Physico-chemical characterization of African traditional cosmetics produced by the Ovahimba tribes of Northern Namibia

A research project submitted to the faculty of Science, University of the Witwatersrand in fulfillment of the requirements for the Masters` degree (Coursework and Research Report)

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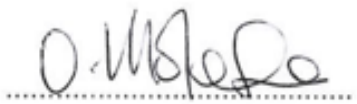
Johannesburg

2015

DECLARATION

I declare that this research is my own, unaided work. It is being submitted for the Degree of Master of Science at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.



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ABSTRACT

Ovahimba people from Kunene region, northern Namibia, are known for covering their bodies with red ochre mixed with clarified butterfat, traditionally known as *otjize* to give them a distinct red appearance. Ochre refers to a clay-like earth pigment which contains some form of iron-containing mineral. A mixture of traditional herbs with clarified butterfat, *otjizumba*, is also applied around the necks as a perfume. This study was prompted by ethnographic interviews amongst the Ovahimba people which revealed functional uses of the traditional cosmetics, specifically the red ochre-derived cosmetic, as a mosquito repellent.

Several analytical techniques were used to determine the presence of mosquito repellent compounds in the red ochre- derived cosmetic and the aromatic plant derived-cosmetic. GC-MS was used to identify the presence of compounds which have previously been found to have mosquito repellent capabilities. GC-MS analysis identified mostly oxygenated compounds which include ketones (2-dodecanone, 2-nanonone, 2-undecanone and 2-tridecanone), aldehydes (heptanal and nonanal) and carboxylic acids (hexanoic acid and heptanoic acid) in dichloromethane extracts of *otjize* and mostly hydrocarbons (o-cymene, α-pinene, limonene, and squalene) and less oxygenated compounds (terpinen-4-ol and α-campholenal) in plant derived cosmetic extracts. The chemical composition of the cosmetics was also analyzed using FTIR. FTIR analysis for organics in both cosmetics showed presence of vibrational motions including O-H, C=O, C-H, C=C and C-C which affirmed the presence of organic functional groups including aldehydes, ketones, esters, alkenes and alkanes. Peak patterns observed using GC-FID showed that the mixture of red ochre and clarified butterfat released higher quantities of volatiles than when individual samples were analyzed.

Mineralogical composition of red ochre was determined by PXRD, supported by FTIR which revealed as significant amount of hematite (Fe_2O_3), the primary mineral responsible for the red hue of the ochre. Other major minerals including quartz (SiO_2), kaolinite ($Al_2(Si_2O_5)(OH)_4$, calcites ($CaCO_3$) and chalconatronite($Na_2Cu(CO_3)_2.3H_2O$) were found to be present in the ochre powder. Elemental analysis of the ochre determined using EDXRF and ICP-OES supported mineralogical composition as

Ovahimba red ochre exhibited high content of iron (Fe) and silicon (Si) and a significant amount of aluminum (Al), calcium (Ca) and copper (Cu). Based on % weight, presence of transition metals in red ochre powder identified using ICP-OES was observed in the descending order; Fe> V> Cu> Au> Ti> Zr. Based on the analysis carried out in this study, it is suggested that red ochre provides catalytic role, due to its diverse metal content especially the presence of transition metals including Fe and Cu, which might be influencing the production of secondary products during autoxidation of fatty acids present in *otjize*, specifically ketones and aldehydes. It was also concluded that the composition of clarified butterfat could be attributed to the release of mosquito repellent compounds in the red ochre derived cosmetic because when animal fat (kudufat) was used as an organic binder, the mixture did not release any of the identified possible mosquito repellent compounds.

Keywords: Aldehydes, autoxidation, clarified butterfat, fatty acids, ketones, mosquito repellents, and red ochre

DEDICATION

To my husband, Moreri Sefako and my loving family

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to Prof Ewa Cukrowska and Prof Luke Chimuka for having given me the opportunity to work under their supervision. Their complete support and guidance has led to the successful completion of my project as well as their constructive criticism and comments on the various drafts of the report.

I would like to thank Dr Riaan Rifkin for providing us with the samples that made it possible to carry out this interesting project.

I would also like to express my sincere appreciativeness to Dr Stanley Moyo for his mentorship and advice throughout my project and for his assistance with the use of most instruments especially GC-FID, XRF and FTIR.

I would also like to thank all the members of the Environmental Analytical Chemistry Research group who helped me cope with all the challenges I have come across through my studies, they made my work in the lab pleasurable through their limitless help and support.

In particular, I would like to thank Dikeledi Mphuthi for her friendship and support, I will forever be indebted for her help.

I would also like to thank my fellow classmates in APES department for their support throughout my coursework.

I would also like to thank Prof D. Billing for allowing us access to use PXRD instrument in his lab and Mr Hlegi Nyoni at University of Johannesburg for the use of their GC-MS equipment.

My studies would have not being possible if it was not for my husband`s generosity and providing the entire funding for my studies and living expenses as well his emotional support, for this I will forever be grateful and I so feel blessed.

Lastly, the LORD, our God is the one who sees us through each and every day and he deserves all the praise.

Table of Contents

LIST OF FIGURES

Chapter 1 Introduction

Chapter 2 Literature review

Chapter 3 Research Methodology

Chapter 4 Results and Discussion

APPENDIX

LIST OF TABLES

ABBREVIATIONS AND ACRONYMS

AA: atomic absorption

B.P: Before Present

EDXRF: Energy dispersive X-ray fluorescence

EXAFS: Extended X-ray absorption fine structure

FID: Flame ionization detector

FTIR: Fourier-transform infrared

GC: Gas chromatography

GC-FID: Gas chromatography-flame ionization detector

GC-MS: Gas chromatography-mass spectrometry

I.C.P-A.E.S: Inductively coupled plasma - atomic emission spectrometry

ICP-MS: Inductively coupled plasma - mass spectroscopy

ICP-OES: Inductively coupled plasma optical emission spectroscopy

MAE: Microwave assisted extraction

min: Minute

MS: Mass spectrometer

MSA: Middle Stone Age

NAA: Neutron activation analysis

PIXE: Particle induced X-ray emission

PUFA: Polyunsaturated fatty acid

PXRD: Powdered X-ray diffraction

RSD: Relative standard deviation

SPE: Solid phase extraction

TLC: Thin layer chromatography

UAE: Ultrasound assisted extraction

WHO: World Health Organization

XPS: X-ray photoelectron spectroscopy

XRD: X-ray diffraction

XRF: X-ray fluorescence spectroscopy

CHAPTER ONE: INTRODUCTION

This chapter provides an overview of the history and archaeological occurrence of ochre around the world and also takes a closer look at uses of traditional cosmetics produced by the Ovahimba tribe of northern Namibia.

1.1 General overview

The cosmetic use of clay-like materials such as ochre in African culture is a long-standing practice extending back to prehistoric times (Matike, Ekosse & Ngole., 2010). Clays were normally used in combination with other natural substances such as plant and animal extracts for a number of reasons ranging from protecting the skin against hostile effects from the sun and insect bites, to beautifying the skin as well as conveying messages of tribal identity (Matike *et al*., 2010). Although the uses of clay pigments may previously have been predominantly identified on the basis of their colour, recent studies have shown that clay exhibits characteristic structures and mineral composition giving it high specific surface area, high sorption ability and high cation exchange capacity (Carretero *et al*., 2006; Lefort, Delonde & Dubois, 2007). Ochre is a material considered to have a great cultural significance to prehistoric people and modern societies since it is found around the world in numerous archaeological sites (Erlandson, Robertson & Descantes, 1999). Ochre in archaeological contexts refers to a clay-like material which include some form of iron-containing mineral (Popelka-Filcoff *et al.*, 2007). It can either exist as an oxide or hydroxide of iron bonded strongly or loosely with other molecules of rocks and minerals in several combinations. There are several mineral combinations that may be identified as ochre including hematite, magnetite, ilmenite, limonite, or goethite, and these can range in colour from red to brown to yellow to purple (MacDonald, 2008). Red, brown and purple ochre comes primarily from the mineral hematite (Fe_2O_3) while orange-yellow ochre is derived from the minerals goethite(FeO(OH)), and limonite FeO(OH)·nH2O) (Popelka-Filcoff *et al.*, 2007; MacDonald, 2008). Hematite is one of the most common iron oxide minerals, and one which is of particular interest to this study. Leet, Judson & Kauffman (1982), describe hematite as having a deep red hue which is consistent in powdered form. It gets its name from Haimaatites, which is Greek for “bloodlike” (Leet *et al*., 1982). References to ochre use exist within a broad range of ethnographic, historical and archaeological literature, demonstrating the diversity of ochre use as a symbolic and utilitarian material (Watts, 2002; MacDonald, 2008; Rifkin, 2012). The work of L. Ström reported on the use of a mixture of red ochre and “grease” by the Ovahimba tribe to protect their skin against the sun as well as to repel insects (Troeng, 1993).

The use of plants as insect repellents has also been reported all over the world. In many African cultures, plant-based repellents have been used for generations either by burning plant materials to create a spatial repellent that drive mosquitoes away (Quinn *et al*., 2007; Maia & Moore, 2011) or by distilling to extract essential oils from the plants to create topical repellents (Quinn *et al*., 2007). Essential oils are mixtures of plants secondary metabolic compounds which comprised of terpenes, terpenic alcohols, oxides, oxygenated compounds e.g. aldehydes, ketones, lactones, phenols esters and ethers (Quinn *et al*., 2007; Kalita, Bora & Sharma, 2013). Most of the plant-based insect repellents currently on the market contain essential oils from one or more of the following plants: citronella (Cymbopogon nardus), cedar (Juniper virginiana), eucalyptus (Eucalyptus maculata), geranium (Pelargonium reniforme), lemon-grass (Cymbopogon excavatus), peppermint (Mentha piperita), neem (Azadirachta indica), soybean (Neonotonia wightii), basil (*Ocimum basilicum),* castor oil (*Ricinus communis*)*,* catnip oil (*Nepeta* species)*,* celery (*Apium graveolens*) (Choochote *et al*., 2007; Patel *et al*., 2012). Most essential oils are only active as repellents for a limited period after application as compared to chemical repellents due to their high volatility.

Recent ethnographic interviews conducted amongst the Ovahimba people by Dr R. Rifkin proved to be enlightening in terms of revealing an extensive functional use of red ochre as a mosquito repellent. Building on this indigenous knowledge, we used modern analytical techniques to analyze red ochre and traditional herbs used by the Ovahimba people as mosquito repellents to better gain a plausible understanding and explanation of such traditional practices.

1.2 The history and archaeological occurrence of ochre around the world and in southern Africa

Ochre is reported to be the earliest pigment used by humans and its use is considered as one of the indications of human evolution as it extends back to earlier hominids (Wreschner, 1980). The presence of ochre in palaeothitic sites in Europe between about 400,000 and 230,000 BP is associated with Homo sapiens and the development of modern human behaviour (Hansen, 2011). Red ochre is the most predominant ochre which appears at the European Upper Palaeolithic sites and its use was mainly

associated with ritual behaviours but other types of ochre also occur in these sites but to a lesser extent (Knight *et al*., 2009; Zilhão *et al*., 2010; Hansen, 2011). Ochre was considered to be an important and highly prized material in many ancient cultures as it played a vital role in cultural expression and health purposes. Although the purpose of this study is not to provide a chronological assay of ochre in prehistoric sites, below is an overview to give the reader a precise insight into the temporal and spatial occurrence and distribution of ochre around the world and more closely in Southern African region.

There seems to be a lack of consensus as to where and when the earliest use of ochre might have occurred, while Troeng (1993), reported that the earliest known ochre use by human is dated to some 400,000 years ago at Wonderwerk Cave in Northern Cape Province in South Africa, Wreschner (1980), stated that archaeological record indicate that ochre may have first appeared among an Acheulian assemblage from an Olduvai Gorge site (BKII) dating back approximately 500,000 BP. Other earliest ochre occurrences include the GnJh-03 site in the Kapthurin Formation of East Africa and the site at Twin Rivers in Zambia which are both dated to be as ancient as nearly 300,000 years old (McBrearty, 2001; Wadley, 2010).

According to Dayet *et al.* (2013), evidence of ochre occurrence and its use in Southern Africa increased greatly in the second part of Middle Stone Age (MSA). The earliest evidence for ochre mining comes from Lion Cavern at Ngwenya in Swaziland, where basal MSA quarry deposits were radio-carbon dated to over 43,000 BP (Dart & Beaumont, 1967; Erlandson *et al.*, 1999). A wide distribution of red ochre in the early African MSA appear to lasts from c. 300 – 250 000 to c. 40 000 years ago (ka), (Barham, 2002; Henshilwood, *et al*. 2002). Ochre found in African MSA sites appears in the same forms as ochre found in the Upper Palaeolithic sites, which is in powder form, as “crayons”, lithics, beads and grindstones stained with ochre, and as either smoothed, polished or cut, and unmodified nodules (d’Errico *et al*., 2005; Henshilwood *et al.*, 2009; Hansen, 2011; Rifkin, 2012), with occurrences dating as far back as the beginning of the MSA (McBrearty, 2001).

1.3 The Ovahimba tribe

The Ovahimba tribe is a traditionally oriented group of native agro- pastoralists found in the harsh and unforgiving conditions of the Kunene region, north of Namibia, on the

other side of the Kunene River (Velo, 1986; Bollig, 1998). The Ovahimba are one of several Herero-speaking groups in south-western Africa, including the Zemba, Hakavona, Kuvale and Kwanyoka. Precise dates for the appearance of the Ovahimba in Namibia are unknown but they are believed to have arrived in Kaokoland in the middle of the 16th century from Angola. They are suspected to either be the Herero who remained in the Kunene Region on a southward migration from the Central Lakes region into central Namibia, or they became the final settlers in the terminal destination on a westward Herero migration into the area (Vedder, 1928; Rifkin, 2012). Recent archaeological explorations indicate that the region has been inhabited by humans since 220 ka (Nicoll, 2010). The Ovahimba of Namibia have been named 'the Reds' because of their distinctive red colour derived from the daily application of red ochre and butter mixture on their entire body (Baeke, 2009).

1.3.1 Uses of red-ochre derived cosmetics

Traditionally, Ovahimba people cover their body, hair and attire with a mixture of red-ochre powder and dairy derived clarified butter, locally known as *otjize* (Rudgley, 2000; Nelda, 2004; Baeke, 2009; Rifkin, 2012). Red ochre is prominently used in initiation ceremonies which include being applied by men during wedding celebrations or when going on extensive journeys and is also applied to human corpses before burials (Tönjes, 1911; Rifkin, 2012). Ethnographic interviews conducted among Ovahimba people had indicated that *otjize* is also used for skin protection against the sun and insect bites, but this indigenous community does not have knowledge about the chemical properties which influence these protective functions of ochre. The ochre is excavated from ochre-rich cliff-faces, mined from deep pits owned by Zemba men or purchased from Zemba agropastoralist merchants in Opuwo. Ochre chunks are processed into powder by grinding and crushing between two stones particularly shaped for this task as observed in **Figure 1.1** (Rifkin, 2012). Clarified butterfat used for making *otjize* is produced by fermenting milk cream in a *Lagenaria* species calabash gourd and boiling the fatty substance to remove water and then storing the grease product in containers traditionally made from cattle horns and leather (Rifkin, 2012).

Figure 1.1: Ovahimba people process red ochre chunks into fine powder (**a**) by grinding between round upper and flat lower grindstones (**b**) after which the powder is mixed with clarified butterfat (**c**) to produce *otjize* (**d**) which is then applied to all parts of the body and personal attire (**e**) (Pictures: courtesy of Dr Riaan Rifkin).

1.3.2 Uses of aromatic plant derived cosmetics

Moreover, information obtained through ethnographic interviews revealed that Ovahimba pastoralists also use traditional cosmetics derived from aromatic plants known as *otjizumba* for protection against insects and also as a "perfume" (Rifkin, 2012). *Otjizumba* is prepared from various species of trees and shrubs called *omumbumbwa*, *orojo*, *otjokwa*, *etyja*, *onjiva, omumbiri* and *omhangwade*. The Kunene region is endowed with numerous species of *Commiphora* endemic to the region (Nott & Curtis, 2005). Ovahimba people have long used these species as the main ingredient for their perfume (Nott & Curtis, 2005). Dry aromatic ingredients are first pounded into a

coarse powder and then ground into a fine dust between round upper and a flat lower grinding stones, specifically *Commiphora wildii* and *C. virgata* resin (Nott & Curtis 2005; Lendelvo, Munyebvu & Suich, 2012). *Otjizumba* is produced by casually mixing the powdered ingredients with clarified butter, at a 1:1 ratio, between the palms of the hands. Since *otjizumba* is most often applied to the neck, personal ornaments and jewellery are also covered with the mixture (**Figure 1.2**).

Figure 1.2: Ovahimba people used traditional herbs (**a**) which they process by grinding between round upper and flat lower grindstones (**b**) after which they are mixed with clarified butterfat (**c**) to produce the "perfume" locally known as otjizumba (**d**) which is then applied over the painted body around the neck (**e**) (Pictures: courtesy of Dr Riaan Rifkin)

1.4 Research questions and hypothesis

1.4.1 Research questions

- Do the red ochre derived cosmetic and aromatic plant- derived cosmetic used by the Ovahimba people contain compounds associated with mosquito repellency?
- Does the combination of fat and red ochre influence the release of mosquito repellent compounds in the red-ochre derived cosmetic and does the type of fat used have any effect on the release of these compounds?

1.4.2 Hypothesis

- Red ochre derived cosmetic and aromatic plant-devired cosmetic used by Ovahimba people contain compounds associated with mosquito repellency
- The combination red ochre and fat influence the release of compounds associated with mosquito repellency and the type of fat used does have an influence in the release of these compounds

1.5 Justification

Despite much research already conducted, mosquitoes still remain a huge burden on society, both as nuisance pests and as vectors of diseases (Hemingway, 2008). Mosquitoes are the primary host in the spread of malaria and other harmful mosquito-borne diseases which are currently on the rise therefore making mosquitoes an important target. Malaria infects approximately 250 million people annually and causes 800,000 deaths particularly among children (WHO, 2008), following the high level of threat malaria poses to humanity, it is has become very necessary to look further for alternative measures to prevent and control malaria. The growth rate of the vector is temperature dependent hence future global warming could lead to a significant increase in malaria cases in densely populated regions of Africa unless control efforts are increased. Although early methods to control vectors which relied on the use of insecticides were highly effective, their dependence on spraying inside houses to kill female mosquitoes has raised environmental and public health concerns (Hemingway, 2008; Dekker *et al*., 2011), and they are also currently expensive, making them less

likely to be purchased by the indigenous poor. This has elevated the need to look into cheaper and safer alternatives with the same level of efficacy as synthetic repellents, which can be incorporated into traditional practices.

There is a relatively large percentage of existing literature suggesting the potential use of red ochre as an insect repellent but little experimental work has been done to explore this prospective. This study builds on the indigenous knowledge on the use of traditional cosmetics produced by the Ovahimba tribe as a mosquito repellent, and uses modern analytical techniques to investigate and identify possible active compounds which may be responsible for eliciting the repellent effect in these traditional cosmetics.

1.6 Aim and Objectives

1.6.1 Aim

- To investigate the chemical composition of red ochre-derived cosmetics and aromatic plant-derived cosmetics used by the Ovahimba tribe in order to determine the presence of compounds associated with mosquito repellency.

1.6.2 Objectives

- To quantify minor and major metals present in Ovahimba red ochre using XRF and ICP-OES;
- To identify the principal mineral in Ovahimba red ochre using XRD and FTIR;
- To identify functional groups present in red ochre, traditional herbs and butterfat used by Ovahimba people using FTIR;
- To determine the presence of volatile organic compounds in red ochre and butterfat mixture using GC-FID and also to determine if the release of these compounds is influenced by the type of fat used;
- To extract and characterize organic compounds in red ochre –derived cosmetic and plant derived cosmetic associated with mosquito repellent activity using GC-MS

CHAPTER TWO: LITERATURE REVIEW

This chapter provides a detailed review of ochre-related literature to illustrate the range of contexts in which ochre is used, it also gives a review on characterization studies to show how certain analytical techniques have been used for geochemical analysis of ochre and finally the chapter also gives a detailed review on compounds associated with mosquito repellent activity.

2.1 Geochemical composition of ochre

Ochre as a weathering product forms where residual concentration and oxidation of iron in iron-rich rocks occur in complex mixtures with other minerals with or without hydration (Watts, 2002). Although it is mainly formed from sedimentary contexts, it can also form in metamorphic and igneous conditions (Bernatchez, 2008). Ochre includes a variety of both igneous and metamorphic ferruginous sedimentary rocks which include hematite, goethite, sandstone specularite and shale (Erlandson *et al*., 1999; Barham, 2002; Popelka-Filcoff *et al*., 2007; Hodgskiss, 2013). Its formation can consequently be affected by environmental conditions (Hradil *et al*., 2003; Hodgskiss, 2013). It usually occurs in complex mixtures with other minerals such as quartz, clay and mica and it usually binds quartz grains together (Jercher *et al*., 1998; Bernatchez, 2008). Organic and inorganic inclusions often occur in ochre which can even alter its colour (Bikiaris *et al*., 1999; Hradil *et al*., 2003; Hodgskiss, 2013).

Ochre is a geochemically heterogeneous material in composition compared to other raw materials such as obsidian and some clays because it always consist of two components; the iron oxide minerals and the accessory minerals such as quartz, calcites, and kaolinites (Helwig, 1998). The type and amount of accessory minerals present in ochre depend on the source of the earth and the degree of processing which the pigment has undergone (Helwig, 1998). The presence of iron oxides contribute to the different colour variations among ochres and very small amounts can affect and change the colour of an ochre piece (Popelka-Filcoff *et al.*, 2007; Bernatchez, 2008; MacDonald *et al*., 2011). The colour of ochre is influenced by the varying proportions of iron oxides and hydroxides. Hematite (Fe_2O_3), hydrous iron oxide; goethite (FeOOH) and limonite ($Fe_2O_3.H_2O$) are the main chromophores for red, yellow and brown colours respectively (Gil *et al*., 2007; MacDonald *et al.*, 2011). This colour variation is produced by the presence of Fe^{3+} ion and reduction reaction between Fe^{3+} and O^{2-} or OH^- ions (Elias *et al.*, 2005). Factors such as mineralogy, crystal structure, particle size and presence of organic and inorganic materials can also influence the appearance and colour of the ochreous material (Bikiaris *et al*., 1999; Hradil *et al*., 2003; Marshall *et al*., 2005; Elias *et al*., 2006). For example, the presence of white pigments made up of aluminum silicate and calcium compounds may cause variations

in colour intensity in ochres (Elias *et al.*, 2005). An example of the different colour variations of ochre from different sources can be observed in **Figure 2.1**.

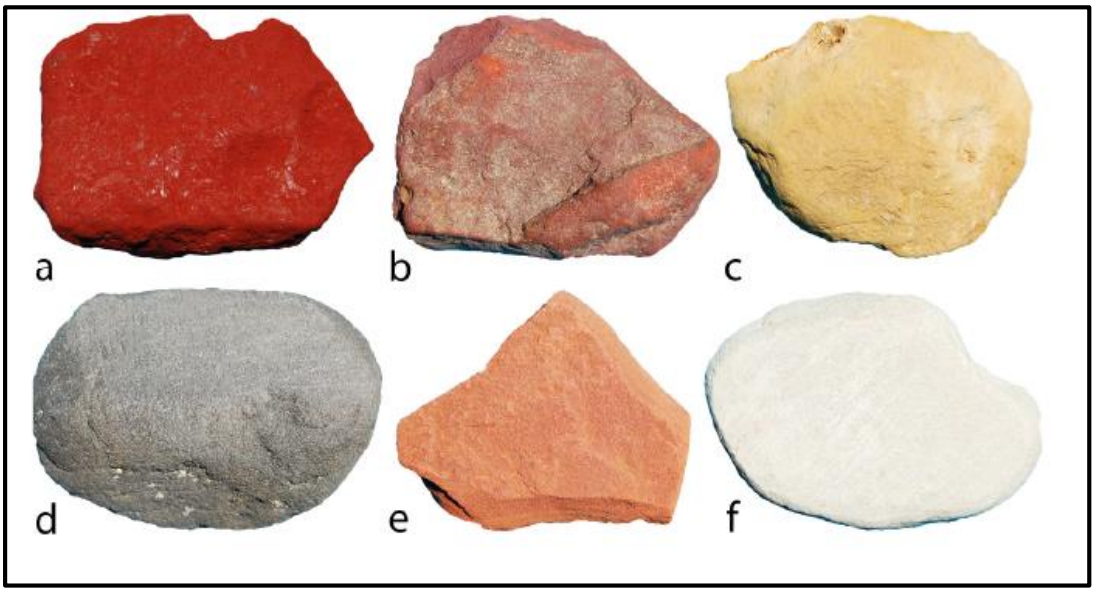


Figure 2.1: Ochre samples showing different colour variations (a) was collected from the Kunene Region in Namibia and the remaining specimens (b, c, d, e and f) derive from the Bokkeveld shale deposits in the Western Cape Province of South Africa (Source: Rifkin, 2012)

Previous work done on the characterization of ochre has indicated that there is significant chemical variability in ochre (Erlandson *et al*., 1998; Polpella-Filcoff *et al*., 2007, 2008; Bernatchez, 2008). Some archaeological material labelled as ochre because of their pigment producing properties may not inevitably be ochre in a geochemical sense hence it is critical to perform elemental and mineralogical analyses on the material to be certain (Erlandson *et al*., 1999).

2.2 Ethnographic uses of ochre

Previously, Erlandson *et al*. (1999) had argued that less work has been done on red ochre use compared to other geological materials such as obsidian and ceramics despite the fact that red ochre was highly valued and widely traded among ancient and historic people around the world. However, MacDonald *et al*. (2011), argue that the use

of ochre and its presence in archaeological contexts is well documented archaeologically, ethnographically and historically. Unlike other geological materials, red ochre does not reveal its use as readily and this has led to many hypotheses about its use which are mostly based on ethnocentric ideas (Northam, 2013). Ochre is considered as a culturally significant material (MacDonald *et al*., 2011), and both symbolic and utilitarian interpretations on its use in MSA have been proposed (Watts, 2009; Rifkin, 2012). Ochre occurrences have created the impression that it has been an integral part of every social and cultural unit since the Upper Paleolithic, with extremely similar transcultural and transhistoric ochre practices observed across communities (Wreschner, 1980). Studies have proposed that high quality ochre with deepest and richest hues were priced and traded over long distances supporting the notion that ochre was put to diverse uses (Erlandson et *al*., 1999). Ochre as an earth pigment appears to have been used since prehistoric times mainly because it is extremely stable, exhibits a high colouring intensity and non-fading ability under various ambient conditions (Bikiaris *et al.*, 1999). Earth pigments such as ochre, are easily excavated from the earth and prepared into final product hence this could be the reason why evidence of their use can be traced back to MSA more than 100 000 years ago (Gil *et al.*, 2007).

2.2.1 Ochre as a paint

Prehistoric evidence and ethnographic reports put forward both symbolic and practical uses of red ochre, especially its use as a pigment for body painting (Rudgley, 2000; Jablonski, 2004; d'Errico, 2008; Rifkin, 2012; Dayet *et al.*, 2013). Ethnographically, red ochre is reported to be the most widely used earth pigment applied to human bodies and artefacts in the course of symbolic practice (Watts, 2002; Rifkin, 2012). Hence, the common assumption made about the large quantities of red ochre found in the MSA, is that red colouring was used mainly for symbolic body decoration (Rifkin, 2012). For example, red body-paint was used for puberty and marriage rituals by girls in Botswana in historic time (Wadley, 2005), was also applied to human corpses before burials and it is still applied by Ovahimba people during wedding celebrations (Galton, 1853; Tönjes, 1911; Erlandson *et al*., 1999; Rifkin, 2012). Wreschner (1980) assumed that red ochre used to cover human corpses was to mask the smell produced by dead bodies. Some tangible evidence for the use of ochre as a 'body cosmetic' comes from the discovery of red ochre residues adhering to shell beads at Blombos Cave (Henshilwood, 2004;

Henshilwood et al., 2004; d'Errico *et al*., 2005; Rifkin, 2012). Body painting and ochre engravings could have been part of traditional practices to show social commitment (Watts 2002; Henshilwood *et al*., 2009). Continued traditional use of ochre for body painting has declined, but cultures such as the Ovahimba of Namibia have retained the practice to date (Rudgley, 2000; Rifkin, 2012).

The archaeological presence of crayon-shaped ochre pieces has also prompted the idea that ochre may have been used to draw on surfaces just like modern crayons (Rifkin, 2012). Since ochre pigments are readily available and chemically stable with the ability to produce a wide range of colours, many distributors of artists' materials still employ this pigments in their iron oxide-based paints (Erlandson *et al*., 1999).

2.2.2 Ochre as medicine

Ideas about the use of ochre by early man had mainly focused on its symbolic value and artistic potential. However the use of ochre as medicine by Gudadja people of North-western Australia suggested the existence of other uses of ochre that are more related to immediate survival (Velo, 1984; Becker, 2000). Several other ethnographic accounts conducted among Ovahimba tribe have also provided an indication on healing properties and medicinal use of red ochre (Velo, 1984; Ellis *et al*., 1997; Becker, 1999; Rifkin, 2011). Scientific evidence has now given an appreciation of the curative properties of ochre, attributing its healing effects to its diverse mineral content which include iron salts and other metal ions (Velo, 1984). Wilcox (1911) reported that iron salts have powerful astringent and styptic effect which tend to halt hemorrhage and have antiseptic and deodorizing properties, suggesting that iron salts in ochre can keep sores free of infections by reducing worst perspiration (Trinkaus, 1984; Velo, 1984). Detailed description of medicinal use of ochre suggest that ochre moistened with water was often used on sores and burns on any part of the body to promote drying in order to speed up the healing process (Velo, 1984; Becker, 2006). Ochre was also believed to cure malignant ulcer (Velo, 1984). Although red (hematite) and yellow (goethite) forms of ochre are reported to both exhibit healing properties, red ochre was the one mostly used by historic people (Velo 1984; Erlandson *et al*. 1999). Since the work by Velo (1984) on medicinal properties of ochre, less work has been conducted to support his work but recently a sensitivity tests study was conducted using ochre from Nigeria which showed that ochre exhibited effective anti-bacterial activity against *Dermatophilus*

congolensis (Dauda *et al*., 2012). The high level of Fe observed in the ochre was speculated to be the factor that contributed to high inhibitory effect against *D. congolensis* because it has been reported that iron in clay kills bacteria by generating radicals that attack their cell components (Williams *et al.*, 2011; Dauda *et al*., 2012).

2.2.3 Ochre as geophagy

Geophagy is the deliberate consumption of non-food substances mainly clays (Young *et al*., 2010; Rifkin, 2012). Ingestion of clays can bring about benefits to human body such as aiding in resolving ionic imbalances (Jones & Hanson, 1985), for providing mineral supplementation with respect to minerals such as Cu, Mg, Se, Zn, I and Fe which are known as brain selective nutrients (Abrahams *et al*., 2006; Brand *et al*., 2009; Cunnane, 2010; Rifkin, 2012), in stabilizing digestive tract pH (Kreulen, 1985), assisting in the inhibition of diarrhoea (Vermeer & Ferrell, 1985), for eradication of intestinal parasites (Knezevich, 1998), as well as aiding in detoxification against enterotoxins (Lozano, 1998; Reynolds, Plumptre & Greenham, 1998; Krief *et al*., 2006; Rifkin, 2011). Alexander von Humboldt and David Livingstone observed that Otomac Indians and native Africans respectively; ate clay and ochre as part of their regular diet especially pregnant people and children (MacDonald, 2008). It can be assumed that the ingestion of ochre also provides the same benefits as suggested for other clay-like materials. A study conducted by Mphuthi (2013), on bioavailability of trace toxic elements in archaeological ochres, led to the conclusion that ochre has relatively low toxicity risk upon internal exposure and therefore considered safe for consumption.

2.2.4 Ochre as hide tanning ingredient

Ethnographic information on the use of ochre in tanning hides has been widely referenced (Audouin & Plisson, 1982; Watts, 2002; Rifkin, 2011; Hodgskiss, 2012). Watts (2002), has argued that ochre is not necessarily needed for hide tanning stating there is no practical reason for its use in hide tanning other than for its coloring purpose, because it has been observed that other tanning methods can be used successfully. However, Audouin & Plisson (1982), demonstrated and established that treating hides with red ochre is beneficial particularly in promoting dehydration and delaying putrefaction (Wadley, 2005; Rifkin, 2011). This is attributed to antiseptic properties of ochre and its ability to inhibit the bacterial activity responsible for production of

collagenase (Velo, 1984; Rifkin, 2011). Therefore, the use of ochre for leather tanning seems to be desirable in circumstances where there appears to be a risk of decay (Wadley, 2005). In their work, Audouin & Plisson (1982) also demonstrated that the difference in moisture content in red and yellow ochre could be what makes red ochre a better tanning ingredient as it produced a pleasingly softer and thinner leather compared to when yellow ochre was used (Wadley 2005). An experimental study by Rifkin (2011) also confirmed that certain types of ochre do preserve animal hide.

2.2.5 Ochre as sunscreen

The use of ochre for skin protection against the sun is the most frequently mentioned alternative functional use for ochre (Troeng, 1993; Wadley, 2005; Matike *et al.*, 2010; Rifkin, 2012). Indigenous African tribes have always used clays to protect their skins from the effects of the sun (Ettagale, 1999; Reed, 2007; Matike *et al.*, 2010). Ultraviolet radiation can penetrate deeply into the skin causing skin ageing and in some cases skin cancer (Juch *et al.*, 1994). Clay minerals such as kaolinite, talc, and smectites have the ability to protect the skin against ultraviolet radiation, as they are capable of absorbing or scattering radiant energy (Hewitt, 1992; Juch *et al*., 1994; Rifkin, 2012). This ability is greatly enhanced by their small particle size which allows them to form a film on the skin (Hewitt, 1992; Rifkin, 2012). Some clays are frequently included in several cosmetic substances to act as sunscreen (Hewitt, 1992; Carretero & Pozo, 2009; Rifkin, 2012). This protective property of clay has also been exploited by indigenous African people including the Ovahimba people who cover their body from head to toe daily with a mixture of red ochre powder and butterfat to block the harmful ultraviolet radiation from penetrating their skin (Troeng, 1993; Rifkin, 2011). Preliminary results in a study conducted by Rifkin *et al.* (2011) on the efficacy of Ovahimba ochre as a sunscreen indicated that red ochre applied by Ovahimba people confers a significant degree of protection against UV rays. This is can be enhanced by the conventional processing method which requires grinding the ochre between upper and lower grinding stones which results in smaller particle sizes with larger surface covering capacities and increasing reflective indices (Rifkin, 2011).

2.2.6 Ochre as insect repellent

Clays such as kaolinite can be used to protect human skin mechanically from external physical agents by forming a film on the skin surface which can prevent insects from piercing through the human epidermal layer (Carretero, 2002; Williams *et al*., 2009; Cunningham *et al*., 2010). Ethnographic accounts have revealed that ochre is known for its use as an insect repellent among the Nuba of Sudan and the Ovahimba of Namibia who apply red ochre all over their bodies for protection against insect bites (Groning, 1998; Tanne, 2000; Rifkin, 2012). At this point, there is no experimental evidence or scientific reports on the mode of action of ochre as a repellent especially against mosquito except for speculations made by Rifkin (2012). He suggested that ochre powder could have equivalent repellency capabilities proposed for kaolinite clay. For example, it could be protecting the skin from mosquito bites by forming a physical barrier. He also suggested that the ability of ochre to inhibit microbial activities on human skin surface might aid in reducing chemical detection by mosquito hence preventing host recognition. This has led to the assumption that an ochre-covered human body may have offered benefits during the evolution of *Homo sapiens* in terms of reducing the vulnerability of humans to mosquito-borne diseases in areas where mosquito-borne diseases were prevalent (Rifkin, 2012).

2.3 Review of analytical techniques used for characterizing ochre

An analytical methodology is considered a requisite for the characterization of earth pigments because the colour alone cannot be considered a safe criterion for earth pigment differentiation (Genestar & Pons, 2005). Previous methodological research has played an important role in determining what is analytically appropriate for geochemical and mineralogical characterization of ochre. X-ray diffraction (XRD), X-ray fluorescence (XRF), inductively coupled plasma-optical emission spectroscopy or mass spectrometry (ICP-OES or ICP-MS), infrared spectroscopy (IR) and gas chromatography (GC) are some of the most important techniques which have been used to characterize chemical composition of iron containing earth pigments. This review will look at principles and applications of the mentioned techniques in notable previous studies which employed their use in characterization of ochre and other earth pigments. It is worth-noting that

these techniques have their own suite of advantages and disadvantages, and have been employed with varying success.

2.3.1 Techniques used for elemental analysis of ochre and other claylike materials

The first step in the characterization of materials is elemental analysis. There are techniques that give information on elemental composition of samples such as XRF, particle induced X-ray emission (PIXE), ICP, neutron activation analysis (NAA), atomic absorption (AA), X-ray photoelectron spectroscopy (XPS). The techniques discussed in this section are XRF and ICP since there were used in this study.

2.3.1.1 X-ray fluorescence spectroscopy (XRF)

XRF is the phenomenon where a material is exposed to X-rays of high energy, and as the X-ray (or photon) strikes an atom (or a molecule) in the sample, energy is absorbed by the atom. Energy Dispersive X-Ray Fluorescence (EDXRF) involves the use of ionizing radiation to excite the sample. This excitation ejects electrons from the atomic shells of the elements in the sample. When a given atom replaces the ejected electron, by taking another electron from an outer atomic shell, X-ray energies are emitted. Since each element generates a specific energy level in this replacement process, these energies are known as characteristic X-rays and are detected by a fluorescence detector. EDXRF spectrometers use an X-ray detector to convert characteristic X-rays into electrical signals. The spectrometer's electronics digitize the signals produced by the detector, and send this information to a PC or internal electronics for display and analysis. An EDXRF spectrometer makes use of the fact that the pulse height of the detector signal is proportional to the X-ray photon energy. These X-rays can be detected and displayed as a spectrum of intensity against energy: the positions of the peaks identify which elements are present in the sample and peak heights can be used to determine how much of each element is present in the sample (Salamó Clapera, 2006). The most important component of the apparatus is the X-ray source, known as an X-ray tube which emits an X-ray beam into the sample being analyzed. This beam excites and displaces electrons and the resulting energy which is characteristic to the element is emitted and is collected by the detection system. X-ray tube filters are also a

critical component since they selectively absorb or transmit some energies of X-rays more than others in order to reduce the counts in the region of interest while producing a peak that is well suited to exciting the elements of interest. The instrument is equipped with a software package which processes the information collected by the detection system (Salamó Clapera, 2006). **Figure 2.2** shows a typical set up of EDXRF instrumentation. XRF analysis is a non-destructive method, but sample preparation maybe required (Repacholi, 1994). XRF has been extensively used to characterize red and yellow ochre samples. Jercher *et al*. (1998) examined a group of ochre samples from six deposits in South and Western Australia known to have been exploited by Aborigines using XRF in order to provide a database containing the chemical and mineralogical characteristics of ochres from these anthropologically important sites. In a study by Popelka-Filcoff *et al*. (2008) the XRF technique was employed to examine the variation in major and minor element patterns of ochre from iron oxide sources in southeastern Missouri to better understand the differences that may occur within and between ochre sources.

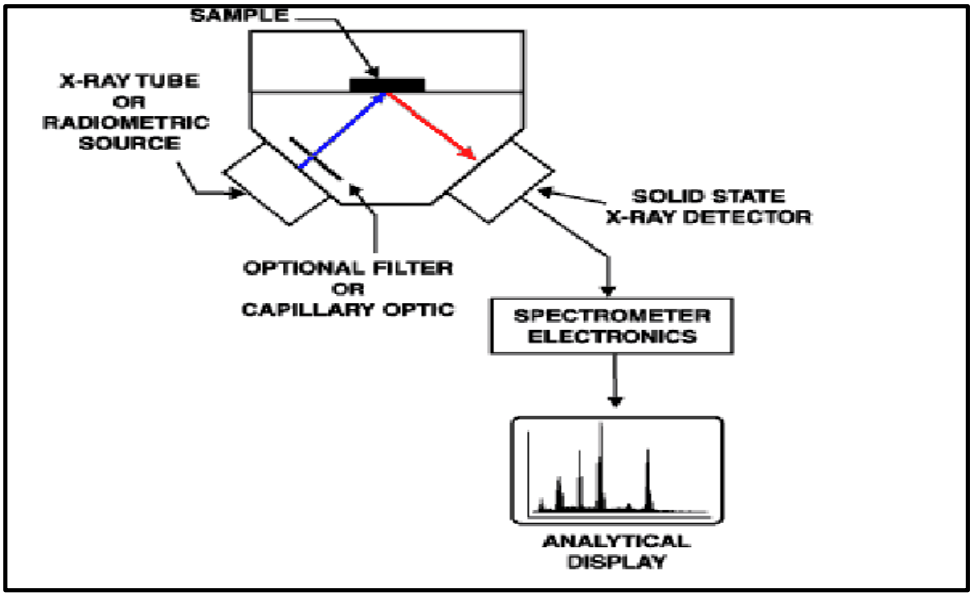


Figure 2.2: Schematic diagram of EDXRF instrumental set up (Source: Salamó Clapera, 2006)

This technique was also employed by d'Errico, Garcia-Moreno & Rifkin (2012), to analyze the elemental composition of Klasies River ochre recovered from layer 14. The

authors concluded that all different earth pigments collected from Layer 14 had a similar elemental composition which comprises a major occurrence of Fe, minor quantities of K, Ca, Mn, Cr, Sr and traces of P, V and Ni. Despite its successful application, XRF has some limitations such as it does not distinguish between ferrous and ferric iron and suffers from poor limits of detection (Repacholi, 1994).

2.3.1.2 Inductively coupled plasma-optical emission spectrometer/mass spectroscopy (ICP-OES/MS)

ICP is a multi-element method that is suitable for the determination of many elements. There are two common types, which are ICP-OES and ICP-MS. They are both used for bulk elemental analysis of any material or substance, with the exception of C, H, O, N, F, Cl and Br (Legodi, 2008). ICP detects elemental concentrations in the range of parts per billion (ppb) and parts per trillion (ppt), with detection limits of about 0.5 ppb and 0.01 - 0.1 ppt for ICP-OES and ICP-MS respectively (Tominson *et al*., 1995). The fundamental operating principle governing the use of ICP-OES involves a sample introduction system which supplies sample to the ICP emission source (plasma), where desolvation, atomization, excitation and light emission occur. During the excitation process, molecular, atomic and ionic species in various energy stages are produced. Energy is released in the form of electromagnetic radiation, which is a characteristic of the emitting analyte. The intensity of an elemental atomic and ion line is used as the analytical signal in quantitative atomic emission spectroscopy. In the case of ICP, excitement of an atom occurs by absorption of thermal energy causing electrons to undergo transition to higher energy orbitals (Skoog, Holler & Nieman, 1992). Each element has a unique emission spectrum derived from its electronic configuration. The emission wavelengths associated with each element are derived from the differences between the energy levels involved in each of the orbitals that partake in the energy transits of the electrons (Montaser, 1998).

Components of the ICP-OES instrument include the peristaltic pump, the nebulizer, the spray chamber, the RF generator, the torch, the optical systems, the detectors and the data processing system. A fluid is passed through the nebulizer through the peristaltic pump where it is nebulized before been fed into the plasma as an aerosol. The nebulizer works in conjunction with the spray chamber. The argon gas is used as a carrier gas through the nebulizer, where a mist formed by the nebulizer and the

saturated argon gas are injected through a spray chamber. In the ICP-OES, the plasma is formed in the torch which contains three concentric tubes of glass. The inner tube directs the nebulizer flow of the sample to the plasma, the middle tube prevents the plasma from getting too close to the nebulizer flow hence making nebulizer injection far easier. The outer tube provides argon for the initial plasma ignition and it also keeps the load coil cool from the immense heat (Montaser, 1998). Upon contact with the plasma, the electron of the analyte become excited and as a result is able to emit element specific spectra in the UV/visible region of 160 to 800 nm which are detected and measured to determine concentration of the elements. The detector produces an electrical signal which is processed by an electronic circuit before being measured by a read-out device. In modern spectrometers the computer controls the operating parameters of the plasma as well as performing the task of sample logging, operation of the auto-samplers, and construction of calibration curves and facilitates the rapid and efficient handling of data (Montaser, 1998). **Figure 2.3** shows a schematic representation of a typical ICP-OES instrumental set up.

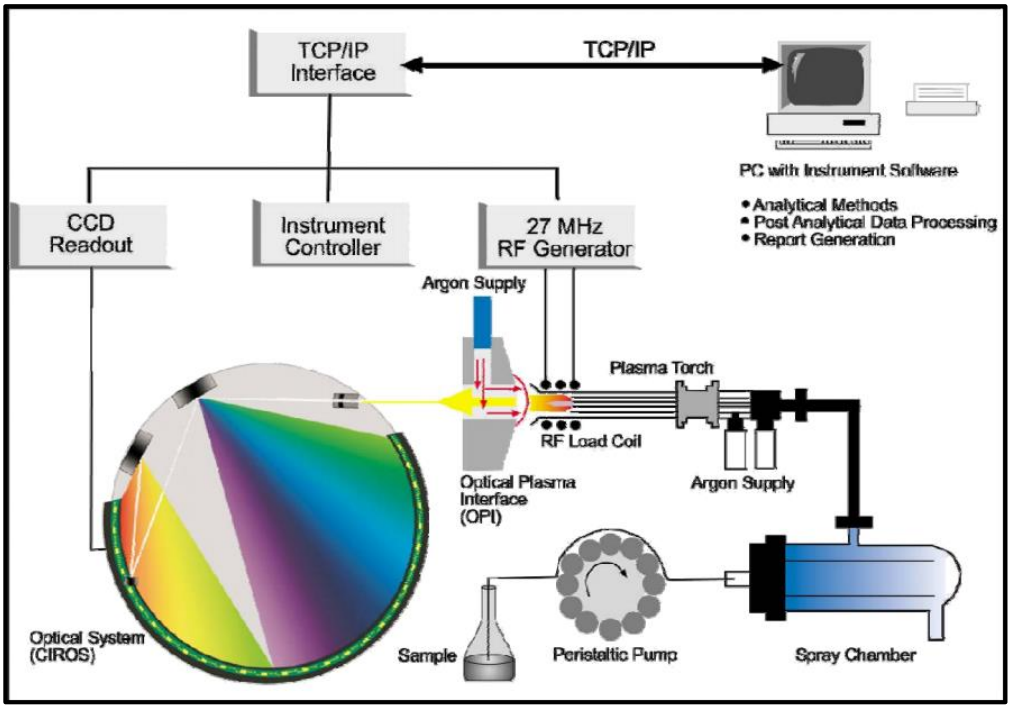


Figure 2.3: A schematic diagram of the SPECTRO ICP-OES instrumental set up (Source: McDowell 2011)

Studies have shown that ICP-MS is suitable for the rapid and simultaneous analysis of multiple elements in geological materials, including soils, organic substances and most rock types, but its application to clay minerals only followed later on (Eggins *et al*., 1997; Kogel & Lewis, 2001). Smith *et al.* (1998) demonstrated that it is possible to match archaeological ochres to geological outcrops using a combination of ICP-MS and SEM-EDS. A study by Marshall *et al*. (2005), used a combination of ICP-OES, IR, XRD and particle size analysis to examine ochres from Clearwell Caves in order to determine the effect of particles size, mineralogical and chemical composition on the colour of the ochre. They observed that the presence of minerals; hematite and goethite, was the main determining factor in red and yellow ochres, respectively, while particle size seemed to play a huge role in purple ochre. Their ICP results showed that the high levels of iron confirmed hematite as the main chromophore in all red ochre samples. They also detected levels of other metals including sodium, potassium, aluminum, magnesium and calcium suggesting the presence of other clay minerals such as smectite, illite and dolomite.

A study by Ellis *et al*. (1997) reported on the use of ICP-OES for elemental analysis of ochre artifacts from Texas and they were able to determine that weathering of some layers of the samples caused alteration in the elemental composition of the samples. Since ICP-OES and ICP-MS require dissolution procedures, these can be time consuming and can induce loss of some volatile elements including As, Pb, Se, Sb and Zn (Dogan *et al*., 2006). An alternative is laser ablation (LA) ICP MS which is non-invasive and requires no sample preparation while still offering low detection limits.

2.3.2 Techniques for mineralogical of ochre and other clay-like materials

At an advanced level of material characterization, it is important to determine in what forms or chemical phases various elements occur in a sample of interest. The techniques available for chemical analysis include XRD, Raman spectroscopy, extended X-ray absorption fine structure (EXAFS) and FTIR. The techniques discussed in this section are XRD and FTIR.

2.3.2.1 X-ray diffraction (XRD)

The three dimensional structure of a crystal materials such as minerals, is defined by regular repeating planes of the atoms that form a crystal lattice. When a focused X-ray beam interacts with these planes of atoms, part of the beam is transmitted, absorbed, refracted and scattered and part is also diffracted (Birkholz, 2006). The interaction of the incident rays with the sample produces constructive interference and a diffracted ray, when conditions satisfy Bragg`s law expressed in **equation 2.1.**

$$n\lambda=2d\sin\theta \qquad \textit{(2.1)}$$

where,

- λ = the wavelength of the x-ray
- d = the spacing of the crystal layers (path difference)
- θ = the incident angle (the angle between incident ray and the scatter plane)
- n = an integer

The law states that when the X-ray is incident onto a crystal surface, its angle of incidence, θ, will reflect back with a same angle of scattering, θ. And, when the path difference, d is equal to a whole number, n, of wavelength, a constructive interference will occur (Bunaciu, Udriştioiu & Aboul-Enein, 2015).

The diffracted X-rays are then detected and processed. By scanning the sample through a range of 2θ angles, all possible diffraction directions of the lattice should be attained due to the random orientation of the powdered sample. Conversion of the diffraction peaks to d spacings allows the identification of the compounds because each compound has a unique set of d spacing (Bunaciu *et al*., 2015). XRD peaks are produced by constructive interference of a monochromatic beam of X-rays scattered at specific angles from each set of lattice planes in a sample. The peak intensities are determined by the distribution of atoms within the lattice. Consequently, XRD pattern is the fingerprint of periodic atomic arrangements in a given material.

X-ray diffractometer consists of an X-ray tube, a sample holder and a detector. A current is applied that heats the filament within the tube and this generate electrons which then hit the target commonly made of copper. When electrons with sufficient energy dislodge inner shells electrons of the target material, characteristic X-ray spectra are produced

consisting of the most common components, namely Kα and Kβ. These X-rays are generated by a cathode ray tube, filtered to produce monochromatic radiation (CuKα = 1.5418 Å) and are directed towards the sample. As the detector and the sample are rotated, the intensity of the reflected X-rays is recorded (Bunaciu *et al.*, 2015). **Figure 2.4** gives an illustration of XRD set up. XRD has a long history of being used as a qualitative and quantitative analytical technique for analysis of various crystalline compounds present in solid and powder materials (Norrish & Taylor, 1962). A study by Tankersley *et al*. (1995) showed that XRD was used successfully to determine that ochre from the Hell Gap site in Wyoming was acquired from the Powars II ochre mine based on its mineralogical composition. Using both XRD and PIXE, Bernatchez (2008), was able to determine the chemical and mineralogical composition of a MSA ochre assemblage from Nelson Bay cave in South Africa. The author was able to determine several chemical groups within the ochre assemblage based on presence of the major colour-bearing minerals in the ochre samples which include hematite, goethite and jarosite and the presence of quartz as well as clay minerals such as kaolinite and illite.

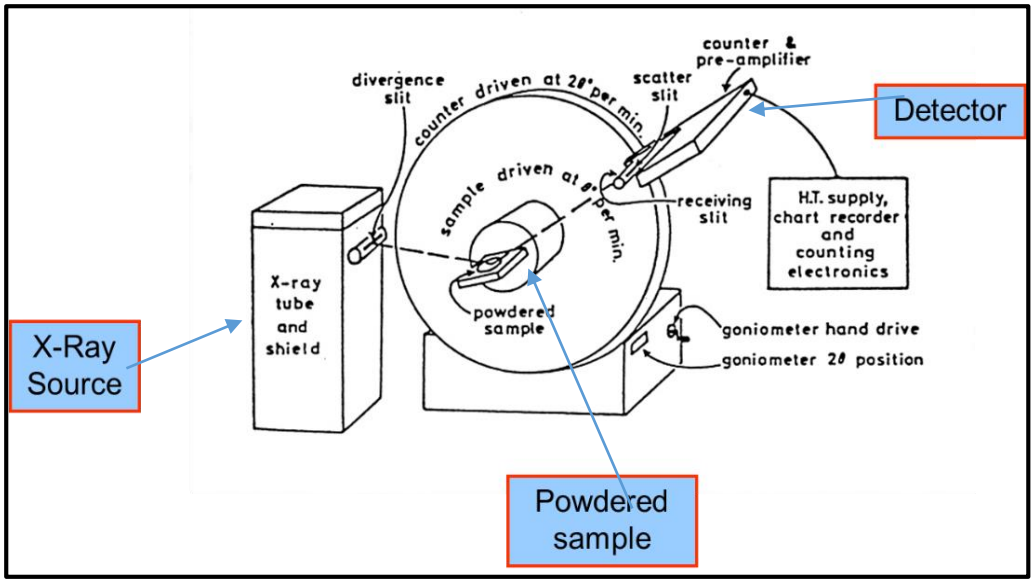


Figure 2.4: Schematic diagram of a typical XRD instrumental set up (Source: Barth 2007)

Roebroeks *et al.* (2012), used XRD to confirm the presence of hematite in small concentrates of red material retrieved during excavations at Maastricht-Belvédère, The

Netherlands. Their results also indicate that there was a strong quartz component in the sediment matrix of the red material. Dayet *et al.* (2013) showed that XRD can be applied for analysis of archaeological ochre from the MSA site in Diepkloof Rock Shelter in South Africa. Using factors including elemental composition, they were able to show that surface analysis can be very useful in assigning samples into mineralogical categories. Although they had no challenge identifying iron oxides using XRD, it was a challenge to distinguish between illite and muscovite clay minerals because they have similar crystallographic structures.

2.3.2.2 Fourier-transform infrared (FTIR)

FT-IR is considered a quick and cheap but quite powerful method of screening archaeological samples including iron earth pigments before subjecting them to the more expensive and time-consuming method of GC–MS (Shillito *et al.*, 2009). Infrared spectroscopy is built on the principle that absorption of photons causes changes in molecular vibrations (Shillito *et al.*, 2009). As a specialized method of molecular spectroscopy, it relies on the principle that molecular rotations and vibrations of chemical compounds absorb specific frequencies of electromagnetic waves. One particular portion of the electromagnetic spectrum, the infrared, is suitable for the detection of molecular vibration. The IR spectrum refers to electromagnetic waves whose wavelengths range from 14286 cm^{-1} to 28.5 cm^{-1}. IR spectrum is ideal for detecting molecular vibrations in liquids and solids. Each of a molecule`s modes of vibration occurs at a specific frequency and will consequently absorb electromagnetic radiation of this frequency. These unique frequency absorptions results in a characteristic spectral profile for a given molecule or molecular compounds (Higdon, 2010).

All FTIR spectrometers in their simplest configuration are composed of a source for providing infrared radiation which is directed by mirrors into the interferometer. The interferometer splits the incoming radiation to produce an interference pattern. An interferometer allows all desired frequencies to be recorded by the detector at each data sampling point which allows faster data collection. The laser is used for controlling the interferometer's moving mirror and data sampling points. Once the IR radiation has passed through the interferometer, it illuminates the sample which either absorbs or transmits frequencies of the spectrum which continue to the detector where the IR

radiation is converted to an analog voltage signal. This voltage signal is then converted to understandable digital data by the signal processor through the application analogue to digital conversion and Fast Fourier Transform (FFT) algorithms (Higdon, 2010). **Figure 2.5** shows a typical set up of FTIR instrumentation. Infrared spectroscopy allows for the determination of functional groups present in a molecule. The IR spectroscopy method can identify a substance with an amorphous or slightly crystalline structure which is the principal components of inorganic pigments such as clay pigments and can differentiate several varieties of yellow, brown, and red inorganic materials (Balakhnina *et al.*, 2011).

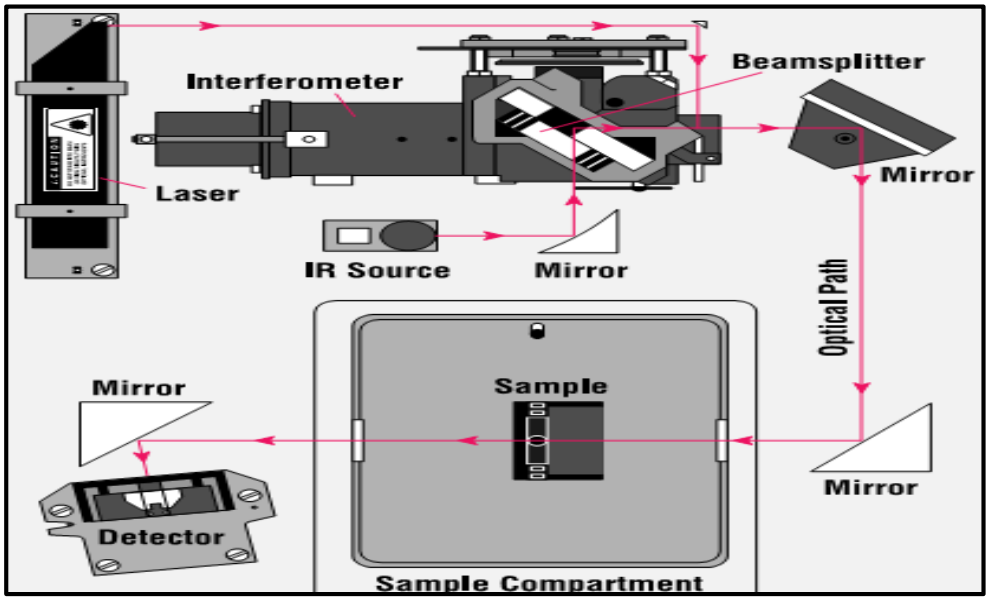


Figure 2.5: Schematic diagram of FTIR instrumentation (Source: Higdon, 2010)

Balakhnina *et al.* (2011), outlined several principal characteristic lines present in the vibrational spectrum of most ochre paints, for example, the line with frequency 466 cm^{-1} corresponds to quartz vibrations; at 914 cm^{-1}, Al–O–H vibrations, the doublet at 1008–1032 cm^{-1} indicates Si–O–Al and Si–O–Si stretching vibrations and play an important role in identifying ochre components (Balakhnina *et al.*, 2011). Whereas the lines at 3150 cm^{-1} (FeOOH) and 3436 cm^{-1} (H_2O) are reported to be characteristic of all types of ochre. Previous work has shown that hematite produces an infrared spectrum with bands at approximately 560 and 480 cm^{-1}, with the more intense band at 560 cm^{-1} (Estep-Barnes, 1972; Balakhnina *et al.*, 2011). FT-IR can also clearly distinguish between organic and non-organic residues (Shillito *et al.*, 2009). Balakhnina *et al*.

(2011) used FTIR to observe the interaction of ochre with a binding medium, (linseed oil) in order to determine if their interaction produced any changes to the vibrational spectra of the two materials. Several changes in vibrational spectra were observed for ochre paint as compared to both linseed oil and ochre alone suggesting that there was an interaction between the molecules of the linseed oil and ochre during paint preparation (Balakhnina *et al*., 2011).

As already mentioned iron earth pigments consist of other accessory minerals and these can dominate the absorptions in the infrared spectrum (Helwig, 1998). Although the presence of these minerals can sometimes make characterization of iron minerals using FTIR challenging, identification of these minerals can sometimes provide information about geological source of the pigment (Helwig, 1998). Genestar & Pons (2005), applied FTIR technique to distinguish between two types of ochres; ochres rich in kaolinite (aluminosilicate) and ochres containing sulphate, and were able to distinguish that the ochres containing kaolinite were sourced from France while the ochre rich in sulphate was mainly from Spain.

2.4 Mosquito repellents

Mosquitoes are not just a nuisance but are also potentially harmful since a bite from mosquito can result in anything from a skin irritation to contracting mosquito borne-diseases such as Malaria, Dengue fever and Lyme disease (Patel *et al.*, 2012). The primary attractant of mosquito to their human host is the carbon dioxide that we breathe out, but as the mosquito gets closer to the host, it is the smell of other compounds that the host emits like lactic acid and ammonia that allow the mosquito to locate and identify its host (Patel *et al.*, 2012). In a nutshell, once the mosquito has located the host, it uses its mouthpart to bite and draw blood from the host, and it is during this process that the infected mosquito can then transmit pathogens such as malaria causing parasite *Plasmodium* into the host and in the process infecting the host (Patel *et al.*, 2012). Strategies for coping with disease pathogens have evolved with humankind comprising of both biological and behavioral mechanisms (Karunamoorthi, Mulelam & Wassie, 2008). The first methods employed to repel insects may have entailed covering the skin with mud or even with aromatic plant extracts (Novak & Gerberg, 2005; Karunamoorthi

et al., 2008), perhaps in most cases, applying repellents to the skin may have been the only feasible way to protect the skin against insect bites.

Mosquito repellents play an important role in disrupting the interaction between mosquitoes and human hosts hence reducing bites and minimizing incidents of malaria transmission (Logan *et al*., 2010). In most cases, a repellent contains an active ingredient that repels mosquito as well as secondary ingredients which assist in delivery of cosmetic appeal (WHO, 2008; Baba, Lawal & Shariff, 2012). For a material to be valuable as a mosquito repellent it must effectively discourage insect attack on the treated area for many hours and on many different types of surfaces, it must work effectively in different environmental conditions and it must be safe for use on human or animal skin (WHO, 2008).

2.4.1 Synthetic repellents

There are numerous commercially available synthetic mosquito repellents which include; DEET (N,N-diethyl-m-toluamide), Icaridin, also known as picaridin, Bayrepel, and KBR 3023 Nepetalactone, also known as "catnip oil" , Permethrin , Bog Myrtle, IR3535 (3-[N-Butyl-N-acetyl]-aminopropionic acid, ethyl ester) (Patel *et al*., 2012). Most of these commercial repellents are prepared using nonbiodegradable synthetic chemicals (Antwi, Shama & Peterson, 2008; Yadav *et al*., 2014). Although the mode of action of most synthetic mosquito repellents is still not well understood, one theory suggests is that repellents such as DEET, act as a vapour barrier that modify and block responses of mosquito`s olfactory receptor neurons (ORNs) normally sensitive to attractants such as lactic acid, preventing host recognition and interaction (Dickens & Bohbot, 2013; Yadav *et al*., 2014). There are quite a number of other comparative efficacy studies pertaining to DEET and other insect repellents but in spite of DEET being effective, it is believed to be unsafe for use (Mark *et al*., 2002; Helio *et al.*, 2004). Toxicology reports concerning DEET exposure have led to new focus on natural insect repellents extracted from plants and other materials as safer alternatives.

2.4.2 Natural repellents

There are many preparations from naturally occurring sources that are used either as repellents or insecticides to certain insects (Patel *et al*., 2012). Several plant species in Africa have been documented to have compounds that are known to reduce mosquito

biting activity when used as repellents (Coker *et al.*, 2000; Omolo *et al*., 2004 Innocent *et al*., 2008; Baba *et al*., 2012). Secondary metabolites produced by plants such as aromatic and aliphatic hydrocarbons and oxygenated compounds such as terpenes are active ingredients responsible for mosquito repellent activity (Coker *et al.,* 2000; Baharum *et al*., 2010; Baba *et al*., 2012). Currently the US Environmental Protection Agency (US-EPA) has registered citronella, lemon and eucalyptus oils as insect repellents (Yadav *et al*., 2014). According to ethnographic accounts, Namibian Myrrh (*Commiphora wildii)* and *Colophospermum mopane; local name, Omutati*, from Namibia also exhibit activities against mosquitoes but no experimental reports have been conducted to support this indigenous knowledge.

Previous studies on long chain aliphatic methyl ketones have shown that they have repellency properties to some blood sucking insects (Blum, 1966; Torr *et al.*, 1996; Gikonyo *et al.*, 2002; Barton, 2003; Roe, 2004). Repellency activities of 2-heptanone to *Iridomyrnex pruinosus* (Blum *et al.*, 1966), 2-octanone to *G. morsitans morsitans* and *G. pallidipes* (Torr *et al.*, 1996), 2-decanone, 2-undecanone and 2-dodecanone to *Glossina morsitans morsitans* (Gikonyo *et al*., 2003), 2-undecanone and 2-tridecanone to *Culex quinquefasciatus* and *Aedes aegypti* mosquitoes (Barton, 2003; Roe, 2004). A study conducted by Barton (2003), observed that 2-undecanone applied at 30% and 40% solution (vol/vol) in isopropyl alcohol to human skin was 100% active for 15 minutes in preventing mosquito bites from Ae*. Aegypti* mosquito. While in another study by Roe (2004), 2-undecanone at 100% and 50% concentration, showed repellent activity for up to 6 hours against *Cx quinquefasciatus* mosquito (Roe, 2004). Innocent *et al*. (2008) conducted a study on long chain aliphatic methyl ketone series of C7-C15, to test their repellency activity against *Anopheles gambiae and* the authors reported that all methyl ketones tested produced a dose dependent repellency response, with 2-tridecanone giving a comparable protection efficacy to DEET.

In olfactometer trials conducted by Logan *et al.* (2010), on three aldehydes; octanal, nonanal and decanal, and two ketones; 6-methyl-5-hepten-2-one and geranylacetone interfered with attraction of mosquitoes to a host. Maximum effect was observed for a 1:1 mixture of 6-methyl-5-hepten-2-one and geranylacetone, which gave repellency exceeding that by DEET. Authors also observed that altering the ratio of these compounds significantly affected the response of the mosquitoes, suggesting that mosquitoes have the ability to detect and respond to specific mixtures and ratios of

natural repellent compounds and that these compounds might function effectively synergistically.

2.5 Review of analytical techniques used in analysis of mosquito repellents

Various chromatographic and spectrometric techniques such as IR, GC, thin layer chromatography (TLC), and column chromatography have been used for separation and characterization of active compounds associated with mosquito repellent activity. Bulugahapitiya & Arachchige (2007), carried out a study to identify mosquito repellent compounds from seed oil of Black cumin, *Nigella sativa*, in which TLC was used to separate compounds in the oil mixture and further analysis was then carried out using IR to determine the functional groups of these compounds and they concluded that the main active compounds in *N. sativa* are terpenoids containing hydroxyl and carbonyl functionalities. The most common technique used for characterization of mosquito repellent compounds is gas chromatography-mass spectrometry (GC-MS) as this is evident through its wide use in many studies (Hwang, 1985; Omolo *et al*., 2004; Innocent *et al*., 2010; Tabanca *et al*., 2010; Padmavathy, 2013). Application of GC-MS for characterization of compounds with mosquito repellent activity is further discussed below as it is also the method applied in this study.

2.5.1 Gas chromatography (GC)

Gas chromatography is an analytical technique that works on the basic principle of separating organic components of a complex mixture into individual molecules based on their volatilities. Hence, only the compounds which can easily vaporize without decomposition are considered suitable for GC analysis. It gives both quantitative and qualitative information for individual organic components in a sample. The components move through a GC column in gaseous form, either because they are generally gases or heated and vaporized into gaseous state. The compounds partition between the mobile phase (carrier gas and analyte) and the stationary phase (the column) and this differential partitioning allows the compounds to be separated in time and space. The components in a mixture will be separated based on the difference in chemical

properties of different molecules in that mixture. The retention time, i.e. the time taken by a molecule to come out of the gas chromatograph, is different for different molecules (Maaqbool, 2014). Carrier gas serves as is the mobile phase which moves the sample from the injector through the column during chromatography. The carrier gas should be inert in order to avoid interaction with the sample being analyzed. Hydrogen, helium and nitrogen are commonly used as carrier gases. The injector is a hollow, heated, glass-lined cylinder where the sample is introduced into the column and vaporized. The samples are injected, vaporized and transported through the column. The column is a stationary phase which is made up of metal, glass or quartz. Two types of column are known, which include the packed column and the capillary column. A detector measures the different components that are separated in the column (Maaqbool, 2014). The two types of GC used were GC-FID and GC-MS. Gas chromatography coupled with flame ionization detector (GC-FID) is a very basic chromatograph technique, which provide primarily information on retention indices that are crucial in analytical chemistry while gas chromatography coupled with mass spectrometry (GC-MS) provides much more reliable qualitative and quantitative analysis of complex samples (Baharum *et al*., 2010).

2.5.1.1 Gas Chromatography-Flame ionization Detector (GC-FID)

In an FID, the sample undergoes a combustion in a hydrogen air flame. Ions and free electrons are formed in the flame. The charged particles produce a measurable current flow in the gap between two electrodes in the detector. The resulting current flow is of greater strength than the signal produced by the pure carrier gas and the fuel gas flame alone. This signal differential provides information about the sample. The current is proportional to the information which depends on the composition of the separated sample. Time and temperature are plotted against x-axis and signal intensity (in terms of peaks height equivalent to the relative amount of different components) along y-axis (Maaqbool, 2014). **Figure 2.6** shows instrumental setup of GC-FID.

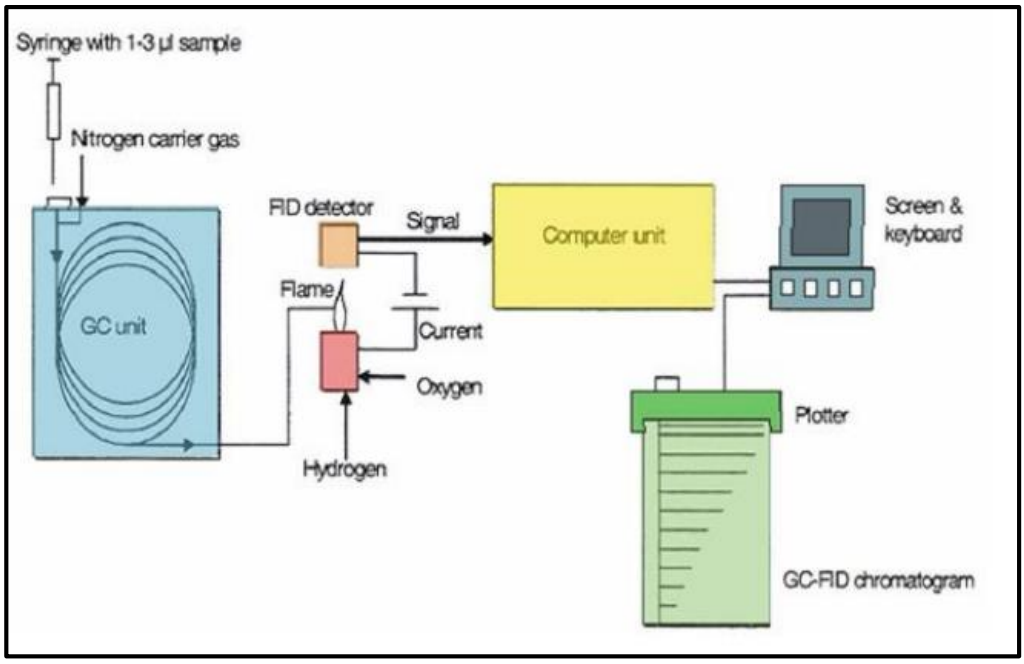


Figure 2.6: Schematic diagram of GC-FID instrumentation (Source: Maaqbool, 2014)

2.5.1.2 Gas Chromatography-Mass spectrometry (GC-MS)

GC-MS is a gas chromatograph (GC) integrated with a mass spectrometer (MS), which helps in the quantification and identification of compounds. The former does the compound separation and the latter is concerned with the identification of components at a molecular level. As soon as a molecule leaves the gas chromatograph, it enters downstream into a mass spectrometer where it is captured, ionized, accelerated and deflected. It is then detected by a detector attached to mass spectrometer. Each molecule that is ionized by mass spectrometer is detected individually by the detector. A PC program is used to record and manage the data (Maaqbool, 2014). **Figure 2.7** shows a typical instrumental setup of GC-MS. There is relatively lack of reference on the use of gas chromatography in analysis of organic compounds in clay-like materials such as ochre but a study conducted by Scott *et al*. (2002), used GC-MS in an attempt to determine if a binding medium was used for rock art painting using red and yellow ochre from the Chumash Indian site of San Emigdio. Their findings using GC-MS revealed that there was no use of a binding material used in the painting techniques employed.

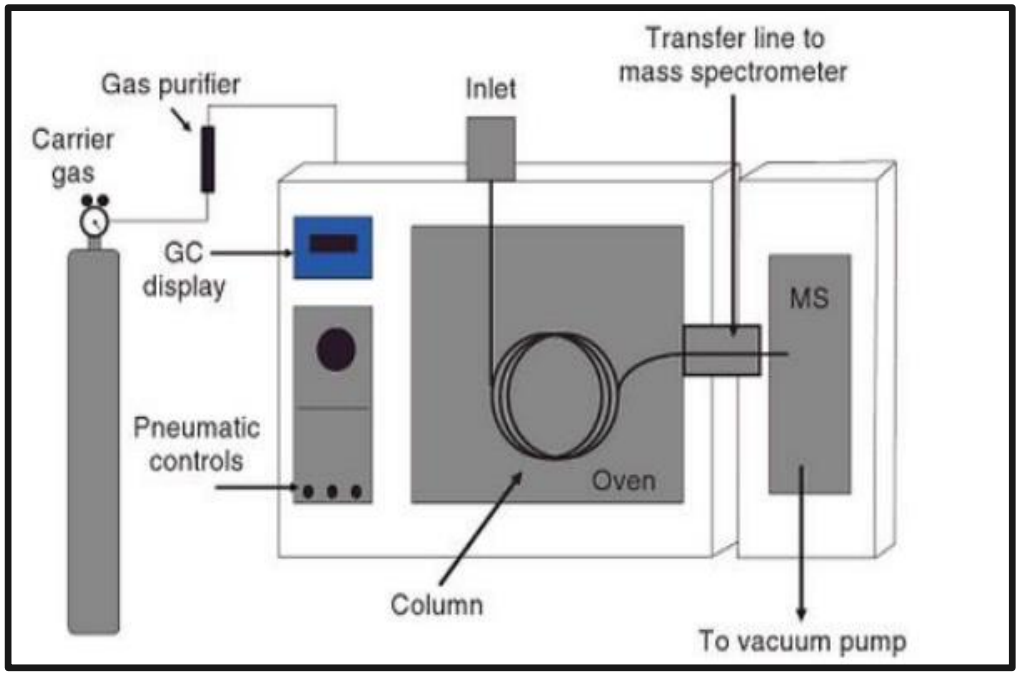


Figure 2.7: Schematic diagram of GC-MS instrumentation (Source: Maaqbool, 2014)

GC-MS is used almost exclusively for the qualitative analysis of the volatiles (Zhao *et al*., 2005; Barahum *et al*., 2010), which makes it the appropriate method for identification of insect repellents as they are volatile organic compounds. There is also a lack of studies on the use of gas chromatography in separating and identifying mosquito repellent compounds from clay-like materials, but GC-MS has been used in many studies for identifying bioactive compounds from plants materials with mosquito repellent capabilities (Hwang, 1985; Pal, Kumar & Tewari, 2011; Padmavathy, 2013). A number of compounds tested as repellents against mosquito *Aedes aegypti* isolated from mugwort *Artemisia vulgaris* were identified using GC-MS comprising mainly of monoterpenoids including linalool, camphor, isoborneol, terpinen-4-ol, and isobornyl acetate and 3-nonanone by Hwang (1985). Terpinen-4-ol was observed as the most active compound and was as effective as the commonly known synthetic repellent, dimethyl phthalate (Hwang, 1985). In a study by Pal *et al*. (2011), GC – MS was used to analyze the chemical composition of the essential oil obtained from the leaves of Plectranthus incanus and the major components of the oil identified were fenchone, piperitone oxide, piperitenone, and piperitenone oxide which were then tested for mosquito repellent activities against mosquito species; *Anopheles stephensi* and *Culex fatigans*. The repellent activity of the essential oil of *P. incanus* was measured by

determining the protection period against the bites of *Anopheles stephensi* and *Culex fatigans* and the oil showed a stronger repellent activity than citronella oil suggesting that the chemical composition of *P. incanus* has a strong insect repellent capability.

2.6 Sample preparation techniques

The purpose of an analytical study is to obtain information about a sample whether it is a solid, a liquid or a gas (Mitra, 2004). Despite the availability of sophisticated analytical techniques, most of them still require extracting a portion of the sample prior to instrumental analysis (Mitra, 2004). Therefore, sample preparation continues to be a crucial step in analytical studies. There are several traditional and modern sample extraction techniques used for solid samples including, pressurized hot water extraction, Soxhlet extraction, solid phase extraction (SPE) microwave assisted extraction (MAE) and ultrasound assisted extraction (UAE).

2.6.1 Microwave assisted extraction (MAE) for elemental analysis

ICP has become an acceptable tool for geochemical analysis but it is a solution based technique which means that solid samples must be digested prior to analysis (Kogel & Lewis, 2001). Microwave sample preparation provides an efficient and clean sample preparation for multi-element analytical techniques such as ICP-OES and ICP-MS. Acid digestion procedures are used in the determination of elements in solids in order to completely transfer the analytes into a solution so that they can then be analyzed in the determination step in a liquid form. Therefore, the goal of every digestion process is the complete decomposition of the solid matrix while avoiding loss and contamination of the analyte. Microwave chemistry is the science of applying microwave irradiation to chemical reactions. The principle of heating using microwave energy is based on the direct effects of microwaves on molecules of the material. Microwaves act as high frequency electric fields which can heat any material containing mobile electric charges, such as polar molecules in a solvent or conducting ions in a solid. The transformation of electromagnetic energy into calorific energy occurs by two mechanisms: ionic conduction and dipole rotation in both the solvent and the sample. In many applications these two mechanisms take place simultaneously, which effectively changes microwave energy to thermal energy (Rostagno & Prado, 2013).

Ionic conduction is due to the electrophoretic migration of ions when an electromagnetic field is applied (Eskilsson & Bjorklund, 2000). The resistance of the solution to this flow of ions and the collisions between molecules due to the direction of ions changing as the field changes sign, results in friction and, hence heating the solution as well increasing solvent penetration into the matrix (Ganzler, Szinai & Salgo, 1990). The dipole rotation is related to alternative movement of polar molecules that have dipole moments which try to line up with the electric field. As the field decreases, thermal disorder is restored which results in the release of thermal energy. At 2450 MHz (used in microwave commercial systems), the alignment of the molecules followed by their return to disorder occurs 4.9 $x10^9$ times per second, leading to rapid heating (Camel, 2000). A typical microwave oven consists of a magnetron tube, a wave guide, a cavity, turnable and mode stirrer (Kingston & Jassie, 1988; Rostagno & Prado, 2013). The magnetron generates microwaves under the influence of a magnetic field and this yield microwave energy is channeled into the microwave cavity by the wave guide (Montaser, 1998). The mode stirrer then distributes this energy throughout the microwave cavity (Roussy & Pearce, 1995) while the turnable, turns the rotor which holds the digestion vessels to expose all vessels to equal amounts of energy and in turn allowing heating to be equal for every vessel in the rotor (Kingston & Jassie, 1988; Montaser, 1998).

Ever since the use of microwave technology for the decomposition of solids was introduced by Abu-Samara, Morris & Koirtyohann (1975), it has become an integral part of modern analytical laboratory (Camel, 2000; Downer, 2008). It offers several advantages to the chemical decomposition of geological material since it produces more efficient sample dissolution through better contact between the acid and the sample (Repacholi, 1994). Numerous papers have been published concerning decomposition of geological samples using a microwave oven (Fischer, 1986; Buresch *et al.*, 1987; Noeltner, 1990; Kuss, 1992; Kogel & Lewis, 2001). Kogel & Lewis (2001), used microwave digestion method which used significantly reduced quantities of HF to digest clay samples for elemental analysis using ICP-MS and reported that this improved elemental detection limits of the method.

2.6.2 Ultrasound assisted extraction (UAE) of organic compounds

Most characterization techniques are solution based and requires some form of sample preparation to solid samples prior to analysis, for example, isolation of organic

compounds from solids is required before analysis using GC. The extraction method of choice for the compounds of interest is something that need to be well thought out considering factors such as the type and nature of the compounds to be extracted (Albu *et al*., 2004).Traditional methods of sample preparation for organic analytes such as Soxhlet extraction and steam distillation are time consuming, requires larger usage of solvents and exposure to heat often caused degradation to thermally liable compounds (Cravotto *et al*., 2008).

Ultrasonic assisted extraction (UAE) is among the newer methods used in extraction technology and its usage is increasingly becoming popular (Vinatoru *et al*., 1997). Mechanical waves formed by ultrasound enable generation of micro-cavitations in the liquid surrounding a solid material resulting in heating the solid material. This occurs through cavitation phenomena which involve physical processes that generate, enlarge, and breakdown micro gas bubbles dissolved in the liquid. The liquid medium is made up of molecules that are held together by attractive forces and as an ultrasound wave passes through the liquid medium, it induces a longitudinal displacement of those molecules, acting like a piston on the surface, resulting from a succession of compression and rarefaction phases (McClements, 1995). These molecules are temporarily dislodged from their original position and during the compression cycle, they collide with the surrounding molecules. During the rarefaction phase, a negative pressure is exerted, pulling the molecules apart, generating a void in the liquid. The voids generated in the medium are the cavitation bubbles which are formed from dissolved gases (Mason *et al*., 2005). When the size of these bubbles reaches a critical point, they collapse during a compression cycle (Flint & Suslick, 1991; Suslick *et al.*, 1999). When the bubbles collapse onto the surface of a solid material, the high pressure and temperature released generate microjets and shock waves directed towards the solid surface. These microjets are useful for the extraction of compounds from solid materials. All ultrasonic systems are composed of a transducer, which converts electrical energy into sound energy by vibrating mechanically at ultrasonic frequencies, generating ultrasound (Povey & Mason, 1998). The generated ultrasound is irradiated by the emitter or the reactor, which also amplifies the waves (Bermúdez-Aguirre, Mobbs & Barbosa-Cánovas., 2011). Most commonly used emitters are the ultrasocnic bath systems and the probe-type sonicators (Bermúdez-Aguirre *et al.*, 2011).

Various studies have proved the efficacy of ultrasound extraction in extracting volatile compounds from different sources especially from plant materials (Mason, Paniwnyk & Lorimer, 1996; Sališová, Toma & Mason, 1997; Vinatoru *et al*., 1997; Paniwnyk *et al.*, 2001; Albu *et al*., 2004; Liang *et al*., 2015). UAE was the method of choice in the study carried by Liang *et al.* (2015), in isolating and identifying bioactive compounds having mosquito larvicidal activity from the seeds of *Amorpha fruticosa.* Ultrasonic extraction uses ultrasonic vibration to ensure intimate contact between the sample and the solvent. The choice of extraction solvent to be used should be taken into consideration as factors such as its polarity, boiling point and reactivity with the extract might affect its efficiency (Albu *et al.*, 2004). Using UAE for the extraction of biologically active compounds has many advantages over other conventional extraction methods since it is simple to use and requires shorter extraction time, less solvents, provide higher extraction rates and high safety (Bjorklund & Eskilsson, 2000; Asikin & Safri, 2012). The effect of ultrasound is dependent of the solvent used (Paniwnyk *et al.*, 2001).

CHAPTER THREE: RESEARCH METHODOLOGY

This chapter addresses experimental procedures of the analytical techniques that were used to determine the mineralogy, and geochemistry of the red ochre samples and the chemical composition plant samples used by Ovahimba pastoralists from the Kunene region of Northern Namibia.

3.1 Study area

Ten ethnographic samples listed and described in **Table 3.1** were provided by Riaan F. Rifkin and Francesco d'Errico. These samples were collected from Okamanga, Otjongoro and Ovinjange villages and the local market in Opuwo located in north of Namibia (**Figure 3.1**).

Table 3.1: List of ethnographic samples obtained from the Kunene region and analyzed during this study

Sample	Origin	Description
1 (8)	Okamanga	Red ochre powder mixed with clarified butter (*otjize*)
2 (15)	Okamanga	Aromatic sample mixed with clarified butter
3 (17)	Okamanga	Uncharred aromatic wood and bark fragments
4 (18)	Okamanga	Composite black ground aromatic powder
5 (20)	Okamanga	Milk derived clarified butter from traditional ochre container
6 (26)	Ovinjange	Finely ground red ochre powder
7 (29)	Ovinjange	Aromatic sample mixed with clarified butter
8 (33)	Opuwo	Ochre and aromatic mixture from ochre container
9 (35)	Opuwo	Aromatic seeds from traditional ochre container
10	Otjongoro	Subcutaneous animal fat from a kudu (*Tragelaphus strepsiceros*) antelope

** Numbers in brackets indicate the original reference numbers assigned during sample collection by Dr. Riaan Rifkin*

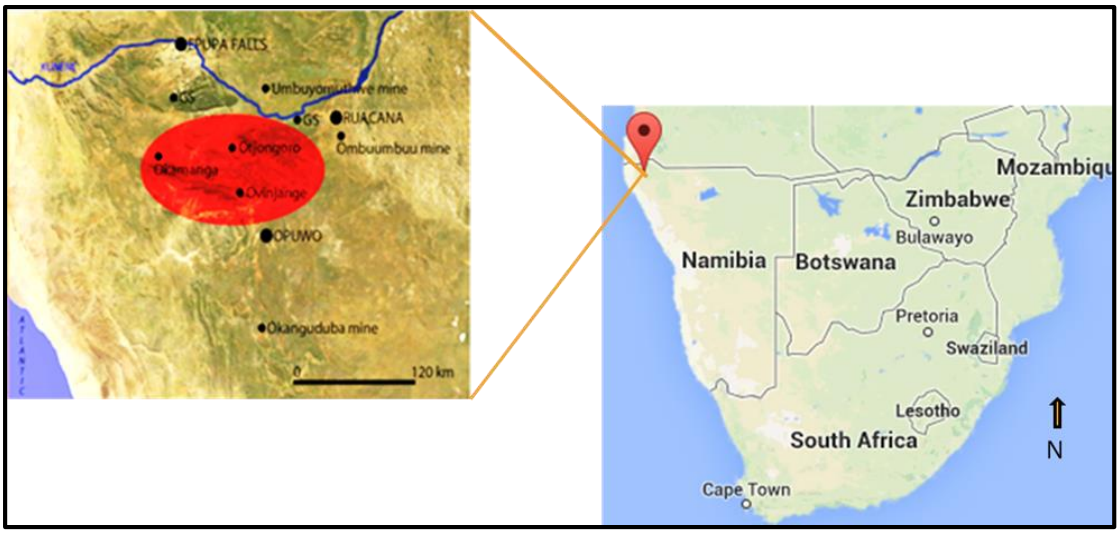


Figure 4.1: The location of the Opuwo, Ovinjange, Okamanga and villages in the Kunene Region of northern Namibia

3.2 Chemicals

The following solvents and reagents were used: dichloromethane (DCM), toluene, nitric acid (55%), boric acid (4%), hydrofluoric acid (40%), and hydrochloric acid (32%) (Sigma-Aldrich, Johannesburg, South-Africa). Certified multi-element standards (DeBruyn Spectroscopic solutions, Johannesburg, South Africa) were used for the calibration of ICP-OES. All chemicals and solvents used were of analytical grade. Deionized water was prepared using Millipore deionizer instrument (Millipore, Massachusetts, USA) with an electrical resistivity of 18.2 MΩ cm. Potassium bromide (KBr) was used for the preparation of KBr pellets for FTIR analysis.

3.3 Sample preparation procedures

Sample preparation was required prior to analyzing samples using ICP-OES and GC which involved the digestion of the red ochre powder using microwave digestion and extraction of organic compounds from red-derived and plant derived cosmetics using ultrasound assisted extraction.

3.3.1 Microwave assisted digestion (MAE)

A typical closed microwave system with build-in pressure and temperature control can be seen in **Figure 3.2**, which shows an Anton Paar Multiwave 3000 microwave reaction system and its consumables that were used for the extraction of elements from the ochre powder.

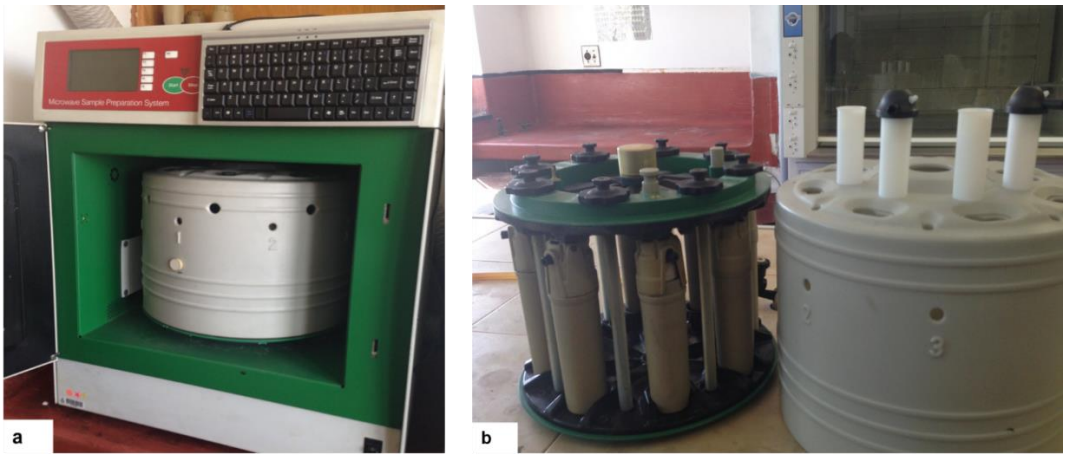


Figure 3.2: An Anton Paar Multiwave 3000, microwave reaction system was used for the extraction of elements from the ochre powder. System seen when a) open and b) shows its consumables including the digestion vessels

For the decomposition of the ochre powder in order to determine the total concentration of the minor or trace and major elements present in the ochre, 0.250 ± 0.005 g of the red ochre powder was accurately weighed using an analytical balance (Precisa 180A, Switzerland) into acid washed digestion vessel, 3 mL of HNO_3 was added, followed by 9 mL of HCl and finally 1 mL of HF was added into the vessel in the fumehood. A blank was also prepared which did not contain ochre powder. The vessels were tightly locked and placed in the jackets and set in the rotor. The set up was then placed in the digestion system with the power programmed as in **Table 3.2**. At the end of the digestion, the vessels were allowed to cool down and taken out of the oven. They were then opened while in fumehood and 6 mL of boric acid was then added to vessels to neutralize HF. The solution was transferred into a 50 mL volumetric flask and diluted to the mark with deionized water. Samples were stored at 4°C in the fridge prior to the determination of the metal concentrations by ICP-OES.

Table 3.2: Microwave power programme

Run	Power (W)	Ramp (min)	Hold (min)
1	800	10:00	10:00
2	600	10:00	10:00
3	0	5:00	5:00

3.3.2 Ultrasound assisted extraction (UAE)

An ElmaTranssonic 460 (Elma, Singen, Germany), ultrasonic bath extractor was used for ultrasonic extraction of target organic compounds from red ochre and plant samples (**Figure 3.3**).

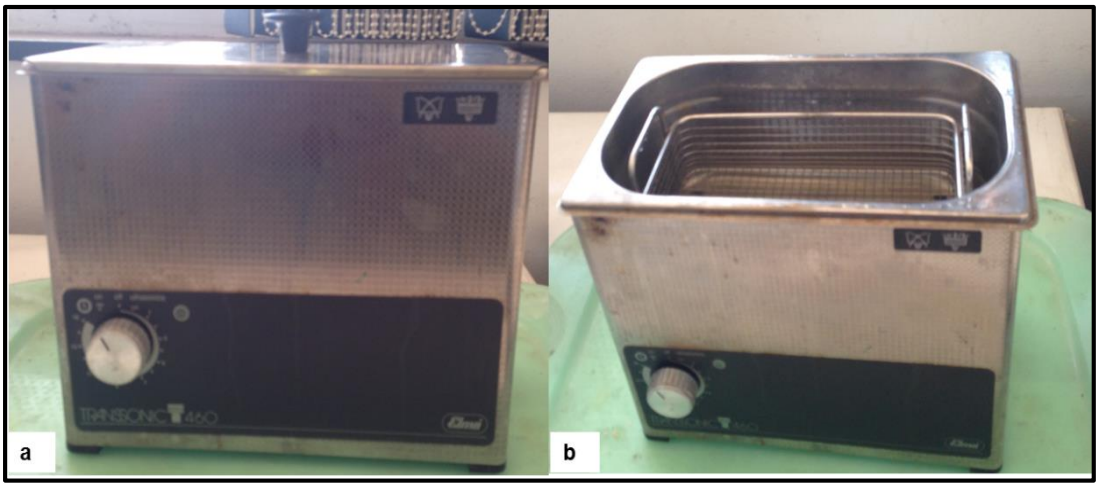


Figure 3.3: ElmaTranssonic 460, an ultrasonic bath extractor which was used for ultrasonic extraction a) closed and b) open showing water bath

Replicated *otjize* samples were prepared by mixing fat and ochre powder in 1:1 ratio to mimic how Ovahimba people prepared their *otjize*. About 0.150 g of red ochre powder sample, fat samples and plant materials were weighed into glass extraction vials and ≈ 0.300 g of replicated *otjize* samples were also weighed into the extraction vials and 2 mL of DCM was added to the samples for extraction of organic compounds. The vials were tightly closed and placed in a beaker half-filled with water. The beaker was placed

in a steel basket of ultrasonic bath and the solution was ultrasonicated for 30 min in 2 sequences taking 15 min each. Water for ultrasonic bath was replaced at each sequence to prevent the boiling of sample solution in the beaker. The solution was filtered using a Nylon 0.45 µm filter through a syringe and filtrates were collected into clean glass vials and analyzed on GC-FID and/or GC-MS. **Table A1** gives the details of actual amounts of samples weighed and used during sample extraction using UAE.

3.4 Elemental and mineralogical analysis procedures

EDXRF and ICP-OES were used for total elemental analysis while PXRD was used to determine the mineral composition of the ochre.

3.4.1 Elemental analysis

EDXRF was used for determining the major elemental composition of the ochre samples. For this, a Bruker Handheld Tracer lll SD ED XRF was employed (**Figure 3.4**). The operating conditions used include a spectrometer equipped with a 4-watt, miniature (<15 mm diameter and <75 mm long) X-ray tube containing an Rh target. The X-ray tube was operated at 40 keV and 10.00 µA.

An ICP-OES instrument from SPECTRO GENESIS End-on-plasma Spectro Analytical Instruments (Pty) Ltd, (Johannesburg, South Africa) as seen in **Figure 3.5** was used for the determination of the metals concentration in ochre powder.

Figure 3.4: A Bruker Handheld Tracer lll SD ED XRF used for elemental analysis of the red ochre

Figure 3.5: An external view ICP-OES instrument from SPECTRO GENESIS End-on-plasma Spectro Analytical Instrument

Sample solutions prepared after digestion were analyzed on ICP-OES for total metals after calibration of the system with certified multi- element standards. The sample

solutions with much higher concentrations were diluted until the concentration was within the calibration range. The analyte concentration in the sample, expressed in mg kg^{-1}, was obtained using the equation Concentration = (C_A) (V_{final}) (D)/ m_{sample}; where C_A is the analyte concentration obtained from the calibration (in mg L^{-1}); V_{final} is the final volume (0.05 L), D is the dilution factor and m_{sample} is the weight (0.25×10^{-3} kg) of the investigated sub-sample. Operating conditions for ICP-OES are shown in **Table 3.3**. The limit of detection (LOD) is the lowest analyte concentration which can be detected by the machine used, it was determined as average concentrations corresponding to three times the standard deviation of the method blank (Hutter, 2011).

Table 3.3: ICP–OES operating parameters and conditions for determination of total metals in Ovahimba red ochre powder

Parameters	**Conditions**
RF Power	1400 W
Coolant Gas Flow	14.00 L min^{-1}
Auxiliary Gas Flow	1.00 L min^{-1}
Nebulizer Gas flow	1.00 L min^{-1}
Sample Pump Flow	2.00 L min^{-1}
Sample Aspiration Rate	2.00 L min^{-1}
Replicates	3
Plasma Torch	Quartz
Spray Chamber	Single pass
Nebulizer	Crossflow
Processing Mode	Area

3.5.2 Mineralogical analysis

A Bruker XRD diffractometer with a Cu anode as a source (**Figure 3.6**), was used for mineralogical composition of Ovahimba red ochre powder. The mineralogical composition of some of the red ochre powder was confirmed using XRD. XRD patterns

were recorded on a Bruker XRD diffractometer with a Cu anode as a source. The identification of the phases was achieved using EVA code.

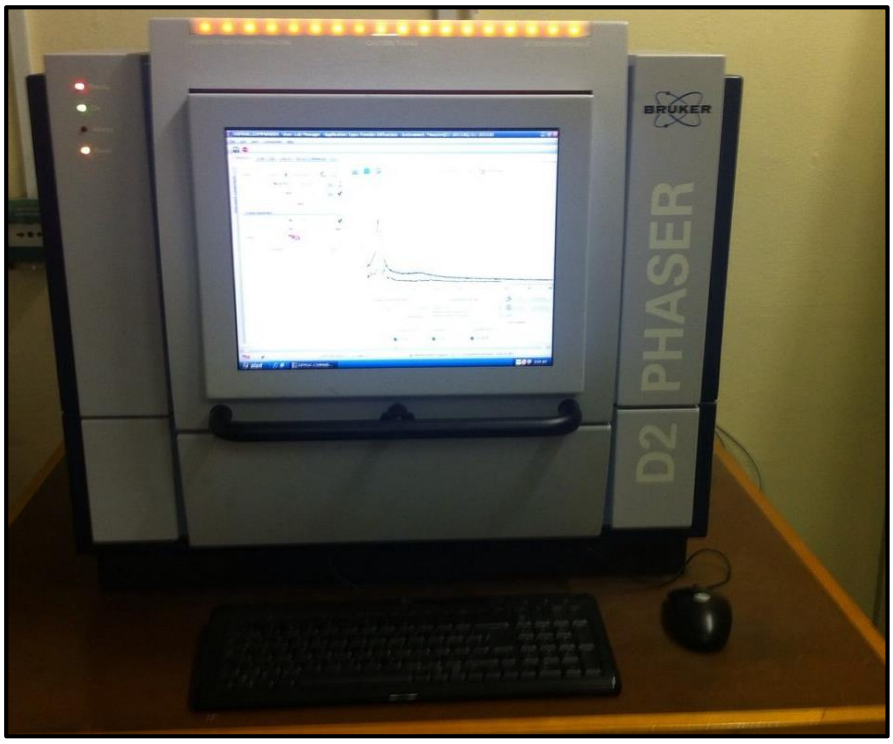


Figure 3.6: Bruker XRD diffractometer with a Cu anode used for mineral composition of red ochre powder

FTIR was used in the determination of chemical composition of red ochre powder in conjunction with PXRD. For FTIR analyses, a Nicolet iS5 spectrometer (Thermo Scientific, USA) shown in **Figure 3.7** was used.

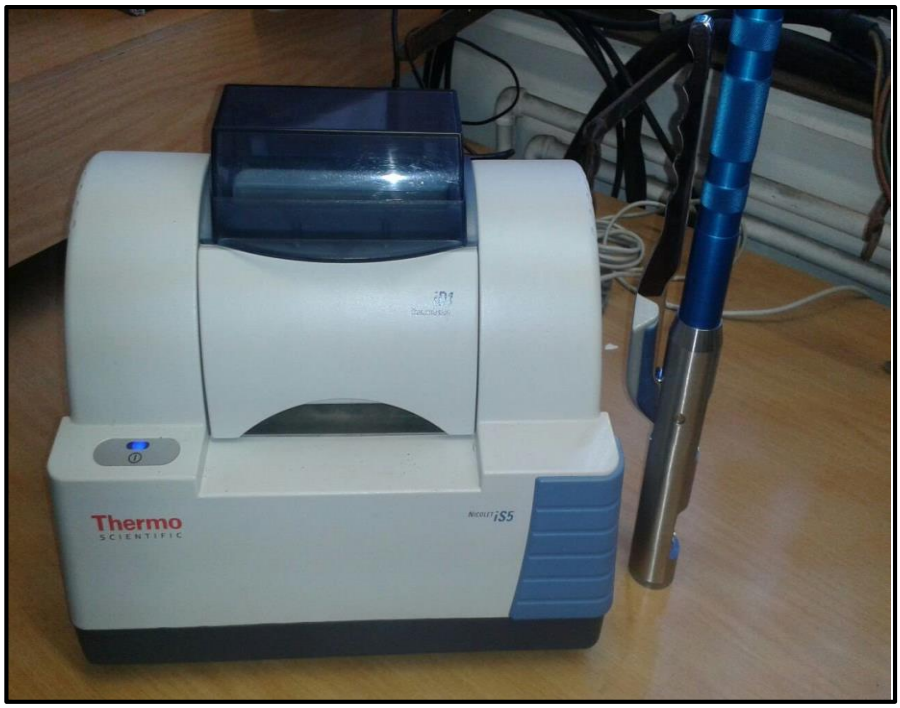


Figure 3.7: Nicolet iS5 spectrometer (Thermo Scientific, USA) was used for the FTIR analysis

The infrared spectra were recorded on KBr (IR grade, Merck, Germany) discs using a Nicolet iS5 spectrometer. Sample preparation was achieved by homogeneously mixing about 2 mg of ochre with 100 mg of anhydrous KBr in an agate mortar. The mixture was then placed under pressure using a hand-operated press and a transparent disk was formed. This disk was then placed in the instrument. FTIR spectra were recorded. FTIR scanning was conducted in ambient conditions and the resolution set at 4 cm^{-1} at a frequency range of 400-4000 cm^{-1}. 16 scans per sample were recorded, averaged and corrected, for each spectrum, against the spectrum with ambient air as background.

3.5 Organic compounds analysis procedures

Functional groups present in the red ochre –derived cosmetic and plant derived cosmetic were also determined using FTIR and the identification of mosquito repellents in the cosmetics was done by GC-MS while the interaction of the butterfat and red ochre was investigated using GC-FID.

3.5.1 Gas Chromatography-Flame ionization Detector (GC-FID)

In this study, analysis was done on an Agilent 7890A Gas chromatograph equipped with Supelco SPBTM-1 Sulphur, fused silica capillary column, 30 m x 0.32 mm x 4.0 µm film thickness connected to FID detector (**Figure 3.8)**. The determination of organic compounds from ultrasonic bath extracts; red ochre, fat, and replicated *otjize* samples, was made on a GC-FID. A Supelco 5 µL manual syringe was used to inject 1 µL of the solvent used as a blank into the column through the inlet. This was followed by analysis of all the samples using the following parameters: the heater of the inlet was set at 250°C and the injection mode was splitless; FID temperature was at 300°C; the initial oven temperature was 60°C for 4 min; the temperature was increased to 200°C at 15°C per minute and held for 10 min resulting in a run time of 23.333 min. Detailed GC-FID conditions used to run the samples are outlined in **Table D1**.

Figure 3.8: An external view of an Agilent 7890A Gas chromatograph with FID detector

3.5.2 Gas Chromatography-Mass spectrometry (GC-MS)

The separation and identification of organic compounds associated with mosquito repellency from ultrasonic bath extracts; red ochre powder, fats, replicated *otjize* samples and plant extracts was carried out on a LECO® GC X GC TOFMS Pegusus 4D

system integrated with a TSQ8000 MS (**Figure 3.9**), using helium as a carrier gas. 1 µL of the solvent used as a blank was injected into the column through the inlet using an automated system. This was followed by analysis of all the samples using the following parameters: the heater of the inlet was set at 250°C and the injection mode was splitless; the initial oven temperature was 55°C for 1 minute; the temperature was increased to 200°C at 10°C per minute and held for 0 min after which the temperature was raised to 320ºC at 7°C per minute for 0.36 minute resulting in a run time of 33.003 min. An Rxi-SSil MS column (30 m x 0.25 mm (ID) x 0.25 µm thickness (df) was used. Detailed GC-MS conditions used to run the samples are outlined in **Table E1**.

Figure 3.9: A LECO® GC X GC TOFMS Pegusus 4D system used for identification of mosquito repellent compounds from red ochre and plant UAE extracts (University of Johannesburg)

3.6 Quality assurance

Several factors were taken into account to ensure the quality of the results. For determination of organic compounds with GC, glassware was thoroughly cleaned with soap and deionized water and dried. They were then rinsed twice with dichloromethane or the organic solvent used for the GC analysis. For MAE, glassware was cleaned with

soap, rinsed with tap water and deionized water. Samples and reagents were weighed on analytical balance and the mass was read at 3 decimals places. Blank samples were used to check for any possible contamination. Extractions were done in triplicates and each extract was also analyzed in triplicates for reproducibility purposes.

CHAPTER FOUR - RESULTS AND DISCUSSION

The results obtained from the geochemical analysis of the ochre samples using EDXRF, PXRD, FTIR and ICP-OES are presented in this chapter. The chapter also presents the results for the characterization of possible mosquito repellent compounds in both red ochre derived cosmetic and aromatic plant derived cosmetic using GC-FID and GC-MS. This chapter also include a discussion of the findings with the help of various references from the literature.

4.1 Geochemical analysis of Ovahimba red ochre powder

This section reports the results from the qualitative and quantitative analysis conducted to determine the geochemical composition of the Ovahimba red ochre. This was achieved by using EDXRF, ICP-OES and PXRD.

4.1.1 Elemental composition analysis of red ochre using EDXRF and ICP-OES

Elemental composition of red ochre samples was analyzed using EDXRF and ICP-OES as described in section 3.7.1.1 and 3.7.1.2 respectively. XRF was mainly used as a screening technique for major elements due to its poor detection limits as compared to ICP-OES. Nonetheless, XRF can allow for detection of most elements in the periodic table with atomic mass above sodium (Kingery-schwartz *et al*., 2013). The results for major metals identified using EDXRF are summarized in **Table 4.1**.

Table 4.1: Major elements identified from red ochre using EDXRF

Ochre samples analyzed	**Identified elements**
Ovahimba ochre powder	Si, K, Ca, Ti, V, Fe, Cu, As, Zr,
Red ochre and clarified butterfat mixture (*otjize*)	Si, K, Ca, Ti, V, Fe, Cu, As, Zr
Ovahimba perfume and paint (*otjizumba* and *otjize*)	Si, K, Ca, Ti, V, Fe, Cu, As

All samples analyzed displayed an identical pattern except that Zr was not detected in the mixture of "perfume" and "paint." Elements identified include Si, K, Ca, Ti, V, Fe, Cu, As and Zr but EDXRF did not provide information on concentrations of the elements. ICP-OES was then used for the determination of metals` concentration in the red ochre powder. **Table 4.1** and **Table 4.2** showed a good agreement between EDXRF and ICP-OES on identification of some elements in the Ovahimba red ochre including Si, K, Ca, Ti, V, Fe, Cu and Zr. The investigation shows that Ovahimba red ochre contains a variety of chemical elements. ICP-OES revealed that Fe had the highest concentration of 4.1×10^5 mg kg^{-1} followed by Si with 1.86×10^5 mg kg^{-1} with the lowest concentrations observed for Mg and Zr at 1.6×10^3 mg kg^{-1} followed by K at 1.2×10^3 mg kg^{-1}

(**Table 4.2**). The relative standard deviation (RSD) were lower than 10% in most cases, demonstrating a good precision for the technique.

Table 4.2: Total metal content of Ovahimba red ochre powder analyzed using ICP- OES (results given as mean ± SD in mg kg^{-1}, *n=3*)

Metals detected	Metal Concentrations (10^3 mg kg^{-1})	Limits of Detection (mg kg^{-1})
Al	14.0 ± 0.5	1.8
As	9.2 ± 2.4	5.4
Au	4.4 ± 0.8	1.8
Ca	5.6 ± 0.8	3.0
Cd	2.4 ± 0.4	1.2
Cu	5.2 ± 0.8	0.6
Fe	410 ±1 9.5	8.4
K	1.2 ± 0.2	8.4
Mg	1.6 ± 0.8	2.4
Si	185.6 ± 8.1	6.0
Sn	6.4 ± 2.0	15.6
Ti	2.0 ± 0.4	1.2
U	37.2 ± 5.2	15
V	62.4 ± 1.4	0.6
Zr	1.6 ± 0.4	11.4

Most of the metals including Au, Ca, Cd, Cu, Mg and Zr were observed to be below 1% with respect to the total weight of the elements detected as a results of high quantities of iron (**Figure 4.1**). Presence of transition metals in red ochre powder in descending order according to their concentration with respect to the total weight of detected elements is as follows; Fe> V> Cu> Au> Ti> Zr. The results to a certain degree agree with what was observed by Grygar *et al*. (2003), who report that red ochre contains higher concentrations of Fe compared to other metals. Red ochre used by the Ovahimba people also exhibited quite a significant level of Si, observed to be the second most abundant element.

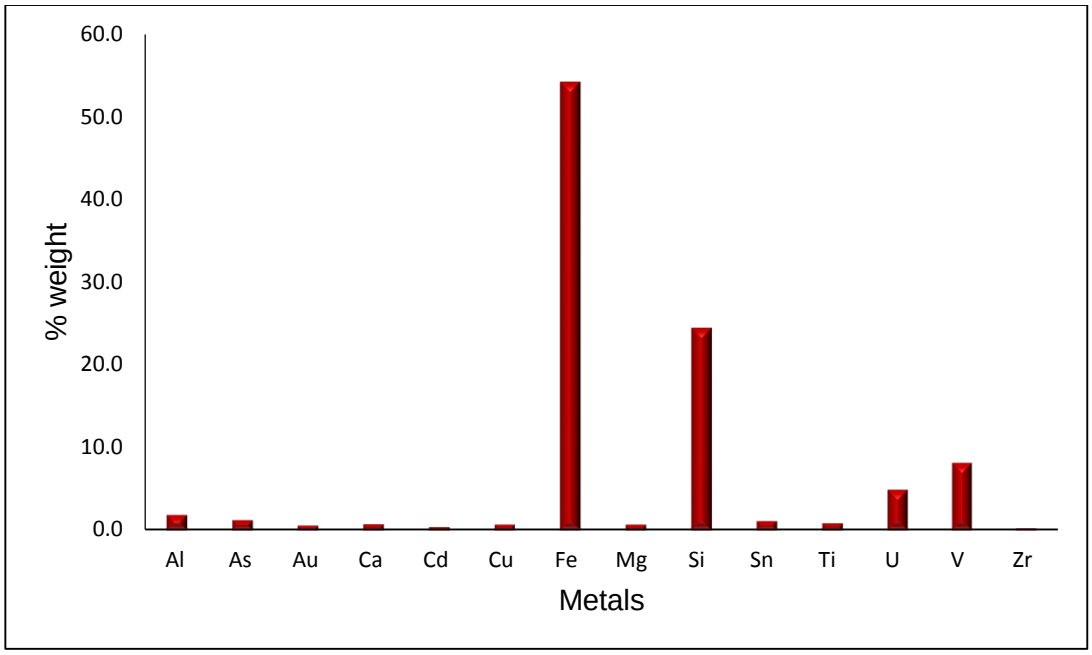


Figure 4.1: Major elemental content with values expressed in percentages with respect to the total weight of detected elements

Unfortunately, there are no published values of elemental analysis of red ochre used by the Ovahimba tribe, therefore quantitative comparison was not possible. However, similar trends have also been reported by other authors including Erlandson *et al.* (1999), who observed that all the eight red ochre samples from western North America were rich in Fe and Si with measurable concentrations for Al, Ca and K while few samples showed measurable percentages for Mg, Ti and Zr.

In general, elemental analysis of ochre always reveals a variation in the concentration of the metals detected in different ochre samples analyzed. These variations could be attributed to difference in weathering conditions, mineral deposits and human activities at sampling sites (Helwig, 1998).

4.1.2 Mineralogical analysis of Ovahimba red ochre using PXRD

The mineralogical composition of Ovahimba red ochre was determined using PXRD as described in Chapter 3 (section 3.7.1.3). XRD analysis showed the presence of intense hematite (Fe_2O_3) peaks indicating a significant amount of this iron oxide in the ochre as shown in **Figure 4.2**. The mineralogical analysis indicates that hematite is the principal mineral in Ovahimba ochre hence it is responsible for the red colour of the ochre. It is

this red hue produced by hematite that gives the Ovahimba people the red distinct colour. XRD analysis showed that Ovahimba ochre is also characterized by a strong presence of quartz (SiO_2) as shown by diffractogram patterns in **Figure 4.2**. The investigation also revealed that the Ovahimba ochre exhibit a moderate amount of kaolinite ($Al_2(Si_2O_5)(OH)_4$ and calcites ($CaCO_3$) with a very low content of chalconatronite ($Na_2Cu(CO_3)_2.3H_2O$).

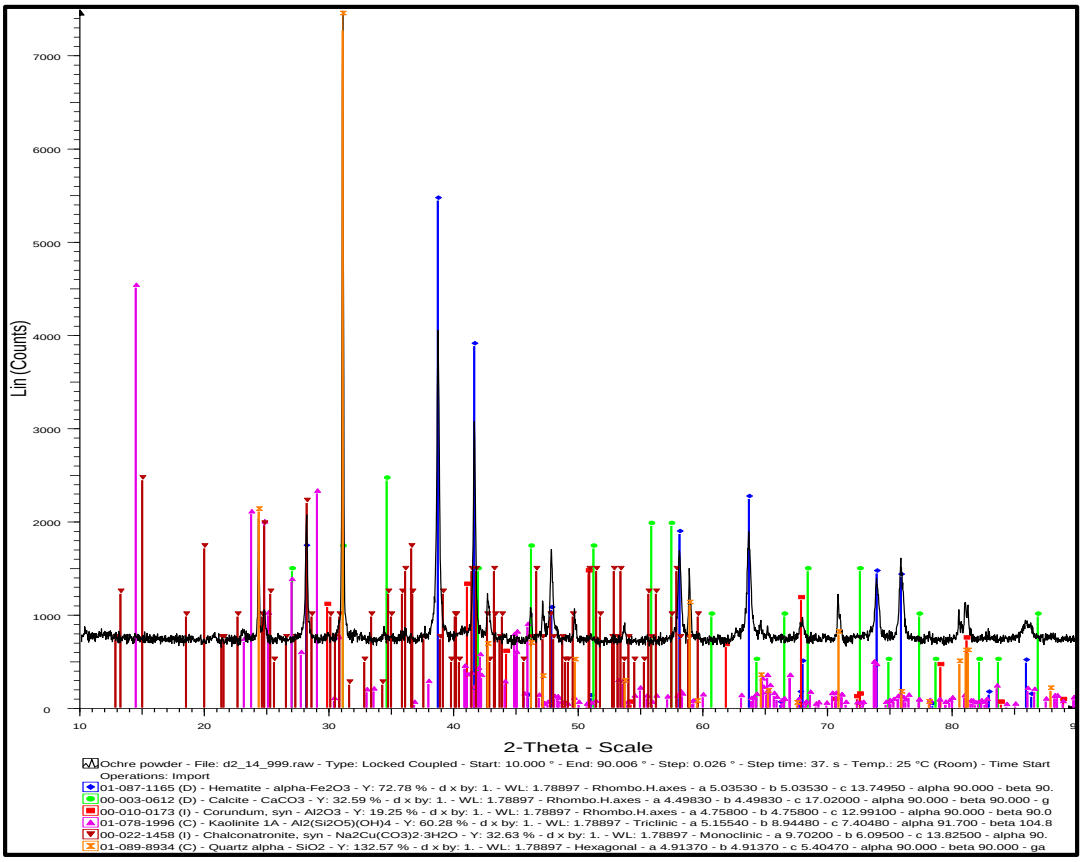


Figure 4.2: XRD diffractogram showing phase composition of red ochre powder from northern Namibia

The results are in agreement with what has been previously observed as red ochre is described as an impure variety of the mineral hematite (Tankersley *et al*., 1995) since it is commonly made up of iron minerals admixed with secondary clays (Helwig 1998). In ochre, iron oxides are often intermixed with various mineral clays and silicates. Calcites, quartz and other clay minerals are always present in varying quantities in ochre samples (Iriarte *et al*., 2009). It is noteworthy that, even if iron oxides are present in lower

quantities than other clay minerals, they turn to produce the colour of the sample due to their high pigmenting power (Helwig, 1998). The results are in keeping with the elemental analysis which identified presence of Fe, Si, Ca, Al and Cu in the Ovahimba red ochre.

4.2 Identification of functional groups in red ochre and plant derived cosmetic used by the Ovahimba people using FTIR

FTIR was a useful technique in identifying organic and inorganic compounds in both the red ochre-derived and aromatic plant-derived cosmetics. The objective of FTIR characterization was to determine functional groups present in red ochre, fat and plant samples as well as to determine any spectral changes; disappearance or appearance of peaks, in the red ochre and fat mixture as compared to the pure samples. **Table 4.3** and **Table 4.4** show the IR bands observed in the spectra and peak assignments for all samples analyzed. Chromatographs were shortlisted as presented in **Figure 4.3 – 4.5** and the rest are presented in **APPENDIX C**.

4.2.1 Identification of functional groups in red ochre derived cosmetic using FTIR

The peak assignments of the bands identified using FTIR for red ochre cosmetics are summarized in **Table 4.3**. The functional groups identified for red ochre and ochre mixed with butterfat are C-H (alkanes), C=C (alkenes), O-H (hydroxyl stretches and/or alcohols), and C=O (aldehydes/ketones/carboxylic acids and/or esters).

Table 4.3: FTIR data for red ochre- derived cosmetics used by Ovahimba people

Sample description	Peak wavenumber (cm^{-1})	Vibrational motion	Functional groups
Finely ground red ochre powder	3408	O-H stretch	hydroxyl stretch
	1438	CO_3	Calcites
	1089,798 & 779	Si-O bends	Quartz /Silicates
	536	Fe-O	Hematite
Red ochre powder mixed with clarified butter (old *otjize*)	2922 & 2852	C-H	aldehyde
	1743	C=O	aldehyde, ester, and/or ketone
	1485	C-C	alkane
	1173	C-O	alcohol or ether
Clarified butterfat	2923 & 2852	C-H	aldehyde
	1744	C=O	aldehyde, ester, and/or ketone
	1465	C-C	alkane
	1173	C-O	alcohol or ether
	722	-C-H	C-H bonds
Freshly prepared red ochre and clarified butter mixture (*replicated otjize*)	2923 & 2852	C-H	aldehyde
	1744	C=O	aldehyde, ester, and/or ketone
	1465	C-C	alkane
	1106	C-O	alcohol or ether
Kudufat	2917 & 2850	C-H	aldehyde
	1742	C=O	aldehyde, ester, and/or ketone
	1465	C-C	alkane
	1160	C-O	alcohol or ether

The FTIR spectrum for red ochre powder is presented in **Figure 4.3**, displaying the characteristic line for hematite observed at 536 cm^{-1}. Most references indicate that the characteristic lines for hematite are usually observed at 560 cm^{-1} and 450 cm^{-1} (Helwig, 1998). These peaks however can shift to a lower energy by approximately 30 cm^{-1} depending on the shape and size of the hematite particles (Helwig, 1998). In this case, it can be assumed that the hematite present in the Ovahimba red ochre exhibits a range of morphology as the peak observed is between this two lines. A broad, strong peak is also observed around 1033 cm^{-1} which is due to the presence of silicates or quartz as also confirmed by the doublet just below 800 cm^{-1} at 798 cm^{-1} and 779 cm^{-1}. These peaks are usually accompanied by a single peak at 460 cm^{-1} but this peak is not appearing on the spectrograph as it fell outside the measurable range. A characteristic peaks for calcites can also be seen at 1438 cm^{-1} accompanied by a peak at ~870 cm^{-1} but this peak is relatively small in this spectrum (**Figure 4.3**).

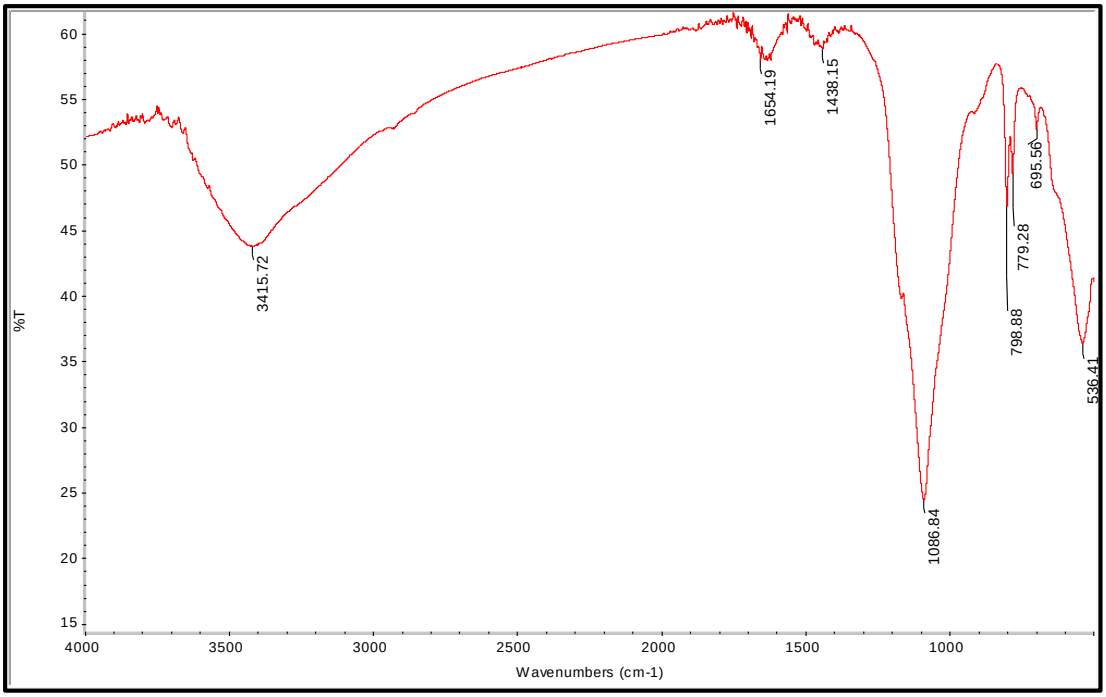


Figure 4.3: Representative transmission FTIR spectrum for red ochre from northern Namibia

It is clear that FTIR can be used as a complementary technique to XRD on investigating clay minerals. Compared to the XRD results, FTIR results obtained did not show the

presence of clay minerals kaolinite and chalconatronite. Most of the minerals absorb bands appear near 1000 cm^{-1} region, therefore if bands for the different minerals are not well resolved, they can overlap and characteristic peaks for other minerals may not be observed if present in low quantities (Djomgoue & Njopwouo, 2013). On the other hand, the use of KBr pellet technique is not suitable for mixture containing kaolinite as the grinding of the sample can have severe irreversible changes to the hydroxyl intensities due to the interaction between KBr and kaolinite and due to these hydroxyl intensity changes, the structural information of kaolinite can be lost (Bell, Citro & Hodge, 1991).

A closer inspection of the **Figure 4.4** which shows the FTIR spectrograph containing both spectra for Ovahimba red ochre, clarified butterfat and *otjize* show some intriguing changes.

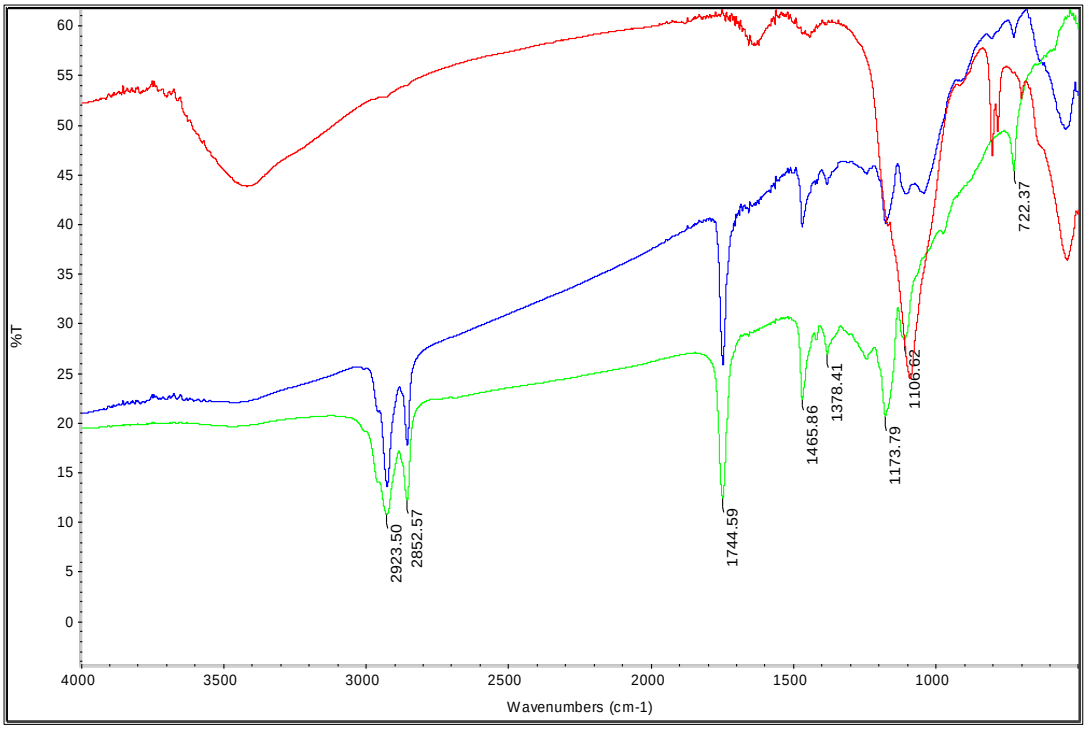


Figure 4.4: Representative transmission FTIR spectrum containing both spectra for Ovahimba red ochre (in red), clarified butterfat (in green) and otjize (in blue)

Although the hematite peak is retained at 530-540 cm^{-1} for all samples, changes were detected on the vibrational peaks for organic groups. Whereas there are no significant changes observed between the spectra for clarified butter and *otjize* except for minimal

peak shifts, the spectrum of *otjize* on the other hand shows clear differences from spectrum of Ovahimba red ochre. The O-H band as well as the sharp inorganic band which was observed at 1066 cm^{-1} from red ochre seems to have disappeared when ochre was mixed with butterfat. This could indicate some form of chemical reaction between the interaction of red ochre and clarified butterfat. The absence of a band at ~3125 cm^{-1} for both butterfat and *otjize*, shows the replacement of a hydrogen bond at a double bond or polymerization which gives rise to aldehydes and ketones shown by appearance of a peak at ~1745 cm^{-1} (Shahidi & Zhong, 2005). There was no difference observed between the peaks observed for kudufat and kudufat mixed with red ochre except the appearance of the hematite peak in the mixture (**Figure C1**). In general, FTIR analysis for both fats shows a similar trend showing groups with carbonyl functionalities (**Table 4.3**).

4.2.2 Identification of functional groups in plant derived cosmetics using FTIR

The spectra analysis of plant materials used by Ovahimba tribe revealed peaks corresponding to functional groups C-H (aromatics), C=C (alkenes), O-H (alcohols), and C=O (aldehydes/ketones/carboxylic acids) as presented in **Table 4.4**. Plants release volatile compounds as a way of attracting pollinators and repelling herbivorous insects. The volatile compounds released by plants that have the ability to repel insects include compounds such as alkaloids, terpenoids, phenolics and flavonoids (Herrman, 2011). The functional groups present in plant materials (**Table 4.4**) can be linked with terpenes and saponins characterized with long chain unsaturated hydrocarbon, poly-hydroxyl alcohols, esters and aromatic rings (Heinrich *et al*., 2005; Gurib-Fakim, 2006).

In the FTIR spectrum of a mixture of *otjize* and *otjizumba*, the absorption band from (a) 3500-3200 cm^{-1} zone showed the presence of O-H stretch, H-bonded for alcohol and phenol at 3431 cm^{-1}, (b) 3200-2700 cm^{-1} zone indicated the presence of C-H, aldehydes and alkyl CH_3 at 2920 & 2820 cm^{-1}, (c) at 2300-2000 cm^{-1} zone, no peaks were observed indicating the absence of C≡C or C≡N, (d) at 1850-1650 cm^{-1}, presence of peaks indicated presence of C=O, ketones, aldehydes and/or esters at 1742 cm^{-1} and (e) 1680-1450 cm^{-1} zone, the presence of a peak at ~1600 cm^{-1} showed the presence of alkenes (**Figure 4.5**).

Table 4.4: FTIR data for aromatic plant derived cosmetics used by Ovahimba people

Sample description	Peak wavenumber (cm^{-1})	Vibrational motion	Functional groups
Aromatic sample mixed with clarified butter (*otjizumba*)	2923 & 2852	C-H	aldehyde
	1744	C=O	ketone, aldehyde, and/or ester
	1466	C-C	alkane
	1173	C-O	alcohol or ether
Ochre and aromatic mixture from ochre container (*otjize* mixed with *otjizumba*)	3431	O-H	alcohol
	2920 & 2820	C-H	aldehyde
	1742	C=O	ketone, aldehyde, and/or ester
	1617	C=C	alkene
	1433	C-C	alkane
	1173	C-O	alcohol or ether
	1089,797 & 778	Si-O bends	Quartz /Silicates
	525	Fe-O	Hematite
Aromatic seeds	1745	C=O	ketone, aldehyde, and/or ester
	1617	C=C	alkene
	1465	C-C	alkane
Uncharred aromatic wood and bark fragments	3432	O-H	alcohol
	1617	C=C	alkene
	1437	C-C	alkane
Composite black ground aromatic powder	3432	O-H	alcohol
	2919 &2890	C-H	aldehyde
	1745	C=O	ketone, aldehyde, and/or ester
	1617	C=C	alkene
	1437	C-C	alkane

The spectral analysis also reveals the sample contains carbonyl and hydroxyl functional groups which can also be attributed to the addition of clarified butterfat used as an organic binder which may contain numerous fatty acids.

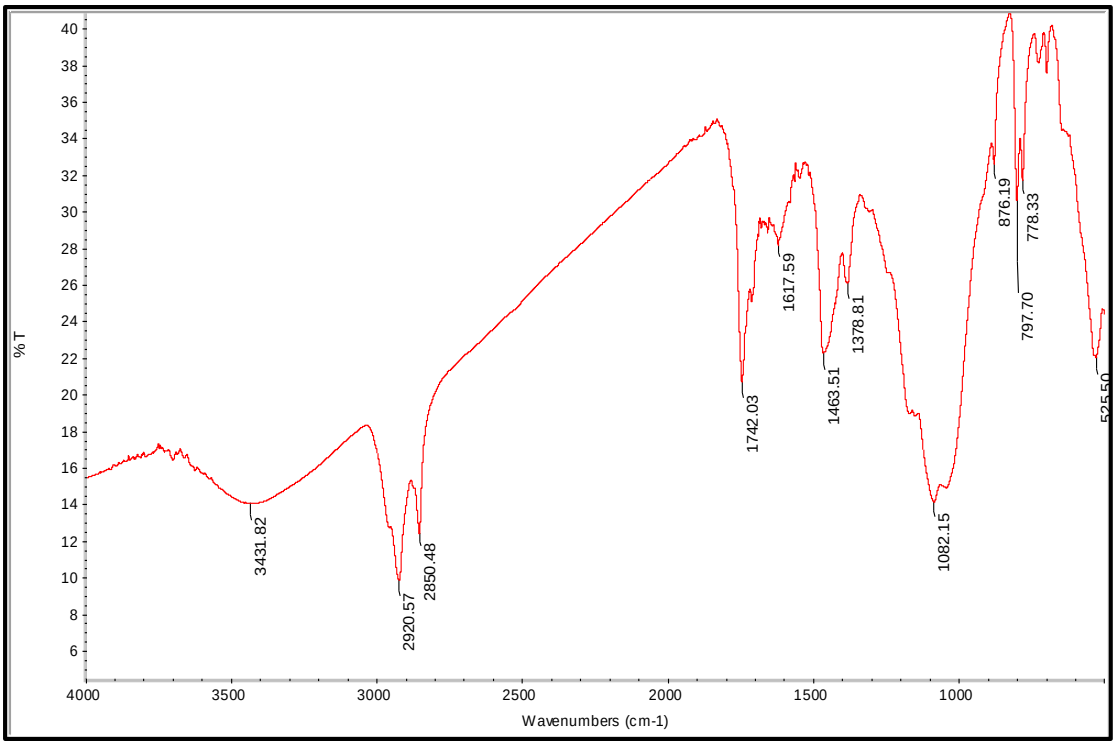


Figure 4.5: Representative transmission FTIR spectrum of a mixture of *otjize* and *otjizumba*

4.3 GC-FID analysis of red ochre derived cosmetics

The UAE extracts of red ochre and fat samples were analyzed using GC-FID as described in Chapter 3 (section 3.7.2.2). This was done to determine peak distribution patterns in order to obtain additional information about the interaction of red ochre and fat samples. GC-FID analysis is essential for indicating presence of volatile and semi-volatile compounds in a mixture. GC-FID chromatograms are presented below in **Figure 4.6-4.10**.

Figure 4.6 show chromatogram for DCM as was chosen a suitable extraction solvent and was run as a blank for the GC-FID analyisis. The signal for DCM can be seen at about 1.2 to 2.2 min accompanied by predominant peaks at 11.8 and 14.5 min and less intense peak is observed at 12.8 min. Some of the signals observed could be attributed to contamination from the instrument and possibly from the solvent as well.

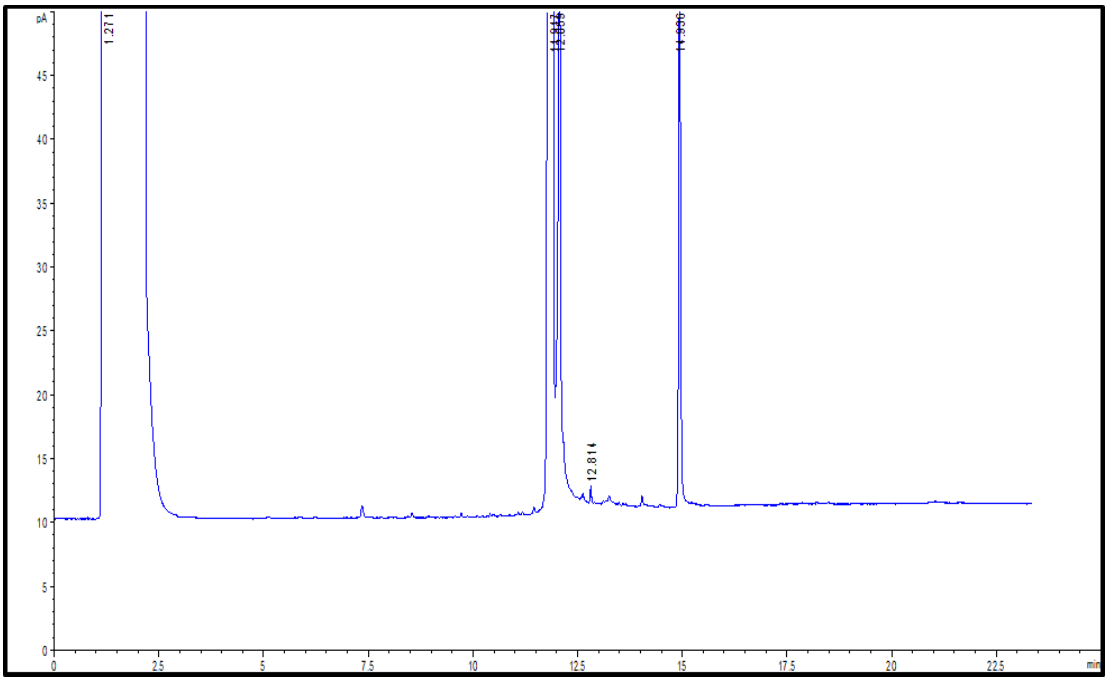


Figure 4.6: Chromatogram of pure DCM (blank)

As observed in **Figure 4.7**, ochre as an inorganic pigment is expected to display fewer numbers of volatile organic compounds. The chromatogram for red ochre displayed weak peaks between 7 to 14 min indicating the presence of organic materials which could a result of contamination during processing by Ovahimba people especially during grinding of the ochre. The signal for DCM which was observed between 11 to 14 min in **Figure 4.6** appears to be suppressed.

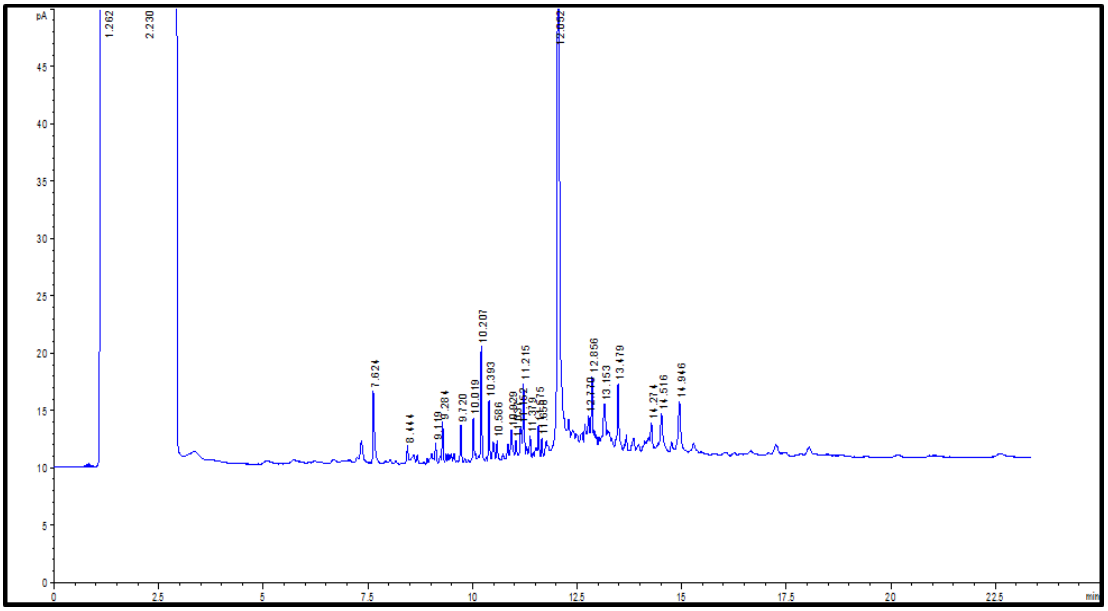


Figure 4.7: Peak distribution for compounds in DCM extracts of Ovahimba red ochre analyzed by GC-FID

Figure 4.8 shows peak distribution for clarified butterfat and replicated *otjize* detected by GC-FID which shows some differences in patterns for the two samples.

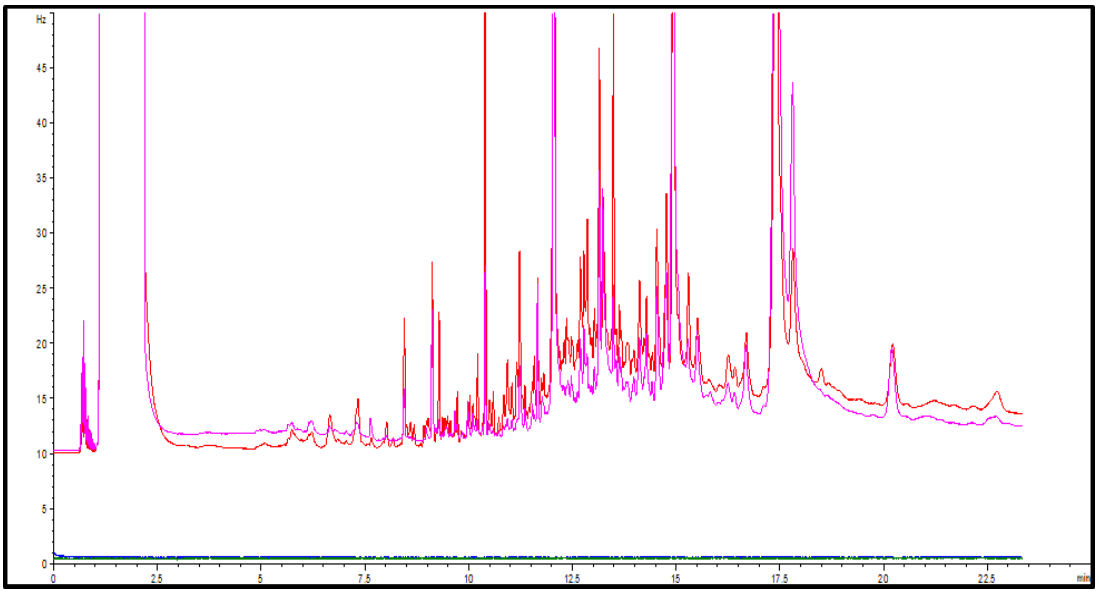


Figure 4.8: Peak patterns for compounds in DCM extracts of Clarified butter (in pink) and replicated *otjize* (in red) analyzed by GC-FID

As expected, GC-FID results from samples containing fats show that many volatile compounds were detected besides the targeted compounds as identified by GC-MS. Although very few new peaks can be observed in the replicated *otjize* which do not appear in the butterfat sample, most peaks in replicated *otjize* show an enhanced signal as observed by an increase in peak heights of numerous peaks. This could indicate that the interaction of clarified butterfat and red ochre either give rise to new volatile compounds as well as increasing the yield of compounds already present in clarified butterfat.

Kudufat was used as control as opposed to using clarified butterfat and **Figure 4.9** shows the signals observed for kudufat and for a mixture prepared using kudufat as a binder.

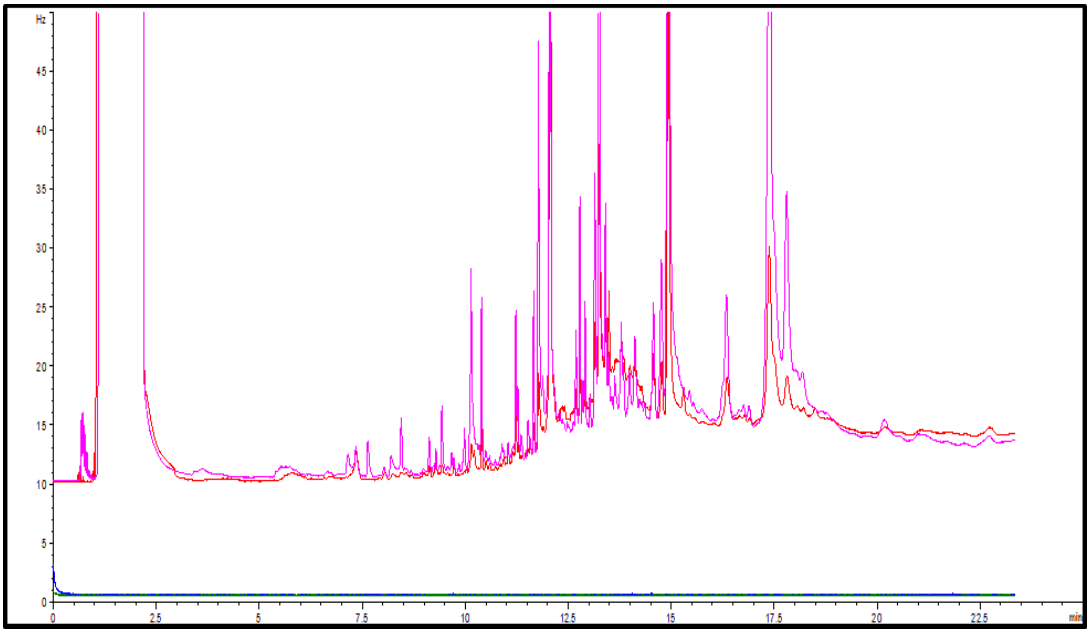


Figure 4.9: Peak patterns for compounds in DCM extracts of kudufat (in pink) and kudufat and red ochre mixture (in red) analyzed by GC-FID

Numerous peaks were also detected for kudufat showing that it does contain volatile compounds, but unlike the interaction of clarified butterfat with red ochre, the mixture of red ochre with kudufat shows very minimal difference between peak patterns of the mixture compared to the signal produced by kudufat alone, suggesting that the

interaction of kudufat and red ochre may not elicit similar effect as compared to the interaction of clarified butter and red ochre.

Figure 4.10 shows peak patterns for the *otjize* mixture that was prepared by the Ovahimba women of Okamanga village.

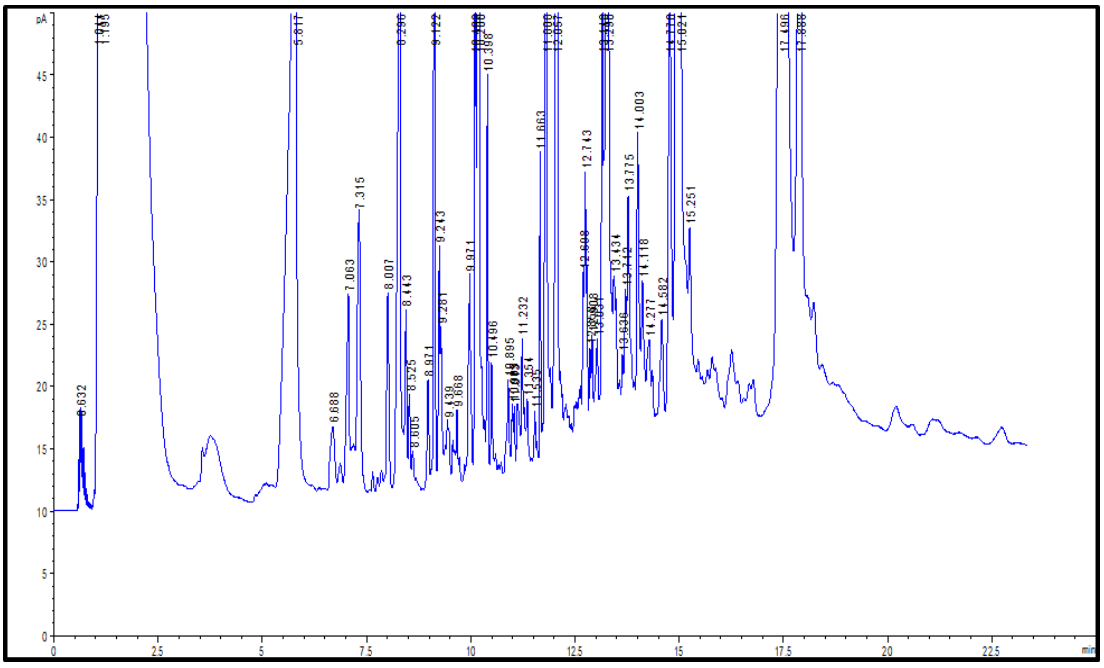


Figure 4.10: Peak patterns for compounds in old red ochre and butterfat mixture (*otjize* prepared by the Ovahimba people)

The old *otjize* mixture showed a strong signal as observed by more peaks with large peak heights compared to the replicated *otjize* seen previously in **Figure 4.9**, suggesting that the release of the volatile compounds could depend on the age of the mixture. The release of more volatiles in an older *otjize* mixture could also be influenced by exposure to environmental conditions such as light and temperature.

4. 4 Determination of mosquito repellent compounds using GC-MS

The application of *otjize* by the Ovahimba tribe maybe viewed predominantly as a symbolic body cosmetic, as “ideal sign of beauty”, but ethnographical accounts have

revealed its use as an insect repellent. Oral testimonies have indicated that the use of red ochre without a binder or butterfat alone elicits no protection against mosquito bites. In an arm in a cage experiment conducted by Rifkin (2015), a similar observation was made whereby replicated *otjize* samples exhibited higher repellency indices compared to ochre powder alone or ochre powder mixed with animal fat. In his study, he hypothesized that both chemical and mechanical repellency capacity could be implicated as mode of action of *otjize*. He suggested that the primary mechanism could be the formation of a structural barrier preventing the insertion of the fascicle into the stratum comeum and the epidermis because less bites were reported for *otjize* treatment suggesting that *otjize* prevented mosquitoes from biting its host again. In this study we propose that chemical repellency capacity is most likely to be the primary mode of action displayed by *otjize* not suggesting that we disregard the mechanical capacity.

4.4.1 Mosquito repellent compounds identified from red ochre derived cosmetics

The GC-MS method described in section 3.7.2.3, was used for identification of compounds in DCM extracts of red ochre-derived cosmetic. Various volatiles have been identified to have mosquito repellent capabilities which include methyl ketones, long chain aldehydes, alcohols and monoterpenes. Red ochre-derived cosmetic was observed to contain numerous compounds identified as mosquito repellents as presented in **Table 4.5** with their molecular structures presented in **Table 4.6.** The complete data set acquired by GC-MS which include the chromatograms for the identified compounds for each sample is presented in **APPENDIX E**.

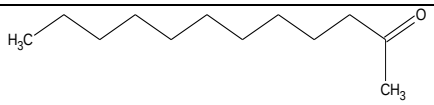
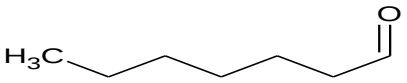
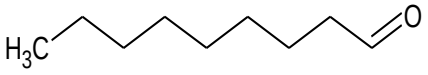
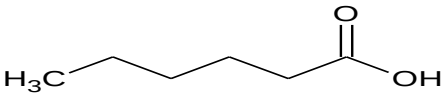
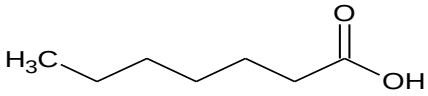
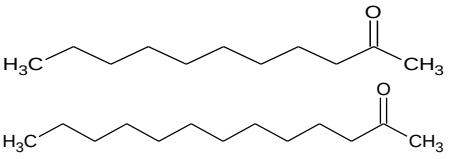
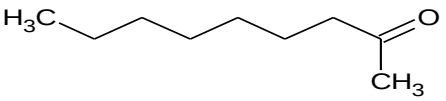
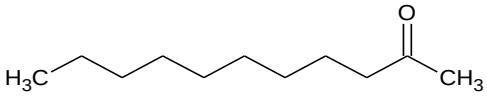
Table 4.5: Possible mosquito repellent compounds identified using GC-MS from red ochre-derived cosmetic used by Ovahimba tribe

Ochre samples	Compounds identified	Molecular formula	Similarity (%)
Finely ground red ochre powder	-	-	-
Old *otjize*	2-Dodecanone	$C_{12}H_{24}O$	87.0
	Heptanal	$C_7H_{14}O$	94.1
	Nonanal	$C_9H_{18}O$	94.2
	Heptanoic acid	$C_7H_{14}O_2$	95.4
Clarified butterfat	2-Dodecanone	$C_{12}H_{24}O$	83.2
	2-Nanonone	$C_9H_{18}O$	89.2
	2-Undecanone	$C_{11}H_{22}O$	83.9
	Heptanal	$C_7H_{14}O$	94.1
	Nonanal	$C_9H_{18}O$	94.0
	Heptanoic acid	$C_7H_{14}O_2$	94.3
Replicated *otjize*	2-Dodecanone	$C_{12}H_{24}O$	83.7
	2-Nonanone	$C_9H_{18}O$	90.6
	2-Undecanone	$C_{11}H_{22}O$	85.6
	2-Tridecanone	$C_{13}H_{26}O$	87.8
	Heptanal	$C_7H_{14}O$	95.5
	Nonanal	$C_9H_{18}O$	93.5
	Heptanoic acid	$C_7H_{14}O_2$	82.0
Kudufat	-	-	-
Red ochre and kudufat mixture	-	-	-

**All of the compounds identified showed similarity percentages higher than 80% when compared to the library*

All the compounds identified in red ochre cosmetics are oxygenated hydrocarbons belonging to functional groups aldehydes, ketones and carboxylic acids.

Table 4.6: Molecular structure of identified mosquito repellent compounds from red ochre-derived cosmetic

Compounds identified	Molecular structure
2-Dodecanone	
Heptanal	
Nonanal	
Hexanoic acid	
Heptanoic acid	
2-Tridecanone	
2-Nonanone	
2-Undecanone	

Previous studies have shown that certain long chain aliphatic methyl ketones, aldehydes, and esters showed repellence to blood sucking insects including mosquitoes (Innocent *et al.*, 2008) and this information is supported by olfactometer experiments using *Ae. aegypti* conducted by Logan *et al.* (2010), which showed that octanal, nonanal, decanal, 6-methyl- 5-hepten-2-one and geranylacetone significantly 'interfered' with host-location. Both aliphatic aldehydes and ketones are known to originate from the oxidation and peroxidation of unsaturated fatty acids (Lea & Piggott, 2012). Aliphatic methyl ketone series with odd carbon atoms including 2-undecanone, 2-tridecanone and 2-pentadecanone were observed by Innocent *et al*. (2008), to be more effective as mosquito repellents than compounds with even carbon atoms such as 2-decanone and 2-dodecanone with 2-tridecanone providing protection efficacy comparable to DEET. Aliphatic methyl ketones with odd carbon atoms including 2-nanonone, 2-undecanone,

and 2-tridecanone as well as aldehydes; heptanal and nonanal were identified in this study using GC-MS based on their high similarity percentages with the library (**Table 4.5** and **Appendix E**). Hexanoic acid and heptanoic acid were also identified which has been documented as one of the carboxylic acids which exhibit high mosquito repellency (Skinner *et al*., 1965; Bernier, Kline & Posey, 2006). Some studies have highlighted the significance of oxygen particularly the presence of hydroxyl –OH group in influencing repellent-receptor interaction resulting in repellency (Fabbro & Nazzi, 2013). The presence of these compounds suggests that *otjize* could provide effective protection against mosquito as the amount of the diversity of these compounds in a mixture could result in high ability and efficacy of these compounds to prevention host recognition by mosquito. Identified compounds are characterized by the presence of long chain hydrocarbon and a carbonyl group and this in agreement with the functional groups determined by FTIR analysis in section 4.2. Ovahimba people mix red ochre with clarified butterfat which they produce through a process which involves heating the milk to remove water and milk solids from the butter to remain with a clear and pure butterfat. This heating process however makes clarified butterfat to be more susceptible to lipid oxidation (Pankaj, 2013). This phenomena causes clarified butterfat to undergo oxidative deterioration which cause rancidity, mainly due to the oxidation of unsaturated glycerides leading to the development of peroxides as well as hydrolysis of glycerides resulting in increased levels of free fatty acids (Kumar *et al*., 2002). The mechanism by which these compounds are formed in heated milk products including butterfat is not well documented but many authors suggest that they arise from autoxidation of lipids. Autoxidation is a non-enzymatic lipid peroxidation in which one free radical induces the oxidation of polyunsaturated fatty acids (Repetto & Boveris, 2012). Autoxidation products formed from unsaturated fatty acids by non-enzymatic autocatalytic oxidation reaction include in the formation of hydroperoxides and these hydroperoxides often dismutate to secondary oxidation products such as aldehydes and ketones. These aldehydes and ketones are responsible for the development of stale off-favour during storage of in heated milk products (Kumar *et al*., 2002). In food production, this is a huge problem as it reduces the life span and quality of clarified butter drastically. However in this case this phenomenon is beneficial as this promotes the release of ketones and aldehydes responsible for insect repellency. Autoxidation is a complex process but it is believed to take place in three steps. The initiation step involves the abstraction of hydrogen from fatty acid reacting with an initiator to form lipid alkyl

radicals (Repetto & Boveris, 2012; Endalew & Kiros, 2014). If the hydrogen atom is adjacent to the double bond it is removed easily while temperature, metal catalysts, ultraviolet and visible light can speed up free radical formation (Min & Boff, 2002). Radicals formed react with other fatty acids to propagate a chain reaction. The peroxide formed during the propagation step is unstable and spontaneously goes to the termination step where acids, alcohols, aldehydes and/or ketones are considered the final products (Endalew & Kiros, 2014). This mechanism is illustrated by the general equations below as suggested by Repetto & Boveris (2012), which involves the formation of hydroperoxide from fatty acids which then decompose to form aldehydes and ketones.

(1) **Initiation step**

$RH \rightarrow R^{\bullet}$ and $H^{\bullet}$ (free radicals) ***(4.1)***

This reaction can be catalyzed by heat, light or metals

(2) **Propagation step**

Molecular oxygen rapidly adds to the carbon-centred radical ($R^{\cdot}$) formed in equation (1), yielding the lipid peroxyl radical (ROO)

$R^{\bullet} + O_2 \rightarrow ROO^{\bullet}$ ***(4.2)***

The formation of peroxyl radicals leads to the production of organic hydroperoxides, which, in turn, can abstract hydrogen from another polyunsaturated fatty acid

$ROO^{\bullet} + RH \rightarrow ROOH + R^{\bullet}$ ***(4.3)***

(3) **Termination step**

Lipid hydroperoxide (ROOH) is the first stable product of the lipid peroxidation reaction. Under conditions where lipid peroxidation is continuously initiated, radical termination occurs, destroying two radicals at once:

$ROO^{\bullet} + ROO^{\bullet} \rightarrow ROH + RO^{\bullet} + {}^{1}O_2$ ***(4.4)***

Transition metal ions trigger autoxidation of unsaturated acyl lipids only in the presence of hydroperoxides. Metal ions are involved in the decomposition of initially formed hydroperoxides (equation ***(4.3)***), into radicals which then propels the radical chain reaction (Belitz *et al*., 2004). ROOH gives rise to the generation of radicals capable of re-initiating the lipid peroxidation by redox-cycling of the metal ions (Repetto *et al*., 2010; Repetto & Boveris, 2012):

$$ROOH + M^{n+} \rightarrow RO^{\bullet} + M^{(n-1)+} \qquad \textbf{(4.5)}$$

$$ROOH + M^{(n-1)+} \rightarrow ROO^{\bullet} + M^{n+} \qquad \textbf{(4.6)}$$

Metals increase the rate of lipid oxidation by reducing the activation of the initiation step in autoxidation (Choe & Min, 2006). They react directly with lipids to produce alkyl radicals and also produce reactive oxygen species and hydroxy radical from hydrogen peroxide (Lee, Koo & Min, 2004). Transition metal ions Fe^{2+} and Cu^{+} stimulate lipid peroxidation through reductive cleavage of hydroperoxides (ROOH) to form corresponding alkoxyl ($RO^{\cdot}$) and peroxyl ($ROO^{\cdot}$) radicals (Repetto *et al.*, 2012). The decomposition of the hydroperoxide can result in the formation of aldehydes and ketones.

Most of the identified aldehydes and ketones with possible mosquito repellent activity were present in both clarified butterfat and in *otjize* except 2-tridecanone which was only observed in replicated *otjize*. This could suggest that the interaction of butterfat with red ochre results in the release of new compounds with mosquito repellency capacity as supported by peak pattern differences shown by the GC-FID data which showed the emergence of new peaks in the replicated *otjize*. The mechanistic details of what could be happening at this stage may still be elusive but it can be hypothesized that it is likely that new compounds as observed with 2-tridecanone are synthesized probably from the autoxidation of fatty acids catalyzed by metal oxides from red ochre.

4.4.1.1 The involvement of red ochre in the release of mosquito repellents in red ochre derived cosmetic

There is a list of catalysts employed in synthesis of aldehydes and ketones during autoxidation which may include but not limited to metal oxides or carbonates including the alkaline earth metals Mg, Ca, and Ba on silica and carbon, the rare-earth element Th and U, as well as the transition metals Fe, Mn, V, Ti, Zr, Ni, Co, and Cu (Jackson & Cermak, 2013). The presence of these metal oxides and carbonates can speed up the rate of autoxidation process and results in higher yields of these secondary products. Therefore, it can be speculated that due to its metal content as shown by elemental analysis using EDXRF and ICP-OES, specifically the presence of transition metals; Fe, Cu, V, Ti, and Zr, red ochre could be serving as a potent heterogeneous catalyst which could be encouraging the release of secondary products of lipid oxidation including those compounds with possible mosquito repellent activities in *otjize*. Concentration limits for Cu and Fe in fat with high content of oleic acid such as butterfat are 0.2 ppm and 2 ppm respectively (Belitz *et al*., 2014). Concentrations higher than the limits will make the fat susceptible to lipid peroxidation. As observed with the red ochre powder, concentrations of Fe and Cu are higher than the concentration limits suggesting that they can be responsible in the autoxidation of lipids in *otjize*. The presence of kaolinite in the ochre could also assist in increasing the protection efficacy of the replicated *otjize* against mosquito bites, as kaolin clay is a well-known insect repellent which function by forming a barrier on a surface (in this case the skin) making the conditions unsuitable for the insect `s feeding (Liang & Liu, 2002).

Numerous other compounds identified by GC-MS (see **APPENDIX E**), could include esterified glycerides which are precursors of methyl ketones synthesis. Oleic acid was observed as one of the fatty acid present in clarified butterfat produced by the Ovahimba people. Nonanal which was identified as one of mosquito repellent compounds in *otjize* (**Table 4.5**), is a secondary product of autoxidation of fatty acid methyl ester of oleic acid (Choe & Min, 2006). It is worth noting that oxidation of polyunsaturated fatty acids (PUFA) can take place in the absence of metal catalyst but the process is usually slow and results in low yield of secondary products such as ketones and aldehydes. As previously observed in the GC-FID peak patterns in **Figure 4.7**, there is an increase in the peak intensities seen for replicated *otjize* compared to peaks in the butterfat alone, which could suggest that there is an enhancement in the

quantities of the compounds released when red ochre is present. Therefore, the presence of mosquito repellent compounds in clarified butterfat alone should not come as a surprise, as it can be assumed that the presence red ochre results in production of higher yield of these compounds in the mixture, resulting in noticeable protection against mosquito when the mixture is applied on the skin.

4.4.1.2 Effect of age of the otjize mixture on the release of mosquito repellents

Interestingly, oral testimonies have also revealed that the Ovahimba people may apply the *otjize* twice in a day and the mixture is prepared just before application and is not kept prepared. This could suggest that the efficacy of the compounds present in a mixture could depend on the age of the mixture. As a result of their volatility and reactivity, ketones and aldehydes can be degraded to produce other compounds such as alcohols and carboxylic acids (Forney *et al*., 1967), which might explain why the old *otjize* mixture seemed to contain less of the compounds identified to have mosquito repellent activity but appeared to have a larger quantity of other volatiles during the GC-FID analysis. Unlike ketones which are more stable and less reactive, aldehydes are very unstable because they are easily oxidized to produce carboxylic acids the presence of a hydrogen atom attached to the carbon-oxygen double bond. As already mentioned, other factors such as light and temperature could also affect the release of mosquito repellents in an older *otjize* mixture.

4.4.1.3 Effect of microbes in the release of mosquito repellent in red ochre cosmetic

Methyl ketones can also be synthesized by microbes such as yeast in response to presence of short chain fatty acids. Moulds such as *Penicilium* and *Asperillius* species can produce very limited quantities of methyl ketones when grown on fatty acid glycerides at favorable temperature around 15-35^{0}C (Choe & Min, 2006). Considering that the processing of butterfat by Ovahimba people does not observe any antiseptic procedures as well as the use of no refrigeration storage conditions, in the hot climatic conditions experienced in northern Namibia, it is worth considering that yeast presence could also have an effect in production of methyl ketones in clarified butterfat used to prepared *otjize*.

4.4.2 Mosquito repellent compounds identified from aromatic plant - derived cosmetics using GC-MS

As already mentioned, Ovahimba people also applied an aromatic plant-derived cosmetic, *otjizumba* around their neck as a perfume. Most all of the plants used in the perfume industry are also used as repellents (Maia & Moore, 2011). Hence, it was worth investigating if the traditional herbs (*C. wildii* and *C. virgate*) used by Ovahimba people as "perfume" could also provide any mosquito repellency, despite the fact that most oral testimonies by Ovahimba people suggest that the protection against mosquitoes is provided by *otjize*. In Africa, several plant species are known to contain several oils which reduce mosquito biting activity when used as repellents. It is likely that they work in several ways either by reducing short range attractive cues, by reducing the evaporation and/or by absorption of repellent actives due to the presence of long-chained fatty molecules or through fatty acids which are known to be repellent to mosquitoes at high concentrations (Maia & Moore, 2011). Several compounds were identified from *otjizumba* which have been reported to have mosquito repellent capabilities (**Table 4.7**) and their molecular structures are presented in **Table 4.8**

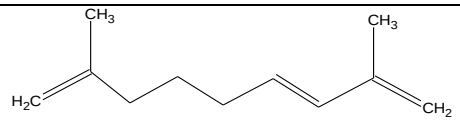
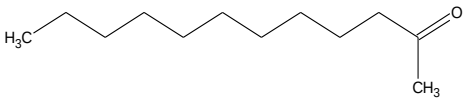
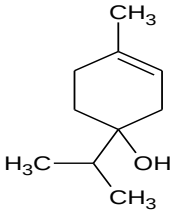
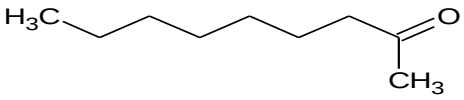
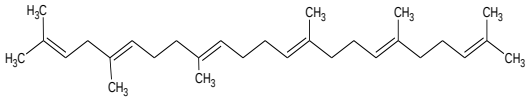
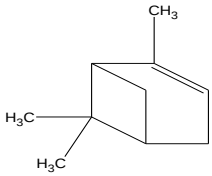
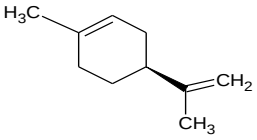
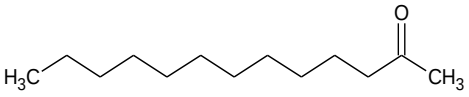
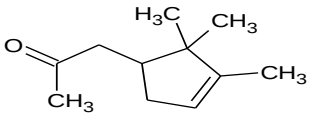
Table 4.7: Molecular structure of identified mosquito repellent compounds from aromatic plant -derived cosmetic

Samples analyzed	Compounds identified	Molecular formula	Similarity (%)
Aromatic sample mixed with clarified butter (*otjizumba*)	o-Cymene	$C_{10}H_4$	95.5
	2-Undecanone	$C_{11}H_{22}O$	88.0
	Terpinen-4-ol	$C_{10}H_{18}O$	90.2
	2-Nonanone	$C_9H_{18}O$	77.4
	α-Pinene	$C_{10}H_{16}$	95.4
	Limonene	$C_{10}H_{16}$	94.0
	Squalene	$C_{30}H_{50}$	86.9
Ochre and aromatic mixture from ochre container (*otjize* mixed with *otjizumba*)	Squalene	$C_{30}H_{50}$	75.8
	2-Tridecanone	$C_{13}H_{26}O$	89.7
	Limonene	$C_{10}H_{16}$	94.2
	α-Campholenal	$C_{10}H_{16}O$	89.4
	α-Pinene	$C_{10}H_{16}$	95.7
Aromatic seeds from traditional ochre container	Squalene	$C_{30}H_{50}$	71.6
Uncharred aromatic wood and bark fragments	Limonene	$C_{10}H_{16}$	93.6
	α-Pinene	$C_{10}H_{16}$	95.2
	Squalene	$C_{30}H_{50}$	75.8
	o-Cymene	$C_{10}H_4$	95.5
Composite black ground aromatic powder	Limonene	$C_{10}H_{16}$	93.1

**All of the compounds identified showed similarity percentages higher than 80% when compared to the library*

Major mosquito repellents identified using GC-MS in the plant parts used by Ovahimba people contained compounds with a variety of functional groups including hydrocarbons; o-cymene, à-pinene, limonene, squalene and oxygenated compounds; terpinen-4-ol and à-campholenal which have been suggested to show strong repellent activities (Park *et al.*, 2005).

Table 4.8: Molecular structures of identified mosquito repellent compounds from aromatic plant-derived cosmetics

Compounds identified	Molecular structure
o-Cymene	
2-Undecanone	
Terpinen-4-ol	
2-Nonanone	
Squalene	
α-Pinene	
Limonene	
2-Tridecanone	
α-Campholenal	

Currently, the use of synthetic chemicals to control insects and arthropods raises several apprehensions related to environment and human health (Nerio *et al.*, 2010). Therefore, numerous essential oils from several plants species have been extensively

tested to assess their repellent properties. Most essential oils with repellent activities contain volatile mixtures of hydrocarbons with a variety of functional groups. These include monoterpenes and sesquiterpenes (Nerio *et al.*, 2010). Monoterpenes such as α-pinene, cineole, eugenol, limonene, terpinolene, citronellal, camphor and thymol are common constituents of a number of essential oils as presenting mosquito repellent activity as described in the literature (Park *et al.* 2005; Sasidharan *et al.*, 2011; Kalita *et al.*, 2013). Plants used by the Ovahimba people of Namibia have never been evaluated chemically despite being extensively used traditionally as a perfume and insect repellents.

The use of other natural products e.g. organic binders, to produce a mixture could increase the protection time, intensifying the repellent effect of some essential oils. Hence the use of clarified butterfat to make *otjizumba* could strengthen the repellent effect for the compounds in the herbs used. Although both red ochre derived and aromatic plant derived cosmetics contain compounds with mosquito repellent capabilities, mosquito repellent compounds in plant cosmetic are observed to mainly comprise of hydrocarbons while otjize contain mostly oxygenated compounds. It could be possible that the mosquito repellents from otjize and otjizumba work in synergy to provide maximum protection against mosquito bites when the two cosmetics are applied together.

CHAPTER FIVE: CONCLUSIONS AND FUTUREWORK

This chapter summarizes the findings of this study on the characterization of traditional cosmetics used the Ovahimba tribe on their mosquito repellent capabilities. Future research areas identified for further investigations are also included in this chapter.

5.1 Conclusions

The primary aim of this study has been to use analytical techniques to characterize traditional cosmetics used by the Ovahimba tribe in order to develop a better understanding of their use as mosquito repellents. Investigations carried out in this study have shown that traditional cosmetics used by the Ovahimba tribe consist of compounds identified by previous studies to have mosquito repellent capabilities. Red ochre derived cosmetic , which is prepared by mixing red ochre with clarified butterfat contained ketones including 2-dodecanone, 2-nanonone, 2-undecanone, 2-tridecanone and aldehydes; heptanal and nonanal and carboxylic acid; hexanoic acid and heptanoic acid. Although some of these compounds were also observed in the clarified butterfat not mixed with ochre, 2- tridecanone was observed only in the mixture but not in the butterfat, suggesting that new compounds could be released by the interaction of clarified butterfat and red ochre powder. Possible mechanism suggested is that the presence of transition metals in red ochre including most importantly the high content of Fe, Cu and others such as V, Ti, and Zr as determined using XRF and ICP-OES could be catalyzing the autoxidation process of the polyunsaturated fatty acids in the butterfat resulting in the synthesis of high yields of ketones and aldehydes mentioned above, identified as possible mosquito repellents. Red ochre which has an intense red hue due to the presence of hematite (Fe_2O_3) as its principal mineral may have been predominantly used as a pigment but this study has shown that its chemical properties may also support its other utilitarian functions.

The study also investigated presence of mosquito repellent compounds from the plant materials used as a perfume by the Ovahimba people. It was observed that the perfume which is mainly prepared from plant parts of *C. wildii* and *C. virgate* had hydrocarbons; o-cymene, α-pinene, limonene, squalene and oxygenated compounds which include terpinen-4-ol and α-campholenal which are considered to be the major common constituents of a number of essential oils found in plants presenting mosquito repellent activity described in the literature. 2-nanonone, 2-undecanone, 2-tridecanone were also identified in the mixture of perfume sample which is prepared by mixing the herbs with clarified butterfat hence as previously discussed these compounds could be as a results of autoxidation of FFA in the butterfat.

The study was able to support the hypothesis that the mode of repellency provided by the red ochre and butterfat mixture is through the release of chemical compounds with mosquito repellents capabilities. The study showed that both *otjizumba* and *otjize* contain mosquito repellent compounds but were observed to be generally different in their structural compositions. The study has shown that indigenous knowledge system can prove valuable in providing information for development of improved scientific formulations. Therefore, it is anticipated that this study can serve as a useful starting point in promoting research aimed at screening for natural sources for new agents for mosquito control based on bioactive compounds from traditional and indigenous sources. It is also expected that the study will help the Ovahimba tribe to preserve its cultural heritage by providing scientific evidence that their body painting practice with red ochre mixture certainly does have utilitarian functionalities to complement the symbolic uses.

5.2 Future work

Further investigations are required to fully understand the mechanism involved in the interaction of clarified butterfat and red ochre to release compounds with possible mosquito repellent capabilities. Free fatty acid (FFA) content and peroxide value (PV) of freshly prepared and stored clarified butterfat prepared by the Ovahimba people should be determined in order to identify the presence and quantity of precursors that could be responsible for the synthesis of aldehydes and ketones with possible mosquito repellent activity. Also FFA composition and peroxide value of kudufat should be determined as well in order to clarify that the difference in the presence of free fatty acids and primary products of lipid oxidation in the two fats could be what contribute to mixture with clarified butter as a binder having possible mosquito repellents than when kudufat is used as an organic binder. Effects of other factors which can influence autoxidation of lipids including pH, light and temperature should be investigated in clarified butterfat and *otjize*. Determination of presence of yeast involved in the synthesis of ketones e.g. *Penicilium* and *Asperillius* species in the clarified butterfat prepared, used and stored by the Ovahimba people can also be carried out. Compounds identified from both cosmetics can be extracted, separated and be subjected to repellent assays using the arm in a cage method for repellent assays to determine their efficacy at different

concentrations against synthetic repellents like DEET. Since the two cosmetics are applied in most cases together, it is worth determining if the combined presence of these mosquito repellents from these traditional cosmetics showed additive or synergistic effect. Considering that no toxicity studies have been conducted on dermal exposure of metals from the red ochre used by the Ovahimba people, bioavailability of metals in red ochre and clarified butterfat mixture through dermal exposure should the assessed, also toxicity studies on the plant derived cosmetics should be carried out. Further investigation is required to fully understand the role of red ochre as a catalyst in the release of mosquito repellents in *otjize.* Specific metal oxides and ions involved during the interaction should be identified e.g. ferric ion complexes measurement using colorimetric detection can be carried to monitor the oxidation of ferrous ion (Fe^{2+}) to ferric ion (Fe^{3+}).

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APPENDIX

Appendix A – Sample preparation

Table A1: Actual weights measured for UAE extractions

Samples weighted	Mass weighted (g)
Ochre powder	0.1510
Clarified butterfat	0.1511
Replicated *otjize*	0.1504/ 0.1518 (0.3022)
Old otjize	0.2792
Kudufat	0.1510
Kudufat/ochre mixture	0.1530/0.1501 (0.3031)
Perfume	0.1501
Otjize & *otjizumba* mixture	0.1508
Perfume powder	0.1523
Uncharred powder	0.1514

Appendix B: EDXRF results

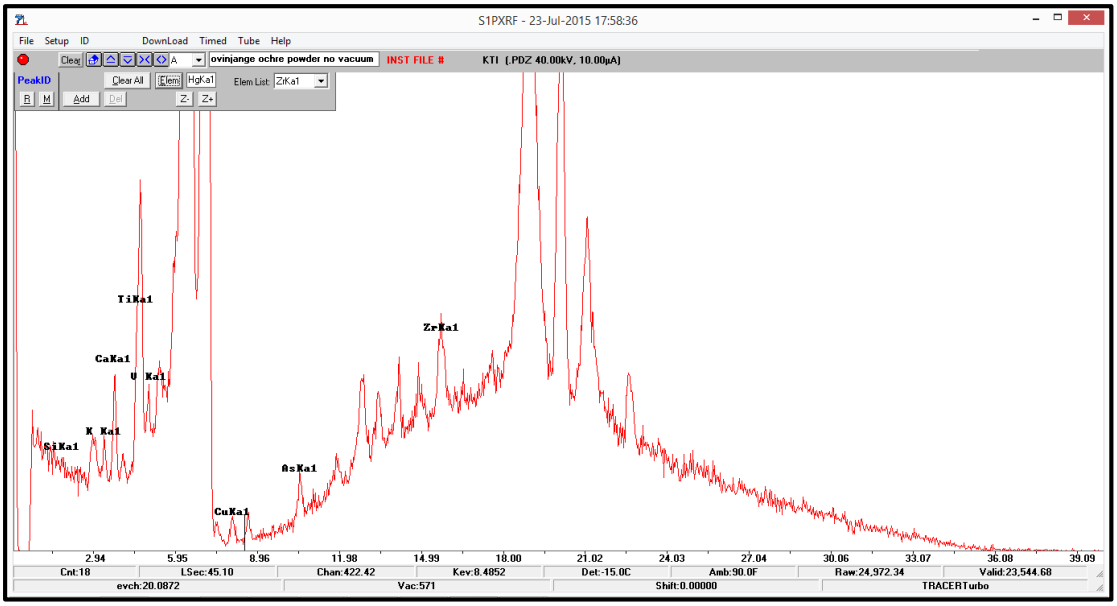

Figure B1: EDXRF spectrogram for Ovahimba red ochre

Table B1: Chan-counts for elements identified by EDXRF in Ovahimba red ochre

Identified elements	Chan- counts
SiKa1	750.20
K Ka1	942.23
CaKa1	1433.95
TiKa1	2325.32
V Ka1	1532.30
FeKa1	660167.67
CuKa1	313.58
ZrKa1	3354.14
AsKa1	837.61
CMPT	38.62

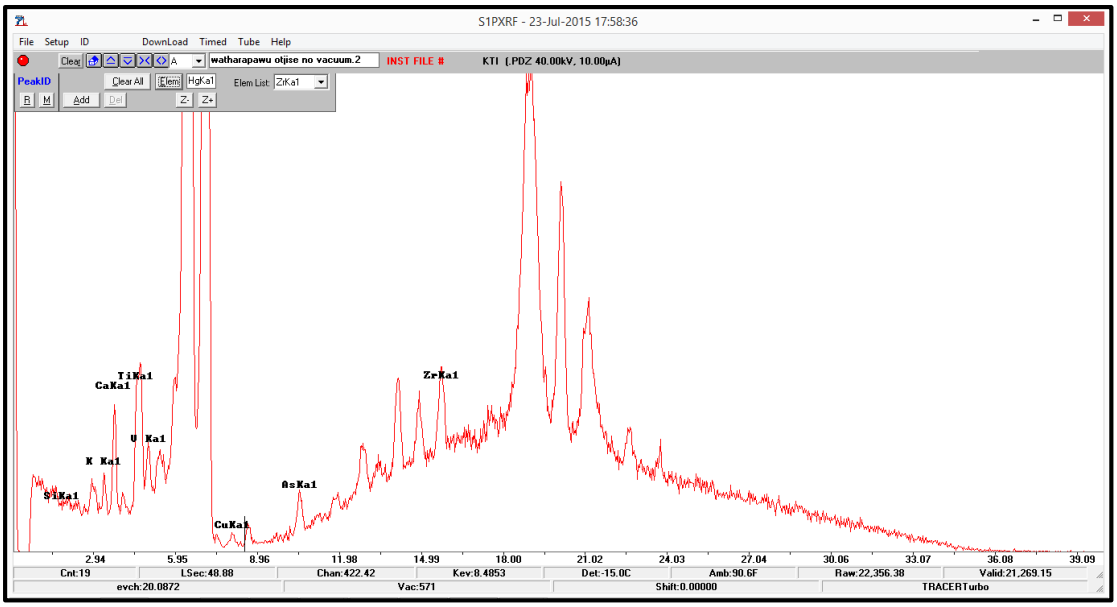

Figure B2: EDXRF spectrogram for otjize

Table B2: Chan-counts for elements identified by EDXRF in otjize

Identified elements	Chan-counts
SiKa1	740.34
K Ka1	1087.79
CaKa1	2017.75
TiKa1	2760.87
V Ka1	1747.57
FeKa1	569603.71
CuKa1	325.32
AsKa1	1189.05
ZrKa1	4500.83
CMPT	40.47

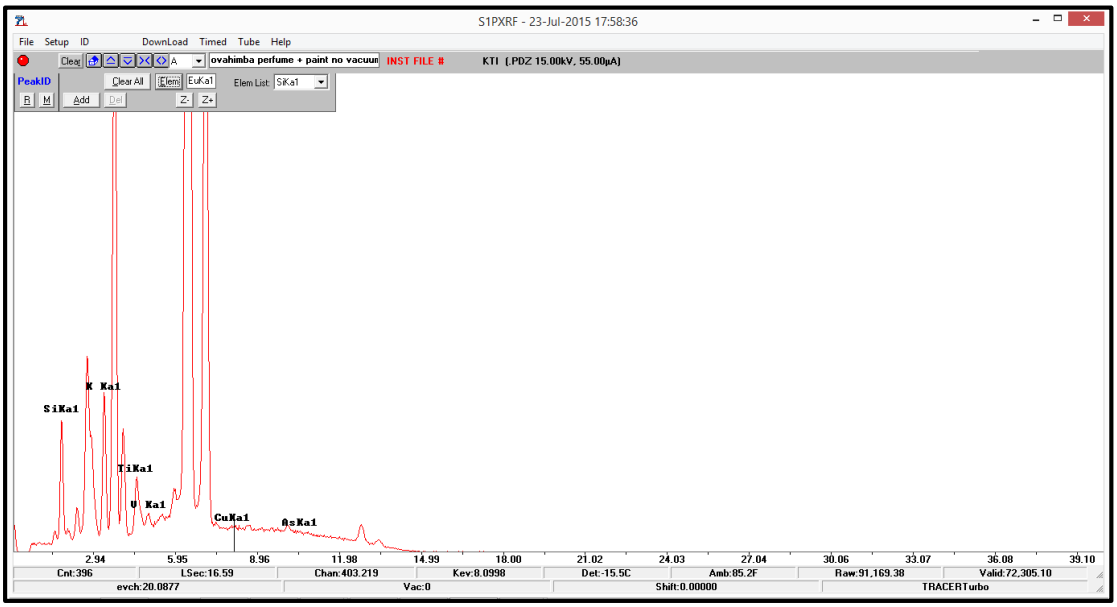

Figure B3: EDXRF spectrogram for Ovahimba perfume and paint

Table B3: Chan-counts for elements identified by EDXRF in Ovahimba perfume and paint

Identified elements	Chan-Counts
SiKa1	12277.34
K Ka1	16726.92
CaKa1	76447.09
TiKa1	9292.80
V Ka1	5446.78
FeKa1	595015.28
CuKa1	4490.34
AsKa1	3939.44
CMPT	190.37

Appendix C - FTIR analysis

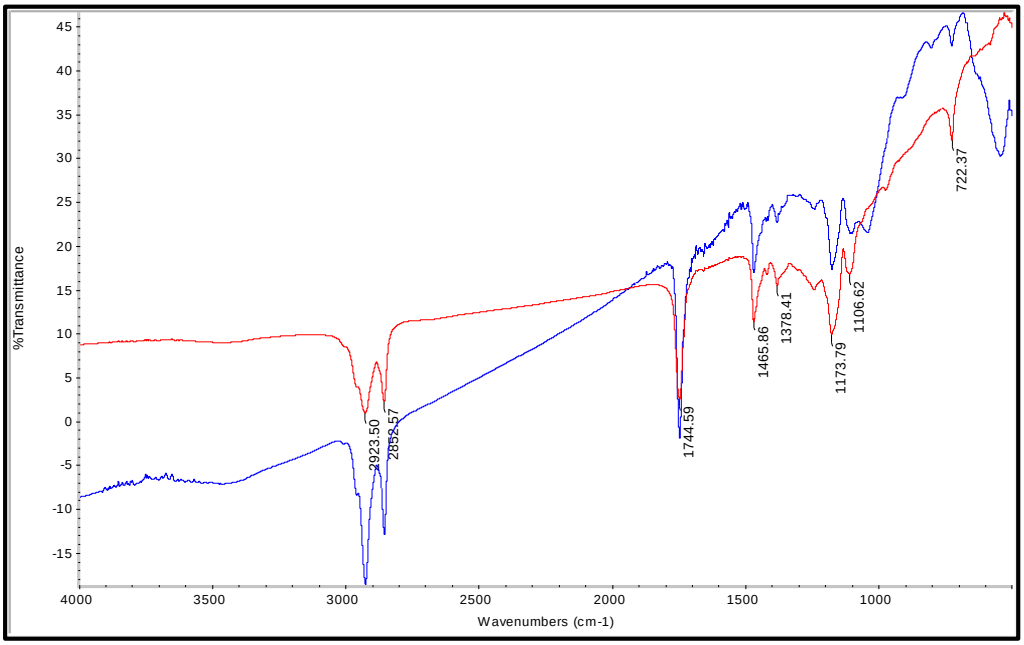

Figure C1: IR spectrum for kudufat (blue) and kudufat and ochre mixture (red)

Table C1: Peak assignment for IR spectrum for kudufat

Functional group	**Wavelengths**	**Vibrational motion**
Aldehyde	2923.90	C-H
	2852.57	
aldehyde,ketone ester, carboxylic acid)	1744.59	C=O
alkane	1485.84	C-C
alcohol or ether	1173.79	C-O

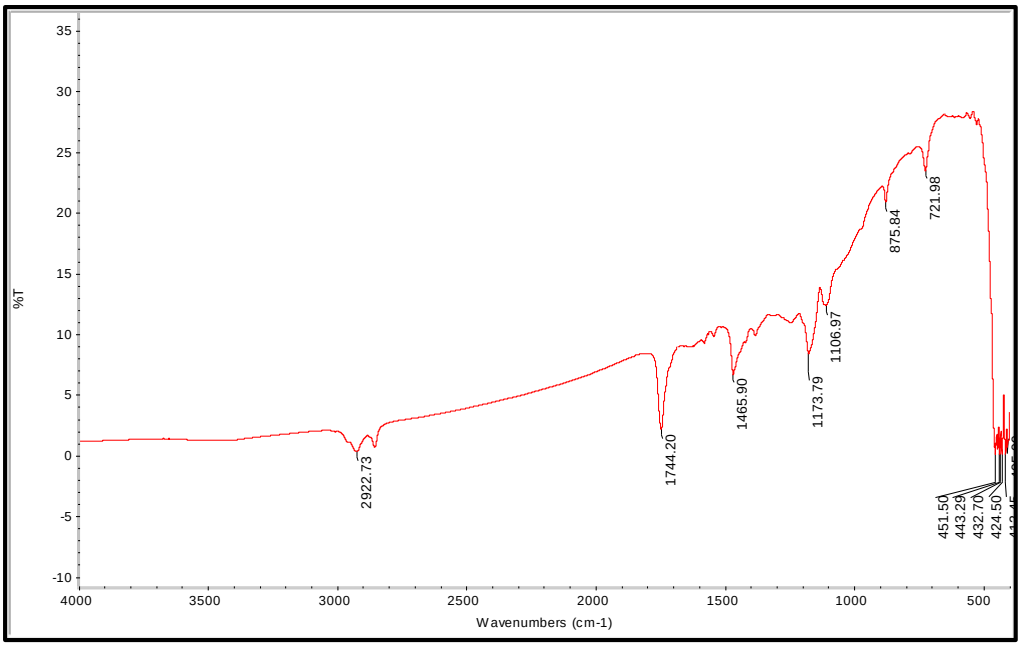

Figure C2: IR spectra for aromatic powder mixed with clarified butterfat

Table C2: Peak assignment for IR spectrum for aromatic powder mixed with clarified butterfat

Functional group	**Wavelengths**	**Vibrational motion**
aldehyde	2922.37	C-H
Aldehyde, ester, carboxylic acid	1744.96	C=O
alkane	1465.90	C-C
alcohol or ether	1173.79	C-O
C-H bonds	875.84, 721.98	-C-H

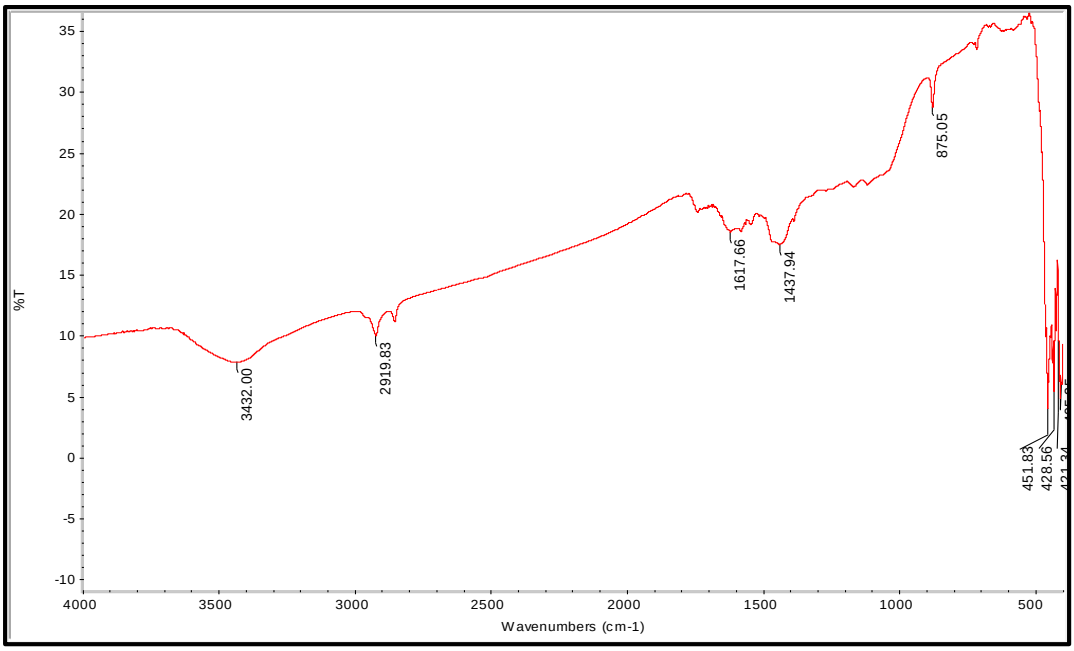

Figure C3: IR spectrum for uncharred aromatic wood and bark fragments (perfume powder)

Table C3: Peak assignment for IR spectrum for uncharred aromatic wood and bark fragments (perfume powder)

Functional group	**Wavelength number (cm^{-1})**	**Vibrational motion**
alcohol	3432.00	O-H
aldehyde	2922.37	C-H
alkene	1617.66	C=C
alkane	1437.94	C-C
C-H bonds	875.05	-C-H

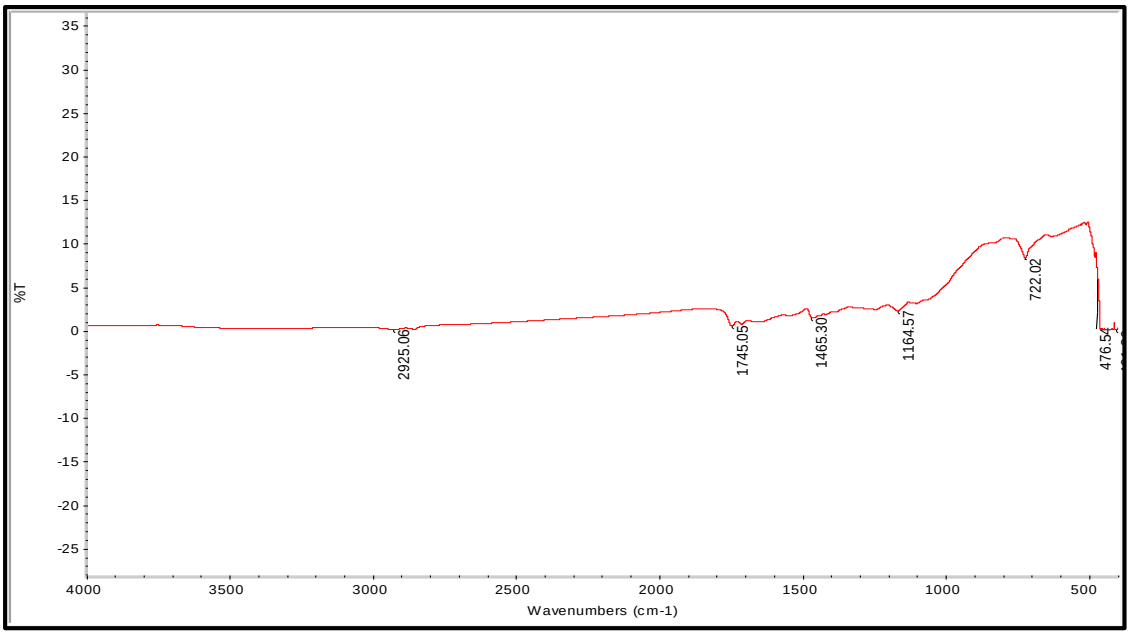

Figure C4: IR spectrum for aromatic seeds

Table C4: Peak assignment for IR spectrum for aromatic seeds

Functional group	**Wavelength number (cm^{-1})**	**Vibrational motion**
ketone, aldehyde, and/or ester	1745.05	C=O
alkane	1465.30	C-C
alcohol or ether	1154.57	C-O
C-H bonds	722.02	-C-H

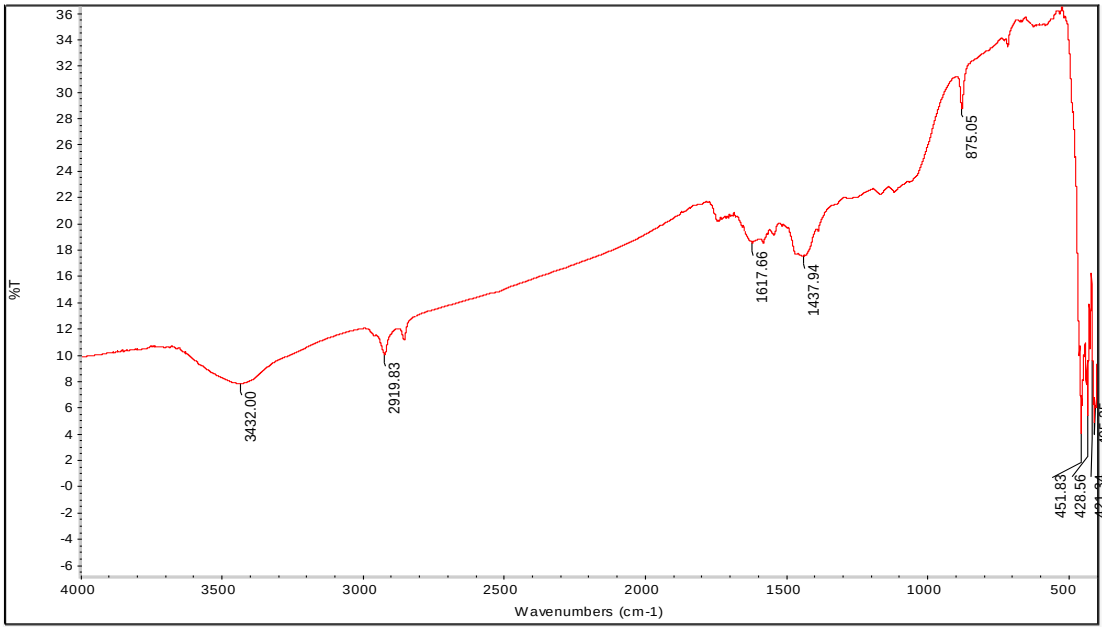

Figure C5: IR spectrum of composite black ground aromatic powder

Table C5: Peak assignment for IR spectrum for composite black ground aromatic powder

Functional group	**Wavelength number (cm^{-1})**	**Vibrational motion**
alcohol	3432	O-H
aldehyde	2919 & 2890	C-H
ketone, aldehyde, and/or ester	1745	C=O
alkene	1617	C=C
alkane	1437	C-C

Appendix D - GC-FID analysis

Table D1: Operating conditions for GC-FID

Oven	Equilibrium time Oven program Run time	0.5 min On 60°C for 4 min then 15°C to 200°C for 10 min 23.333 min
Front injector	Front SS inlet N2 Mode Heater Pressure Total flow Septum purge flow Gas saver Purge flow to split vent	 Splitless On 250°C On 9.2723 psi On 44 mL/min On 2 mL/min Off 40 mL/min at 1 min
Column #1	HP-5 5% Phenyl Methyl Siloxan: HP 5	30m x 320 µm x 0.25 µm Maximum temperature : 325°C

Appendix E- GC-MS analysis

Table E1: Operating conditions for GC-MS

Oven	Primary oven Equilibrium time Oven program Run time Secondary oven	 0.5 min 55°C for 1 min then 10°C /min to 200°C for 0 min and 7°C /min to 320°C to 0.36 min 33.003 min Conditions similar to primary oven but all the temperatures are kept at 5°C higher
Carrier gas	Helium Volume Heater	1 mL/min 1 µL 250°C
Column	Rxi-SSil MS Rxi-175Sil MS	30 m x 0.25mm (ID) x 0.25 µm (df) Min. Temp. 320°C Maxi. Temp. 350°C 1.18 m x 0.15 mm (ID) x 0.15 µm (df) Min. Temp 340°C Maxi. Temp 360°C

Tables below show all compounds identified by GC-MS for all samples but mosquito repellent compounds in each sample are highlighted in yellow

Table E2: Compounds identified by GC-MS for Ovahimba ochre

Sample	Peak #	Name	Weight	R.T. (s)	Area	Library	Similarity	S/N	Noise	UniqueMass
Ovahimba ochre	87	3,3-Diethylpentadecane	268	1118.9	2675953	mainlib	681	789.2	44.843	197
Ovahimba ochre	66	3,3-Diethylpentadecane	268	959.9	27504006	mainlib	619	11040	30.96	243
Ovahimba ochre	16	Carbonic acid, hexadecyl phenyl ester	362	580.9	646394	mainlib	785	254.95	210.06	94
Ovahimba ochre	64	Cholest-5-en-3-ol (3á)-, carbonochloridate	448	952.2	770510	replib	704	484.12	30.515	260
Ovahimba ochre	83	Cholesta-4,6-dien-3-ol, (3á)-	384	1104.3	152980	mainlib	703	351.69	25.809	366
Ovahimba ochre	57	Cholesterol	386	881.6	1738828	mainlib	839	454.46	42.318	188
Ovahimba ochre	6	Cyclohexasiloxane, dodecamethyl-	444	452.1	161110	replib	834	455.67	31.349	341
Ovahimba ochre	4	Cyclopentasiloxane, decamethyl-	370	353.9	329479	replib	821	791.2	40.017	267
Ovahimba ochre	1	Cyclotetrasiloxane, octamethyl-	296	258.6	827229	replib	906	1581.9	44.176	281
Ovahimba ochre	48	Dibutyl phthalate	278	767.7	4431264	mainlib	940	4028.6	83.544	149
Ovahimba ochre	67	Diisooctyl phthalate	390	972.5	3729750	replib	812	3920.9	83.544	149
Ovahimba ochre	5	Dodecane	170	397.8	11056483	mainlib	950	470.19	2101.1	57
Ovahimba ochre	14	Dodecanoic acid, methyl ester	214	572.8	7753044	replib	900	2549	261.59	74
Ovahimba ochre	53	Eicosane, 2,4-dimethyl-	310	840.4	10186246	mainlib	912	1524.4	530.99	85
Ovahimba ochre	68	Eicosane, 2-methyl-	296	976.3	117978	mainlib	866	344.83	26.732	309
Ovahimba ochre	73	Eicosanoic acid, 2,3-bis(acetyloxy)propyl ester	470	995	940791	mainlib	636	299.39	175.55	103
Ovahimba ochre	91	Eicosanoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl es	470	1137	7726572	mainlib	681	6956.6	30.96	243
Ovahimba ochre	88	Glycerol tricaprylate	470	1119.9	528826	mainlib	514	269.21	27.116	385
Ovahimba ochre	36	Heneicosane	296	701.4	6952316	replib	927	1222.7	530.99	85
Ovahimba ochre	56	Heptacosane	380	858.8	3386011	replib	942	583.89	530.99	85
Ovahimba ochre	72	Heptacosane	380	991.8	3842931	replib	904	737.65	530.99	85
Ovahimba ochre	74	Heptacosane	380	1024.2	4227220	replib	902	452.47	530.99	85
Ovahimba ochre	26	Heptadecane, 2,6,10,15-tetramethyl-	296	657.2	1754340	mainlib	875	269.82	530.99	85
Ovahimba ochre	20	Hexadecane	226	610.7	10950759	replib	927	1771.2	530.99	85
Ovahimba ochre	79	Hexadecanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl este	330	1054.4	2311349	mainlib	643	257.45	146.26	133
Ovahimba ochre	33	Sulfurous acid, hexyl tetradecyl ester	362	679.7	11154237	mainlib	866	1833.9	530.99	85
Ovahimba ochre	59	Sulfurous acid, hexyl tetradecyl ester	362	911	8267728	mainlib	869	1313.9	530.99	85
Ovahimba ochre	63	Unknown 1	233	944.2	206829	mainlib	493	493.04	34.36	233
Ovahimba ochre	78	Unknown 2	237	1040.1	654891	mainlib	485	235.29	104.31	145
Ovahimba ochre	84	Unknown 3	341	1111.9	2536057	mainlib	458	497.62	106.62	152
Ovahimba ochre	70	n-Butyric acid tetrahydrofurfuryl ester	158	986	1275279	mainlib	578	615.67	72.498	163
Ovahimba ochre	49	n-Hexadecanoic acid	256	768.7	64111431	replib	915	8135.7	467.57	73

Table E3: Compounds identified by GC-MS for clarified butterfat

Sample	Peak #	Name	Weight	R.T. (s)	Area	Library	Similarity	S/N	Noise	UniqueMass
Clarified butterfat	19	2-Dodecanone	184	451.5	21040683	mainlib	832	984.47	1638.6	58
Clarified butterfat	39	2-Dodecanone	184	656.5	66791207	mainlib	868	3240.4	1638.6	58
Clarified butterfat	31	2-Dodecanone	184	559.5	11207389	mainlib	897	1008.6	871.84	59
Clarified butterfat	27	2-Hexen-1-ol, 2-ethyl-	128	539.3	14147807	replib	762	1411.4	709.6	86
Clarified butterfat	82	2-Methyl-4-phenylthiolane, 1,1-dioxide	210	1020	5277143	mainlib	569	1976.5	193.4	104
Clarified butterfat	7	2-Nonanone	142	331.5	16462503	replib	892	654.58	1638.6	58
Clarified butterfat	11	2-Nonenal, (E)-	140	375	66652324	replib	935	1168.9	4495.7	70
Clarified butterfat	23	2-Nonenal, (Z)-	140	481.8	6836758	mainlib	745	2707.6	180.21	139
Clarified butterfat	16	2-Sec-Butylcyclohexanone	154	425.5	3949994	mainlib	768	693.21	335.08	125
Clarified butterfat	24	2-Undecenal	168	492.4	14301026	mainlib	909	4092.3	274.58	121
Clarified butterfat	26	Acetic acid, 2-phenylethyl ester	164	533.8	1824895	replib	909	687.24	193.4	104
Clarified butterfat	17	Acetic acid, 2-phenylethyl ester	164	432	3000587	replib	893	973.75	193.4	104
Clarified butterfat	15	Benzaldehyde, 2,5-dimethyl-	134	413.9	5240799	replib	902	1809.5	143.58	134
Clarified butterfat	12	Benzoic acid	122	382.8	27957664	replib	851	2481.5	221.17	122
Clarified butterfat	68	Butanoic acid, 2,3-dihydroxypropyl ester	162	852	963164	mainlib	609	630.79	50.868	215
Clarified butterfat	20	Cyclohexasiloxane, dodecamethyl-	444	452.1	1794893	replib	840	4827.4	34.475	341
Clarified butterfat	10	Cyclopentasiloxane, decamethyl-	370	353.9	2490819	replib	824	4986.6	46.905	267
Clarified butterfat	3	Cyclotetrasiloxane, octamethyl-	296	258.6	2577280	replib	891	4365.7	50.4	281
Clarified butterfat	37	Decanoic acid, ethyl ester	200	606.5	13331543	replib	876	4615.3	264.55	88
Clarified butterfat	46	Decanoic acid, ethyl ester	200	697.5	47837973	replib	876	15432	264.55	88
Clarified butterfat	25	Decanoic acid, ethyl ester	200	506	9329989	replib	905	3134.2	264.55	88
Clarified butterfat	60	Dibutyl phthalate	278	767.6	10375758	replib	950	7206.3	108.93	149
Clarified butterfat	79	Diisooctyl phthalate	390	972.1	4068043	replib	717	3399.5	108.93	149
Clarified butterfat	35	Dodecanoic acid	200	593.5	10827658	replib	925	4289.7	109.59	157
Clarified butterfat	72	Eicosanoic acid, 2,3-bis(acetyloxy)propyl ester	470	931.1	3274313	mainlib	720	754.76	285.72	103
Clarified butterfat	99	Heptacosane	380	1188.2	929824	replib	714	1180.3	34.109	243
Clarified butterfat	2	Heptanal	114	215	18302945	replib	941	1179.3	709.6	86
Clarified butterfat	6	Heptanoic acid	130	321.8	54916280	replib	943	1299.5	1549.4	60
Clarified butterfat	100	Hexadecane	226	1193.5	883495	replib	677	755.42	41.349	229
Clarified butterfat	81	Hexadecanoic acid, 2,3-bis(acetyloxy)propyl ester	414	996.1	4471215	mainlib	734	4184.3	71.701	158
Clarified butterfat	61	Hexadecanoic acid, ethyl ester	284	780.5	117312960	mainlib	884	38385	264.55	88
Clarified butterfat	55	Hexadecanoic acid, methyl ester	270	753.1	5527581	replib	898	737.57	537.25	74
Clarified butterfat	4	Hexanoic acid	116	269.8	972986964	replib	913	9778.1	1549.4	60
Clarified butterfat	95	Hexanoic acid, 4-tridecyl ester	298	1134.2	5069409	mainlib	618	5587.6	47.572	211
Clarified butterfat	8	Nonanal	142	340.5	2508967	replib	940	887.78	211.01	114
Clarified butterfat	74	Nonanoic acid, [tetrahydro-2-furanyl]ethyl ester	242	940.8	11094876	mainlib	530	4931.5	31.456	271
Clarified butterfat	43	Octadecane, 2,6-dimethyl-	282	679.4	713982	mainlib	866	1223.4	51.66	197
Clarified butterfat	59	Octadecane, 5-methyl-	268	763.6	540763	mainlib	845	1007.8	39.521	225
Clarified butterfat	67	Octadecanoic acid	284	848.8	9450366	replib	896	11334	39.895	241
Clarified butterfat	69	Octadecanoic acid, ethyl ester	312	856.2	14657358	replib	833	4817.9	264.55	88
Clarified butterfat	13	Octanoic acid	144	383.2	172040518	mainlib	887	3507.8	1549.4	60
Clarified butterfat	92	Octanoic acid, 2-ethylhexyl ester	256	1119.4	8214951	mainlib	601	920.76	101.86	191
Clarified butterfat	77	Octanoic acid, 2-phenylethyl ester	248	953.7	1943669	mainlib	599	667.24	193.4	104
Clarified butterfat	14	Octanoic acid, ethyl ester	172	394.2	5154917	replib	836	1633.1	264.55	88
Clarified butterfat	86	Octanoic acid, octyl ester	256	1057	81102650	mainlib	623	855.24	1549.4	60
Clarified butterfat	70	Oxirane, [(hexadecyloxy)methyl]-	298	894	4303003	mainlib	803	928.5	424.83	75
Clarified butterfat	34	Pentadecane, 5-methyl-	226	586.9	714319	mainlib	882	660.99	93.604	168
Clarified butterfat	48	Pentadecanoic acid	242	711.8	854170	mainlib	886	915.76	61.409	199
Clarified butterfat	50	Pentadecanoic acid	242	727.4	1282658	mainlib	901	2045.6	35.964	242
Clarified butterfat	49	Pentadecanoic acid	242	715.4	3519932	mainlib	785	3090.3	70.4	185

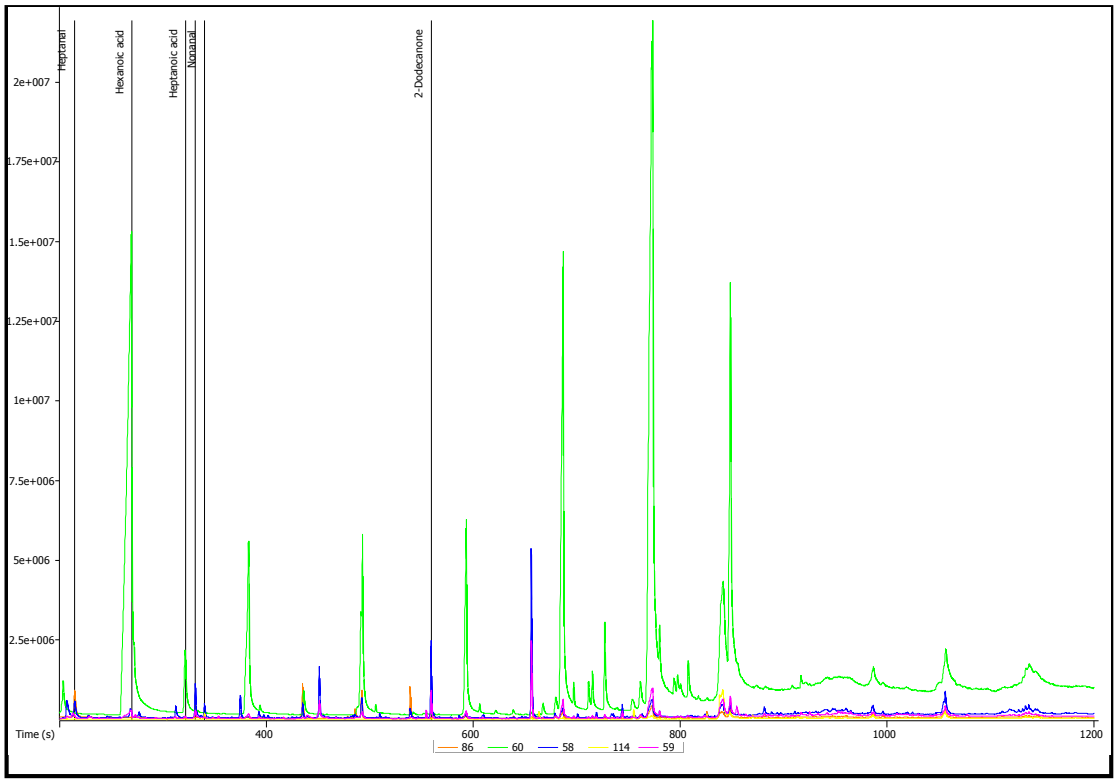

Figure E1: Chromatogram for clarified butterfat

The chromatogram is expressed according to the unique mass of the identified compounds, heptanoic acid (60) Nonanal (96) 2-dodecanone (58) hexanoic acid (60) 2-nonanone (58) heptanal (86)

Table E4: Compounds identified by GC-MS for replicated *otjize*

Sample	Peak #	Name	Weight	R.T. (s)	Area	Library	Similarity	S/N	Noise	UniqueMass
Replicated otjize	15	2-Decenal, (Z)-	154	436	4465832	mainlib	930	1185.2	296.47	107
Replicated otjize	38	2-Dodecanone	184	657.3	38159193	mainlib	837	2410.1	1252.9	58
Replicated otjize	25	2-Hexen-1-ol, 2-ethyl-	128	540.6	6069286	replib	761	682.5	568.8	86
Replicated otjize	82	2-Methylbenzyl cyanide	131	1021.8	3381249	replib	607	845.94	186.23	104
Replicated otjize	10	2-Nonenal, (E)-	140	375.7	4313179	replib	932	629.35	501.04	93
Replicated otjize	29	2-Tridecanone	198	560.2	16533671	replib	878	1042.4	1252.9	58
Replicated otjize	20	2-Undecenal	168	493	6187911	mainlib	919	2205.7	233.86	121
Replicated otjize	37	2-Undecene, 5-methyl-	168	629.2	4249454	mainlib	617	611.18	90.787	158
Replicated otjize	78	Butanoic acid, 2,3-dihydroxypropyl ester	162	988	2954716	replib	560	1776.5	48.7	212
Replicated otjize	77	Butanoic acid, 2,3-dihydroxypropyl ester	162	975.1	1317958	mainlib	539	485.05	144.19	145
Replicated otjize	67	Cholesterol	386	890.9	12110018	mainlib	821	1356.5	69.634	160
Replicated otjize	19	Cyclohexanone, 2-ethyl-	126	482.8	2736181	replib	743	1430	133.33	139
Replicated otjize	16	Cyclohexasiloxane, dodecamethyl-	444	452.3	913668	replib	754	2214.5	37.387	341
Replicated otjize	8	Cyclopentasiloxane, decamethyl-	370	354.1	1674142	replib	824	3073.5	50.047	267
Replicated otjize	2	Cyclotetrasiloxane, octamethyl-	296	258.8	2013369	replib	919	3268	52.623	281
Replicated otjize	23	Decanoic acid, ethyl ester	200	506.6	4627299	replib	789	1713.7	229.71	88
Replicated otjize	58	Dibutyl phthalate	278	768.4	13506781	mainlib	938	10364	108.49	149
Replicated otjize	35	Diethyl Phthalate	222	608	1803190	replib	789	1186.7	108.49	149
Replicated otjize	100	Diethylmalonic acid, heptyl tetrahydrofurfuryl ester	342	1197.8	4117632	mainlib	547	1231	144.19	145
Replicated otjize	76	Diisooctyl phthalate	390	973.4	5969725	replib	784	3429.8	108.49	149
Replicated otjize	73	Dodecane, 2,6,10-trimethyl-	212	949.5	8424339	replib	657	2600.6	37.431	271
Replicated otjize	32	Dodecanoic acid	200	593.9	80317909	replib	920	2767.5	1365.2	60
Replicated otjize	51	Dodecanoic acid, ethyl ester	228	740.6	2930407	replib	753	1086.7	229.71	88
Replicated otjize	34	Dodecanoic acid, ethyl ester	228	607.3	7035695	replib	791	2756.9	229.71	88
Replicated otjize	33	Fumaric acid, 4-chlorophenyl ethyl ester	254	599	9092401	mainlib	785	1397.9	463.27	127
Replicated otjize	1	Heptanal	114	215.3	7772236	mainlib	955	613.26	568.8	86
Replicated otjize	5	Heptanoic acid	130	322.2	20190913	replib	820	460.33	1365.2	60
Replicated otjize	53	Heptanoic acid, methyl ester	144	754	3347859	replib	741	559.45	431.75	74
Replicated otjize	80	Hexadecanoic acid, 2,3-bis(acetyloxy)propyl ester	414	997	6112741	mainlib	708	1150.6	248.79	103
Replicated otjize	99	Hexadecanoic acid, 2,3-bis(acetyloxy)propyl ester	414	1193.7	1450917	mainlib	568	543.74	144.19	145
Replicated otjize	59	Hexadecanoic acid, ethyl ester	284	781.2	68462183	mainlib	865	24832	229.71	88
Replicated otjize	3	Hexanoic acid	116	267.4	380644232	replib	954	6302.2	1365.2	60
Replicated otjize	14	Hexanoic acid, 4-pentenyl ester	184	417.4	12012444	mainlib	665	632.21	1694.5	68
Replicated otjize	75	Hexanoic acid, 4-tridecyl ester	298	963	13827621	mainlib	674	4765.3	38.991	243
Replicated otjize	4	Hexanoic acid, ethyl ester	144	272.9	5358733	replib	934	1860.2	229.71	88
Replicated otjize	26	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	430	541.5	516630	mainlib	664	814.44	52.623	281
Replicated otjize	83	Hexasiloxane, tetradecamethyl-	458	1030.4	2615234	mainlib	672	2735.6	50.769	221
Replicated otjize	89	Hexasiloxane, tetradecamethyl-	458	1078.1	3435811	mainlib	713	1189.8	121.09	147
Replicated otjize	84	Methacrylaldehyde, tert-butyl isopropyl acetal	186	1034.2	1201568	mainlib	516	673	37.431	271
Replicated otjize	60	Methoxyacetic acid, 3-tridecyl ester	272	818.4	2724134	mainlib	755	462.57	524.32	61
Replicated otjize	6	Nonanal	142	341.1	4861639	replib	935	615.26	585.56	96
Replicated otjize	43	Octadecane, 2,6-dimethyl-	282	680.2	759461	mainlib	869	1238.8	55.995	196
Replicated otjize	72	Sulfurous acid, 2-propyl undecyl ester	278	947.6	3057394	mainlib	608	1975.2	121.09	147
Replicated otjize	24	Tetradecane	198	510.5	334612	replib	938	565.71	54.059	198
Replicated otjize	40	Tetradecanoic acid	228	669.2	769130	mainlib	758	503.12	79.968	185

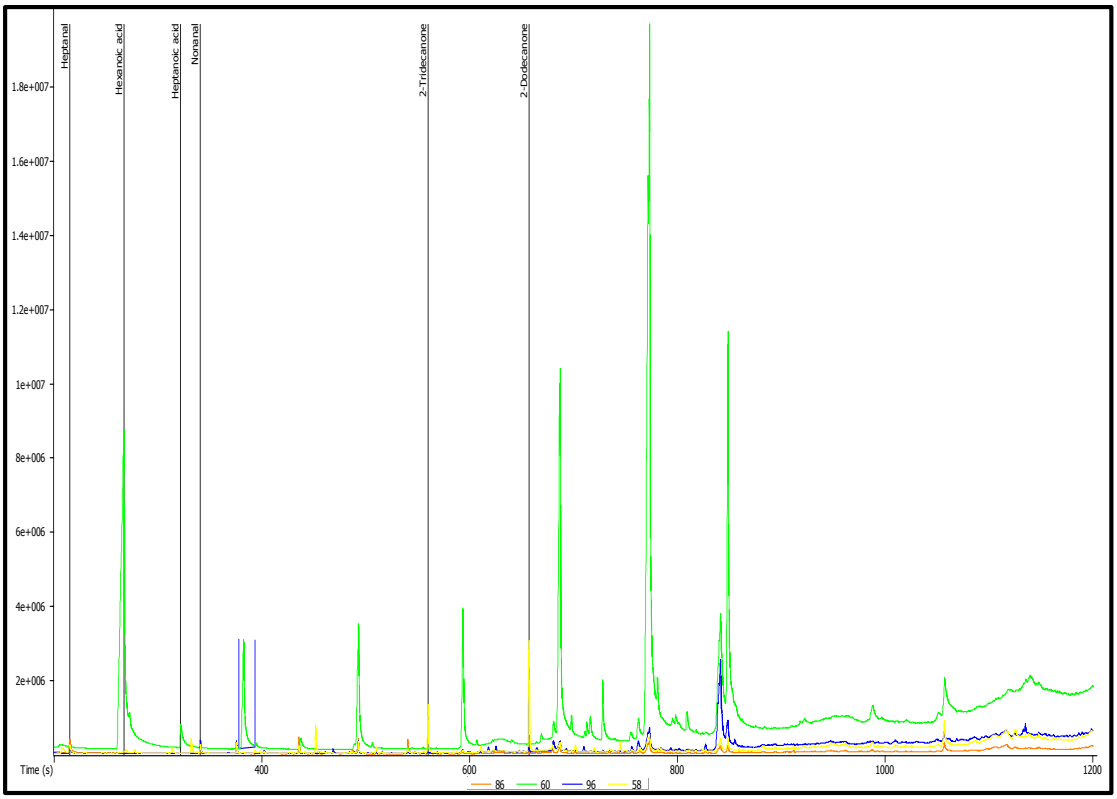

Figure E2: Chromatogram for replicated *otjize*

The chromatogram is expressed according to the unique mass of the identified compounds, heptanoic acid (60) Nonanal (96) 2-dodecanone (58) hexanoic acid (60) 2-nonanone (58) heptanal (86) 2-tridecanone (58)

Table E5: Compounds identified by GC-MS for kudufat

Sample	Peak #	Name	Weight	R.T. (s)	Area	Library	Similarity	S/N	Noise	UniqueMass
Kudufat	34	1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	278	728.3	6149616	replib	928	6784.3	71.283	149
Kudufat	78	1,2-Ethanediamine, N,N'-diethyl-	116	955.8	2509719	replib	873	439.02	514.39	58
Kudufat	58	1,3-Diphenyl-4H-1,2,4-triazoline-5-thione	253	840.5	195133	mainlib	591	332.57	31.924	252
Kudufat	67	1,E-11,Z-13-Octadecatriene	248	902.7	222143	mainlib	694	348.69	71.283	149
Kudufat	99	1-Bromo-4-bromomethyldecane	312	1163.2	4560333	mainlib	689	1430.5	37.966	219
Kudufat	84	1-Monolinoleoylglycerol trimethylsilyl ether	498	1024.4	659136	mainlib	537	459.98	30.543	355
Kudufat	100	1-Monolinoleoylglycerol trimethylsilyl ether	498	1185.4	4119586	mainlib	614	477.17	85.397	147
Kudufat	57	17-Octadecynoic acid	280	840.1	816309	mainlib	806	511.18	42.296	199
Kudufat	77	2,2-Dimethylpropanoic acid, tridec-2-ynyl ester	280	950	2585625	mainlib	622	410.15	184.04	94
Kudufat	96	2,3-Octanedione	142	1138	2815025	replib	563	1895.2	29.819	243
Kudufat	15	2,5-Cyclohexadiene-1,4-dione, 2,6-bis(1,1-dimethylethyl)-	220	546.5	302268	mainlib	844	471.45	54.328	177
Kudufat	25	2,6-Diisopropylnaphthalene	212	669.8	248615	mainlib	794	510.74	40.729	197
Kudufat	26	2,6-Diisopropylnaphthalene	212	672.7	487951	mainlib	862	899.29	40.729	197
Kudufat	27	2,6-Diisopropylnaphthalene	212	675.2	485499	mainlib	857	823.37	40.729	197
Kudufat	9	2-Decenal, (Z)-	154	435.4	6289533	mainlib	936	496.28	856.82	83
Kudufat	89	2-Piperidinone, N-[4-bromo-n-butyl]-	233	1110.5	3258591	mainlib	652	2317.3	29.819	243
Kudufat	81	2-Piperidinone, N-[4-bromo-n-butyl]-	233	982.2	913222	mainlib	789	437.36	42.565	215
Kudufat	69	2H-Pyran-2-one, tetrahydro-6-nonyl-	226	913.7	767809	mainlib	679	540.39	86.993	114
Kudufat	56	2H-Pyran-2-one, tetrahydro-6-propyl-	142	837.9	24467609	replib	821	2591.8	624.81	99
Kudufat	31	3,5-di-tert-Butyl-4-hydroxyacetophenone	248	708.4	231662	replib	608	558.34	31.59	233
Kudufat	30	3,5-di-tert-Butyl-4-hydroxybenzaldehyde	234	687.5	389962	mainlib	710	809.23	37.966	219
Kudufat	62	3-Hexanone, 4-methyl-	114	869.9	447337	mainlib	623	446.54	86.993	114
Kudufat	91	4-Hydroxy-4-methylhex-5-enoic acid, tert.-butyl ester	200	1121.3	1866845	mainlib	650	731.12	26.279	291
Kudufat	36	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	276	748.9	1102593	replib	907	2058.4	43.086	205
Kudufat	87	9-Octadecenamide, (Z)-	281	1055.2	30491681	replib	867	7782.2	195.54	59
Kudufat	52	9-Octadecenoic acid (Z)-, methyl ester	296	821.9	8850715	replib	918	3816.2	211.17	74
Kudufat	7	Benzaldehyde, 2,4-dimethyl-	134	414.9	3391021	mainlib	905	595.53	146.43	133
Kudufat	41	Benzene, (1-butylnonyl)-	260	758.2	5840055	mainlib	848	926.54	527.66	91
Kudufat	47	Benzene, (1-ethyldecyl)-	246	774.7	14339284	replib	800	2350.2	527.66	91
Kudufat	50	Benzene, (1-methyldecyl)-	232	791.4	49513998	mainlib	894	13521	325.36	105
Kudufat	53	Benzene, (1-methylundecyl)-	246	830.8	1539114	mainlib	833	384.81	325.36	105
Kudufat	37	Benzene, (1-methylundecyl)-	246	749.8	12465047	mainlib	877	3209.1	325.36	105
Kudufat	44	Benzene, (1-propyldecyl)-	260	764.3	8198200	mainlib	841	1445.6	527.66	91
Kudufat	2	Benzene, 1,3-dichloro-	146	290.6	1386602	mainlib	918	523.9	68.552	146
Kudufat	4	Benzenemethanol, à,à-dimethyl-	136	332.4	859774	replib	789	311.86	138.48	121
Kudufat	80	Bis(2-ethylhexyl) phthalate	390	971.7	20206918	replib	904	15952	71.283	149
Kudufat	64	Cholesterol	386	879	1660573	mainlib	805	400.45	61.014	178
Kudufat	10	Cyclohexasiloxane, dodecamethyl-	444	452	787599	replib	811	2452.3	29.811	341
Kudufat	6	Cyclopentasiloxane, decamethyl-	370	353.8	1325025	replib	823	3546.8	35.898	267
Kudufat	1	Cyclotetrasiloxane, octamethyl-	296	258.5	1588345	replib	912	3516.2	40.931	281
Kudufat	45	Dibutyl phthalate	278	767.3	28519483	mainlib	949	31809	71.283	149
Kudufat	21	Diethyl Phthalate	222	607.1	1387293	replib	869	1246.9	71.283	149
Kudufat	72	Ethanol, 2-ethoxy-	90	925	2779028	replib	611	1006.8	195.54	59
Kudufat	65	Fumaric acid, 2-dimethylaminoethyl hexyl ester	271	888.4	2519348	mainlib	847	380.27	514.39	58

Table E6: Compounds identified by GC-MS for Ovahimba ochre and kudufat mixture

Sample	Peak #	Name	Weight	R.T. (s)	Area	Library	Similarity	S/N	Noise	UniqueMass
Ovahimba ochre + Kudufat	71	3,3-Diethylpentadecane	268	964.1	29285070	mainlib	662	8994.7	36.317	243
Ovahimba ochre + Kudufat	90	4-Butoxy-2,4-dimethyl-2-pentene	170	1079.6	1914423	mainlib	539	702.42	39.822	327
Ovahimba ochre + Kudufat	70	5-Iodo-nonane	254	950.6	19794054	mainlib	700	5314.6	35.97	271
Ovahimba ochre + Kudufat	35	7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	276	749.9	1170236	replib	925	1973.4	52.455	205
Ovahimba ochre + Kudufat	76	7-Methyl-Z-tetradecen-1-ol acetate	268	999.8	370993	mainlib	726	408.02	36.317	243
Ovahimba ochre + Kudufat	63	9-Octadecenamide, (Z)-	281	918	1914159	replib	656	468.47	260.26	59
Ovahimba ochre + Kudufat	51	9-Octadecenoic acid (Z)-, methyl ester	296	822.6	8039988	replib	920	2182.1	302.5	74
Ovahimba ochre + Kudufat	21	Benzophenone	182	633.8	6928708	mainlib	934	1028.5	345	105
Ovahimba ochre + Kudufat	97	Butanoic acid, 2,3-dihydroxypropyl ester	162	1147	2395073	mainlib	541	1508.6	84.475	163
Ovahimba ochre + Kudufat	84	Butyric acid, 3-tridecyl ester	270	1056.2	66238998	mainlib	595	18344	123.25	145
Ovahimba ochre + Kudufat	28	Cyclohexane, (1-methylethyl)-	126	688.5	515884	replib	680	884.5	42.929	219
Ovahimba ochre + Kudufat	9	Cyclohexasiloxane, dodecamethyl-	444	452.3	773322	replib	837	1880	37.952	341
Ovahimba ochre + Kudufat	53	Cyclopentaneundecanoic acid, methyl ester	268	832.1	4267739	mainlib	857	1330.8	302.5	74
Ovahimba ochre + Kudufat	5	Cyclopentasiloxane, decamethyl-	370	354	2828134	replib	822	6046.2	45.562	267
Ovahimba ochre + Kudufat	50	Cyclotetrasiloxane, 2,4,6,8-tetramethyl-	240	815	287283	replib	553	646.73	27.341	351
Ovahimba ochre + Kudufat	1	Cyclotetrasiloxane, octamethyl-	296	258.7	2626964	replib	919	4829.9	50.871	281
Ovahimba ochre + Kudufat	57	Decane, 2,4-dimethyl-	170	841.2	376271	mainlib	839	523.49	38.992	252
Ovahimba ochre + Kudufat	66	Decanoic acid, 2-hydroxy-1-(hydroxymethyl)ethyl ester	246	942.1	6850238	mainlib	623	1825.4	39.822	327
Ovahimba ochre + Kudufat	43	Dibutyl phthalate	278	768.3	32771456	mainlib	943	29520	87.289	149
Ovahimba ochre + Kudufat	18	Diethyl Phthalate	222	607.8	1000331	replib	824	839.42	87.289	149
Ovahimba ochre + Kudufat	80	Dihydro-isosteviol methyl ester	334	1037.3	536637	mainlib	652	439.87	39.464	257
Ovahimba ochre + Kudufat	72	Diisooctyl phthalate	390	973.3	20928651	replib	823	20755	87.289	149
Ovahimba ochre + Kudufat	85	Dodecanamide	199	1056.7	18001599	mainlib	690	1985.1	260.26	59
Ovahimba ochre + Kudufat	15	Dodecane, 2,6,10-trimethyl-	212	555.3	1118492	replib	603	1119.6	86.24	159
Ovahimba ochre + Kudufat	29	Eicosane	282	701.8	54546780	replib	926	783.33	6234.5	57
Ovahimba ochre + Kudufat	96	Eicosanoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl es	470	1140.1	12740433	mainlib	684	7630.9	36.317	243
Ovahimba ochre + Kudufat	30	Glutaric acid, di(2-isopropylphenyl) ester	368	709.4	153186	mainlib	548	339.6	36.625	233
Ovahimba ochre + Kudufat	49	Hexadecanal	240	793.4	3171250	replib	904	588.96	523.38	82
Ovahimba ochre + Kudufat	31	Hexadecanal	240	710	3177482	replib	853	421.61	523.38	82
Ovahimba ochre + Kudufat	19	Hexadecane	226	611.1	61284537	replib	938	904.17	6234.5	57
Ovahimba ochre + Kudufat	37	Hexadecanoic acid, methyl ester	270	753.6	18960957	replib	926	6032.4	263.08	87
Ovahimba ochre + Kudufat	64	Hexanedioic acid, bis(2-ethylhexyl) ester	370	925	9099958	mainlib	877	3574.3	230.71	129
Ovahimba ochre + Kudufat	7	Hexanoic acid, 4-pentenyl ester	184	417.3	2672338	mainlib	674	363.34	630.99	68
Ovahimba ochre + Kudufat	13	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	430	541.4	246123	mainlib	675	406.4	50.871	281
Ovahimba ochre + Kudufat	32	Isopropyl myristate	270	711	1426204	replib	756	786.21	148.02	102
Ovahimba ochre + Kudufat	41	Octadecane, 4-methyl-	268	764.2	557050	mainlib	854	1086.6	40.205	225
Ovahimba ochre + Kudufat	58	Octadecanoic acid	284	847.8	22289753	replib	906	5239.1	230.71	129
Ovahimba ochre + Kudufat	94	Octanoic acid, 1-methyltridecyl ester	340	1123	135210371	mainlib	608	10412	262.01	127
Ovahimba ochre + Kudufat	22	Undecanoic acid, methyl ester	200	667.9	2365099	replib	751	682.49	302.5	74
Ovahimba ochre + Kudufat	25	Unknown 1	356	678.8	48803520	mainlib	444	1664.1	123.25	145
Ovahimba ochre + Kudufat	46	Unknown 2	258	777.3	166872	mainlib	481	381.84	36.317	243
Ovahimba ochre + Kudufat	79	Unknown 3	127	1031.9	7682382	replib	489	534.11	262.01	127
Ovahimba ochre + Kudufat	44	n-Hexadecanoic acid	256	770.5	184397429	replib	912	14856	582.86	73
Ovahimba ochre + Kudufat	56	trans-13-Octadecenoic acid	282	840.4	620929	mainlib	862	782.7	34.359	246

Table E7: Compounds identified by GC-MS for old *otjize*

Sample	Peak #	Name	Weight	R.T. (s)	Area	Library	Similarity	S/N	Noise	UniqueMass
Old otjize	41	2-Dodecanone	184	656.5	49402684	mainlib	870	1862.3	2177.4	58
Old otjize	9	2-Nonenal, (Z)-	140	375.1	24733823	mainlib	936	927.38	2061.7	69
Old otjize	37	2-Pentanone, 4-cyclohexylidene-3,3-diethyl-	222	617.2	1605974	mainlib	583	1597.2	84.763	193
Old otjize	100	2-Piperidinone, N-[4-bromo-n-butyl]-	233	1183	1433782	mainlib	745	1968.8	43.863	211
Old otjize	19	2-Undecenal	168	492.4	15050840	mainlib	910	3583.5	318.04	121
Old otjize	33	2H-Azepin-2-one, hexahydro-1-methyl-	127	597.4	14295693	replib	736	2519	396.99	127
Old otjize	15	Butanoic acid, 2,3-dihydroxypropyl ester	162	443.4	3662229	replib	804	898.23	186.16	131
Old otjize	69	Cholesterol	386	879.9	4383638	mainlib	829	2032.2	27.641	386
Old otjize	21	Copaene	204	504	3011775	replib	896	3244.1	78.187	161
Old otjize	16	Cyclohexasiloxane, dodecamethyl-	444	452.1	3059611	replib	842	8223	34.479	341
Old otjize	8	Cyclopentasiloxane, decamethyl-	370	353.8	5856576	replib	825	11463	47.84	267
Old otjize	2	Cyclotetrasiloxane, octamethyl-	296	258.5	3556732	replib	905	6430.2	51.589	281
Old otjize	22	Decanoic acid, ethyl ester	200	506	6144222	replib	799	1985.6	279.03	88
Old otjize	58	Dibutyl phthalate	278	767.5	19329238	mainlib	937	12082	105.18	149
Old otjize	67	Dodecanamide	199	857.3	20132267	mainlib	839	1433.9	1122.4	59
Old otjize	32	Dodecanoic acid	200	596.1	38431388	replib	920	12427	94.599	157
Old otjize	35	Dodecanoic acid, ethyl ester	228	606.6	6864561	replib	844	2156.9	279.03	88
Old otjize	63	Eicosane, 2,4-dimethyl-	310	840.5	943958	mainlib	795	1453.5	39.218	253
Old otjize	89	Eicosanoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl es	470	1049.7	1621141	mainlib	673	2423.7	33.214	243
Old otjize	97	Eicosanoic acid, 2-(acetyloxy)-1-[(acetyloxy)methyl]ethyl es	470	1138	51750493	mainlib	680	29454	64.518	173
Old otjize	82	Ethyl Acetate	88	953.4	618778	replib	502	1068.3	33.619	257
Old otjize	1	Heptanal	114	214.7	71987057	mainlib	941	1089.9	2856.6	55
Old otjize	4	Heptanoic acid	130	325.6	253020001	replib	954	1975.5	3223.1	60
Old otjize	23	Hexadecane	226	510	825610	replib	940	1545.6	48.624	198
Old otjize	86	Hexadecanoic acid, 2,3-bis(acetyloxy)propyl ester	414	995.8	9328505	mainlib	727	1129	413.53	103
Old otjize	79	Hexadecanoic acid, butyl ester	312	921.2	1326109	replib	626	2181.9	33.619	257
Old otjize	52	Hexadecanoic acid, ethyl ester	284	739.8	3576300	replib	697	1140.7	279.03	88
Old otjize	3	Hexanoic acid	116	275.8	2.094E+09	replib	938	7386.9	3223.1	60
Old otjize	90	Hexanoic acid, cyclohexyl ester	198	1074.7	1785294	replib	548	1909.3	52.313	187
Old otjize	45	Hexanoic acid, thio-, S-butyl ester	188	694.5	1848047	mainlib	771	1247.9	78.527	159
Old otjize	25	Hexasiloxane, 1,1,3,3,5,5,7,7,9,9,11,11-dodecamethyl-	430	541.2	837557	mainlib	661	2148.1	34.321	327
Old otjize	81	Hexasiloxane, tetradecamethyl-	458	946.6	4139020	mainlib	668	5689.3	46.959	221
Old otjize	88	Hexasiloxane, tetradecamethyl-	458	1029.5	7701998	mainlib	721	9495.8	46.959	221
Old otjize	38	Hexasiloxane, tetradecamethyl-	458	620.6	875234	replib	736	2236.7	33.793	355
Old otjize	92	Hexasiloxane, tetradecamethyl-	458	1076.7	8471016	mainlib	725	9434.8	46.959	221
Old otjize	40	Longiverbenone	218	654.1	11025887	mainlib	783	6640.2	117.73	147
Old otjize	71	Methoxyacetic acid, 2-tridecyl ester	272	881.4	783704	mainlib	802	1075	60.385	182
Old otjize	99	Methyl hydrogen phthalate	180	1157	3771298	mainlib	519	5427.8	28.97	296
Old otjize	39	Naphthalene, 1,6-dimethyl-4-(1-methylethyl)-	198	652.7	4588732	replib	869	5608.6	60.22	183
Old otjize	6	Nonanal	142	340.5	60966250	replib	942	1523.9	2856.6	55
Old otjize	14	Nonanoic acid	158	438.6	10413559	mainlib	875	1411.3	250.19	129
Old otjize	43	Octadecane, 2,6-dimethyl-	282	679.5	2384702	mainlib	871	4300.6	44.645	197
Old otjize	57	Octadecane, 5-methyl-	268	763.5	1699388	mainlib	858	3532.1	37.806	225
Old otjize	65	Octadecanoic acid	284	852.2	21198306	mainlib	870	23640	32.716	284
Old otjize	66	Octadecanoic acid, ethyl ester	312	856.4	20811324	replib	681	6480.9	279.03	88
Old otjize	10	Octanoic acid	144	386.9	617783787	mainlib	934	3883.7	3223.1	60
Old otjize	87	Octanoic acid, 2-phenylethyl ester	248	1020.3	4853362	mainlib	619	2415.9	212.97	104
Old otjize	11	Octanoic acid, ethyl ester	172	394.2	2656708	replib	663	855.92	279.03	88
Old otjize	73	Oxalic acid, isobutyl hexadecyl ester	370	894.2	20741729	mainlib	796	3648.7	448.13	75
Old otjize	30	Pentadecane, 5-methyl-	226	586.9	2415608	mainlib	874	2635.3	80.874	168
Old otjize	70	Pentadecane, 8-hexyl-	296	881.3	597202	mainlib	865	945.86	49.249	196

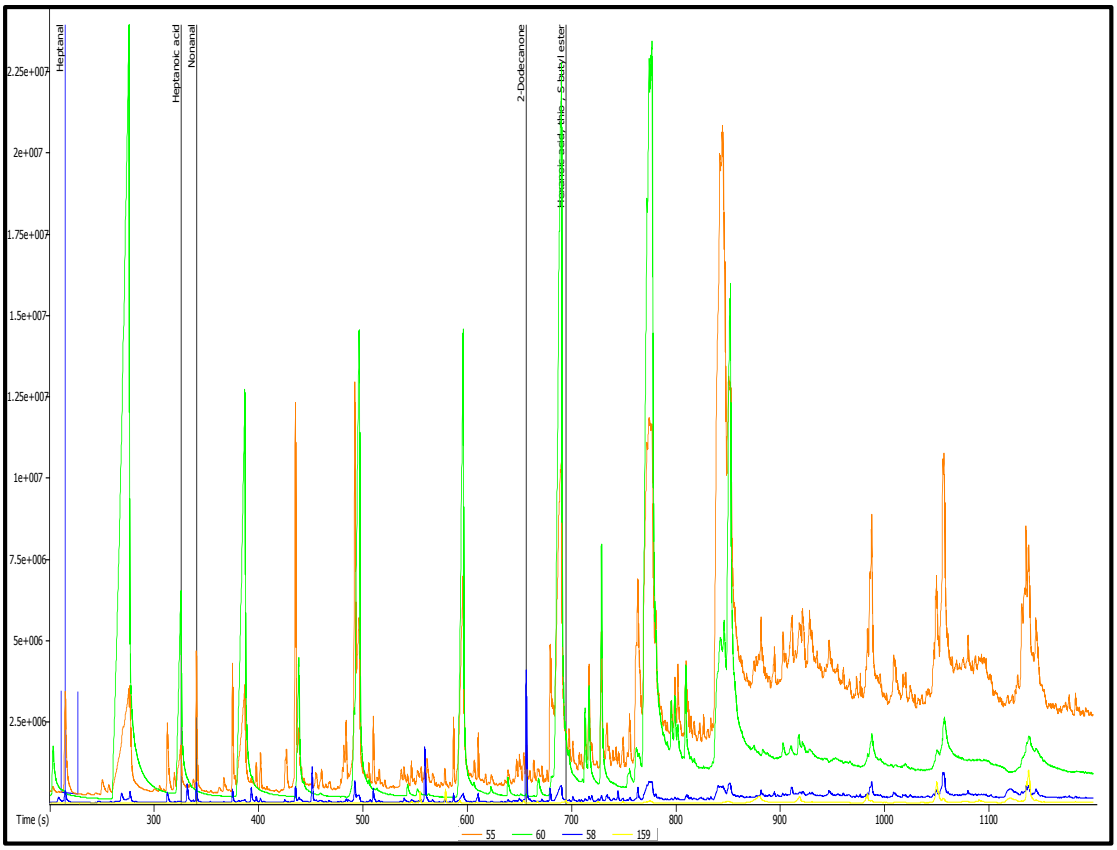

Figure E3: Chromatogram for old *otjize*

The chromatogram is expressed according to the unique mass of the identified compounds, heptanoic acid (60) Nonanal (55) 2-dodecanone (58) hexanoic acid (60) 2-nonanone (58) heptanal (55)

Table E8: Compounds identified by GC-MS for perfume

Sample	Peak #	Name	Weight	Library	R.T. (s)	Similarity	S/N	Area	UniqueMass
Aromatic sample mixed with clarified butter	81	Diisooctyl phthalate	390	replib	979	597	637.92	2586633	167
Aromatic sample mixed with clarified butter	18	Isolongifolene, 9,10-dehydro-	202	mainlib	499.4	827	1258.4	2849561	187
Aromatic sample mixed with clarified butter	34	trans-calamenene	202	mainlib	580.5	691	979.77	2977159	159
Aromatic sample mixed with clarified butter	10	Terpinen-4-ol	154	replib	392.9	902	416.32	3472473	111
Aromatic sample mixed with clarified butter	4	Hexanoic acid, ethyl ester	144	replib	271.9	874	659.74	3735400	88
Aromatic sample mixed with clarified butter	13	Benzene, 2-methoxy-4-methyl-1-(1-methyle	164	mainlib	423.6	889	963.99	4095085	149
Aromatic sample mixed with clarified butter	26	Humulene	204	replib	548.4	934	430.85	4193650	93
Aromatic sample mixed with clarified butter	1	à-Pinene	136	mainlib	235.3	954	495.04	4272645	93
Aromatic sample mixed with clarified butter	86	Unknown 2	258	mainlib	1013.9	428	2832.5	4345706	215
Aromatic sample mixed with clarified butter	16	Cyclohexasiloxane, dodecamethyl-	444	replib	449.1	843	893.61	6766492	341
Aromatic sample mixed with clarified butter	59	Benzene, (1-methyldodecyl)-	260	replib	751.2	788	760.62	6810450	105
Aromatic sample mixed with clarified butter	66	Hexano-dibutyrin	330	mainlib	793.5	723	524.11	6919420	99
Aromatic sample mixed with clarified butter	17	2-Undecanone	170	replib	452.6	880	821.04	7086908	58
Aromatic sample mixed with clarified butter	9	Cyclopentasiloxane, decamethyl-	370	replib	350.9	845	1093.8	7398571	267
Aromatic sample mixed with clarified butter	53	Benzene, (1-methylundecyl)-	246	replib	707.8	884	862.75	9272161	105
Aromatic sample mixed with clarified butter	54	Benzene, (1-pentyloctyl)-	260	mainlib	714.3	878	537.43	9286285	91
Aromatic sample mixed with clarified butter	71	Hexanoic acid, anhydride	214	replib	862.5	791	1044.7	9601904	99
Aromatic sample mixed with clarified butter	42	Benzene, (1-ethylnonyl)-	232	mainlib	644	866	618.46	9740933	91
Aromatic sample mixed with clarified butter	30	2-Tridecanone	198	replib	560.9	897	1324.6	1.1E+07	58
Aromatic sample mixed with clarified butter	68	(E)-9-Octadecenoic acid ethyl ester	310	mainlib	848.9	899	2117	1.1E+07	88
Aromatic sample mixed with clarified butter	3	á-Pinene	136	mainlib	264	939	1253.3	1.1E+07	93
Aromatic sample mixed with clarified butter	5	o-Cymene	134	mainlib	292.9	955	1335.4	1.1E+07	119
Aromatic sample mixed with clarified butter	60	Hexadecanoic acid, methyl ester	270	replib	754.6	901	998.44	1.1E+07	74
Aromatic sample mixed with clarified butter	62	2H-Pyran-2-one, tetrahydro-6-nonyl-	226	mainlib	760.9	868	1403.2	1.6E+07	99
Aromatic sample mixed with clarified butter	11	Benzene, 2-methoxy-4-methyl-1-(1-methyle	164	mainlib	414.2	932	4411.1	1.9E+07	149
Aromatic sample mixed with clarified butter	70	Ethanol, 2-ethoxy-	90	mainlib	861.1	831	1385.2	2E+07	59
Aromatic sample mixed with clarified butter	6	Limonene	136	replib	295.6	940	1823.2	2.2E+07	68
Aromatic sample mixed with clarified butter	27	rotundene	204	mainlib	552.7	870	8236.4	4E+07	108
Aromatic sample mixed with clarified butter	93	Squalene	410	replib	1071.2	869	1917.5	4.2E+07	81
Aromatic sample mixed with clarified butter	12	Benzene, 2-methoxy-4-methyl-1-(1-methyle	164	mainlib	417.7	947	9016.1	4.2E+07	149
Aromatic sample mixed with clarified butter	64	Hexadecanoic acid, ethyl ester	284	mainlib	781.7	895	13238	5.5E+07	88

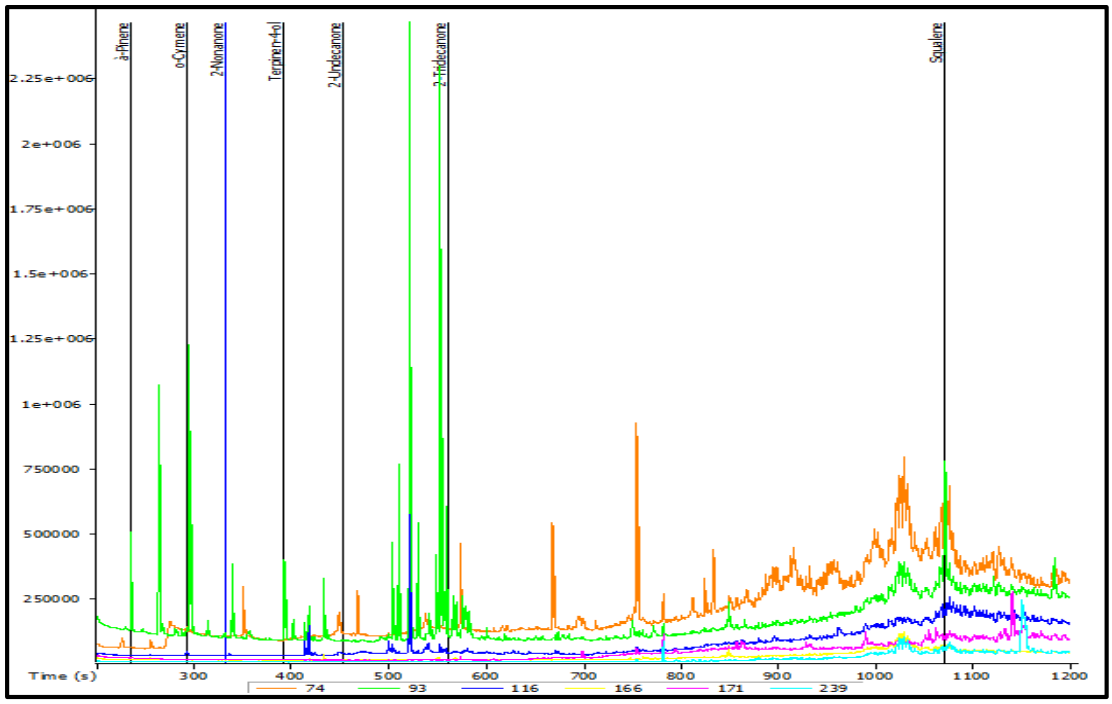

Figure E4: Chromatogram for perfume

The chromatogram is expressed according to the unique mass of the identified compounds, a-pinene (93), o-cymene (114), 2-undecanone (58) squalene (81) 2-tridecanone (58), limonene (68), terpinen-4-ol (111) and squalene (238)

Table E9: Compounds identified by GC-MS for black ground aromatic powder

Sample	Peak #	Name	Weight	Library	R.T. (s)	Similarity	S/N	Area	UniqueMass
Composite black ground aromatic powder	43	Pyrido[3,4-d]pyrimidin-4(3H)-one, 6,8-dime	175	mainlib	714.6	724	276.76	470682	175
Composite black ground aromatic powder	64	Hexasiloxane, tetradecamethyl-	458	mainlib	897.4	596	220.95	566695	221
Composite black ground aromatic powder	30	Benzaldehyde, 4-hydroxy-3,5-dimethoxy-	182	replib	652	748	345.79	599999	182
Composite black ground aromatic powder	5	Benzene, 2-methoxy-4-methyl-1-(1-methyle	164	mainlib	414.7	921	215.37	631712	149
Composite black ground aromatic powder	12	rotundene	204	mainlib	553.2	781	223.12	709007	108
Composite black ground aromatic powder	100	Unknown 11	199	mainlib	1193.6	481	283.65	758520	158
Composite black ground aromatic powder	56	Tetradecanoic acid, ethyl ester	256	replib	782.1	684	280.76	777473	88
Composite black ground aromatic powder	62	Heptadecane, 2-methyl-	254	replib	882	790	251.88	808896	114
Composite black ground aromatic powder	94	5,5,7,7-Tetraethylundecane	268	mainlib	1145	584	472.56	883564	382
Composite black ground aromatic powder	85	1-Monolinoleoylglycerol trimethylsilyl ether	498	mainlib	1056.7	503	351.68	947402	271
Composite black ground aromatic powder	34	Benzene, (1-pentylheptyl)-	246	mainlib	671.8	895	598.35	962979	175
Composite black ground aromatic powder	20	Dodecanoic acid, ethyl ester	228	replib	607.8	741	357.62	1060822	88
Composite black ground aromatic powder	76	Unknown 4	220	mainlib	995	452	754.28	1231872	204
Composite black ground aromatic powder	50	Hexadecanoic acid, methyl ester	270	replib	755.4	881	450.22	2570233	74
Composite black ground aromatic powder	15	Dodecanoic acid, methyl ester	214	replib	574	832	423.81	2606393	74
Composite black ground aromatic powder	97	Unknown 10	369	mainlib	1183.3	455	1648.3	2674214	296
Composite black ground aromatic powder	58	Eicosane, 2,4-dimethyl-	310	mainlib	841.4	740	353.78	3111038	85
Composite black ground aromatic powder	2	Limonene	136	replib	295.9	931	387.02	3496616	68
Composite black ground aromatic powder	37	Hexadecane, 4-methyl-	240	mainlib	679.8	860	415.46	3761565	85
Composite black ground aromatic powder	65	2-Hexadecanol	242	replib	927.4	600	303.81	3897963	73
Composite black ground aromatic powder	7	Cyclohexasiloxane, dodecamethyl-	444	replib	448.9	835	806.53	4084210	341
Composite black ground aromatic powder	70	1-Monolinoleoylglycerol trimethylsilyl ether	498	mainlib	969.2	596	265.14	4449887	73
Composite black ground aromatic powder	38	Benzene, (1-propylnonyl)-	246	replib	680.1	902	1386.6	1.3E+07	91
Composite black ground aromatic powder	78	1,9(Z)-Octadecadiene, 1-methoxy-	280	mainlib	998.9	523	2016.5	1.3E+07	215
Composite black ground aromatic powder	54	Dibutyl phthalate	278	replib	773.1	945	4523.2	1.5E+07	149
Composite black ground aromatic powder	42	Benzene, (1-methylundecyl)-	246	replib	708.4	880	2356.8	1.5E+07	105
Composite black ground aromatic powder	1	Cyclotetrasiloxane, octamethyl-	296	replib	255.5	925	2667.3	1.6E+07	281
Composite black ground aromatic powder	29	Benzene, (1-ethylnonyl)-	232	mainlib	644.5	857	1630.3	1.6E+07	91
Composite black ground aromatic powder	27	Benzene, (1-propyloctyl)-	232	mainlib	633.8	893	1943.4	1.9E+07	91
Composite black ground aromatic powder	90	3,3-Diethyltridecane	240	mainlib	1121.6	559	1442.6	1.9E+07	127
Composite black ground aromatic powder	32	Benzene, (1-methyldecyl)-	232	replib	662.7	883	3274.9	2.1E+07	105
Composite black ground aromatic powder	72	Undecane, 2-methyl-	170	replib	978.9	577	1138	2.1E+07	127

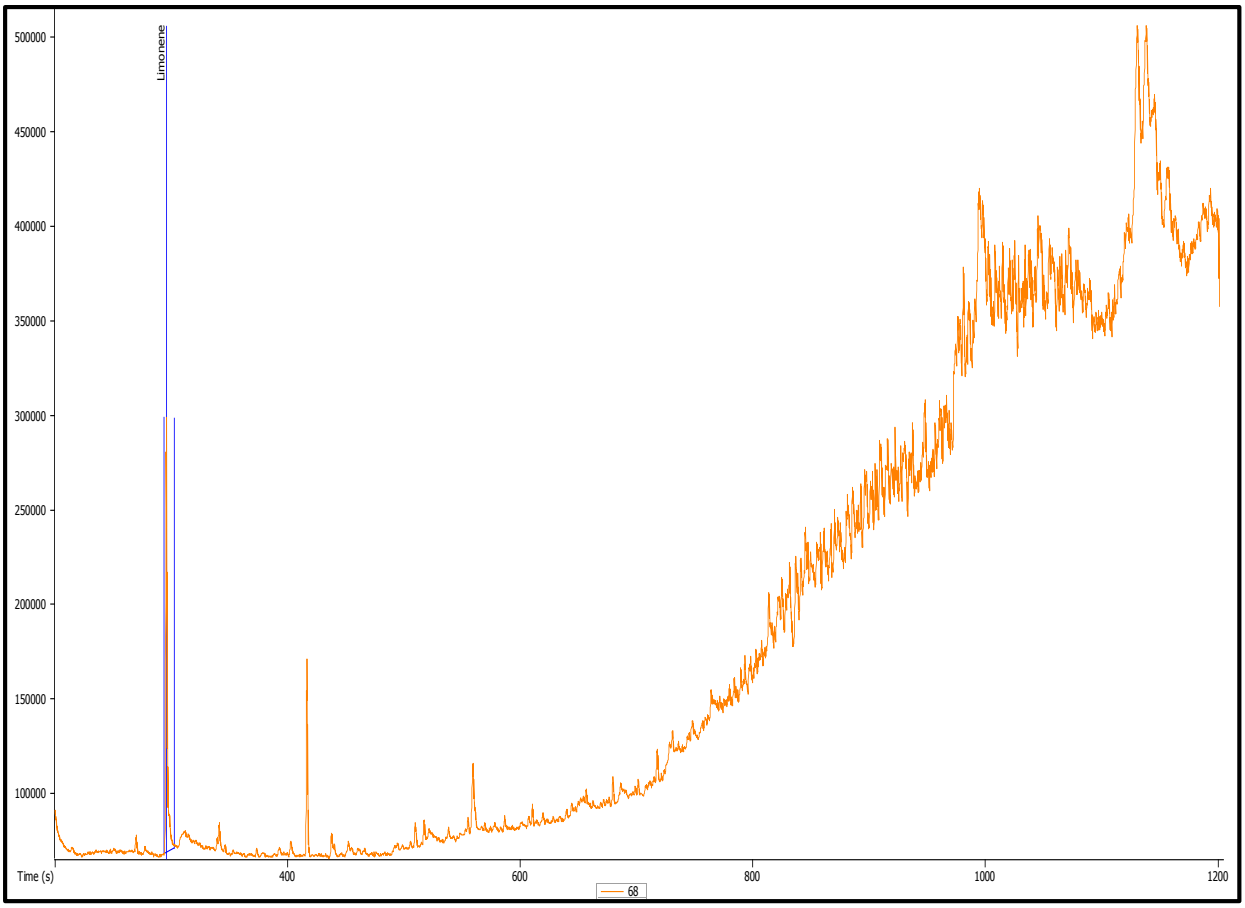

Figure E5: Chromatogram for black ground aromatic powder

The chromatogram is expressed according to the unique mass of limonene (239)

Table E10: Compounds identified by GC-MS for *otjizumba* mixed with *otjize*

Sample	Peak #	Name	Weight	Library	R.T. (s)	Similarity	S/N	Area	UniqueMass
Ochre and aromatic mixture from ochre container	90	Unknown 9	481	mainlib	1156.1	410	549.02	152773	481
Ochre and aromatic mixture from ochre container	53	n-Hexadecanoic acid	256	mainlib	947.7	752	719.73	163482	495
Ochre and aromatic mixture from ochre container	86	Unknown 8	481	mainlib	1136.6	374	646.9	196658	481
Ochre and aromatic mixture from ochre container	55	Unknown 1	481	mainlib	950.7	400	443.31	215945	407
Ochre and aromatic mixture from ochre container	92	Silane, tetramethyl-	88	replib	1158.8	515	662.71	289106	481
Ochre and aromatic mixture from ochre container	83	Unknown 7	478	mainlib	1116.3	414	982.89	290556	481
Ochre and aromatic mixture from ochre container	77	Hentriacontane	436	replib	1065.2	817	704.36	397106	233
Ochre and aromatic mixture from ochre container	75	Isosteviol acetate	360	mainlib	1060.9	567	594.95	406281	318
Ochre and aromatic mixture from ochre container	98	Unknown 11	407	mainlib	1184.2	418	730.25	496763	407
Ochre and aromatic mixture from ochre container	45	Octadecahydro-cyclopenta[e]pyrene	258	mainlib	808	583	747.39	536716	258
Ochre and aromatic mixture from ochre container	57	Unknown 3	208	replib	958.3	391	630.44	556073	257
Ochre and aromatic mixture from ochre container	39	Phthalic acid, butyl tridec-2-yn-1-yl ester	400	mainlib	733.7	820	450.08	1926043	149
Ochre and aromatic mixture from ochre container	76	Squalene	410	replib	1061.4	669	2130.3	2038901	344
Ochre and aromatic mixture from ochre container	49	Octadecanoic acid, ethyl ester	312	replib	859.1	523	588.49	2263379	88
Ochre and aromatic mixture from ochre container	51	Hexasiloxane, tetradecamethyl-	458	mainlib	941.3	660	1615.2	2479058	221
Ochre and aromatic mixture from ochre container	59	Phthalic acid, di(2-propylpentyl) ester	390	mainlib	964.7	688	846.25	2518407	167
Ochre and aromatic mixture from ochre container	58	Tryptamine	160	replib	958.6	573	952.23	2627306	130
Ochre and aromatic mixture from ochre container	33	2-Methyl-1-(ethyl(dimethyl)silyloxy)cyclohe	200	mainlib	566.2	585	1152	2881546	171
Ochre and aromatic mixture from ochre container	97	Cyclohexanone, 2,6-bis(2-methylpropyliden	206	mainlib	1183.3	525	1664.8	2945506	206
Ochre and aromatic mixture from ochre container	36	Tetradecanoic acid, ethyl ester	256	replib	699.5	815	730.54	3030140	88
Ochre and aromatic mixture from ochre container	73	Alloaromadendrene oxide-(1)	220	mainlib	1040.1	753	2594.9	3602782	287
Ochre and aromatic mixture from ochre container	40	Benzoic acid, 2-phenylethyl ester	226	mainlib	738.8	913	1194.4	7657364	104
Ochre and aromatic mixture from ochre container	41	Hexadecanoic acid, methyl ester	270	replib	755.7	863	935.96	8608470	74
Ochre and aromatic mixture from ochre container	63	Diisooctyl phthalate	390	replib	981.5	839	609.57	8797881	92
Ochre and aromatic mixture from ochre container	38	2,4-Dimethylpentan-2-ol decanoate	270	mainlib	717.6	517	444.75	10017622	155
Ochre and aromatic mixture from ochre container	13	Triethyl phosphate	182	mainlib	356.3	786	2487.3	10850630	155
Ochre and aromatic mixture from ochre container	22	Cyclohexanone, 2-(2-bromo-4,4,4-trichlorol	334	mainlib	486.9	718	947.11	11049156	98
Ochre and aromatic mixture from ochre container	12	Cyclopentasiloxane, decamethyl-	370	replib	350.9	857	1608.2	11286196	267
Ochre and aromatic mixture from ochre container	6	2,6-Dimethyl-1,3,5,7-octatetraene, E,E-	134	mainlib	271.7	892	589.6	11597823	119
Ochre and aromatic mixture from ochre container	7	2,6-Dimethyl-1,3,5,7-octatetraene, E,E-	134	replib	281	905	1067.1	12663810	134
Ochre and aromatic mixture from ochre container	9	Limonene	136	replib	296.1	942	1108.6	13139481	68
Ochre and aromatic mixture from ochre container	14	à-Campholenal	152	replib	359	894	1313.9	13509708	108
Ochre and aromatic mixture from ochre container	68	9,12,15-Octadecatrienoic acid, ethyl ester, (	306	replib	998.7	727	1195.3	13748744	94
Ochre and aromatic mixture from ochre container	74	Fumaric acid, myrtenyl octyl ester	362	mainlib	1050.8	748	775.64	14341144	92
Ochre and aromatic mixture from ochre container	47	2H-Pyran-2-one, tetrahydro-6-nonyl-	226	mainlib	846.4	857	1548.5	15254995	99
Ochre and aromatic mixture from ochre container	5	á-Pinene	136	mainlib	264.3	927	785.02	16044984	93
Ochre and aromatic mixture from ochre container	67	12,15-Octadecadiynoic acid, methyl ester	290	mainlib	989.3	710	2321.7	17224959	106
Ochre and aromatic mixture from ochre container	81	Hentriacontane	436	replib	1107.3	950	1469	46493890	85
Ochre and aromatic mixture from ochre container	64	Bis(2-ethylhexyl) phthalate	390	replib	981.9	916	38410	51235615	279
Ochre and aromatic mixture from ochre container	100	Squalene	410	replib	1190.6	758	1513.2	54003618	119
Ochre and aromatic mixture from ochre container	88	3,3-Diethyltridecane	240	mainlib	1145.4	540	2292.8	68666743	99
Ochre and aromatic mixture from ochre container	8	Benzene, 1-methyl-3-(1-methylethyl)-	134	replib	293.7	957	3796.3	90738626	119
Ochre and aromatic mixture from ochre container	2	à-Pinene	136	mainlib	235.6	957	4416.2	91432161	93

Table E11: Compounds identified by GC-MS for aromatic powder

Sample	Peak #	Name	Weight	Library	R.T. (s)	Similarity	S/N	Area	UniqueMass
Uncharred aromatic wood and bark fragments	20	Benzophenone	182	replib	638.8	830	301.58	494806	182
Uncharred aromatic wood and bark fragments	38	à-Pinene	136	mainlib	235.3	954	495.04	4272645	93
Uncharred aromatic wood and bark fragments	14	Quinoline, 2,7-dimethyl-	157	replib	592.3	593	245.8	631373	157
Uncharred aromatic wood and bark fragments	54	Tributyl acetylcitrate	402	replib	879.8	879	513.49	654607	186
Uncharred aromatic wood and bark fragments	79	1-Monolinoleoylglycerol trimethylsilyl ether	498	mainlib	1022.1	589	497.85	655351	429
Uncharred aromatic wood and bark fragments	69	5,5-Dimethyl-1-oxa-5-silacyclononanone-9	186	mainlib	942.4	531	208.15	708896	145
Uncharred aromatic wood and bark fragments	76	Oleic Acid	282	replib	997.9	768	235.65	713208	98
Uncharred aromatic wood and bark fragments	67	Hexasiloxane, tetradecamethyl-	458	mainlib	941	595	690.91	1101898	221
Uncharred aromatic wood and bark fragments	11	Dodecanoic acid, methyl ester	214	replib	574.2	785	224.44	1286522	74
Uncharred aromatic wood and bark fragments	74	Oleic Acid	282	replib	991.3	632	253.27	1348456	98
Uncharred aromatic wood and bark fragments	80	Oxacyclododecan-2-one	184	mainlib	1023.1	812	335.43	1387708	98
Uncharred aromatic wood and bark fragments	48	7H-Furo[3,2-g][1]benzopyran-7-one, 4-met	216	mainlib	828.7	881	204.79	2091814	50
Uncharred aromatic wood and bark fragments	12	Benzene, (1-butylhexyl)-	218	mainlib	581.1	803	207.64	2093749	91
Uncharred aromatic wood and bark fragments	2	o-Cymene	134	mainlib	293.5	934	253.78	2134496	119
Uncharred aromatic wood and bark fragments	16	Benzene, (1-methylnonyl)-	218	mainlib	614.9	911	387.98	2164816	105
Uncharred aromatic wood and bark fragments	24	Methyl tetradecanoate	242	mainlib	669.3	888	468.89	2341794	74
Uncharred aromatic wood and bark fragments	39	Hexadecanoic acid, methyl ester	270	replib	755.4	848	523.75	2346170	87
Uncharred aromatic wood and bark fragments	35	Phthalic acid, hex-3-yl isobutyl ester	306	mainlib	733.4	763	915.31	2800943	149
Uncharred aromatic wood and bark fragments	32	Squalene	410	replib	1061.4	669	2130.3	2038901	344
Uncharred aromatic wood and bark fragments	66	Oleic Acid	282	replib	938	658	235.25	2874496	73
Uncharred aromatic wood and bark fragments	46	Methoxsalen	216	mainlib	819.2	874	412.61	2893397	89
Uncharred aromatic wood and bark fragments	34	Benzene, (1-propyldecyl)-	260	mainlib	724.2	800	263.24	2926546	91
Uncharred aromatic wood and bark fragments	3	Cyclopentasiloxane, decamethyl-	370	replib	350.8	851	827.37	18700320	73

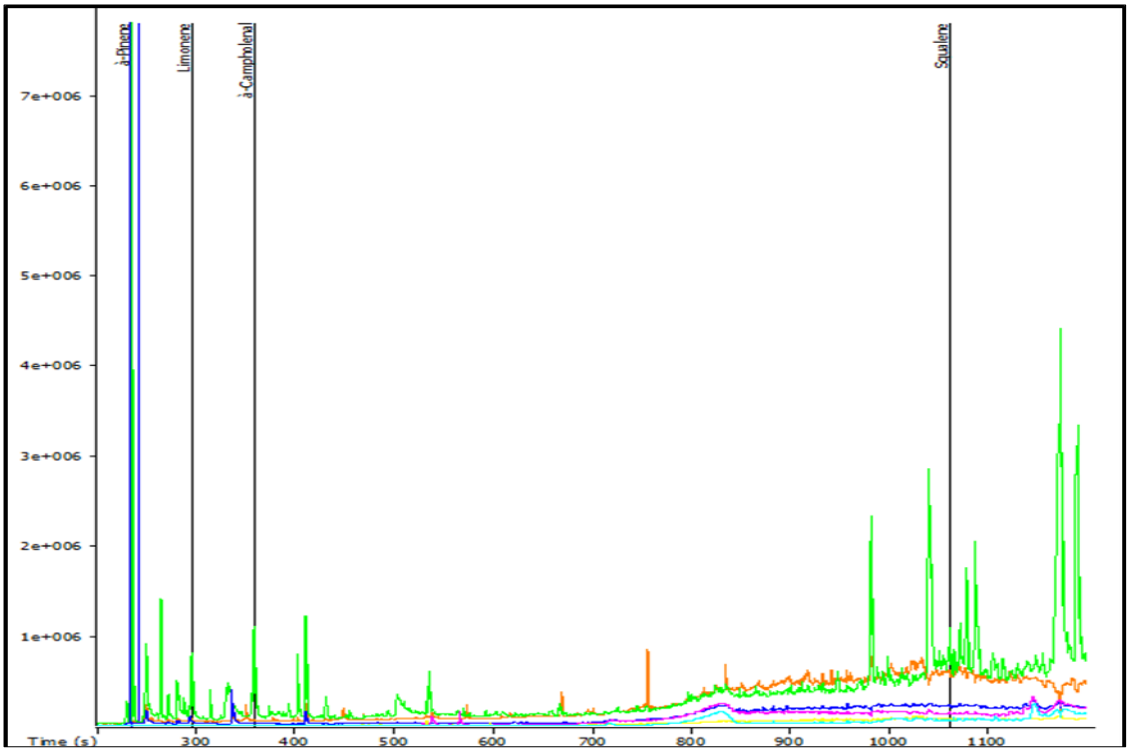

Figure E6: Chromatogram for aromatic powder

The chromatogram is expressed according to the unique mass of the identified compounds, a-pinene (93), o-cymene (114), 2-undecanone (58) squalene (81) 2-tridecanone (58), limonene (68), terpinen-4-ol (111), à-campholenal (108) and squalene (239)

Table E12: Compounds identified by GC-MS for aromatic seeds

Sample	Peak #	Name	Weight	Library	R.T. (s)	Similarity	S/N	Area	UniqueMass
Aromatic seeds from traditional ochre container	79	Unknown 8	478	mainlib	1099.4	375	396.61	100183	482
Aromatic seeds from traditional ochre container	61	Unknown 5	422	mainlib	997.1	377	466.96	165245	375
Aromatic seeds from traditional ochre container	39	1-Hexyl-2-nitrocyclohexane	213	mainlib	844.6	524	570.73	183623	475
Aromatic seeds from traditional ochre container	60	Oleic Acid	282	mainlib	996	651	654.84	244682	421
Aromatic seeds from traditional ochre container	22	1-Iodo-2-methylundecane	296	mainlib	655.1	731	314.86	275820	236
Aromatic seeds from traditional ochre container	32	DL-Arabinose	150	replib	823.4	614	321.83	306911	185
Aromatic seeds from traditional ochre container	53	Oleic Acid	282	replib	958.3	740	522.08	351409	257
Aromatic seeds from traditional ochre container	75	Hexasiloxane, tetradecamethyl-	458	replib	1068.6	649	355.44	591061	430
Aromatic seeds from traditional ochre container	45	Hexasiloxane, tetradecamethyl-	458	mainlib	898.1	556	452.43	614536	429
Aromatic seeds from traditional ochre container	69	1,3,6,10-Dodecatetraene, 3,7,11-trimethyl-	204	replib	1038.2	600	904.55	659396	299
Aromatic seeds from traditional ochre container	21	Unknown 1	415	mainlib	622.2	475	713.02	673047	415
Aromatic seeds from traditional ochre container	96	Squalene	410	replib	1175.9	716	312.94	731023	199
Aromatic seeds from traditional ochre container	42	n-Hexadecanoic acid	256	mainlib	865.7	660	859.44	788782	226
Aromatic seeds from traditional ochre container	57	1-Monolinoleoylglycerol trimethylsilyl ether	498	mainlib	982.4	683	653.36	794946	430
Aromatic seeds from traditional ochre container	70	9-Hexadecenoic acid, hexadecyl ester, (Z)-	478	mainlib	1044.9	522	637.54	801300	423
Aromatic seeds from traditional ochre container	20	Hexasiloxane, tetradecamethyl-	458	replib	617.6	652	530.92	959481	221
Aromatic seeds from traditional ochre container	100	Octadecane, 1-chloro-	288	replib	1190.7	596	386.81	1011634	187
Aromatic seeds from traditional ochre container	67	Oxalic acid, cyclohexyl dodecyl ester	340	mainlib	1028.5	527	393.3	1027693	100
Aromatic seeds from traditional ochre container	16	Squalene	410	replib	577.1	754	449.83	1035894	299
Aromatic seeds from traditional ochre container	76	9-Tetradecenal, (Z)-	210	mainlib	1078.1	798	312.46	1053149	125
Aromatic seeds from traditional ochre container	98	Tetrasiloxane, 1,1,3,3,5,5,7,7-octamethyl-	282	mainlib	1180.9	555	422.24	1285471	143
Aromatic seeds from traditional ochre container	36	9,12,15-Octadecatrienoic acid, methyl ester	292	mainlib	827.8	833	2804	1315480	292
Aromatic seeds from traditional ochre container	15	rotundene	204	mainlib	553.9	849	351.88	1339435	108
Aromatic seeds from traditional ochre container	64	Unknown 7	464	mainlib	1005.5	444	1022.9	1346788	407
Aromatic seeds from traditional ochre container	93	Unknown 9	176	replib	1168.1	479	493.43	1402204	243
Aromatic seeds from traditional ochre container	44	2,4,5-Trioxoimidazolidine	114	replib	884.1	626	569.45	1503916	114
Aromatic seeds from traditional ochre container	25	Pentadecanoic acid, methyl ester	256	replib	714.3	813	368.06	1917247	74
Aromatic seeds from traditional ochre container	97	Allyl Isothiocyanate	99	replib	1179.6	527	569.67	1956623	400
Aromatic seeds from traditional ochre container	54	Unknown 3	367	mainlib	958.5	494	769.14	2040675	130
Aromatic seeds from traditional ochre container	82	Spiro(1,3-dioxolane)-2,3'-(5'-androsten-16'-	332	mainlib	1113.4	586	1059.1	3826548	316
Aromatic seeds from traditional ochre container	58	Methoxyacetic acid, octyl ester	202	mainlib	989.6	653	950.58	4144474	112
Aromatic seeds from traditional ochre container	99	Unknown 10	383	mainlib	1186.2	475	2330.4	4192711	296
Aromatic seeds from traditional ochre container	86	Z-10-Tetradecen-1-ol acetate	254	mainlib	1120.9	592	495.93	4219951	98
Aromatic seeds from traditional ochre container	14	Humulene	204	replib	549.6	911	523.53	4269063	93
Aromatic seeds from traditional ochre container	41	7-Nonenamide	155	mainlib	864.7	613	564.63	4643050	59
Aromatic seeds from traditional ochre container	81	Heptacosane	380	replib	1108	845	707.89	4733322	113
Aromatic seeds from traditional ochre container	7	2-Decenal, (E)-	154	replib	438.2	796	1007.1	4984968	110

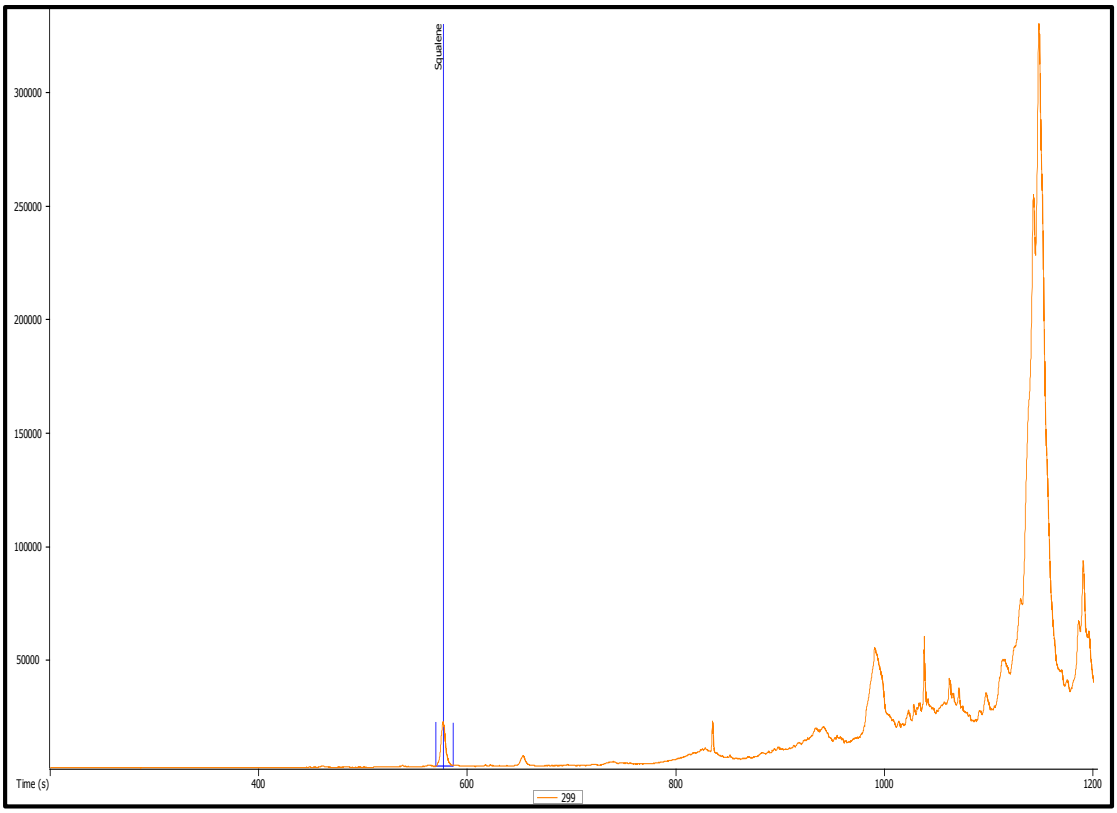

Figure E7: chromatogram for aromatic seeds

The chromatogram is expressed according to the unique mass of squalene (81)