EXTRACTION, PHYSICO-CHEMICAL CHARACTERIZATION AND STABILITY MONITORING OF ESSENTIAL OIL FROM *Matricaria recutita* L. GROWN IN SELECTED AREAS IN KENYA

FREDRICK MZEE MWAZIGHE

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE

DEGREE OF MASTER OF SCIENCE IN ENVIRONMENTAL CHEMISTRY

AT THE

UNIVERSITY OF NAIROBI

MAY 2013

DECLARATION

This thesis is my original work and has not been submitted for examination for a degree in any other university.

Submitted by: …….

MWAZIGHE FREDRICK MZEE

I56/60158/2010

……

DATE

This thesis has been submitted for examination with our approval as university supervisors:

……..	……
Prof. Geoffrey N. Kamau	Dr. Joseph M. Mwaniki
Department of Chemistry	Department of Chemistry
University of Nairobi	University of Nairobi

……

Dr. H.N. Njenga

Department of Chemistry

University of Nairobi

DEDICATION

This work is dedicated to my parents, Norman and Margaret Mwazighe, and my siblings.

ACKNOWLEDGEMENTS

I am sincerely grateful to my supervisors Prof. Geoffrey N. Kamau, Dr. Joseph M. Mwaniki and Dr. Hellen Njenga, for their invaluable advice and support throughout the research.

I extend my thanks to The Gandhi Smarak Nidhi Trust Fund for paying my fees which made it possible for me to pursue the degree programme.

I am thankful to SEANAC-Botswana for a research grant which made it possible for me to carry out GC-MS analyses of samples at Makerere University in Uganda.

I am immensely grateful to the staff of the Department of Chemistry, Makerere University, especially Dr. Justus Kwete, Dr. John Wasswa, George and Stephen for their guidance and hospitality during my stay at their department.

I am indebted to John Kiratu of the South Dakota State University for doing some GC-MS analyses of my samples. I am also thankful to the Jomo Kenyatta University of Agriculture and Technology Department of Chemistry staff and students for their assistance in the preliminary GC-MS analyses.

My gratitude is also extended to my colleagues and fellow postgraduate students for their moral support and invaluable suggestions throughout my work.

I also thank my parents and siblings for their support throughout my work.

I am eternally grateful to God for life and curiosity.

Table of Contents

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS AND ACRONYMS

µL	Microlitre
^{13}C	Carbon-13 isotope
^{1}H	Proton
Approx.	Approximately
ASE	Accelerated Solvent Extraction.
DCM	Dichloromethane
GC	Gas Chromatography
GC-MS	Gas Chromatography and Mass Spectrometry
GmbH	Gesellschaft mit beschränkter Haftung (company with limited liabilities)
HPLC	High Pressure Liquid Chromatography
IR	Infrared
IUPAC	International Union of Pure and Applied Chemistry
m/e	Mass to charge ratio
mg	milligrams
mL	milliliter
MS	Mass spectra

NMR	Nuclear Magnetic Resonance
ppm	parts per million
R_F	Retardation factor
R.T.	Retention time
S.D	Standard deviation
SECD	Solvent Extraction and Clevenger Distillation combined.
SECD-8	SECD with 8-day soaking
SECD-P	SECD with percolation
SFE	Supercritical Fluid Extraction
TLC	Thin Layer Chromatography
UV	Ultraviolet light
UV-Vis	Ultraviolet-Visible
v/v	Volume by volume
w/w	Weight by weight
δ	Chemical shift
λ_{MAX}	Wavelength of maximum absorbance

ABSTRACT

Extracts were obtained from *Matricaria recutita* L. flowers by cold solvent extraction using different solvents, Clevenger distillation and solvent extraction combined with Clevenger distillation (SECD); this was done to compare the yield and quality of the different extracts providing information for optimization of extraction and the composition of flowers from selected regions. The extracts were characterized using TLC, UV-Vis spectrometry, IR spectrometry and GC-MS. UV-Vis scanning and measurement of refractive index (R.I) of the chamomile essential oil were carried out at monthly and/or bi-monthly intervals to determine if they could be used to monitor the stability and changes in quality of the essential oil. Isolation of components of chamomile essential oil by preparative TLC was also done to obtain standards that can be used for future characterizatio. *Matricaria recutita* L. flowers from Kangari, Njabini and Kibwezi were used for the extraction of essential oil by Clevenger distillation. The flowers from Kibwezi gave the highest yield of essential oil at 0.60% w/w and the flowers from the two other regions gave a yield of 0.44% w/w each. GC-MS analysis of the essential oils from the three flower samples revealed that the Kibwezi sample had the highest chamazulene content (10.382%). All the samples were also found to have high α-bisabolol oxide A content with the Kangari sample having the highest content (61.142%). The quality of the essential oil extracted by Clevenger distillation appeared to be affected by the duration of storage of the flowers and the extraction temperature. Some components of the essential oil increased in percentage with increase in extraction temperature while others decreased. Storage of flowers over a period of seven months also seemed to reduce some components of the essential oil. In cold extraction, water gave the highest yield of extract (17.31% w/w) though it had a poor quality compared to the extracts of organic solvents, as established from the smell and TLC (it had fewer components

on TLC). Hexane had the least amount of extract (2.02% w/w). UV-Vis analysis of the extracts revealed that they were characterized by strong absorption in the range 270-400 nm and a λ_{MAX} at around 660 nm.

In Solvent Extraction combined with Clevenger Distillation (SECD) using different solvents, ethyl acetate gave the highest yield of extract (0.3268% w/w), while hexane/acetone gave the lowest (0.2363% w/w). All SECD extracts were blue in colour and most had the characteristic smell of chamomile essential oil. However, the hexane extract did not have the characteristic smell. They were all characterized by a λ_{MAX} at 605 nm though the absorbances varied. SECD extraction where percolation was used in the solvent extraction step, as opposed to 8-day soaking, was found to give higher percentages of most of the main components except for α-bisabolol oxide A. The hexane SECD extract had the poorest quality in terms of the percentages of most of the main components. UV-Vis monitoring of chamomile essential oil over a period of five months showed a decrease in absorbance in the region 270 to 390 nm (about 15% decrease per month). This showed that UV-Vis can be used as a tool in monitoring the quality of the essential oil. The refractive index of the essential oil measured over six months, was found to be an average of 1.48724 ± 0.00515. There was no trend observed in the refractive indices measured over the six months, which meant that refractive index is an unreliable parameter in monitoring the quality of the essential oil. The blue component in chamomile essential oil was successfully isolated by preparative TLC and it was determined to be pure by TLC and GC-MS. Its identity was confirmed to be chamazulene by a spectral search in the MS library. The molar absorptivity of chamazulene was determined to be 360.87 $cm^{-1}M^{-1}$ (The literature value for the molar absorptivity of chamazulene is 436.50 $cm^{-1}M^{-1}$).

CHAPTER ONE

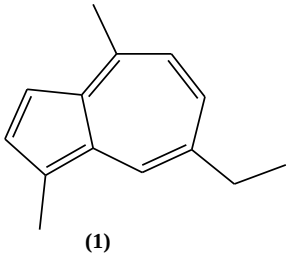
1.0 INTRODUCTION

German chamomile is a widely recognized herb in western culture whose medicinal usage dates back to the times of Hippocrates, Galen and Asclepius (Thorne Research, 2008). The ancient Egyptians considered it as a gift from the 'sun god' and used it to alleviate fever and sun stroke. In the sixth century, it was used to treat insomnia, back pain, neuralgia, rheumatism, skin conditions, indigestion, flatulence, headaches, and gout (Gardiner, 1999). It is considered a "cure all" in Europe and is referred to as "alles zutraut" in Germany which means "capable of anything" (Berry, 1995).

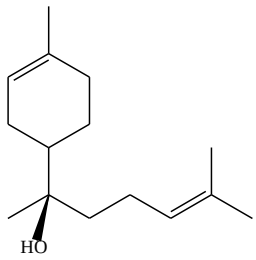
There are numerous varieties of chamomile but the two most popular in traditional herbalism are, *Matricaria chamomilla (Chamomilla recutita;Matricaria recutita;* German chamomile*;* Hungarian chamomile) and *Chamaemelum nobile* (*Anthemis nobilis;*Roman chamomile; English chamomile) which belong to the Asteraceae/Compositae family and are similar in physical appearance, chemical properties and general applications (Thorne Research, 2008). German chamomile is also referred to as wild chamomile, or simply true chamomile (Grieve, 1994; Mc Guffin *et al*, 1997). It is an apple-pineapple scented, smooth, branched annual and grows two to three feet tall. The flower head is one inch in diameter and has a hollow conical center covered with tiny yellow florets surrounded by silver-white to cream coloured florets. It has erect branching with finely divided leaves. Roman chamomile on the other hand, is an aromatic creeping perennial which grows only one foot in height. Its flower heads are one inch in diameter, with a broad conical disk that is covered in yellow florets surrounded by white florets. It has many freely branching hairy stems and finely divided leaves (Gardiner, 1999). German

chamomile is considered the more potent of the two and has received more scientific evaluation and is more cultivated than Roman chamomile. The plant is indigenous to northern Europe and grows wild in central European countries; it is especially abundant in Eastern Europe. It is also found in western Asia, the Mediterranean region of northern Africa, and the United States of America. It is cultivated in many countries (OAU-STRC, 1985;Youngken, 1948; CSIR, 1953; Leung, 1996; Bruneton, 1995; BHMA, 1990; Tyler *et al*, 1988). In Kenya, chamomile is grown in, among other areas, the Aberdares region, Naivasha and Kibwezi. It is grown for sale to herbal shops which either blend it with tea and sell it as chamomile tea or just sell the flowers for further blending by other traders.

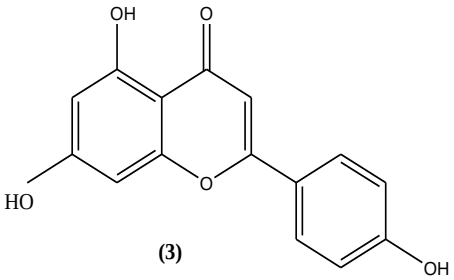
The flowers are the medicinal part of the plant. The flowers contain an essential oil (0.4-1.5%), which has an intense blue colour owing to its chamazulene **(1)** content (1-15%). Other major constituents include α-bisabolol **(2)** and related sesquiterpenes (up to 50% of the oil). Apigenin **(3)** and related flavonoid glycosides constitute upto 8% (dry weight) of the drug (Bruneton, 1995)



(1)



(2)



(3)

However, research has shown that flowers grown in different regions give varying yields of essential oil (Orav *et al*, 2001; Presibella *et al*, 2006; Raal *et al*, 2003;Shams-Ardakani *et al*, 2006) and the essential oils have different concentrations of the active components. This is bound to have an effect on the biological activity of the essential oil. One of the factors that

affect the essential oil content of the flowers is the drying process. The drying process of chamomile can degrade more than 50% of essential oil (Costa, 2001; Muller *et al*, 1996) and such losses compromise the effectiveness of its medicinal usage considering that the minimum content of essential oils in chamomile flowers is expected to be 0.4% by some pharmacopoeia (Brazilian Pharmacopoeia 1996). Storage of the dried flowers also affects the oil content and the composition of the oil (Falzari & Menary, 2003). This research sought to determine the oil yield of chamomile flowers from three different regions in Kenya and also determine the composition of the oils. This was meant for comparison with chamomile essential oils from other regions of the world from literature and to see if the oil composition was as recommended by various pharmacopoeia.

Chamomile is known to act as an antiinflammatory, spasmolytic, vulnerary, antimicrobial, sedative, calming restlessness and tensions, particularly as it affects the viscera, as a peripheral vasodilator and analgesic (Bradley, 1992; Hoffman, 1990; Mills, 1994).

Chamomile has been applied in treating flatus, colic, abdominal distension and spasms, premenstrual irritability and spasmodic dysmenorrhoea. It has also been used in treating infantile convulsions from colic, teething, earache, anxiety, insomnia, gastritis, gingivitis, inflammed sore eyes, sore throats, nasal catarrh, wounds and swelling, tension headaches and fever management (Bradley, 1992; BHMA, 1990; Hoffman, 1990; Mills, 1994).

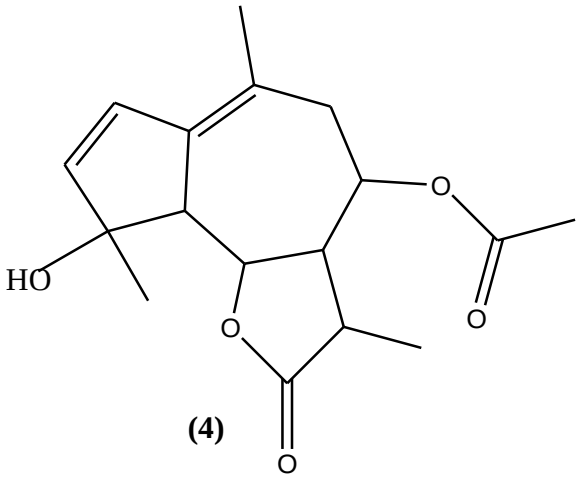
Hydrodistillation, classified into either Clevenger distillation (for small scale) or steam distillation (large scale), is the most common method of extracting essential oil from chamomile flowers. Solvent extraction methods have also been used in obtaining an extract from chamomile; these include among others, supercritical fluid extraction (SFE) and accelerated

solvent extraction (ASE). In SFE, carbon dioxide is widely used because it is cheap, simple to use and shows great affinity to lipophilic compounds to be extracted (Reverchon & Senatore, 1994). The chamomile oil produced by SFE has a yellow colour because the extraction process does not produce any thermal transformation like in steam distillation which leads to formation of chamazulene from matricin. Its viscocity is also lower because paraffins are absent as a result of the separation of the waxes which follows the extraction. ASE needs shorter extraction time and consumes less solvent. It is also automated and the extracted samples do not need further filtration (Zuloega *et al*., 2000). The traditional methods of extraction like solvent extraction and hydrodistillation have drawbacks such as high temperature (in hydrodistillation) and extended concentration steps (in solvent extraction) which can result in loss or degradation of volatile components of the extract. Solvent extraction using organic solvents may also lead to contamination of the extract when some solvent remains in it (Kotnik *et al*., 2007).

A study on the factors affecting yield of essential oil in Clevenger distillation (Mwaniki & Kamau, 2003) revealed that the yield of the essential oil is dependent on non-stoppage of distillation time, absence of an organic solvent in the distillation flask, optimum quantity of flowers and water, and the distillation not being performed under reduced pressure. The study also showed that introduction of an inert organic solvent in the collecting column of the clevenger apparatus improved the yield of essential oil by preventing the dispersion of the oil in the collecting column. This present study sought to determine the effect of extraction temperature on the components of the essential oil.

Mwaniki and Mbugua (2007), used a method of extraction where they combined solvent extraction and Clevenger distillation in obtaining an extract similar to the blue essential oil from steam distillation. The method was called SECD (Solvent Extraction-Clevenger Distillation).

Acetone was used to soak a given weight of flowers for 8-10 days after which the extract was concentrated, put into a distillation flask into which an optimum volume of water and boiling chips were added and the mixture subjected to 4.5 hours of Clevenger distillation. The extract obtained was blue just like essential oil from direct clevenger distillation and the yield was comparable to that of Clevenger distillation. GC-MS analysis of the extract showed that the quantity of chamazulene obtained using this method was 4% higher than that from the Clevenger distillation method. Probably because the use of acetone rather than water extracts more matricin **(4)** from chamomile flowers which is subsequently transformed to chamazulene in the high temperatures of Clevenger distillation. The GC chromatograms also showed that the two extracts had varying amounts of components from their varying peak heights.



(4)

From NMR, the oil from direct clevenger distillation had a higher concentration of aromatic compounds in the NMR ppm range δ 6-8 than that from the SECD method. On the other hand, the SECD extract has a higher proportion of the aliphatic compounds in the ppm range δ 0.8-2.4 (Mwaniki & Mbugua, 2007). Having few aromatics is a disadvantage because the aromatics like flavonoids in the oil are the ones responsible for most of the medicinal properties of the

chamomile oil. This work sought to use different solvents in the SECD method to study the differences in the extracts in terms of quantity and quality.

1.1 PROBLEM STATEMENT

Herbal medicine has become very popular in Kenya (NCAPD, 2008) and chamomile as chamomile tea is becoming popular as well. People take herbal medicine expecting it to be potent in specific areas, but if the medicine lacks in potency then patients end up using it without any effect.

There's need for scientific evaluation of the herbal medicine sold by herbal shops to determine that the content of active components is as recommended by various pharmacopoea in the world.

Storage conditions also affect the potency of any medicine and it is always indicated that a certain medicine is potent upto a certain period. There's need to determine the stability of the essential oil obtained from chamomile.

There's constant need for optimization of essential oil extraction to ensure that a good quality and quantity of essential oil is obtained.The SECD method, in which acetone was used, offers a good method of obtaining essential oil from chamomile flowers, in that a large amount of flowers can be used at once, but the extract was found to have some drawbacks like a low concentration of aromatics which are the biologically active components.

1.2 JUSTIFICATION

Chamomile owes its biological activity to the active compounds in its essential oil. Various pharmacopoea recommend an essential oil content above 0.4% in the flowers. The higher the concentration of the active compounds in the essential oil the more biologically active it is.

This study will provide information on the essential oil content of flowers from different parts of Kenya and analysis of the essential oil will provide a profiling to see the percentages of the active components in each oil. Optimization of extraction processes will ensure that a good quality and quantity of essential oil is obtained.

1.3 OBJECTIVES

1.3.1 Main objective

The overall objective was to extract chamomile essential oil from chamomile flowers grown in Kenya using different extraction methods with a view to studying the characteristics of the oil and variation in quality and quantity.

1.3.2 Specific objectives

The specific objectives of the current study were to:

i. Investigate the effect of using different types of solvents in obtaining an extract from chamomile flowers.

ii. Study the variation in yield and quality of chamomile oil with temperature changes in Clevenger distillation.

iii. Characterize the different chamomile extracts.

iv. Compare the yield and quality of chamomile oil from flowers grown in different parts of Kenya.

v. Use different cold extraction procedures in the solvent extraction step of SECD, with a view to improving the quality of the SECD extract.

vi. Study the quality of the extracted essential oil as a function of storage time of flowers.

vii. Monitor the stability of the extracted chamomile oil.

viii. Obtain standards from the chamomile essential oil, which can be used for future characterization of chamomile extracts.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Matricaria recutita* L.

The best known botanical name for german chamomile, also used in the pharmacopoeias, is *Matricaria recutita* (syn. *Matricaria chamomilla*, L., *Chamomilla recutita* (L.) Raushert). The Latin name 'recutitus' refers to the petals, meaning truncated, trimmed. The name of 'chamomilla' may well originate from Dioscoride and Plinius the Elder who - due to the pomaceous odor – called the plant 'chamaimelon'. Chamaimelon means, more or less, 'low growing apple tree' (Greek: chamai = low, melon = apple) (Franke & Schilcher, 2005).

German chamomile does not have scale-like palets between the flowers of the capitulum. Capitulum is bottom cone-shaped long and hollow. This plant has white ligulate flowers, smells pleasantly of chamomile (typical chamomile smell) and is annual, grows 10 to 80 cm high. The plant has thin spindle-shaped roots. The stem is in an upright position, mostly heavily ramified, bare, round, and filled with marrow. The leaves are alternate, double to triple pinnatipartite, with narrow-linear prickly pointed sections being hardly 0.5 mm wide. The golden yellow tubular florets with five teeth are 1.5 to 2.5 mm long, ending always in a glandulous tube. The white ligulate flowers are 6 to 11 mm long and 3.5 mm wide (Franke & Schilcher, 2005).

The chamomile has varieties of diploid and tetraploid. The varieties of diploid have shorter growth and less brushwood height than the varieties of tetraploid (Moghaddasi, 2011). The glandular hairs of *M. chamomilla* are multicellular and biseriate with two basal cells, two peduncle cells and a secretory head composed of six cells. The histochemical tests show that the

glands are positive for lipids, essential oils, sesquiterpene lactones and peptic like substances (Andreucci *et al*., 2008).

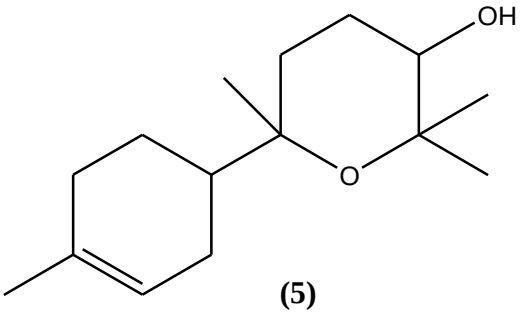
German chamomile requires cool, temperate conditions to grow well, and temperatures of 7 to 26 ^{0} C are required. The plant can survive cold winter nights as low as -12 ^{0} C. To be able to grow well vegetatively and produce a lot of flowers, chamomile needs long summer days, full sun and high heat units to produce optimum oil yields (Alberts, 2009).

The German chamomile should be harvested when most of the flowers have grown. Early or late harvest would reduce the quality of effective materials (Moghaddasi, 2011). The flowers have maximum amounts of essential oils when ray florets are in mood; afterwards, the amount of essential oils decreases. Weather determines whether the plant would flower once or twice; that is why, the amount of the product is really variable, as it is with other plants (Sharafzadeh & Alizadeh, 2011).

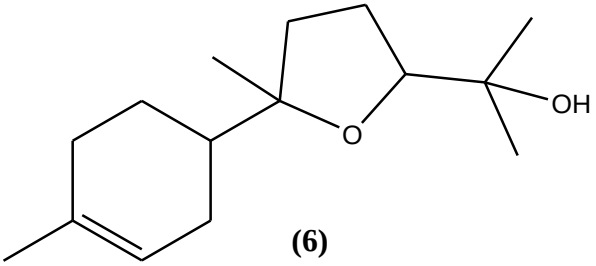
2.2 Active Chemical Constituents of *Matricaria recutita*, L.

Chamomile contains a large number of therapeutically interesting and active compound classes. The most important ones are the components of the essential oil and the flavonoid fraction. Mucins, coumarins, phenol carboxylic acids (phenyl substituted carboxylic acids), amino acids, phytosterols, choline, and mineral substances have also been detected and characterized in chamomile (Franke & Schilcher, 2005). The chamomile constituents are best categorized according to their lipophilicity (Schilcher, 1987). The lipophilic fraction includes individual components of the essential oil, coumarins, methoxylated flavones aglyca, phytosterols, and "lipidic and waxy substances" (Franke & Schilcher, 2005). The hydrophilic fraction consists of flavonoids, mucilage, phenyl carboxylic acids, amino acids, and choline.

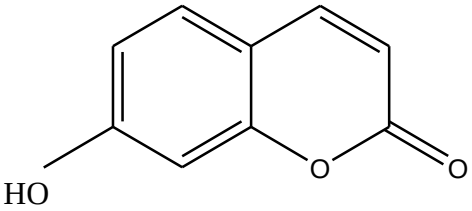
Active principles of German chamomile are terpenoids: α-bisabolol **(2)**, α-bisabolol oxide A **(5)** and B **(6)**, chamazulene **(1)**, sesquiterpenes; coumarins: umbelliferone **(7)**; flavonoids: luteolin **(8)**, apigenin **(3)**, quercetin **(9)**; spiroethers: en-yn dicycloether and other components such as tannins, anthemic acid, choline, polysaccharides and phytoestrogens (Bagchi *et al*., 2001; Karbalay-Doust *et al*., 2010; McKay & Blumberg, 2006; Newall *et al*., 1996).



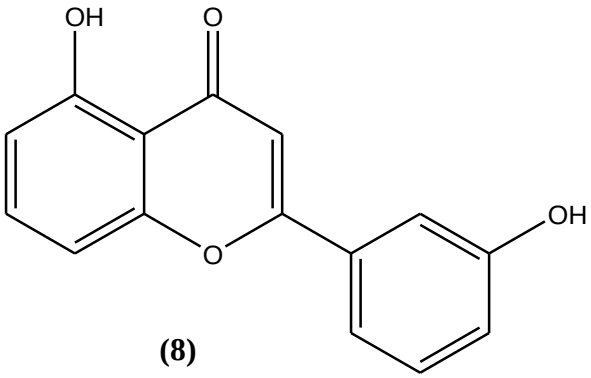
(5)



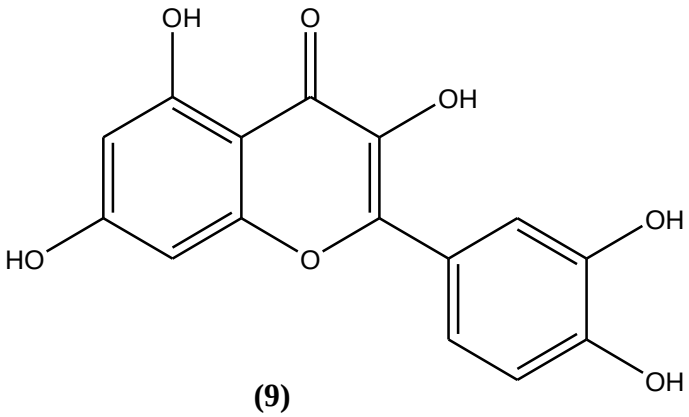
(6)



(7)



(8)



(9)

2.3 Main Active Constituents

2.3.1 The Essential Oil and its Constituents

The essential oil is present in all organs of the chamomile plant, with only the roots containing small quantities. The flowers and flower heads are the main organs of the production of essential oil. The composition of the essential oil in roots differs from that in flowers. The oil content changes during ontogenesis, reaching a maximum of 0.3 – 1.5% in flowers just before full bloom (Schilcher, 1987). Remarkably chamomile flower oil mainly consists of sesquiterpene derivatives (75 – 90%) but contain only traces of monoterpenes (Motl & Repcak, 1979). The oil contains up to 20% of polyalkynes.

In 1863, the French chemist Piesse isolated a blue substance from the essential oil of chamomile. He characterized the compound as a hydrocarbon and called it azulene (Piesse, 1863). The antiphlogistic effect of chamazulene **(1)** had been known long before its constitution was found to be 1,4-dimethyl-7-ethyl-azulene in 1953 (Meisels & Weizmann, 1953; Sorm *et al*., 1953). It had been assumed to be 1,4-dimethyl-7-isopropyl azulene (Stahl, 1954a). The structure elucidation was actually done on chamazulene isolated from *Artemisia arborescens* L. (Meisels

& Weizmann, 1953). The presence of chamazulene precursors had been reported for quite a long time until Cekan and co-workers finally isolated a substance. The structure of the compound that they called matricin was assigned as (3S,3aR, 4S, 9R, 9aS, 9bS)-4-acetyloxy-3a,4,5,9,9a,9b-hexahydro-9-hydroxy-3,6,9-trimethylazuleno[4,5-b]furan-2(3H)-one in 1956 (Cekan *et al*., 1954a; Cekan *et al.*, 1954b;Cekan *et al.*, 1957). Matricin is present in ligulate florets and tubular florets of chamomile only, but not in the bottom of the flower heads. In 1982, the constitution of matricin was confirmed by Flaskamp *et al*. (1982), using modern spectrometric methods.

Matricin is very unstable and decomposes visibly by turning blue after a short time, particularly in aqueous solution (Schmidt *et al*., 1991). This colour reaction also occurs during steam distillation of chamomile oil (Schilcher, 1987). It is caused by decomposition to chamazulene. The matricin content in chamomile varies considerably between cultivars. "Mabamille", a proazulene and (-)-α-bisabolol rich cultivar of Martin Bauer GmbH and Robugen GmbH (both in Germany), contains up to 0.05% in dried flowers (Hempel, 2001). Of all chamomile extracts, the extract obtained using supercritical carbon dioxide has the highest matricin content (approx. 0.2%) (Hempel, 2001). The immediate precursor of chamazulene is chamazulene carboxylic acid (CCA) **(10)**. It is formed from matricin by the elimination of water and acetic acid and decarboxylates to chamazulene as shown in figure 1 below.

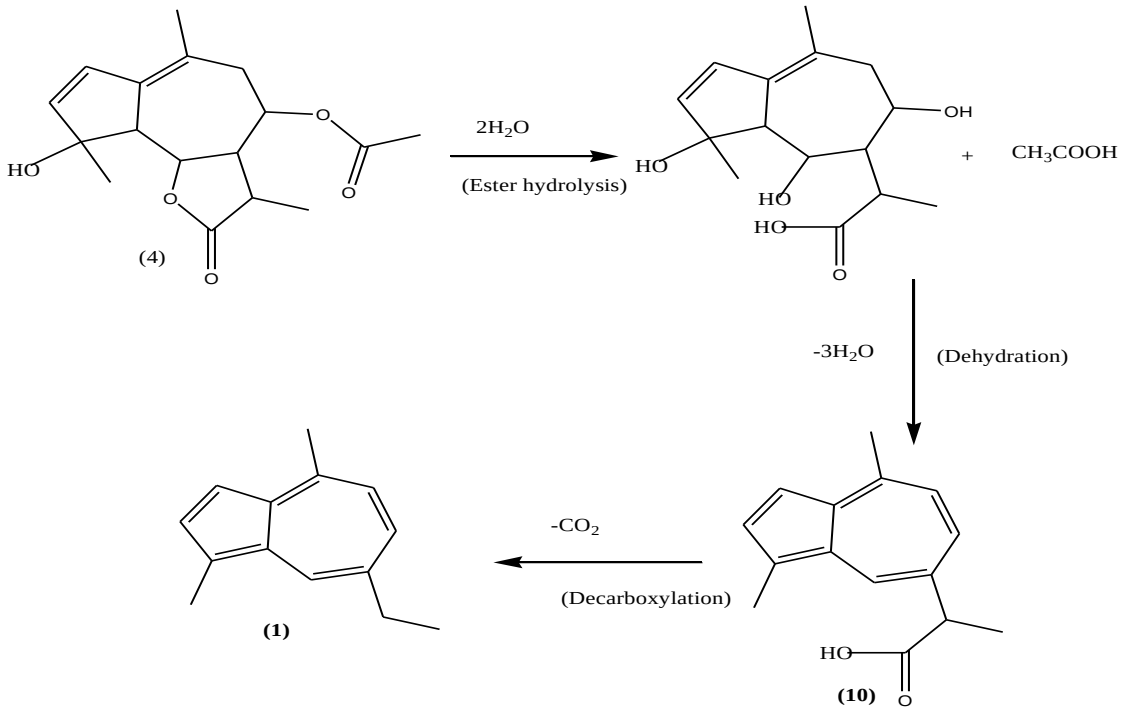
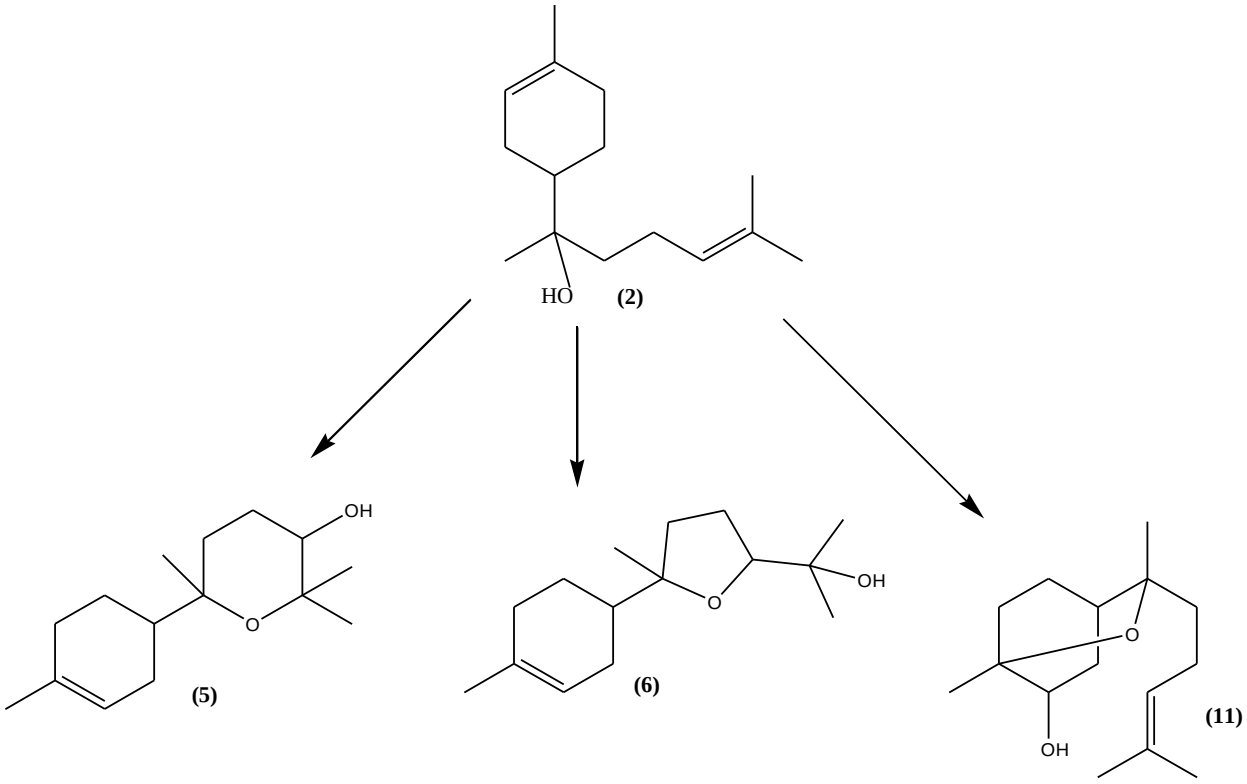


Figure 1: Degradation of matricin to chamazulene carboxylic acid and further decarboxylation to chamazulene.

Chamazulene carboxylic acid was first isolated by E. Stahl from chamomile and yarrow (*Achillea millefolium*) in 1954 (Stahl, 1954b). Its constitution was confirmed by Cuong *et al.* (1979) using mass spectrometry and NMR. The compound was forgotten until 2000, when Imming recognized it to be a natural profen, constitutionally similar to synthetic antiphlogistic compounds like ibuprofen and naproxen (Franke & Schilcher, 2005). It was again isolated from a chamomile cultivar with a high proazulene content ("Mabamille"), extensively characterized physico-chemically and shown to be more stable than originally reported, especially in neutral and weakly basic aqueous solutions (Goeters, 2001). However, in aqueous acid and in aprotic organic solvents it rapidly loses carbon dioxide.

Sorm *et al*. (1951) isolated another essential constituent of chamomile oil, the monocyclic tertiary sesquiterpene alcohol (-)-α-bisabolol (Levomenol). The constitution of bisabolol from chamomile was confirmed (Herout *et al*., 1953; Holub *et al*, 1955) by comparing spectroscopic data with synthetic bisabolol already described by Ruzicka *et al*. (Ruzicka & Capato, 1925; Ruzicka & Liguori, 1932). The isopropylidene structure of natural (-)-α-bisabolol was proved through infrared (IR) and nuclear magnetic resonance spectra (Isaac *et al*, 1968). Four optical isomers of α-bisabolol are possible. Three of them were isolated from different plants and distinguished because of their optical rotation (Isaac, 1979). In 1977, Kergomard and Verscambre (1977) determined the absolute configuration of (-)-α-bisabolol isolated from chamomile by stereoselective synthesis of the corresponding diastereoisomers and comparison of the NMR spectra. (-)-α-bisabolol has (5R,6S) configuration atom numbering as in β-bisabolol, isolated from cotton buds (Minyard *et al*., 1968). This was confirmed by Knoll and Tamm (1975). The bisaboloids' stereochemistry has been fully established except that of bisabolol oxide C **(11)**. Contrary to earlier assumptions, all steric centres of bisabolol oxides A and B, (-)-α-bisabolol and bisabolone oxide A **(12)** have an S-configuration. The identical stereochemistry of all bisaboloids is also shown by the fact that some bisaboloids are interconvertible (Flaskamp *et al*., 1981).



In 1951, (-)-α-bisabolol oxide A was isolated by Sorm *et al*. Some years later, Sampath *et al*. determined its structure (Sampath *et al*, 1969a) and isolated the isomeric α-bisabolol oxide B from chamomile (Sampath *et al*, 1969b).

(-)-α-bisabolol oxide A ($C_{15}H_{26}O_2$): molecular mass 238, syrupy $[\alpha]_D = -42.2^0$.

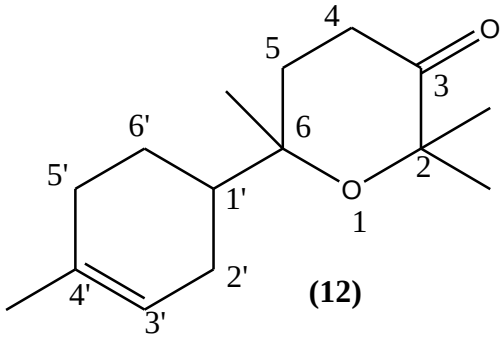
(-)-α-bisabolol oxide B ($C_{15}H_{26}O_2$): molecular mass 238, $[\alpha]_D = -46.95^0$.

These two isomeric oxides of α-bisabolol, imply that four cyclic structures are possible: two containing a tertiary hydroxyl group and two with a secondary hydroxyl group (Franke & Schilcher, 2005). While testing chamomile material collected in Turkey, Hozl and Demuth found a bisaboloid unknown so far. Its constitution was determined using IR and NMR spectroscopy and through the products of oxidation and reduction found to be an α-bisabolone oxide **(12)**

(Hozl & Demuth, 1975). α-Bisabolol oxide A can be transformed to it or can be obtained from it by reduction. The bisabolone oxide isolated from plant material showed a different optical rotation compared to material prepared from (-)-α-bisabolol oxide A:

$[\alpha]_D = -6,2^0$ (Sampath *et al*, 1969a).

$[\alpha]_D = +3,5^0$ (Hozl & Demuth, 1975).



(12)

The nomenclature of the chamomile bisaboloids should be as follows according to IUPAC guidelines:

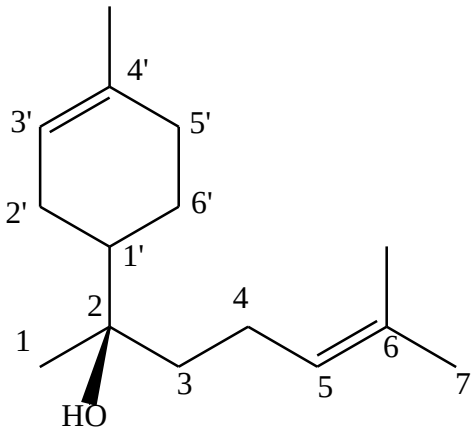
(-)-α-bisabolol: (-)-(1‘S, 2S)-6-methyl-2-(4-methyl-3-cyclohexene-1-yl)-hepten(e)-2-ol.

(-)-bisabolol oxide A: (-)-(1‘S, 3S, 6S)-tetrahydro-2,2,6-trimethyl-6-(4-methyl-3-cyclohexen(e)-1-yl)-2H-pyran(e)-3-ol.

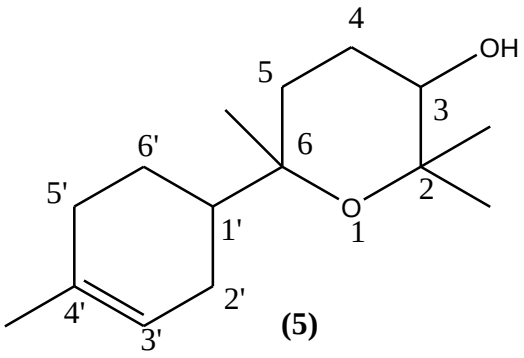
(-)-bisabolol oxide B: (-)-(1“S, 2’S, 5’S)-1-methyl-I-[tetrahydro-5-methyl-5-(4-methyl-3-cyclohexen(e)-1-yl)-furan(e)-2-yl]-ethanol.

(+)-bisabolone oxide A: (+)-(1‘S,6S)-tetrahydro-2,2,6-trimethyl-6-(4-methyl-3-cyclohexen(e)-1-yl)-2H-pyran(e)-3-on.

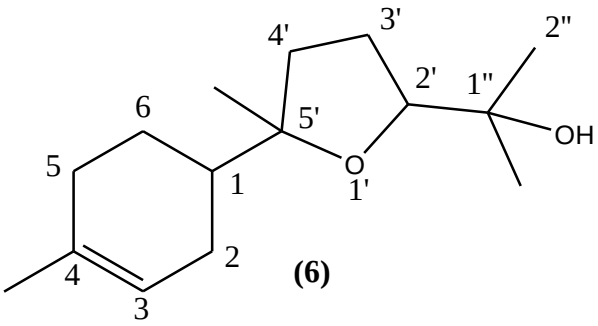
The structures below show the numbering to illustrate the nomenclature of chamomile bisaboloids.



(2)



(5)

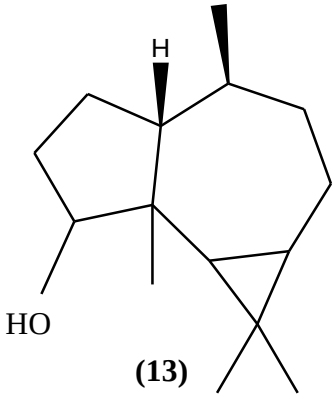


(6)

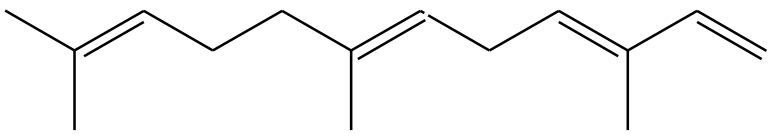
α-Bisabolol oxide A, B, and sometimes bisabolone oxide A are the main constituents of the chamomile essential oil. Schilcher (1973) established a classification in chemotypes based on the composition of the essential oil. There are several chamomile chemocultivars, depending on the active principles, which could, for example, be specified as *M. recutita*, L.cv. "rich in bisabolol" or as *M. recutita*, L. cv. "rich in bisabololoxide" (Franke & Schilcher, 2005).

Motl *et al*. (1977), isolated the azulenogenic sesquiterpene alcohol, spathulenol **(13)**. Its constitution was determined by ^{1}H and ^{13}C NMR and by IR (Juell *et al*, 1976). Its constitution was corroborated by regio- and stereoselective synthesis from (+)-aromadendrene (Van Lier *et al*, 1985). In 1979, Lemberkovics identified two farnesene isomers, trans-β- **(14)** and trans-α- **(15)** farnesene (Lemberkovics, 1979). According to her findings, β-farnesene appeared to be the main component, whereas α-farnesene was present in traces. Reichling *et al*. (1979), were not able to detect α-farnesene in the essential oil of chamomile flowers.

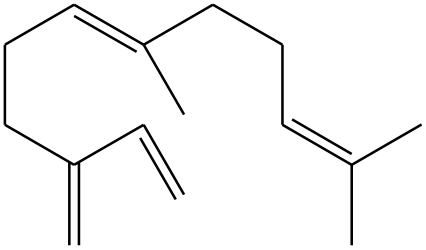
Two spirocyclic polyynes, the isomeric cis **(16)** and trans **(17)** enyne dicycloethers, were found in the petroleum ether extract of chamomile flowers by Bohlmann *et al*. (1961) and reinvestigated in 1982, including a discussion of NMR data (Bohlmann & Zdero, 1982). The cis spiroether, according to Sorm *et al*., (1951), cis-2-[hexadiyne)-(2,4)-ylidene]-1,6-dioxaspiro-[4,4]-nonene), is the major component in most plant specimens (Schilcher, 1985). The trans spiroether was predominant only in certain commercially available chamomile flowers (Schilcher, 1985). Both compounds were found in petroleum ether extracts and in freshly distilled essential oil (Breinlich, 1966).



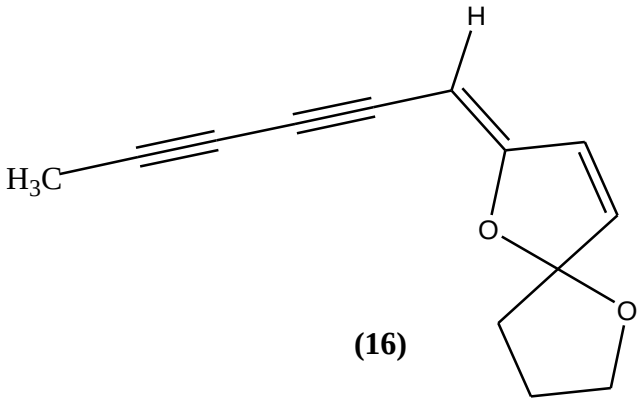
(13)



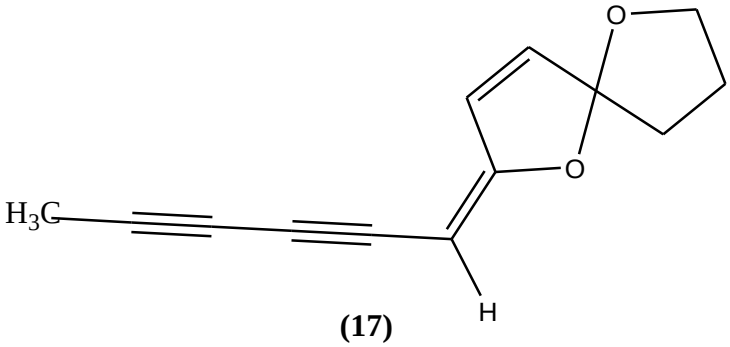
(14)



(15)



(16)



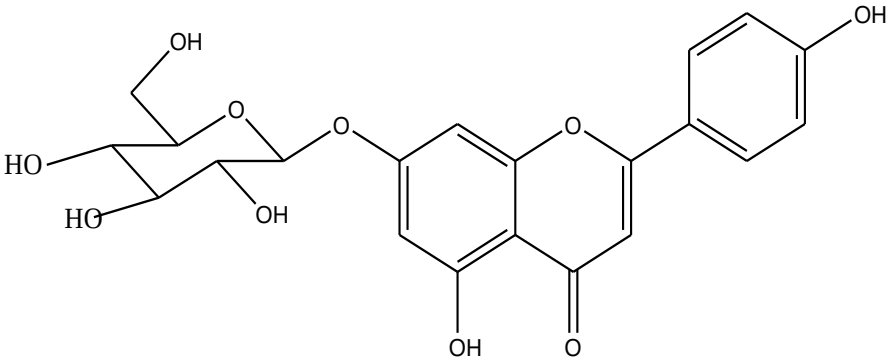
(17)

The literature highlights a number of compounds which have been isolated and characterized.

2.3.2 Other Constituents in Chamomile

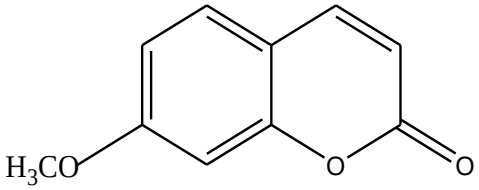
Flavonoid glycosides represent the major fraction of water-soluble components in chamomile. Apart from the glycosides, flavonoid aglyca were found in great variety among the lipophilic constituents. Chamomile flavonoids were recognized to be spasmolytic and antiphlogistic and are therefore of great interest (Franke & Schilcher, 2005). Apigenin was the first flavone to be isolated from chamomile in 1914 (Power & Browning, 1914). Its constitution was however

elucidated as late as 1952 (Sorm *et al*., 1952). Lang and Schwandt successfully isolated and identified apigenin-7-glucoside **(18)** from ligulate florets (Lang & Schwandt, 1957).

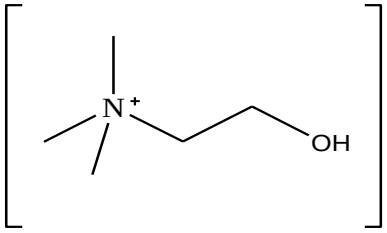


(18)

Both the 7-methoxy-coumarin herniarin **(19)** and the 7-hydroxy-coumarin umbelliferone are of analytical and pharmacological interest. Herniarin is mainly present (Franke & Schilcher, 2005). Analysis of material of different origins resulted in a range of 37.4-98.5 mg of herniarin (Schilcher, 1985) and 6-17.8 mg of umbelliferone per 100 g of chamomile flowers both detected in ligulate and tubular florets. The average content in ligulate florets was significantly higher (Schilcher, 1985; Schilcher, 1987). In herbal chamomile preparations sold in Italy, the preparations containing ligulate florets showed higher content of coumarin when compared to other parts of the anthodia (flower heads). The ratio of umbelliferone:herniarin was <1:5 (Tosi *et al*., 1995).



(19)



(20)

The content of herniarin decreased during anthodia ontogenesis in both diploid and tetraploid varieties, the tetraploid cultivar showing a higher concentration (Repcak *et al*., 1998). Umbelliferone was identified as a stress metabolite of the plant (Repcak *et al*., 2001).

Bayer *et al*. observed up to 0.3% choline **(20)** (Bayer *et al*., 1958a; Bayer *et al*., 1958b). Choline is very likely to participate in the antiphlogistic activity of total extracts, aqueous preparations and infusions (Franke & Schilcher, 2005).

2.3.3 Percentage of Active Components in Chamomile Essential Oil

Different cultivars have different amounts of the active components. However, their chemical composition is affected by the local ecological conditions and the cultivation method as well (Franke & Schilcher, 2005).

A study of the main sesquiterpenes of chamomile essential oil revealed the major components to be chamazulene (19.9%), α-bisabolol (20.9%), A and B α-bisabolol oxides (21.6% and 1.2%, respectively) and β-farnesene (3.1%). Among the minor components was spathulenol (Costescu *et al*., 2008).

An Iranian experiment studied four cultivars of German chamomile, Bodegold (tetraploid), Germania (diploid), Bona (diploid) and Goral (tetraploid). The results showed that plant height of Goral and Bodegold were significantly higher than Germania and Bona. Goral produced the highest anthodia yield. The lowest dry anthodia yield was produced by Bona. The highest essential oils content (0.627% w/w) extracted from Bona in the first harvest but Germania

produced the lowest essential oils (0.627% w/w) at the third harvest. Chamazulene content of the cultivars ranged between 9.6-14% (Azizi, 2006).

The essential oils of *M. recutita* L. cultivated in Estonia were isolated and thirty seven components were identified. The main components were α-bisabolol oxide A (20-33%) and B (8-12%), bisabolone oxide A (7-14%), (E)-farnesene (4-13%), α-bisabolol (8-14%), chamazulene (5-7%), and en-yn-dicycloether (17-22%) (Orav *et al*., 2001).

A recent investigation in Estonia indicated that the main constituents of the essential oils were as follows: α-bisabolol oxide A (39.4%), bisabolone oxide A (13.9%), (Z)-en-yne-dicycloether (11.5%), α-bisabolol oxide B (9.9%), α-bisabolol (5.6%), and chamazulene (4.7%) (Raal *et al*., 2011).

A study regarding the responses of young plants of diploid and tetraploid *M. chamomilla* cultivars to abiotic stress (within an interval from 6 hr before to 54 hrs after spraying the leaf rosettes with aqueous $CuCl_2$ solution) revealed that the content of herniarin in the treated plants rose approximately 3 times. The highest amounts of umbelliferone in stressed plants exceeded 9 times and 20 times those observed in control plants of the tetraploid and diploid cultivar, respectively. Due to stress, the concentration of ene-yne-dicycloether in leaves decreased by more than 40% (Eliasova *et al*., 2004).

An Iranian study in Isfahan indicated essential oil components of German chamomile isolated by hydrodistillation of the aerial parts of the plant. Sixty three components were characterized, representing 86.21% of the total oil components detected; α-bisabolol oxide A (25.01%) and α-bisabolol oxide B (9.43%), spathulenol (8.49%), cis-en-yn-dicycloether (7.42%) and α-

bisabolene oxide A (7.17%) were the major constituents of the oil. Chamazulene was 3.28% and α-bisabolol was 6.01% (Shams-Ardakani *et al*., 2006).

2.3.4 Biological Activities of the Main Components in Chamomile

Several pharmacological actions have been documented for German chamomile, based primarily on in-vitro and animal studies. Such actions include antibacterial, antifungal, anti-inflammatory, antispasmodic, anti-ulcer, anti-viral and sedative effects (Thorne Research, 2008).

The constituents of chamomile known to have antimicrobial properties include α-bisabolol, luteolin, quercitin, and apigenin. Herniarin may also have antibacterial and antifungal properties in the presence of ultraviolet light. Preliminary in-vitro studies on the antimicrobial activity of chamomile have yielded promising results. Chamomile oil, at a concentration of 25 mg/mL, demonstrates antibacterial activity against such gram-positive bacteria as *Bacillus subtilis*, *Staphylococcus aureus*, *Streptococcus mutans*, and *Streptococcus salivarius*, as well as some fungicidal activity against *Candida albicans* (Aggag & Yousef, 1972; Berry, 1995; Cinco *et al*., 1983). Whole plant chamomile extract at 10 mg/mL demonstrates a similar effect, completely inhibiting growth of group B *Streptococcus* in vitro (Cinco *et al*., 1983).

In addition, chamomile extract blocks aggregation of *Helicobacter pylori* and various strains of *Escherichia coli* (Aggag & Yousef, 1972; Annuk *et al*., 1999; Cinco *et al*., 1983). Chamomile extract has also been shown to inhibit the growth of polio virus and herpes virus. German chamomile esters and lactones demonstrate activity against *Mycobacterium tuberculosis* and *Mycobacterium avium*. Chamazulene, α-bisabolol, flavonoids, and umbelliferone display

antifungal properties against *Trichophyton mentagrophytes* and *Trichophyton rubrum* (Turi *et al*., 1997).

The high α-bisabolol content in chamomile oil is credited for providing the majority of antibacterial, antifungal, anti-inflammatory, and anti-ulcer activity although the precise mechanism of action is unclear (Isaac, 1979; Isaac & Thiemer, 1975; Szelenyi *et al*., 1979).

In-vitro, chamomile extract inhibits both cyclooxygenase and lipoxygenase, and consequently prostaglandins and leukotrienes (Hormann & Korting, 1994). Other anti-inflammatory effects are thought to occur via the influence of azulenes (chamazulene, prochamazulene, and guaiazulene) on the pituitary and adrenals, increasing cortizone release and reducing histamine release (Berry, 1995). When bisabolol oxides were tested on dextran-induced rat paw oedema, they showed the highest antiinflammatory activity (Verzar *et al*., 1979).

Chamomile extracts exhibit antispasmodic properties. Apigenin, α-bisabolol, and the cis spiroethers appear to provide the most significant antispasmodic effects. When tested on spasms of isolated guinea pig ileum induced by barium chloride, 10 mg of apigenin provided the antispasmodic activity roughly equivalent to 1 mg of papaverine (an opioid antispasmodic). Similar results were observed with α-bisabolol and the cis-spiroether (Achterrath-Tuckermann *et al*., 1980; Breinlich & Scharnagel, 1968; Hozl *et al*., 1986; Mann & Staba, 1986). Bisabolol oxides A and B also show papaverine-like spasmolytic action but half as potent as α-bisabolol (UNESCO, 1986). Other flavonoids and the small amount of coumarins contribute to smooth muscle relaxation, but to a lesser degree. In vitro studies demonstrate α-bisabolol inhibits gastric ulcer formation induced by indomethacin, ethanol, or stress (Szelenyi *et al*., 1979). Oral administration of chamomile oil to rats at doses ranging from 0.8-80 mg/kg α-bisabolol

demonstrate significant protective effect against gastric toxicity of 200 mg/kg acetylsalicylic acid (Torrado & Agis, 1995).

Regarding sedative activity, one study using intraperitoneal administration of chamomile extract in mice concluded that apigenin functions as a ligand for benzodiazepine receptors, resulting in anxiolytic and mild sedative effects, but no muscle relaxant or anticonvulsant effects (Viola *et al*., 1995). In contrast to diazepam, apigenin does not cause memory impairment. A lyophilized infusion of chamomile, also administered intraperitoneally in mice, elicited a depressive effect on the central nervous system (Loggia *et al*., 1982).

Research is exploring the antiproliferative and apoptotic effects of chamomile extract in various human cancer cell lines. One preliminary study observed in vitro exposure to chamomile results in differential apoptosis in cancer cells but not in normal cells at similar doses; apigenin and apigenin glycosides appear to be the key components responsible for those effects (Srivastava & Gupta, 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Sampling Methods

Chamomile flowers from Njabini and Kangari were bought from an organic shop in Kangari, while the ones from Kibwezi were obtained from the University of Nairobi farm in Kibwezi. One kilogram of flowers was obtained from each region.

3.2 Sampling Sites

Njabini is located in Nyandarua county of Kenya and lies west of Aberdares ranges. Its coordinates are 0.72 ^{0}S and 36.67 ^{0}E. It receives a significant amount of rain and is in the cold part of the country.

Kangari is in Murang'a county, east of the Aberdare ranges. Its coordinates are 0.78 ^{0}S and 36.89 ^{0}E. It is also a wet and cold region.

Kibwezi is in Makueni county with coordinates 2.42^{0}S and 37.97^{0}E. It is located in a hot and dry region of Kenya.

The map below (Figure 2) shows the sampling sites, A being the Aberdares region around which, Njabini (C) and Kangari (D) are situated and B is Kibwezi.

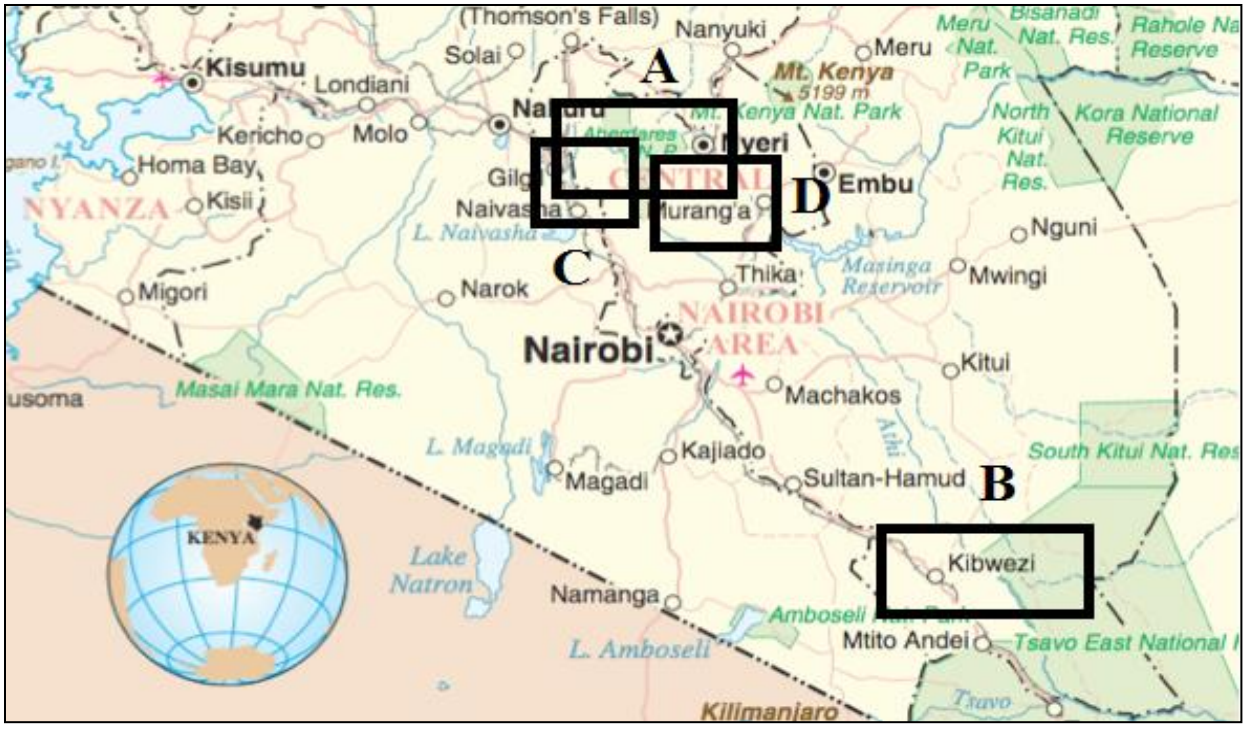


Figure 2: Part of the map of Kenya indicating sampling sites: B (Kibwezi), C (Njabini) and D (Kangari).

3.3 Chemicals

All the solvents (hexane, acetone, methanol, ethanol, DCM, ethyl acetate) used in the solvent extractions were general purpose grade and were distilled before use. These were supplied by Scielab Chemical Supplies. However, for GC-MS analysis HPLC grade n-hexane was used (The instrument used for GC-MS was an Agilent Technologies 6890N Network GC system with a 5975 Inert XL Mass Selective Detector and a 7683B Series Injector). The anhydrous sodium sulphate used in the drying of the chamomile essential oil was general purpose grade as well. The solvents used as mobile phases in TLC and preparative TLC were of general purpose grade and had also been distilled before use.

3.4 Materials Preparation

The dry flowers were crushed and passed through a sieve to get rid of stalks and petals. The sieved flowers were stored in air tight polythene bags to avoid any losses of essential oil with storage.

The chamomile essential oil used in TLC with cold extracts and UV-Vis analysis is the one obtained through Clevenger distillation.

The chamazulene that was analysed using UV-Vis and GC-MS was the one obtained through preparative TLC.

3.5 Cold Solvent Extraction

About 1 gram of the ground flowers were put into a conical flask and 50 ml of solvent added. The mixture was agitated for about 3 minutes and then left undisturbed. The flask was covered with aluminium foil to avoid loss of solvent, for 48 hours. The following solvents and solvent mixtures were used:

Water; Ethanol (absolute); Water/ Ethanol (1:1 mixture); Hexane; Dichloromethane; Acetone; Ethyl acetate; Methanol; Hexane/Acetone (1:1 mixture); Ethyl acetate/Dichloromethane (1:1 mixture).

After 48 hours, the contents of the flask were filtered through filter paper (Whatman) and the filtrate was rotary evaporated under reduced pressure to remove the solvent and concentrate the extract. The amount of extract for each solvent and solvent mixture was determined

gravimetrically using an analytical balance. The volume of solvent recovered in the concentration of the extract was also determined in order to find the percentage of the solvent recoverable.

3.6 Clevenger Distillation

A given amount of ground flowers was put in a round bottomed flask and then an optimum volume of distilled water which had been warmed for ease of mixing was added. For 20 grams of flowers, a 500 ml flask was used and 250 ml of distilled water added to it. For 40 grams of flowers, a 1000 ml flask was used and 500 ml of distilled water added.

A Clevenger apparatus and a condenser were then attached to the flask with the flask in an oil bath on an electric mantle. The water-flower mixture was then subjected to distillation for an optimum number of hours which was determined to be 6 hours. In the first 30 minutes once the oil had started collecting in the collecting column of the Clevenger apparatus, about 1 ml of hexane was put through the condenser to wash down any oil which had stuck to the walls of the condenser and also to avoid the oil from sticking to the walls of the condenser and the collecting column. The Clevenger apparatus was wrapped with cotton wool held together by aluminium foil, to avoid any heat losses and the collecting column was wrapped in aluminium foil to avoid the oil extract from being degraded by light in the period of extraction.

After 6 hours of distillation, the electric mantle was put off and the water left to run through the condenser until the collecting column had cooled off. Any oil still stuck on the walls of the condenser and the collecting column was washed down using small amounts of hexane until all

had been washed into the hexane layer in the collecting column. The hexane and water layers were then drained from the collecting column into a separating funnel from which the lower water layer was discarded after extraction with two portions of hexane. The hexane layer was drained into a clean dry beaker where it was dried using anhydrous sodium sulphate. In drying the hexane layer, a little sodium sulphate was added and the beaker swirled. This was repeated until sodium sulphate stopped forming clumps. The extract was then left for 5 to 10 minutes after which it was transferred to a preweighed vial using a Pasteur pipette. The extract was concentrated first by rotary evaporator under reduced pressure and then the minimal solvent left to evaporate on its own. The yield, determined gravimetrically, was reported as percentage weight of extract per weight of dry flower sample used. The vial was finally corked and wrapped in aluminium foil before storage in a refrigerator until analysis.

3.6.1 Comparison of Essential Oil Yield of Flowers from Different Regions

The essential oil yield of flowers from Kangari, Njabini and Kibwezi was determined by carrying out a series of Clevenger distillations for each flower sample. The essential oil yield was calculated by dividing the weight of extract by the weight of dry flowers used and finding the percentage.

3.6.2 Determination of the Optimum Distillation Time

20 grams of ground flowers were put in a 500 ml round bottomed flask and 250 ml of warm distilled water added. A Clevenger apparatus and a condenser were attached and distillation carried out for one hour. After the one hour, the heating mantle was turned off and the 1 hour

extract removed as described in Clevenger distillation above. This extraction was repeated for 2, 3, 4, 5, 6, and 7 hours using a fresh sample of 20 grams of ground flowers each time. The optimum time was the shortest time that gave the highest yield.

3.6.3 Extraction at Different Heat Source Temperatures

To determine the best temperature to carry out Clevenger distillation, extraction of the chamomile essential oil was carried out at 115 °, 130 °, 140 °, 145 °, and 150 °C. These were the temperatures of the heating oil in which the distillation flask was dipped during the distillation process. About 10 g of the ground chamomile flowers and 250 ml of distilled water was used in each extraction and the extractions were carried out for 6 hours. The optimum temperature was determined by looking at the extract's colour (to see if it was the expected blue colour or different), its weight and smooth running of the extraction process.

The extracts were also analysed using GC-MS.

3.6.4 Extraction of Oil on Different Months

A sample of dry ground flowers was set aside for monthly extractions. 10 grams of this sample was used for obtaining an essential oil sample through Clevenger distillation from February 2011 to July 2011. The flower sample was put in a polythene bag which was tightly closed and stored.

The experiment was meant to determine if there was any loss in the quality of oil obtained from flowers stored over a period of time. To determine the quality of the oil obtained in all the months, GC-MS was used in analysing the oil samples.

3.7 TLC of Chamomile Oil and Cold Extracts

The mobile phase used for development was DCM:Ethyl acetate (98:2) and 5.5cm by 8cm analytical TLC plates (with silica gel stationary phase) were used. The solutions of the blue chamomile oil, acetone extract, hexane extract, DCM extract, ethyl acetate extract, ethyl acetate-DCM extract, hexane-acetone extract, methanol extract, water extract, water-ethanol extract and ethanol extract were spotted 0.5 cm from the 5.5 cm side of the 5.5 by 8 cm TLC plate. They were then developed with 98:2 DCM:ethyl acetate mobile phase. The spots were viewed first under UV light and then the developed plate was left in Iodine for some time to make visible the UV inactive spots.

3.8 Combined Solvent Extraction and Clevenger Distillation (SECD)

The solvents whose extracts had shown more spots in the TLC were used for solvent extraction in the SECD method. To determine the best method of solvent extraction, percolation and soaking for 8 days or more (which was the original solvent extraction method used in SECD) were tested.

3.8.1 Solvent Extraction with Clevenger Distillation preceded by Percolation (SECD-percolation)

About 20 grams of ground flowers were soaked in 200 ml acetone in a conical flask which was then covered with aluminium foil to prevent loss of solvent. After 24 hours, the resultant solution was filtered into a concentrating flask and then concentrated with a rotary evaporator under

reduced pressure. The recovered solvent was returned to the flowers and soaking continued for another 24 hours. This was repeated until a constant weight of extract was attained. The extract was then transferred to a distillation flask where 250 ml of distilled water was added and the extract was subjected to Clevenger distillation for six hours. The weight of the resultant extract from this extraction was determined.

3.8.2 Solvent Extraction with Clevenger Distillation preceded by 8-day Soaking (SECD-8)

About 20 grams of ground chamomile flowers were soaked in 200 ml acetone in a conical flask which was then covered with aluminium foil and left undisturbed for 8 days. After the 8 days the extract was concentrated, its weight determined and then transferred to a distillation flask where it was subjected to a 6 hour Clevenger distillation. The weight of the extract from this extraction was also determined and compared to the one from SECD-percolation.

3.8.3 SECD using Different Solvents

20 grams samples of the ground chamomile flowers were soaked in 140 ml of each of the following solvents: acetone, hexane, hexane/acetone (1:1 v/v), DCM, ethyl acetate, DCM/ethyl acetate (1:1 v/v), and methanol. The conical flasks were then covered with aluminium foil to stop loss of solvent. They were left undisturbed for 8 days after which the solutions were filtered, concentrated and then transferred to a distillation flask where 250 ml of distilled water was added. Boiling chips were then added and a Clevenger apparatus together with a condenser were attached and distillation carried out for 6 hours. At the end of the extraction period, the extract was removed, dried, its weight determined and stored as in Clevenger distillation above.

3.9 UV-Vis Analysis of SECD Extracts

0.0100 grams of each SECD extract was weighed and dissolved in 25 ml of hexane. In weighing the extracts a light weighing bottle was used. The weighing bottle was first weighed and the balance tared. A glass rod was touched on the surface of the oily extract and then carefully touched to the inside of the weighing bottle. The weight of the oil was then reduced using a thin spatula until the desired weight was attained. The solutions were then scanned using a UV-Vis spectrophotometer model UV-1700 Pharmaspec (Shimadzu) from 700 nm to 200nm.

3.10 UV-Vis Analysis of Cold Extracts and Chamomile Essential Oil

0.0200 grams of the cold extracts were weighed and dissolved in 25 ml of their respective solvents mentioned in 3.6.3. 0.0200 grams of the blue chamomile essential oil (obtained by Clevenger distillation) was also dissolved in 25 ml of absolute alcohol. The resultant solutions were scanned using a UV-Vis spectrophotometer model UV-1700 Pharmaspec (Shimadzu) from 750 nm to 200 nm.

3.11 IR Analysis of the Cold Extracts

0.1 g of each of the chamomile extracts which had been extracted using cold extraction were dissolved in the respective solvents used to extract them apart from the methanol and ethanol extracts which were dissolved in DCM because the two solvents would have dissolved the NaCl cells which were used for scanning with IR. This was done because of the tendency of methanol and ethanol of having traces of water. The IR spectrophotometer model 500 Buck Scientific Inc. was used for the analysis.

The IR scans of the solvents were first obtained by scanning from 4000 cm^{-1} to 600 cm^{-1}. Then each extract solution was also scanned under IR from 4000 cm^{-1} to 600 cm^{-1} as well. For the IR spectra of each extract solution, the solvent spectrum was subtracted through the spectral subtract feature of the software used, to obtain the spectra for the extract.

3.12 Determination of the Refractive Index of the Chamomile Essential Oil

An Abbe refractometer was used for the determination of the refractive index. It consists of hinged prisms with highly polished faces where a few drops of the sample are placed. When the prisms are pressed back together, the sample spreads across the polished faces. The prisms are usually harboured in a water jacket which can be used to set the temperature of the prisms. The water jacket has an inlet and an outlet of water; it is connected to a thermostated water-bath whose temperature was set at 25°C for this determination. A window allows light to pass through the prism and it can be observed through a lens. Through the lens one can see a circle with cross hairs and a scale. A knob is used for adjustment until two distinct regions can be seen at the circle. Another knob is used to align the line of distinction with the center of the cross hairs. Then the reading can be taken from the scales. The refractive index for the given sample of chamomile oil was determined for six months once each month. After each reading the sample was stored in a refrigerator. The determination was meant to establish if the refractive index can be used to monitor any changes in the quality of the chamomile essential oil.

3.13 Visual Monitoring of Essential Oil

Chamomile oil samples were put in three vials labeled:

FM-WNDW- for the sample that was placed at the window.

FM-CPBD- for the sample that was placed on top of a cupboard away from direct sunlight.

FM-LCKR- for the sample that was placed inside a locker.

The vials were stoppered, but no aluminium foil covered around them since they were meant to monitor any changes with time at different exposure to sunlight. Any changes in colour of the essential oil was noted and recorded once every month.

3.14 UV-Vis Monitoring of Essential Oil

0.0200 g of chamomile essential oil was dissolved in 5 ml of absolute ethanol and then scanned with UV-Vis spectrophotometer model UV 1700 Pharmaspec. At first this monitoring was done at a two months interval and then subsequent ones were done after a one month interval. This experiment was carried out to establish if UV-Vis spectrophotometry could be used as a tool in monitoring the changes in the quality of chamomile essential oil stored over a period of time.

3.15 Separation of the Chamomile Oil

3.15.1 Preparative TLC

The blue chamomile oil (0.9063 g) was spotted on preparative TLC plates and developed with DCM: ethyl acetate (98:2). The bands resulting from the development were scraped, soaked in

DCM and filtered. The purity of the separated bands was checked by spotting their solutions on analytical TLC plates and developing with solvent systems of increasing polarity: starting with hexane and increasing the polarity with ethyl acetate. The bands which gave fewer spots on TLC were respotted on preparative TLC plates and developed with solvent systems which had separated them.

The purified bands were scraped off, soaked in DCM and filtered. The filtrate was concentrated and the isolated extracts stored in the refrigerator awaiting analysis by UV-Vis and GC-MS.

3.15.2 UV-Vis Analysis of Blue Component (Chamazulene) Isolated from Chamomile Essential Oil

0.0006g of chamazulene (weighed by difference), which had been isolated from chamomile essential oil by preparative TLC, was dissolved in 25 ml hexane and scanned from 700 to 200 nm. The wavelength of the maximum peak as well as its absorbance was noted.

3.16 GC-MS Analysis of Chamomile Essential Oil Samples

Preliminary GC-MS analyses were done at Jomo Kenyatta University of Agriculture and Technology (Kenya), and the final analyses were carried out at Makerere University in Kampala, Uganda.

The samples which were analysed on GC-MS were:

- Essential oil extracted from flowers of different regions (Njabini, Kangari, and Kibwezi).

- Essential oil extracted from a flower sample on different months.
- Essential oil extracted at different temperatures.
- SECD extracts.
- Extracts obtained through SECD preceded by eight day soaking or percolation.
- Isolated compound 4P and blue component.

For all the samples, except the isolated compounds, about 5 mg was dissolved in 1 ml HPLC grade n-hexane (For the isolated compounds, 40 microliters of a solution of the compound in hexane were drawn and further diluted to 1ml). At the GC-MS 1 µL of the sample solution was injected. Analysis was done using an Agilent Technologies 6890N Network GC system with a 5975 Inert XL Mass Selective Detector and a 7683B Series Injector. The column used was a ZB-5MSi which was 30 m long, had 0.25 mm internal diameter and 0.25 µm film thickness. The carrier gas was Helium with the split ratio 1:8 and flow rate 1ml/min (37cm/s). The injector temperature was 280^0C and the MS source temperature was 200^0C. The MS detector was operated in the Electron Impact mode 70 eV at a scan rate 2 scans/sec with an acquisition mass range of 40-500 amu. The temperature programming was 60^0C for 1 minute then up to 110^0C at 10^0C/min. The temperature was then held for 2 min at 110^0C, then up to 250^0C at 10^0C/min and then held at 250^0C for 13 minutes.

Identification of the components in a gas chromatogram was done through a library search in the instrument's data base. Quantitative analysis of the components was done using the peak areas of the components as percentages of the total peak areas. This was done by a function in the instruments software called Enhanced Data Analysis.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

4.1 Cold Solvent Extraction

Table 1, below shows the results for extracts obtained with cold solvent extraction, which was carried out for 48 hours for each solvent.

Table 1: Performance of various solvents used in cold extraction experiments.

Solvent used	Amount of flowers used (g)	Amount of extract (g)	Percentage extract (%)	Volume of solvent used (ml)	Recovered solvent (ml)	Percentage solvent recovered (%)
Water	1.0098	0.1748	17.31	50	32	64
Ethanol	1.0198	0.0982	9.63	50	36	72
Water-Ethanol (1:1)	1.0170	0.1700	16.71	50	34	68
Hexane	1.0037	0.0203	2.02	50	32	64
DCM	1.0047	0.0513	5.10	50	27	54
Acetone	1.0691	0.0423	3.96	50	33	66
Ethyl acetate	1.0640	0.0923	8.68	50	33	66
Methanol	1.0103	0.1348	13.34	50	30	60
Hexane-Acetone (1:1)	1.0027	0.0447	4.46	50	34	68
Ethyl acetate-DCM (1:1)	1.0025	0.1717	17.13	50	35	70

The results represent averages of experiments which were done in triplicate. The solvents were evaluated in terms of the amount of extract obtained, the quality of the extract and the recoverability of the solvent.

Water seemed to give the highest percentage of the extract at 17.31%, followed by ethyl acetate-DCM 1:1 mixture at 17.13% and the water-ethanol 1:1 mixture which gave 16.71% extract. The other single solvent apart from water which gave a high percentage of extract was methanol which gave 13.34% extract. The less polar solvents like hexane and DCM seemed to give higher percentages of extracts on mixing them with polar solvents. The hexane-acetone 1:1 mixture gave an extract of 4.46% which was an improvement from the percentage extracts of the single solvents (hexane, 2.02% and acetone, 3.96%). Ethyl acetate-DCM 1:1 mixture gave 17.13% extract which was also an improvement from the single solvents (ethyl acetate, 8.68% and DCM, 5.10%). The results show that polar solvents extract more from chamomile flowers which means that the main components are polar. The fact that water gave the highest percentage suggests the additional influence of the polarity by hydrogen bonding. Addition of ethanol lowered the hydrogen bonding. This high percentage in water is advantageous since water is the solvent used to make herbal tea; it implies that the herbal tea contains most of the components of chamomile.

All the extracts had a characteristic sweet smell probably as a result of the volatile components in them, except for the water extract. The extract with the strongest sweet smell was that of methanol, with that of hexane, DCM, and ethyl acetate having a faint sweet smell.

The colour of the extracts ranged from dark brown to yellowish brown. The methanol, ethanol, and water-ethanol extracts had a dark brown colour while the acetone, ethyl acetate, and ethyl acetate-DCM extracts had a brown colour. The hexane extract was yellowish-brown in colour

and the DCM extract was green in colour. The differences in the colours of the extracts could be as a result of the varying amounts of the components extracted by each solvent.

The percentage solvent recovered ranged from 54% for DCM to 72% for ethanol. The low solvent recovery could be explained by losses due to leakages in the recovery apparatus and some of the solvent remaining in the ground flowers. Although the solvent mixtures were recovered, they were not reusable since the mixing ratio was bound to have changed because of the constituent solvents' varying boiling points. Only the single solvents recovered were reused. The high boiling point of water also made it uneconomical to recover it and it was also unreasonable since it is readily available. It took longer as well to recover methanol, ethanol and ethyl acetate which have boiling points of 65˚C, 78˚C, and 77˚C, respectively (Furniss et al., 1978). Another reason for the former two solvents taking longer to distill could be their tendency of having traces of water in them, hence raising their boiling points. DCM on the other hand took a shorter time to distil relative to the other solvents because of its low boiling point (40˚C). However, the low boiling point could have resulted in the low recovery of solvent due to losses in possible leaks and evaporation.

4.2 Clevenger Distillation

4.2.1 Determination of the Optimum Distillation Time

Clevenger distillation at varying times was performed to determine the time that gave the best yield (Table 2).

Table 2: Change of yield of essential oil with increase in distillation time.

Time (hrs)	% Yield			Average	S.D
	First trial	Second trial	Third trial		
1	0.1570	0.1550	0.1580	0.1567	0.0015
2	0.2755	0.2813	0.2734	0.2767	0.0041
3	0.3250	0.3320	0.3297	0.3289	0.0036
4	0.4264	0.4311	0.4255	0.4277	0.0030
5	0.5012	0.5016	0.5008	0.5012	0.0004
6	0.5043	0.5039	0.5044	0.5042	0.0003
7	0.5045	0.5052	0.5046	0.5048	0.0004

The 7 hours distillation time gave the highest average yield of 0.5048% though it was not much different from the yield of 6 hours 0.5042%. Although the 7 hours distillation time gave more yield, the resulting essential oil was greenish-blue in colour and not the characteristic blue colour of chamomile essential oil. There was also a problem of burnt flowers, may be as a result of reduced water with the long distillation time. The quality of the oil could be attributed to long exposure to high temperatures for it was noted that as the distillation continued the collecting column where the oil collected became hot. In subsequent distillations, 6 hours was used as the standard distillation time because it gave a good yield of the essential oil and the quality was a good one since it had the characteristic blue colour. The graph below shows clearly how the yield of essential oil changed with time (Figure 3). The figure shows four linear cases which point to the fact that as extraction proceeds, different components are extracted in different proportions. Some are extracted in considerable proportions after a number of hours, with some having been extracted earlier.

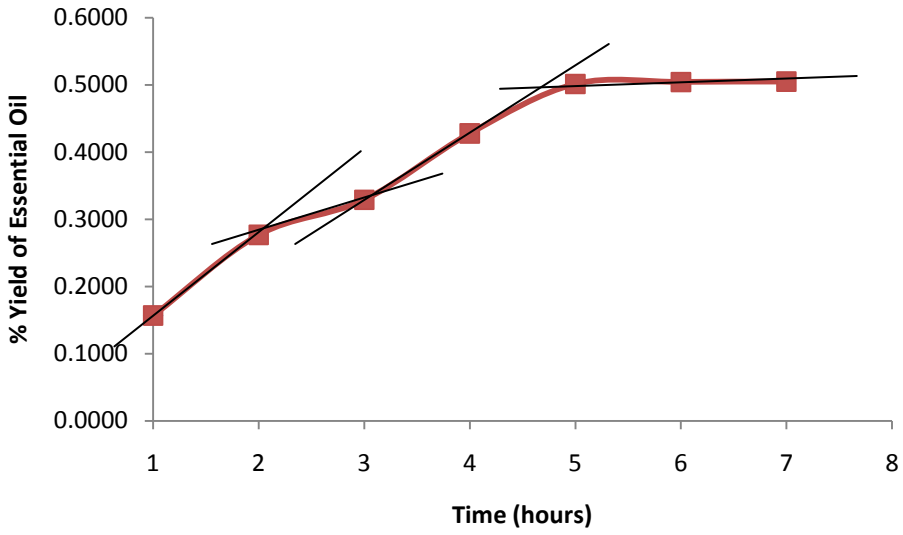


Figure 3: Graph of yield of essential oil at varying distillation times

4.2.2 Comparison of Yield of Essential Oil of Flowers from Different Regions

The yield of essential oils of flowers from different areas in Kenya was also determined (Tables 3, 4, and 5). This experiment was aimed at determining the effect of a given environment on the overall yield of extracted essential oil.

Table 3: Percentage yield of essential oil of flowers from Kangari.

Trial	Sample (g)	Extract (g)	Percentage Yield (%)
1	20.0000	0.0777	0.39
2	20.0000	0.1044	0.52
3	20.0000	0.1007	0.50
4	20.0030	0.0878	0.44
5	20.0271	0.0852	0.43
6	20.0000	0.0785	0.39
7	20.0000	0.0794	0.40
8	20.0408	0.0874	0.44
9	20.0041	0.0995	0.50
10	20.0000	0.0839	0.42

11	20.0375	0.0927	0.46
12	20.0127	0.0870	0.43
13	20.0070	0.0859	0.43
14	20.0001	0.0822	0.41
15	20.0000	0.0782	0.39
Average			0.44±0.04

Table 4: Percentage yield of essential oil of flowers from Njabini.

Trial	**Sample (g)**	**Extract (g)**	**Percentage yield (%)**
1	20.0539	0.1007	0.50
2	20.9094	0.0984	0.47
3	20.0260	0.0876	0.44
4	20.0000	0.0886	0.44
5	20.0451	0.0799	0.40
6	20.0215	0.0811	0.41
Average			0.44±0.03

Table 5: Percentage yield of essential oil of flowers from Kibwezi.

Trial	**Sample (g)**	**Extract (g)**	**Percentage Yield (%)**
1	20.0694	0.1088	0.54
2	20.0090	0.1086	0.54
3	20.0394	0.1245	0.62
4	20.0336	0.1366	0.68
Average			0.60±0.07

The flowers from Kangari gave an average yield of essential oil of 0.44% which was similar to that of the flowers from Njabini. The flowers from Kibwezi gave an average yield of 0.60% hence the highest yield. There are a number of factors that determine the amount of essential oil

in chamomile flowers. Research has shown that herbal products can have differences in their content and composition of essential oil depending on the soil where they grow (Salamon, 1992). Genotype and environmental conditions determine essential oil accumulation and its composition; Schilcher separated chemotypes based on the composition of the chamomile oil (Schilcher, 1987). Kangari and Njabini happen to be in the cold region of Kenya, which receives a lot of rain which might explain the similarity in yields of essential oil of flowers from the two regions. While Kibwezi is in a hot region.

High average temperature and longer insolation have been shown to reduce essential oil content (Seidler-Lozykowska, 2010). However, Salamon (1994) who tested diploid cultivar 'bona' in 3 locations in Slovakia did not find any influence of various growing conditions on the oil composition. This means that weather conditions don't affect all cultivars of chamomile.

Salinity and drought stress have also been shown to reduce the yield of essential oil in chamomile (Razmjoo *et al*, 2008). Drying and storage of the flowers could also affect the yield of essential oil. Thus the difference in the yield of essential oil could be a result of one or a combination of the above factors.

4.2.3 Extraction at Different Heat Source Temperatures

Extractions which had been carried out while varying the temperature of the heat source, with the temperature at 115^{o}, 130^{o}, 140^{o}, 145^{o}, and 150^{o}C, gave extracts which had varying characteristics as shown in table 6 below.

Table 6: Effect of extraction temperature on quality, quantity of extract, and distillation process.

Temperature of oil bath (˚C)	Time taken for distillate to start collecting (mins)	% Yield of essential oil	Colour of extract
115	25:38.58	0.33	Blue
130	13:18.46	0.44	Blue
140	12:03.17	0.52	Bluish-green
145	9:40.63	0.59	Green
150	8:17.89	0.59	Green

The times and yields represent average values of three replicates.

The effect of the extraction temperature can be shown graphically as shown below. Figure 4 shows clearly that the time that elapses before the distillate starts collecting in the collecting column of the Clevenger apparatus (breakthrough time) reduces with the increase in the extraction temperature as expected. A higher temperature was therefore shown to be good for a speedy extraction process or a shorter breakthrough time. This is because high temperatures ensure faster volatilization of components.

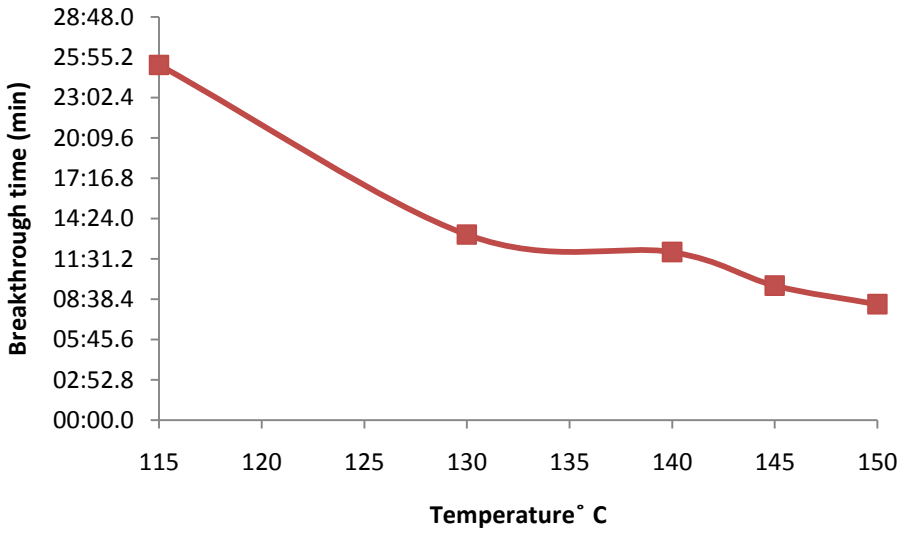


Figure 4: Graph of breakthrough time in Clevenger distillation against extraction temperature

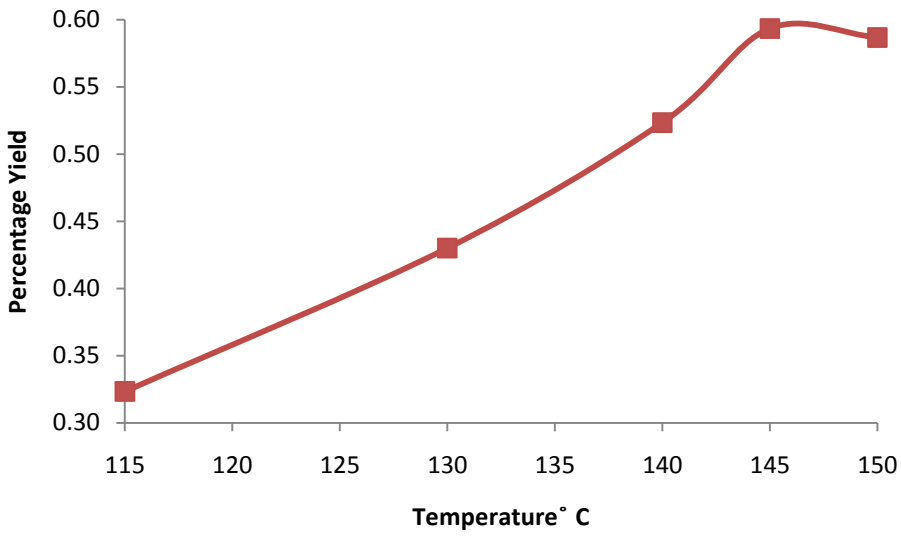


Figure 5: Change in yield of essential oil with change in extraction temperature

Figure 5 shows that an increase in extraction temperature also affects the yield of essential oil. An increase in extraction temperature leads to an increase in the yield of essential oil until it reaches 145^{o}C, after which the yield remains constant.

Extraction at 115˚C did not provide enough heat for the essential oil to start collecting in a short time. Most of the oil extracted at this temperature stuck in the Clevenger apparatus, because it had not attained a high enough temperature for it to condense only in the collecting column. This necessitated washing of the whole Clevenger apparatus with hexane to obtain the extract at the end of the extraction. Losses were inevitable in the process of washing the apparatus because of the nature of the apparatus and a yield of 0.33% was attained. It also took a long time, 25 minutes and 38 seconds for the distillate to start collecting at this extraction temperature. The extract at this temperature was the characteristic blue colour of chamomile essential oil. At 130˚C the temperature was high enough for collection to take place at the collection column and a yield of essential oil of 0.44% was attained. It took 13 minutes and 18 seconds for the distillate

to start collecting in the collecting column at this temperature. The extract at this temperature was also blue in colour.

At 140˚C the extract was bluish-green in colour though no flowers were burnt at the bottom of the flask; this observation was made because in other experiments it was noted that when the flowers were burnt at the bottom of distillation flask, the extract would be bluish-green in colour or even green. At this temperature, it took 12 minutes and 3 seconds for the distillate to start collecting. This temperature gave a yield of essential oil of 0.52%.

The heating source temperature of 145˚C, made it possible for the distillate to start collecting after 9 minutes and 40 seconds. The essential oil obtained was also slightly higher in percentage yield at 0.59%, though it was green in colour.

At 150˚C it took 8 minutes and 17 seconds for the distillate to start collecting but the yield of essential oil plateaued at 0.59%. The extract at this temperature was also green in colour. The high temperature resulted in the flowers coming up the Clevenger apparatus. This problem was solved using boiling chips which regulated the boiling process.

4.3 TLC of Chamomile Oil and Cold Extracts

Chamomile oil and the cold extracts gave varying number of spots when developed on a TLC plate using DCM/ethyl acetate (98:2) as the mobile phase. Table 7 highlights the R_F values for components of cold extracts and chamomile oil obtained by Clevenger distillation.

Table 7: R_F values of the spots of the various chamomile extracts.

	R_F values of spots							
Extract	1	2	3	4	5	6	7	8
Chamomile oil		0.27	0.36	0.42	0.48	0.60	0.72	0.91
Acetone	0.00	0.08	0.14	0.24	0.30	0.41	0.69	0.94
Hexane		0.05	0.14	0.23	0.30	0.41	0.68	0.92
Ethyl acetate	0.00	0.05		0.24		0.41	0.68	0.94
DCM	0.00	0.05		0.23	0.30	0.41	0.68	0.94
Ethyl acetate-DCM	0.00	0.06	0.14	0.23	0.30	0.39	0.67	0.90
Hexane-Acetone	0.00	0.06	0.12	0.24	0.30	0.41	0.71	0.94
Methanol	0.00	0.06	0.15	0.24	0.30	0.41	0.70	0.94
Water						0.39		
Water-Ethanol				0.23		0.39	0.71	
Ethanol	0.00	0.05		0.23		0.39	0.71	

The effectiveness of the various solvents in cold extraction was measured by the number of spots their extracts produced on TLC (table 8). The extracts of acetone, ethyl acetate-DCM, hexane-acetone, and methanol gave the highest number of spots, which were 8, probably as a result of the solvents and solvents mixtures being polar. Chamomile oil, hexane and DCM extracts gave seven spots although the R_F values of the spots varied with only a few comparable. All the extracts except the chamomile oil had spots with comparable R_F values. The chamomile oil, water and water-ethanol extracts did not have the spot of R_F value = 0.05/0.06, which was suspected to be that of matricin because it has been reported in literature as a compound that remains close to the base line when TLC is carried out using different mobile phases (Franke & Schilcher, 2005). In the TLC of chamomile extracts matricin gives an R_F value of 0.00, 0.06 and 0.03 when the mobile phases are benzene or toluene: ethyl acetate (95:5 v/v), DCM: ethyl acetate (98:2 v/v) and chloroform: benzene or toluene (75:25 v/v) respectively and are developed over 12 centimeters. The fact that chamomile oil lacks the spot at R_F value 0.05/0.06 makes the

suspicion stronger that it is that of matricin since chamomile oil does not contain matricin which is usually converted to chamazulene during steam distillation.

The water and the water-ethanol extracts had the least number of spots 1 and 3, respectively, possibly because most of the compounds in chamomile are lipophilic and thus insoluble in water.

Figure 6 below illustrates the different components for the various solvents used in cold extraction.

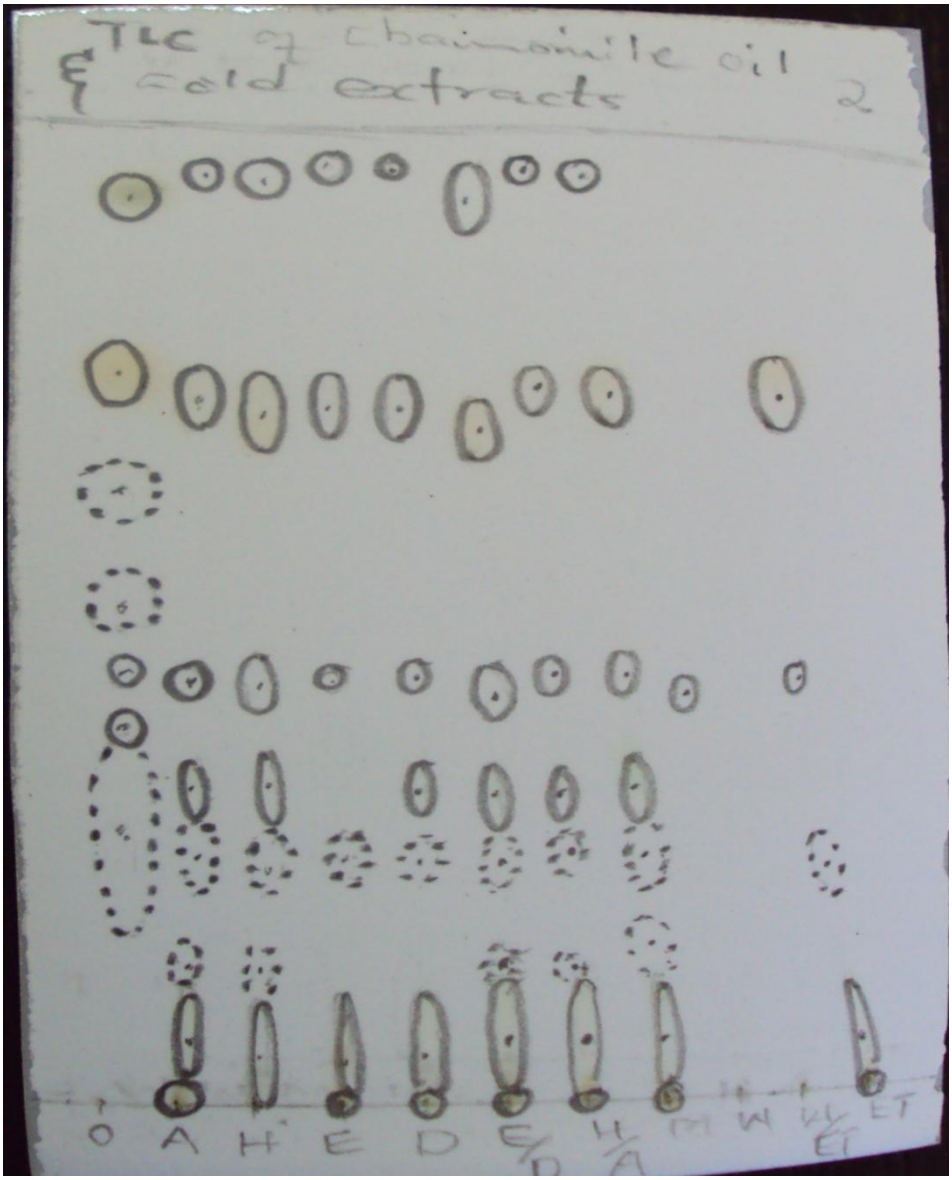


Figure 6: TLC of chamomile oil and cold extracts

The spots on the figure above represented the following:

O- Chamomile oil.

A- Acetone extract.

H- Hexane extract.

E- Ethyl acetate extract.

D- DCM extract.

E/D- Ethyl acetate/DCM extract.

H/A- Hexane/Acetone extract.

M- Methanol extract.

W- Water extract.

W/ET- Water/Ethanol extract.

ET- Ethanol extract.

Table 8: Effectiveness of different solvents and solvents mixtures in cold extraction.

Solvent used in cold extraction	Number of spots for extract on TLC
Acetone	8
Hexane	7
Ethyl acetate	6
DCM	7
Ethyl acetate/DCM	8
Hexane/acetone	8
Methanol	8
Water	1
Water/ethanol	3
Ethanol	5

4.4 Combined Solvent Extraction and Clevenger Distillation (SECD)

The solvents whose extracts showed a good number of spots (6-8) in their TLC were used in the solvent extraction step of the SECD method of obtaining chamomile essential oil. The number of spots was used as an indicator of the effectiveness of a given solvent in extracting most of the components in chamomile flowers. The solvents chosen were acetone, hexane, methanol, DCM, ethyl acetate, hexane/acetone, and ethyl acetate/DCM whose extracts showed 8, 7, 8, 7, 6, 8, and 8 spots respectively.

4.4.1 SECD-P and SECD-8

In addition to using different solvents in SECD, the method used in the solvent extraction step was varied using acetone; percolation and 8-days soaking was used in this step. The yields of extracts were compared, before subjecting them to Clevenger distillation and the final yield was also compared (Table 9).

Table 9: Amount of extract from percolation and 8 day soaking solvent extraction.

Solvent extraction method	Percolation	8 days soaking
Mass of flowers used (g)	20.0500	20.1385
Mass of solvent extraction product (g)	1.319	0.5736
Percentage of solvent extraction product (%)	6.58	2.85
Mass of SECD extract (g)	0.0440	0.0438
Percentage of SECD extract (%)	0.2195	0.2174

The results above (table 9) show that solvent extraction by percolation gave a larger amount of cold extract (6.58%) than that of 8-day soaking (2.85%).However when the extracts were subjected to Clevenger distillation, the amount of essential oil obtained was similar. Due to the cumbersome nature of the percolation method and fear of losing volatile components in the many concentration steps of the percolation method, 8-day soaking was used in subsequent SECD extractions.

4.4.2 SECD using Different Solvents

Different solvents had different yields of extracts in SECD extraction (Table 10).

Table 10: Yields of SECD extracts of different solvents.

Solvent used	Weight of flowers used (g)	Weight of extract (g)	Yield (%)
Hexane	20.5959	0.0640	0.3107
Acetone	20.0036	0.0503	0.2515
DCM	20.3494	0.0546	0.2683
Ethyl acetate	20.0724	0.0656	0.3268
Methanol	20.0193	0.0539	0.2692
Hexane/Acetone (1:1)	20.0200	0.0474	0.2368
Ethyl acetate/DCM (1:1)	20.3734	0.0567	0.2783

Ethyl acetate and hexane had the highest yields of the SECD extracts with percentage yields of 0.3268% and 0.3107% respectively. However, the hexane SECD extract did not have the characteristic smell of the chamomile essential oil though its smell was sweet, an indicator that some volatile components were missing or were in small amounts. The rest of the extracts had the characteristic smell of chamomile essential oil. All the SECD extracts had the characteristic blue colour of chamomile oil, showing that all the solvents used extracted matricin from the chamomile flowers over the 8 day period of soaking.

4.5 UV-Vis Analysis of SECD Extracts

UV-Vis spectra of all the SECD extracts showed peaks at 280 nm and 310 nm. A maximum which was picked by the peak pick function of the instrument was also evident in all the extracts at 605 nm. The absorbance of the maxima at 605 nm varied from one extract to another.

When a blue component, which was isolated from chamomile essential oil, was analysed under UV-Vis it also showed the same maximum peak at 605 nm. The peak being in the visible range was most likely a measure of the intensity of the blue colour in the extracts or in other words a measure of the amount of the blue component in the extracts. The absorbances of the different extracts at 605 nm were as follows (Table 11):

Table 11: Absorbances of the SECD extracts at 605 nm.

SECD extract	Absorbance at 605 nm
Ethyl acetate extract	0.019
Acetone extract	0.018
Hexane extract	0.009
Hexane-Acetone extract	0.022
Methanol extract	0.018
DCM extract	0.025
Ethyl acetate-DCM extract	0.025

From Beer's Law which states that absorbance is directly proportional to the concentration of the chemical species responsible for the absorbance (Skoog *et al*., 1998), it can be said that the extracts had varying concentrations of chamazulene, the component responsible for the blue colour (Franke & Schilcher, 2005). From the absorbances, DCM and ethyl acetate-DCM extracts had the highest concentrations with absorbances at 0.025, followed by the hexane-acetone extract with the absorbance at 0.022. Ethyl acetate extract followed with the absorbance at 0.019. The methanol and acetone extracts had similar absorbances at 0.018 suggesting similar concentrations of chamazulene. The hexane extract had the least absorbance at 0.009 suggesting hexane had the least concentration of chamazulene. This could be explained by the fact that matricin, a precursor for chamazulene (structure **(4)**), being a polar compound is not effectively extracted by hexane which is a non polar solvent.

4.6 UV-Vis Analysis of Cold Extracts and Chamomile Essential Oil

All the products of cold extraction had a maximum peak at around 660 nm, which varied in absorbance from one extract to another (table 12). The spectra were also characterized by strong absorption in the range 270 – 400 nm. However, the cold extracts did not have a peak at 603 nm (Figure 7), which is attributed to chamazulene which is a product of decomposition of matricin at high temperatures.

The UV spectrum of the chamomile essential oil also showed strong absorption between 270 and 400 nm and had a maximum peak at 603 nm.

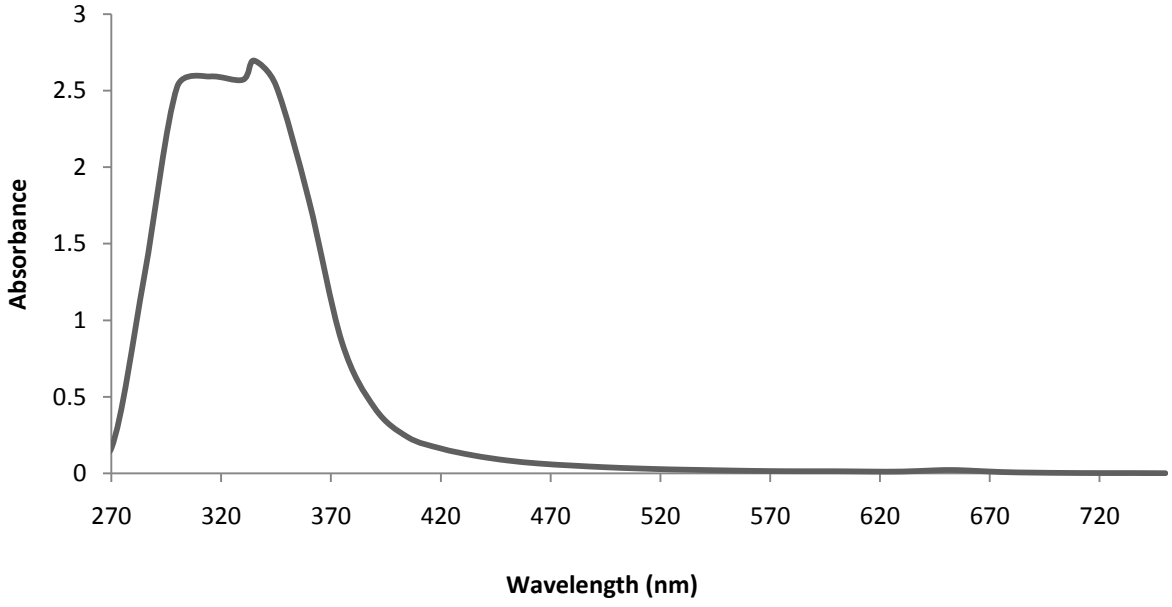


Figure 7: UV-Vis spectrum of 20 mg of ethanol extract in 25 ml ethanol.

Table 12: Maximum UV/Vis absorbances of the cold extracts and chamomile essential oil.

Extract	Peak wavelength (nm)	Absorbance
Acetone extract	663	0.040
	606	0.013
	348	2.786
DCM extract	645	0.047
	305	2.962
Ethyl acetate extract	662	0.013
	308	3.043
Ethanol extract	652	0.023
	335	2.696
Ethanol-water extract	316	2.684
Hexane-acetone extract	663	0.036
	606	0.011
	534	0.016
	332	3.101
Ethyl acetate-DCM extract	665	0.014
	321	2.683
Hexane extract	312	2.985
Methanol extract	653	0.031
	601	0.015
	310	2.783
Chamomile essential oil	603	0.137
	317	2.840

The differences in absorbances at some wavelengths from one extract to another indicates differences in the capabilities of the different solvents in extracting the components responsible for the absorbance at those wavelengths.

4.7 IR Analysis of Cold Extracts

IR analysis of cold extracts revealed differences as evident in absorption bands for each extract. The IR spectra were characterized by the bands shown by tables 13-20 below.

Table 13: Characteristic IR bands for acetone cold extract.

Frequency (cm^{-1})	Intensity/thickness of band	Functional group
1377.54	Medium, sharp	C-H rock alkanes
1445.72	Medium, sharp	C-C stretch (in ring) aromatics
1474.70	Medium, sharp	
1722.95	Strong, sharp	C=O stretch
1737.45	Strong, sharp	C=O stretch
1766.67	Strong, sharp	
2871	Medium, sharp	C-H stretch alkanes
2939.28	Medium, sharp	

Table 14: Characteristic IR bands for DCM cold extract.

Frequency (cm^{-1})	Intensity/thickness of band	Functional group
735.41	Strong, sharp	C-H aromatic
900.75	Weak	
1027.13	Weak	
1246.40	Medium	
1270.40	Medium	
1377.54	Weak	
1737.45	Medium	C=O stretch
1747.19	Medium	
2360.34	Weak	
2384.57	Weak	
2876.10	Medium	C-H stretch alkanes
2949.07	Medium	
3085.43	Weak	

Table 15: Characteristic IR bands for ethanol cold extract.

Frequency (cm^{-1})	Intensity/thickness of band	Functional group
681.72	Medium	
779.12	Strong	C-H aromatic
832.57	Strong	
861.79	Strong	
929.97	Medium, sharp	O-H bend carboxylic acids
1635.30	Strong	
2300.15 - 1951.73	Weak, broad	C≡C stretch alkynes
3712.78 - 3021.95	Strong, broad	O-H stretch, H-bonded

Table 16: Characteristic IR bands for hexane cold extract.

Frequency (cm^{-1})	Intensity/thickness of band	Functional group
1581 - 910	Medium, broad	
1746	Weak, sharp	
2702	Medium	
2761	Medium	
2872.99	Strong	
3028.57	Medium	=C-H stretch alkenes

Table 17: Characteristic IR bands for ethyl acetate cold extract.

Frequency (cm^{-1})	Intensity/thickness of band	Functional group
740.16	Strong, narrow	C-H aromatic
832.57	Medium	
871.53	Strong, sharp	
895.77	Strong	
968.94	Medium	
1071.10	Weak	
1144	Weak, broad	
1589 - 1445.72	Medium, broad	
1601.32	Medium	
1737.45	Medium	C=O stretch
1825.11	Medium	
1883.55	Weak, sharp	
1941.99	Weak, sharp	
2355 - 2078	Medium, broad	

Table 18: Characteristic IR bands for ethyl acetate-DCM cold extract.

Frequency (cm^{-1})	Intensity/thickness of band	Functional group
629.23	Weak, narrow	
702.16	Medium, narrow	
779.84	Strong, narrow	C-H aromatic
848.02	Strong, narrow	
930.69	Medium	Aryl-O-CH_2
1003.86	Medium	
1032.84	Medium	
2093.56	Weak	C≡C stretch alkynes
2171.48	Weak	
2872.66	Medium, narrow	C-H stretch alkanes
3499.69	Weak	
3601.85	Weak	

Table 19: Characteristic IR bands for hexane-acetone cold extract.

Peak (cm^{-1})	Intensity/thickness of band	Functional group
1124.37	Medium	
1284.69	Medium	
1459.72	Medium, sharp	C-H bend alkanes
1513.09	Medium, sharp	
1556.72	Medium, sharp	
1663.00	Medium, sharp	-C-C- stretch (in ring) aromatics
1726.00	Medium, sharp	
2013.26 - 1833	Medium, broad	
2270.82 - 2163	Medium, broad	
2353.36	Medium	
2382.53	Medium	
2498.98	Medium	
2552.58	Medium	
2702.94 - 2605.94	Medium	H-C=O : C-H stretch aldehydes
2785.71	Medium	
2950.78	Medium	C-H stretch alkanes
2989.67	Medium	
3455.94	Weak	
3659.90	Medium	
3786.00	Medium	

Table 20: Characteristic IR bands for methanol cold extract.

Peak (cm^{-1})	Intensity/thickness of band	Functional group
691.46	Medium	
774.13	Strong, narrow	C-H aromatics
866.54	Weak	
895.77	Strong, narrow	
978.67	Weak	
1017.40	Weak	
1168.25	Weak	
1250.92	Weak	
1285.13	Weak	
1416.50	Medium	C-C stretch (in-ring) aromatics
1445.72	Medium	
1615.81	Medium	
1640.28	Medium	-C=C- stretch alkenes
1713.21	Medium	

1737.45	Medium	C=O stretch
2063.62	Weak, narrow	
2151.05	Weak	
2326.13	Medium, narrow	
2428.52	Weak, narrow	
2545.16	Weak, narrow	
2715.49	Weak, narrow	
2880.84	Medium, narrow	C-H stretch alkanes
2953.77	Medium	
3085.15	Medium	=C-H stretch alkenes

The peak appearing between 1770 cm^{-1} and 1720 cm^{-1}, whose intensity ranges from medium to strong, is evident in most of the spectra hence is characteristic of the chamomile extract.

4.8 Determination of Refractive Index of Chamomile Essential Oil

The determination of the refractive index in one month intervals was meant to determine if it could be used as a monitoring parameter.

Table 21: Refractive index of chamomile essential oil measured at monthly intervals.

Date	**Refractive Index at 25°C**
01/02/11	1.49830
16/02/11	1.48277
02/03/11	1.48478
04/04/11	1.48577
04/05/11	1.49100
03/06/11	1.48375
04/07/11	1.48430
Average	1.48724 ± 0.00515

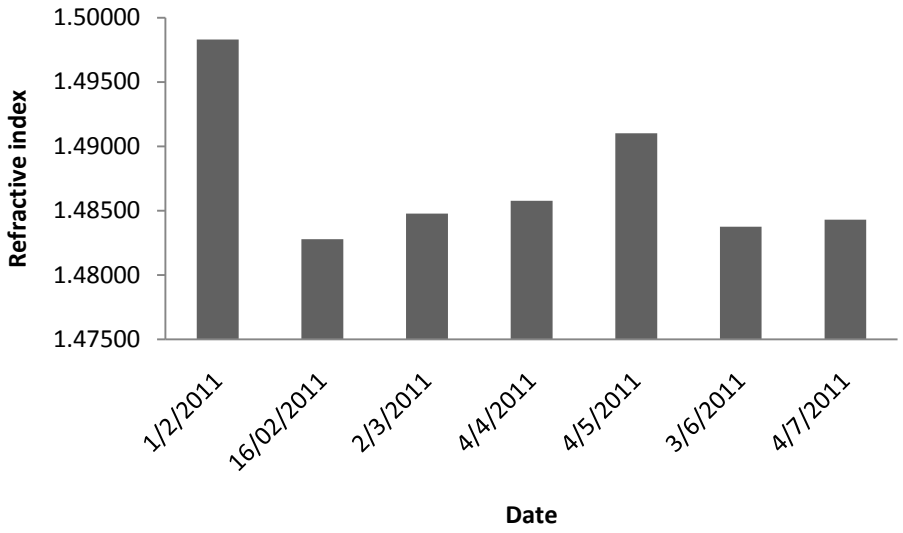


Figure 8: Refractive index of chamomile oil at different months.

The refractive index of chamomile essential oil has been variously reported to range from 1.480 to 1.505 (Chamomile blue pure essential oil) and 1.440 – 1.450 (Cedar wood oil) when measured at 20^0C. It has also been reported to range between 1.480 and 1.5055 when measured at 25^0C (*Chamomilla recutita* Rauschert/ German chamomile). The refractive index that was measured in the six months ranged from 1.49830 to 1.48277 which was comparable to the values reported above. The trend of the refractive indices (Figure 8) shows that the measurement of this parameter is not reliable in monitoring any changes in the quality of the chamomile essential oil. It gave an average of 1.48724 ± 0.00515 over a period of six months.

4.9 Visual Monitoring of Chamomile Essential Oil

Upon extraction at optimum temperature, the essential oil was blue in colour. But on exposure to different degrees of light over a period of time the colour went through a series of changes as shown below (Table 22).

Table 22: Physical changes of chamomile essential oil with different exposures to light.

Date	FM-WNDW	FM-CPBD	FM-LCKR
28-03-2011	The sample had stayed for 2 days but still had the characteristic ***blue*** colour of chamomile oil	The sample had stayed for 2 days but still had the characteristic ***blue*** colour of chamomile oil	The sample had stayed for 2 days but still had the characteristic ***blue*** colour of chamomile oil
28-04-2011	The colour had changed to ***green***.	Still had the ***blue*** colour.	Still had the ***blue*** colour similar to FM-CPBD.
28-05-2011	Colour had changed to ***yellowish-brown***.	Still had the ***blue*** colour.	Still had the ***blue*** colour similar to FM-CPBD.
28-06-2011	Yellowish-brown in colour.	Greenish-blue in colour	Greenish-blue in colour
28-07-2011	Yellowish-brown in colour	Green in colour	Green in colour
28-08-2011	Yellowish-brown in colour	Green in colour	Green in colour
28-09-2011	Yellowish-brown	Green	Green
28-10-2011	Yellowish-brown	Green	Green
28-11-2011	Yellowish-brown	Green	Green
28-12-2011	Yellowish-brown	Green	Green

FM-WNDW: vial set by the window; **FM-CPBD**: vial set on the cupboard; **FM-LCKR**: vial set in the locker.

After a couple of days of exposure to light, the three samples retained the original blue colour. After a month's exposure to light, the sample by the window (FM-WNDW) changed to green while the other two samples still had the blue colour.

In the second month the sample by the window changed to yellowish brown in colour while the other two were still blue in colour. However, in the third month the sample on the cupboard (FM-CPBD) and the one in the locker (FM-LCKR) changed colour to greenish-blue then to green in the fourth month while the sample by the window remained yellowish brown through the monitoring period. This showed clearly that direct exposure to light hastens the degradation of the chamomile oil. It was also noticed that the samples lost the characteristic smell of chamomile oil, which is a proof of loss of quality, although they remained tightly corked throughout the period.

4.10 UV-Visible Monitoring of Chamomile Essential Oil With Time

Monitoring of the oil at a two month interval showed a reduction in the absorption between 270 – 390 nm as shown in figure 7 below. At 310 nm, the absorbance reduced by 29.7% from 3.806 in February, 2011 to 2.675 in April, 2011. There was also a reduction in absorbance at 360 nm by 27.8% from 3.612 in February, 2011 to 2.605 in April, 2011 (Figure 9).

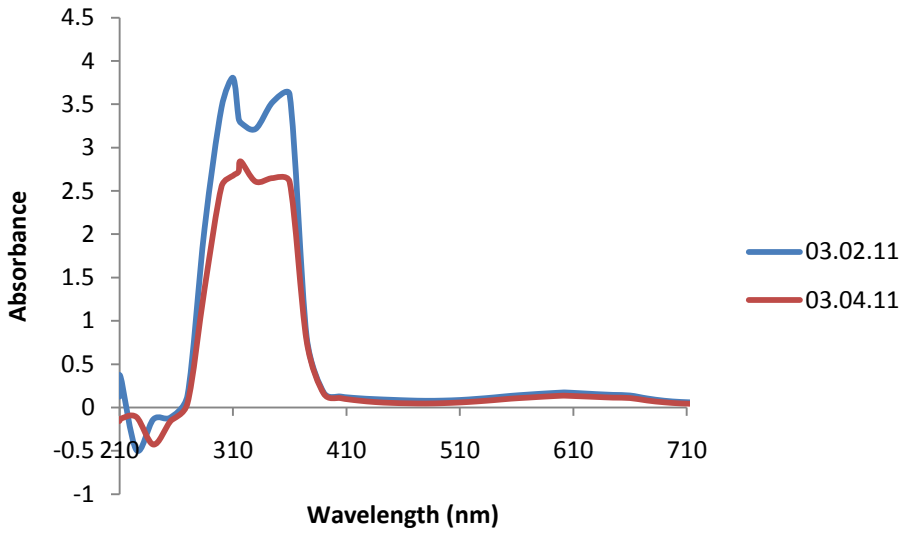


Figure 9: UV-Vis monitoring of chamomile oil in February and April 2011.

Monitoring of the oil at a one month interval also showed some differences in absorption (Figure 10). Note that figure 10 concentrates on the region 510 nm to 710 nm, hence the difference in the absorption curve.

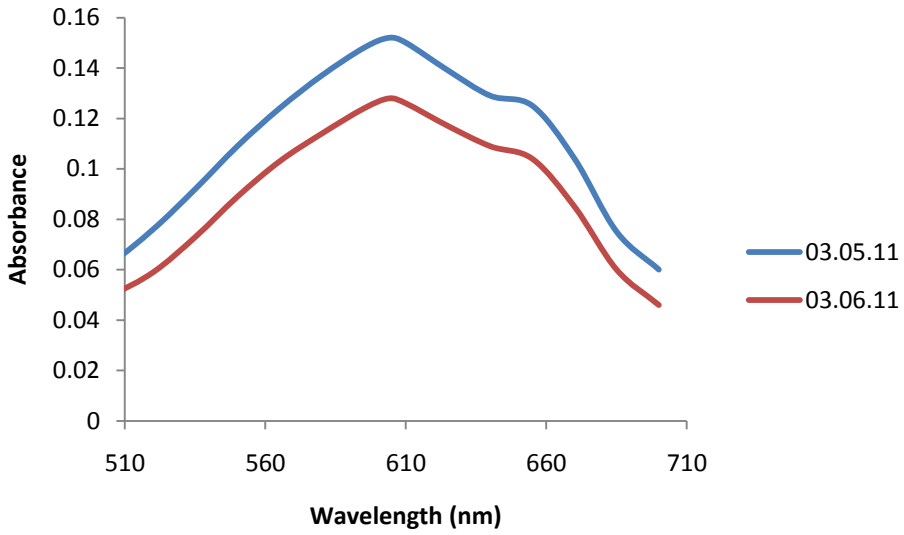


Figure 10: UV-Vis monitoring of chamomile oil in the months of May and June 2011.

For example at 604 nm there was a 15.8% reduction in absorbance from 0.152 in May to 0.128 in June. The reduction in absorbances in the region 270 – 390 nm could be attributed to the

reduction in concentrations of the compounds responsible for the absorption in that region which are most likely volatile components.

Absorbance at 603/604 nm shows very little difference most likely because it is caused by absorption by less volatile components. This maximum peak having been observed in the isolated blue component (chamazulene) could be attributed to it.

4.11 Separation of the Chamomile Oil

4.11.1 Preparative TLC

The blue chamomile oil was spotted on preparative TLC and developed with DCM:ethyl acetate (98:2 v/v). This solvent system was used because it had shown good separation of the components. The TLC showed five bands, but only 3 were clearly separated when viewed under UV light. The bands were scraped off the plate, soaked in DCM and filtered. The filtrates of the clearly separated bands were labeled compounds 1, 2 and 4.

The purity of 1 was checked by spotting on an analytical TLC plate and developing using hexane. The TLC showed two spots, one of the desired blue component of $R_F = 0.59$ and another spot of $R_F = 0.74$ showing that it was not pure. The impure compound 1 was respotted on preparative TLC and developed using 100% hexane. The blue component was scraped off the preparative plate and the silica removed through dissolution in DCM and filtration using a filter paper. When the purity of compound 1 was checked by TLC, it gave a single spot suggesting absolute purity. The solution was left to concentrate in a vial which was then corked, wrapped in aluminium foil and stored in a refrigerator awaiting analysis. (See Appendix VI for TLCs).

When compound 2 was spotted on an analytical TLC and developed using hexane:ethyl acetate (95:5 v/v), two spots were visible: a major one of R_F = 0.37 and a minor one of R_F = 0.25. Preparative TLC of compound 2 was done using hexane: ethyl acetate (95:5 v/v). Compound 2 could not be purified any further. Compound 4 on the other hand showed a single spot on analytical TLC at R_F of 0.61 when developed with hexane:ethyl acetate (85:15 v/v) (See Appendix VI). Compound 4P was scraped from the plate and dissolved in DCM. The silica was removed as in the case of compound 1. The solution was left to concentrate and the vial corked, wrapped in aluminium foil and stored in a refrigerator awaiting analysis.

4.11.2 UV-Visible Analysis of Blue Component (Chamazulene) Isolated from Chamomile Essential Oil

The UV-Visible spectrum of the blue component had a λ_{MAX} at 605 nm with absorbance of 0.047. Using Beer's law $A = \varepsilon bC$ (Skoog *et al*., 1998), the molar absorptivity of the component was calculated as follows.

A= absorbance.

ε= Molar absorptivity of chemical species ($cm^{-1}M^{-1}$).

C= concentration of the chemical species (M).

b= path length or the thickness of the cell (cuvette) containing the analyte solution (mostly 1 cm)

When molar absorptivity is made the subject of the formula, it becomes:

$\varepsilon = \frac{A}{bC}$……Equation 1.

C (concentration in molarity) = $\frac{moles\ of\ analyte}{volume\ of\ solution\ in\ liters}$……Equation 2.

Moles of analyte is given by: $\frac{Mass\ of\ analyte\ (g)}{Molar\ mass\ of\ analyte\ in\ g/mol}$……Equation 3.

The analyte in this case is chamazulene, hence since 0.0006g of chamazulene was used in the analysis and the molar weight of chamazulene is 184.28 g/mol (Roth & Rupp, 1995), the moles of chamazulene is:

Moles of chamazulene = $\frac{0.0006\ g}{184.28\ g/mol}$ = 3.2559×10^{-6} moles.

In a 25 ml hexane solution there were 3.2559×10^{-6} moles, this implies that in 1 liter (1000 ml) there would be:

$\frac{1000}{25} \times 3.2559 \times 10^{-6} = 1.3024\times10^{-4}$ M= C.

Therefore, ε= $\frac{A}{bC}$ = $\frac{0.047}{1cm\times1.3024\times10^{-4}\ M}$ = 360.87 $cm^{-1}M^{-1}$.

From literature (Roth & Rupp, 1995), $\log_{10}\varepsilon_{605\ nm}$ = 2.64 (ε = 436.5). ε = 360.87 $cm^{-1}M^{-1}$ gives $\log_{10}\varepsilon_{605\ nm}$ to be 2.56. In literature, cyclohexane was used, which could be the reason for the difference in the molar absorptivity

4.12 GC-MS Analysis of Chamomile Oil Samples

After running each sample on GC-MS, peak identification was done by doing a library search using NIST MS search 2.0 and the percentage of the components was done by obtaining a percentage report using Enhanced Data Analysis. The results of each sample analysed were as shown below.

4.12.1 Chamomile Essential Oil of Flowers from Different Regions

Table 23: Percentage of main components in chamomile oil of flowers from different regions, determined by GC-MS.

Component	Retention time (min)	Percentage by peak area (%)		
		KA	NJA	KIB
Unknown	10.359	-	-	0.348
Unknown	11.046	-	-	1.095
n- Decanoic acid	12.554	-	0.711	-
β-farnesene	13.989	3.781	3.960	5.165
Spathulenol	15.779	0.689	0.745	0.322
Unknown	16.338	-	-	0.399
T-cadinol	16.528	1.062	1.928	-
α-Bisabolol oxide B	16.702	6.175	5.157	24.277
Unknown	16.789	1.072	1.483	0.869
α-Bisabolol	16.989	2.223	3.586	6.533
Unknown	17.052	15.683	24.723	2.883
Chamazulene	17.641	2.115	0.913	10.382
α-Bisabolol oxide A	17.805	61.142	53.454	42.649
Cis-enyne dicycloether	19.189	4.407	1.992	2.637
Trans-enyne dicycloether	19.287	1.275	1.347	0.659

KA-Kangari; **NJA**-Njabini; **KIB**-Kibwezi; - Not detected.

The amount of β-farnesene was highest in the Kibwezi sample at 5.165%. Njabini and Kangari samples had 3.960% and 3.781% of β-farnesene respectively. Spathulenol on the other hand was highest in the Njabini sample at 0.745%. Compared to foreign samples, β-farnesene has been reported to be 4 to 13% in Estonian chamomile (Orav *et al*., 2001). Spathulenol has been

reported to be 1.4-3.9% (Raal *et al*., 2003) and even as high as 8.49% in chamomile grown in Iran (Shams-Ardakani *et al*., 2006).

The Kibwezi oil sample had the highest amount of α-Bisabolol oxide B at 24.277%, while the Kangari and Njabini oils had 6.175% and 5.157%, respectively. This has been reported to range between 8-12% in essential oil from Estonia (Orav *et al*., 2001) and range between 5.7-24.8% in essential oils from other regions in Europe with the oil from Great Britain having the highest at 24.8% (Raal *et al*., 2003). α-Bisabolol was highest in the Kibwezi sample at 6.533% and lowest in the Kangari sample at 2.223%. α-Bisabolol was reported highest in an oil sample from Hungary at 23.6% which was tagged α-Bisabolol-rich and lowest in an oil sample from Great Britain at 0.4% (Raal *et al*., 2003).

The Kibwezi oil sample, with a chamazulene content at 10.382%, was tagged chamazulene-rich compared to the ones from Kangari (2.115%) and Njabini (0.913%). The Great Britain sample was also found to be chamazulene-rich with a 13.8% content. Chamazulene content in other oils has been reported to be 5-7% (Orav *et al*., 2001) and even as low as 1.2% in a sample from Belgium (Raal *et al*., 2003). In 2011, Estonian oil was found to have 4.7% chamazulene-content (Raal *et al*., 2011) which was different from an earlier study on Estonian oil which found a chamazulene-content above 5% (Orav *et al*., 2001). A study on Iranian chamomile found a chamazulene content of 3.28% (Shams-Ardakani *et al*., 2006).

α-Bisabolol oxide A content was highest in the Kangari essential oil at 61.142%. Essential oils from Njabini and Kibwezi had α-bisabolol oxide A content of 53.454% and 42.649%, respectively. The three samples had a higher content of α-bisabolol oxide A than that found in Iranian chamomile (25.01%) (Shams-Ardakani *et al*., 2006). In Estonian chamomile, α-bisabolol

oxide A was reported to be 39.4% (Raal *et al*., 2011) and 20-33% (Orav *et al*., 2001). In a study of oils from different areas of Europe, α-bisabolol oxide A was found to be 43.2% (Estonia), 54.6% (France), 12.6% (Hungary), 47.3% (Belgium), and 19.7% (Great Britain) (Raal *et al*., 2003).

The Kangari sample had the highest amount of cis-enyne dicycloether at 4.407% and the Njabini sample had the lowest at 1.992%. These percentages were lower than those reported elsewhere; Iranian chamomile had 7.42% (Shams-Ardakani *et al*., 2006) and Estonian chamomile was found to have a cis enyne dicycloether content of 11.5% (Raal *et al*., 2011). In chamomile samples from different regions in Europe, the cis enyne dicycloether content was found to be ranging between 13.0% and 22.2% with the Belgian sample having the highest percentage (Raal *et al*., 2003).

The Njabini chamomile oil had the highest content of the trans enyne dicycloether at 1.347%, followed by the Kangari oil at 1.275%. The Kibwezi oil had 0.659% of the trans enyne dicycloether. The above percentages of the trans enyne dicycloether are higher than those reported in oil from European chamomile which was an average of 0.3% (Raal *et al*., 2003).

The above results show a variation of the components in the essential oil from the three geographical areas; this could be a result of the differences in the ecological conditons of the growing areas and also post-harvest handling of the flowers like drying and storage.

4.12.2 Chamomile Oil Extracted at Different Temperatures

Different components of chamomile essential oil were obtained from chamomile flowers (from Kangari) when the oil was extracted at varying temperatures in Clevenger distillation (Table 24).

Table 24: Percentage of the main components in chamomile oil extracted at different temperatures

Component	Retention time (min)	Percentage by peak area at temperature: 115^0C	130^0C	140^0C	145^0C	150^0C
β-farnesene	13.989	1.258	2.182	2.335	2.226	2.912
Spathulenol	15.774	0.583	0.522	0.466	0.464	0.426
Unknown	16.338	-	-	0.324	0.312	-
T-cadinol	16.531	1.736	1.586	1.457	1.361	1.277
α-Bisabolol oxide B	16.697	3.797	3.680	3.207	3.010	2.770
Unknown	16.789	1.860	1.707	1.634	1.616	1.682
α-Bisabolol	16.986	2.728	2.172	2.047	1.979	1.867
Unknown	17.046	15.535	14.251	12.776	11.953	11.274
Chamazulene	17.641	2.460	2.444	2.491	2.248	2.324
α-Bisabolol oxide A	17.813	66.399	66.794	64.809	63.809	65.952
Cis-enyne dicycloether	19.196	1.839	3.203	5.664	7.271	5.748
Trans-enyne dicycloether	19.292	1.804	1.459	1.791	1.809	1.698
n-Hexadecanoic acid	19.820	-	-	-	0.527	0.704
n-Hexadecanoic acid ethyl ester	20.153	-	-	-	0.342	-
Unknown	21.846	-	-	0.439	0.397	0.536
n-Pentacosane	25.579	-	-	0.560	0.675	0.831

- Not detected.

In addition, the percentages of the different components tend to vary with the increase in extraction temperature (Figure 11).

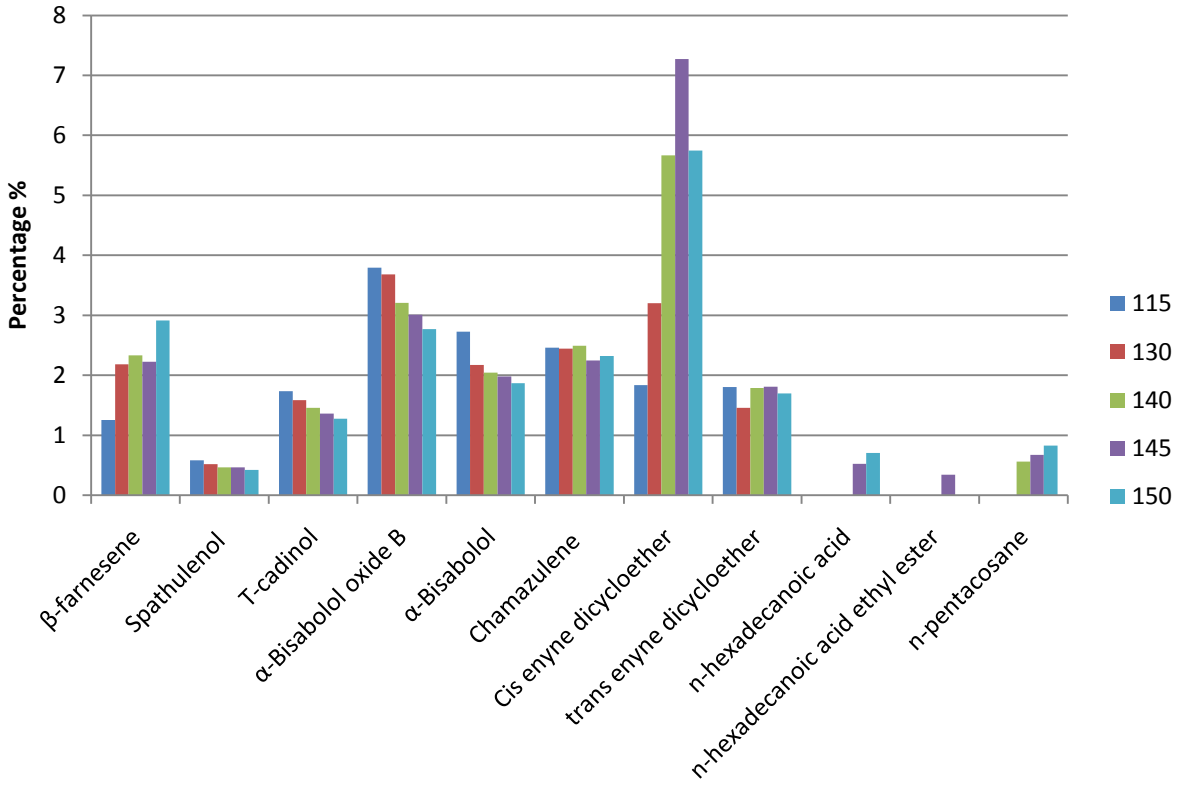


Figure 11: Change in percentage of main components with increasing temperature (^{o}C) in Clevenger distillation.

Change in the extraction temperature seemed to affect the percentage of the main components differently with some increasing and others decreasing with increase in temperature. The components that showed an increasing trend were β-farnesene, cis enyne dicycloether, and n-pentacosane. However, cis enyne dicycloether decreased in percentage at 150^{0}C with the percentage moving from 7.271% at 145^{0}C to 5.748% at 150^{0}C. There were some components that were only detectable in samples extracted at high temperatures; n-hexadecanoic acid was

detected in the 145^{0}C extract and showed an increase in the 150^{0}C extract. The percentage rose from 0.527% to 0.704%; n-hexadecanoic acid ethyl ester was only detected in the 140^{o}C extract while n-pentacosane, first detected in the 140^{0}C extract, increased with increase in temperature. The increase in percentage of these components could point to their being less volatile.

The components that had a decreasing trend with increase in extraction temperature were spathulenol, T-cadinol, α-bisabolol oxide B and α-bisabolol. The decrease in concentration could be a result of degradation of the components in the high temperatures. There were yet other components that did not have a particular trend in the percentage change. These were chamazulene, trans enyne dicycloether and α-bisabolol oxide A. No clear pattern was obtained for the variation of percentage of α-bisabolol oxide A with increase in temperature (Figure 12).

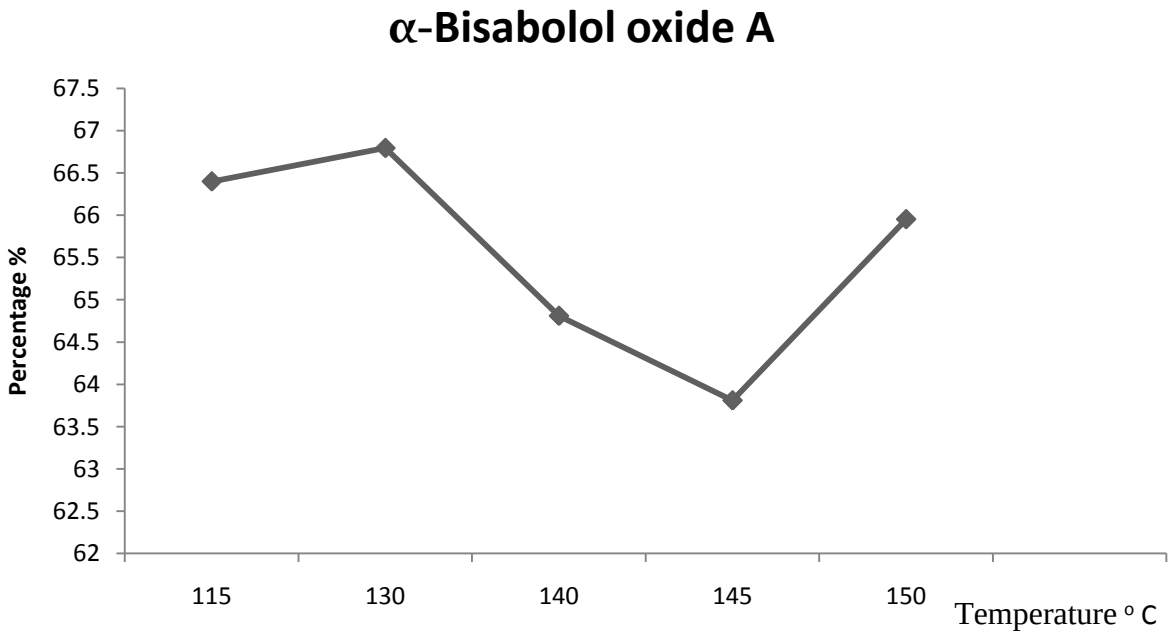


Figure 12: Variation in the percentage of α-Bisabolol oxide A with increase in extraction temperature in Clevenger distillation.

4.12.3 Chamomile Oil Extracted on Different Months

The amount of different components extracted showed variation with storage time of the chamomile flowers (Table 25). Kangari flowers were used for these experiments.

Table 25: Variation of the components of chamomile oil extracted from a flower sample on different months of 2011.

Component	Retention time (min)	Percentage by peak area on date (2011): 2 Feb	3 Mar	6 April	3 May	3 Jun	5 Jul
β-farnesene	13.989	2.921	2.897	2.617	2.289	1.919	2.406
Spathulenol	15.779	0.861	0.861	0.542	0.717	0.655	0.555
Unknown	16.256	0.390	0.426	-	0.406	0.369	-
T-cadinol	16.533	2.103	2.036	1.678	1.840	1.773	1.495
α-Bisabolol oxide B	16.697	5.351	5.199	3.989	4.379	3.988	3.367
Unknown	16.795	1.623	1.728	1.763	2.339	2.339	2.409
α-Bisabolol	16.994	-	-	2.220	2.688	2.407	-
Unknown	17.053	25.304	23.267	15.728	16.026	14.952	15.513
Chamazulene	17.641	2.630	2.560	2.495	2.476	2.352	1.992
α-Bisabolol oxide A	17.812	55.751	56.115	64.923	60.513	63.773	66.824
Cis-enyne dicycloether	19.189	1.657	3.05	1.85	3.593	2.855	4.259
Trans-enyne dicycloether	19.290	1.409	1.859	2.194	1.925	2.032	0.605

- **Not detected**

Some components showed a decreasing trend with storage of the flowers over the months; these were β-farnesene, spathulenol, T-cadinol, α-Bisabolol oxide B, and chamazulene. The graph below illustrates this (Figure 13). However, there was an anomaly with some components whose

percentage would decrease and then increase after some months. This could be explained by the fact that the component's concentration remained constant with respect to others whose concentration went down hence the apparent increase. The variation in percentage is probably a result of loss of the volatile components with storage time, although the flower sample was stored in the same condition throughout the six months (in an air tight polythene bag). α-Bisabolol oxide B shows significant decrease compared to the other components, which points to the fact that it is more volatile than the other components at the storage conditions or degrades rapidly. The results show that quality of the flowers is lost with storage even when stored in an air tight polythene bag.

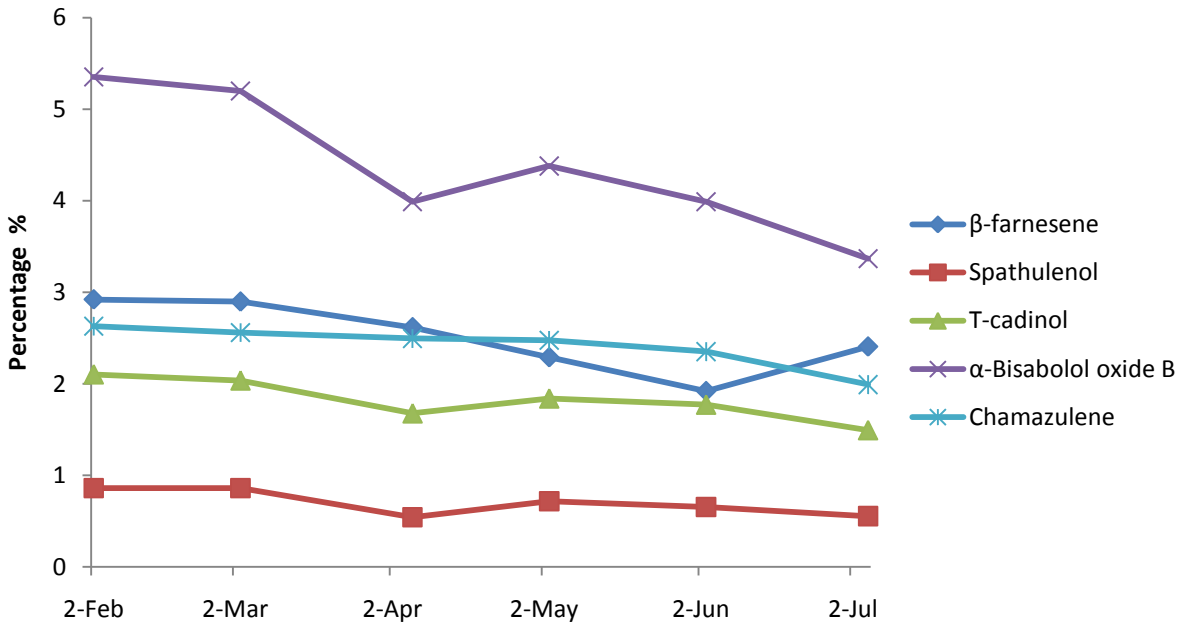


Figure 13: Variation in the percentage of some components with the storage of flowers over six months in the year 2011.

4.12.4 Chamomile Oil Extracted by SECD using Different Solvents

n-Decanoic acid was detected in the acetone, ethyl acetate and ethyl acetate/DCM SECD extracts at 0.565%, 0.383% and 0.388% respectively. The DCM extract had the highest amount of β-farnesene at 6.326% and the acetone extract had the lowest amount at 1.323%. Spathulenol was highest in the DCM extract at 0.977% and lowest in the hexane extract at 0.555%.

The highest percentage of T-cadinol was in the ethyl acetate extract at 2.293% and the lowest was in the hexane extract at 0.789%. α-Bisabolol oxide B was highest in the hexane/acetone and ethyl acetate extracts at 5.904% and 5.894% respectively. The extract with the lowest amount was again the hexane extract with a percentage of 3.063% (Table 26).

Table 26: Percentages of the main active components in chamomile oil extracted by SECD using different solvents

Component	**Reten-tion time (min)**	**Percentage by peak area using (solvent):**						
		Hex	**H/AC**	**Ace**	**DCM**	**EA**	**E/D**	**Meth**
n- Decanoic acid	12.544	-	-	0.565	-	0.383	0.388	-
β-farnesene	13.988	3.602	3.462	1.323	6.326	4.123	1.930	3.763
Spathulenol	15.774	0.555	0.570	0.607	0.977	0.934	0.816	0.725
Unknown 1	16.348	-	-	-	-	0.625	0.554	-
T-cadinol	16.533	0.789	0.941	1.784	1.561	2.293	1.893	0.890
α-Bisabolol oxide B	16.697	3.063	5.904	4.471	4.860	5.894	5.302	5.713
Unknown 2	16.789	0.749	1.396	2.026	2.499	1.677	2.054	1.193
α-Bisabolol	16.994	-	1.773	2.589	-	-	-	2.198
Unknown 3	17.050	7.607	13.766	16.165	13.207	16.396	13.707	13.449
Chamazulene	17.641	0.859	2.390	1.758	1.328	1.744	1.654	1.680
α-Bisabolol oxide A	17.814	38.548	68.474	67.787	62.431	55.974	64.197	65.831
Cis-enyne dicycloether	19.189	0.681	0.641	-	0.685	-	-	1.788
Trans-enyne dicycloether	19.285	1.021	0.684	-	0.704	-	-	0.916

n-Hexadecanoic acid	19.825	-	-	-	0.818	1.876	1.527	0.222
n-Hexadecanoic acid ethyl ester	20.164	0.513	-	-	-	2.738	1.529	-
Linoleic acid, methyl ester (Z,Z)	21.158	8.197	-	-	0.533	-	-	-
Linoleic acid Methyl ester (Z,Z,Z)	21.220	9.743	-	-	0.687	-	-	-
Octadecanoic acid methyl ester	21.425	1.186	-	-	-	-	-	-
Unknown 4	21.764	-	-	-	-	0.968	0.510	-
Unknown 5	21.847	9.505	-	0.469	0.667	1.712	1.263	1.084
n-Tricosane	23.046	-	-	-	0.392	0.522	0.581	-
n-Pentacosane	25.579	0.604	-	-	0.842	1.173	1.262	-

Hex-hexane, **H/Ac**-hexane/acetone, **Ace**-acetone, **EA**-ethyl acetate, **E/D**-ethyl acetate/DCM, **Meth**-methanol, – Not detected.

Figure 14 shows the variation in the percentages of the main components in the different SECD extracts.

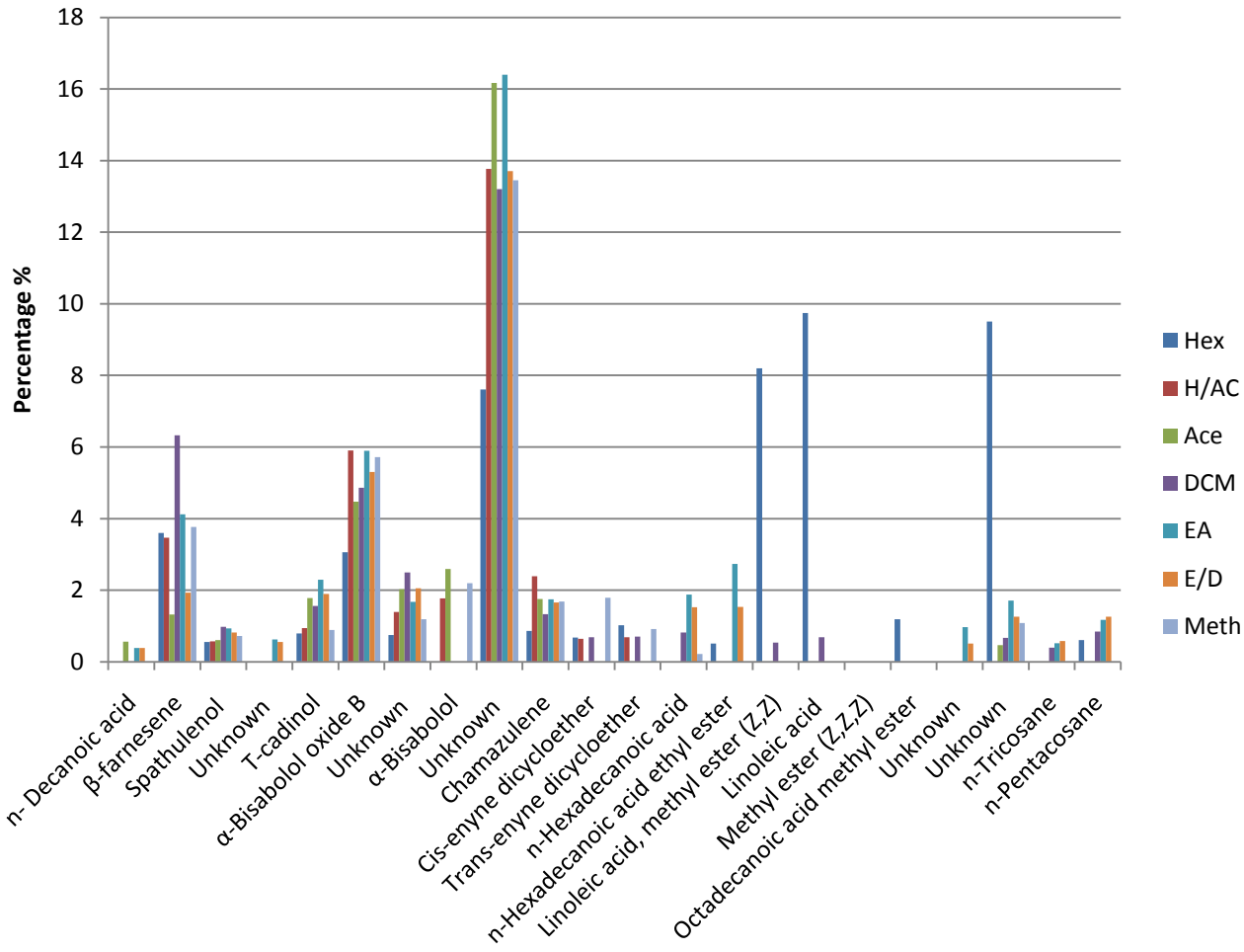


Figure 14: Comparison of the components in chamomile oils extracted by SECD method using different solvents

Only in three of the seven extracts was α-bisabolol detected. These were the hexane/acetone, acetone and methanol extracts. The acetone extract had the highest percentage of α-bisabolol at 2.589% while the lowest was in the hexane/acetone extract at 1.773%. Acetone seemed to improve the capacity of hexane to extract α-bisabolol.

The hexane/acetone extract had the highest percentage of chamazulene at 2.390%, while the hexane extract had the lowest at 0.859%. The low amount of chamazulene in the hexane SECD

extract could be explained by the fact that hexane being a non-polar solvent extracted less of the chamazulene precursor, matricin.

α-Bisabolol oxide A percentage was highest in the hexane/acetone SECD extract at 68.474%, followed closely by the acetone extract at 67.787%. The hexane extract had the lowest percentage of the α-bisabolol oxide A at 38.548% probably as a result of hexane being non-polar.

The cis enyne dicycloether was detected in the hexane, hexane/acetone, DCM and methanol SECD extracts with the methanol extract having the highest percentage at 1.788%. Of these the extract with the lowest percentage of the spiroether was the hexane /acetone extract at 0.641%. The trans enyne dicycloether was also only present in the hexane, hexane/acetone, DCM and methanol SECD extracts. The highest percentage of the trans spiroether was in the hexane SECD extract at 1.021% and the lowest percentage was in the hexane/acetone extract.

Figure 15 illustrates the percentage α-bisabolol oxide A in the SECD extracts. The results show that hexane/acetone (1:1) is the best solvent mixture of the solvents studied that optimizes the extraction of α-bisabolol oxide A.

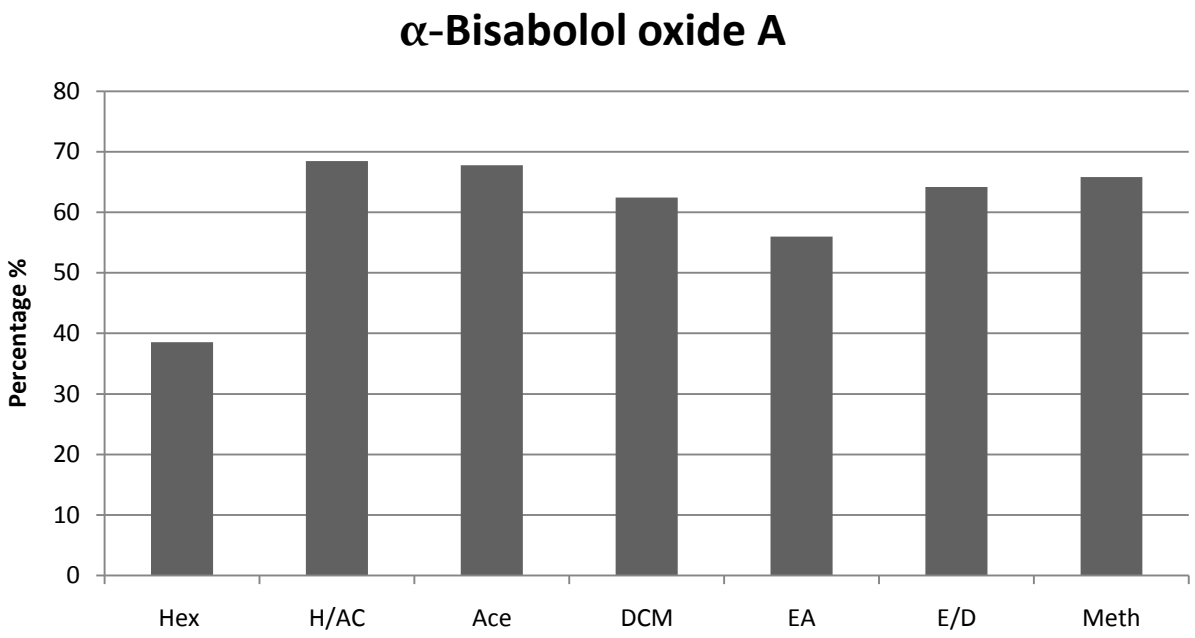


Figure 15: Percentages of α-Bisabolol oxide A in the SECD extracts.

4.12.5 Chamomile Oil Extracted by SECD preceded by Percolation and 8 day Soaking.

Two experiments which were carried out using the SECD method of extraction, involved varying the solvent extraction step. In one, soaking was done through percolation and in the other the flowers were soaked for 8 days. The final extracts had different percentages of the main components as shown below (Table 27).

The SECD-P extract had the highest percentages of all the major components except α-bisabolol oxide A which was highest in the SECD-8 extract at 67.787%; this could mean that the 8-days soaking extracts more of the α-bisabolol oxide A, and the many concentration steps in SECD-P reduce the amount of the component. In the SECD-P extract α-bisabolol oxide A was 53.031%. The spiroethers were the main components which were not detected in both of the extracts. This could possibly be as a result of the concentration step(s) in the solvent extraction part of SECD.

Table 27: Percentage of main components in chamomile oil extracted by SECD with percolation and 8-day soaking.

Component	Retention time (min)	Percentage by peak area with:	
		SECD-8	**SECD-P**
n- Decanoic acid	12.554	0.565	-
β-farnesene	13.984	1.323	6.150
Spathulenol	15.779	0.607	0.989
T-cadinol	16.533	1.784	2.407
α-Bisabolol oxide B	16.697	4.471	5.952
Unknown	16.789	2.026	2.455
α-Bisabolol	16.995	2.589	2.608
Unknown	17.056	16.165	23.073
Chamazulene	17.641	1.758	2.076
α-Bisabolol oxide A	17.810	67.787	53.031
Unknown	21.846	0.469	-

- **Not detected**

Figure 16 illustrates comparison of the amounts of different components in SECD-8 and SECD-P extracts.

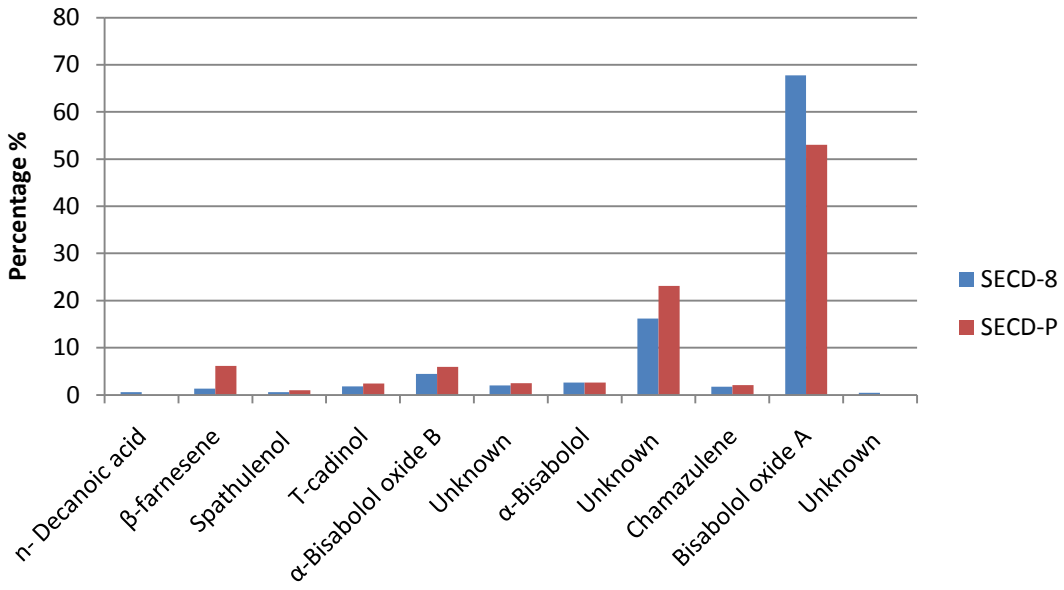


Figure 16: Comparison of percentages of main components in SECD-8 and SECD-P extracts.

4.12.6 GC-MS Analysis of the Blue Component Isolated from the Chamomile Essential Oil

The blue component which was isolated by Preparative TLC was analysed by GC-MS for identification purposes and determination of its purity (Table 28). The chromatogram (Figure 17) for the blue component had a peak at retention time 17.641 min. The percentage report showed that the peak at this retention time was at 100% which is an indicator of its absolute purity.

Table 28: Percentage report for blue component's chromatogram

Peak	R.T.	Height	Area	Percent Total
1	17.641	9727276	1.74E+08	100

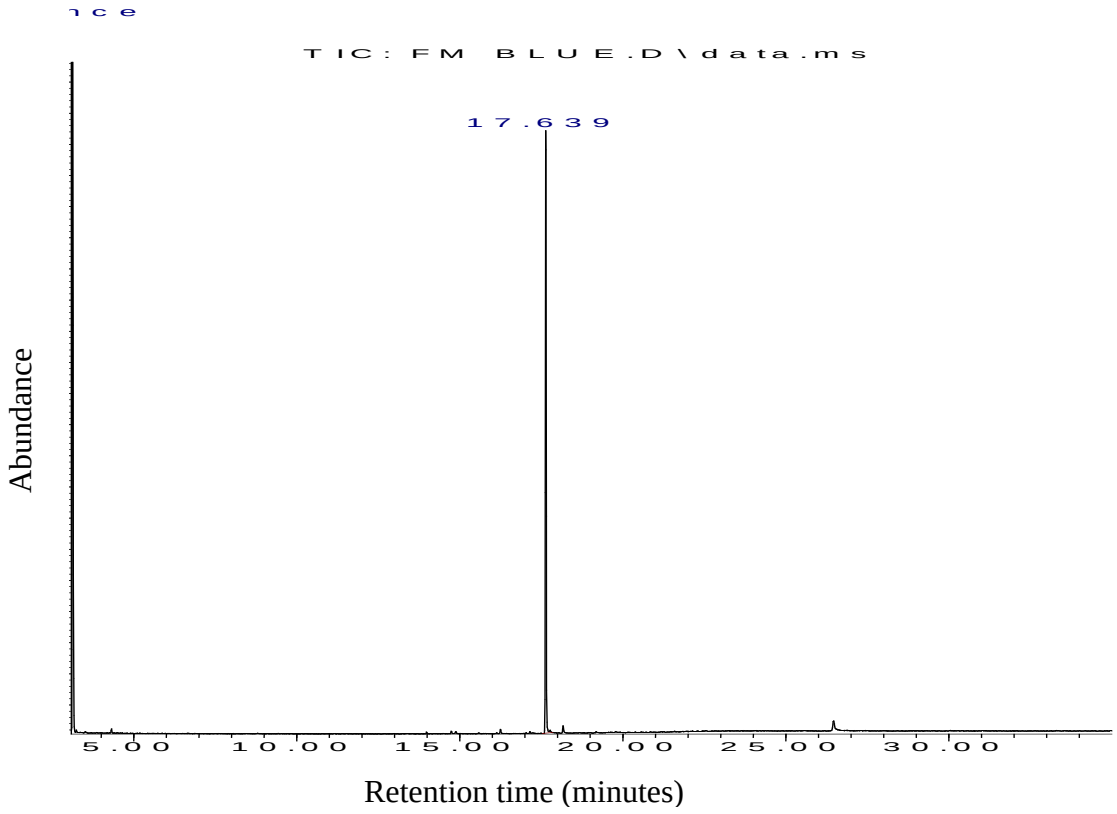
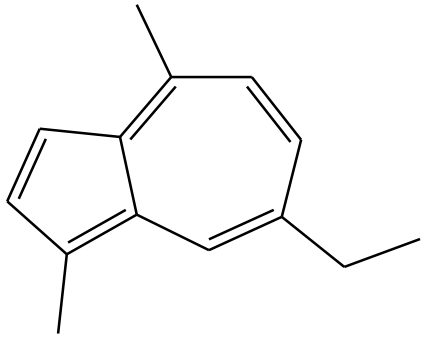


Figure 17: Chromatogram of isolated blue component of chamomile essential oil.

The mass spectrum for the blue component was as shown below (Figure 18). A spectral search in the MS library matched this mass spectrum to that of chamazulene **(1)** (Figure 19) hence confirming its identity.



(1)

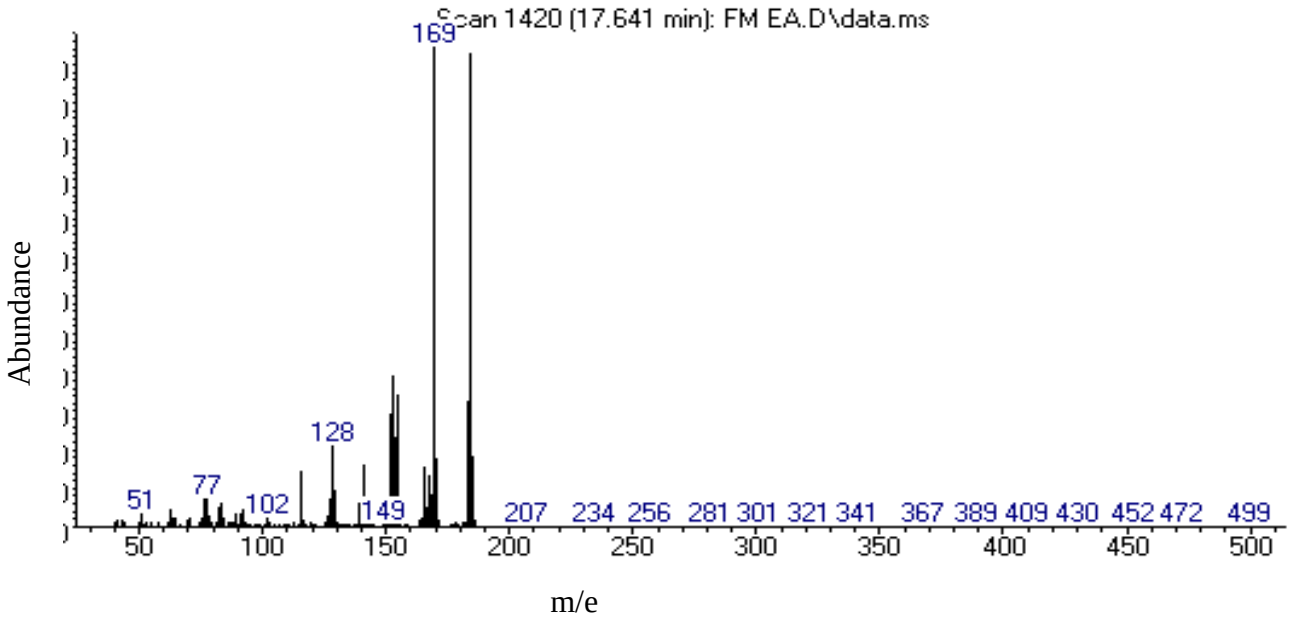


Figure 18: Mass spectrum of the blue component of chamomile essential oil.

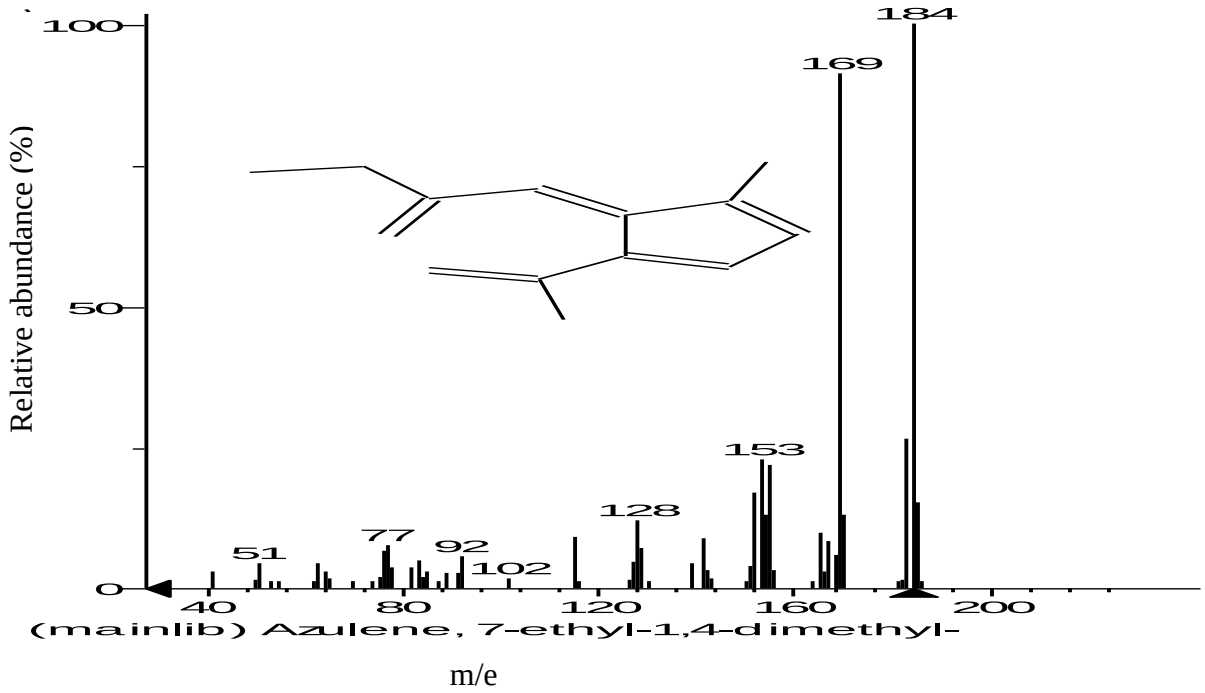


Figure 19: MS library mass spectrum of chamazulene.

4.12.7 GC-MS Analysis of Compound 4P

The GC-MS analysis of compound 4P gave the following chromatogram (Figure 20).

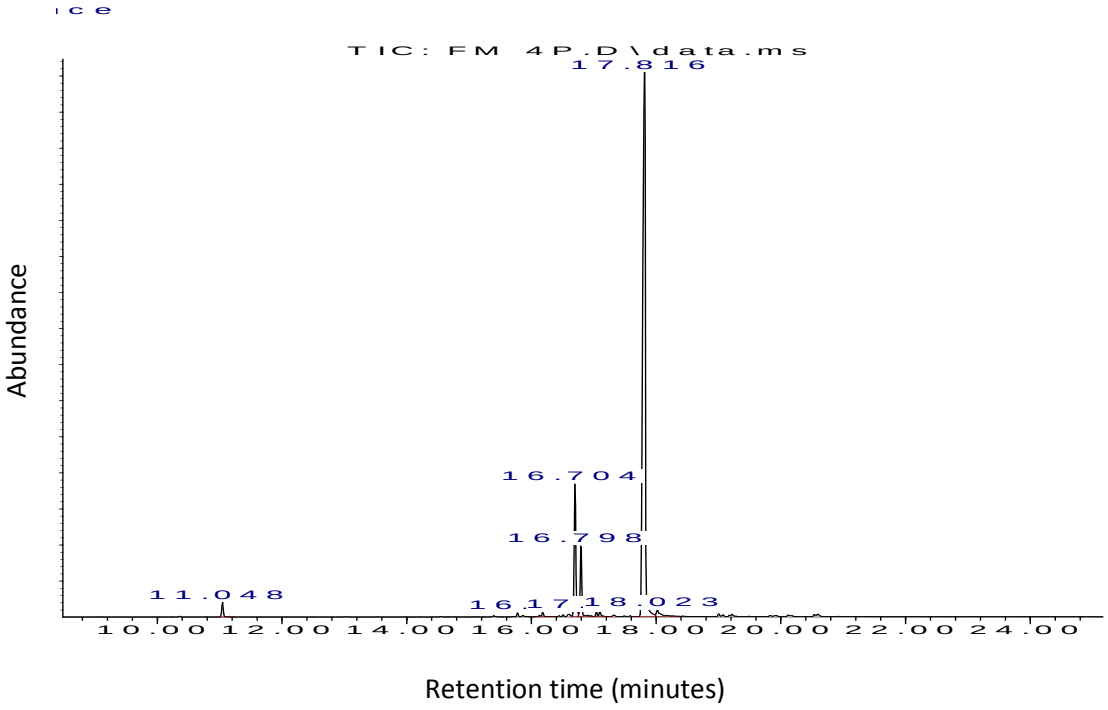


Figure 20: Chromatogram of compound 4P.

From the chromatogram, it was clear that the compound was not pure, although there was a major component at retention time 17.815 minutes, whose percentage was 78.204% as shown in the percentage report below (Table 29). The seven components of 4P at different retention times are shown in Table 29.

Table 29: Percentage report for compound 4P's chromatogram.

Peak	R.T.	Area	Percent Total
1	11.046	73475690	1.204
2	16.184	36159802	0.592
3	16.707	6.99E+08	11.444
4	16.799	3.85E+08	6.303
5	17.097	55043342	0.902

6	17.815	4.77E+09	78.204
7	18.02	82469128	1.351

The peak at retention time 17.815 minutes yielded the following mass spectrum (Figure 21).

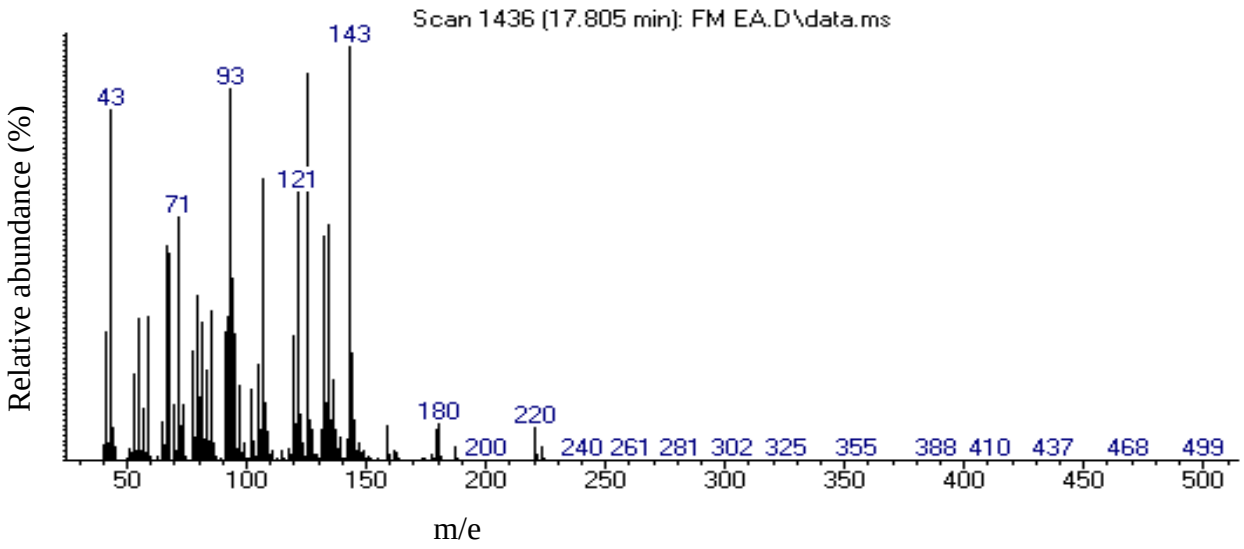
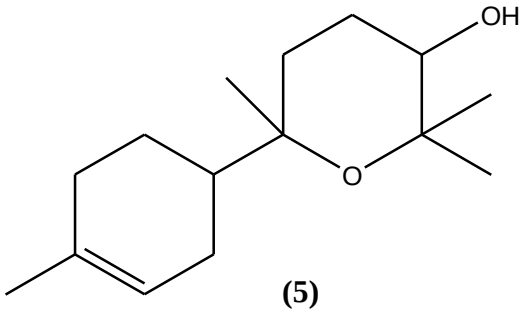


Figure 21: Mass spectrum of the major peak of compound 4P.



(5)

A search of the MS library matched the above spectrum to that of α-bisabolol oxide A **(5)** shown in figure 22. Hence compound 4P was confirmed to be mainly α-bisabolol oxide A.

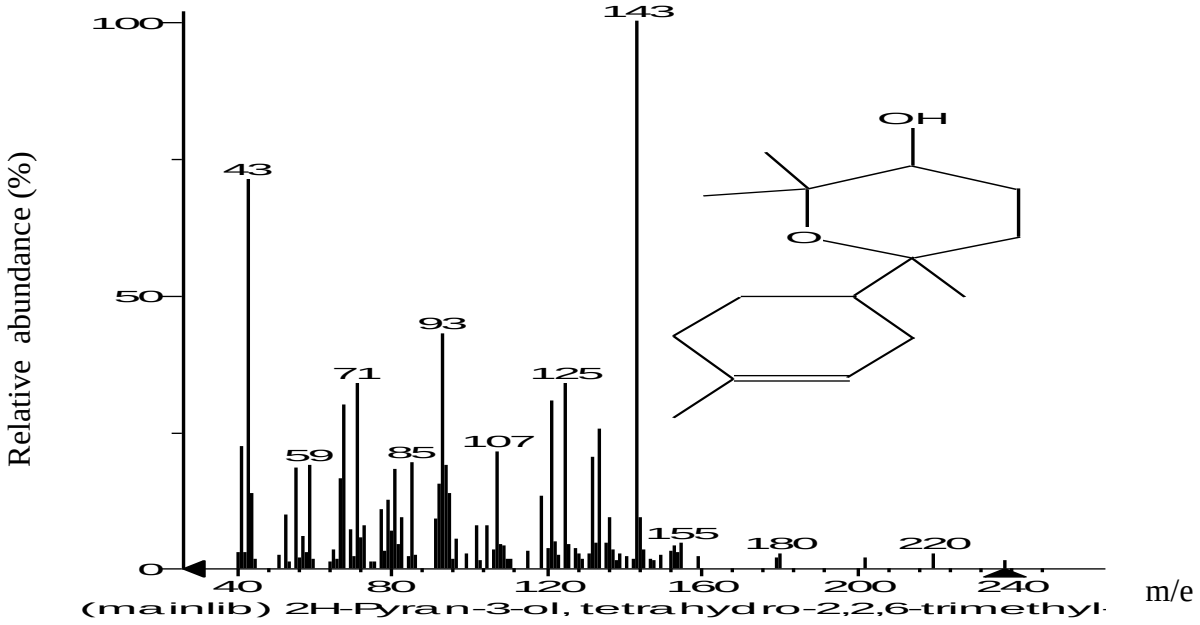
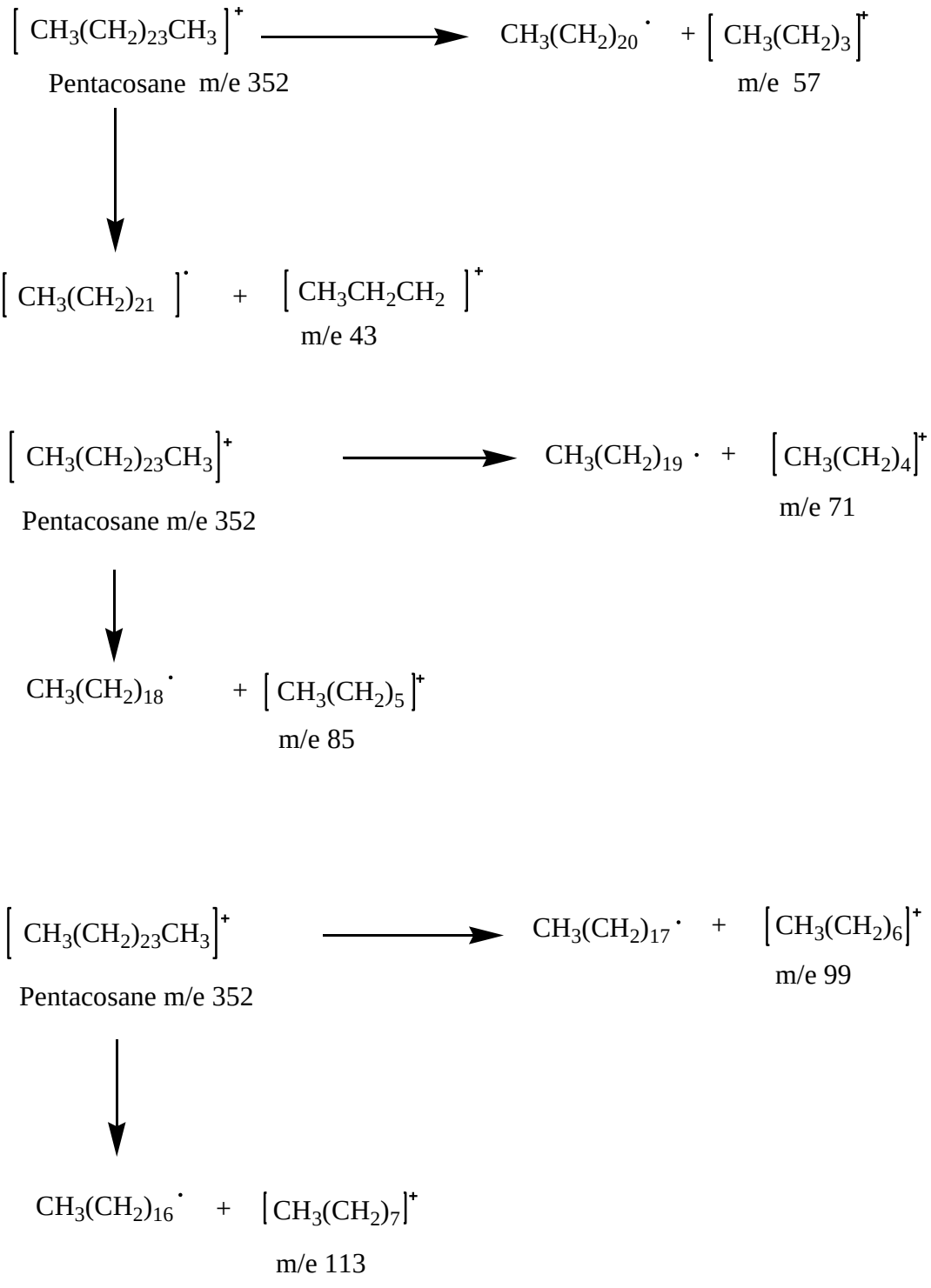


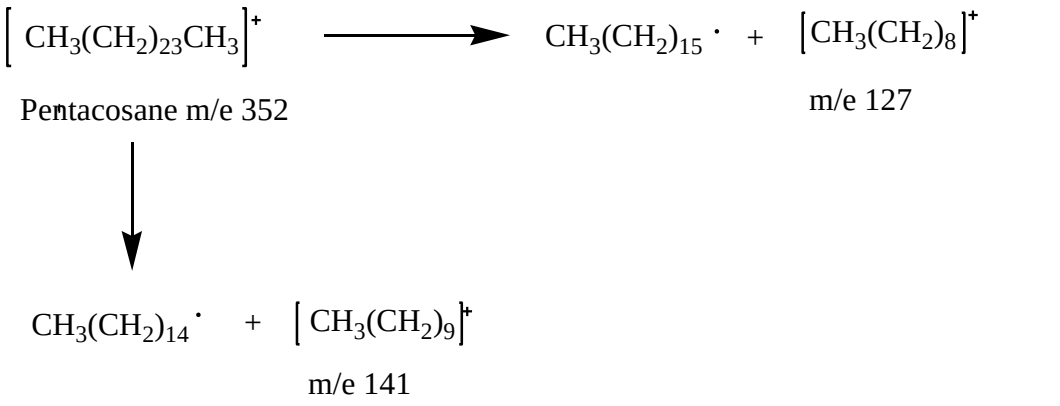
Figure 22: MS library mass spectrum of α-bisabolol oxide A.

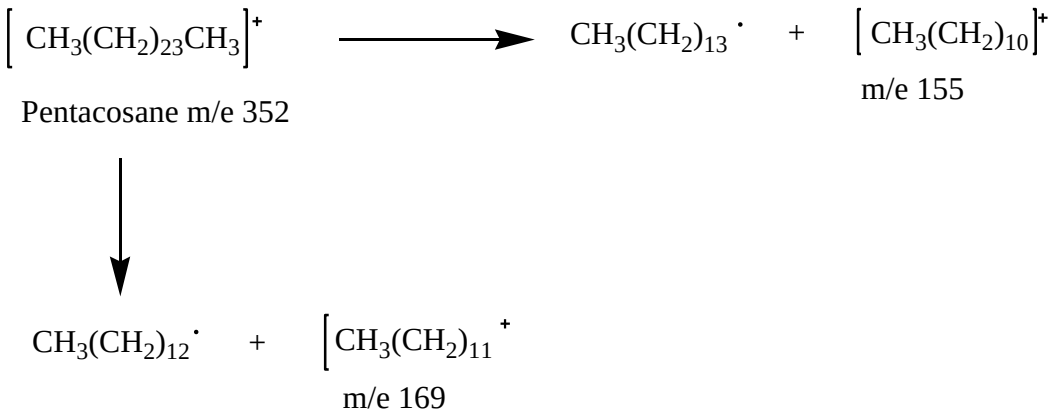
4.13 Mass Fragments of Some of the Components in Chamomile Essential Oil

n-Pentacosane (Peak at R.T. 25.579 minutes)

Mass spectrum of GC peak at R.T. 25.579 minutes shows peaks at m/e: 43, 57, 71, 85, 99, 113, 141, 169, 352 (see appendices for intensities). The molecular ion peak of pentacosane (m/e 352) can be seen in the mass spectrum although it is of low intensity. The spectrum shows the shorter fragments to be having more intense peaks than the longer ones, which is characteristic of long chain hydrocarbons. This is mainly so because the longer chains can easily stabilize the radical by delocalization of the single electron and the positive charge is more stable on shorter chains hence their high intensities (Silverstein *et al*., 1981).


[CH3(CH2)23CH3]+
Pentacosane m/e 352
CH3(CH2)20 ·
+ [CH3(CH2)3]+
m/e 57
[CH3(CH2)21]·
+ [CH3CH2CH2]+
m/e 43
[CH3(CH2)23CH3]+
Pentacosane m/e 352
CH3(CH2)19 ·
+ [CH3(CH2)4]+
m/e 71
CH3(CH2)18 ·
+ [CH3(CH2)5]+
m/e 85
[CH3(CH2)23CH3]+
Pentacosane m/e 352
CH3(CH2)17 ·
+ [CH3(CH2)6]+
m/e 99
CH3(CH2)16 ·
+ [CH3(CH2)7]+
m/e 113





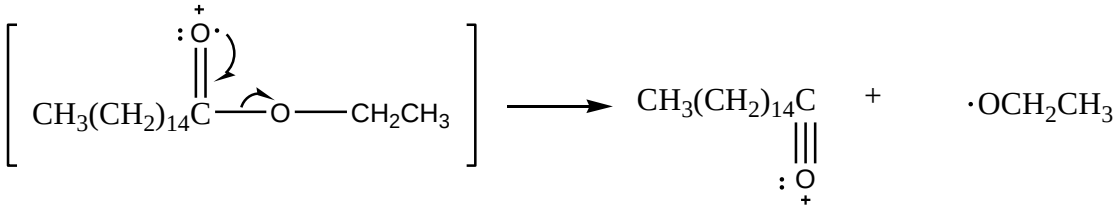
The base peak (peak with the highest intensity) for n-pentacosane is at m/e 57.

Tricosane (peak at R.T. 23.046 minutes)

Mass spectrum of the GC peak at R.T. 23.046 minutes had peaks at m/e: 43, 57, 71, 85, 99, 113, 141, 169, 324. These peaks were similar to those of pentacosane which is expected because they are all long chain hydrocarbons. The main distinguishing peak is the molecular ion peak for tricosane which was at m/e 324. The molecular ion peak results from the ion formed by loss of one electron by the compound molecule (Silverstein *et al*., 1981).

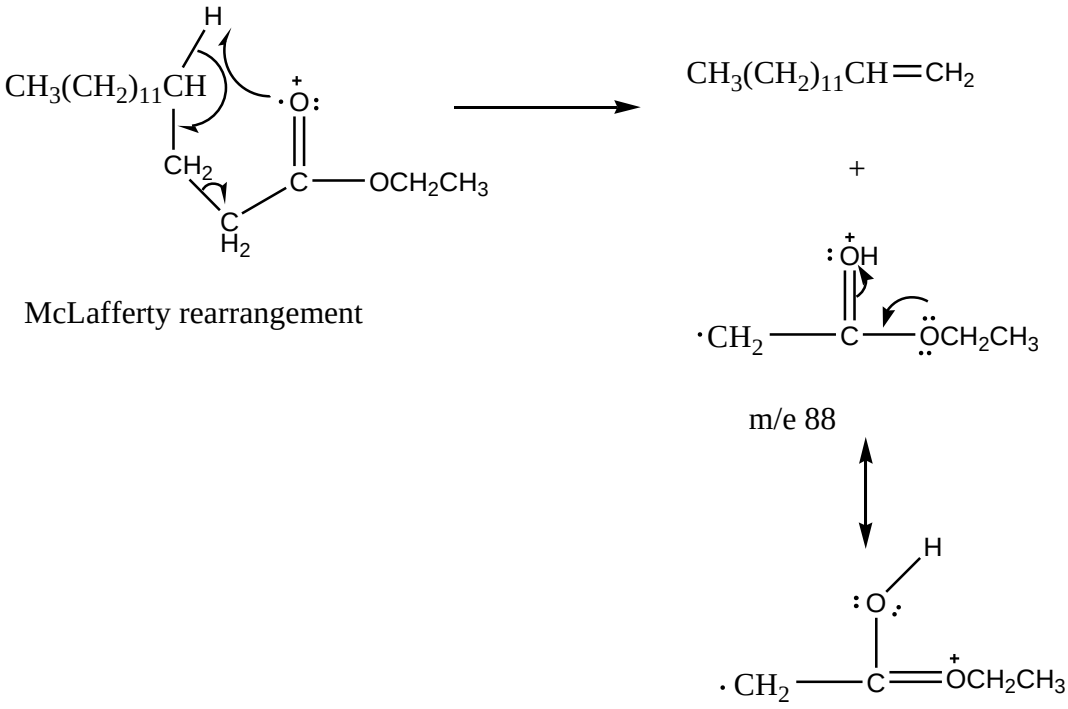
Hexadecanoic Acid Ethyl Ester (peak at R.T. 20.153 minutes)

The mass spectrum of the GC peak at R.T. 20.153 minutes had peaks at m/e: 43, 55, 67, 73, 88, 101, 115, 135, 157, 177, 199, 239, and 284. The molecular ion peak of hexadecanoic acid ethyl ester is present at m/e 284 though it's not prominent. The peak at m/e 88 is the most intense (base peak) because the fragment is resonance stabilized as shown below.



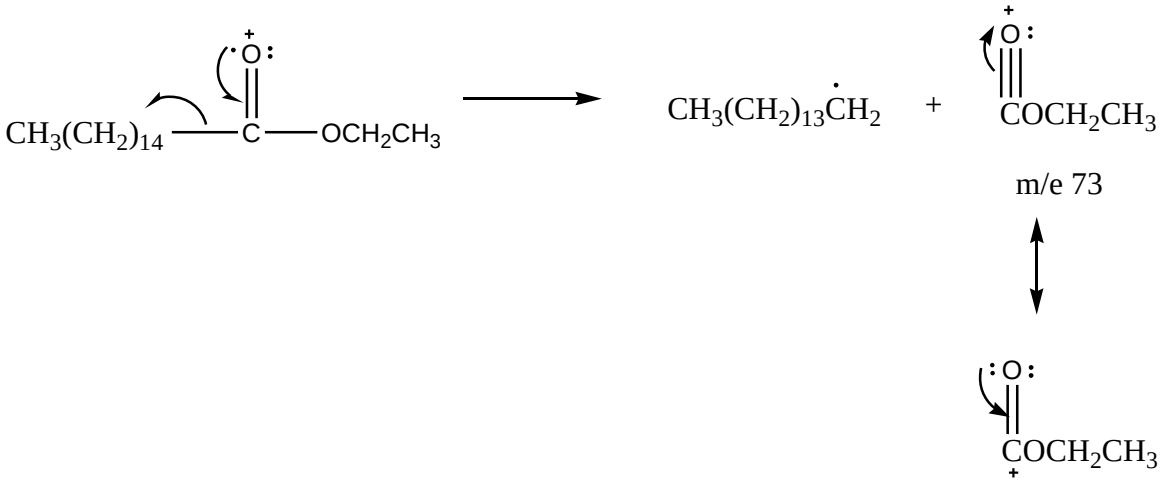
m/e 284
molecular ion peak

m/e 239



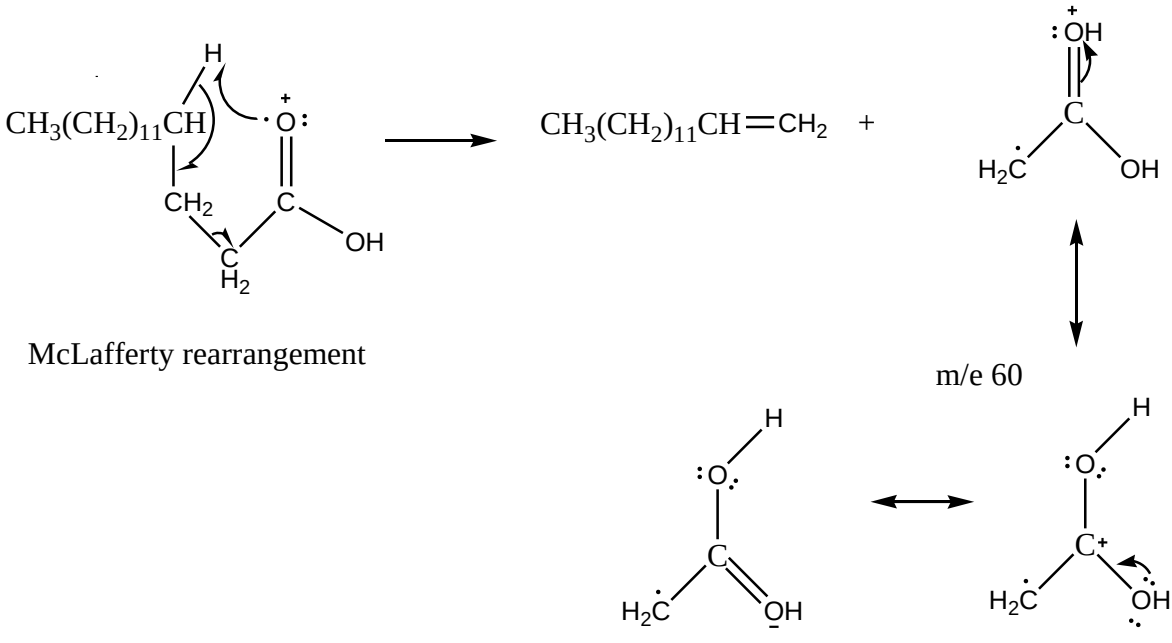
McLafferty rearrangement

m/e 88



n-Hexadecanoic Acid (peak at R.T. 19.825 minutes)

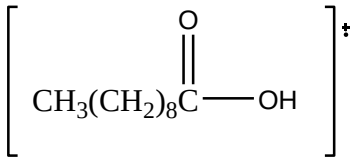
The mass spectrum of the GC peak at R.T. 19.825 minutes had peaks at m/e: 43, 60, 73, 97, 115, 129, 157, 185, 199, 213, and 256. The molecular ion peak of n-hexadecanoic acid at m/e 256 was quite prominent. The peak at m/e 60 was also quite intense, probably as result of the resonance stabilization shown below.



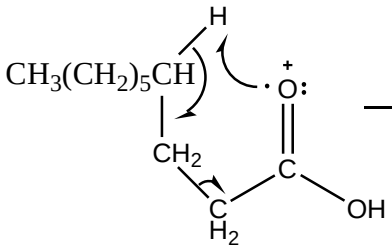
McLafferty rearrangement

n-Decanoic Acid (peak at R.T. 12.554 minutes)

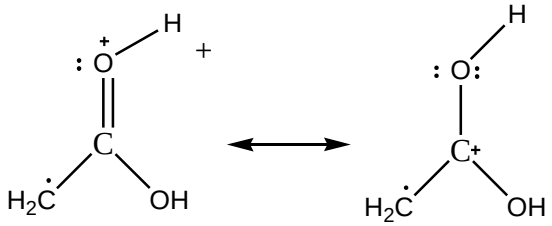
The mass spectrum of the GC peak at R.T. 12.554 minutes had peaks at m/e: 41, 60, 73, 101, 129, 151, and 172. The peak with m/e 60 was intense probably as a result of the stability of the fragment which arises from the resonance stabilization shown below.



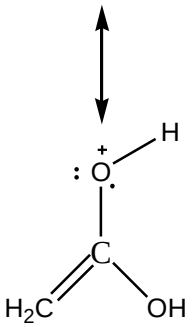
m/e 172
molecular ion peak



McLafferty rearrangement

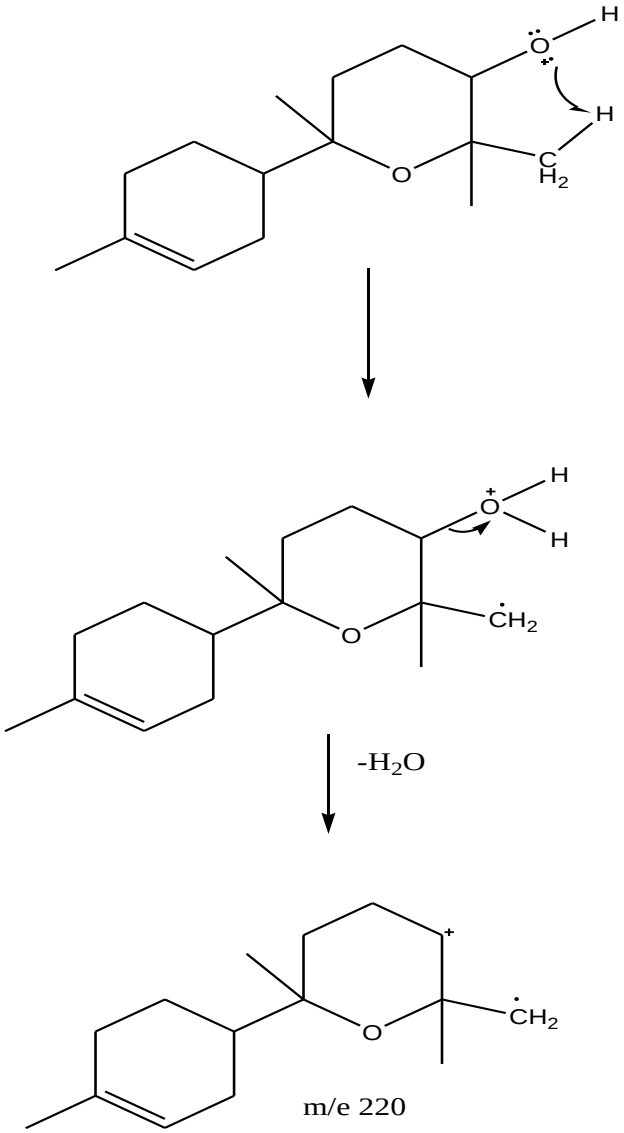


m/e 60



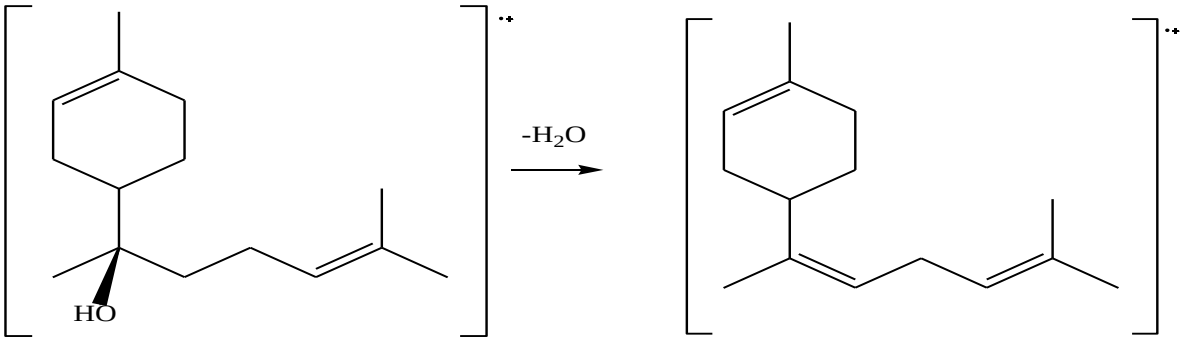
α-Bisabolol Oxide A

The mass spectrum for the GC peak at R.T. 17.805 minutes had peaks at m/e: 43, 71, 93, 107, 121, 125, 143, 180, and 220. The molecular ion peak for α-bisabolol oxide A (m/e 238) is not visible in the mass spectrum, but the fragment with m/e 220, which is M-18 (molecular weight minus 18), could be formed as shown below.

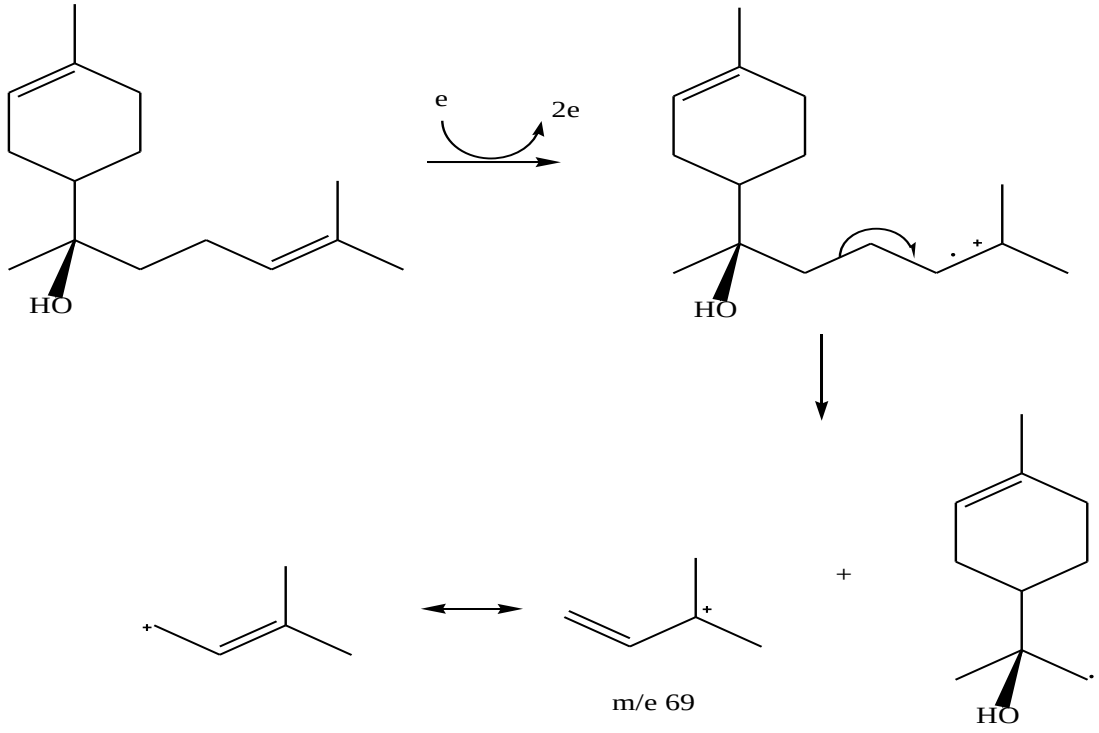


α-Bisabolol

The mass spectrum for the GC peak at R.T. 16.994 minutes had peaks with m/e: 43, 69, 81, 93, 109, 119, 134, 161, and 204. The molecular ion peak for α-bisabolol (m/e 222) was not present in the mass spectrum. However the peak resulting from the loss of a water molecule (m/e 204) was quite noticeable. The peak at m/e 69, formed as shown below, was quite prominent probably as result of resonance stabilization.



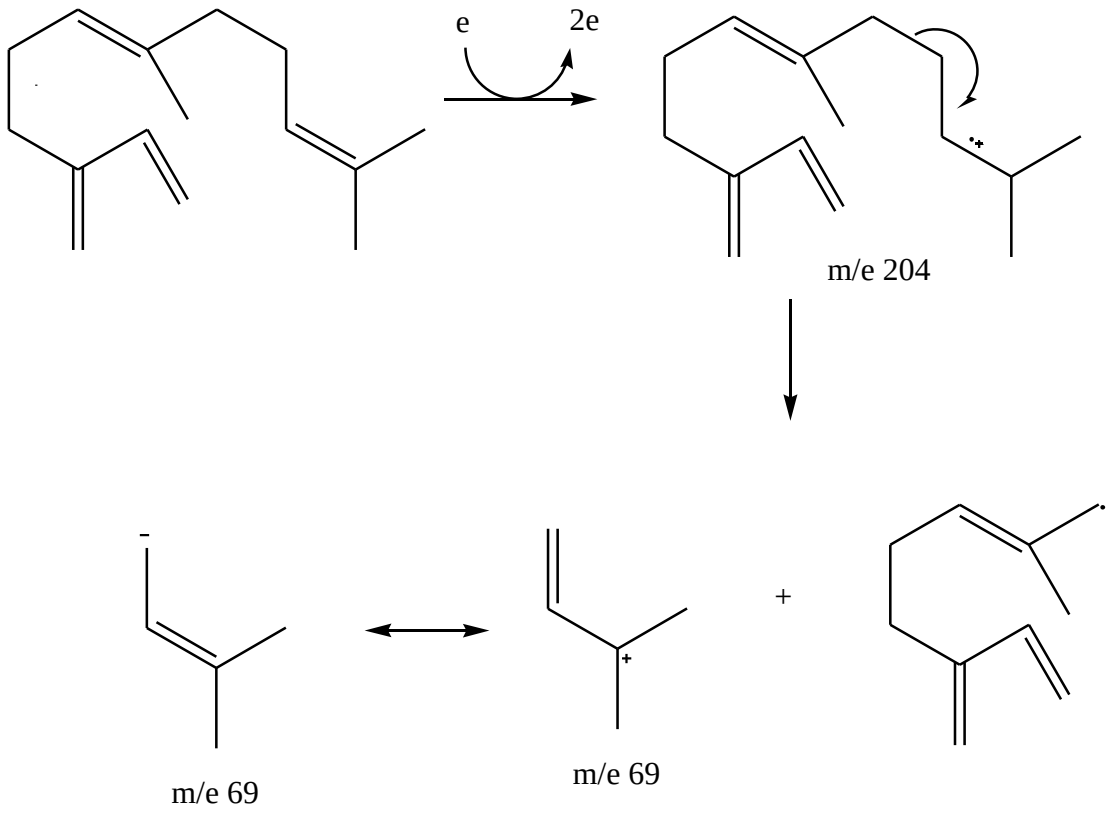
m/e 204



m/e 69

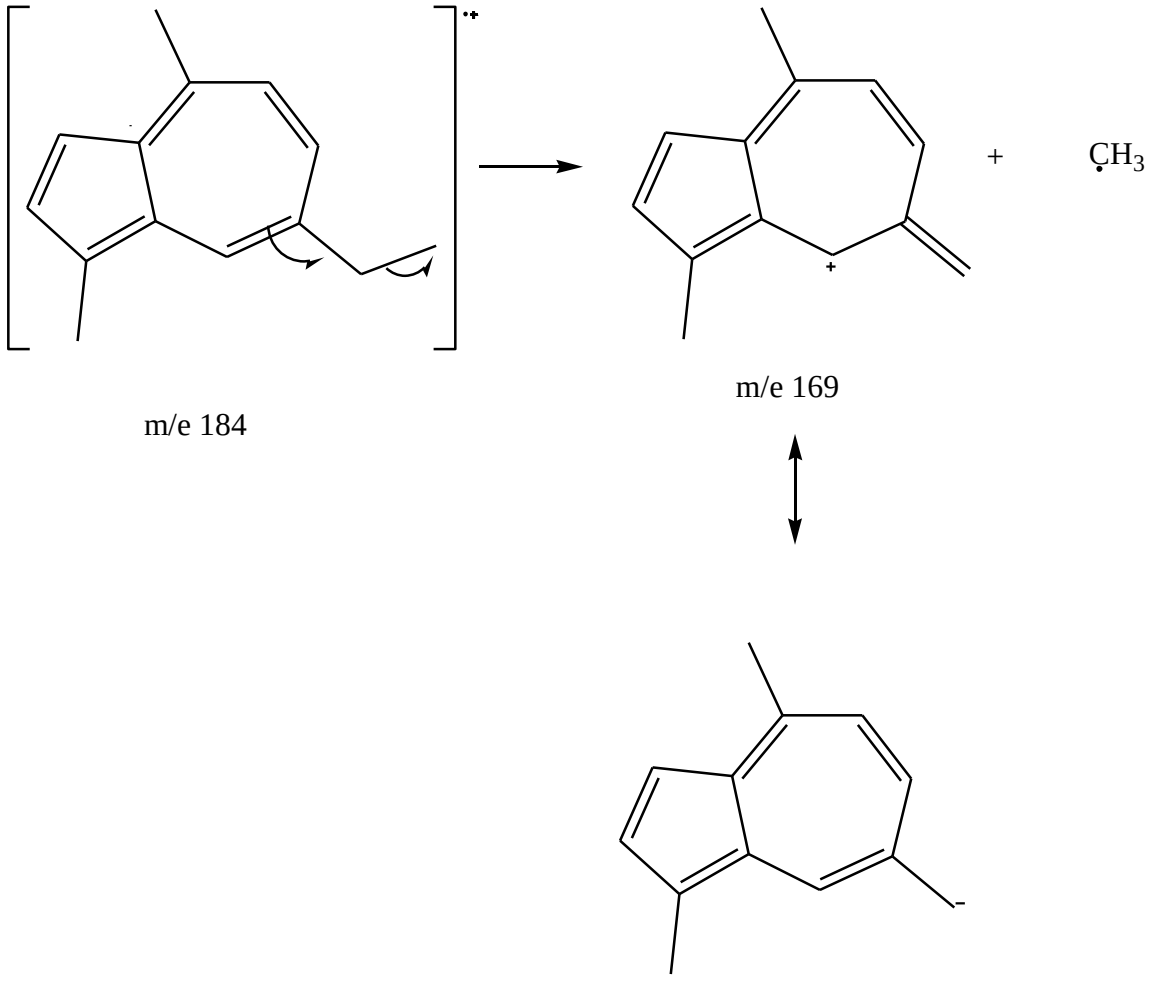
E-β-Farnesene

The mass spectrum of the GC peak at R.T. 13.979 minutes had peaks at m/e: 41, 69, 93, 133, 161, and 204. The molecular ion peak for E-β-farnesene (m/e 204) was noticeable in the spectrum. The base peak (m/e 69) could be explained by the fact that the fragment responsible for the peak was resonance stabilized. The library search of the MS data base matched the mass spectrum of this compound to that of Z-β-farnesene, which is its isomer, explaining why the base peak for the sample and library spectrum vary; the base peak for the sample is at m/e 69, while that of the library spectrum is at m/e 41.



Chamazulene

The most prominent peaks in the mass spectrum of the GC peak at R.T. 17.641 minutes were at m/e 169, and 184. The molecular ion peak for chamazulene (m/e 184) is quite prominent as a result of stabilization of the ion by the aromatic system. The fragmentation of the molecular ion leads to the formation of a fragment which is resonance stabilized hence very stable as revealed by the prominence of its peak (m/e 169).



m/e 184

m/e 169

CHAPTER FIVE

5.0 CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

(i) In cold extraction experiments, the different solvents gave extracts that varied in quality and quantity. Water had the highest amount of extract (17.31% w/w) although it did not have the characteristic sweet smell of the other chamomile extracts, suggesting that water does not extract the volatile components responsible for the sweet smell. Hexane gave the least amount of extract (2.02% w/w). Mixing some of the solvents in a 1:1 ratio seemed to improve the quantity of extract except for the water/ethanol mixture which did the opposite possibly because both are polar compounds. Comparing the chamomile extracts from cold extraction using TLC showed that extracts from organic solvents had some similar components. They also had some components which compared in R_F values to some components in the essential oil. The water and water/ethanol extracts had the least number of spots with 1 and 3 spots, respectively.

(ii) The flowers from Kangari and Njabini gave similar yields of essential oil (0.44% w/w) while flower samples from Kibwezi gave 0.60% w/w. Visually, the essential oil from Kibwezi flowers was more blue than the other samples, which is an indicator of high chamazulene content. This was confirmed by GC-MS analysis for the Kibwezi sample having 10.382% of chamazulene. The Kangari and Njabini samples had 2.115% and 0.913% of chamazulene, respectively. All the samples from the three areas had high α-bisabolol oxide A content, ranging from 42.649% to 61.142% with the Kangari sample having the highest and the Kibwezi sample having the lowest.

(iii) Increase in extraction temperature in Clevenger distillation affected the extraction process, yield and quality of essential oil. Increase in the temperature reduced the breakthrough time of the extraction process and affected positively the yield of essential oil. Maximum yield was achieved at 145^0C. However, high temperatures seemed to affect the quality of the essential oil with its colour changing from blue to bluish-green to green with increase in temperature.

(iv) GC-MS analysis of extracts at different temperatures also revealed differences in the quality of the essential oils. Some components increased in percentage with increase in the extraction temperature.These were β-farnesene, cis enyne dicycloether, n-hexadecanoic acid and n-pentacosane. However, the percentage of cis enyne dicycloether increased up to 145^0C after which it decreased. Spathulenol, τ-cadinol, α-bisabolol oxide B and α-bisabolol showed a decrease in percentage with increase in extraction temperature.

(v) Solvent extraction by percolation seemed to give more extract (6.58% w/w) compared to solvent extraction by 8-day soaking (2.85% w/w). When the extracts were subjected to Clevenger distillation in SECD, they gave similar percentages of SECD extracts, 0.2195% and 0.2174% respectively. GC-MS analysis of the SECD-P and SECD-8 revealed that SECD-P had the highest amounts of the major components except that of α-bisabolol oxide A which was highest in SECD-8. The dicycloethers, some of the main components in chamomile essential oil, were not detected in the SECD-P and SECD-8 extracts, maybe as a result of their loss in the concentration steps of the solvent extraction part of SECD.

(vi) Storage of flowers for a number of months seemed to reduce the percentages of some of the components in the essential oil extracted from the flowers. The components which decreased in percentage were β-farnesene, spathulenol, τ-cadinol, α-bisabolol oxide B and chamazulene.

(vii) In SECD extraction using different solvents, ethyl acetate had the highest percentage of extract at 0.3268% w/w while hexane/acetone had the lowest at 0.2368% w/w. The percentages of the SECD extracts were below the 0.44% w/w obtained by Clevenger distillation.

(viii) UV-Vis analysis of the SECD extracts revealed that they all had a maximum absorption at 605 nm. However, the absorbances at this wavelength varied. The DCM and ethyl acetate/DCM SECD extracts had the highest absorbance at 0.025 and the hexane SECD extract had the lowest at 0.009. Isolated chamazulene also had a maximum absorbance at 605 nm and the low absorbance for the hexane extract meant that it had a low amount of chamazulene which was confirmed by GC-MS results. GC-MS analysis of the SECD extracts revealed that the extracts had differences in the amounts of the active components. α-Bisabolol oxide B was found to be highest in the hexane/acetone and ethyl acetate SECD extracts with 5.904% and 5.894%, respectively. α-bisabolol was only detected in hexane/acetone, acetone and methanol extracts, with the acetone SECD extract having the highest percentage at 2.589%. The hexane/acetone SECD extract had the highest percentage of chamazulene (2.390%). The hexane SECD extract had low percentages of most of the main components. α-Bisabolol oxide A was highest in the hexane/acetone SECD extract at 68.474%.

(ix) UV-Vis analysis of cold solvent extraction products revealed a maximum peak at around 660 nm. They also had strong absorption in the range 270-400 nm.

(x) The refractive index of chamomile essential oil ranged from 1.48277 to 1.49830 when measured at 25^0C which was comparable to literature values. However, it could not be used as a monitoring parameter for it showed no particular trend when measured over a six months period.

(xi) Visually, chamomile essential oil was observed to change from blue to green to yellowish-brown. The rate of change of the colour was dependent on the degree of exposure to light with the sample exposed to direct light changing faster.

(xii) UV-Vis spectrometry was found to be an important tool in the monitoring of the stability of the chamomile essential oil because over time there was a marked decrease in absorption in the region 270-390 nm.

(xiii) A pure blue component, whose identity was confirmed to be chamazulene and purity determined to be 100% by GC-MS, was successfully isolated by preparative TLC and can be used as a standard for future characterization of chamomile essential oil. The method used in the isolation can also be used in the future to get more of it.

5.2 Recommendations

(i) More isolation of compounds from chamomile essential oil be done using column chromatography to isolate more components.

(ii) Determination of conditions (temperature and soil conditions) in Njabini, Kangari and Kibwezi should be done to establish what might be the cause of differences in the yield of the essential oil.

(iii) Farmers should be sensitized on the best practices in drying and storage of chamomile flowers to avoid losses in essential oil.

(iv) The exact concentrations of the active components in chamomile extracts, which might require the use of internal standards in GC-MS, should be determined.

(v) The stability of chamomile oil should be monitored using UV-Vis over a longer period at constant intervals to help in determining the decomposition rates.

(vi) Percentage recovery experiments should be carried out to determine the efficiency of the optimized analytical procedures developed in this study.

REFERENCES

Achterrath-Tuckermann, U., Kunde, R., & Flaskamp, E. (1980). Pharmacological investigations with compounds of chamomile,V. Investigations on the spasmolytic effect of compounds of chamomile and Kamillosan on the isolated guinea pig ileum. *Planta Med.* ***39*** , 38-50.

Aggag, M. E., & Yousef, R. T. (1972). Study of antimicrobial activity of chamomile oil. *Planta Med.,* ***22*** , 140-144.

Alberts, W. G. (2009). *German Chamomile Production.* Pretoria, 0001 South Africa: Directorate Agricultural Information Services. Private Bag X144.

Andreucci, A. C., Ciccarelli, D., Desideri, I., & Pagni, A. M. (2008). Glandular hairs and secretory ducts of *Matricaria chamomilla* (Asteraceae): Morphology and Histochemistry. *Ann. Bot. Fennici: Vol* ***45*** , 11-18.

Annuk, H., Hirmo, S., & Turi, E. (1999). Effect on cell surface hydrophilicity and susceptibility of Helicobacter pylori to medicinal plant extracts. *FEMS Microbiol. Lett.,* ***172*** , 41-45.

Azizi, M. (2006). Study of four improved cultivars of *Matricaria chamomilla* L. in climatic condition of Iran. *Iranian J. Med. Aroma. Plants,* ***22*** , 386-396.

Bagchi, D., Das, D. K., & Tosaki, A. (2001). Benefits of resveratrol in women's health. *Drugs Exp. Clin. Res. Vol* ***27*** , 233-248.

Bayer, I., Katona, K., & Tardos, L. (1958a). Adatok a chamomillae flos cholin-tartalmahoz. *Acta Pharm. Hung.,* ***28*** , 164.

Bayer, J., Katona, K., & Tardos, L. (1958b). Betrage Zum Cholingehalt der Bluten von *Matricaria chamomilla L. Naturwiss.,* ***45*** , 629.

Berry, M. (1995). The chamomiles. *Pharm J.*, ***254*** , 191-193.

Bohlmann, F., & Zdero, C. (1982). Sesquiterpene lactones and other constituents from *Tanacetum parthenium*. *Phytochemistry,* ***21*** , 2543-2549.

Bohlmann, F., Herbst, P., Arndt, C., Schonowski, U., & Gleinig, H. (1961). Polyacetylenverbindungen, XXXIV. Über einen neuen Typ von Polyacetylenverbindungen aus verschiedenen Vertretern des *Tribus Anthemideae* L. *Chem. Ber.*, ***94*** , 3193.

Bradley, P. R. (1992). *British Herbal Compendium Volume I.* British Herbal Medicine Association.

Brazilian Pharmacopoeia . (1996). Sao Paolo: Atheneu, pp 13.

Breinlich, J. (1966). Zur Chemie und Pharmakologie der en-in-dicycloaether der *Matricaria chamomilla* II. *Dtsch. Apoth. Ztg.*, ***106*** , 698.

Breinlich, V. J., & Scharnagel, K. (1968). Pharmacological characterisitcs of the en-yn-dicycloethers from *Matricaria chamomilla*. *Arzneimittelforschung,* ***18*** , 429-431.

British Herbal Medicine Association. (1990). *British herbal pharmacopoeia.* London: BHMA.

Bruneton, J. (1995). *Pharmacognosy, phytochemistry, medicinal plants.* Paris: Lavoisier.

Cedar wood oil. Retrieved January 24, 2012, from Essential oils india: http://www.essentialoils-india.com/cedar-wood-oil.html

Cekan, Z., Herout, V., & Sorm, F. (1954a). Isolation and properties of the pro-chamazulene from *Matricaria chamomilla* L., a further compound of the guaianolide group. *Collect Czechoslov. Chem. Commun.*, ***19*** , 798.

Cekan, Z., Herout, V., & Sorm, F. (1954b). O terpenech LXII. O isolacia vlastnostech prochamazulenu z. hermanku (*Matricaria chamomilla* L). *Chem. Listy, **48*** , 1071-1077.

Cekan, Z., Herout, V., & Sorm, F. (1957). Die Struktur von Matricin, ein Guajanolid aus der Kamille (*Matricaria chamomilla*). *Collect Czechoslov. Chem. Commun., **22*** , 1921.

Chamomile blue pure essential oil. Retrieved January 24, 2012, from Lalaessentialoils: http://www.lalaessentialoils.com/chamomile-blue-pure-essential-oil.html

Chamomilla recutita Rauschert/ German chamomile. (2012). Retrieved January 24, 2012, from heotc: http://www.heotc.com/available/*chamomilla-recutita*-rauschert-german-chamomile

Cinco, M., Banfi, E., & Tubaro, A. (1983). A microbiological survey on the activity of a hydroalcoholic extract of camomile. *Int. J. Drug Res., **21*** , 145-151.

Costa, M. A. (2001). *Agricultural Production Process of the Culture of Chamomile in the Municipality of Mandirituba, PR. Dissertation (MSc in Agronomy).* Curitiba, Brazil: Agricultural Sciences, Federal University of Parana.

Costescu, C. I., Hadaruga, N. G., Rivis, A., Hadaruga, D. I., Lupea, A. X., & Parvu, D. (2008). Antioxidant activity evaluation of some *Matricaria chamomilla* L. extracts. *Journal of Agroalimemtary Processes and Technologies, **14*** , 417-432.

Council of Scientific & Industrial Research (CSIR) (1953). *The Indian Pharmaceutical Codex. **Vol I**. Indigenous drugs.* New Delhi.

Cuong, B. N., Gacz-Baitz, E., Radics, L., Tamas, J., Ujszaszy, K., & Verzar-Petri, G. (1979). Achilliah, The first proazulene from *Achillea millefolium*. *Phytochemistry **18*** , 331-332.

Eliasova, A., Repcak, M., & Pastirova, A. (2004). Quantitative Changes of Secondary Metabolites of *Matricaria chamomilla* by Abiotic Stress. *Z. Naturforsch,* ***59*** , 543-548.

Falzari, L. M., & Menary, R. C. (2003). *Chamomile for Oil and Dried Flowers.* Tasmania: RIRDC.

Flaskamp, E., Nonnenmacher, G., & Isaac, O. (1981). Stereochemistry of the bisaboloids from *Matricaria chamomilla L. . Z. Naturforsch.* ***36b*** , 1023-1030.

Flaskamp, E., Zimmermann, G., Nonnenmacher, G., & Isaac, O. (1982). Untersuchungen zur Charakterisierung des Prochamazulens Matricin aus Matricaria. *Z. Naturforsch.* ***37 b*** , 508.

Franke, R., & Schilcher, H. (2005). *Chamomile Industrial Profiles.* Boca Raton: CRC Press, LLC. pp 49-82

Furniss, B. S., Hannaford, A. J., Smith, P. W., & Tatchell, A. R. (1978). *Vogel's Textbook of Practical Organic Chemistry, 4th Edition.* London: Longman Group Limited.

Gardiner, P. (1999). *Chamomile (Matricaria recutita, Anthemis nobilis).* Longwood Herbal Task Force pp 1-21.

Goeters, S. (2001). *Chemische und pharmakologische Charakterisierung eines anti-inflammatorischwirksamen Pflanzeninhaltsstoffs, isoliert aus Asteraceen. Dissertation* . Philipps-Universität Marburg: Marburg/Lahn, Germany.

Grieve, M. (1994). *A Modern Herbal* . Tiger, Great Britain.

Hempel, B. (2001). Private communication (Robugen GmbH).

Herout, V., Zaoral, M., & Sorm, F. (1953). Synthesis of 2 tetrahydrobisabolols. *Collect Czechoslov. Chem. Commun.,* ***18*** , 122-126.

Hoffman, D. (1990). *The New Holistic Herbal.* Element: Dorset.

Holub, M., Herout, V., & Sorm, F. (1955). Synthesis of Alpha-bisabolol- A Spasmolytically active sesquiterpenic alcohol. *Czechoslov. Farm.,* ***3*** , 129.

Hormann, H., & Korting, H. (1994). Evidence for the efficacy and safety of topical herbal drugs in dermatology: part I: anti-inflammatory agents. *Phytomedicine,* ***1*** , 161-171.

Hozl, J., & Demuth, G. (1975). Influence of ecological factors on the composition of essential oil and flavonoids in *Matricaria chamomilla* of different origin . *Plant Med.,* ***27*** , 37.

Hozl, J., Ghassemi, N., & Hahn, B. (1986). Preparation of 14C-spiro ethers by chamomile and their use by an investiagtion of absorption. *Planta Med,* ***52*** , 553.

Isaac, O., Schneider, H., & Eggenschwiller, H. (1968). Beitrag Zur Kenntnis des Kamillen-Bisabolols. *Dtsch. Apoth. Ztg.,* ***108*** , 293-298.

Isaac, O., & Thiemer, K. (1975). Biochemical studies on camomile components/III. In vitro studies about the antipeptic activity of (-)-alpha-bisabolol. *Arzneimittelforschung,* ***25*** , 1352-1354.

Isaac, O. (1979). Pharmacological investigations with compounds of chamomile i. on the pharmacology of alpha-bisabolol and bisabolol oxides. *Planta Med.,* ***35*** , 118-124.

Juell, S. M., Hansen, R., & Jork, H. (1976). Substances first isolated from the essential oils of two artemisia-species, 1. Spathulenol, an azulenogenic C15-alcohol. *Arch. Pharm.,* ***309*** , 458.

Karbalay-Doust, S., Noorafshan, A., Dehghani, F., Panjehshahin, M. R., & Monabati, A. (2010). Effects of hydralcoholic extract of *Matricaria chamomilla* on serum testosterone and estradiol levels, spermatozoan quality, and tail length in rat. *Iran J. Med. Sci. Vol **35*** , 122-128.

Kergomard, A., & Verscambre, H. (1977). Synthese et configuration absolue de terpenes naturels: (+) uroterpenol, (+) et (−) α-bisabolols, (−) α-bisabololone . *Tetrahedron, **33*** , 2215.

Knoll, W., & Tamm, C. (1975). Biosynthese der Verrucarine und Roridine. Teil 5. Synthese von zwei Diastereoisomerenpaaren des [1,8-14C]-α-Bisabolols und Versuche zu deren Einbau in Verrucarol Verrucarine und Roridine, 32. Mitteilung [1]. *Helv. Chim. Acta, **58*** , 1162.

Kotnik, P., Skerget, M., & Zeljko, K. (2007). Supercritical Fluid Extraction of Chamomile Flower Heads: Comparison with Conventional extraction, Kinetics and Scale up. *Journal of Supercritical Fluids **43*** , 192-198.

Lang, W., & Schwandt, K. (1957). Assessment of the Glycoside content of chamomile. *Dtsch. Apoth. Ztg., **97*** , 149.

Lemberkovics, E. (1979). Farnesene Isomers in Chamomile Oil. *Sci. Pharm., **47*** , 330-332.

Leung, A. & Foster, S (1996). *Encyclopedia of common natural ingredients used in food, drugs and cosmetics, 2nd ed.* New York: John Wiley. 296.

Loggia, R. D., Traversa, U., Scarcia, V., & Tubaro, A. (1982). Depressive effects of *Chamomilla recutita* (L.) rausch. tubular flowers, on central nervous system in mice. *Pharmacol Res Commun **14*** , 153-162.

Mann, C., & Staba, E. J. (1986). The chemistry, pharmacology, and commercial formulations of chamomile. In L. E. Craker, & J. E. Simon, *Herbs, Spices, and Medicinal Plants. Recent*

*Advances in Botany, Horticulture, and Pharmacology Vol **1*** (pp. 235-280). Binghamton, NY: Haworth Press.

Mc Guffin, M., Hobbs, C., Upton, R., & Goldberg, A. (1997). *American Herbal Products Association- Botanical Safety Handbook.* CRC Press.

McKay, D. L., & Blumberg, J. B. (2006). A review of the bioactivity and potential health benefits of chamomile tea (*Matricaria recutita L.*). *Phytother Res. Vol **20*** , 519-530.

Meisels, A., & Weizmann, A. (1953). The Structure of Chamazulene. *J.Am. Chem. Soc. Vol **75*** , 3865-3866.

Mills, S. (1994). *The Complete Guide to Modern Herbalism.* Thorsons, Great Britain.

Minyard, J. P., Thomson, A. C., & Hedin, P. A. (1968). Constituents of the cotton bud. VIII. .beta.-Bisabolol, a new sesquiterpene alcohol . *J. Org. Chem., **33*** , 909-911.

Moghaddasi, S. M. (2011). Study on cammomile (*Matricaria chamomilla* L.) Usage and Farming. *Adv. Environ. Biol. Vol **5.*** , 1446-1453.

Motl, O., & Repcak, M. (1979). New Components from Camomile essential oil. *Planta Med., **36*** , 272-273.

Motl, O., Felklova, M., Lukes, V., & Jasikova, M. (1977). Zur GC Analyse und zu chemischen Typen von Kamillenöl . *Arch. Pharm., **310*** , 210.

Muller, J., Koll-Weber, M., & Kraus, W. (1996). Drying behaviour of chamomile (*Chamomilla recutita* L. Rauschert). *Journal of Medicinal and Aromatic Plants **1**(3)* , 104-110.

Mwaniki, J. M., & Kamau, G. N. (2003). Factors Affecting yield of Essential oil from Clevenger Distillation of Chamomile Flowers. *International Journal of Biochemiphysics Vol **11&12** (1&2)* , 27-31.

Mwaniki, J. M., & Mbugua, S. N. (2007). Combined Solvent Extraction-Clevenger Distillation method for producing blue essential oil from *Matricaria chamomilla* flowers. *Journal of the Kenya Chemical Society Vol **4** No.1* , 12-26.

National Coordinating Agency for Population and Development. (NCAPD) (2008). *Seeking Solutions for Traditional Herbal Medicine: Kenya Develops a National Policy: Policy Brief No. 1.*

Newall, C. A., Anderson, L. A., & Phillipson, J. D. (1996). *Herbal Medicines: a guide for health-care professionals.* London: Pharmaceutical Press.

Orav, A., Kailas, T., & Ivask, K. (2001). Volatile constituents of *Matricaria recutita* L. from Estonia. *Proc. Estonian Acad. Sci. Chem., **50*** , 39-45.

Organisation of African Unity-Scientific, Technical & Research Commission.(OAU-STRC) (1985). *African pharmacopoeia, 1st ed.* Lagos.

Piesse, S. (1863). Comptes Rend. hebdom. *Seances Acad. Sciences Vol **57*** , 1016.

Power, F., & Browning, H. J. (1914). The Constituents of the flowers of *Matricaria chamomilla*. *J. Chem. Soc., London, **105*** , 2280.

Presibella, M. M., Villas-Boas, L. D., Belletti, K. M., Santos, C. A., & Weffort-Santos, A. M. (2006). Comparison of Chemical Constituents of *Chamomilla recutita* L. Rauschert Essential Oil

and its Antichemotactic Activity. *Brazilian Archives of Biology and Technology Vol.* ***499*** *No. 5* , 717-724.

Raal, A., Arak, E., Orav, A., & Ivask, K. (2003). Comparison of essential oil content of *Matricaria recutita* L. from different origins. *Ars Pharmaceutica* ***44****(2)* , 159-165.

Raal, A., Kaur, H., Orav, A., Arak, E., Kailas, T., & Muurisepp, M. (2011). Content and composition of essential oils in some Asteraceae species. *Proceedings of the Estonian Academy of Sciences,* ***60*** , 55-63.

Razmjoo K., Heydarizadeh P., Sabzalian M. R. (2008). Effect of Salinity and Drought Stresses on Growth Parameters and Essential Oil Content of *Matricaria chamomilla*. *Int.J. Agri. Biol.:* ***10*** , 451-454.

Reichling, J., Beiderbeck, R., & Becker, H. (1979). Comparative Studies on Secondary Products from Tumors, Flowers, Herb and Roots of *Matricaria chamomilla* L. *Planta Med.,* ***36*** , 322.

Repcak, M., Eliasova, A., & Ruscancinova, A. (1998). Production of herniarin by diploid and tetraploid *Chamomilla recutita*. *Pharmazie,* ***53*** , 278-279.

Repcak, M., Imrich, J., & Franekova, M. (2001). Umbelliferone, a stress metabolite of *Chamomilla recutita* (L). Raushert. *J. Plant Physiol.,* ***158*** , 1085-1087.

Reverchon, E., & Senatore, F. (1994). Supercritical Carbon Dioxide Extraction of Chamomile Essential Oil and its analysis by Gas Chromatography-Mass Spectrometry . *J. Agric. Food Chem* ***42*** , 154-158.

Roth, L., & Rupp, G. (1995). *Roth Collection of Natural Products Data.* New York: VCH Publishers.

Ruzicka, L., & Capato, E. (1925). Höhere Terpenverbindungen XXIV. Ringbildungen bei Sesquiterpenen. Totalsynthese des Bisabolens und eines Hexahydro-cadalins. *Helv. Chim. Acta, **8*** , 259.

Ruzicka, L., & Liguori, M. (1932). Polyterpene und Polyterpenoide LXIII. Über eine neue Synthese des Bisabolens. *Helv. Chim. Acta, **15*** , 3.

Salamon, I. (1992). Effect of different plant densities on the essential oil quantity and quality of chamomile (*Chamomilla recutita* L. Rauschert). *Sbornik UVITZ Zahradnictvi, **19** (2)* , 95-99.

Salamon, I. (1994). Growing Conditions and the essential oil of Chamomile, *Chamomilla recutita* (L.) Rausch. *J. Herbs Spice Med. Plants, **2*** , 31-37.

Sampath, V., Trivedi, G. K., Paknikar, S. K., & Bhattacharyya, S. C. (1969a). Structure of Bisabolol Oxide A. *Indian J. Chem., **7*** , 100.

Sampath, V., Thakar, M. R., Paknikar, S. K., Sabata, B. K., & Bhattacharyya, S. C. (1969b). Structure of d- and l- isomers of a-Bisabolol oxide B. *Indian J. Chem., **7*** , 1060.

Schilcher, H. (1973). Neuer erkenntnisse bei der Qualitatsbeurteilung von Kamillenbluten bzw. Kamillenol-Einteilung der Handelskamille in vier chemische Typen. *Planta Med., **23*** , 132-144.

Schilcher, H. (1985). *Zur Biologie von Matricaria chamomilla, syn. "Chamomilla recutita (L.) Rauschert," Research report 1968-1981.* Berlin: Inst. Pharmakognosie and Phytochemie of FU.

Schilcher, H. (1987). *Die Kamille - Handbuch fur Arzte, Apotheker und andere Naturwissenshaftlex.* Stuttgart, Germany: Wissenschaftl Werlagsgesellschaft.

Schmidt, P. C., Weibler, K., & Soyke, B. (1991). Kamillenblüten und -extrakte:Matricin- und Chamazulenbestimmung - Vergleich von GC, HPLC und photometrischen Methoden . *Dtsch. Apoth. Ztg.*, ***131****(5)* , 175-181.

Seidler-Lozykowska, K. (2010). Effect of the Selected Weather Conditions on essential oil, a-bisabolol and Chamazulene Content in Flower Heads of Chamomile (Chamomilla recutita L. Rauschert). *Journal of Essential Oil Research,* ***22*** , 45-47.

Shams-Ardakani, M., Ghannadi, A., & Rahimzadeh, A. (2006). Volatile Constituents of *Matricaria chamomilla* L. from Isfahan, Iran. *Iranian J. Pharma. Sci.,* ***2*** , 57-60.

Sharafzadeh, S., & Alizadeh, O. (2011). German and Roman Chamomile. *Journal of Applied Pharmaceutical Science 01 Vol* ***10*** , 01-05.

Silverstein R. M., Bassler G.C., & Morrill T. C. (1981). *Spectrometric Identification of Organic Compounds, 4th Edition*. John Wiley & Sons. New York. pp 15

Skoog, D. A., Holler, J. F., & Nieman, T. A. (1998). *Principles of Instrumental Analysis, 5th Edition.* Hartcourt Brace & Company. pp 139.

Sorm, F., Zaoral, M., & Herout, V. (1951). On the Composition of the Oil of *Matricaria chamomilla* L. . *Collect. Czechoslov. Chem. Commun.,* ***16*** , 626-638.

Sorm, F., Zekan, Z., Herout, V., & Raskova, H. (1952). Isolation of Spasmolytic Substances from *Matricaria chamomilla*. *Chem. Listy,* ***46*** , 308-309.

Sorm, F., Nowak, J., & Herout, V. (1953). The Composition of Chamazulene. *Coll. Czech. Chem. Commun.* ***18*** , 527-529.

Srivastava, J. K., & Gupta, S. (2007). Antiproliferative and apoptotic effects of chamomile extract in various human cancer cells. *J. Agric Food Chem, **55** ,* 9470-9478.

Stahl, E. (1954a). Über das Cham-Azulen und dessen Vorstufen, I. Mitteil.: Cham-Azulencarbonsäure aus Schafgarbe . *Chem. Ber., **87** ,* 202-205.

Stahl, E. (1954b). Zur Struktur der Cham-Azulencarbonsäure. *Naturwiss., Vol **41** ,* 257.

Szelenyi, I., Isaac, O., & Thiemer, K. (1979). Pharmacological experiments with compounds of chamomile III Experimental studies of the ulcerprotective effect of chamomile. *Planta Med., **35** ,* 218-227.

Thorne Research, Inc. (2008). *Matricaria chamomilla*. *Alternative Medicine Review, **13(1)** ,* 58-62.

Torrado, S., & Agis, A. (1995). Effect of dissolution profile and (-)-alpha-bisabolol on the gastrotoxicity of acetylsalicylic acid. *Pharmazie, **50** ,* 141-143.

Tosi, B., Romagnoli, C., Menziani-Andreoli, E., & Bruni, A. (1995). Characterization of coumarins in chamomile preparations sold in Italy. *Int. J. Pharmacognosy, **33** ,* 144-147.

Turi, M., Turi, E., Koljalg, S., & Mikelsaar, M. (1997). Influence of aqueous extracts of medicinal plants on surface hydrophobicity on *Escherichia coli* strains of different origin. *APMIS, **105** ,* 956-962.

Tyler, V. E., Brady, L. R., & Robbers, J. E. (1988). *Pharmacognosy, 9th ed.* Philadelphia: Lea & Febiger.

UNESCO. (1986). *Matricaria chamomilla L.* International Centre for Chemical Studies, University-Industry Cooperation. pp 106-109.

Van Lier, F. P., Hesp, T. G., Van der Linde, L. M., & Van der Weerdt, A. J. (1985). First preparation of (+)-spathulenol. Regio- and stereoselective oxidation of (+)-aromadendrene with ozone. *Tetrahedron Lett.*, ***26*** , 2109-2110.

Verzar, P., Gizella, Szegi, J., & Marczal, G. (1979). Pharmacological effect of certain chamomile compounds. *Acta. Pharm. Hung.* ***49****(1)* , 13-20.

Viola, H., Wasowski, C., & Levi de Stein, M. (1995). Apigenin, a component of *Matricaria recutita* flowers, is a central benzodiazepine receptors-ligand with anxiolytic effects. *Planta Med*, ***61*** , 213-216.

Youngken, H. W. (1948). *Textbook of pharmacognosy, 6th ed.* Philadelphia: Blakiston pp 1063.

Zuloega, O., Etxebarria, N., Fernandez, L. A., & Madariaga, J. M. (2000). Optimization and Comparison of MAE, ASE and Soxhlet Extraction for the Determination of HCH in soil samples. *Fresenius J Anal Chem* ***367*** , 733-737.

APPENDICES

APPENDIX I: UV SPECTRA OF COLD EXTRACTS

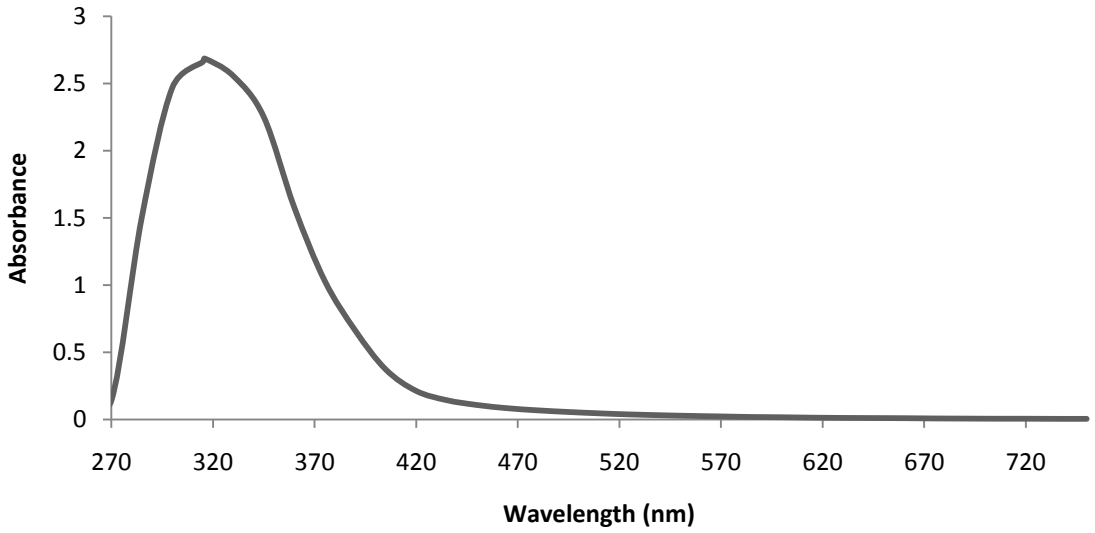


Figure 23: UV-Vis spectrum of 20 mg ethanol-water extract in 25 ml ethanol-water mixture.

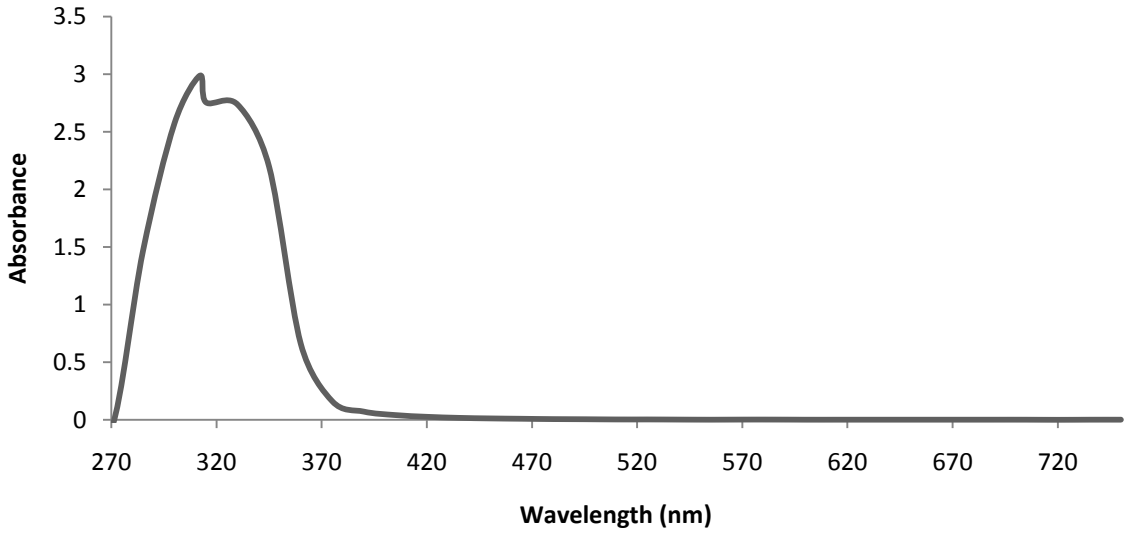


Figure 24: UV-Vis spectrum of 20 mg of hexane extract in 25 ml hexane.

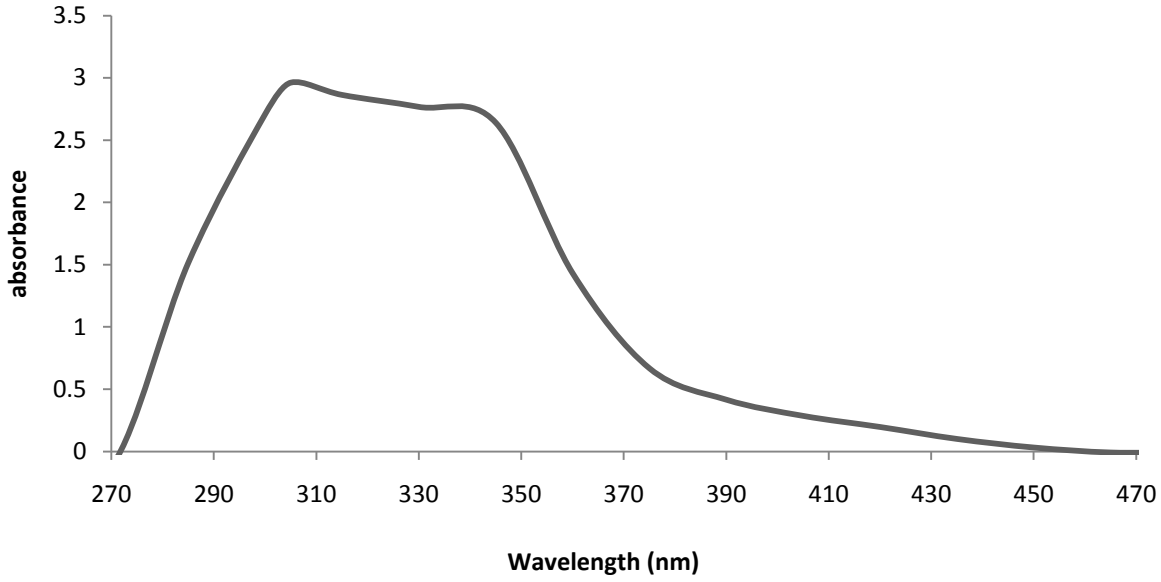


Figure 25: UV-Vis spectrum of 20 mg DCM extract in 25 ml DCM.

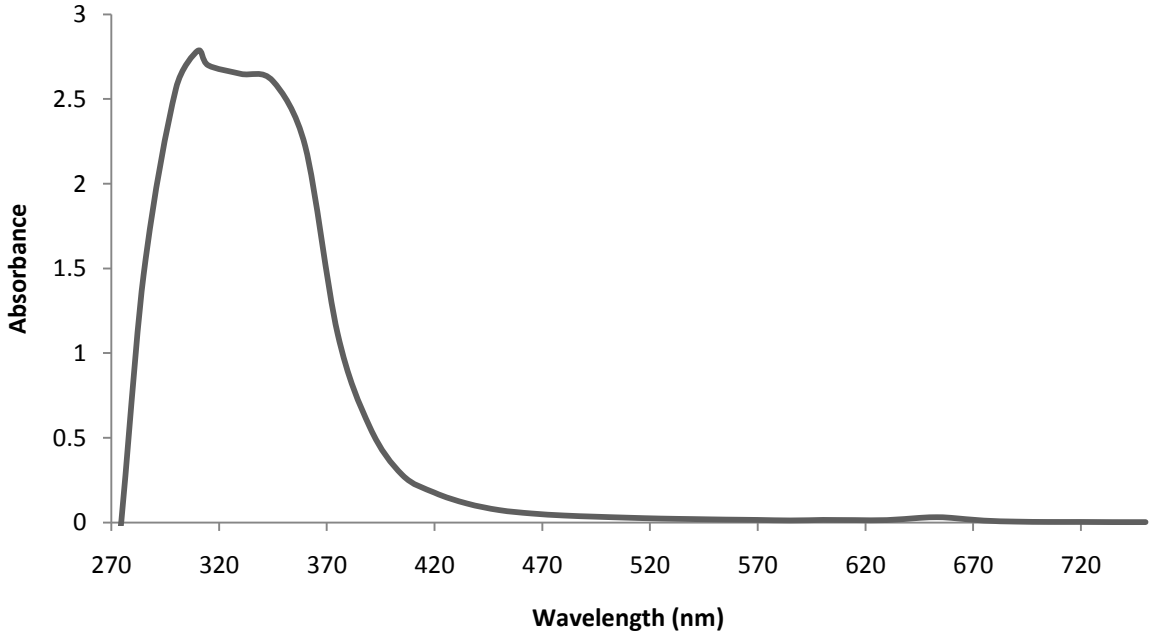


Figure 26: UV-Vis spectrum of 20 gm methanol extract in 25 ml methanol.

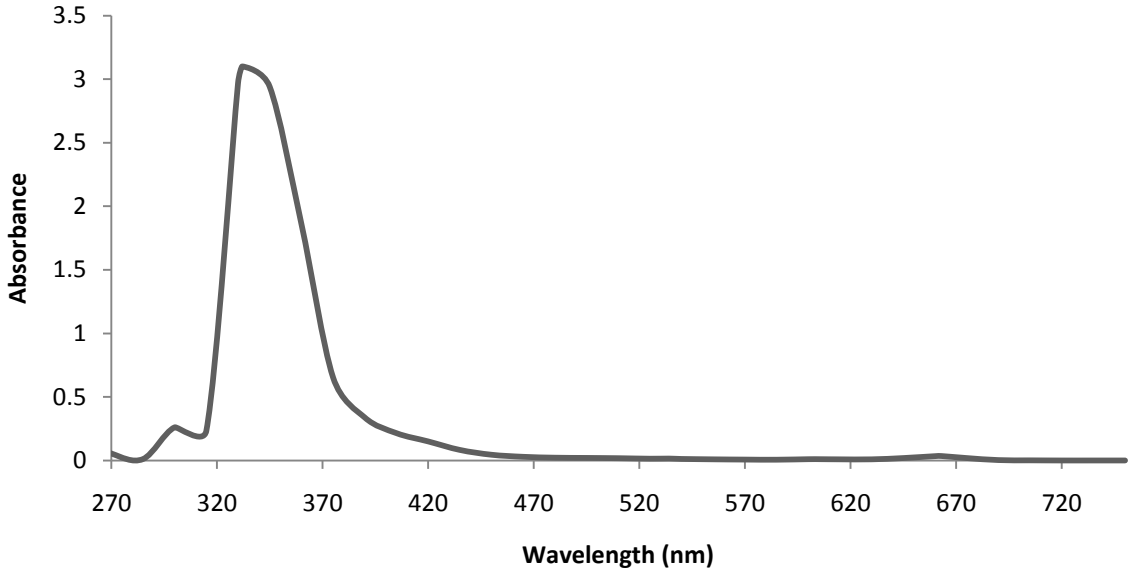


Figure 27: UV-Vis spectrum of 20 mg of hexane-acetone extract in 25 ml hexane-acetone.

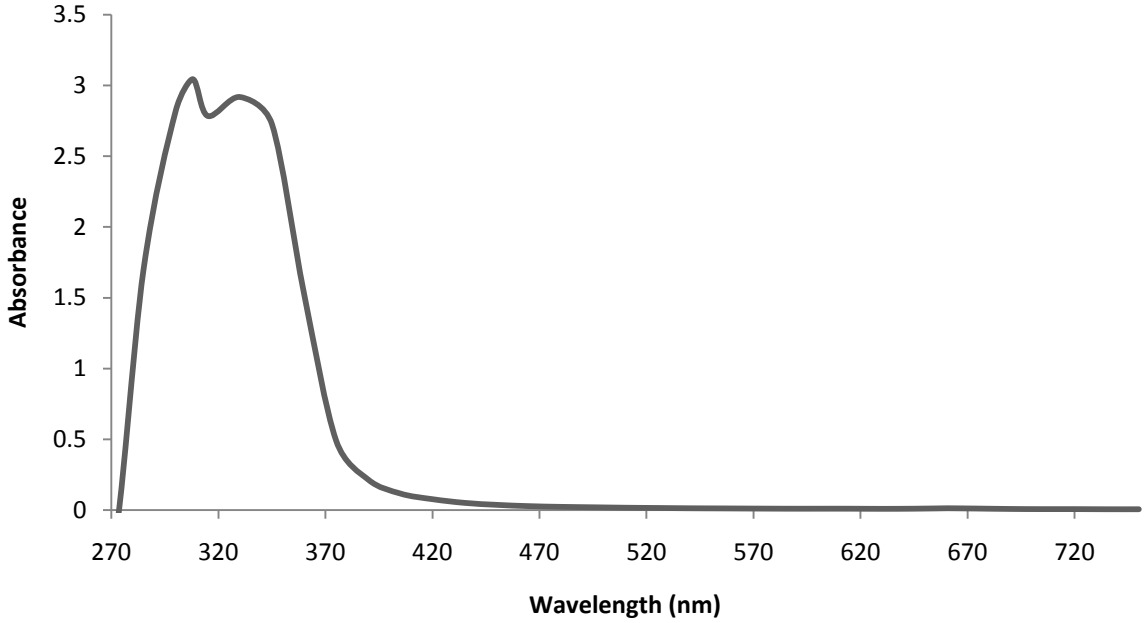


Figure 28: UV-Vis spectrum of 20 mg ethyl acetate extract in 25 ml ethyl acetate.

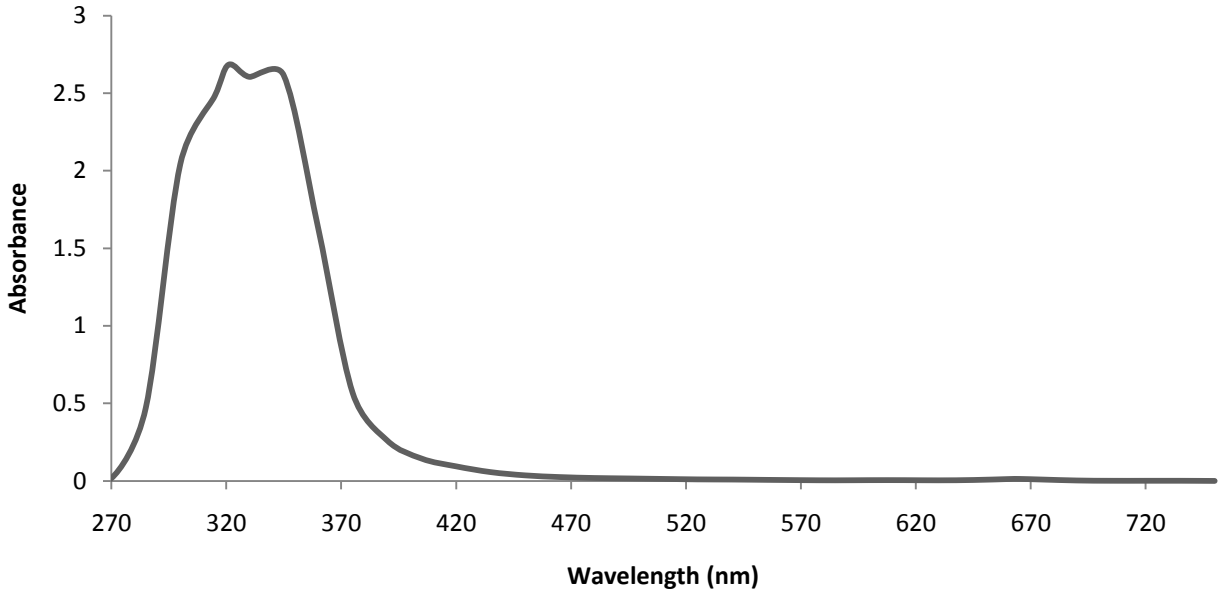


Figure 29: UV-Vis spectrum of 20 mg ethyl acetate-DCM extract in 25 ml ethyl acetate-DCM.

APPENDIX II: UV-VIS SPECTRA OF SECD EXTRACTS AND CHAMOMILE OIL

Note that the spectra do not show the peak due to chamazulene at λ_{MAX} = 605 nm because of dilution which was carried out to enable showing the part of the spectra between 280 nm and 400 nm.

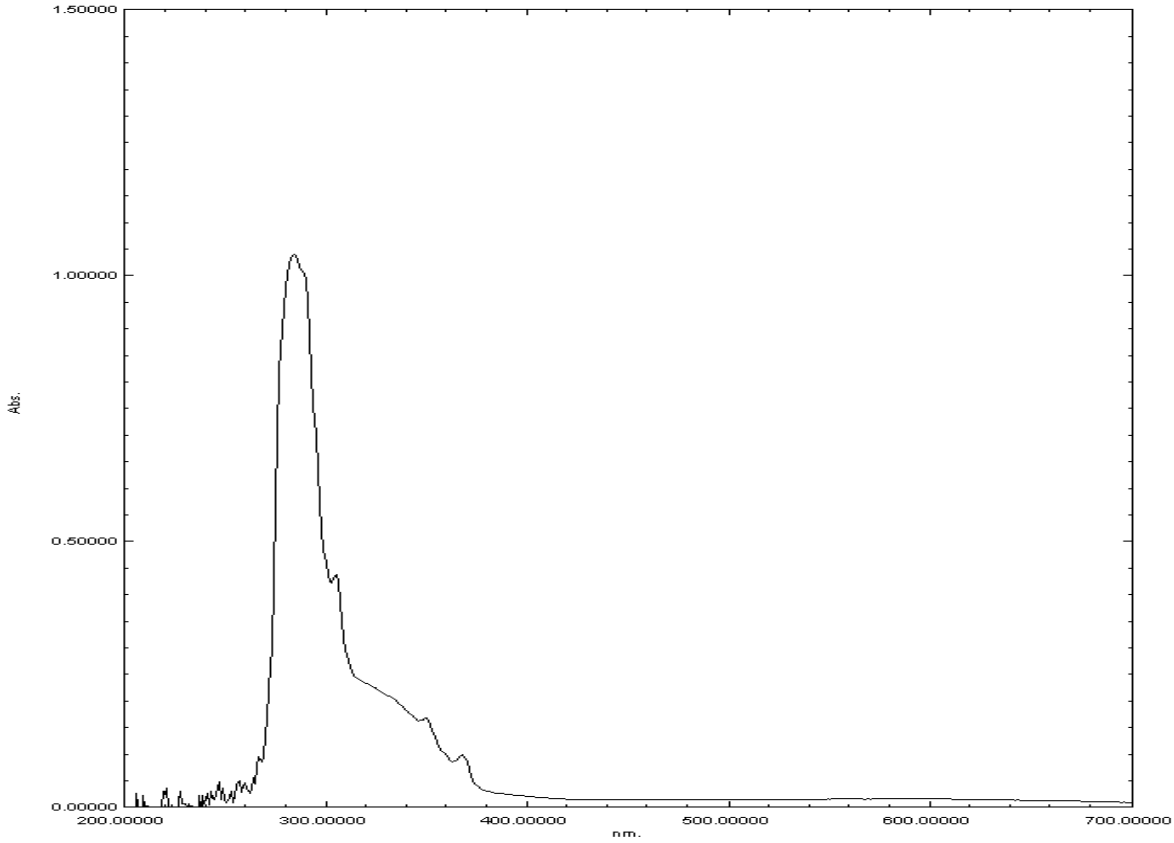


Figure 30: UV-Vis spectrum for acetone SECD extract

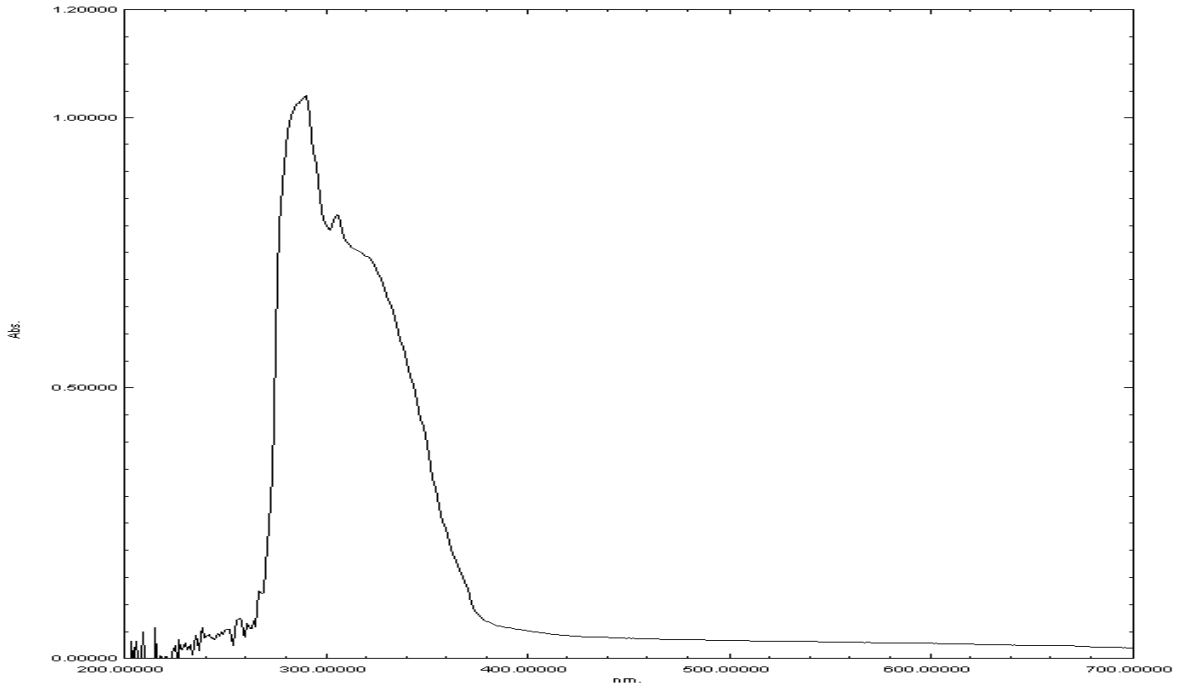


Figure 31: UV-Vis spectrum of DCM SECD extract.

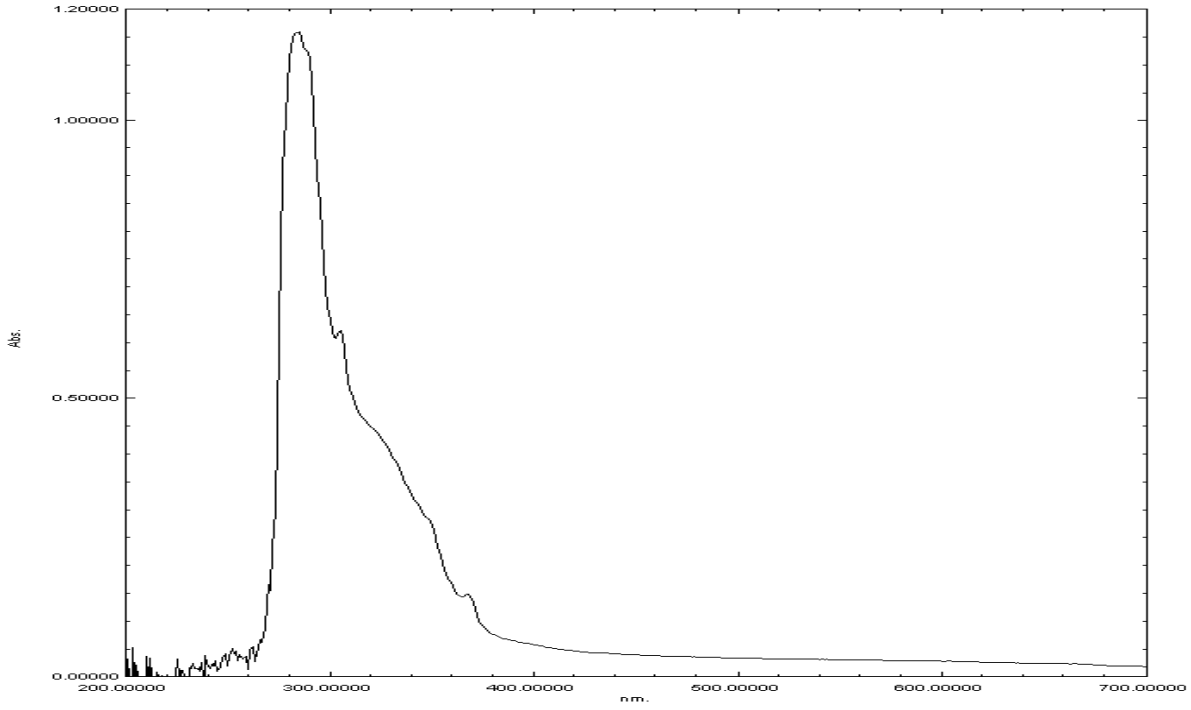


Figure 32: UV-Vis spectrum of Ethyl acetate-DCM SECD extract.

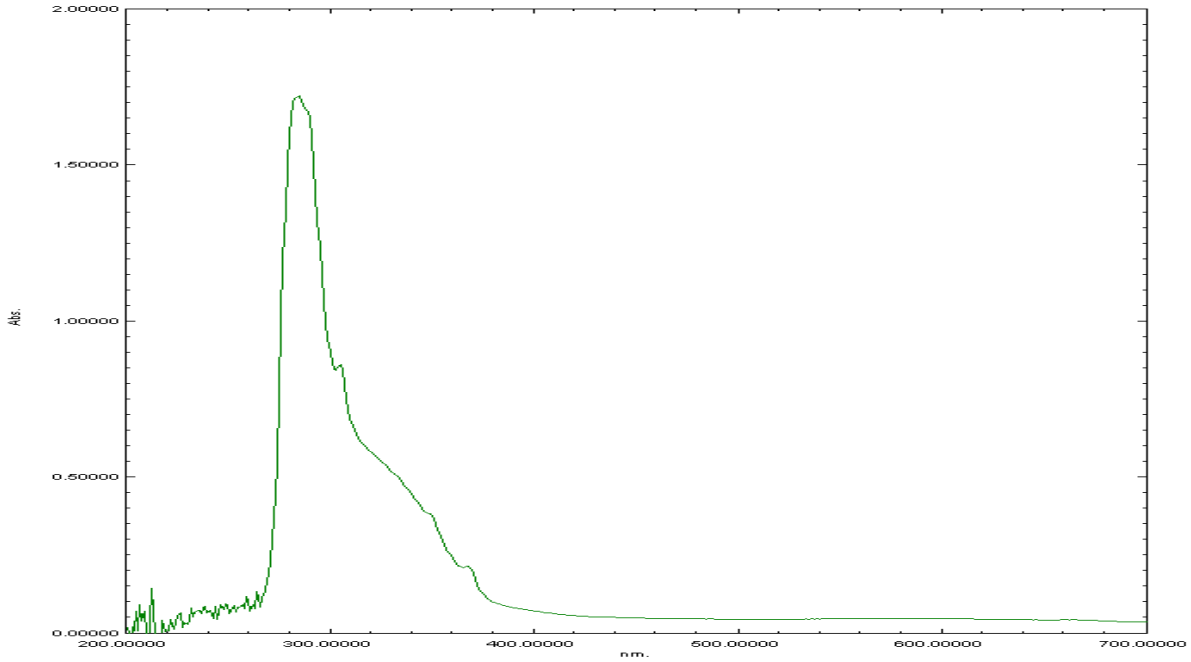


Figure 33: UV-Vis spectrum of ethyl acetate SECD extract.

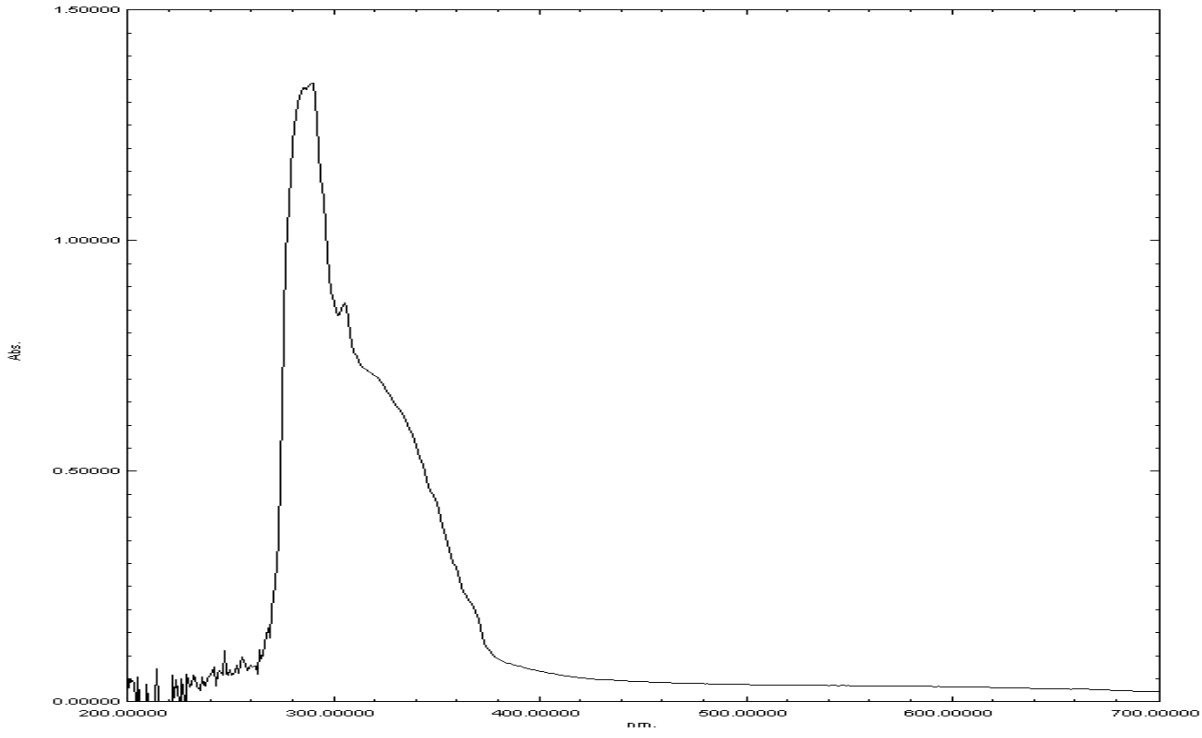


Figure 34: UV-Vis spectrum of hexane-acetone SECD extract.

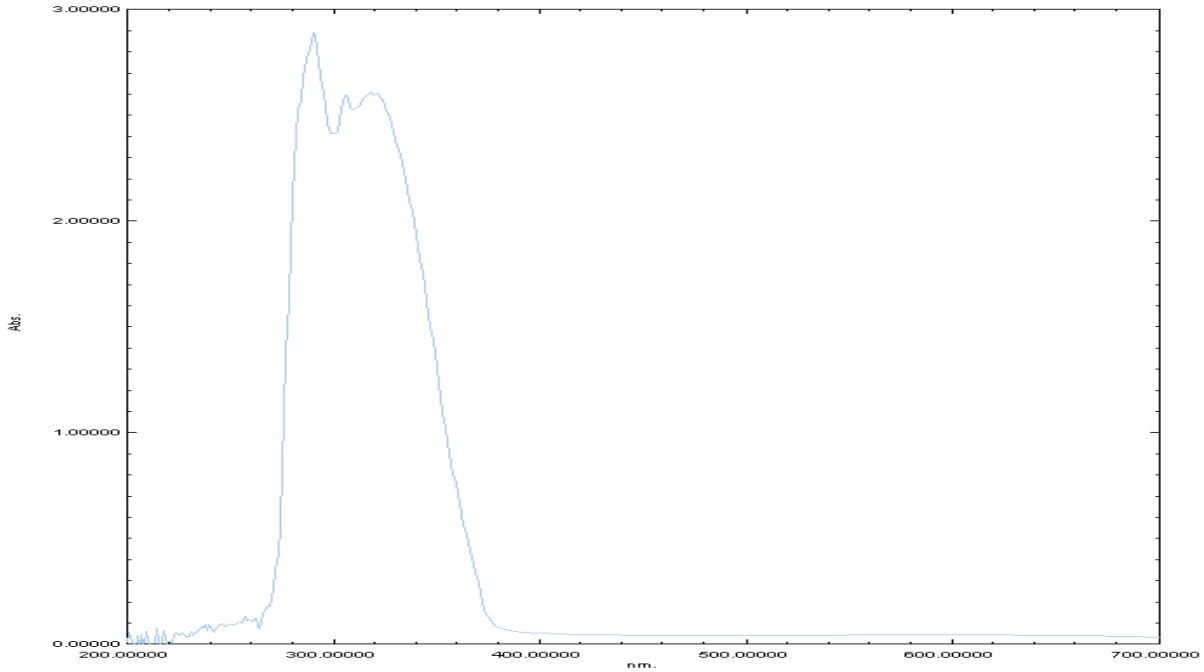


Figure 35: UV-Vis spectrum of hexane SECD extract.

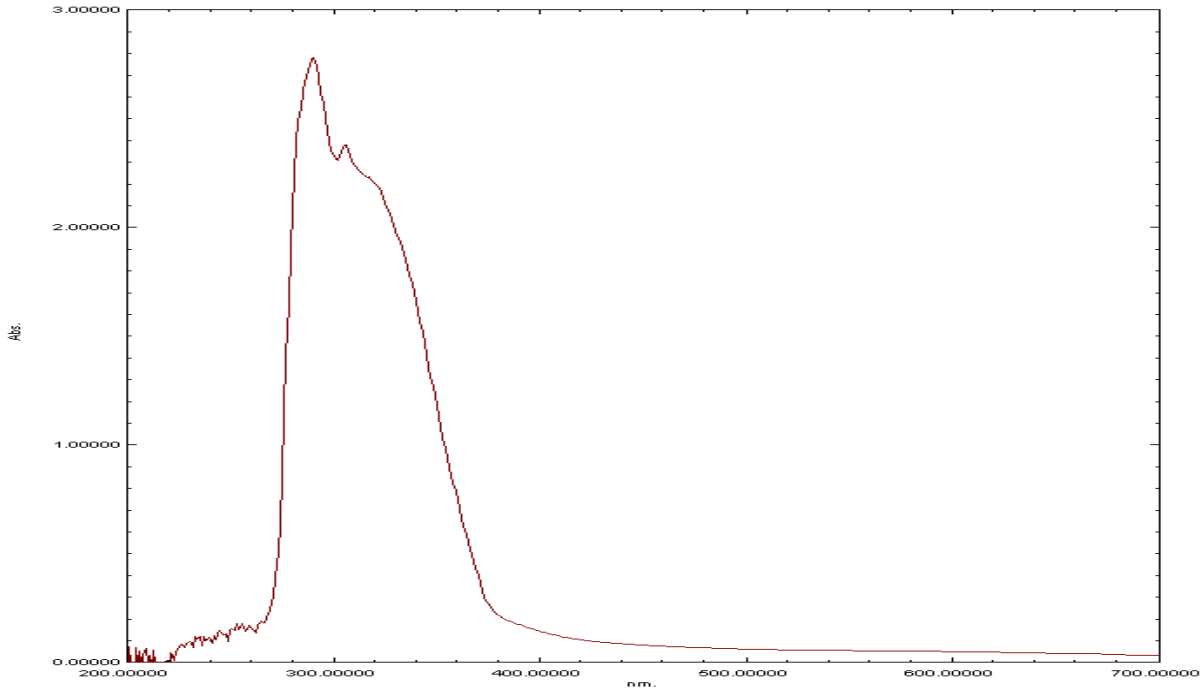


Figure 36: UV-Vis spectrum of methanol SECD extract.

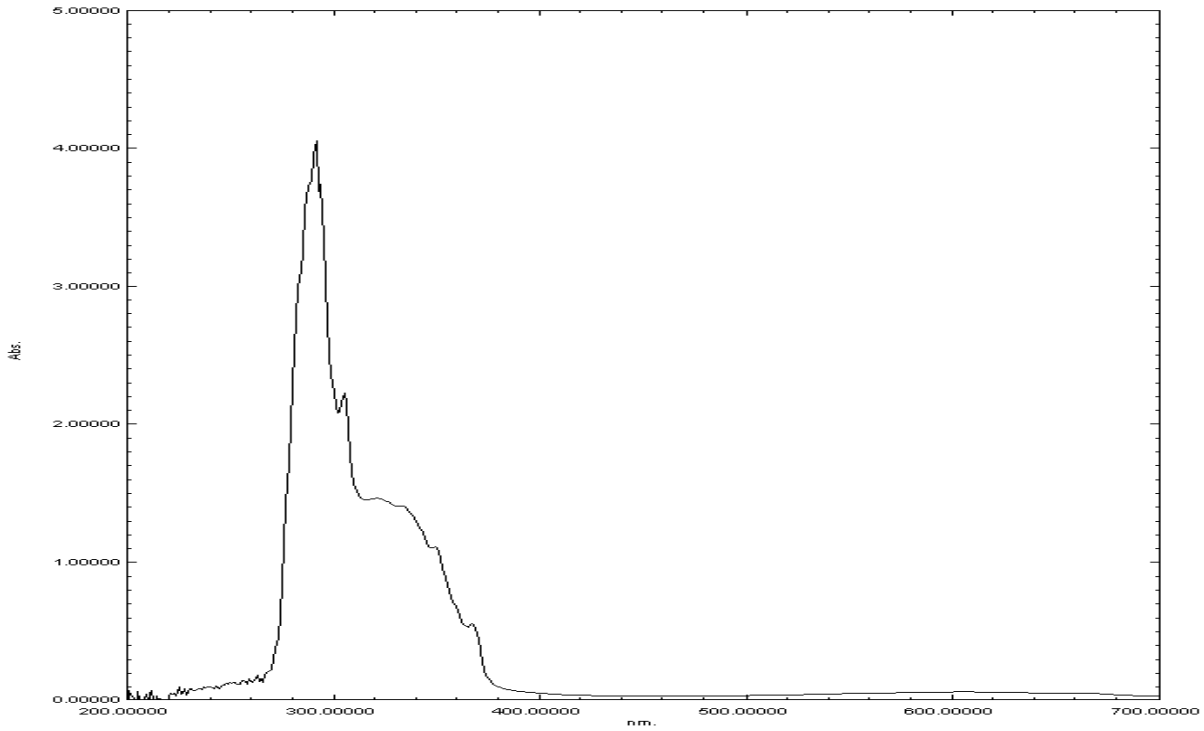


Figure 37: UV-Vis spectrum of Clevenger distilled chamomile essential oil.

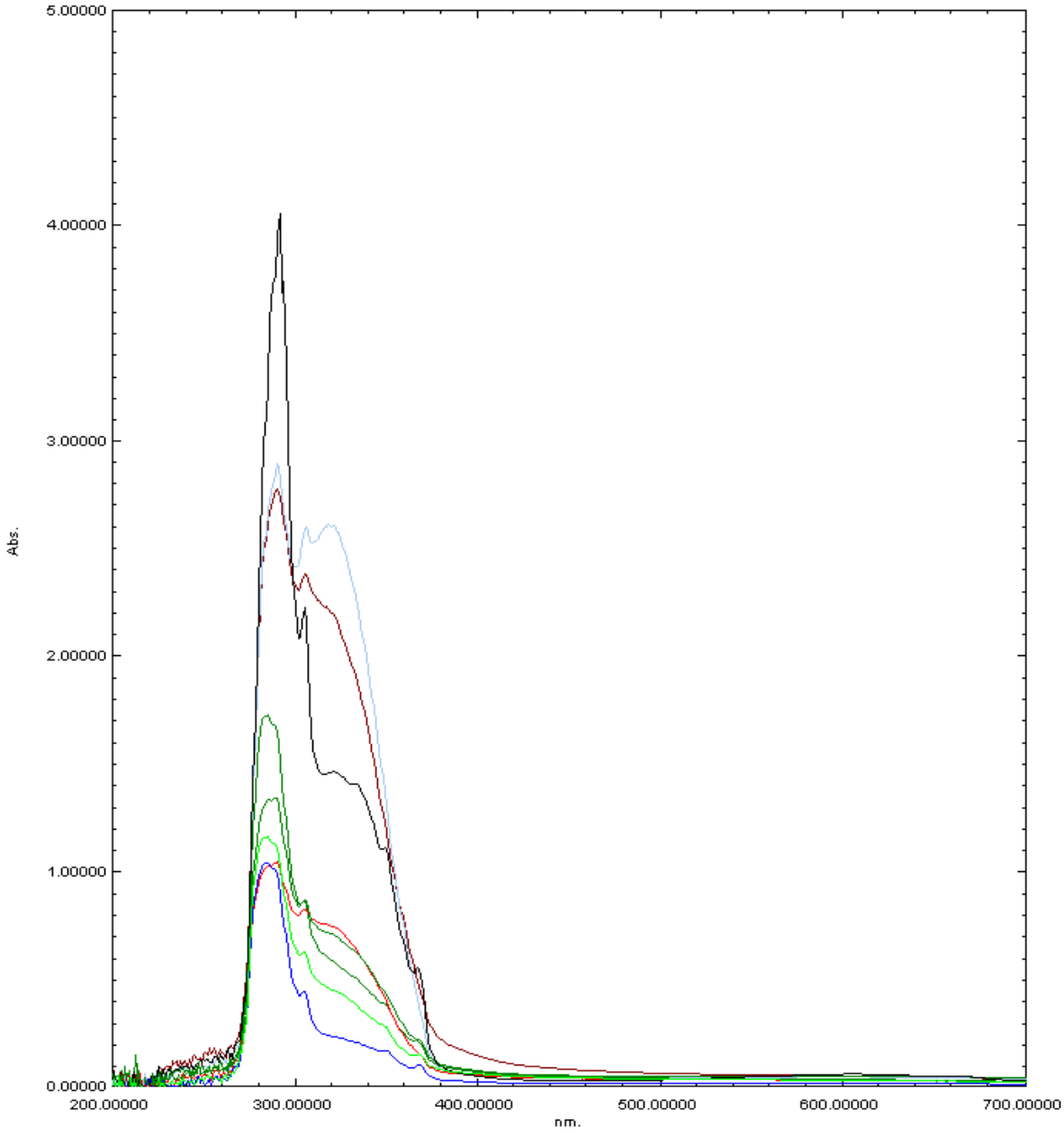


Figure 38: An overlay of UV-Vis spectra of chamomile essential oil and SECD extracts.

APPENDIX III: IR SPECTRA OF EXTRACTS OBTAINED BY COLD EXTRACTION USING DIFFERENT SOLVENTS

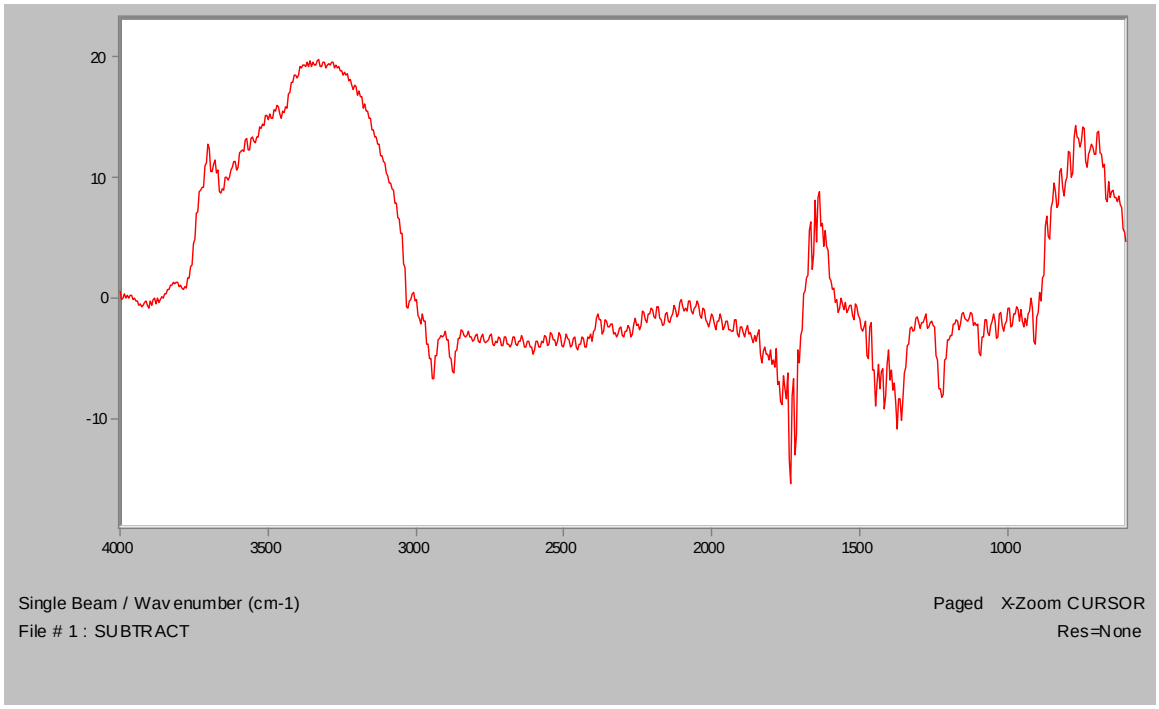


Figure 39: IR spectrum of cold acetone extract.

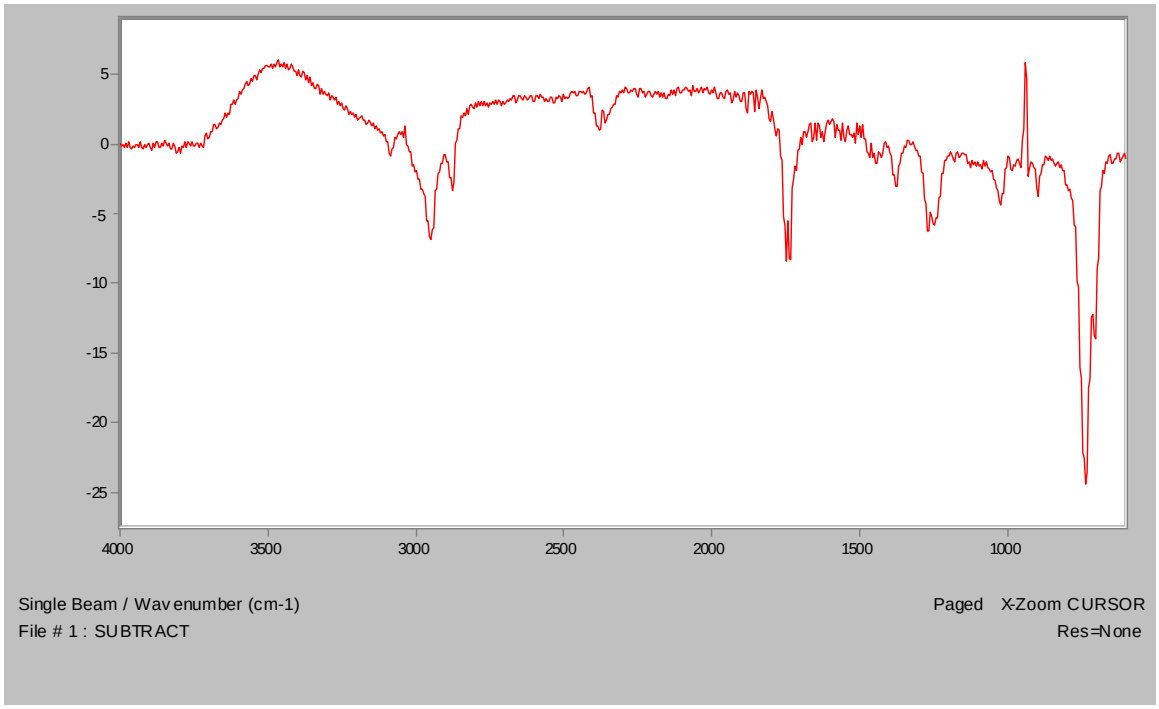


Figure 40: IR spectrum of cold DCM extract.

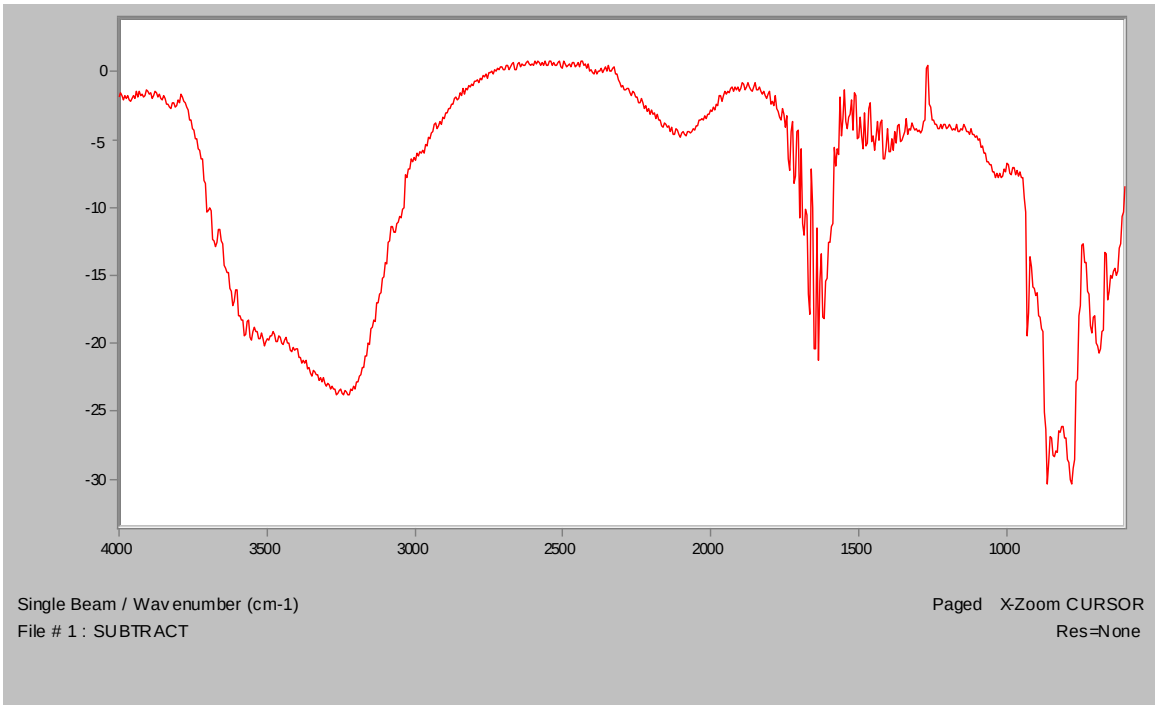


Figure 41: IR spectrum of cold ethanol extract.

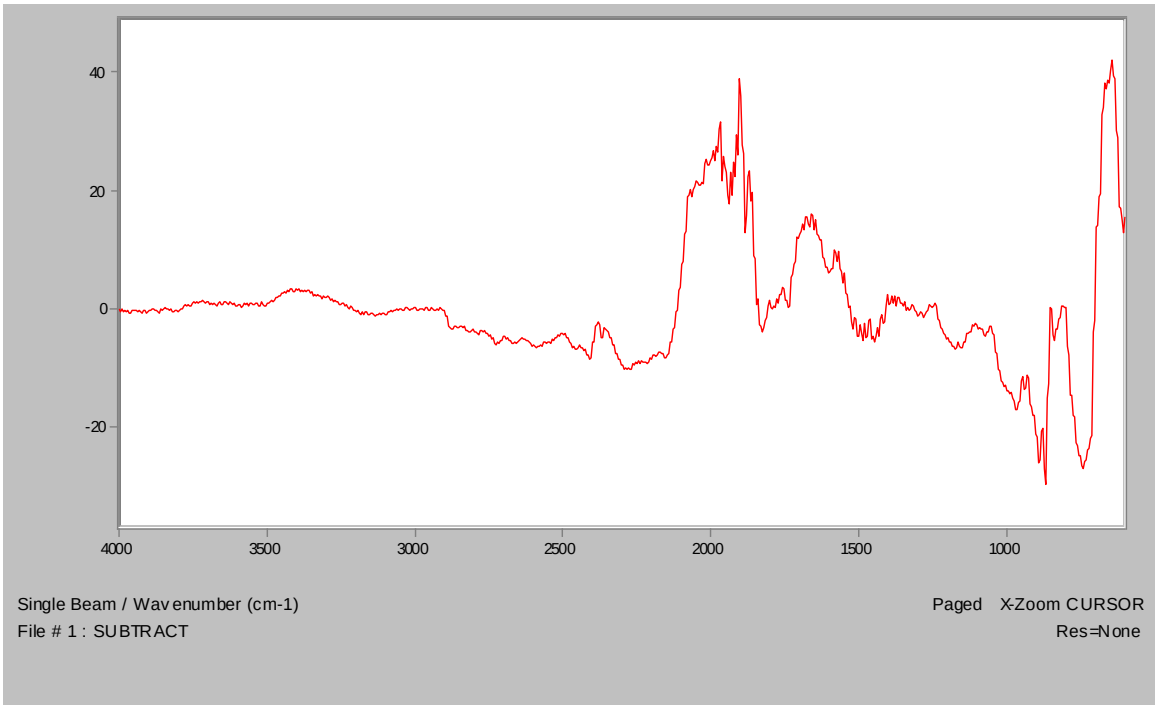


Figure 42: IR spectrum of cold ethyl acetate extract.

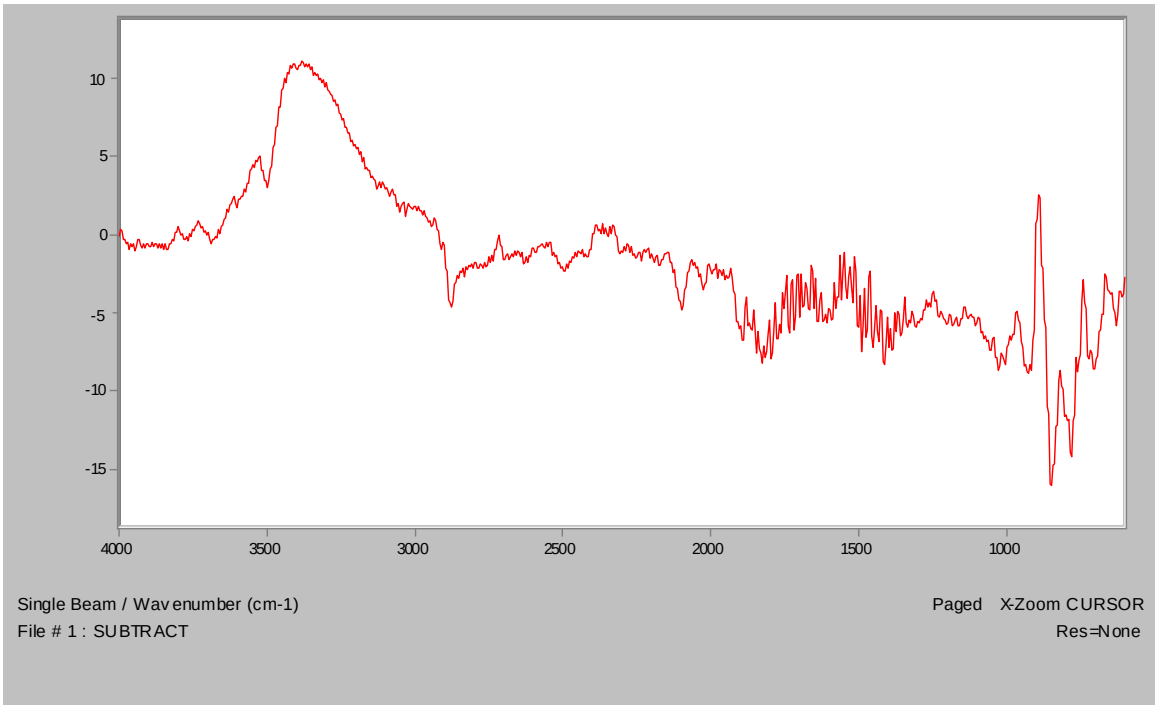


Figure 43: IR spectrum of cold ethyl acetate/DCM extract.

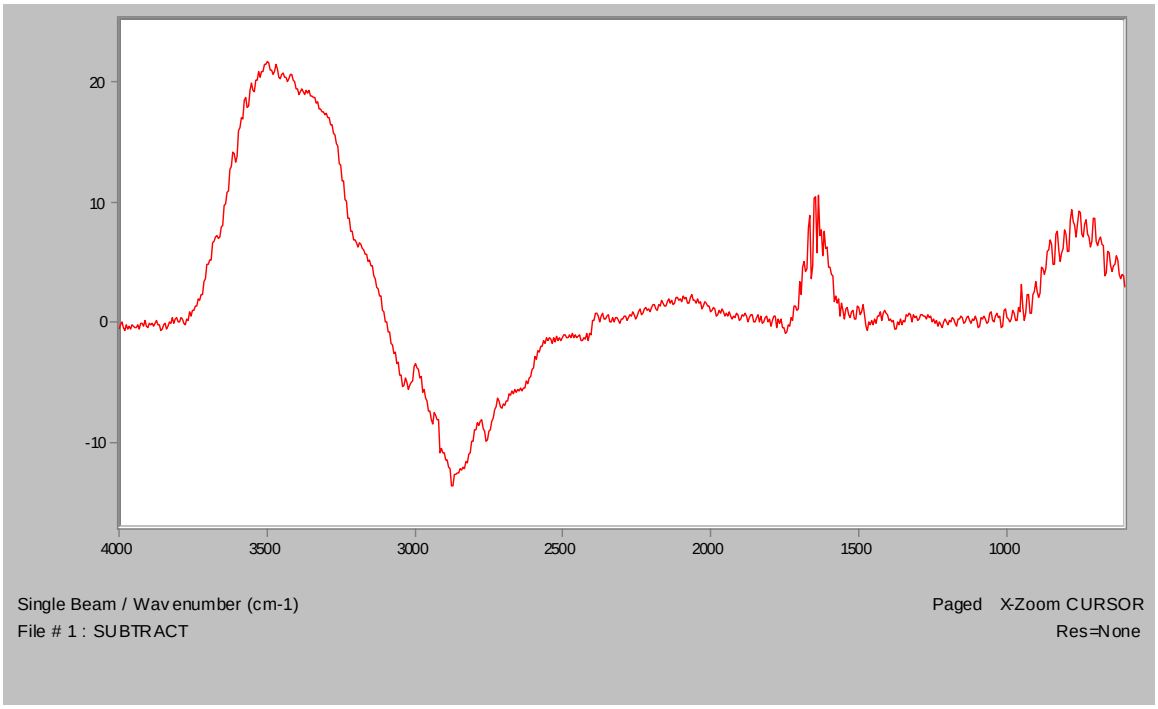


Figure 44: IR spectrum of cold hexane extract.

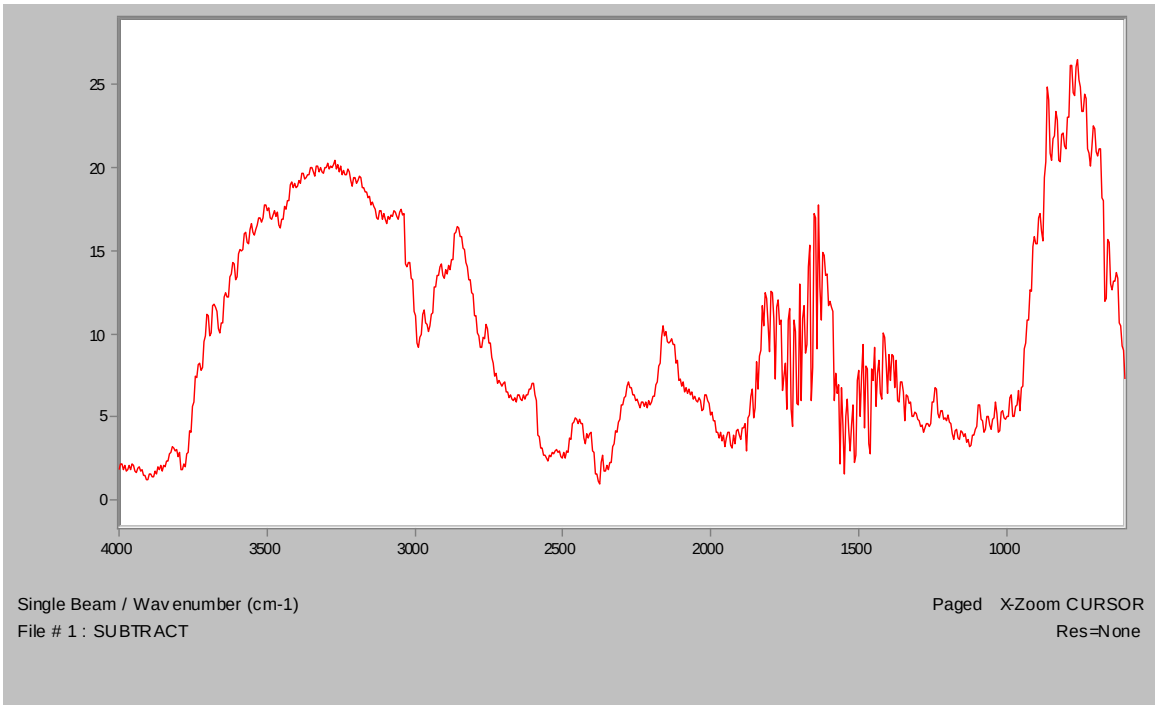


Figure 45: IR spectrum of cold hexane/acetone extract.

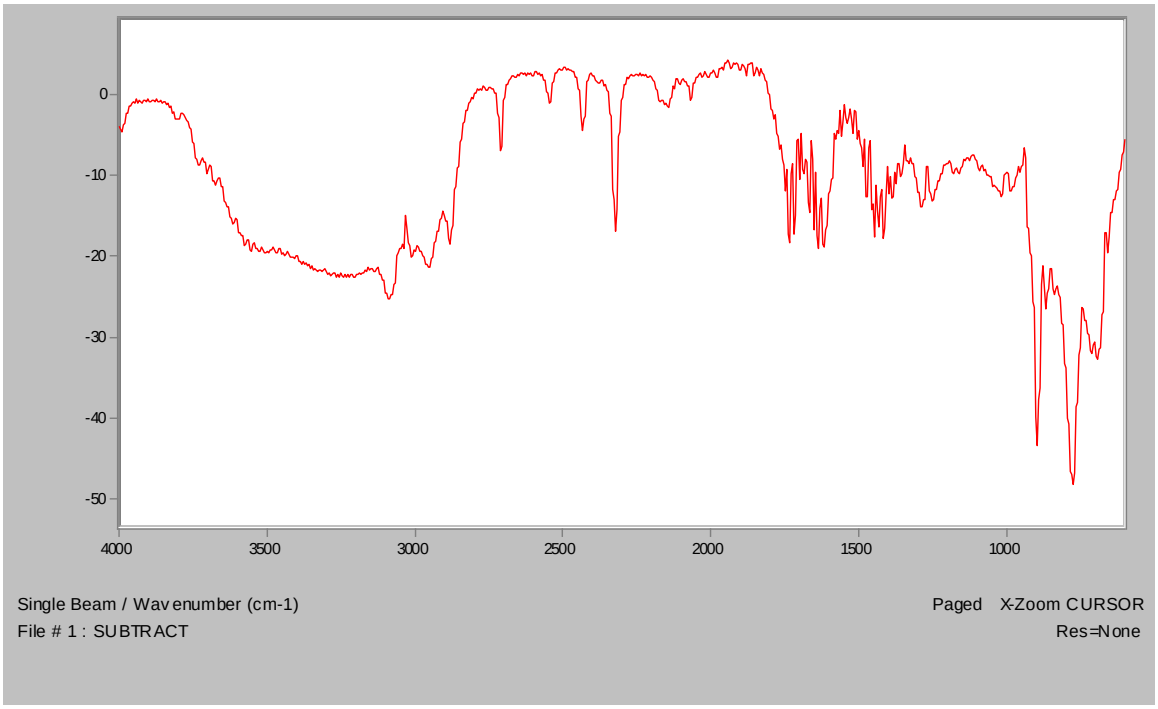


Figure 46: IR spectrum of cold methanol extract.

APPENDIX IV: GC CHROMATOGRAMS OF ESSENTIAL OIL FROM KANGARI, NJABINI, AND KIBWEZI CHAMOMILE FLOWERS

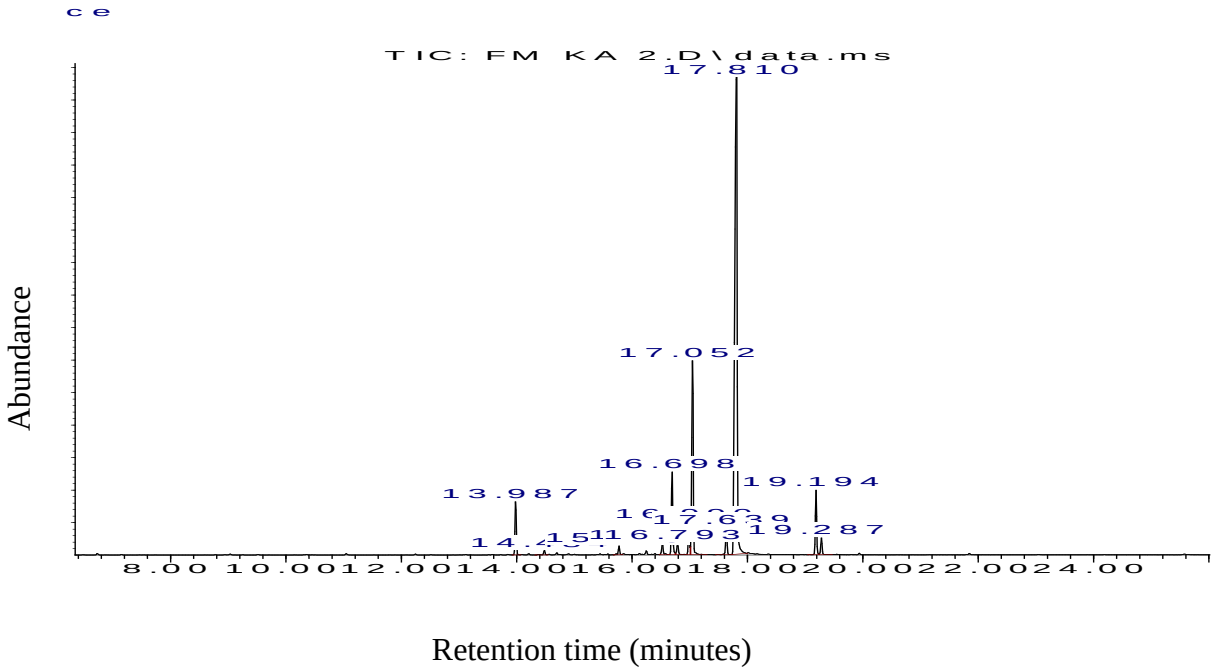


Figure 47: GC chromatogram of essential oil from Kangari chamomile flowers.

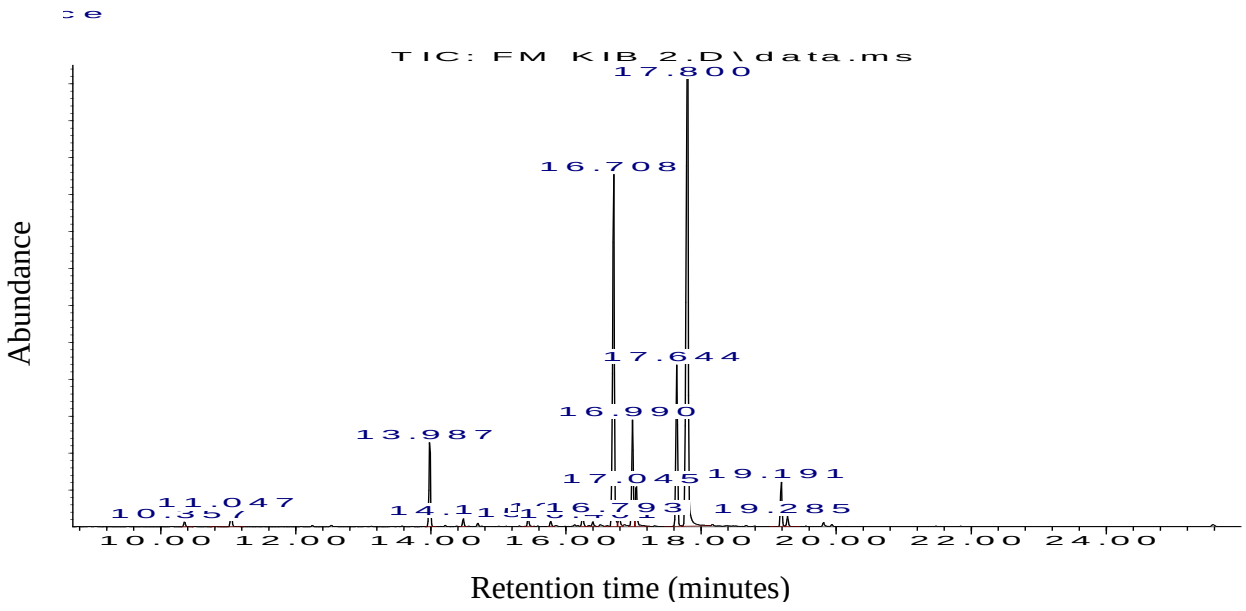


Figure 48: GC chromatogram of essential oil from Kibwezi chamomile flowers.

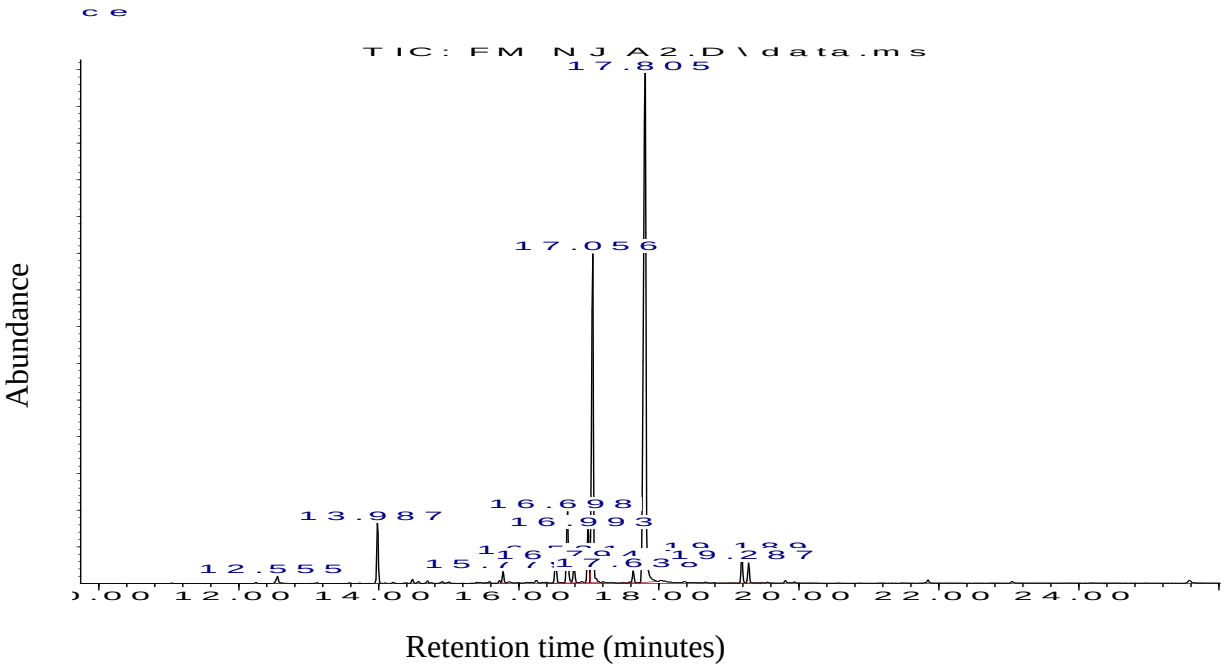


Figure 49: GC chromatogram of essential oil from Njabini chamomile flowers.

APPENDIX V: MASS SPECTRA OF IDENTIFIED GC PEAKS AND THEIR MATCHES FROM THE MS LIBRARY

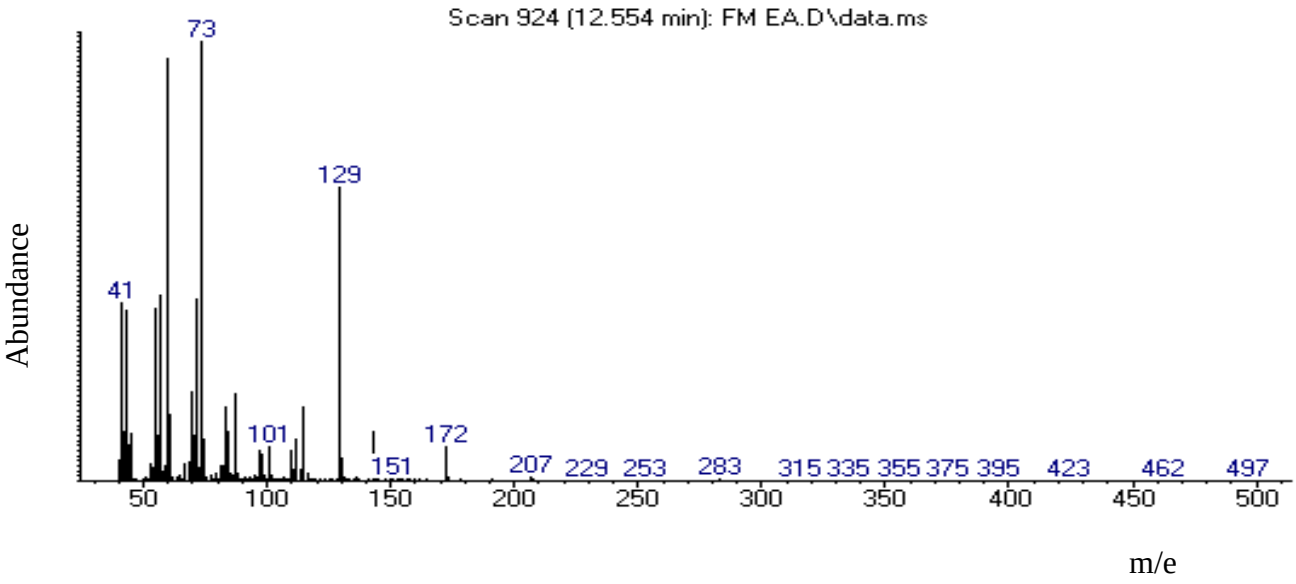
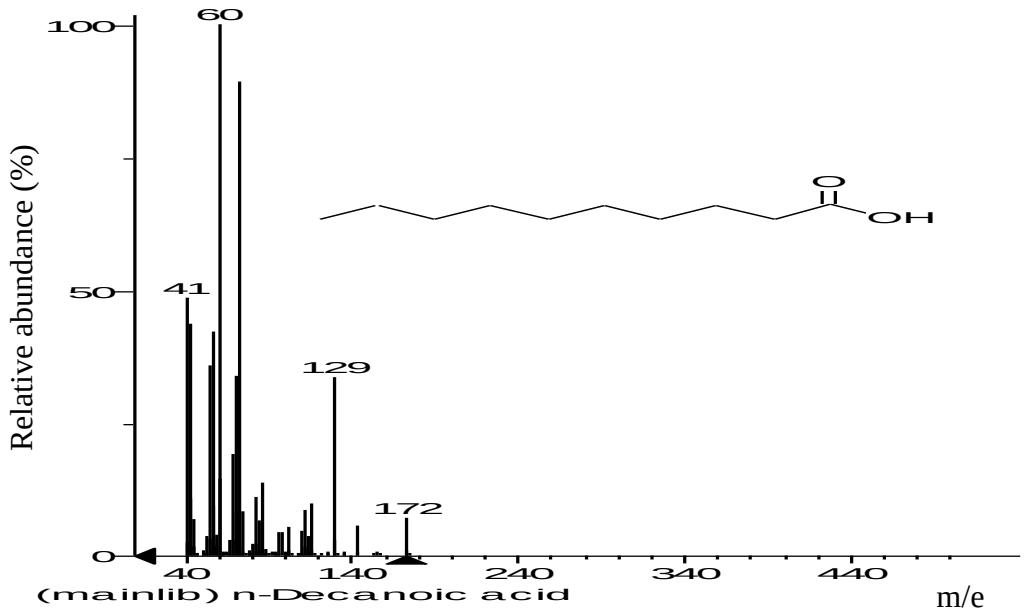


Figure 50: Mass spectrum of peak at R.T. 12.554 minutes.



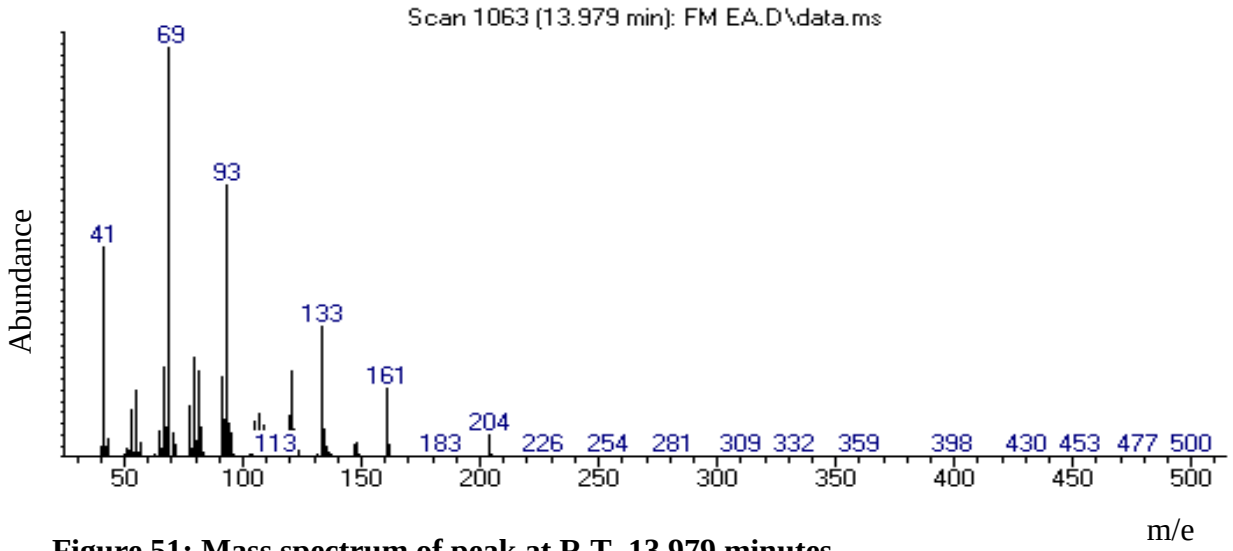
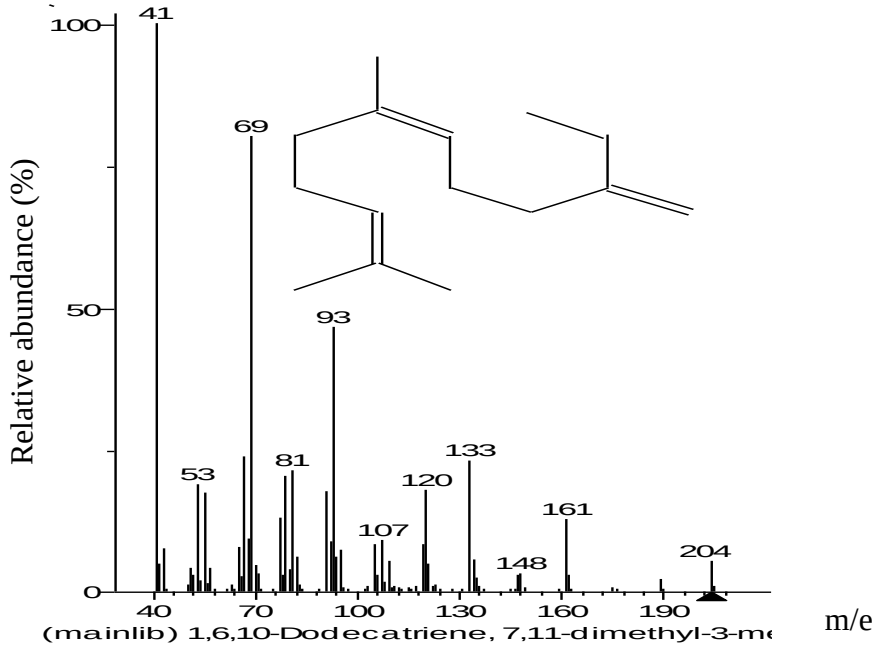


Figure 51: Mass spectrum of peak at R.T. 13.979 minutes.



Z-Beta-farnesene

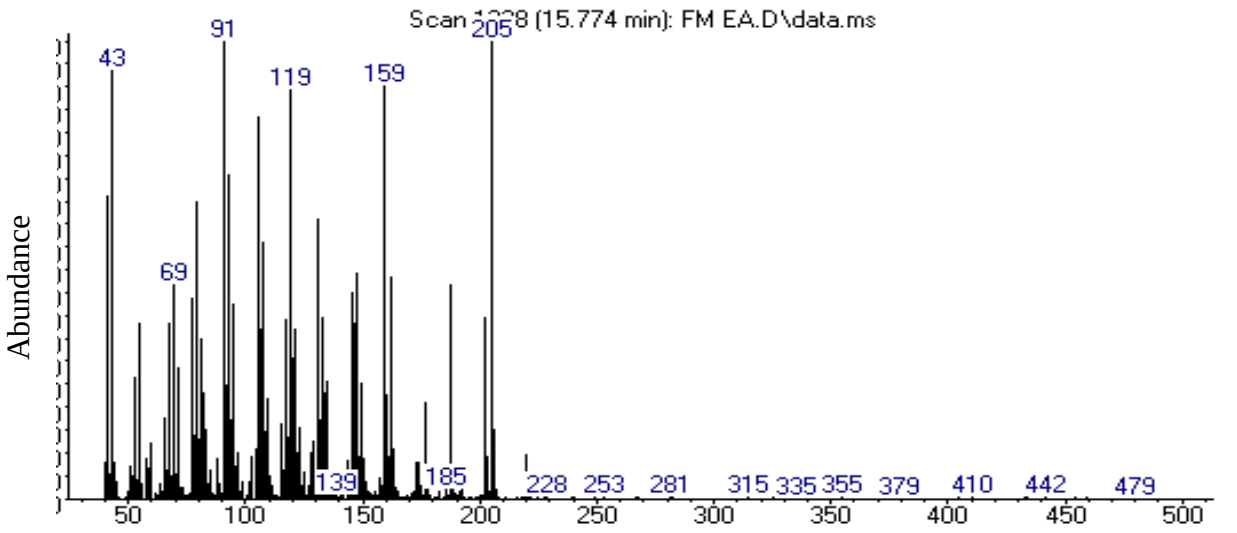
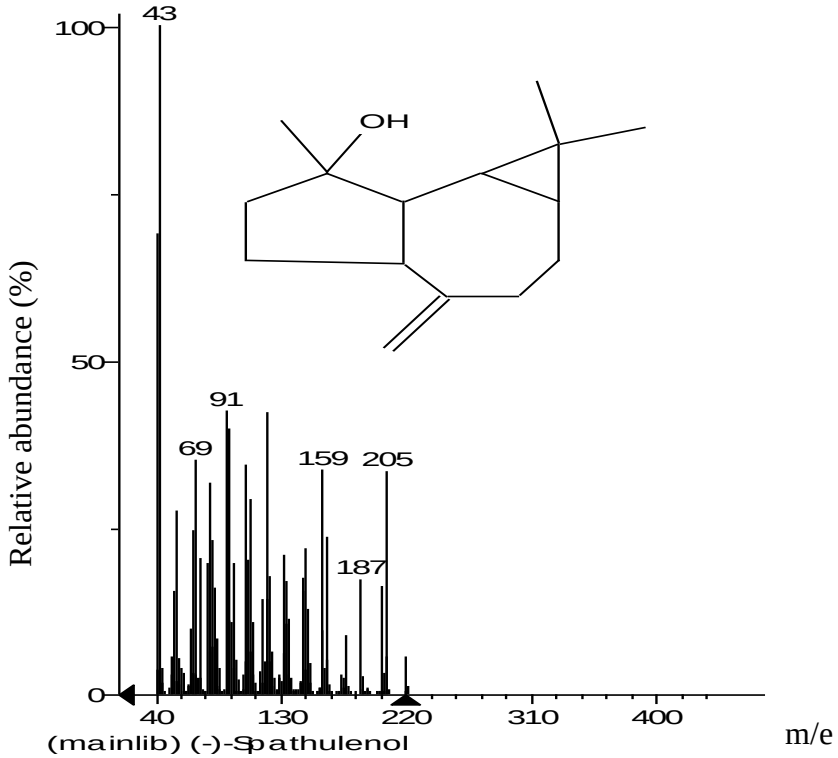


Figure 52: Mass spectrum of peak at R.T. 15.774 minutes.



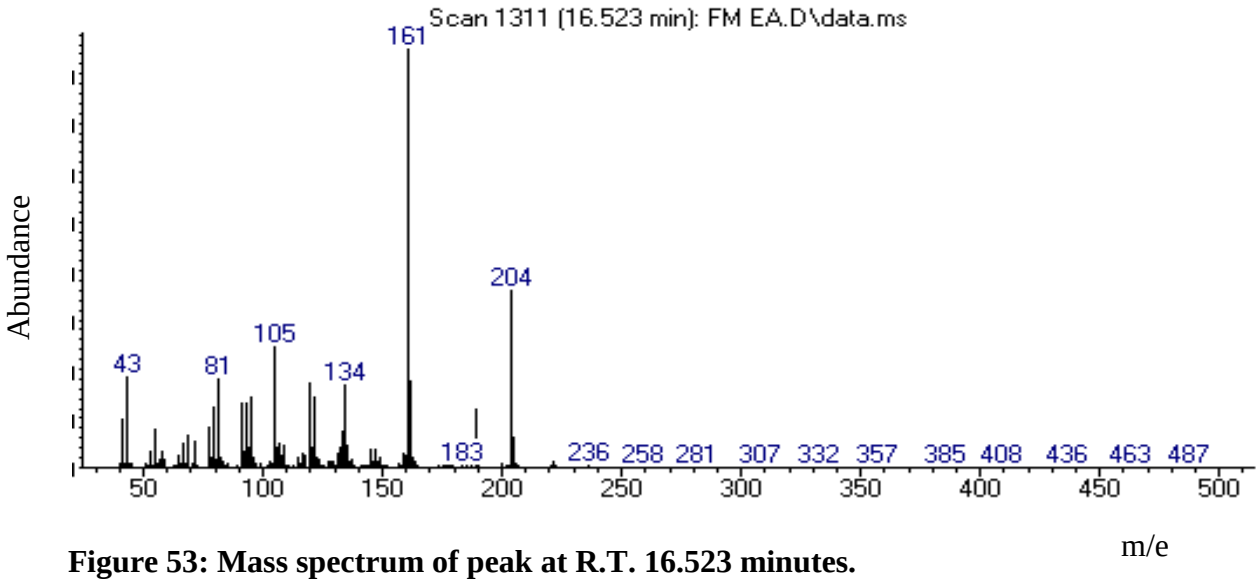
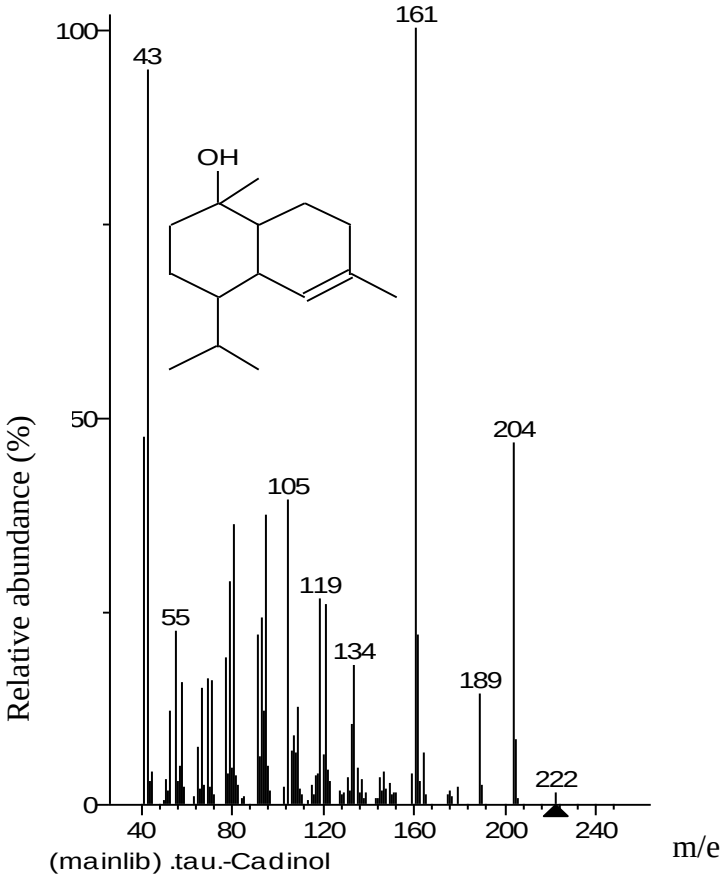


Figure 53: Mass spectrum of peak at R.T. 16.523 minutes.



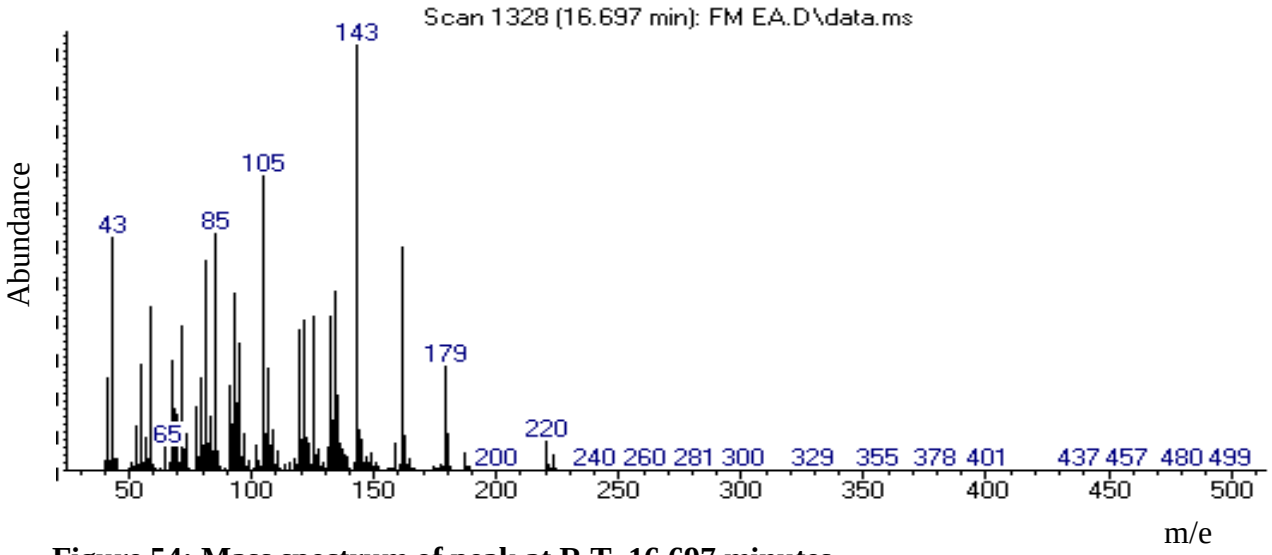
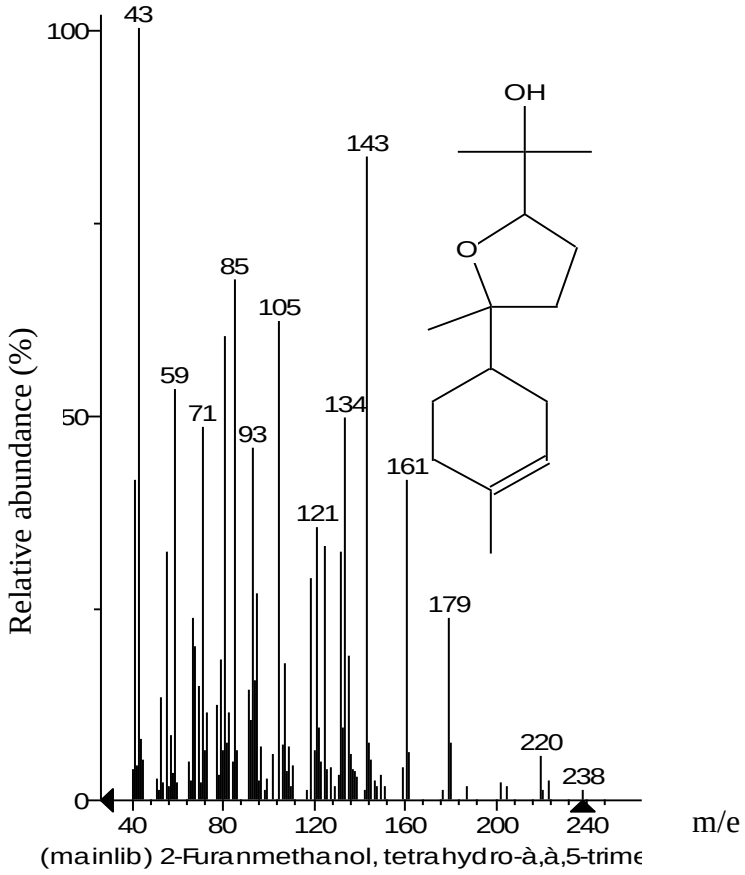


Figure 54: Mass spectrum of peak at R.T. 16.697 minutes.



α-Bisabolol oxide B

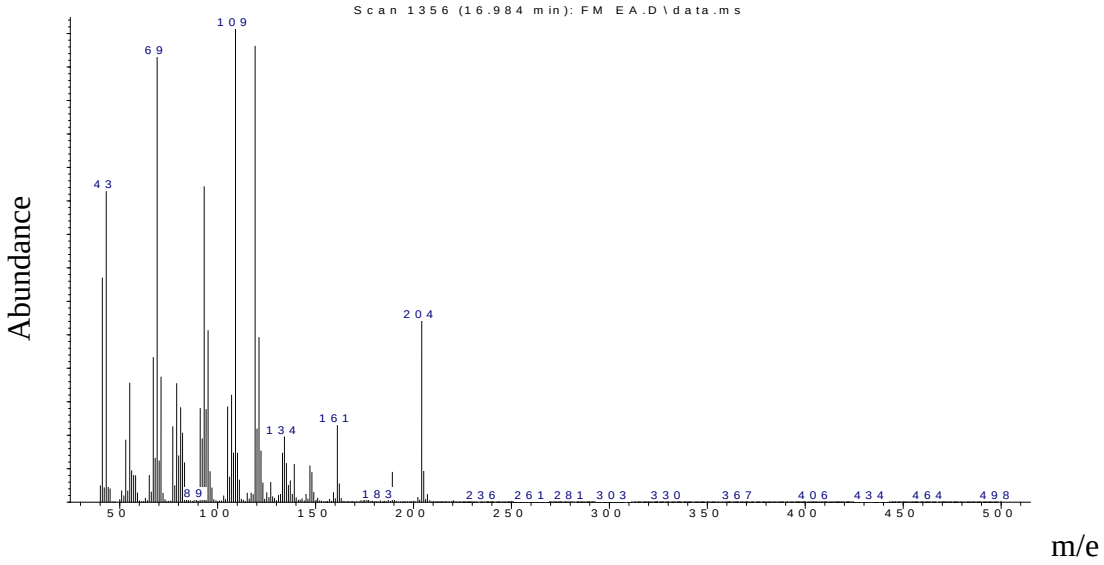
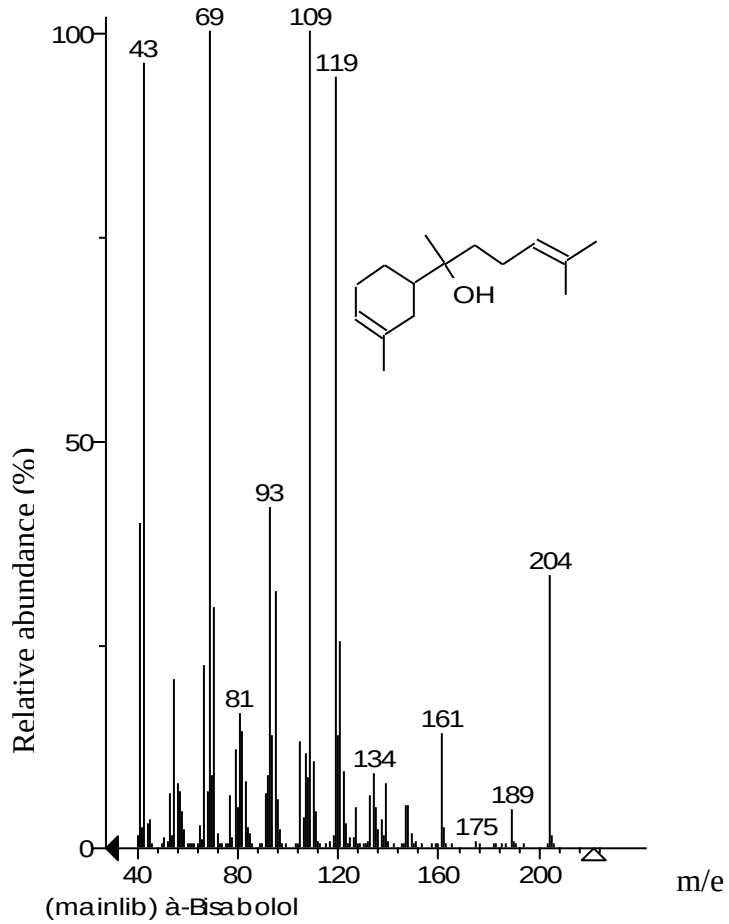


Figure 55: Mass spectrum of peak at R.T. 16.994 minutes.



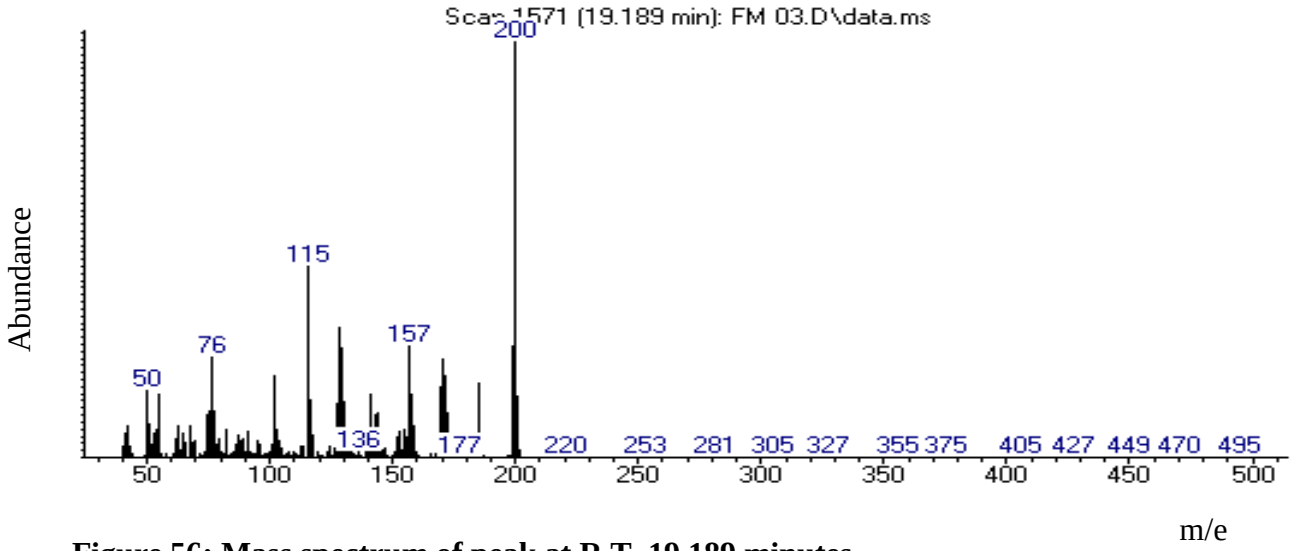
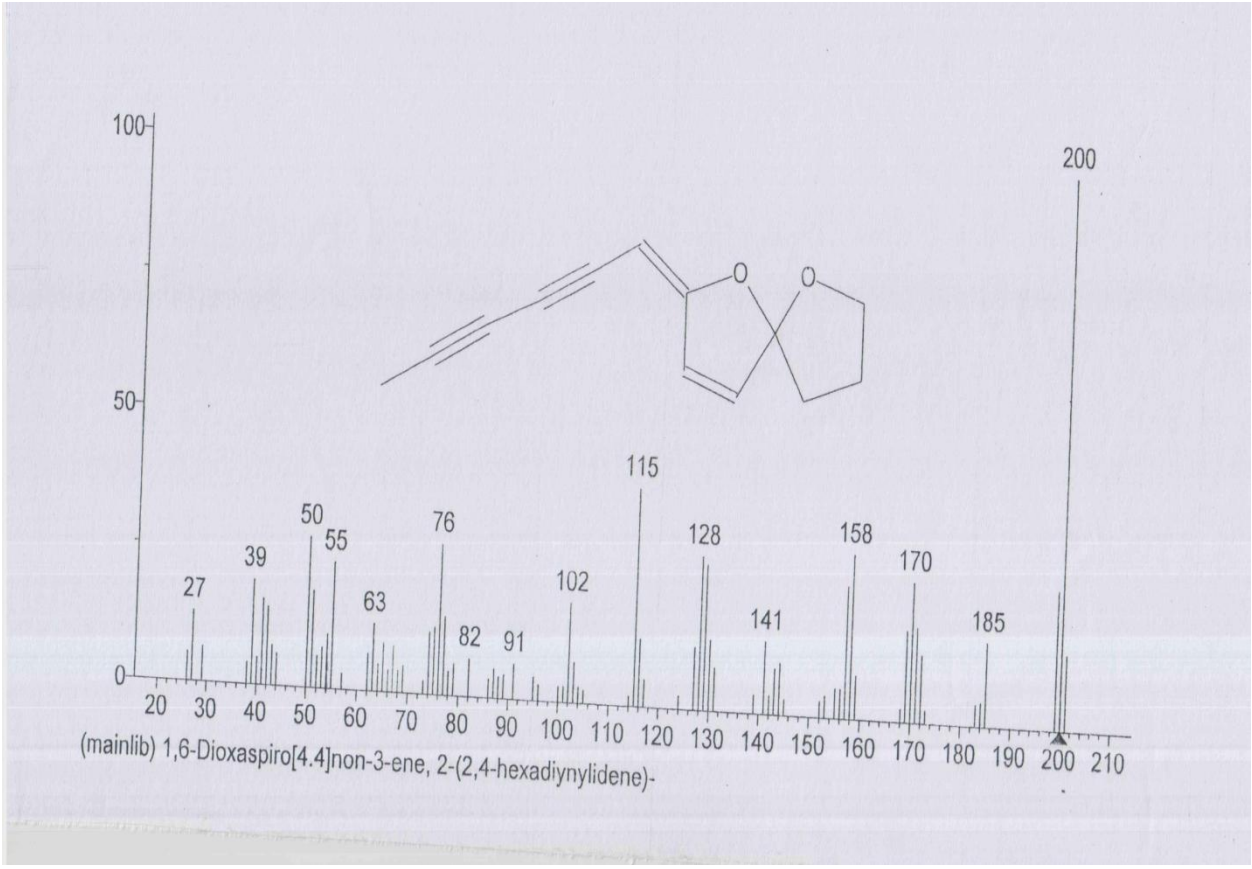


Figure 56: Mass spectrum of peak at R.T. 19.189 minutes.



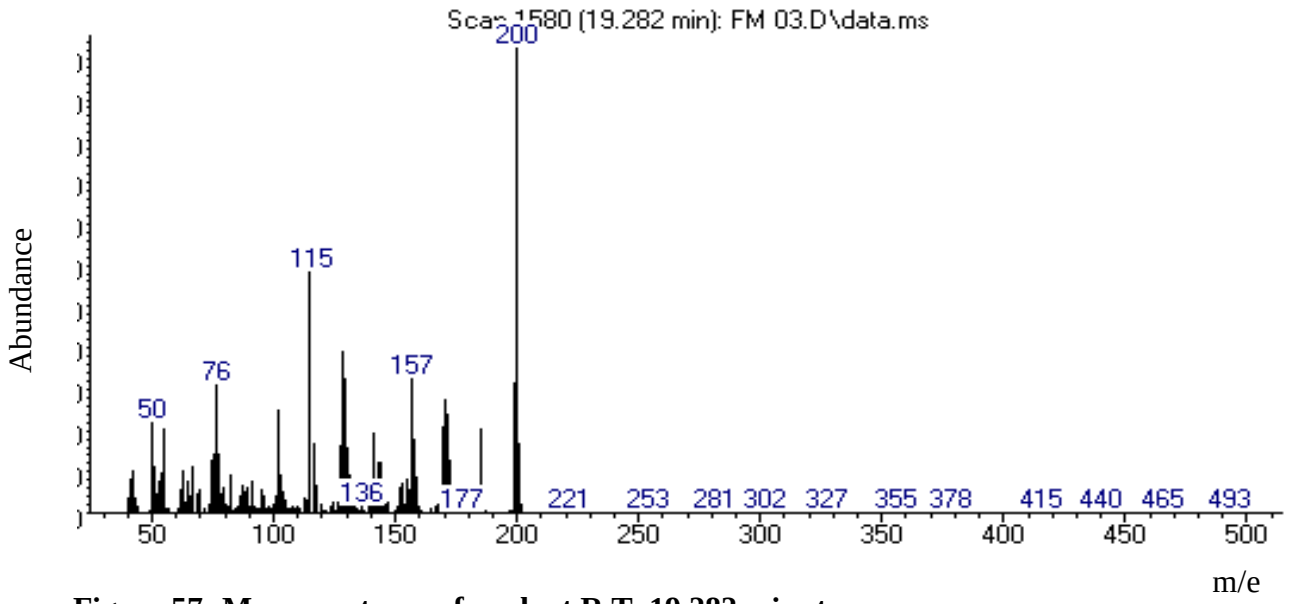
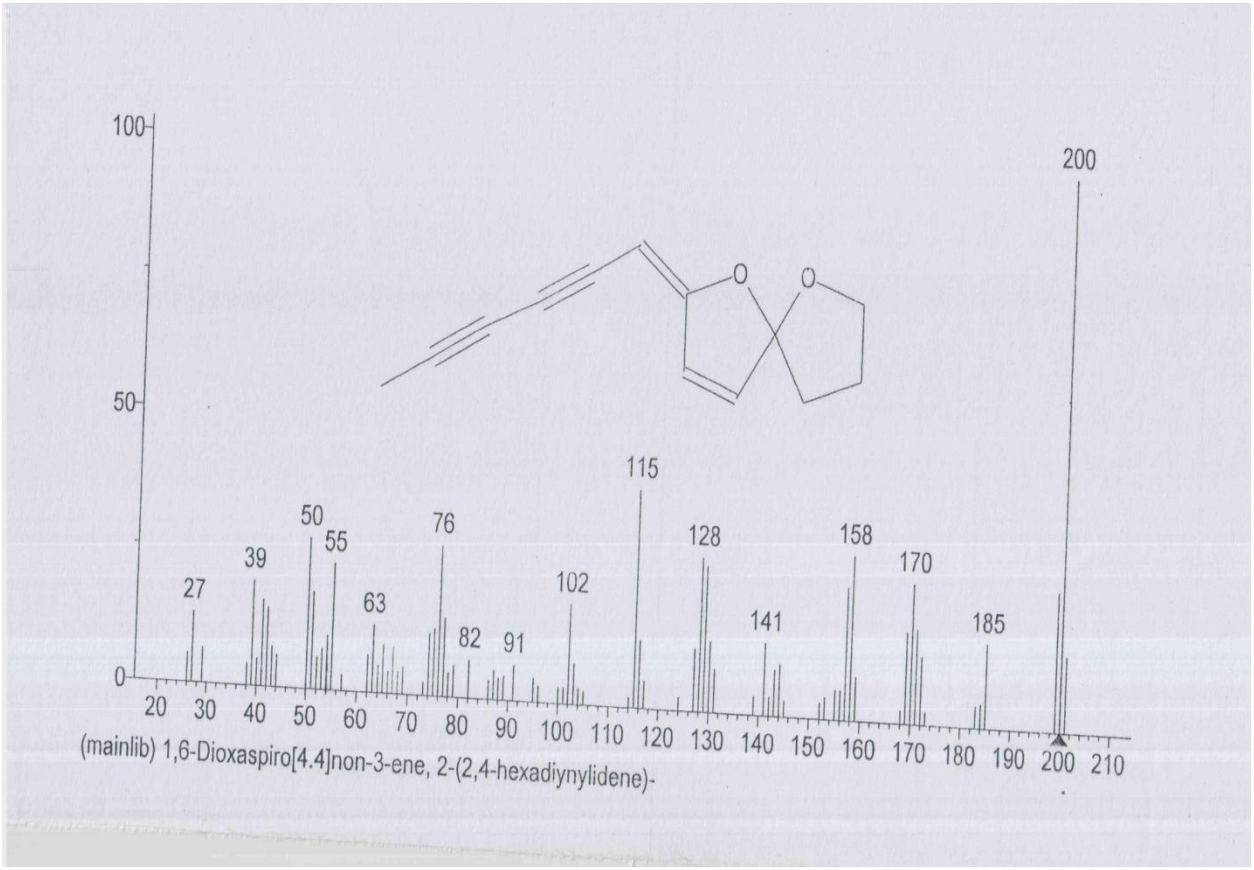


Figure 57: Mass spectrum of peak at R.T. 19.282 minutes.



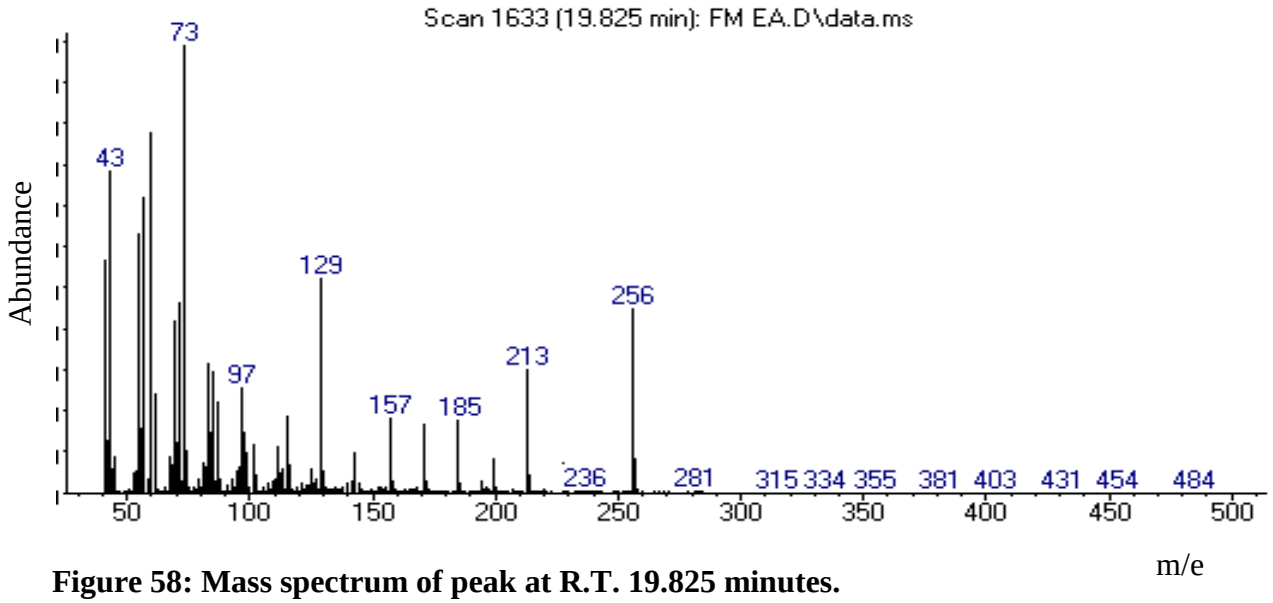
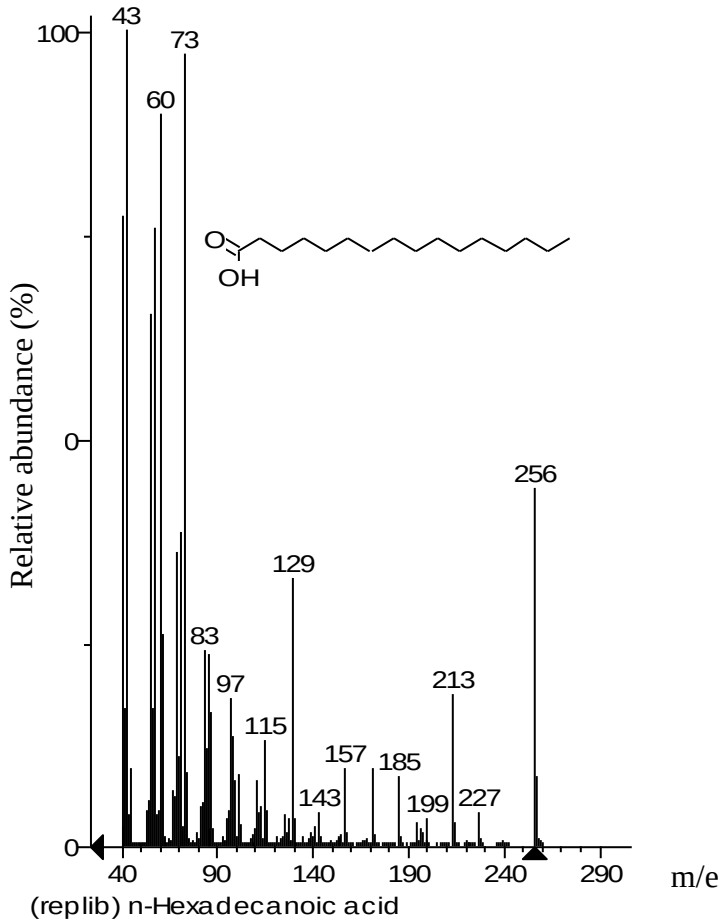


Figure 58: Mass spectrum of peak at R.T. 19.825 minutes.



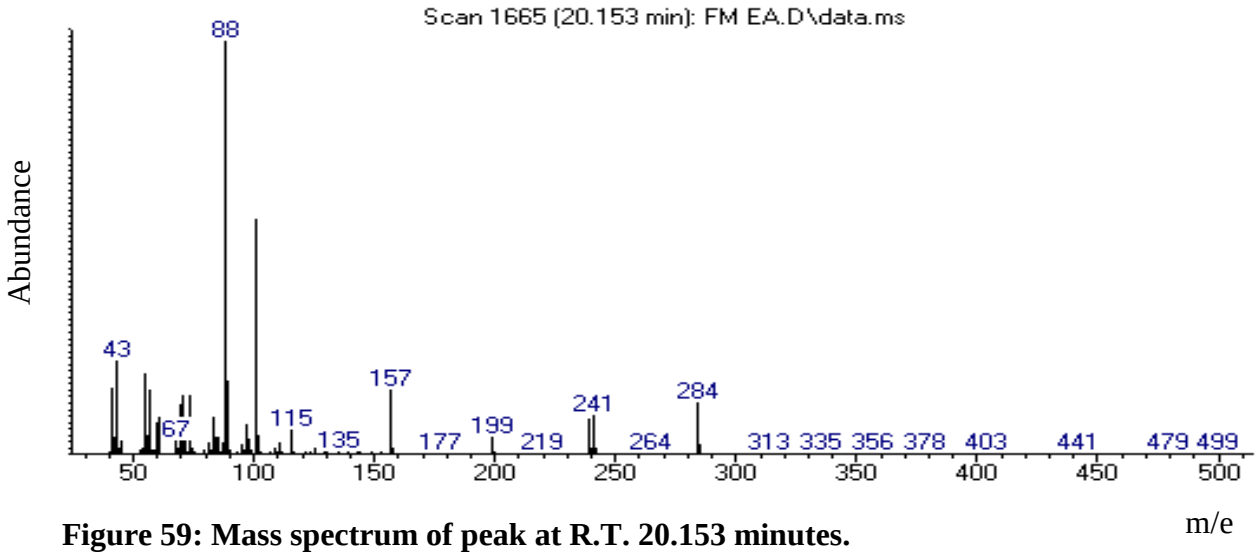
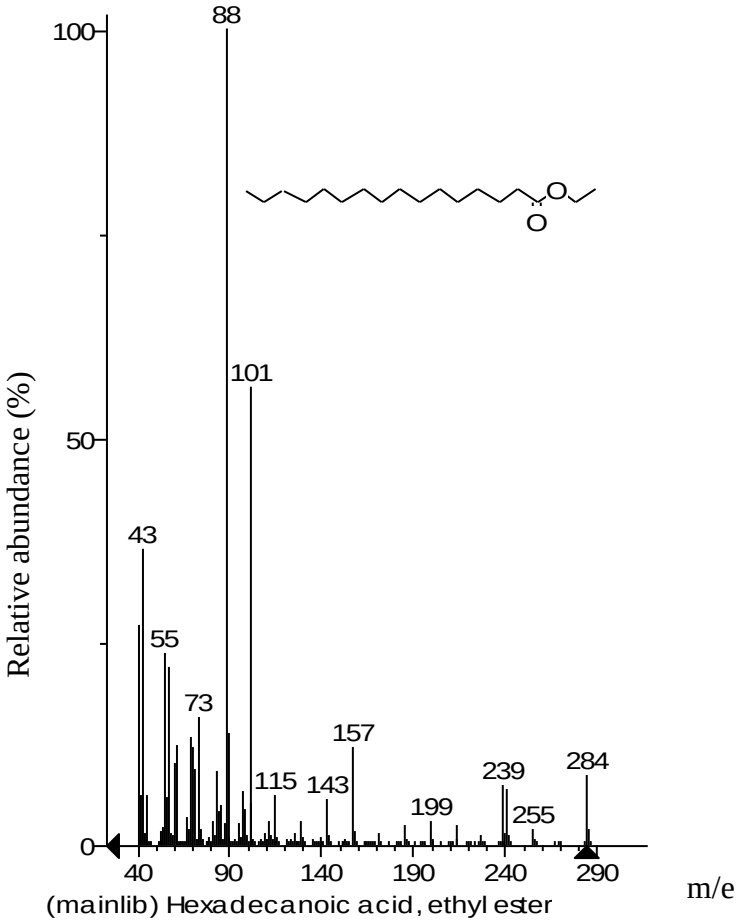


Figure 59: Mass spectrum of peak at R.T. 20.153 minutes.



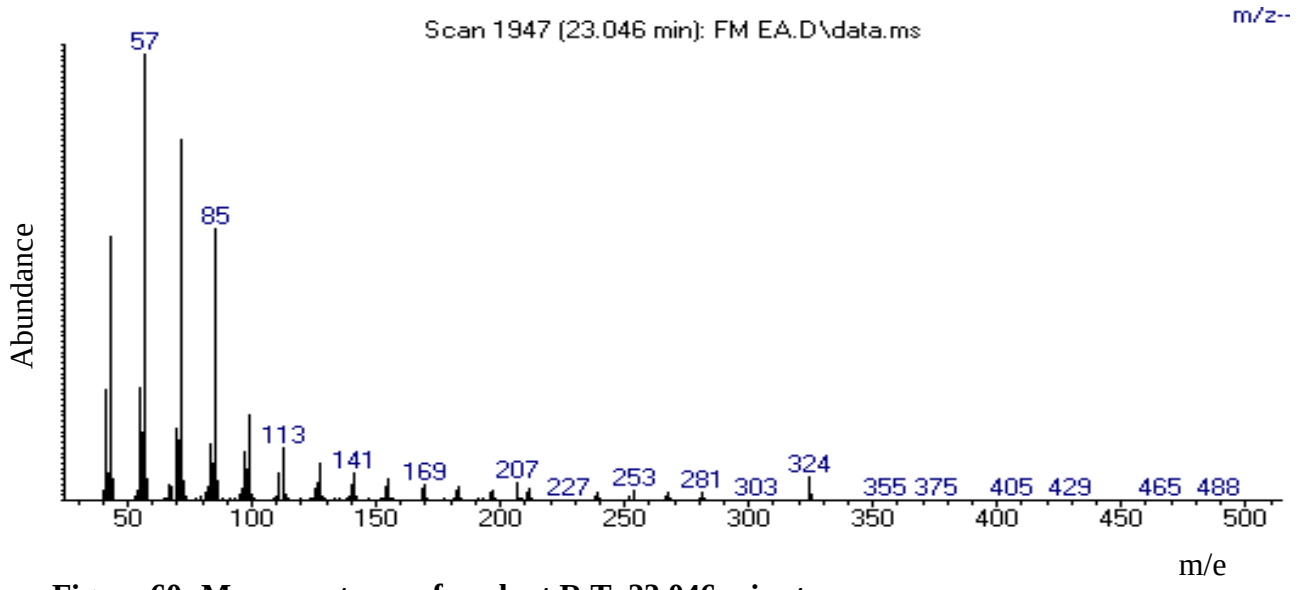
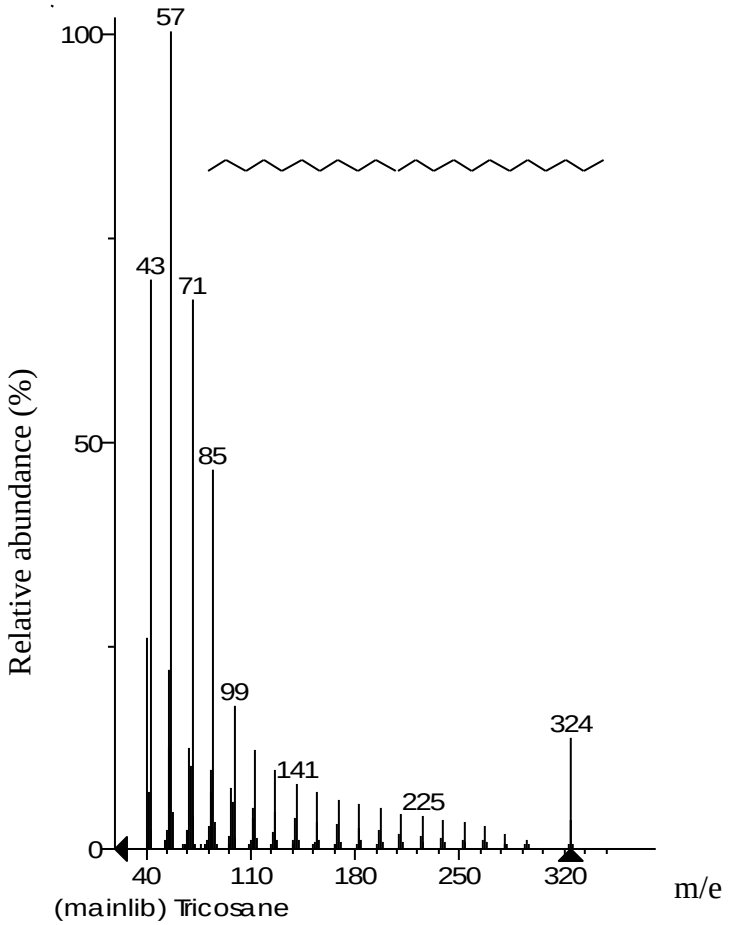


Figure 60: Mass spectrum of peak at R.T. 23.046 minutes.



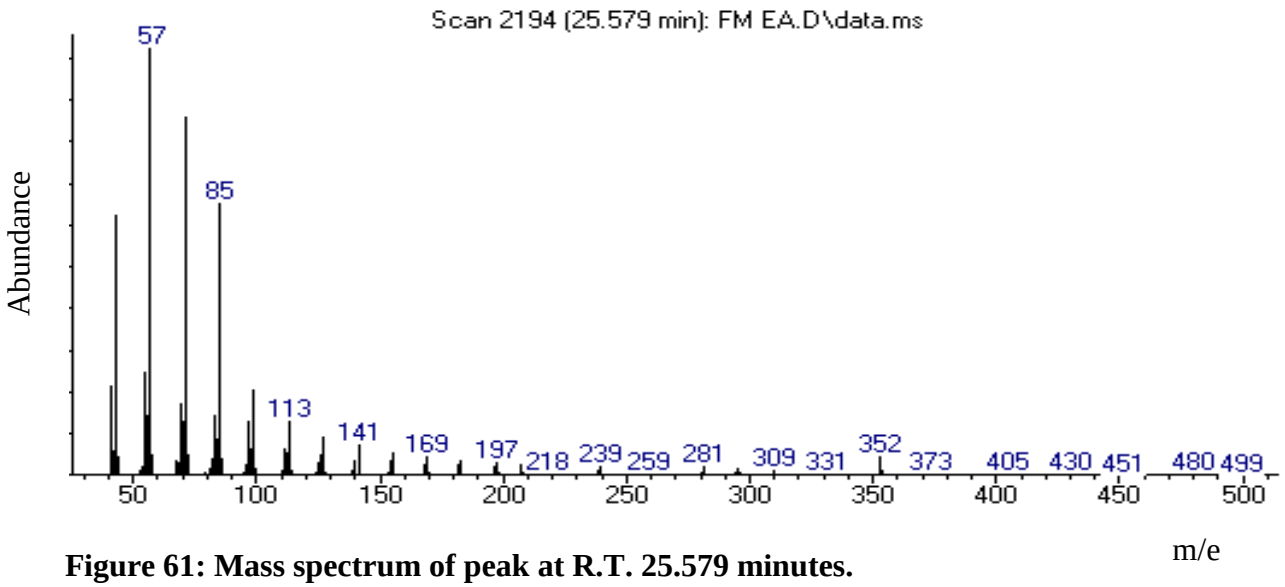
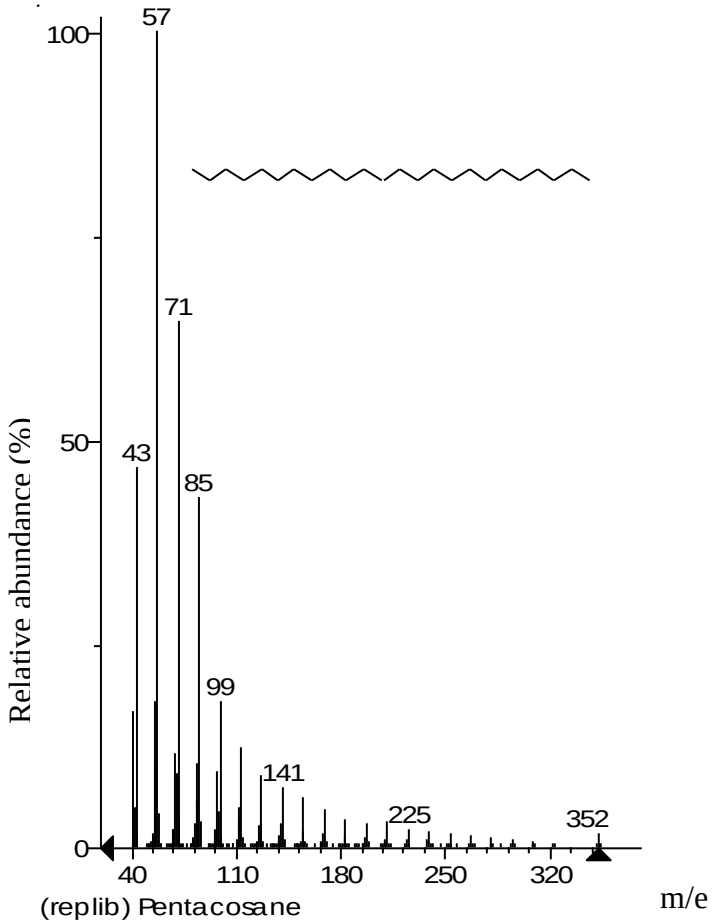


Figure 61: Mass spectrum of peak at R.T. 25.579 minutes.



APPENDIX VI: TLC OF ISOLATED COMPONENTS OF CHAMOMILE ESSENTIAL OIL.

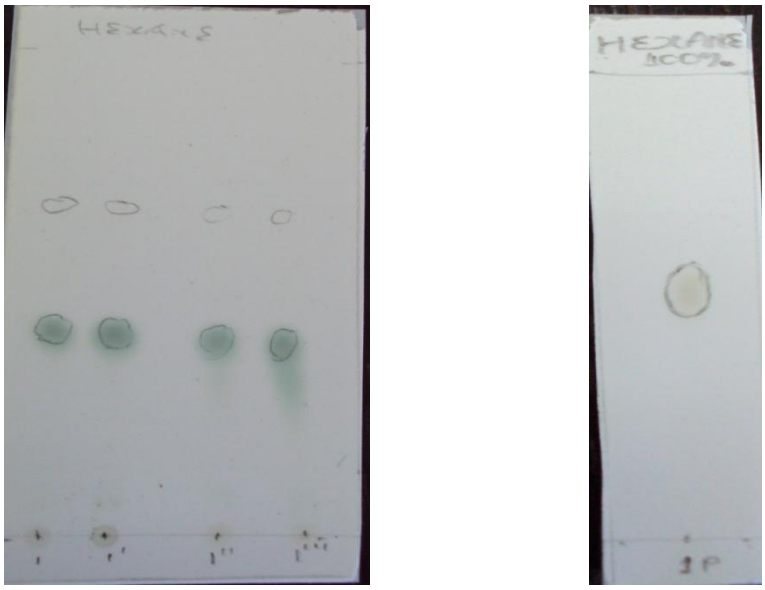


Figure 62: TLC of impure and purified compound 1 developed using hexane.

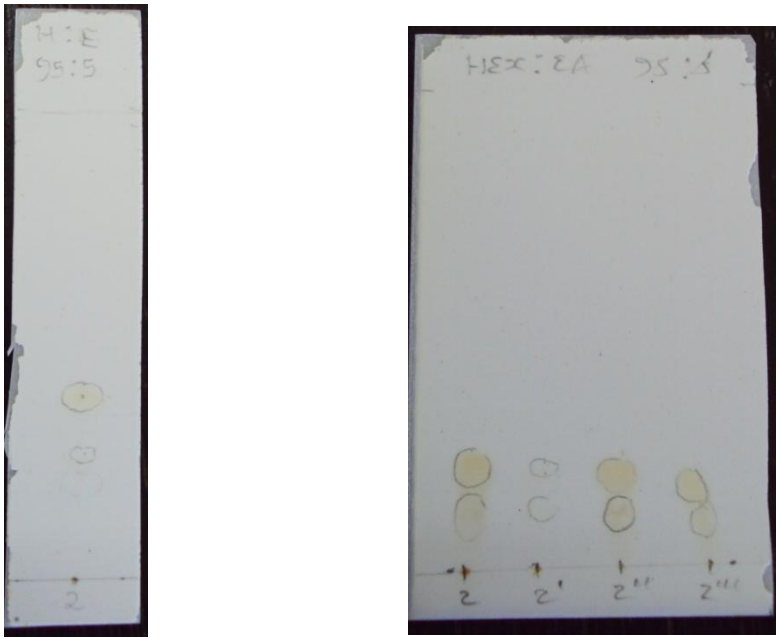


Figure 63: TLCs of compound 2, developed using hexane/ethyl acetate (95:5) mixture.

Figure 64: TLC of compound 4P developed using hexane/ethyl acetate (85:15) mixture.

APPENDIX VII: SOME APPARATUS AND INSTRUMENTATION.

Figure 65: The set up for Clevenger distillation.

Figure 66: Agilent Technologies 6890N Network Gas Chromatograph used in the GC-MS analysis.