Chemical profiling and determination of antioxidant and antibacterial properties of selected essential oils

MAMOKETE BETTY MOHALE

MASTER OF HEALTH SCIENCES: SOMATOLOGY

Department of Health Science

at the

Central University of Technology,
Free State

Supervisor: Prof. N.J. Malebo (Ph.D. Microbiology)
Co-supervisor: Prof. S.S. Mashele (Ph.D. Medicinal Chemistry)
Co-supervisor: Dr M.B. Komane (D-Tech Pharmaceutical Sciences)

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DECLARATION OF OWN WORK

I, **Mamokete Betty Mohale**, hereby declare that this research project submitted to the **Central University of Technology, Free State,** in fulfilment of the requirements for the degree of **MASTER OF HEALTH SCIENCES: SOMATOLOGY** is my own work and has not been submitted before to any institution by myself or any other person in fulfilment of the requirements for the attainment of any qualification.

.....	.....
Signature	Date

DEDICATIONS

The thesis is dedicated to the following individuals:

My daughter: **Tshimologo Thatohatsi Qhanya**

My parents: **Mr Maibi Mohale** and **Mrs Alice Mohale**

My partner: **Mr Lehlohonolo Benedict Qhanya**

My late grandmother: **Mrs Malefaso Mokoena**

My angel in hevean: **Kwandanise Junior Lebohang**

My angel in heaven: **Tsoarelo Rearabetse Mohale**

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LIST OF ABBREVIATIONS

ATCC:	American Type Culture Collection
B. cereus:	*Bacillus cereus*
BHA:	Butylated hydroxyanisole
BHT:	Butylated hydroxytoluene
°C:	Degree Celsius
CH:	Clevere Hydrodistillation
CO_2:	Carbon dioxide
CLP:	Classification, labelling, and packaging
DPPH:	2.2-diphenyl-1-picrylhydazyl
DMSO:	Dimethyl sulfoxide
***E. coli*:**	*Escherichia coli*
EOs:	Essential oils
GC-MS:	Gas chromatography mass spectrometry
IC_{50}:	Half-maximal inhibitory concentration
LPS:	Lipopolysaccharides
MAE:	Microwave assisted extraction
MAH:	Microwave assisted hydrodistillation
mg:	Milligrams
MHB:	Muller Hinton broth
MIC:	Minimum inhibitory concentration
ml:	Millilitre
mm:	Millimetre
OM:	Outer membrane
PH:	Potential hydrogen
PVC:	Polyvinyl chloride
REACH:	Registration, evaluation, authorization, and restriction of chemicals
ROS:	Reactive oxygen species
RNS:	Reactive nitrogen species
S. aureus:	*Staphylococcus aureus*
TBZ:	Thiabendazole

TBHQ:	Tert-butyl hydroquinone
µL:	Microlitre
m:	Micrometre
nm:	Nanometer

CHAPTER 1

BACKGROUND

1.1 Introduction

Aromatherapy is a form of therapeutic intervention that complements medical health care treatments and involves the use of essential oils to attain physical, mental, and spiritual well-being (Hedaoo & Chandura, 2019). It was introduced by a range of scientists from several nations (Ali *et al*., 2015). Essential oils (EOs) have been used for centuries, but the pharmaceutical, food, and cosmetic industries have recently paid increasing attention to their applications. These oils have various aromatic properties that can be extracted from various parts of plants, including their roots, barks, wood, flowers, seeds, resins, and stems (Battaglia, 2003; Ebadollahi *et al*., 2020). Steam distillation, maceration, expression, supercritical fluid extraction, enfleurage, and other procedures are used to extract these oils from plants (Sarkic & Stappen, 2018; Ebadollahi *et al*., 2020). Somatology, which is associated with the application of EO, is the study of holistic and other non- invasive treatments such as facial massage, aromatherapy, and other similar treatments (Richter & Jooste, 2013).

1.2 Aromatherapy and Somatology

EOs are widely used in the Somatology profession for a variety of treatments, of which the most common are massage and freshening treatments that are available in salons and spa environments. Topical applications using body creams, lotions, compressors, and bath water can all be used to administer EO (Shin *et al.,* 2017; Sarkic & Stappen, 2018). The effects of these oils can also be administered by means of inhalation equipment such as vaporisers, humidifiers, saunas (Sarkic & Stappen, 2018), steam rooms, and rasuls (Alharbi *et al*., 2021). Essential oils have a variety of effects and are used for their antioxidant, antimicrobial, anti-inflammatory, calming/relaxing, antiseptic, analgesic, and sudoferic properties (Adorjan & Buchbauer, 2010; Vella *et al*., 2020). Due to their wide applications, the chemical composition of each of these oils is of great importance for the determination of its bioactivity and for the quality control of its active ingredients.

1.3 Gas Chromatography Mass Spectrometry

The gas chromatography mass spectrometer (GC-MS) instrument is a common analytical tool that is used to determine the composition of EOs (Matulyte *et al*., 2019). This process not only discloses the ingredients these oils, but also reveals their unique molecular structures and varied bioactivities (Matulyte *et al*., 2019). The GC-MS is a useful tool for analysing volatile compounds and is well known for its ability to identify and quantify known and unknown compounds in EO. Gas chromatography is a widely used and highly sensitive detector that is capable of both quantitative and qualitative profiling of EO (Adebo *et al*., 2021).

Determining the composition of EO is important because the antioxidant, antibacterial, antifungal, carminative, and other properties that are inextricably tied to them are required in a variety of sectors for various reasons. For example, their antibacterial and antioxidant characteristics are essential in the field of somatopathology as they can help with the management and improvement of a variety of diseases and conditions such as hyperpigmentation, accelerated ageing, dehydration, acne, and other skin disorders. It is imperative that high-quality oils be used in treatments to address aesthetic and health concerns (Sarkic & Stappen, 2018). It is also crucial that, prior to using these oils, a patch test is performed to detect any skin irritancy and hypersensitivity response (Sarkic & Stappen, 2018). However, aesthetic disorders are not the only problem in the beauty industry, as disease transmission is a serious issue that should be considered and attended to (Alharbi *et al*., 2021).

1.4 The Antioxidant and Antibacterial Properties of Essential Oils

It has been scientifically demonstrated that essential oils are effective against common bacterial strains found in health care clinics. This means that the antibacterial properties of essential oils can be beneficial if used in health care clinics to prevent disease spreading. Therefore, since EOs are an integral part of the field of Somatology, it is imperative to assess their antioxidant and antimicrobial activities to advance treatments and prevent the spread of diseases. The aim of this study was to investigate components of EO and to determine their antioxidation and antimicrobial activities against *Staphylococcus aureus, Bacillus cereus,* and *Escherichia coli*.

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CHAPTER 2
LITERATURE REVIEW

2.1 Introduction

Aromatherapy is a complementary health therapy that uses essential oils extracted from various parts (roots, leaves, flowers, bark, stems, twigs) of aromatic plants. To extract the oil from an aromatic plant, a variety of extraction procedures are used. Plants are heat- and/or pH-sensitive and these factors are considered when their oils are extracted. Analytical techniques such as gas chromatography- mass spectrometry can be used to determine the composition and characteristics of EOs. Thus, chemical profiling and determination of the antioxidant and antibacterial activities of selected oils was the focus of the current research. Essential oils can be administered topically and through inhalation routes during various treatment regimens in both the Somatology and medical fields. The route of administration should be carefully chosen to maximise the quality and benefit of the oil used (Hedaoo & Chandukar, 2019).

2.2 History of Essential Oils

Aromatherapy is a treatment field that is used alone or as a complement to medical intervention. It involves the use of EOs to achieve physical, mental, and spiritual well-being (Hedaoo & Chandura, 2019). Aromatherapy was introduced when a group of scientists from a wide spectrum of health care fields combined their skills and knowledge to naturally improve people's well-being (Ali *et al*., 2015). Scientists from Greece, Egypt, China, France, and India contributed to the concept of aromatherapy and the application of EO in the cosmetics and medical sectors. The Greeks thought that their ancient gods possessed an understanding of perfume and fragrance (Hedaoo & Chandurkar, 2019), and a well-known Greek scientist invented myrrh fragranced perfume which introduced the concept of aromatherapy as a cosmetic treatment. In 1987, Rene-Maurice Gattafossie, a chemist, established the field of medical aromatherapy. During World War II, a French surgeon named Jean Valnet utilised and recorded the healing properties of various essential oils to treat soldiers' wounds (Hedaoo & Chandurkar, 2019). In fact, as far back as ancient times the Egyptians and Indians discovered and reported the mood-raising properties of EOs (Hedaoo & Chandukar, 2019).

2.3 Defining Essential Oils

An essential oil is a volatile, extremely aromatic essence that is produced from various parts of plants (leaves, seeds, fruits, wood, gum, twigs, roots, and berries) (Ebadollahi *et al*., 2020). All EOs are semi soluble in water and soluble in lipids and pure alcohol due to their hydrophobic properties. These oils can be degraded by light, heat, and moisture (Jugreet *et al*., 2020). Somatology benefits from the use of EO and so do the food and pharmaceutical industries (Herman *et al*., 2019).

In this study, various EOs were considered for investigation. *P. crispum* and *O. bacilicum* are generally used as culinary herbs and are therefore less frequently used in the cosmetics field. *Artemesia afra* and *P. crispum* oil contain harmful compounds (especially when used by pregnant women) as they encourage menstruation. They contain compounds such as thujone, myristicin, and thujene, but it was considered important to investigate their characteristics. *Lavendula angustofolia* and *M. chamomilla* are generally considered adulterated oils. They are popular in the aromatherapy field because of their calming and soothing properties. *Citrus aurantium* var. *bergamia* and *C. aurantium* var. *amara* are considered unstable as they contain limonene, which is easily oxidised. However, proper storage of these latter oils will improve their shelf life.

2.3.1 Neroli essential oil (*C. aurantium* var. *amara)*

Neroli EO, also known as *C. aurantium* var. *amara,* is known as sour orange and originates from the Rosaceae family. The genus is an umbrella of citrus fruits such as lemon, lime, grapefruit, mandarins, and citrons. *Citrus aurantium* var. *amara* is an evergreen tree that can grow to a height of five (5) meters (Maksoud *et al*., 2021). This popular tree bears white, scented flowers and originates from Syria, Eastern Africa, and the United States of America. It is also cultivated in countries such as Spain and Italy. *Citrus aurantium* var. *amara* is believed to be a good source of antioxidant properties due to its citric nature.

Maksoud *et al*. (2021) stated that *C. aurauntium* exhibits a potent radical scavenging activity. *Citrus aurantium* var. *amara* was investigated in this study for its antioxidant properties that could be of great importance in the treatment of skin ailments/conditions in the somatology industry. *Citrus aurantium* var. *amara* also possesses antibacterial activity against both Gram-positive and Gram-

negative bacterial strains (Li *et al*., 2019). The *Staphylococcus aureus* bacterium is mostly linked to skin infection, while *E. coli* is linked to gastrointestinal infection that could be contracted from public places such as toilets; hence, the oil could be used as a surface disinfectant.

There is an increasing demand for *Citrus aurantium* var. *amara* products due to the multitude of health benefits this plant offers, such as treatment of insomnia and anxiety (Suryawanshi, 2011; Bora *et al*., 2020). The neroli oil is non-irritant but should be avoided during pregnancy as it contains synephrine (Battaglia, 2003). Synephrine is a vasoconstrictor that is beneficial for the relaxation of bronchial smooth muscles and thus improves nasal drainage (Suryawanshi, 2011), but it has the risk of interfering with heart function.

Figure 2.1: Image of the *Citrus aurantium* var. *amara* (neroli) leaves and flowers
Source: Edris (2007)

2.3.2 Lavender essential oil *(L. angustofolia)*

The most commonly known species of lavender is *Lavendula officinalis.* Other species are *Lavendula augustifolia, L. latifolia,* and *L. stoechas* (Ali *et al*., 2015). Lavender is an aromatic evergreen sub-shrub with linear or lance-shaped leaves. The *Lavendula* genus has approximately 30 species that grow around the world (Fernández-Sestelo & Carrillo, 2020). The four main species of lavender are *L. augustifolia*, *L. latifolia* (a spike lavender), *L. x intermedia,* and *L. stoechas,* which is known as maritime lavender (Battaglia, 2003).

Lavendula angustofolia has antiviral properties and is capable of fighting viral infections, particularly when blended with other EOs (Ali *et al*., 2015). *Lavendula anustofolia* can be used as an antiseptic due to its antimicrobial properties and is thus beneficial for the treatment of wounds to enhance the healing process (Battaglia, 2003; Ali *et al*., 2015). The oil of this plant also has antidepressant properties and is therefore an excellent mood booster. In addition, Igarashi (2013) reports that this oil is considered non-toxic, non-sensitizing, and non-irritant and is thus safe for use in the cosmetic industry.

Lavendula angustofolia is also known for its analgesic properties and, due to its antimicrobial properties, its oil is used for pain alleviation and for disinfecting the scalp and skin. Its calming effect also makes it useful in managing nervous tension. Furthermore, the oil improves blood circulation, combats, and even prevents viral infections, elevates the mood, reduces high blood pressure, and aids in digestive problems (Henley, 2007). *Lavendula angustofolia* is commonly known for its sedative, relaxant, and carminative properties (Igarashi, 2013). It is safe and non-toxic. However, studies have reported that the oil possesses toxic properties due to one of its main components, namely linalyl acetate (Prashar *et al*.,2004). Linalyl acetate and linalool compounds in *L. angustofolia* oil reportedly have *in vitro* cytotoxic activity in human skin cells. In fact, it has been suggested that linalyl acetate is the most toxic compound in this oil (Prashar *et al.*, 2004). However, Igarashi (2013) opposes the foregoing view, arguing that *L. angustofolia* can be used by pregnant women because it contains linalyl acetate and linalool at low concentrations.

Figure 2.2: Image of the *Lavendula angustofolia* (Lavender) flower.
Source: Fascella *et al*., 2020

2.3.3 Bergamot essential oil (*Citrus aurantium* var*. bergamia*)

Bergamot oil is scientifically known as *Citrus aurantium* var*. bergamia*. It originates from the Rosaceae family, which is popularly known as the umbrella name for all citrus genera (Avila-Sosa *et al*., 2016). The Bergamot tree bears blossoms in the shape of smallish white flowers and bears light yellow fruits, commonly known as bitter oranges. *Citrus aurantium* var. *bergamia* is closely related to lime, sour orange (neroli), and grapefruit. It is cultivated primarily in Riggio Calabria in southern Italy (Valussi *et al*., 2021). The skin of the bergamot fruit is smooth, thin, tough, green, and yellowish when it is ripe. The bergamot fruit is not edible because the pulp is sour. The oil is extracted from the peels of nearly ripe fruit that are greenish in colour and have a rich, sweet and fruity smell. It has an oily, herbaceous, and balsamic body and is known for its richness in flavonoids. The colour of the oil changes when it becomes rancid. It is used primarily in the perfume and food industries and is also used to flavour juices and jams. *Citrus aurantium* var. *bergamia* oil is ideally extracted by cold pressing, steaming, and water distillation. The process must be well managed as the extractants

preferably, should not be subjected to oxygen or hydrolysis. High temperatures, the presence of water, and an acidic pH could also compromise the oil composition (Valussi *et al*., 2021). According to a study by Nabiha *et al.* (2010), the oil of *Citrus aurantium* var. *bergamia* contains linalool, linalyl acetate, and limonene compounds that contribute to its diverse properties. According to a study by Nabiha *et al.* (2010), the oil of *Citrus aurantium* var. *bergamia* contains linalool, linalyl acetate, and limonene compounds that contribute to its diverse properties. compound, linalyl acetate, is present in low concentrations, which makes the oil safe for use by humans. The oil sedates, stimulates the nervous system, and can be used to treat cystitis and wounds due to its antiseptic, anti-inflammatory, and antimicrobial properties (Navarra *et al.,* 2015). Bergapten, which is present in this oil, is phototoxic, therefore studies recommend avoiding exposure to the sun after the oil has been applied for cosmetic benefits (Battaglia, 2003).

Figure 2.3: Image of *Citrus aurantium* var. *bergamia* (Bergamot) leaves and fruits.
Source: Gioffre *et al.,* 2020

2.3.4 Sweet basil essential oil (*O. basilicum*)

Sweet basil is popularly known as *Ocimum bacillicum* and originates from the Labiate family. It is a green, leafy herb that is widely used in the food industry. *Ocimum bacillicum* generally grows in tropical regions in Asia, Africa, and South America (Simon *et al*., 1990). Steam distillation is the most common method used to extract oil from this plant. It has compounds such as linalool, methyl

chavicol, and eugenol that collectively have antimicrobial, antifungal, and antiseptic properties (Nurzyńska-Wiedak *et al*., 2012; Da Silva *et al*., 2020).

According to Simon *et al*. (1990), basil species that originate from different parts of the world differ slightly in composition. However, various authors have found the quality of the basil oil extracted from these various plants quite good (Simon, 1990; Calderón Bravo *et al*., 2021). This aspect is important for quality assurance, prevention of adulteration of the oil for cosmetic use is vital. Mkaddem Mounira *et al*., 2022, states that the quality of *Ocimum bacillicum* oil is generally influenced by geographical factors that may result in varying percentages of compounds present in it, and this could affect its efficacy for some applications.

The *Ocimum bacilicum* herb is widely cultivated due to its antioxidant, anti-inflammatory, antiviral, and antibacterial properties (Shahrajabian *et al*., 2020). In addition, the herb reduces fever and alleviates coughs, flu, asthma, bronchitis, influenza, and diarrhoea (Shahrajabian *et al.,* 2020). The properties of this oil are highly recommended in the field of Somatology where it can be used for various treatment regimens. The key products of this herb are basil seed oil and basil herb oil. Basil seed oil has not been widely investigated, but according to Calderón Bravo *et al*. (2021), the oil can be used to treat indigestion, diarrhoea, ulcers, kidney disorders, and sore throat.

Figure 2.4: Image of *Ocimum bacillicum* (Basil) leaves.
Source: Aflatooni *et al*., 2019

2.3.5 Blue Mountain sage essential oil (*S. stenophylla*)

Hamidpour *et al*. (2014) report that *Salvia stenophylla* is commonly known as blue mountain sage and belongs to the Labiatae / Loraaceae family. It has a large number of species as about 900 are found worldwide (Zaccardelli *et al*., 2020). It is mostly cultivated in African countries and oil is extracted from its dried leaves mainly by steam distillation (Zaccardelli *et al*., 2020). Due to the various aromatic compounds linked to this genus, *Salvia* species are recognised and appreciated for their bisabalool properties; therefore, these species are considered medicinally and cosmetically significant (Viljoen *et al*., 2006). The benefits of the oil include antimicrobial, anti-inflammatory, antioxidant, antiseptic, and antibacterial properties that are effective against tuberculosis, in particular. Additionally, it can be used to treat dermatitis, sores, ulcers, and night sweats (Fisher, 2006; Viljoen *et al*., 2006; Gono-Bwalya, 2003; Sienkiewicz, 2015).

The oil extracted from *S. stenophylla* is not commonly used in the beauty industry. The literature on this oil is sparse, and thus its use in the cosmetic industry is limited. In this study, the composition, and applications of *S. stenophylla* were investigated to expand the literature, as the oil has beneficial properties that can be of great significance to the aromatherapy and cosmetic industries for skin and body care regimens.

Figure 2.5: Image of *Salvia stenophylla* (Blue Mountain sage) ariel.
Source: Hamidpour *et al*., 2014

2.3.6 German chamomile essential oil (*M. chamomilla*)

The plant that produces chamomile oil is scientifically known as *Matricaria chamomilla* and belongs to the Asteraceae family, which is known as a treasure of flavonoids and a major source of blue oil (Singh *et al*., 2011; Srivastava *et al*., 2010). The herb is commonly cultivated in Germany and India but is also found in other European countries (Singh *et al*., 2011). *Matricaria chamomilla* grows in a hollow, bright gold cone-packed shape with disc-like florets that are ringed with about 15 white-lingulate florets (Srivastava *et al*., 2010).

The properties of the essential oil extracted from this plant are attributed to the presence of terpenoids and flavonoids. *Matricaria chamomilla* is effective in the management of ailments such as inflammation, insomnia, muscle spasm, hayfever, wounds, gastrointestinal disorders, haemorrhoids, and rheumatic pain (Singh *et al*., 2011; Srivastava *et al*., 2010). The composition of *Matricaria chamomilla* is not only composed of terpenoids and flavonoids, but also contains a large number of compounds such as coumarin, herniarin, and umbelliferon. Most studies investigated oil extracted from the florets, but Srivastava *et al.* (2010) investigated oils extracted from various parts of the plant. The presence of coumarin and umbelliferon in these oils was extensively investigated as these compounds amplify the presence of some beneficial properties that could be useful in the cosmetic industry (Molnar *et al*., 2017). Coumarins have antimicrobial properties, while umbelliferon has antioxidant properties that are believed to inhibit the proliferation of various human tumour cell lines (Molnar *et al*., 2017).

Antioxidant agents are often used in sunscreen as they absorb ultraviolet (UV) light at different wavelengths. Ultraviolet light is a concern and a challenge in the field of Somatology as the sun rays adversely contribute to various skin problems such as premature ageing and hyperpigmentation (Lobo *et al*., 2010). Thus, *Matricaria chamomilla* oil is considered for facial treatments and massages due to the properties ascribed to it. Facial products rich in antioxidants are manufactured for professional and home treatment regimens. Therefore, it was crucial to affirm the significance of chamomile oil by investigating its antioxidant properties.

Shankar *et al*. (2010) used supercritical fluid extraction for this oil, while other methods such as maceration, hydrodistillation, and soxhlet extraction have been explored. However, the latter three

extraction methods have limited application in this case as they operate with heat and could thus alter the composition of the oil.

Matricaria chamomilla is considered safe for children, as its toxicity level is very low *(*Singh *et al.,* 2011). Roman chamomile is a different type of chamomile, and German chamomile is considered slightly stronger compared to other species (Murti *et al*., 2012). The oil of the latter plant is used in food preparations and for medicinal and therapeutic purposes. For example, the *M. chamomilla* herb is used for pain, muscle spasm, hay fever, inflammation, rheumatic pain, haemorrhoids, peptic ulcers, and irritable bowel syndrome (Srivastava *et al.*, 2010). *Matricaria chamomilla* has spiro ether and bisabolol, which are compounds with antispasmodic properties known to ease tension, aching muscles, and ease the pain caused by menstruation (Srivastava *et al.*, 2010). Furthermore, these compounds have a relaxing effect on the smooth muscle lining of the digestive tract (Srivastava *et al.*, 2010).

The properties ascribed to essential oils differ among various plant species, and this information is significant for the field of Somatology. For example, somatologists and their clients often overlook the difference between seed and herb oils. *Chamaemumelum nobile* is an oil that can easily be confused with *Matricaria chamomilla*. These two oils are often mistaken for the same because the plants look the same and are reported under one *unani Babura* name in the literature. However, reputable studies of the two oils revealed a significant difference in their composition (Singh *et al*., 2011).

Figure 2.6: Image of *Matricaria chamomilla* (German chamomile) flowers.
Source: Singh *et al.*, 2011

2.3.7. Parsley essential oil (*P. crispum*)

Petroselinum crispum is commonly known as parsley. It is a plant of herbs that is grown in tropical and subtropical regions. It is a green leafy herb with serrated edges and is also known as rock celery (Daradkeh and Essa, 2016). The herb is widely used in the cosmetic, pharmacology, and food industries. *Petroselinum crispum* oil has been in use for a long time because of its beneficial properties. Essential oil extracted from this herb has antioxidant and antimicrobial properties that are considered beneficial for use in the cosmetic and food industries (Azevedo *et al.*, 2020). The composition of the oil extracted from *Petroselinum crispum* (parsley seed) is quite distinct and of great value to the cosmetic industry, where it is used to address allergic reactions and hypersensitivity.

Figure 2.7: Image of *Petroselinum crispum* (Parsely) leaves.
Source: Daradkeh and Essa, 2016

2.3.8 Wormwood essential oil (*A. afra*)

Artemisia afra is popularly known as African wormwood. It originates from the Comositetae family, which has 500 species (Liu *et al*., 2009). It is a popular Southern African medicinal plant, as it is known to treat ailments such as coughing, diabetes, colds, and malaria, to name a few (Liu *et al*., 2009). *Artemisia afra* is antiparasitic and therefore also alleviates gastrointestinal symptoms, anorexia, and indigestion (Nigam *et al*., 2019). The composition of the different species varies, which means that they possess slightly different properties (Nigam *et al*., 2019). For example, *A. absinthium* is used in alcohol beverages and dietary supplements (Nigam *et al*., 2019). Therefore, it is important for a therapist in the health and skin care industry to be knowledgeable about wormwood when performing a treatment.

This genus has gained attention because of the various properties of the different parts of its aromatic plants. For example, *Artemesia afra* gained popularity in the management of the corona virus during the global COVID-19 pandemic (Kozloff *et al*., 2020; Dandara *et al*., 2021; Orege *et al*., 2021). Some of the *Artemisia* derivatives (*A. annua*) are also known to be antimicrobial (Orege *et al*., 2021), and for this reason it is used as an ingredient in some antimicrobial drugs. Plants of the genus *A. afra* are generally used for the treatment of respiratory complications and diabetes (Liu *et al*., 2009; Dandara *et al*., 2021; Orege *et al*., 2021). For salon use, *A. afra* plants are used as herbal tea, a component in massage oils and creams, in steam baths, in the sauna and Jacuzzi, and in humidifiers (Patil &

Chandra, 2011). These methods also allow for the absorption of the oil through inhalation in cases where topical application is inappropriate due to skin allergies.

Somatologists are now aware of the properties of EOs, as they have been highlighted in various studies, but the different methods of administering these EOs still need to be explored in depth. For example, taking advantage of the antimicrobial properties of EOs will be beneficial in addressing the presence of microbes that abound in shared facilities such as toilets, as they can transfer infections from one individual to the other. It is also proposed that clients could receive leftover EO blend after treatment to continue using them at home for extended benefit.

Figure 2.8: Image of *Artemisia afra* (Wormwood) plant.
Source: Adebowale *et al*., 2020

2.4 Adulteration and Contaminants associated with EOs.

Adulteration is a process by which the composition of certain substances, including those in EOs, is altered and diluted with synthetics to maximise their quantity for increased profit (Choudhary *et al*., 2020). Some EOs lose their quality during the extraction process due to exposure to high temperatures and an extended extraction time (Abifarin *et al*.,2020). Therefore, not only do extraction methods contribute to the degradation of oil quality, but also adulterate the oil when the quantity is maximised (Ng *et al*., 2016). Adulteration has a positive impact on profit margins, but it poses a

serious health threat to consumers if inappropriate substances are added to increase the quantity. Moreover, alteration of oil composition can also occur during the extraction process and even during the preparation stage, such as when the most suitable drying method is selected (Thamkaew *et al.,* 2021). During the extraction process itself, some methods expose plant materials to high temperatures that contribute to the degradation of some constituents that carry certain properties (Abifarin *et al*., 2020).

Adulteration is a challenging issue in the field of Somatology because some adulterants are reportedly toxic and therefore have a negative impact on the endocrine system (Johnson & Boren, 2013). The most popular adulterants that are added to EO include benzyl alcohol, lavandin oil, and the compound of linalyl acetate (Ali *et al.*, 2015; Beale *et al*., 2017). Essential oils have been adequately demonstrated to lose their initial properties once foreign chemicals are introduced to them (Vargas-Jentzsch *et al*., 2019). Therefore, it becomes a threat when somatologists select oils for treatments due to their known properties but are unaware of the impact of the adulterants in them. Some contaminants include pimaric acid, abietic acid, and dipropylene glycol. Pimaric acid and abietic acid are not naturally found in oils but rather in resins. Another compound that is not naturally found in EO is dipropylene glycol, which is an adulterant used in plasticisers and polyester alkyd resins (Lawrence, 2002).

Adulterants are generally added to expensive and high-demand EO. The most common adulterated oils include lavender, chamomile, and frankincense. The EOs are mixed with these adulterants in a way that will not easily be detected by GC-MS scanning. Adulterations have thus continuously been a constant challenge in the Somatology industry because therapists are trained to use the oils rather than assess their quality; therefore, should a client react adversely to a treatment, it may be a challenge for the therapist to track the cause of the adverse reaction. Furthermore, the information regarding the addition of adulterants is not well documented; thus, somatologists will find it difficult to trace the cause of an adverse reaction of a client. Thus, more studies are crucial to expose and curb the inappropriate adulteration of EOs. Such findings should be documented and made accessible to the Somatology industry to ensure that therapists are aware of the negative impact of adulterants.

In addition, in-depth knowledge of the extraction methods used for EOs is generally not considered important in the cosmetic industry; hence the effect of the adulteration of these oils tends to 'slip through the cracks. This phenomenon has sparked the need for better scientific knowledge of the adulteration of these oils in the cosmetic industry. Eliyemmi *et al*. (2019) confirms that EO extraction methods play a significant role in altering their compositions. The latter author further demonstrated that oils that were extracted using microwave-assisted hydrodistillation (MAH) and clevere distillation (CH) showed a qualitative difference in some constituents. Furthermore, the latter study revealed that MAH yields oils that have a much lower quantity of monoterpene hydrocarbons and a higher level of oxygenated hydrocarbons than those obtained from CH. Confirming this information was beyond the scope of the current study but was used to confirm the importance of factors that play a role in composition profiling.

Essential oils are used due to the health benefits they have for humans, which means that they are not limited to the cosmetic industry but are also used in other industries such as preservatives in food and for medicinal purposes. Their application in the medical field is mainly due to their antibacterial properties; hence, they have gained so much attention that the demand has influenced manufacturers to add adulterants to these oils in order to make the products somewhat cheaper while also improving profit margins.

2.5 Essential Oil Extraction Methods

Different extraction methods are used to extract EO from parts of plants (Eliyemmi *et al*., 2019). These methods include steam/water distillation, expression, supercritical fluids, soxhlet, maceration, and pressurised liquid extraction (Rasul, 2018; Chemat *et al*., 2020). Generally, the advantages of conventional extraction methods (maceration, steam/water distillation, and expression) are minimized as they are time-consuming, and they are also exacerbated by the excessive use of solvents, high costs, and the fact that they yield limited amounts of oil (Rasul, 2018). Thorough preparation is required prior to the actual extraction process as some factors may affect the yield. The first important step is drying the sample using air, an oven, or microwaves. Factors that should be taken into consideration with these processes are temperature, contamination potential, sample size, and the state (fresh or dry) of the plant, as all these factors could influence the final composition of the extracted oil (Abd Aziz *et al*., 2021). Dried samples are preferred, as they have more flavonoids

compared to fresh plants. Additionally, dried plants interact optimally with solvent analytes (Azwanida, 2015). Fresh samples can, of course, be used, but they need to be used quickly (within about three hours of harvesting) to ensure inhibition of microbial contamination potential. Therefore, fresh samples are not recommended, while dry samples are preferred because this state minimises contamination. The particle size of the sample must not be smaller than 0.5 mm to ensure a complete interaction between the sample and the solvent analytes (Azwanida, 2015).

2.5.1 Distillation extraction system

A popular extraction method is steam distillation, which is commonly used for the extraction of EO from aromatic plants (Chemat & Boutekedjiret, 2015). During the extraction process, the selected plant samples are exposed to steam or heated water, as the vapour allows the oil to evaporate into a condenser (see Figure 2.9). This condenser has cold flowing water to cool the process, and ultimately the vapour released from the plant material is converted to a liquid state (Chemat & Boutekedjiret, 2015). Due to their different densities, water and oil will separate immediately after dipping into a receiver. This is the final stage where the oil will be collected and filtered for use (Chemat & Boutekedjiret, 2015). The challenges with this type of extraction are degradation and vapour pressure that may occur when the flow rate is too high (Akdag & Özturk, 2018).

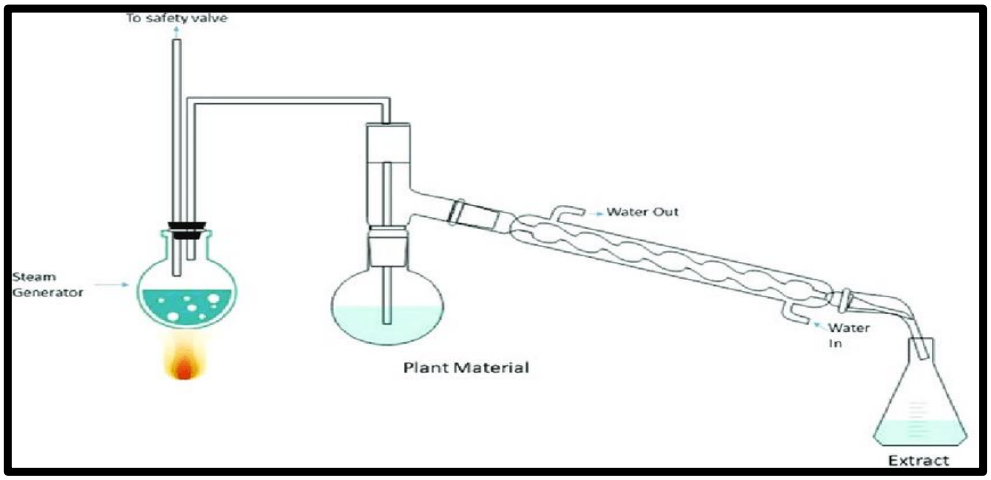

Figure 2.9: Illustration of a steam distillation system. The three (3) flasks serve various purposes. The first one presents a steam or vapour producer (filled with a limited quantity of water), while the second one represents the plant material and the last one, labelled 'extracts', is the collector.
Source: Silori *et al*., 2019

2.5.2 Soxhlet extraction

The distillation method to extract components from plant material has been used since ancient times, but methods such as soxhlet extraction (Abd Aziz *et al.,* 2021) have been developed recently as extraction technology has been advanced and modernised. The latter is an automatic extraction method that operates at high speed and temperature but can result in degradation of the EO (Zhang *et al.*, 2018). Soxhlet does the opposite of what occurs when microwave-assisted extraction is used. It is more time-consuming than MAE, requires the use of more solvent than MAE, does not favour thermo liable constituents, and leads to a decreased oil yield when compared to MAE (Azwanida, 2015; Akhtar *et al*., 2019; Chemat *et al.,* 2020; Abd Aziz *et al*., 2021). Figure 2.10 illustrates this process.

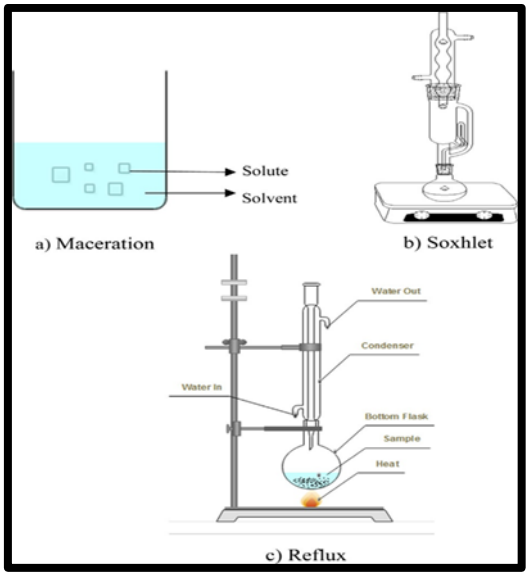

Figure 2.10: Illustration of Soxhlet extraction
Source: Abd Aziz *et al.,* 2021

2.5.3 Microwave-assisted extraction

Microwave-assisted extraction (Abd Aziz *et al*., 2021) involves the use of microwave energy to induce solvents to interact with a sample. It encourages analytes to be partitioned from the matrix for better interaction with the solvent (Eskilsson & Bjorkklund, 2020). The diagram shows an example of sandalwood plants soaked in a water flask to extract essential oil from sandalwood. The flask that is

positioned outside the microwave is responsible for the separation of wastewater or by-products while the EO (marked in yellow) floats on top.

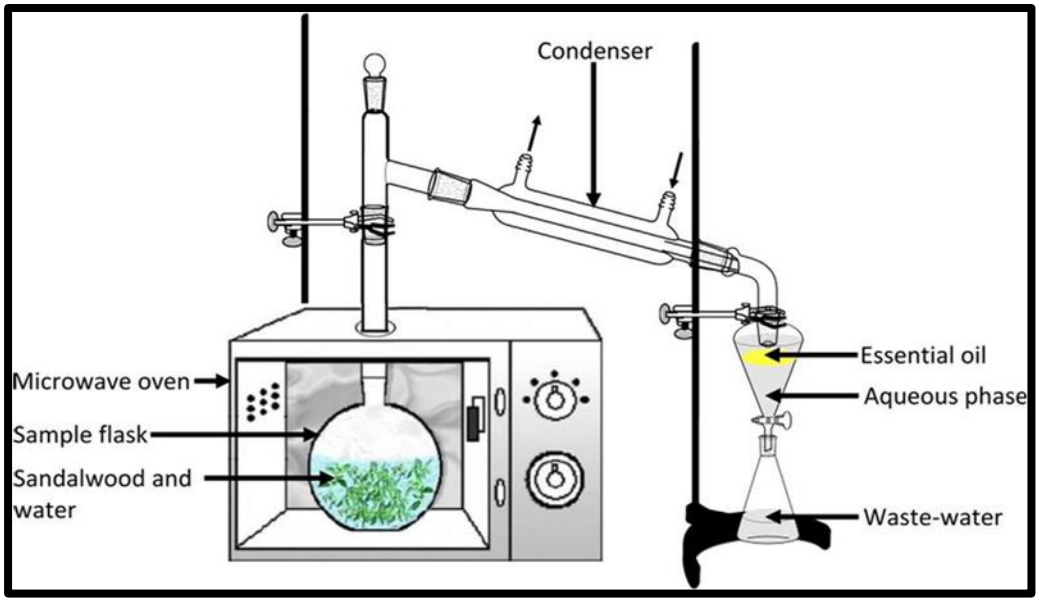

Figure 2.11: A figure outlining the microwave-assisted extraction process.
Source: Abd Aziz *et al*., 2021

2.5.4 Enfleurage extraction

The enfleurage method is generally performed when steam distillation is not possible due to samples that are sensitive to high temperatures. This method is not commonly used due to its prohibitive costs (Parab *et al*., 2020). Enfleurage is suitable for flower top material that is covered by a layer of fat in the extraction process (Parab *et al*., 2020). The layer will absorb after a certain period and then alcohol will be used to separate the essential oil from the fat. Essential oil can be collected once the alcohol has evaporated (Parab *et al*., 2020).

Figure 2.12: A figure outlining the enfleurage extraction method.
Source: Parab *et al*., 2020

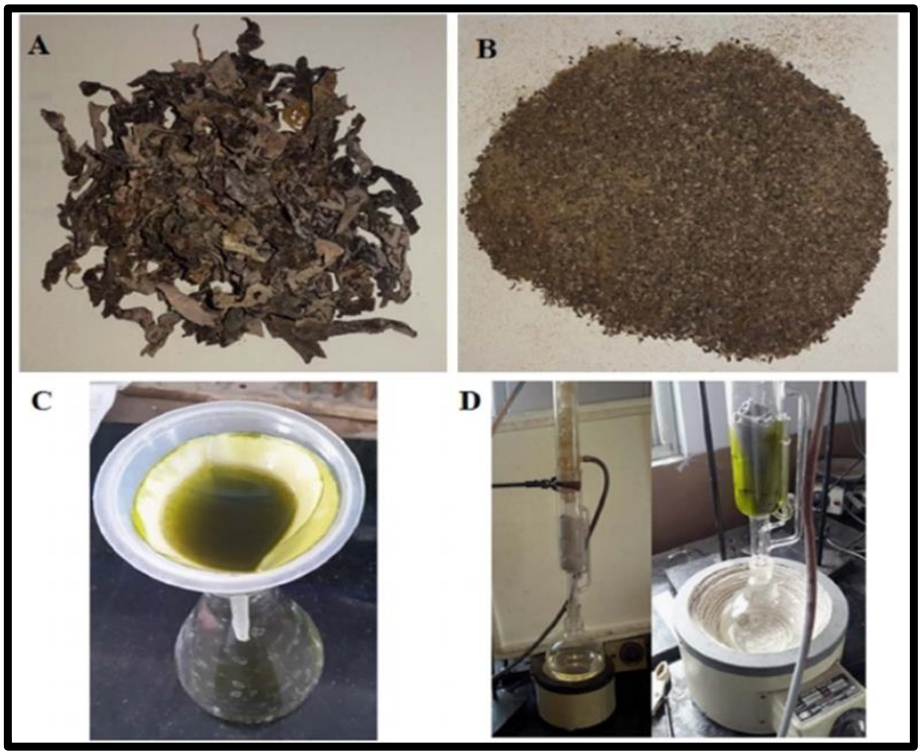

Figure 2.13: Images to illustrate the maceration and soxhlet extraction methods. **A: D**ried plant material being prepared for extraction. **B:** Ground/powdered sample that will be macerated with a suitable solvent to achieve extraction. **C:** Filtration process. **D:** Soxhlet extraction from a flask loaded with plant material.
Source: Venkatesan *et al*., 2017

2.5.5 Cold pressed extraction

Cold pressing works well with plants that originate from the citrus family (Rasul, 2018). The plant material from various parts is chopped and placed in a container filled with the chosen solvent (methanol or acetone). The mixture is normally left to brew for 3-7 days with frequent agitation or

shaking. When the soaking process is completed, the mixture will undergo a decantation phase (Rasul, 2018). The process becomes longer if water is chosen as solvent (Rasul, 2018).

Pressurised liquid extraction is also known as solvent extraction or accelerated solvent extraction. This process occurs at a high temperature with the intention of speeding it up by increasing the diffusivity of the selected solvent. The high pressure keeps the solvent in a liquid state without boiling it so that penetration into the matrix pore is improved (Rasul, 2018). Figure 2.14 illustrates the extraction of canola seeds that are mashed under pressure to release the canola oil.

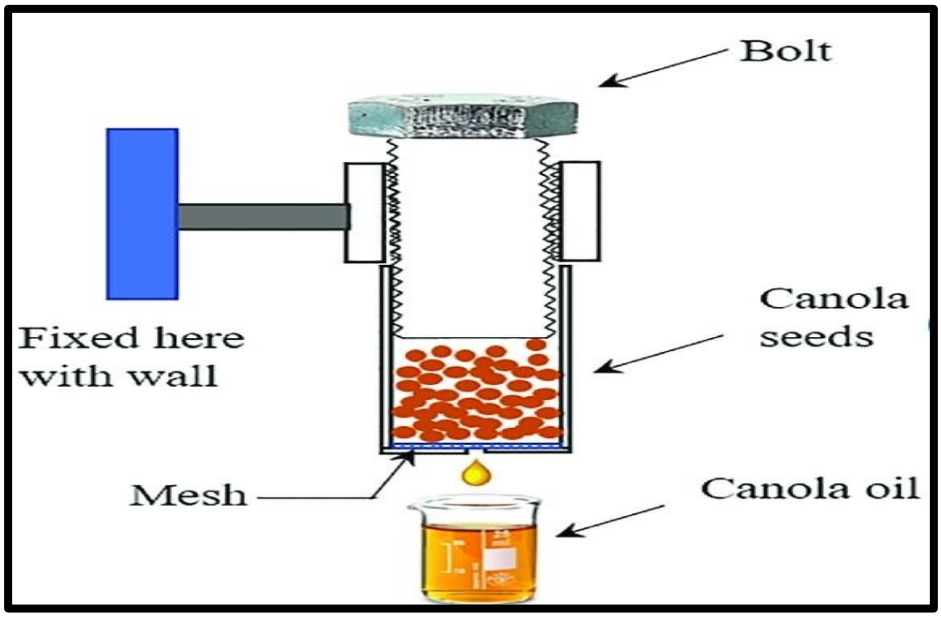

Figure 2.14: Illustration of the cold pressing extraction system
Source: Saleem & Ahmad, 2018

2.6 Applications and Mechanisms of EOs

There are various options to administer EO. They can be, for instance, administered topically or orally or inhaled. Researchers have various points of view regarding the topical and oral applications of essential oils. Some argue that they cannot be ingested orally (Battaglia, 2003) as they may have a detrimental effect on internal organs. However, Igarashi (2013) states that EO toxicity is determined by its chemical composition rather than concentration, and therefore EOs should not be consumed in excess of the recommended application prescribed by a medical professional. practitioner. Based

on the results of the second researcher, it seems necessary to conduct more extensive research to close the scientific data gap on the safety of EOs for oral consumption, particularly since they are commonly administered by health professionals other than medical doctors. Essential oils have been extensively researched for their qualities and properties, and they are used in a variety of ways for a variety of reasons, with inhalation being the most prevalent application approach after topical application (Bagttalia, 2003; Hedaoo & Chandurkar, 2019).

The focus of this study was on determining the chemical composition and antioxidant and antibacterial capabilities of selected EOs, but the purpose was also to emphasise the importance of selecting the best route of administration, which is always related to the goal of any treatment. It is noteworthy that the absorption of EOs can nowadays be facilitated by using newly developed nanotechnology. This means that essential oil nanoparticles are encapsulated for enhanced uptake (Koyama & Heinbockel, 2020).

Essential oils are currently used as a massage medium in the field of Somatology as they release a pleasant odour into the atmosphere. Their ability to serve as antioxidant agents has also been demonstrated in various studies (Carpena *et al*., 2021). In the cosmetics industry, the antioxidant properties ascribed to EOs are of great importance when performing facial treatments to reduce hyperpigmentation. (Alharbi *et al*., 2021). The most common application methods of these oils are discussed below.

2.6.1 Inhalation

Aćimović (2021) argues that the EO inhalation method is safe as it has not been reported to have had any negative health effects in humans. One procedure is inhalation of EO vapours directly from a receptacle. This approach seems by far the safest; however, clients who are prone to allergies should avoid using this technique, as recommended by Matsumuto *et al.* (2013). The inhalation of an EO from a receptacle is not the only option available in the field of Somatology. The use of saunas and/or steam rooms is also an option of inhalation technique to experience the optimal benefits of various aromas of EO (Vella *et al*., 2020). A sauna is an enclosed room with a high temperature and high humidity while dry heat is released from the coals when water is poured on them. The water used to cool the coals can be infused with essential oils. The main difference between a steam room

and a sauna is that, in a steam room, oils can be added to the water for wet steam inhalation (Vella *et al*., 2020; Alharbi *et al*., 2021).

Aromatherapy is one of the most effective natural remedies for boosting immunity and has been used to treat a variety of illnesses (Ali *et al*., 2015). Aromatherapy is referred to as a 'complementary' health therapy and has therefore gained momentum in the medical sector due to its ability to heal various ailments when used in conjunction with medications. Furthermore, bacteria, fungi, and viruses can be treated with the application of EOs that complement the prescribed medication (Hamid *et al*., 2011; Ali *et al*., 2015). Aromatherapy is the therapeutic use of volatiles or scents to prevent or treat disorders through the inhalation technique (Hamid *et al*., 2011). Essential Oils are used in such treatments as they have beneficial effects when applied through aromatherapy massage, which is known to improve physical and mental well-being. When aromatherapy is used in conjunction with prescribed medications, it often helps combat chronic diseases, as well as viral and bacterial infections (Huang, 2017). This treatment helps relieve tension, anxiety and even despair (Huang, 2017). Aromatherapy is administered in a variety of ways in the Somatology profession, including massaging. Other methods include ingesting oils orally; however, the oral ingestion technique remains questionable as a safe remedy for human use. Currently, the direct inhalation technique from a receptacle is considered the most effective procedure, but techniques such as compressions and steam room or sauna inhalation are also widely used methods (Johnson & Boren, 2013; Tisserand & Young 2013; Farrar & Farrar, 2020).

Additional benefits associated with the inhalation of EO vapours include pain relief, detoxification, unclogging pores, improving blood circulation, and muscle relaxation before or after visiting a gym or doing physical exercise (Farrar & Farrar, 2020). If a person's health prevents them from using a sauna or steam room, a vaporiser is a good alternative as it allows easy and effective inhalation (Sarkic & Stappen, 2018). A diffuser is a device that converts a substance into vapour, and EO can be added to a diffuser for inhalation benefits (Farrar & Farrar, 2020). The olfactory system encompasses the nasal cavity that connects to the olfactory bulb, which is associated with the transmission of aroma signals because the olfactory system is the closest to the brain. Some chemical compounds can pass directly through the axon of sensory nerve cells to the central nervous system (CNS), triggering an emotional reaction that affects the person's mood. This method should

be considered when the intention of the treatment is to relax, calm, soothe and sedate the client using EO properties that are related to emotions and mood (Koyama & Heinbockel, 2020).

Inhalation (mostly when inhaling EO vapour straight from the container) affects the respiratory system, but the pathways involved are not the same as those of the olfactory system. The olfactory system bundles all the routes involved in the inhalation method, while the respiratory system is concerned with gas exchange, which implies that diffusion can be considered because it is directly linked to vapour. Vapour molecules dissolve in the respiratory system's epithelium and get absorbed into the bloodstream (Koyama & Heinbockel, 2020).

Sufficient scientific evidence has demonstrated that the effectiveness of EOs depends on their composition and application mechanisms that enhance absorption. For example, inhaling vapour from *L. angustofolia*, *C. aurantium* var. *bergamia,* and *M. chamommila* oils are known to have a relaxing, calming, antidepressant and analgesic effect, which means that the technique supersedes the topical massage technique. All of the above properties were observed in *in vivo* studies conducted by various authors (Koyama & Heinbockel, 2020). In the latter study, anxiolytic properties were confirmed using the inhalation technique because the oils showed a reduction in salivary cortisol and the participants showed reduced alertness toward an activity when performing certain tasks, suggesting that the effect of the oils induced calmness.

2.6.2 Topical application of Essential Oils

Another application of essential oils is the topical procedure. They can be added to bath water, used in scalp treatments, included in body cream/oil products and compressors, and in massage creams/oils (Barcan, 2014; Vella *et al*., 2020). Essential Oils can also be used for aromatherapy facials using cold or warm compressors that are effective in reducing inflammation and body aches and pains (Shin *et al.*, 2017; Vella *et al*., 2020). The antibacterial characteristics of EO can also help with skin infections, while their antioxidant capabilities can help prevent premature ageing, skin dryness, and hyperpigmentation (Huang *et al*., 2017). Essential oils have been used topically by somatologists for many years, whereas other route of application have been avoided due to safety concerns (Battaglia, 2003). In addition, the inhalation technique is overlooked in the cosmetics sector, and this has left a huge gap in this industry. The topical application of EO is directly related to the

skin, which is the largest organ of the body, as it covers the entire body externally (Abdo *et al*.,2020). The epidermis, dermis, and hypodermis are the three layers that make up the structure of the skin (Lawton, 2019 & 2020; Abdo *et al*., 2020). All these layers have diverse purposes, such as protecting cells (keratinocytes, melanocytes, Langerhans cells and Merkel cells), while the dermis and hypodermis are much deeper layers that contain hair follicles, blood vessels, sensory neurones, collagen fibres, sweat glands and are attached to muscles (Lawton, 2019 & 2020).

The skin's functions include protecting the body from microorganism invasion, acting as a barrier against the sun's harmful effects, and acting as a link between small proline-rich proteins and larger proteins. The plasma membrane also has a hydrophobic layer on the outer surface that allows it to be receptive to substances such as those derived from EOs. The function of the plasma membrane is to control and maintain homeostasis and regulate sensory, endocrine, and exocrine systems, as well as body temperature (Lawton, 2019; 2020). Intrinsic and extrinsic factors that influence skin health include the environment, age, medicine, sun exposure, hormones, food ingested, and skin products used (Levakov *et al*., 2012; Ponte *et al*., 2012; Lawton, 2019 & 2020).

Collagen and elastin in the skin deteriorate as we age, and once they lose flexibility, the skin sags and begins to exhibit outward signs of ageing. Wrinkles, dryness due to dehydration, sagginess, and a leathery texture are all indicators of the ageing process (Levakov *et al*., 2012; Ponte *et al*., 2012; Mohiuddin, 2019). In addition, the sebaceous glands become less active as people age. As a result, the sebum distribution changes. Ageing skin can be caused by old age and/or excessive sun exposure that induces photo and premature ageing (Levakov *et al*., 2012; Ponte *et al*., 2012; Mohiuddin, 2019).

Pigmentation, which can range from hypo- to hyperpigmentation, is another challenge that affects the appearance and health of the skin. Hypopigmentation is caused by a lack of production of melanin pigments at specific locations on the skin (Prćić *et al*., 2011; Bergqvist & Ezzedine, 2020). An example is vitiligo (Davis & Callender, 2010, Goswami & Sharma, 2020), which is not a common phenomenon (Prćić *et al*., 2011; Bergqvist & Ezzedine, 2020). Hyperpigmentation is characterised by an excess of melanin in certain areas of the skin (Davis & Callender, 2010; Goswami & Sharma, 2020). Examples of such inflictions are chloasma, melasma, and lentigines (Plensdorf *et al.,* 2017). Due to illnesses, poor nutrition, reaction to medications, excessive chemical exposure, or even too

much sun exposure, hyperpigmentation has remained an issue for majority of individuals and accelerates the onset of premature ageing of the skin.

In most cases, antioxidants help in the management of hyperpigmentation, dryness, skin ageing, and a variety of other skin disorders through their free radical attack property that prevents a chain reaction of reactive oxygen and nitrogen species (ROS) and nitrogen reactive species (ROS) (Azevedo *et al*., 2020). Antioxidants act as a reservoir that balances or neutralises free radicals (Carpena *et al*., 2021). Food, cosmetics, plants, and EO contain antioxidants. Essential Oils that are commonly used as preservatives in the food industry can also be incorporated in facial treatments that target indications of ageing, pigmentation, and other skin disorders (Azevedo *et al*., 2020; Fernandes *et al*., 2020).

2.6.3 Dilution ratios of Essential Oils

Numerous EO application techniques have been proposed, but dilution regimens should always be followed to ensure safe and successful treatment. According to Lane (2019), who is a certified aromatherapist, different concentrations or ratios are used at different stages of treatment depending on the client's skin condition, health, and age. A dilution chart is used which recommends a safe dilution ratio for infants and children under the age of 2, ranging between 0.25-0.5%. Additionally, 1% is the recommended dose for children older than 12 years, pregnant women, individuals with sensitivity, and the elderly. Furthermore, a 3-10% dose is preferred for short-term use, while 2- 2.5% is recommended for topical use such as massages and other topical applications (Lane, 2019). **Figure 2.15** below shows different concentrations of EO on a dilution chart.

1 oz (30 ml) of Carrier Oil

1 oz = 6 teaspoons or 2 tablespoons

0.5% Dilution: 3 drops total

1% Dilution: 6 drops total

2% Dilution: 12 drops total

3% Dilution: 18 drops total

4% Dilution: 24 drops total

5% Dilution: 30 drops total

Figure 2.15: Dilution chart for EOs
Source: Lane, 2019 [Loving Essential Oils]

2.6.4 Essential oil compounds and their effects

Essential oils contain a variety of compounds that have beneficial effects on human health (Matulyte *et al*., 2019). It should be noted that environmental factors such as climate, origin, seasonal changes, harvesting time, extraction method, and even adulteration can affect the composition of EOs (Fejér *et al.*, 2018). In addition, various factors such as the potency of the components present in the oil (Fung *et al*., 2021) and the mode of administration determine the effectiveness of EO. An optimal combination of EO compounds that are responsible for the delivery of specific qualities can be used to fully exploit the properties of these oils, such as anxiolytic and antidepressant effects (Koyama & Heinbockel, 2020).

Most oils contain compounds such as linalool, pinene, and limonene that have been shown to have anxiolytic and antidepressant qualities as they interact with neurotransmitter pathways such as gamma aminobutyric acid (GABA) receptors and transient receptor potential (TRP) channels (Koyama & Heinbockel, 2020). Research has shown that the best technique for administering is inhalation, which is associated with the desired characteristics of these oils (Hedaoo & Chandurkar, 2019; Koyama & Heinbockel, 2020).

Lavendula angustofolia is an example of an oil that has the aforementioned compounds (Koyama & Heinbockel, 2020; Hedaoo & Chandurkar, 2019). *Lavendula angustofolia* is reported to have antiseptic, antibacterial, culminative, and sedative properties. A major goal in achieving effective sedative and soothing effects is to reduce high blood pressure and a high rate of respiration during panic or distress episodes. EO sedates the body by interacting with the hypothalamus pituitary adrenal axis (HPA) to lower cortisol levels in the blood. Scented molecules are usually the first to

reach olfactory receptors, while chemicals absorbed by breathing reach the brain considerably faster than through the skin (Koyama & Heinbockel, 2020).

2.7 Microbial Contamination in the Salon Environment

Bacteria are an integral part of the human body but are also linked to various illnesses (Krüger *et al*., 2019). Microbes are associated with a variety of disorders, some of which are highly contagious (Li *et al*., 2019). For example, *Staphylococcus aureus* is found naturally on the skin and easily spreads from person to person. It is also present in the nasopharynx (Berger *et al*., 2018). *Escherichia coli* is found in the gastrointestinal tracts of humans and other warm-blooded mammals and is easily contracted due to infested surfaces. The natural habitat of *Bacillus cereus* is soil, but its spores can be widely isolated from food, plants, and invertebrates (Bağcıoğlu *et al*., 2019; Denamur *et al*., 2021).

Staphylococcus aureus, a human pathogen that causes skin infections, is an organism that has been extensively studied (Tong *et al*., 2015). It poses the risk of cross contamination in the Somatology industry, as it occurs naturally on the skin. So does *Escherichia coli*, which lives in the intestines and causes urinary tract infections (Denamur *et al*., 2021). In addition, *Bacillus aureus* is a bacterium that can cause diarrhoea, vomiting, and nausea. It is found mainly in soil and in vegetables and is responsible for eye and respiratory tract infections.

All the organisms mentioned above have an impact on the beauty business, as they can cause skin and other infections that can adversely affect the outcome of a therapist's treatment regimen. Unhygienic surfaces on the workstation could result in cross contamination, and thus carry the risk of infection transfer. *Bacillus cereus* and *E. coli* are both dangerous bacteria that can spread from one person to another through shared facilities. These organisms pose the risk of cross-contamination between workers and clients in the Somatology industry due to contaminated surfaces (Alharbi *et al*., 2021). This is a critical problem that puts clients at risk of catching diseases from a spa or shared facilities, and this emphasises the importance of comprehensive spa cleaning.

Bacteria such as *E. coli*, *S. aureus*, and *B. cereus* easily spread in spas, resorts, hotels, and beauty salons as these facilities are generally shared. This suggests that *E. coli* bacteria can lurk not only in hospitals, but also in public restrooms, saunas, steam rooms, sun beds, bathrooms, and swimming

pools (Miller & Diep, 2008; Dalman *et al*., 2019; Abney *et al*., 2021). These organisms can proliferate in leisure areas and beauty salons and pose a major threat to clients and employees. Beauty salons are often not tested for the presence of pathogens or the danger of transmitting them. Clients share facilities such as jacuzzis, rasuls, saunas, steam rooms, and lockers, and this alone can contribute to cross-contamination. The sites indicated above can be considered hotspots for infection transmission between clients or between clients and therapists. Essential oils can be used to prevent the spread of diseases/infections and can also be used in vaporisers, humidifiers, and diffusers to reduce the presence of organisms in the air and on the interior surfaces of a building (Alharbi *et al*., 2021; Farrar & Farrar, 2020). Although it has not been proven that this option reduces microbes in the air, Madsen (2018) argues that microorganisms such as *S. aureus* can be found in indoor air, so exploring the application of EOs is pertinent as they can be beneficial in implementing preventive methods in the beauty industry.

Essential oils are hydrophobic and can easily penetrate the cells of Gram-positive bacteria, but not those of Gram-negative bacteria (Man *et al*., 2019). Gram-negative bacteria have significantly thicker peptidoglycan than Gram-positive bacteria and, due to their complicated cell structure, they are more resistant to most essential oils than their Gram-negative counterparts (Lopez-Romeo *et al*., 2015). Gram-negative bacteria have a peptidoglycan of 2-3 nm and 20% dry cell content (Berger *et al*., 2018). Gram-positive bacteria have cell walls that allow hydrophilic molecules, such as those of Eos, to flow through and reach the cytoplasm. This is due to the presence of the phenolic compound in the EOs, which enables them to exert antibacterial activity on Gram-positive bacteria by triggering cell death (Man *et al*., 2019).

2.8 Rationale for the Study

The potential proliferation of harmful bacteria in the beauty industry has emerged as a major concern, with beauty salons particularly vulnerable due to the high volume of consumers who visit health and skin care clinics and receive their treatment in the same workstation environment as other clients. Somatologists have limited access to documented research on harmful microorganisms and their impact on the human body. These therapists are mainly performing tactile therapy, and many seem unaware of the impact of microorganisms that are directly linked to the majority of skin conditions contracted unknowingly by their clients. This presents a challenge when it comes to treating or

correcting different skin disorders and conditions. The issue of contaminated surfaces that result in skin and other infections linked to microorganisms is an overlooked topic in Somatology and the larger beauty industry. These concerns have not been explicitly expressed enough to spark interest and attract the attention of researchers and scientists. Skin disorders (particularly hyperpigmentation) that require treatment are routinely addressed in the beauty industry using facial products from a variety of companies, and not all products contain EO components. Hyperpigmentation is a skin problem that is a challenge for both clients and somatologists due to the high cost of most skin lightening treatments. Even home regimens that are regarded as successful are very expensive. Conversely, EOs are cost effective and have antioxidant properties that could be used to manage hyperpigmentation.

As adulteration in the production of EO products has become quite common, it is vital to consider the composition of EO and the impact of the adulterants that are added to these products for use in the beauty industry. Another reason to examine the chemical composition of EOs is that the findings may simplify EO selection, as the applicability of these oils to specific treatments may be linked to their compounds.

The objective of the study was to highlight the importance of the use of EOs or their compounds in the cosmetics industry for the treatment of a variety of skin disorders. Data on the properties of EOs have been scientifically obtained and are well documented. There is strong evidence that EOs have antibacterial and antioxidant properties that may be useful to the cosmetic industry for the treatment of various skin conditions and to reduce the presence of surface bacteria in the salon environment. It is difficult to identify areas of contamination if therapists and somatologists do not have sufficient information on the nature of or threats posed by organisms that could contaminate treatment products and surfaces that are publicly used. Cross contamination between clients and therapists, or even among clients, is a reality, as therapists work with clients on a daily basis in the same environment.

There is a wealth of literature on the use of EOs and their properties in the field of Somatology, but there is a dearth of scientific information that will inform somatologists about the risks associated with microorganisms and cross-contamination in the salon environment. More specifically, indigenous knowledge about the use of EOs to South Africa (*S. stenophylla* and *A. afra*) is sparse and topics based on Somatology and the use of EOs extracted from plants grown in South Africa have rarely

been researched. Research has evaluated the quality of EOs, but few studies have tested their efficacy in treating skin ailments and reducing surface contamination in the salon environment.

2.9. Aim of the Study

The goal of this study was to use gas chromatography–mass spectrometry (GC-MS) to establish the chemical compositions of various essential oils and to assess their antibacterial and antioxidant properties.

2.10 Objectives of the Study

The key objectives were to:

- Assess the chemical composition of the selected EOs.
- Assess the antioxidant activity of the selected EOs; and
- Assess antibacterial activity of the selected EOs.

2.11 Structure of the Thesis

This thesis consists of six chapters. Apart from Chapter 1, Chapter 2, and Chapter 6 that are the introduction, literature review, and closing chapters respectively, each chapter consists of an abstract, introduction, literature review, materials and methods, results, discussion, and conclusion section.

The chapters are structured as follows:

Chapter 1: Introduction

Chapter 2: Literature review

Chapter 3: Chemical profiling of selected essential oils using GC-MS

Chapter 4: Assessing the antioxidant activity of each of the selected oils against DPPH free radical.

Chapter 5: Assessing the antibacterial activity of each of the selected oils against *S. aureus*, *B. cereus,* and *E. coli.*

Chapter 6: Recommendations and closing remarks.

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CHAPTER 3

CHEMICAL PROFILING OF SELECTED ESSENTIAL OILS

3.1 Abstract

Aromatherapy is a complementary therapy that is popularly known as massage treatment in the field of Somatology. Its holistic effects on emotional and physical well-being have gained interest in the cosmeceutical industry. Various studies have been conducted to search for advanced novel compounds with improved chemical composition and properties. The purpose of the present study was to assess the chemical composition of the essential oils extracted from *Citrus* var. *arauntium, Lavendula augustofilia, Citrus arauntium* var. *bergamia, Artemisia afra, Petroselinum crispum, Matricaria chamomilla, Salvia stenophylla,* and *Ocimum basilicum*. The composition of the oils of these plants was analysed using gas chromatography mass spectrometry (GC-MS). The results revealed a range of between 13-49 compounds in the eight oils tested, and these compounds represented between 97.24-100% of the oils. Some of the same compounds were detected in the eight the oils, but the percentages of their presence differed greatly. Some compounds were dominant in some oils, while they were present in minimal quantities in others. The overall profile of the compounds is as follows:

- thujone (69%), thujene (14.8%), and pinene (0.18-0.87%) were detected in ***A. afra.***
- phenethyl alcohol (26.23%), linalyl anthranilate (26.56%), and D-limonene (7.33-27.4%) were detected in ***C. aurantium* var. *amara***;
- myrcene (0.17-0.27%), bisabalol (0.29-10.50%), cymene (0.19-2.81%), and eucalyptol (0.59-11.86%) were detected in ***S. stenophylla***;
- linalyl acetate (48.43%), D-limonene, and sabinene (0.14-0.53%) were detected in ***C. aurantium* var. *bergamia;*** while
- linalyl butyrate (29.1%) was detected in lavender.
- Both ***Ocimum bacilicum*** and ***Lavendula angustofolia*** contained estragole (79%) and linalool (3.9-27.9%); while
- ***Petriselinum crispum*** contained salinene (6.14-26.33%) and apiole (11.82%).

- ***M. chamomilla*** had 9-Amino-1-phenyl-3,6-diazahomoadamantane (10.59%) and some unknowns. The detection of these unknown dominant compounds could mean that the oil was adulterated or that the compounds were not available in the GC-MS library.

The findings of these analyses may serve as a guide for the selection of ideal oils for an intended treatment. The chemical composition of oils extracted from lavender, neroli, bergamot, chamomile, and sweet basil have been investigated in earlier studies as well, and adulterants like linalool, linalyl acetate, terpineole, pimaric acid, and abietic acid have been reported.

3.2 Introduction

Aromatherapy is one of the most popular holistic therapies for boosting immunity and it has been widely used to prevent and manage various ailments (Ali *et al*., 2015). Aromatherapy has gained increased attention in the medical field due to the properties ascribed to the EOs used in such treatments when used in conjunction with prescribed medications (Hamid *et al*., 2011; Ali *et al*., 2015). Various ailments linked to fungi, viruses (herpes simplex virus type1) and bacteria are believed to be well managed when using EOs in conjunction with medical intervention. Aromatherapy, when combined with appropriate medication, assists in the treatment of chronic diseases, tension, anxiety, and despair (Huang, 2017). Essential oils also possess antibacterial properties that can help cure skin infections, especially if they are used in health and beauty clinics. The antioxidant properties of EOs can be exploited extensively in the health and beauty industries to improve premature ageing, skin dryness, and hyperpigmentation (Huang *et al*., 2015).

Furthermore, epilepsy can be improved with the application of EOs, especially when administered through the inhalation method (Hamid *et al*., 2011). Various authors agree that EOs have beneficial properties if administered by an appropriate application method, particularly inhalation or topical application (Hamid *et al*., 2011). The latter is widely used in the field of Somatology to access the properties of EOs through aromatherapy treatments. Other techniques include compressions and the addition of oils to the water that is used in steam rooms and saunas. However, in the latter cases, the safety of the use of EO use for humans has not been substantiated by scientific evidence (Johnson & Boren, 2013; Tisserand & Young, 2013).

EOs contain a variety of compounds that can be negatively affected by factors such as climate, origin, seasonal changes, harvest period, extraction method, and even adulteration that can impact their composition (Duarte *et al.,* 2010; Virendra & Diwaker, 2007; Fejer *et al.*, 2018; Matulyte *et al*., 2019). Adulteration of EO is a challenge in the field of Somatopathology because it has a negative impact on the outcome of treatments. Some adulterants are reportedly harmful, and others are believed to be endocrine disruptors (Boren *et al*., 2015). Benzyl alcohol, lavandin, linalyl acetate (which is considered an adulterant), and other adulterants that were added to EO affected the results in studies such as that of Beale *et al*. (2017). Clearly, adulteration degrades EO quality as the addition of foreign substances results in the loss of their original properties. Such findings should serve as a guide for therapists when selecting EOs for treatments (Vargas-Jentzsch *et al*., 2019).

EOs are by far the most complicated compounds in plants, and determining their chemical composition requires the use of special analytical procedures. The most frequently used approach for analysing EOs is the use of the gas chromatography mass spectrometry (GC-MS) instrument. The device is extremely useful in determining the number of compounds within a sample (Matulyte *et al*., 2019). The technique is used not only for the characterisation of EO, but also to determine the components of other complicated chemicals that require profiling or characterisation (Matulyte *et al*., 2019).

3.3 Materials and Methods

The oils *of Citrus aurantium* var. *amara* (neroli), *Lavendula angustofilia* (lavender), *Citrus arauntium* var. *bergamia* (bergamot), *Artemisia afra* (wormwood), *Petroselinum crispum* (parsely), *Matricaria chamomilla* (German chamomile), *Salvia stenophylla* (blue mountain sage), and *Ocimum basilicum* (sweet basil) were analysed using GC-MS according to the following procedures:

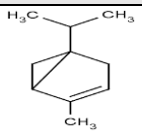
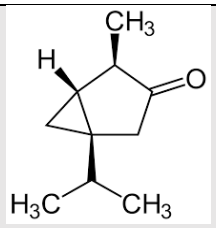
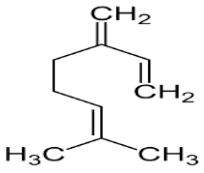
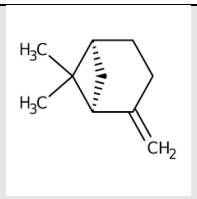
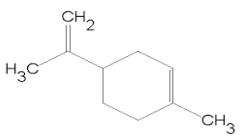
An Agilent 7890B GC system that was coupled directly to a 5977A mass spectrometer was used. A volume of 1ml 0.5% solution (v/v sample/hexane) was injected using a split ratio of 150:1 with an autosampler at 24.79 psi and an inlet temperature of 250°C. The gas chromatogram (GC) system was equipped with an Agilent 19091S 433 UI: HP5-MS UI (30m x 250µm x 0.25µm) column. The oven temperature was ramped from 60°C to 280°C at various incremental and was held for 2 minutes. Helium was used as carrier gas at a constant flow of 0.67 mL/min. Spectra were obtained

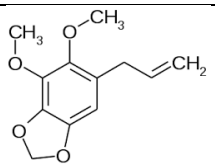
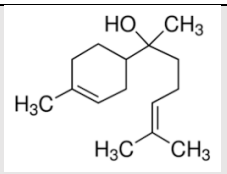
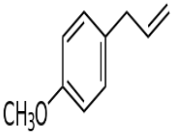
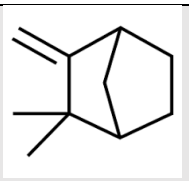
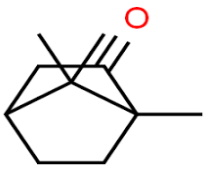
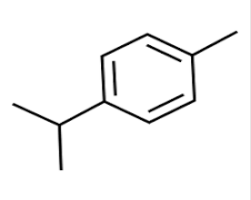
by electron impact at 70 eV, scanning from 35 to 550 m/z. The peak areas of the selected GC constituents were individually expressed as percentages of the total of all the total ion count (TIC) peak areas as determined by mass spectrometry detection (MSD, 250°C) without using correction factors. The compounds were identified using the NIST11 and Flavor2 mass spectra libraries.

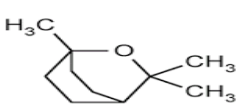
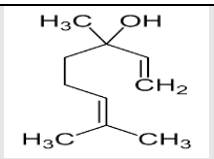
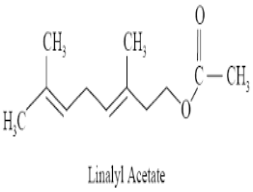
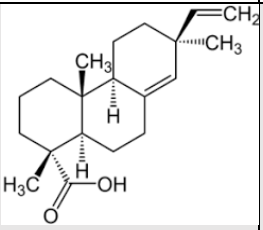
3.4 Summary of the Studied Essential Oils and their Known Properties

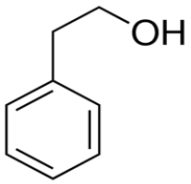
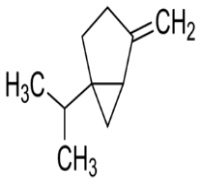
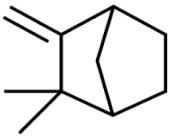
An overview of the dominant components that were previously detected in the EOs under investigation is presented in **Table 3.1.** The goal is to highlight the therapeutic properties of dominant compounds generally found in EOs and to emphasise the importance of understanding these features. Having a good understanding of the dominant compounds in EOs and their therapeutic qualities can assist in the selection of oils that will address certain challenges in the Aromatherapy and Somatology fields. **Table 3.1** indicates the principal components found in earlier studies in the oils under investigation as well as their therapeutic qualities and molecular and structural formulae.

Table 3.1: Summary of components previously detected in the EOs under study.

Component	Therapeutic properties	Molecular formulae	Structural formulae	Plants used for essential oil extraction	References
A-Thujene	Anti-convulsant, neutrotoxic, toxic	$C_{10}H_{16}O$		wormwood	Nóbrega *et al.,* 2011
β Thujone	Sedative, antiviral, spasmolytic, neurotoxic	$C_{10}H_{16}$		wormwood	Sharafzadeh & Alizadeh, 2011, Djilani & Dicko, 2012
β-Myrcene	Anti-inflammatory	$C_{10}H_{16}$		wormwood, bergamot, blue mountain sage, basil, neroli, lavender	Ren *et al.,* 2017; Djilani & Dicko, 2012
β-Pinene	Detoxifying, antioxidant	$C_{10}H_{16}$		wormwood, bergamot, blue mountain sage, parsley, neroli, lavender	Vokk *et al.,* 2011; Djilani & Dicko, 2012
D-Limonene	Antibacterial, antioxidant, anticancer	$C_{10}H_{16}$		bergamot, neroli, parsley,	Djilani & Dicko, 2012; Sarkic &

				blue mountain sage, basil	Stappen, 2018
Apiole	Detoxifying	$C_{12}H_{14}O_4$		parsley	Kamatou & Viljoen, 2010; Tisserand and Young, 2013
Bisabalol	Anti-inflammatory, healing, antimicrobial, soothing	$C_{15}H_{26}O$		German chamomile, lavender	Sarkic & Stappen, 2018
Estragole	Sedative	$C_{10}H_{12}O$		basil	Djilani & Dicko, 2012
Camphene	Antibacterial	$C_{10}H_{16}$		wormwood, blue mountain sage	Girola *et al*., 2015
Camphor	Antimicrobial	$C_{10}H_{16}O$		wormwood, lavender, blue mountain sage	Garg & Jain, 2017
Cymene	Anti-inflammatory, antioxidant, antimicrobial	$C_{10}H_{14}$		neroli, wormwood, bergamot, lavender, blue	Marchese *et al*., 2017

				mountain sage	
Eucalyptol	Anti-inflammatory	$C_{10}H_{18}O$		wormwood, lavender, blue mountain sage, basil	Djilani & Dicko, 2012; Bhowal & Gopal, 2015; Brown *et al.*, 2017
Linalool	Antimicrobial	$C_{10}H_{18}O$		lavender, bergamot, chamomile, neroli, basil	Kim *et al.*, 2017; Djilani & Dicko, 2012; Sarkic & Stappen, 2018
Linalyl acetate	Anti-spasmolytic, anti-inflammatory, sedative, antifungal	$C_{12}H_{20}$		bergamot	Djilani & Dicko, 2012; Api *et al.*, 2015; Kim *et al.*, 2017
Pimatic acid	Antibacterial, anti-inflammatory	$C_2OH_{30}O_2$		German chamomile	Ali *et al.*, 2015

Phenyl alcohol	Antimicrobial, antiseptic	$C_8H_{10}O$		neroli	Sirilun *et al.*, 2017
Sabinene	Antimicrobial, anti-tumor, antiviral	$C_{10}H_{16}$		wormwood, lavender, German chamomile	Djilani & Dicko, 2012
Myristicin	Antioxidant, antimicrobial	$C_{11}H_{12}O_3$		parsley	Agyare *et al.*, 2017

3.5 Results and Discussion

3.5.1 Neroli (*Citrus aurauntium var amara*)

In the current study, 22 compounds were identified in the neroli oil when compared against the data in the mass spectroscopy library (**Table 3.2).** The data indicated that 99.9% of the overall compounds in this EO that had been extracted from the flowers of *Citrus arautium* var. *amara* was detected. Moreover, Linalyl anthranilate (26,6%) and phenethyl alcohol (26,2%), which are appreciated for their antimicrobial properties, were detected in this oil. Linalool (15,7%), which is a monoterpene, demonstrated antibacterial activity against *Staphylococcus aureus* and other bacterial strains in a study conducted by Kamatou and Viljoen (2008). The antibacterial activity of this compound is of immense significance in the Somatology field to combat skin infections caused by *S. aureus*. Haj-Ammar (2012) affirms that limonene is one of the most prevalent antibacterial compounds in this oil. The current study affirmed that linalyl anthranilate and phenethyl alcohol, which have antibacterial properties, were predominant in the neroli oil that was examined. The origin of the oil is a significant differentiating trait, and the observed differences in the percentages of its components suggest that the oil's attributes can be affected environment. Neroli was also one of the oils that exhibited antibacterial properties in a study conducted by Wang *et al*. (2016), who argue that linalyl anthranilate is an antimicrobial agent that can also be utilized as a preservative. Due to the preponderance of

linalool in this oil, it has been argued that neroli essential oil has strong antimicrobial activity (Brandt, 1990; González-Mas *et al.*, 2019). However, a minimal percentage of this component (15.7%) was detected in the current investigation. D-Limonene is a common terpene with a short half-life in humans, meaning it can linger in the body for 12 to 24 hours before being eliminated through urine (Sun, 2007). Limonene belongs to a class of hydrocarbons that are susceptible to oxidation (resulting in composition changes) when exposed to light if proper storage is not maintained (Tisserand & Young, 2013). However, this study detected only 7.33% of this compound.

A factor that needs to be taken into consideration is oil storage as some compounds, such as limonene that was detected in this oil, are readily oxidized. Skin irritations are common as a result of oxidation. Linalool, limonene, and pinene are monoterpenes found in neroli (González-Mas *et al*., 2019). Therefore, even though this oil has antimicrobial properties, it might irritate the skin if it is experience excessive exposed to light or if the oil has not been stored appropriately (Tisserand and Young, 2013). EOs should be stored properly because light can alter their qualities and cause their properties to degrade. This will result in oxidation which leads to compound degradation that causes skin irritation (Tisserand and Young, 2013). The findings on this oil are critical in the Somatology field as they will sensitize somatologists to the risks associated with this oil and thus better inform their decision to use it.

Table 3.2: EO composition of *C. aurantium* var. *amara*

Retention Time (min)	Compound Name	% Area
7. 99	α-Pinene	0.19
10.34	Sabinene	0.18
10.5	β-Pinene	1.14
11.54	β-Myrcene	0.27
13.46	p-Cymene	0.45
13.69	D-Limonene	7.33
15.39	γ-Terpinene	0.47
17.6	Linalool	15.71
18.19	Phenethyl alcohol	26.23

19.12	Terpinen-1-ol	0.16
19.58	β-Terpineol*	0.64
20.44	β-Terpineol*	0.15
21.60	Linalyl anthranilate	26.56
21.89	Hydroxycitronellal	7.09
24.33	Methyl anthranilate	2.72
25.46	α-Terpineol	5.79
27.33	Terpinolene	1.77
28.26	Neryl acetate	0.23
28.65	Geranyl acetate	0.36
52.15	Unknown#	2.55
	Total	**99.99**

Compounds marked with an Asterix (*) (β-Terpineol*) are stereoisomers that were not identified in the library data. Refer to **Tables 3.2, 3.5,** and **3.6** that indicate that no positive identification of these detected compounds was possible when they were compared with the data in the GC-MS library.

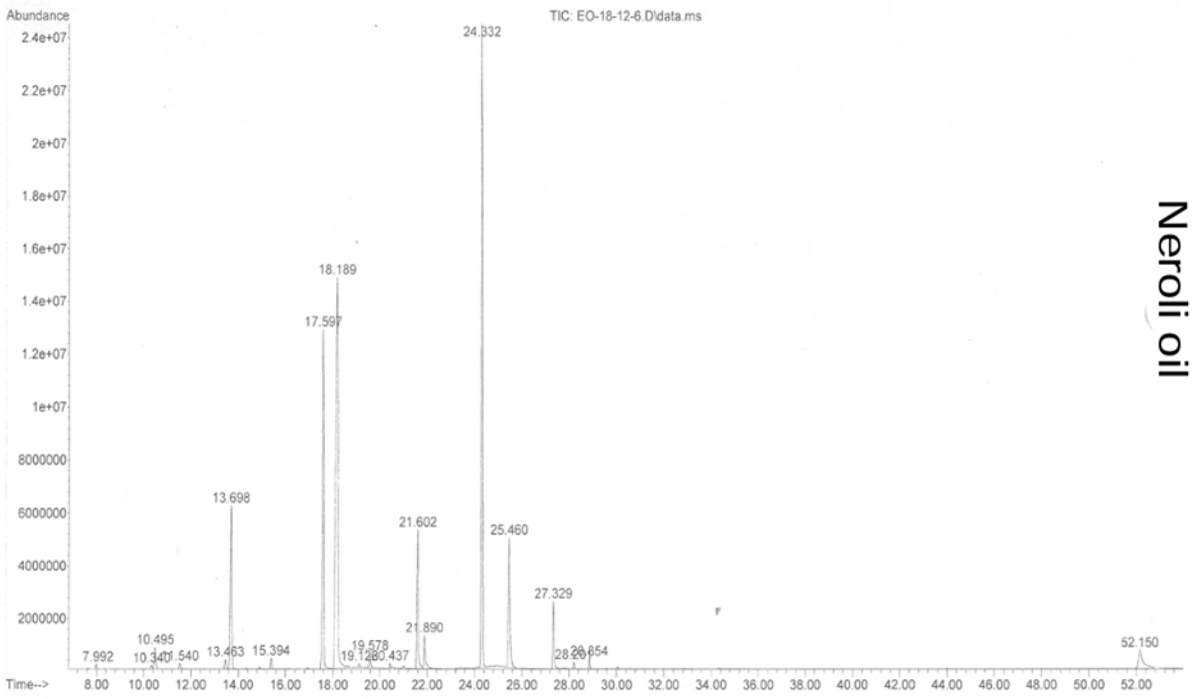


Figure 3.1: Parts of *C. aurantium* var. *amara* chromatogram representing abundance of various components with peaks complemented by retention time (RT).

3.5.2 Lavender (*Lavendula angustofolia*)

Mass spectroscopy chromatograms are presented in **Figure 3.2** indicating retention time and relative abundance, while percentages of the profiled lavender essential oil are presented in **Table 3.3.** Gas Chromatography-Mass Spectrometry analysis was conducted to assess the composition of lavender oil. 35 compounds were identified that represented 97% of the total oil extracted from *Lavendula augustofolia*. Linalyl butyrate was the most prevalent compound as it accounted for 29% of the oil, followed by linalool (27%), and 1,8 cineole (18%). *Lavendula angustofolia* is a potent antibacterial agent due to the dominant presence of linalyl butyrate, linalool, and 1,8 cineole (monoterpenes) in its oil. Linalyl butyrate, which was detected as the most abundant compound in this oil, reportedly has stomachic properties, while linalool possesses antibacterial properties (Beale *et al*., 2017; Gaonkar *et al*., 2018; Sarkic & Stappen, 2018; Białoń *et al*., 2019). The literature indicates that 1,8 cineole is a monoterpene which is claimed to have antimicrobial properties, hence it is assumed that this oil is a strong antimicrobial agent (Şimşek & Duman, 2017).

Based on these findings, the fact that the two dominant compounds were detected at roughly the same percentages implies that the lavender under study possessed antibacterial and stomachic properties. It is important to note that there are many species of lavender that have distinct chemical compositions. They all have identical primary compounds, but the percentages of their presence vary in these plants which affects the properties of each species (Cavanagh & Wilkinsons, 2002). For instance, *Lavendula angustofolia* is claimed to be the safest oil to use, but this claim is questionable as it has been reported to be an emmenagogue (Cavanagh & Wilkinsons, 2002). Information such as this should be made explicit to Somatologists so that the implications of utilising oils from different species of plants, even those of the same family, are fully understood.

Lavendula augustofolia is popularly known for its calming and relaxing properties. However, the diuretic property of *L. anustofolia* has not been widely investigated or even considered for use in the Somatology field. *Lavendula stoechas* is mostly used for headaches, which means it has analgesic properties (Cavanagh & Wilkinsons, 2002; Malcom & Tallian, 2017). It is thus vital that Somatologists are familiar with plants' scientific names and properties as this will guide them to differentiate among the oils extracted from various species of plants, such as *lavendula*. This will make it easier to select an oil that will be most effective for the intended treatment.

Linalool provides *Lavendula angustofolia* with its strong antibacterial property (refer to Chapter 5), while linalyl acetate has strong antioxidant action (refer to Chapter 4). The significance of such information should be noted in the Somatology industry. Although some oils are from the same species, their compounds differ owing to a variety of factors such as climate, origin, and environmental conditions (Raveau *et al*., 2020).

Table 3.3: EO composition of *Lavendula angustofolia*

Retention Time (min)	Compound Name	% Area
7.99	α-Pinene	0.87
8.76	Camphene	0.44
10.34	Sabinene	0.23
10.49	β-Pinene	0.86
11.53	β-Myrcene	0.54
12.59	γ-Terpinene	0.23
13.46	p-Cymene	0.27
13.79	1,8-Cineole	11.86
14.35	cis-β-Ocimene	0.79
14.91	trans-β-Ocimene	0.25
16.92	α-Terpinolene	0.32
17.62	Linalool	27.85
18.23	1-Octen-3-yl-acetate	0.21
19.49	Camphor	9.21
20.48	Isoborneol	2.17
20.65	Lavandulol	0.20
21.01	(-)-Terpinen-4-ol	2.38
21.59	α-Terpineol	1.25
21.77	Hexyl butanoate	0.40
23.75	Hexyl n-valerate	0.17
24.32	Linalyl butyrate	29.10

25.61	(R)-Lavandulyl acetate	3.41
27.03	Hexyl angelate	0.12
28.19	Neryl acetate	0.27
28.85	Geranyl acetate	0.60
29.09	α-Zingiberene	0.13
30.06	β-Caryophyllene	1.84
30.58	α-Bergamotene	0.16
31.25	trans-β-Famesene	1.07
32.05	Germacrene-D	0.46
33.07	α-Muurolene	0.66
33.35	Calamenene	0.13
35.15	Caryophyllene oxide	0.21
36.8	(+)-epi-Bicyclosesquiphellandrene	0.33
37.99	α-Bisabolol	0.29
	Total	97.24

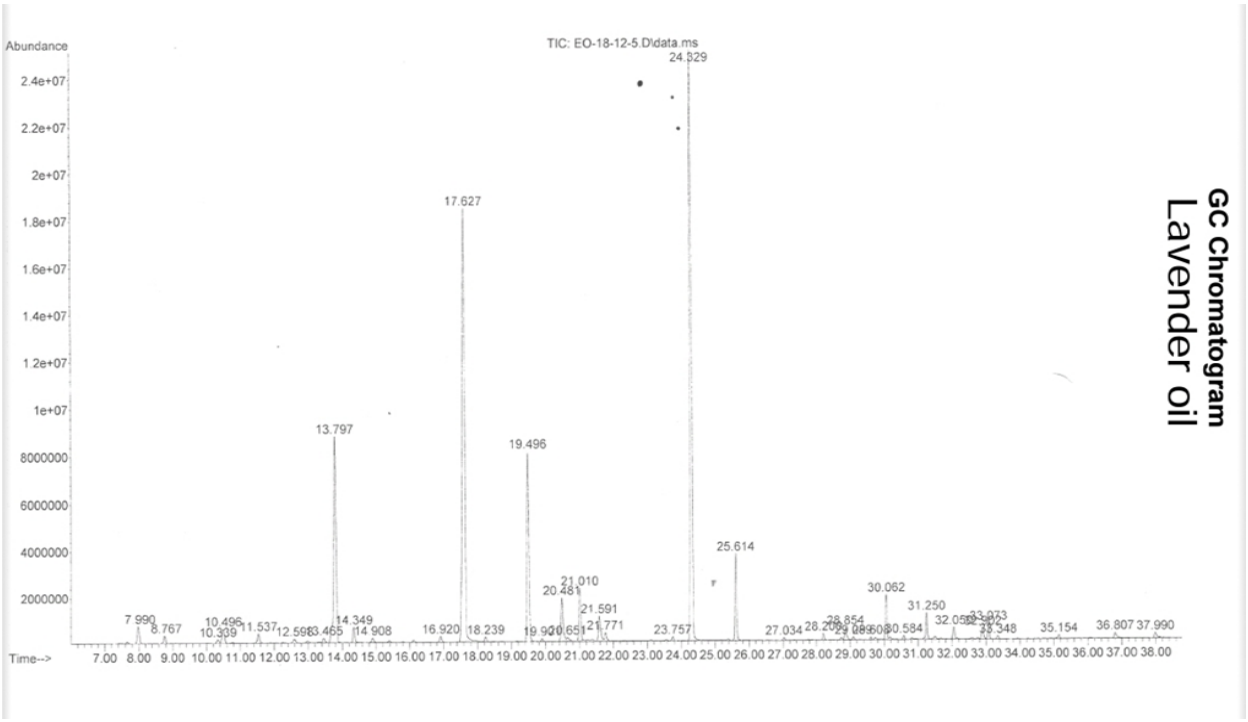


Figure 3.2: Parts of *Lavendula agustofolia* chromatogram representing abundance of various components with peaks complemented by retention time (RT).

3.5.3 Bergamot (*Citrus aurantium* var. *bergamia*)

The retention time and percentages of compounds in bergamot oil are presented in **Table 3.4** while **Figure 3.3** indicate the total ion chromatogram (TIC) *for C. aurantium var bergamia* indicating the relative abundance of the identified compounds. The highest peak identified on the TIC was linalyl butyrate. All these compounds are indicated in the MS data. The 14 identified components represented 99% of the total oil of *C. aurantium* var. *bergamia*. The oil contained three types of compounds, namely monoterpene hydrocarbons, oxygenated monoterpenes, and sesquiterpenes. The predominant monoterpene hydrocarbon in the bergamot oil was limonene (27%). The oil also contained 48% linalyl acetate, which is a key component of oxygenated hydrocarbons. Linalool accounted for about a quarter of the total (10%) of *Citrus aurantium* var. *bergamia* that was investigated by Nabiha *et al*. (2010). The latter study detected bisabalene, which was not found in the bergamot that was tested in the current study. Linalool has been shown to be cytotoxic on epithelial cells and fibroblasts (Orchard & van Vuuren, 2017), while linalyl acetate is reportedly more hazardous separately than in the entire oil (Prashar *et al*., 2004).

Citrus aurantium var. *bergamia* comprised of 48% linalyl acetate, which is believed to be an adulterant that can possibly contribute to irritation of the skin when administered topically. Several studies have shown that linalyl acetate is antispasmodic, sedative, antifungal, and anti-inflammatory (Lis-Balchin, 2012; Djilani & Dicko, 2012; Api *et al*., 2015; Kim *et al*., 2017). All the above-mentioned properties are of paramount significance in the Somatology field as the industry specialises in topical treatments. It is for this reason that the method of application should be carefully selected to avoid adverse reactions such as irritation or over sensitivity.

Table 3.4: EO composition of *C. aurantium* var. *bergamia*

Retention Time (min)	Compound Name	% Area
07.99	α-Pinene	0.6
10.31	Sabinene	0.54
10.50	β-Pinene	3.07

11.56	β-Myrcene	0.51
13.47	p-Cymene	0.65
13.70	D-Limonene	27.4
15.39	γ-Terpinene	0.97
17.55	Linalool	9.66
23.66	β-Citral	0.94
24.36	Linalyl acetate	48.43
24.81	α-Citral	1.47
25.22	Limonene acetate	0.38
27.67	Terpinolene	3.06
27.74	2-Bornene	0.92
	Total	**98.6**

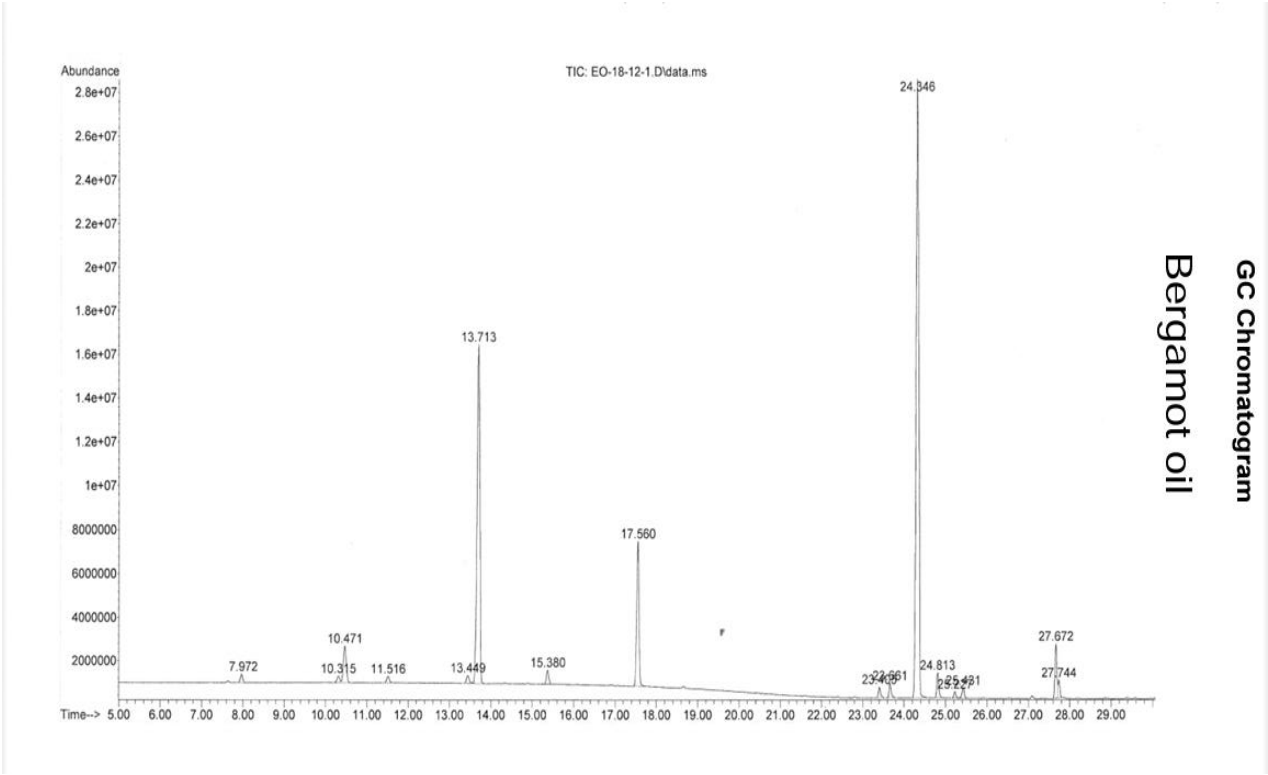


Figure 3.3: Parts of *C. aurantium* var. *bergamia* chromatogram representing abundance of various components with peaks complemented by retention time (RT).

3.5.4 Wormwood (*Artemisia afra*)

Mass spectroscopy chromatograms are presented in **Figure 3.4** indicating retention time and relative abundance, while percentages of the profiled *A. afra* essential oil are presented in **Table 3.5.**
The GC-MS results revealed 14 constituents, representing 99% of the total oil of *Artemisia afra*. The *Artemisia afra* oil contained 69% α-thujone, which was the predominant compound, while β-thujone was detected at 14%. Both these compounds are classified under ketones which have sedative, antiviral, anti-spasmolytic, and neurotoxic properties (Djilani & Dicko, 2012; Sharopov *et al*., 2012). Pinene was one of the predominant compounds, but it was found in trace amounts. According to the literature, the unknown compounds that were detected could have been cis-pinacamphane and trans-pinocamphone. The two suspected compounds had previously been detected in *A. afra* oil by Sharafzadeh and Alizadeh (2011), Djilani and Dicko (2012). Isomer identities are clarified by comparison for compounds marked with an asterisk (refer to **Tables 3.3, 3.6, 3.7** and **3.8**).

Artemesia afra contains a large quantity of ketones that have beneficial effects such as reducing cough, heartburn, colds, asthma, and bronchitis. They are known to improve diabetes (Nigam *et al*., 2019) but are also neurotoxic (Lachenmeier, 2010). To avoid responses such as hallucinations, hyperexcitability, and epileptic fits, it is recommended that the oil be carefully diluted and used in moderation (Lachenmeier, 2010).

Table 3.5: EO composition of *A. afra*

Retention *Time* (min)	Compound Name	% Area
7.99	α-Pinene	0.31
8.77	Camphene	0.39
10.34	Sabinene	0.22
11.54	β-Myrcene	0.19
12.09	3,3,6-Trimethyl-1,4-heptadien-6-ol	0.23
13.46	p-Cymene	1.10
13.79	1,8-Cineol	8.61
14.35	β-Ocimene	0.22

17.83	α-Thujone*	69.08
18.29	β-Thujone*	14.78
19.49	Camphor	1.16
20.96	Unknown[1]	2.57
32.08	α-Curcumene	0.08
35.00	Spatulenol	0.39
	Total	**99.33**

The * indicates that the compounds differed in structure even though they had the same name.

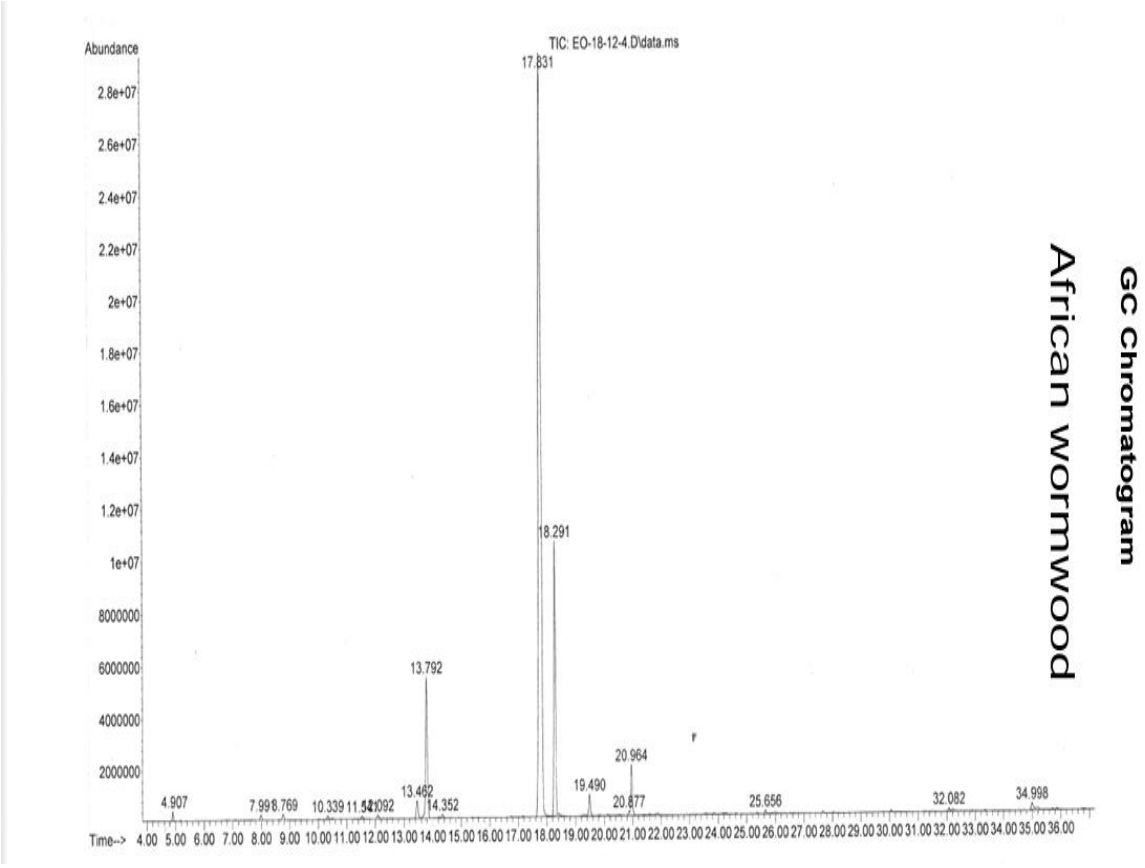


Figure 3.4: Parts of *A. afra* chromatogram representing abundance of various components with peaks complemented by retention time (RT).

3.5.5 Parsley (*Petroselinum crispum*)

Mass spectroscopy chromatograms are presented in **Figure 3.5** indicating retention time and relative abundance, while percentages of the profiled *Petroselinum crispum* essential oil are presented in

Table 3.6. Thirteen constituents were detected in the parsley oil, representing 98% of this oil. Aliphatic esters, alcohols, and hydrocarbons were present to a large extent. The dominant compounds were Selinene (26,3%) and D-limonene (15.79%). The unknown compound that was part of the predominant components was either myristicin or sabinene according to the literature (Vokk *et al*.,2011). Vokk *et al*., (2011) argued that the unknown compound that was detected in that study represented 30% of the oil, which is close to the 29% that was detected in the current study. According to Djilani and Dicko (2012), myristicin can function as a sedative while also regulating monthly periods in females. However, its emmenagogue property poses a risk to pregnancy as it can lead to termination or preterm labour.

Menstruation is shedding of the uterine wall, and if it occurs during pregnancy, it can disrupt the placenta and lead to a miscarriage (Mtawali *et al*., 1997). Therefore, as one of the dominant compounds in parsley is suspected to be myristicin or sabinene, it is advisable to use parsley oil under the supervision of a physician throughout pregnancy. According to Djilani and Dicki (2012) and Agyare *et al*. (2017), both the aforementioned components (myristicin and sabinene) are capable of antibacterial activity.

Table 3.6: EO composition of *P. crispum*

Retention Time (min)	Compound Name	% Area
5.14	β-Pinene	0.37
10.25	D-Limonene	15.79
13.72	trans-Limonene oxide	0.18
16.10	Unknown*	2.43
19.27	Unknown*	29.05
19.62	2-Isopropylidene-5-methyl-hex-4-enal	0.21
24.05	β-Elemene	1.19
25.95	β-Caryophyllene	0.13
26.81	4,5-di-epi-Aristolochene	1.08
29.15	γ-Selinene + unknown	3.50
30.06	β-Selinene	26.33

31.68	α-Selinene	6.14
31.93	Apiole#	11.82
	Total	**98.22**

Compounds marked with an asterix (*) could be positively indicated by GC-MS.

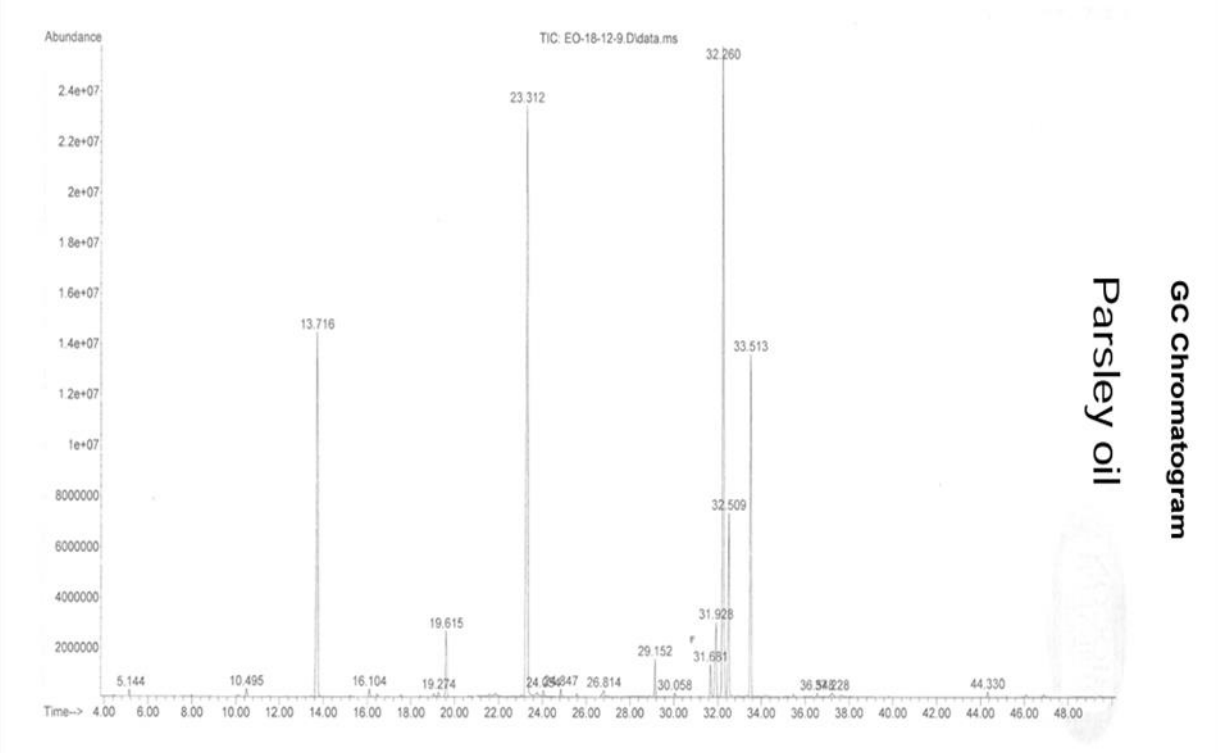


Figure 3.5: Parts of *P. crispum* chromatogram representing abundance of various components with peaks complemented by retention time (RT).

3.5.6 German chamomile *(Matricaria chamomilla)*

Abundance of components in *Matricaria chamomilla* is presented in **figure 3.5**, with relative retention times relating to the peaks. The total of 35 compounds (representing 95% of the oil) was identified and is presented in **Table 3.7**. The results revealed the presence of methyl dihydroabietate at 12,35% and 9-amino-1-phenyl-3,6, diazahomoadamantane at 10,59% as the predominant compounds. Terpenoids, bisabalol, bisabalol oxide, and a & b chamazulene were among the active compounds identified in this oil by Sharafzadeh and Alizadeh (2011). Some of the compounds found in the latter study form part of coumarins, luteolin, umbelliferone, and flavonoids that are examples of

sesquiterpenes, but they were not found in this oil in the current study. When this study's results are compared to those of Sharafzadeh and Alizadeh (2011), they are not in agreement when it comes to composition, as the dominant compounds found in the two studies varied greatly. The unknown compounds (marked with superscript a, b, and c) included secondary compounds that made up the entire oil, while adulterants such as dipropylene glycol, pimaric acid, and abietic acid, which are normally found in resins, were also detected.

Dipropylene glycol is an adulterant used in plasticizers and polyester alkyd resins, and it is another compound that does not naturally occur in EOs (Lawrence, 2002). Although *M. chamomilla* reportedly possesses powerful anti-inflammatory qualities, it was impossible to establish results that would support this claim due to the long list of unknown compounds observed (Miguel, 2010). Many of the compounds that were detected at a noticeable percentage appear on the list of unknown or unidentified compounds. The long list of unknown compounds that were detected emphasises the need for somatologists to verify products' validity and suppliers' reliability.

Table 3.7: EO composition of *M. chamomilla*

Retention Time (min)	Compound Name	% Area
8.47	Sabinene	0.14
11.28	α-Terpinene	0.12
11.88	1,1'-Oxybis-2-propanol[a] + unknown	1.27
13.15	1,1'-Oxybis-1-propanol[a]	0.87
13.53	2-(2-Hydroxypropoxy)-1-propanol[a]	0.49
14.38	γ-Terpinene	0.19
17.22	β-Terpineol	0.23
17.62	Linalool	3.89
23.06	Terpinen-4-ol	0.34
27.06	Geraniol	3.44
33.69	1-Decene	2.94
40.76	α-Guaiene	0.13
44.42	(+)-α-Longipinene	0.13

44.84	α-Bulnesene	0.15
53.07	Patchouli alcohol	0.33
56.24	α-Santalol	0.16
59.57	Guaiazulene	0.15
69.75	4-(2-Furyl)-6-(1-piperidinyl)-1,3,5-triazin-2-amine	0.15
78.31	1α,2α,3α-1,3-Dimethyl-2-[2-[3-(1-methylethyl)phenyl]ethyl]-methylcyclohexane carboxylate	0.23
80.31	Methyl dihydroabietate[b]	0.11
81.18	9-Amino-1-phenyl-3,6-diazahomoadamantane[c]	10.59
81.547	9-Amino-1-phenyl-3,6-diazahomoadamantane[c]	3.36
82.171	Unknown 1[b]	0.47
82.494	Unknown 2[b]	1.34
82.753	Unknown 3[b]	0.15
82.983	Unknown 4[b]	0.86
83.100	Unknown 5[b]	0.61
83.33	Unknown 6[b]	1.98
83.765	Unknown 7[b]	6.33
84.118	Unknown 8[b]	4.33
84.312	Unknown 9[b]	2.32
84.594	Unknown 10[b]	32.18
84.847	Unknown 11[b]	1.62
85.394	Methyl dihydroabietate[b]	12.35
85.871	Unknown 12[b]	1.43
	Total	**95.38**

Compounds marked [a, b, c] indicate the possible presence of adulterants such as pimaric, abietic acid, methyl dehydroabietic, and Dipropylene glycol.

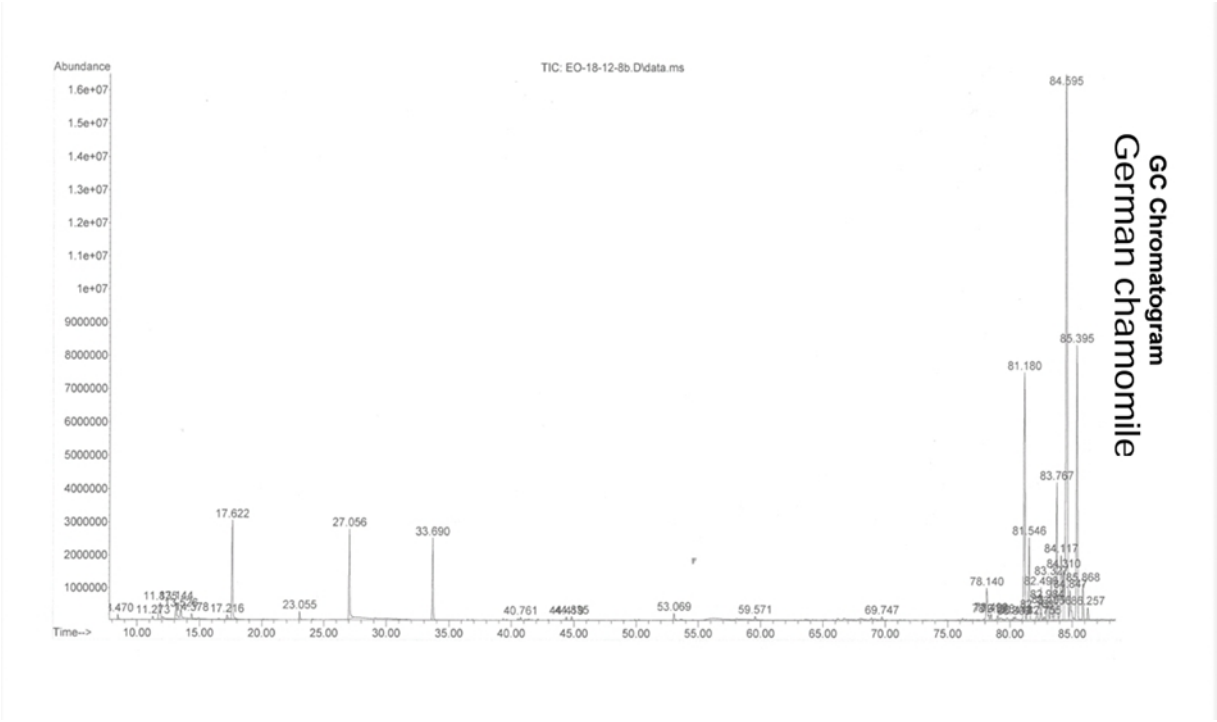


Figure 3.6: Parts of *M. chamomilla* chromatogram representing abundance of various components with peaks complemented by retention time (RT).

3.5.7 Blue Mountain sage (*Salvia stenophylla*)

Mass spectroscopy chromatograms are presented in **Figure 3.7** indicating retention time and relative abundance, while percentages of the profiled *Salvia stenophylla* essential oil are presented in **Table 3.8.** Fort-nine components were identified, representing 98% of the total oil of *S. stenophylla*. The dominant compounds detected were myrcene (14%), which has anti-inflammatory properties, and D-limonene (10,7%), which is an antibacterial and potent anti-oxidative agent. These were followed by bisabalol (10,5%) which has healing, calming, anti-inflammatory, and antibacterial properties, -bisabalol (47.6%), limonene (1.6%), carene (7.0%), -terpinene (0.2%), p-cymene (0.1%), and nerolidol (57%). The latter were also listed as chemicals found in blue mountain sage oil by Fisher (2006). The findings showed considerable commonalities in the compounds, while a significant difference was spotted in the percentages indicating their presence. Camphene, camphor, and carene were also observed, and these are reported to possess antibacterial properties (see **Table 3.1**). The findings suggest that, while blue mountain sage is a powerful anti-inflammatory agent, it is still crucial to choose the method of administration carefully, as inhalation is not the best option if the

inflammation is not external or on the skin. When using oils topically, proper dilutions are crucial. Overall, the findings will contribute to the advancement of the Somatology industry as they can be applied to ensure better and safe outcomes.

Table 3.8: EO composition of *S. stenophylla*

Retention Time (min)	Compound Name	% Area
7.28	Unknown 1[1]	1.07
7.42	γ-Terpinene[1]	0.33
7.66	α-Phellandrene[1]	0.28
7.99	α-Pinene	5.57
8.77	Camphene	6.60
10.5	β-Pinene	2.34
11.56	β-Myrcene	14.01
12.25	α-Phellandrene	0.62
12.6	δ-3-Carene	8.87
13	α-Terpinene	0.23
13.33	p-Cymene[2]	0.19
13.47	p-Cymene[2]	2.81
13.7	D-Limonene	10.64
13.79	1,8-Cineol	1.44
14.35	trans-β-Ocimene	2.92
14.91	cis-β-Ocimene	0.78
15.39	γ-Terpinene	0.95
16.81	Terpinolene[3]	0.14
16.91	Terpinolene[3]	0.25
17.55	Linalool	0.22
19.49	Camphor	6.11
20.48	Isoborneol	0.57
21	Terpinen-4-ol	0.40
27.46	(-)-Nicotine	0.22

28.59	α-Copaene	0.20
29.74	α-Gurjunene	0.27
30.06	β-Caryophyllene	6.18
30.58	α-Bergamotene	0.81
30.69	Aromadendrene	0.84
31.17	Humulene	1.40
31.25	β-Famesene	0.55
31.9	γ-Muurolene	0.16
32.22	β-Selinene	0.15
32.49	β-Patchoulene	0.41
32.63	(-)-Aristolene	0.21
32.89	β-Bisabolene	1.83
33.07	α-Muurolene	0.76
33.35	δ-Cadinene	0.91
33.71	(+)-Valencene	0.12
33.92	Selina-3,7(11)-diene	0.35
34.53	Nerolidol	0.20
34.99	(-)-Spathulenol	0.25
35.15	Caryophyllene oxide	2.14
35.92	Humulene epoxide II	0.37
36.07	Cadine-1,4-diene	0.23
36.81	(+)-epi-Bicyclosesquiphellandrene	1.09
37.22	Bisabolol oxide B	0.36
38.06	α-Bisabolol	10.50
47.43	Manool	0.33
	Total	**98.18**

Compounds marked with a superscript of [2](p-Cymene[2]) are those that were not available in the library and those that have a superscript of (α-Phellandrene[1]) and (Terpinolene[3]) are those that were incorrectly identified. According to Lawrence (2013), they were most likely (Z)-Salvene, (E)-Salvene, Tricyclene, or α-Thujene. Additionally, the GC-MS could not accurately spot a difference between these mentioned compounds.

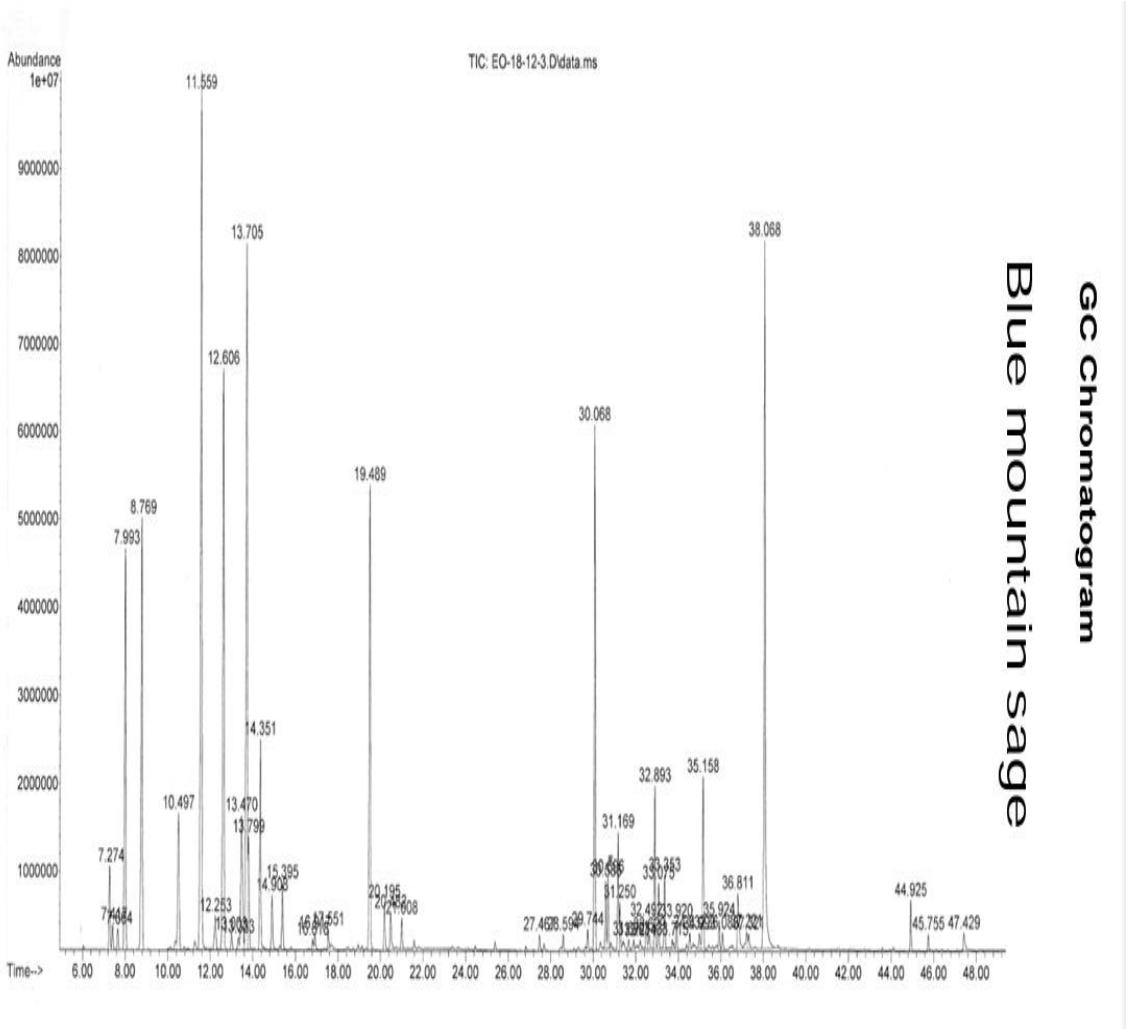


Figure 3.7: Parts of *S. stenophylla* chromatogram representing abundance of various components with peaks complemented by retention time (RT).

3.5.8 Sweet basil (*Ocimum basilicum*)

Mass spectroscopy chromatograms are presented in **Figure 3.9** indicating retention time and relative abundance, while percentages of the profiled *Ocimum bacilicum* essential oil are presented in **Table 3.8.** The GC-MS results revealed 14 components representing 100% of the total of the *O. basilicum* oil. Methyl chavicol at 79% and linalool at 17,5% were found to be the most prevalent components in this oil. Methyl chavicol is a potent anti-inflammatory agent according to Djilani and Dicko (2012), Bhowal and Gopal (2015), and Brown *et al*. (2017), hence it was concluded that the sweet basil oil detected in this study would have a strong anti-inflammatory activity. Furthermore, the presence of

linalool is linked to antimicrobial characteristics (Kim *et al*., 2017; Sarkic & Stappen, 2018). However, methyl chavicol, according to Clarke (2008), has carcinogenic effects when used in large quantities, but if the oil is adequately diluted, an unpleasant reaction is unusual, and it could then be applied effectively in Aromatherapy treatments. EOs with anti-inflammatory qualities are used to relieve and combat skin inflammation or any other condition related to inflammation. To avoid adverse reactions, it is always advisable to dilute EOs with carrier oils (Nurzyńska-Wierdak *et al*., 2012).

Table 3.9: EO composition of *O. bacilicum*

Retention Time (min)	Compound Name	% Area
11.23	Sulcatone	0.13
11.75	β-Myrcene	0.17
13.66	D-Limonene	0.47
13.80	1,8-Cineol	0.59
14.91	γ-terpinene	0.23
17.60	Linalool	17.49
19.98	l-Menthone	0.12
22.06	Methyl chavicol (Estragole)	79.11
23.68	β-Citral	0.30
24.81	α-Citral	0.39
30.06	β-Caryophyllene	0.26
30.58	α-Bergamotene	0.34
31.16	Humulene	0.07
33.92	Bisabolene m	0.33
	Total	**100**

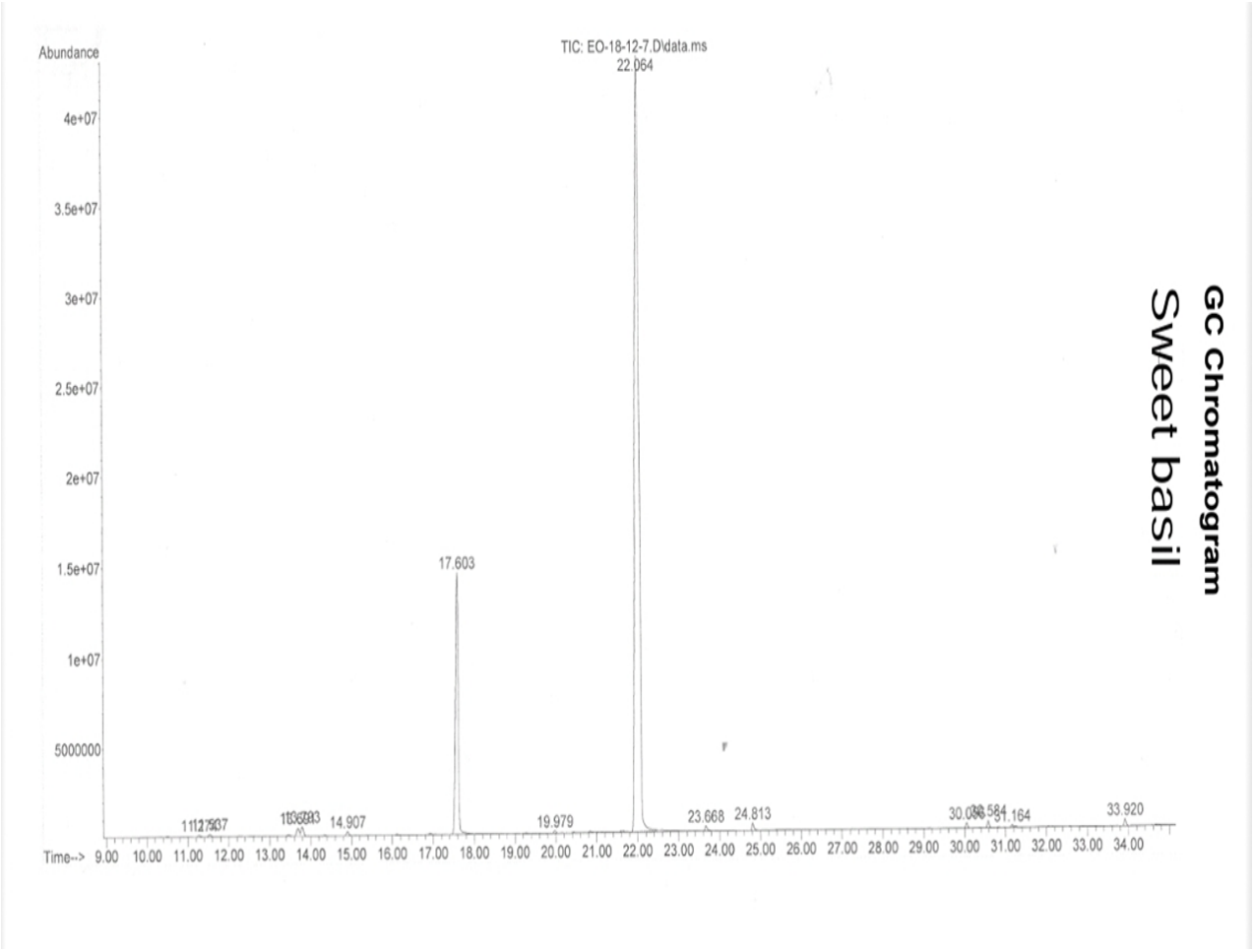


Figure 3.8: Parts of *O. bacilicum* chromatogram representing abundance of various components with peaks complemented by retention time (RT).

3.6 Conclusion

An objective of the study was to determine the chemical composition of the selected oils, and this objective was achieved successfully. Compounds of the same nature were detected in various samples, but their percentages varied from those presented in the literature. This could imply that an abundance of certain compounds in EO contributes to some of their properties. Oil characterization is of paramount significance in Somatology as it can assist in selecting oils of good quality for effective treatments.

Although the most dominant *M. chamomilla* oil compounds were also found in other samples, the assessment of this oil was particularly interesting as most of its dominant compounds were unknown.

It is highly likely that this oil contained adulterants that could have been abietic acid, pimaric acid, as well as dipropylene glycol, which claimed a large percentage of the entire oil. The presence of these unknown compounds confirmed the suspicion that some oils are in high demand, such as *M. chamomilla* and *Citrus aurantium* var. *bergamia,* are commonly adulterated. Significant amounts of linalyl acetate were detected, which is one of the most commonly used adulterants. The addition of adulterants to EOs has a place, but is also fraught with risk, as they can compromise or degrade the quality of an oil and, as a result, will no longer fulfil its purpose when applied in treatments (Vargas Jentzsch *et al*., 2019).

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CHAPTER 4

ANTIOXIDANT PROPERTIES OF SELECTED ESSENTIAL OILS

4.1 Abstract

Free radicals are produced as a result of air pollution, smoking, X-rays, and regular physiological activities. They can contribute to hyperpigmentation, dehydration, accelerated ageing, and other degenerative diseases in humans. This has remained an issue in the Somatology industry that has argued that the application of EOs, which have antioxidant characteristics, may be of importannce in treating and curbing of such diseases. An objective of the study was to determine the antioxidant activity of selected essential oils. This was achieved using the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) free radical assay. In general, *O. basilicum* exhibited a high DPPH scavenging activity with an IC_{50} value of 0.013±0.016 mg/mL. This rate was followed by *S. stenophylla* (0.027±0.019 mg/mL), *A. afra* (0.029±0.022 mg/mL), *L. angustofolia* (0.033±0.013 mg/mL), *P. crispum* (0.039±0.024 mg/mL), *C. aurantium* var. *amara* (0.109±0.131 mg/mL), *M. chamomallia* (0.155±0.134 mg/ mL), and *C. auratium* var. *bergamia* (0.319±0.331 mg/mL). These results demonstrated that EOs such as *O. basilicum, S. stenophylla, A. afra,* and *L. angustofolia* could be considered when the intention is to minimise or prevent the effects of hyperpigmentation, dehydration, ageing, and other challenges related to free radicals. However, further investigations will have to be conducted to study their efficacy *in vivo*.

4.2 Introduction

2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) is a stable free radical with a delocalised electron that can be stabilised by an antioxidant. An electron's delocalisation gives it a deep violet or purple colour, which is absorbed by ethanol at 520 nm. The efficiency of an antioxidant used is usually shown by a change in colour. The colour yellow indicates a high level of scavenging activity, whereas purple indicates a low level of activity (Kedare & Singh, 2011).

Synthetic antioxidants such as butylated hydroxyanisole (BHA), butylated hydrotoluene (BHT), propyl gallate (PG), and tert-butyl hydroquinone (TBHQ) have long been employed in the food industry due

to their superior stability, high performance, accessibility, and affordability. However, various health issues associated with their presence have been raised, such as an increase in the incidence of skin allergies, gastrointestinal issues, and cancer risk. The demand for natural antioxidants grew as a result of the conclusions reached by studies on health issues. Natural antioxidants can be found in a variety of foods such as vegetables, fruits, herbs, and spices (Lourenço *et al*., 2019). Plant chemicals that include antioxidants also have antibacterial and antifungal properties, which make them suitable for use in Somatology where natural antioxidants in the form of EOs are used. Incorporating EOs in facial treatments to manage hyperpigmentation, premature ageing, and many other skin disorders linked to oxidative stress can considerably improve the quality of the services offered by therapists if they use the aforementioned features (Lourenço *et al.*, 2019).

Antioxidants have been employed in the food business for centuries because their beneficial characteristics. They are widely used for food preservation and other biological qualities that are linked to the treatment and prevention of numerous skin problems such as premature ageing, hyperpigmentation, and other lifestyle-related illnesses caused by reactive oxygen and nitrogen species (i.e., ROS and RNS) (Miguel, 2010; Manisha *et al*., 2017; Carocho *et al*., 2018; Ahmed *et al*., 2019). EOs are thus in high demand because they have many qualities, including antioxidant, antibacterial, antiviral, and antifungal capabilities, all of which are supported by distinct compounds (Miguel, 2010).

However, EOs should be used with caution as they may have negative side effects such as irritation and sensitization. Sindle and Martin (2020) argue that adverse reactions triggered by EOs are not comparable to those caused by non-EO facial products and other chemicals used in thefielf of Somatology. Essential oils are recognised as effective antioxidants, and they are carminative, antimicrobial, and anticancer agents when used in conjunction with medication. As a result, they have the capacity to stop the chain of reaction caused by unstable free radicals (Sindle & Martin, 2020).

The antioxidant properties of EOs can be exploited to prevent and manage diseases such as Parkinson's disease and heart problems (Gautam *et al*., 2014; Saljoughian *et al*., 2018; Abd Rashed *et al*., 2021). Additionally, they can improve hyperpigmentation and accelerated ageing because they are anti-inflammatory (Flora, 2009; Foe *et al*., 2016; Adwas *et al*., 2019). According to Vashi and Kundu (2013), hyperpigmentation is induced by damage or trauma to the dermis and epidermis

(examples include acne, eczema, and psoriasis), and such damage results in the accumulation of melanocytes or pigment at the trauma site (Foe *et al*., 2016). Antioxidants can also reduce the detrimental effects of environmental pollutants such as UV light, atmospheric pollutants, and X- and Y-rays. All of these can lead to undesirable outcomes and can be related to lifestyle-related disorders (Foe *et al*., 2016).

Overproduction of melanin pigment in some parts of the skin, particularly the face, is known as hyperpigmentation. Most people who contract this affliction experience dark sports, melasma, and chloasma and suffer a variety of other hyperpigmentation conditions (Andrei *et al*., 2018). All of the above can be congregated as hyperpigmentation, which is of great concern in the Somatology industry (Andrei *et al*., 2018). The conditions mentioned above are a global epidemic, and for this reason EOs are gaining popularity because they have shown antioxidant activity in several studies (Campa & Baron, 2018; Andrei *et al*., 2018). The anti-melotogenic and anti-inflammatory effect they cause can also be beneficial in treatment of hyperpigmentation induced by acne, eczema, psoriasis, and many other skin conditions (Miguel, 2010; Foe *et al*., 2016; Campa & Baron, 2018; Andrei *et al*., 2018).

However, despite the availability of natural therapies, many people with hyperpigmentation and uneven skin tone resort to bleaching treatment as a possible solution (Andrei *et al*., 2018; Sarkic & Stappen, 2018). To curb such practices, it should be noted that EOs are cost effective and that they have minimal side effects compared to bleaching treatment (Ong & Bashir, 2012). Bleaching products contain harmful chemicals that encourage skin inflammation, which can be treated with natural products such as EOs (Ong & Bashir, 2012).

Essential oils are commonly used in the field of Somatology for massages, but recent research has showed that they can be used for various purposes other than massages and perfumes. Natural therapies help to reduce dehydration on skin, and they improve its health (Battaglia, 2003; Sarkic & Stappen, 2018). This suggests that EOs can also be used in moisturising facial treatments (Sarkic & Stappen, 2018). However, exploring the latter in depth was beyond the scope of the study. The next objective was to explore the antioxidant effects of selected EOs.

4.3 Materials and Methods

4.3.1 Sourcing the selected EOs

Most of the EOs extracted from *Citrus auratium* var. *amara* (neroli), *Lavendula officinalis* (lavender), *Citrus auratium bergamia* (bergamot), *Ocimum basilicum* (basil), *Salvia stenophylla* (blue mountain sage), *Artemisia afra* (wormwood), *Matricaria chamomallia* (chamomile), and *Petroselinum crispum* (parsley) were supplied by Thitapoho farm in Tweespruit, South Africa, while others were supplied by Emmegenes Body Shop in Bloemfontein. The results of the chemical analyses of all these oils were reported in Chapter 3, and reference to these results will be made in the current chapter.

4.3.2 Chemical reagents for antioxidant evaluation

The antioxidant activity of each essential oil was determined by means of the free radical-scavenging method using a stable radical 2,2-dipheny-1-picrylhydrazyl (DPPH) assay with slight modification (Bektas *et al*., 2016). A DPPH stock solution was prepared, and 10-fold dilutions of the samples were performed in methanol. These were plated out in triplicate in a 96-well plate. 2,2-dipheny-1-picrylhydrazyl (96 µM) was added to the test samples while methanol was added to the controls. The plate was incubated in the dark for 30 min at room temperature before measuring absorbance using a Spectramax M2^{e} ascorbic acid served as a positive control.

4.3.3 Spectrophotometric measurements

A fresh DPPH was prepared at a concentration of 0.004% w/v, and this was mixed with ethanol. The spectrometer was zeroed using ethanol as a blank solution. The first solution of the series concentration was DPPH with ethanolic solution only, and absorbance was measured at 517 nm using Spectramax M2^{e}.

4.3.4 Percentage inhibition activity of DPPH

The percentages of the antioxidant activity of the essential oils and ascorbic acid were calculated using the following formula:

Percentage of inhibition of DPPH activity (%) = (A-B)/A ×100%

Where: A = optical density of the blank,

B = optical density of the sample.

The antioxidant half maximal inhibitory concentration (IC_{50}) for the plant samples and the standard were calculated using line regression graphs that were plotted.

4.3.5 Data analysis

The antioxidant activity of each tested EO was calculated as a percentage of inhibition and the inhibition rates of the different oils, along with those of the ascorbic acid, were presented in different concentrations and further plotted and tabulated. The IC_{50} values were calculated using the regression line that was plotted.

4.4 Results

Table 4.1: Antioxidant activity of the eight EOs against DPPH free radical

Concentration (mg/ml)	**% DPPH scavenging activity**								
	Citrus auratium var. *amara*	*Lavendula officinalis*	*Citrus auratium* var. *bergamia*	*Artemisia afra*	*Ocimum basilicum*	*Salvia stenophylla*	*Matricaria chamomallia*	*Petroselinum crispum*	Ascorbic Acid (Positive control)
2.21	45.30±4.81	36.40±2.27	44.43±0.36	46.39±2.04	48.62±3.45	60.26±0.54	31.62±1.33	48.56±7.17	88.42±0.247
0.212	33.75±0.45	26.98±5.77	24.22±5.77	25.32±13.24	45.45±5.52	31.35±1.04	27.95±1.52	22.81±1.54	80.8±0.83
0.0212	26.75±0.77	21.53±8.67	19.99±3.45	15.51±4.31	35.65±4.69	21.04±0.32	18.30±2.00	17.31±5.08	77.25±1.69
0.00212	22.27±0.19	15.83±6.38	15.11±5.27	13.24±11.25	30.30±1.59	19.17±0.89	15.90±0.42	11.65±2.29	73.47±2.28
0.000212	16.65±0.75	11.41±8.62	9.88±3.49	6.15±2.04	22.21±4.68	11.00±0.33	8.25±1.96	7.19±5.03	71.39±4.68
0.0000212	12.25±0.19	5.82±6.39	7.99±0.85	5.20±7.04	20.34±1.65	9.15±0.89	5.89±0.43	1.64±2.30	59.75±3.56
IC_{50} (Average)	0.109±0.131	0.033±0.013	0.319±0.331	0.0279±0.022	0.013±0.016	0.027±0.019	0.155±0.134	0.039±0.024	0.003±0.0001

Data presented as mean (± SD n=3)

4.5 Discussion

The antioxidant activity of each of the selected EO samples was assessed by DPPH assay at six (6) different concentrations ranging from 0.0000212 mg/mL to 2.21 mg/mL. The ability of the sample to donate an electron, or act as an antioxidant to a free radical, was observed if the colour changed from purple to yellow. However, antioxidant activity is not always determined by colour change as some of the oils have an original deep blue or yellow colour. The antioxidant activity was expressed in scavenging percentage (%) (**Table 4.1)** and 50% concentration (IC_{50} mg/ml**) (Figures 4.1-4.4).** The lowest IC_{50} represented a strong activity while the highest demonstrated weak or low activity. The mid-point between low and strong activity represented mild activity. The scavenging activities were expressed in percentages, where a high percentage indicated strong scavenging activity and a low percentage indicated weak activity. The results obtained from the tested samples were expressed as ascorbic acid equivalents.

As was earlier affirmed, environmental factors may influence the composition of EOs, which results in some oils containing sesqiterpenes or non-isoprenoid compounds while others may contain monoterpene hydrocarbons. The presence of these molecules may endow each oil with qualities such as antioxidant activity. According to the literature, *S. stenophylla* contains pinene, selinene, limonene, myrcene, linalool, and many other monoterpenes that render it a strong antioxidant agent (Marin *et al*., 2016; Ahmed *et al*., 2019). These compounds were detected in *S. stenophylla* in the current study. Sequitepertenes are known for their antimalarial, antibacterial, and analgesic properties, and are thus useful in Somatology for various treatments. The aforementioned compounds (see Chapter 3 **Table 3.1**), including apiole, were observed in *P. crispum;* however, the scavenging activity varied, which was also observed by Tisserand and Young (2014).

Ascorbic acid was used as a standard chemical and the scavenging activity of all the examined compounds was found to be half that of ascorbic acid (88.42±0.247 to 59.75±3.56). This observation does not imply that the samples were devoid of antioxidant compounds; rather, it implies that an increase in sample concentration was required to raise activity. **Table 4.1** shows that *S. stenophylla* (60.260.54 to 9.150.89) had the highest percentage of scavenging activity when compared to that of the other oils, followed by *O. bacilicum* (48.62±3.45 to 20.34±1.65).

The activity of each of the others was as follows: *Citrus auratium* var. *amara* (45.30±4.81 to 12.25±0.19*), A. Afra* (46.39±2.04 to 5.20±7.04), *C. auratium* var. *bergamia* (44.43±0.36 to 7.99±0.85), *L. angustofolia* (36.40±2.27 to 5.82±6.39), *P. crispum* (48.56±7.17 to 1.64±2.30), and *M. chamomilla* (31.62±1.33 to 5.89±0.43).

Conversely, *Ocimum. basilicum* exhibited a higher IC_{50} value at 0.013±0.016 mg/mL, followed by *S. stenophylla* (0.027±0.019 mg/mL), *A. afra* (0.0279±0.022 mg/mL), *L. angustofolia* (0.033±0.013 mg/mL), *P. crispum* (0.039±0.024 mg/mL), *C. aurantium* var. *amara* (0.109±0.131 mg/mL), *M. chamomallia* (0.155±0.134 mg/mL), and *C. auratium* var. *bergamia* (0.319±0.331 mg/mL). Ascorbic acid had the highest activity with an IC_{50} of 0.003±0.0001 mg/mL, while all the investigated oils showed a substantially lower scavenging capability than the reference sample. Ascorbic acid showed a strong scavenging activity at the lowest concentration.

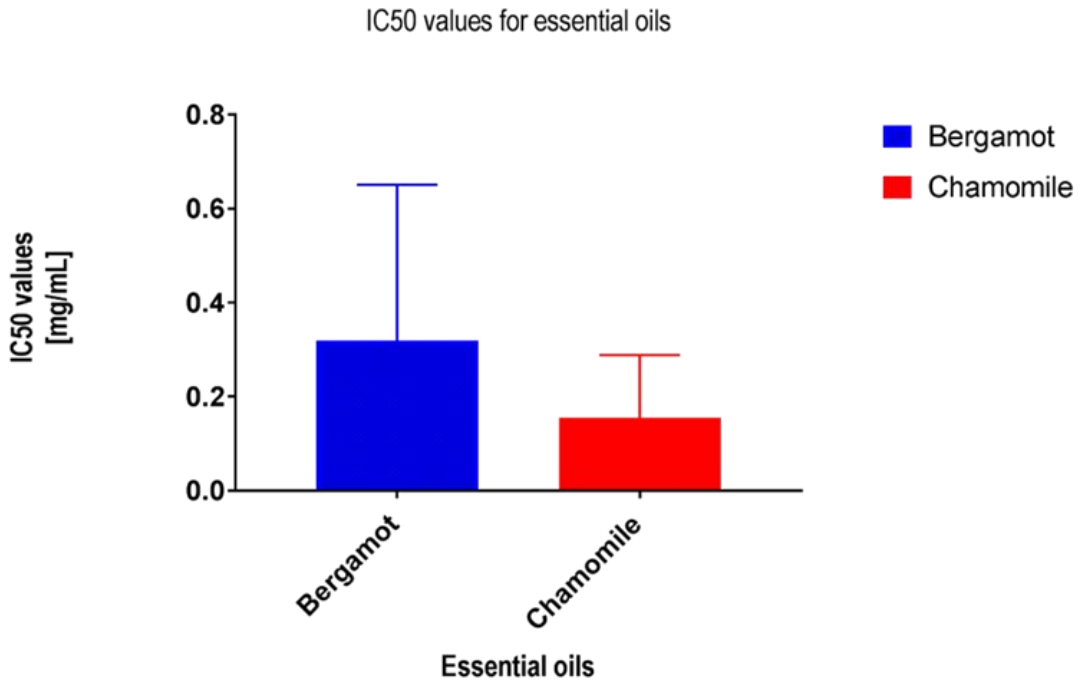

Figure 4.1: Bar graph illustrating the average IC_{50} values for bergamot (*C. arauntium* var. *begarmia*) and chamomile (*M. chamomilla*) EOs.

Figure 4.1 shows the average IC_{50} values of *C. arauntium* var. *bergamia* and *M. chamomilla*. When comparing bergamot oil to chamomile oil, the IC_{50} of *C. arauntium* var. *bergamia* oil was greater. As a result, it was concluded that bergamot oil had weak antioxidant activity compared

to that of the other evaluated samples and ascorbic acid. *Matricaria chamomilla* was found to have strong antioxidant activity in a study by Firat *et al.* (2018), which is contrary to the findings of the current study. This difference could be attributed to the variation in *M. chamomilla* oil observed in the current study (see Chapter 3, GC-MS data). According to Xing *et al*. (2019), the *C. arauntium* var. *bergamia* oil used in their study exhibited a strong antioxidant activity, which means that the earlier study's findings are not in agreement with those of the current study.

Several factors that contributed towards the abnormal absorbance readings were discovered in the experiment, resulting in the misinterpretation of data. This necessitated a sample blank to eliminate the colour of the sample. Abnormal absorbance readings were detected because the intensified purple colour differed in every oil, especially in those that had colour. The absorbance of the DPPH radical can be influenced by various factors such as light, oxygen, incubation period, and pH (Oczelik *et al*., 2003; Firat *et al*., 2018). For example, the deep blue colour of the *M. chamomilla* that was used in the current study resulted in higher absorbance which made it impossible to calculate the scavenging activity. Moreover, earlier studies demonstrated that incubation at 90 minutes produced positive outcomes compared to incubation at 30 minutes (Firat *et al*., 2018). In the current study, the incubation duration was 30 minutes, which may have influenced the findings.

Another factor that could have affected the quality of the oils was their chemical composition. When GC-MS analysis (see Chapter 3) was performed on *M. chamomilla*, a lengthy list of unknown compounds was revealed, and these could have been connected to its weak scavenging activity. The long list of unidentified compounds could thus explain why the current findings differed from those of Firat *et al.* (2018) and Piri *et al*. (2019). -Bisabolol oxide A and B, (E) -farnesene, -bisabolone oxide A, chamazulene, and -bisabolol were reported by Firat *et al*. (2018). Piri *et al*. (2019) highlight that *M. chamomilla* possesses -bisabolone oxide A (45.64–65.41%), (E)- and (Z)-bisabolene (42.76 and 40.08% respectively), oxygenated sesquiterpenes (53.31–74.52%), and oxygenated sesquiterpene. These results by Piri *et al*. (2019) agree with those of Firat *et al*. (2018), and they thus both found that *Matrcaria chamomilla* exhibited strong antioxidant activity. However, this was not corroborated by the current study. The compounds that were mentioned possibly encouraged the strong scavenging activity observed in those studies, whereas their absence might have encouraged weak activity in the current study.

However, a fraction of bisabalol was detected in the current study. Upon reflection, it is argued that the antioxidant capabilities of *M. chamomilla* oil could have been influenced by the foreign compounds detected in its composition. It can thus be concluded that *M. chamomilla* showed weak scavenging activity connected to the $IC_{50\ as}$ reported in **Table 4.1**. Therefore, if the objective of a Somatology therapist is to apply bergamot and chamomile topically, the application of a high concentration of these EOs may not be feasible.

In the current study, the highest IC_{50} was observed for *C. aurantium* var. *bergamia*, thus indicating poor antioxidant activity. This suggests that the oil was active against radicals at its highest concentration. This finding can be connected to the limonene that was detected in *C. aurantium bergamia*, which is claimed to be unstable (Ren *et al*., 2017). D-limonene is known as a potent antioxidant agent, but due to its instability, a weak scavenging activity was observed in the present study as well as in the studies by Djilani and Dicko (2012) and Sarkic and Stappen (2018). The above concerns could be linked to its long shelf life, as the oil was purchased in 2017 while the study was conducted in 2021. According to a study by Kokina *et al*. (2019), the activity of EOs that have been stored for more than 12 months is compromised as their properties are affected. Keeping EOs on the shelf for a long time will thus have a negative impact on their properties as some compounds, like limonene, become unstable with time.

Kokina *et al*. (2019) affirm the importance of paying attention to the shelf life of EOs, as they found that those that had been stored for more than 12 months had reduced therapeutic value and aromatic quality. A similar observation was made in the current study, as *C. aurantium* var. *amara* and *C. aurantium* var. *bergamia* had weak antioxidant activity. Therefore, the more intact its composition, the more effective an oil becomes (Kokina *et al*., 2019). Inappropriate shelf life resulting in unstable compounds is common in citrus oils, thus their antioxidant activity is easily compromised (Kokina *et al*., 2019).

Citrus. aurantium var. *amara* exhibited weak scavenging activity (see Chapter 3, GC-MS analysis). Components such as cymene, D-limonene, and -Pinene (that were detected in minimal quantities in the current study) are antioxidants found in neroli oil. According to Sarrou *et al*. (2013), the antioxidant activity of *C. aurantium* var. *amara* oils taken from various parts of the plant revealed that oil extracted from old leaves had weak antioxidant activity whereas oil

extracted from fresh leaves had substantial antioxidant activity. Sarrou *et al*. (2013) concluded that the antioxidant activity of oil extracted from the old leaves may have been impaired due to metabolite depletion. In terms of shelf life affecting the potential of oils to display antioxidant capabilities, the latter authors' findings are similar to those of the current study as well as those reported by Kokina *et al*. (2019).

The findings of Sorrou *et al*. (2013) were used as reference for comparison with the results of *C. aurantium* var. *amara.* It was revealed that the neroli oil possessed geriniol, -terpinene, and terpenene, which were also observed by the former author. These compounds are claimed to have potent antioxidant activity even at low concentrations; however, this depends on their validity (Sarrou *et al*., 2013). All the compounds referred to were discovered in both studies, but the outcomes were different.

The above-mentioned points may raise uncertainties about the quality of EOs. However, they highlight the importance of their compounds and the therapeutic qualities associated with them. The outcomes of this study can be attributed to small quantities of free radical scavenging compounds used and the failure to follow storage instructions. *Ocimum basilicum, L. angustofolia, A. afra, S. stenophylla, C. aurantium* var. *amara,* and *P. crispum* all reached IC_{50} values that indicated their ability to serve as weak to strong antioxidant agents in comparison to ascorbic acid (Tan *et al*., 2018; Forni *et al*., 2019).

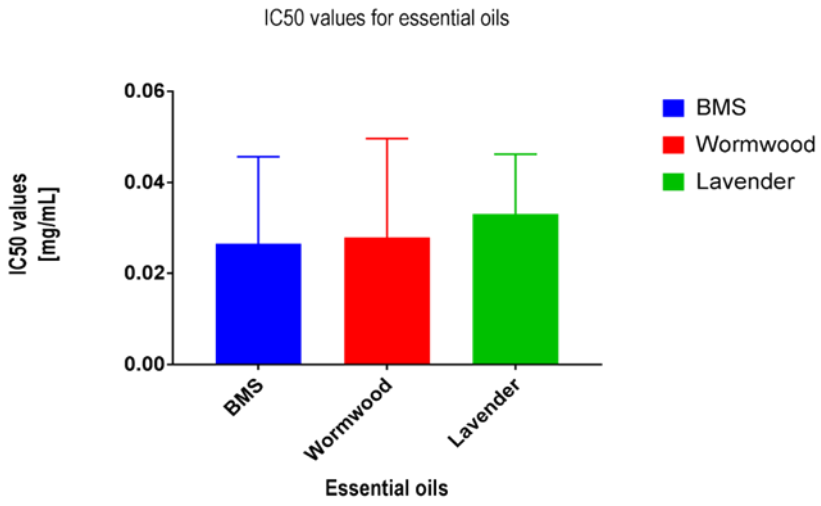

Figure 4.2: Bar graph that illustrates the average IC_{50} values for wormwood (*A. afra*), lavender (*L. angustofolia*), and blue mountain sage (*S. stenophylla*) EOs.

L. angustofolia showed antioxidant activity by suppressing DPPH radical with an IC_{50} of 0.0330±013 (see **Figure 4.2**). A discrepancy among the plotted EOs was evident (see **Figure 4.2**) as *L. angustofolia* showed mild scavenging activity compared to *A. afra* and *S. stenophylla*. The IC_{50} finding suggests that *L. angustofolia* interacted with DPPH at a relatively moderate concentration. This moderate scavenging activity was different to the activity reported by Nurzyńska-Wierdak *et al*. (2016), because they found that *L. angustofolia* had a strong antioxidant activity. Additionally, Silva *et al*. (2015) report that antioxidant activity was observed in *L. angustofolia* oil that was concentration dependent, which is a trend that was observed in the current study as well (refer to **Table 4.1**) because the activity of the oils against the DPPH increased as oil concentration increased. These results were possibly influenced by the composition (see Chapter 5) of the *L. angustofolia* EO, as it was observed that its dominant compounds (1,8 cineole 7.3%, linalool 23.9-29.9%, and linalyl acetate 22.3-32.1%) were inclined towards antibacterial activity.

According to El Euch (2019), the antioxidant activity of the *Salvia* species appears to be powerful against DPPH due to their composition and richness in chemicals such as thujone, camphor, and thujene 1,8 cineole, which were discovered in the assessed *Salvia* samples. The compounds indicated above were also found in *S. stenophylla*, but in different proportions. The antioxidant activities of blue mountain sage and wormwood were found to be quite strong, and their IC_{50} was found to be comparable to that of *O. bacilicum*. The activity that was observed suggests that the oils should be considered when preparing antioxidant-rich blends, particularly for facial treatments in the Somatology field. The observed activity was consistent with that in a study by Kane *et al*. (2019), who detected phenols and flavonoids in the EO of *O. bacilicum*. Flavonoids and phenols are powerful antioxidants that were presented in high concentrations in the study conducted by Kane *et al*. (2019). This emphasizes the necessity of purchasing oils from reliable suppliers who consider the cultivation environment. These variables are significant because they influence the properties and composition of EOs (refer to Chapter 3, GC-MS data).

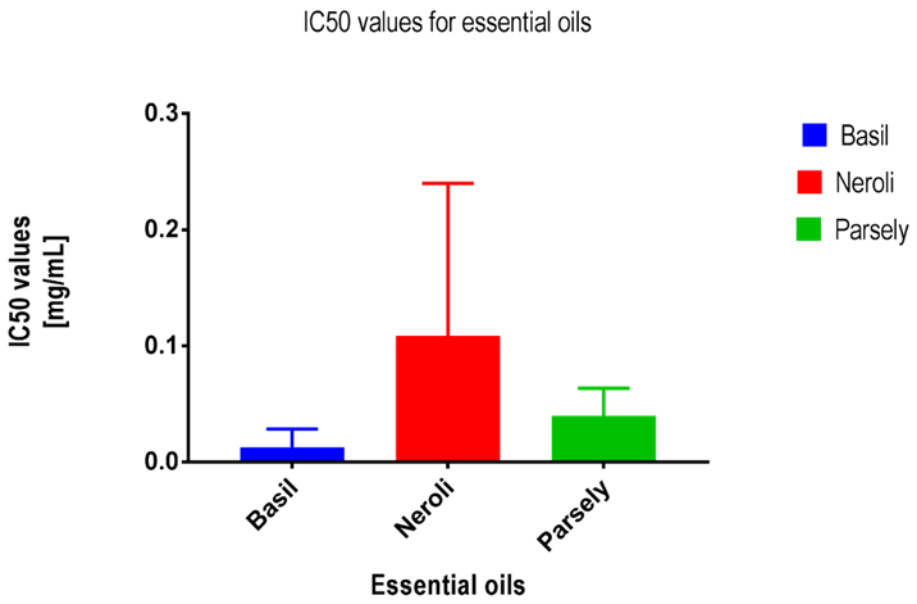

Figure 4.3: Bar graph that illustrates the graphic IC_{50} of basil (*O. bacilicum*), neroli (*C. aurantium* var. *amara*), and parsley (*P. crispum*) EOs.

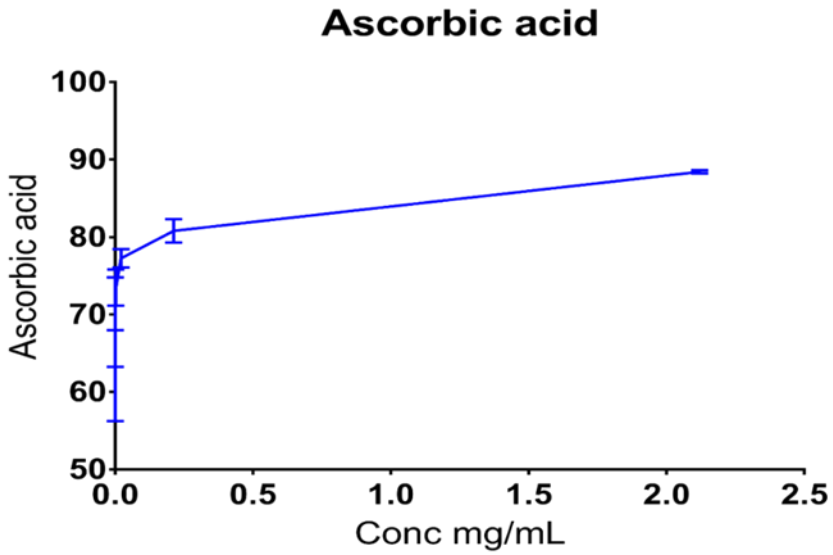

Figure 4.4: Graph illustrating concentration versus inhibition percentage of ascorbic acid against DPPH radical.

Most of the oils under study showed varying antioxidant activity against the DPPH radical. Ascorbic acid was used as a positive control, and it showed purple elimination at all concentrations. *Ocimum basillicum* had the lowest IC_{50} (0.013±0.016) compared to the other oils. High content of estragole (79%) was detected in *O. bacillicum,* signifying strong antioxidant activity. This finding was similar to that of Avetisyan *et al*. (2019), who observed 57% estragole,

which suggested strong antioxidant activity. Researchers such as Ahmed *et al*. (2019) and Avetisyan *et al*. (2017) found that *O. basilicum* oil had reasonably strong antioxidant properties, but its activity was not as strong as that of BHT, which was utilised as a positive control in their study. The dominant compounds of *O. basilicum* in the current study were similar to those reported by Ahmed *et al*. (2019) and Avetisyan *et al*. (2017), but the percentages of their presence varied. Both studies indicated that linalool (17-57% in both studies) and estragole (31-68% in both studies) were the dominant compounds. In earlier studies, D-limonene (16%) and beta Selinene (26%) were detected in *P. crispum* and were claimed to be significant antioxidant agents (Patil & Chandra *et al*, 2017; Sarkic & Stappen, 2018). These claims were corroborated by the current study because parsley had an IC_{50} of 0.039±0.024, which indicated that it could fight free radicals.

The findings discussed above imply that the EOs under study can be safely utilised in the Somatology field to prepare blends that will prevent oxidative stress from causing cell damage. The compositions of *P. crispum* in the study and in the literature slightly differed, probably as the species the oil had been extracted from came from different countries. According to Marin *et al*. (2016), the *P. crispum* oil in their study possessed the following compounds: myristicin (36%), apiole (21%), and pinene respectively. Myristicin ranged between 13-23%, apiol between 17-27%, and pinene. The literature reports that environmental factors also affect the composition of EOs. Therefore, when purchasing these oils, aspects such as harvest time, season, and extraction method should be considered (Sampaio & Da Costa, 2018).

The results of the current study are significant because they highlight the antioxidant properties found in the EOs of *O. bacillicum*, *S. stenophylla*, *A. afra*, *C. auratium* var. *amara*, *L. angustofolia*, and *P. crispum*. This confirms that they can be used for oxidative stress-related illnesses and hyperpigmentation treatments. They can also be considered for use in blend preparations for various beauty treatments such as massages and facials. These are significant findings that should be considered by somatologists who should not randomly use just any oils for massage and relaxation. Furthermore, the use of EOs in treatment regimens can reduce unnecessary costs and make health and beauty treatments also accessible for those who cannot afford expensive salons for laser or bleaching treatments.

The beauty industry does not need to look further for new cures because EOs are already available. However, it is necessary to research their properties so that they may be safely and effectively used in the beauty industry and for the prevention and cure of body ailments in humans. This can be accomplished by reintroducing them to the market using a different approach that considers their antioxidant and antibacterial properties. As the study's findings demonstrated that essential oils have antioxidant capabilities, the beauty community should look beyond the notion of mere relaxation and learn more about these oils' properties and composition to understand how these should affect their choice of treatment.

4.6 Conclusion

One objective of the study was to explore the antioxidant activity of selected EOs. The majority of the oils that were tested demonstrated promising antioxidant qualities, which means that they can collectively be used in the beauty industry as natural cures, mostly for facial treatments, to combat ageing, hyperpigmentation, skin dryness, and other skin-related disorders. Essential Oils are far more than just a massage medium as they may be utilized to treat a variety of problems. The findings encourage the use of essential oils in topical treatments specifically. Most importantly, the study provides scientific evidence that these oils can act as antioxidants that are useful for facial and other treatments to address skin afflictions such as hyperpigmentation, dehydration, and premature ageing. However, in order to assess their potential applications in the Somatology field, the oils' activity must be tested extensively *in vivo*.

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CHAPTER 5

ANTIBACTERIAL PROPERTIES OF SELECTED ESSENTIAL OILS

5.1 Abstract

The use of EO extends beyond its use for relaxation and massage within the field of Somatology. The literature indicates that EOs have antioxidant and antimicrobial properties, therefore, it is imperative to confirm the antimicrobial activity of selected EOs used in the Somatology industry to diversify their application. An objective of the study was to evaluate the antibacterial activities of selected EOs against *Escherichia coli*, *Staphylococcus aureus,* and *Bacillus cereus*. The antibacterial activity of the selected oils was assessed using the agar dilution method and microdilution assay at six different concentrations. The results revealed that *O. bacilicum* and *C. aurantium* var. *bergamia* had strong (≥26 mm) antibacterial activity against *E. coli*. Mild antibacterial activity (≥16 mm) was observed with *L. angustofolia, C. aurantium* var. *amara,* and *S. stenophylla* when tested against *S. aureus*. *Escherichia coli* was resistant to some of the oils except to *L. angustofolia*, *S. stenophylla*, *O. bacilicum,* and *C. aurantium* var. *bergamia,* while *B. cereus* showed susceptibility to the oils, except *C. aurantium* var. *amara, C. aurantium* var. *bergamia* and *M. chamomilla. Staphylococcus aureus* showed resistance to *P. crispum* and *M. chamomilla.* Antibacterial activity was observed for *L. angustofolia, C. aurantium bergamia, S. stenophylla,* and *O. bacilicum.* This suggests that these oils could be considered for use in antibacterial blends for application in the Somatology industry.

5.2 Introduction

The concept of microorganisms emerged in the early ages when they were primarily connected with illnesses (Krüger *et al*., 2019). However, studies have revealed that commensal bacteria play an important role in preventing pathogenic bacteria from causing harm to the host (Dykhuizen, 2005). Despite this, microorganisms are still related to a variety of diseases such as meningitis, diarrhoea, bacteremia, and food poisoning (Thompson *et al*., 2021). *Staphylococcus aureus* is generally found in the human body, primarily on the skin and in the nasopharynx (Berger *et al*., 2018). Furthermore, various skin infections have been linked to *S. aureus*, whereas

urinary tract infections are triggered by *E. coli* (Berger *et al*., 2018). *Escherichia coli* is found in the gastrointestinal tract of humans and warm-blooded mammals (Denamur *et al*., 2021), but *B. cereus* is found in the soil and its spores can be isolated from food, plants, and invertebrates (Bağcoiğlu *et al*., 2019).

Common strains that contribute to cross-contamination between human clients in the beauty industry are *S. aureus*, *E. coli,* and *B. cereus*. Furthermore, the stains mentioned above have been isolated from contaminated surfaces in spas, resorts, hotels, and beauty salons. The proliferation of *E. coli* occurs in shared or public facilities such as saunas, steam rooms, bathrooms, spas, resorts, and swimming pools where it results in contamination and the spread of diseases (Miller & Diep, 2008; Dalman *et al*., 2019; Wibmann *et al*., 2021; Abney *et al*., 2021). Bacterial species are becoming more resistant to conventional antibiotic treatment; therefore, there is an increased demand for alternative, efficacious antibacterial agents. Fortunately, in the field of Somatology, most treatments offered to clients use a combination of EOs that have an antimicrobial effect on clients when they are treated appropriately (Warnke *et al*., 2013; Yap *et al*., 2014).

The most integral characteristic of EOs is their hydrophobic nature, which allows them to partition between the lipid layers of the cell membrane of a bacterium to reach the cytoplasm (Dhifi *et al*., 2016). Essential oils possess compounds such as terpenes, terpenoids, phenols, ketones, and many others that allow them to penetrate the bacterial cell wall and interact with various components, ultimately leading to cell death (Nazzaro *et al*., 2013; Dhifi *et al*., 2016). Several studies have reported that the phenolic compounds in EOs contribute to their antibacterial properties. Phenolic compounds can interfere with energy-producing enzymes at low concentrations, while they contribute to protein denaturation at higher concentrations (Man *et al*., 2019). Furthermore, the hydrophobic nature of EOs allows them to penetrate through cells of Gram-positive bacteria and not through those of Gram-negative bacteria (Man *et al*., 2019). Gram-positive bacteria have a significantly thicker peptidoglycan than Gram-negative bacteria (**Figure 5.1)**. Despite their thick peptidoglycan layer, Gram-positive bacteria have a smooth, single-layered cell wall that allows hydrophobic molecules to flow through and reach the cytoplasm. Therefore, antibacterial activity of EOs on Gram-positive bacteria can be attributed to the presence of phenolic compounds, which causes cell death through leakage of the cell

contents (Lobiuc *et al*., 2023; Man *et al*., 2019). Gram-negative bacteria tend to be resistant to most EOs because they have a more complex cell structure compared to Gram-positive cells (Lopez-Romero *et al*., 2015).

Gram-negative bacteria have a peptidoglycan of 2-3 nm and dry cell content (Beger *et al*., 2018). Gram-negative bacteria possess a wavy, double-layered cell membrane that provides integrity to the cell and houses the porins and lipopolysaccharides (Vollmer *et al*., 2008). Porins are situated on the membrane where they control osmolarity and the movement of salts and nutrients that are enter and/or exit the cell. Additionally, they modulate cellular permeability and antibiotic resistance (Choi & Lee, 2019). Lipopolysaccharides (LPS) create a barrier that prevents hydrophobic solutes from accessing the internal environment of the cell. Hence, resistance occurs to some EOs because of hydrophobicity. Lipopolysaccharides possess endotoxins that give Gram-negative cells a pathogenic characteristic (Maldonado *et al*., 2016).

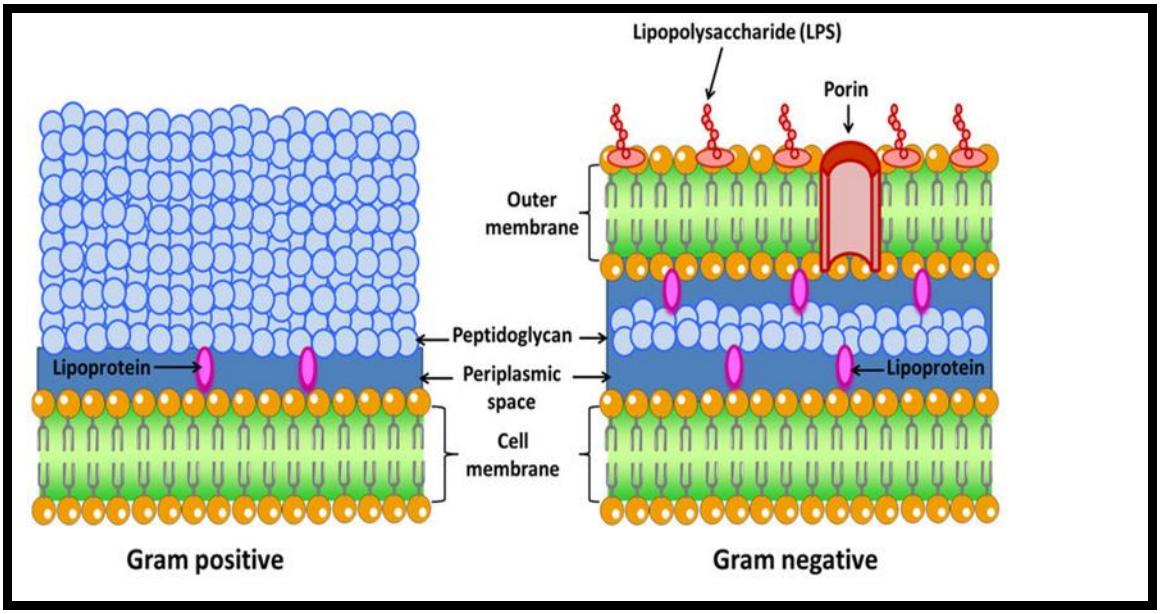


Figure 5.1 Shows a difference between Gram-positive and Gram-negative bacteria. Porins, wavy doubled layered membrane, outer and inner membrane are present on gram negative bacteria and absent on gram positive bacteria. A difference in thickness of both diagrams is clearly demonstrated.
Source: Jiménez-Jiménez *et al*., 2022

It is a typical practice in the Somatology industry to use EOs primarily for room fragrance or massages rather than to prevent cross contamination in treatment rooms (Werner, 2009). The

potential and applicability of EOs in Somatology will be limited until somatologists have adequately explored the additional benefits of EOs, such as their antibacterial properties and the effects of alternative routes of administration. Currently, air fresheners, humidifiers, and vaporizers are used to fragrance the rooms in spas and saunas. This practice can have enhanced effects through the preparation of antibacterial blends that will reduce the presence of bacteria in the air (indoors) and limit the potential for cross-contamination within the spa environment. This will ensure the safety of clients and therapists against bacterial species. It was against this backdrop that it was crucial to determine and evaluate the antibacterial action of commonly used EOs.

5.3 Materials and Methods

The objective of this chapter was to assess the antibacterial properties of *Citrus aurantium* var. *amara (*neroli), *Lavendula angustofilia* (lavender), *Citrus aurantium* var. *bergamia* (bergamot), *Artemisia afra* (wormwood), *Petroselinum crispum* (parsley), *Matricaria chamomallia* (German chamomile), *Salvia stenophylla* (blue mountain sage), and *Ocimum basilicum* (basil). The selected oils were supplied by Emmagenes body shop (Bloemfontein) and Thitapoho farm in Tweespruit. The oils were dissolved in 96% ethanol, 10% dimethyl sulfoxide (DMSO), and p-Iodonitrotetrazolium chloride (INT). Stock solutions of the EOs were adjusted to 64 mg/ml while the bacterial suspension was adjusted to 0,5 McFarland standard. The essential oils of choice were dissolved in both DMSO and ethanol, where the first group of EOs was dissolved in 10% DMSO, while the second group was dissolved in ethanol. Group A1 of Kirby-Bauer assay comprised concentrated (pure) EOs, while Group A2 of the EOs was dissolved in 96% ethanol (diluted).

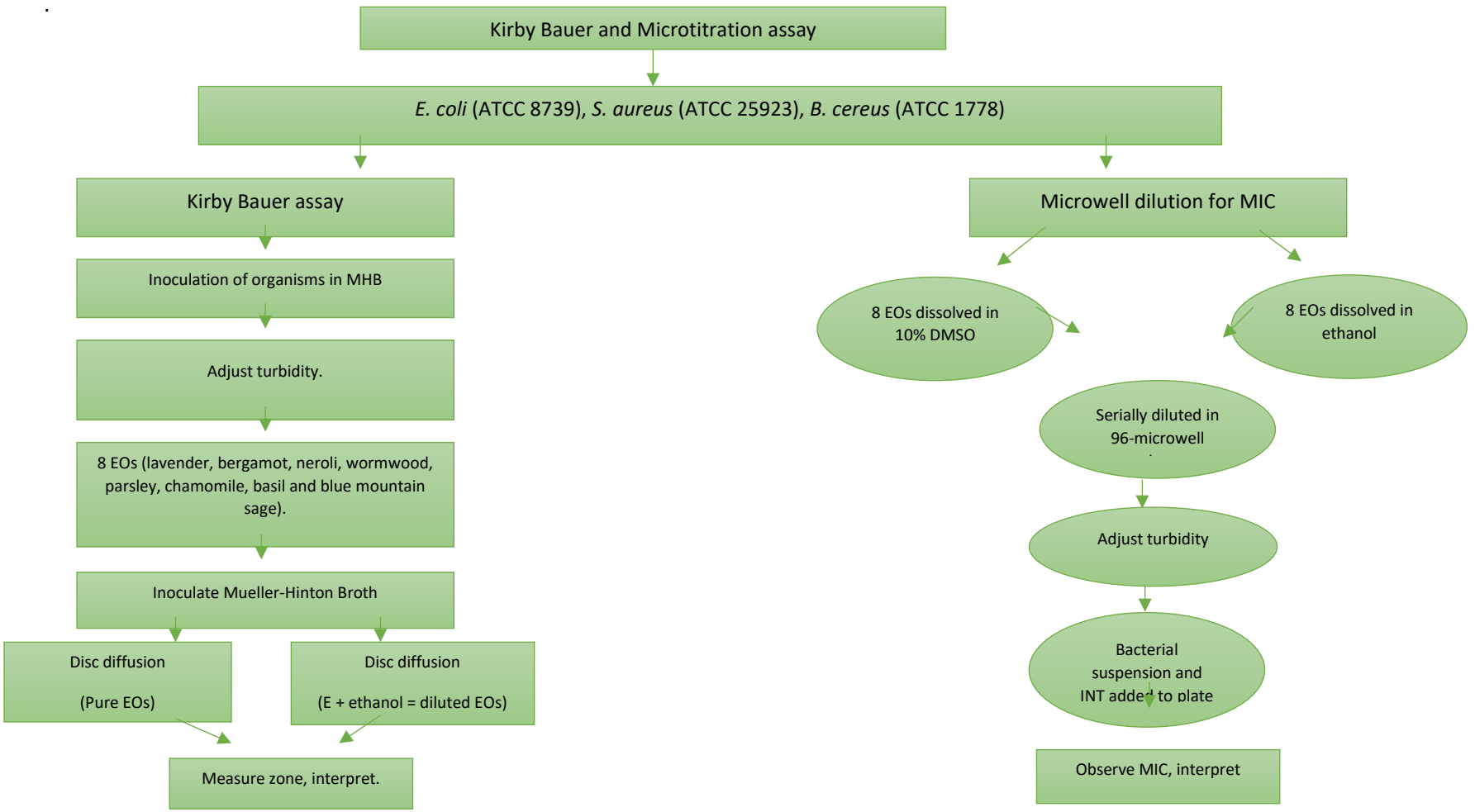


Figure 5.2: Evaluation process to determine the antibacterial activity of the selected EOs

5.3.1 Antibacterial assay (microwell dilution assay)

The antibacterial activity of the EOs was assessed against *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778), and *Escherichia coli* (ATCC 8739) using the microwell dilution assay method (Eloff, 1998). All microorganisms used in the minimum inhibitory concentration (MIC) assays were grown on MHB for 24 hours at 37°C. Microdilution assay was performed in a sterile 96-well microtiter plate. Concisely, a final bacterial suspension of $5x10^5$ mg/mL was prepared using MHB and reciprocated into a 96-well plate. A stock solution of 10% DMSO EOs (first group) and ethanol (second group) was added in the 96-well plate. Into the first 96-well microtitre plate, 100µL sterile MHB and 100µL diluted EO were added. Serial 2-fold dilution tests were performed to reach final concentrations of 100, 50, 25, 12.5, 6.25, 3.125 mg/mL. In each well 10µL of inoculum was added and the plates were incubated for 24 hours at 37°C to determine the MIC values. p-Iodonitrotetrazolium chloride (40 µl of 0.04% [w/v]) was added directly to the culture medium of each well for colour change and incubated for 6 hours. Bacterial growth was indicated by colour ranging from pink to violet. Three replicates were run for each oil. Thiabendazole (TBZ) was used as a positive control.

5.3.2 Antibacterial bioassay (disk diffusion assay)

Disk diffusion assay (the Kirby-Bauer method with slight modification) was used to screen for antibacterial activity of the selected EOs against Gram-negative bacteria *Escherichia coli* (ATCC 8739), Gram-positive bacteria *Staphylococcus aureus* (ATTC 25923), and *Bacillus cereus* (ATCC 11778). Each lyophilized bacteria disk was transferred to test tubes containing Muller Hinton broth (MHB) and incubated overnight at 37°C. One bacteriological loop from each broth was streaked on MHB plates and incubated for 48 hours at 37°C. After the incubation period, a single colony was removed, streaked on a Muller Hinton agar plate, and incubated at 37°C for an additional 24 hours. The organisms were re-grown in broth to allow the correct adjustment of turbidity to that of 0.5 McFarland standard. Then 0.5cm diameter of a hole was created on the center of the petri dish and Essential oils of *Citrus aurantium* var. *amara*, *Lavendula angustofilia, Citrus aurantium* var. *bergamia, Artemisia afra*, *Petroselinum crispum, Matricaria chamomilla, Salvia stenophylla*, and *Ocimum basilicum* were poured and incubated for 24 hours. The inhibition zone and results were then recorded in accordance with the measured diameter (mm) as illustrastrated on **figure 5.3** sketch 3.

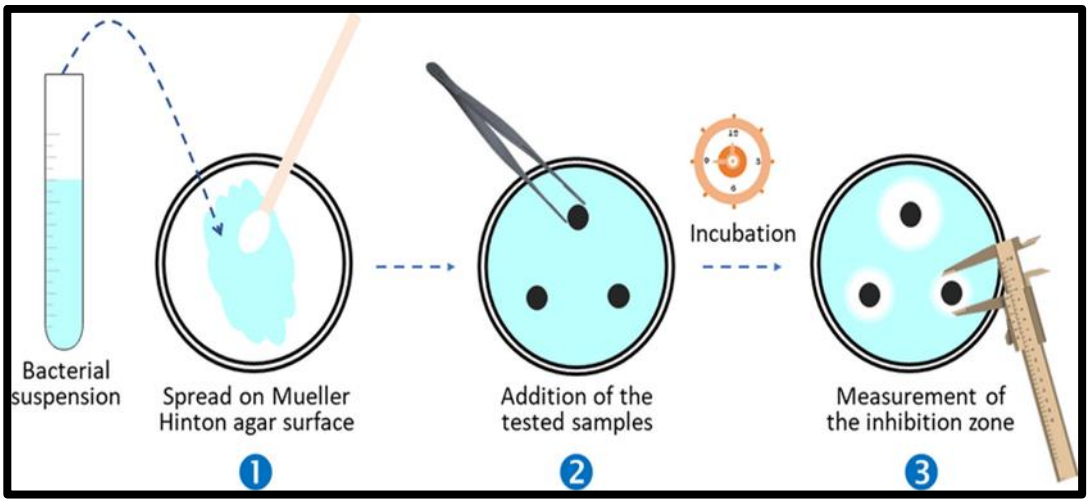


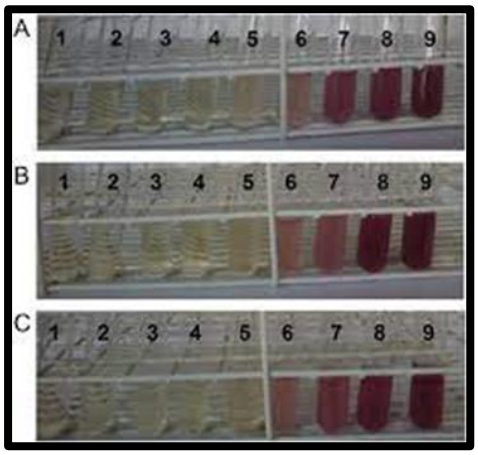
Figure 5.3: Schematic diagram of antibacterial assay (Kirby-Bauer)
Source: El Guerraf *et al*., 2022.

5.4 Results and Discussion

5.4.1 Microwell results of EOs dissolved in ethanol.

Minimum inhibitory concentration (MIC), also known as a microdilution assay, has been popularly used for the determination of the lowest concentration that completely inhibits the growth of tested microorganisms (Chouhan *et al*., 2017). **Figure 5.4** illustrates the process of microdilution that is performed in test tubes. The MIC was determined by observing the various colours as seen in the figure, where the different shades of pink (**6-9**) represent growth and a clear colour (**1-5**) represents inhibition. In this instance, the MIC of test tube 5 was observed. **Figure 5.5** (below) demonstrates the microdilution performed in the 96-well plates. Minimum inhibitory concentration values were similarly determined by the colour change that is visible in this figure. **Image A** in **Figure 5.5** is an example of the breakdown of an MIC determination. **Image A**, **column B** represents a positive control, while **column C** represents a negative control. The deep pink colour in **Image B** represents bacterial growth, while the clear colour shows inhibited growth. The pink discolouration of the negative control (**column B**) shows growth, while an inhibitory effect is exhibited on the positive control (**column C**) which is indicated by a clear colour. **Rows A to C** represent inhibition, while rows **E-H** represent bacterial growth at different densities.
Ethanol was used as a negative control while Thiabendazole was used as a positive control. The use of Thiabendazole as a possible positive control was unlike many other studies that incorporated antibiotics

as a positive control. Thiabendazole is a fungal agent that was investigated by researchers such as Ansari *et al*. (2009) and Tahlan *et al*. (2019) for its antibacterial properties. Ansari *et al*. (2009) report that this drug demonstrated antibacterial property against both Gram-negative and Gram-positive bacteria, hence it was used in the current study for lack of specificity between Gram-negative and Gram-positive bacteria.



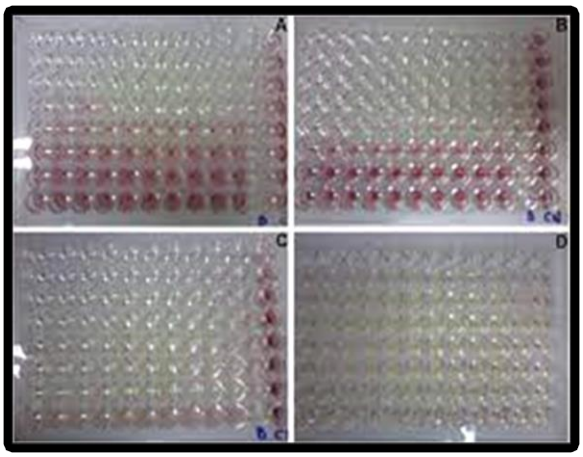


Figure 5.4: Microdilution performed in test tubes **Figure 5.5:** Microdilution in 96-well microtiter plates

Source: Veiga *et al*., 2019

Table 5.1: Antibacterial results (microdilution assay) of EOs against *E. coli.*

Treatment/EO	***Escherichia coli* (Gram negative)**					
Concentrations (mg/ml)	**100**	**50**	**25**	**12.5**	**6.25**	**3.125**
Artemisia afra	++	++	++	++	++	++
Citrus arautium* var. *amara	++	++	++	++	++	++
Citrus arauntium* var. *bergamia	++	++	++	++	++	++
Lavendula augustofilia	-	-	+	++	++	++

Matricaria chamomilla	++	++	++	++	++	++
Ocimum basilicum	++	++	++	++	++	++
Petroselinum crispum	++	++	++	++	++	++
Salvia stenophylla	-	-	+	++	++	++
Negative control (ethanol)	++	++	++	++	++	++
Positive control Thiabendazole	-	-	-	-	+	+

Key: (-) = no growth; (+) = minor growth; (++) = strong growth
Minimum inhibition concentration ≥ 100 mg/ml

With microdilution assay, the *P. crispum* and *M. chamomilla* oils demonstrated no inhibitory activity against any of the tested strains. A similar pattern was observed with the oils dissolved in DMSO. Growth inhibition of *E. coli* was observed only with higher concentrations of the *L. angustofolia* and *S. stenophylla* EOs. Organisms such as *E. coli* are known to be resistant to most EOs and antibiotics (Nazzaro *et al*., 2013). Furthermore, Man *et al*. (2019) observed that *L. angustofolia* was one of the oils that exhibited good antibacterial activity against both Gram-positive and Gram-negative bacteria. This similarity was observed even though the study designs were differed. In the current study, no growth inhibition against any of the strains was demonstrated on ethanol as a negative control in microdilution. Thiabendazole exhibited a strong inhibitory effect on all tested strains, which supports its lack of specificity between Gram-negative and Gram-positive bacteria. None of the results of the tested oils were comparable to those of TBZ.

Table 5. 2: Antibacterial results (microdilution assay) of EOs against *B. cereus*

Treatment/ EO	*Bacillus cereus* (Gram positive)					
Concentrations (mg/ml)	**100**	**50**	**25**	**12.5**	**6.25**	**3.125**
Artemisia afra	-	++	++	++	++	++
Citrus arautium* var. *amara	++	++	++	++	++	++
Citrus arauntium* var. *bergamia	-	++	++	++	++	++
Petroselinum crispum	++	++	++	++	++	++
Lavendula angustofilia	-	-	-	+	++	++
Matricaria chamomilla	++	++	++	++	++	++
Ocimum basilicum	-	+	++	++	++	++
Salvia stenophylla	-	-	-	+	++	++
Negative control (ethanol)	+	+	+	+	+	+
Positive control Thiabendazole	-	-	-	-	+	+

Key: (-) = no growth; (+) = minor growth; (++) = strong growth
Minimum inhibition concentration ≥ 100 mg/ml

Growth inhibition of *B. cereus* was observed at high concentrations of the *A. afra, C. aurantium* var. *amara, and O. bacilicum* EOs. Strong inhibitory effect was observed on the oils of *L. angustofolia* and *S. stenohylla* with an MIC of 25 mg/mL. Additionally, Kačániová *et al*. (2020) observed weak activity from *C. aurantium* var. *amara*, while Baldim *et al*. (2018) observed strong inhibitory effect from *O. bacilicum* on

B. cereus. Neither of these findings agree with the findings of the current study because *C. aurantium* var. *amara* showed mild effect instead of weak, while *Ocimum bacilicum* achieved a mild instead of strong inhibitory effect. This discrepancy may be due to the different designs that were employed by the two studies.

Table 5.3: Antibacterial results (microdilution assay) of EOs against *S. aureus*

Treatment/ EO	***Staphylococcus aureus* (Gram positive)**					
Concentrations (mg/ml)	**100**	**50**	**25**	**12.5**	**6.25**	**3.125**
Artemisia afra	-	-	+	++	++	++
Citrus arauntium* var. *amara	-	-	++	++	++	++
Citrus aurantium* var. *bergamia	-	-	-	+	++	++
Lavendula angustofilia	-	-	+	++	++	++
Matricaria chamomilla	++	++	++	++	++	++
Petroselinum crispum	++	++	++	++	++	++
Ocimum basilicum	-	-	++	++	++	++
Salvia stenophylla	-	-	-	+	++	++
Negative control (ethanol)	++	++	++	++	++	++

Positive control Thiabendazole	-	-	-	-	+	+

Key: (-) = no growth; (+) = minor growth; (++) = strong growth
Minimum inhibition concentration ≥ 100 mg/ml

The inhibitory effect on *S. aureus* was demonstrated at 50 mg/mL in the EOs of *A. afra, C. aurantium* var. *amara,* and *O. bacilicum.* A strong inhibitory effect was observed in the oils of *S. stenophylla* and *C. aurantium* var. *bergamia*. These observed effects can be related to the composition of the oils, as they possess terpenoids that are associated with antimicrobial properties. According to Hossain (2017), *C. aurantium* var. *bergamia* contains linalool, myrcene, and linalyl acetate, which are strong antibacterial compounds (Nazzaro *et al*., 2013). However, opposite results were observed in this study, which were attributed to the presence of limonene, which is claimed to be unstable and short-lived with a mild antibacterial effect (Nazzaro *et al*., 2013; Mukhtar *et al.,* 2018).

Lavendula angustofolia contains linalool, eucalyptol, and myrcene as its major compounds, thus complementing the results, by expressing antibacterial property towards tested strains. The identified compounds were similar to those reported by various authors (Zengin & Baysal., 2014; Guimarães *et al*., 2019; Man *et al*., 2019), which suggests that oils that contain the listed compounds possess good antibacterial properties. *Lavendula angustofolia* showed consistency in inhibiting the growth of both Gram-positive and Gram-negative organisms at a concentration of 25 mg/mL.

Matricaria chamomilla showed no antibacterial activity against any of the tested strains. As previously mentioned, the presence of unknown compounds that could not be identified using the GC-MS library suggests that the oil might have been adulterated. Adulteration was suspected because several authors have reported *M. chamomila* as oil most adulterated due to its demand for commercial purposes (Singh *et al*., 2011; Avonto *et al*., 2012; Mahgoub *et al*., 2020). The oil appeared viscous and could not dissolve in the DMSO and olive oil, and it had to be vortexed and centrifuged before it dissolved in the ethanol. These observations can be attributed to unknown compounds that were possibly adulterants added to the oil. The pimaric acid and other adulterants that were detected are not naturally occurring in EOs, and their presence could have been the reason for the solubility and polarity challenges that were encountered.

Growth was present at all concentrations when *P. crispum* was evaluated using microdilution. This implies that the oil has antibacterial properties, but a much higher concentration may be required to inhibit the growth of the organisms. Another factor that could have contributed to weak activity could be the microdilution process itself, as some compounds were diluted by the solvents. *Petroselinum crispum* oil demonstrated much stronger antibacterial activity in the studies by Linde *et al*. (2016) and Semeniuc and Semenuic *et al*. (2017). The findings in the literature were therefore, not in agreement with those of the current study, as no activity was observed in the latter due to suspected adulteration. The observed differences could also be attributed to the composition of the EOs used in the study. It was concluded that the activity of *P. crispum* in the current study was influenced by its chemical composition, as it varied greatly from its composition reported in the literature. *Salvia stenophylla* was active against all organisms. *Salvia stenophylla* exhibited MIC as its values were comparable to those of *L. angustofolia*. *Lavendula agustofolia* and *S. stenophylla* contained linalool, myrcene, camphor and eucalyptol (see **Table 3.1**, GC-MS results), which contributed to their antibacterial activity.

Artemesia afra possesses ketones such as α and β thujone, which suggesting that it has antibacterial properties; however, the current results showed mild inhibition in *S. aureus* and *B. cereus*. Other researchers also observed compounds (Juergens, 2014), such as alpha- and beta-thujone and camphor. The difference observed was that the *A. afra* oil used in the current study showed mild activity, as growth was completely inhibited at the highest concentration. Therefore, the results were not comparable to those reported in the literature because strong inhibition was revealed. The outcomes can be connected to the mechanisms required for the oil to bypass the cell wall to get to the internal contents of the bacteria.

The composition of the EOs that were used played a significant role, but it does not necessarily confirm that the oils will achieve potent results. Factors such as storage period and instructions should be taken into consideration when EOs are used, as the composition of their compounds depends on them. According to Turek and Stintzing (2013), thujene and thujone tend to be unstable when storage recommendations are not adhered to adequately. Storage recommendations have a negative impact on the shelf life of citrus oils, particularly *C aurantium* var. *amara* and *C. aurantium* var. *bergamia,* as they contain limonene (Mukhtar *et al.,* 2018). Limonene is a terpene that has a short shelf life, as it is unstable (Kim *et al.*, 2017). It was detected in high concentrations in both listed citrus oils, and it may have affected the ability of the oils to perform optimally. *Citrus aurantium* var. *amara* possesses linalool, phenyl alcohol,

and linalyl anthranilate, which contribute to its antibacterial properties. This is an important point to highlight, especially when oils that have been kept too long are used topically, as they can cause adverse reactions if their composition has been altered. In addition, compounds such as linalyl acetate evaporate quickly from products such as creams and citrus oils even if they were dominant compounds in the oil from which they were extracted (Skőld *et al*., 2008).

Ocimum. bacilicum oil contained high percentages of estragole, linalool, myrcene and eucalyptol (see **Table 3.1**, GC-MS analysis), which reportedly possess antimicrobial properties (Silva-Alves *et al*., 2013). Estragole is a known antimicrobial compound, and this explains the antibacterial activity observed against the two Gram-positive bacteria (Silva-Aves *et al*., 2013). The composition and activity of *O. basilicum* suggest that the oil has antibacterial properties. The current results are presented in a period when consumers opt for more natural remedies, and thus the use of oils with strong antibacterial activity can find traction in the Somatology industry. The use of these oils needs attention when preparing blends that are intended to minimize bacterial growth in and around spa areas.

5.4.2 Microwell results of EOs dissolved in DMSO.

Table 5.4: Antibacterial results (microdilution assay) of EOs against *E. coli*

Treatment/EO	***Escherichia coli* (Gram negative)**					
Concentrations (mg/ml)	**100**	**50**	**25**	**12.5**	**6.25**	**3.125**
Artemisia afra	++	++	++	++	++	++
Citrus aurantium* var. *amara	++	++	++	++	++	++
Citrus aurantium* var. *bergamia	++	++	++	++	++	++
Lavendula angustofilia	++	++	++	++	++	++

Matricaria chamomilla	++	++	++	++	++	++
Ocimum basilicum	++	++	++	++	++	++
Petroselinum crispum	++	++	++	++	++	++
Salvia stenophylla	++	++	++	++	++	++
Negative control (ethanol)	++	++	++	++	++	++
Positive control Thiabendazole	-	-	-	-	+	+

Key: (-) = no growth; (+) = minor growth; (++) = strong growth
Minimum inhibition concentration ≥ 100 mg/ml

Table 5.5: Antibacterial results (microdilution assay) of EOs against *B. cereus.*

Treatment/EO	***Bacillus cereus* (Gram positive)**					
Concentrations (mg/ml)	**100**	**50**	**25**	**12.5**	**6.25**	**3.125**
Artemisia afra	++	++	++	++	++	++
Citrus aurantium* var. *bergamia	++	++	++	++	++	++
Citrus aurantium* var. *amara	++	++	++	++	++	++
Petroselinum crispum	++	++	++	++	++	++
Lavendula angustofilia	++	++	++	++	++	++

Matricaria chamomilla	++	++	++	++	++	++
Ocimum basilicum	++	++	++	++	++	++
Salvia stenophylla	++	++	++	++	++	++
Negative control (ethanol)	++	++	++	++	++	++
Positive control Thiabendazole	-	-	-	-	+	+

Key: (-) = no growth; (+) = minor growth; (++) = strong growth

Table 5.6: Antibacterial results (microdilution assay) of EOs against *S. aureus*

Treatment/ EO	***Staphylococcus aureus* (Gram positive)**					
Concentrations (mg/ml)	**100**	**50**	**25**	**12.5**	**6.25**	**3.125**
Artemisia afra	++	++	++	++	++	++
Citrus aurantium var amara	++	++	++	++	++	++
Citrus aurantium var bergamia	++	++	++	++	++	++
Lavendula angustofilia	++	++	++	++	++	++
Matricaria chamomilla	++	++	++	++	++	++

Ocimum basilicum	++	++	++	++	++	++
Petroselinum crispum	++	++	++	++	++	++
Salvia stenophylla	++	++	++	++	++	++
Negative Control (ethanol)	++	++	++	++	++	++
Positive control Thiabendazole	-	-	-	-	+	+

Key: (-) = no growth; (+) = minor growth; (++) = s strong growth

Table 5.4 to **Table 5.6** present the MIC results for EOs dissolved in DMSO. It was revealed that none of the EOs demonstrated inhibition against any of the strains. Additionally, the oils generally did not dissolve in the 10% DMSO. This conclusion was drawn after the oils had developed a layer that floated in the solution; hence, it was necessary to employ ethanol as a solvent to rectify the polarity challenges. *Salvia stenophylla*, however, formed a cloudy solution that was rectified using a centrifuge. These processes may have resulted in suppressed antibacterial activity of all the tested EOs. Thielmann *et al.* (2019) encountered a similar challenge when the EOs in the study would not dissolve in DMSO.

The results amplify the significance of compatibility when preparing blends for aromatherapy treatments. Incompatibility of the carrier products and the EO can be a contributing factor towards compromised activity and properties in the blend. The properties of EOs are directly linked to the solubility of a blend; thus, careful selection is of paramount significance in Somatology. The importance of dilution of EOs needs to be explicit as it helps to prevent toxicity while minimising the risk of unwanted reactions (Orchard *et al.*, 2019). Essential oils must be mixed with a suitable carrier oil before they will disperse optimally, and this is necessary to avoid reactions such as irritation and sensitisation (Michalak, 2018). Some carrier products or oils that are used in Somatology are more cost-effective and compatible with some EOs than others, and this needs to be considered when blends are prepared. In the field of Somatology, many manufacturers focus more on profit margins than on the preservation of human health. Therefore,

imperative to ensure the safety of clients by following correct procedures, using compatible products, and ensuring the quality of the EOs that are selected.

Incompatibility between carrier oil and EO was encountered in the current study when *M. chamomilla* and olive oil were used. Both oils are viscous in consistency, so it was difficult to mix them as they split and separated. This inability to blend may be attributed to the addition of adulterants and the viscosity of the oils, which means that solubility is a challenge that somatologists must be aware of. This challenge can be avoided if more in-depth research is conducted to offer solutions that will limit adverse reactions. Moreover, centrifuge equipment is not used in spas; therefore, the introduction of equipment that will mimic vortex and centrifuge will be important to ensure optimal solubility. In general, the results highlight the importance of using quality oils and the possible challenges that can be caused by adulterated oils. Investigating the incompatibility of olive oil and *M. chamomilla* was beyond the scope of this study, and therefore future studies should explore this phenomenon in depth.

Positive results were observed in the study, and this highlights the need to identify and understand organisms that may be harboured in Somatology health clinics. The antibacterial activity of the tested oils can be exploited in the field of Somatology for various treatments and the prevention of bacterial transmissions and infections from one person to the other, especially between a somatologist and a client. It is argued that the introduction of EOs as surface disinfectants will minimise the bacterial presence in salons and health clinics, as their antibacterial activity has been reported in earlier studies. Areas such as jacuzzis, rasuls, saunas, and steam rooms carry a large volume of bacteria/microorganisms; therefore, the introduction of antibacterial EOs is desirable.

The Kirby-Bauer assay (to achieve supplementary results) was performed to verify the challenges of solubility and polarity. Opposite activities were expressed as concentrated oils were compared to those diluted in ethanol. Therefore, the TBZ was absent from the Kirby-Bauer assay because it impeccably dissolved in 10% DMSO. Thiabendazole demonstrated strong results that were comparable to those of oils dissolved in ethanol.

5.4.3 Antibacterial bioassay (Kirby-Bauer or disc diffusion assay)

The Kirby-Bauer assay was performed and the inhibition zones around the embedded disc of the EO were measured after 24 hours of incubation. An inhibitory effect was indicated with a clear zone that had formed around the oil-saturated disc (**Figure 5.3**). The scale that was used to measure the inhibition zone for the Kirby-Bauer assay was as follows: Strong inhibitory effect ≥ 28 mm; mild inhibitory effect ≤ 16 mm, and low inhibition ≤12 mm. Both concentrated and diluted EOs demonstrated varying levels of activity against the selected bacterial strains. Additionally, the results observed with the concentrated oil group differed from those that were diluted in ethanol. Kirby-Bauer was performed as a supplementary assay because polarity and solubility challenges were encountered with oils dissolved in 10% DMSO in microwell dilution. The challenge was only observed with EOs and not with the positive control (TBZ). Hence, only concentrated and diluted EO (ethanol as a solvent) EOs were compared in this assay.

Table 5.7: Results of antibacterial screening of concentrated EOs against some bacterial strains

Treatment/ concentrated EO	*S. aureus*	*B. cereus*	*E. coli*
Artemisia afra	0.0±0.0	0.0±0.0	0.0±0.0
Citrus aurantium* var. *amara	0.0±0.0	21.0±2.0	12.33±3.06
Citrus aurantium* var. *bergamia	6.33±5.51	0.0±0.0	33±2.0
Lavendula angustofilia	26±3.61	3.33±5.78	1.67±2.89
Matricaria chamomilla	0.0±0.0	0.0±0.0	0.0±0.0
Ocimum basilicum	60.67±4.04	61.0±2.0	38.0±2.65

Petroselinum crispum	0.0±0.0	0.0±0.0	0.0±0.0
Salvia stenophylla	25.0±5.0	0.0±0.0	0.0±0.0
Negative control (ethanol)	0.0±0.0	0.0±0.0	0.0±0.0

Data are means of three replicates (n=3) ± standard deviation

The results presented in **Table 5.7** reveal that the concentrated *O. basilicum* had a strong inhibitory effect against all the strains tested, with the largest zone detected in *B. cereus* (61.0±2.0), followed by *S. aureus* (60.67± 4.04) and *E. coli* (38.0±2.65). *Citrus aurantium* var. *amara* showed weak activity against *B. cereus* and *E. coli*, while no activity was observed in *S. aureus*. *Citrus aurantium* var. *bergamia* showed a significantly strong inhibitory activity on *E. coli* (33±2.0), while no activity was observed on *B. cereus* and *S. aureus*. *Salvia stenophylla* exhibited a mild inhibitory effect in *S. aureus* (25.0±5.0), while no inhibitory activity was observed with *B. cereus* on *E. coli*. *Lavendula angustofolia* showed a mild inhibitory effect on S. *aureus* (26±3.61) and weak inhibitory activity on *B. cereus* and *E. coli*. Finally, none of the strains examined were inhibited by concentrated *M. chamomilla*, *Petroselinum crispum*, or *Artemisia afra*.

Table 5.8: Results for the agar diffusion antibacterial screening test of diluted EOs against some bacterial strains

Treatment/ diluted EO	***S. aureus***	***B. cereus***	***E. coli***
Artemisia afra	19.0±2.0	20.0±3.61	12.67±2.08
Citrus aurantium* var. *amara	0.0±0.0	0.0±0.0	0.0±0.0
Citrus aurantium* var. *bergamia	15.67±4.04	10.33±1.53	8.67±3.25

Lavendula angustofilia	17.67±3.06	18.67±3.21	13.33±12.58
Matricaria chamomilla	0.0±0.0	0.0±0.0	0.0±0.0
Ocimum basilicum	10.67±9.29	8.67±7.57	7.67±6.61
Petroselinum crispum	15.33±4.04	11.0±2.0	1.67±2.87
Salvia stenophylla	9.67±1.51	11.33±5.51	9.0±2.0
Negative control (ethanol)	0.0±0.0	0.0±0.0	0.0±0.0

Data are means of three replicates (n=3) ± standard deviation

Diluted *M. chamomilla* and *C. aurantium* var. *amara* showed did not show activity in any of the tested strains, while *A. afra, S. stenohylla,* and *O. bacilicum* showed mild activity against all the bacterial strains. *Lavendula angustofolia* (17.67±3.06) showed mild activity on *B. cereus* (18.67±3.21) and weak activity in *E. coli* (13.33±2.58). *Petroselinum crispum* demonstrated weak activity in both *B. cereus* and *E. coli,* while showing mild activity on *S. aureus*. This observation indicated an unusual trend in *P. crispum* (15.33±4.04), as it did not show activity on any of the assays performed. *Citrus aurantium* var. *bergamia* showed mild effect in *S. aureus* (15.67±4.04), while no evident activity was observed on *B. cereus* and *E. coli.* Semeniuc and Rotar (2017) explored the antibacterial activity of various EOs, including *C. aurantium* var. *bergamia* and *C. aurantium* var. *amara*. Growth inhibition of Gram-negative bacteria was observed, and the oils further interfered with the structure of the cell walls of the bacteria. The latter study explored both microdilution and the Kirby-Bauer assay, but the methods and study design were slightly different from those of the current study as it used concentrated oils; therefore, the results differed slightly. However, similarity that was observed with *C. aurantium* var. *amara* was that it demonstrated antibacterial activity against *E. coli,* which is known to be resistant to most EOs. Additionally, both *C. aurantium* var. *bergamia* and *P. crispum* possess compounds such as D-limonene, salinene, pinene, and linolool, which are claimed to be antibacterial (Patterson *et al*., 2019). Although the presence of these compounds differed in percentage, they were detected mostly in *P. crispum.* They also showed weak inhibition against

B. cereus and *E. coli. Citrus aurantium* var. *amara* had similar compounds that possess antibacterial properties, but it did not show consistency on both the assays used.

According to Inoue *et al*. (2004), Damjanović-Vratnica *et al*. (2011), and Şimşek *et al*. (2017), linalool and 1,8 cineol are terpenoids that possess antibacterial properties, and these listed compounds were also observed in the current study. The composition of *L. angustofolia* contributed to the antibacterial activity in both Gram-positive and Gram-negative bacteria. This could have been due to the presence of multiple compounds possessing antibacterial properties in *L. Angustofolia* oil. *Artemisia afra* possesses alpha and beta thujone, which are ketones claimed to have a mild antibacterial effect; therefore, this oil expressed mild activity throughout, although no activity was observed for Kirby-Bauer (Nazzaro et al., 2013; Juergens *et al*., 2014). The above findings may possibly imply that some oils work better with Kirby-Bauer, while the activities of others rely more on microdilution. Additionally, it could be that the major compounds within *A. afra* were unable to induce the permeability of the cell wall to reach the cytoplasm.

The activity of *O. bacilicum* was constant in all strains tested with Kirby-Bauer. The strong activity can be linked to the composition of *O. basilicum* that was applied to the strains in its purest state, because this trend was not observed in the microdilution. *Ocimum bacilicum* contains terpenes and terpenoids such as linalool, estragole, myrcene, and eucalyptol, which all possess antibacterial properties related to phenolic terpenoid and delocalised electrons (Inoue *et al*., 2004; Damjanović-Vratnica *et al*., 2011; Nazzaro *et al*., 2013; Şimşek *et al*., 2017). The inhibitory activity can possibly be reduced by the addition of solvents, and hence reduced or weak activity was articulated.

According to a study conducted by Bin-Masalam *et al*. (2021), *O. bacilicum* oil showed strong activity against both Gram-positive and Gram-negative organisms, which is a phenomenon that was also observed in the current study. Furthermore, the activity might have been due to the presence of phenolic components such as estragole, which are found in *O. basilicum* that was used in the study. The only difference between the results reported by Bin-Masalam *et al*. (2021) and those of the current study is that a concentrated *O. basilicum* showed strong effect against *E. coli*.

Matricaria chamomilla did not show activity throughout and this could have been due to the presence of suspected adulterants (pimaric acid, abietic acid, methyl dehydroabietic, and dipropylene glycol) (refer to Chapter 3). The presence of unknown compounds was a challenge that affected the conclusions that

could be drawn from the results. Clearly, such compounds could have interfered with the antibacterial properties of the oil. Adulteration is thus a factor that could negatively impact antimicrobial effects.

The findings do not imply that oils that did not demonstrate any activity are less effective, but they could suggest that other variables such as composition, oil viscosity, and movement on the agar plate should be further investigated. The results are important because various factors will negatively impact the results of treatments and the ability of oils to disperse their properties effectively. Furthermore, the results imply that, in the context of aromatherapy, a carrier product can play a significant role in facilitating or limiting the movement of an oil in attempt of the therapist to reach the targeted areas.

5.5 Conclusion

The objective of the results reported in the current chapter was to investigate the antibacterial activity of the eight selected EOs. This was achieved successfully, and the results were presented in different tables. An important observation was that, even though some of the oils produced positive results, this does not mean that these oils will always be active against all bacteria. It should be noted that some bacteria may have mechanisms that make them resistant to some EOs. However, weak activity does not, suggest that the use of EOs should be regarded as unimportant. After conducting this research, it became evident that its application should not be limited to massages only. The other properties of EOs can be explored by investigating the effects of different methods of administration, such as vaporisers if the intention is to disinfect indoor areas. This means that the activity and safety of EOs used in a vaporized state will need to be assessed.

The results of this study demonstrated that *L. angustofolia* and *S. stenophylla* had the highest antibacterial activity compared to the other EOs. Therefore, these two EOs be considered for aromatherapy massages if the intention is to take advantage of their demonstrated antibacterial property. Additionally, they can be considered as surface disinfectants to prevent the multiplication of microorganisms in beauty salons, spas, and beauty clinics. Essential oils can be included in sanitising solutions, as ethanol will enhance their antimicrobial property.

Generally, antibacterial activity was observed for Gram-positive and not for Gram-negative bacteria, probably because of the differences in the cell structure of the bacteria and the composition of the EOs. However, some oils expressed activity against Gram-negative bacteria, and this should be investigated further. The antibacterial activity observed against some of the EOs could have been due to the presence of phenolic compounds, terpenes, terpenoids, and others. Some EOs could be considered for the preparation of antibacterial blends for treatments. This can be explored in future research work as the current study evaluated antimicrobial activity *in vitro*. Additionally, EOs may be of immense importance in minimising the presence of microbes in spa facilities such as rasuls, jacuzzis, sauna rooms, steam rooms, and bathrooms. The isolation of various microbes is required to confirm the existence and proliferation of bacteria in these environments.

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CHAPTER 6
CONCLUSION AND RECOMMENDATIONS

6.1 Introduction

Aromatherapy is a complementary therapy that involves the use of essential oils to preserve physical, mental, and spiritual well-being in humans (Hedaoo & Chandurkar, 2019). Aromatherapy was discovered by a group of scientists from several nations (Ali *et al*., 2015). Essential oils (EOs) have been around for millennia, but the pharmaceutical, food, and cosmetic industries have recently started to pay increasing attention to them. This study investigated various properties of EOs in the quest to fill identified gaps in the pool of knowledge and address various challenges experienced in the beauty industry. This was an experimental and quantitative study and focused on assessing the activities and actions of selected EOs against three bacterial strains, namely *S. aureus, B. cereus* and *E. coli*. The oils were also assessed for their antioxidant activities against the DPPH radical. Somatologists, who have started to play a key role in the beauty industry, need to explore different applications of EOs besides applying them as massage mediums. A pivotal challenge in the field of Somatology for skin conditions, such hyperpigmentation, which are currently treated with bleaching products. The threat of a proliferation of microorganisms in venues such as beauty salons and spas also prompted the need to explore the various properties of EOs.

For the current study, some oils were sourced from a company in Bloemfontein (Emmegenes), while others were collected from the Thitapoho farm in Tweespruit. The findings and recommendations presented in this chapter stem from the results of the experimental work and the findings that addressed the objectives and the aim of the study. The results based on the data were compared with those obtained from available literature. The research focused on chemical profiling and an investigation of the antioxidant and antibacterial activities of essential oils. The oils were sourced from *Citrus aurantium* var. *amara* (neroli), *Lavendula angustofolia* (lavender), *Citrus auratium* var. *bergamia* (bergamot), *Ocimum basilicum (basil), Salvia stenophylla* (blue mountain sage), *Artemisia afra* (wormwood), *Matricaria chamomallia* (chamomile) *and Petroselinum crispum* (parsley). The study was driven by the following objectives:

- To assess the chemical composition of the abovementioned oils.
- To assess the antioxidant activity of the selected oils; and

- To assess the antibacterial activity of the selected oils from a Somatology perspective.

6.2 Concluding Discussion

Essential oils have been used by societies for centuries (Battaglia, 2003; Ebadollahi *et al*., 2020), and their various applications have been widely explored and investigated (Adorjan & Buchbauer, 2010; Vella *et al*., 2020). The properties of these oils have been exploited by many industries that use them as natural remedies, but unfortunately, the drive for profits tends to compromise the composition of these oils when they are adulterated with the addition of inappropriate additives. It was against this background that it was deemed important to explore options that will extend the use of essential oils for diverse applications and methods of administration, particularly in the Somatology field.

The oils selected for this study were tested for their antioxidant properties and, in general, demonstrated antioxidant activity. The results suggest that most of the tested EOs may be safely used for their antioxidant properties in the field of Somatology. This implies that the current focus on EO application as a message medium can be extended as these oils may also be incorporated in blends for facials to treat hyperpigmentation, premature ageing, dryness, and other skin conditions that may require antioxidant agents.

The current study focused on the activities of essential oils. The possibilities for improvement in their application were outlined in Chapter 3. The effects of essential oils are also explored to verify their potential in the treatment of hyperpigmentation and other skin conditions. The findings were positive and will therefore expand options when EOs are selected, and their ideal application method is considered. Areas of concern were also highlighted, which will guide appropriate selection and application practises. For example, the findings suggest that the EOs under study may be used for facial massage and application under facial masks in steam treatments. A word of caution is that the suggested options may be used only when necessary and when oil products have been carefully sourced from reputable providers.

When acne is treated, knowledge of the antibacterial properties of the EOs that will be used is essential, especially if bacteria are involved in acne breakout. Treatment of acne is notoriously challenging, and somatologists must be mindful of their personal and environmental hygiene.

It was scientifically demonstrated that some of the tested EOs had activity that curbed the growth of *S. aureus* and *B. cereus* (which are Gram positive), and surprisingly, some oils demonstrated activity against *E. coli* (which is Gram negative). The results thus suggest that the tested EOs have potential for application against Gram negative strains, and this is supported in the literature (refer to Chapter 3) that highlights their ability to act as antibacterial agents. However, even though most oils showed both antioxidant and antibacterial activity, it was revealed that chamomile oil was not active and produced no results for the above activities. The reason for this conclusion is that unknown components (it was assumed that these could have been resins such as pimaric acid, abietic acid, methyl dehydroabietic and dipropylene glycol) were detected in its chemical profile. Most of these components are not naturally found in EO and they could thus have been used as adulterants, which might have played a role in the weak activity observed in *M. chamomilla*.

Another challenge that was highlighted was the need to adhere to the guidelines and rules for the purchase and storage of EOs, as inappropriate handling plays a role in their quality and composition. Quality is linked to the adulteration of EOs as was discussed in Chapter 3. For instance, *M. chamomilla* had resins that do not naturally form part of its composition, and therefore this oil could reveal neither antibacterial nor antioxidant properties in the current study. As adulteration is common in the EO market, it is important to pay attention to the labelling, packaging, and other information that will indicate the authenticity and quality of an oil. In most cases, the manufacturers will not disclose adulterants, but the results indicated that adulteration is practised. Somatologists commonly buy oils from wherever they will find them in the market, and many do so without considering the quality and risks associated with low-grade products. The findings should thus serve as a warning that the properties of EOs are directly dependent on their composition. Therapists should therefore be well informed, consider the composition of the products they purchase, and buy from reputable suppliers that comply with legislative regulations in the cosmetic industry.

According to Barbieri and Borsotto **(**2018), the rules governing EOs should be followed by adhering to the REACH and CLP (registration, evaluation, authorisation, and restriction of chemicals) framework. All chemicals that are distributed should be approved before they are introduced to consumers. In essence, the regulations protect against false advertising and labelling. The latter should state the following: name of the product; its characteristics/functions; and the company name, address, and telephone number. If the product contains hazardous compounds, they should be clearly indicated on the label. Weight,

storage instructions, volume, and expiration should also be indicated to preserve quality. All of this information will help should a manufacturer be approached with enquiries or queries. The specifications of cosmetic products should be available in case a product contains mutagenic, carcinogenic, or any other chemical that can be harmful to living beings. The adhesion to this rule is important because EOs are also used as components in cosmetic products.

Regulations are implemented to protect human and animal health and the environment and maintain high standards in the EO market to ensure that no harmful chemicals are added. South Africa should strictly adhere to the authentication and authorisation processes to avoid the harmful addition of contaminants to beauty and complementary treatment products (Refer to the GC-MS profile of chamomile in Chapter 3). It is regrettable that EOs still contain contaminants because they are amongst the products that are not registered with the European Economic Area (EEA).

6.3 Limitations

Some limitations were identified in the study in terms of sample collection, time, and available finances:

- Some of the EOs that were sourced were ready-made, while others were bought from a reputable supplier. Some oils were difficult to source, hence the researcher settled on ones that were available at a store that sells body products.
- The original scope of the study was much larger, but due to time and financial constraints, additional chapters had to be removed, such as the one on toxicity. A future project will highlight this phenomenon.

6.4 Generic Recommendations

The researcher offers the following recommendations regarding the chemical composition of EOs and their antioxidant and antibacterial activity:

- The composition of an oil needs to be carefully considered for better application results and to avoid adverse reactions. It is also advisable to assess the quality of oils purchased from other countries by testing their efficacy in terms of different skin conditions.

- Information about the cultivation season of the plant from which an oil was extracted should give a clear indication whether the composition depends on the origin, season, or other factors. Filtering this information will provide evidence of reliable products that can be safely used safely for various applications.
- A patch test must be performed 24 hours or a few days before treatment to avoid adverse reactions from oils that contain added chemicals.
- Different notes in the aroma of EOs need to be considered, as they help in harmonising a blend. For an example, top notes evaporate quickly while middle notes take a few minutes. On the other hand, base notes, on the other hand, linger for hours before evaporation because they are generally viscous.
- A full consultation is required before treatment to obtain comprehensive information on the history of the client. This information is essential for oil selection and the treatment of possible complications that may require intervention of a doctor.
- Expiration must be noted as EOs may become compromised after a period of time, and they then have negative effects on human skin.
- Infections are treated as contraindications, but exploring other methods of administration, such as inhalation, may help.
- It is important to consider the application regimen of a selected EO, as there may be more options than mere massage. Inhalation may be considered as a standard treatment for clients with contraindications that prohibit topical application.
- Humidifiers, diffusers, and other electrical methods can be used in crowded areas to minimise the presence of bacteria in salons or other public spaces. These options have yet to be fully explored in future studies.
- The effectiveness of an oil should be assessed when blended with a carrier oil to check if whether compatibility does play a role in its performance. Additionally, carrier oils must be evaluated to determine if they play a role in minimising toxicity in EOs.
- All surfaces should be regularly assessed in beauty salons and spas to verify for the presence of harmful microorganisms.

6.5 Recommendations for Future Projects

- Future studies should focus on cytotoxicity tests.
- Most cosmetic manufacturers use essential oils as ingredients in beauty products and therefore, it is therefore important to assess the ability and effectiveness of these oils in aromatherapy versus an actual beauty product to compare the results. This relates to compatibility as an important factor to consider when choosing EOs and carrier products that are suitable for better penetration.
- Samples of EO sourced from different countries need to be tested to verify quality and efficacy. Factors such as geographical conditions should also be considered when testing such product.
- The effectiveness of EOs in improving skin conditions such as hyperpigmentation, dehydration, oiliness, dryness, and acne should be verified.
- Researchers should explore the efficacy of a wide range of EOs for application in the Somatology industry.
- The effectiveness of antifungal oils should be explored in depth to profile various selected cultures. This is recommended because antibiotics are produced from fungi; therefore, it might be possible to combat bacteria with antifungal oils to broaden options in a spa or salon.
- The effectiveness of essential oils on acne secretions (pustules) on the skin and the microbes/bacteria in them should be further investigated. It is also important to obtain samples for such investigations from lesions of various subjects of different ages, races, and gender.
- Investigations into the potency of anti-inflammatory oils need to be tested to determine if they have a positive impact when treating photo-inflammation that commonly occurs as premature ageing and hyperpigmentation and compromised barrier.

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