ANSAM JAWAD KADHIM THE ESSENTIAL OIL COMPOSITION OF *PTERONIA INCANA* MASTER THESIS 2024

NEAR EAST UNIVERSITY
INSTITUTE OF GRADUATE STUDIES
DEPARTMENT OF PHARMACOGNOSY

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M.Sc. THESIS

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Declaration

I confirm that the data, records, analysis, and findings in this thesis were gathered and presented in compliance with the Near East University Institute of Graduate Studies' academic and ethical criteria. I further declare that I have properly attributed and referenced any data and material that are not special to this work, as required by these rules of conduct.

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Abstract

Pteronia incana, belongs to the Asteraceae family, is a herb which grows naturally in South Africa. As an ethnopharmacological relevance of *Pteronia incana* is utilised traditionally to cure a variety of renal diseases, backaches, fever, and influenza. The essential oil or extract of this plant is used as a preservative, antibiotic, and antifungal. The main objective of this study was to evaluate the chemical compositions of commercially sold *Pteronia incana* essential oils obtained from different batches during the production which were produced by a company in South Africa and compare it with that of other previous studies. In general, myrcene (7.8-12.1%), α-pinene (15.2%), β-pinene (18.2-29.6%), limonene (5.8-9.0%), 1,8- cineole (8.3-13.5%) and *p*-cymene (8.1-31.9%) were found to be the major components of the essential oils obtained from three different batches of *P. incana*. The chemical composition of Sample A (Batch no: SAO33) and C (Batch no: SA028) was similar though Sample B (Batch no: SA027) was slightly different. When it was compared with the previous studies, although all the samples for previous studies were taken from the same areas of South Africa and under different climatic conditions, such as changing seasons or from different parts of the plant, and the percentage fluctuated, they all show that essential compounds are almost present in most of previous studies and the presence of β-pinene as a major compound of essential oils with high percentage is common. This study is important because it assists in the quality, and similarities or differences in the commercially sold essential oil composition of *P. incana* and its comparison of the previous literature which were the samples collected from its natural habitats. Its main components and overall chemical makeup suggest that it may find use in a range of sectors, such as aromatherapy, herbal products, and the pharmaceutical industry.

Keywords: *Pteronia incana*, essential oil, South Africa, 1,8-cineole, myrcene, α-pinene, β-pinene, limonene.

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Table of Contents

List of Tables

List of figures

CHAPTER I

Introduction

Plants have historically played a vital role in the development of medicine, largely due to their ability to produce secondary metabolites with potentially significant biological effects. Traditional medicine has relied on plants to treat a wide range of diseases. According to the World Health Organization (WHO), more than 80% of the world's population still relies on traditional and folk medicine, most of which is plant-based. Medications derived from plants used in traditional medicine are often more affordable, readily available, and exhibit fewer side effects compared to their synthetic counterparts. Even in developed nations like the United States, herbal drugs account for a notable percentage of the total pharmaceutical intake (Jafarinia & Jafarinia, 2019). Recent analysis of traditional medicinal plants using modern methods has led to the discovery of numerous promising compounds. These plant-derived substances can be utilised to enhance existing drugs or develop entirely new ones (Rolnik et al., 2021). Many commonly used drugs, such as morphine, aspirin, quinine, and digitoxin, have their roots in herbal sources (Jafarinia & Jafarinia, 2019).

The largest family is Asteraceae, which consists of more than 23,000 currently accepted species, spread across 1,620 genera and 12 subfamilies, (Rolnik & Olas 2021). Most members of this family have therapeutic uses and boast a long history in traditional medicine, with some species being cultivated for over 3000 years for nutritional and medicinal purposes including anti-inflammatory, antimicrobial, antioxidant, and hepatoprotective properties (Rolnik et al., 2021). Asteraceae family members are known and distributed worldwide, most prevalent in arid and semi-arid regions of the subtropics.

The focus on the chemical compounds found in plants primarily stems from their utilisation in medicine and pharmaceuticals. The composition of herbs can vary depending on factors such as the season, agricultural practices, and the growth stage of the plant or its specific part (like inflorescence, leaf, stem, root, fruit, or seed) (Bessada et al., 2015).

The various medicinal effects of these plants are universally recognized, leading to their inclusion in the national pharmacopoeias of various countries such as Germany, the Czech Republic, France, and Switzerland **(**Achika et al., 2014)**.** Due to the presence of a wide range of bioactive compounds such as flavonoids and phenols, which contribute to its therapeutic potential, bioactive secondary metabolites such as flavonoids, phenolic acids, coumarins, terpenes (mono-, sesquiterpenes, diterpenes, and triterpenes), essential oils, lignans, saponins, polyphenols, polysaccharides, and sterols have been frequently reported from the Asteraceae family (Achika et al., 2014; Rolnik et al., 2021). These compounds, classified as secondary metabolites, serve to shield the human body from the harmful impacts of oxidative stress (Bessada et al., 2015).

Polyphenolic compounds are particularly noteworthy among the active substances in plants, as they play a crucial role in the biological activities of the plant material (Bessada et al., 2015). Phenolic compounds (such as phenolic acids and flavonoids), for example, offer anti-inflammatory, anti-cancer or anti-atherosclerotic activities (among other biological effects), as a result of their antioxidant activity (Krishnaiah, 2011; Niki, 2010; Bessada et al., 2015) aiding in free radical scavenging (Piątkowska et al., 2022). Phenolic compounds like chicoric acid, kaempferol derivatives, and luteolin derivatives are prevalent in these plants (Rolnik et al., 2021).

Flavonoids are ubiquitous in plants and contribute to the sensory qualities of foods, and play a key role in influencing the immune system (Jafarinia & Jafarinia, 2019). Flavonoids are categorized into different classes, including flavones, flavanones, isoflavones, flavonols, flavanols, flavanonols, and anthocyanins. Flavan-3-ol and anthocyanins are major classes of flavonoids found in plants. The antioxidant activity of flavonoids depends on their chemical structure, especially the position of the hydroxyl groups in the molecule (Jaison et al., 2023).

The genus *Pteronia* L., a flowering plant species belonging to the Asteraceae family, is represented by 70 species worldwide (Hulley et al., 2010). The Hottentotís and Khoi-San people were among the first to use *Pteronia* species for their anti-infective properties. Oddly, the genus has been used so extensively historically that so little is known about it (Shearing, 1997). The ÑKhoiSaní people have documented using some *Pteronia* species, referred to as "*èboegoebossieî*" and "*èlaventelbossieî*", as natural cosmetics and ethnomedicines to cure stomach disorders (Shearing, 1997; Coovadia, 2008). One of the species in this genus is *Pteronia incana* (Burm.) DC, which is commonly known as blue dog, blue bush, ash bush, or wild lavender bush in English, and by various names such as "*asbos, asbossie, bloubos, laventelbossie, kraakbos*" in Africans. It is Indigenous to the Karoo regions of South Africa. Among the 70 species within the genus *Pteronia* L. of small woody shrubs, *P. incana* is widely distributed in South Africa and well-recognized (Germishuizen and Meyer, 2003; Leistner, 2000). The plant is distinguished by its small silvery leaves covered in a woolly whitish indumentum, solitary terminal flower heads **(Fig. 1**), and involucral bracts that are glabrous outside (Hulley et al., 2010). *P. incana* is a notable plant species known for its diverse essential oil variations, distinct leaf anatomy, and traditional ethnobotanical uses (Hulley et al., 2010).

Figure 1. Botanical illustration of *Pteronia incana* flowers

A leaflet that the Montagu Museum distributes summarises the traditional medical use of *P. incana* (1998). Its use for treating renal diseases, backaches, fever, and influenza are mentioned. The plant is quite fragrant and yields a volatile oil, just as other *Pteronia* species (Bruns and Meiertoberens, 1987; Mayekiso, 2006; Webber, 1999). Mayekiso et al., 2008 observed that multicellular glands released essential oil after studying the Morphology and ultrastructure of glandular and non-glandular trichomes (Hulley et al., (2010).

P. incana, a plant species considered a weed in the Eastern Cape of South Africa covers around 60,000 hectares and is unappealing to livestock (Webber, 1999). Research has shown that plants like *P. incana* produce secondary metabolites as a defense mechanism against herbivores and microbes (Wink 2003; Koschier, 2002; Werker, 1993; Duke, 1993; Wagner, 1991; Bell, 1981; Reynolds and Rodriguez, 1984; Croteau, 1977). This unpalatability is thought to be a defence strategy. *P. incana* is unique in that it thrives without the need for extensive cultivation, unlike many other plants (Bruns & Meiertoberens 1987). Professor Graven and colleagues at the University of Fort Hare have explored the commercial potential of the essential oil of *P. incana*, identifying key constituents for potential commercial use (Mayekiso, et al., 2006).

1.1. The Asteraceae family

The Asteraceae family of plants has a remarkable ecological and economic importance, and is a medicinal plant widely distributed throughout the world except Antarctica (Rolnik et al., 2021). They are found with particular importance in the Mediterranean, Eastern Europe and Asia Minor and have been used since ancient times (Bessada, et al., 2015). The name "Asteraceae" comes from aster, the most prominent genus in the family, and is derived from the Greek name "ἀστήρ" meaning star, and is related to the shape of its star-shaped flowers. The term "Compositae" is more ancient but still valid, obviously referring to the fact that the family is one of the few families of angiosperms that have compound flowers. Asteraceae (Compositae) is an advanced and botanically highly specialised family of mainly herbaceous plants (Commonly referred to as aster, daisy, or sunflower family) (Stevens, 2001; Jeffrey, 2007). The group has more than 23.000 currently accepted species, spread across 1620 genera and 12 subfamilies (Rolnik et al., 2021). The main feature of the family is the composite flower type in the form of capitula surrounded by involucral bracts. Asteraceae may represent up to 10% of the native flora in many regions of the world. The largest composite genera are *Senecio* (1000 species), *Vernonia* (1000 species), *Centaurea* (700 species), *Cuscinia* (600 species), *Helichrysum* (550 species), and *Artemisia* (550 species) (Stevens, 2001). Most members of the Asteraceae are herbaceous, but there are a large number of shrubs, vines, and trees (Barkely et al., 2006; Brouillet & Strother, 2006)

The Asteraceae family is known for its vast range of habitats, exhibits a diverse range of morphologies and varied members, most of which are woody shrubs or rosette-trees, with most being perennial herbs but some appearing as annuals, varying in height from 1 to 3 metres, however, some species can grow into large trees exceeding 30 metres in height (Rolnik, et al., 2021) or climbing plants (Olorode, 1984; Adedeji, & Jewoola, 2008). The leaves of Asteraceae plants display a wide variety of forms, from large to small and spiny, with some species having minimal or no leaves, as the function is performed by the green stem. In most species, the leaves are simple and pinnately lobed; in other genera, they may be alternating, opposite, or prickly (Adedeji & Jewoola, 2008). Most leaves are characterized by an indumentum and hairs of different lengths and colours. The plants typically produce flat clusters of small flowers in a range of colors (Hulley et al., 2010). Usually, the inflorescence develops into a capitulum, bearing actinomorphic or zygomorphic flowers that might be neuter, female, actinomorphic, or hermaphrodite. An involucre of bracts often envelops the inflorescence (Adedeji & Jewoola, 2008).

The Asteraceae family of plants has distinct reproductive morphology that includes (Bessada et al., 2015)**:**

- Flowers: The tiny, closely packed flowers, known as florets, are arranged into heads known as capitula. Phyllaries are an involucre of bracts that encircle these capitula. The heads may be arranged in spikes, racemes, cymes, corymbs, or panicles, or they may be solitary.
- Receptacle: The floret-producing receptacle is usually convex, although it can sometimes infrequently be concave, conical, or cylindrical. It might be hairy, scaly, smooth, or nude.
- Florets: There are one or two types of florets on each head, which can be perfect, unisexual, neuter, or sporadically dioecious. While inner florets are tubular and devoid of ligules(disc florets), outside florets are frequently ligulate (also known as ray florets). All fleurs may be tubular, ligulate, or a combination of the two (**Fig 2)**.
- Calyx: The pappus of persistent or soon-falling hairs, bristles, awns, or scales that crowns the ovary is the calyx, which is superior. There are instances where it is only a ring or nonexistent.
- Corolla: The corolla has a long or short tube and is gamopetalous. It is 4- or 5-lobed (disc florets), occasionally 2-lipped and uneven, and can be regular ligulate (ray florets).
- Androecium (Stamens): The corolla tube has five (rarely four) stamens. The anthers connate form a tube around the style, occasionally remaining free, and the filaments are free. The two-celled anthers unfold longitudinally and frequently contain appendages on both ends.
- Gynoecium (Ovary): The ovary has a single basal ovule and is inferior, having one cell. Perfect or pistillate flowers typically have two parts that might be smooth, hairy, or papillose in different forms.

- Fruits: The fruit is an indehiscent, generally dry, 1-celled, 1-seeded achene. It could have a beak, and the pappus that never goes away usually crowns it. Seeds lacking endosperm.

Types of florets (Meikle, 1985):

a. **Disk florets:** In most members of Asteraceae, the central florets have a radially symmetrical, tubular corolla, with 5 short lobes. These florets are called disk florets. They form the central disk of the capitulum in typical daisies. Disk florets are often perfect flowers. The entire inflorescence may be composed of disk florets only (a condition referred to as homogamous); when so, the inflorescence is said to be discoid.

b. **Ray florets:** Surrounding the disk florets are sometimes an outer ring of ray florets. These have zygomorphic symmetry, and are usually either sterile or pistillate, with 3 apical teeth. Ray florets are strap-shaped, imperfect, and never occur alone in the inflorescence (a condition referred to as heterogamous). They are always associated with disk florets (although disk florets may occur without ray florets), and form a circle around the margin of the head, the centre filled with disk florets.

c. **Ligulate floret:** the best known weedy composite, the common dandelion, has a third kind of floret, a ligulate floret. These resemble ray florets with their zygomorphic symmetry, but ligulate florets are perfect (bisexual), and have 5 apical teeth at the end of the strap.

d. **Bilabiate floret:** These florets have zygomorphic symmetry, but not so much as the ray or ligule. They have 3-4 lobes on the lower (long) lip, and 1-2 lobes on the upper (shorter) lip. They are also bisexual, can be represented by thistles, and are relatively rare in the U.S.

There are many economically important members of this family such as used for ornamental, food and/or medicinal purposes (Meikle, 1985):

a. Ornamental: Aster, *Calendula, Chrysanthemum*, Dahlia, sunflower (*Helianthus*), marigolds (*Tagetes*), Zinnia, Gaillardia, Coreopsis.

b. Weeds: *Taraxacum* (dandelion), *Sonchus* (sow-thistle), *Cirsium* (thistle), *Ambrosia* (ragweeds), *Xanthium* (cocklebur).

c. Food: *Lactuca* (lettuce), *Helianthus* (sunflower and Jerusalem artichoke), *Cichorium* (chicory), *Carthamus* (safflower oil), artichokes, salsify

d. Medicine: *Echinacea*, *Anthemis* (chamomile), *Artemisia* (wormwood), *Achillea* (yarrow), *Solidago* (goldenrod).
e. Insecticides: pyrethrins are obtained from Chrysanthemums.

Herbal medicines are composed of various active components that can have therapeutic effects. These medicines have a long history of use spanning thousands of years due to their natural origins and perceived minimal side effects (Jafarinia & Jafarinia 2019). Historical records indicate that herbal medicine usage dates back over 5000 years.

Members of this family have been used traditionally as astringent, antipyretic, anti-inflammatory, antibacterial, antifungal, insecticide, antitumor, hepatoprotective, diaphoretic in fevers, smooth muscle relaxant, nerve tonics, laxatives, and for the treatment of wounds, bleedings, headache, pains, spasmodic diseases, flatulence, dyspepsia dysentery, lumbago, leucorrhoea, haemorrhoids, gangrenous ulcer and disorders causing cachexia (Achika,et al., 2014 ; Bessada, et al., 2015). Phytochemical investigations of the Asteraceae family have revealed that many components from this family are highly bioactive, as proven in research works with extracts (of roots, stems, bark, leaves, flowers, fruits and seeds) should be highlighted the medicinal properties of plants belonging to the Asteraceae family are recognized worldwide. Plant parts were used as medicine shows variation. Leaf (63.63%) are the leading part used in a majority of medicinal plants followed by the stem (13.63%), root (45.45%), whole plant (47.72%), flower (25%), bark (2.27%), seed (2.27%) and fruit (2.27%), (Easmin et al., 2021).

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Various plant species have been historically utilised in traditional medicine for a range of ailments. For example, *Carduus* sp. has been employed as antihemorrhoidal and cardiotonic remedies, while *Onopordum tauricum* has been used for liver disease (Rolnik et al., 2021). *Onpordum acanthium* was utilised for its antipyretic and diuretic properties, and *Centaurea solstitialis* is a folk medicine remedy in Turkey for stomach issues, abdominal pain, herpes infections, and the common cold (Rolnik & Olas, 2021). *Tanacetum parthenium*, known as feverfew, has been used for headaches, migraines, nausea, rheumatism, and inflammation (Koc et al., 2015). *Bidens pilosa*, also called Spanish needles, has been utilized in subtropical regions for liver issues and hypertension, and is a key component in Taiwanese herbal infusions (Rolnik & Olas, 2021). *Carthamus tinctorius* is used in Korean herbal medicine for rheumatism and osteoporosis, while *Emilia sonchifolia* root juice is used in Chinese and

Nepalese medicine for dysentery and diarrhea. *Cichorium intybus*, or chicory, is used for inflammatory conditions, liver disorders, gallstones, gout, rheumatism, and appetite loss (Achika et al., 2014). Chicory roots contain acids like caffeic acid and chlorogenic acid, abundant in Asteraceae plants, possesses antiviral, antioxidant, and anti-inflammatory properties (Nwafor et al., 2017; Petropoulos et al., 2019). *Achillea aleppica* and *Achillea biebersteinii* teas are recommended for abdominal pain, while *Chrysophthalmum montanum* is applied topically to wounds and ingested for high blood pressure. *Matricaria aurea* is suggested for bronchitis, sore throat, and cough, and Notobasis syriaca seeds are used for liver disease (Arasan et al., 2016). While *Taraxacum* spp. are a source of triterpenes such as *taraxasterol* and various acids like malic acid and citric acid (Rolnik & Olas, 2021). *Arctiin*, derived from *Arctium lappa*, exhibits cytotoxic, antiproliferative, and anti-inflammatory properties by inhibiting inflammatory mediators. Acetylenes, found in various tribes of the Asteraceae family, demonstrate diverse biological activities including cytotoxic and anti-inflammatory effects (Rolnik & Olas, 2021). *Cynara cardunculus*, commonly known as artichoke, has been consumed for centuries. In ancient times, the immature flowers were considered high-quality vegetables for special occasions by wealthy Greeks and Romans, while mature flowers were used as milk coagulants in cheese production. Presently, artichoke flowers are consumed as frozen and canned delicacies and are used in plant-based milk and cheese production (Rolnik & Olas, 2021). While Jerusalem artichoke remedies include tea for immune support and tubers for obesity diets (Rolnik & Olas, 2021). The flowers of *Tagetes erecta*, known as Mexican marigold, are utilized as food colorants and are added to poultry feed to reduce egg cholesterol levels and enhance egg yolk pigmentation (Rolnik & Olas, 2021). Dandelion roots used for producing high fructose syrup and dandelion leaf preparations added to health products. Dandelion exhibits antiplatelet activity and can enhance hepatic regenerative capacity (Achika et al., 2014). Extracts from *Emilia sonchifolia* and compounds from *Taraxacum* species exhibit anti-inflammatory effects by inhibiting cytokine production and suppressing inflammatory pathways (Rolnik & Olas, 2021).

The Asteraceae family includes many species that can be part of a healthy diet. A study by *García-Herrera et al*. revealed that the protein content in these plants ranges from 0.4 to 6.13 g per 100 g of edible parts, with fiber content ranging from 2.55 to 13.44 g (Piątkowska et al., 2022). The roots, leaves, and flowers of these plants are rich sources of essential minerals like sodium (Na), potassium (K), calcium (Ca), and magnesium (Mg), as well as vitamins A, B, C, and D. Most Asteraceae plants have a low fat content (Piątkowska et al., 2022).

1.2 The genus *Pteronia* L.

The genus *Pteronia*, originating in southern Africa, consists of about 80 species within the family Asteraceae (Zdero et al., 1990). There are about 24 native *Pteronia* species recorded in Namibia, with 12 species in the Fynbos Biome. Some Pteronia species are also found growing in Angola, Lesotho, Swaziland, and Zimbabwe [(1)]. *Pteronia* species are known to dominate the plant communities they inhabit. The morphology of *Pteronia* is diverse, with significant differences seen among species in terms of habit, flowers, and leaf shapes. *Pteronia* is closely related to *Engleria* but is isolated within the African Asteraceae family. While *Engleria* has radiate capitula, *Pteronia* has discoid capitula. Initially placed in the genus *Engleria*, *Pteronia* falls under the subtribe Solidagininae of the tribe Asteraceae (Kakembo, 2009).

The name "*Pteronia"* is derived from the Greek word for 'a wing', while the specific epithet "*incana*" means 'grey', referring to the appearance of the leaves (Fig 2) [(1)]. It is known for its rare characteristics such as spiny habit, opposite and succulent leaves, and beaked achenes (Bremer 1994; Kakembo, 2009).

(Fig 2) *Pteronia incana*, leaves.

1.3 The botanical characteristics of the genus *Pteronia*

Here are some general botanical characteristics of the genus *Pteronia* (Kakembo, 2009):

1. Leaves: *Pteronia* species often have short, thin leaves that resemble needles or lanceolate shapes. They can be placed along the stems in an opposing or alternating order.

2. Flowers: At the tips of the stems, *Pteronia* plants usually have little, daisy-like flowers called inflorescences or clusters. Typically, the petals of the blooms are purple, white, or yellow.

3. Inflorescence: *Pteronia* species have a variety of shaped and sized inflorescences, although they frequently take the form of dense clusters of flower heads held above the leaves.

4. Habit: Most *Pteronia* species are shrubby or herbaceous plants. While some species can grow up to many feet in height, others may thrive as low-growing mats.

5. Habitat: *Pteronia* plants are frequently found in dry or semi-arid areas in a range of habitats, such as grasslands, scrublands, and rocky slopes.

6. Ecology: A wide variety of *Pteronia* species have evolved to withstand hard circumstances, such as poor soil and little water supplies. Certain plants are well-known for their capacity to flourish in rocky or sandy soils and their resistance to drought.

7. Perfume: The fragrant leaves of certain *Pteronia* species are well-known for giving off a pleasing perfume when crushed or touched. Because of this characteristic, several species are now utilised in the manufacturing of essential oils and in conventional medicine.

1.4 Phytochemical constituents of the genus *Pteronia* species

The genus *Pteronia* contains several species that are known to have various phytochemical constituents. Some of the phytochemicals that have been identified in *Pteronia* species include:

1. Essential oils: Many *Pteronia* species are known for their aromatic foliage, which contains essential oils. These oils often contain compounds such as monoterpenes (e.g., limonene, α-pinene, and β-pinene) and sesquiterpenes (e.g., β-caryophyllene, germacrene D), which contribute to the characteristic fragrance of the plants. (Hulley et al. 2010; Achika et al., 2014)

Pteronia species are aromatic plants native to South Africa, known for their fragrant essential oils. These oils are extracted from the leaves, stems, and flowers of the plant through steam distillation or cold pressing (Robert & Rodney, 2014)
Some common species of *Pteronia* that are used to produce essential oils include:

- *P. pallens* (L.) C.A.Sm. – Also known as the camphor bush, this species produces an essential oil with a strong camphor-like aroma. It is commonly used for its calming and soothing properties (Coovadia, 2008).

- *Pteronia incana* (Sond.) C.A.Sm. – Known as the grey bush, this species produces an essential oil with a sweet, floral scent. It is often used in aromatherapy for its uplifting and mood-boosting effects (Hulley et al. 2010).

- *Pteronia tagetoides* DC. – This species is commonly known as the yellow bush and produces an essential oil with a citrusy, fruity aroma. It is often used for its energising and rejuvenating properties.

These essential oils from *Pteronia* species can be used in aromatherapy, massage therapy, skincare products, and household cleaning products. They are prized for their therapeutic properties and unique scents.

2. Flavonoids: Flavonoids are a group of secondary polyphenolic metabolites that are present in plants. Chemically, flavonoids are composed of two phenyl rings and a heterocyclic ring (the ring with the inserted oxygen), which together make up a 15-carbon skeleton. Flavonoids are a class of plant secondary metabolites that are a diverse group of phytochemicals with antioxidant anti-inflammatory, antimicrobial properties and other potential health benefits such as improving cardiovascular health, and protecting against oxidative stress (Rolnik & Olas, 2021). Several *Pteronia* species have been found to contain flavonoids such as quercetin, kaempferol, luteolin and apigenin. Many of the flavonoids that have been discovered are found in foods such fruits, vegetables, and beverages. These compounds are responsible for the colourful pigments in flowers and fruits of *Pteronia* plants (Panche et al., 2016)

3. Phenolic compounds: Phenolic compounds are a group of secondary metabolites found in various plant species, including *Pteronia* species. Phenolic compounds, including phenolic acids and tannins, are commonly found in Pteronia species. Some specific phenolic compounds found in *Pteronia* species include quercetin, kaempferol, gallic acid, and ellagic acid. These compounds have antioxidant, anti-inflammatory, and antimicrobial properties, and play a role in the plant's defence mechanisms against environmental stresses and pathogens (Rolnik et al., 2021).

4. Terpenoids: *Pteronia* species are known to contain various terpenoids, including diterpenes and triterpenes, which have been studied for their potential pharmacological activities.

Terpenoids are a diverse class of organic compounds found in many plant species, including those belonging to the genus *Pteronia*.

Terpenoids, also known as isoprenoids, are derived from isoprene units and exhibit remarkable structural diversity. They can range from simple structures like monoterpenes (containing two isoprene units) to complex molecules like diterpenoids and triterpenoids (containing four or more isoprene units) (Achika et al., 2014).

Terpenoids play essential roles in plants, serving as defence compounds against herbivores and pathogens, as well as contributing to the plant's aroma and flavour. Additionally, some terpenoids have been found to possess pharmacological activities, including antimicrobial, anti-inflammatory, and anticancer properties (Achika et al., 2014).

Industrial Applications of Terpenoids from *Pteronia* species and other plants have industrial applications, particularly in the fragrance, flavour, and pharmaceutical industries.

5. Alkaloids: Some *Pteronia* species have been found to contain alkaloids, which are nitrogen-containing compounds with diverse biological activities (Rolnik et al., 2021).

- Pteronine: This alkaloid is found in *Pteronia* species and is known for its insecticidal properties.

- Pteropodine: Another alkaloid found in *Pteronia* species, pteropodine has been studied for its potential antimicrobial and anti-inflammatory properties.

- Pterosin: This alkaloid has been isolated from certain *Pteronia* species and has shown potential as an anti-inflammatory agent.

- Pterolobine: Found in *Pteronia* species, pterolobine has been studied for its potential anti-cancer properties.

- Pteropine: This alkaloid is found in *Pteronia* species and has shown activity against certain types of cancer cells.

- Pteronitol: Another alkaloid found in *Pteronia* species, pteronitol has been shown to have anti-inflammatory properties.

- Pterogenin: This alkaloid has been isolated from *Pteronia* species and has shown potential as an anti-tumor agent.

6. Saponins: Saponins are glycosides found in many plant species, including some *Pteronia* species. They have been studied for their potential health benefits, including cholesterol-lowering, immune-modulating properties and anti-inflammatory effects.
Saponins have a characteristic structure consisting of a hydrophilic (water-attracting) sugar moiety attached to a hydrophobic (water-repelling) triterpene or steroid backbone. This unique structure gives saponins their ability to form stable foams when agitated in water.
Pteronia species containing saponins have been used traditionally in herbal medicine for their expectorant, diuretic, and tonic properties, also it has been used to treat respiratory conditions, digestive disorders, and skin ailments (Rolnik et al., 2021). However there is no previous work on the saponin content of *P. incana*.

7. Coumarins: Coumarins are another group of phytochemicals found in some *Pteronia* species. Coumarins are often found in the form of glycosides in plants and are known for their potential pharmacological properties. Some of the *Pteronia* species have been studied for their coumarin content, and these compounds have attracted interest due to their potential health benefits and industrial applications such as anticoagulant, anti-inflammatory, antioxidant properties, antimicrobial and anticancer activities (Achika et al., 2014). Additionally, coumarins are known for their pleasant aroma and are often used in the fragrance and flavour industries (Zdero et al., 1990). However there is no previous work on the coumarin content of *P. incana*.

1.5 Classification of *P. incana* (Roskov et al., 2018):

Order*: Asterales*

Kingdom*: Plantae*

Phylum*: Streptophyta*

Class*: Equisetopsida*

Subclass*: Magnoliidae*

Family: Asteraceae (Compositae)

Genus: *Pteronia* L.

Botanical name: *Pteronia incana* (Burm.) DC

1.6 Synonyms [(2)]

Heterotypic Synonyms of *Pteronia incana* (POWO, 2024)

Athanasia rigida Scop.

Chrysocoma incana Burm.

Eupatorium cinereum L.

Pteronia rigida P.J. Bergius

Pteronia xantholepis DC.

1.7 **Distribution and habitat of *Pteronia incana***

(Fig.3) The distribution map of *P. incana (POWO, 2024)* [(3)]

Pteronia incana is a species endemic to South Africa, primarily found in the arid, winter rainfall regions (Roskov, et al., 2018). Its distribution spans from the Eastern Cape Province to the Namibian border (Fig. 3), encompassing areas like the Little Karoo, Robertson Karoo, Namaqualand, and parts of the Overberg region especially along the broader region of the Breede river valley (Hulley et al., 2010). Thriving in sandy areas with clay soils on flat or gently sloping terrain,

Typically associated with the Cape Floristic Region, particularly renosterveld vegetation, *P. incana* is a diverse genus characterised by variations in size, flower colours, and leaf textures. These perennial woody shrubs, ranging from 0.3 to 1.5 metres in height, bloom in spring months with flowers of white, yellow, and pink buds depending on the species (Hulley et al., 2010).

The leaves can be smooth and glabrous or hairy, arranged oppositely or alternately (Shearing, 1997). *P. incana* features small, light grey, woolly, fragrant leaves, forming low, dense bushes. These aromatic leaves are utilised for various medicinal purposes, highlighting the species' significance beyond its ecological role in the region [(1)].

P. incana, can be propagated through seeds or cuttings. Seeds require a well-draining soil mix and consistent moisture for germination, while cuttings from semi-hardwood stems should be planted in a similar soil mix. They thrive in warm, bright locations with regular watering [(1)].

1.8 **Botanical Characteristics of *P. incana***

P. incana, (also called Grey Pteronia, among many other common names), belongs to the Asteraceae family, native to Southern Africa. It is a low-growing, tiny, perennial shrub that resembles a bush (Webber et al., 1999) with an average height that ranges between 0.5 and 1 m [(1)]. It has thin, woolly, greyish fragrant leaves, about 5 mm long and 3 mm broad, and has a white, felt-like substance on their surface. The leaves are oblong to obovate in form and arranged oppositely with sometimes ramified branches that reach a height of about 100 cm (Bruns & Meiertorens, 1987; Webber et al., 1999).

P. incana often has a single, short stem that allows branches to grow up in several directions from the base of the plant. But occasionally, the main stem might be hidden or just partially exposed, giving an appearance that the plant is developing as a multi-stemmed shrub forming

a fine, rounded, mounding shape. Dry, woody, dark-brown stems and branches that are pubescent when young and become brown-grey with time make up this structure [(1)].
Mid-spring to early summer sees *P. incana* produce flower heads with a diameter of around 15 mm. These flowerheads are made up of yellow disc florets that have a pleasant coconut aroma (Fig. 4). A flowerhead typically has ten flowers. Smooth, non-sticky, yellow-green bracts encircle the florets (Mayekiso et al., 2008).

The fruit is an obovate, compressed achene that is about 5 mm long (Fig 5). An achene is a dry fruit with one seed that does not open to release the seed. The seed is a small, dark brown achene oval-shaped rosette of basal leaves [(1)].

It has taken over an estimated 60,000 hectares of semi-arid ground in the Eastern Cape of South Africa. Since this plant is regarded as a weed in this area, it has taken up a lot of space that might be used for agriculture. It is said that this plant's exudates make plant cultivation superfluous and are inappropriate for a range of cattle (Mayekiso et al., 2006).

***Pteronia incana* in flower [(4)]**

(Fig. 4)

The ultrastructure of trichomes in *P. incana* leaves and stems reveals the presence of both non-glandular and glandular trichomes. Non-glandular trichomes protect the grooved epidermal surface, while glandular trichomes accumulate essential oils and phytotoxic compounds as a defence mechanism against herbivores. Glandular trichomes have plasmodesmata for material movement between cells and cuticular sacs that release essential oils when ruptured. These trichomes are adapted to arid conditions, impacting transpiration and potentially influencing water economy and temperature regulation. The glandular trichomes are characterised by dense cytoplasm, osmophilic droplets, and various organelles. The secreted material accumulates in a cavity and is released when the cavity ruptures. These compounds make *P. incana* unpalatable to livestock (Mayekiso et al., 2008).

Figure 5: Pteronia incana in fruit [(1)]

1.9 **Biological activities of *Pteronia incana***

Essential oils have long been used to improve appearance, health, and defence against environmental harm (Carvalho et al., 2015). Women in ancient Egypt used oils for cosmetic purposes; Cleopatra was one of the most famous users. These practices extended to Greek and Roman cultures. Essential oils derived from different plants are prized in cosmetics and perfumery. An EU survey conducted between 2002 and 2009 revealed a 3% annual growth in the essential oils market.

Essential oils are complicated mixtures of substances derived from a variety of plant groups that are present in fragrant plants. They are mostly produced in plant cells and kept in various plant sections (Carvalho et al., 2015). Cold pressing and steam distillation are two extraction techniques. They are mostly composed of monoterpenes, sesquiterpenes, and diterpenes, however they serve a variety of organic purposes. About 300 essential oils, valued for their flavour, smell, and biological properties, are derived from 2000 plant species and are used commercially. Their aroma makes them popular in cosmetics, but their antifungal and antibacterial qualities also make them potentially lessening the need for preservatives in goods (Carvalho et al., (2015).

The main applications for essential oils are in skin care, hair care, and perfumery goods. With skin care accounting for 23 percent of all sales in 2009, skin care is the largest sector in the global beauty business. However, because of their volatility and sensitivity to heat, light, moisture, and oxygen, essential oils have a limited shelf life. Microencapsulation is thought to be a useful method for overcoming these difficulties since it provides controlled-release distribution and better EO management (Carvalho et al., 2015).

Research into essential oils from aromatic plants in the Eastern Cape/Karoo region of South Africa has shown insect-repelling properties and can combat fungi and insects, providing natural alternatives to synthetic pesticides. With the rising demand for organic foods, essential oils are being explored as safer alternatives for food preservation (Mayekiso et al., 2008).

According to a study (Coovadia, 2008**)** a vast and amazing variety of medicinal plants may be found across Africa, caused by indigenous knowledge and ancestral experience. *Securidaca longipedumalata*, for example, is a common plant in Africa. Tanzanians utilise the aerial portions and roots as a purgative for problems of the neurological system. For two weeks, one cup of a root decoction is given every day, which causes the intestines to empty it and trigger a purgatory reaction. The use of traditional medicine is quite flexible. The same leaves of *Securidaca longipedumalata* are used to cure a variety of conditions in East Africa, including snakebites, wounds, ulcers, coughs, and sexual infections. On the other hand, the leaves are used for relieving headaches in Malawi. The dried leaves are used in Nigeria to cure a variety of skin conditions, such as dermatitis and eczema. In Angola, the dried root is utilised as aphrodisiac. While the root bark is used to treat epilepsy in Ghana, the same dried roots and leaves have religious importance in Guinea-Bissau and are said to have psychoactive effects. This only illustrates how a single plant may serve a variety of functions across the continent.

Pteronia species have a history of being used for their anti-infective properties by indigenous groups like the Hottentot and Khoisan people (e.g. a well-known traditional healer or bossiedokter, the late Doortjie Dories) (P.M. Burger, pers. comm. to B-EVW) (Hulley et al., 2010). *Pteronia* species were among the earliest plants utilised by the Hottentotís for their anti-infective properties. The Khoi-San people also employed *Pteronia* species as a "buchu remedyî, or to cure stomach illnesses ranging from cramping to nausea (Shearing, 1997). Ironically, despite *Pteronia*'s long history of usage, little is known about its phytochemistry and antibacterial qualities. One additional well-researched feature of *Pteronia*, which is found in many different species, is that it is extremely poisonous to ungulate herbivores- particularly *Pteronia* 15 pallens, which is fatal to cattle. Because the species is tasty to the population of sheep and cattle, it is generally not well grazed. Additionally, it has been demonstrated that the species has insecticidal qualities (Shearing, 1997; Coovadia, 2008).

1- Preservatives effect

The effectiveness of essential oil from *Pteronia incana*, as a preservative in topical treatments was assessed (Varvaresou et al., 2009). The major constituents identified in the oil were 1,8-cineole and pinene, with several significant components exclusive to particular oils (Varvaresou et al., 2009). Challenge tests showed that when bacteria were decreased by more than 99% after two days and yeasts and molds by more than 99% after fourteen days, the goods were sufficiently maintained (Varvaresou et al., 2009). According to Muyima et al., three distinct concentrations of each oil—0.5, 1.0, and 1.5% (w/w)—were used as the only preservative in a cosmetic cream (Mangena and Muyima., 1999; Varvaresou et al., 2009). Although the activity varied depending on the test organism species, oil type, and oil concentration, it demonstrated microbial reduction characteristics (Varvaresou et al., 2009). The essential oil of *P.incana* may offer interesting preservation potential for industrial applications due to their wide range of antibacterial activity (Mangena and Muyima, 1999). The oil may be recommended as an active component in pharmaceutical formulations as well as a potential natural conservation agent for the food and/or cosmetics sectors (Mangena and Muyima, 1999).

2- Antibacterial properties

Mangena and Muyima, 1999 reported that the antibacterial activity of *P. incana* oil was quite wide, especially at high concentrations. In order to assess the potential therapeutic benefits of this species, scientists looked at potential antibacterial activity against a range of four respiratory infections (Hulley et al., 2010). The results demonstrated that the most effective extracts were methanol:dichloromethane (MeOH:CH2Cl2) against both *Cryptococcus neoformans,* a yeast pathogen with MIC values as low as 0.5–2.0 mg/ml, and *Mycobacterium smegmatis,* a Gram-positive bacterium with MIC values as low as 0.5–0.8 mg/ml. With a particularly notable activity of 0.3 mg/ml, the essential oils demonstrated the strongest antibacterial activity against *Cryptococcus neoformans*. For example, *Klebsiella pneumoniae* exhibited a moderate level of susceptibility, with two MeOH: H2O extracts having MIC values ranging from 1.5 to 2.0 mg/ml (Hulley et al., 2010).

The bacteria that responded to *P. incana* oil the most were *Staphylococcus aureus* and *Enterobacter cloacae*. Oil was more effective against bacteria than against yeast. *S. aureus, E. chrysanthemi,* and *E. cloacae* all seemed to be more susceptible to *P. incana* oil (Mangena & Muyima, 1999). *Erwinia amylovora*'s growth was greatly inhibited by α -pinene, whereas bacteria were inhibited by β -pinene, particularly at larger bacterial populations (Scortichini and Rossi 1991; Mangena & Muyima, 1999). However, it was shown that high concentrations of sesquiterpenes and/or monoterpene hydrocarbons reduced the antibacterial action of essential oils (Chalchat et al. 1997, Mangena & Muyima, 1999). Research has emphasised the chemical makeup of *Pteronia incana* essential oils, with particular attention paid to elements like *p*-mentha-8-ene-2-ol that are present in the oil (Varvaresou et al., 2009; Mangena & Muyima, 1999).

It's significant to note that the oils shown antimicrobial action against bacteria including salmonellas, shigellas, and staphylococci that are important for food deterioration and/or poisoning as well as bacteria that are important to medicine (Mangena, & Muyima, 1999).

3- Antifungal properties:

Pteronia incana has anti-fungal and anti-yeast strain properties including *Epidermophyton, Microsporum, Malassezia and Trichophyton* strains (Muyima, & Nkata, 2005). The main ingredient in the oil, 1, 8-cineole, led to its significant antifungal effects, even though *M. furfur* CBS 1878 was less sensitive to the oils than *M. furfur* 4163 (Muyima & Nkata, 2005). Depending on the test organism and concentration, the oil's antifungal properties changed. The findings showed that *P. incana* oil is highly efficient against both kinds of bacteria and dermatophyte fungus, which are linked to dandruff and scalp problems (Muyima & Nkata, 2005). Because of their strong antifungal qualities, these essential oils have potential for usage in industrial settings. They may be utilised as an alternative to treat dermatophytes that cause dandruff and other scalp disorders, or as an element in products for scalp care.

The study found that *H. burtonii* UOFS YC608, *S. cerevisiae* UOFS Y0149, and *T. delbruckii* UOFS Y0159 were most affected by *P. incana* oil (Muyima & Nkata, 2005). However, only half of the yeasts tested were sensitive to the undiluted form of this oil (Muyima & Nkata, 2005).

4- Other Properties of *P. incana* essential oils

P. incana, a different species, is still traditionally utilized to treat renal diseases, backaches, influenza, and fever **(**Hulley et al., 2010).
Due to its unique chemical makeup, *P. incana*'s intensely fragrant oil is an excellent choice for fragrance in perfumery [(5)]. *P. incana* yields oil that is yellowish and strongly scented. The main reason for interest in this oil has been its distinct smell, which makes it appropriate for use as a fragrance (Bruns & Meiertoberens 1987; Mangena & Muyima, 1999).

1.10 Safety of Essential oil of the *P. incana* (Robert Tisserand & Rodney Young, 2014)

- Hazards: *Pteronia incana* essential oil may be potentially carcinogenic due to its methyl eugenol content and can cause skin sensitization if oxidised.
- Contraindications: Oral ingestion of the oil is not recommended.
- Maximum dermal use levels: EU recommends 0.003%, IFRA recommends 0.006%.
- Regulatory guidelines: IFRA suggests a maximum concentration of 0.0004% methyleugenol in leave-on products, while the SCCNFP equivalent is 0.0002%.
- Safety advice: A dermal maximum of 0.3% is recommended based on a7.2% methyleugenol content with a dermal limit of 0.02%.
- Organ-specific effects: Limited information is available on adverse skin reactions. Autoxidation products of limonene and α-pinene may lead to skin sensitization.
- Systemic effects: No data on acute toxicity or carcinogenic potential. Methyleugenol is a rodent carcinogen with high oral exposure, while limonene and α-caryophyllene exhibit anticarcinogenic properties.
- Comments: *Pteronia* oil has limited shelf life, may polymerize and oxidise, and is not widely available.

Understanding and addressing these safety considerations are essential for the safe use and application of *Pteronia incana* essential oil in various contexts.

1.11 Purpose of the Study

P. incana is an important South African plant growing naturally and the leaf oils were produced and sold in the markets. Moreover, the essential oil of the plant has been used for various purposes in the traditional medicine; there was not much research on the essential oil composition of the *P. incana*. This study aims to investigate the commercially sold essential oil composition of leaves of *P. incana* obtained in different batches during the production. By conducting a detailed analysis of the essential oil composition, the study intends to identify the specific volatile compounds that contribute to the unique aroma of *P. incana* essential oil. Understanding the chemical diversity and variations in essential oil composition within this species can provide valuable insights into its potential applications across various industries, including pharmaceuticals, fragrances, and cosmetics.

Overall, this study seeks to enhance our understanding of the medicinal and aromatic properties of *P. incana* and explore its potential as a valuable resource for the development of novel products in the pharmaceutical, fragrance, and cosmetic sectors.

1.12 Research Questions/Hypothesis

Research Questions: This study aims to address the following research questions:

1. What are the chemical constituents of the essential oils of the leaves of *P. incana*?
2. Are there significant chemical differences in the essential oil composition in different batches?
3. Are there any specific compounds that could lead producers and/or researchers in order to help them to define the essential oils of *P. incana*?
4. Is there a significant chemical difference between the previous literatures on the essential oil composition of *P. incana*?

Hypothesis: The hypothesis for this study on *P.incan*a is as follows:

The essential oils produced from the leaves of *P.incana* will be the same in the essential oil composition in all batches for its quality.

1.13 Limitations of the study

Researching the chemical composition of *P. incana* essential oil from diverse sources in Africa presents various challenges and constraints, including sample variability, plant material availability, external variables such as climate shifts, time and resource limitations, and issues related to generalizability of findings.

Furthermore, it was not possible to plant material in its nature due to being growing in only the South Africa region.

CHAPTER II

Literature Review

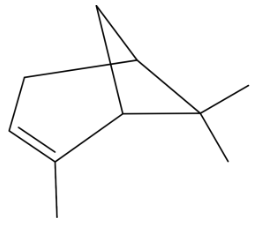
Pteronia incana, a heather-like perennial bush, has been the focus of agricultural research under the CENTOIL project in South Africa (Hulley et al., 2010). Its essential oil, known as 'Blue Bush Oil', is extracted through steam distillation from the leaves and stalks (Hulley et al., 2010). *P. incana* can be harvested multiple times annually without hindering plant growth, ensuring a continuous oil supply. The essential oil of *P. incana* is rich in myrcene, *α*- pinene, *β*-pinene, sabinene, *α*-terpinene, 1, 8-cineole, and limonene as its main constituents.

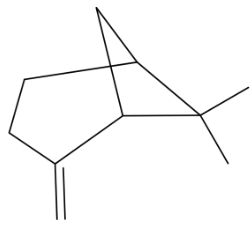
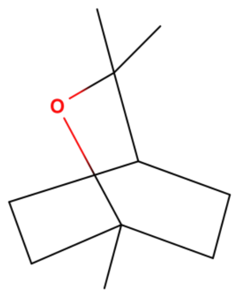
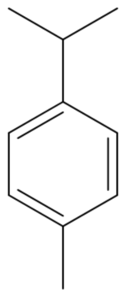
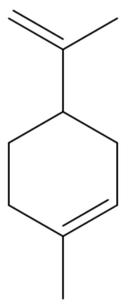
A number of diterpenes and other phenolic compounds have been isolated from *P. incana* and several other *Pteronia* species (Zdero et al., 1990), including an unusual lactone called incanapteroniolide, which was found in *P. incana*. (Hulley et al., 2010)

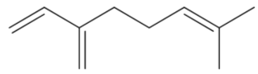
Even though this plant has been cultivated for essential oil production in South Africa, there isn't too much search on the chemical composition of essential oil of *P. incana* and its variations depending on different plant parts and varieties. The reported major compound of the *P. incana* essential oil was given in table 1.

Major compounds identified in *Pteronia incana* essential oil table 1

Pteronia incana is variable mixtures of terpenoids, specifically monoterpenes [C10]**.**

Name of the compound	Structure of the compound
α-Pinene	

β-Pinene	
1,8-Cineole	
ρ-Cymene	
Limonene	

Myrcene	

In a study, the essential oil obtained from leaves of *P. incana* collected for four different months in seasons (December, March, June, September) from a place of the Eastern Cape of South Africa was explored by Mayekiso, et al., 2006. The aim of that study was to investigate the best harvesting time of this plant material for various purposes. The GC/MS analysis has shown that the spectrum of the essential oil of *P. incana* sample varies with the season of analysis. As a result of their research, the oil yield was higher in the flowering period and it was reduced in drought periods. The GC spectrum of the oil sample in June and

December had the characteristic peaks which appeared throughout the year. These peaks seemed to represent the compounds; *α*-pinene (12.57-22.34%) in June, *β*-pinene (25.26%) in December, *β*-myrcene+sabinene (16.99%) in December and 1, 8-cineole (12.10-24.78%) in different months. However the spectrum of the samples obtained between March and August appeared to be characterised by the additional terpenoids like, *β*-thujene, limonene, α- terpinene and δ-4-carene.

In another study, the essential oil obtained from leaves and stalks of *P. incana* collected from a place of the Eastern Cape of South Africa was explored by Bruns, et al., 1987. Depending on the growth stage and on the leaf-to-stalk ratio, the yield of oil totals between 0.4% and 1.1% of the plant's dry weight. The aim of that study is to recover its essential oil commercially. The oil is described as having a relatively strong odour, which is comparable to that of juniper oil which makes it suitable for use as a fragrance. The GC/MS analysis has shown that the spectrum of the essential oil of *P. incana* sample has monoterpene fraction, sesquiterpene fractions and unidentified components in the sesquiterpene fraction. In general, it has been dominated by monoterpenes; they are considered the main compounds such as, *α* pinene (14.2%), *β*-Pinene + Sabinene (29.8%), myrcene (17.7%), 1,8-cineole + limonene (14.0%), *p*-cymene (2.3%), and terpinolene (9.4%). The sesquiterpene fraction displays an extremely complex composition with far more than 100 constituents, which contains not only C15 substances but, owing to their boiling behaviour, also a number of oxygenated monoterpenes.

In another study, the essential oil obtained from fresh leaves of *P. incana* collected from a place of the Eastern Cape of South Africa was explored by Muyima et al., 2002. Essential oils of aromatic plants have been reported to possess not only unique fragrance properties but also broad antimicrobial activities against Gram Positive and Gram-negative bacteria as well as yeasts. The purpose of this study was to evaluate the preservative capabilities of the essential oils of two South African indigenous aromatic plants including *P. incana*. The GC/ MS analysis showed that all the oils were constituted by more than 10 different chemical compounds. One of the major components of *P. incana* oil was *p*-mentha-8-ene-2-ol with a percentage of 16.3%. The other major compounds were myrcene (14.4%), *α*-pinene (12.2%), and *β*-pinene (20.3%). *P. incana* oil showed moderate antimicrobial activity against all the test organisms, with *Pseudomonas aeruginosa* being the least sensitive. However, the oil has a unique aroma, which makes it a good candidate for use as a fragrance ingredient. The results show the potential of tested essential oils including *P. incana*, could be used as natural preservatives against *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Ralstonia pickettii*, *Candida albicans*, and *Aspergillus niger* in aqueous cream formulations and similar cosmetic products.

Similarly, a study has been done by Mangena & Muyima, 1999 the essential oil *P. incana* was collected from a natural habitat in South Africa. The oil was extracted from the fresh leaves of the samples by water distillation. The aim of this study was to carry out a comparative analysis of the antimicrobial activities of essential oils of two aromatic plants indigenous to the Eastern Cape, namely *A. afra* and *P. incana*, and *R. officinalis*, an aromatic plant from the Mediterranean region grown in the Eastern Cape. The chemical composition of the essential oils from *Artemisia afra*, *Pteronia incana* and *Rosmarinus officinalis* as identified by GC-MS analysis, revealed that all the oils analysed contained 1,8-cineole, with *R. officinalis* containing the highest percentage. Show the peak concentrations of *P. incana*, *o*-cymene (4.47%), *p*-cymene (18.90%), limonene+1, 8-cineole (20.82%), myrcene (0.49%), α-pinene (19.08%), and β-pinene (32.20%). *Pteronia incana* was the only oil having *o*- and *p*-cymene amongst its constituents. *Pteronia incana* oil had a higher content of *β*-Pinene, and lower content of myrcene. *α*-Pinene significantly reduced growth of *Erwinia amylovora* while *β*-pinene was inhibitory to bacteria, especially at higher bacterial populations (Scortichini and Rossi 1991). Large amounts of monoterpene hydrocarbons and/or sesquiterpenes were, however, found to lower the antimicrobial activity of essential oil (Chalchat *et al*. 1997). The results of the antibacterial tests indicated that *Pteronia incana* oil also displayed a fairly broad spectrum of antibacterial activity, particularly at higher concentrations. *Enterobacter cloacae*, *Erwinia chrysanthemi* and *Staphylococcus aureus* appeared to be more sensitive to *P*. *incana* oil.

In a study, the essential oil obtained from dried leaf material of *P*. *incana* was collected at three localities, namely Worcester [33° 19° CB], Herolds Bay [34° 22° AB] and near Alice at the University of Fort Hare campus [32° 26° DD] was explored by Hulley et al., 2010**.** The purposes of this *P*. *incana* study were to: (1) precisely record the medicinal applications of this species; (2) examine the anatomy of the leaf, with a particular emphasis on secretory structures; (3) determine the chemical makeup and regional variations of the essential oil; and
(4) look into potential antimicrobial activity. The main compounds of essential oil samples from eleven individual plants of *Pteronia incana* collected at three localities, as identified by GC–MS. As a result of their research, the yields were variable, ranging from 0.01% to 1.08% on a dry weight basis. The variation seems to be unrelated to provenance and date of collection. A total of 62 volatile components were identified in the eleven samples studied. The major compounds are several monoterpenes as well as sesquiterpenes. *β*-pinene (0.3- 10.2%), limonene (0.8-10.9%), 1,8-cineole (0.4-18.5%), myrcene (0.2-8.0%), spathulenol
(2.9-22.9%), *p*-cymene (4.2-36.7%) and methyleugenol (1.0-10.9%) are main compounds present in all or most of the samples, with smaller amounts of *α*-pinene, sabinene, *γ*- terpinene, terpinen-4-ol, bicyclogermacrene, globulol and *α*-bisabolol in several of the samples.

CHAPTER III

Methodology

The aim of this study was to determine the essential oil composition of commercially sold *P. incana* because of its possible therapeutic benefits as a medicinal herb that is frequently used in traditional medicine. In addition, to check the quality of the product obtained from different batches. A number of analytical methods, including gas chromatography-mass spectrometry (GC-MS), were used to evaluate the resultant essential oil in order to identify and measure the phytochemical components that were present.

3.1 Materials and Methods

Essential oils

The essential oils of *P. incana* were taken from the company in South Africa named Parceval (Pty) Ltd Wellington and the batch numbers of the samples were SA033 (Sample A) where it was harvest date and distilled on 9th June 2022, Mid-winter, winter rainfall, growth stage dormant no flowers, these leaves, stems were taken from location Matjiesrivier, GPS (33°14'30" S - 19°38'45" E), altitude 908m, oil kg 0.100, yield 0.242%. SA027 (Sample B) where it was harvest date 10th Feb 2022 and distilled on 11th Feb 2022, mid-summer, summer rainfall where it was active growth, flowering, these leaves, stems were taken from location Peddie GPS (33°11'10" S - 27°10'18" E), altitude 326m, oil kg 0.133, yield % 0.316%. And SA028 (Sample C) where it was harvest date 28th March 2022 distilled on 29th Mar 2022, late summer, winter rainfall, growth stage dormant no flowers, these leaves, stems were taken from location Uniondale GPS (33°31'20" S - 23°06'11" E), altitude 890m, 0.040 oil kg, yield 0.072%. The analysis was done in Alvaro at TUT as well as Marietjie Stander's lab at University Stellenbosch. The essential oils were stored at 4°C until further analyses. The essential oil of *P.incana* is considered rare in South Africa (Ulrich Feiter, 2022).

3.2 *GC-FID* analysis

The GC/MS analysis was carried out with an Agilent 5977B GC-MSD system. Innowax FSC column (60 m x 0.25 mm, 0.25 mm film thickness) was used with helium as carrier gas (0.8 mL/min). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min, and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. Split ratio was adjusted at 40:1. The injector temperature was set at 250° C. Mass spectra were recorded at 70 eV. Mass range was from *m/z* 35 to 450. FID results were used to report the relative percentages (%) characterised compounds.

3.3 *GC/MS* analysis

The analysis was carried out using an Agilent 7890B GC system. FID detector temperature was 300°C. To obtain the same elution order with GC/MS, simultaneous auto-injection was done on a duplicate of the same column applying the same operational conditions. Relative percentage amounts of the separated compounds were calculated from FID chromatograms. Identification of the essential oil components was carried out by comparison of their relative retention times (RRT) with those of authentic samples or by comparison of their linear retention index (LRI) to a series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, NIST Library) (McLafferty & Stauffer, 1989; Hochmuth, 2008) and in- house “Başer Library of Essential Oil Constituents” built up by genuine compounds and components of known oils, as well as MS literature data (Joulain & König, 1998; ESO 2000) was used for the identification.

3.4 Identification of components

Identification of the essential oil components were carried out by comparison of their relative retention times with those of authentic samples or by comparison of their relative retention index (RRI) to a series of *n*-alkanes. Computer matching against commercial (Wiley GC/MS Library, Adams Library, MassFinder 3 Library) (McLafferty & Stauffer 1989; Koenig, et al 2004), and in-house “Başer Library of Essential Oil Constituents” built up by genuine compounds and components of known oils, as well as MS literature data (Joulain & König, 1998) , was used for the identification.

CHAPTER IV

Results and Discussion

In this section a comprehensive analysis of the chemical compositions of *P. incana* (Sample A, Sample B and Sample C) essential oils are carried out. A detailed examination of the components, their percentages is entailed in the chapter. In Table 2, the essential constituents of all samples are given. Table 2 presents a detailed comparison of the chemical composition of the three samples, Sample A, Sample B and sample C obtained from Gas Chromatography-Mass Spectrometry (GC/MS) analysis carried out. It lists various chemical components identified in these samples and provides the percentage of each component. Additionally, the table includes a notation for "tr," indicating trace amounts of certain components (typically less than 0.1%).

In general, a total of 50 elements, representing 99.9% of the total essential oil sample A, a total of 51 elements representing 100% of the total essential oil sample B , and 55 elements representing 99.9% of total essential oil sample C. The composition of the Sample A and C was similar though the Sample B was slightly different. The major compounds of the analysed essential oils were *β*-pinene (18.2-29.6%); myrcene (7.8-12.1%); 1, 8-cineole (8.3- 13.5%); *p*-cymene (8.1-31.9%) and limonene (5.8-9.0%). In addition, *α*-pinene was one of the major compounds in only one of the analysed oils which were Sample B (15.2%).

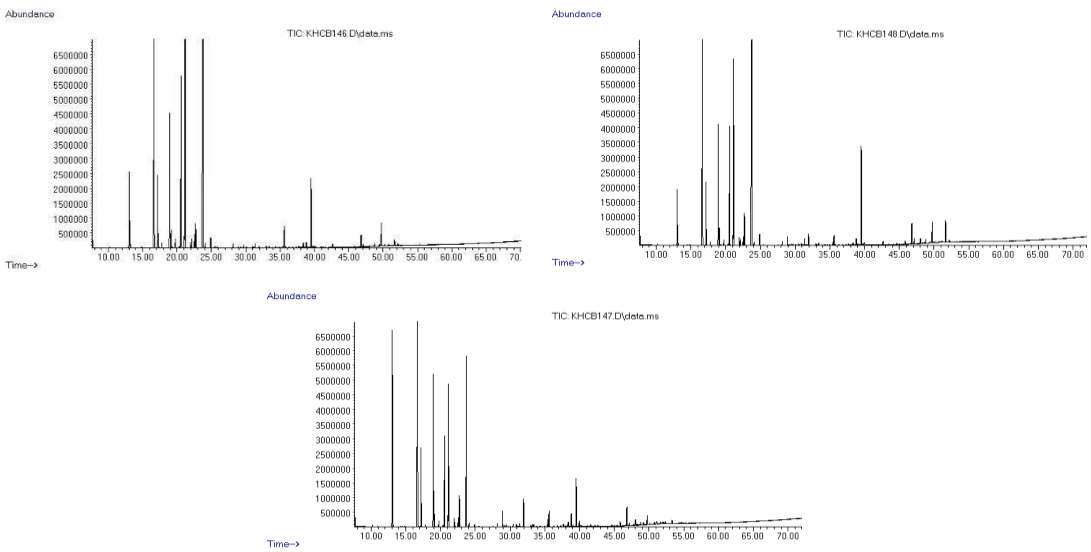


Figure 6: Gas Choromatography Chromatograms of the 3 Samples

Table 2. The commercially sold essential oil compositions of the *Pteronia incana* from South Africa

LRI	Compound Name	Relative Percentage Amounts (%)		
		SAMPLE A	SAMPLE B	SAMPLE C
988	α-pinene	3.9	**15.2**	3.7
992	α-thujene	0.1	0.1	0.1
1037	Camphene	0.0	0.0	0.0
1085	β-pinene	**18.2**	**29.6**	**19.1**
1099	Sabinene	3.5	5.2	3.8
1113	1-undecene	0.3	0.2	0.2
1139	Myrcene	**7.8**	**12.1**	**8.5**
1143	α-phellandrene	0.7	0.7	0.8
1156	α-terpinene	0.3	0.3	0.3
1168	dehydro-1,8 cineole	0.1	0.1	0.0
1174	Limonene	**9.0**	**5.8**	**7.3**
1184	β-phellandrene	0.5	0.7	0.5
1187	1,8-cineole	**13.5**	**8.3**	**10.4**
1207	(*Z*)-β-ocimene	0.3	0.5	0.5
1211	3-methylpentyl acetate	0.4	0.1	0.3
1221	γ-terpinene	0.5	0.5	0.4
1225	(*E*)-β-ocimene	1.1	1.9	1.8
1250	*p*-cymene	**31.9**	**8.1**	**29.9**
1258	Terpinolene	0.2	0.2	0.2
1278	*(Z)*-2,6 dimethyl-1,3,7-nonatriene	0.5	0.2	0.7
1292	(*Z*)-3 hexenyl-acetate	0.1	-	0.0
1301	3-methyl-1-pentanol	0.1	-	-
1360	Methyl octanoate	0.2	0.1	0.2
1380	Presilphiperfol-7-ene	0.0	0.7	0.4
1389	Perillene	0.0	0.1	0.0
1395	7-(α)-(H)-Silphiperfol-5-ene	-	0.1	0.0
1400	Ethyl octanoate	0.1	-	0.1
1411	(α)-*p*-dimethylstyrene (=*p*-cymenene)	0.0	0.0	0.0

1422	7-(β)-(H)-Silphiperfol-5-ene	-	0.1	0.5
1450	Bicycle Elemene	0.2	0.2	0.3
1465	Silphiperfol-6-ene	0.1	1.1	-
1496	Modhephene	0.1	-	0.1
1508	α-Isocomene	0.0	0.1	0.2
1571	β-Caryophyllene	0.1	0.4	0.2
1577	Terpinolene-4-ol	0.9	0.8	0.5
1600	4-terpinenyl acetate	0.1	0.0	0.1
1645	α-humulene	-	0.2	0.1
1645	(*Z*)-Ocimenol	0.1	-	-
1666	Ledene	0.1	-	0.1
1669	α-terpinyl acetate	0.2	0.2	0.1
1683	Germacrene D	0.3	0.8	0.4
1707	Bicyclogermacrene	2.3	2.1	4.1
1716	Piperitenone	0.1	0.0	0.1
1723	δ-cadinene	0.0	0.3	0.1
1820	(*E*)-geranyl acetone	0.2	-	0.2
1893	Silphiperfolan-7-β-ol	-	0.1	0.1
1942	Cameroonian-7-α-ol	-	0.2	0.1
1980	Methyl eugenol	0.4	0.7	0.7
1992	(*E*)-nerolidol	0.2	0.1	0.3
2029	Presilphiperfolan-8-ol	-	0.2	0.2
2054	Globulol	0.0	-	0.1
2061	Viridiflorol	0.1	0.1	0.2
2089	Unidentified Compound	0.1	-	0.1
2099	Spathulenol	0.7	0.4	0.8
2153	Dimyrcene II A	-	0.1	0.1
2176	α-bisabolol	0.3	0.1	0.9
2191	2-himachalen-7-ol	-	0.1	-
2198	*trans*-α-bergamotol	0.1	-	0.1
2205	α-cadinol	-	0.1	0.1
2239	Zingiberenol 1	-	0.2	-

2285	(6S,7R)-bisabolene	-	0.0	-
	Total	99.9	100.0	99.9

RRI: Relative retention indices calculated against *n*-alkanes; % calculated from total ion chromatogram (TIC); tr: Trace (tr<0.1 %)

The data in (Table 2) reveals information about the diverse array of compounds in these samples, which can impact their fragrance, therapeutic potential, and suitability for various applications, such as perfumery or pharmaceuticals.

Comparative Analysis of the Chemical Composition in Sample A, Sample B and C

The comparative analysis of the chemical composition in Sample A, B, and C below reveals a rich and intricate interplay of chemical components. Each component contributes to the unique aroma, therapeutic potential and industrial applications of these samples. In this in- depth discussion, we will explore the role of each component, its potential implications, and the significance of variations between Samples A, Sample B, and sample C.

Comparative Analysis of α-Pinene

Sample A: This sample contains *α*-pinene at a substantial level, constituting 3.9% of its composition. *α*-Pinene is a well-known monoterpene with a fresh, pine-like aroma.

Sample B: In stark contrast, Sample B boasts a much higher percentage of α-pinene, comprising a remarkable 15.2% of its composition. This abundance of α-pinene could make Sample B a sought-after ingredient for fragrances and medicinal applications where α- pinene's therapeutic properties hold significance.

Sample C: This sample contains α-pinene at a substantial level, constituting 3.7% of its composition.

Comparative Analysis of *β*-pinene

Sample A: *β*-pinene is detected in Sample A at a notable concentration of 18.2%.

Sample B: In Sample B, *β*-pinene is also detected, though at a higher level of 29.6%. The higher concentration of this component may impact the potential therapeutic applications of Sample B compared to Sample A and sample C .

Sample C: *β*-pinene is detected in Sample C at a notable concentration of 19.1%.

Comparative Analysis of Myrcene

Sample A: Myrcene is prominent in Sample A, making up 7.8% of its composition.

Sample B: While myrcene is also present in Sample B, it accounts for a higher percentage (12.1%). These variations in content can influence the overall fragrance profile and applications of these samples.

Sample C: In sample C the composition of myrcene is 8.5%.

Comparative Analysis of Limonene

Sample A: Limonene is prominent in Sample A, making up 9.0% of its composition.

Sample B: While limonene is also present in Sample B, it accounts for a lower percentage (5.8%).

Sample C: In sample C the composition of limonene is 7.3%.

Comparative Analysis of 1, 8-cineole

Sample A: 1, 8-cineole is prominent in Sample A, making up 13.5% of its composition.

Sample B: While 1, 8-cineole is also present in Sample B, it accounts for a lower percentage (8.3%).

Sample C: In sample C the composition of 1, 8-cineole is 10.4%.

Comparative Analysis of *p*-cymene

Sample A: *p*-cymene is prominent in Sample A, making up 31.9 % of its composition.

Sample B: While *p*-cymene is also present in Sample B, it accounts for a lower percentage (8.1%).

Sample C: In sample C the composition of *p*-cymene is 29.9%.

The percentages of *α*-pinene, *β*-pinene, myrcene, limonene, 1, 8-cineole, *p*-cymene and other compounds vary between the samples. This supports the hypothesis that the essential oils produced from the leaves of *Pteronia incana* will be the same in the essential oil composition in all batches for its quality. The ratios are almost identical to those of previous studies with minor changes.

Discussion

This discussion will provide a comprehensive view of the chemical makeup of samples A, B, and C and highlight the significance of variations in their composition. These variations may have profound implications for these samples' aroma, therapeutic properties, and potential industrial applications. Furthermore, this section will also incorporate a comparative analysis with previous research, when available, to identify similarities and differences in the chemical composition of our samples compared to similar or related samples studied in the past. Such comparisons will enrich our understanding of the uniqueness of Sample A and Sample B in the broader context of chemical profiling research. The discussion will aim to address the implications of our findings, both in terms of the individual chemical components and the overall composition. The major compounds of the *P. incana* essential oil reported previously have been given in Table 2. In Table 3, it was summarized that the major compounds of *P. incana* reported previously.

Table 3. The major compounds reported previously for the essential oil compositions of *P. incana*

Country	Plant Part	Major Compounds (%)	References
Eastern Cape region of South Africa	Fresh leaves	*o*-Cymene (4.47) *p*-Cymene (18.90) Limonene+1,8-cineole (20.82) Myrcene (0.49) α-Pinene (19.08) β-Pinene(32.20)	Mangena & Muyima, 1999
Eastern Cape region of South Africa	Aerial parts	β-Pinene (32.5) α-Pinene (18.6) *p*-Cymene (11.3) Myrcene (10.3) 1,8-Cineole (9.0) Sabinene (7.4)	Tisserand & Rodney, 2014

		Methyleugenol (7.2) Limonene (7.0) Terpinen-4-ol (5.3) β-Caryophyllene (3.2) Aromadendrene (3.1) d-Cadinene(2.3)	
Eastern Cape region of South Africa	leaves and stalks	α-Pinene (14.2) β-Pinene + Sabinene (29.8) Myrcene (17.7) 1 ,8 Cineole + Limonene (14.0) *p*-Cymene (2.3) Terpinolene (9.4)	Bruns & Meiertoberens, 1987
Eastern Cape region of South Africa	leaves	α-pinene (22.34) top rate in June. β-pinene in March (36.37). Myrcene in December (16.99). 1,8-cineole in June (24.78).	Mayekiso et al., 2006
Eastern Cape region of South Africa	fresh leaves	β -Pinene (20.3) p-Mentha-8-ene-2-ol (16.39) β -myrcene (14.4) α-Pinene (12.2)	Muyima, et al., 2002
Eastern Cape region of South Africa	dried leaf	β-Pinene (0.3-10.2) limonene (0.8-10.9) 1,8-cineole (0.4- 18.5) Myrcene (0.2-8.0) spathulenol (2.9- 22.9) *p*-cymene (4.2-36.7) methyleugenol (1.0-10.9)	Hulley et al., 2010

These comparisons illustrate both similarities and variations in the chemical composition of *P. incana*. The presence α-pinene, β-pinene, myrcene, Limonene, 1,8-cineole, *p*-cymene as major components appears to be a consistent trend across different studies, but the percentage composition may vary, likely due to edaphic factors such as, seasonal variations, genetic factors, different plant parts and methodological differences.

In a previous study, the essential oil obtained from leaves of *P. incana* was explored by Mayekiso, et al., 2006. As a result of their research, the GC spectrum of the oil sample in June had the characteristic peaks which appeared throughout the year. These peaks seemed to represent the compounds; *α*-pinene, *β*-pinene, *β*-myrcene, sabinene and 1,8-cineole. The percentage of *α*-pinene, which was also one of the major compounds in the present study sample B (15.2%); was slightly changed in all samples collected from different months (12.57-22.34%); and reached the top rate in June. The percentage of *β*-pinene in the present study sample A&B (18.2-29.6%); was slightly changed in the sample collected in March (36.37%). The percentage of myrcene in the present study sample A&B (7.8-12.1%); was slightly high in the sample collected in December (16.99%). The percentage of 1, 8-cineole in the present study sample A&B (8.3-13.5%), was slightly changed in the sample collected from different months (12.10-24.78 %). However, the samples obtained between March and August by Mayekiso et al., 2006 appeared to be characterised by the additional terpenoids like, *β*-thujene, limonene, *α*-terpinene and δ-4-carene.

In a study, the essential oil obtained from leaves and stalks of *P. incana* was explored by Bruns et al., 1987. Depending on the growth stage and on the leaf-to-stalk ratio, the yield of oil totals between 0.4% and 1.1% of the plant's dry weight. The GC/MS analysis has shown that the spectrum of the essential oil of *P. incana* sample has monoterpene fraction, sesquiterpene fractions and unidentified components in the sesquiterpene fraction. Compounds identified in the monoterpene fraction of *Pteronia incana* contain 18 components, these peaks seemed to represent the compounds; *a*-pinene , *β*-pinene , myrcene
, limonene , 1,8-cineole , *p*-cymene, and terpinolene. The percentage of *α*-pinene, which was also one of the major compounds in the present study sample B (15.2%); was slightly changed in the sample collected (18.6%). The percentage of *β*-pinene in the present study sample A& B (18.2-29.6%) was slightly changed in the sample collected (32.5%). The percentage of *Myrcene* in the present study sample A&B (7.8-12.1%); was slightly different in the sample (10.3%). The percentage of *p-cymene* in the present study sample A&B (8.1- 31.9%); was slightly changed in the sample collected (11.3 %).

In a study, the essential oil obtained from fresh leaves of the above plants of *P. incana* collected from a place of the Eastern Cape of South Africa was explored by Muyima et al., 2002. GC/MS analysis showed that the oils were constituted by more than 10 different chemical compounds. These were *p*-mentha-8-ene-2-ol, as a major component of *P. incana*. Constituents of the essential oils (given as peak area % of GC–MS analysis) of *P. incana* were *β*-pinene 20.3%, *p*-mentha-8-ene-2-ol 16.3%, *β*-myrcene 14.4%, α-pinene 12.2%. The percentage of *α*-pinene in the present study sample B (15.2%), was slightly changed in the sample collected (12.2%). The percentage of *β*-pinene in the present study sample A&B (18.2-29.6%); was slightly changed in the sample collected (20.3%). The percentage of myrcene in the present study sample A&B (7.8-12.1%); was slightly different in the sample (14.4%).

Similarly, study has been done by Mangena and Muyima, 1999; the essential oil *P. incana* was collected from South Africa. The oils were extracted from the fresh leaves of the plants by water distillation. Composition of the essential oil *Pteronia incana* as identified by GC/MS analysis revealed that all the oils tested contained 1, 8-cineole. Show the peak concentrations of *P. incana*, *o*-cymene 4.47%, *p*-cymene 18.90%, limonene + 1,8-cineole 20.82%, mycerene 0.49%, α−pinene 19.08%, and β-pinene 32·20%. *P. incana* oil had a higher content of β-pinene, and lower content of myrcene. The percentage of *α*-pinene in the present study sample B (15.2%); was slightly changed in the sample collected (19.08%). The percentage of β-pinene in the present study sample A&B (18.2-29.6%); was slightly changed in the sample collected (32.20%). The percentage of myrcene in the present study sample A&B (7.8-12.1%); was slightly high in the sample collected (0.49%). The percentage of *p*- Cymene in the present study sample A&B (8.1-31.9%); was slightly changed in the sample collected (18·90%). In the present study the percentage of limonene in samples A&B (5.8- 9.0%) and *1, 8*-cineole in samples A&B (8.3-13.5%); was slightly high in the sample collected (20.82%).

In a study, the essential oil obtained from dried leaf material of *P. incana* was explored by Hulley et al., 2010.The main compounds of essential oil samples from eleven individual plants of *P. incana,* as identified by GC/MS. As a result of their research, the yields were exceptionally variable, ranging from 0.01% to 1.08% of dry weight. A total of 62 volatile components were identified in the eleven samples studied. The major compounds are several monoterpenes as well as sesquiterpenes. *β*-pinene (0.3- 10.2); limonene (0.8-10.9%), 1,8-cineole (0.4-18.5%); myrcene (0.2-8.0%); spathulenol (2.9-22.9%); *p*-cymene (4.2-36.7%) and methyleugenol (1.0-10.9%) are main compounds present in all or most of the samples, with smaller amounts of *α*-pinene, sabinene, *γ*-terpinene, terpinen-4-ol, bicyclogermacrene, globulol and *α*-bisabolol in several of the samples.

The percentage of *α*-pinene, which was also one of the major compounds in the present study sample B (15.2%); was low in the samples collected at three localities, Worcester Herolds Bay and Alice, was arranged from 0.9 to 6.7%. The percentage of *β*-pinene in the present study sample A&B (18.2-29.6%); was low in the samples collected at three localities , Worcester Herolds Bay and Alice, was arranged from 0.3 to 10.2%. The percentage of *myrcene* in the present study sample A&B (7.8-12.1%); was low in the samples collected at three localities, Worcester Herolds Bay and Alice, was arranged from 0.2 to 8.0%. The percentage of *1 ,8*-cineole in the present study sample A&B (8.3-13.5%); was slightly high in the most samples collected at three localities, Worcester Herolds Bay and Alice, was arrange from 0.2 % to 18.5 %.The percentage of *p*-cymene in the present study sample A&B (8.1-31.9%); was slightly high in the most samples collected at three localities, Worcester Herolds Bay and Alice, was arrange from 4.2 % to 36.7%.

The percentage of limonene in the present study sample A&B (5.8-9.0%); was slightly high in the most samples collected at three localities, Worcester Herolds Bay and Alice, was reported as 0.8 % to 10.9%.

CHAPTER V

Conclusion

In conclusion, the chemical composition of Sample A and C was similar though Sample B was slightly different. The comprehensive analysis of the components in Sample A, Sample B, and Sample C highlights the intricate nature of their chemical composition. The variations in the presence and percentage of these compounds contribute to the unique fragrance profiles and potential applications of these samples. These differences offer opportunities for tailored use in perfumery, aromatherapy, and various industries where the precise composition of compounds is pivotal for product development and optimization. Further research and experimentation are essential to understand and harness these chemical profiles' potential fully. Our research shows that *P. incana* essential oil stands out as a strong contender with a variety of possible advantages. It could be used for various pharmacological effects such as analgesic, antibacterial, anti-inflammatory and antioxidant, etc. due to the dominance of monoterpenes, particularly *β*-pinene, (18.2-29.6%); *α*-pinene (15.2%); myrcene (7.8-12.1%); 1,8-cineole (8.3-13.5%); *p*-cymene (8.1-31.9%); and limonene (9 - 5.8%), are principally responsible for this range of effects, as well as the plant's potent medicinal properties. As a result of our results, there is still a need for optimization and an increase in the quality and repeatability of the commercially sold essential oil product of *P. incana* due to the changes in chemical composition of different batches. This research might also serve as a starting point for more research into the biological effects and potential health advantages of *P. incana* essential oil.

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1 CHAPTER I Introduction Plants have historically played a vital role in the development of medicine, largely due to their ability to produce secondary metabolites with potentially significant biological effects. Traditional medicine has relied on plants to treat a wide range of diseases. According to the World Health Organization (WHO), more than 80% of the world's population still relies on traditional and folk medicine, most of which is plant-based.

Medications derived from plants used in traditional medicine are often more affordable, readily available, and exhibit fewer side effects compared to their synthetic counterparts. Even in developed nations like the United States, herbal drugs account for a notable percentage of the total pharmaceutical intake (Jafarinia & Jafarinia, 2019).

Recent analysis of traditional medicinal plants using modern methods has led to the discovery of numerous promising compounds. These plant-derived substances can be utilised to enhance existing drugs or develop entirely new ones (Rolnik et al., 2021). Many commonly used drugs, such as morphine, aspirin, quinine, and digitoxin, have their roots in herbal sources (Jafarinia & Jafarinia, 2019).

The largest family is Asteraceae, which consists of more than 23,000 currently accepted species, spread across 1,620 genera and 12 subfamilies, (Rolnik & Olas 2021). Most members of this family have therapeutic uses and boast a long history in traditional medicine, with some species being cultivated for over 3000 years for nutritional and medicinal purposes including anti-inflammatory, antimicrobial, antioxidant, and hepatoprotective properties (Rolnik et al., 2021).

Asteraceae family members are known and distributed worldwide, most prevalent in arid

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graduate courses you have given in the last 2 years.

Academic Year	**Semester**	**Course**	
2022-2023	Fall	İLAÇ HAMMADDESİ YÖNÜNDEN ÖNEMLİ FAMİLYALAR	AA
		DOĞAL BİLEŞİK ANALİZLERİNDE KROMATOGRAFİK TEKNİKLER	BB
		ÖNEMLİ AROMATİK BİTKİLER	AA
	Spring	İLERI UÇUCU YAĞLAR	AA
		ARAŞTIRMA YÖNTEMLERİ VE ETİK	BA
		İNGLİZCE DİLİ DESTEĞİ	S
2023-2024	Fall	BİYOİSTATİSTİK	CC
		ADVANCED ANALYTICAL CHEMISTRY	BA
		SEMINAR	S
	Spring	THESIS	