EXTRACTION AND CHARACTERIZATION OF INSECT REPELLING ESSENTIAL OIL FROM PLANTS. A CASE STUDY CHAMOMILE, GARLIC AND CUSTARD APPLE.

BY

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A research project submitted in partial fulfilment of the requirement for the undergraduate degree in Bachelor of Science in

CHEMISTRY

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Declaration

Declaration by the Candidate

The project is my original work and has never been presented for a degree in any other university or for any other award.

KEMBOI J. LINDA

BS01/034/2013 Signature Date

Declaration by the Supervisor

This project has been forwarded with my approval as the University Supervisor

DR. ALOYS MOSIMA OSANO

Supervisor Signature date
Dedication.

This project is dedicated with profound admiration and appreciation to God almighty for giving me strength, My lovely Dad and Mum, my lovely sister and my lovely brothers for their moral support. Great appreciation also goes to my supervisors Dr, Alloys Mosima Osano and Mr. John Mining and all my lectures, course mate and the entire Maasai Mara University fraternity who made it a success through their constant support supervision, encouragement and moral support throughout out this project.
Acknowledgement.

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Secondly, am thankful to the friendly lab technicians in various laboratory I was able to interact with in the course of my project, with whom I worked with hand to hand during my practical sessions. Without their support this endeavour wouldn’t be a success.

Last but not least am grateful to my fellow students and course mates; Christine Nyaundi , and Olal for cooperation in many activities that made me grow in experience more so to those who contributed ideas to aid me in this understanding.
Abstract

Plant-based repellents have been used for generations in traditional practices as a personal protection measure against host-seeking insects like mosquitoes. Knowledge on traditional repellent plants obtained through ethnobotanical studies, is a valuable resource for the development of natural and environmental safe products. Recently, commercial repellents containing plant-based ingredients have gained increasing popularity among customers. These are commonly perceived as ‘safe’ in comparison to long-established synthetic repellents, although this is sometimes misconceptions. To date, insufficient studies have followed standard with pesticides evaluation scheme guideline for repellent testing. There is need for further standardized studies in order to better the evaluation of repellent compounds and develop new product that offer high repellence as well as good customers safety. In this study, essential oils were extracted from chamomile, garlic and custard apple as insect repellent. The plants samples were randomly collected from the market places where they are available, packaged and brought to the laboratory ready for extraction and analysis. The essential oils as insect repellents was extracted from camomile, garlic and custard apple using hydro distillation, the essential oils was characterised and quantified using FTIR. Also, the insect repelling properties of the extracted oils was determined using a double choice method. The percentage yield for the essential oils from the three species was calculated from the relation between the essential oil mass obtained and the raw material used. FTIR analysis of the extracted essential oils showed the presence of various functional groups. The field repellent results indicated difference in susceptibility of volatile chemicals among the species of the tested insects.
# TABLE OF CONTENT

Declaration .................................................................................................................................................. ii  
Dedication .................................................................................................................................................. iii  
Acknowledgement ......................................................................................................................................... iv  
Abstract ...................................................................................................................................................... v  

## CHAPTER ONE: INTRODUCTION

1.0 Background of the Study ................................................................................................................................. 1  
1.1 Repellent Properties ........................................................................................................................................ 2  
1.1.1 Chamomile *(Chamaemelum nobile)* ............................................................................................................ 3  
1.1.2 Garlic *(Allium sativum)* .................................................................................................................................. 5  
1.1.3 Custard Apple *(Annona squamosa)* ............................................................................................................. 6  
1.2 Statement of the Problem .................................................................................................................................. 8  
1.3 Objectives ......................................................................................................................................................... 8  
1.4 Significance of the study .................................................................................................................................... 9  
1.5 Justification ...................................................................................................................................................... 9  

## CHAPTER TWO: LITERATURE REVIEW

2.0 SYNTHETIC PRODUCTS ................................................................................................................................. 10  
2.1 INSECT REPELLENTS OF NATURAL ORIGIN ............................................................................................ 11  
2.2.1 Osage orange ............................................................................................................................................... 13  
2.2.2 Catnip ........................................................................................................................................................ 14  

## CHAPTER THREE: METHODOLOGY

3.0 Distillation ....................................................................................................................................................... 15  
3.2 FTIR Analysis .................................................................................................................................................. 18  
3.3 Bioassay .......................................................................................................................................................... 19  
3.3.1 Field test ...................................................................................................................................................... 20  
3.3.2 Attractant/repellent bioassay for housefly .................................................................................................. 20  

## CHAPTER FOUR: RESULTS AND DISCUSSIONS

4.0 Extraction data ................................................................................................................................................ 22  
4.1 FTIR Data ....................................................................................................................................................... 24  
4.1.1 Chamomile essential oils FTIR analysis ........................................................................................................ 24  
4.4.2 Garlic essential oils FTIR analysis .............................................................................................................. 26  
4.2 Repellence data .............................................................................................................................................. 28  

## CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.0 Conclusion .................................................................................................................................................... 31
5.2 Recommendations ........................................................................................................32
REFERENCES..................................................................................................................33
Appendix A. Experimental run Data Analysis..................................................................37
A.1 FTIR Data...................................................................................................................37
   A.1.1 FTIR spectrum of garlic essential oils .................................................................37
   A1.3 FTIR Spectrum for chamomile essential oils .......................................................39
CHAPTER ONE: INTRODUCTION

1.0 Background of the Study

Among the multitude of plants species, some are known as aromatic because of volatile compounds they contain, which gives them scent and characteristic flavour. Aromatic plants are closely associated with origins of western civilization, many of which have been mentioned for their uses as spices, pot herbs and medical plants, additionally these plants have found a wide application in perfumery, cosmetic and detergents industries, pharmacology and fine chemistry as well as aromatics for the food industry. (Regnault-Roger & Hamraoui, 1995). One of the fraction of aromatic plant, mostly used for industrial application and which have shown the promise for insect pest management (IPM) is the fraction of volatile fragrance compounds commonly known as essential oils.

Therefore, essential oils can be defined a fragrant essence of plants, usually volatile obtained from an odoriferous, single species of plant. Most essential oils are primarily composed of terpenes and their oxygenated derivatives and are obtained by steam distillation or solvent extraction of different parts of aromatic plant including, buds, flowers, leaves, seeds, stem, bark etc.

Essential oils of many plant species have been found to have toxic and repellent effect against different insect (Curtis, J.B, L., & A, 1991). Insect repellent is an alternative to the use of insecticides. This insect repellent may be applied to the skin to protect an individual from the bites of mosquitos, mites, ticks, lice’s etc. or it can be used to exclude insect from the area of interest such as in packaging to prevent infestation of stored products (Bakkalie, Averbeck, & M, 2008). With increasing problem of insecticides resistance, and the public concern regarding pesticides safety, the coming of new, safer, active ingredients are becoming
necessary to replace the existing compounds in the market. Natural product is a perfect alternative to synthetic insecticides as a means to reduce negative impact to human health and the environment.

Essential oils are usually obtained via steam distillation of aromatic plant especially those used in fragrance and flavouring in the perfume and the food industry respectively and more recently for aromatherapy and as herbal medicine. Plant essential oils are produced commercially from several botanical sources, many of which are members of mint family (Lamiaceae). In recent years, several essential oils have been founded to have repellent properties, these include citronella, cedar, verbena, geranium lavender, pine, cinnamon, rosemary, basil, thyme and peppermint.

Therefore, the purpose for this study is to extract, characterise essential oils from different plant species and to evaluate their repellence against crawling insects.

1.1 Repellent Properties.
Repellent are by their physical and chemical properties liquid substances, with a characteristic scent that evaporates at room temperature the relation between chemical structure of the substances and repellent efficacy is still under because repellent belonging to various group of chemicals compounds it is believed that the biological efficacy requires the presence of amide, imides, alcoholic and phenol groups of groups.

Efficacy of the repellent can also be assessed by determining their boiling points on which it evaporates and the active substance that affect the olfactory cells (some substances acting mechanically through contact of insect’s taste)

Volatile repellents molecules behave according to the Frick’s diffusion model and evaporates into atmosphere and penetrates into the skin. Due to this process, there is a decline in its concentration sink condition on the skin surface and the weakening of biological response.
Therefore, it is important to understand the mechanism of repellent from entomological and toxicological point of view. If the repellent evaporates faster from the application site then it is absorbed, it will be more effective and the duration of its response will depend on many external and internal factors. Absorbed through the skin, the repellent reaches the systematic circulation and are further transported in to their organs and tissue which can lead to adverse effect on the user’s body.

1.1.1 Chamomile (*Chamaemelum nobile*)

Chamomile also known as camomile a traditional medical herb native to Western Europe, India and western asia.it has become abundant in the United States where it has escaped cultivation to grow in pastures, cornfield roadsides and other places which are well drained. The generic name chamomile is derived from Greek ‘khamai’ meaning the ground and melon meaning apple. The official medical chamomile is the German chamomile which was considered a sacred herb by the ancient Saxons. The Egyptians valued the herbs as the cure for malaria and dedicated chamomile to their sun god. Two species of these sweet-scented plant Roman chamomile and German chamomile because of their similar appearance and medical uses.

Roman chamomile, *Chamaemelum nobile* is a member of the Asteraceae or daisy family. It is hairy, low growing perennial. Because of the creeping roots and compact mat like growth of these species it is sometimes called lawn chamomile. Roman chamomile releases a pleasant, apple scent when walked upon. It is used as a strewing herb during the Middle Ages to scent floors and passage ways in the home and to deter insects. This fragrant evergreen is a garden favourite. It also causes the physical effect on the other herb as a companion.

The health benefits of the camomile are thought to be many.it is believed to have been used to treat insomnia, skin problem as well as the temporary relief from asthma. It is sometimes
used to regulate menstrual cycle and to treat ailments specifically related to female productive organs. It is also claimed to treat cancer, sore throat, diabetes and also headache. It is probably known as stress reliever.

Planting and growing chamomile is not difficult. German chamomile can be planted directly from seeds in early spring, either broadcast or rows. The seeds need exposure of light in order to germinate. German chamomile is annual. It needs to be watered daily. At the perennial, it tends to grow in branches. They can be dug up periodically, separated at the roots and planted in new regions. Roman chamomile is very hardy and it is always found in lawn and it is sometimes seen as a wide plant. It can also be planted as ground cover.

Figure 1.1 Chamomile plant
1.1.2 Garlic (*Allium sativum*)

Garlic is a strongly aromatic bulb crop that has been cultivated for thousands of years. Garlic is species of plant of the genus *Allium* which includes onions, leeks and chives among others species. The name garlic come from the Anglo-Saxon word ‘garlie’ meaning ‘spear’ in reference to its spear shaded leaves.

<table>
<thead>
<tr>
<th>Class</th>
<th>Equisetopsida</th>
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<tr>
<td>Subclass</td>
<td>Magnollidae</td>
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<tr>
<td>Order</td>
<td>Asparagales</td>
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<tr>
<td>Family</td>
<td>Amaryllidaceae</td>
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<tr>
<td>Genus</td>
<td>Allium</td>
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<tr>
<td>Species</td>
<td>Sativum</td>
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</table>

The garlic plant is believed to have originated from a wild ancestor (*A.longicuspis*) in the south central Asia, an area occasionally referred to as the garlic crescent.

Garlic has been cultivated in middle- east and far- east and such as been the valuable source of food and medicine, as well as prised component of specific rituals, for many different societies. Like onions and other alliums, garlic bulbs that develop entirely underground and the plant may or may not spring. Garlic may develop in a wide assortment of different soils, yet it grows best in rich, deep loams with minimum amount of water.

Garlic has long been known for its medical properties. Garlic is prominent remedy for many ailments ranging from common cough and cold to stomach problems. It is also believed that garlic expelled intestinal worms and skin parasites, protected against venomous animals, neutralised internal and external inflammations, relieved toothaches.
1.1.3 Custard Apple (*Annona squamosa*)
Custard is a shrub or a small tree of American origin. The species is widely grown as a commercial fruit tree both within its native range and in tropical region around the world, and although less common than other Annona species production area are increasing. The species is listed as cultivation escape, naturalised weed. In global compendium of weeds (Randall, 2012) it is known to escape cultivation, often naturalizing and even becoming invasive in some places.

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae</th>
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<tr>
<td>Phylum</td>
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<td>Class</td>
<td>Dicotyledonae</td>
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<td>Order</td>
<td>Annonales</td>
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<td>Genus</td>
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<tr>
<td>Species</td>
<td>Annona squamosa</td>
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</table>
Annona squamosal is indigenous to tropical South America and West Indies according to Wester (1912).

Custard apple is popular seasonal fruit with great economic importance in a number of countries. It is also used as an agroforestry species as a source of food and for honey bees’ cultivation. The fruit is also utilised commercially as flavouring for ice cream and can also be made into sherbet.

The leaves, bark, roots, seeds and fruit of Annona squamosal have various important medical uses. The green fruit and seeds have effective veridical and insecticidal properties and are used as astringents in a diarrhoea and dysentery. Crushed leaves are applied as an effective cure for ulcers and malignant sores. A poultice from fresh leaves is used for dyspepsia and when mixed with oil is used for diseases of the scalp. Crushed leaves are applied to the nasal area in case of fainting spells. The astringent bark, leaves and unripe fruits and seeds can be used as a source of the alkaloids anonaine (R.S., 1975). An extract is also used as a drastic purgative (Coronel R.E., 1983).
1.2 Statement of the Problem.

Plant-based repellents have been used for generations in traditional practices as a personal protection measures against host seeking insects like mosquitoes. Knowledge on traditional repellent plants obtained through ethnobotanical studies, is a valuable resource for the development of natural and environmental safe products. Recently, commercial repellents containing plant based ingredients have gained increasing popularity among customers. These are commonly perceived as ‘safe’ in comparison to long-established synthetic repellents, although this is sometimes misconceptions. To the date insufficient studies have followed standard with pesticides evaluation scheme guideline for repellent testing. There is need for further standardized studies in orders to better the evaluation of repellent compounds and develop new product that offer high repellence as well as good customers safety. The current study is geared towards extracting, and characterising essential oils from camomile, garlic and custard apple, and finally determine its insect repelling ability.

1.3 Objectives

Main objectives

To extract and characterise essential oils from camomile, garlic and custard apple, and analyse its insect repelling ability.

Specific objectives

- To extract essential oils from camomile, garlic and custard apple plants using hydro distillation.
- To characterise and quantify essential oils from camomile, garlic and custard apple plants using FTIR.
To determine the insect repelling properties of essential oils from camomile, garlic and custard apple using double choice method.

1.4 Significance of the study

The study produces new environment friendly and eased used product as insect repellent.

This study therefore will reduce public health problems associated with asthma, and also it will help reduce insects such as cockroaches and houseflies.

1.5 Justification

The existing commercial products are poisonous and they degrade the environment. Most insects have grown resistance to, also these commercial products are expensive, and therefore this study is geared to come up with cheap, environment friendly and sustainable products as this is a green insect repellent.
CHAPTER TWO: LITERATURE REVIEW.

2.0. SYNTHETIC PRODUCTS

The use of repellents compounds date back to antiquity when various plant oils, smoker, tar etc. were used to displace or kill insects. Before the second world war, there were four principle repellents; oil of the citronella, which sometimes used for hair dressing for head lice, dimethyl phthalate discovered in 1929 and Rutgers612, which become available in 1939. At the outbreak of world war 2 the latter three components were combined into formulation for use by the military.

Other military repellent formulae for use on clothing were developed during the war, but they failed to provide desired protection of military personnel deployed around the world. As the result by 1956, the United States government had screened over 20,000 potential mosquito’s repellent compound. In 1953 the insect repellent properties of N-N diethyl-Toluamide (DEET) was discovered and the first DEET produce was introduced in 1956 (Dogan, 1999). DEET is still the most widely used mosquito repellent. In short it has been regarded safe, but toxic effect has been recorded including 5encephalopathy to children, urticarial syndrome ((Howard I. M, 1985)) anaphylaxis, hypertension and decreased heart rate.

Several other compounds have been evaluated for repellence activities but none have had the commercial success of DEET.A13-3720 OR 1-3 cyclohexane-Ltyl carbonyl 2 methyl piperidine CIC4 or 2 hydroxyl methyl cyclohexyl acetic acid lactone(Frances 1998) and A13-35765 or 1-3 cyclohexane -1-yl carbonyl pyridine ((Klun J A Ma, 2000)) and references in have reported to have activity similar to DEET against several important species of diptera and acarina. Permethrin is used in mosquito bed net(( Robet L.L, 1991) and permethrin and cypermtrin has been shown to repel to western subterranean termine. Retcticulitermes heserus
(Rust M. K, 1993). However, the acute toxicity of pyrethroids to insect is the principle mode of action.

2.1 INSECT REPELLENTS OF NATURAL ORIGIN

Natural ingredients are included in some formulations of repellents of 65 formulations of non-US products insect repellents, 33 contain DEET and the reminder contains natural oils (Leonhardt B.A, 1991) of all substances (872 synthetic and 29 botanical oils) tested for repellence to four species of domiciliary cockroaches by US Department of Agriculture between 1953, and 1974, 127 repelled 94% or more of German cockroaches 61 repelled 100% of all four species tested. None of those 13 was a botanical extract but many was analogy of natural products 1-4 naphthoquinone has many substituted forms that occur in nature (klun el al 2000) found that anopheles mosquito was differtially by isomer of some piperidines.

In US, citronella, is a popular botanical ingredient insect repellent formulation. The insecticidal properties of this oil were discovered in 1901, and it was used for time as a hair dressing for the control of fleas and lice. Despite popular conceptions (Lindsay L.R., 1996) reported that citronella candles of incense were ineffective for reducing the biting of mosquitoes in the field as a citronella candle three commercial products were recently evaluated for repellence in laboratory olfactometer against aedes aegypti. Buzz away containing [citronella, cedar woods, eucalyptus and lemon grass] green ban [containing citronella, cajuput, and lavender safrole- free sassafras peppermint and bergaptene bergamot oil] and skin soft [consisting many types of oils and stearates] failed cause any repellence in the olfactometer through DEET procedures (Chou J.T, 1997).

Neem oil, from Azedarach Indica, when formulates as 2% in coconut oil yielded absolute protection for instance no bites, for twelve hours from anopheles’ mosquito (Sharm el at
A neem extract proprietary product AG1000 has been indicated to be repellent to the biting midge which can spread cattle diseases (Braverman y.Chizov-Ginzburg, 1999).

Quwenling, a popular eucalyptus based repellents product, consist of a mixture of p-methane-3-8 diol(PMD) isopulegone and citronella quwenling has largely replaced dimethyl thiolate as an insect repellent of choice in china (Trigg J.K, 1996)). Eucalyptus oil itself, the ingredients of which PMD gave protection comparable to DEET in repelling anopheles mosquito In field studies (Trigg J.K, 1996) however repellent from culicoides inpectatus eucalyptus oil was attractive to C. imicola (Braverman y.Chizov-Ginzburg, 1999).

A common exercise is to place a red cedar block or sachet to repel clothing moths. This is probably why many chest drawers are made of red cedar for protection on heirloom clothing. Processed red cedar flake boards were found to be repellent to the German cockroach, but not to American brown banded cockroach (Appel A. G., 1989).

Over the past several years the laboratory has performed research investigating insect repellents of natural origin. Insect repellents for protection of human being from biting arthropod, mainly mosquitos make up to the lion share of insect repellents solid in the united states.it is believed that many applications of insect’s repellent technology are under-utilized at this time. The use of repellent barrier strips to prevent any entry of insects into sensitive areas is largely untried approach. Parathyroid insecticides re sometimes used in the manner, but the acute toxicity of these compounds to insects is the principle mode of action of these compounds. Also, impregnation of repellents into packaging to prevent insect infestations of stored or shipped products is also not commonly used. The recent research therefore addresses some of the methods and materials that may be employed in screening potential new repellent in novel application.
The recent research has focused on insect repellents derived from plant species, the Osage orange (*maclura pommifera*) and catnip (*Nepeta-cataria*).

### 2.2.1 Osage orange

The fruit of the orange has been utilised as an insect repellent for many years. Pioneers in the America West placed the ripe fruit of these tree cupboard to repel cockroaches and other insects (S, 1991). The scientific validity of this well popularised practice has been little studied, (Karr, 1991) found the fragments of Osage orange fruit as well as its hexane and methanol extract, were significantly repellent to the cockroaches. Later research in laboratory showed that the dichloromethane extract of Osage orange was also repellent to the maize weevils.

The previous study made no attempt to determine volatile components of the Osage components extract, volatility is viewed by many as being the essential to repellent activity, although a compound irritating to the feet of the insect will cause the insect to spend time less no the treated area. The next study employed the use of gas chromatography and gas chromatography coupled with mass spectrometry to identify volatile compounds of Osage orange essential oils, and test the oil and its constituents in repellence trial. Numerous sesquiterpenoids were determined to be present in the oil, and many of them were repellent to the German cockroaches, and this was the first examinations of volatile compounds of Osage oil. Because all the components identified are well known and some are available from other sources and because there is only a small quality of essential oil in the Osage orange, it may be more economical to extract active compounds from other sources. Whether the compounds are synthetically enhanced in mixtures or if the compounds would be effective singly is currently under investigation.
2.2.2 Catnip

Catnip has been noted for many years for the intoxicating effect on cats. Nepetalactone has been associated at the active effect on the catnip with two isomers being present in the plant essential oil Cis and Trans (E, Z) modern nomenclature denotes that Cis –trans catnip has traditional applications as an insect repellent, some of which has been confirmed scientifically.

Hot water extract from catnip prevented fleas, beetles in one study and fresh catnip repelled black ants (Riotte, 1975) and has been found to be repellent to member of 13 families of insects (Eisner, 1964)

Nepetalactone is also an important component of defensive secretions of the coconut tick (Smith J.L., 1979) and lubber grasshopper (Snook, 1993). It was found that the E, Z isomers of nepetalactone was more active than the Z, E isomers. The structures of the nepetalactones isomers differ on in the orientation of single chemical bond. E, Z isomers has a greater action than the Z, E isomer at the receptors. Very little is known however, about the receptors responsible for the repellent responses in cockroaches. It is not known if the receptor specific for repellent even exist. In all likelihood, the receptors involved are specific for other compounds and the action of repellents at these receptors is secondary.
CHAPTER THREE: METHODOLOGY.

3.0 Distillation

Hydro distillation has been used for hundreds of years and today remains one of the most favourably methods of extracting essential oils. The technique of hydro distillation permits the separation of volatile components from non-volatile materials without raising temperature of the distillation above 100 °C.

The steam carries essential oils to a condenser and then re-liquefies and the lighter essential oils float on top. The water and the oil is then separated and the water portion is referred to as the hydrosol, hydrolat or flower of floral water, and the oil portion, is the essential oil.

Plant materials

The plants custard apple, garlic and chamomile will be collected from local market around Maasai Mara University in January 2017.

Reagents and Apparatus.

500 ml round bottomed flask (distillation pot)

Separating funnel

Conical flasks

Measuring cylinder

Test tubes and test tube racks

Universal indicator

Weigh balance

Claisen connecting tube
Aluminium foil.

Funnel

Source of heat

Water clamp and stand

Dichloromethane

Custard apple seeds

Chamomile

Garlic

In this research, the separation process chosen is hydro distillation. Essential oils was extracted from each plant by hydro distillation, 125 grams to 750 grams of fresh plant material will be cut into small pieces and placed in distillation flask with approximately five times as much water, and ten glass beads.

Vaporised water and essential oil

Vapour is cooled, steam condenses into water and essential oils which do not mix

Essential oils

Flower water
3.1 OIL SEPARATION

Essential oils and water condensate are known to have different densities and form an immiscible two liquid phase mixture at room temperature. The separation of essential oils from condensate hence utilises this densities and immiscibility advantages for the two to be separated from each other.

** Determination of the mass of the decanter contents. **

In separating the water from the oil, the water later was carefully run out from the bottom of the separating funnel by opening the tap until the meniscus is at the calibration mark. The content that will remain inside the separating funnel was oils.

**Procedure.**

1. The collected plants samples was washed to remove dirt on the surface of the sample, it is also to make sure no any other impurities are present.

2. The excess moisture on the samples surface was absorbed using paper towel. The extraction was carried out only on fresh samples.

3. 200g of fresh samples was weighed. Plant samples will be cut into small pieces using scalpel then the cut plant samples will be poured into reweighed beaker as to obtain the exact mass.

4. The small pieces of plant samples was poured into 500ml round bottomed flask and 250ml of distilled water was added and is then allowed to soak for 15 minutes as the distillation apparatus is being set

5. Heating is initiated with heating mantle; cooling water was sent to the condenser and the cloudy distillate was captured.
6. After 2 hours’, hydro distillation was shut down and the distillate will be transferred into separating funnel. Extraction was done using 10ml methylene chloride. If the two layers fails to separate out 2ml of saturated sodium chloride was added and the distillate was swirled gently.

7. A large size of test tube was weighed. A portion of decanted organic layer will be transferred into weighed test tube, a steam of air was used to evaporate off the solvent.

8. The test tube containing the sample was reweighed and the mass is determined for the recovered oil. Percentage recovery of oil was then calculated from the amount of plant species used.

9. The number of distillation session depended on the yield of essential oil. The extracted oil was stored refrigerated in about 4 degrees until their insect repellent activities was tested.

3.2 FTIR Analysis.

The FTIR spectrum of essential oils was obtained using Spectrum FTIR in Maasai Mara University and the functional groups was determined with the help of IR correlation chart. The IR spectra was reported in percentage transmittance. The wavelength region for analysis was 4000-400 cm\(^{-1}\) (in the mid infrared range).

The flow sheet for the extraction of essential oils is summarized below:
3.3 Bioassay

Is a type of scientific experiment that involved the use of animals, tissues or a bacteria with the essence of determining the biological activity of substance such as hormone or drug. This kind of experiment are usually conducted to measure the effect of substance on living organism and are essential in the development of new drugs and monitoring environmental pollutants. This biological assay was performed against German cockroaches and houseflies.

**Apparatus**

Clear bottles

Scalpel

Food substance

Cockroaches

Houseflies

Vaseline
3.3.1 Field test

A clear plastic bottled with the open top was employed in the test for repellence. All the walls for the four bottles was smeared with Vaseline to prevent escape of cockroaches. The food (substrate) was placed at the middle of the middle of the bottle. The test repellent was applied by dropping it on the food substance whereas controlled experiment was untreated with the essential oils repellent.

The adult cockroaches was caught and released to each smeared bottles containing three different repellent at equal dosage. The bottles was placed in the house surrounded by cloths and curtains to keep the dark environment and to prevent disturbance from the surrounding. The cockroaches located in the treated and controlled area was carefully observed for 3 hours after treatment.

Repellence against cockroaches was calculated with the following equation

\[ \text{Repellence } \% = 100 - \frac{\text{T} \times 100}{\text{N}} \]

Where T stands for number of cockroaches located in the treated area

N stand for the number of total cockroaches.

3.3.2 Attractant/repellent bioassay for housefly

Clear, flat container was cleaned using distilled water and dried using paper towels to remove excess moisture. 10 grams of food substances was weighed and placed on each container, one drop of essential oil was put in each container containing food substance. In another container with 5 grams of food, no treatment was applied and therefore it was used as a control,

The assay was replicated with an increase if the amount of each essential oil used. The number of flies attracted to each container containing the food substance treated with
essential oils as well as the control experiment were counted after every interval of 30 minutes for a period of 2 hours for the calculation of percentage repellence. The results was expressed in terms of attraction/ repulsion (%R) were calculated by using

\[ \text{%R} = \left[ \frac{100(C-T)}{C} \right] \]

Where C stand for the number of flies trapped in the controlled container,

T stand for the number of flies trapped in the treated containers.
CHAPTER FOUR: RESULTS AND DISCUSSIONS

4.0 Extraction data

The aim of this experiment was to extract the essential oils of three different plants species, garlic, custard apple oil and chamomile through hydro distillation. The quantity of the essential oils was determined by use of top loading analytical balance, which determine their mass within the accuracy of 0.01 gram.

<table>
<thead>
<tr>
<th>Time(min)</th>
<th>Solvent type</th>
<th>Solvent volume(ml)</th>
<th>Garlic mass(grams)</th>
<th>Collected volume of water +oil(ml)</th>
<th>Collected volume of oil(grams)</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>Water</td>
<td>200</td>
<td>50</td>
<td>160</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>Water</td>
<td>200</td>
<td>50</td>
<td>148</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>Water</td>
<td>300</td>
<td>100</td>
<td>167</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>Water</td>
<td>400</td>
<td>150</td>
<td>240</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>Water</td>
<td>400</td>
<td>200</td>
<td>300</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>Water</td>
<td>400</td>
<td>200</td>
<td>326</td>
<td>Total=4.6</td>
</tr>
</tbody>
</table>

*Table 4.1 Garlic essential oils extraction data*

<table>
<thead>
<tr>
<th>Time(min)</th>
<th>Solvent type</th>
<th>Solvent volume(ml)</th>
<th>Custard apple mass(grams)</th>
<th>Collected volume of water+ oil(ml)</th>
<th>Collected volume of oil(gram)</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>Water</td>
<td>200</td>
<td>20</td>
<td>150</td>
<td></td>
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<tr>
<td>120</td>
<td>Water</td>
<td>200</td>
<td>25</td>
<td>168</td>
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</tr>
<tr>
<td>120</td>
<td>Water</td>
<td>300</td>
<td>30</td>
<td>247</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>Water</td>
<td>400</td>
<td>50</td>
<td>316</td>
<td>Total=3.4</td>
</tr>
</tbody>
</table>

*Table 4.2 Custard apple essential oil data*

<table>
<thead>
<tr>
<th>Time(min)</th>
<th>Solvent type</th>
<th>Solvent volume(ml)</th>
<th>Chamomile mass (grams)</th>
<th>Collected volume of water+ oil(ml)</th>
<th>Collected volume of oil(gram)</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>Water</td>
<td>200</td>
<td>20</td>
<td>180</td>
<td></td>
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<tr>
<td>120</td>
<td>Water</td>
<td>200</td>
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</tr>
<tr>
<td>120</td>
<td>Water</td>
<td>300</td>
<td>30</td>
<td>220</td>
<td></td>
</tr>
<tr>
<td>120</td>
<td>Water</td>
<td>400</td>
<td>50</td>
<td>318</td>
<td>Total=2.3</td>
</tr>
</tbody>
</table>

*Table 4.3 chamomile essential oil data*
The total percentage of essential oils produced for garlic, custard apple seeds and chamomile were 0.7%, 2.72%, 1.8% respectively.

The yield of the essential oils for all the three species were calculated from the relation between the essential oil mass obtained and the raw material used in the extraction.

\[
\text{Percentage yield}\% = \frac{\text{Essential oils}}{\text{Raw material}} \times 100
\]

The essential oils obtained from all the plant species was pale yellow oily liquid with garlic and custard apple seed oil having a pungent smell, while chamomile produced very fragrant essential oils. The samples was weighed in different masses and different volume of distilled water and was extracted at different times.

Hydro distillation is one of the simple, oldest process for obtaining essential oils from plants and mostly used by small production of essential oils. In hydro distillation the plants is almost entirely covered with water as suspension in the flask which is placed in hot plate.

In hydro distillation, the surface contact between biomass and boiled water will cause less effectiveness to obtain high yield because some of the oil not condense (Mulvaney, 2012). The contact between the leaves seeds and flowers and the water may cause some of the essential oil to be trapped inside the water and does not leave as steam towards the condenser.

**Disadvantages**

- The process is slow and distillation time is much longer thereby consuming more power making the process uneconomical.
- Variable rate of distillation due to difficult control of heat
Extraction of plant species is not always complete.

Prolonged action of hot water can cause hydrolysis of some constituents of the essential oils such as esters, which react with water at high temperature to form acids and alcohols.

Not suitable for large capacity/commercial scale distillations and not suitable for high boiling hard roots/woody plants materials.

4.1 FTIR Data.

4.1.1 Chamomile essential oils FTIR analysis

FTIR is used to analyse the functional group like alkanes, alkenes, carboxylic acids, phenols, alcohols, nitro groups, amides, amines etc. present in plant extract. The graph obtained with peaks represent the presence of functional groups.

The FTIR spectrum of the essential oils obtained with hydro distillation using dichloromethane as extracting solvent. The band at ~1735.96 cm\(^{-1}\) present in IR spectrum belongs to C=O stretching vibration and is attributed to the presence of matricine. The C=O stretching vibration appears as an intense band between 1800-1600 cm\(^{-1}\) (Steven 1999). When the intense band at 1735.93 cm\(^{-1}\) is combined with two bands at ~1242.16 cm\(^{-1}\) (ester C-C-O stretch at 1238 cm\(^{-1}\) in figure 4) and 1028 cm\(^{-1}\) (at 1018.41 cm\(^{-1}\) in the spectrum in figure 4 ester O-C-C stretch), the spectrum follows the rule of three which is a feature of a saturated ester.

Furthermore, apigenin 7- glycoside feature also a C=O functional group in the chemical structure, therefore the functional group attribute the intense band to apigenin 7- glycoside as well, in addition to matricine. The band at ~1165.00 cm\(^{-1}\) and at ~1111.00 cm\(^{-1}\) is assigned to the di-cycloether since ether give rise to two or more band at 1210-1070 cm\(^{-1}\) (asymmetric C-O-C).
Lastly the FTIR spectrum of chamomile essential oils appear one sharp peaks positioned at 1450.47 cm\(^{-1}\). It is known that isopropyl and gem- dimethyl groups give rise to a split umbrella mode with two peaks in the IR spectrum positioned at 1385 cm\(^{-1}\) to 1365 cm\(^{-1}\) (Stevens, 1999). The splitting is caused by vibrational interaction between umbrella modes of the 2 methyl groups.

In addition to the peak at ~1396.46 cm\(^{-1}\) it also indicate the presence of a CH\(_3\) or CH\(_2\) or both groups. The chemical structure of \(\alpha-7\) bisabolol consist of an isopropyl group. Alternatively the band at 1452.47 and 1396.46 cm\(^{-1}\) is attributed to the isopropyl group of bisabolol. The band positioned 3000-800 cm\(^{-1}\) correspond to the asymmetric and symmetric C-H stretches (CH\(_3\) and CH\(_2\)). the band in the region 3500-3200 cm\(^{-1}\) belongs to the O-H vibrations (Andoni A, 2015)

<table>
<thead>
<tr>
<th>Frequency cm(^{-1})</th>
<th>Functional group</th>
</tr>
</thead>
<tbody>
<tr>
<td>717.52</td>
<td>Aromatic</td>
</tr>
<tr>
<td>1018.41</td>
<td>Cyclohexane ring, methylene</td>
</tr>
<tr>
<td>1111.00</td>
<td>Dicycloether</td>
</tr>
<tr>
<td>1165.00</td>
<td>Dicycloether</td>
</tr>
<tr>
<td>1342.46</td>
<td>Sulphonates</td>
</tr>
<tr>
<td>1396.46</td>
<td>Trimethyl or tert butyl</td>
</tr>
<tr>
<td>1450.47</td>
<td>Carbonate</td>
</tr>
<tr>
<td>1651.07</td>
<td>Imino alkenyl</td>
</tr>
<tr>
<td>1735.93</td>
<td>Aldehyde</td>
</tr>
<tr>
<td>2137.13</td>
<td>Isothiocynate</td>
</tr>
<tr>
<td>2600.04</td>
<td>Thiol</td>
</tr>
<tr>
<td>2831.50</td>
<td>Methyl ether</td>
</tr>
<tr>
<td>3402.43</td>
<td>Amide</td>
</tr>
</tbody>
</table>
4.4.2 Garlic essential oils FTIR analysis

From the FTIR of dichloromethane extraction of essential oils showed the presence of functional groups like hydroxyl, carboxylic and amide.

The broad peak at ~3000 cm⁻¹ is due to the O-H stretching of hydroxyl group which indicates the presence of polyhydroxy compounds such as flavonoids, non-flavonoids and saponins. The peak at 2831.50cm⁻¹ and 2854.65cm⁻¹ is due to the asymmetric stretching of C-H of aromatic compounds. The peak at 2121.70 cm⁻¹ indicate C≡C which corresponds to terminal alkynes (monosubstituted). The peak at ~1643.35cm⁻¹ is corresponding to C=C, C=N stretching which usually weak or strong if conjugated. The peak at ~1450 cm⁻¹ is corresponding to C=C-C which in turn indicate the presence of aromatic ring stretch. The peak at ~1273.02cm⁻¹ and 1134.14 cm⁻¹ indicates the C-O stretching strong alcohol ether and ester which in turn revealed the presence of flavonoids, tannins, saponins, and glycosides.

<table>
<thead>
<tr>
<th>Frequency cm⁻¹</th>
<th>Bond</th>
<th>Functional groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>3000</td>
<td>O-H</td>
<td>Hydroxyl</td>
</tr>
<tr>
<td>2831.50</td>
<td>C-H</td>
<td>Aromatic compound</td>
</tr>
<tr>
<td>2854.65</td>
<td>C-H, O-CH₃</td>
<td>Asymmetric</td>
</tr>
<tr>
<td>2121.70</td>
<td>C≡C</td>
<td>Terminal alkyne</td>
</tr>
<tr>
<td>1643.35</td>
<td>C=C, C=N</td>
<td>Amide, Alkenyl</td>
</tr>
<tr>
<td>1134.14</td>
<td>O-H</td>
<td>Alcohol</td>
</tr>
</tbody>
</table>

The sample given for FTIR analysis for custard apple essential oils showed the presence of several peaks functional groups;
The peak at 2939.52cm⁻¹, 2908.65cm⁻¹, 1743.65cm⁻¹ and 1651.07cm⁻¹ indicate the presence of O-H stretch, C=O stretch, and −c=c stretch and the functional groups such as alcohols, phenols, alkanes, ketones, saturated aliphatic, and alkenes respectively.

The peaks formation at ~1234.44cm⁻¹, 848.68cm⁻¹ represent C-O-O stretch and O-H bend. The peak at 3417.86cm⁻¹ shows the presence of high concentration of amines, phenols, and alcohols. The peak at 3001.24cm⁻¹ and 956.69cm⁻¹ shows high concentration of alkenes.

The peak at 1651.07 shows the C=O and N-H and is attributed to high concentration of esters and amides.

The presence of N-H and C-H group at 1450.47cm⁻¹ shows high concentration of secondary amines and alkanes.

*Table 4.6. FTIR peak values of custard apple essential oils*

<table>
<thead>
<tr>
<th>Frequency</th>
<th>Bond</th>
<th>Functional group</th>
</tr>
</thead>
<tbody>
<tr>
<td>3417.86</td>
<td>O-H, N-H</td>
<td>Phenols, amines, Hydroxyl.</td>
</tr>
<tr>
<td>3001.24</td>
<td>C-H</td>
<td>Alkanes</td>
</tr>
<tr>
<td>2954.95</td>
<td>C-H</td>
<td>Methyl</td>
</tr>
<tr>
<td>2939.52</td>
<td>C=O</td>
<td>Carbonyl</td>
</tr>
<tr>
<td>2908.65</td>
<td>C-H</td>
<td>Methyl</td>
</tr>
<tr>
<td>2854.65</td>
<td>C-H</td>
<td>Methyl</td>
</tr>
<tr>
<td>2677.20</td>
<td>C=O</td>
<td>Carbonyl</td>
</tr>
<tr>
<td>1743.65</td>
<td>C=O</td>
<td>Aldehydes</td>
</tr>
<tr>
<td>1651.07</td>
<td>C=C, N-H</td>
<td>Alkenyl, secondary amine</td>
</tr>
<tr>
<td>1450.47</td>
<td>N-H, C-H</td>
<td>Secondary amines</td>
</tr>
<tr>
<td>1234.44</td>
<td>O-H</td>
<td>Aromatic ester, aryl O-stretch</td>
</tr>
<tr>
<td>956.69</td>
<td>C-H</td>
<td>Trans C-H</td>
</tr>
<tr>
<td>848.68</td>
<td>C-H</td>
<td>1, 4 Di-substituted para</td>
</tr>
<tr>
<td>609.51</td>
<td>OH</td>
<td>Alcohol</td>
</tr>
</tbody>
</table>
The FTIR analysis of the three essential oils obtained from different plants obtained many peaks, which represented the presence of various functional group which include; amides, amines phenols, alkanes, alkenes carboxylic acid, esters aldehydes ketones etc.

4.2 Repellence data

The field repellence of cockroaches

<table>
<thead>
<tr>
<th>Name of essential oils</th>
<th>Number of cockroaches introduced</th>
<th>Number of cockroaches repelled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control experiment</td>
<td>5</td>
<td>None</td>
</tr>
<tr>
<td>Garlic essential oils</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>Custard apple essential oils</td>
<td>5</td>
<td>3</td>
</tr>
<tr>
<td>Chamomile essential oils</td>
<td>5</td>
<td>none</td>
</tr>
</tbody>
</table>

Table 4.7 Repellence data of essential oils against cockroaches

The field repellent results indicated difference in susceptibility of volatile chemicals derived from essential oils among the species of the tested cockroaches. The two essential oils, garlic essential oils and custard apple seed essential oils in this study provided better repellence against German cockroaches that is 80% and 60% respectively. On the other hand chamomile essential oils showed no repellence at all. This method of bioassay was selected because of reliability among several attempt have been made previously (Tharava et al 2007).

Several commercial essential oils from plant species *Boesembeigia rotunda*, *Citrus hydstrix*, *Curcuma longa L*, *Linya cubeba* *Piper nigrum L*, *Psidium guava L*, and *Zingiber officiale* were evaluated for cockroaches under laboratory conditions (Faujan et al 1995).

The toxic and repellent properties of the nine major constituents of essential oils, comprising benzene derivatives against cockroaches were evaluated, verified and analysed in many
earlier experiments. Many earlier studies focused on cockroaches repellent which caused the movement of insect pest away from treated area (Stettenkamp et al 1992).

The repellence is useful in difficult to reach hidden areas such as electrical and plumbing systems, which may serve as the runways for cockroaches and facilitate their disposal between the apartments. Furthermore, non-toxic and relatively volatile repellents may be applied on the surface.

Through cleaning solution that can be applied in the surface which protect the machinidise in the transport and storage equipment from being disrupted from pest. For such applications, repellent must have low mammalian toxicity and relatively low residual activity.

**Attractant /repellent Bioassay**

In the attractant repellence bioassay the use of concentration for all the three essential oils was 0.1.

In this assay, garlic essential oils showed 66.67% repellence higher than the custard apple essentials oils with repellence of 50.00%. Chamomile essential oils showed no repellence for *M.domestica*.

<table>
<thead>
<tr>
<th>Name of essential oils</th>
<th>Mean number of flies attracted to control</th>
<th>Mean number of flies attracted to treated samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Garlic essential oils</td>
<td>7,5,6=6</td>
<td>4,1,1=2</td>
</tr>
<tr>
<td>Custard apple oils</td>
<td>6,3 ,2=4</td>
<td>3,1,2=2</td>
</tr>
<tr>
<td>Chamomile oils</td>
<td>4,4,4=4</td>
<td>7,3,4=5</td>
</tr>
</tbody>
</table>

*Table 4.8. Repellence data of essential oils against houseflies*

In attraction/repellent bioassay. Garlic showed higher repellent activity as compared to custard apple essential oils. Chamomile on the other showed no repellence at all. In relative studies the significance of repellents effect of garlic and insecticidal activity of custard apple essential oils has been known from the work of others author as well (part et al 2005) determined the repellent efficiency of 5 monoterpenes (C, P cymene L alpha terpenes and T
derived from the essential oils of T vulgaries) against the mosquito species. The essential oils obtained from *Ocimum glatissimum* L, T seryplum L showed 100% repellence activities at 2% concentration (Signh and Signh 1990). In others study the essential oils of *M pipenta* exhibited 98.6% repellence, which that of zotticalis, 84.9% followed by *C yerum*, 77.9% and the essential oils was still good. Though significantly lower i.e. 63% at the concentration of 1% Present study also shows agreement with the others.
CHAPTER FIVE: CONCLUSION AND RECOMMENDATION

5.0 Conclusion

The essential oils of garlic, custard apple, and chamomile was successfully extracted using hydro distillation. Even though hydro distillation is the simplest and the efficient method used for small scale production of oils, it has several limitations. For instance the process is slow and the distillation time is much longer thereby consuming much power making the process uneconomical. Secondly, there is a variation rate of distillation due to difficult control of heat. Thirdly, prolonged action of hot water can cause hydrolysis of some constituents of the essential oils such as esters which react with water at high temperatures to form acids and alcohols and lastly the extraction of various plant parts are not always complete.

The anticipated results were obtained and at some point have confirmed the belief that essential oils extracted from garlic, custard apple seed and chamomile plants actually carried the scent of the plant and therefore can be used on industrial scale to make various finished products which includes repellents, cosmetic room spray.

From biological assay performed I can confidently ascertain the essential oils extracted from garlic and custard apple seeds could be used in chemical industry in the manufacture of repellent.

The experimental raw data obtained from these experiments for FTIR analysis of all the three essential oils were determined and are summarized and shown in Appendix A.

My project research findings has definitely provided a basis for further investigations on similar area, as well as toxicity aspect of essential oils. I hope that what is provided here will be pursued extensively in the scientific world.
### 5.2 Recommendations

In the course of my project I met some hiccups at different point of my research that made me come up with the following recommendation this research would have gone very far if it were not for the unavailability of various analysis instruments in the school and also time was a factor:

I challenge chemistry students to do further and extensive studies on the extraction and analysis of various essential oils since I did not manage to do a detailed analysis of the essential oils I extracted, I call upon any interested party to carry out more detailed analysis and find out various components responsible for sweet or bad scent and repellent properties exhibited by the essential oils it may be probably accepted or published.

I also call upon the Kenyan Government to channel its resources to help the students taking this field of science as this will help in the development of new products in the chemical, cosmetic and perfume industries and also development of new repellents to help in fight against insect pest contaminations.

Lastly I want to encourage and motivate every student taking this field of science to work tirelessly with the aim of making the world a better place through vast discoveries in the field of chemistry.
REFERENCES


FrancesS. P., C. A. (n.d.). Laboratory and field evaluationof repellents ,deet,CIC-4 and A13-37220 against anopheles mosquito.


APPENDICES
Appendix A. Experimental run Data Analysis

A.1 FTIR Data.

As described in chapter 3 of the experimental procedure for the analysis using FTIR, the functional groups for each essential oil was reported in terms of peaks and the spectrum trace. Each of essential collected during the extraction period were smeared separately on the polythene paper to identity the different functional grounds contained in them.

A.1.1 FTIR spectrum of garlic essential oils
A1.2 FTIR Spectrum of custard apple essential oils.

![FTIR Spectrum of custard apple essential oils](image)

<table>
<thead>
<tr>
<th>Peak</th>
<th>Intensity</th>
<th>Corr. Intensity</th>
<th>Base (H)</th>
<th>Base (L)</th>
<th>Area</th>
<th>Corr. Area</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>609.51</td>
<td>23.53</td>
<td>1.43</td>
<td>532.65</td>
<td>509.21</td>
<td>6138.957</td>
<td>62.982</td>
</tr>
<tr>
<td>2</td>
<td>729.23</td>
<td>11.21</td>
<td>15.92</td>
<td>810.10</td>
<td>632.05</td>
<td>14221.121</td>
<td>892.071</td>
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<tr>
<td>3</td>
<td>848.99</td>
<td>27.80</td>
<td>2.81</td>
<td>902.69</td>
<td>810.10</td>
<td>5626.202</td>
<td>144.518</td>
</tr>
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<td>4</td>
<td>956.69</td>
<td>27.11</td>
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<td>902.69</td>
<td>5543.235</td>
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<td>321.506</td>
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<td>2.09</td>
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<td>5939.399</td>
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</tr>
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<td>3.36</td>
<td>6.66</td>
<td>1456.18</td>
<td>1404.18</td>
<td>4314.405</td>
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<td>10</td>
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<td>22.36</td>
<td>3.63</td>
<td>1686.90</td>
<td>1512.19</td>
<td>6743.878</td>
<td>-156.648</td>
</tr>
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<td>11</td>
<td>1743.96</td>
<td>2.24</td>
<td>35.54</td>
<td>1905.67</td>
<td>1685.50</td>
<td>14995.907</td>
<td>1628.069</td>
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<td>12</td>
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<td>49.35</td>
<td>3.92</td>
<td>2708.06</td>
<td>2414.68</td>
<td>12090.700</td>
<td>147.270</td>
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<td>60.29</td>
<td>2646.93</td>
<td>2705.09</td>
<td>8062.736</td>
<td>2337.375</td>
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<td>51.76</td>
<td>12.35</td>
<td>2602.06</td>
<td>2646.93</td>
<td>6491.119</td>
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</tr>
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A1.3 FTIR Spectrum for chamomile essential oils.