CHAPTER 2

PRODUCTION AND ISOLATION OF ESSENTIAL OILS

2.1 Introduction

Essential oils have been known to mankind for centuries. For all these years, extracting the odours from plants has been an important occupation. This has developed into a large modern industry. Essential oils are important raw materials for many industries where they play a number of roles. By definition essential oils contain highly volatile substances that are isolated by a physical method or process from plants of a single botanical species[1]. The oils normally bear the name of the plant species from which they are derived. Essential oils are so termed as they are believed to represent the very essence of odour and flavour.

Essential oils are composed of different chemical groups of terpenic hydrocarbons and their oxidized derivatives such as aldehydes, esters, ketones and alcohols. Terpenes represent a large group of natural compounds that do not contribute much to flavour, fragrance or odour of the oil[2]. Very often the hydrocarbon terpenes represent a large percentage of the components of essential oils of plants and can be found in a remarkable variety of closely related structures. As a common feature, essential oils carry the essence of a plant, the identifiable aroma, flavour or other characteristics that may have some practical use. They are used for different purposes such as[3]:

1. In pharmaceutical products the oils are used for medicinal and cosmetic purposes.
2. In the perfume industry, they are used as the constituents of expensive fragrances.
3. In the food industry, they are used as food preservatives and flavour enhancers.

Often rare and expensive pure oils in the market are diluted with lower quality commercial-grade oils or synthetic chemicals to increase the volume and therefore the profit margin, a fact not usually revealed on the label[4]. This problem can be addressed by developing reliable analytical methods for the detection of adulterated oils in order to discourage or penalise this action.

2.2 Isolation techniques of essential oils

The recovery of essential oil (the value added product) from the raw botanical starting material is very important since the quality of the oil is greatly influenced during this step. There are a variety of methods for obtaining volatile oils from plants. Steam distillation, aqueous infusion, solvent-extraction, cold or hot expression and supercritical fluid extraction (SFE) with carbon dioxide are methods often used. The chemical composition of the oil, both quantitative and qualitative, differs according to the technique used to remove the oil from the plant[5]. A comprehensive review of various techniques employed to recover the essential oil from the materials in which they occur was prepared by Weurman[6]. These techniques are briefly explained in this chapter.

2.2.1 Steam distillation

The vast majority of true essential oils are produced by the steam distillation method. However, there are various distillation methods that are used. In all of the distillation processes, water is heated to produce steam that carries the most volatile aromatic materials along with it. These aromatic components are then cooled in a condenser and collected in the resulting distillate[4]. During distillation the boiling water penetrates the plant tissue and dissolves a part of the essential oil present in the oil containing structures. This aqueous solution diffuses through the cell membranes and, upon its arrival at the surface, the oil is immediately vaporized[5]. This process continues until all the enclosed volatiles are removed from the cells.

Koedem and co-workers[7,8] investigated the influence of the length of the distillation period on the composition of the essential oils in the seed of the *Unbelli Fenous* species. It was found that the time required to remove the oils from the plant material will have an influence on the quality of the final oil. They established that the composition of the oil changes indirectly during the distillation process. The higher boiling oxygenated compounds are the first to appear in the distillate, whereas the amount of the lower boiling hydrocarbons gradually increases as the distillation process proceeds.

A modified dimension in the recovery of essential oils with the distillation method was the development of a simultaneous distillation/solvent-extraction apparatus by Likens and Nickerson[9]. The device has the major advantage of a many thousand-fold concentration of volatiles from aqueous media in a single step. The greatest interest in this apparatus is evidenced by its wide application in numerous laboratories. Several modified version of this method have been constructed[10]. As the oxygenated oil constituents are much more soluble in boiling water than their hydrocarbon analogues, the latter remain associated with the plant material to a greater extent. In conclusion, it was established that the components of the essential oils are liberated according to their degree of water solubility rather than following their order of boiling point[5].

Also the presence of trace metal was found to influence the quality of the oil[11,12]. Essential oils come from various parts of plants - the seeds, bark, leaves, stems, roots, flowers, and fruit. The oils can be distilled from the plant material or extracted. The majority of essential oils are distilled using this method. The key to producing a therapeutic-grade essential oil is to preserve as many of the delicate aromatic compounds within the essential oil as possible - elements that are very fragile and destroyed by high temperature and high-pressure. Contact with chemically reactive metals (i.e., copper or aluminum) is another danger to the fragile aromatic compounds in oils[13]. To ensure a high grade of essential oil, it is imperative to use stainless steel cooking equipment at low pressure and low temperature for long periods of time.

2.2.2 Solvent-Extraction

In the solvent-extraction method of essential oils recovery, an extracting unit is loaded with perforated trays of essential oil plant material and repeatedly washed with the solvent. Hexane is often used. All the extractable material from the plant is dissolved in the solvent[5]. This includes highly volatile aroma molecules as well as non-aroma waxes and pigments. The extract is distilled to recover the solvent for future use. The waxy mass that remains is known as the *concrete*. The concentrated concretes are further processed to remove the waxy materials which dilute the pure essential oil. To prepare the *absolute* from the *concrete*, the waxy concrete is warmed and stirred with alcohol (ethanol). During the heating and stirring process the concrete breaks up into minute globules. Since the aroma molecules are more soluble in alcohol than the waxes, an efficient separation of the two results.

Solvent-extraction of essential oils from plant materials using low boiling solvents has an advantage over distillation because the temperature remains relatively low. Usually temperatures below 50 ^{o}C are used during most processes[5]. The oils that result from solvent extraction often have a more “natural” composition compared to distilled oils, which may have undergone thermally induced alterations. The formation of artefacts may negatively influence the quality of extracted volatiles. Schnelle and Horster[14] detected several artefacts among volatiles extracted from the essential oil of a mint species when a mixture of petroleum ether and acetone was used for extraction. It was discovered that these substances were generated by the reaction of the acetone used in the extraction procedure with non-terpenoids present in the plant material.

2.2.3 Supercritical fluid extraction

Supercritical fluid extraction (SFE) is a solvent extraction process that uses a supercritical fluid as the extraction solvent. The low viscosity of supercritical fluids (SFs) combined with high diffusion rates are ideal for the extraction of diffusion-controlled matrices such as plant tissues[15]. Supercritical extraction is faster than liquid extraction and the supercritical fluid solvents are more easily removed. Recovery can be effected by reducing the pressure to release the solvent from the extracted analytes.

Mostly, CO_2 is used as the extraction solvent. CO_2 has the desirable property that it behaves like a solvent and can be manipulated to obtain differential or sequential fractions. Because of its high vapour pressure at room temperature and atmospheric pressure, all traces of gas can easily be removed from the volatile oil. The low critical temperature of CO_2 is particularly useful when extracting oils that contain heat labile compounds[16]**.** Supercritical fluid CO_2 is inert and does not introduce artifacts. These desirable properties ensure that essential oils are produced that have organoleptic properties closely resembling those of the plant from which they were extracted[17.]

The solvating power of supercritical CO_2 may be improved by the addition of a more polar modifier such as methanol, but it is generally not necessary for the extraction of essential or volatile oils. When temperatures below the critical temperature of CO_2 are used, liquid CO_2 may be used as a very inert, safe liquid solvent which will extract the aroma molecules in a process similar to solvent-extraction[18].

2.3 Essential oil plants

Many plants are grown by South African farmers for the production of essential oils. These include exotic species such as *Cymbopogon* (lemongrass) and *Tagetes minuta* (kakiebos) or indigenous species such as *Artemisia afra* (wilde als) and *Pelargonium* (geranium). The analysis of these oils is important for quality control purposes and also to find specific compounds that have biological activity, for their isolation and pharmaceutical application. South Africa also has a treasure of uncharted botanical riches. New methods need to be developed to expedite the bio-prospecting of our botanical diversity for novel plants with commercial value as future essential oils or other value added products.

In this thesis the essential oils of *Cymbopogon* (lemongrass) *Artemisia afra* (wilde als), *Tagetes minuta* (kakiebos) and *Pelargonium* (geranium) were studied using a novel analytical technique called comprehensive multidimensional supercritical fluid and gas chromatograpy (SFCxGC).

2.3.1 Artemisia afra **(Wilde als)**

Artemisia afra is one of the oldest known indigenous medicinal plants in Southern Africa. Its common names in South Africa are: wild wormwood, African wormwood (Eng.); wilde-als (Afr.); umhlonyane (Xhosa); mhlonyane (Zulu); lengana (Tswana); zengana (Southern Sotho). *Artemisia afra* grows in thick, bushy, slightly untidy clumps, usually with tall stems up to 2 m high, but sometimes as low as 0.6 m. The stems are thick and woody at the base, becoming thinner and softer towards the top. Many smaller side branches shoot from the main stems. The stems are ribbed with strong swollen lines that run all the way up. The soft leaves are finely divided, almost fern-like. The upper surface of the leaves is dark green whereas the undersides and the stems are covered with small white hairs, which give the shrub the characteristic overall grey colour. *A. afra* flowers in late summer, from March to May[19]. It is used to cure diseases such as the common cold, diabetes mellitus, bronchial complaints and stomach disorders[21]. The main components of

Artemisia afra from the literature are 1,8-cineol and two ketones (α- and β-) thujone, camphor and borneol[21]. Figure 3.1 shows the typical *Artemisia afra* plant.

Figure 2.1 *Artemisia afra* plant[20].

2.3.2 *Tagetes minuta* (kakiebos)

Tagetes minuta is an annual member of the *compositae* family. The plant grows to a height of 50-150 cm with a single stem highly branched at the top. It is of South American origin although it has been introduced to Europe, Australia and Eastern and Southern Africa. In the southern countries, *Tagetes minuta* oil has numerous applications, as an insect repellent and in treatment of certain illnesses such as smallpox, earache, colds and to reduce fevers. In addition, it has been recognised to possess hypotensive, spasmolytic, anti-inflammatory, antimicrobial and antifungal properties[22]. The main oil components such as dihydrotagetone, (Z)- and (E)- tagetones and (Z)- and (E)- tagetenones have interesting aroma properties. Inconsistency and diversity of the composition of *Tagetes minuta* oil has been reported to depend highly on external factors such as place of harvest and different development stages of the plant during harvest[21].

Figure 2.2 *Tagetes minuta* plant[20]

2.3.3 Pelargonium (Geranium)

More than 200 *Pelargonium* species have been identified. *Pelargonium radens* and P. *capitatum* are some of the *Pelargonium* species indigenous to South Africa. Hybrids of these species are cultivated from which the commercially important geranium oil is extracted. The aromatic plant is also cultivated for extraction of commercial rhodinol (mixture of linalool, citronellol and geraniol). Figure 2.3 shows a typical plant of pelargonium. Some of the main constituents found for pelargonium are α-pinene, α-phellandrene, p-cymene, γ-terpinene, β-caryophyllene, guia-6,9-diene, germacrene D, ς-cadiene, citronellol, terpinen-4-ol, 10-epi-y-eudesmol, citronellyl formate and unidentified sesquiterpenes. The loss of oxygenated constituents in distillation has been observed:

which makes the aroma of the oil incomplete in terms of organoleptic richness and fullness[23].

Figure 2.3. *Pelargonium capitatum* plant[20]

2.3.4 *Cymbopogon* (lemongrass)

Lemongrass, a perennial herb widely cultivated in the tropics and subtropics, designates two different species, *Cymbopogon flexuosus* and *C. citratus*. The plant grows in dense clumps and may reach diameters of up to 2 centimeters. The leaves may be up to 1 metre long. The plant needs a warm, humid climate and full sun. The quality of lemograss oil is generally determined by the content of citral, the aldehyde responsible for the lemon odour[24]. Citral consists of the *cis*-isomer, geranial, and the trans isomer, neral. These two are normally present in a ratio of about 2 to 1. *Cymbopogon flexuosus* has a far higher citral content than *C. citratus*. Also *C. flexuosus* has a detectable amount of caryophyllene, which is absent in *C. citratus* [25].

Some of the constituents of the essential oil are myrcene, citronellol, methyl heptanone, dipentene, geraniol, limonene, nerol and farnesol. Citral, extracted from the oil, is used in flavoring soft drinks, in scenting soaps and detergent, as a fragrance in perfumes and cosmetics, and as a mask for disagreeable odours in several industrial products[5].

Figure 2.4. Lemongrass plant[20].

2.4 Conclusion

The advantages of a particular isolation method of essential oils compared to others is closely related to the objectives of our study of the essential oils composition of given material. If the purpose is only to demonstrate the improvement of a particular technique of separation or sample injection, then the composition of the essential oil is of rather limited importance. However, if the intention is to study the true composition of the oil for purposes such as quality control or biosynthetic pathway of the oil, then the isolation technique plays an important role as shown by the discussions of the isolation methods for essential oils.

2.5 References

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