadensis* Miller*)* on cutaneous wounds. The injuries of the treated animals demonstrated a better alignment, less inflammatory cell infiltration and fundamentally enhanced biomechanical properties on day 20 ($P < 0.05$). These effects proposed that application of *Aloe vera* aqueous extract on open wounds triggers significant wound contraction and quickens healing (Oryan *et al.*, 2010).

2.5.2 Anti-Ulcer Activity of *Aloe vera*

The anti-ulcer effect of *Aloe vera* in non-steroidal anti-inflammatory drug (indomethacin) induced peptic ulcer was observed in animals. *Aloe vera* showed statistically significant anti-ulcer activity compared to the standard drug omeprazole (Borra *et al.*, 2011)

2.5.3 Laxative Effect of *Aloe vera*

The laxative effect of *Aloe vera* is found to be due to the presence of anthranoid glycoside derivatives, mainly aloin. Research report indicated *Aloe ferox*resin extract increased gastrointestinal motility (Celestino *et al.,* 2013).

2.5.4 Antitumor Activity of *Aloe vera*

Antitumor action of 50% ethanol extract of *Aloe vera* was assessed against Ehrlich ascites carcinoma (EAC) tumor in mice. Drug with *Aloe vera* restored the serum biochemical parameters towards typical levels and diminished the levels of lipid peroxidation and expanded the levels of decreased glutathione and other cancer prevention compounds. The 50% ethanol extract of *Aloe vera* demonstrated antitumor

impact by adjusting lipid peroxidation and enlarging cell reinforcement barrier (Naveena and Selvasubramanian, 2011).

2.5.5 Anti-diabetic Effect of *Aloe vera*

A study was designed to evaluate the anti-diabetic, anti-hyperlipidemic and anti-oxidative activities of *Aloe vera* gel extract in diabetic and control rats. The results of this research showed that an *Aloe vera* gel extract contained an appreciable amount of (Cr, Mn and Zn) which potentiates the anti-diabetic activity of this plant (Ali and Mohamed, 2011).

2.5.6 Anti-oxidant Activity of *Aloe vera*

The effects of exudate of *Aloe barbadensis* Miller leaves on oxidative stress and some anti-oxidant status of streptozotocin induced-diabetic rats were tested. This study demonstrated that high glucose led to increased oxidative stress and exudates of *Aloe barbadensis* Miller leaves showed antioxidant action as were indicated by increased scavenging superoxide dismutase (SOD) activity and decreased in lipid peroxidation levels (Nwanjo, 2006).

2.5.7 Antimicrobial Effect of *Aloe vera*

Antimicrobial effect by a research group using *Aloe vera* was assessed by the presence of zones of inhibition. In a particular study, both the gel and the leaf inhibited the growth of *Staphylococcus aureus* and only the gel repressed the development of *T. mentagrophytes* while the leaf extract alone showed inhibitory activity against both *Pseudomonas*

aeruginosa and *Candida albicans* (Agarry *et a.l.,* 2005). In a different study, antibacterial activity of *Aloe vera* was tested against bacterial strains; *Escherichia coli, Bacillus subtilius, Salmonella typhi, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Staphylococcus epidermidis*. The methanolic extract of *Aloe vera* showed the greatest antibacterial effect as compared to other solvent extracts (ethanolic and aqueous extracts) (Agarry *et al.*, 2005). In a similar development, the following solvents; aqueous, ethanol and acetone were used to extract the bioactive compounds from the leaves of *Aloe vera* to screen for antimicrobial action against selected human clinical pathogens by agar diffusion technique. The greatest antimicrobial action were observed in acetone extracts (Kumar and Muthuselvam, 2009). It has also been reported that, *Aloe vera* leaf gel can restrain the development of two gram positive microscopic organisms *Shigella flexneri* and *Streptococcus pyogenes*. Particular plant compound, for example, anthroquinones, dihydroxy-anthroquinones and saponins have been proposed to have direct antimicrobial activity (Mariappan and Shanthi, 2012).

2.5.7.1 Antiviral Activity of *Aloe vera*

In this study antiviral activity of a crude hot glycerine extract of *Aloe vera* gel against herpes simplex virus type-2 (HSV-2) replication in Vero cell line was conducted. The extract showed antiviral activity against HSV-2 not only before attachment and entry of the virus into the Vero cells, but also in post attachment stages of virus replication (Chatterjee *et al.*, 2013).

2.5.7.2 Antifungal Activity of *Aloe vera*

Aloe vera leaves gel was evaluated for their antifungal activity at 0.15%, 0.25% and 0.35% concentration against five plants pathogenic fungi viz., *Aspergillus niger, Aspergillus flavus, Alternaria alternata, Drechslera hawaiensis and Penicillum digitatum*. *Aloe vera* gel fully inhibited the development of *Drechslera hawaiensis* and *Alternaria alternate* (Sitara *et al*., 2011).

2.5.8 Toxicity and Adverse Reactions of *Aloe vera*

The dried latex from the superficial pericycle cells of *Aloe vera* has the same side effects as different peristalsis stimulating laxatives; however Aloe has a more extraordinary irritant action than Senna. Aloe is contra-indicated throughout pregnancy, menstruation and hemorrhoids because of hyperemia of the pelvic organs. An overdose may cause extreme stomach agony, bleeding gastritis and inflammatory kidney diseases. However, the fresh aloe juice/gel ordinarily does not produce any side effects. Sometimes the local application of Aloe gel may cause an intense skin rash, which usually vanishes with continued use (Juneby, 2009).

2.6 Plant metabolites

Plant metabolites are compounds that facilitate the growth and development of plants. However, some of them are not required for plant survival. Each plant family, genus, and species produces a characteristic mix of these chemicals, and they can sometimes be used as taxonomic parameters in classifying plants. Plant metabolites are divided into three

main classes namely: alkaloids, phenolics and terpenoids. Thousands of metabolites have been isolated from plants, and many of them have demonstrated powerful physiological effects in humans and are used as medicines. Many medicinal herbs have been used for hundreds of years to treat respiratory complaints such as bronchial inflammation, pneumonia, expectoration and cough, in Traditional Chinese Medicines (TCM) and have shown less or no side effects as compared to synthetic drugs (Shang *et al.,* 2010). Similar research also reported that, plant metabolites serve as weapons against bacteria, fungi, insects, other plants and other large animals. Most secondary metabolites are toxic or repellant to other organisms, especially microbes and herbivores. They also exhibit allelopathy, that is, when a chemical signal secreted by the roots of one plant blocks the germination of nearby seeds or inhibits the growth of a neighboring plant (Peter and Raven, 2005). This was confirmed in subsiquent studies which suggest that plant metabolites have antibacterial, antifungal, anti-inflammation, antidiabitic, antiarthritic and antioxidant properties hence their ability to serve as protection for the plant (Gracelin *et al*., 2013). By this antimicrobial ability, many of these compounds are also used by humans as medicines, flavoring and recreational agents such as alkaloids nicotine and cocaine (Gracelin *et al*., 2013).

Table 2.2: Applications of some plant metabolites

Application	Compound	Species
Pharmaceuticals	Atropine	Atropa
	Scopolamine	Datura
	Quinine	Cinchona
	Cardenolides	Digitalis
	Tramadol	Latifolia
	Codeine	Papaver
Fragrances	Rose oil	Rosa
	Lavender oil	Lavendula
Flavours	Vanillin	Vanilla
	Capsaicin	Capsicum
Colours	Indigo	Indigofera
	Shikonine	Erythrorhyzon
Poisons	Coniine	Conium
	Strychnine	Strychnos
Stimulants	Caffeine	Coffea
	Theophylline	Thea
	Nicotine	Nicotiana
Hallucinogens	Cocaine	Erythroxylon
	Cannabinol	Cannabis
Insecticides	Nicotine	Nicotiana
	Pyrethrin	Pyrethrum
	Piperine	Piper

Source: (Wink, 1999)

2.7 Extraction

Extraction is a process of removing the desired plant metabolite by using a solvent. It is the first step of any medicinal plant study and plays a significant and crucial role on the final outcome of the study (Harborne, 1998).

Extractions are often carried out for one of the following reasons;

a. For unknown bioactive compounds. Plants may be reported to exhibit bioactivity and this may interest a chemist to identify the compound exhibiting such properties. These compounds may serve as pure therapeutic substances for the purported ailment or condition or may serve as moieties for modification into more useful form.
b. Extraction may also be carried out to retrieve a known compound present in plant for a special purpose.
c. Identification of all secondary metabolites of one natural source (e.g. plant) that are not produced by different "controlled" source. For example, two species of the same genius or the same species grown under different conditions. This gives evidence of the influence of the environment in the synthesis of secondary metabolites in plants.
d. Extraction may also be carried out to identify the structural relationship between compounds within the same organism.
e. Identification of all secondary metabolites present in an organism for chemical fingerprinting or metabolomics study. Metabolomics refers to the scientific study of chemical processes involving metabolites. Metabolome on the other hand refers to the collection of all metabolites in a biological cell, tissue, organ or

organism, which are the end products of cellular processes (Harborne, 1998; Sarker and Satyajit, 2006).

Recently, analysis of bioactive compounds have been made easier than before with the introduction of modern spectrometric and chromatographic techniques but to a large extent, the success still depends on the extraction methods, exact nature of plant part and input parameters (Pavia *et al.*, 1988). The extraction time, type of solvent, temperature and the metric properties of plant part are said to be the most common factors affecting extraction process (Ganza, 2014). It is only possible to conduct further separation, identification, and characterization of bioactive compounds if the extraction process has been appropriately done (Ganza, 2014). Bioactive compounds from plant materials can be extracted by various classical extraction techniques. Most of these techniques are based on the extracting power of different solvents used and the application of heat and/or mixing. The commonly used classical techniques are: soxhlet extraction, maceration and hydro distillation to obtain a crude extract which is then concentrated using a rotary evaporator (Azmir *et al*., 2013; Ganza, 2014).

2.8 Column chromatography

The most modern and sophisticated methods of separating mixtures available to the organic chemist involve chromatography. Chromatography is defined as the separation of a mixture of two or more different compounds (in some cases, ions) by distribution between two phases, one of which is stationary and one of which is mobile (Pavia *et al.,* 1988). In column chromatography, the stationary phase (a solid adsorbent) is placed in a

vertical glass column and the mobile phase (a liquid) is added to the top and flows down through the column (by either gravity or external pressure). Column chromatography is generally used as a purification technique to isolate desired compounds from a mixture (Kenkel, 2003).

Column chromatography is a technique based on both adsorptivity and solubility. It is a solid-liquid phase–partitioning technique where the solid may be almost any material that does not dissolve in the associated liquid phase. The solids most commonly used are silica gel (SiO_2 .xH_2O), also called silicic acid, and alumina ($Al_2O_3.xH_2O$). These compounds are used in powdered or finely ground; usually 200- to 400-mesh forms (Pavia *et al.,* 1988). The crude extract to be purified by column chromatography is applied at the top of the column. The liquid solvent (the eluent) is passed through the column by gravity or by the application of air pressure. Equilibrium is established between the solute adsorbed on the adsorbent and the eluting solvent flowing down through the column. Because the different components in the mixture have different interactions with the stationary and mobile phases, they will be carried along with the mobile phase to varying degrees and a separation will be achieved. The individual components, or elutants, are collected as the solvent drips from the bottom of the column (Harvey, 2000). Factors that affect column chromatography include; adsorbent chosen, polarity of solvent chosen, size of column relative to the amount of extract to be chromatographed and rate of elution. The polarity of the solvent which is passed through the column affects the relative rates at which compounds move through the column (Harvey, 2000; Pavia *et al*., 1988). Polar solvents can compete more effectively with the polar molecules of a mixture for the polar sites on the adsorbent surface and will also

solvate the polar constituents better. Consequently, a highly polar solvent will move even highly polar molecules rapidly through the column. If a solvent is too polar, movement becomes too rapid, and little or no separation of the components of a mixture will result. If a solvent is not polar enough, no compounds will elute from the column (Pavia *et al.,* 1988). Proper choice of an eluting solvent is thus crucial for the successful application of column chromatography as a separation technique. Often a series of increasingly polar solvent systems are used to elute a column. A non-polar solvent is first used to elute the less-polar compounds. Once the less-polar compound is off the column, a more-polar solvent is added to the column to elute the more-polar compounds (Kenkel, 2003).

The column size and the amount of adsorbent must be selected correctly to separate a given amount of sample well. As a rule of thumb, amount of adsorbent should be 25 to 30 times, by weight, the amount of material to be separated by column chromatography. The column should have a height- to- diameter ratio of about 8:1 (Pavia *et al.,* 1988). The flow rate of solvent through the column is very significant in the effectiveness of a separation. In general, the time the extract remains on the column is directly proportional to the extent of equilibrium between stationary and moving phases. Thus, similar compounds eventually separate if they remain on the column long enough. The time an extract remains on the column depends on the flow rate of the eluent (Pavia *et al.,* 1988).

2.9 High performance liquid chromatography

High performance liquid chromatography is basically a highly improved form of column chromatography. Instead of a solvent being allowed to drip through a column under

gravity, it is forced through under high pressures of up to 400 atmospheres that makes it move faster. It also allows the use of a smaller particle size for the column packing material which gives it a greater surface area for interactions between the stationary phase and the molecules flowing through it. This allows a much better separation of the components of the mixture (Harvey, 2000). Preparative high-performance liquid chromatography (HPLC) has become a favourite method of natural product isolation and purification. The various modes available (e.g., normal-phase, reversed-phase, size exclusion, and ion-exchange) to date can be used to purify most classes of natural products. Although preparative HPLC is very similar to analytical HPLC, instead of injecting a small amount of sample to maximize the resolution, the amount of feed is very high in order to maximize the purification productivity and minimize the amount of solvent used (Mbaveng *et al.*, 2008).

2.10 Thin-Layer Chromatography

Thin-layer chromatography (TLC) is a very important technique for the rapid separation and qualitative analysis of small amounts of material. The technique is closely related to column chromatography; it is actually considered as simply column chromatography in reverse, with the solvent ascending the adsorbent rather than descending (Pavia *et al.,* 1988). The TLC technique is often used to analyze the fractions obtained from column chromatography to determine if the fraction contains a mixture of components (different compounds) and if fractions can be combined without affecting their purity (Kenkel, 2003). The separation by TLC depends on the relative affinity of compounds towards stationary and mobile phase. The compounds move over the surface of the stationary

phase under the influence of the mobile phase. During this movement, the compounds with higher affinity to the stationary phase (less polar compounds) travel slowly while those with less affinity to the stationary phase more polar compounds) travel faster. This leads to separation of components in the mixture. The individual components are then visualized as spots on the plate after staining with iodine vapour (Harvey, 2000).

2.11 Mass spectrometry

Mass spectrometry differs from other types of spectroscopy in that a mass spectrum is not a record of the energy absorbed by a molecule in moving from one energy level to another. Instead, it allows for the determination of the molecular mass and the molecular formula of a compound, as well as certain structural features (Seyhan, 1988). A small sample of the compound is vaporized and then ionized when bombarded with a high-energy electron. Usually, the collision knocks an electron out of the molecule resulting in a radical cation (molecular ion). Many of the molecular ions fragment into smaller cations, radicals, neutral molecules, and other radical cations due to instability (McMurry, 2000; Seyhan, 1988). These fragments of the molecules are detected individually on the basis of their mass-to-charge ratios (m/e) by a detector (Pavia, *et al.*, 2001). The details of exactly how these positively charged fragments are separated and detected differ according to the specific design of the mass analyzer portion of the instrument (Pavia, *et al.*, 2001). However, the information acquired and displayed by the mass spectrum allows for the reconstruction and identification of the original molecule. Besides the significant applicability to molecular compound identification, mass

spectrometry also finds application in elemental analysis, such as to determine what isotopes of an element might be present in a sample (Kenkel, 2003).

2.12 Nuclear magnetic resonance (NMR) spectroscopy

Nuclear magnetic resonance (NMR) spectroscopy is an instrumental technique that allows the number, type, and relative positions of certain atoms in a molecule to be determined. This type of spectroscopy applies only to those atoms that have nuclear magnetic moments because of their spin properties (Pavia *et al.,* 1988). The NMR spectroscopy technique depends on the absorption of energy when the nucleus of an atom is excited from its lowest energy spin state to the next higher one. Many elements are difficult to study by NMR, and some cannot be studied at all. Fortunately though, the two elements that are the most common in organic molecules (carbon and hydrogen) have isotopes (^{1}H and ^{13}C) capable of giving NMR spectra that are rich in structural information (Pavia *et al*, 2001). A proton nuclear magnetic resonance (^{1}H NMR) spectrum tells us about the environments of the various hydrogen atoms in a molecule; a carbon-13 nuclear magnetic resonance (^{13}C NMR) spectrum does the same for the carbon atoms as seen in Table 2.3. Together, ^{1}H and ^{13}C NMR are used in determining the molecular structure of a substance. It is often used in conjunction with other spectrometric techniques such as Fourier Transform Infra-Red (FT-IR) and mass spectrometry (Carey, 2000).

Table 2. 3: ^{13}C data

Type of carbon	Chemical shift (ppm)
R—CH_3	8–25
R—CH_2—R	20–45
R_3—CH	40–60
R4—C	36–45
R—CH_2—X	15–80
R3C—NH_2	35–70
R—CH_2—OH	50–90
RC ≡CR	75–95
R_2C =CR_2	110–150
RCOOH	160–185

Source: VCAA 2015, *VCE Chemistry Data Book*, VCAA, Melbourne, p. 7.

2.13 Gas Chromatography-Mass Spectrometry (GC-MS)

In the gas chromatography-mass spectrometry (GC-MS) technique, the gas stream emerging from the gas chromatograph is admitted through a valve into a tube where it passes over a molecular leak. Some of the gas stream is thus admitted into the ionization chamber of the mass spectrometer where the mass spectrum of every component in the sample injected into the gas chromatograph is obtained. In this technique, the instrument must determine the mass spectrum of each component in the mixture before the next component exits from the gas chromatography column so that, one component is not contaminated by the next fraction before its spectrum is obtained (Pavia *et al.,* 2001).With the GC-MS system, a mixture of compounds can be analyzed and a library search conducted on each component. If the components are known compounds, they can

be identified tentatively by comparisons with compounds found in the computer library. In this way, a "hit list" is generated which reports on the probability that the compound in the library matches the known substance (Pavia *et al*., 2001).

2.14 Infrared spectroscopy

Almost any compound having covalent bonds, whether organic or inorganic, absorbs various frequencies of electromagnetic radiation in the infrared region (4000-400 cm^{-1}) of the electromagnetic spectrum. As with other types of energy absorption, molecules are excited to a higher energy states when they absorb infrared radiation. The absorption of infrared radiation corresponds to energy changes on the order of 8-40 KJ/mole which corresponds to the range encompassing the stretching and bending vibrational frequencies of the bonds in most covalent molecules (Pavia *et al*, 2001). During the absorption, frequencies of infrared radiation which match the natural vibrational frequencies of the molecule under study are absorbed and the energy absorbed serves to increase the amplitude of the vibrational motions of the bonds in the molecule. However, only bonds that have a dipole moment that changes as a function of time are capable of absorbing infrared radiation. Infrared spectroscopy is used to determine structural information about a molecule or compound. The absorption of each type of bond (N-H, C-H, O-H, C-X, C=O, C-O, C-C, C=C, C≡C, C≡N, etc.) as seen in Table 2.4 are regularly found only in certain small portions of the vibrational infrared region. Two types of infrared spectrometers are in common use in the organic laboratory: dispersive and Fourier transform instruments (Pavia *et al,* 2001).

Table 2.4: Characteristic range for infrared absorption

Bond	Wave number (cm^{-1})
C—C1	700–800
C—C	750–1100
C—O	1000–1300
C=C	1610–1680
C=O	1670–1750
O—H (acids)	2500–3300
C—H	2850–3300
O—H (alcohols)	3200–3550
N—H (primary amines)	3350–3500

Source: VCAA 2015, *VCE Chemistry Data Book*, VCAA, Melbourne, p. 7.

2.15 Log P and pKa

Partition coefficient P, is a parameter that characterizes the relative affinity of a compound in its un-ionized form, for water and an immiscible model lipid solvent (usually Octanol) because it most closely simulates the properties of biological membranes (Avdeef, 1992).

Determination of P (or log P) values involves the placing of a drug compound along with the two immiscible solvents in a separation funnel. Molecules of the solute will distribute in each phase until equilibrium is established. The ratio of the two concentrations is the partition coefficient or distribution coefficient P, i.e. Partition Coefficient, P = [Organic] / [Aqueous] Where [] = concentration. Log P= log10 (Partition Coefficient). Log P values are very useful for improving the bioavailability and pharmacological action of drugs.

NOTE:

Log P > 0 means 10:1 Organic: Aqueous

Log P = 0 means 1:1 organic: Aqueous

Log P < 0 means 1:10 organic: Aqueous (Avdeef, 1992, Paschke *et al*., 2004).

The pKa or 'Dissociation Constant' is a measure of the strength of an acid or a base. The pKa allows you to determine the charge on a molecule at any given pH. Bronsted Lowry was the first to show the advantage of expressing the ionization of both acids and bases on the same scale. He made an important distinction between Strong and weak bases:

Strong acids and bases - defined as completely ionized in pH range 0-14

Weak acids and bases - defined as incompletely ionized in pH range 0-14

The pKa or ionization constant is defined as the negative logarithm of the equilibrium coefficient of the neutral and charged forms of a compound (Avdeef *et al.*, 1993; Tomlinson, 1982).

2.16 Antimicrobial Resistance

The discovery of antibiotics in the mid-twentieth century revolutionized the management and treatment of infectious diseases caused by bacteria. Infections that would normally have been fatal were now curable. Since then, antimicrobial agents (antibiotics and related medicinal drugs acting on bacteria, viruses, fungi and parasites) have saved the lives and eased the suffering of millions of people. Today, antibiotics are crucial not only for the treatment of bacterial infections, but also for prophylactic coverage of high risk patients for example those in intensive care, organ transplants, cancer chemotherapy and

prenatal care. However, these gains are now seriously jeopardized by the rapid emergence and spread of microbes that are resistant to antimicrobials (Jouda, 2013).

The mass production of penicillin in 1943 dramatically reduced illnesses and deaths from infectious diseases caused by bacteria. However, within four years, bacteria began appearing that could resist the action of penicillin. Pharmaceutical companies fought back by developing other types of antibiotics. After more than 50 years of widespread use of these "miracle drugs", antibiotics are no longer as effective as they once were. Virtually all important bacterial infections throughout the world are becoming resistant (L. Johnson, 2006). Even though pharmaceutical industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Nascimento, 2000).

2.16.1 *Escherichia coli (E. coli)*

Escherichia coli is a common inhabitant of the gastrointestinal tract of humans and animals which was first isolated from the feaces of a child in 1885 by the Austrian pediatrician Theodor Escherich (Escherich, 1885). There are *E. coli* strains that are harmless commensals of the intestinal tract and others that are major pathogens of humans and animals. The pathogenic *E.coli* are divided into those strains causing disease inside the intestinal tract and others capable of infection at extra-intestinal sites (Kaper *et al.*, 2004). *Escherichia coli* can be found secondarily in soil and water as the result of fecal contamination. Classically, its detection has been used as an indicator of poor water quality (Rodney and Welch, 2006).

2.15.1.1 Morphology and identification

Escherichia coli are Gram-negative, non-spore forming bacilli. They are approximately 0. 5µmin diameter and 1.0–3.0 µm in length. Within the periplasm is a single layer of peptidoglycan. The peptidoglycan has a typical sub-unit structure where the N-acetyl muramic acid is linked by an amide bond to a peptide consisting of L-alanine, D-glutamic acid, meso-diamino pimelic acid and finally D-alanine (Rodney, 2006). *Escherichia coli* are commonly motile in liquid by means of peritrichous flagella. Swarming behavior and differentiation into hyper flagellated and elongated bacilli typical of that seen with the Proteus species can be observed on some solid media (Harshey, 1994).

2.15.1.2 Epidemiology

Escherichia coli are common inhabitants of the terminal small intestine and large intestine of mammals. They are often the most abundant facultative anaerobes in this environment. They can occasionally be isolated in association with the intestinal tract of non-mammalian animals and insects. The presence of *E. coli* in the environment is usually considered to reflect fecal contamination and not the ability to replicate freely outside the intestine. There is evidence however to suggest that *E. coli* may freely replicate in tropical fresh water (Bermudez and Hazen, 1988).

2.15.1.3 Infection

One of the most notable features of *E. coli* is broad diversity of disease-causing genotypes. These different genotypes and their disease-causing abilities lead to categories of *E. coli* often referred to as pathotypes. There are six intestinal and two extra

intestinal pathotypes currently recognized (Schreiber and Donnenberg, 2002 ; Kaper *et al*., 2004). Enterotoxigenic *E. coli* (ETEC) is a frequent cause of diarrhea in both humans and animals and are estimated to cause 600 million cases of human diarrhea and 800,000 deaths worldwide principally in children under the age of 5 (WHO, 1999). ETEC cause watery diarrhea that can be mild in nature or in some instances can be a severe, cholera-like illness where rapid dehydration can be life-threatening. Enteropathogenic *E. coli* (EPEC) are a significant cause of infant diarrhea in developing nations. They are currently defined as those diarrheagenic *E. coli* strains that cause attaching and effacing (A/E) lesions on intestinal epithelium but which lack Shiga toxins. EPEC disease is generally the result of growth of EPEC in the small intestine. EPEC cause a watery diarrhea that may contain mucus but typically does not have blood in it. Vomiting, fever, malaise and dehydration are also associated (Rodney, 2006).

Enterohemorrhagic *E. coli* (EHEC) cause diseases of the large intestine that may present as simple watery diarrhea and then progress to bloody stools with ulcerations of the bowel. The transmission of EHEC disease in humans is through ingestion of contaminated beef or foods contaminated with cattle feaces. Children under the age of five are the major victims of EHEC disease, although the elderly may also exhibit bloody diarrhea (Rodney, 2006).

Uropathogenic *E. coli*; Urinary tract and bloodstream *E. coli* (UPEC) are recognized as having the potential to cause both invasive neonatal diseases and urinary tract infections (Johnson *et al.*, 2001).These strains are the principal causes of morbidity and mortality from either community or hospital-acquired *E. coli* infections. Approximately 60% of adult women will have a UTI in their lifetime. As much as 90% of all community-

acquired UTIs and greater than 30% of the hospital-acquired UTIs are caused by *E. coli* (Haley *et al*., 1985).

2.16.2 *Staphylococcus aureus (S. aureus)*

Members of the genus Staphylococcus (Staphylococci) are gram positive cocci that tend to be arranged in grape-like clusters (Ryan and Ray, 2004).

2.16.2.1 Morphology and identification

Staphylococci are spherical cells about 1 micro meter diameter arranged in irregular clusters. Single cocci, pairs, tetrads, and chains are also seen in liquid cultures. Young cocci stain strongly gram-positive; on aging, many cells become gram-negative. Staphylococci are non-motile and do not form spores (Brooks *et al.,* 2007).

Staphylococcus aureus is a facultative anaerobe that grows at an optimum temperature of 37 ºC and an optimum pH of 7.5. *S. aureus* produces white colonies that tend to turn a buff-golden colour with time, which is the basis of the species epithet aureus (golden). Most, but not all, strains show a rim of clear β-hemolysis surrounding the colony (Ryan and Ray, 2004). On a nutrient agar, following aerobic incubation for 24 hours at 37 ºC, colonies are 1 – 3 mm in diameter, have a smooth glistening surface, an entire edge and an opaque pigmented appearance. In most strains, pigmentation is golden with orange, yellow and cream varieties. On McConkey agar, colonies are small to medium in size and pink or pink-orange in colour (Mckie and McCartney, 1989).

2.15.2.2 Epidemiology

Staphylococci are highly successful colonizers of human and animals. They reside mainly on the skin, particularly in moist areas such as the anterior nares (nose), axilla and groin. Between one-third and three-quarters of individuals carry these organisms at any one time. Staphylococcal infections occur worldwide and newly emerging hyper-virulent or multi-resistant strains spread rapidly over wide geographical areas. The bacteria survive in the air, on objects or in dust for days; therefore they can contaminate environments (such as hospitals) and continue to be transmitted over long periods of time. Some individuals may shed the organism more heavily than others. Staphylococcal infections are acquired from either self (endogenous) or external (exogenous) sources (Irving *et al*., 2006).

2.15.2.3 Infections

S. aureus causes serious infections of the skin, soft tissues, bone, lung, heart, brain or blood (Irving *et al*., 2006). Including pneumonia, bacteremia leading to secondary pneumonia and endocarditis, osteomyelitis secondary to bacteremia and septic arthritis, seen in children and in patients with a history of rheumatoid arthritis. Diseases caused by Staphylococcal toxins include scalded skin syndrome and toxic shock syndrome (Sherris, 1984).

2.15.2.4 Antimicrobial Susceptibility

Resistance to penicillin G can be predicted by a positive test for β-lactamase; approximately 90% of *S aureus* produce β-lactamase. Resistance to nafcillin, oxacillin and methicillin occurs in about 35% of *S aureus* isolates (Brooks *et al.*, 2007). Alternative antibiotics for resistant organisms (e.g. MRSA) include vancomycin, erythromycin and gentamicin. Some strains become resistant to multiple antibiotics (Irving *et al.*, 2006).

2.16.3 *Klebsiella pneumoniae (K. pneumoniae)*

Klebsiella pneumoniae is among the most important causative organisms of nosocomial infections and the major population at risk is neonates and immuno-compromised hosts. The pneumonia caused by this bacteria is often very difficult to treat (Aount and Klastersky, 1991; Pierce and Sanford, 1974). *Klebsiella* became resistant to broad-spectrum β-lactam antibiotics due to the emergence and spread of plasmid-mediated β-lactamases such as extended spectrum β-lactamases (Tenover and Hughes, 1996).

2.15.3.1 Morphology and Identification

Klebsiella pneumoniae is a facultative anaerobic, non-motile, rod-shaped, gram-negative bacterium with a prominent polysaccharide capsule. This capsule encases the entire cell surface, accounts for the large appearance of the organism on gram stain, and provides resistance against many host defense mechanisms (Sikarwar and Batra, 2011). *Klebsiella*

strains grow as yellow mucoid colonies. Other members of the Enterobacteriaceae either do not grow or produce small colorless, pink, red or orange colonies (Brisse *et al.*, 2006).

2.15.3.2 Epidemiology

Klebsiella pneumoniae can be found as a commensal in the mouth and upper respiratory tract, it is also found in moist environments, particularly in the intestinal tract of humans and animals. These are also found in plants, water and soil. Recent epidemiological studies indicate that the frequency of nosocomial infections caused by *klebsiella* species have increased substantially over the last twenty years (Mc.Gowan, 1985).

2.15.3.3 Infections

Infections with *Klebsiella* are caused mainly by *K. pneumoniae* and *K. oxytoca* in a proportion estimated at 2 to 1 (Bauernfeind *et al.*, 1981). *Klebsiella* causes pneumonia, urinary infections, septicemia and other pyogenic infections. Sometimes it also causes diarrhea. *K. pneumoniae* is generally associated with lower respiratory tract infections and middle ear infections (Bauernfeind *et al.*, 1981).

2.16.4 *Pseudomonas aeruginosa (P. aeruginosa)*

Pseudomonas aeruginosa is a classic opportunist pathogen belonging to the genus Pseudomonas (Mckie and McCartney, 1989).

2.15.4.1 Morphology and Identification

It is obligate aerobe, motile, rod-shaped, and measuring about 0.6 x 2 µm. It is gram-negative and occurs as single bacterium, in pairs, and occasionally in short chains, sometimes producing a sweet or grape-like or corn taco-like odor (Brooks *et al.,* 2007). Its production of blue, yellow, or rust-colored pigments differentiates it from most other Gram-negative bacteria. The blue pigment, pyocyanin, is produced only by *P. aeruginosa*. Fluorescin, a yellow pigment that fluoresces under ultraviolet light is produced by *P. aeruginosa* and other free-living less pathogenic Pseudomonas species. Pyocyanin production and fluorescein combined produce a bright green color that diffuses throughout the medium (Ryan and Ray, 2004). *P. aeruginosa* grows well at 37–42 °C; its growth at 42 °C helps differentiate it from other *Pseudomonas* species. It does not ferment carbohydrates, but many strains oxidize glucose (Brooks *et al.*, 2007).

2.15.4.2 Epidemiology

P. aeruginosa normally inhabit soil, water, and vegetation and can be isolated from the skin, throat, and stool of healthy persons. They often colonize hospital food, sinks, taps, mops, and respiratory equipment. Spread is from patient to patient via contact with fomites or by ingestion of contaminated food and water (Baron, 1996).

2.15.4.3 Infections

Pseudomonas aeruginosa causes infections in healthy individuals and those who are hospitalized or have a compromised immune system as a result of other diseases. A variety of human infections are commonly associated with this bacterium:

- Urinary tract infections
- Ventilator-associated pneumonia
- Surgical site infection
- Respiratory infections
- Ocular infections
- Ear infections (external otitis, malignant external otitis)
- Skin and soft tissue infections, including hot tub folliculitis, and osteomyelitis
- Burn sepsis

Individuals with compromising conditions, such as HIV/AIDS, cystic fibrosis, chemotherapy-related neutropenia, and diabetes have an increased risk of acquiring an infection and developing complications (Trautmann and Halder, 2008).

2.15.4.4 Antimicrobial Susceptibility

Pseudomonas aeruginosa is frequently resistant to many commonly used antibiotics. Although many strains are susceptible to gentamicin, tobramycin, colistin, and amikacin, resistant forms have developed, making susceptibility testing essential (Baron, 1996).

2.16.5 *Candida albicans (C. albicans)*

Candida albicans is unicellular yeast though it can be a multicellular mold. It is a ubiquitous fungi organism which has been one of the many eukaryotic organisms that work in symbiotic existence with humankind for a long time. However, when the delicate balance of coexistence that keeps each one at bay is disrupted, it becomes an opportunistic pathogen by causing superficial infections, such as oral or vaginal candidiasis or life-threatening infections (Calderone and Clancy, 2012).

2.16.5.1 Mophology and Identification

Candida are thin-walled, small yeast (4 to 6 microns) that reproduce by budding and are one of the most common causes of opportunistic mycoses worldwide. *C. albicans* exhibits a wide range of different morphological phynotypes due to phenotypic switching and bud to hypha transition. The yeast to hyphae transition is a rapid process and induced by environmental factors (Slutsky *et al.*, 1985).

2.15.5.2 Epidimiology

Candida albicans is present on the skin and in the oral cavity and gastrointestinal and urogenital tracts of most healthy individuals. In the healthy host, *C. albicans* normally exists as a benign commensal organism. However, as an opportunistic pathogen, this fungus can cause superficial infections, such as oral or vaginal candidiasis, or life-threatening systemic infections in immune-compromised patients. *C. albicans* infections also constitute the most common fungal infections in AIDS patients(Kim, and Sudbery, 2011; Lim *et al.*, 2012).

2.15.5.3 Infection

Candidiasis; a disease caused by *C. albicans*, is the cause of thrush; an infection in the mouth creating a whitish film on the palate, tongue, and inside cheeks. When thrush extends into the throat it causes Esophagitis. Candidiasis is also the cause of Cutaneous Candidiasis, commonly known as diaper rash, which can be problematic anywhere in the folds of the skin such as armpits, hands, groins, buttocks, and under breasts. Many other illness have been found to be related to the *Candida* organism such as acne, sinusitis, bronchitis, UTIs, ear infections, chronic fatigue syndrome, depression, athlete's foot, constipation, and diarrhea (Guler *et al.*, 2006; Vidigal and Svidzinski, 2009). The common illness of *C. albicans* is Vaginal Yeast Infection. Even though Candidiasis is not known to be a sexually transmitted disease, it still has been one of the concerns for the Centers for Disease Control (CDC) and continues to be included in their sexually transmitted diseases (STD) guidelines due to the high rate of occurrences reported (Anaissie *et al.*, 2009).

2.17 Ointments

These are semisolid systems which usually behave as viscoelastic materials when shear stress is applied. They generally contain medicaments and are intended to be applied externally to the body or to the mucous membrane. (Shelke Usha, *et al.*, 2015). All ointments consist of a base which chiefly acts as a carrier for the medicaments. The nature of the base also controls its performance. Hence selecting an ointment base is a very important aspect of their formulation (Lachman *et al.,* 1976). An ointment is used topically on a variety of body surfaces such as the nose, anus, skin, mucus membrane of

eyes, they are used topically as protectors, antiseptic, emollient, antipruritic, keratolytics and astringents and it may or may not be medicated (Shelke-Usha, *et al.,* 2015).

Herbal drugs are also formulated in the form of ointment. The ointment base is prepared and the ointment is formulated by incorporating the active ingredients in the base at most effective ratio by trituration. Medicated ointments contain a medicament dissolved, suspended or emulsified in the base (Shelke-Usha, *et al.*, 2015).

2.17.1 Characteristics of an ideal ointment

- It should be physically and chemically stable
- The base of the ointment should possess no therapeutic action
- In ointment base, finely divided active ingredients should be evenly distributed.
- The ointment should be sooth and free from grittiness (Aulton, 1988).

2.17.2 Polyethylene Glycol (PEG) ointment bases

Polyethylene glycols (PEGs) are neutral polymers available in a wide range of molecular weight and highly soluble in water (Plaut and Federman, 1985). These are relatively inert, non-volatile, water soluble or water-miscible liquids or waxy solids (Martindale, 2009). PEGs do not readily penetrate the skin, although the polyethylene glycols are water-soluble and are easily removed from the skin by washing, making them useful as ointment bases (Rowe *et al.,* 2009). Penetration experiments indicate that the type and amount of PEG will greatly affect the penetration time (Ugrine *et al.,* 1989). Polyethylene

glycols, can be used as a vehicle, better results often are obtained by using blends of high and low- molecular weight glycols (Mabry *et al.,* 2015). PEG 4000 in the presence of equal amount of PEG 400 has a good texture and might be a suitable ointment for tropical and subtropical areas (Ugrine *et al.*, 1989).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1. Equipment/Apparatus

Measuring cylinder, TLC plates, test tubes, calibrated ruler, plates, sterilized wire loop, column tube, beakers, cotton wool, aluminum foil, 5mm borer, glass bottles, Bunsen burner, pipette, capillary tubes, graduated dropper, pipette filler, Microtech Gloves, Watsman filter paper, glass wool, spreader, bleach, Electric balance – precision standard. 300g maximum weight, OHAUS brand, USA, Refrigerator – Kendro Laboratory Products. Model number ULT2586-3-w37. Lowest temperature -80 ^{o}C. USA, Incubator – Thermolyne. Subsidiary of Sybron, Model number 142040, USA, Fourier Transform Infra-Red Spectrophotometer (Shimadzu model) device, Japan (Kwame Nkurmah University of Science and Technology-Ghana), Ultra violet spectrophotometer, C-13/P-NMR Machine-500 MHZ Bruker ADVANCE III HD NMR, Japan (University of Ghana-Legon, Ghana). GC-MS machine-Perkin-Elmer Gas Chromatography clarus 500-Mass spectrometry device, Japan (Ghana Standards Authority), Oven, Autoclave machine, Digital pH meter, Stop watch, Incubator.

3.2 List of Reagents

Nutrient agar from oxoid limited, Basingstoke, Hampshire, England, Muller-Hinton agar from oxoid limited, Basingstoke, Hampshire, England. Nutrient Broth from oxoid limited, Basingstoke, Hampshire, England, Ethanol – analytical grade of 96 % purity from BDH Laboratory Supplies, England, n – Hexane – laboratory reagent of 95 % purity

from Thomas Baker (chemicals) Limited, England, Chloroform – laboratory reagent of ≥ 99 % purity from Koch-Light Laboratories Limited, England, Ethyl acetate – analytical reagent of 85 % purity from BDH Laboratory Supplies, England, n – Butanol – General Laboratory Reagent (GLR) of 99 % purity from PS Park Scientific Limited, UK, Methanol – Harris reagent of 95 % purity from Philip Harris Limited, England, Silica gel – laboratory use (60 – 120 mesh for column chromatography) from BDH Laboratory supplies, England, Fehling solutions – laboratory use from PS Park Scientific Limited, UK, Polyethylene glycol 4000 from BDH chemicals limited, England, Polyethylene glycol 400 from BDH chemicals limited, England, Iodine.

All the chemicals and reagents were purchased from Benburto Chemicals Limited, Accra (Primary source: BDH Laboratories Supplies, England).

3.3 Plant Collection, Identification and Preparation

3.3.1 Collection of Plant Material

Sample of *Aloe barbadensis* Miller was collected in the month of May, 2015 from Parks and Gardens in Kumasi in the Ashanti Region of Ghana, West Africa.

After collection of plant sample, it was taken to the Department of Applied Biology, University For Development Studies, Navrongo-Ghana where the plant was identified as *Aloe barbadensis* Miller by Doctor Isaac Sackey.

3.3.2 Preparation of Leaf of *Aloe barbadensis* Miller

The leaves were transported in sacks to the laboratory where they were washed under running tap water to get rid of soil residues. The leaves were dried under sun for four weeks to ensure that they were free from moisture. The dried leaves were then pounded with the aid of mortar and pestle to obtain the powdered form of the plant material which was stored in an air-tight container prior to use.

3.4 Extraction of the Leaf of *Aloe barbadensis* Miller

Powdered leaf sample (1.5 kg) was macerated in absolute ethanol for 72 hours at room temperature (27^{o}C). The mixture was periodically shaken to enhance the extraction of the bioactive phytochemicals. The mixture was then filtered and the solvent was evaporated using rotary evaporator at temperature of 40^{o}C, to give a dark gummy residue of 155.61g. The product was then transferred into a glass tube container and labeled ethanol extract, wrapped with aluminum foil and kept inside a desiccator for total evaporation of the rest of the solvent. The dried crude extract was then stored in a refrigerator until further use.

3.4.1 Partitioning of Crude Extract

The crude extract, 51.87 g of *Aloe barbadensis* Miller was partitioned between equal volumes of n-hexane and water using separator funnel. The solvent of each fraction was then evaporated using a rotary evaporator. The extract of the n-hexane fraction was determined to be 5.5 g which was kept for further application. The aqueous portion was determined to be 4.3 g. The fraction of the aqueous portion was further extracted several

times with ethyl acetate to give 3.2 g product after removal of the ethyl acetate using rotary evaporator.

3.5 Column Chromatography of the n-hexane fraction of *Aloe barbadensis* Miller

Silica gel H of (60- 120 μm mesh size) (100 g) was carefully packed using wet method in a column of dimensions 680 mm (high) ×15 mm (width). The column was allowed to stand for 4 hours to allow the silica gel (stationary phase) settle well. The product from the n-hexane fraction, 5.5 g, was loaded onto the silica gel and was allowed to equilibrate for 4 hours before elution began.

Starting with n-hexane 100% as the initial eluent, polarity was increased as follows: n-hexane: ethyl acetate 9:1, 4:1, 7:3, 3: 2, and 1:1. Fractions of 20 ml each were collected and a total of 51 fractions were realized which were allowed to evaporate at room temperature. Each fraction was numbered in accordance with how they were collected. The column fractions were monitored on TLC and visualizing with iodine vapor after the spots were clearly separated to detect colored compounds. Fractions that showed same spot, color and at the same R_f values were recombined in one beaker.

3.5.1 Isolation of Compound G

Fractions 31-41 (green compounds) were recombined to give 338.9 mg and further ran on a column using chloroform followed by (5:95) methanol/chloroform and (10:90) methanol/chloroform. A total of 13 fractions were obtained and fractions 6-10 were recombined (57.7 mg). Further purification was carried out on this compound using preparative thin layer chromatography with a solvent system of chloroform/methanol (9:1).

The filtrate was then placed inside clean beaker and allowed to evaporate under room temperature to give 49.1 mg of Compound G.

Salkowski reaction: A few milligrams of Compound G was dissolved in chloroform and a few drops of concentrated sulphuric acid were added to the solution. A reddish brown color was formed in the upper chloroform layer (Harborne, 1998) indicating the presence of a steroid.

3.6 Column Chromatography of the Ethyl Acetate Fraction of *Aloe barbadensis* Miller

Similar procedure as above was applied here. Approximately 3.2 g of the ethyl acetate fraction was loaded onto the silica gel column and allowed to stabilize for 4 hours before elution began. This fraction was eluted with solvents of increasing polarity (0-60, 10-50, 20-40, 30-30 40-20) v/v_methanol/ ethyl acetate. Fractions of 20 ml each were collected and a total of 44 fractions were obtained which were allowed to evaporate at room temperature. Each fraction was numbered in accordance with how they were collected. The column fractions were monitored on TLC, and visualized with iodine vapour to detect colorless compounds. Fractions that showed same spot, colour and at the same R_f value were recombined in one beaker.

3.6.1 Isolation of Compound A

Fractions 22-27 (pink coloured fractions) were recombined to give 0.9 g and this was further eluted with chloroform/methanol (90: 10) v/v giving a total of 30 fractions. Fractions 9-18 were further recombined and the solvent evaporated to give 0.2466 g. The residue was chromatographed with chloroform, followed by solvent polarity of methanol:

chloroform 10/90, 20/80 and 30/70 $CHCl_3$/MeOH. A total of 22 fractions were collected and fractions 2-8 were recombined to give 0.2131 g residue. This residue was again chromatographed by eluting with methanol/chloroform (10/90, 20/80) giving a total of 15 fractions. Fractions 2-7 (yellow fractions) were recombined to give 0.0749 g product. This product was further purified with methanol/chloroform (5/95, 10/90 MeOH/$CHCl_3$). A total of 11 fractions were realized. Fractions 9-10 (yellow fractions) were recombined (39.6 mg) as a pure compound designated as Compound A.

3.7 Characterization of Isolated Compounds

3.7.1 Infrared Analysis

The functional groups of the isolated compounds (Compound A and G) were determined on Fourier Transformed Infrared Spectrophotometer (Shimadzu model, No: 94133) device. The results of same are shown in Tables 4.3 and 4.5 respectively.

3.7.2 Proton and carbon-13 Nuclear Magnetic Resonance (NMR) Analysis

^{1}H and ^{13}C NMR spectra were acquired on 500 MHz Bruker ADVANCE III HD NMR spectrometer, equipped with a 5mm broadband multinuclear z-gradient (BBO) probe head for both compounds A and G. The results of same are shown in Tables 4.4 and 4.6 respectively.

3.7.3 Determination of LogP and pKa of Isolated Compounds

3.7.4 Determination of Maximum Absorption of Isolated Compounds

For the present study JASCO double beam UV/Visible spectrophotometer (model-V630) was used with, spectral bandwidth of 20 nm, wavelength accuracy ±1 nm and a pair of 1-cm matched quartz cells was used to measure absorbance of the resulting solution. Weighing was done on electronic balance (Model ShimadzuAUW-220D). Octanol was selected as solvent for developing spectral characteristics of the drugs after assessing the solubility of both drugs in different solvents and in an attempt to mimic the biological system.

3.7.5 Preparation of Standard Stock Solutions of the Isolated Compounds

Standard stock solutions of both compounds were prepared by dissolving 10 mg of each drug in 2 ml of Octanol in 100 ml separate volumetric flasks. Final volume was made up to 100 ml with Octanol to get working standard solution of 100 µg/ml each. These stock solutions were serially diluted to obtain solutions with variable concentrations 5-30 µg/ml and 5-100 µg/ml for Compound G and Compound A respectively.

3.7.6 Determination of Absorption Maxima

Using appropriate dilution of standard stock solution of both drugs with Octanol, solutions containing 20 µg/ml of each drug were scanned separately in the range of 300-700 nm. Wavelength of maximum absorption was determined for both drugs to be 360 nm.

3.7.7 Determination of Standard Curves

The linearity of measurement was evaluated by analyzing different concentrations of the standard solution of Compound G and Compound A. For both drugs, the Beer- Lambert's law was obeyed in the concentration range of 5-30 µg/ml and 5-100 µg/ml for Compound G and Compound A respectively. The correlation coefficient was found to be 0.996 at 360 nm for Compound G and 0.991 at 360 nm for Compound A. The entire process was repeated to ensure reproducibility.

3.8 Preparation of Phosphate Buffer Solutions

3.8.1 Preparation of stock solution A: 0.1 M potassium dihydrogen phosphate

Potassium dihydrogen phosphate (KH_2PO_4), 13.61g was dissolved in 1000 ml distilled water.

3.8.2 Preparation of stock solution B: 0.1 M disodium hydrogen phosphate

Disodium hydrogen phosphate dihydrate ($Na_2HPO_4.2H_2O$), 17.6 g was dissolved in 1000 ml distilled water. Buffer solutions ranging from pH 4 to 9 were prepared by appropriately mixing stock solution A and stock solution B. In most cases, the pH of the buffer had to be adjusted using a pH meter and a drop-wise addition of 1 M HCl and 1 M NaOH.

3.8.3 Partitioning of Drug G between n-Octanol and Buffer Solutions

Drug G, 2.6 mg was partitioned between 50 ml each of n-Octanol and buffer solution of pH4 in a separatory funnel to give an initial concentration of 26 µg/ml. Octanol was chosen as the model lipid phase because it most closely simulates the properties of biological membranes. The absorbance of the Octanol layer was measured on a UV spectrophotometer after every 30 minutes and its corresponding concentration estimated using the standard curve plot. Immediately after each measurement, the mixture was shaken very well to ensure uniform mixture of the two solvents. The mixture was then allowed to stand for 30 minutes until there was clear separation between the two immiscible solvents. The process was repeated at 30 minutes interval for a total of 150 minutes when equilibrium was established. The experiment was repeated to ensure reproducibility. The entire process was then repeated using buffer solutions of pH 5, 6, 7, 8 and 9. Equilibrium curves were drawn using concentration (µg/ml) against time (minutes). The pH of each buffer was taken before and after each partitioning process to ensure there were no significant changes in the buffer as in the case of the blood/aqueous phase of a biological system.

3.8.4 Partitioning of Drug A between n-Octanol and Buffer Solutions

Drug A, 5 mg, was partitioned between 50 ml each of n-Octanol and buffer solution of pH 4 in a separatory funnel to give an initial concentration of 50 µg/ml. The same process was applied to Compound A as in Compound G (above).

3.8.5 Determination of pH of Compound G and Compound A

Each of the compounds, 2 mg was dissolved in 25 ml of methanol in separate clean beakers to give solutions of concentrations 80 µg/ml of Compound G and 80 µg/ml of Compound A. The ball-end of a digital pH meter was dipped into each solution and the readings taken. From the readings, Compound G was found to be basic whilst that of compound A was acidic.

3.8.6 Determination of Hydrogen Ion Concentration [H^+] of Isolated Compounds

The [H^+] of both compounds were estimated by using the equation pH = -$\log_{10}$ [H^+] and substituting in the pH values of the various buffer solutions before the partitioning since there were no significant difference between the pH values before and after partitioning.

3.8.7 Determination of Drug Concentrations in the Aqueous Layer

The concentrations of each compound in the Octanol layer at equilibrium for each partitioning were taken on the 120th minute when the equilibrium curve began to assume linearity. For Compound G, these concentrations were subtracted from 26 µg/ml to give the corresponding concentrations of Compound G in the aqueous layer and again subtracted from 50 µg/ml to give the corresponding concentrations of Compound A in the aqueous layer.

3.8.8 Estimation of Apparent Partition Coefficient (P_{app}) of Isolated Compounds

Apparent partition coefficient (P_{app}) of Compound G and Compound A were estimated using the equation, P_{app}= [drug in octanol layer]/ [drug in buffer layer].

Where [ ] =concentration.

3.8.9 Estimation of log_{10} P of Isolated Compounds

For Compound G, a graph of $1/P_{app}$ against $[H^+]$ was plotted since it is a basic drug and for Compound A, a graph of $1/P_{app}$ against $1/[H^+]$ was plotted since it is an acidic drug. (Paschke *et at*., 2004). P values were then estimated from the graphs. Where P = Partition coefficient. The log_{10} of P value was then calculated to give the log P of the isolated compounds.

3.8.9.1 Estimation of pk_a of Isolated Compounds

The pKa of Compound G was estimated using the equation, slope = 1/ (p * Ka) since it is a basic drug and the pKa of Compound A was estimated using the equation, slope = Ka / p since it is an acidic drug (Paschke *et al.*, 2004). Where Ka = acid dissociation constant. Substituting the value of p from above and the slopes from the graphs into each equation, the Ka for both compounds were calculated. The pKa for both compounds were estimated using the equation, $pKa = -\log_{10}Ka$. Where pKa = antilog of Ka

3.9 Antimicrobial Activities of the Isolated Compounds

3.9.1 Test Organisms

The test organisms used for this analysis were clinical isolates of bacteria and viruses obtained from Tamale Teaching Hospital, Ghana. The isolates were; *Staphylococcus*

aureus, Escherichia coli, Klebsiella pneumoniae, pseudomonas aeruginosa, Candida albicans and Talaromyces fluvus.

3.9.2 Culture Media

The culture media used for the analysis include Mueller Hinton agar (MHA), Nutrient broth (NB) and nutrients agar. The mentioned media were used for sensitivity test, determination of minimum inhibitory concentration (MIC), minimum bactericidal concentration (MBC). All the media were prepared according to manufacturer's instructions and sterilized by autoclaving at 121°C for 15 minutes.

3.9.3 Preparation of the Standard Inocula of the Microorganisms

The test microorganisms were prepared by streaking on a freshly prepared nutrient agar plates to obtain discrete colonies. A colony was picked with a sterile wire loop and transferred aseptically into a small bijou bottle containing sterile normal saline. It was then shaken to dissolve completely and the turbidity was compared with that of 0.5 McFarland standard equivalent to a bacterial cell density of 1.5×10^8 CFU.

3.9.4 Preparation of Plant Isolates Concentrations

The concentrations of the isolated compounds were prepared using dimethyl sulphoxide (DMSO). Stock solutions, 40 mg/ml concentration were prepared by adding 40 mg of each compound in 1 ml of DMSO in sterile containers. Serial dilutions were carried out to obtain concentrations 20, 10, 5, 2.5, 1.25, 0.63, 0.32, 0.16, 0.08 and 0.04 mg/ml isolates concentration.

3.9.5 Preparation of Polyethylene Glycol (PEG) Ointment

Applying the modified method of Donkor *et al* (2014), Poly (ethylene glycol) 4000 (PEG 4000) and Polyethylene glycol 400 (PEG 400), 30 g each were weighed into a beaker and melted on a thermostatic water bath at 75℃ until liquefied. It was then stirred with a glass rod under tap water at room temperature until congealed (Donkor *et al.,* 2014).

3.9.6 Preparation of Plant Isolate-PEG Ointment Formulations

Compound G and A isolated from *Aloe barbadensis* Miller leaf extract were separately formulated together with PEG ointment. Equal quantities (40, 20, 10, 5, 2.5, 1.25, 0.63, 0.32, 0.16, 0.08 and 0.04 mg) of each plant isolate were weighed into appropriately labeled separate beakers and 1.0 g of the PEG ointment was then added to each beaker and warmed at 70℃ while stirring continuously with a glass rod for about 30 minutes for uniform mixture. The mixtures were allowed to cool at room temperature to produce a plant isolate- PEG ointment formulation at varying concentrations of 40, 20, 10, 5, 2.5, 1.25, 0.63, 0.32, 0.16, 0.08 and 0.04 mg/g (Donkor *et al.*, 2014).

3.10 Determination of Antimicrobial Activity of the Isolated Compounds Using Agar Well Diffusion Method.

The antimicrobial activity of the plant isolates was evaluated using Agar Well Diffusion Method. Molten Mueller Hinton agar (OXOID, Basingstoke, Hamphire, England) was

prepared and sterilized as instructed by the manufacturer and was poured aseptically into sterile petri dishes (about 4 mm thick) and allowed to solidify. The bacterial and fungal strains in normal saline were swabbed on the solidified Mueller Hinton agar. Wells of 5 mm diameter were punched into the agar plates with the help of sterilized cork borer (5 mm). Using a micropipette, 1.0 ml of the various concentrations of the plant isolates were added to the wells made in the plates. Chloramphenicol (10 mg/ml) and fluconazole (10 mg/ml) were used as positive control for bacterial and fungal strains respectively whilst DMSO was used as negative control. Tests were replicated and plates were allowed to stand for 40 minutes at room temperature and finally incubated aerobically in inverted positions at 37°C for 24 hours.

Antimicrobial activities of the isolated compounds were evaluated by measuring the zone of inhibition (mm) (Romasi *et al.,* 2011). For the plant isolate-PEG ointment formulation, the same 5 mm wells were created on different petri dishes and filled with same quantity of different concentrations (20, 10, 5, 2.5, 1.25 mg/g) and incubated after complete diffusion at 37°C for 24 hours**.** The entire process was repeated on a different day to ensure reproducibility.

3.10.1 Determination of Minimum Inhibitory Concentration (MIC)

Minimum inhibitory concentration (MIC) of the pure isolated compounds was carried out using the agar well diffusion method. Concentrations of 1.25, 0.63, 0.32, 0.16, 0.08 and 0.04 mg/ml were used. Molten Mueller Hinton agar was prepared and sterilized as instructed by the manufacturer and was poured aseptically into sterile petri dishes to a

depth of about 4 mm. Care was taken while pouring the media into the plate in order to obtain a uniform depth and surface and allowed to solidify. Test organisms in normal saline were spread on the solidified agar. Wells of 5 mm diameter were created with a sterile cork-borer at wide enough intervals and the wells were filled with the various concentrations of the plant isolates. Same quantity of the negative control (99% DMSO) and positive control (Chloramphenicol/fluclonazole) was also drawn into a well each on the same plate. Plates were left to stand for about 40 minutes until complete diffusion into the medium at room temperature (27^{o}C) and finally incubated in inverted positions at 37°C for 24 hours. Tests were carried out in duplicates after which they were observed for minimum inhibitory concentrations for the unformulated plant isolates. The entire process was repeated to ensure reproducibility. This process was repeated and the wells were filled with the plant isolate-PEG ointment formulations (1.25, 0.63, 0.32, 0.16, 0.08 and 0.04) mg/g to determine the minimum inhibitory concentrations for the formulated compounds.

3.10.2 Determination of Minimum Bactericidal Concentration (MBC)

The tube diffusion method was employed for the determination of MBCs. Minimum bactericidal concentrations of the isolated compounds were prepared by using sterilized transparent bottles for the different test organisms cultured. Normal saline solution, 2 ml, containing the test organisms was each dispensed into the sterilized transparent bottles. A 0.1 ml of various drug concentrations (40, 20, 10, 5 and 2.5) mg/ml was each dropped into sterile transparent bottles containing the test organisms. The bottles were corked and incubated aerobically at 37°C for 24 hours. Sterile nutrient agar plates were inoculated

with sample from each of the transparent bottles. The plates were further incubated for 24 hours at 37°C and subsequently observed for bacterial growth. The lowest concentration that killed 100% of the inoculum bacteria, thus, no growth on plate was recorded as the MBC. The entire process was repeated to ensure reproducibility. The above process was then repeated with the isolated compound-PEG ointment formulations (40, 20, 10, 5 and 2.5) mg/g in order to determine the MBC for the formulated forms of the drugs.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Results

4.1.1 Chromatography Results

The present study was conducted to isolate stable compounds from the leaves of *Aloe barbadensis* Miller crude extract of n-hexane and aqueous mixture. The hexane fraction was subjected to column chromatography using different solvent systems to give one major compound, Compound G (Figure 4.1). The aqueous fraction was further partitioned with ethyl acetate and the ethyl acetate fraction subjected to column chromatography using different solvent systems to give another major compound, Compound A (Figure 4.2). On the basis of ^{1}H-NMR, ^{13}C-NMR, and IR, the isolated compounds were identified as Aloin A and a phytosterol known as stigmasterol.

Figure 4.1:TLC of Compound G (Stigmasterol) (MeOH:CHCl3=1:9)

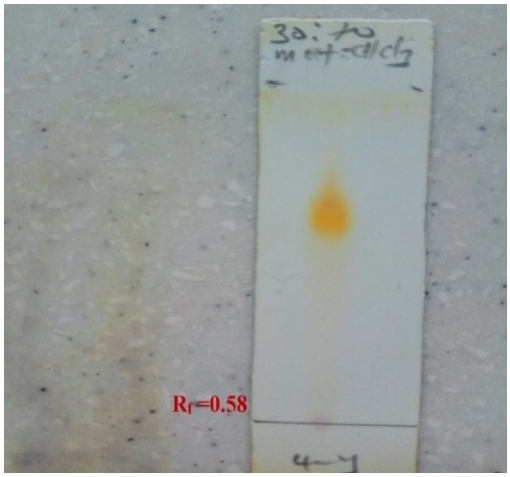


Figure 4.2: TLC of Compound A (Aloin A) (MeOH:$CHCl_3$=3:7)

Table 4. 1: Physical Characteristics of Crude Extract of *Aloe barbadensis* Miller

Plant	Solvent	Yield (g)	Yield (%)	Colour	Odour	Nature
Aloe vera	ethanol	155.61	10.37	Deep brown	odourless	Gummy

The results in Table 4.1 indicated that, the total mass of crude extract from the plant using ethanol was 155.61g, representing 10.37% of the total mass of powdered *Aloe barbadensis* Miller leaf (1500 g) used in this research. The crude extract obtained was an odourless gummy deep brown substance.

Table 4. 2: Physical Characteristics of Isolated Compounds from *Aloe barbadensis* Millar

Plant isolate	Yield (mg)	Yield (%)	Colour	Odour	Nature
Compound G	149.3	0.1	Green	odourless	Solid
Compound A	121.6	0.078	Yellowish-brown	odourless	Crystalline

Results in Table 4.2 indicated that, two compounds were isolated from the leaf of the plant in this research and were arbitrary named as Compound A and Compound G prior to characterization and identification. Percentage yield of Compound A and G were determined to be 0.1 and 0.078 % respectively (Table 4.2). Both isolated compounds were odourless but whilst compound A was yellow-brown in colour, that of Compound G was green by physical observation. Both compound were solid in nature but in addition, Compound A was crystalline.

4.1.2 Results of Isolated Compound A

4.1.2.1 Results of Fourier Transformed Infrared analysis of isolated Compound A

The results obtained from Fourier Transformed Infrared analysis of isolated Compound A and the suggested functional groups are presented in Table 4.3 and Appendix 4 respectively.

Table 4.3: FTIR assignment for Compound A

Peak	Wavelength (cm^{-1})	Type of vibration	Functional group
1	3308.35	OH stretch H-bonded	Phenol
2	2922.43	CH stretch in alkane	methylene -CH_2-
3	2323.56	CH stretch in alkane	methylene -CH_2-
4	2050.79	CH stretch in alkane	methylene -CH_2-
5	1635.12	C=C Stretch	Alkene
6	1615.36	C=C Stretch	Alkene
7	1601.63	NO_2 stretch conjugation	Ketone RCOR
8	1487.37	Ring aromatic stretch (4p)	Aromatic
9	1455.23	Ring aromatic stretch (4p)	Aromatic
10	1425.41	Ring aromatic stretch (4p)	Aromatic
11	1285.08	C-O-C stretch alkyl aril ether	ether ROR
12	1218.35	C-O-C stretch alkyl aril ether	ether ROR
13	1185.73	C-C(OH)-C stretching aliphatic	Secondary Alcohol R-OH
14	1164.23	C-C(OH)-C stretching aliphatic	Secondary Alcohol R-OH
15	1075.45	C-C(OH)-C stretching aliphatic	Secondary Alcohol R-OH
16	1011.2	C-C(OH)-C stretching aliphatic	Secondary Alcohol R-OH

The distinct peaks in isolated Compound A sample (Appendix 4) are presented in Table 4.3 which corresponded to O-H, C-H, C =O, C=C and C-O-C stretching vibrations respectively. The FT-IR spectra of isolated Compound A (Appendix 4) revealed a broad characteristic vibration band and weak vibration bands at 3308.35 cm-1 and (2050.79-2323.56) cm^{-1} respectively, corresponding to -OH group, and methylene (-CH_2-) groups respectively. The C = C stretching vibrations were located at peaks 1635.12, and 1615.36 cm^{-1} as weak absorptions (Appendix 4) whilst the sharp peak at 1601 cm^{-1} (Appendix 4) was an indication of the presence of a ketone group in the compound. The FT-IR spectra also exhibited the presence of ring aromatic stretching by showing medium absorptions at 1425.41 and 1487.37 cm^{-1}, this is an indication that the compound contains aromatic groups. The presence of sharp peaks at 1011.20, 1164.23 and 1185.73 cm^{-1} on the FT-IR spectrum is an indication of the presence of secondary alcohols in Compound A whilst the presence of a sharp peak at 1218.86 cm^{-1} is an indication of the presence of an ether group in the compound.

4.1.2.2 Results of NMR analysis of isolated Compound A from *Aloe barbadensis* Miller

The results obtained from proton, ^{1}H and ^{13}C NMR of isolated Compound A and the suggested type of protons and carbons are presented in table 4.4 below. Same results can be seen in Appendix 5 and 6 respectively.

Table 4.4: NMR assignment for Compound A

#	^{1}H (ppm)	Type of proton	^{13}C (ppm)	Type of carbon
1	11.73	phenolic proton	163.4	Phenolic carbon
2	6.86	Aromatic proton	114.5	Aromatic ring carbon
3			151.5	Aromatic ring carbon
4	7.03	Aromatic proton	119.2	Aromatic ring carbon
5	7.02	Aromatic proton	119.9	Aromatic ring carbon
6	7.47	Aromatic proton	137.0	Aromatic ring carbon
7	6.83	Aromatic proton	116.8	Aromatic ring carbon
8	11.76	Phenolic proton	162.9	Phenolic carbon
9			195.6	Ketone
10	4.56	Alkenyl proton	45.9	Aliphatic carbon
11	4.64	methylene proton	64.6	Benzylic methylene group
12			143.3	Aromatic ring carbon
13			118.7	Aromatic ring carbon
14			145.6	Aromatic ring carbon
15			117.7	Aromatic ring carbon
1^{1}	3.38	sugar proton	86.7	C-glucose moity
2^{1}	3.01	sugar proton	71.9	C-glucose moity
3^{1}	3.23	sugar proton	80.0	C-glucose moity
4^{1}	2.89	sugar proton	72.1	C-glucose moity
5^{1}	2.91	sugar proton	81.7	C-glucose moity
6^{1}	3.54	sugar proton	63.3	C-glucose moity

Table 4.4 shows results of the ^{1}H and ^{13}C NMR spectra of isolated Compound A from *Aloe barbadensis* Miller. The ^{1}H NMR spectrum of Compound A (Appendix 5) had peaks characteristic of an aromatic compound with an attached sugar (Table 4.4). The aromatic region had five protons, i.e., H-7 (δH 6.83, *d*, *J* = 8.6 Hz), H-2 (δH 6.86, *d*, *J* = 1.5 Hz), H-5 (δH 7.02, *d*, *J* = 1.5 Hz), H-4 (δH 7.03, *d*, *J* = 8.6 Hz), H-6(δH 7.47 (1H, *t*, *J* = 7.9), which are expected for Aloin A (Adushan, 2008). The splitting pattern of the proton attached to C-10 (δH 4.69, *d*, *J* = 2.4 Hz) confirmed a *C*-glucosidic linkage (Adushan, 2008). The signals for the sugar protons H-5' (δH 2.84, *m*), H-4' (δH 2.85, *t*, *J* = 9.2 Hz), H-2' (δH 2.96, *t*, *J* = 9.2 Hz), H-3' (δH 3.28, *t*, *J* = 9.2 Hz), H-6'b (δH 3.33, *m*), H-1' (δH 3.33, *t*, *J* = 9.2 Hz) and H-6'a (δH 3.44, *m*) are well resolved and allow for unambiguous assignment (Adushan, 2008). The methylene proton H-11 (δH 4.56, *d*) shift corresponds to that of a benzylic primary alcohol. The phenolic hydroxy protons 8-OH (δH 11.35, br *s*, D2O exchangeable) and 1-OH (δH11.68, br *s*, D2O exchangeable) of Aloin A can also be clearly seen (Adushan, 2008).

The ^{13}C NMR spectrum of Compound A (Appendix 6) displayed a total of 21 carbon atoms. Two CH_2 carbons, eleven CH carbons and nine non-protonated carbons were identified (Table 4.4). In the ^{13}C NMR spectrum of isolated Compound A, six signals (δC 63.3, 71.9, 72.1, 80.0, 81.7, 86.7) were characteristic of a *C*-glucose moiety, five aromatic signals (δC 114.5, 116.8, 119.2, 119.9, 137.0,), four signals (δC 117.7, 118.7, 143.3, 145.6) and a signal depicting a benzylic methylene group (δC 64.5). Two signals characteristic of phenolic carbons (δC 162.9, 163.4) and one of a ketone (δC 195.5) were also observed for Compound A, clearly depicting Aloin A (Adushan, 2008).These results confirm the presence of aromatic protons, phenolic protons, aromatic protons, and

methylene protons present in the ^{1}H NMR spectra of Compound A (Appendix 5). Same results also confirm the presence of ketone, phenol, methylene and aromatic functional groups the FTIR spectra of the compound (Appendix 4).

4.1.2.3 Results of Ultra Violet spectroscopy of isolated Compound A

Maximum absorption of isolated Compound A from the *Aloe vera* plant occurred at 360 nm on JASCO double beam UV/Visible spectrophotometer (model-V630), as shown in Figure 4.3 which implies, the compound is more sensitive and minimizes deviations from Beer Lambert's law at 360 nm.

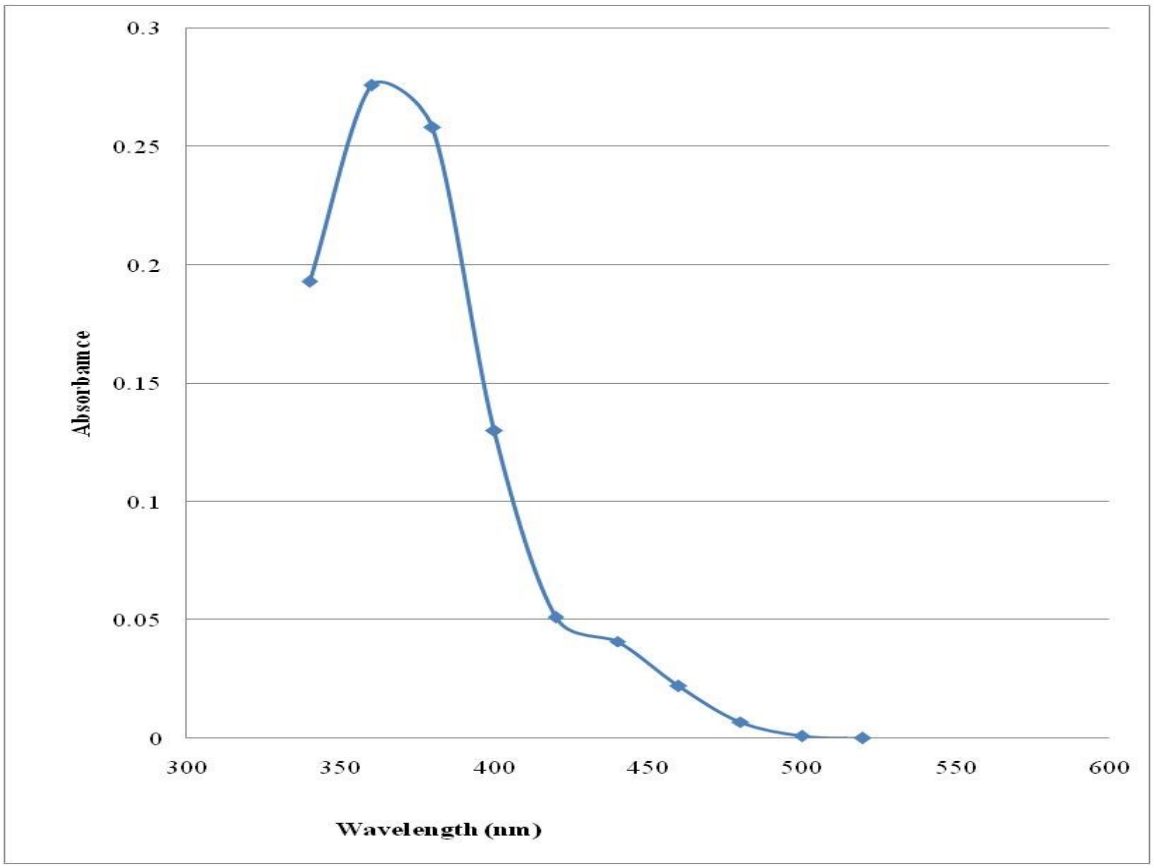


Figure 4. 3: UV maxima for Compound A at concentration 20 µg/ml in Octanol

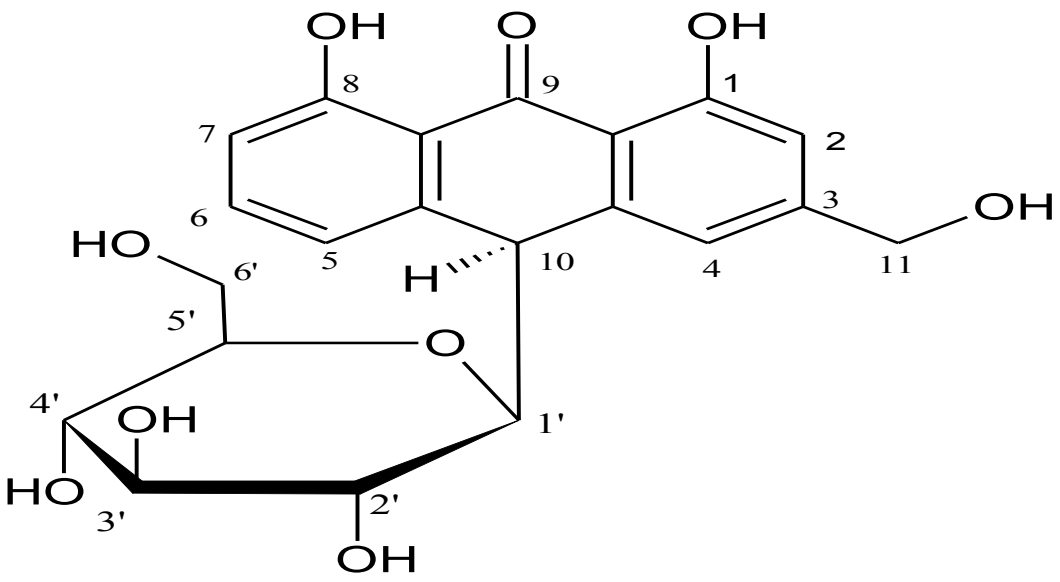


Figure 4. 4: Chemical Structure of Aloin A (Compound A)

4.1.3 Results of Isolated Compound G

4.1.3.1 Results of Fourier Transformed Infrared analysis of isolated Compound G

The results obtained from Fourier transformed Infrared analysis of isolated Compound G and the suggested functional groups are presented in Table 4.5. Same results can be seen in Appendix 4.

Table 4.5: FTIR assignment for Compound G

Peak	Wavelength (cm^{-1})	Type of vibration	Functional group
1	3362.95	OH stretch H-bonded	Hydroxyl-OH
2	2922.1	CH stretch in alkane (aliphatic)	methyl $-CH_3$

3	1742.48	C=C vibration	Alkene C=C
4	1463.62	CH stretch in alkane	methylene -CH_2-
5	1416.58	CH stretch in alkane	methylene -CH_2-
6	1164.75	C-C(OH)-C stretching aliphatic	Secondary Alcohol R-OH
7	1090.92	C-C(OH)-C stretching aliphatic	Secondary Alcohol R-OH

The distinct peaks in isolated Compound G sample (Appendix 4) are presented in Table 4.5 which corresponded to O-H, C-H, C=C and C-C(OH)-C stretching vibrations respectively. The FTIR spectra of isolated Compound G (Appendix 4) revealed an intensely broad characteristic vibration band and sharp stretching vibration band at 3362.95 and 2922.1 cm^{-1} respectively, corresponding to -OH group, and methyl (-CH_3-) group respectively. The C = C stretching vibrations was located at peak 1742.48 cm^{-1} as a sharp absorptions (Appendix 4). The presence of medium to sharp peaks at 1164.75 and 1090.92 cm-1 on the FTIR spectrum is an indication of the presence of secondary alcohol in Compound G.

4.1.3.2 Results of NMR analysis of isolated Compound G from *Aloe barbadensis* Miller

The results obtained from ^{1}H and ^{13}C NMR of isolated Compound G and the suggested type of protons and carbons are presented in Table 4.6. Same results can be seen in Appendix 5 and 6 respectively.

Table 4.6: NMR assignment for Compound G

S/N	^{1}H (ppm)	Type of proton	^{13}C (ppm)	Type of carbon
1				
2				
3	7.30	Phenolic proton	71.9	Phenolic carbon
4			57.1	Saturated carbon
5			140.9	Olefinic carbon
6	5.14	Olefinic proton	121.89	Olefinic carbon
7				
8				
9				
10			39.9/40.7	Saturated carbon
11				
12			25.6	Saturated carbon
13			42.4	Saturated carbon
14			42.5	Saturated carbon
15				
16				
17			50.4	Saturated carbon
18			21.3	Saturated carbon
19	1.25	Methyl proton (R-CH_3)	19.8	Methyl carbon
20			32.9	Alkenyl carbon
21			31.9	Alkenyl carbon
22				

23			29.1	Methylene carbon
24	0.97	Methyl proton (R-CH_3)	13.5	Methyl carbon
25				
26	1.03	Methyl proton (R-CH_3)	19.2	Methyl carbon
27	0.99	Methyl proton (R-CH_3)	12.3	Methyl carbon
28			21.5	Methyl carbon
29	0.90	Methyl proton (R-CH_3)	19.2	Methyl carbon

The ^{1}H NMR spectrum of Compound G (Appendix 5) shows a total of 12 peaks characteristic of a steroid. On The ^{1}H-NMR spectrum of Compound G (Appendix 5), it was seen that H-3 proton appeared at δ3.51 as a triplet of a doublet of doublet and H-6 olefinic proton showed a multiplet at δ5.14 which confirm the presence of alkene functional group in the FTIR spectra (Table 4.5) . Moreover, six methyl protons appeared at δ1.25, δ1.17, δ1.03, δ0.99, δ0.97 and δ0.90 (3H each, s, CH_3) for H-19, H-18, H-26, H-27, H-21, and H-29 respectively for methyl group (Table 4.6) which confirm the presence of methyl group in the FTIR spectra. The ^{13}C NMR spectrum of isolated Compound G (Appendix 6) displayed a total of 29 carbon atoms. Nine CH_2 carbons, six CH_3 carbons eleven CH carbons and two non-protonated carbons were identified (Table 4.6) which also confirm the presence of these functional groups and protons in the FTIR and ^{1}H NMR spectra (Table 4.5 and 4.6). In the ^{13}C NMR spectrum, six signals (δC 12.3, 13.5, 19.2, 19.2, 19.8, 21.5) were characteristic of a methyl group, one methylene signal

(δC 29.1), two aliphatic alkenyl signals (δC 31.9, 32.9) and two cyclo alkenyl carbons (δC 121.9, 140.9) as reported by Rajput and Rajput (2012) for stigmasterol.

The physical and chemical data (IR, ^{1}H and ^{13}C NMR chemical shift assignments) of Compound G were in accordance with the spectral data published by Rajput and Rajput (2012) for stigmasterol. Hence the compound could be one of the phytosterols (Stigmasterol) as shown in Figure 4.6.

4.1.3.3 Results of Ultra Violet spectroscopy of isolated Compound G

Maximum absorption of isolated Compound G from the *Aloe vera* plant occurred at 360nm, on JASCO double beam UV/Visible spectrophotometer (model-V630). These results are shown in Figure 4.5 which implied, the compound was very sensitive and minimized deviations from Beer Lambert's law at 360 nm.

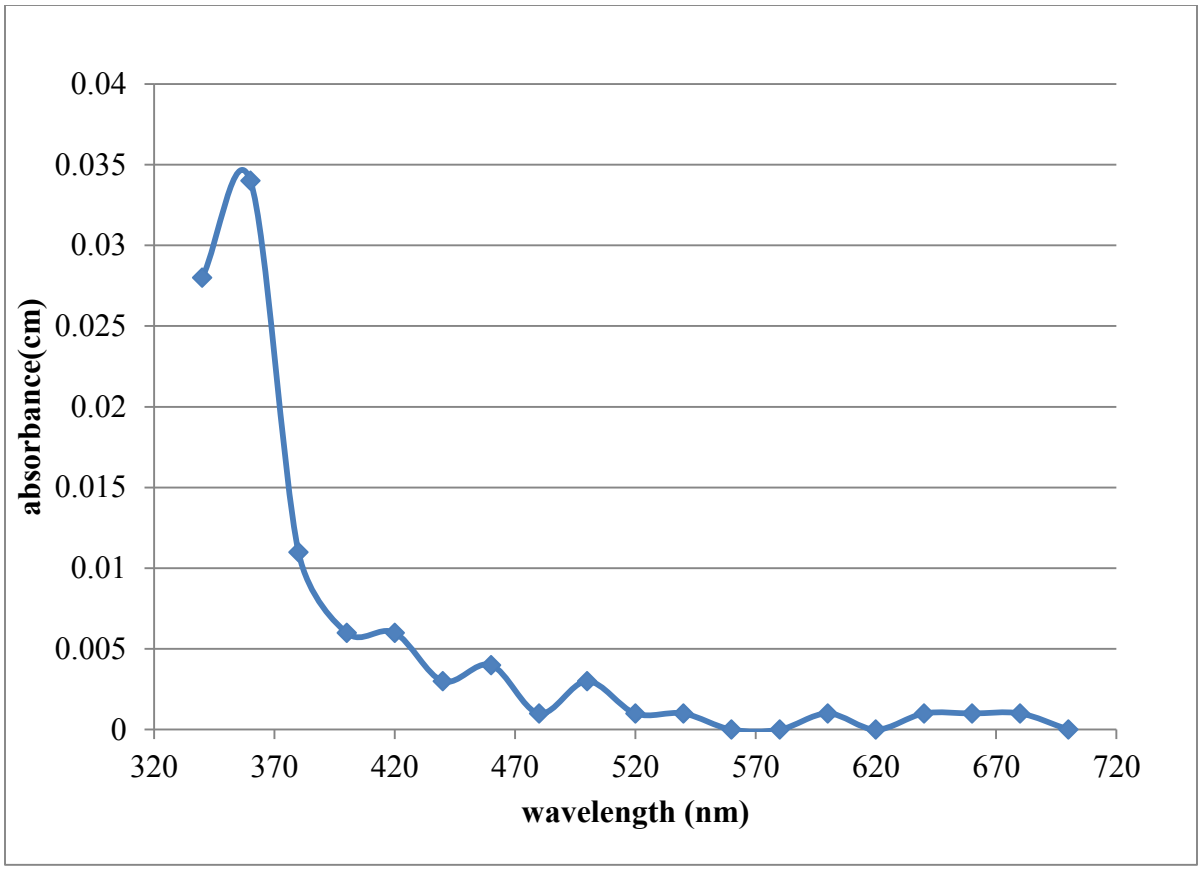


Figure 4. 5: UV maxima for Compound G at concentration 20 µg/ml in Octanol

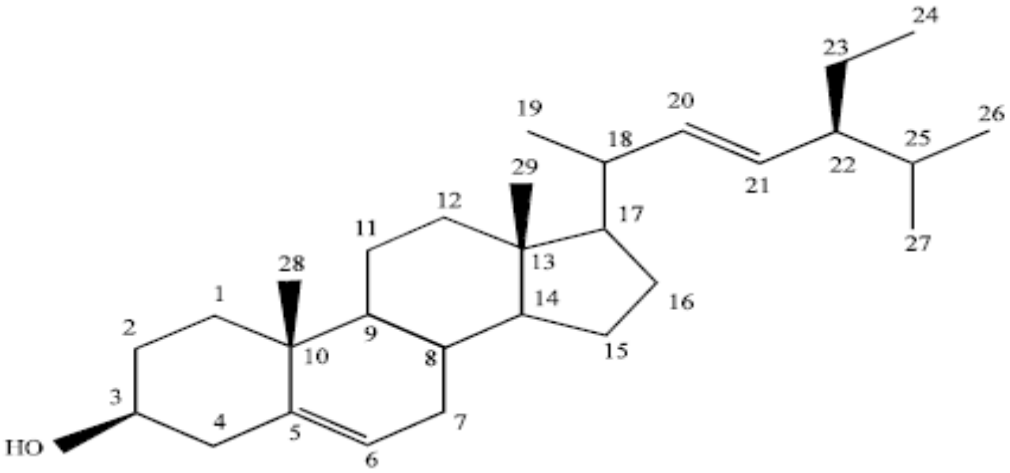


Figure 4. 6: Chemical structure of Compound G (Stigmasterol)

4.1.4 GC-MS analysis of isolated compounds from *Aloe babardensis* Miller

The mass spectrum of the isolated compounds did not provide much information as it is difficult to identify any notable fragmentation patterns. These results can be seen in (Appendix 3)

4.1.5 Results of physicochemical analysis of isolated compounds from *Aloe barbadensis* Miller

Table 4.7: Some parameters determined for the isolated compounds from *Aloe barbadensis* Miller

Optical characteristics of Isolated compounds	Compound A	Compound G
Retention factor (R_f)	0.58 (MeOH:$CHCl_3$ = 3:7)	0.67 (MeOH:$CHCl_3$ = 1:9)
Log P	0.7304570558	0.190642
pKa	6.667452953	3.889612
Suggested name of compound	Aloin A	Stigmasterol

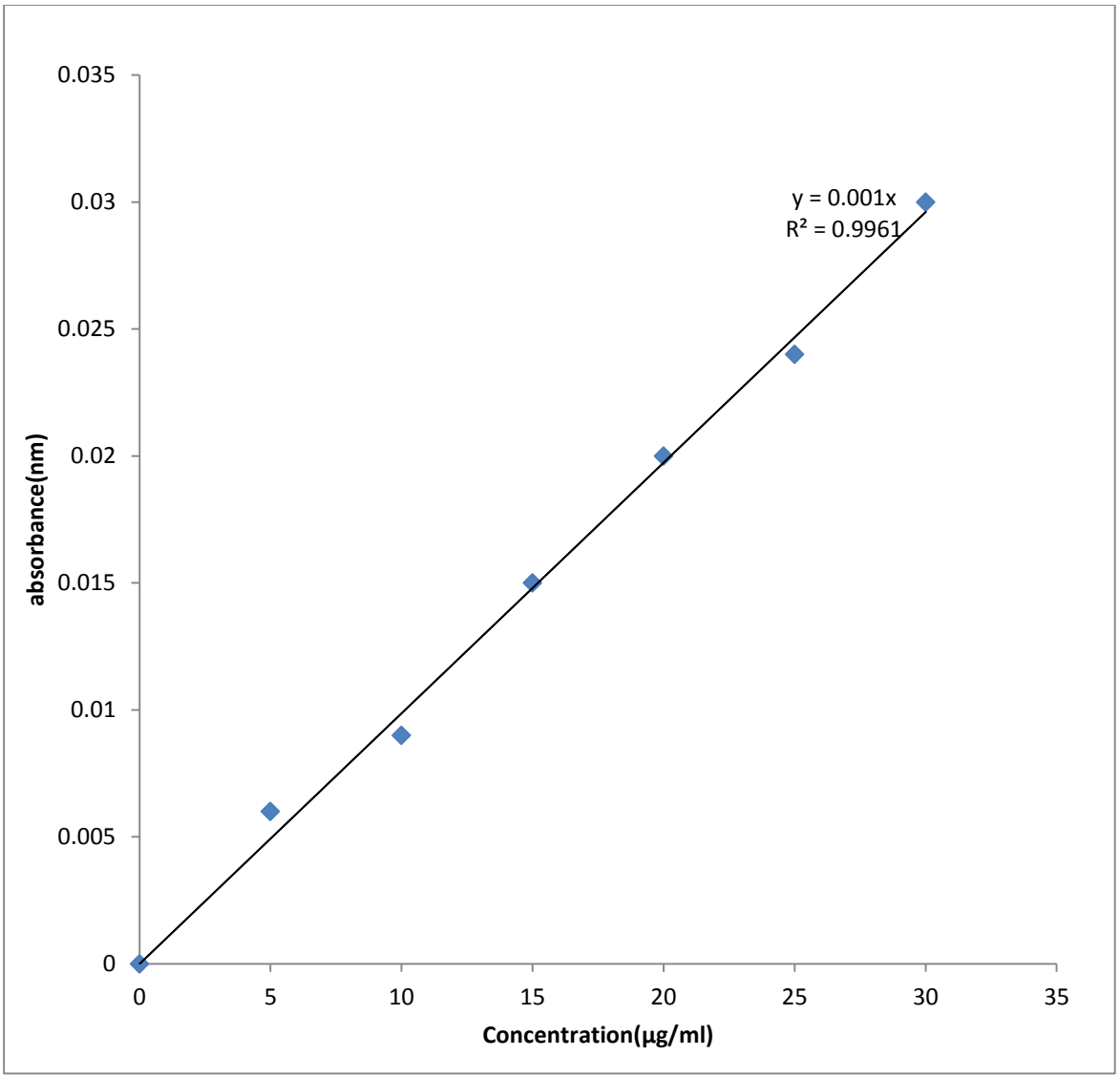


Figure 4.7: Standard curve for Compound G at 360 nm in Octanol

Figure 4.7 is a linearity curve for isolated Compound G which obeyed the Beer Lamberts's law within the concentration range of 5-30 µg/ml in octanol. Same figure showed the regression equation for Compound G to be Y= 0.001X and the correlation coeffient (R^2) value as 0.996.

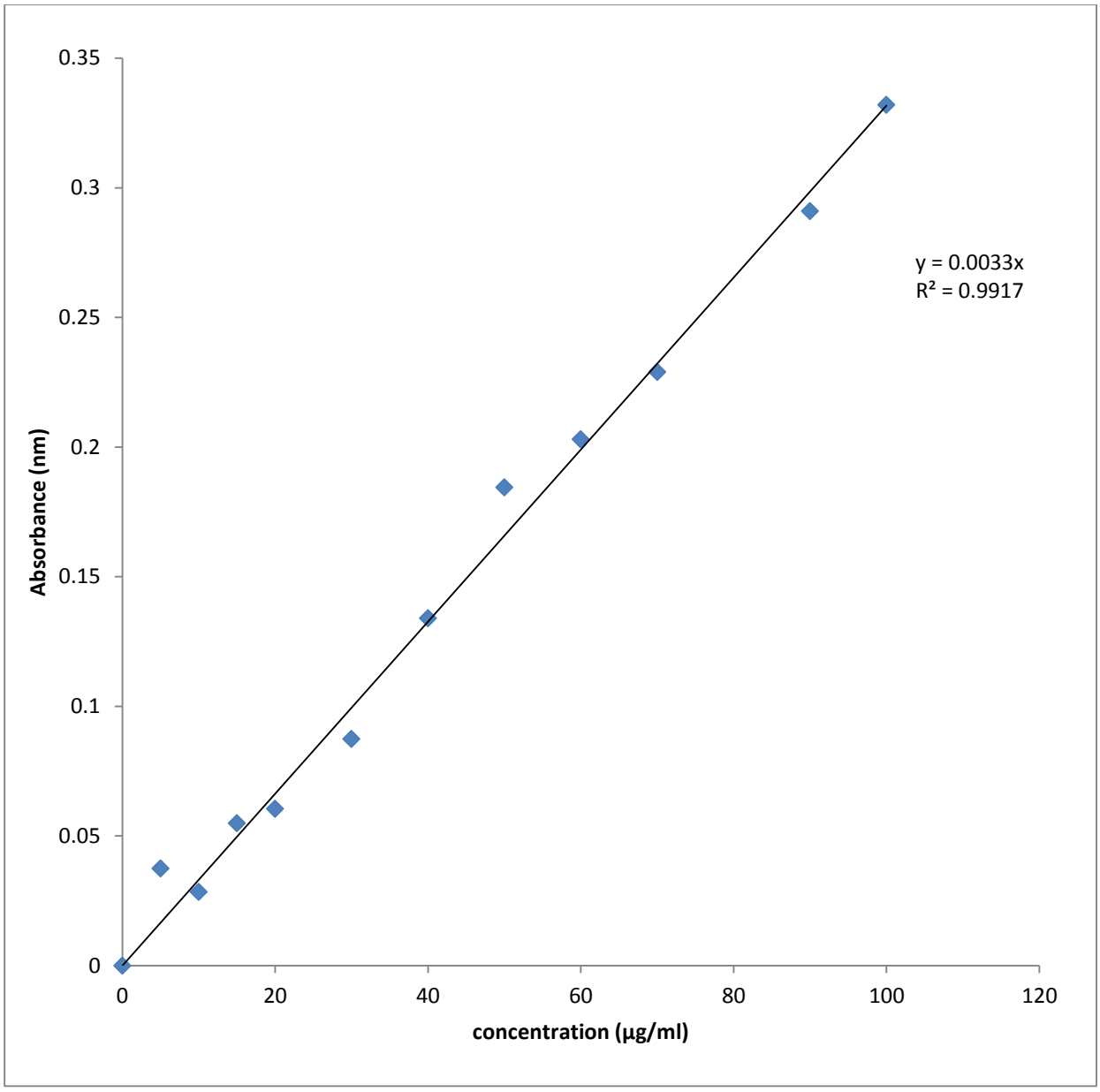


Figure 4.8: Standard curve for Compound A at 360 nm in Octanol

Figure 4.8 is a linearity curve for isolated Compound A which obeyed the Beer Lamberts's law within the concentration range of 5-100 µg/ml in octanol. Same figure showed the regression equation for Compound A as Y= 0.0033X and the correlation coeffient (R^2) value as 0.992.

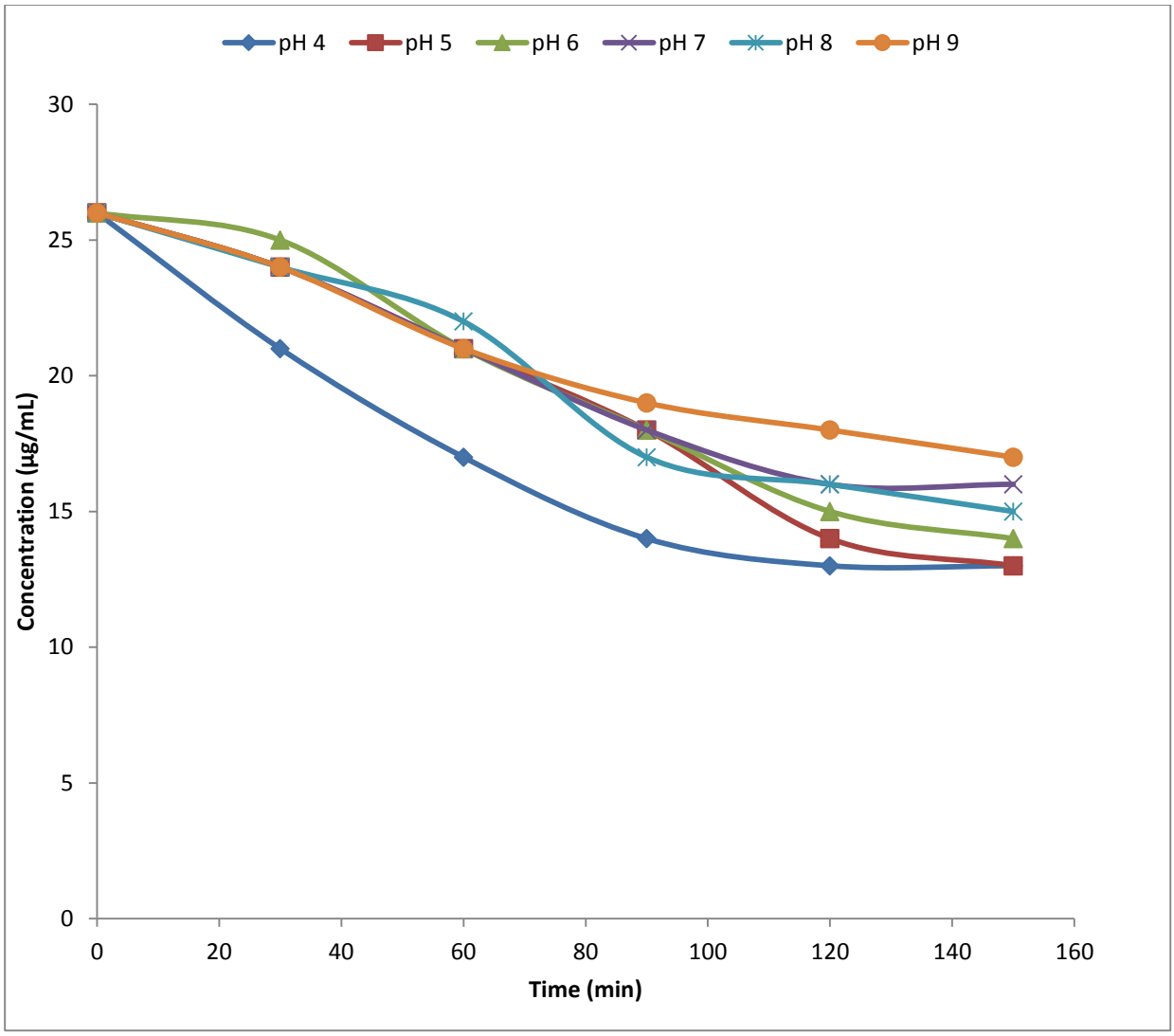


Figure 4.9: A graph of concentration vs time (Equilibrium curves of Compound G (26 µg/ml) Partitioned between Octanol and buffer solutions at varying pH).

Figure 4.9 shows equillibrium curves for Compound G (26 µg/ml) when it was partitiopned between octanol and buffer at varying pH values (pH 4, pH 5, pH 6, pH 7, pH 8, pH 9). From the graph it was realized that, the concentration of the isolated compound in the octanol medium began to reduce as time increased until at the 120th minute when it approximately became constant. This suggested that, the compound at

this point had fully partitioned itself between the lipid medium (octanol) which mimiced the human cell membrane and the aqueous medium (buffer) which mimiced the human blood. Octanol was chosen as the model lipid phase because it most closely simulates the properties of biological membranes (Paschke *et al*., 2004).

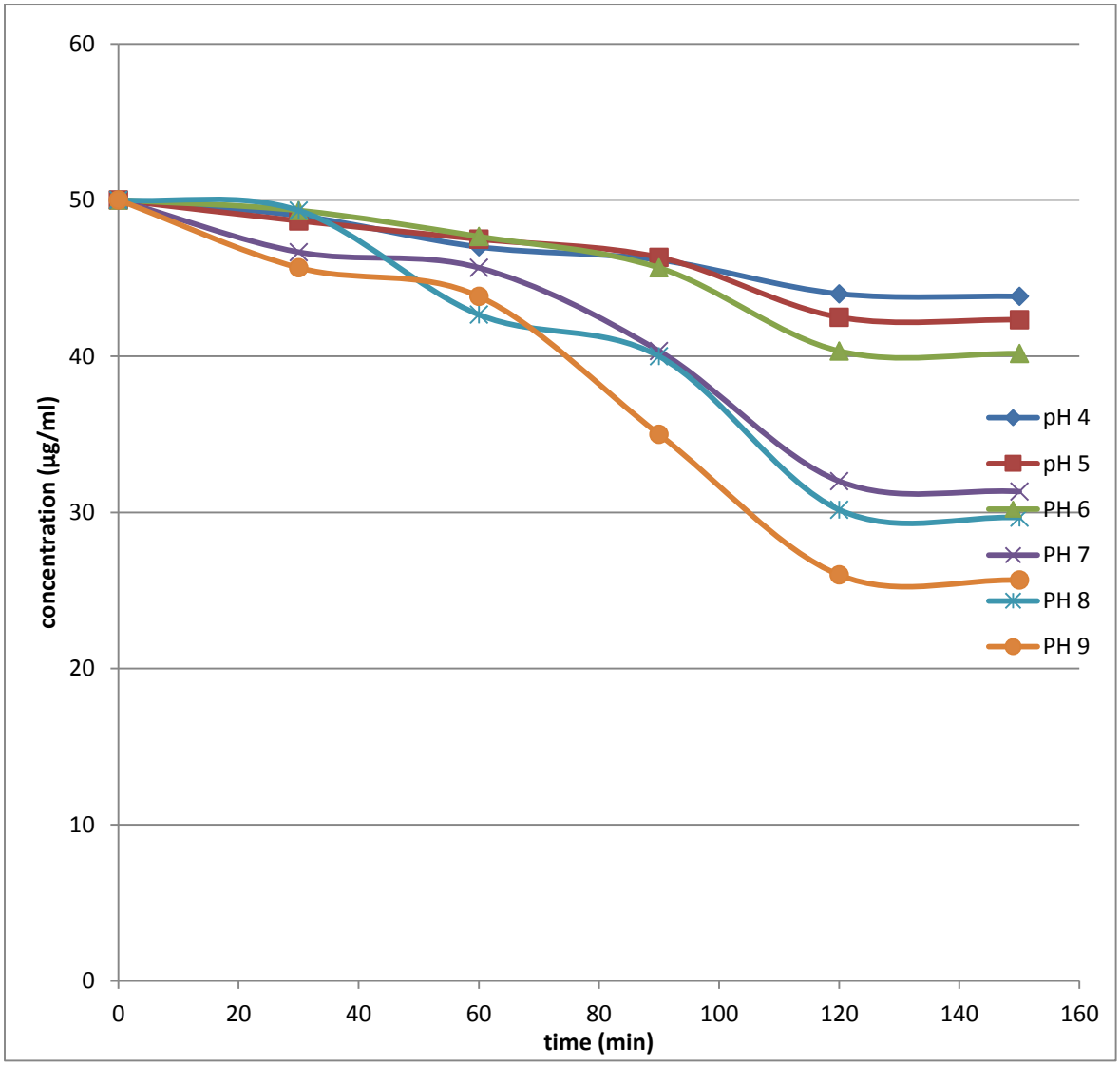


Figure 4.10: A graph of concentration vs. time (Equilibrium curves of Compound A (50µ g/ml) Partitioned between Octanol and buffer solutions at varying pH).

Figure 4.10 show equillibrium curves for Compound A (50 µg/ml) when it was partitioned between octanol and buffer at varying pH values(pH 4, pH 5, pH 6, pH 7, pH 8, pH 9). From the graph it was realized that, the concentration of the isolated compound in the octanol medium began to reduce as time increased until at the 120th minute when it approximately became constant. This suggested that, the compound at this point had fully partitioned itself between the lipid medium (octanol) which mimics the human cell membrane and the aqueous medium (buffer) which mimics the human blood *(Paschke et al.*, 2004).

4.1.6 Results of logP and pKa of isolated compounds from *Aloe barbadensis* Miller

The pH values of isolated Compound A and G were determined to be 6.2 and 8.1 respectively indicating that, Compound A was weakly acidic whilst Compound G was weakly basic. Figure 4.11 and Figure 4.12 are graphs for the estimation of logP and pKa values for Compound G and Compound A respectively. The lopP values for isolated Compound G and A were estimated to be 0.19 and 0.73 respectively (Table 4.7). This suggested that, both compounds are lipophilic once they both have positive lopP values. However, Compound A is more lipophylic than Compound G since its logP value is more positive than that of Compound G (Paschke *et al*., 2004). From Figure 4.11 and Figure 4.12, the pKa values of the isolated Compounds G and A were estimated to be 3.89 and 6.67 respectively (Table 4.7).

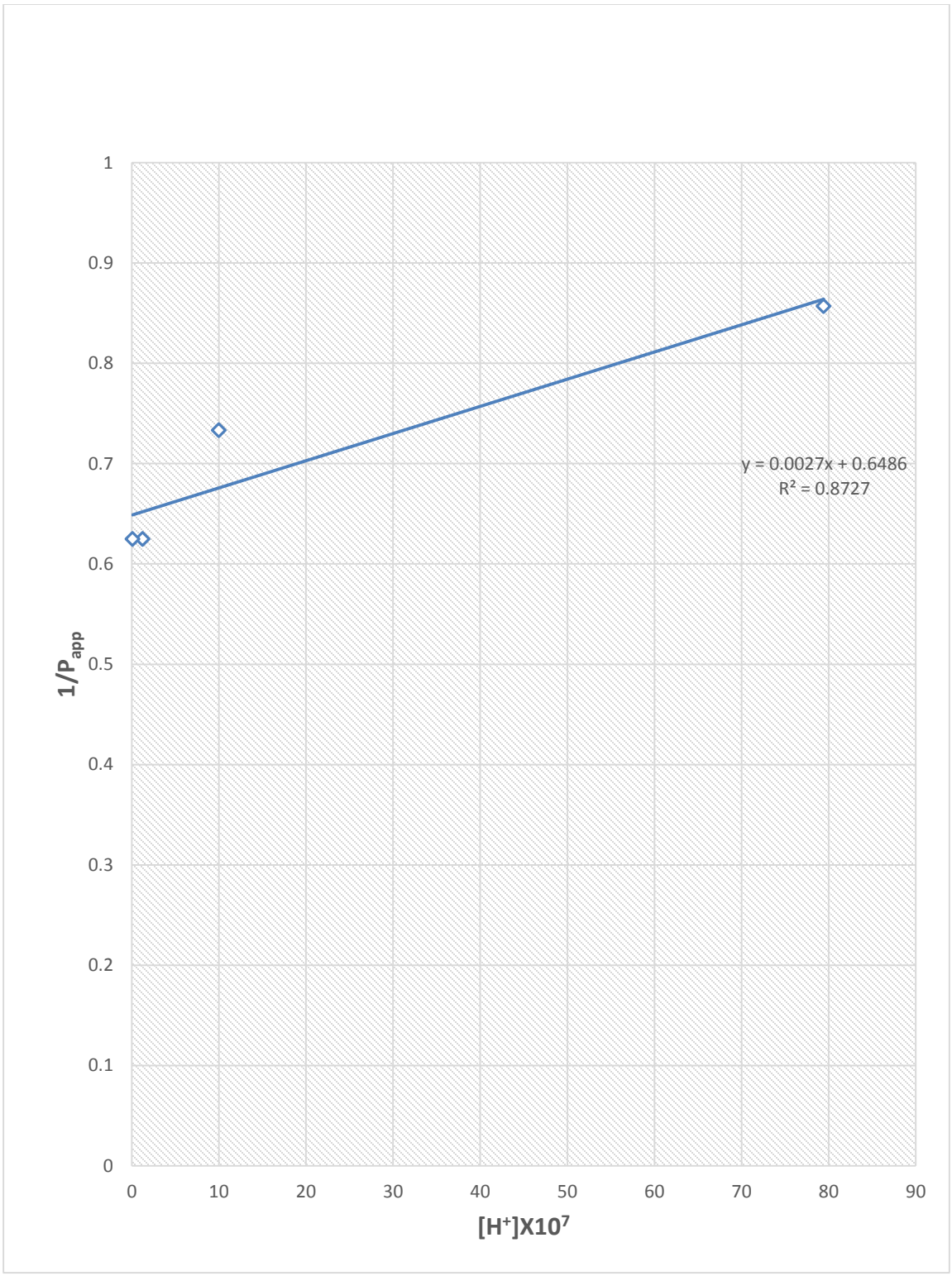


Figure 4.11: Graph of 1/Papp vs hydrogen ion concentration for determining log P and pka of isolated Compound G

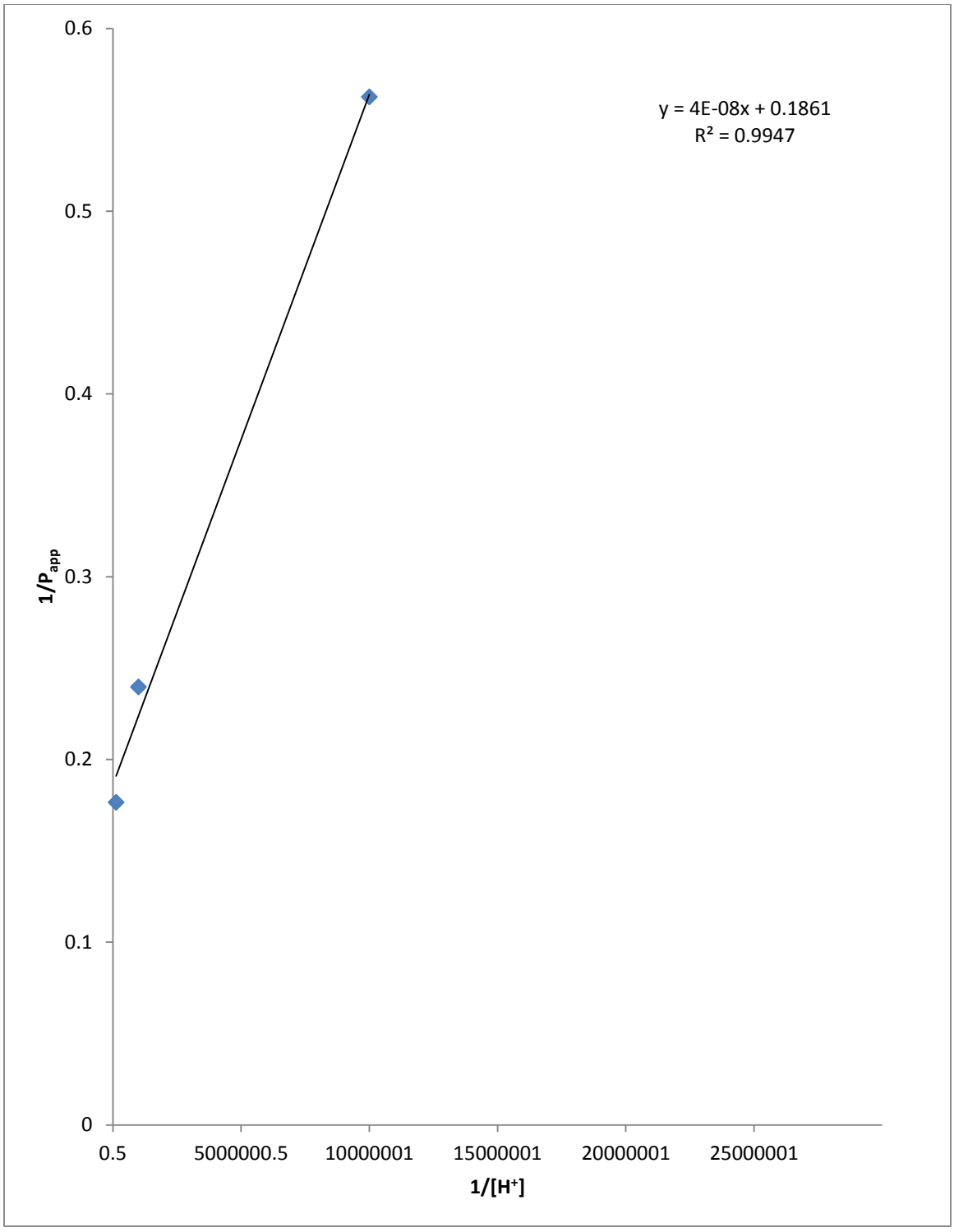


Figure 4.12: Graph of 1/Papp vs. the inverse of hydrogen ion concentrations for determining log P and pKa of isolated Compound A

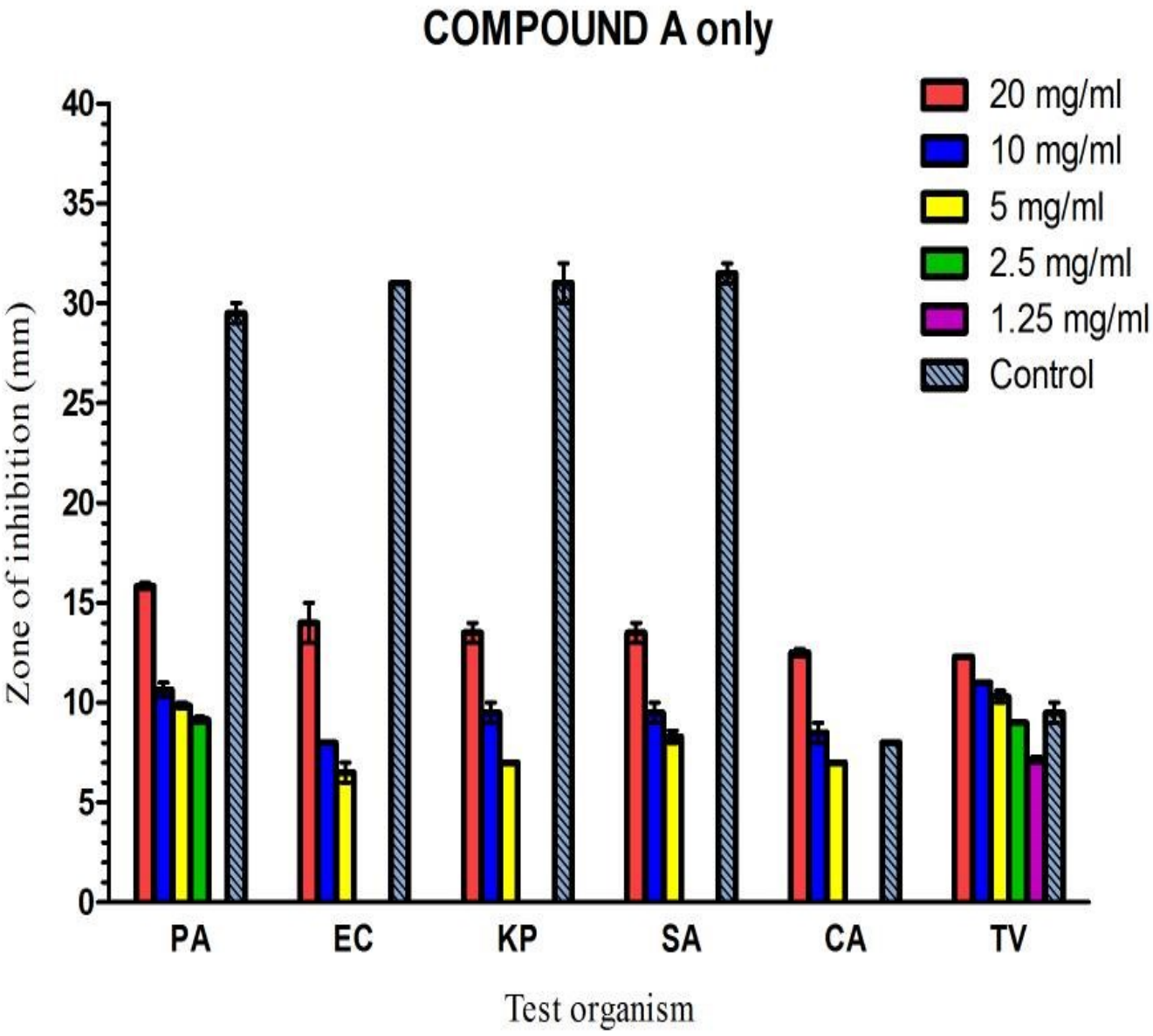
4.1.7 Antimicrobial activity of isolated compounds from *Aloe barbadensis* Miller

The results in Table 4.8 indicate that all the test microorganisms were suppressed by isolated Compound A only with zone of inhibition ranging from 6-15.9 mm for all the selected microorganisms at varying concentrations of (1.25, 2.5, 5, 10, 20) mg/ml. *P. aeruginosa* was susceptible to isolated Compound A with diameter zone of inhibition range of 9.2-15.9 mm at the varying concentrations. *E. coli* was susceptible to the compound with diameter zone of inhibition in the range of 6.5-15 mm at the varying concentrations. Similarly *K. pneumoniae* exhibited zone of inhibition in the range of 7-13.5 mm at the varying concentrations**.** *S. aureus* was susceptible to isolated Compound A from *Aloe barbadensis* Miller with zone of inhibition in the range of 8.3-13 mm at the varying concentrations. The isolate exhibited inhibition against the fungi, C. *albicans* and *T. fluvus* with inhibition zone ranges of 7-12.5 and 7.2-12.3 mm respectively at the varying concentrations. Same results can be seen on Figure 4.17. From the results, it was realized that Compound A only was more effective against *T. fluvus* than the other test organisms since even at the lowest concentration of the compound (1.25 mg/ml) in this test, inhibition was observed for *T. fluvus*. Whilst the positive control for the bacterial strains, chloramphenicol (CL) exhibited very high potency against all the test organisms with zones of inhibition ranging from 29.0-31.5 mm, the positive control for the fungal strains, fluconazole (FL), rather exhibited lower potency against the fungi with zones of inhibition range of 8.0-9.5 mm. The negative control, DMSO was detected not to have any effect on the growth of all the test organisms since it exhibited no inhibition (0.0 mm) on all test organisms (Table 4.8).

Table 4.8: Antimicrobial sensitivity test of Isolated Aloin A (Compound A) only at varying concentrations (mg/ml)

Test Organisms	Control	Standard		Diameter zone of inhibition (mm)				
				Concentration (mg/ml)				
	Negative	Positive		20 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml
	DMSO	CL	FL					
ı	0.0 ± 0.00	29.5 ± 0.71	NA	15.9 ± 0.21	10.7 ± 0.49	9.9 ± 0.21	9.2 ± 0.21	0.0 ± 0.00
	0.0 ± 0.00	31.0 ± 0.00	NA	15.0 ± 0.41	8.0 ± 0.00	6.5 ± 0.71	0.0 ± 0.00	0.0 ± 0.00
ıe	0.0 ± 0.00	31.0 ± 0.1.4	NA	13.5 ± 0.71	9.5 ± 0.71	7.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
	0.0 ± 0.00	31.5 ± 0.71	NA	13.0 ± 0.71	9.5 ± 0.71	8.3 ± 0.42	0.0 ± 0.00	0.0 ± 0.00
	0.0 ± 0.00	NA	8.0 ± 0.00	12.5 ± 0.28	8.5 ± 0.71	7.0 ± 0.00	0.0 ± 0.00	0.0 ± 0.00
	0.0 ± 0.00	NA	9.5 ± 0.71	12.3 ± 0.00	11.0 ± 0.00	10.3 ± 0.42	9.0 ± 0.00	7.2 ± 0.21

ıamphenicol (20 mg/ml), FL = Fluconazole (20mg/ml), NA = Not applicable, Data expressed as mean ± STDEV, n = 2



PA = *P. aeruginosa*, EC = *Escherichia coli*, KP = *K. pneumoniae*, SA = *S. aureus*, CA = C. *albicans*, TV = *T. fluvus*

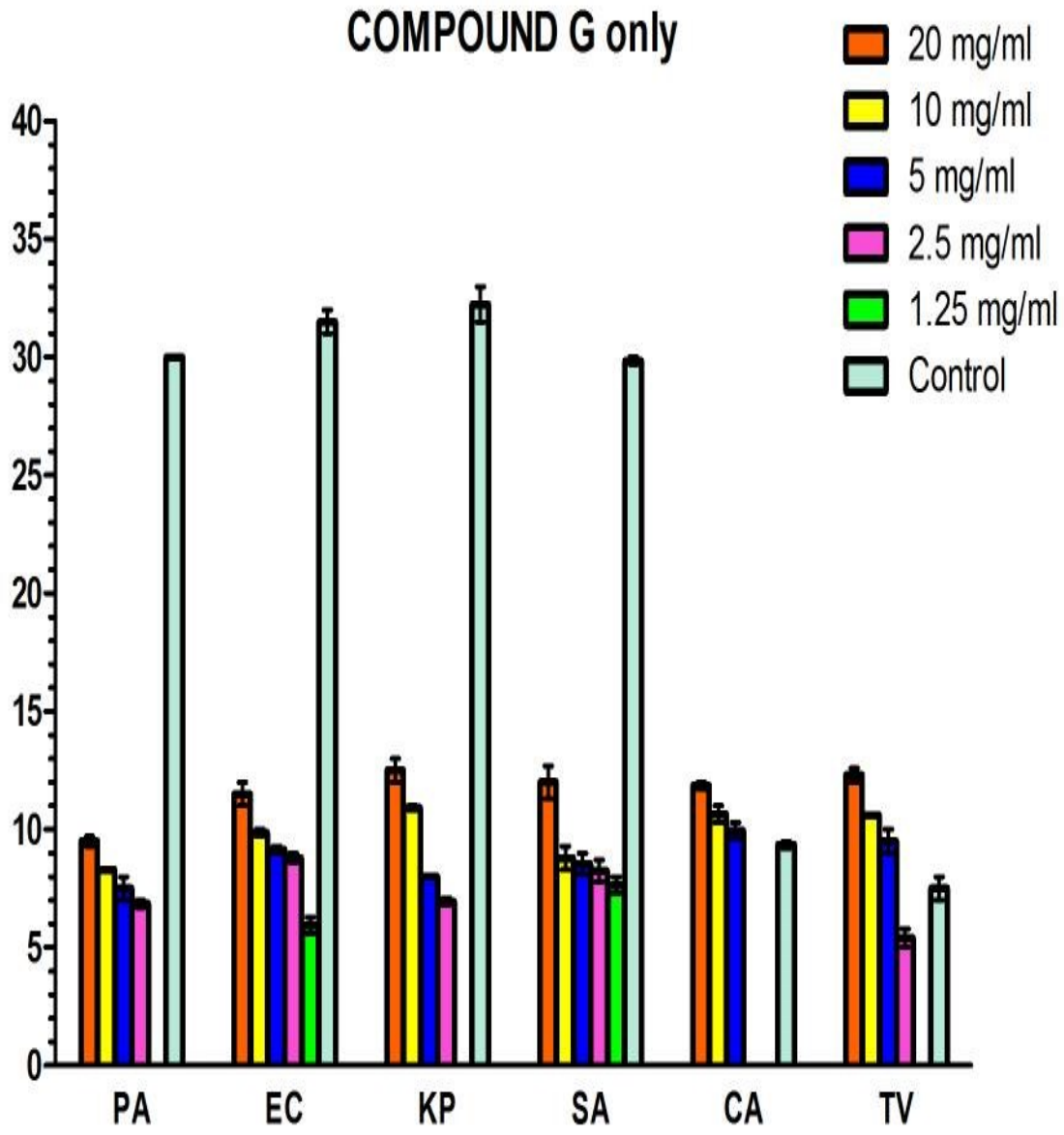
Figure 4.13: Antimicrobial activity of isolated Aloin A (Compound A) from *Aloe barbadensis* Miller on selected test organisms at varying concentrations.

Table 4.9: Antimicrobial sensitivity test of isolated Stigmasterol (Compound G) at varying concentrations (mg/ml)

Test	Control	Standard		Diameter zone of inhibition (mm)				
				Concentration (mg/ml)				
	Negative	Positive		20 mg/ml	10 mg/ml	5 mg/ml	2.5 mg/ml	1.25 mg/ml
	DMSO	CL	FL					
	0.0 ± 0.0	30.0 ± 0.00	NA	9.5 ± 0.28	8.3 ± 0.00	7.5 ± 0.71	6.9 ± 0.21	0.0 ± 0.00
	0.0 ± 0.0	31.5 ± 0.71	NA	11.5 ± 0.71	9.9 ± 0.21	9.0 ± 0.21	8.8 ± 0.28	6.0 ± 0.49
ıe	0.0 ± 0.0	32.4 ± 0.85	NA	12.5 ± 0.71	10.9 ± 0.14	8.0 ± 0.00	7.0 ± 0.21	0.0 ± 0.00
	0.0 ± 0.0	29.85 ± 0.21	NA	12.0 ± 0.99	8.8 ± 0.71	8.6 ± 0.64	8.3 ± 0.64	7.7 ± 0. 49
	0.0 ± 0.0	NA	9.3 ± 0.21	11.9 ± 0.21	10.7 ± 0.49	10.0 ± 0.49	0.00 ± 0.00	0.0 ± 0.00
	0.0 ± 0.0	NA	7.5 ± 0.71	12.3 ± 0.42	10.6 ± 0.00	9.5 ± 0.71	5.4 ± 0.57	0.0 ± 0.00

ıamphenicol (mg/ml), FL = Fluconazole (mg/ml), NA = Not applicable, Data expressed as mean ± STDEV, n

The results in Table 4.9 indicate that all the test microorganisms were susceptible to isolated Compound G from *Aloe barbadensis* Miller with zone of inhibition ranging from 5.4-12.5 mm for all the selected microorganisms at varying concentrations (1.25, 2.5, 5, 10, 20) mg/ml. *P. aeruginosa* was susceptible to Compound G only with diameter zone of inhibition range of 6.9-9.5 mm at the varying concentrations. *E. coli* was susceptible to the compound with diameter zone of inhibition range of 6-11.5 mm at the varying concentrations. Similarly *K. pneumoniae* had zone of inhibition range of 7-12.5 mm at the varying concentrations. Also *S. aureus* were susceptible to isolated compound G only from *Aloe barbadensis* Miller with zone of inhibition range of 7.7-12 mm at varying concentrations. The plant isolate G exhibited inhibition against *C. albicans* and *T. fluvus*, fungi with zone of inhibition ranges of 10-11.9 and 5.4-12.3 mm respectively at varying concentrations. Same results can be seen on Figure 4.14. From the results, it was realized that Compound G only was more effective against *S. aureus* followed by *E. coli* than the other test organisms since even at the lowest concentration of the compound (1.25 mg/ml) in this test, inhibitions were observed as 7.7 and 6.0 mm respectively for *S. aureus* and *E. coli*. Very similar to compound A, whilst the positive control for the bacterial strains, chloramphenicol (CL) exhibited very high potency against all the test organisms with zones of inhibition ranging from 29.85-32.4 mm, the positive control for the fungal strains, fluconazole (FL), rather exhibited lower potency against the fungi with zones of inhibition range of 7.5-9.3 mm. The negative control, DMSO was detected not to have any effect on the growth of all the test organisms since it exhibited no inhibition (0.0 mm) on all test organisms (Table 4.9).



PA = *P. aeruginosa*, EC = *Escherichia coli*, KP = *K. pneumoniae*, SA = *S. aureus*, CA = C. *albicans*, TV = *T. fluvus*

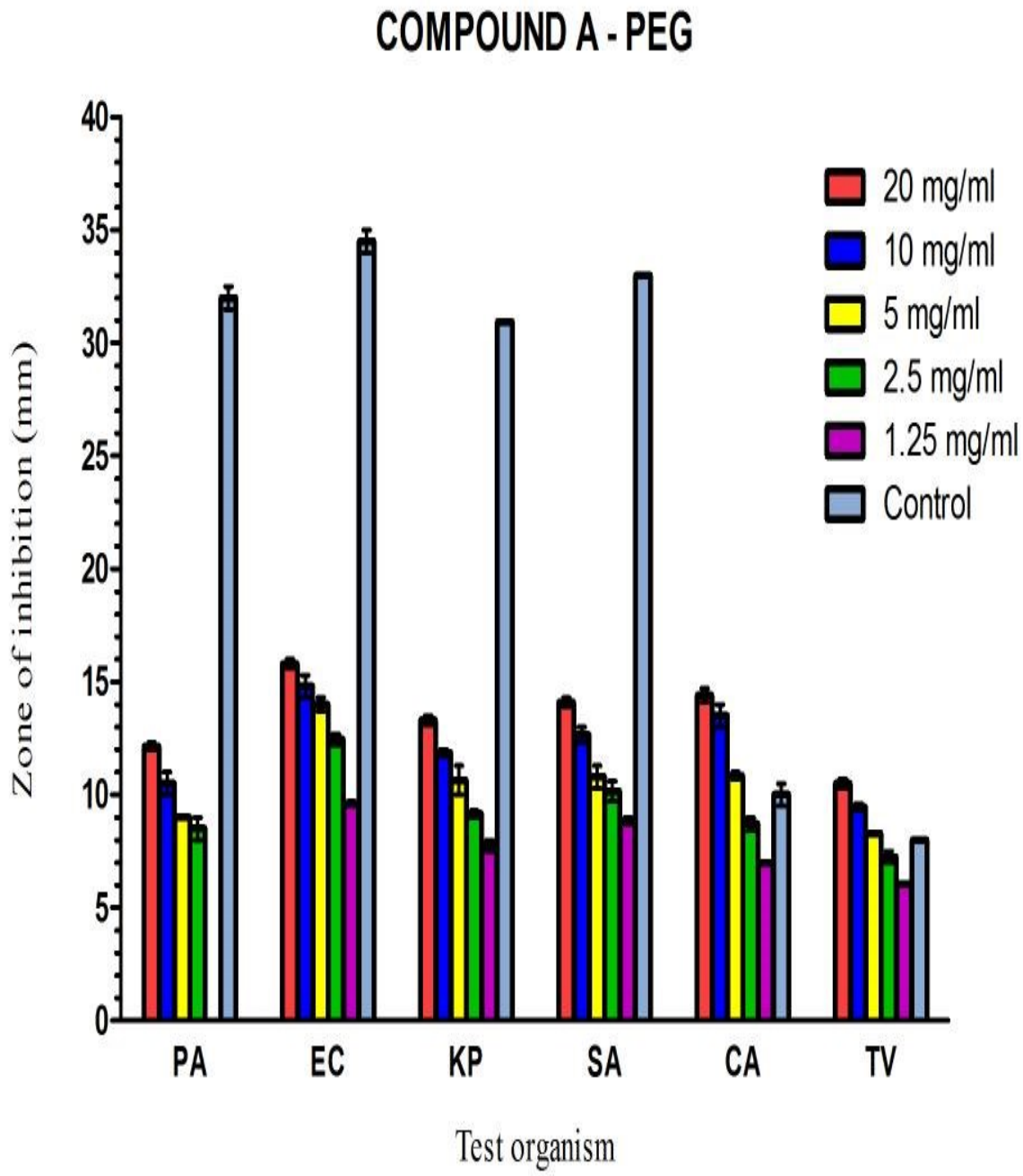
Figure 4. 14: Antimicrobial activity of isolated Stigmasterol (Compound G) from *Aloe barbadensis* Miller on selected test organisms at varying concentrations.

Table 4.10: Antimicrobial sensitivity test of isolated Aloin A-PEG (Compound A-PEG) formulation at varying concentrations (mg/g)

Test c	Control		Standard	Diameter zone of inhibition (mm)				
	Negative		Positive	Concentration (mg/g)				
	DMSO	CL	FL	20 mg/g	10 mg/g	5 mg/g	2.5 mg/g	1.25 mg/g
P. aer	± 0.00	32.0 ± 0.71	NA	12.2 ± 0.21	10.5 ± 0.71	9.0 ± 0.00	8.5 ± 0.71	0.0 ± 0.00
E. coli	± 0.00	34.5 ± 0.71	NA	15.8 ± 0.28	14.8 ± 0.71	14.0 ± 0.42	12.5 ± 0.35	9.6 ± 0.14
K. pne	± 0.00	30.5 ± 0.07	NA	13.3 ± 0.28	11.9 ± 0.21	10.7 ± 0.92	9.2 ± 0.21	7.8 ± 0.35
S. aur	± 0.00	33.0 ± 0.00	NA	14.1 ± 0.28	12.7 ± 0.49	10.8 ± 0.71	10.2 ± 0.64	8.9 ± 0.21
C. alb	± 0.00	NA	10.0 ± 0.71	14.4 ± 0.42	13.5 ± 0.71	10.9 ± 0.71	8.7 ± 0.42	6.9 ± 0.21
T. flu	± 0.00	NA	8.0 ± 0.00	10.5 ± 0.38	9.5 ± 0.21	8.3 ± 0.00	7.3 ± 0.35	6.1 ± 0.07

ethylene glycol, CL = Chloramphenicol (mg/g), FL = Fluconazole (mg/g), NA = Not applicable, Data expressed as mean ± STDEV, n = 2

Table 4.10 results show that the formulated Compound A isolated from *Aloe barbadensis* Miller is a good antimicrobial agent as all the tested microorganisms were susceptible to Compound A-PEG formulation with zone of inhibition ranging from 6.1-15.8 mm for all the selected microorganisms at varying concentrations of (1.25, 2.5, 5, 10, 20) mg/g. *P. aeruginosa* showed susceptibility to the isolated Compound A-PEG ointment with diameter zone of inhibition range of 8.5-12.2 mm at the varying concentrations. *E. coli* similarly showed susceptibility to isolated Compound A-PEG ointment with diameter zone of inhibition range of 9.6-15.8 mm at the varying concentrations. Similarly *K. pneumoniae* showed susceptibility to the isolated Compound A-PEG ointment with the zone of inhibition range of 7.8-13.3 mm at varying concentrations. Also *S. aureus* showed susceptibility to plant isolate A-PEG ointment with zone of inhibition range of 8.9-14.1 mm at the varying concentrations. Similarly, the plant isolate A-PEG ointment exhibited inhibition against fungi, (*C. albicans* and *T. fluvus*) with inhibition zone ranges of 6.9-14.4 and 6.1-10.5 mm respectively at the varying concentrations. Same results can be seen on Figure 4.15. However very similar to compound A only, whilst the positive control for the bacterial strains, chloramphenicol (CL) exhibited very high potency against all the test organisms with zones of inhibition ranging from 30.5-33.0 mm, the positive control for the fungal strains, fluconazole (FL), rather exhibited lower potency against the fungi with zones of inhibition ranging from 8.0-10.0 mm. The negative control, DMSO was detected not to have any effect on the growth of all the test organisms since it exhibited no inhibition (0.0 mm) on all test organisms (Table 4.10).



PA = *P. aeruginosa*, EC = *Escherichia coli*, KP = *K. pneumoniae*, SA = *S. aureus*, CA = C. *albicans*, TV = *T. fluvus*

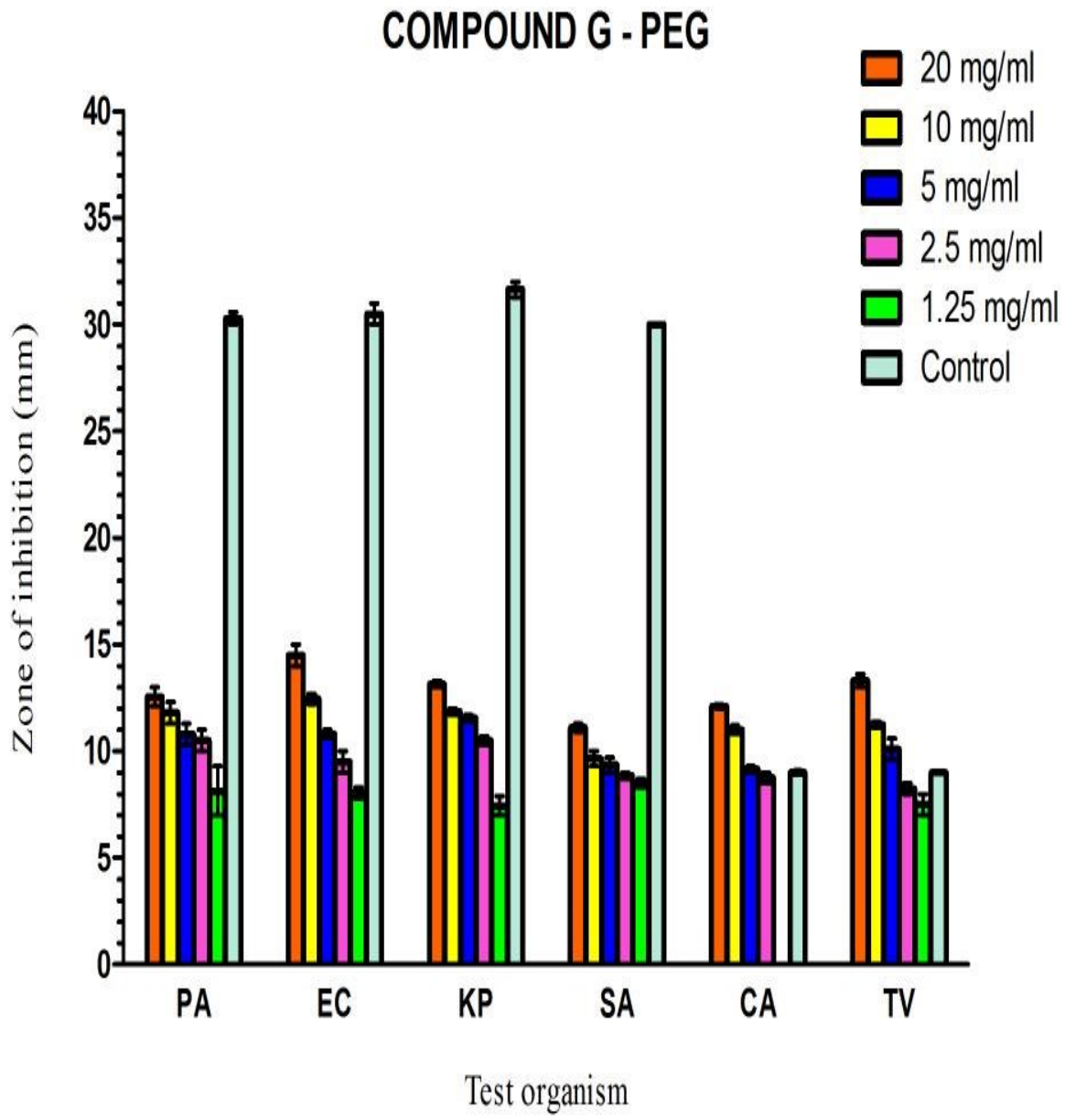
Figure 4. 15: Antimicrobial activity of isolated Aloin A-PEG (Compound A-PEG) formulation from *Aloe barbadensis* Miller on selected test organisms at varying concentrations.

Table 4.11: Antimicrobial sensitivity test of isolated Stigmasterol-PEG (Compound G –PEG) formulation at varying concentrations (mg/g)

Control	Standard		Diameter zone of inhibition (mm)				
			Concentration (mg/g)				
Negative	Positive						
DMSO	CL	FL	20 mg/g	10 mg/g	5 mg/g	2.5 mg/g	1.25 mg/g
0.0 ± 0.00	30.3 ± 0.42	NA	12.6 ± 0.32	11.8 ± 0.35	10.8 ± 0.35	10.5 ± 0.35	9.5 ± 0.14
0.0 ± 0.00	30.5 ± 0.71	NA	14.5 ± 0.71	12.5 ± 0.35	10.8 ± 0.28	9.5 ± 0.71	8.1 ± 0.35
0.0 ± 0.00	31.7 ± 0.49	NA	13.2 ± 0.21	11.9 ± 0.21	11.5 ± 0.21	10.5 ± 0.28	7.5 ± 0.64
0.0 ± 0.00	30.0 ± 0.00	NA	11.1 ± 0.28	9.7 ± 0.49	9.4 ± 0.49	8.9 ± 0.21	8.5 ± 0.28
0.0 ± 0.00	NA	9.0 ± 0.14	12.1 ± 0.14	11.0 ± 0.28	9.2 ± 0.21	8.8 ± 0.35	0.0 ± 0.00
0.0 ± 0.00	NA	9.0 ± 0.00	13.3 ± 0.42	11.3 ± 0.21	10.1 ± 0.71	8.3 ± 0.35	7.5 ± 0.71

PEG = Polyethylene glycol, CL=Chloramphenicol (mg/g), FL = Fluconazole (mg/g), NA = Not applicable, Data expressed as mean ± STDEV, n = 2

Table 4.11 results show that the plant isolate-G ointment is a good antimicrobial agent as all the tested microorganisms were susceptible to Compound G-PEG ointment with zones of inhibition ranging from 7.5-14.5 mm for all the selected microorganisms at varying concentrations of (1.25, 2.5, 5, 10, 20) mg/g. *P. aeruginosa* showed susceptibility to isolated Compound G-PEG ointment with diameter zone of inhibition range of 9.5-12.6 mm at the varying concentrations. *E. coli* similarly showed susceptibility to isolated Compound G-PEG ointment with diameter zone of inhibition range of 8.1-14.5 mm at the varying concentrations. Similarly *K. pneumoniae* showed susceptibility to the isolated Compound G-PEG ointment with the zone of inhibition range of 7.5-13.2 mm at varying concentrations. Also *S. aureus* showed susceptibility to plant isolate ointment with zone of inhibition range of 8.5-11.1 mm at the varying concentrations. Similarly, the plant isolate ointment exhibited inhibition against fungi, *C. albicans* and *T. fluvus* with ranges of 8.8-12.1 and 7.5-13.3 mm respectively at the varying concentrations. Same results are shown in Figure 4.16. However very similar to Compound G only, whilst the positive control for the bacterial strains, chloramphenicol (CL) exhibited very high activity against all the test organisms with zones of inhibition ranging from 30.5-31.7 mm, the positive control for the fungal strains, fluconazole (FL), rather exhibited lower potency against the fungi with zone of inhibition of 9.0 mm for both fungi. Similar to Compound G only, the negative control (DMSO) exhibited no inhibition (0.0 mm) on all test organisms (Table 4.11).



PA = *P. aeruginosa*, EC = *Escherichia coli* KP = *K. pneumoniae*, SA = *S. aureus*, CA = C. *albicans*, TV = *T. fluvus*

Figure 4.16: Antimicrobial activity of isolated Stigmasterol –PEG (Compound G-PEG) formulation from *Aloe barbadensis* Miller on selected test organisms at varying concentrations.

Table 4.12: MIC and MBC/MFC for Isolated compounds on test microorganisms (mg/ml)

es	*P. aeruginosa*		*E. coli*		*K. pneumoniae*		*S. aureus*		*C. albicans*		*T. fluvus*	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MFC**	**MIC**	**MFC**
A	2.5	AF	5	AF	5	AF	5	AF	5	AF	1.25	AF
G	2.5	AF	1.25	AF	2.5	AF	0.32	AF	2.5	AF	2.5	AF

mum Inhibitory Concentrations, MBC = Minimum Bactericidal Concentrations, MFC = Minimum Concentration, AF = Above 40 mg/ml

The MIC of isolated Compound A only was determined and the results found to be 2.5, 5, 5, 5, 5 and 1.25 mg/ml for *P. aeruginosa, E. coli, K. pneumoniae, S. aureus, C. albicans* and *T. fluvus* respectively (Table 4.12). The MBC/MFC of isolated compound A was investigated but was undetermined at 40mg/ml hence, the MBC/MFC of isolated Compound A may be above 40mg/ml as can be observed in (Table 4.12).

Similarly, the MIC for isolated Compound G only was determined to be very low as compared to Compound A. The results were found to be 2.5, 1.25, 2.5, 0.32, 2.5, and 2.5 mg/ml for *P. aeruginosa, E. coli, K. pneumoniae, S. aureus, C. albicans* and *T. fluvus* respectively (Table 4.12). This suggest that, the bioactivity of Compound G is higher than that of Compound A. Similar to Compound A, the MBC/MFC for Compound G may be above 40 mg/ml as it was undetermined at 40 mg/ml when tested against the selected micro-organisms.

Table 4.13: MIC and MBC/MFC for Isolated compounds-PEG formulation on microorganisms (mg/g)

ıtes	*P. aeruginosa*		*E. coli*		*K. pneumoniae*		*S. aureus*		*C. albicans*		*T. fluvus*	
	MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MFC**	**MIC**	**MFC**
ı-PEG	2.5	AF	0.63	AF	0.63	AF	0.32	AF	0.32	AF	1.25	AF
ı-PEG	0.08	AF	0.63	AF	1.25	AF	0.63	AF	1.25	AF	0.16	AF

ethylene glycol, MIC = Minimum Inhibitory Concentrations, MBC = Minimum Bactericidal ons, MFC = Minimum Fungicidal Concentration, AF =Above 40 mg/g

The MIC of isolated Compound A-PEG formulation appeared to be much lower than the Compound A only when tested on the selected microorganisms. The MIC values were found to be 2.5, 0.63, 0.63, 0.32, 0.32 and 1.25 mg/g for *P. aeruginosa, E. coli, K. pneumoniae, S. aureus, C. albicans* and *T. fluvus* respectively (Table 4.13) for Compound A-PEG as against 2.5, 5, 5, 5, 5, 1.25 mg/ml for *P. aeruginosa, E. coli, K. pneumoniae, S. aureus, C. albicans* and *T. fluvus* respectively (Table4.12) for Compound A only. This therefore suggests that, the formulated form of Compound A is more effective against the selected test organisms than the pure form of the compound. The MBC/MFC of isolated Compound A-PEG was investigated but was undetermined at 40 mg/g hence, the MBC/MFC of isolated Compound A-PEG may be above 40 mg/g as can be observed in (Table 4.13) above.

Similarly, the MIC for isolated Compound G-PEG formulation on each test organism was determined to be lower as compared to Compound G only. The results were found to be 0.08, 0.63, 1.25, 0.63, 1.25 and 0.16 mg/g for *P. aeruginosa, E. coli, K. pneumoniae, S. aureus, C. albicans* and *T. fluvus* respectively (Table 4.13) for Compound G-PEG as against 2.5, 1.25, 2.5, 0.32, 2.5 and 2.5 mg/ml for *P. aeruginosa, E. coli, K. pneumoniae, S. aureus, C. albicans* and *T. fluvus* respectively (Table 4.12) for Compound G only. This showed that, the formulated form of Compound G was more effective against the test organisms as compared to the compound only. Similar to Compound A-PEG formulation, the MBC/MFC for Compound G-PEG formulation may be above 40 mg/g as it was undetermined at 40 mg/g when treated against the selected test organisms.

4.2 Discussion

4.2.1 Isolation and identification of Stigmasterol and Aloin A

Aloe barbadensis Miller is indigenous to Ghana and has been used by the indigenous people as a source of therapy for various illnesses. There exist many similarities in the traditional medicinal uses between *Aloe barbadensis* Miller and other members of the genus, Isolation and purification of many compounds have been successfully accomplished from Aloe species. The two compounds isolated and purified have been known to occur in *Aloe barbadensis* Miller. However, isolation of these two compounds, **l-1,8-dihydroxy-3- hydroxymethyl-9- antracenone (Aloin A)** and **5,22-Cholestadien-24-ethyl-3β-ol (Stigmasterol)** although not novel compounds, their formulation with polyethylene glycol (PEG) as an ointment against wound pathogenic microorganims may have been accomplished in this work for the first time.

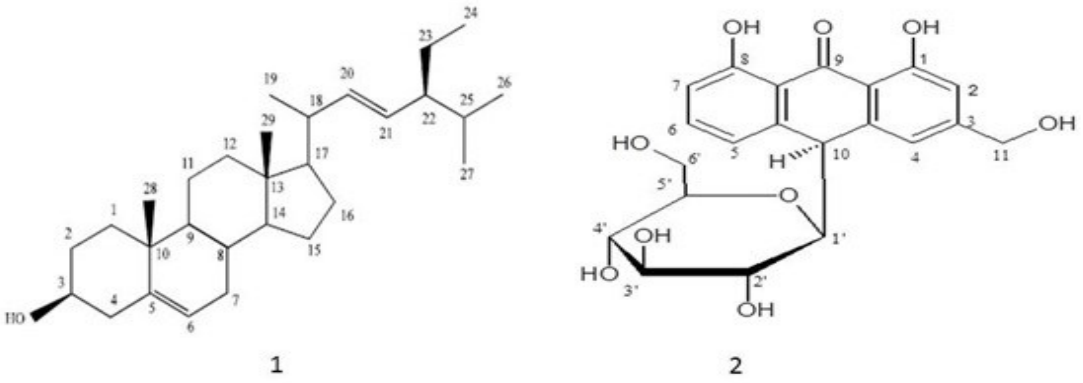


Figure 4. 17: Chemical structures of Stigmasterol (1) and Aloin A (2) isolated from *Aloe barbadensis* Miller.

Stigmasterol (1) and Aloin A (2) as shown in (Figure 4.17) were isolated from hexane/water branch extract of *Aloe barbadensis* Miller leaf through chromatographic processes using different solvent systems with yields ranging from 0.078 to 0.1% (Table 4.2).

The physical and chemical data (IR, ^{1}H and ^{13}C NMR chemical shift assignments) of **compound 1** are shown below IR (KBr, cm^{-1}): 3362.92, 2922.1, 1742.48, 1463.62, 1416.58, 1164.75, 1090.92, 720.49 cm^{-1}; ^{1}H NMR ($CDCl_3$, 400 MHz) δ7.30 (s, OH-3), 5.14 (m, 1H, H-6), 1.25 (s, 3H, H-19), 1.03 (s, 3H, H-26), 0.99 (s, 3H, H-27), 0.97 (s, 3H, H-24), 0.90 (s, 3H, H-29) and were in accordance with the spectral data published by Rajput and Rajput (2012) for stigmasterol. ^{13}C-NMR ($CDCl_3$) δ 140.9 (C-5), 121.89 (C-6), 71.9 (C-3), 57.1 (C-4), 50.40 (C-17), 42.4 (C-13), 40.7 (C-10), 32.9 (C-20), 31.9 (C-21), 29.1 (C-23), 25.6 (C-12), 21.3 (C-18), 19.8 (C-19), 12.3 (C-27) Appendix 4, 5 and 6. GC-MS data was not conclusive and the spectra are shown in the appendix 3.

Compound (2); Chemical data were in accordance with Coopoosamy and Magwa (2006) and Adushan (2008) for Aloin A. IR (KBr, cm^{-1}) 3308.35, 2922.43, 1635.12, 1573.82, 1487.37cm; ^{1}H NMR (CD3OD): δ 7.47 (1H, t, J=8.0 Hz, H-6), 7.03 (1H, s, H-4), 7.02 (1H, d, J=8.8 Hz, H-5), 6.86 (1H, s, H-2), 6.83 (1H, t, J=8.0, H-7), 4.64 (2H, d, J=3.6 Hz, 3-CH2-OH), 4.56 (1H, s, H-10), 3.38 (1H, dd, J=9.2, 2.0 Hz, H-1'), 3.01 (1H, t, J=9.2, H-2'), 3.23 (1H, t, J=8.8 -Hz, H-3'), 2.89 (1H, t, J=8.8 Hz, H-4'), 2.91 (1H, m, H-5'), 3.54 (1H, dd, J=1.6, 11.6 Hz, H-6'), 3.40 (1H, dd, J=4.0, 9.6 Hz, H-6). ^{13}C NMR (CD3OD) :δ 163.4 (C-1), 114.5 (C-2), 151.5 (C-3), 64.6 (3-CH_2-OH), 119.2 (C-4), 119.9 (C-5), 137.0 (C-6), 116.8 (C-7), 162.9 (C-8), 195.6 (C-9), 45.9 (C-10), 117.7 (C-1a), 143.3 (C-4a),

146.6 (C-5a), 118.7 (C-8a), 86.7 (C-1'), 71.9 (C-2'), 80.0 (C-3'), 72.1 (C-4'), 81.7 (C-5'), 63.3 (C-6'). Appendix 4, 5 and 6.

4.2.2 Physicochemical Screening of isolated compounds from *Aloe barbadensis* Miller

Log P is used in the pharmaceutical/biotechnology industries to understand the behaviour of drug molecules in the body. Drug candidates are often screened according to logP, among other criteria, to help guide drug selection and analog optimization. This is because lipophilicity is a major determining factor in a compound's absorption, distribution in the body, penetration across vital membranes and biological barriers, metabolism and excretion (Leo *et al*., 1971). Note;

Log P > 0 means 10:1 Organic phase: Aqueous phase

Log P = 0 means 1:1 Organic phase: Aqueous phase

Log P <0 means 1:10 Organic phase: Aqueous phase (Leo *et al.*, 1971).

The lopP values for isolated Compound G (stigmasterol) and A (aloin A) were estimated to be 0.19 and 0.73 respectively (Table 4.7). This suggests that, both compounds are lipophilic once they both have positive lopP values. However, Compound A is more lipophylic than Compound G since its logP value is more positive than that of Compound G. From Figure 4.11 and Figure 4.12, the pKa values of the isolated Compounds G and A were estimated to be 3.89 and 6.67 respectively (Table 4.7). The logP and pKa results for aloin A in this research were however at variance with values reported by Adushan (2008) : 1.86 ± 0.71 and 9.51 respectively.

4.2.3 Antimicrobial Studies of isolated compounds from *Aloe barbadensis* Miller

The antimicrobial sensitivity test of the isolated compounds on three Gram negative bacteria: *E. coli, K. pneumoniae, P. aeruginosa,* one Gram positive bacterium: *S. aurous*; one unicellular fungus: *C. albicans* and one multi-cellular fungus: *T. fluvus* which are associated with various form of diseases such as skin, wound, urinary tract infections among others were considered in this research.

4.2.3.1 Antimicrobial Studies of isolated Aloin A

Antimicrobial agent if in contact with any organism that is susceptible to it at a concentration cidal or static to the organism should make the population of the organism to reduce gradually until such a time that the medium may become sterile (Sabo, 2015). It has also been reported that plant extracts are more active against Gram positive bacteria than Gram negative bacteria (Jigna and Sumitra, 2006). Similar research also reported that, gram-negative bacteria have an outer phospholipids membrane with the structural lipopolysaccharide components, which make their cell wall impermeable to antimicrobial agents (Nikaido and Vaara, 1985). In this current research, antimicrobial activity of isolated Aloin A from the leaf of *Aloe barbadensis* Miller against six (6) selected clinical isolates (Gram positive/Gram negative bacteria, fungi) revealed that the isolated Aloin A was active against the entire microorganisms used in this research (Table 4.8 and Figure 4.13) since the compound was able to inhibit the growth of all test organisms at varying concentrations. All the test microorganisms showed high sensitivity towards the compound with maximum activity recorded against *P. aeruginosa* with zone

of inhibition of 15.9 mm at concentration 20 mg/ml and the least activity was recorded for *E. coli, K. pneumoniae* and *C. albicans* with zone of inhibition of 6.5, 7.0 and 7.0 mm respectively (Table 4.8). The observed results further revealed that the activity of the isolated Aloin A showed dose dependence against all the test microorganisms (*P. aeruginosa, K. pneumoniae, C. albicans, S. aureus, E. coli,* and *T. fluvus*). There was no significant difference in the potency of the compound against *K. pneumoniae, C. albicans, S. aureus, E. coli* and *T. fluvus* ($p > 0.05$) when the concentration of Aloin A was raised from 1.25 to 2.5 mg/ml. However, *P. aeruginosa* witnessed a significant difference ($p < 0.05$) in the potency of the compound when the concentration was raised from 1.25 to 2.5 mg/ml (Appendix 1). When the concentration of the compound was however doubled to 5 mg/ml, there was significant difference in the activity of the compound against each organism ($P < 0.05$) **(**Table 4.8**).** Similar differences in the bioactivity of the compound were recorded when the concentration of the compound was further increased to 10 and 20 mg/ml ($p < 0.05$). These results therefore confirmed the research work reported by Oluduro and Omoboye, (2010): The activity of plant crude extract against test microorganisms is greatly influenced by increased concentration of plant extracts. Further analysis of the results revealed that, there was significant effect of the isolate at a concentration of 1.25 mg/ml on *T. fluvus* ($p < 0.05$), a multi-cellular fungus compared with that of *C. albicans*. It is interesting to note that, both selected fungal strains (*C. albicans* and *T. fluvus*) were more susceptible to the isolated Aloin A than the standard drug (fluconazole) used in this research. This can be seen in (Table 4.8 and Figure 4.13). The isolate expressed significantly higher activity than the standard drug against *T. fluvus and C. albicans* ($p < 0.05$) at varying concentrations. This suggests

that, the compound could be a better alternative antifungal drug than the standard antibiotic, fluconazole. However, the standard drug (chloramphenicol) showed pronounced antimicrobial activity than Aloin A against test bacteria; *E. coli, S. aureus, K. pneumoniae*, *P. aeruginosa* ($p < 0.05$). It is worth noting that, the negative control used (DMSO) showed no activity against all test microorganisms.

4.2.3.2 Antimicrobial Studies of isolated stigmasterol

The results of isolated phytosterol (stigmasterol) from *Aloe barbadensis* Miller leaf (Table 4.9, Figure 4.14) indicated that all the test microorganisms were susceptible to the compound at varying concentrations. All the test microorganisms showed high sensitivity towards the compound with maximum zone of inhibition of 12.5 mm at a concentration of 20 mg/ml on *S. aureus* and the least activity was recorded for *T. fluvus* of inhibition zone 5.4 mm at concentration 2.5 mg/ml (Table 4.9). Similar to results obtained for Aloin A, the observed results for isolated stigmasterol further revealed that the activity of the compound showed dose dependence against all selected test organisms ($p < 0.05$). When the concentration was raised from 1.25 to 2.5 mg/ml, there was significant difference in the potency of the compound on *P. aeruginosa, K. pneumoniae, E. coli* and *T. fluvus* ($p < 0.05$) but interestingly the gram positive bacteria *S. aureus* and the unicellular fungus *C. albicans* showed no significant difference in the bioactivity of the compound within same concentrations ($p > 0.05$). However when the concentration was quadrupled from 1.25 to 5 mg/ml, there was an observed significant increase ($p < 0.05$) in the bioactivity level of the compound on all the test organisms except *S. aureus* which still recorded no significant difference in activity of the drug at this varied concentrations. It is worth

noting that even when the concentration of the compound was raised to 10 mg/ml, the compound still did not exhibit dose dependence ($p > 0.05$) on *S. aureus* until it was further raised to 20 mg/ml ($p < 0.05$). This suggested that, the isolated phytosterol activity could be dose independent on the gram positive bacterium, *S. aureus*. Generally, these results further validate research work reported by Azu and Onyeagba (2007), which indicated efficacy of most plant extract activity is dose dependent. Similar to that of the isolated Aloin A, the activity of isolated stigmasterol was more significantly pronounced than the standard drug (fluconazole) used against both fungal strains; *C. albicans and T. fluvus* ($p < 0.05$) used in this research. However for all the test bacteria (*S. aureus, P. aeruginosa, E. coli, K. pneumoniae*), the activity of the standard drug (chloramphenicol) was significantly pronounced ($p < 0.05$) than stigmasterol at the concentrations used in this research. The negative control used (DMSO) showed no activity against all test microorganisms.

4.2.3.3 Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal/Fungicidal Concentration (MBC/MFC) of isolated compounds from *Aloe barbadensis* Miller.

Agar well diffusion method was used to determine the minimum inhibitory concentration (MIC) for the isolated compounds from the plant. Low MIC values are an indication of high potency of plant extracts against the said microorganisms (Doughari and Okafor, 2007).

The MIC values of isolated Aloin A against the test microorganisms were determined to be 2.5, 5.0, 5.0, 5, 5, 1.25 mg/ml for *P. aeruginosa, E. coli K. pneumoniae, S. aureus, C. albicans* and *T. fluvus* respectively (Table 4.12). *T. fluvus and P. aeruginosa* recorded the lowest MIC values (1.25, 2.5 mg/ml respectively). This suggests that, isolated Aloin A showed substantial activity against *T. fluvus* and *P. aeruginosa* compared with the other test microorganisms. With regards to stigmasterol, the MIC values were determined to be 2.5, 1.25, 2.5, 0.32, 2.5 and 2.5 mg/ml against *P. aeruginosa, E. coli, K. pneumoniae, S. aureus,* C. *albicans* and *T. fluvus* respectively (Table 4.12). These results revealed that, *S. aureus* and *E. coli* recorded the least MIC values of 0.32 and 1.25 mg/ml respectively. This suggests that, stigmasterol showed greater activity against *S. aureus* and *E. coli* than the other test microorganisms (*P. aeruginosa, K. pneumoniae,* C. *albicans* and *T. fluvus*). Further analysis of the MIC results revealed that, stigmasterol showed stronger activity against all the test microorganisms than Aloin A based on the lower MIC values found compared with Aloin A (Table 4.12), except for *T. fluvus* where Aloin A had a lower MIC value than stigmasterol. The MBC/MFC for the isolated compounds from *Aloe barbadensis* Miller were determined and it is worth noting that, at concentration 40 mg/ml the MBC and MFC for both compounds were undetected within the concentrations used in this research.

4.2.4 Antimicrobial Studies of Formulated compounds from *Aloe barbadensis* Miller

Antimicrobial sensitivity test of formulated compound–PEG Ointment was conducted on the selected microorganisms using Agar well diffusion method.

4.2.4.1 Antimicrobial Activity Studies of formulated Aloin A-PEG Ointment

The antimicrobial effect of formulated Aloin A-PEG ointment on the selected microorganisms showed substantial activity against all the microorganisms with average zone of inhibition greater than 10 mm at 20 mg/g. The activity of Aloin A - PEG ointment was recorded at various concentrations (Table 4.10, Figure 4.15). The formulated ointment exhibited dose dependence against all test microorganisms. When the concentration of Aloin A - PEG ointment was raised from 1.25 to 2.5 mg/g, there was no significant difference in diameter zone of inhibition against all test microorganisms ($p > 0.05$) except *P. aeruginosa* which exhibited significant difference($p < 0.05$). However, when the concentration of the formulated ointment was doubled (5 mg/g), there was high significant difference ($p < 0.05$) in diameter zone of inhibition of all test microorganisms. Similar trends were recorded as the concentration of the ointment was raised to 10 mg/g and 20 mg/g ($p < 0.05$). Similar to the activity of Aloin A alone, its formulated ointment recorded significantly higher activity against both fungi (*C. albicans, T. fluvus*) than fluconazole which is the standard drug used in this research. However, chloramphenicol (positive control for bacteria) expressed very high activity against all test bacteria (*E. coli, K. pneumoniae, P. aeruginosa, S. aureus*) than the formulated ointment. This suggests that, PEG is a good ointment base which could easily release the bioactive compound in contact with the microorganism for the enhanced activity.

4.2.4.2 Antimicrobial Activity Studies of formulated stigmasterol-PEG Ointment

Evaluation of formulated stigmasterol-PEG Ointment for its antimicrobial activity against the test microorganisms revealed that the ointment exhibited activity against all the test microorganisms at variable concentrations (Table 4.11, Figure 4.16). The bioactivity recorded at 20 mg/g showed that the ointment was highly active against the entire microorganisms with average inhibition zone of 12 mm. This suggests that the ointment base could release the active component (stigmasterol) for smooth contact with the microorganisms. The results further revealed that, the ointment expressed dose dependent activity against *C. albicans, K. pneumoniae, P. aeruginosa* ($p < 0.05$) but recorded no significant difference in activity against the fungi (*T. fluvus, C. albicans*) and the only gram positive bacterium, *S. aureus* ($p > 0.05$) when the concentration was raised from 1.25 to 2.5 mg/g. However, when the concentration was further raised to 5 mg/g there was a significant difference in diameter zone of inhibition of the entire microorganisms ($p < 0.05$). Similar trends were recorded as the concentration of the ointment was increased to 10 mg/g and 20 mg/g ($p < 0.05$) indicating dose dependency of the ointment. Interestingly, the formulated ointment of stigmasterol exhibited significantly higher activity against all test microorganisms at the various concentrations than its corresponding unformulated compound. The activity expressed by the formulated ointment could be attributed to enhanced exposure and easy contact of the active compound to the test microorganisms by the delivery agent, PEG. The choice of ointment base is crucial as it influences the release and bioactivity of drugs.

4.2.4.3 Minimum Inhibitory Concentrations (MIC) and Minimum Bactericidal/Fungicidal Concentrations (MBC/MFC) of formulated compound-PEG ointment.

The activity of Aloin A- PEG-ointment against the test microorganisms was stronger and more effective than the pure Aloin A alone. This was observed from the low MIC values recorded; 2.5, 0.63, 0.63, 0.32, 0.32 and 1.25 mg/g for *P. aeruginosa, E. coli, K. pneumoniae, S. aureus, C. albicans* and *T. fluvus* respectively (Table 4.13) for Aloin A-PEG against 2.5, 5, 5, 5, 5 and 1.25 mg/ml for *P. aeruginosa, E. coli, K. pneumoniae, S. aureus, C. albicans* and *T. fluvus* respectively (Table 4.12) for Aloin A only. This could be attributed to the high ability of the delivery agent, PEG to release the bioactive compound to come into close contact with the microorganisms. Similar to the activity of Aloin A-PEG ointment, the activity of stigmasterol-PEG ointment on the test microorganisms resulted in very low MIC values as compared to stigmasterol only. The MIC values obtained were as follows: 0.08, 0.63, 1.25, 0.63, 1.25, and 0.16 mg/g for *P. aeruginosa, E. coli, K. pneumoniae, S. aureus, C. albicans* and *T. fluvus* respectively (Table 4.13) for stigmasterol-PEG against 2.5, 1.25, 2.5, 0.32, 2.5 and 2.5mg/ml for *P. aeruginosa, E. coli, K. pneumoniae, S. aureus, C. albicans* and *T. fluvus* respectively (Table 4.12) for stigmasterol only. Both formulated ointments exhibited a broad spectrum effect as they were active against gram positive and gram negative bacteria as well as both unicellular and multicellular fungi employed in this research. Although the formulated ointment of both compounds recorded very low MIC values for all test microorganisms, both ointments could not completely eliminate the microorganisms at the concentration used this experiments.

CHAPTER FIVE

5.0 SUMMARY OF FINDINGS, CONCLUSION AND RECOMMENDATIONS

5.1 Summary of findings

The salient findings from the thesis are as follows:

1. The mass of crude extract from the *Aloe barbadensis* Miller plant using ethanol was 155.61g, representing 10.37% of the total mass of the powdered leaf sample (1500 g).
2. Aloin A and Stigmasterol were characterized from the isolation process on basis of ^{1}H-NMR, ^{13}C-NMR, and IR with a percentage yields of 0.1 and 0.0785 % respectively.
3. Both isolated compounds were odourless. Aloin A was found to be yellow-brown in colour whilst that of Stigmasterol was found to be green by physical observation. Both compound were solid in nature with Aloin A being crystalline.
4. Aloin A was found to be acidic (pH 6.2) whilst Stigmasterol was found to be basic (pH 8.1).
5. The *logP* values for isolated Aloin A and stigmasterol were estimated to be 0.73 and 0.19 respectively. This suggested that, both compounds are lipophilic once they both have positive *logP* values. In addition, the pKa values of the isolated Aloin A and stigmasterol were estimated to be 6.67 and 3.89 respectively.
6. From the antimicrobial activity tests, it was found that, all the test microorganisms (*Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae, pseudomonas aeruginosa, Candida albicans and Talaromyces fluvus* were suppressed by both

Aloin A and Stigmasterol and their corresponding formulated ointments at varying concentrations (1.25, 2.5, 5, 10, 20) mg/ml.

5.2 Conclusion

The two compounds isolated from the leaf of *Aloe barbadensis* Miller are Stigmasterol and Aloin A and have been characterized using FTIR, $^{1}H/^{13}C$ NMR, log P and pKa. The two compounds though not novel, but their formulation with polyethylene glycol (PEG) as an ointment against multi-resistant wound infectious microorganisms may have been accomplished in this research for the first time. In this research, the formulated ointments showed higher activity with lower MIC values than their corresponding pure isolated compounds. The findings justify the use of *Aloe barbadensis* Miller in traditional medicine against several illnesses. These formulated ointment products are highly promising agents against skin diseases and wound infecting pathogens.

5.3 Recommendations

Toxicity studies on the characterized compounds are recommended, so as to establish their levels of safety. Mechanisms of action of the two compounds are also recommended in order to establish the biochemical interactions through which the compounds produce their pharmacological effects. The plant isolate-PEG should be explored further in vivo to determine the effectiveness of these ointments for possible potential use for treating infectious diseases. It is recommended that, pharmaceutical companies should venture into Aloin A and Stigmasterol in their anti-infective drug formulations against multi-drug resistant microorganism

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APPENDICES

Appendix 1: Statistical Analysis of Aloin A on test organism

ANOVA Table for Aloin A and Aloin A-PEG (mg/ml and mg/g)

	DF	Sum of Squares(SS)	Mean Squares(MS)	F-value	P-Value
Treatment (between columns)	71	10005	140.9	618.8	0.0001
Residual (within columns)	72	16.4	0.2277	618.8	0.0001

Means tables for Aloin A (mg/ml) on test organisms at varying concentrations

Means table for Aloin A (mg/ml) at varying concentrations on *P. aeruginosa* (PA)

Effect: Treatment

Concentration	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
20mg/ml	2	15.85	0.2121	0.15	13.94	17.76
10mg/ml	2	10.65	0.495	0.35	6.203	15.1
5mg/ml	2	9.85	0.2121	0.15	7.944	11.76
2.5mg/ml	2	9.15	0.2121	0.15	7.244	11.06
1.25mg/ml	2	0	0	0	0	0

Means table for Aloin A (mg/ml) at varying concentrations on *E. coli* (EC)

Effect: treatment

Concentration	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
20mg/ml	2	14	1.414	1	1.294	26.71
10mg/ml	2	8	0	0	8	8
5mg/ml	2	6.5	0.7071	0.5	0.1469	12.85
2.5mg/ml	2	0	0	0	0	0
1.25mg/ml	2	0	0	0	0	0

Means table for Aloin A (mg/ml) at varying concentrations on *K. pneumoniae* (KP)

Effect: treatment

Concentration	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
20mg/ml	2	13.5	0.7071	0.5	7.147	19.85
10mg/ml	2	9.5	0.7071	0.5	3.147	15.85
5mg/ml	2	7	0	0	7	7
2.5mg/ml	2	0	0	0	0	0
1.25mg/ml	2	0	0	0	0	0

Means table for Aloin A (mg/ml) at varying concentrations on *S. aurous* (SA)

Effect: treatment

Concentration	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
20mg/ml	2	13.5	0.7071	0.5	7.147	19.85
10mg/ml	2	9.5	0.7071	0.5	3.147	15.85
5mg/ml	2	8.3	0.4243	0.3	4.488	12.11
2.5mg/ml	2	0	0	0	0	0
1.25mg/ml	2	0	0	0	0	0

Means table for Aloin A (mg/ml) at varying concentrations on *C. albicans* (CA)

Effect: treatment

Concentration	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
20mg/ml	2	12.5	0.2828	0.2	9.959	15.04
10mg/ml	2	8.5	0.7071	0.5	2.147	14.85
5mg/ml	2	7	0	0	7	7
2.5mg/ml	2	0	0	0	0	0
1.25mg/ml	2	0	0	0	0	0

Means table for Aloin A (mg/ml) at varying concentrations on *T. Fluvus* (TV)

Effect: treatment

Concentration	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
20mg/ml	2	12.3	0	0	12.3	12.3
10mg/ml	2	11	0	0	11	11
5mg/ml	2	10.3	0.4243	0.3	6.488	14.11
2.5mg/ml	2	9	0	0	9	9
1.25mg/ml	2	7.15	0.2121	0.15	5.244	9.056

Means tables for Aloin A-PEG Ointment (mg/g) at varying concentrations

On tests organisms.

Means table for Aloin A-PEG ointment (mg/g) at varying concentrations on *P. aeruginosa* (PA)

Effect: treatment

Concentration	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
20mg/g	2	12.15	0.2121	0.15	10.24	4.147
10mg/g	2	10.5	0.7071	0.5	4.147	16.85
5mg/g	2	9	0	0	9	9
2.5mg/g	2	8.5	0.7071	0.5	2.147	14.85
1.25mg/g	2	0	0	0	0	0

Means table for Aloin A-PEG ointment (mg/g) at varying concentrations on *C. coli* (EC)

Effect: treatment

Concentration	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
20mg/g	2	15.8	0.2828	0.2	13.26	18.34
10mg/g	2	14.8	0.7071	0.5	8.447	21.15
5mg/ml	2	14	0.4243	0.3	10.19	17.81
2.5mg/g	2	12.45	0.3536	0.25	9.273	15.63
1.25mg/g	2	9.6	0.1414	0.1	8.329	10.87

Means table for Aloin A-PEG ointment (mg/g) at varying concentrations on *K. pneumoniae* (KP)

Effect: treatment

Concentration	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
20mg/g	2	13.3	0.2828	0.2	10.76	15.84
10mg/g	2	11.85	0.2121	0.15	9.944	13.76
5mg/g	2	10.65	0.9192	0.65	2.391	18.91
2.5mg/g	2	9.15	0.2121	0.15	7.244	11.06
1.25mg/g	2	7.75	0.3536	0.25	4.573	10.93

Means table for Aloin A-PEG ointment (mg/g) at varying concentrations on *S. aurous* (SA)

Effect: treatment

Concentration	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
20mg/g	2	14.1	0.2828	0.2	11.56	16.64
10mg/g	2	12.65	0.495	0.35	8.203	17.1
5mg/g	2	10.8	0.7071	0.5	4.447	17.15
2.5mg/g	2	10.15	0.6364	0.45	4.432	15.87
1.25mg/g	2	8.85	0.2121	0.15	6.944	10.76

Means table for Aloin A-PEG ointment (mg/g) at varying concentrations on *C. albicans* (CA)

Effect: treatment

Concentration	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
20mg/g	2	14.4	0.4243	0.3	10.59	18.21
10mg/g	2	13.5	0.7071	0.5	7.147	19.85
5mg/g	2	10.85	0.2121	0.15	8.944	12.76
2.5mg/g	2	8.7	0.4243	0.3	4.888	12.51
1.25mg/g	2	7	0	0	7	7

Means table for Aloin A-PEG ointment (mg/g) at varying concentrations on *T. fluvus* (TV)

Effect: treatment

Concentration	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
20mg/g	2	10.5	0.2828	0.2	7.959	13.04
10mg/g	2	9.45	0.2121	0.15	7.544	11.36
5mg/g	2	8.3	0	0	8.3	8.34
2.5mg/g	2	7.23	0.3536	0.25	4.073	10.43
1.25mg/g	2	6.05	0.7071	0.05	5.415	6.685

Fishers PLSD for Aloin A (mg/ml, mg /g)

Effect: Treatment

Significant level: 5%

95% Confidence level difference

ALOIN A VS ALOIN A-PEG OINTMENT ON TEST ORGANISMS

Aloin A vs Aloin A-PEG ointment on *P. aeruginosa* (PA)

	Mean Difference	Critical Difference	P < 0.05	95% Confidence level	
				Lower	Higher
A (20mg/ml), A-PEG (20mg/g)	3.7	10.97	s	1.611	5.789
A (10mg/ml), A-PEG (10mg/g)	0.15	0.4445	ns	-1.939	2.239
A (5mg/ml), A-PEG (5mg/g)	0.85	2.519	ns	-1.239	2.939
A (2.5mg/ml), A-PEG (2.5mg/g)	0.65	1.926	ns	-1.439	2.739
A (1.25mg/ml), A-PEG (1.25mg/g)	0	0	ns	-2.089	2.089

Aloin A vs Aloin A-PEG ointment on *E. coli* (EC)

	Mean Difference	Critical Difference	P < 0.05	95% Confidence level	
				Lower	Higher
A(20mg/ml), A -PEG (20mg/g)	-1.8	5.335	ns	-3.889	0.2890
A (10mg/ml), A -PEG (10mg/g)	-6.8	20.15	s	-8.889	-4.711
A (5mg/ml), A -PEG (5mg/g)	-7.5	22.23	s	-9.589	-5.411
A (2.5mg/ml), A -PEG (2.5mg/g)	-12.45	36.9	s	-14.54	-10.36
A (1.25mg/ml), A -PEG (1.25mg/g)	-9.6	28.45	s	-11.69	-7.511

Aloin A vs Aloin A-PEG ointment on *S. aurous* (SA)

	Mean Difference	Critical Difference	P < 0.05	95% Confidence level	
				Lower	Higher
A (20mg/ml), A-PEG (20mg/g)	-0.6	1.778	ns	-2.689	1.489
A (10mg/ml), A-PEG (10mg/g)	-3.15	9.335	s	-5.239	- 1.061
A (5mg/ml), A-PEG (5mg/g)	-2.5	7.409	s	-4.589	- 0.4110
A (2.5mg/ml), A-PEG (2.5mg/g)	-10.15	30.08	s	-12.24	- 8.061
A (1.25mg/ml), A-PEG (1.25mg/g)	-8.85	26.23	s	-10.94	- 6.761

Aloin A vs Aloin A-PEG ointment on *K. Pneumoniae* (KP)

	Mean Difference	Critical Difference	P < 0.05	95% Confidence level	
				Lower	Higher
A (20mg/ml), A -PEG (20mg/g)	0.2	0.5927	ns	-1.889	2.289
A (10mg/ml), A -PEG (10mg/g)	-2.35	6.965	s	-4.439	-0.2611
A (5mg/ml), A -PEG (5mg/g)	-3.65	10.82	s	-5.739	-1.561
A (2.5mg/ml), A -PEG (2.5mg/g)	-9.15	27.12	s	-11.24	-7.061
A (1.25mg/ml), A -PEG (1.25mg/g)	-7.75	22.97	s	-9.839	-5.661

Aloin A vs Aloin A-PEG ointment on *C. albicans* (CA)

	Mean Difference	Critical Difference	P < 0.05	95% Confidence level	
				Lower	Higher
A (20mg/ml), A-PEG (20mg/g)	-1.9	5.631	ns	-3.989	0.1890
A (10mg/ml), A-PEG (10mg/g)	-5.5	14.82	s	-7.089	- 2.911
A (5mg/ml), A-PEG (5mg/g)	-3.85	11.41	s	-5.939	- 1.761
A (2.5mg/ml), A-PEG (2.5mg/g)	-8.7	25.78	s	-10.79	- 6.611
A (1.25mg/ml), A-PEG (1.25mg/g)	-7	20.75	s	-9.089	- 4.911

Aloin A vs Aloin A-PEG ointment on *T. fluvus* (TV)

	Mean Difference	Critical Difference	P < 0.05	95% Confidence level	
				Lower	Higher
A (20mg/ml), A -PEG (20mg/g)	1.8	5.335	ns	-0.289	3.889
A (10mg/ml), A -PEG (10mg/g)	1.55	4.594	ns	-0.539	3.639
A (5mg/ml), A -PEG (5mg/g)	2	5.927	ns	-0.08897	4.089
A (2.5mg/ml), A -PEG (2.5mg/g)	1.75	5.186	ns	-0.339	3.839
A (1.25mg/ml), A -PEG (1.25mg/g)	1.1	3.26	ns	-0.989	3.189

Appendix 2: Statistical Analysis of Stigmasterol (Compound G) on test organisms

ANOVA Table for Stigmasterol (mg/ml and mg/g)

	DF	Sum of Squares (SS)	Mean Squares (MS)	F-value	P-Value
Treatment (between columns)	71	8293	116.8	463.4	0.0001
Residual (within columns)	72	18.15	0.2521	463.4	0.0001

Means tables for Stigmasterol (mg/ml) on test organisms at varying concentrations

Means table for Stigmasterol (Compound G) (mg/ml) on *P. aeruginosa* (PA) at varying concentrations

Effect: Treatment

Concentration	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
G (20mg/ml)	2	9.5	0.2828	0.2	6.959	12.04
G (10mg/ml)	2	8.3	0	0	8.3	8.3
G (5mg/ml)	2	7.5	0.7071	0.5	1.147	13.85
G (2.5mg/ml)	2	6.85	0.2121	0.15	4.944	8.756
G (1.25mg/ml)	2	0	0	0	0	0

Means table for Stigmasterol (Compound G) (mg/ml) on *E. coli* (EC) at varying concentrations

Effect: Treatment

Concentration	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
G (20mg/ml)	2	11.5	0.7071	0.5	5.147	17.85
G (10mg/ml)	2	9.85	0.2121	0.15	7.944	11.76
G (5mg/ml)	2	9.15	0.2121	0.15	7.244	11.06
G (2.5mg/ml)	2	8.8	0.2828	0.2	6.259	11.34
G (1.25mg/ml)	2	5.95	0.4950	0.35	1.503	10.4

Means table for Stigmasterol (Compound G) (mg/ml) on *K. pneumoniae* (KP) at varying concentrations

Effect: Treatment

Concentration	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
G (20mg/ml)	2	12.5	0.9071	0.5	6.147	18.85
G (10mg/ml)	2	10.9	0.1414	0.1	9.629	12.17
G (5mg/ml)	2	8	0	0	8	8
G (2.5mg/ml)	2	6.95	0.2121	0.15	5.044	8.856
G (1.25mg/ml)	2	0	0	0	0	0

Means table for Stigmasterol (Compound G) (mg/ml) on *S. aurous* (SA) at varying concentrations

Effect: Treatment

Concentration	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
G (20mg/ml)	2	12	0.9899	0.7	3.106	20.89
G (10mg/ml)	2	8.8	0.7071	0.5	2.447	15.15
G (5mg/ml)	2	8.55	0.6364	0.45	2.832	14.27
G (2.5mg/ml)	2	8.25	0.6364	0.45	2.532	13.97
G (1.25mg/ml)	2	7.65	0.4950	0.35	3.203	12.1

Means table for Stigmasterol (Compound G) (mg/ml) on *C. albicans* (CA) at varying concentrations

Effect: Treatment

Concentration	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
G (20mg/ml)	2	11.85	0.2121	0.15	9.944	13.76
G (10mg/ml)	2	10.65	0.4950	0.35	6.603	15.1
G (5mg/ml)	2	9.95	0.4950	0.35	5.503	14.4
G (2.5mg/ml)	2	0	0	0	0	0
G (1.25mg/ml)	2	0	0	0	0	0

Means table for Stigmasterol (Compound G) (mg/ml) on *T. fluvus* (TV) at varying concentrations

Effect: Treatment

Concentration	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
G (20mg/ml)	2	12.3	0.4243	0.3	8.488	16.11
G (10mg/ml)	2	10.6	0	0	10.6	10.6
G (5mg/ml)	2	9.5	0.7071	0.5	3.147	15.85
G (2.5mg/ml)	2	5.4	0.5657	0.4	0.3175	10.48
G (1.25mg/ml)	2	0	0	0	0	0

Means tables for formulated Stigmasterol (Compound G –PEG) Ointment (mg/g) at varying concentrations on test organisms

Means tables for formulated Stigmasterol (Compound G –PEG) Ointment (mg/g) at varying concentrations on *P. aeruginosa* (PA)

Effect: Treatment

Concentration	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
G-PEG(20mg/ml)	2	12.55	0.6364	0.45	6.832	18.27
G-PEG(10mg/ml)	2	11.8	0.7071	0.5	5.447	18.15
G-PEG(5mg/ml)	2	10.8	0.7071	0.5	4.447	17.15
G-PEG(2.5mg/ml)	2	10.5	0.7071	0.5	4.147	16.85
G-PEG(1.25mg/ml)	2	9.5	1.626	1.15	-6.462	22.76

Means tables for formulated Stigmasterol (Compound G –PEG) Ointment (mg/g) at varying concentrations on *E. coli* (CA)

Effect: Treatment

Concentration	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
G-PEG(20mg/ml)	2	14.5	0.7071	0.5	8.147	20.85
G-PEG(10mg/ml)	2	4.45	0.3536	0.25	9.273	15.63
G-PEG(5mg/ml)	2	10.8	0.2828	0.2	8.259	13.34
G-PEG(2.5mg/ml)	2	9.5	0.7071	0.5	3.147	15.85
G-PEG(1.25mg/ml)	2	8.05	0.3536	0.25	4.373	11.23

Means tables for formulated Stigmasterol (Compound G –PEG) Ointment (mg/g) at varying concentrations on *K. pneumonia* (KP)

Effect: Treatment

Concentrations	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
G-PEG(20mg/ml)	2	13.15	0.2121	0.15	11.24	15.06
G-PEG(10mg/ml)	2	11.85	0.2121	0.15	9.944	13.76
G-PEG(5mg/ml)	2	11.55	0.2121	0.15	9.644	13.46
G-PEG(2.5mg/ml)	2	10.5	0.2828	0.2	7.959	13.04
G-PEG(1.25mg/ml)	2	7.45	0.6363	0.45	1.732	13.17

Means tables for formulated Stigmasterol (Compound G –PEG) Ointment (mg/g) at varying concentrations on *S. aurous* (SA)

Effect: Treatment

Concentration	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
G-PEG(20mg/ml)	2	11.1	0.2828	0.2	8.559	13.64
G-PEG(10mg/ml)	2	9.65	0.4850	0.35	5.203	14.1
G-PEG(5mg/ml)	2	9.35	0.4950	0.35	4.903	13.8
G-PEG(2.5mg/ml)	2	8.85	0.2121	0.15	6.944	10.76
G-PEG(1.25mg/ml)	2	8.5	0.2828	0.2	5.959	11.04

Means tables for formulated Stigmasterol (Compound G –PEG) Ointment (mg/g) at varying concentrations on *C. albicans* (CA)

Effect: Treatment

Concentrations	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
G-PEG(20mg/ml)	2	12.1	0.1414	0.1	10.83	13.37
G-PEG(10mg/ml)	2	11	0.2828	0.2	8.459	13.54
G-PEG(5mg/ml)	2	9.15	0.2121	0.15	7.244	11.06
G-PEG(2.5mg/ml)	2	8.75	0.3536	0.25	5.573	11.93
G-PEG(1.25mg/ml)	2	0	0	0	0	0

Means tables for formulated Stigmasterol (Compound G –PEG) Ointment (mg/g) at varying concentrations on *T. fluvus* (TV)

Effect: Treatment

Concentrations	Count	Mean	Standard Deviation	Standard Deviation Error	95% Confidence level	
G-PEG(20mg/ml)	2	13.3	0.4243	0.3	9.488	17.11
G-PEG(10mg/ml)	2	11.25	0.2121	0.15	9.344	13.16
G-PEG(5mg/ml)	2	10.1	0.7072	0.5	3.747	16.45
G-PEG(2.5mg/ml)	2	8.25	0.3536	0.25	5.073	11.43
G-PEG(1.25mg/ml)	2	7.5	0.7071	0.5	1.147	13.85

Fishers PLSD for Stigmasterol (Compound G) (mg/ml, mg/g)

Effect Treatment

Significant level: 5%

95% Confidence level difference

STIGMASTEROL (G) VS FORMULATED STIGMASTEROL (G –PEG) OINTMENT ON TEST ORGANISMS

Stigmasterol (G) vs formulated Stigmasterol (G-PEG) ointment on *P. aeruginosa* (PA)

	Mean Difference	Critical Difference	P < 0.05	95% Confidence level	
				Lower	Higher
G (20mg/ml), G-PEG (20mg/g)	-3.05	8.591	s	-5.248	-0.8521
G (10mg/ml), G-PEG (10mg/g)	-3.5	9.859	s	-5.698	-1.302
G (5mg/ml), G-PEG (5mg/g)	*	*	*	*	*
G (2.5mg/ml), G-PEG (2.5mg/g)	-3.65	10.28	s	-5.848	-1.452
G (1.25mg/ml), G-PEG (1.25mg/g)	-8.15	22.96	s	-10.35	-5.952

Stigmasterol (G) vs formulated Stigmasterol (G-PEG) ointment on *S. aurous* (SA)

	Mean Difference	Critical Difference	P < 0.05	95% Confidence level	
				Lower	Higher
G (20mg/ml), G-PEG (20mg/g)	0.9	2.535	ns	-1.298	3.098
G (10mg/ml), G-PEG (10mg/g)	-0.85	2.394	ns	-3.048	1.348
G (5mg/ml), G-PEG (5mg/g)	-0.3	0.845	ns	-2.498	1.989
G (2.5mg/ml), G-PEG (2.5mg/g)	-0.6	1.69	ns	-2.798	1.598
G (1.25mg/ml), G-PEG (1.25mg/g)	-0.85	2.394	ns	-3.048	1.348

Stigmasterol (G) vs formulated Stigmasterol (G-PEG) ointment on *E. coli* (EC)

	Mean Difference	Critical Difference	P < 0.05	95% Confidence level	
				Lower	Higher
G (20mg/ml), G-PEG (20mg/g)	-3	8.45	s	-5.198	-0.8021
G (10mg/ml), G-PEG (10mg/g)	-2.6	7.323	s	-4.798	-0.4021
G (5mg/ml), G-PEG (5mg/g)	-1.65	4.648	ns	-3.848	0.5479
G (2.5mg/ml), G-PEG (2.5mg/g)	-0.7	1.972	ns	-2.898	1.498
G (1.25mg/ml), G-PEG (1.25mg/g)	-2.1	5.915	ns	-4.298	0.09794

Stigmasterol (G) vs formulated Stigmasterol (G-PEG) ointment on *K. pneumoniae* (KP)

	Mean Difference	Critical Difference	P < 0.05	95% Confidence level	
				Lower	Higher
G (20mg/ml), G-PEG (20mg/g)	-0.65	1.831	ns	-2.848	1.548
G (10mg/ml), G-PEG (10mg/g)	-0.95	2.676	ns	-3.148	1.248
G (5mg/ml), G-PEG (5mg/g)	-3.55	9.999	s	-5.748	-1.352
G (2.5mg/ml), G-PEG (2.5mg/g)	-3.55	9.999	s	-5.748	-1.352
G (1.25mg/ml), G-PEG (1.25mg/g)	-7.45	20.98	s	-9.648	-5.252

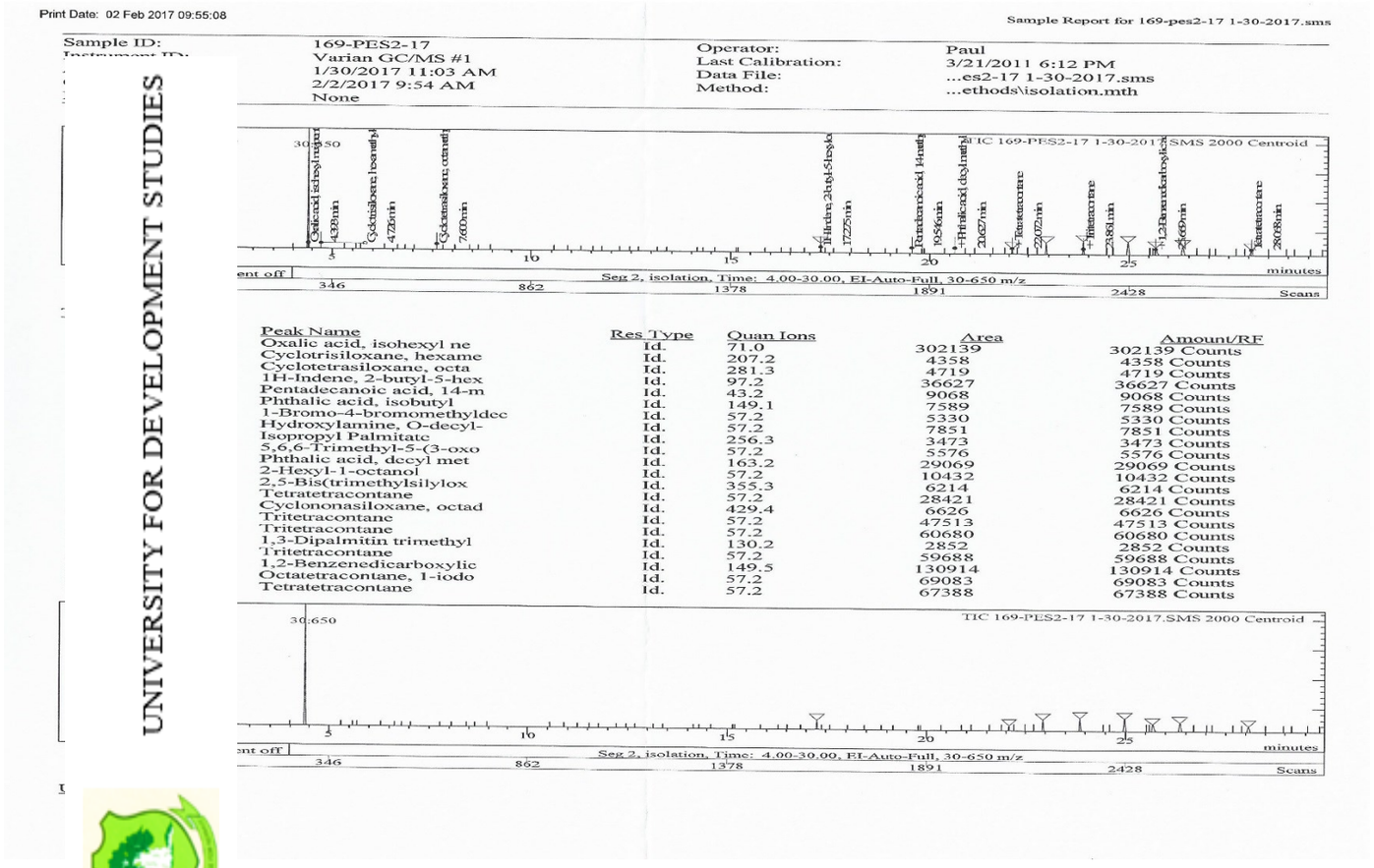
Stigmasterol (G) vs formulated Stigmasterol (G-PEG) ointment on *C. albicans* (CA)

	Mean Difference	Critical Difference	P < 0.05	95% Confidence level	
				Lower	Higher
G (20mg/ml), G-PEG (20mg/g)	-0.25	0.7042	ns	-2.448	1.948
G (10mg/ml), G-PEG (10mg/g)	-0.35	0.9859	ns	-2.548	1.848
G (5mg/ml), G-PEG (5mg/g)	0.8	2.253	ns	-1.398	2.998
G (2.5mg/ml), G-PEG (2.5mg/g)	-8.75	24.65	s	-10.95	-6.552
G (1.25mg/ml), G-PEG (1.25mg/g)	0	0	ns	-2.1198	2.198

Stigmasterol (G) vs formulated Stigmasterol (G-PEG) ointment on *T. fluvus* (TV)

	Mean Difference	Critical Difference	P < 0.05	95% Confidence level	
				Lower	Higher
G (20mg/ml), G-PEG (20mg/g)	-1	2.817	ns	-3.198	1.198
G (10mg/ml),G-PEG (10mg/g)	-0.65	1.831	ns	-2.848	1.548
G (5mg/ml), G-PEG (5mg/g)	-0.6	1.69	ns	-2.798	1.598
G (2.5mg/ml), G-PEG (2.5mg/g)	-2.85	8.028	s	-5.048	-0.6521
G (1.25mg/ml), G-PEG (1.25mg/g)	-7.5	21.13	s	-9.698	-5.302

Appendix 3: Gas Chromatography- Mass Spectrometry (GC-MS) spectra of isolated compounds (Aloin A, Stigmasterol)



Print Date: 02 Feb 2017 09:55:08

Sample Report for 169-pes2-17 1-30-2017.sms

Sample ID:	169-PES2-17	Operator:	Paul
	Varian GC/MS #1	Last Calibration:	3/21/2011 6:12 PM
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	2/2/2017 9:54 AM	Method:	...ethods\isolation.mth
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TIC 169-PES2-17 1-30-2017.SMS 2000 Centroid

Seg 2, isolation, Time: 4.00-30.00, EI-Auto-Full, 30-650 m/z

Peak Name	Res Type	Quan Ions	Area	Amount/RF
Oxalic acid, isohexyl ne	Id.	71.0	302139	302139 Counts
Cyclotrisiloxane, hexame	Id.	207.2	4358	4358 Counts
Cyclotetrasiloxane, octa	Id.	281.3	4719	4719 Counts
1H-Indene, 2-butyl-5-hex	Id.	97.2	36627	36627 Counts
Pentadecanoic acid, 14-m	Id.	43.2	9068	9068 Counts
Phthalic acid, isobutyl	Id.	149.1	7589	7589 Counts
1-Bromo-4-bromomethyldec	Id.	57.2	5330	5330 Counts
Hydroxylamine, O-decyl-	Id.	57.2	7851	7851 Counts
Isopropyl Palmitatc	Id.	256.3	3473	3473 Counts
5,6,6-Trimethyl-5-(3-oxo	Id.	57.2	5576	5576 Counts
Phthalic acid, decyl met	Id.	163.2	29069	29069 Counts
2-Hexyl-1-octanol	Id.	57.2	10432	10432 Counts
2,5-Bis(trimethylsilylox	Id.	355.3	6214	6214 Counts
Tetratetracontane	Id.	57.2	28421	28421 Counts
Cyclononasiloxane, octad	Id.	429.4	6626	6626 Counts
Tritetracontane	Id.	57.2	47513	47513 Counts
Tritetracontane	Id.	57.2	60680	60680 Counts
1,3-Dipalmitin trimethyl	Id.	130.2	2852	2852 Counts
Tritetracontane	Id.	57.2	59688	59688 Counts
1,2-Benzenedicarboxylic	Id.	149.5	130914	130914 Counts
Octatetracontane, 1-iodo	Id.	57.2	69083	69083 Counts
Tetratetracontane	Id.	57.2	67388	67388 Counts

TIC 169-PES2-17 1-30-2017.SMS 2000 Centroid

Seg 2, isolation, Time: 4.00-30.00, EI-Auto-Full, 30-650 m/z

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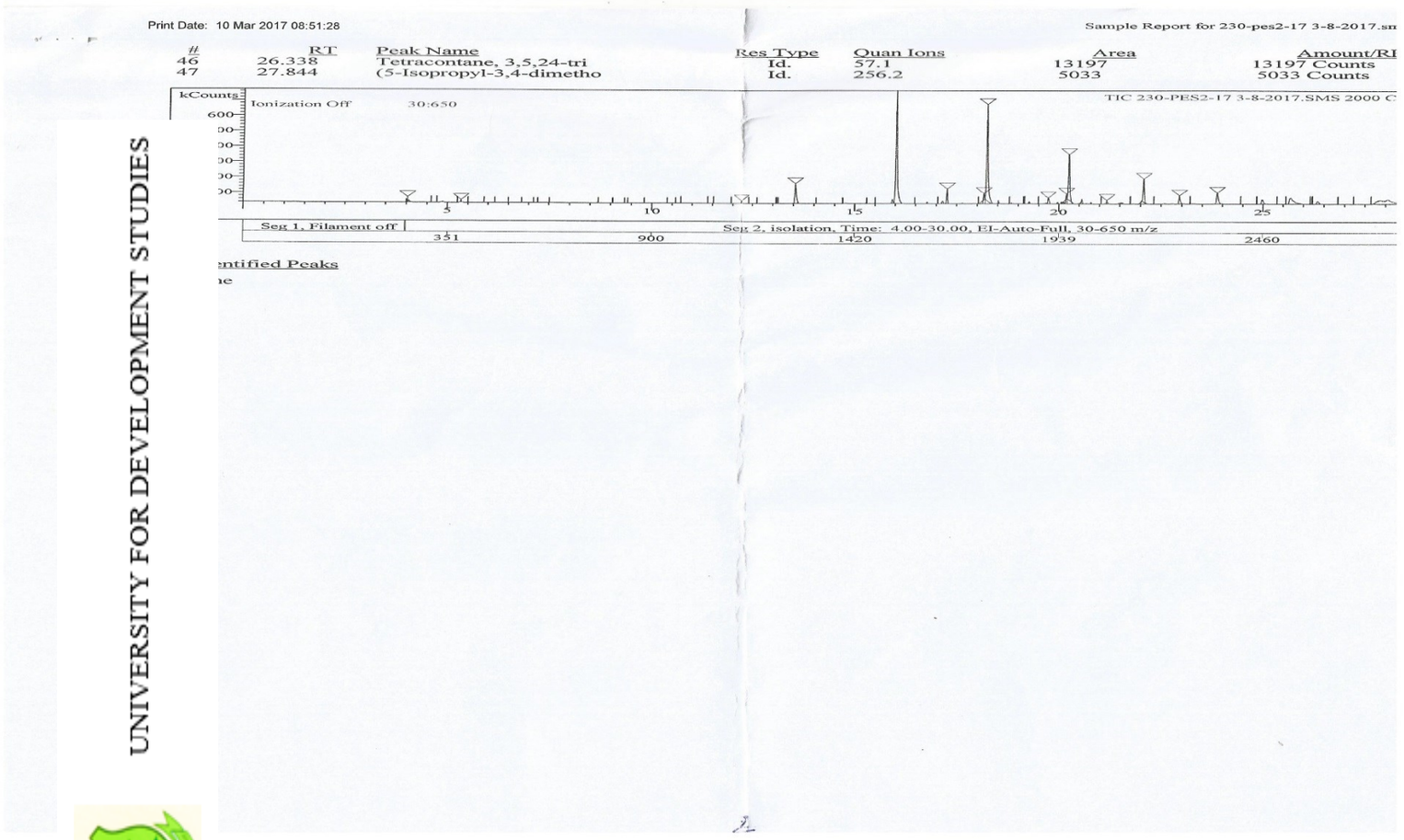
GC-M r Stigmasterol



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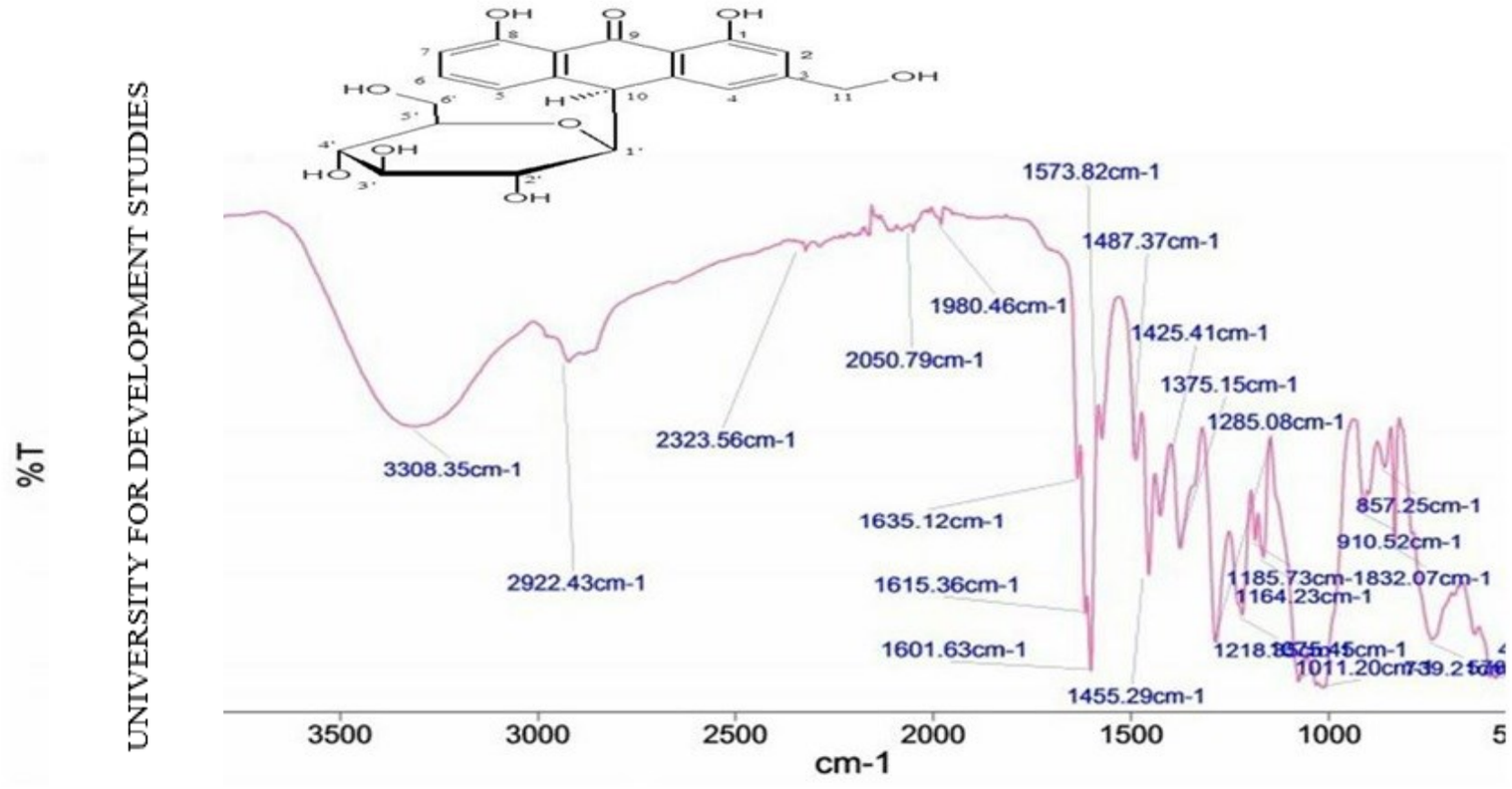
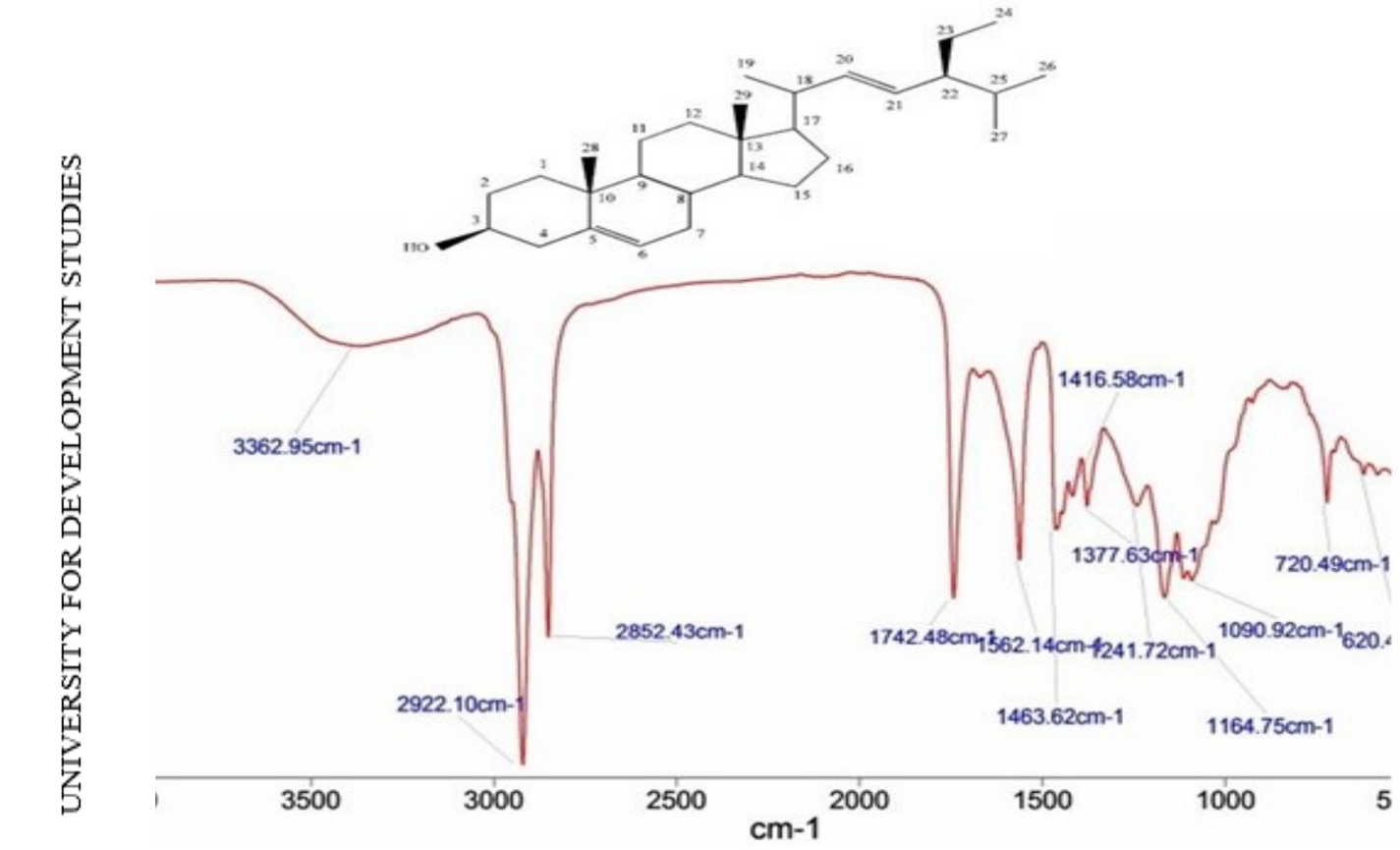
GC-M r Aloin A

Appendix 4: Fourier Transform Infrared (FT-IR) Spectra of isolated compounds (Aloin A, Stigmasterol)

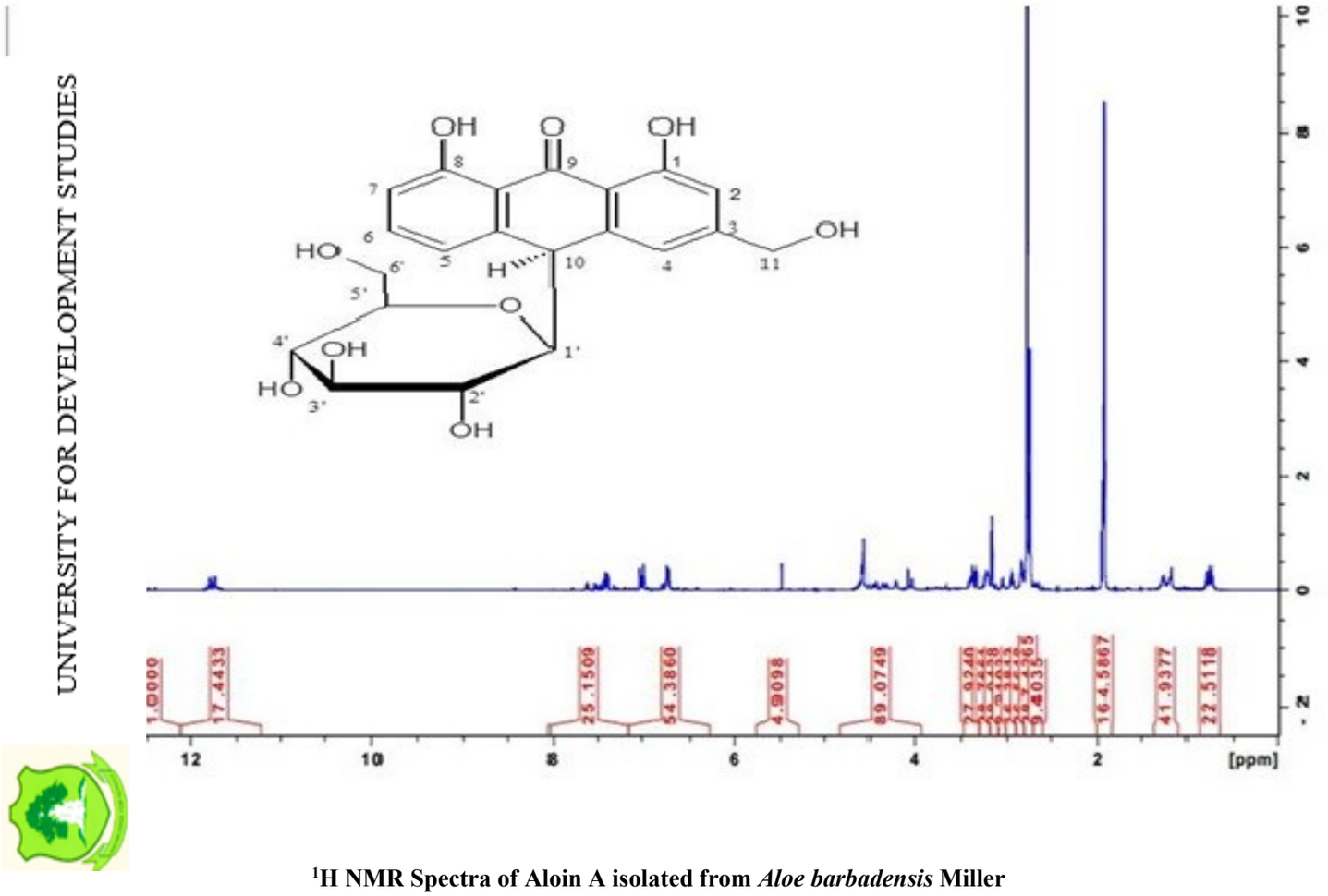


FT-IR Spectra of Aloin A isolated from *Aloe barbadensis* Miller

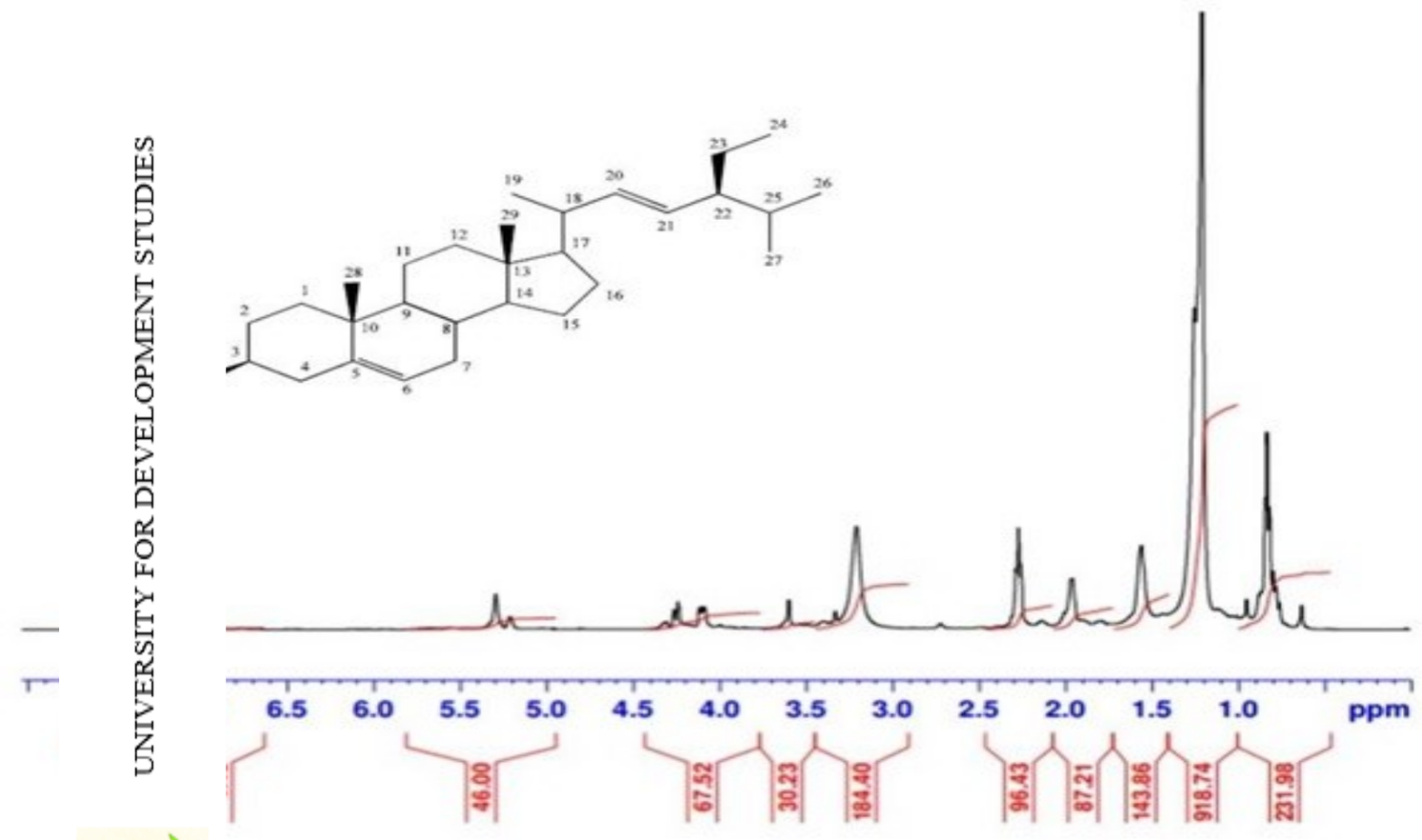


FT-IR Spectra of stigmasterol from *Aloe barbadensis* Miller

Appendix 5: ^{1}H NMR Spectra of isolated compounds (Aloin A, Stigmasterol) from *Aloe barbadensis* Miller

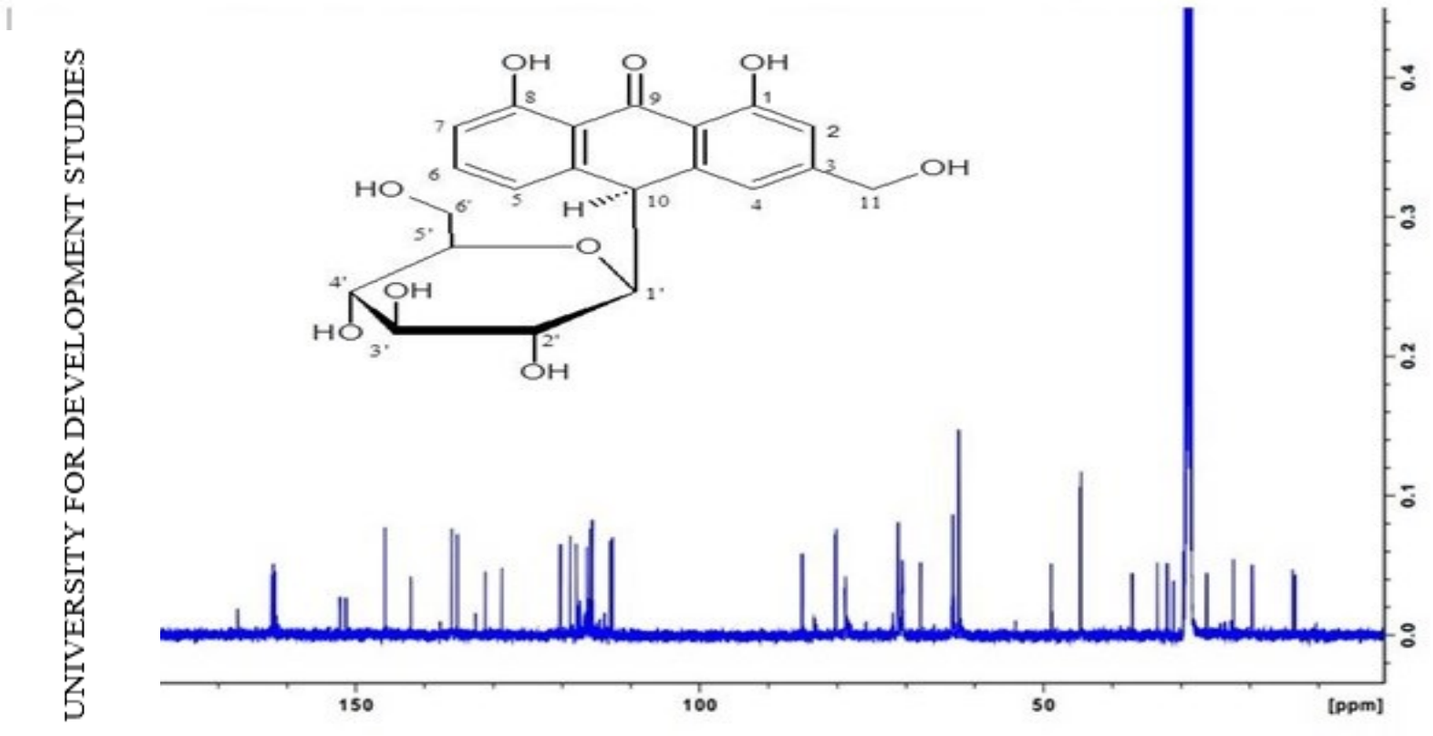


^{1}H NMR Spectra of Aloin A isolated from *Aloe barbadensis* Miller

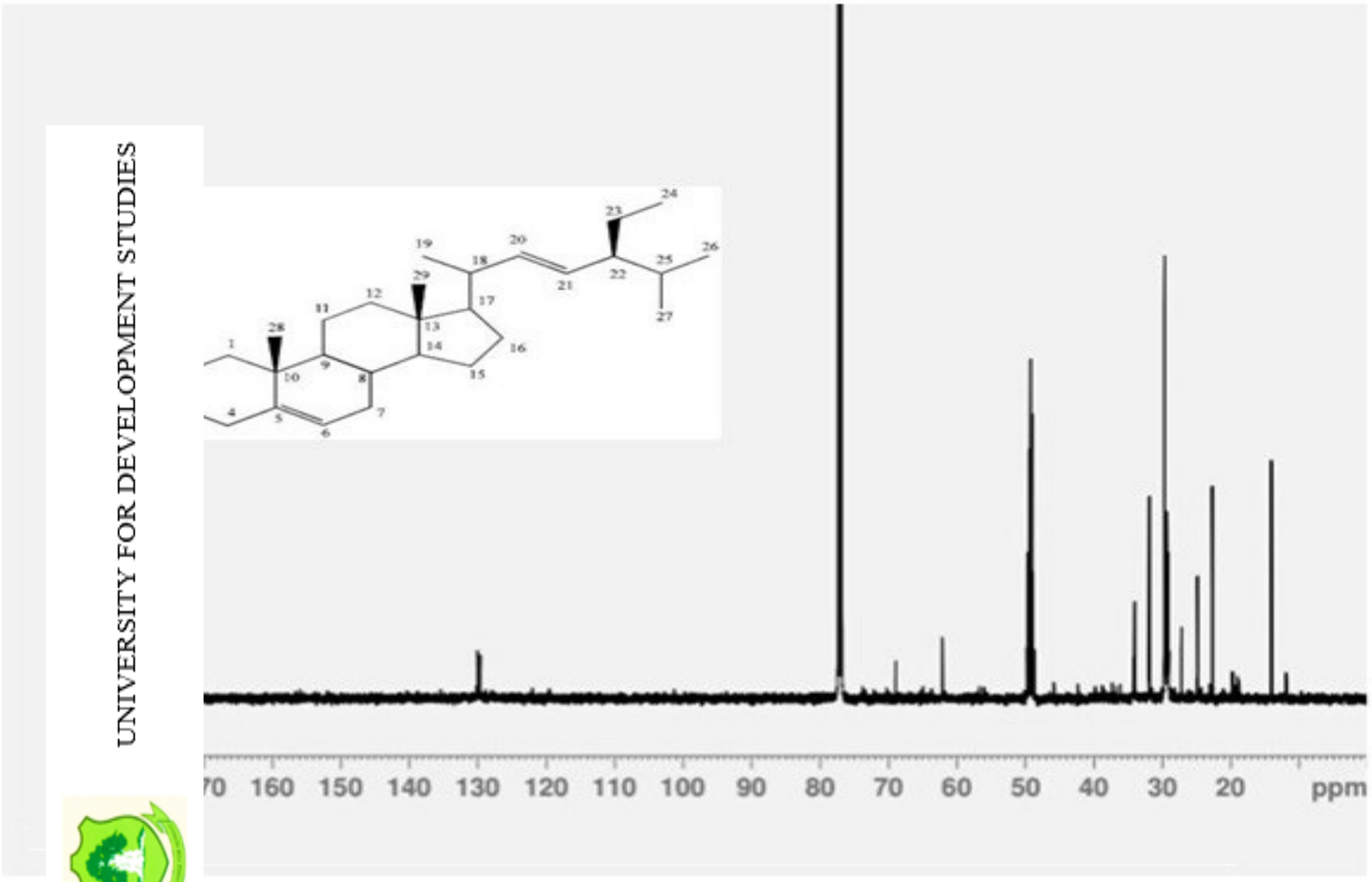


^{1}H N f isolated Stigmasterol from *Aloe Barbadensis* Miller

Appendix 6: ^{13}C NMR Spectra of isolated compounds (Aloin A, Stigmasterol) from *Aloe barbadensis* Miller



^{13}C NMR Spectra of Aloin A isolated from *Aloe barbadensis* Miller



^{13}C N d Stigmasterol from *Aloe barbadensis* Miller

Appendix 7: Raw data recorded for Aloin A (Compound A) on test organisms

Zone of Aloin A (mm) on test organisms at varying concentrations

Con	PA		EC		KP		SA		CA		TV	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
20 m	16	15.7	15	13	13	14	13	14	12.3	12.7	12.3	12.3
10 m	10.3	11	8	8	9	10	10	9	9	8	11	11
5 mg	10	9.7	6	7	7	7	8.6	8	7	7	10.6	10
2.5 m	9	9.3	0	0	0	0	0	0	0	0	9	9
1.25	0	0	0	0	0	0	0	0	0	0	7.3	7

Zones of inhibition of Aloin A-PEG Ointment (mm) on test organisms at varying concentrations

Concentration	PA		EC		KP		SA		CA		TV	
	est 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
20 mg	12	12.3	16	16.6	13.5	13.1	14.3	13.9	14.7	14.1	10.3	10.7
10 mg	10	11	15.3	14.3	11.7	12	13	12.3	14	13	9.6	9.3
5 mg/	9	9	14.3	13.7	10	11.3	11.3	10.3	11	10.7	8.3	8.3
2.5 m	8	9	12.7	12.2	9	9.3	10.6	9.7	9	8.4	7	7.5
1.25 r	0	0	9.5	9.7	7.5	8	8.7	9	7	7	6	6.1

Appendix 8: Raw data recorded for Stigmasterol (Compound G) on test organisms

Zone s of Stigmasterol (mm) on test organisms at varying concentrations

Conce	PA		EC		KP		SA		CA		TV	
	st 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
20 mg	3	9.7	12	11	13	12	12.7	11.3	11.7	12	12	12.6
10 mg	3	8.3	10	9.7	11	10.8	8.3	9.3	10.3	11	10.6	10.6
5 mg/		7	9	9.3	8	8	8.1	9	9.6	10.3	10	9
2.5 m		6.7	8.6	9	6.8	7.1	7.8	8.7	0	0	5	5.8
1.25 r		0	5.6	6.3	0	0	7.3	8	0	0	0	0

Zones of inhibition of formulated Stigmasterol (Compound G-PEG) Ointment (mm) on test organisms at varying concentrations

Conc	PA		EC		KP		SA		CA		TV	
	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2	Test 1	Test 2
20 mg	12.1	13	15	14	13.3	13	10.9	11.3	12	12.2	13	13.6
10 mg	11.3	12.3	12.2	12.7	12	11.7	9.3	10	11.2	10.8	11.1	11.4
5 mg/	10.3	11.3	10.6	11	11.4	11.7	9	9.7	9	9.3	9.6	10.6
2.5 m	10	11	10	9	10.3	10.7	8.7	9	9	8.5	8.5	8
1.25 r	9.3	7	7.8	8.3	7	7.9	8.7	8.3	0	0	7	8