UNIVERSITY OF NAIROBI

EVALUATION OF THE COMPOSITION, PHYSICO-CHEMICAL CHARACTERISTICS, SURFACTANT AND ANTI-MICROBIAL POTENTIAL OF *COMMIPHORA ABYSSINICA* GUM RESIN

WAWERU JAMES GITAU

I56/81398/2012

THE THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN INDUSTRIAL CHEMISTRY OF THE UNIVERSITY OF NAIROBI

2015

DECLARATION

I declare that this thesis is my original work and has not been submitted to any other university.

Signature …….. Date……

WAWERU JAMES GITAU

I56/81398/2012

This thesis has been submitted for examination with our approval as University supervisors.

Signature…… Date……

PROF. HELLEN NJERI NJENGA

DEPARTMENT OF CHEMISTRY

UNIVERSITY OF NAIROBI

Signature……. Date……

DR. LEONIDAH KERUBO OMOSA

DEPARTMENT OF CHEMISTRY

UNIVERSITY OF NAIROBI

DEDICATION

This work is dedicated to my parents, the late Mr. and Mrs. Waweru for the strong foundation that they built for the family.

ACKNOWLEDGEMENT

My sincere gratitude goes to my supervisors, Prof. Hellen Njeri Njenga and Dr. Leonidah Kerubo Omosa for their invaluable advice, professional guidance and tireless support throughout the period of my research work. I am thankful to the University of Nairobi for offering me the MSc. Scholarship that enabled me to conveniently pursue my studies.

I would also like to recognize the National Council for Science, Technology and Innovation (NACOSTI) who funded the research project within which this study was undertaken and KEN-02 project which provided some consumables for the work. I am grateful to the University of Nairobi's Departments of Chemistry, Physics, Biochemistry, Land Resources Management and Agricultural Technology (LARMAT), Pharmaceutical Sciences and Centre for Bioinformatics and Biotechnology (CEBIB) for generously offering me free access to their facilities. I specifically extend my thanks to their technical staff for their guidance and tolerance. I also acknowledge the Kenya Industrial Research and Development Institute (KIRDI) and the Kenya Medical Research Institute (KEMRI) Centre for Respiratory Diseases Research (CRDR) for allowing me to carry out parts of my research work in their laboratories. I immensely thank Dr. Beth Ndunda and Dr. Moses Langat for assisting me to carry out GC-MS analysis at the University of Surrey, UK.

I would also like to thank my postgraduate colleagues in the Department of Chemistry for their encouragement throughout period of this study. Lastly, am thankful to my siblings for their generous financial and moral support.

Because of you all, I did not give up; your kindness and conscientiousness has been undeniable and I remain highly indebted to you. All glory is to the Almighty God for making everything to be possible.

ABSTRACT

In this study, the exudates of *C. abyssinica* were identified and collected from different geographical locations in Kajiado County. Air-dried gum resin was ground and then macerated using different solvent systems with varying polarities (hexane, methanol and ethanol) in order to target different classes of compounds. The resultant extracts were then subjected to antimicrobial assays including anti-bacterial and anti-fungal assays. The physico-chemical parameters of the gum resin from different populations of *C. abyssinica* were determined to establish their potential application in the agro-chemical industry. The oleo gum resin from the various locations was found to have low moisture, ash and protein contents of 9.15-10.58%, 2.24-3.04% and 9.06-11.13% respectively. This resin contained 1.31-1.87% volatile oil, 26-47% resin and 82.25-84.50% water soluble matter. The ethanol extracts had saponification values ranging from 171 to 176 mg KOH/g and an acid value of approximately 4 mg KOH/g. This extract exhibited good foaming and surface tension reduction capabilities, comparable to conventional synthetic detergent surfactants, and a low critical micelle concentration (CMC) value of 36-40 mg/100ml at 22°C. The gum formed a highly viscous solution and was found to stabilize emulsions at a concentration of 0.1%, and the stability increased with increase in temperature to 60°C. The crude extracts obtained using hexane and methanol were found to consist mainly of sesquiterpene compounds while those extracted with ethanol contained sterols. The extracts were active against gram positive bacteria (*Staphylococcus aureus* and methicilin resistant *Staphylococcus aureus*- MRSA) and one fungus (*Trycophyton mentagrophytes*). Apart from the yield of ethanol extract, all the other properties had insignificant variations, and therefore the mean values of the gum resin samples studied from the different locations can be used to describe the general characteristics of the *C. abyssinica* gum resin. Formulation of surfactant systems

using the ethanol extract can now be undertaken. A toxicological study should be carried out so as to affirm the suitability of substituting some applications of gum Arabic emulsifier with the water soluble gum extract from *C. abyssinica*.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS AND NOTATIONS

AOAC: Association of Official Analytical Chemists

ASAL: Arid and semi-arid lands

ATCC: American type culture collection

AV: Acid value

CDE: Cocamide diethanolamide

CLSI: Clinical Laboratory Standard Institute

CMC: Critical micelle concentration

CRDR: Centre for Respiratory Diseases Research

DCM: Dichloromethane

DMSO: Dimethyl sulphoxide

FAO: Food and Agriculture Organization

FFA: Free fatty acids

GA: Gum arabic

GARA: Gum Arabic and Resin Association

GC: Gas chromatography

GC-ECD: Gas chromatograph-electron capture detector

GC-FTIR: Gas chromatograph-Fourier transform infrared spectrometry

GC-MS: Gas chromatograph-mass spectrometry

HLB: Hydrophile- lipophile balance

KEFRI: Kenya Forestry Research Institute

KEMRI: Kenya Medical Research Institute

KIRDI: Kenya Industrial Research Development Institute

LABSA: Linear alkyl benzene sulfonic acid

L-G: Liquid-gas

L-L: Liquid-liquid

L-S: Liquid-solid

MIC: Minimum inhibitory concentration

MRSA: Methicin-resistant *Staphylococcus aureus*

NWFP: Non-wood forest products

o/w: Oil-in-water

PDA: Potato dextrose agar

SLS: Sodium laurylether sulphate

w/o: Water-in-oil

NOTATIONS

cc: Cubic centimeters

cP: Centipoise

g: Grams

kg/m^3: Kilograms per cubic meter

Macfarland: 3.0×10^8 Colony forming units per milliliter

mg/ml: Milligrams per milliliter

mg: Milligrams

ml: Milliliters

mN/m: Milli Newtons per meter

nm: Nanometers

pH: Hydrogen ion concentration

ppm: Parts per million

spp.: Species

w/v: Weight per volume

µl: Micro liters

$^\circ$C: Degrees Celsius

α: Alpha

γ: Gamma

β: Beta

δ: Delta

ψ: Psi

LIST OF APPENDICES

CHAPTER ONE

INTRODUCTION

1.1 General Introduction

The chemical industry heavily relies on petrochemical building blocks for the production of various synthetic industrial and household products (Dunford, 2012). Despite their manifold applications, these building blocks are however unsustainable because they are up to 95% dependent on fossil fuels which are limited in supply and their use lead to emission of environmentally unfriendly carbon dioxide and to the production of majorly non-biodegradable products (Dunford, 2012). Gradual replacement or complementation with plant-based raw materials is the viable option.

Plants use solar energy to synthesize both primary and secondary metabolites which offer a vast potential for the production and development of sustainable raw materials which can be utilized in the commodity and fine chemical sectors (Europa Publications Limited, 2001). The secondary metabolites also referred to as natural products are synthesized by plants to enable them interact with the surrounding environment. For instance, some are used in defense strategies against herbivores and microbes while others act as plant pheromones thus aiding in pollination (Veberic, 2010).

Plants produce a large variety of raw materials for the agro-based industries. Non-wood forest products include rubber, resins, gums, gum resins, oleo-gum resins, essential oils, waxes, natural fibers, wild fruits, tannins, medicines, sugars and honey among others. These materials have been exploited as raw materials in pharmaceutical, textile, bio-plastic, bio-energy, dyes, pigments, fibers, polymers and fuels production (Benkeblia, 2011).

Plant exudates are by-products or products of certain metabolic pathways. At times they are part of the normal plant metabolism, but in most cases are attributed to gummosis. This is a pathological oozing of sap as a result of physical injury, microbial and pest infestation, diseases and environmental stress. The organized materials in the cell wall undergo metamorphosis into unrecognizable materials exhibited as gums or resins (Nussinovitch, 2010). They ooze from the stems and barks of trees and shrubs in form of tears, nodules or lumps which harden after exposure to the sun forming hard, glassy substances. These appear in varying colors from white, yellow, amber, brown to dark brown depending on the tree species and chemical composition (Gachathi and Eriksen, 2011).

Plant resinous exudates, exemplified by frankincense, myrrh, benzoin, Dragon's blood and ferulae resina are important sources for traditional medicines. Myrrh, originally from Arabia, is the exudate produced by the secretory tissue in the bark of *Commiphora* species (Shen et al., 2012). They are commonly used as perfume and incense, and their medicinal values have been applied since ancient times (Langenheim, 2003). They are used in indigenous medicines for the management of a number of ailments including wounds, pain, arthritis, fractures, obesity, parasitic infection and gastrointestinal diseases (Shen et al., 2012). The centuries-old utilization of plant derivatives in medicinal applications manifests man's ingenuity.

Previous phytochemical work on plants from *Commiphora* genus has yielded diverse classes of secondary metabolites mainly terpenoids, steroids, flavonoids, sugars and lignins (Hanus et al., 2005). Antiproliferative, anti-inflammatory, antimicrobial, hepatoprotective and cardiovascular properties of the purified compound and the crude extracts were investigated by El Ashry et al. (2003), and some of the compounds were found to exhibit interesting activities.

There is scanty information on the phytochemistry and physico-chemical characteristics of the gum resin from *Commiphora abyssinica*.

1.2 Gums and Resins and Their Industrial Applications

Gums, resins and oleo-gum resins are the dried exudates from the stems and branches of various species in some genus from the family Burseraceae, namely *Commiphora*, *Acacia* and *Boswellia* (Lemenih and Kassa, 2011). They are metabolic products that compose a vital and significant class of non-wood forest derivatives.

Gums are high molecular weight hydrophilic polysaccharides (carbohydrate polymers). Industrial gums include gum arabic, agar, guar gum, karaya gum, gum tragacanth, xantham gum, glucomannan and alginate among others. They are readily soluble in water and are capable of increasing solution viscosities by orders of magnitude even at low concentrations (Damodaran and Paraf, 1997). They are therefore widely used in the food industries as thickeners, stabilizers and emulsifiers.

Resins are solid to semi-solid amorphous exudates from some plants especially conifers. They are flammable substances and are mostly used in varnishes and paints, in organic synthesis and sometimes as incense (Boer and Ella, 2000). The term oleo-gum resin describes exudates composed of essential oils, a water-soluble gum and an alcohol soluble resin. Since ancient times, these resources have been widely utilized for both household and industrial purposes. They have an established international market providing an invaluable income supplement to millions of largely marginalized and poor rural communities (FAO, 1990). They are vital industrial commodities, serving as major raw materials in the basic chemical, food and pharmaceutical industries owing to their spreading, thickening and emulsifying properties. Specific industries include cosmetic, pharmaceutical, textile, paper, beverage,

food, paints and varnishes, soaps and detergents, agro-chemical, plastic and mining (Benkeblia, 2011).

The major commercial gum and resins are gum arabic from *Acacia senegal* and *Acacia seyal*, myrrh from *Commiphora myrrha*, frankincense from *Boswellia neglecta*, and opoponax from *Commiphora holtziana* (Gachathi and Eriksen, 2011). True myrrh is obtained from *C. myrrha*, though the exudates from some other *Commiphora* species such as *C. abyssinica* are also classified as myrrh. The largest myrrh market is in China where the oil extracted from the resin is used in traditional medicines. There is also an established market in the USA and Europe especially France, where the resins are used as industrial raw materials (Singh, 2010). Kenya has not significantly exploited these forest resources. However, initiatives by the Kenya Forestry Research Institute (KEFRI) and Gum Arabic and Resin Association (GARA) have made the country to emerge as an exporter of gum Arabic. GARA initiatives include enhancing sound production of quality gums and resins, linking the collectors to the export market and lobbying for favorable government policies to boost this sector. KEFRI has been offering training programmes to the collectors to boost the production. However, their initiatives have mainly focused on commercially established gums and resins such as gum arabic and myrrh from *C. myrrha*. Consequently, many other gum and resin producing woodlands have been under degradation due to poverty-driven exploitation.

1.3 Quality and Standardization of Gum Resin

Commercialization of gums depends on their quality, which is normally determined using some physical properties such as moisture, total ash content, volatile matter, nitrogen content, optical rotation and metal composition. The assessment of gum quality in the world market relies on international specifications which are based on some physicochemical parameters of the Sudan gum from *Acacia Senegal variety Senegal* which are listed in Table 1.1 (FAO, 1990).

Table 1.1: International gum arabic quality specifications

Property	**Range**
Moisture (%)	13- 15
Ash content (%)	2- 4
Volatile matter (%)	51- 65
Internal energy (%)	30- 39
Nitrogen content (%)	0.26- 0.39
Optical rotation (°)	(-26)- (-34)
Cationic composition (ash at 550 °C) (ppm): Iron	730- 2490
Manganese	69- 117
Zinc	45- 111
Copper	52-66

These properties may however vary depending on age of trees, exudation time, season, storage type and climate (Montenegro et al., 2012). In addition, some other parameters such as total soluble fiber, refractive index, intrinsic viscosity and pH are also important.

Moisture content facilitates solubility of the hydrophilic carbohydrates and the hydrophobic proteins; cationic composition gives an assessment of specific concentrations of heavy metals and the ash content indicates the critical foreign matter levels, total matter insoluble in acid and salts of calcium, magnesium and potassium. The volatile matter indicates the type and level of polymerization of the sugar components which highly determines their use as emulsifiers and stabilizers in the manufacture of pharmaceuticals such as cough syrups. Internal energy reflects the quantity of energy required to produce a quantity of carbon by heating at 500°C to release carbon dioxide. Nitrogen content shows the number of amino acid

constituents, while optical rotation identifies the nature and source of the sugars present (Lelon et al., 2010; Montenegro et al., 2012). These parameters can form the basis for determining the quality of a gum resin and also its evaluation as a potential substitute for gum arabic in formulations.

1.4 Problem Statement

Gum and resin producing tree species are widely distributed in the arid and semi-arid areas (ASAL). Natural gums and resins are invaluable components of non-wood forest products (NWFP) especially among the ASAL communities. As such, the industry is an indispensable source of income for local communities where local and international trade on the products exists. The communities in *C. abyssinica* gum resin production areas in Kenya are however characterized by low standards of living as exhibited by poverty, lack of social services and poor infrastructure. This drives them to engage in unsustainable methods of exploitation of the tree species such as charcoal burning which exacerbates their plight.

To mitigate these risks in the gum and resin industry, a comprehensive study on the properties and potential of these NWFP as sources of building blocks for the chemical industry, is necessary. This move can position them in the local and international market. Intensive research has been done on some gum producing species such as *Acacia senegal* and *Acacia seyal* as well as gum resin producing species of genus *Commiphora* such as *C. myrrha, C. opobalsamum, C. mukul* and *C. molmol*. Many of their benefits have been revealed resulting in an established market structure that significantly benefits the respective local communities. No such studies have been carried out for *C. abyssinica.* Unlike other gum producing countries such as Ethiopia and Sudan, Kenya has not established strategies either for collection and quality maintenance or models of commercialization of gums and gum resins.

1.5 Hypotheses

i. The physical chemical parameters of *C. abyssinica* gum resin are independent of location.

ii. The extracts of *C. abyssinica* are a potential source of surfactant ingredients.

1.6 Objectives

1.6.1 Main objective

The main objective of this study was to characterize *C. abyssinica* oleo-gum resin from Kajiado in terms of quality-related physical properties and also to study the yield, composition and performance characteristics of its extracts with the view of establishing the potential of its industrial utilization.

1.6.2 Specific objectives

The specific objectives of this study were:

i. To determine the physico-chemical properties of *C. abyssinica* gum resin from Kajiado County.

ii. To investigate the yields of essential oils, ethanol and water soluble components of the gum resin.

iii. To determine the surfactant properties of the ethanol and water soluble extracts and compare them with commercial surfactants.

iv. To establish the compounds found in the crude extracts.

v. To evaluate the bioassay of the extracts.

1.7 Justification

Commiphora abyssinica tree species are found in abundance in many semi-arid parts of Kenya including Kajiado. Past research has revealed surfactant properties in *C. abyssinica's* gum resin solutions (Mwendwa, 2007; Chesori, 2008). The gum component of the exudates has been found to have some characteristics including emulsion stabilizing properties comparative to those of gum arabic but this has not been quantified to warrant industrial application.

Proper classification, grading and extraction are essential components to production of quality marketable products from this potential resource. A complete understanding of the physical properties, composition and structure of the *C. abyssinica* gum resin components as well as the yields of various extracts can influence its eventual utilization in products such as detergents, emulsifiers, fragrants and dispersants. Substitution of synthetic surfactants with natural surfactants from resins will enhance formulation of eco-friendly products due to guaranteed biodegradability. The study will also aid in quality standardization of *C.abyssinica* gum resin. The project is expected to contribute to feasibility studies on the potential application of *C. abbysinica* gum resin in various sectors of the chemical industry. Given the abundance of this forestry resource in Kenya, positive results can promote local industry and trade in the commodity. This would consequently result in equitable development, poverty alleviation, job creation hence curbing rural-urban migration and rehabilitation of indigenous forests.

CHAPTER TWO

LITERATURE REVIEW

2.1 Botanical Information

Commiphora abyssinica (Berg) Engl belongs to the family Burseraceae of flowering plants from the order Sapindales. This family consists of about 800 resinous tree and shrub species from 18 genera. They are widely distributed in the tropical and sub-tropical regions. The flagrant resins produced from the bark of these trees are of considerable economic, cultural and medicinal value (Langenheim, 2003).

2.1.1 Genus *Commiphora*

The plant study belongs to the genus *Commiphora* consisting of more than 200 plant species distributed in the tropical and sub-tropical regions especially Northeastern Africa, Southern Arabia, India and Madagascar (Hanus et al., 2005; Shen et al., 2012). These species are short trees or shrubs with thorny branches (Fig. 2.1).

Figure 2.1: *Commiphora myrrha* tree species

They produce resinous exudates commonly known as myrrh from their stem bark. These exudates contain polysaccharides, steroids, sterols, proteins and terpenes. Previous phytochemical investigations on the *Commiphora* resins have led to the identification of diverse secondary metabolites such as terpenoids, steroids, flavonoids, sugars and lignins (Hanus et al., 2005). The main myrrh producing species are *C. abyssinica*, *C. schimperi* and *C. myrrha*.

2.1.1.1 Commiphora abyssinica

Commiphora abyssinica (Berg.) Engl. is known by several other names such as *Commiphora madagascarensis*, Arabian myrrh and Abyssinian myrrh. It is a bush shrub mainly found in savannah woodland and drier parts of tropical Africa, especially in the northeastern regions namely Kenya, Somalia, Ethiopia and Sudan (Maradufu and Warthen, 1988). In Kenya it is widely distributed in the arid and semi- arid lands especially in Kajiado, Laikipia and Isiolo regions. Fig 2.2 represents the *C. abyssinica* tree species with dried tears.

Figure 2.2: *Commiphora abyssinica* tree species

Commiphora abyssinica produces a yellow to brown exudate specifically referred to as oleo-gum resin as it is composed of a volatile oil, alcohol-soluble resin and a water-soluble gum component. The actual composition is 3-8% essential oil, 30-60% gum and 25-40% resin (Singh, 2010). The gum is a complex mixture of hydrophobic protein component which acts as an emulsifier by adsorbing onto the surface of oil droplets and a hydrophilic carbohydrate component which inhibits flocculation through steric repulsions in formulations (Lelon et al., 2010). The alcohol soluble component contains bioactive molecules from the plant which may include terpenoids, flavonoids, saponins, steroids, tannins or alkaloids. These molecules possess antimicrobial and antifungal properties for defense after injury as the gum seals the wound (Kalia and Avérous, 2011). Eagleson (1994) described the *Commiphora abyssinica* exudate as containing 2.5 to 10% essential oil, a variable amount of resin and gum. The oil is used to add aroma in perfumes.

2.2 Ethno-Medicinal Application and Pharmacological Information on the Genus *Commiphora*

Myrrh extracts have demonstrated vast *in vitro* and *in vivo* pharmacological effects such as antioxidant, anti-inflammatory, antimicrobial, antiproliferative and inhibitory effect on tumor cells. They have also remained an excellent source of traditional medicines against microbial infection, inflammation, arthritis, tumor, obesity and gastrointestinal diseases (Shen et al., 2012).

The medicinal utilization of fragrant oleo-gum resins is one of man's oldest therapies (Tadesse et al., 2007). Medicinal use of myrrh extracts dates back to as far as Biblical times when it was used for wound treatment (Hanus et al., 2008). From ancient times, it has been used widely as medicine especially in China, Greece, Egypt and Rome (Shen et al., 2012). In traditional Chinese medicine, it was used for treatment of arthritis, fractures, trauma and blood-stagnation complications. It was also used in other traditional medicines as an anti-

inflammatory, emmenogogue, antiseptic and carminative agent (Omer et al., 2011). Myrrh and frankincense still find wide therapeutic applications in North Africa and China (Tadesse et al., 2007).

As a result of the effective antimicrobial properties, the extracts have been used in the treatment of gingivitis, mouth ulcers, sinusitis, brucellosis, glandular fever and also as an anti-parasitic agent (Mohamed et al., 2014). They have also received scientific validation resulting in applications in contemporary medications most of which are similar to the traditional therapies. This has been further encouraged by their significant pharmacological activity, unique chemical composition and non-toxicity (Tadesse et al., 2007).

The local community, the Maasai, boils the resin together with bone soup. They believe it helps to increase the blood level, facilitate smooth blood flow, and treat back pain. This mysteriously coincides with the traditional Chinese medicinal use in blood stagnation complications (Shen *et. al*., 2012). It has been documented that myrrh increases the level of white blood cells through promotion of local leukocytosis (Capasso, 2003). The ever emergence of new killer diseases and drug-resistant disease vectors further exacerbated by the inefficient and expensive drug discovery process has called for urgency in discovery of new medicinal agents, notably from plants.

2.3 Physical Characteristics of *Commiphora* Gum Resins

Myrrh gum resin contains water-soluble gum, alcohol soluble resin and a volatile oil component. It also contains various levels of impurities such as dried leaves, tree bark, soil or insects which stick on the exudates as it dries. The composition is about 2-10% essential oil, 25-40% ethanol-soluble matter and 30-60% water soluble gum for most species, while the impurities are normally between 3-4% (Kennedy et al., 1996; Evans, 2009).

According to Abourashed and Khan (2013) *C. mukul* normally contains 1.5-17% oil, though a similar resin reported by Hanus et al., (2005) produced a much lower essential oil content of approximately 0.4% which was composed mainly of myrcene and dimyrcene. Chiteva et al., (2013) quantified a gum resin from a Kenyan *Commiphora holtziana* and obtained 9.1-9.2% essential oils, 41-44% solvent soluble resin and 39.8-40.2 water soluble gum.

A resinoid isolated from *C. mukul* had the physicochemical properties shown in Table 2.1.

Table 2.1: Physicochemical properties of *C. mukul* resinoid

Property	**Ethyl acetate extract**	**Ethanol extract**
Appearance	Brownish semi-solid	Brownish semi-solid
Acid value (mg KOH/g)	10.20-11.88	13.16-15.77
Saponification value (mg KOH/g)	219.94-225.36	230.32-233.94
Ester value (mg KOH/g)	209.74-213.48	217.16-218.17
Iodine value (g/g)	98.34-100.34	100.26-100.48

(Siddiqui and Mazumder, 2012)

Another *Commiphora* resinoid from *C. wightii* exhibited the properties shown in Table 2.2.

Table 2.2: Physicochemical properties of *C. wightii* resinoid

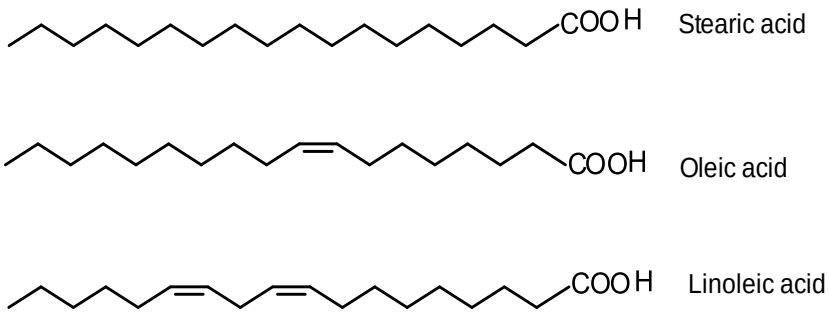
Property	**Ethyl acetate extract**	**Ethanol extract**
Appearance	Brown- dark brown semi-solid	Brown- dark brown semi-solid
Acid value (mg KOH/g)	6.63-15.07	8.20-14.65
Saponification value (mg KOH/g)	222.02-252.54	235.14-270.02
Ester value (mg KOH/g)	215.39-237.47	226.94-255.37
Iodine value (g/g)	98.75-103.08	100.79-103.98

(Siddiqui and Mazumder, 2012)

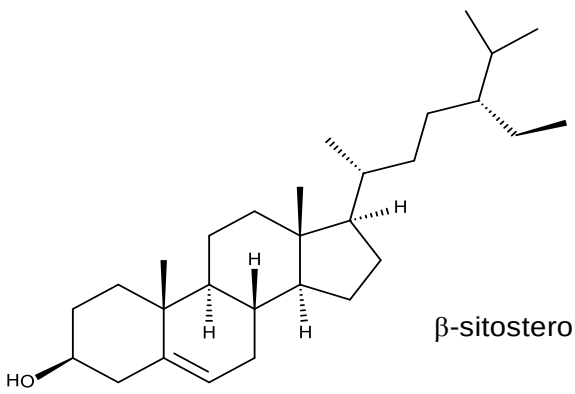
2.4 Surface Active Compounds of Non-Wood Forest Products (NWFP)

Surface active compounds can be isolated from all constituents of wood. The water-soluble cellulose derivatives for instance are widely applied as dispersing agents. Cellulose and hemi-cellulose are utilized in oil-in-water emulsions as steric stabilizers. Resin, fatty acids, sterols and sterol ethoxylates are invaluable surface active compounds isolated from forest products (Kjellin and Johansson, 2010).

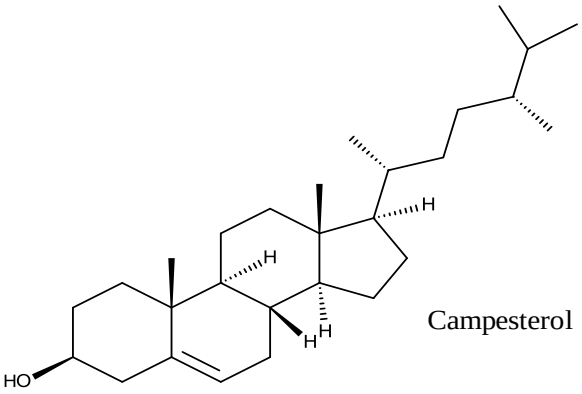
Coniferous woods are the main industrial source of these surface active compounds, since their oleoresin contains 70-80% resin acids. All trees contain fatty acids, mainly as either constituents in steryl esters or triglycerides. Trees contain a large number of fatty acids with chain lengths varying from C_{12} to C_{24} (Kjellin and Johansson, 2010). Linoleic and oleic acids account for the bulk of these fatty acids. Some hardwoods also contain large amounts of long chain fatty acids varying from C_{24} to C_{30}. Some of these fatty acids occur as surface active monoglycerides (Killops and Killops, 1993).



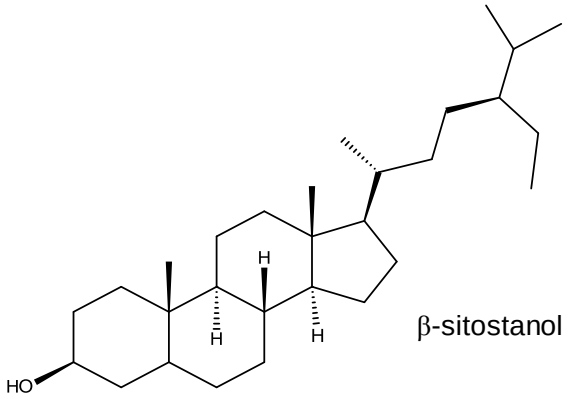
Both softwood and hardwood trees also contain sterols in form of fatty acid esters. An example is sitosterol and its saturated analogues such as campesterol and sitostanol. Some triterpenyl alcohols are also found in varying amounts.



β-sitosterol



Campesterol



β-sitostanol

Through the Kraft pulping process (sulfate pulping process), the resin and fatty acids are converted to sodium soaps which are recovered as sulfate soaps after the cooking process (Kjellin and Johansson, 2010). Steryl esters are largely hydrolysed to sterols and fatty acids, after which the sterols obtained are integrated in sodium soap.

Plant sterols and stanols are collectively called phytosterols, and are incorporated worldwide in a wide range of functional products. Together with their derivatives, they are widely used in cosmetic formulations owing to their favourable effects on biological tissues (Holmberg, 2004). For instance in the 1980s, the catalyzed reaction of phytosterol with ethylene oxide produced phytosterol ethoxylates which are mainly used as emulsifiers. Phytosterol ethoxylates have currently solicited profound interest in cosmetic formulation applications. The higher ethoxylates can serve as typical oil-in-water co-emulsifiers while the lower

ethoxylates have potential use as water-in-oil co-emulsifiers (Holmberg, 2004; Kjellin and Johansson, 2010).

Abietic and dehydroabietic acids have also been utilized as surfactants in form of soap or polyoxyethylene derivatives. Polyoxyethylene sterols have mostly been utilized in personal care products due to their ameliorating effects on skin and strong interfacial and emulsifying properties (Holmberg, 2004).

2.5 Surfactants

The term 'surfactants' is an acronym for 'surface active agents'. This is a class of molecules with ability to modify the interfacial properties of a liquid in which they are present (Broze, 1999). They are amphipathic organic compounds, meaning they have both hydrophobic and hydrophilic groups. In most cases, the hydrophobic group (tail) is a linear hydrocarbon chain which attaches to organic compounds while the hydrophilic group (head) attaches to molecules of water in interfaces involving a water liquid phase. Consequently, they are soluble in organic solvents as well as in water. Surfactants have the tendency of adsorbing at surfaces and interfaces dramatically altering the surface properties. They lower the interfacial tension between two surfaces through adsorption when present even at low concentrations (Rosen and Kunjappu, 2012). Such interfaces could be between the liquid and a gas (L-G) or a solid and liquid (L-S) or and another immiscible liquid (L-L).

Surfactants are versatile commercial chemicals, either as products or invaluable performance ingredients of the chemical industry, and virtually every activity involves them in one way or another (Fainerman et al., 2001). They are commonly used in industries dealing with detergents, emulsions, cosmetics, textiles, paints, paper, latex, petroleum, plastics, mining (especially in oil recovery), pharmaceuticals, agrochemicals and leather (Karsa and Porter, 1995). They are also finding applications in high technology areas such as ultra-thin film

coating and in nanotechnology (Zaikov, 2004). Specifically, they are used as emulsifiers, dispersants, wetting agents or frothers. In oil-in-water emulsions, they adsorb on the interface during homogenization significantly reducing the interfacial tension therefore disrupting droplet size and forming a coating around oil molecules thus preventing their aggregation (Yang et al., 2013). This is a very desirable property particularly in cosmetics, pharmaceuticals and petroleum recovery processes.

Surfactants are broadly divided into two groups depending on source; petrochemical or synthetic surfactants from crude oil sources and oleochemical surfactants from plant oils and animal fats (Kjellin and Johansson, 2010). Sourcing of surfactant raw materials from petrochemical distillates and oleochemicals was at a balance globally in early 2000 (Myers, 2006). Specific figures however vary geographically depending on availability of raw materials, for instance as at 2003, petrochemicals had a dominant share of 80% in Europe while in the South East Asian countries oleochemicals had a larger share of 55-65% (Chakrabarty, 2003). The natural fats and fatty acids are versatile materials in production of cationic, anionic and non-ionic surfactants since these molecules have the characteristic hydrophilic chain and the hydrophobic carboxyl group which can be selectively modified to make the variety of surface active agents (Chakrabarty, 2003).

Some surfactants have been classified as environmentally hazardous due to slow biodegradability, necessitating a shift to more linear synthetic surfactants with proven ability to degrade faster, and to biosurfactants. Surfactants from renewable sources are commonly called green surfactants as they generally follow the two-point criteria; biodegradability and low toxicity in aquatic environments (Biresaw and Mittal, 2013). The rate of biodegradation is a function of the chemical nature of the surfactant, and contrary to the obvious perception, the source of the raw material is not the key determinant (Mendelson, 2005). Following

extensive research, some general rules correlating the molecular structure of a surfactant and its susceptibility to biodegradation have been developed (Myers, 2006):

i. The primary factor is the chemical structure of the hydrophobic group: High degree of branching (especially at the alkyl terminus) limits biodegradation.
ii. The chemical nature of the hydrophilic group has limited effect on the rate of biodegradation.
iii. The rate of primary degradation is proportional to the distance between the terminus of the hydrophobe and the hydrophilic group.

Most of the modern synthetic surfactants are manufactured having linear lyophobic chains and they thereby meet the criteria. The main advantages of oleochemical building blocks is their sustainability, local availability, easier processing, rather stable prices due to flexible production and the aesthetic appeal of "naturalness". Moreover, the possibility of exhaustion of petrochemicals coupled with the high costs of exploration and extraction have instigated research into renewable building blocks (Glew, 2012). *C. abyssinica* is not a food commodity and therefore its potential industrial use even in large scale cannot bring about economic disequilibrium.

An ideal surfactant should be multifunctional, ecologically safe, possess good thermal and chemical stability, be highly biocompatible, have good surface activity and viscosity enhancement, be based on renewable raw materials and efficiently function at relatively low temperatures (Myers, 2006).

Characteristics evaluated to determine the suitability of an oleochemical for application in surfactant production are specific gravity, colour, viscosity, acid, saponification, ester and iodine values. However, the specific typical characteristics depend on the particular application of the surfactant.

For industrial purposes, an empirical trial and error method is preferably chosen over a fundamental research in the principles of interfacial interactions. This only serves to come up with a suitable and effective product, but limits the prospects of improvement. Modern formulations seek a surfactant that can help attain similar or better performance whilst consuming less mechanical energy, function at low wash temperatures, usage of less quantities of water and possess faster rates of degradation (Myers, 2006).

The efficiency of a surfactant is determined by some various characteristics such as critical micelle concentration (CMC), surface tension, solubility, foaming capacity, wetting and detergency power. Consequently, for a particular surfactant to be considered suitable for detergency applications, it should have a low CMC, stable pH, be biodegradable and possess desirable foaming properties (Myers, 2006).

2.5.1 Classification of Surfactants

Surfactants are mainly classified depending on the type of charge carried by the head. As such, there are four main groups namely anionic, cationic, non-ionic and amphoteric surfactants.

2.5.1.1 Anionic surfactants

This class consists of surfactants with a negatively charged hydrophile. This polar head group is mainly a sulfonate (RSO_3^-), sulfate ($ROSO_3^-$), carboxylate ($RCOO^-$) or a phosphate ($ROPO_3^-$). Examples of such surfactants are alkyl sulfate, sulfated oils, alkyl ether sulfate, alkyl ether carboxylate and alkyl aryl sulfonate. They are very efficient in removal of particulate solids and they also posssess superior detersive action (Broze, 1999). They are the most abundant group of surfactants, accounting for over 60% of world production of surfactants. Linear alkylbenzene sulfonates (LAS) are synthetic detergents that comprise the bulk of detergents due to their relative cost effectiveness, and they continue to dominate the

global detergent industry (Fainerman, 2001). An example is alkylethercarboxylate shown in Fig 2.3.

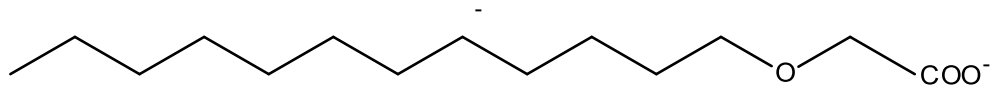


Figure 2.3: Alkylethercarboxylate

2.5.1.2 Cationic surfactants

These surfactants are characterized by a positively charged head group. They are mainly based on amino or quartenary ammonium groups (R_4N^+). Fig 2.4 is an illustration of a fatty amine salt.

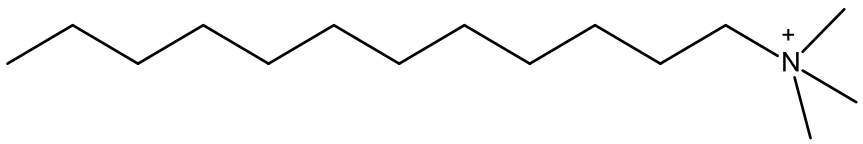


Figure 2.4: Fatty amine salt

2.5.1.3 Non-ionic surfactants

These are surfactants with an uncharged hydrophilic head group since it does not dissociate into ions. They have ether or a polyhydroxyl polar group that enhances water solubility. Examples are poly-ethoxy ethers (POE), poly-hydroxy surfactants and ethoxylated fatty alcohol (Fig 2.5).

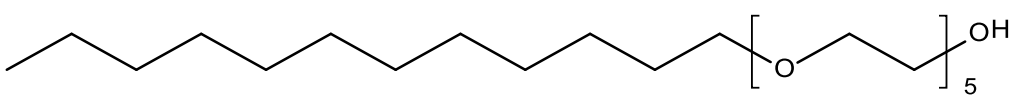


Figure 2.5: Ethoxylated fatty alcohol

2.5.1.4 Amphoteric surfactants

Amphoteric surfactants, also known as zwitterionic surfactants, normally contain both anionic and cationic charges. An example is betaine (Fig 2.6).

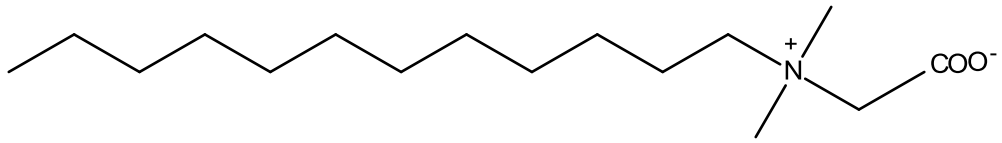


Figure 2.6: Betaine

2.5.2 Production of Surfactants

The different types of surfactants are produced through definite chemical processes that involve raw materials and various specialty chemicals. The main raw materials used in the production are alcohols, glycerides, fatty acids, oils, amines and alkanol amides. These are reacted with specialty chemicals such as ethylene oxide, propylene oxide, epichlorohydrin, acrylic acid and dimethyl sulphate through chemical processes such as condensation, alkoxylation, sulphonation, sulphation, polymerization, phosphation and quartenisation (Elementis Specialties, 2011).

2.5.3 Theory of Surface Action

Surfactants generally function through adsorption at the interface. The hydrophilic group (head) is directed towards the aqueous phase while the hydrophobic part (tail) is oriented towards the second phase. The molecules gradually cover the surface with increase in concentration until a point reaches when there is no more space on the surface, at a concentration called the critical micelle concentration. The adsorption power largely depends on three factors

(i) Chemical nature of the surfactant: This is determined by the type of charge on the polar head group and the nature of the hydrophobe (type, length and degree of branching).

(ii) Nature of the surface: Both chemical (charge, polarity and pH) and physical properties such as temperature affect the efficiency of the adsorption process (Myers, 2006).

A range of definite chemical interactions at the interface between the adsorbate (surfactant) and the adsorbent (surface) are involved such as ion exchange, ion binding (pairing), dispersion and hydrophobic bonding. Adsorption that involves charge-charge interactions is very sensitive to variations in external conditions like pH and electrolyte concentration (Myers, 2006). However, the adsorption mechanism keeps changing as the process proceeds.

2.5.4 Properties of Surfactants

2.5.4.1 Micellization

This is the process of aggregation of surfactant molecules in solution to form micelles. This normally happens at a very low concentration, called critical micelle concentration (CMC). Micelles are therefore dispersed surfactant molecules phase in a liquid colloid. Micelles behave like macromolecules in an aqueous solution, and they influence viscosity and the solubility of organic molecules such as hydrocarbons and oils. Above CMC, the micelles can therefore act as emulsifiers (Kunjappu and Rosen, 2013).

2.5.4.2 Detergency

Detergency is the most significant property of surfactants. Detergency refers to the cleaning process; the removal of dirt, grease or oil from a solid surface. This requires a surfactant with good wetting properties to enhance proper contact with the solid surface, dirt removal, and suspension in the bulk media. This is achieved through lowering of the surface tension of the

liquid solution which consequently lowers the interfacial tension between the interfaces or the two media (water/stain, stain/fabric and air/water). The hydrophilic head pulls the dirt or stain particle from the fabric towards the water. The surfactant molecules then suspend them in the wash water. For oily molecules, they may either be emulsified or solubilized by the surface active agent (Toedt et al., 2005).

2.5.4.3 Emulsification

This is the process of formation of an emulsion of two or more immiscible liquids. Surfactant molecules are sometimes added to enhance the stability of the emulsion in a way that resembles micellar solubilization, though in this case the resultant droplets are much bigger. For instance, when an oil-water mixture is agitated, various sizes of droplets are formed (Myers, 2006). The two liquids are immiscible and they will therefore exert different attractive forces on a molecule at the interface which increases the interfacial tension. Addition of a surfactant will tend to stabilize the emulsion through the following:

(i) Reduction of interfacial tension: This works through thermodynamic stabilization. On dispersion, there is reduction in interfacial area, and therefore reduced interfacial free energy. The surfactant molecules adsorb on the interface with the hydrophilic head towards the water and the hydrophobic tail towards the oil, thereby enhancing miscibility.

(ii) Formation of an interfacial film: This acts by forming a mechanical barrier that alters the coalescence rate of the droplets. In this case, the concentration of the emulsifier must be sufficiently high to form a film between the immiscible phases that prevents adhesion and also emulsifies the droplets.

(iii) Formation of an electrical double layer: This layer acts by forming an electrical barrier between approaching molecules. The emulsifier forms a film that produces electrical repulsion forces that keeps the droplets apart (Glass, 1989).

2.5.5 Characterization of Surfactants

2.5.5.1 Surface tension and critical micelle concentration *(CMC)*

Surface tension is the tendency of the surface of a liquid to behave like a thin elastic sheet. This is caused by unequal attractive forces on a molecule on the surface of a liquid, leaving a net force downwards. Contrary to this, the molecules in the bulk have equal forces of attraction from all the sides and therefore experience zero net force. Surface active agents adsorb at the air/water interface, thereby lowering the surface tension of the aqueous solution. This makes it easier to remove dirt or other materials from a surface and suspend them in the aqueous media.

At very low concentrations, surfactant molecules are present in the solution as monomers surrounded by liquid molecules. For an ionic surfactant, the ions are free in the solution and it behaves like an electrolyte. However, with further addition, the monomers tend to aggregate to form micelles. This process commonly called micellization takes place at a low surfactant concentration called critical micelle concentration (CMC). At this concentration, the surface is saturated and there is no significant change in surface tension with further increase in surfactant concentration. The equilibrium before and after the CMC is illustrated in Fig.2.7.

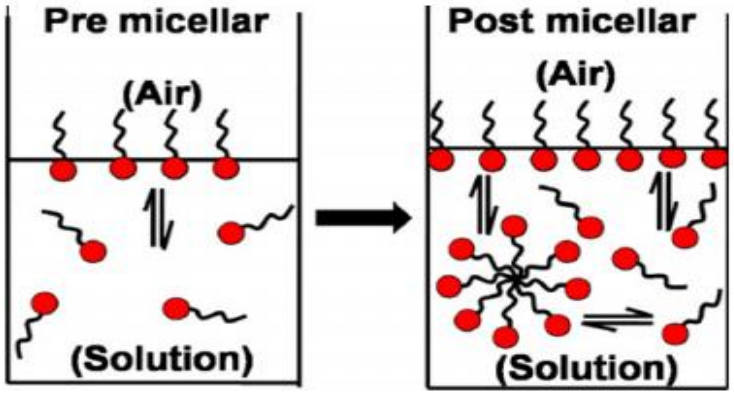


Figure 2.7: Schematic representation of equilibriums at the pre- and post- CMC zones (Mukherjee et al., 2013)

Any observed change could be due to either desorption or further adsorption of monomers at the surface or interface as the micelles (both neutral and ionic) themselves are not surface active (Mukherjee et al., 2013). Above the CMC, the micelles act as emulsifiers and can therefore solubilize insoluble particles (Fig 2.7).

CMC is usually used to assess the efficiency of a surfactant (Barbosa et al., 2013). Low CMC value is an indication of superior quality surfactants, since saturation can be achieved while using minimal amounts and the micelles are more stable. The nature of the hydrophobic group affects the structure of the micelle, especially in ionic surfactants where an increase in the chain length increases the size of the micelle and consequently a lower CMC. Aggregation number is the average number of monomers in a micelle.

Surface and interfacial properties are commonly investigated using the electrical conductivity method, though it can only be relevant in ionic surfactants. Tensiometry remains the most versatile method for such investigations due to resistance to charge interference. After obtaining a series of values of surface tension γ for a given concentration C, a plot of γ-C or γ-log C can be used to explain the behavior of surface properties. Both plots are mainly sigmoid in nature. An illustration is shown in Fig. 2.8.

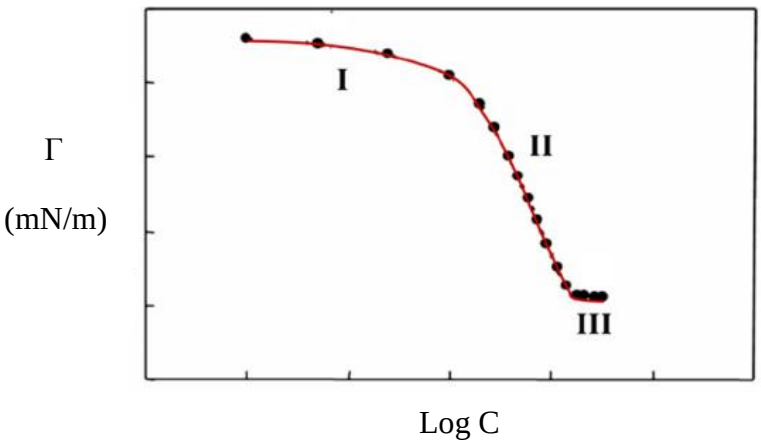


Figure 2.8: A simple tensiometric profile

Referring to Fig. 2.8, region (I) illustrates the initial stage of low surfactant concentration and there is little reduction in surface tension. From the onset of region (II), there is a proficient amphiphile adsorption at the interface which sharply reduces the surface tension.

The break at region (III) shows the beginning of the process of micellization. At this stage the surface is saturated with surfactant monomers and further adsorption hardly occurs. This leads to a practically constant surface tension despite increase in concentration. The break at the onset of region (III) is commonly taken as the CMC, but a more exact value is obtained at the point of intersection of tangent lines drawn at region (I) and (II). If a stalagmometer was used to determine the surface tension using the drop weight method, the peak in the plot of drop increment with concentration ($\delta n/\delta C$) versus C gives a more exact CMC (Hadkar, 2008). Surface tension is a function of temperature. Increase in temperature reduces the surface tension, reaching zero at critical temperature (Pruppacher and Klett, 1997).

Chen et al. (2010) investigated detergent abilities of the saponins from *Camellia Oleifera* and reported a reduction in surface tension of water to 50mN/m by crude saponin extract, 41.7mN/m by Tween 80 and 35.6mN/m by SLS at 25^{o}C. This is illustrated in Figure 2.9.

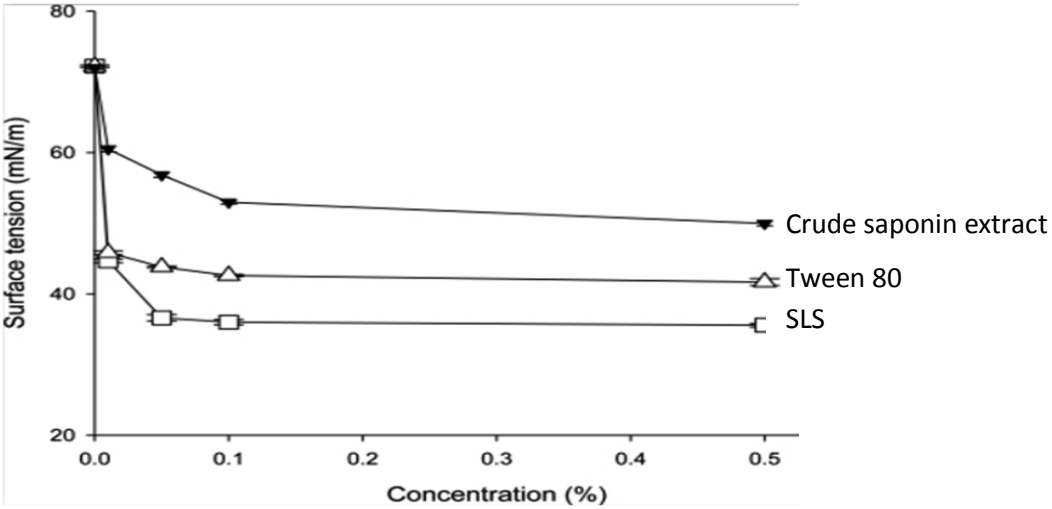


Figure 2.9: Variation of the surface tension of *Camellia Oleifera* crude saponin extract, Tween 80 and SLS vs concentration.

2.5.5.2 Foaming capacity/power and foam stability

The ease of foam formation or its stability is independent of the surface tension reduction capabilities of the surfactant. Rather, they depend on the tendency of the surface active agent to concentrate in the interface and its inherent special properties in a concentrated surface film (NPCS Board Consultants and Engineers, 2009). Foaming generally depends on the type of fatty acid, concentration and the CMC. The foaming power is however decreased by the presence of salts of calcium, magnesium and iron. Even good surfactants therefore tend to lather less in hard water. This is due to formation of insoluble salts, commonly called scum (Eqns 2.1, 2.2).

$2RCOONa + Ca^{2+} \rightarrow (RCOO)_2Ca\downarrow + 2Na^{+}$ ……**(Eqn. 2.1)**
(Soluble) (Precipitate)

$2RCOONa + Mg^{2+} \rightarrow (RCOO)_2Mg\downarrow + 2Na^{+}$ ……..**(Eqn. 2.2)**
(Soluble) (Precipitate)

Foams are very unsteady thermodynamic systems and therefore decay very easily. The stability of foam is defined by the rate of decay with time. Where foaming is an undesirable property, antifoamers and defoamers are used to counter the effect. Generally, the lower the CMC, the more efficient the surfactant is as a foamer. Foam height normally increases with increase in concentration of a surfactant below the CMC, and then levels off near the CMC (Lai, 2006; Kunjappu and Rosen, 2013). The foam height still increases further above the CMC with different detergents exhibiting different heights. This determines the effectiveness of a surfactant system and depends on its surface tension reduction capability and the strength of the intermolecular forces. Foam height and surface tension reduction have been found to be correlated at CMC, while foam stability and surface tension are correlated at the air-water interface. Foaming has a very limited effect on dirt removal but helps to attract customers since they tend to judge a detergent by its foaming power.

The foam capacity can be evaluated with or without soil. The shake foam test also called inverted cylinder test is a common method of evaluation conducted by placing the solution in a graduated cylinder and then shaking or inverting for a fixed number of times or a fixed duration of time (Lai, 2006). Another method developed by Ross and Miles involves placing a portion of a solution under investigation in a jacketed cylinder and then adding a second portion of 200ml through a standardized orifice from a height of 90cm leading to foam generation (Chen et al., 2010). The immediate foam height indicates the foam power of the surfactant solution. The foam stability is defined by a parameter R5, which is the ratio of the foam height after 5 minutes to the initial foam height (Lunkenheimer and Malysa, 2003).

Chen et al. (2010) found that the crude saponin extract from *Camellia oleifera* had moderate foam power ability. Results for 0.5% solutions were as represented in Figure 2.10.

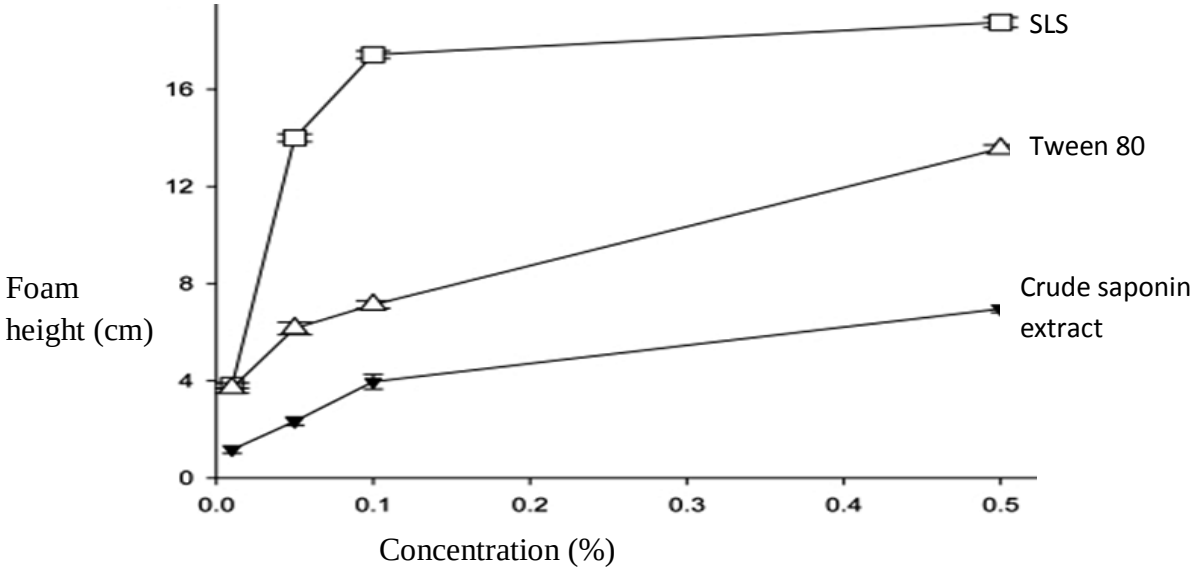


Figure 2.10: Graph of foam power of of the crude saponin extract, tween 80 and SLS versus concentration

This crude saponin extract gave 86.0 % foam stability. The most stable foam was by Tween 80 (96.3 %) followed by SLS with an R5 value of 93.6 %.

2.5.5.3 Hydrophile- lipophile balance (HLB)

The hydrophile- lipophile balance determines the properties and applications of a particular surfactant. The emulsification power, foaming, wetting, detergency and solubilisation properties depend on the hydrophile- lipophile balance (Pilemand, 2002). Anionic surfactants with short chain hydrophobic alkyl groups are predominantly wetting agents while longer chain analogues exhibit superior detergency properties. Thus, HLB is a critical factor in determining the properties of a surfactant since a high value means more solubility in water and low HLB value means more solubility in oil. This HLB system was initially developed by Griffin and is only applicable to non-ionic surfactants to indicate the ratio of hydrophilic to hydrophobic groups (Bieleman, 2000).

2.5.5.4 Cloud point

This is the temperature at which phase separation of a surfactant solution of a specific concentration occurs. At this temperature, the micelles aggregate to form a highly concentrated and cloudy phase. Diminished solubility hence cloudiness with increased temperatures is common with non-ionic surfactants due to hydration and agglomeration of micelles (Slade, 1998). The other phase is essentially depleted of detergent molecules. Detergency of a surfactant solution is correlated to its cloud point. This property is of significant consideration since it is undesirable for a liquid detergent product to turn cloudy on points of sale or while under use by the consumers (Lai, 2006).

Cloud point is determined using various techniques such as visual observation, turbidimetry, light scattering, viscometry, thermo-optical and particle counting methods (Eliassi and Parach, 2007).

2.6 Soaps

Soaps are sodium or potassium salts of higher chain fatty acids. They are a type of surfactants produced through the chemical conversion of the respective fatty acids mainly from fats and oils using alkalis such as NaOH or KOH. The process is called saponification or the alkaline hydrolysis of fats and oils. Soaps belong to a narrow class of anionic detergents since they have a negatively charged carboxylate functional group. They have the general formula R-$COO^-(Na^+/K^+)$, where R represents the long chain alkyl group (C_{12}-C_{20}). The R group is lipophilic while the –COO(Na/K) part is hydrophilic. Their respective solubility determines the cleansing action of the soap.

Modern soaps contain some property modifying additives such as emollients, perfumes, antioxidants, preservatives, abrasives, deodorants and colourings, for example TiO_2 for white soaps (Toedt et al., 2005). Despite soaps being excellent cleansers, their effectiveness is limited to soft water since they interact with mineral ions in hard water to form insoluble salts. They are however relatively non-toxic and highly biodegradable.

The component necessary for soap manufacture is fatty acid. Fats, oils, resins, waxes and balsams contain free fatty acids in varying ratios. The specific content together with other physical and chemical properties determines individual applicability in the chemical industry. Though commonly used as blends, fats and oils used in soap making have generally been classified as either lauric or non-lauric. This is based on their tendency to have either low (non-lauric) or high (lauric) levels of C_{12} alkyl chain material (Kirk-Othmer, 2013). Lauric oils find a relatively limited application in commercial soap production, and majorly comprise of coconut and palm kernel oil. Non-laurics are much widely used, with tallow and palm oil accounting for the bulk. Their shortages have led to exploitation of various other oils depending on local availability such as lard, sunflower and castor oil (non-lauric), rosins and

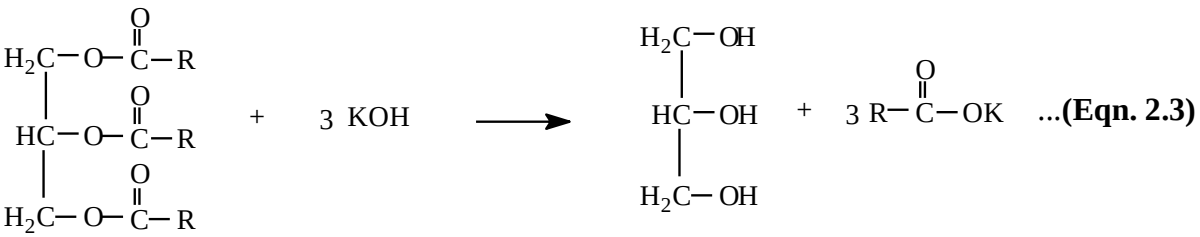
other natural raw materials. Laurics control lather but are uneconomical due to high water solubility of the bar (soap), therefore an appropriate ratio is necessary (Kirk-Othmer, 2013).

Fatty acids available for saponification may be derived from sterol esters, free fatty acids, glycolipids, phospholipids, partial glycerides or triglycerides (triacylglycerols) (Nielsen, 2010). The composition, quality and the purity of a particular chemical substance are very crucial and are determined using a series of chemical and physical tests.

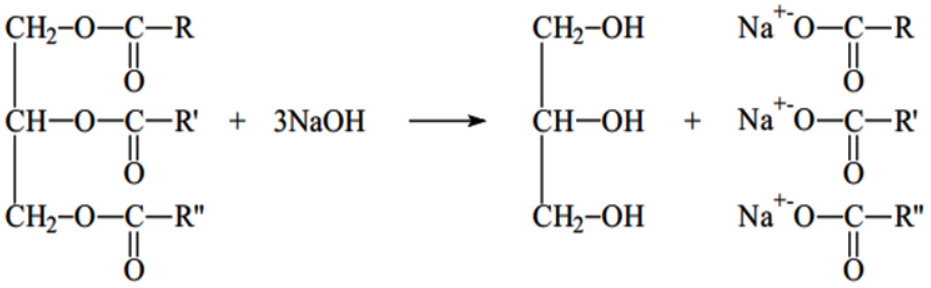
2.6.1 Chemical Parameters Used to Characterize Fatty Acids

2.6.1.1 Saponification value (SV)

This is defined as the quantity of potassium hydroxide (KOH) in milligrams required to completely saponify and neutralize the free fatty acids present in one gram of the fat or oil. The saponification process is also called alkaline hydrolysis of fats and oils because one of the products is the sodium or potassium salt of the higher fatty acid (soap). Saponification value is a very important parameter in the soap making process since it gives the quantity of alkali needed to saponify a specific quantity of oil. A schematic representation of saponification using KOH and lye is shown in equations 2.3 and 2.4 respectively.



...(Eqn. 2.3)



.....(**Eqn. 2.4)**

It is an index of the mean molecular weights of the triglycerides present in the fats or oils. Each triglyceride molecule releases 3 molecules of fatty acid salts. Hence, the mean molecular weight of the component fatty acids can be approximated by dividing the mean molecular weight of the triglyceride by 3. Lower saponification values will therefore reflect longer average fatty acid chain lengths (Nielsen, 2010) while high saponification values indicates a higher percentage of short chain fatty acids. This is explained by the lower number of carboxylic groups per unit mass. Generally, higher quality soaps are obtained from triglycerides with relatively higher saponification values.

2.6.1.2 Acid value (AV)

Acid value/ number is defined as the mass of potassium hydroxide (KOH) in milligrams (mg) that is required to fully neutralize the free fatty acids in 1 gram of a substance (Tawde et al., 2013). Also known as the acid number or neutralization number, it is an indicator of the degree of oxidation. Just as exemplified by corrosion, oxidation leads to deterioration of the good properties of a substance. The number serves as a quality test as it helps to determine the rate of deterioration.

2.6.1.3 Ester value (EV)

It is defined as the mass of potassium hydroxide (KOH) in milligrams required to combine with fatty acids that are present in form of glycerides in one gram of the fat or oil. It therefore gives a measure of the saponifiable glycerides in a fat or oil sample. It excludes the mass of

potassium hydroxide that is consumed in the neutralization process and is thereby calculated as the difference between the saponification and acid value for that sample.

2.6.1.4 Free fatty acids

Free fatty acids result from the breakdown of the fat or oil. The higher the quantity of free acids, the faster the oil or fat will undergo rancidity. The rancidity is caused by the hydrolytic or oxidative cleavage of triglycerides leading to the formation of free fatty acids (Mandhavi et al., 1995). This results in various breakdown products such as organic acids, alcohols, ketones and aldehydes which mainly renders a food commodity defective. This is mostly because the substance adopts a different flavor and aroma. Usually it becomes sour and foul smelling, but in special cases as in cheese ageing it gives desirable properties. Therefore variation in the value with storage is likely to happen as an indication of further hydrolysis of the triglycerides. The acids are naturally found in most fats and oils though in very low quantities. The value is mainly significant in qualifying frying oils, where a maximum of 5 is recommended. Free acids also interfere with trans-esterification. Therefore the number is used to assess the biodiesel yield of potential raw materials as well as a quality test for the biodiesel.

Usually, the percent free fatty acid value is calculated in terms of oleic acid. 1000g of an oil sample contains 282g of oleic acid, therefore:

$$\%\,FFA = \frac{(\mathrm{v}-\mathrm{b})\times \mathrm{N}\times 28.2}{W} \quad \textbf{(Eqn. 2.5)}$$

The difference between the test volume (v) and the blank (b) will give the volume of hydrochloric acid of normality N equivalent in saponifying a mass W of the oil.

From the acid value computation, we had

$$AV = \frac{(\mathrm{v}-\mathrm{b})\times \mathrm{N}\times 56.1}{\mathrm{W}} \quad \textbf{(Eqn. 2.6)}$$

Therefore for a particular type of fatty acid containing substance, we can easily calculate the percentage of free fatty acid by combining the two equations

$\% FFA = 0.503 \times AV$ ..... **(Eqn. 2.7)**

This gives an estimate of the amount of fatty acids that needs to be removed in the refining steps. Most fats and oils used in soap production have low values of % FFA, for example tallow has 2-4% FFA while coconut oil has a maximum of 3% FFA.

2.6.1.5 Glycerol

This is computed from the ester value. From the saponification reaction, 3 molecules of KOH (168g) produce a glycerin molecule (92g). Therefore, the percent glycerin can be calculated as:

$\% Glycerol = \frac{92 \times 100 \times \mathrm{EV}}{168 \times 1000} = 0.0547 \times \mathrm{EV}$ ..... **(Eqn. 2.8)**

Where EV is the ester value.

Dileesh et al. (2013) analyzed the saponification and acid values of various edible oils used in soap preparation and calculated the ester, percent free fatty acid and glycerol in them (Table 2.3).

Table 2.3: Saponification value, acid value, ester value, %FFA and % of glycerol of various oils

Oil (1 gm)	Saponification Value (mg)		Acid value (mg)	Ester value (mg)	% FFA	% Glycerol
	Obtained	Standard	Obtained			
Coconut oil	252	250-265	1.866	250.33	0.94	13.69
Sunflower oil	193.2	185-198	1.866	191.33	0.94	10.47
Olive oil	194.6	184-196	3.733	190.86	1.88	10.44
Mustard oil	166.6	166-175	3.733	162.86	1.88	8.90
Gingely (gesame) oil	190.4	188-193	9.333	181.07	4.72	9.90

2.6.1.6 Iodine value

Fats and oils are usually classified depending on the level of saturation of the fatty acid acyl groups; saturated, mono-unsaturated and poly-unsaturated. Iodine value or iodine number is used to express the specific level of unsaturation, and it gives the mass of iodine in grams that is taken up by 100g of the chemical substance. The principle behind this is that iodine saturates the fatty acid, therefore the higher the iodine value, the higher the number of double bonds and consequently the higher the level of unsaturation (Dunn, 2010). Iodine value helps to predict the effect of the chemical substance on the hardness of the soap, such that the lower the value the harder the soap. In soap manufacture, both saturated and unsaturated fatty acids are used, mostly as blends to achieve some desired hardness which is defined by the purpose (Kirk-Othmer, 2013).

2.6.2 Physical Parameters Used to Evaluate Fat and Oils

The main physical constants are pH, viscosity and density. The pH is directly related to the acid value of the material. These parameters are not of high consideration since they can be easily adjusted through incorporation of additives to achieve some specific desired formulation characteristics.

2.7 Detergents

These chemical products were introduced after World War II due to acute shortage of fats and oils, which were the predominant raw materials for the manufacture of soaps (Stamell, 2008). Detergents are complete cleaning products containing one or more surfactants, though the term is commonly used to refer to synthetic surfactants. Detergents have a better cleaning ability as compared to ordinary soaps, and can be used effectively on hard water since they dissolve all metal ions and are not detrimental to soft fabrics (no formation of hydroxyl ions on hydrolysis). However, they are generally less biodegradable (Srivastava and Jain, 2009).

Detergency is the main significance of surfactants, accounting for 15-25% of the total surfactant production (Fainerman et al., 2001). A detergent is composed of a surfactant and a wide range of additives to optimize the cleansing ability. These include:

i. Surfactants to cut greases and wet surfaces
ii. pH modifiers to stabilize other ingredients
iii. Abrasives to scour
iv. Builders to remove metal ions that form precipitates thus softening the water and prevent their deposition on fabric or machine.
v. Oxidants for bleaching action.
vi. Enzymes such as proteases, amylases and lipases to enhance degradation of organic stains.
vii. Foam enhancers to stabilize or counteract foam
viii. Aesthetic additives such as optical brighteners, fabric softeners, colours and perfumes.
ix. Fillers to fill up the weight after the appropriate dose of active ingredient.

2.8 Emulsions

An emulsion is a disperse system of two or more immiscible liquids. In other words, they are heterogeneous systems composed of at least one immiscible liquid finely dispersed in form of droplets into another. They are inherently thermodynamically unstable and therefore the components will tend to lower their interfacial energy by maintaining a minimum interfacial area (Particle Sciences- Drug development services, 2011). It is therefore very difficult to mix the liquids. When the heterogeneous mixture is shaken, spherical droplets are formed in order to maintain a minimum surface area and an interfacial tension is maintained between the component liquids.

Addition of an emulsifier lowers this interfacial tension thus enabling the miscibility of the liquids. They are often added to various formulations to enhance the ease of emulsion formation or to promote its stability. If the emulsifier is a surface active agent, its molecules will tend to have the polar ends oriented in the polar phase and the non polar ends in the non-polar phase. This will in turn lower the interfacial tension resulting in the miscibility of the component liquids (Totten et al., 2003).

Macromolecules such as gums and proteins also serve as emulsifiers since they greatly increase the stability of an emulsion. Hydrocolloids are amphiphilic polysaccharides such as modified starch and gum arabic. They are widely used to stabilize beverage emulsions through steric hindrance, viscosity effects and electrostatic interactions. They may therefore increase the viscosity of the solution or partition at the interface thereby forming a physical barrier that prevents coalescence. Fine solid particles can also adhere onto the surfaces of a lipid droplet establishing steric hindrance that stabilizes the emulsion (Chanamai and McClements, 2002). Gum arabic is a commonly used industrial emulsifier. It is used as a thickening, emulsifying, suspending and stabilizing agent in food, drink, pharmaceutical, cosmetic, paint and textile industries (Tadesse et al., 2007).

There are two main types of emulsions; water-in-oil (w/o) and oil-in-water (o/w), depending on the component comprising the continuous phase. However, an o/w emulsion may invert to a w/o emulsion after heating to the phase inversion temperature (PIT) (Chanasattru, 2008). Emulsions are widely employed in agricultural, food, petroleum, polymer, textile, paints and cosmetic industries. For the emulsion to achieve its performance requirements, maintaining its stability is a very important consideration in any formulation. Instability of an emulsion is caused by some physical properties such as coalescence, flocculation, creaming, Ostwald ripening and sedimentation (Chanasattru, 2008) as shown in Fig. 2.11.

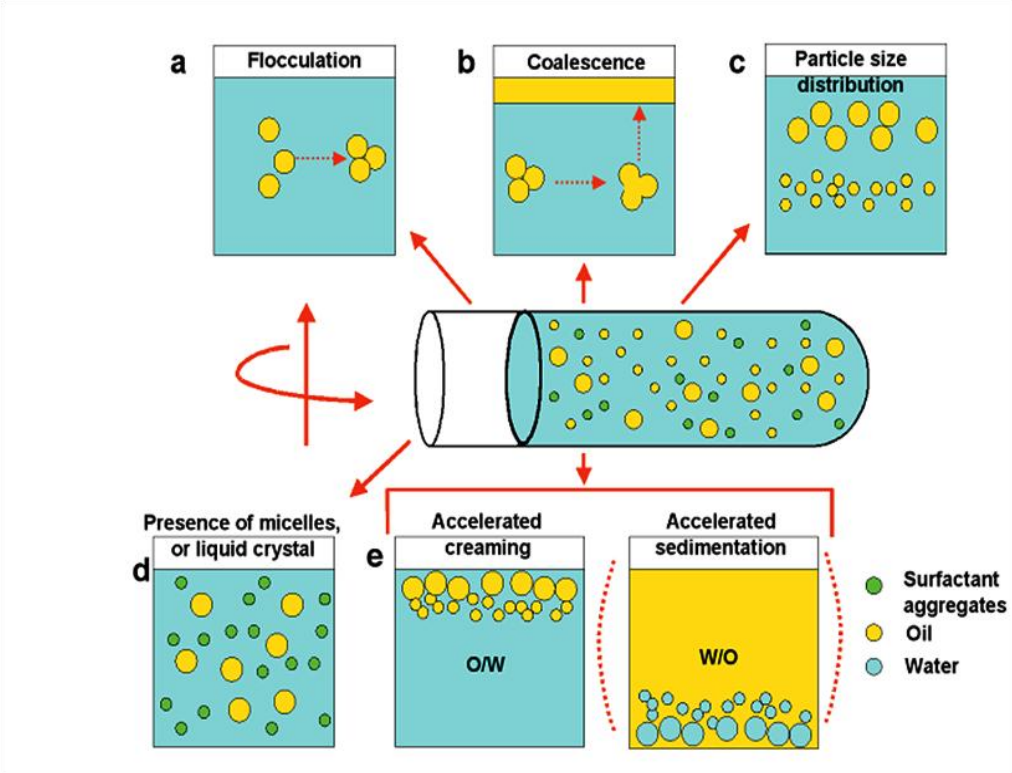


Figure 2.11: Possible physical instability properties after acceleration through centrifugation (Andre et al., 2003).

The resulting destabilization of a product exhibited by the separation of the respective phases will alter its performance which in turn may inflict major losses to the manufacturer as a result of consumer dissatisfaction, loss of brand loyalty, health effects, environmental concerns and product recall.

Despite being time consuming and often precisely incorrect, determination of the physical stability of the emulsion formulation is therefore quite necessary. This is done to assess both the short-term and long-term stability of the emulsion. The emulsifier concentration, pH and the emulsification temperature affects the overall stability of an emulsion.

The stability increases with increased emulsification temperature and pH increase between 3 and 6. Since according to Stoke's law (Eqn 2.9) the sedimentation rate of a droplet **(v)** is proportional to the square of its radius **(r)**, the stability of an emulsion against gravitational

separation **(g)** can be enhanced by shearing the emulsion through homogenization (Shachman, 2005). The densities **(D)** of the components should also be preferably close.

$$v = \frac{2gr^2(Do-Dw)}{9\mu} \quad \textbf{(Eqn. 2.9)}$$

Where **Do** is the density of the oil phase, **Dw** is the density of water phase and μ is the viscosity of the medium.

An emulsion has an extra energy compared to the non-emulsified components which brings about the thermodynamic instability. This is the reversible work (ΔW) done through emulsification process and is equal to the product of the interfacial tension (σ) and interfacial area (ΔA) as shown in equation 2.10.

$$\Delta W = \sigma \times \Delta A \quad \textbf{(Eqn. 2.10)}$$

Stability is achieved if **σ** and **ΔA** approaches zero.

The main methods used to determine stability are the measurement of zeta potential, analytical ultracentrifugation, laser light scattering, UV absorbance measurement and visual observation of phase separation. The choice of method depends on the type of emulsion and availability of pieces of equipment (Chiu and Jiang, 1999; Zahra et al., 2007). UV absorption and laser light scattering determines stability based on particle size distribution. In light scattering, a detector with a parallel monochromatic laser beam illuminates the emulsion through the fraunhofer diffraction principle (Zahra et al., 2007).

Chiu and Jiang (1999) analyzed the long-term stability of a vitamin E emulsion in a surfactant at room temperature using both UV measurement and light scattering methods. Absorption peaks were reportedly 293-299 nm before leveling off. A gradual increase in absorbance was observed with increase in concentration of the emulsifier and concentration of sodium chloride.

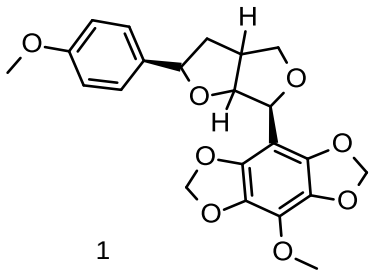
2.9 Myrrh Phytochemistry

Myrrh is the bitter resinous and aromatic exudates from the bark of a variety of species in the genus *Commiphora*. It has a distinct odour, which is due to the presence of furano-sesquiterpenes which are specifically found in this genus (Hanus et al., 2005). The chemical composition of myrrh oil has been studied extensively and monoterpenes, oxygenated sesquiterpenes and sesquiterpene hydrocarbons which differ consistently from one species to another have been identified. GC-based techniques mostly GC-ECD and GC-FTIR are commonly used in the analysis of myrrh essential oil (Mohamed et al., 2014).

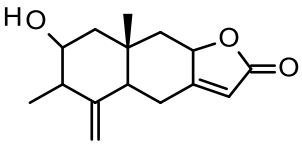
Tree resins generally contain terpenes and terpenoids, majorly consisting of diterpenoids and triterpenoids. The two types of compounds occur separately thus making resins to differ in properties. Myrrh and frankincense are characterized by triterpenoid compounds (Hanus et al., 2005).

Myrrh resin contains sterols, proteins and resin acids such as α-, β- and γ- commiphoric acid, commiphorin **(1)**, commiferin **(2)**, commiphorinic acid, β-heerambomyrrhol and heeraboresene (Iwu, 2013). Abourashed and Khan, (2013) also reported presence of α-heerambomyrrhol, campesterol, cholesterol, β-sitosterol **(4)**, α-amyrone and 3-epi- α-amyrin. The resin acids are very specific to the botanical source of a particular resin.

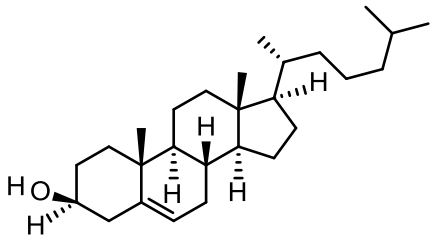
According to Selvamani (2010), latex from *Commiphora abyssinica* contains 6.5% sterols. Compounds isolated through fractionation include cholest-5-en-3β-ol **(3)**, β-sitosterol **(4)** and Δ-campestan-3β-ol **(5).**



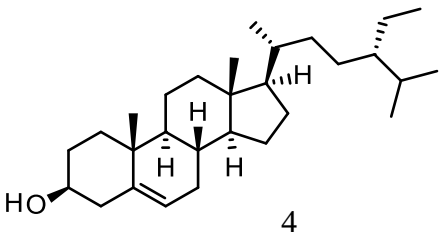
1



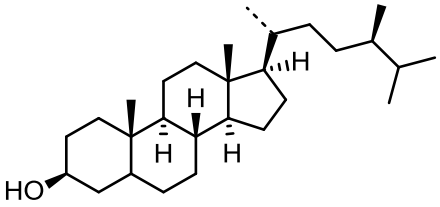
2



3

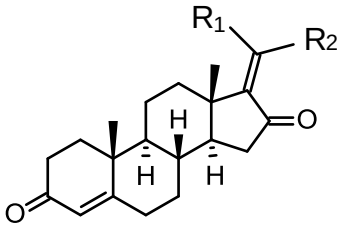


4



5

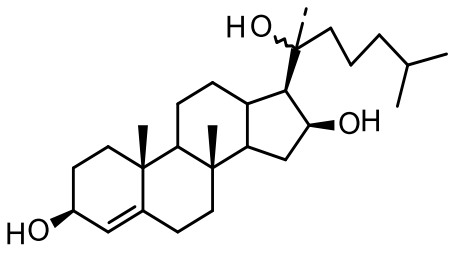
Other steroids that have been isolated from *C. mukul* resin, and the isomers Z- and E-guggulsterones **(6 and 7)** have received more focus due to their potent antitumor, anti-inflammatory and hypolipidemic qualities (Shen et al., 2012).



6 R_1=H, R_2=CH_3

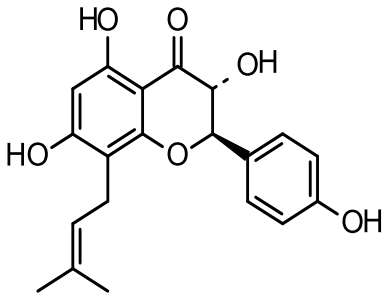
7 R_1=CH_3, R_2=H

Guggulsterol II (Cholest-5-ene-3,16,20-triol) **(8)** was isolated from the methanol extract of *C. mukul* resin.

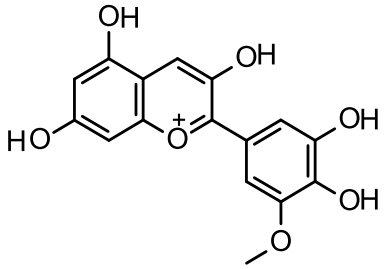


8

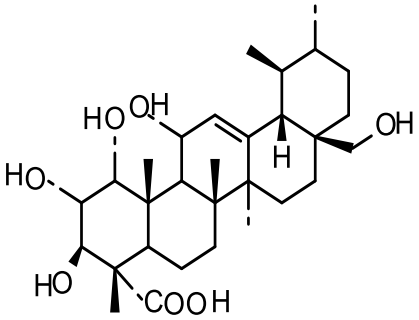
Some flavanones including phellamurin (from *C. africana*) **(9)** and 3,3',4',5,7-pentahydroxy-5'-methoxyflavylium (1+) from *C. Angolensis* **(10)** and methyl esters such as 1,2,3,11,28-pentahydroxy-12-ursen-23-oic acid (from *C. holziana*) **(11)** and 3-hydroxy-12-ursen-23-oic acid (From *C. pyracanthoides*) **(12)** have also been isolated from some *Commiphora* species.



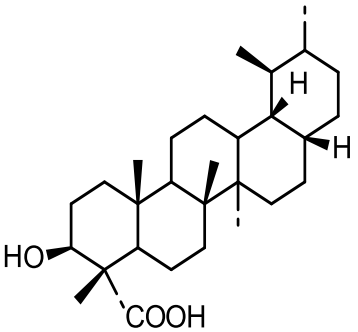
9



10



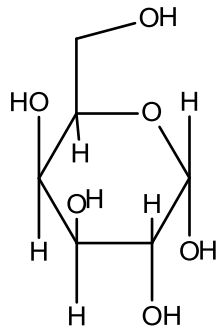
11



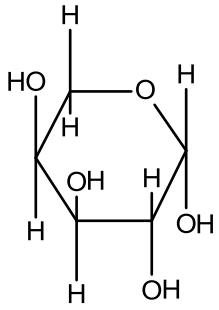
12

Commiphora resin generally has low toxicity due to volatile constituents, which involves allergy, nausea and locomotor activity (Shen et al., 2012).

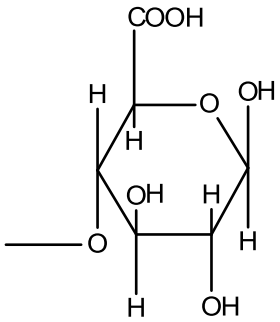
The water soluble gum consist of proteins and carbohydrates in form of polysaccharides such as D-galactose **(13)**, L-arabinose **(14)**, 4-O-methylglucuronic acid **(15)** and xylose **(16)** (Hanus et al., 2005; Cassileth et al., 2010; Iwu, 2013).



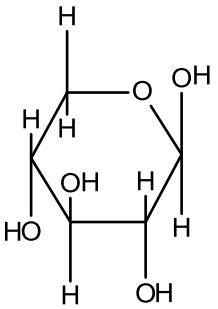
13



14



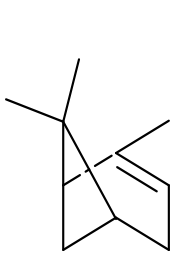
15



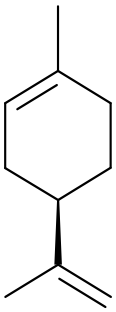
16

2.9.1 Myrrh volatile oil

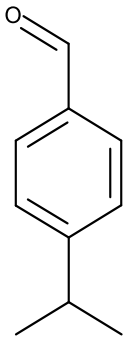
The analysis of the volatile oil from *Commiphora* species has identified monoterpenoids such as α-pinene, camphene, β-pinene, myrcene and limonene (Hanus et al., 2005). The first researchers on myrrh essential oil reported α-pinene **(17)**, limonene **(18)**, cuminaldehyde **(19)**, cinnamic aldehyde **(20)**, eugenol **(21)**, m-cresol **(22)** among others (Hanus et al., 2005). Current research has noted monoterpenoids including, camphene, pinene and myrcene. However, significant disparities have been observed in the composition of the volatile oil from various *Commiphora* species (Shen et al., 2012).



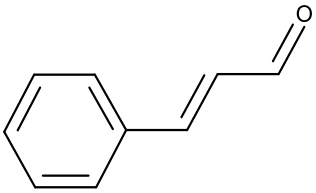
17



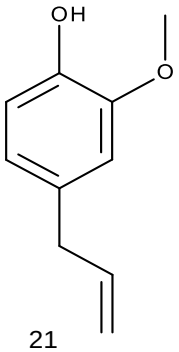
18



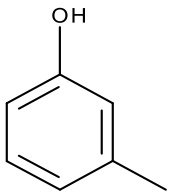
19



20



21



22

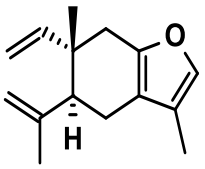
Singh (2010) stated that for myrrh to be considered authentic, it must contain at least 50 % furanoudesma-1,3-diene and 15 % lindestrene. According to his research, the other abundant compounds are furanodiene, germacrene B, isofuranogermacrene, 2-methoxy-furanodiene and β-elemene in concentrations of 8.8, 6.6, 6.1, 4.6 and 3.8 %, respectively.

A recent GC-MS analysis of *Commiphora Kerstingii* revealed the following composition: Octadecanoic acid, sucrose, α-camphorenal, nerolidolisobutyrate, diisopropenyl-1-methyl-1-vinyl cyclohexane, abietic acid, oleic acid, verbenol, naphthalene, limonene, 2,6-dimethylhepta-1,5-diene, 7-hexadecenal and 10-methyl-8-tetradecen-1-ol acetate (Ameh, 2014).

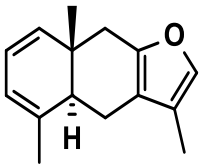
Various authors (Hanus et al., 2005; Abourashed and Khan, 2013) have reported the following compositions of myrrh essential oil as illustrated in Table 2.4.

Table 2.4: Composition of myrrh essential oil

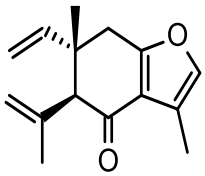
Compound	**% Composition**
Curzerene (23)	11.9
furanoeudesma-1,3-diene (24)	12.5
curzerenone (25)	11.7
2-methoxy-4,5-dihydrofuranodien-6-one (26)	0.2
dihydropyrocurzerenone (27)	1.1
lindestrene (28)	3.5
1,10(15)-furanodien-6-one (29)	1.2
3-methoxy-10-methylenefuranogermacra-1-en-6-one (30)	0.9
3-methoxy-10(15)-dihydrofuranodien-6-one (31)	1.5
3-methoxyfuranoguaia-9-en-8-one (32)	0.1
furanodien-6-one (33)	0.4



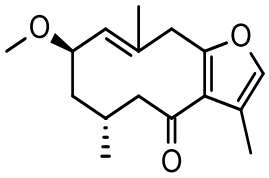
23



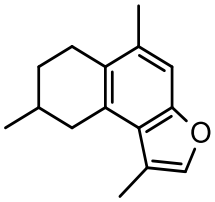
24



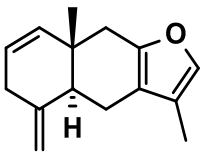
25



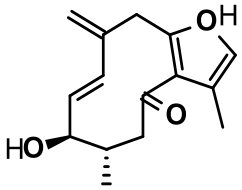
26



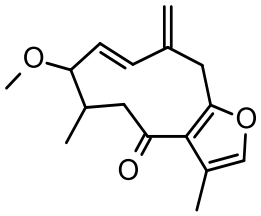
27



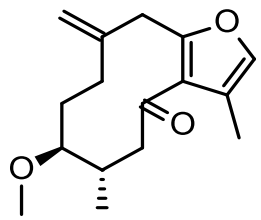
28



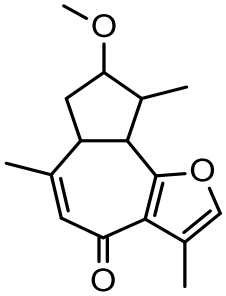
29



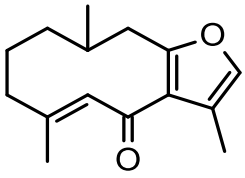
30



31



32



33

Hanus et al. (2005) reported a yield of 3.1% volatile oil from the hydrodistillation of myrrh from *Commiphora myrrha* (Nees) Engl. var. molmol. GC-MS analysis of this oil is given in Table 2.5.

Table 2.5: Composition of *C. myrrha* volatile oil

Compound	% Composition
δ-elemene	0.5
β-bourbonene	0.6
β-elemene	8.4
β-caryophyllene	0.7
γ-elemene	2.6
α-humulene	0.3
dehydroaromadendrane	0.1
9-epi-caryophyllene	0.4
γ-muurolene	0.3
alloaromadendrene	1.7
Curzerene **(23)**	40.1
γ-cadinene	0.8

Table 2.5 continued

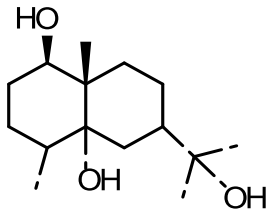
Compound	**% Composition**
δ-cadinene	0.3
β-sesquiphellandrene	0.2
selina-3,7(11)-diene	0.2
elemol	0.2
caryophyllene alcohol	0.4
caryophyllene oxide	0.2
cis-β-elemenone	0.8
furanoeudesma-1,3-diene	15.0
γ-eudesmol	2.7
furanodiene	1.1
7-epi-α-eudesmol	2.2
2-O-methyl-8,12-epoxy- germacra-1(10),4,7,11-tetraene, isomer I*	0.5
2-O-methyl-8,12-epoxy- germacra-1(10),4,7,11-tetraene, isomer II*	3.9
2-hydroxyfuranodiene	0.2
10-epi-γ-eudesmol acetate	0.3
2-O-acetyl-8,12-epoxy-germacra-1(10),4,7,11-tetraene, isomer I*	6.5
2-O-acetyl-8,12-epoxy-germacra-1(10),4,7,11-tetraene, isomer II*	0.3

*indicates that the correct isomeric form was not determined.

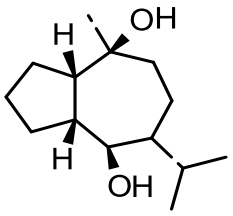
2.9.2 Sesquiterpenoids from myrrh

Sesquiterpenenoids from genus *Commiphora* are commonly structurally classified into germacrane, guaiane, eudesmane, elemane, cadinane, oplopane and bisabolane groups. Sesquiterpenoids such as β-elemene, α-copaene, α-humulene, β-selinene and germacrene

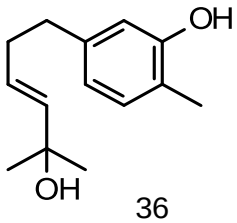
have been widely reported in myrrh essential oil. Examples are 1,5,11-eudesmanetriol from *C. opobalsamum* **(34)**, 4(15)-guaiene-6,10-diol from *C. guidotti* **(35)** and 1,3,5,9-bisabolatetraene-2,11-diol from *C. kua* resin **(36)** (Shen et al.,2012).



34

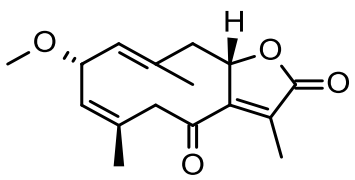


35

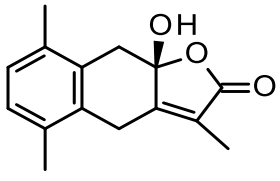


36

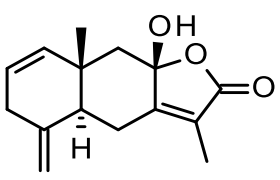
Several furanosesquiterpenoids including furanogermacrane, furanoeudesmanes, furanoguaiane, furanocadinane and furanoelemane have also been detected. In more recent research (Shen et al.,2012), some sesquiterpenoid lactones such as germacranolide **(37)**, eudesmanolides **(38 and 39)**, guaianolide **(40)**, elemanolide **(41)**, and cadinanolide **(42 and 43)** have been isolated from *C. opobalsamum* and *C. myrrha* and was a major advance in the phytochemistry of this genus. Diterpenoids are commonly isolated from *C. mukul* but of late some diterpenoids such as pimarane diterpenoid, sandaracopimaric acid **(44)** and abietane diterpenoids such as abietic acid **(45)** and dehydroabietic acid **(46)** have also been isolated from *C. myrrha* (Shen et al.,2012).



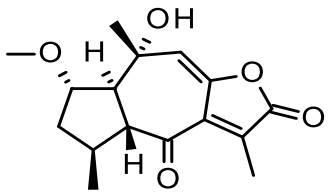
37



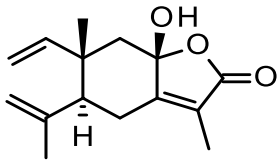
38



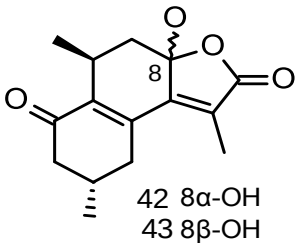
39



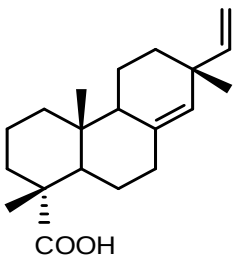
40



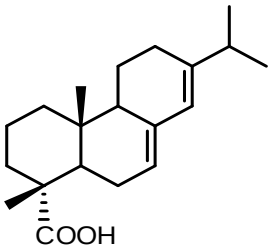
41



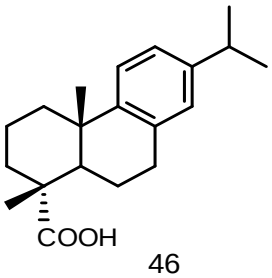
42 8α-OH
43 8β-OH



44

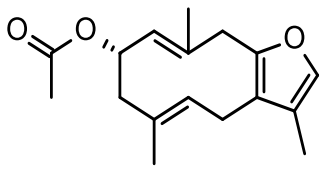


45

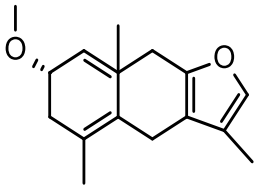


46

Other sesquiterpene compounds isolated from *C. myrrha* include furanodiene (isofuranodiene), and furanosesquiterpenoids include 2-O-acetyl-8,12-epoxygermacra-1(10),4,7,11-tetraene (2-acetoxyfuranodiene) **(47)** and 2-O-methyl-8,12-epoxygermacra-1(10),4,7,11-tetraene (2-methoxyfuranodiene) **(48)**.



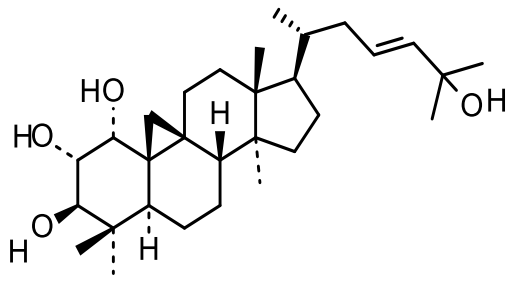
47



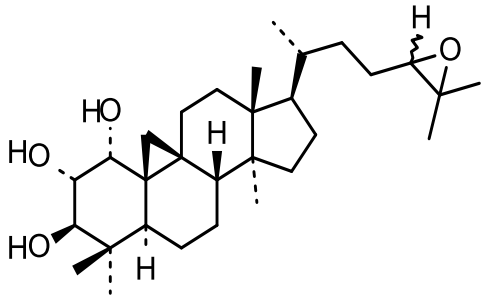
48

2.9.3 Triterpenoids from myrrh

Triterpenoids form the bulk of the secondary metabolites from *Commiphora* species and they include dammarane, cycloartane, polypodane, octanordammarane, ursane, oleanane, lupane, and lanostane. Compounds **(49)** and **(50)** are examples of cycloartane triterpenoids isolated from *C. myrrha* and *C. opobalsamum* (Shen et al., 2012).

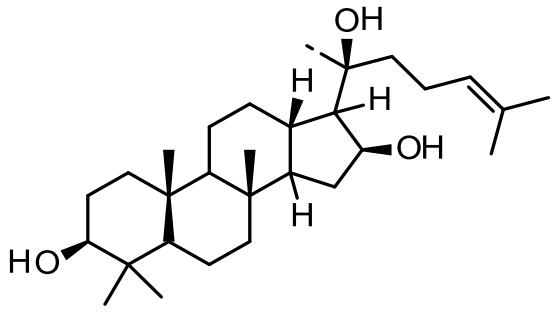


49



50

Dammar-24-ene-3,12,16,20-tetrol **(51)** is a dammarene triterpenoid that has been isolated from *C. confusa* and *C. kua*.



51

2.10 Anti-Microbial Activity of Myrrh Extracts

Plant-derived compounds offer a potential source of anti-microbial agents and other pharmaceuticals (Abdallah et al., 2009). The active compounds present in an extract exercise their anti-microbial effects through causing structural and functional destruction on the cell membrane of the microbe. Consequently, the composition, structure and functional groups in an extract are the key determinants of anti-microbial activity (Mohamed et al., 2014). Many oils also show anti-pathogenic properties (Hamid et al., 2011). Secondary metabolites such as alkaloids, flavonoids, terpenes, coumarins and phenolics are responsible for the bioactivity in medicinal plants. The demonstrated presence of these secondary metabolites scientifically validates the popular medicinal utilization of a plant (Swayamjot et al., 2005).

Myrrh exudates were widely used traditionally for medicinal applications in arthritis, hyperlipidemia, pain, wounds, fractures, blood stagnation and in formulation of Ayurvedic and Chinese medicine (Shen et al., 2012). These workers extensively compiled the pharmacological data from current research on this genus and reported a variety of bioactive molecules responsible for several effects such as anti-inflammatory, anti-tumor, antioxidant and antimicrobial activities.

Myrrh essential oil and crude extracts have exhibited various anaesthetic, cytotoxic, anti-inflammatory and anti-microbial biological activities (Mohamed et al., 2014). Beside the resin, the leaf, stem and bark from genus *Commiphora* also demonstrates anti-microbial potential (Shen et al., 2012). Various *Commiphora* species have considerable inhibitive activity against both gram positive and gram negative bacteria (El-Ashry et al., 2003). Abdallah et al. (2009) reported that methanol extracts of *C. molmol* exhibited significant inhibition of *S. aureus* and various strains of MRSA, with MIC values of 31.25 and 31.25-250 μg/ml, respectively, depending on the MRSA strain in question.

Due to their biological activity, they are used as additives in perfumes, cosmetics, phytotherapy, nutrition and pesticide formulating industries. Myrrh is also used therapeutically in aromatherapy. It is also added as an astringent in mouthwashes to treat mouth infections, ulcers, sore throat and inflammation of the pharynx (Capasso, 2003).

In this study, the methanolic, ethanolic and hexane extracts have been investigated for anti-bacterial effects against both gram-negative (*S. typhi, E. coli* and Shigella) and gram-positive bacteria (*S. aureus* and MRSA), and for anti-fungal effects against *Candida albicans, Trichophyton mentagrophytes* and *Microsporum gypseum* fungal strains.

CHAPTER 3

METHODOLOGY

3.1 Study Area

Gum resins used in this study were collected from Nolokwa, Kilonito and Kudu Hills, Central Division, Kajiado Constituency, Kajiado County (Fig. 3.1).

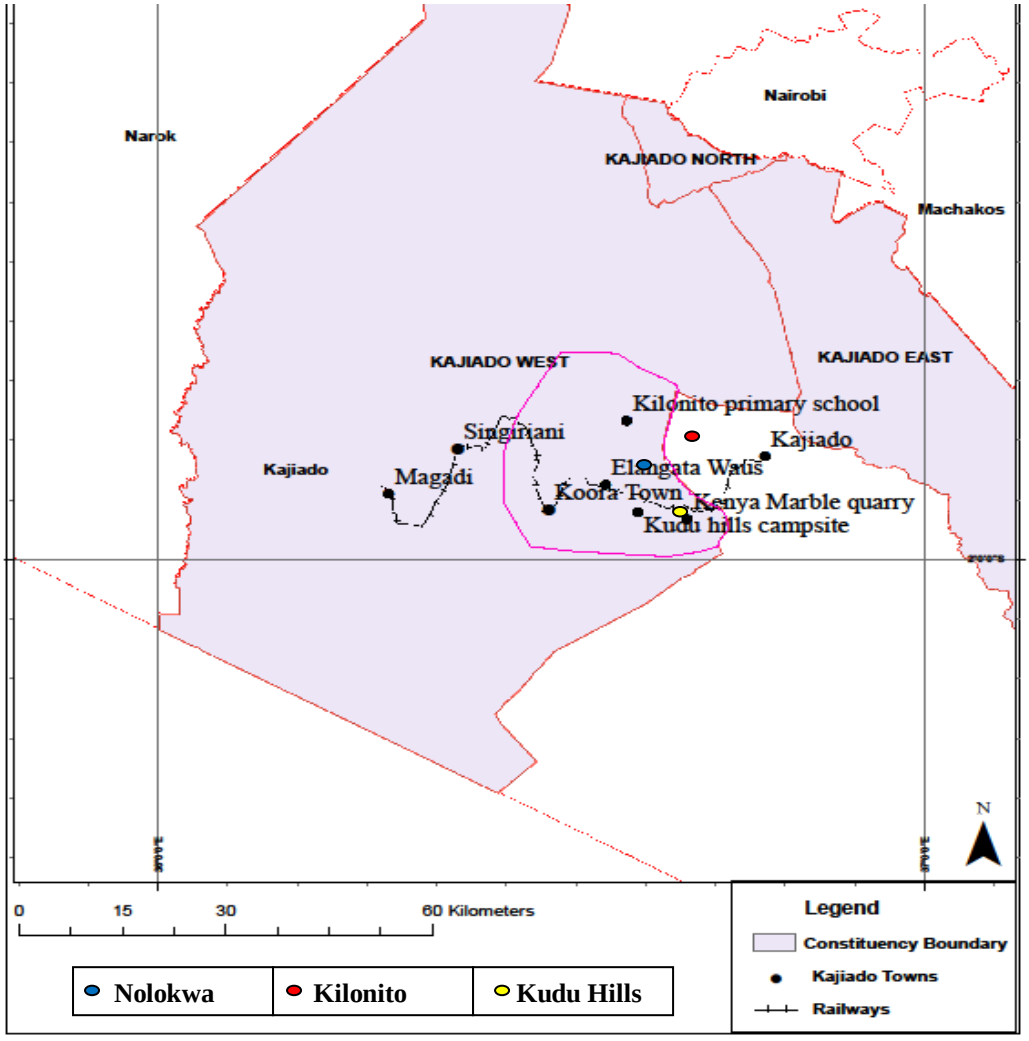


Figure 3.1: Map of the location of the sampling sites in Kajiado County, Kenya

Specific study sites were Nolokwa in Singirane Location, Kilonito in Kilonito Location and Kudu Hills in Elangata Waus Location. The sampling area was between latitudes E36°33′43′′ and E36°37′46′′ and between longitudes S01°49′02′′ and S01°55′24′′. It is predominantly a low-lying plain surrounded by hills and characterized by a hot and dry climate hence classified under arid and semi- arid lands (ASAL) of Kenya. Rainfall is generally low and erratic with an average of about 500 mm, occurring in two seasons; long rains between March and May and short rains between October and December (O'Mara, 2005).

This area is principally composed of black cotton soils (Bille, 1980). Despite a significant presence of medium to high fertility soils, the arable potential is greatly reduced by the hostile rainfall-temperature regime. The main economic activities are pastoralism and wildlife conservation to a small extent. There are few perennial streams and swamps that provide water for the Maasai community, their cattle and wildlife during the long dry season (Woodhouse et al., 2000). Selection of the study sites was done based on occurrence and geographical distribution of *Commiphora abyssinica* tree species. Two samples, one which was a more recent exudate and the other an old exudate (labeled Nolokwa 2) were collected from the Nolokwa study area.

3.1.1 Description of the sampling sites

The sampling sites are as described in tables 3.1, 3.2 and 3.3.

Table 3.1: Nolokwa sampling site

Elevation	4437 ft
GPS readings of location	E36°3′43′′, S01°53′00′′
General topography	Gentle slope
Area description	Located 35m from Kajiado- Namanga railway line, traversed by River Nolokwa.

Table 3.2: Kilonito sampling site

Elevation	4448 ft
GPS readings of location	E36°36′23′′, S01° 49′02′′
General topography	Gentle slope
Area description	Located 3 km from Kilonito Township

Table 3.3: Kudu Hills sampling site

Elevation	4448 ft
GPS readings of location	E36°37′46′′,S01°55′24′′
General topography	Gentle slope
Landmark	Located 100m West of Kudu Hills Camping Site.

3.2 Plant Material

Commiphora abyssinica oleo- gum resin exudates were randomly harvested from the trees. (See Fig. 3.2). They were in the form of light yellow to dark brown tears varying from slightly sticky to dry tears. The samples were packed in transparent polyethylene bags, sealed and coded.

3.2.1 Sample Preparation

Some of the gum resins were used as collected from the field (Fig. 3.2).

Figure 3.2: *C. abyssinica* tears

For some analysis, samples were air dried and then ground mechanically into a coarse product (Fig 3.3). Some of the ground gum resin was further ground into a powder using pestle and mortar and then mixed thoroughly to make a homogeneous sample stock. All samples were coded and stored in tightly sealed polyethylene bags at room temperature.

Figure 3.3: Ground and coded coarse sample

3.3 Analysis of Physicochemical Properties of the Gum Resin

3.3.1 Moisture content

Analysis of moisture content was performed according to Association of Official Analytical Chemists (AOAC) methods using clean, dry and pre-weighed watch glass. Approximately 5.000g of sample from each location was weighed on a watch glass in triplicate and dried in an oven (Memmert U400) at 105±2°C for 5 to 6 hours to constant weight.

$$\%moisture = 100\frac{W_0 - W_1}{W_0} \quad \text{..... (Eqn. 3.1)}$$

Where;

W_o= Original weight of sample in grams

W_1 = Weight of sample after drying

3.3.2 Ash content

Each oven-dried sample from the moisture determination was transferred onto platinum crucibles and placed in a programmable furnace (Nabertherm, LH15/14). The temperature was raised to and maintained at 550 °C for 1 hour. The sample was then cooled in a dessicator, weighed and reheated in the furnace for another 30 minutes. This cycle was repeated until the variation between two successive weights was less than 1mg. The final weight was recorded.

$$Ash\ content = 100\frac{W1}{W0} \text{..... (Eqn. 3.2)}$$

Where;

W_o= Initial weight of sample in grams

W_1= Final weight of the ash in grams

3.3.3 Nitrogen content

Nitrogen content was determined according to AOAC (1990). Approximately 1.000g of the pulverized sample was accurately weighed into micro Kjeldahl digestion flasks and about 2.000g of mixed catalyst (K_2SO_4, $CuSO_4$ and selenium) was added into the flask followed by 10ml concentrated sulphuric acid. The mixture was digested in a digestion block at 420°C for 2 to 3 hours until a clear solution was obtained. After cooling, the solution was diluted to 100ml using de-ionized water and 10ml of the diluted solution distilled with 8ml 40% NaOH using a nitrogen distiller (Gerhardt-Vapodest) as illustrated in Fig. 3.4. The ammonia released was bubbled into a receiver flask containing 10ml of 4% boric acid and some few drops of methyl red indicator. Presence of ammonia gas was indicated by change of the pink colour to blue. The solution in the receiver flask was then back titrated with 0.01N H_2SO_4.

$$\%N = \frac{(V_{H_2SO_4} - V_{BK}) \times 0.01\boldsymbol{N}\ H_2SO_4\ \times 14.007}{1g} \quad \text{..... (Eqn.3.3)}$$

Figure 3.4: Nitrogen distillation

3.3.4 Protein content

Protein content was determined on the basis of total nitrogen content (N). N is multiplied by a factor specific for the substrate under consideration, and where it's not available a factor of 6.25 is generally acceptable. In this case there was no data on amino acid analysis of *C. abyssinica* gum resin, and therefore a Jones factor of 6.25 was used as recommended (FAO, 2002) as shown in equation 3.4.

$$\% \, Protein = 6.25 \times \% \, N \quad \text{..... (Eqn. 3.4)}$$

3.3.5 Elemental analysis

For metal content determination, 5.000g of each sample was first ashed as in Sec. 3.3.2. The grey ash was then digested in 20ml 1:1 mixture of HCl and HNO_3 for about 1 hour in the hood until the solution was clear. The solution was diluted to 100ml with de-ionized water and filtered. Potassium, sodium, calcium, magnesium, manganese, iron, copper and zinc were then analyzed using atomic absorption spectrophotometer (Bulk Scientific *Model* 210VGP-USA).

3.3.6 Solubility in water

5.000g of the pulverized sample was dissolved in 20ml of distilled water. About 0.500g was then added successively with continuous shaking up to a total of 8gms, after which the addition was reduced to 0.200g. The saturation point was noted.

3.3.7 Physical properties

Five 1% aqueous solutions were prepared by dissolving 1.000g of pulverized sample in 100ml of de-ionized water. It was then filtered through Whatman No.1 filter paper. It was used for the analysis of pH, density, refractive index, optical rotation and viscosity as follows:

pH*:* A glass electrode microprocessor pH meter (HANNA 240) was first calibrated using buffer solutions. It was then used to measure the pH values of the gum resins solutions.

Density: A pycnometer (2ml) was cleaned, dried and weighed. It was then rinsed, filled up with the solution and reweighed. The pycnometer volume was confirmed by use of a 1000µl micropipette.

Refractive index: An Abbe refractometer (Zeiss) was used to determine the refraction of light by the solution.

Specific optical rotation: The specific optical rotation of the different gum resins was determined by analyzing the 1% (w/w) gum resin solutions at 20^{o}C in an ADP220 polarimeter (Bellingham+Stanley Ltd) using a 10 cm cell length.

Viscosity: An Ostwald viscometer (Technico) was used. The solution was sucked up the vertical capillary tube and then allowed to drop with gravity. The time taken for the level to pass point the mark was recorded. The kinematic viscosity was then calculated as in equation 3.5.

$$\eta_s = \frac{\eta_w \times \rho_s \times t_s}{\rho_w \times t_w} \quad \text{..... (Eqn. 3.5)}$$

Where,

η_w- dynamic viscosity of water (Pa.S)

η_s- dynamic viscosity of sample (Pa.S)

ρ_w- density of water (kg/m^3)

ρ_s- density of sample (kg/m^3)

t_w- drop time of water (s)

t_s- drop time of sample (s)

3.4 Extraction of Essential Oils, Resin and Gum Components

3.4.1 Essential oil extraction by hydrodistillation in a clevenger apparatus

About 200g of the ground gum resin of *C. abyssinica* was accurately weighed and transferred to a round-bottomed flask. 300ml distilled water was added and the flask connected to a clevenger apparatus fitted with a condenser as shown in Fig. 3.5. The flask was subjected to constant heating using a heating mantle for 6 hours. The apparatus was then rinsed with dichloromethane (DCM) and this was added to the distillate. The oil-water mixture was extracted with hexane and partitioned in a separating funnel. The aqueous phase was re-extracted twice with fresh aliquots of hexane for maximum extraction. The solvent was then removed using a rotary evaporator (Rotavapor 11, Buchi, Switzerland). The weight of the oil obtained was then determined. This was then repeated for samples from the other locations.

Figure 3.5: Hydrodistillation in a clevenger apparatus

3.4.2 Extraction of essential oil by steam distillation

About 200g ground gum resin was accurately weighed and placed in a round bottomed flask. A steam generator containing water was placed on a hot plate and the apparatus connected as shown in Fig 3.6. The condensed mixture of volatile oil and water was collected for six hours. The oil was separated using hexane as described in Sec 3.4.1. This was repeated for the other gum resin samples.

Figure 3.6: Steam distillation

3.4.3 Ethanol soluble matter

About 200g of the coarsely ground gum resin sample was macerated in 200ml ethanol. The mixture was heated in a water bath maintained at 80 $^{\circ}$C for 5 hours under reflux. The extract was decanted and another 200ml of ethanol added and the mixture heated for 3 more hours. This was repeated a third time for 2 hours, until no significant change in the colour of the solvent was observed. The extracts were then pooled, filtered through Whatman No.1 filter paper and the ethanol was separated by use of a rotary evaporator (Rotavapor 11, Buchi,

Switzerland). The extract was transferred to a pre-weighed evaporating dish to evaporate remaining solvent. The weight of the ethanol extract was then determined.

The above procedure was repeated with the finely ground samples except that only two extractions were done for 3 and 2 hours respectively as the extraction was completed under those conditions.

3.4.4 Water soluble matter

10.000g of the raw gum resin (finely homogenized) was dissolved in 100ml distilled water. It was then filtered using Whatman No.1 filter paper. The cake was re-extracted with 100ml water and filtered again. This was repeated for a third time and the filtrates were pooled and dried to constant weight at 105 $^{\circ}$C in a Memmert U400 oven. The percentage yield was then evaluated.

3.4.5 Water and ethanol insoluble matter

10.000g of the raw gum resin (finely homogenized) was dissolved in 200ml 50 % ethanol and soaked for 2 days. It was then filtered using a pre-weighed Whatman No.1 filter paper and the filter paper dried to constant weight at 105 $^{\circ}$C in a Memmert U400 oven. The mass of the insoluble matter was then evaluated as the difference between the final weight of the dried filter paper and the initial weight.

3.5 Physical Properties of the Ethanol Extracts

3.5.1 pH, density, refractive index, specific rotation and viscosity

These properties were measured as described in Section 3.3.7 for gum resin solutions.

3.6 Fatty Acid Characteristics of the Ethanol Extract

3.6.1 Determination of acid value (AV)

0.500 g samples were weighed into 250 ml conical flasks. Into each flask was added 50ml of neutralized alcohol, warmed gently and shaken well to dissolve the solid sample. Some few drops of phenolphthalein indicator were added and the solution titrated with 0.1N KOH. Acid value was then calculated as in equation 3.6

$$AV = V \times N \times \frac{56.1}{W} \quad \text{..... (Eqn. 3.6)}$$

where,

V is the amount of KOH (ml) consumed by the oil sample at the equivalent point

N is the normality of KOH

W is the weight of the sample

56.1 is the equivalent mass of KOH

3.6.2 Determination of saponification value (SV)

For each sample, 2.000g was dissolved in 5ml ethanol in a round bottomed flask after which 25ml of 0.5 N alcoholic KOH was added and the mixture shaken. The solution was then heated in a water bath under reflux for about 1 hour until it cleared. A blank was also refluxed. Some few drops of phenolphthalein indicator were added and the hot excess alkali titrated against 0.5 M HCl.

Saponification value (SV) was then determined using equation 3.7.

$$SV = (B - A) \times 0.5 \times \frac{56.1}{W} \text{..... (Eqn. 3.7)}$$

The difference between the test volume (A) and the blank (B) gives the volume of 0.5 M HCl equivalent to KOH used in saponifying a mass W of the oil while 56.1 is the molar mass of KOH.

3.6.3 Determination of ester value (EV)

Ester value was determined from the difference between the saponification value and the acid value as in equation 3.8.

$$EV = SV - AV \quad \text{(Eqn. 3.8)}$$

3.7 Comparison of Surfactant Properties of the Ethanol Extract and those of some Synthetic Detergent Ingredients

Three synthetic surfactants were used to investigate the amphiphilic and physical properties of the resin extract.

Linear alkyl benzene sulfonic acid (LABSA) popularly known as sulphonic acid is the most commonly used detergent ingredient. It is a strong organic acid which is reacted with a base such as caustic soda to form the sulphonate as shown in equation 3.9.

$$RC_6H_4SO_3H + NaOH \rightarrow RC_6H_4SO_3Na + H_2O \quad \text{(Eqn. 3.9)}$$

It is a powerful wetting agent and highly biodegradable. It also possesses strong foam power though the foam is not very stable and therefore foam boosters are often added in formulations containing the sulphonate.

Sodium lauryl ether sulphate/ sodium laureth sulphate (SLS) has a lower wetting power than LABSA.

Cocamide diethanolamide/ cocamide DEA (CDE) is a coconut fatty acid diethanolamide, prepared by reacting fatty acids from coconut oil with diethanolamine. It is mostly used as a foam booster and stabilizer as well as to increase the viscosity of a detergent formulation.

3.7.1 Surface tension

For each of the samples, 100ml of 1mg/ml solutions was prepared by dissolving 100 mg of the sample in 100ml water in a 100ml volumetric flask. Serial dilutions were then made to obtain solutions of 80, 60, 40, 20, 10 and 5 mg/100ml. The solutions were aged for 30 minutes and then the surface tension was determined at 22°C using a stalagmometer. Surface tension was calculated from the number of drops using the formula illustrated in equation 3.10.

$$\Upsilon_1 = \Upsilon_2 \frac{\rho_1}{\rho_2} \times \frac{n_2}{n_1} \quad \text{(Eqn. 3.10)}$$

Where ρ_1 is the density of sample, ρ_2 is the density of water, n_1 is the number of drops of the sample, n_2 is the number of drops of water, Υ_1 is the surface tension of the sample and Υ_2 is the surface tension of water at 22 °C.

3.7.2 Critical micelle concentration (CMC)

Surface tension was plotted against concentration to obtain a smooth curve. Two extreme tangents to the curve were drawn and their point of intersection determined as the critical micelle concentration point. However due to difficulties in this method, another technique was adopted. The increment of the number of drops with concentration ($\Delta n/\Delta c$) was evaluated and plotted against concentration (C). The first derivative was obtained as a peak and this was taken as the CMC (Hadkar, 2008).

3.7.3 Foam analysis

The shake foam test conducted without soil was used to test the foam volume (Lai, 2006). 10ml of solutions of varying concentrations (0.005 %- 0.1 %) were placed in a 100ml graduated cylinder and shaken vigorously for one minute. The foam volume was observed immediately and after five minutes and recorded in cubic centimeters.

Foaming capacity (power) or foaminess (E_f) was calculated as in equation 3.11.

$$E_f = \frac{V_f - V_1}{V_f} \qquad \text{..... (Eqn. 3.11)}$$

Where V_f is the total volume after foaming and V_l is the initial volume

Foam stability was obtained as the fraction of the foam height after 5 minutes to the maximum foam height, commonly referred as the R5 value (Lunkenheimer and Malysa, 2003).

3.7.4 Spreadability

Floratech test method and AOCS Official method for spreadability testing (Method number APP09) by determining surface area was used. A drop of distilled water was applied on a clean filter paper (Whatman No. 1, diameter 125mm) using the common laboratory dropper on a smooth glass surface. The average diameter after spreading for 60 seconds was recorded and used to calculate the wetted area. Spreadability was calculated as a fraction of the area of each of the solutions relative to that of water.

3.7.5 Cloud Point

1% w/v solution was put in a boiling tube immersed in a water bath. A thermometer was inserted in the boiling tube and the temperature of the water bath gradually raised until the solution turned cloudy.

3.8 Emulsifying Properties of Ethanol-Insoluble Water-Soluble Gum

In the preparation of the emulsion, the dry gum method was used (Fig 3.7).

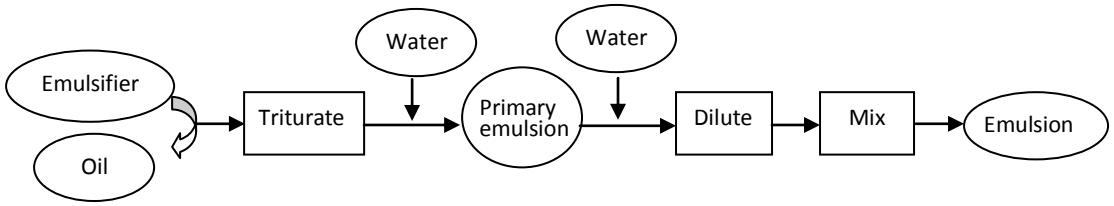


Figure 3.7: Flow-chart of the emulsion preparation by the dry gum method (Courtesy of Shenyang Pharmaceutical University Pharmaceutical labs)

Pure sunflower oil (Rinsun- Kapa Oil Refineries Ltd) was thoroughly mixed with distilled water and the water extract in the ratio 4:2:1, respectively. This solution was then diluted to 100ml to make the primary o/w emulsion. After homogenization, 10 ml was drawn using a pipette into a volumetric flask and diluted to 100ml to make a solution of 0.1 %. This was then serially diluted to obtain various concentrations: 0.05, 0.025 and 0.0125 %. These solutions were homogenized through vigorous shaking and the absorbance was then analyzed after 24 hours using a UV/VIS/NIR spectrophotometer (Solidspec-3200 DUV) at the range 200-500nm. The peak absorbance was observed between 200 and 214 nm. The emulsion stability index (ESI) was calculated as the ratio of each solution's peak absorbance to the peak absorbance of a freshly prepared sample (0.1 %) at room temperature.

To establish the effect of pH and temperature, the stability of the 0.1 % sample was tested as above at pH 2 and 12 and after heating to 40, 60 and 80 $^{\circ}$C respectively.

3.9 GC-MS Characterization of the Crude Extracts

3.9.1 Sample preparation

Three, 10.000±0.001 g samples were accurately weighed into three 250 ml conical flasks. The samples were macerated for three days separately with 150ml of three different solvents namely methanol, ethanol and hexane in order of decreasing polarity. The extracts were then filtered through Whatman filter paper No.1 before being concentrated in a rotary evaporator

(Rotavapor RII, Buchi, Switzerland). The resinoids were left open to allow any remaining solvent to evaporate. The samples were later transferred to vials and stored for crude extract analysis.

3.9.2 GC-MS analysis

The GC-MS analysis was carried out at the Department of Chemical and Process Engineering, University of Surrey, UK, using an Agilent Technologies 7890A GC system incorporated with an Agilent Technologies 5975C XL EI/CI MSD mass spectrometer with a triple axis detector. The GC column used was an HP-SMS of length 30m, internal diameter of 0.25 nm and film thickness of 0.25 microns. 2 µl of the *C. abyssinica* analyte were injected in the ion source maintained at a temperature of 250°C. The detector temperature was set at 230°C. Helium was used as the carrier gas.

The components of the analytes were identified through matching their retention indices and mass spectra to those of standards from the National Institute of Science and Technology (NIST) library database.

3.9.3 Sample preparation

About 100g of the coarsely crushed sample was macerated in separate conical flasks in methanol, ethanol and hexane in order of decreasing polarity. The extraction was carried out at room temperature for 48 hours with occasional shaking. The extracts were then filtered through Whatman No. 1 and the solvent separated by use of a rotary evaporator (Rotavapor RII, Buchi, Switzerland).

3.9.4 Bioassay isolates

The bioassay evaluation was carried out in the mycology laboratory, Centre for Respiratory Diseases Research (CRDR), Kenya Medical Research Institute (KEMRI). Test extracts were

tested against reference and clinical isolates of selected pathogenic bacteria and fungi. The selection was done depending on susceptibility to *Commiphora* extracts and availability. The micro-organisms for the evaluation were from the American Type Culture Collection (ATCC) as well as collections of culture from KEMRI. The selected test strains were the common pathogens associated with infectious diseases such as *E. coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, *Candida albicans* ATCC 90028 and clinical isolates of *Trichophyton mentagrophytes, S. typhi*, Shigella and *Microsporum gypseum.* A methicilin-resistant *Staphylococcus aureus* (MRSA) strain was also included. The clinical isolates were culture collections at the mycology laboratory, KEMRI.

3.9.5 Disk diffusion susceptibility testing (Kirby-Bauer method)

In-vitro antimicrobial activity was done according to Kirby Bauer disk diffusion method (Bauer et al., 1966) according to the Clinical Laboratory Standard Institute (CLSI) quality controlled procedure (NCCLS, 2002). A solution of 0.500 g of each crude extract dissolved in 1ml of dimethyl sulphoxide (DMSO) was prepared. Mueller Hinton agar was used as the bacterial culture medium. 19.000g of the medium was dissolved in 500 ml distilled water and then autoclaved at 121^{o}C for about 15 minutes for sterilization. After cooling, 20 ml of the media was then distributed evenly in sterilized Petri dishes. This preparation was used to culture bacteria for 24 hours while potato dextrose agar (PDA) was used to culture fungi for 72 hours.

0.5 Macfarland suspension standards were prepared using the test organisms which were then inoculated aseptically onto the plate containing Mueller Hinton agar and PDA, respectively by swabbing with a sterile cotton swab. Using a sterilized paper punch, 6mm diameter discs were made from a Whatman No. 3 filter paper and placed on an empty sterile Petri dish. They were then impregnated with 10µl of the crude extracts and aseptically placed

onto the inoculated media using sterilized forceps. Sterilization was achieved by rinsing in ethanol followed by heating. Chloramphenicol was used as the positive control for antibacterial and nystatin for antifungal assays. DMSO and distilled water were used as the negative control for both assays respectively. The plates were left for some time to diffuse and then incubated in an inverted manner for 24 hours at 37°C for bacteria and 72 hours at 30 °C for fungi.

The zones of inhibition were measured in millimeters on the plate underside using a ruler and the readings recorded. These were used as indicators of activity.

3.9.6 Minimum inhibition concentration (MIC)

The extracts which exhibited significant activity were subjected to MIC assay using the micro-well dilution method (Karaman et al., 2003). The wells in the first row were inoculated with the stock solution of 0.5g/ml. Serial dilution was then done using nutrient broth to make solutions ranging from 500.00-7.81mg/ml in the wells in the consequent rows. The microplates were then incubated at 37°C for 24 hours under aerobic conditions. The MIC was taken as the least concentration to show activity.

CHAPTER 4

RESULTS AND DISCUSSION

4.1 Physicochemical Parameters

4.1.1 Moisture content

The moisture content from the four study areas was in the range 8.90-10.59 % as shown in Table 4.1. The two gum resins from Nolokwa had values 8.90±0.40 and 9.15±0.35 % slightly lower than those from Kilonito (10.59±0.60 %) and Kudu Hills (10.28±0.40 %).

Table 4.1: Moisture content of the raw gum resins

Location	% moisture content		
	After collection	**After 3 months storage**	**After 4 months storage**
Nolokwa	9.15±0.35	7.80±0.15	6.54±0.25
Nolokwa 2	8.90±0.40	7.52±0.30	6.48±0.20
Kilonito	10.59±0.60	7.58±0.34	6.10±0.08
Kudu Hills	10.28±0.40	7.87±0.45	6.87±0.62
Average ±SD	9.73±0.59	7.69±0.12	6.50±0.01

The variations can be attributed to other factors such as the degree of vitrification, environmental conditions and picking time (López-Franco et al., 2011). These values are consistent with values from the same gum resin as obtained by Mwendwa, 2007 (10.07 %) and Kyalo, 1998 (10.33 %). A study on a gum resin from a related species, *Commiphora africana*, reported a moisture content of 10.6±0.04 % (Gundidza et al., 2011). Low moisture content is typical of gum resins. However, gums have relatively higher moisture contents, for example gum arabic has a recommended moisture range of 13-15 % (FAO, 1990).

Investigation of the moisture content of a potential industrial material is of economic significance since its application will highly depend on the optimization of the relevant production procedures such as drying, grinding and packaging. (Gundidza et al., 2011).

When the gum resins were ground and stored at room temperature, they were found to lose moisture with time to an average value of 7.69 % and 6.50 % after 3 and 4 months respectively. The low values further underscores reduced susceptibility of the gum resin to microbial degradation.

4.1.2 Ash content

The ash content represents the quantity of inorganic matter. The inorganic matter content is a good quality parameter and the low value characterizes a high degree of purity (Glicksman, 1969). As shown in Table 4.2, the gum resin from Kudu Hills had the highest ash content of 3.04±0.10 %. The three other resins had values between 2.24 and 2.56% (Nolokwa 2.31±0.05, Nolokwa 2 2.56±0.09 and Kilonito 2.24±0.05 %).

Table 4.2: Ash content of the raw gum resins

Location	**Sample (g)**	**Ash (g)**	**% Ash content**
Nolokwa	5.000	0.1155±0.0025	2.31±0.05
Nolokwa 2	5.000	0.1279±0.0045	2.56±0.09
Kilonito	5.000	0.1122±0.0025	2.24±0.05
Kudu Hills	5.000	0.1520±0.0049	3.04±0.10
Average ±SD			2.54±0.02

These values show consistency with those of Kyalo, 1998 (2.98 %) on the same species. The high ash content in the Kudu Hills gum resin is attributed to the relatively high content of insoluble matter as will be seen later in the yield comparison.

Ash content is used to assess the purity of gum arabic and the range given according to international specifications is 2-4 %. Following this criteria, the *C. abyssinica* gum resin from the study areas can be said to be clean since the impurity levels are within the lower limit. They illustrate a low content of foreign matter, salts of potassium, calcium and magnesium, and acid insoluble matter. The ash content is below the maximum recommended limit for use in food and pharmaceutical formulations (≤4 %) (FAO, 1990).

4.1.3 Nitrogen and protein content

The nitrogen content and the corresponding protein values are shown in Table 4.3. When compared to moisture content given in Table 4.1, the protein content decreased as moisture content increased.

Table 4.3: Nitrogen content and Protein value of the raw gum resins

Location	**Vol H_2SO_4 Sample (ml)**	**Vol H_2SO_4 Blank (ml)**	**Vol H_2SO_4 (ml)**	**Value (%)**	
				Nitrogen	**Protein**
Nolokwa	11.7±0.3	0.3	11.4±0.3	1.60±0.04	10.00±0.25
Nolokwa 2	13.1±0.4	0.4	12.7±0.4	1.78±0.05	11.13±0.31
Kilonito	10.7±0.4	0.3	10.4±0.4	1.45±0.05	9.06±0.31
Kudu Hills	11.7±0.4	0.3	11.4±0.4	1.59±0.05	9.94±0.31
Average ±SD				1.61±0.12	10.03±0.78

Nitrogen content was in the range 1.45±0.05 to 1.78±0.05%. Nolokwa 2 gum resin had the highest nitrogen content of 1.78±0.05 %, while Kilonito had the lowest value (1.45±0.05%). Nitrogen content obtained was converted to % protein after multiplying the nitrogen content by a Jones factor of 6.25 (FAO, 2002). This was consistent with the results on myrrh resin from *Commiphora myrrha* by Yasser et al., (2013), where he reported a nitrogen value of

1.69% and a protein content of 10.45%. A protein content of 10% from myrrh resin has also been reported (Wiendl et al., 1995). *Commiphora abyssinica* gum resin was therefore found to have similar nitrogen content as myrrh from *Commiphora myrrha*.

4.1.4 Elemental analysis

A total of 8 metal elements were analyzed and their concentrations were as shown in Table 4.4. The metal composition in the four gum resins was fairly consistent. Calcium and potassium content were conspicuously high, with average values of 310.66 and 81.36 mg/kg.

Table 4.4: Results of elemental analysis of the raw gum resins

Cation	Composition (mg/kg)			
	Nolokwa	**Nolokwa 2**	**Kilonito**	**Kudu Hills**
Magnesium	4.48	4.48	4.48	4.51
Potassium	63.67	113.56	45.37	102.82
Calcium	399.04	398.14	201.65	243.81
Zinc	1.93	0.94	ND	0.10
Iron	1.70	1.26	2.55	4.00
Copper	0.70	1.12	0.62	0.63
Manganese	1.00	1.08	2.43	1.77
Sodium	3.01	3.09	2.27	2.67

ND: Not detected

This can be attributed to the nature of the soils in the area. Limestone is mined in this area which explains the high calcium value. Kilonito which was the farthest location from the limestone mining area had the lowest concentration of the two metals.

The high content of calcium and potassium coupled with the low ash content implies minimal amounts of foreign matter and acid insoluble matter. The concentration of magnesium was

also relatively high, with an average value of 4.49 mg/kg. The three metals (Ca^{2+}, K^{+} and Mg^{2+}) improve dental remineralization thus preventing formation of caries (Limeback, 2012). Use of this gum in food formulations would therefore have an added benefit. The other metals that were analysed were found to be in relatively low concentrations; Na (2.27-3.09 mg/kg), Zn (ND-1.93 mg/kg), Mn (1.00-2.43 mg/kg) and Fe (1.26-4.00 mg/kg). Such quantities are satisfactory since minerals are important for maintenance of health and development. Iron for instance is an important constituent of blood and muscles.

4.1.5 Physical properties

Other physical parameters analyzed included pH, optical rotation, refractive index, density and viscosity of 1 % solutions as well as solubility in water. Results for these physical properties are shown in Table 4.5.

4.1.5.1 pH

The gum resins formed weak acidic solutions, within a pH range of 5.20-5.31. These results were consistent with 5.35 obtained by Mwendwa (2007) and 5.39 by Chesori (2008).

4.1.5.2 Solubility

The gum resins showed high solubility (average 49 %), though lower than the 60 % w/v for gum arabic (Ahmed et al., 2009). Previously, a solubility of 46% w/v has been reported for this gum resin (Kyalo, 1998; Mwendwa, 2007). This is similar to the solubility of the Kudu Hill resin, and therefore the differences could be due to variations in locations.

4.1.5.3 Viscosity

The viscosity of the aqueous gum resin solutions in all locations increased from that of water by a small margin, averaging to 9.867×10^{-3} Pa.s

Table 4.5: Physical properties of gum resin

Location	Physical Property					
	pH	**Specific rotation (^{0})**	**Refractive index**	**Density (kg/m^3)**	**Viscosity (Pa.s)×10^{-3}**	**% solubility in water**
Nolokwa	5.31	-49	1.334	998.4	9.889±0.013	50.00±0.00
Nolokwa 2	5.31	-51	1.334	998.4	9.672±0.021	50.00±0.00
Kilonito	5.26	-44.5	1.334	998.3	9.754±0.019	50.00±2.25
Kudu Hills	5.20	-49.5	1.334	998.3	10.154±0.040	46.00±2.00
Average±SD	5.27	-48.5	1.334	998.4	9.867±0.138	49.00±2.00
Distilled water	5.82	0	1.333	997.1	8.937	N/A

4.1.5.4 Optical rotation

All the gum resins were optically active, and were found to be levorotatory. The values were in the range -44.5^0 to -51^0 which was consistent with a previous report (-45.5^0) on the same resin (Kyalo, 1998). Thus, *Commiphora abyssinica* gum resin has a higher specific rotation than gum arabic, whose specifications is from -26^0 to -34^0.

4.2 Extraction Yields

4.2.1 Essential oil

The yield of essential oil using steam distillation (1.31-1.87%) was higher than through hydrodistillation (1.12-1.51 %). These results are shown in Table 4.6.

Table 4.6: Yield of essential oil

Location	Sample (g)	Mass of oil (g)		% yield	
		Hydro-distillation	Steam distillation	Hydro-distillation	Steam distillation
Nolokwa	200.00	2.6398±0.1174	3.2437±0.2346	1.32±0.06	1.62±0.12
Nolokwa 2	200.00	3.0186±0.2389	3.4412±0.1782	1.51±0.12	1.72±0.09
Kilonito	200.00	2.7350±0.2221	3.7434±0.3328	1.37±0.11	1.87±0.17
Kudu Hills	200.00	2.2374±0.1656	2.6282±0.2167	1.12±0.08	1.31±0.11
Average ±SD				1.33±0.13	1.63±0.06

This was consistent with the documented fact that steam distillation is a better method of extraction than hydro-distillation in terms of quantity and quality (Swift, 2002). The yield is a function of location, with Kudu Hills producing a gum resin with the lowest content of essential oils by both techniques. The maximum yield from *C. abyssinica* gum resin from the study area is less than the 2-10 % from most myrrh producing species (Hanus et al., 2005) which indicates a lower quality gum resin from *Commiphora abyssinica*. The variations in yield with method and location are illustrated in Fig 4.1.

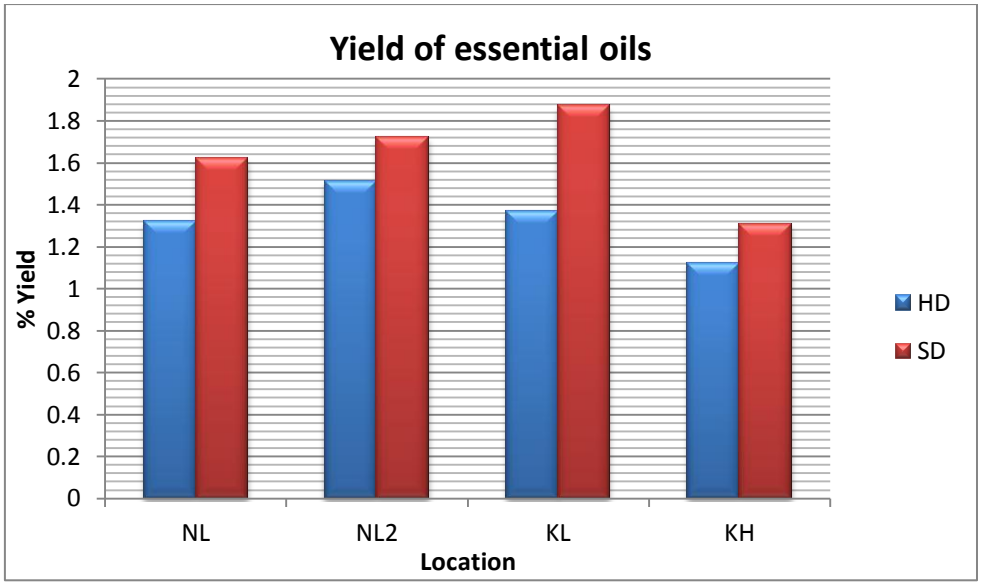


Figure 4.1: Yield of essential oil from the various locations by hydro-distillation and steam distillation

However this is not odd since another species from the same category (*C. mukul*) was found to contain 0.4 % essential oils (Hanus et al., 2005). Both distillation methods yielded average values higher than the 1.1 % reported for *Commiphora abyssinica* from Yemen (Ali et al., 2009).

4.2.2 Ethanol soluble matter

Small particle size speeds up the rate of extraction therefore, the yields from the fine samples could be taken as the maximum yield. Significant variations in composition of ethanol soluble resin were observed in the various study sites. The highest yields of ethanol resinoid were observed for Nolokwa 2 (47.79 %) and Kilonito (46.52 %). Nolokwa had a lower yield of 37.65 %, while Kudu Hills had a relatively low content of 26.37 %. This is illustrated in Table 4.7.

Table 4.7: Percentage of resinoid

Location	Mass of sample (g)	Mass of extract (g)		% yield	
		Coarse sample	Fine sample	Coarse sample	Fine sample
Nolokwa	200.00	66.48±1.32	75.30±2.07	33.24±0.66	37.65±1.04
Nolokwa 2	200.00	94.82±2.23	95.58±1.94	47.41±1.12	47.79±0.97
Kilonito	200.00	88.26±1.39	93.04±1.35	44.13±0.70	46.52±0.68
Kudu Hills	200.00	49.56±1.21	52.74±1.44	24.78±0.61	26.37±0.72
Average ±SD				37.39±7.09	39.58±5.80

The inconsistencies on gum properties with location and/or tree have been reported and according to Chikamai and Banks (1993), the type of soils, age of tree and local climate are some of the influencing factors. The same trend when using coarse samples was observed, with individual levels of embrittlement on heating accounting for the difference in percentage yield. Nolokwa 2 gum resin lumps which had the least moisture content completely disintegrated and the yields were similar for fine and coarse samples. All the others (Nolokwa, Kilonito and Kudu Hills) did not fully disintegrate even after 10 hours of heating. Nolokwa gum resin had the highest variation in yield of the coarse and fine samples with 33.24 and 37.65 %, respectively. The comparisons are illustrated in Fig. 4.2.

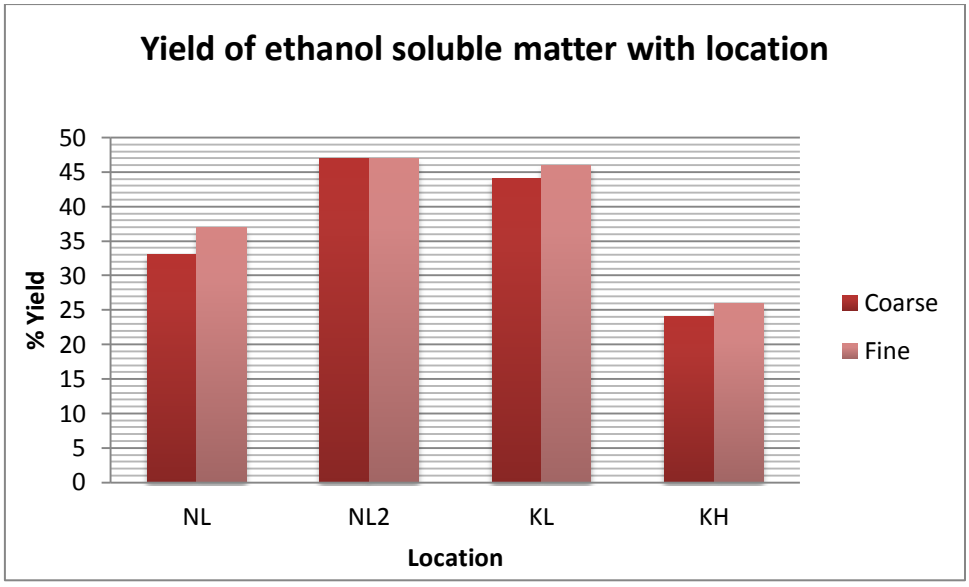


Figure 4.2: The yield of resinoid for coarse and ground gum resin

Typical resin compositions of myrrh gum resins are 25-40 %. The values from two study sites (Nolokwa and Kudu Hills) fell within this range but the other two (Nolokwa 2 and Kilonito) were higher. Some *Commiphora* species have been found to contain high levels of ethanol soluble resin. For instance, *Commiphora holtziana* has a yield of 41-44 % (Chiteva et al., 2013).

Coarse sample extraction took 10 hours of heating while maximum extraction of fine sample took 5 hours. This translates to double heat energy consumption in extraction of unground samples. On the other hand, mechanically grinding the lumps is also an energy-consuming process since the dried gum resin lumps are very tough and sticky. Grinding the gum resin improved extraction of the ethanol soluble matter by an average of 2.19 %, therefore from an economic perspective, extracting coarse material is recommended at an industrial scale. Sun-drying the gum resin to the quality of Nolokwa 2 is also recommended for the raw material.

4.2.3 Water soluble matter

The water soluble matter was obtained as described in section 3.4.4. This was found to be in the range 82.25±0.50 to 84.50±1.00 % as shown in Table 4.8.

Table 4.8: Yield of water soluble matter

Location	Mass of sample (g)	Mass of extract (g)	% yield
Nolokwa	10.000	8.450±0.100	84.50±1.00
Nolokwa 2	10.000	8.375±0.125	83.75±1.25
Kilonito	10.000	8.425±0.075	84.25±0.75
Kudu Hills	10.000	8.225±0.050	82.25±0.50
Average ±SD			83.69±0.04

Among the study sites *C. abyssinica* gum resin from Kudu Hills was found to have the least water soluble matter. The water soluble gum on other *Commiphora* species has been reported at 60% (Singh, 2010). In this case, the high value has been attributed to dissolution of both the gum and some percentage of the resin in water. Minor variations in properties of the species were observed in the different geographical areas. The Kenyan *C. abyssinica* gum resin was found to be highly water-soluble, and therefore water can be a solvent of choice to isolate the extraneous impurities.

4.2.4 Water and ethanol insoluble matter

The water and ethanol insoluble matter is a measure of contamination by foreign matter. It was determined as described in section 3.4.5. Kudu Hills gum resin had the highest impurity level of 3.77%, followed by Kilonito (3.54%), Nolokwa 2 (3.11%) and Nolokwa (2.27%) as shown in Table 4.9.

Table 4.9: Percent impurities

Location	Mass of sample (g)	Mass of impurities (g)	% content
Nolokwa	10.000	0.227±0.0053	2.27±0.05
Nolokwa 2	10.000	0.311±0.0068	3.11±0.07
Kilonito	10.000	0.354±0.0116	3.54±0.12
Kudu Hills	10.000	0.377±0.0074	3.77±0.07
Average ±SD			3.17±0.04

All the values were consistent with the typical levels of 3-4 % (Evans, 2009) except Nolokwa 2 which was lower. The main impurities are tree bark and soils, and therefore the low value could indicate that the gum resin was very clean or non-representative. Clean gum resins are obtained from natural exudates (as opposed to incisions) that are extracted directly from the trees while cautiously avoiding scrapping the tree bark.

4.3 Comparison of the Physical Properties of the Raw Gum Resin, Ethanol and Water Extracts

The density, pH, surface tension, foaming power and stability for the various components were determined and compared.

4.3.1 Density

Density is not a significant quality parameter but it is an important physical parameter in characterization. 1%w/v water extract solutions had the highest densities (998.5-998.7) at 25°C in comparison with similar solutions of the raw gum resins (998.3-998.4) and the ethanol extracts (998.1), respectively. The details are shown in Table 4.10.

Table 4.10: Comparison of density

Solution		**Value at 25°C (kg/m^3)**
Distilled water		997.1
Raw gum (See Table 4.5)	Nolokwa	998.4
	Nolokwa 2	998.4
	Kilonito	998.3
	Kudu Hills	998.3
Water extract	Nolokwa	998.7
	Nolokwa 2	998.5
	Kilonito	998.6
	Kudu Hills	998.5
Ethanol extract	Nolokwa	998.1
	Nolokwa 2	998.1
	Kilonito	998.1
	Kudu Hills	998.1

The density differences among the extracts was consistent were insignificant but the densities were higher than that of water.

4.3.2 pH

The ethanol extracts were more acidic (4.04-4.22) compared to the raw gum resin (5.20-5.31) and the water extracts (5.12-5.19) as shown in Table 4.11.

Table 4.11: pH of the raw gum and the extracts

Solution		**Value at 25°C**
Raw gum	Nolokwa	5.31
	Nolokwa 2	5.31
	Kilonito	5.26
	Kudu Hills	5.20
Water extract	Nolokwa	5.18
	Nolokwa 2	5.19
	Kilonito	5.16
	Kudu Hills	5.12
Ethanol extract	Nolokwa	4.21
	Nolokwa 2	4.22
	Kilonito	4.12
	Kudu Hills	4.04

The pH of the water extract was slightly lower than that of the raw substance, a difference which can be as a result of a higher concentration of the acidic components. The acidity of the various aqueous solutions could be attributed to the presence of acidic sugars. There is a consistent variation in acidity with location. Kudu Hills samples generally showed lower acidity, followed by Kilonito, Nolokwa and Nolokwa 2. The pH was generally moderate and therefore if the components were to be used in the same state in detergent formulations, the products would be mild and friendly to the skin.

4.3.3 Viscosity

Viscosity assessment deals with the flow properties of a particular solution. All the components increased the viscosity of distilled water. 1%w/v water soluble gum exhibited relatively high viscosities in the range 1.0202-1.0917 cP as shown in Table 4.12. This was slightly higher than that of the raw gum and the ethanol extract.

Table 4.12: Comparison of viscosity

Solution		**Value at 25°C (cP)**
Distilled water		0.8937
Raw gum	Nolokwa	0.9889±0.0013
	Nolokwa 2	0.9672±0.0021
	Kilonito	0.9754±0.0019
	Kudu Hills	1.0154±0.0040
Water extract	Nolokwa	1.0433±0.0011
	Nolokwa 2	1.0202±0.0027
	Kilonito	1.0456±0.0039
	Kudu Hills	1.0917±0.0032
Ethanol extract	Nolokwa	0.9321±0.0025
	Nolokwa 2	0.9512±0.0028
	Kilonito	0.9307±0.0039
	Kudu Hills	0.9257±0.0011

In the manufacture of detergents, high viscosity is not a prerequisite for the active matter since thickeners can be added to achieve the desired product thickness. However, the ability to form viscous solutions at low concentrations makes gums such as gum arabic suitable for

use as emulsion stabilizers. Therefore the water extract need to be evaluated as a potential emulsion stabilizer.

4.3.4 Surface Tension

A consistent trend was observed in the surface activity of all the samples irrespective of location meaning that the gum resins from all the study sites have similar interactions with water molecules. At normal wash water temperatures of 22°C, the surface tension of 0.1% w/v ethanol extract (average- 43.31mN/m) was superior to that of the raw gum (56.55mN/m) and that of the water extract (74.9mN/m) as shown in Table 4.13.

Table 4.13: Surface tension of raw gum resin and the extracts

Solution		**Mean no. of drops at 22°C**	**Surface tension (mN/m)**
Distilled water		228	72.44
Raw gum	Nolokwa	291	56.58±0.39
	Nolokwa 2	294	56.00±0.58
	Kilonito	296	55.63±0.37
	Kudu Hills	284	57.98±0.60
Water extract	Nolokwa	218	75.54±0.34
	Nolokwa 2	219	75.19±0.17
	Kilonito	220	74.84±0.34
	Kudu Hills	222	74.17±0.33
Ethanol extract	Nolokwa	388	43.38±0.11
	Nolokwa 2	387	43.68±0.12
	Kilonito	389	43.09±0.22
	Kudu Hills	375	43.08±0.23

The raw gum resins showed moderate surface activity at the experimental temperature and concentration. However, all the water extracts surprisingly produced higher values than that of pure water, where Nolokwa water extract had the highest surface tension value of 75.54±0.34 mN/m. Fig 4.3 is an illustration of the surface tension variations.

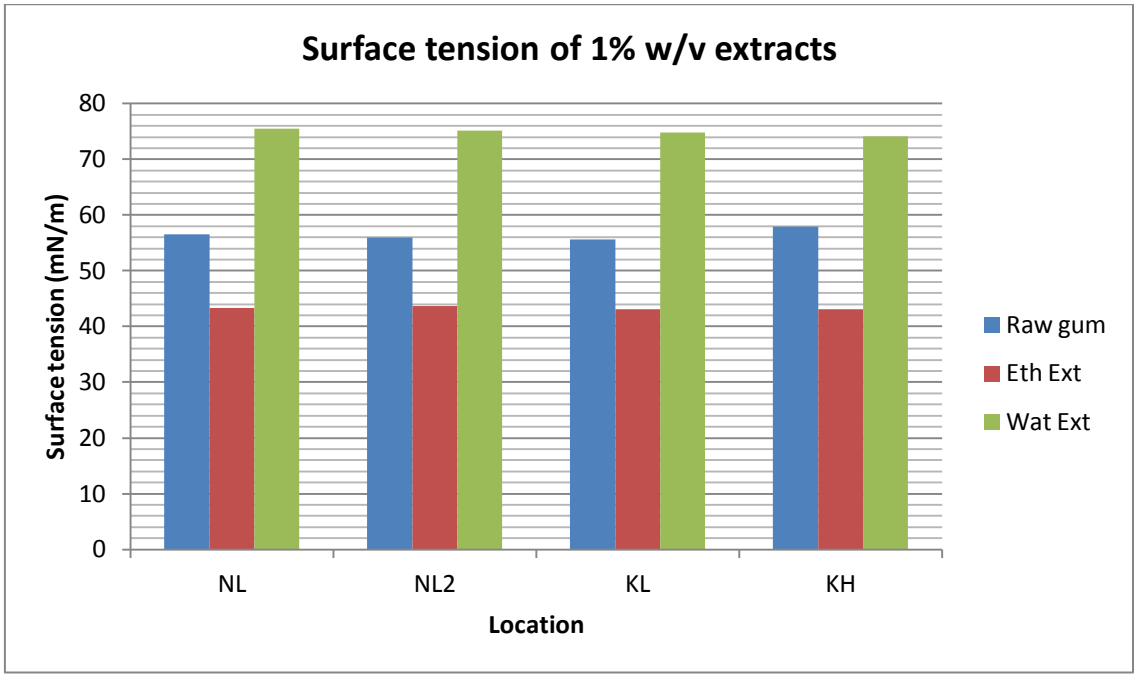


Figure 4.3: Comparison of surface tension of the raw gum and its water and ethanol extracts.

According to documented facts on surface tension, only very few substances which include mercury have a higher surface tension than water. However, this anomalous behaviour has also been previously observed by Lee et al. (2012) in a biopolymer solution of xantham gum (74.0 mN/m at 0.1% w/v) using the du Nuoy ring method and by Watanabe et al. (2003) on sodium alginate (du Nuoy ring method) and carboxymethylcellulose biopolymers (drop weight method). According to the theorem of surface thermodynamics (Gibbs adsorption isotherm), the surface tension of a biopolymer solution at low concentration should be close to that of pure water and should typically decrease with increased concentration. This is because at high concentrations, the biopolymer preferentially accumulates at the surface rather than in the bulk of the water (Lee et al., 2012).

The significant reduction in surface tension even at low concentrations is a critical prerequisite for a surfactant. The ethanol extract was therefore a better low temperature surfactant than the raw gum resin or the water soluble gum.

4.3.5 Foaming power and foam stability

The foaming properties are presented in Table 4.14.

Table 4.14: Foaming power (E_f) and foam stability (R5) values

Solution		**Initial volume, V_l (cc)**	**Final Volume, V_f (cc)**	**Volume after 5 minutes**	**Foaming Capacity, E_f (%)**	**Foam stability, R5 (%)**
Raw gum	Nolokwa	20	54	54	62.96	100
	Nolokwa 2	20	56	54	64.29	96.43
	Kilonito	20	55	53	63.64	96.36
	Kudu Hills	20	56	55	64.29	98.21
Water extract	Nolokwa	20	27	27	25.93	100
	Nolokwa 2	20	26	26	23.08	100
	Kilonito	20	28	28	28.57	100
	Kudu Hills	20	26	26	23.08	100
Ethanol extract	Nolokwa	20	89	84	77.53	94.38
	Nolokwa 2	20	88	84	77.27	95.45
	Kilonito	20	86	82	76.74	95.35
	Kudu Hills	20	86	83	76.74	96.51

The foaming properties of the three components showed a significant consistency. This further illustrates similar structural characteristics. There was no significant or consistent variation in properties with location. Among the three components, 1 % w/v ethanol extract exhibited the highest foaming power in the range 76.74 to 77.53 %. The raw gum

demonstrated a foaming power of 62.96-64.29 % and the water extract (after alcohol extraction) of approximately 25 %. The results show that 1 % *Commiphora abyssinica* gum resin ethanol extract possess excellent foaming power while the raw gum resin possess moderate foam power. The water extract is a poor foamer. This is illustrated in Fig. 4.4.

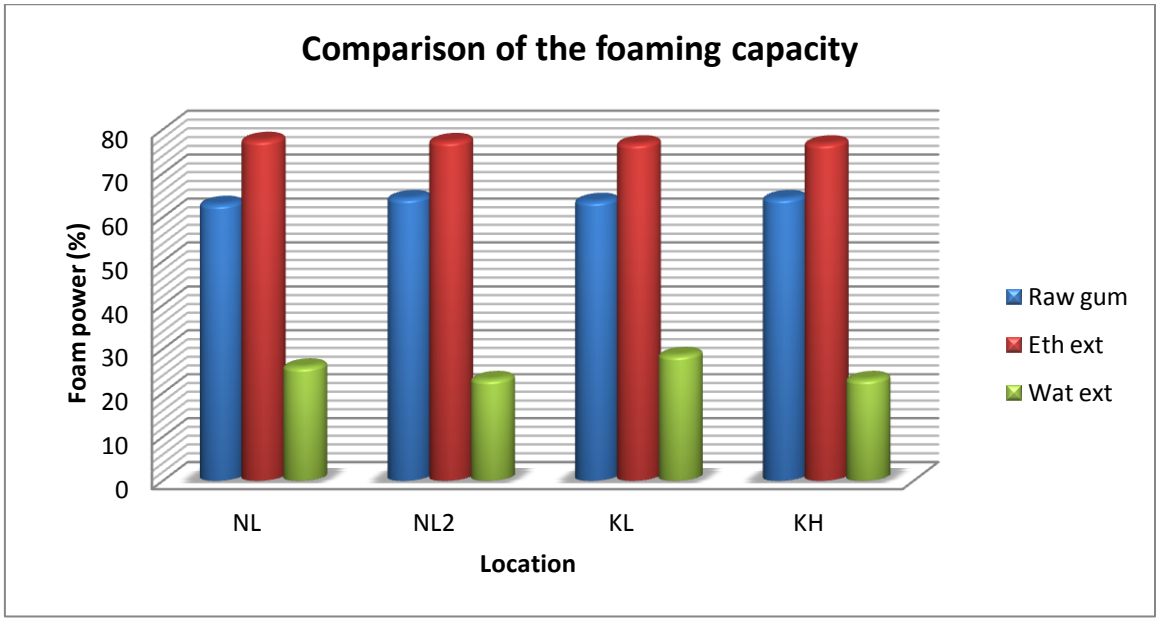


Figure 4.4: Foaming capacity of the raw gum, ethanol and water extracts

The foam heights of the raw gum and the two extracts are shown in Figure 4.5.

Figure 4.5: Foam height of the ethanol extract, raw gum and the water extract respectively.

In foam stability analysis, aqueous solutions with R5 ≥ 50% are considered metastable (Lunkenheimer and Malysa, 2003). In this study, all the components demonstrated high metastability. Though foaming power has little to do with the cleaning ability of a detergent, it adds confidence to consumers. The ethanol extract therefore is a good potential ingredient in detergent production.

4.4 Fatty Acid Characteristics of the Ethanol Extract

The saponification values of the *C. abyssinica* ethanol extract were between 170.8 and 176.4 mg KOH/g. The resins from Nolokwa gave slightly higher values (176.72mg/g) than Kudu Hills (173.91mg/g) and Kilonito (171.11mg/g). This is shown in Table 4.15.

Table 4.15: Saponification value, acid value, ester value, % FFA and % of glycerol of the ethanol extract

Sample	Saponification Value (mg/g)	Acid value (mg/g)	Ester value (mg/g)	% FFA	% Glycerol
Nolokwa	176.72	3.92	172.80	1.97	9.45
Nolokwa 2	176.72	3.92	172.80	1.97	9.45
Kilonito	171.11	4.48	166.63	2.25	9.11
Kudu Hills	173.91	4.48	169.43	2.25	9.27
Average ±SD	174.62±1.49	4.20±0.20	170.42±1.69	2.11±0.10	9.32±0.09

The values are high enough to warrant application in the cosmetic industry. The saponification values indicate higher average molecular weights of the triglycerides present. However, the values are lower than for the ethanol extract from *C. mukul* (230.32-233.94) and *C. wightii* (235.14-270.02) gum resins (Siddiqui and Mazumder, 2012).

Lauric oils are sourced from tallow and palm kernel but are extremely limited, hence they are also being sought from synthetics and rosin (Kirk-Othmer, 2013). According to Panda (2002), typical gum rosins have saponification values between 170 and 176 mg KOH/g, which happens to be the same range as that of ethanol extract from *C. abyssinica* gum resin obtained in this study. Therefore, *C. abyssinica* is a potential source of saponifiable resin.

The acid value is also referred to as the neutralization number, and it is a standard measure of the acid or alkaline content of a substance. From the results, the acid values of the *C. abyssinica* resin are within the range 3.92 to 4.48. The low values indicate low rancidity. Acid values for typical gum rosins range from 160-170 mg KOH/g (Panda, 2002). *C. abyssinica* resin therefore has relatively small amounts of undesirable free fatty acids. They are also lower than the values obtained for the ethanol extracts from some other *Commiphora*

species; *C. mukul* gum resin (13.16-15.77 mg KOH/g), *C.wightii* gum resin (8.20-14.65 mg KOH/g) (Siddiqui and Mazumder, 2012).

It should be noted that the acid value could have been even lower than obtained since the resin was stored in non-evacuated containers, and further oxidation might have taken place leading to contamination and hence a higher value (Tawde et al., 2013). The invariability of the properties of the extracts shows that the properties of the resin were not significantly affected by location.

Ester values were found to be 166.63-172.80 mg KOH/g. They were generally high which indicated a high content of saponifiable glycerides. These values were also lower than for the other two *Commiphora* species discussed above (*C. mukul*: 230.32-233.94 and *C. wightii:* 226.94-255.37) (Siddiqui and Mazumder, 2012).

The percentages of free fatty acids and of glycerol ranged from 1.97 to 2.25 and 9.11 to 9.45, respectively. Most of the fats and oils used in soap manufacture have free fatty acids below 5 %: tallow 2-4 %, coconut oil maximum 3 % and palm kernel oil maximum 3 % (Ahmad et al., 2013).

Although saponification number is generally expressed in terms of mg KOH, however, NaOH is the one widely used in oil saponification especially in the production of toilet soap. This necessitates an alternative scale in terms of milligrams of NaOH to find the exact quantity needed. This is obtained by multiplying the KOH values by the molar weight ratio of the two alkalis, which is 0.7129. Table 4.16 gives the saponification values of the *C. abyssinica* resin in terms of NaOH.

Table 4.16: Representation of saponification values in KOH and NaOH scales

Sample	Saponification Value	
	KOH (mg/g)	**NaOH (mg/g)**
Nolokwa	176.72	125.98
Nolokwa 2	176.72	125.98
Kilonito	171.11	121.98
Kudu Hills	173.91	123.98
Average ±SD	174.62±1.49	124.48±1.91

From the table, it is clear that a relatively lower amount of caustic lye is needed to saponify a given quantity of the resin than potash.

4.5 Comparison of the Ethanol Extract with Common Synthetic Detergents

4.5.1 Physical properties

The pH, specific gravity and viscosity were determined at 25^{o}C and are presented in Table 4.17.

Table 4.17: Physical properties of the ethanol extracts and commercial surfactants

Location	pH	Density (kg/m^3)	Viscosity (cP)
Nolokwa	4.21	998.1	0.9321
Nolokwa 2	4.22	998.1	0.9512
Kilonito	4.12	998.1	0.9307
Kudu Hills	4.04	998.1	0.9257
Average	4.15	998.1	0.9269
LABSA	2.44	998.1	0.9499
SLS	7.13	998.5	0.9734
CDE	9.67	997.1	1.7709
Distilled water	5.85	997.1	0.8937

The pH of the ethanol extracts was mildly acidic (4.04-4.22). The synthetic standards used varied in acidity; LABSA- highly acidic (2.44), SLS- neutral (7.13) and CDE basic (9.67). However, significant similarities in specific gravity were observed, with the density of the resin extracts being in the same range as two of the standards, LABSA and SLS (998.1-998.5kg/m^3). The addition of 1% ethanol extracts did not have a large effect on the viscosity of pure water. LABSA and SLS also did not raise the viscosity of water significantly (0.9499 and 0.9734 cP, respectively), but CDE exhibited a relatively superior viscosity enhancement, almost doubling the value for the ethanol extracts (1.7709 cP) which explains why CDE is used in detergent formulations for this purpose.

4.5.2 Amphiphilic properties

4.5.2.1 Surface tension and CMC

The surface tension reduction of the *C. abyssinica* gum resin ethanol extract was compared with LABSA and SLS. Table 4.18 shows the decrease in surface tension at various concentrations of the different surfactants.

Table 4.18: Variation of surface tension with concentration indicating location of CMC

Solution	Concentration (c, mg/100ml)	Mean no. of drops (n)	Surface tension, 22°C (mN/m)	Δn	Δc	Δn/Δc
Nolokwa	0	230	72.44	0	0	-
	5	234	71.95	4	5	0.80
	10	239	70.44	5	5	1.00
	20	267	63.02	28	10	2.80
	40	334	50.40	67	20	3.35
	60	356	47.29	29	20	1.45
	80	376	44.77	20	20	1.00
	100	388	43.38	12	20	0.6
Nolokwa 2	0	231	72.44	0	0	-
	5	235	71.93	4	5	0.80
	10	240	70.44	5	5	1.00
	20	254	66.55	14	10	1.40
	40	322	52.49	68	20	3.40
	60	352	48.02	30	20	1.50
	80	372	45.45	20	20	1.00
	100	387	43.68	15	20	0.75

Table 4.18 continued

Solution	**Concentration (c, mg/100ml)**	**Mean no. of drops (n)**	**Surface tension, 22^{o}C (mN/m)**	**Δn**	**Δc**	**Δn/Δc**
Kilonito	0	229	72.44	0	0	-
	5	233	71.96	4	5	0.80
	10	237	70.70	4	5	0.80
	20	247	67.88	10	10	1.00
	40	324	51.74	77	20	3.85
	60	352	47.57	28	20	1.40
	80	373	44.95	21	20	1.05
	100	389	43.09	16	20	0.8
Kudu Hills	0	223	72.44	0	0	-
	5	225	71.80	2	5	0.40
	10	228	69.62	7	5	1.40
	20	248	65.14	16	10	1.60
	40	316	51.12	68	20	3.40
	60	342	47.23	26	20	1.30
	80	362	44.63	20	20	1.00
	100	375	43.08	6	20	0.30

Table 4.18 continued

Solution	**Concentration (c, mg/100ml)**	**Mean no. of drops (n)**	**Surface tension, 22^{o}C (mN/m)**	**Δn**	**Δc**	**Δn/Δc**
LABSA	0	226	72.44	0	0	
	5	228	71.81	2	5	0.40
	10	235	69.68	7	5	1.40
	20	284	57.66	49	10	4.90
	40	371	44.14	87	20	4.35
	60	415	39.45	44	20	2.20
	80	428	38.25	13	20	0.65
	100	432	37.91	4	20	0.20
SLS	0	231	72.44	0	0	-
	5	233	71.82	2	5	0.4
	10	235	71.20	2	5	0.4
	20	242	69.14	7	10	0.70
	40	281	59.55	39	20	1.95
	60	318	52.62	37	20	1.85
	80	348	48.09	30	20	1.5
	100	358	46.75	10	20	0.5

The surface tension initially reduced minimally with increase in concentration. With further increase in concentration, a point was reached where there was a sharp reduction in surface tension. This marked the critical micelle concentration, and after that the curve flattened. This is shown in Figure 4.6.

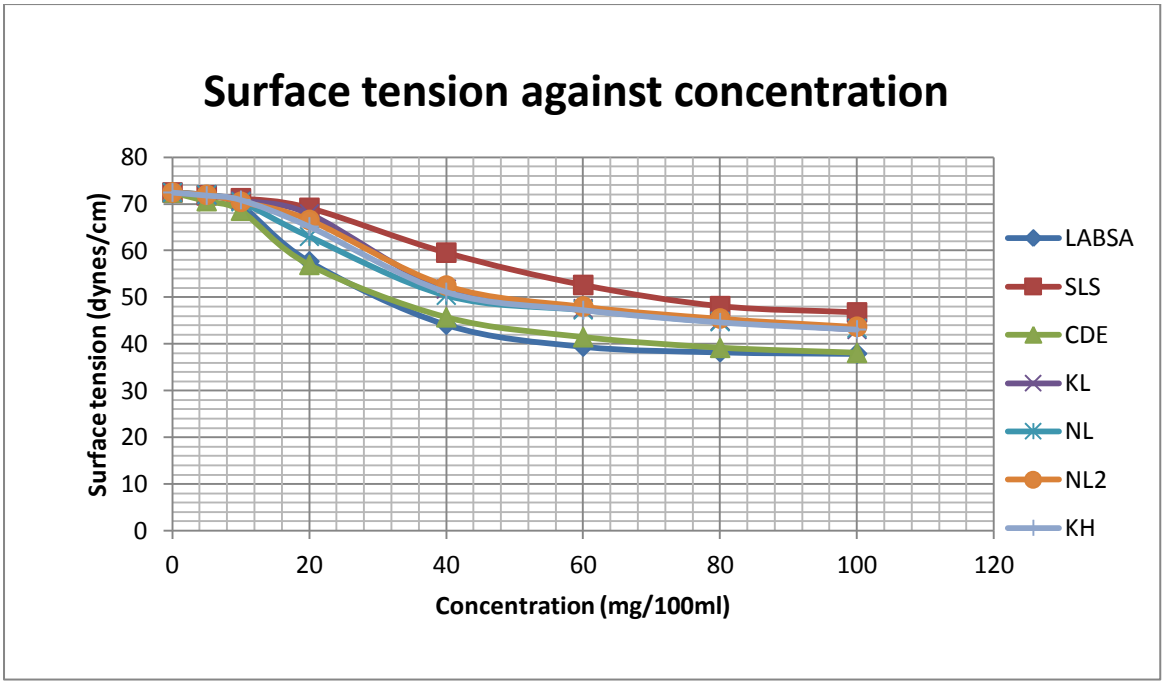


Figure 4.6: Surface tension versus surfactant concentration

Figure 4.7 is a plot of Δn/Δc vs concentration which was used for CMC determination.

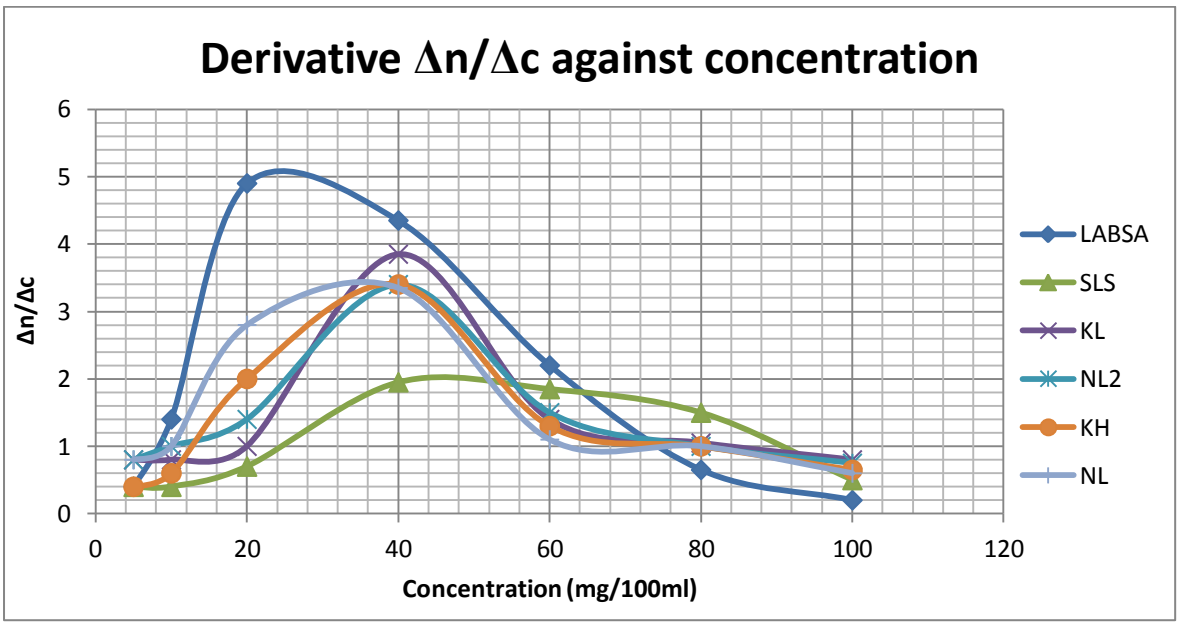


Figure 4.7: Derivative Δn/Δc plotted against concentration with peak points representing CMC

The critical micelle concentration of the crude ethanol extracts from three study sites (Nolokwa 2, Kilonito and Kudu Hills) was 40mg/l. However, Nolokwa resin extract had a

slightly lower CMC value of 36mg/l which indicates that it is a better source of a low temperature detergent (See Table 4.19).

Table 4.19: CMC values of surfactants

Solution	**Value at 22°C (mg/100ml)**
Nolokwa	36
Nolokwa 2	40
Kilonito	40
Kudu Hills	40
LABSA	24
SLS	46

The low temperature CMC was compared experimentally with that of common detergent ingredients and it was found to be in the order: sulphonic acid (24mg/100 ml) < ethanol extracts (39mg/100ml) < sodium lauryl ether sulphate (46mg/100ml). The 43.31 mN/m surface tension value for 0.1% w/v aqueous solutions showed that the ethanol extract was more effective than SLS (46.75 mN/m) but less than LABSA (37.91 mN/m) at 22°C (Table 4.18). Mwendwa (2007) reported a surface tension value of 41.33 mN/m and a CMC value of 30mg/100ml for 0.1% w/v aqueous solution of the same extract at 25°C. This observation was in agreement with the general observation that within the range 20-40°C, surface tension reduces with increase in temperature (Pruppacher and Klett, 1997; Chesori, 2008).

CMC is directly related to the length of the hydrophilic chain and is inversely proportional to the length of the hydrophobic chain (Emmanuel et al., 2014). Therefore the ethanol extract from *C. abyssinica* has a shorter hydrophobic chain than sulphonic acid but longer than sodium laureth ether sulphate.

4.5.2.2 Foam analysis

Both foaming power and foam stability were analyzed to determine the efficiency and effectiveness of the resin extract as a foamer and the results compared with that of three commercial synthetic detergents namely, LABSA, SLS and CDE.

Foam height demonstrates the efficiency of a surfactant as a foamer. All the ethanol extracts showed high foam effectiveness (76.74-77.53 %) at a concentration of 0.1%, which was more or less equal to that of SLS (77.77 %) but slightly lower than that of the common synthetic foaming agent, CDE (80 %). Table 4.20 shows the values of foam height and foam stability for 0.1% w/v solutions obtained.

Table 4.20: Foaming capacity and foam stability values of the surfactants at 1% concentration.

Solution	Initial (cc)	Maximum, 0 minutes (cc)	5 minutes	Foaminess (%)	Foam stability, R5
Nolokwa	10	44.5	42	77.53	94.38
Nolokwa 2	10	44	42	77.27	95.45
Kilonito	10	43	41	76.74	95.35
Kudu Hills	10	43	41.5	76.74	96.51
Average±SD	10.0±0.0	43.6±0.3	41.6±0.3	77.07±0.14	95.42±0.02
LABSA	10	52	49	80.77	94.23
SLS	10	45	42	77.77	93.33
CDE	10	50	49	80.00	98.00

The common synthetic wetting agent, LABSA had 80.77% foam effectiveness. Surfactant solutions with an R5 value above 50% are considered to be metastable (Lunkenheimer and Malysa, 2003). The foam stabilities of over 90% observed for all the resin extract samples were comparable to those of the commercial surfactants, suggesting that the resin extracts are viable alternatives in this regard. SLS was found to have the lowest stability value of 93.3%,

which was consistent with the value of 93.6% obtained by Chen et al. (2010). Though foaming properties of a surfactant have little to do with the cleaning power of a detergent, it is highly satisfying to users.

The foam height variation with concentration was also investigated. Generally, the foam properties of the various resin extracts were the same which further confirmed similar physical properties irrespective of location. Foam height increased with increase in concentration of an aqueous surfactant solution. Table 4.21 shows how foam height varied with concentration and time.

Table 4.21: Variation of foaming properties with concentration and time

Solution	Concentration (mg/100ml)	Initial (cc)	Final (cc)	After 5 min (cc)
Nolokwa	5	10	16	16
	10	10	17.5	17.5
	20	10	19	19
	40	10	21	21
	60	10	22	22
	80	10	22.5	22.5
	100	10	22.5	22.5
Nolokwa 2	5	10	15	15
	10	10	17	17
	20	10	19.5	19.5
	40	10	21	21
	60	10	22	22
	80	10	22	22
	100	10	22	22

Table 4.21 continued

Solution	Concentration (mg/100ml)	Initial (cc)	Final (cc)	After 5 min (cc)
Kilonito	5	10	14	14
	10	10	16	16
	20	10	17	17
	40	10	19.5	19.5
	60	10	21	21
	80	10	21	21
	100	10	21	21
Kudu Hills	5	10	14	14
	10	10	16	16
	20	10	17.5	17.5
	40	10	19.5	18.5
	60	10	20	20
	80	10	21	21
	100	10	21	21
LABSA	5	10	24	22
	10	10	31	28
	20	10	40	38
	40	10	44	41
	60	10	47	44
	80	10	48	44
	100	10	48	44

Table 4.21 continued

Solution	Concentration (mg/100ml)	Initial (cc)	Final (cc)	After 5 min (cc)
SLS	5	10	23	19
	10	10	30	27
	20	10	36	34
	40	10	40	38
	60	10	42	40
	80	10	42	40
	100	10	42	40
CDE	5	10	18	15
	10	10	25	25
	20	10	33	33
	40	10	38	38
	60	10	42	42
	80	10	42	42
	100	10	42	42

From the plot of foam height against concentration (Appendix 1.1), it was observed that foam stability was related to CMC. When the concentration of the *C. abyssinica* resin solution was increased from 0.1 to 1% there was significant improvement in foaming power which more or less matched that of the synthetic detergent ingredients at 0.1% concentration which was not very different from that at 1% (Fig 4.8). According to Kunjappu and Rosen (2013), the foam height peaks at the CMC but continues to increase depending on the inherent properties of the substance. The increase in foaming power with concentration above the CMC as depicted by the ethanol extracts implies that more quantity of the extract will be required to

enhance the foaming properties of a conventional detergent formulated using the resin compared to the synthetic surfactants used in this study.

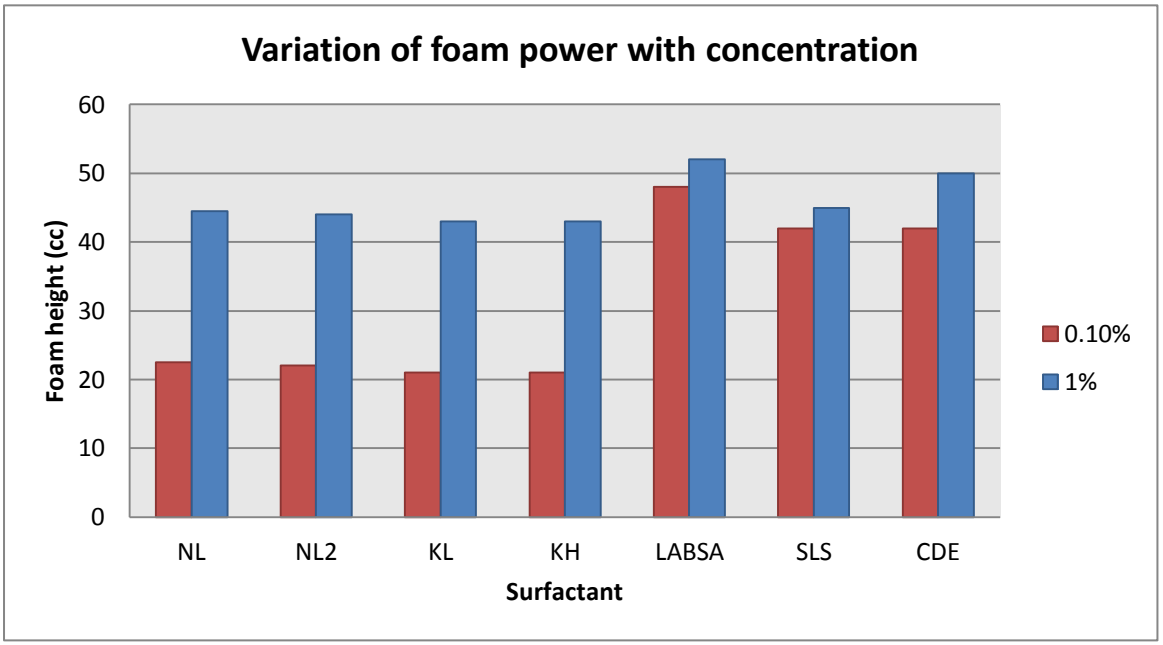


Figure 4.8: Foam power in 0.1% and 1% surfactant solutions

4.5.2.3 Spreadability

Spreadability demonstrates the wetting properties of a surfactant. The spreadibility of Kilonito resin was the same as for SLS while Nolokwa, Nolokwa 2 and Kudu Hills demonstrated higher values. LABSA exhibited the best wetting properties while CDE had no effect on the wetting power of distilled water. These are shown in Table 4.22. The results further underscore the potential use of the ethanol extract as a surfactant.

Table 4.22: Spreadability of the surfactant solutions

Solution	Diameter (cm)	Area (cm^2)	% Spread by area
Distilled water	3.0	7.070	100
Nolokwa	3.15	7.794	110.2
Nolokwa 2	3.15	7.794	110.2
Kilonito	3.10	7.549	106.8
Kudu Hills	3.15	7.794	110.2
Average±SD			109.4±0.60
LABSA	3.20	8.044	113.8
SLS	3.10	7.549	106.8
CDE	3.0	7.070	100

4.5.2.4 Cloud point

A significant variation in the turbidity of the aqueous solutions with increase in temperature was observed as illustrated in Table 4.23. Nolokwa resin solution turned cloudy at 26°C, Nolokwa 2 at 36°C, Kilonito at 39°C and Kudu Hills at 47°C. A cloud point of 27°C had been reported earlier (Chesori, 2008).

Table 4.23: Cloud point temperature of the surfactant solutions

Location	Temperature (°C)
Nolokwa	26
Nolokwa 2	36
Kilonito	39
Kudu Hills	47
LABSA	Cloudy at room temp
SLS	Not observed
CDE	Not observed

LABSA was turbid at the room temperature (22°C) while SLS and CDE did not become turbid even at the boiling point of water. The surfactants should be used in temperature ranges below their respective cloud points due to diminished solubility and hence less detergency above that temperature.

4.6 Emulsifying Properties of Water Soluble Gum

A fairly consistent trend on stability of the *C. abyssinica* gum and gum arabic o/w emulsions was observed. The emulsion stability under various conditions was as shown in Table 4.24.

Table 4.24: Emulsion stability for Kudu Hills, Nolokwa and Gum arabic (GA)

Variable			**Emulsion stability index (ESI)**		
C (%)	**T (°C)**	**pH**	**Kudu Hills**	**Nolokwa**	**Gum arabic**
0.1	22	6.0	0.78	0.66	0.76
0.05	22	6.0	0.71	0.41	0.26
0.025	22	6.0	0.29	0.20	0.18
0.0125	22	6.0	0.10	0.10	0.11
0.1	40	6.0	1.35	1.00	0.96
0.1	60	6.0	1.35	1.00	1.31
0.1	80	6.0	1.35	0.85	1.31
0.1	22	2.0	1.22	0.97	1.50
0.1	22	12.0	0.37	0.29	0.36

C- Concentration, **T**- Temperature

After 24 hours, the emulsion stability index (ESI) was found to decrease significantly with decrease in concentration up to approximately 0.10 for 0.0125 % gum emulsion. This is illustrated in Fig. 4.9. According to Stokes' law, the reduced viscosity with dilution was bound to accelerate instability.

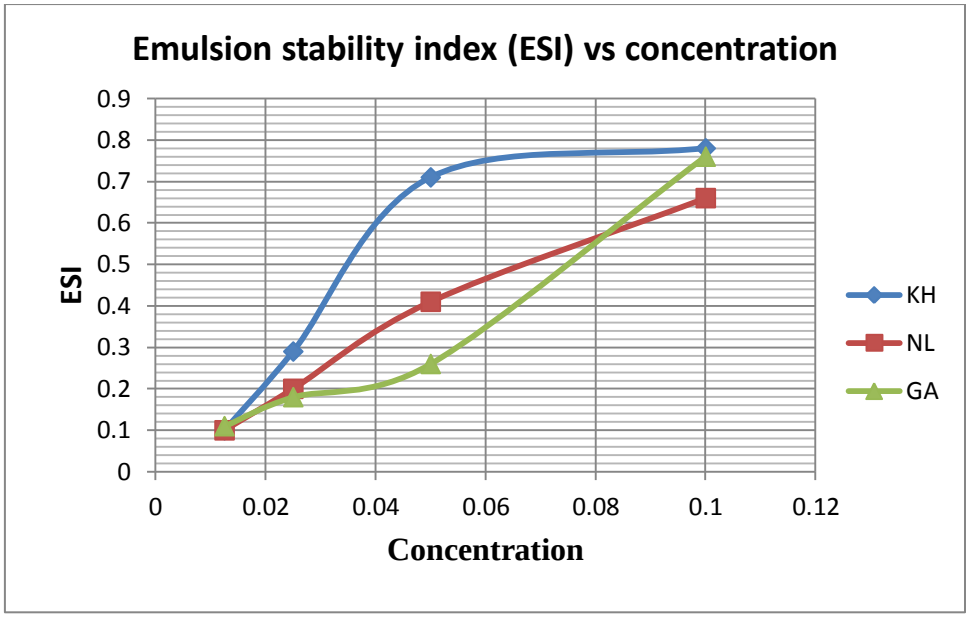


Figure 4.9: Emulsion stability index variation with concentration

There was however a remarkable improvement in the emulsion stability when the 0.1% emulsion was heated to between 40 and 80 $^{\circ}$C prior to storage as shown in Fig. 4.10.

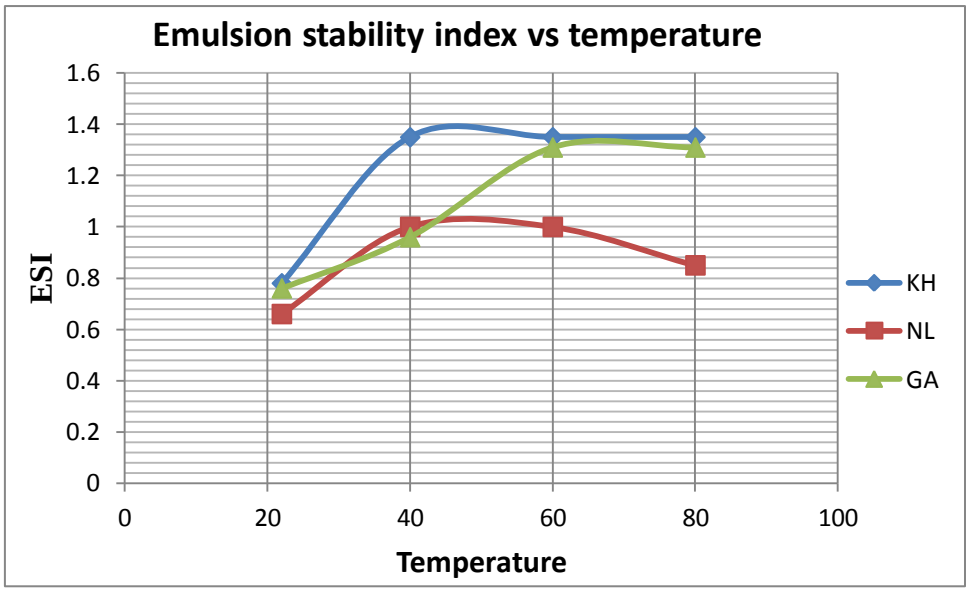


Figure 4.10: Emulsion stability index variation with temperature

With regard to pH, the reference emulsions were set at pH 6.0. It is therefore clear from Fig 4.11 that increased acidity enhanced the stability of the emulsions especially for gum arabic while increased alkalinity destabilized all of them.

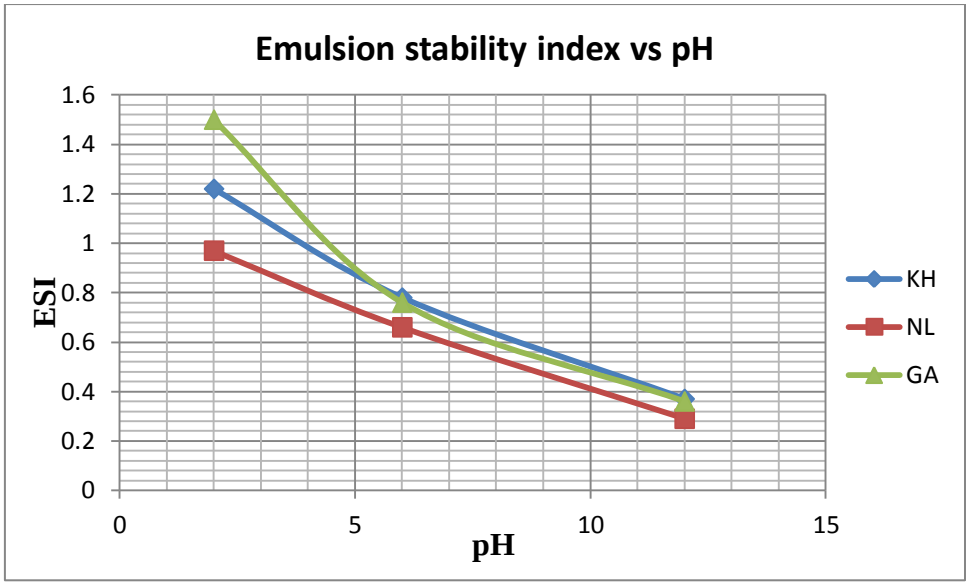


Figure 4.11: Emulsion stability index variation with pH

These findings are in agreement with those reported by Branen et al. (1990) who found that emulsion stability increases with increased emulsification temperature, degree of shear and increase in pH in the range 3-6.

The surface tension of a 0.5 % w/v gum arabic solution has been reported at 46.9 mN/m at 25 $^{\circ}$C (Huang et al., 2001) and remains fairly the same with increase in concentration. The strong emulsifying properties of gum arabic have been attributed to a probable arabino-galactan complex (Phillips et al., 1990). On the other hand, the surface tension of *C. abyssinica* gum solution was found to be higher than that of water. The emulsion stabilizing capacity of the gum resin may probably be arising from similar molecular interactions as those of gum arabic. Steric hindrance as a result of the fine gum particles in the colloidal dispersion adhering to the surfaces of the lipid droplets could also be contributing to emulsion stability.

4.7 GC-MS Analysis

Identification of constituents was done by comparing the MS spectra and the retention indices of the compounds with the reference data in the MS library. In this analysis, the library search was done using NIST MS search 2.0. The name, molecular weight and structural formula

were ascertained and compared with those previously identified from other *Commiphora* species. The results are illustrated in Table 4.25. Appendices 2.1 to 4.5 give the mass spectra of the analytes and the corresponding match from the NIST library database.

Table 4.25: The characterized compounds from the crude extracts by name, molecular formula and structure

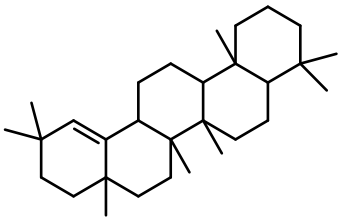
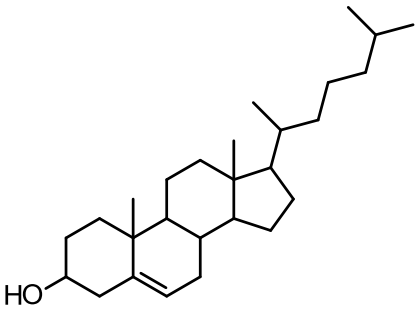
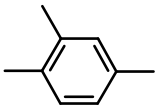
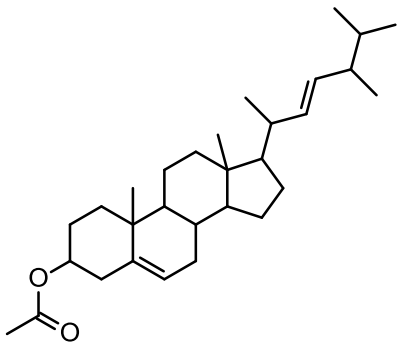
Name	**Mol. Wght**	**Structural Formula**
Olean-18-ene	$C_{30}H_{50}$	
Cholest-5-en-3-ol (3β)	$C_{27}H_{46}O$	
ψ-cumene	C_9H_{12}	
Ergosta-5,22-dien-3-ol, acetate, (3β,22E)	$C_{30}H_{48}O_2$	

Table 4.25 continued

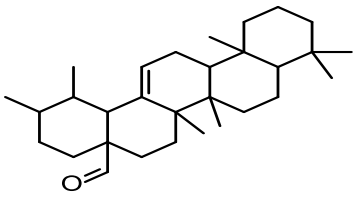
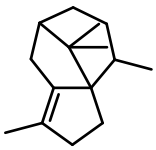
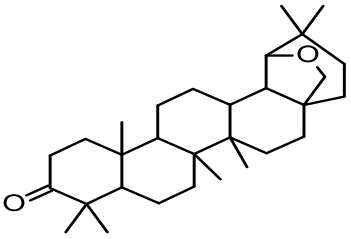
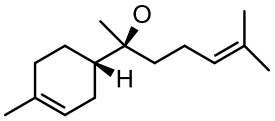
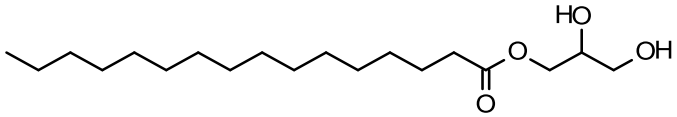
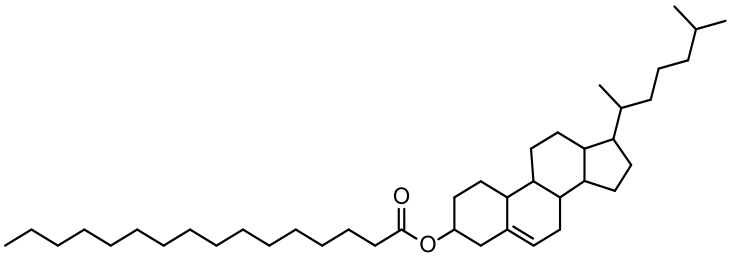
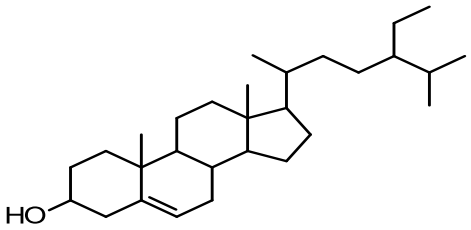
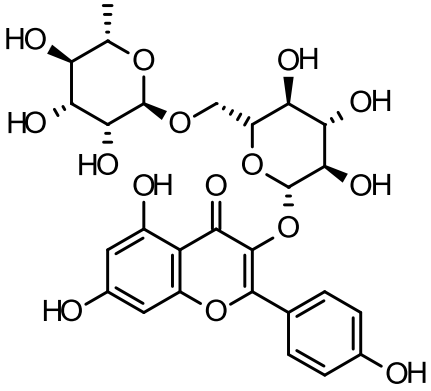
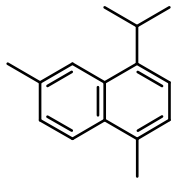
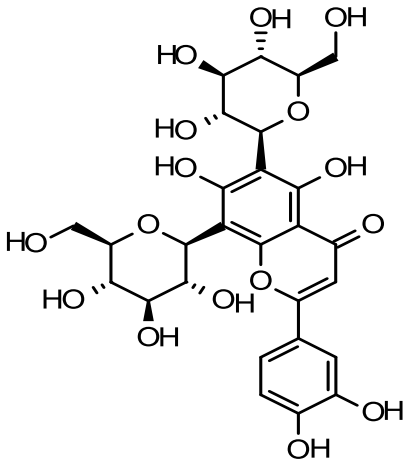
Name	**Mol. Wght**	**Structural Formula**
Urs-12-en-28-al	$C_{30}H_{48}O$	
Cyperene	$C_{15}H_{24}$	
3-Oxoallobetulane	$C_{30}H_{48}O_2$	
α-Bisabolol	$C_{15}H_{26}O$	
Hexadecanoic acid (Glycerol-1 palmitate)	$C_{19}H_{38}O_4$	
Cholesterinpalmitat	$C_{43}H_{76}O_2$	

Table 4.25 continued

Name	**Mol. Wght**	**Structural Formula**
Stigmast-5-en-3-ol	$C_{29}H_{50}O$	
Kaempferol 3-O-rutinose	$C_{27}H_{30}O_{15}$	
Cadalene	$C_{15}H_{18}$	
Lucenin 2	$C_{27}H_{30}O_{16}$	

4.8 Bioassay of *C. abyssinica* extracts

The in-vitro anti-microbial evaluation of the crude *C. abyssinica* extracts (methanol, ethanol and hexane) against three Gram negative, two Gram positive and three fungi strains was carried out. The antimicrobial activities are shown in Table 4.26.

Table 4.26: Microbial susceptibility to crude extracts

Extract	Inhibition zone (mm)							
	Gram negative test strains			Gram positive test strains		Test fungi test strains		
	St	**Sh**	**Ec**	**Sa**	**MRSA**	**Ca**	**Tm**	**Mg**
1A	8	7	6	6	**9**	6	**22**	6
1B	7	6	7	6	**9**	6	**23**	6
1C	6	6	6	6	**7**	6	**8**	6
2A	6	6	6	6	**9**	6	**21**	6
2B	**8**	**6**	**7**	**16**	**18**	**8**	**24**	6
2C	6	6	6	6	6	6	**16**	6
3A	7	6	6	6	6	6	**25**	6
3B	7	6	6	6	6	6	**23**	6
3C	6	6	6	6	6	6	6	6
4A	7	6	6	6	6	6	**22**	6
4B	6	6	6	6	6	6	**25**	6
4C	6	6	6	6	6	6	**12**	6
Nystatin						12,11	17,15	16,15
Chloramphenicol	12,11	12,13	26,25	22,24	15,16			
Ref numbers	Clinical	Clinical	ATCC 25922	ATCC 25923	Clinical	ATCC 90028	Clinical	Clinical
DMSO	6	6	6	6	6	6	6	6

1-Nolokwa, **2**- Nolokwa 2, **3**- Kilonito, **4**- Kudu hills, **A**- Ethanol, **B**- Methanol, **C**- n-hexane **St**- *Salmonella typhi*, **Sh**- *Shigella*, **Ec**- *Escherichia coli*, **Sa**- *Staphylococcus aureus*, **MRSA**- Methicilin resistant *Staphylococcus aureus*, **Ca**- *Candida albicans*, **Tm**- *Trichophyton mentagrophytes*, **Mg**- *Microsporum gypseum*

4.8.1 Disk diffusion susceptibility testing

The results obtained from this evaluation exhibited significant differences in anti-microbial activity in relation to the solvent used, with methanol extracts generally showing relatively high activity and hexane extracts generally inactive. This trend was also observed by Munyendo et al. (2011) and Mohamed et al (2014). This might be due to the effects of polarity, with the highly polar methanol extracting the different types of terpenes, sesquiterpenoids, saponins and sterols more effectively (Mohamed et al., 2014). Most of the extracts were inactive against Gram negative bacteria namely *Shigella spp*. and *E. coli*. The activity against *S. typhi* and *Shigella* is illustrated in Fig 4.12.

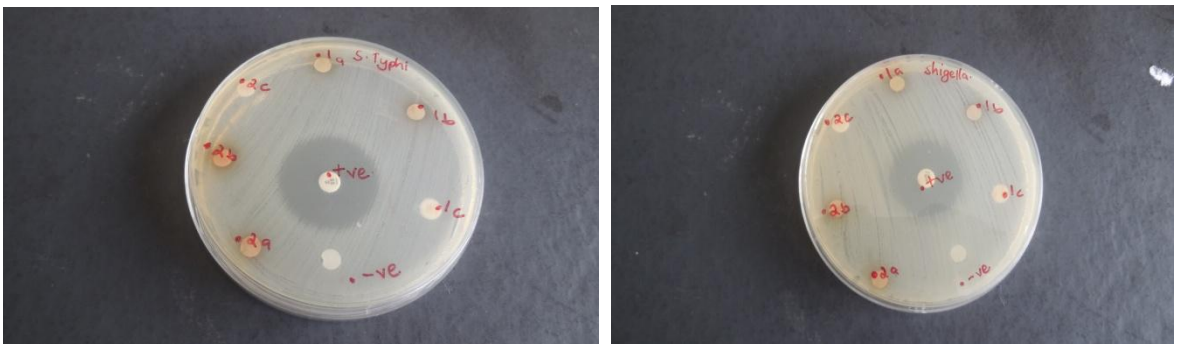


Figure 4.12: Antibacterial activity against *S. typhi* (1) and *Shigella* (2)

However, the extracts from Nolokwa demonstrated bacteriostatic activity against both *S. typhi* and MRSA. Of major interest is the methanol extract from Nolokwa 2 which showed significant antibacterial activity. This extract was inactive against one Gram negative strain, *Shigella spp*., exhibited bacteriostatic activity against the other Gram negative bacteria *S. typhi* (8mm) and *E. coli* (7mm), and was bacteriocidal against Gram positive bacteria *S. aureus* (16mm) and MRSA (18mm), the latter being higher than Chloramphenicol (15mm), the standard drug used. The 16mm activity against *S. aureus* was also significant when compared with the control antibiotic (22mm). This was consistent with the results of Musa, (2008), who conducted an antimicrobial evaluation on *C. kerstingii* Engl. extracts and found

significant bacterial inhibition with the highest inhibition being on *S. aureus*. Abdallah et al. (2009) also demonstrated that methanol extracts have the highest antibacterial activity against *S. aureus*.

The selective activity towards Gram positive bacteria is attributed to their morphological differences. Gram negative bacteria are characterized by an outer phospholipidic membrane that carries lipopolyssacharide and lipoprotein structural components hence making the cell wall impermeable to lipophilic compounds. On the contrary, Gram positive bacteria only have a peptidoglycan outer layer which does not effectively bar the permeability of solutes (Nostro et al., 2000; Mohamed et al., 2014). This structural complexity enhances the resistance of Gram negative bacteria against anti-microbial compounds compared to Gram positive bacteria (Munyendo et al., 2011).

As shown in Table 4.25, the extracts were inactive towards two fungal strains *C. albicans* and *M. gypseum*, with the exception of Nolokwa 2 methanol extract which exhibited some limited activity against *C. albicans* (8mm). *C. albicans* is suspected to form biofilms which enhances its resistance to antifungal agents (Mohamed et al., 2014). With the exception of n-hexane extract from Kilonito, all the others were active against *T. mentagrophytes* at varying degrees. These other n-hexane extracts exhibited some limited activity while the ethanol and methanol extracts showed superior activity to nystatin, the standard drug used. The ability of an agent to eliminate the fungal isolate determines its successful treatment of a fungal infection (Barros et al., 2007). The remarkable activity against this common dermatophyte illustrates potential use of *C. abyssinica* oleo-gum resin in combating nail infections like onychomycosis and skin fungal infections such as athlete's foot, ringworms and jock itch. It can also be included in formulation of pet cleaners since *T. mentagrophytes* is a common fungus especially in rabbits.

4.8.2 Minimum inhibition concentration

The methanol extract from Nolokwa 2 was found to be highly effective against Gram positive bacteria MRSA and *S. aureus*. These were therefore subjected to MIC assay and the results obtained were recorded in Table 4.27.

Table 4.27: MIC on *S. aureus* and MRSA

Concentration (g/ml)	MRSA	*S. aureus*
0.5	18	17
0.25	14	15
0.125	14	14
0.062	14	10
0.031	12	

The highest concentration used was 500mg/ml. The minimum inhibitory concentration against MRSA was 31 mg/ml while that against a clinical isolate of *S. aureus* was 62 mg/ml. This is higher than the MIC of crude methanol extract of *C. kerstingii* Engl. on *S. aureus*, which was 5mg/ml (Musa, 2008). The activities of the extracts were found to increase with concentration. Sesquiterpenes isolated from *Commiphora molmol* were found to be the secondary metabolites responsible for the inhibition of *S. aureus* (Gibbons, 2004). The remarkable inhibition of MRSA shows that the methanol extract can be used as a source of resistance modifying agents (RMA). Despite MRSA being the resistant version of *S. aureus*, higher relative activity against it was observed. This anomaly coincided with a case highlighted by Gibbons (2004), where an anti-staphylococcal agent (Synercid) was developed and recommended for anti-MRSA treatment but was later found not to be resistant to a clinical isolate of *S. aureus*. This bacterium is responsible for a wide range of diseases from

cutaneous infections such as boils to extreme cases of pneumonia, osteomyelitis, endocardiditis and septicemia (Dunkle et al., 2009).

The study confirms potential antimicrobial agents in *C. abyssinica* oleo-gum resin that can be used in medicinal and food preservation applications. Further research to identify and possibly isolate the active compounds as well as to ascertain the specific toxicity and safety levels should be undertaken prior to applicability.

CHAPTER 5

CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

The physico-chemical properties of the oleo-gum resin from *Commiphora abyssinica* in the study area were determined. They were found not to be dependent on location. The ethanol and water extracts of gum resin from the various locations were also found to possess similar properties further confirming the uniformity in properties. However, the composition of the respective components was found to be dependent on location.

The ethanol extract was found to have superior amphiphilic properties when compared to the raw gum and the water extract, and therefore is the best component for use in detergent formulation. This component had a low CMC value and satisfactory surface tension reduction capabilities and can therefore be used as a substitute for synthetic surfactants used in some detergent formulation. This was because it was found to produce better amphiphilic properties than SLS and also possessed powerful, metastable foam. It had relatively high saponification values and hence can be saponified to produce soap.

Use of *C. abyssinica* resin (ethanol extract) in formulation of cleaning agents therefore would have multiple benefits; effective cleaning power even at low temperatures, good foaming properties, eco-friendly and protecting the skin against some bacterial and fungal infections.

The primary results of emulsion stabilizing effects of the *Commiphora abyssinica* gum and gum arabic are comparable, thereby indicating viable applicability as an industrial food or non-food stabilizer. However, rheological and toxicological studies are necessary for establishing the actual flow behavior and the specific safety levels respectively to affirm its commercial value.

Using GC-MS analysis, several compounds were characterized. Among these were monoterpenes such as ψ-cumene, sesquiterpenes such as cadalene, α-amyrin, α-bisabolol and (-)-cyperene, triterpenes such as olean-18-ene as well as steroidal saponins such as urs-12-en-28-al and cholest-5-en-3-ol. Flavone glycosides such as kaempferol-3-O-rutinoside and lucenin 2 were also characterized.

In the antimicrobial analysis, a greater selectivity by the crude extracts was exhibited against the gram positive bacteria and fungi than against the gram negative bacteria. This is a common observation due to reduced susceptibility of the gram negative bacteria which is attributed to their morphology. The methanol extract was especially active against *S. aureus* which causes boils. The extract can therefore be used as a source of a potent antimicrobial agent. However, high concentrations were used and it is expected to be much lower if pure compounds were to be used.

5.2 Recommendations:

i. Column chromatography should be carried out to obtain pure isolates and confirm the characterized compounds.

ii. The water soluble gum was found to become highly viscous in high concentrations. Following the positive results on emulsion stability, further studies on shear stress are recommended to characterize the stabilizing effects with concentration.

iii. Toxicological study of the water soluble gum for use as food additives or in formulation of industrial products such as agrochemicals

iv. Gram positive bacteria and yeast bioassay should be done using pure gum resin isolates.

v. Formulation of detergents through synergistic combination of the ethanol extract and common industrial detergent ingredients.

REFERENCES

Abdallah, E.M., Khalid, A.S. and Ibrahim, N. (2009). Antibacterial activity of oleo-gum resins of *Commiphora molmol* and *Boswellia papyrifera* against methicillin resistant *Staphylococcus aureus* (MRSA). Sci. Res. Essays **4**(4): 351–356.

Abourashed, E. A. and Khan, I. A. (2013). *Leung's encyclopedia of common natural ingredients: Used in food, drugs and cosmetics*. Hoboken, N.J: Wiley.

Ahmad, M., Khan, M. A., Zafar, M., and Sultana, S. (2013). *Practical Handbook on Biodiesel Production and Properties*. Boca Raton, Florida: CRC Press.

Ahmed, S. E., Mohamed, B. E. and Karamalla, K. A. (2009). Analytical studies on the gum exudates from Anogeissus leiocarpus. Pakistan Journal of Nutrition **8**(6): 782-786.

Ali, N.A.A., Wurster, M. and Lindequist, U. (2009) Chemical composition of Essential oil from the Oleogum resin of *Commiphora habessinica* (Berg.) Engl. From Yemen. Journal of Essential Oil Bearing Plants. ISSN0972-060X.

Ameh, P. O. (2014). Physicochemical characterization and inhibitive performance evaluation of *Commiphora kestingii* gum exudates in acidic medium. International Journal of Physical Chemistry **9**(8): 184-198.

Andre, V., Willenbacher, N., Debus, H., Börger, L., Fernandez, P., Frechen, T. and Rieg, J. (2003). "Prediction of emulsion stability: Facts and myth" *Cosmetics and Toiletries Manufacture Worldwide*. Karlsruhe: Aston Publishing Group.

Barbosa, S. A., Coelho, D. F., Silveira, E., Tambourgi, E. B. and De Souza, R. R. (2013). Biosurfactant production by *Pseudomonas aeruginosa* and *Arrowia lipolytica* and its use for detergent formulations. Journal of Chemical Engineering **7:** 767-773.

Barros, M. E. S., Santos, D. A. and Hamdan, J. S. (2007). Evaluation of susceptibility of *Trichophyton mentagrophytes* and *Trichophyton rubrum* clinical isolates to antifungal drugs using a modified CLSI microdilution method (M38-A). Journal of Medical Microbiology **56:** 514-518.

Bauer, A. W., Kirby, W. M. M., Sherris, J. C. and Turck, M. (1966). Antibiotic susceptibility testing by standardized single disk method. Am. J. Clin. Pathol. **45:** 493-496.

Benkeblia, N. (2011). *Sustainable agriculture and new biotechnologies*. Boca Raton, FL: CRC Press.

Bieleman, J. (2000). *Additives for coatings.* Weinheim: Wiley-VCH.

Bille, J. C. (1980). *Observations from Elangata Wuas group ranch in Kajiado District (revised)*, Nairobi, Kenya: International Livestock Centre for Africa.

Biresaw, G. and Mittal, K. L. (2013). *Surfactants in tribology: Volume 3*. Boca Raton, Fla: CRC.

Boer, E., and Ella, A. B. (2000). *Plant resources of South-East Asia- Plants producing exudates.* Leiden: Backhuys Publishers.

Broze, G. (1999). *Handbook of detergents: Part A*. New York: M. Dekker.

Capasso, F. (2003). *Phytotherapy: A quick reference to herbal medicine*. New York: Springer.

Cassileth, B. R., Yeung, K. S. and Gubili, J. (2010). *Herb-drug interactions in oncology*. New York: McGraw-Hill Medical.

Chakrabarty, M. M. (2003). *Chemistry and technology of oils and fats*. New Delhi: Allied Publishers.

Chanamai, R. and McClements, D. J. (2002). Comparison of gum arabic, modified starch and whey protein isolate as emulsifiers: influence of pH, $CaCl_2$ and temperature. J. Food Science **67**(1): 121-125.

Chanasattru, W. (2008). Influence of water-soluble poly-ols on globular protein functionalities. PhD thesis, University of massachusetts, Amherst.

Chen, Y. F., Yang, C. H., Chang, M. S., Ciou, Y. P. and Huang, Y. C. (2010). Foam Properties and Detergent Abilities of the Saponins from *Camellia oleifera*. International Journal of Molecular Sciences **11:** 4417-4425.

Chesori, C.R. (2008). Study of effects of age on surfactant properties of the gum resin of *Commiphora schimperi* and characterisation of sodium derivative of the ethanol extract, 4th year Project Report, Department of Chemistry, University of Nairobi.

Chikamai, B. N. and Banks, W. B. (1993). Gum arabic from *Acacia Senegal*: Wild in Kenya. Food Hydrocolloids **7**(6): 521-534.

Chiteva, R., Yenesew, A., Chikamai, B. and Wanjohi, J. (2013). Phytochemical investigation of resins from Kenyan Commiphora holtziana. International Journal of current research **5**(7): 1791-1793.

Chiu, Y. C. and Jiang, F. C. (1999). Determination of long-term stability of vitamin E emulsion and formation of micro-emulsions by using laser light scattering and UV absorption measurements. Journal of Dispersion Science and Technology **20**(2): 449-465.

Damodaran, S. and Paraf, A. (1997). *Food proteins and their applications*. New York: Marcel Dekker.

Dileesh, S., Adithya M., Sankar, A. and Peter, C. V. (2013). Determination of saponification, acid and ester values; percentage of free fatty acids and glycerol in some selected edible oils: Calculation of concentration of lye needed to prepare soap from these oils. Research Scholar ISSN 2249-6696.

Dunford, N. T. (2012). *Food and industrial bioproducts and bioprocessing*. Ames, Iowa: Wiley-Blackwell.

Dunkle, J. W., Bressler, J., Brown, E. L. and McCurdy, S. (2009). *Characterization of Staphylococcus aureus isolates associated with pediatric carriage in Houston, Texas*, Masters Abstracts International.

Dunn, K. M. (2010). *Scientific soapmaking: The chemistry of the cold process*. Farmville, VA: Clavicula Press.

Eagleson, M. (1994). *Concise encyclopedia chemistry*. Berlin: Walter de Gruyter.

El-Ashry, E., Rashed, N., Salama, O. M. and Saleh, A. (2003). Components, therapeutic value and uses of myrrh. Pharmazie **3:** 163-168.

Elementis Specialties (2011). Specialty surfactant products (LR). Langestraat, EE Delden-Netherlands.

Eliassi, A. and Parach, A. (2007). Determination of cloud points of poly(propylene glycol) aqueous mixtures using particle counting method. Journal of Chemical and Engineering Data **53**(1): 4010-4012

Emmanuel, O. O., Bhartia, P. G., Tytler B. A. and Adikwu, M. U. (2014). Surface activity and hydrophile-lipophile balance of hydrophilic polymers from exudates of cashew and khaya plants. International Journal of Biological and Pharmaceutical Research **5**(5): 443-448.

Europa Publications Limited (2001). *The environment encyclopedia and directory 2001*. London: Europa.

Evans, W. C. (2009). *Trease and Evans' Pharmacognosy*. London: Elsevier Health Sciences UK.

Fainerman, V. B., Mobius, D. and Miller, R. (2001). *Surfactants: Chemistry, Interfacial properties, applications*. Amsterdam: Elsevier.

FAO (2002). "Food energy- methods of analysis and conversion factors" Food and nutrition paper **77**. ISSN 0254-4725.

FAO Rome (1990). Food and Nutrition paper **49**: 23.

Gachathi, F. N. and Eriksen, S. (2011). Gums and resins: The potential for supporting sustainable adaptation in Kenya's drylands. *Climate and Development* **3**(1): 59-70.

Gibbons, S. (2004). Anti-staphylococcal plant natural products. Nat. Prod. Rep. **21**(2) 263-277.

Glass, J. E. (1989). *Polymers in aqueous media: Performance through association*. Washington, DC: American Chemical Society.

Glew, D. W. (2012). *Validating low carbon bio-renewable alternatives to petrochemicals: Exploring end of life impacts*. PhD thesis, University of New York.

Glicksman M. (1969): *Gum technology in food industry*. New York: Academic printers.

Gundidza, M., Mmbengwa, V., Sibambo, S. R., Magwa M. L., Mushisha, O., Benhura, M.A., Gundidza, E. and Samie, A. (2011). Rheological, moisture and ash content analyses of a gum resin from *Commiphora Africana*. African Journal of Food Science **5**(4): 188 – 193.

Hadkar, U. B. (2008). *Practical Physical Pharmacy and Physical Pharmaceutics.* New Delhi: Nirali Prakashan.

Hamid, A.A., Aiyelaagbe, O.O. and Usman, L.A. (2011). Essential Oils: Its medicinal and pharmacological uses. International Journal of Current Research **33**(2): 086-098.

Hanus, L. O., Rezanka, T., Dembitsky, V. M. and Moussaieff, A. (2005). Myrrh- Commiphora chemistry. Biomed. Papers **149**(1): 3–28.

Hanus, L. O., Rosenthal, D., Rezanka, T., Dembitsky, V. M. and Mousssaief, A. (2008). Fast and easy GC/MS identification of myrrh resins. Pharmaceutical Chemistry Journal **42(**12): 719-720

Holmberg, K. (2004). Novel Surfactants - Preparation, Applications, and Biodegradability. SOFW Journal **130:** 91-91.

Huang, X., Kakuda, Y. and Cui, W. (2001). Hydrocolloids in emulsions: Particle size distribution and interfacial activity. Food Hydrocolloids **15(**4): 532-544.

Iwu, M. M. (2013). *Handbook of African Medicinal Plants, Second Edition*. Hoboken: Taylor and Francis.

Kalia, S. and Avérous, L. (2011). *Biopolymers: Biomedical and environmental applications*. Hoboken, N.J: Wiley.

Karaman, I., Sahin, F., Gulluce, M., Ogutcu, H., Sengul, M. and Adiguzel, A. (2003). Antimicrobial activity of aqueous and methanol extracts of *Juniperus oxycedros*. Journal of Ethnopharmacology **85**: 231-255.

Karsa, D. R. and Porter, M. R. (1995). *Biodegradability of surfactants*. London: Blackie Academic and Professional.

Kennedy, J. F., Phillips, G. O. and Williams, P. A. (1996). *The chemistry and processing of wood and plant fibrous material: Cellucon '94 Proceedings*. Cambridge: Woodhead Publishing Ltd.

Killops, S. D. and Killops, V. J. (1993). *An introduction to organic geochemistry*. Harlow, Essex, England: Longman Scientific and Technical.

Kirk-Othmer (2013). *Chemical technology of cosmetics*. Hoboken, New Jersey: Wiley.

Kjellin, M. and Johansson, I. (2010). *Surfactants from renewable resources*. Chichester, U.K: Wiley.

Kunjappu, J. T. and Rosen, M. J. (2013). *Surfactants and interfacial phenomena*. Hoboken, N.J: Wiley.

Kyalo, S.K. (1998). Analysis of water soluble gum of acacia variety. 4th Year Research Project, Department of Chemistry, University of Nairobi.

Lai, K. Y. (2006). *Liquid detergents*. Boca Raton: Taylor and Francis.

Langenheim, J. H. (2003). *Plants Resins: Chemistry, Evolution, Ecology, and Ethnobotany.* Portland- Cambridge: Timber Press.

Lee, B., Chan, E., Ravindra, P. and Khan, T. A. (2012). Surface tension of viscous biopolymer solutions measured using the du Nuoy ring method and the drop weight method. Polym. Bull. **69**: 471-489.

Lelon, J. K., Jumba, I. O., Keter J. K., Chemuku, W. and Oduor, F. D. O. (2010). Assessment of physical properties of gum arabic from *Acacia senegal* varieties in Baringo District, Kenya. African Journal of Plant Science **4**(4): 95-98.

Lemenih, M. and Kassah H. (2011). *Opportunities and Challenges for sustainable production and marketing of gums and resins in Ethiopia*. Bogor, Indonesia, Center for International Forestry Research (CIFOR). 105p ISBN: 978-602-8693-57-58.

Limeback, H. (2012). *Fluoride therapy in Comprehensive Preventive Dentistry*. West Sussex, UK: Wiley.

Lomborg, B. (2013). *How much have global problems cost the world? A scorecard from 1900 to 2050*. Cambridge: Cambridge University Press.

López-Franco, Y. L., Córdova-Moreno, R.E., Goycoole, F.M., Valdez, M.A., Juárez-Onofre, J. and Lizardi-Mendoza, J. (2011). Classification and physicochemical characterization of mesquite gum (*Prosopis spp*.). Food hydrocolloids **26**(1) 159-166.

Lunkenheimer, K. and Malysa, K. (2003). Simple and generally applicable method of determination and evaluation of foam properties. J. Surf. Deterg. **6**: 69–74.

Madhavi, D. L., Deshpande, S. S. and Salunkhe, D. K. (1995). *Food anti oxidants: Technological, toxicological and health perspectives.* New York: Marcel Dekker.

Maradufu A. and Warthen, D. J. (1988). Furanosesquiterpenoids from *commiphora myrrha* oil. Plant Science **57:** 181-184

Mendelson, C. (2005). *Laundry: The home comforts book of caring for clothes and linens*. New York: Scribner.

Mohamed, A. A., Ali, S. I., El-Baz, F. K., Hegazy, A. K. and Kord, M. A., (2014). Chemical composition of essential oil and in vitro antioxidant and antimicrobial activities of crude extracts of Commiphora myrrha resin. Industrial Crops and Products **57:** 10-16.

Montenegro, A. M., Boiero, M. L., Valle L. and Borsarell, C. D. (2012). Gum Arabic: More Than an Edible Emulsifier. Products and Applications of Biopolymers **51:** 953- 978

Mukherjee, I., Satya P., Moulik, S. P., Animesh K. and Rakshit, A. K. (2013). Tensiometric determination of Gibbs surface excess and micelle point: A critical revisit. Journal of Colloid and Interface Science **394:** 329–336.

Munyendo, W. L. L., Orwa, J.A., Rukunga, G.M. and Bii, C.C. (2011). Bacteriostatic and Bactericidal Activities of *Aspilia mossambicensis*, *Ocimum gratissimum* and *Toddalia asiatica* extracts on selected pathogenic bacteria. Research Journal of Medicinal Plant **5:** 717-727.

Musa, A. A. (2008). Antioxidant and antibacterial activity of *Commiphora kerstingii* Engl. stem bark extract. Research Journal of Phytochemistry **2:** 106-111.

Mwendwa, F.M. (2007). Surfactant properties of *Commiphora Schimperi* gum resin. 4th Year Project Report, Department of Chemistry, University of Nairobi.

Myers, D. (2006). *Surfactant science and technology*, 3rd Edition. Hoboken, New Jersey: J. Wiley.

National Committee for Clinical Laboratory Standards (2002). Broth dilution and susceptibility testing of yeasts approved standard method M27-A. Villanova, PA.

Nielsen, S. S. (2010). *Food analysis*. New York: Springer.

Nostro, A., Germano, M. P., D'Angelo, V., Marino, A. and Cannatelli, M.A. (2000). Extraction methods and bioautography for evaluation of medicinal plant antimicrobial activity. Letters of Applied Microbiology **30**(5) 379-384.

NPCS Board Consultants and Engineers (2009). *Soaps, detergents and disinfectants technology handbook*. Delhi: NIIR.

Nussinovitch, A. (2010). *Plant gum exudates of the world: Sources, distribution, properties, and applications*. Boca Raton, FL: CRC Press/Taylor and Francis.

O'Mara, F. P. (2005). *International Grassland Congress: Offered papers*. Wageningen: Academic Publishers.

Omer, S. A., Adam, S. E. I. and Mohammed, O. B. (2011). Antimicrobial activity of *Commiphora myrrha* against some bacteria and Candida albicans isolated from gazelles at King Khalid wildlife research centre. Research Journal of Medicinal Plant **5**(1): 65-71.

Panda, H. (2002). *The complete technology book on natural products (forest based)*. Delhi, India: Asia Pacific Business Press.

Particle Sciences- Drug development services (2011). *Technical brief*, volume 2.

Philips G. O., Wedlock D. J. and Williams, P. A. (1990). *Gums and stabilisers for the food industry*. Oxford: IRL Press.

Pilemand, C. (2002). *Surfactants: Their abilities and important physico-chemical properties.* Copenhagen: Miljø og luftveje, Arbejdsmiljøinstituttet.

Pruppacher, H. R., and Klett, J. D. (1997). *Microphysics of clouds and precipitation*. Dodrecht: Kluwer Academic Pulbishers.

Rosen, M. J. and Kunjappu, J. T. (2012). *Surfactants and Interfacial Phenomena*. Hoboken: John Wiley and Sons.

Selvamani, P., Gupta, J. K. and Jyoti D. (2010). "Plants of The Genus: Commiphora- Their Chemistry" *Comprehensive Bioactive Natural Products, Volume 6*. Studium Press LLC, USA.

Shachman, M. (2005). *The soft drinks companion: A technical handbook for the beverage industry*. Boca Raton, FL: CRC Press.

Shen, T., Li, G. H., Wang X. N. and Lou H. X. (2012). The Genus Commiphora: A review of its traditional uses, phytochemistry and pharmacology. Journal of Ethnopharmacology **142:** 319-330.

Siddiqui, M. Z. and Mazumder, P. M. (2012). Comparative study of hypolipidemic profile of resinoids of *Commiphora mukul/ Commiphora wightii* from different geographical locations. Indian J Pharm Sci **74**: 422-427.

Singh, B. P. (2010). *Industrial crops and uses*. Wallingford, Oxfordshire, UK: CABI.

Slade, P. E. (1998). *Handbook of fiber finish technology*. New York: Marcel Dekker.

Srivastava, A. K. and Jain, P. C. (2009). *Chemistry Vol (1 and 2)*. New Delhi: V. K. (India) Enterprises.

Stamell, J. (2008). *Excel HSC chemistry*. Glebe, N.S.W: Pascal Press.

Swayamjot, K., Husheem, M., Saroj, A., Pirkko, L.H. and Subodh- Kumar, K. (2005). The in-vitro cytotoxic and apoptotic activity of Triphala- an Indian herbal drug. Journal of Ethnopharmacology **97**(1): 15-20.

Swift, K. A. D. (2002). *Advances in flavours and fragrances: From the sensation to the synthesis*. Cambridge: Royal Society of Chemistry.

Tadesse, W., Desalegn, G. and Alia, R. (2007). Natural gum and resin bearing species of Ethiopia and their potential applications. Investigación Agraria: Sistemas y Recursos Forestales **16**(3): 211-221.

Tawde, S. R., Kunawwar, N. A. and Shende, A. T. (2013). Acid value of various domestic uses oil. International Journal of Engineering Science and Technology (IJEST). **5(**5S): 1-2.

Toedt, J., Koza, D. and Cleef-Toedt, K. V. (2005). *Chemical composition of everyday products*. Westport: Greenwood Press.

Totten, G. E., Westbrook, S. R. and Shah, R. J. (2003). *Fuels and lubricants handbook: Technology, properties, performance, and testing*. West Conshohocken, PA: ASTM International.

Veberic, R. (2010). *Bioactive compounds in fruit plants*. Ljubljana: Biotehniska fakulteta.

Watanabe, H., Matsuyama, T. and Yamamoto, H. (2003). Experimental study on electrostatic atomization of highly viscous liquids. *Journal of Electrostatics* **57**(2): 183-197.

Wiendl, R. M., Müller, B. M. and Franz, G. (1995). *Proteoglycans from the gum exudate of myrrh*. Carbohydrate Polymers **28**(3): 217-226.

Woodhouse, P., Bernstein, H. and Hulme, D. (2000). *African enclosures? The social dynamics of wetlands in drylands*. Oxford: James Currey.

Yang, Y., Leser, M. E., Sher, A. A. and McClements D. J. (2013). Formation and stability of emulsions using a natural small molecule surfactant: Quillaja saponin (Q-Naturale). Food Hydrocolloids **30**(2): 589-596.

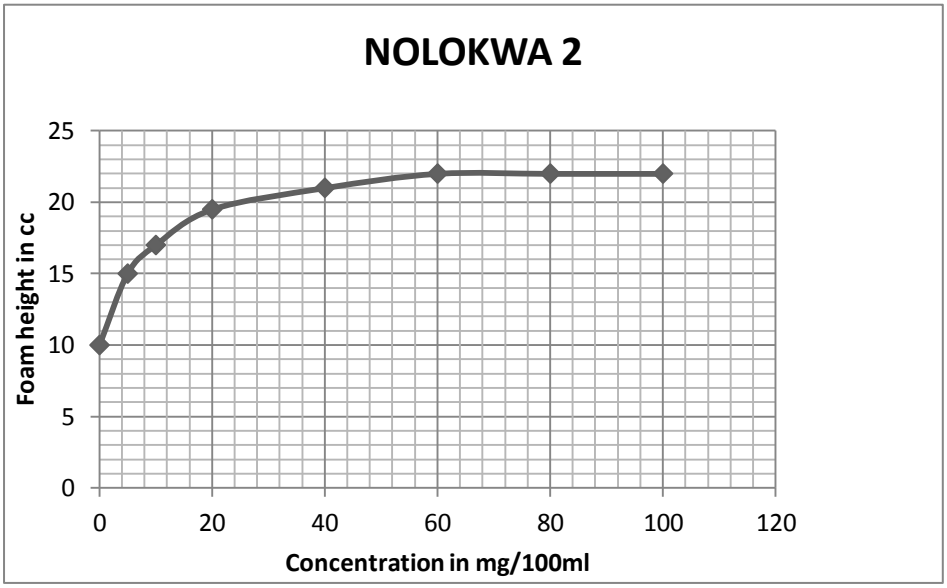
Yasser, A. J., Muneer, A., Abdelhafid, B., Daoudi, C. S. and Hammadi, L. A. (2013). Chemical and Phytochemical analysis of some anti diabetic plants in Yemen. Int. R. J. Pharm. **4**(9): 72-76.

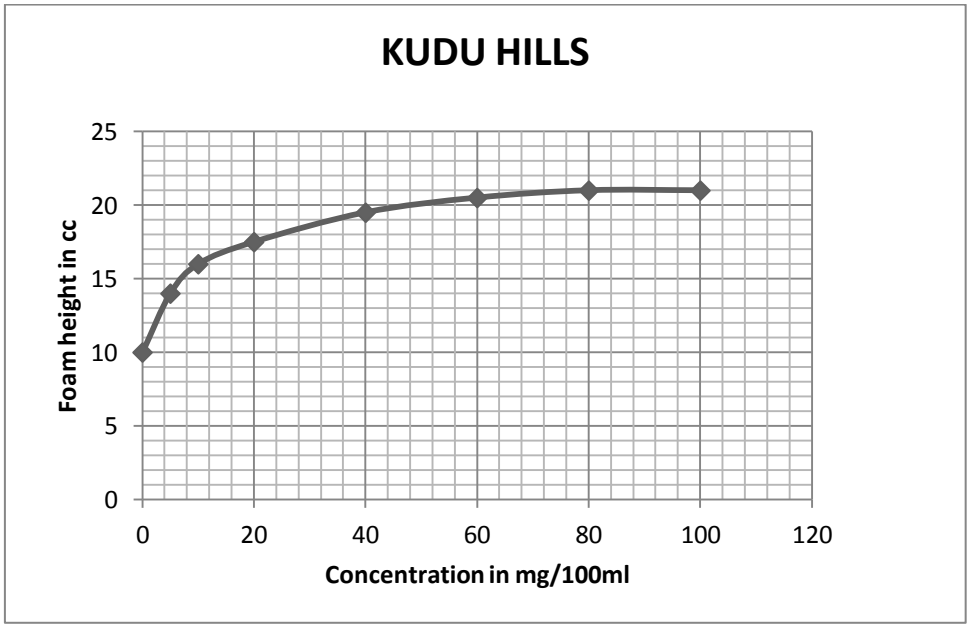
Zahra, E., Mohammad, S., Mohammad, M. and Babak, G. (2007). Physicochemical and emulsifying properties of Barijeh (*Ferula gumosa*) gum. Iran J. Chem. Eng. **26(**3): 81-88.

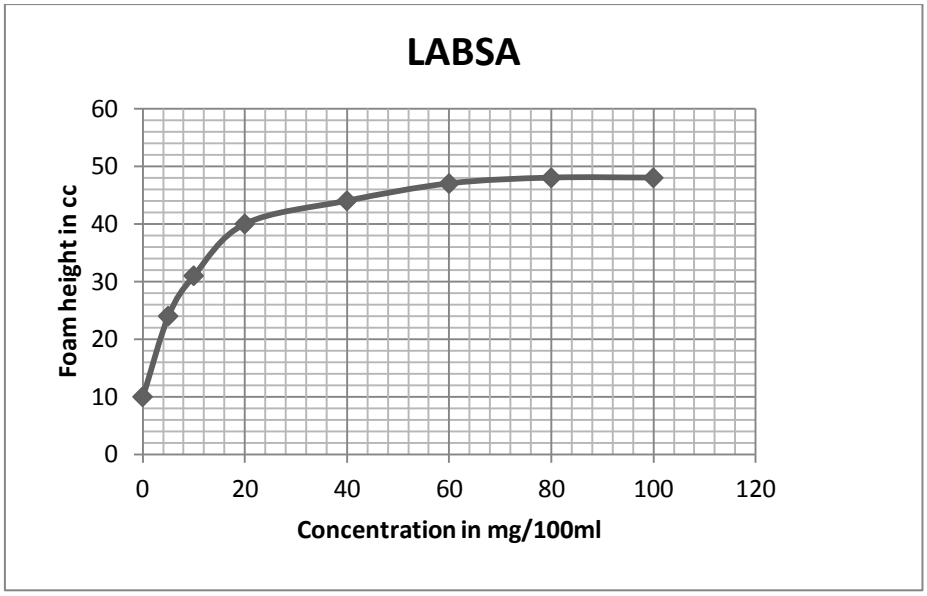
Zaikov, G. E. (2004). *Biocatalytic technology and nanotechnology*. New York: Nova Science.

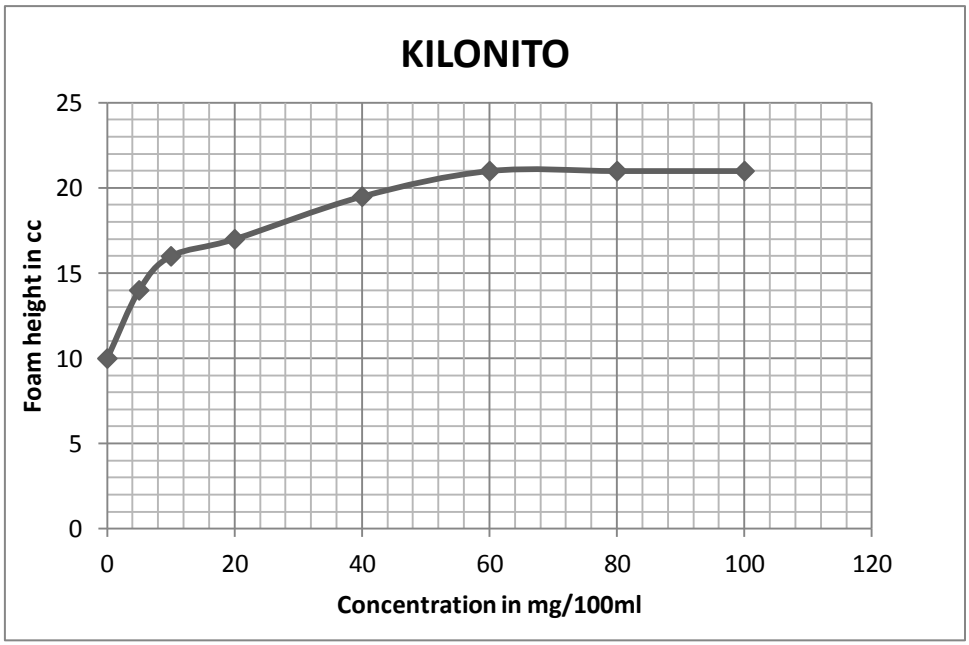
APPENDICES

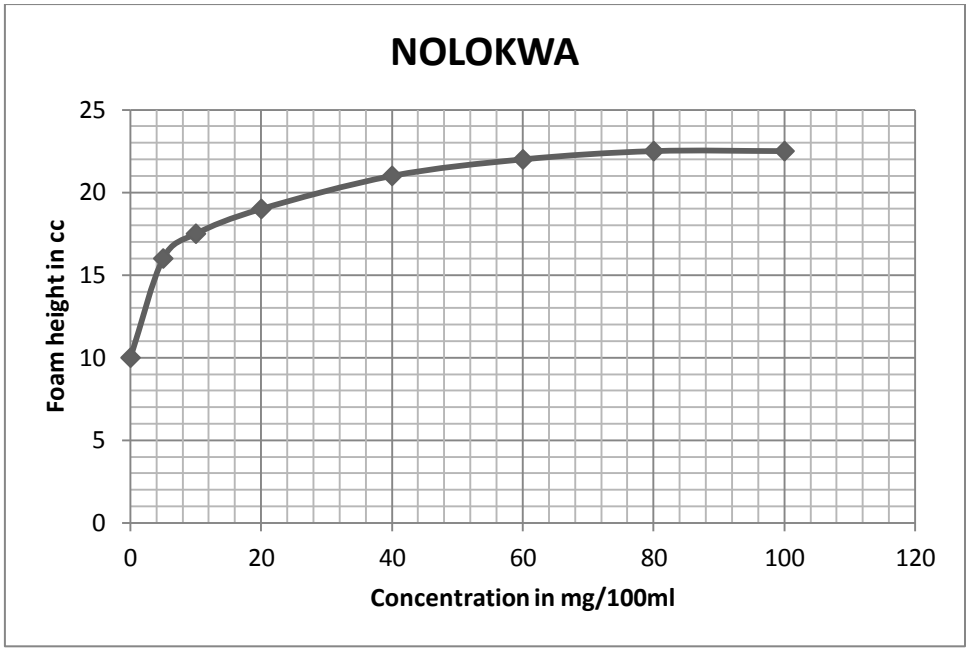
Appendix 1: Foam Height Variation with Concentration for Nolokwa 2, Kudu Hills, LABSA, Kilonito, Nolokwa and CDE.

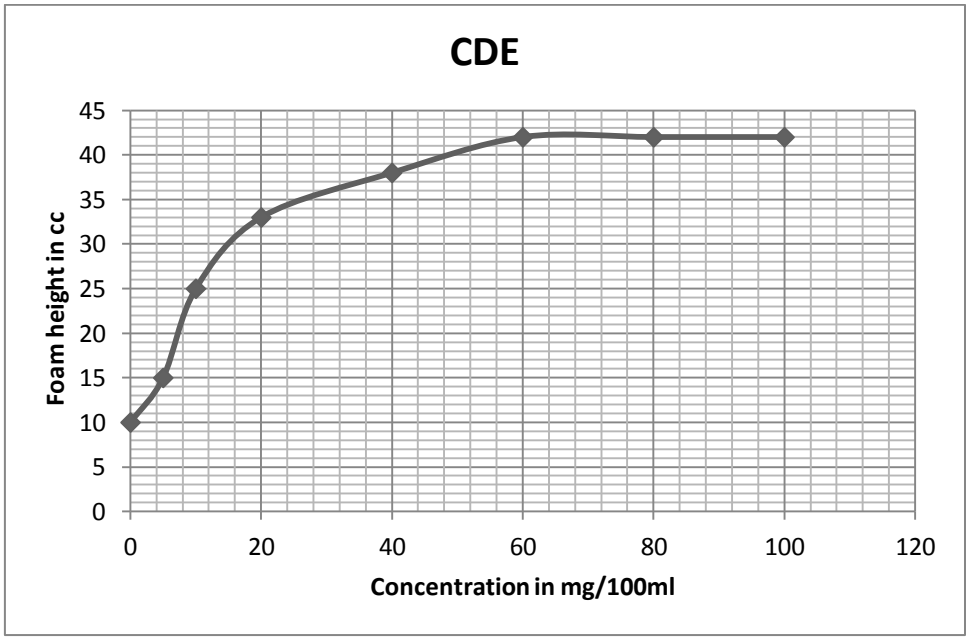





LABSA
Foam height in cc
60
50
40
30
20
10
0
0
20
40
60
80
100
120
Concentration in mg/100ml

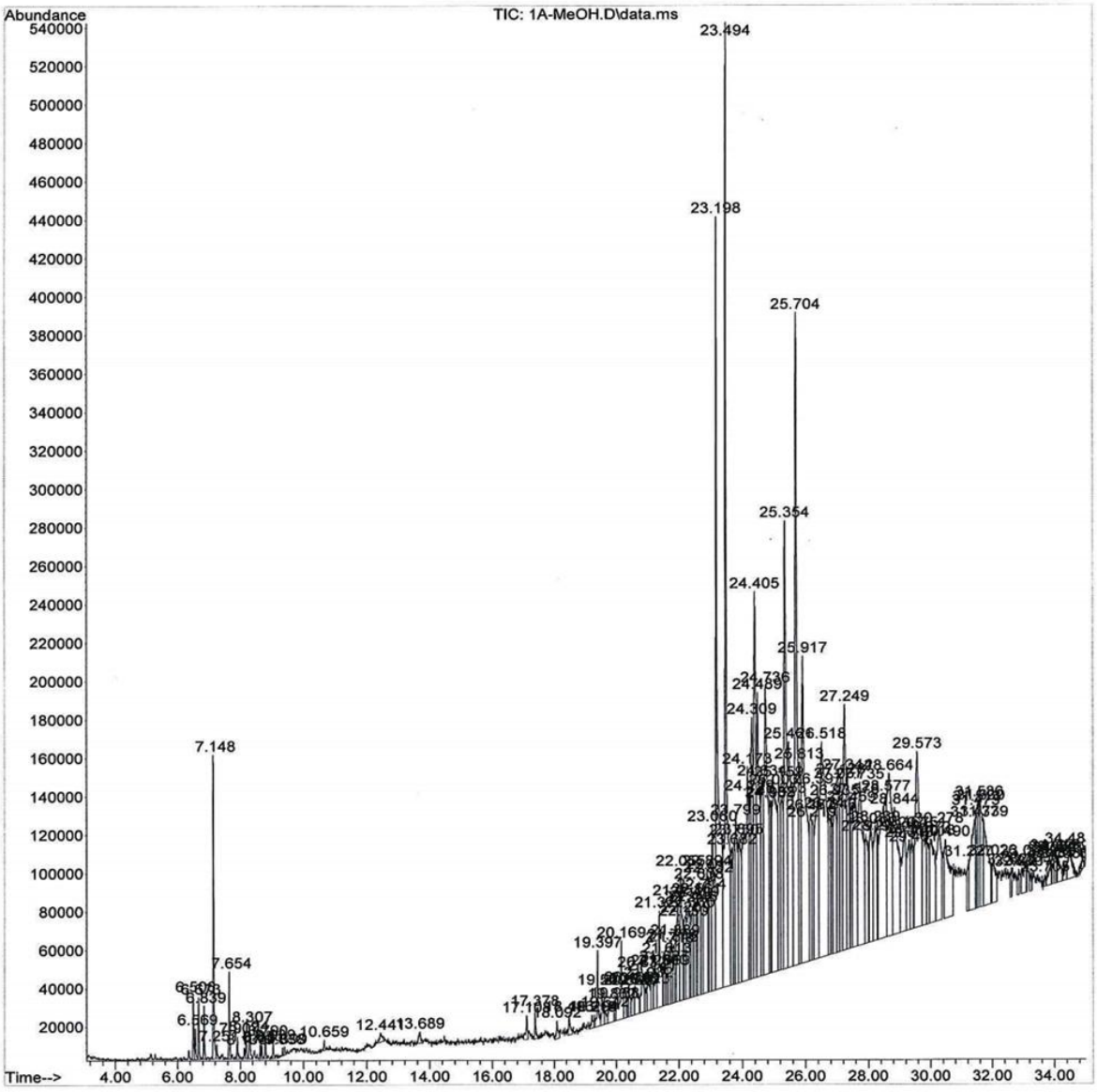

KILONITO
Foam height in cc
25
20
15
10
5
0
0
20
40
60
80
100
120
Concentration in mg/100ml


NOLOKWA
Foam height in cc
25
20
15
10
5
0
0
20
40
60
80
100
120
Concentration in mg/100ml

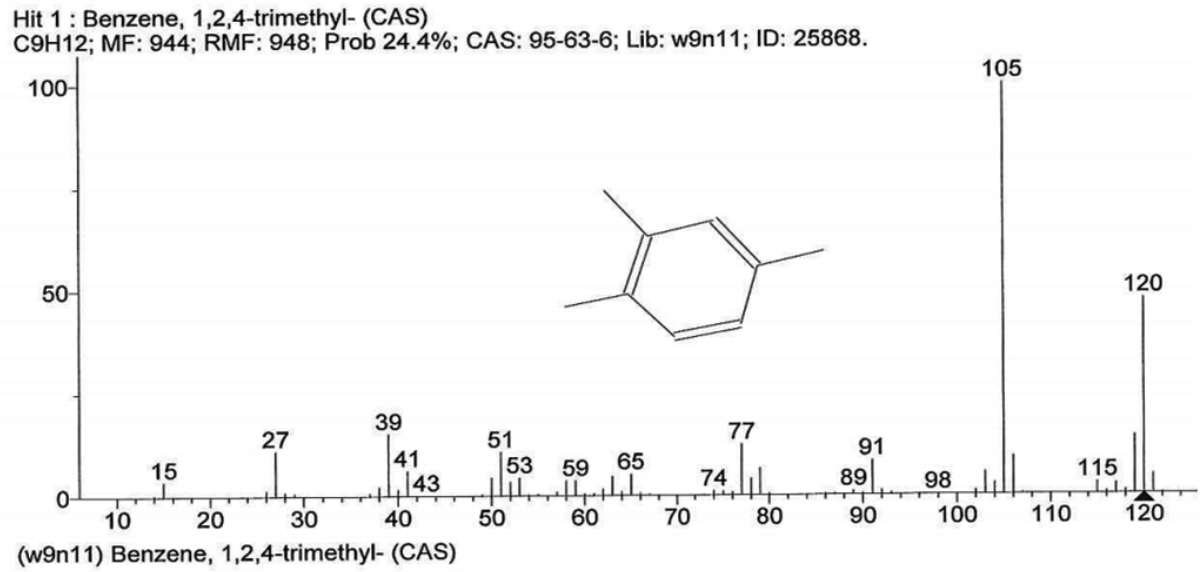

CDE
Foam height in cc
45
40
35
30
25
20
15
10
5
0
0
20
40
60
80
100
120
Concentration in mg/100ml

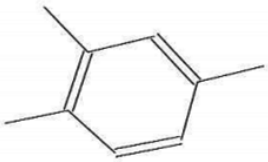
Appendix 2: GC-Chromarogram of Nolokwa Crude Methanol Extract

```
File       :C:\msdchem\1\data\Students\Postgrads\B_Ndunda\1A-MeOH.D
Operator   :
Acquired   : 24 Feb 2014  21:46     using AcqMethod LONG STANDARD.M
Instrument :   Agilent GC-MS
Sample Name: 1A-MeOH
Misc Info  :
Vial Number: 4
```



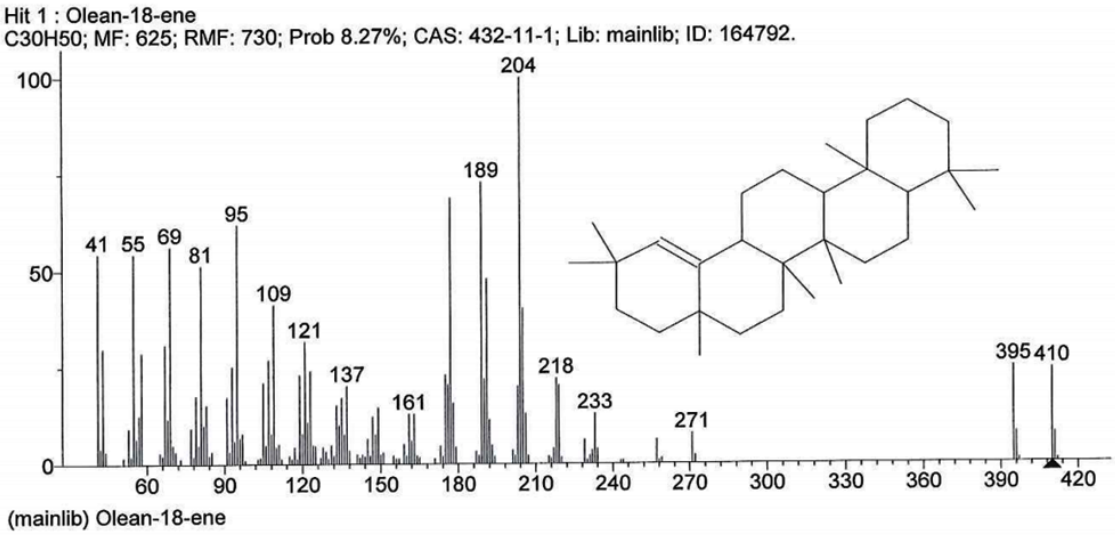
Appendix 3: Mass Spectra of Compound 1, Cumene

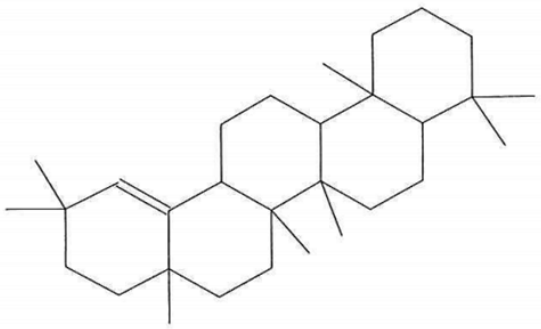




10 largest peaks:
105 999 | 120 475 | 39 151 | 119 141 | 77 124 | 27 110 | 51 107 | 106 91 | 91 83 | 79

Appendix 4: Mass Spectra of Compound 2, Oleanene



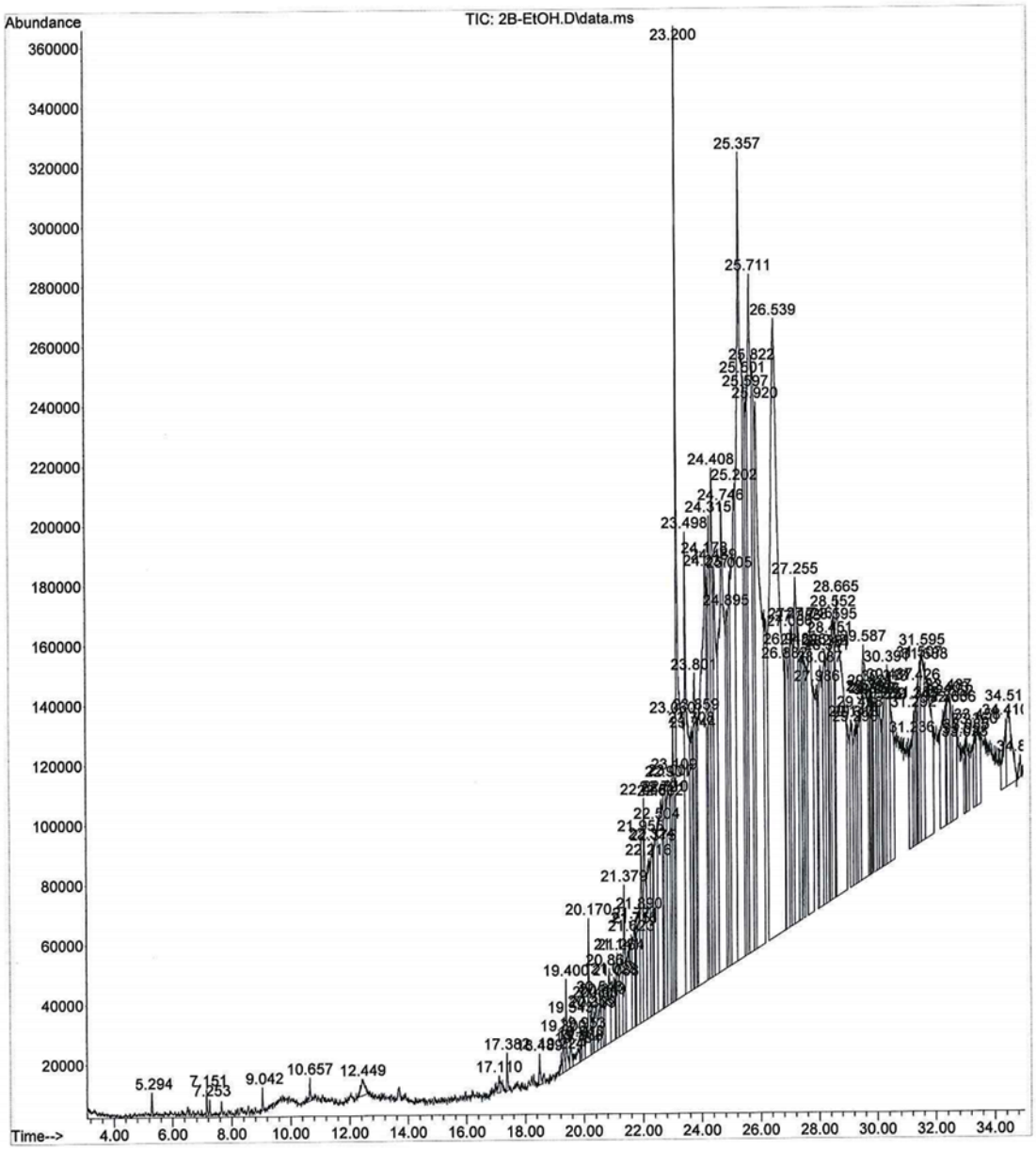


10 largest peaks:

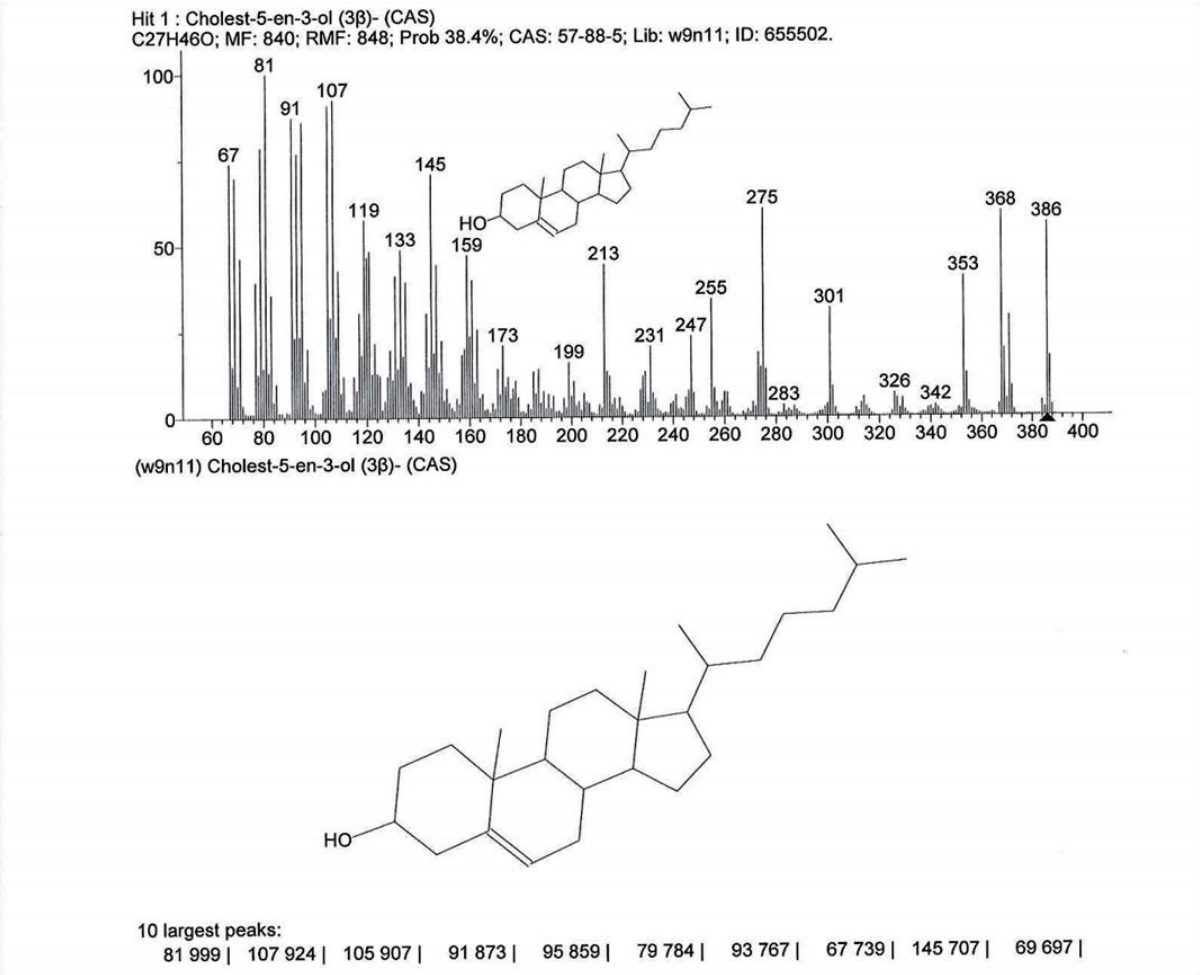
204 999 | 189 728 | 177 687 | 95 619 | 69 562 | 41 543 | 55 543 | 81 513 | 191 478 | 109 413 |

Appendix 5: GC-Chromatogram of Nolokwa 2 Crude Ethanol Extract

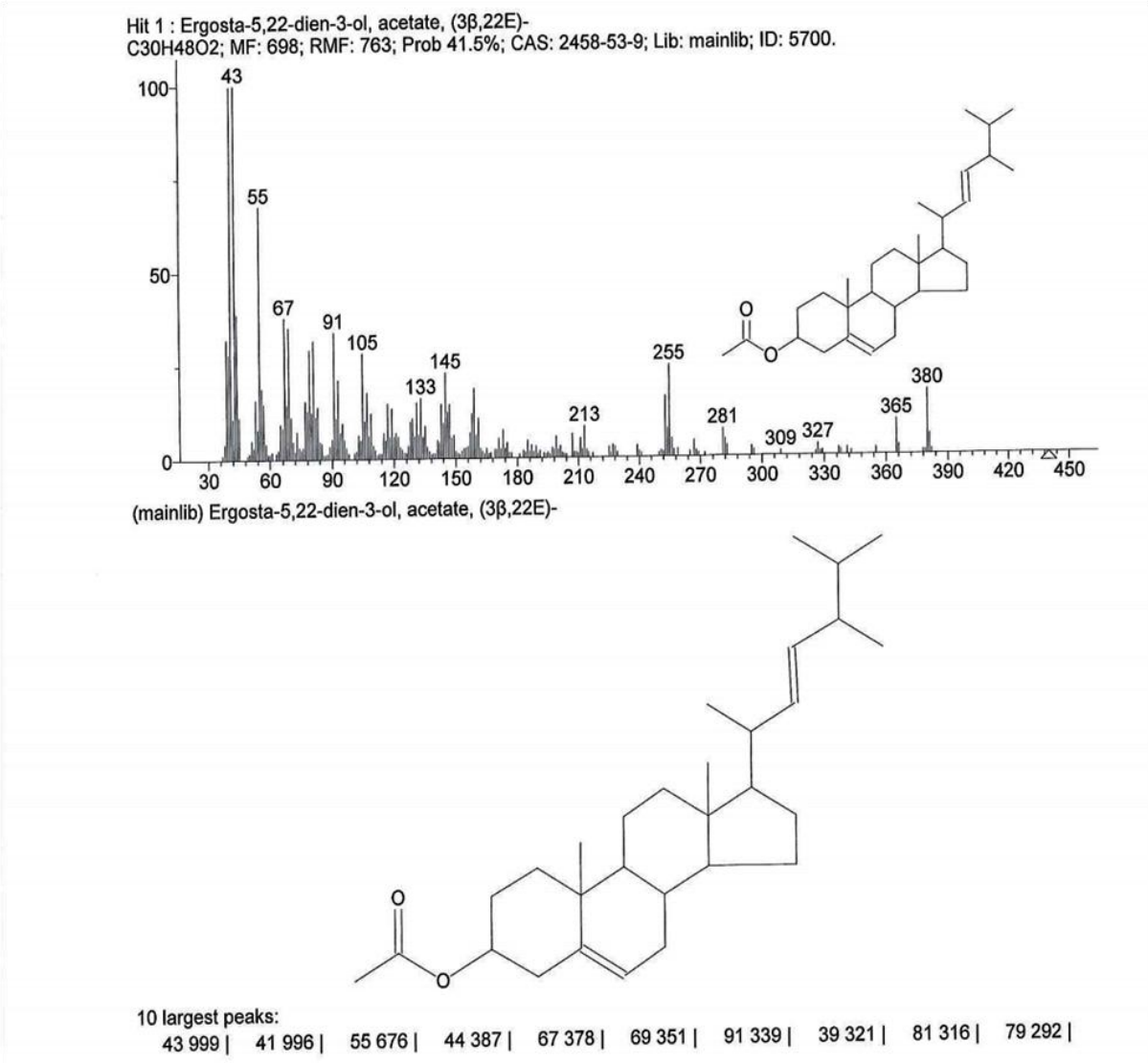
```
File       :C:\msdchem\1\data\Students\Postgrads\B_Ndunda\2B-EtOH.D
Operator   :
Acquired   : 24 Feb 2014  23:51     using AcqMethod LONG STANDARD.M
Instrument :   Agilent GC-MS
Sample Name: 2B-EtOH
Misc Info  :
Vial Number: 7
```



Appendix 6: Mass Spectra of Compound 1

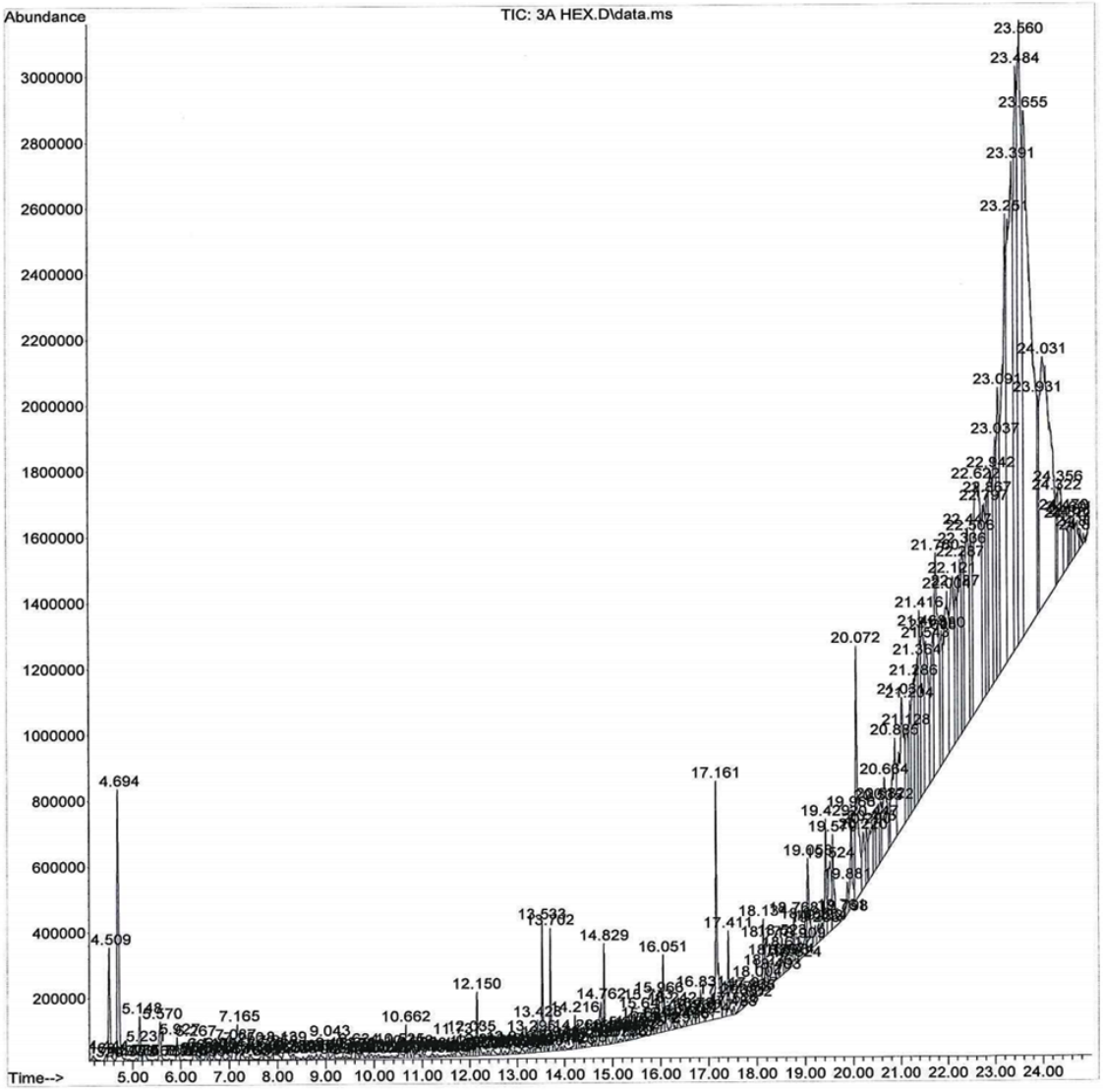


Appendix 7: Mass Spectra of Compound 2

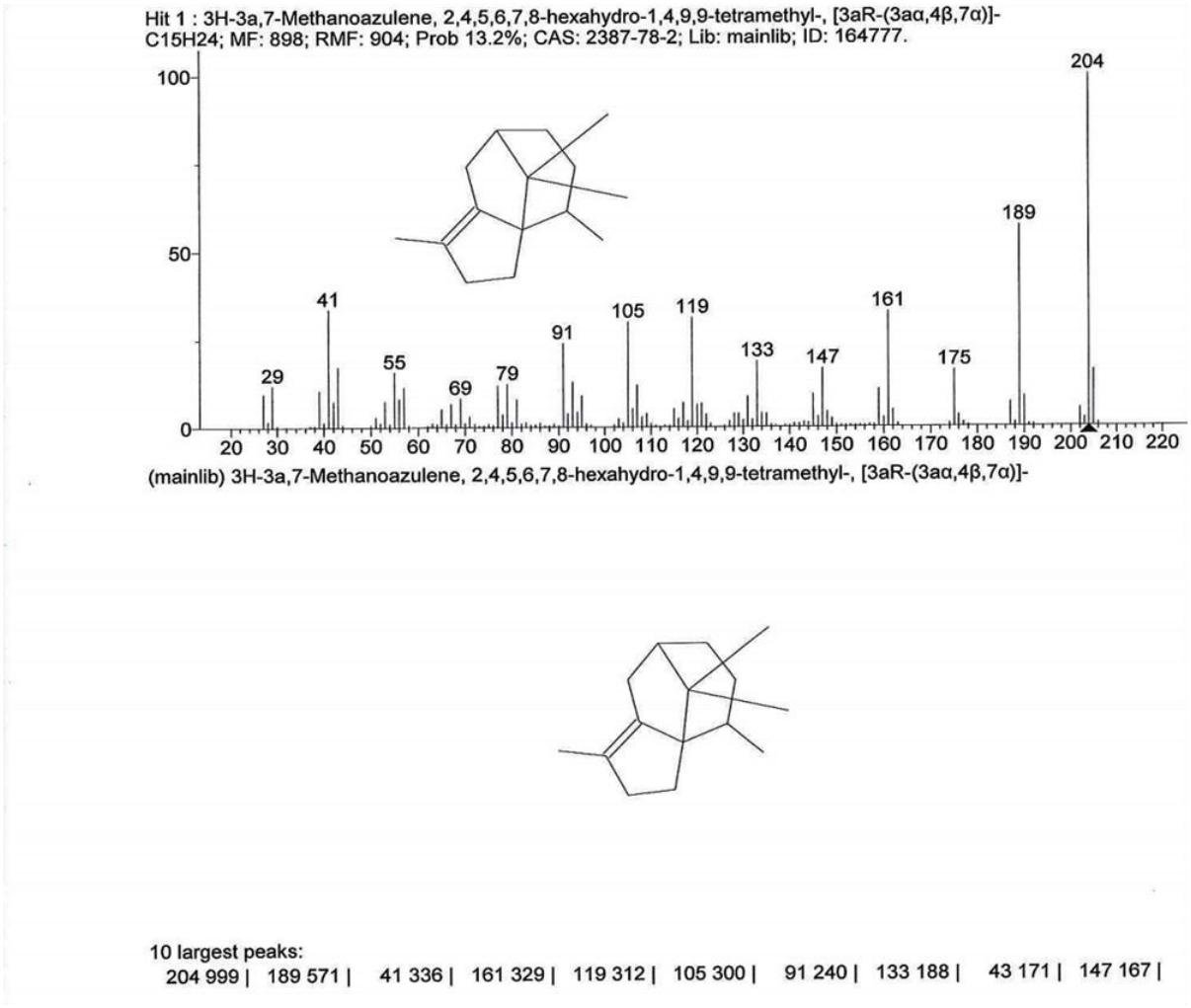


Appendix 8: GC-Chromatogram of Nolokwa Crude Hexane Extract

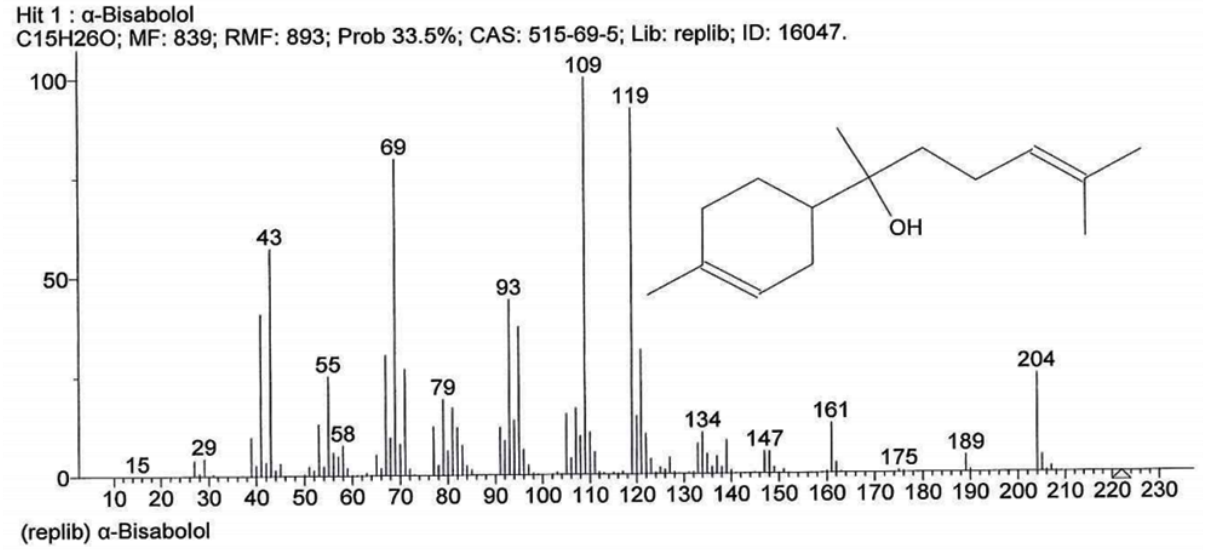
```
File       :C:\msdchem\1\data\Students\Postgrads\B_Ndunda\3A HEX.D
Operator   :
Acquired   : 26 Feb 2014  13:50      using AcqMethod STANDARD.M
Instrument :   Agilent GC-MS
Sample Name: 3A HEX
Misc Info  :
Vial Number: 7
```

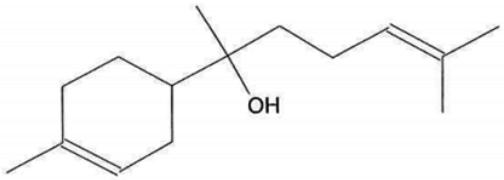


Appendix 9: Mass Spectra of Compound 1, Cyperene



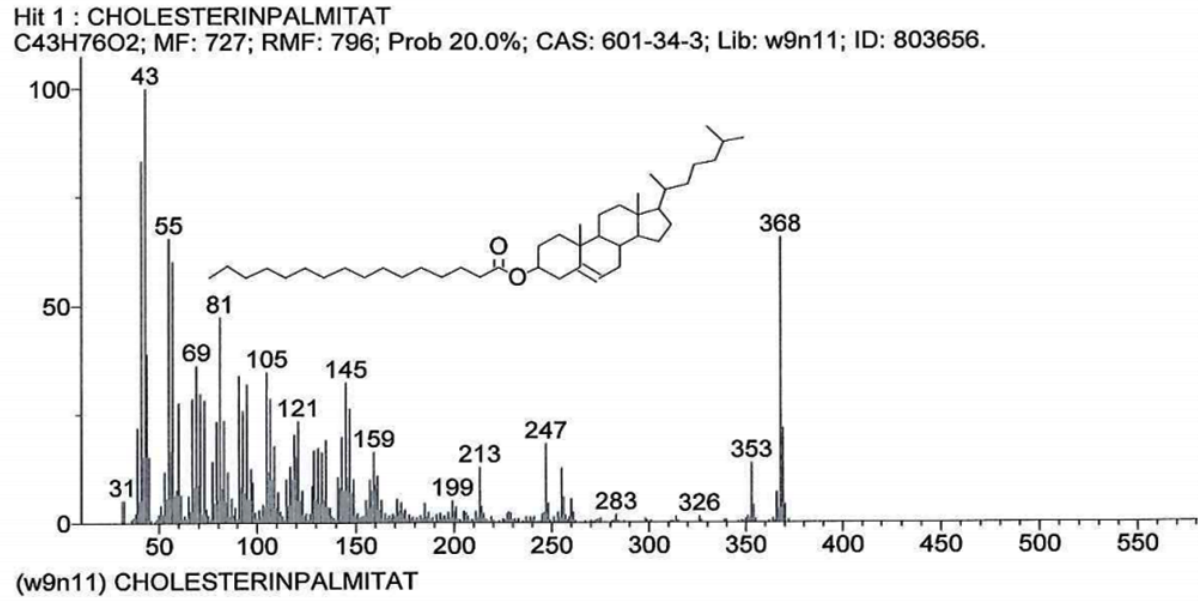
Appendix 10: Mass Spectra of Compound 2, Bisabolol

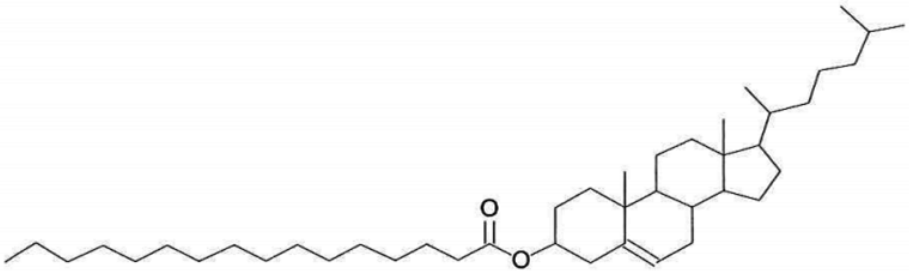




10 largest peaks:
109 999 | 119 920 | 69 796 | 43 572 | 93 442 | 41 407 | 95 372 | 121 313 | 67 303 | 71 267 |

Appendix 11: Mass Spectra of Compound 3, Cholesterinpalmitat

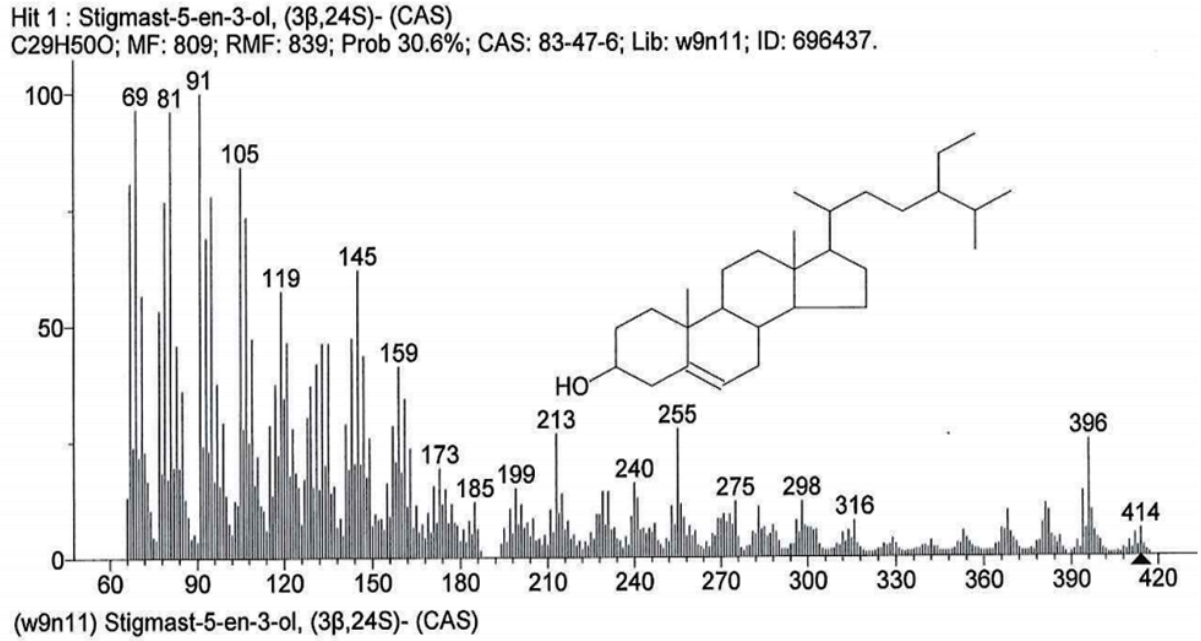




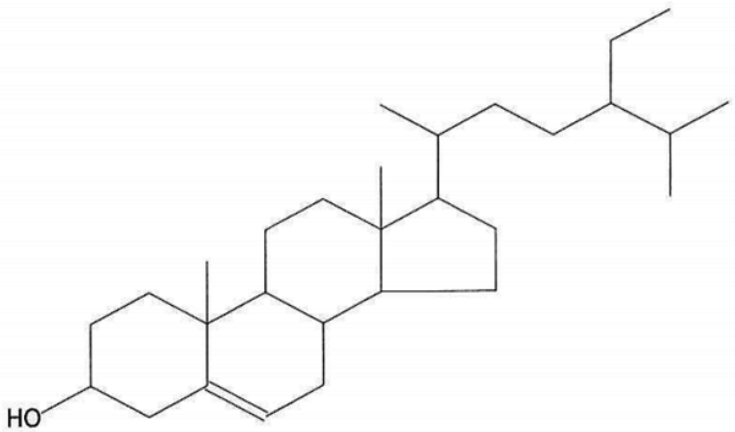
10 largest peaks:

43 999 | 41 831 | 368 655 | 55 654 | 57 600 | 81 473 | 44 389 | 69 362 | 105 347 |

Appendix 12: Mass Spectra of Compound 4, Stigmastenol



(w9n11) Stigmast-5-en-3-ol, (3β,24S)- (CAS)



10 largest peaks:

91 999 | 69 964 | 81 960 | 105 841 | 67 805 | 95 778 | 79 766 | 107 733 | 93 688 | 145 619 |