Resource Accounting and Irrigation Requirements for the Cultivation of *Artemisia afra* Jacq. Ex. Wild as a Conservation Strategy

Report to the
Water Research Commission

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WATER RESEARCH COMMISSION

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EXECUTIVE SUMMARY

Medicinal plants are known to play an important role in primary healthcare through traditional medicine, and they are sold in the informal markets and commercial sectors of South Africa. The ever-increasing demands by a growing global population who prefer healthcare and dietary supplements from natural sources have led to an expanding medicinal plant industry, thereby opening new opportunities. *Artemisia afra* Jacq. ex Willd. is one of the medicinal plants that have traditionally been used to treat various ailments, with several studies mentioning its use to treat and/or manage upper respiratory infections and cancers. The continued wild harvesting of *A. afra* may increase harvesting pressure on its wild populations and will have a long-term impact on its natural ecosystem. The cultivation of *A. afra* has received little attention, although it is mentioned in many publications as one of the South African medicinal plants with commercial potential.

This project sought to contribute to the conservation of *A. afra* by investigating remote sensing as a potential monitoring tool and by developing cultivation information, with a focus on water management.

The objectives of the project were:

- To investigate the use of remote sensing technology in mapping the distribution of *A. afra* wild populations to monitor its population dynamics
- To identify the best chemotype and develop propagation and cultivation techniques for the identified chemotype in three provinces
- To document the growth performance and water requirement of cultivated *A. afra* for optimized biomass production and bioactive secondary metabolites

To achieve the objective of mapping the distribution of *A. afra*, suitable climatic conditions for growth of the species were identified. Soil samples were collected from three populations in the Maluti-A-Phofung local municipality in the Free State Province. The soil samples were analysed at an accredited laboratory for physical and chemical properties, carbon source utilization (microbial functional diversity), enzyme activities, and the total microbial count. To identify suitable climatic conditions for the natural areas of growth and distribution of *A. afra*, an existing database from the South African National Botanical Institute (SANBI) was consulted, together with a literature search conducted and personal communication, to identify various regions to visit for physical collection of GPS points. The maximum entropy model (MaxEnt model, version 3.4.4) was used to create maps of suitable areas for the growth of *A. afra*. Furthermore, to estimate the effect of climate change on *A. afra* distribution, monthly long-term average interpolated weather surfaces were derived from the ARC Weather Station Network for the period 1991–2020. The averages of six different dynamically downscaled Coupled Global Climate Model (CGCM) projections of future climate change were then used for the 2021–2050 and 2051–2080 periods. Finally, the median values of changes from the current situation (1991–2020) to the periods of

2021–2050 and 2051–2080 were applied to the long-term average surfaces derived from the ARC weather station network.

To investigate the potential of remote sensing for monitoring wild populations of *A. afra*, the collected distribution points were used to determine the most suitable populations or areas where it is distributed. The criteria used were to select areas with more plants closer to each other for uniformity within a 10 m radius to accommodate the potential extension of analysis of space-borne remote sensing techniques. Spectral signatures of *A. afra* were taken with an Analytical Spectral Devices (ASD) FieldSpec 4 Hi-Res spectroradiometer. Additionally, spectral measurements of other wild and cultivated plant species were taken for species comparison purposes. The spectral signatures were compared with pixel values of the different bands of the Sentinel-2 Multispectral Imager, which has 12 bands at different spectral and spatial resolutions.

Artemisia afra samples were collected from seven wild populations across three different provinces and subjected to phytochemical analysis to investigate the best chemotype. To develop *in vitro* propagation techniques, nodal explants (1.5 - 2 cm long) from middle lateral branches were excised and surface-decontaminated before culturing in growing media supplemented with different cytokinin concentrations. Another propagation method evaluated involved stem cuttings of 3-5 cm in length, which were planted in different growing media, following dipping in various plant growth regulators and at different stages. Studies on the irrigation of *A. afra* were also conducted. The first study, which was a pot trial under glasshouse conditions, consisted of two factors, *viz.* factor 1 = harvesting periods (HP) with two separate harvests (harvesting at 30 days and 60 days; HP_1 and HP_2), and factor 2 = terminal water stress (TWS) at two levels (no terminal water stress and one-week of terminal water stress; TWS_0 and TWS_1). The second study, conducted under a rainout shelter, had four predetermined water deficit levels, expressed as a percentage of plant-available water allowed to deplete before irrigating back to field capacity (allowable depletion level, ADL). The applied deficit levels were 80–85% ADL (T1, severe stress), 60–65% ADL (T2, moderate stress), 40-45% ADL (T3, mild stress), and 20–25% ADL (T4, well-watered). A UPLC-QTOF-MS (Waters Synapt XS QTOF with ACQUITY UPLC I-Class PLUS), with a Waters Acquity UPLC BEH C18 column (100 mm, 2.1 mm, 1.7 mm particle size) and a MassLynx 4.1 Waters Connect software, with Waters UNIFI Scientific Information System as the database, were used for phytochemical analysis and metabolite profiling of cultivated *A. afra* samples.

In summary, the results showed that 61% of the soil samples were clay loam soils, 17% sandy loam, another 17% sandy clay loam, and the last 5% were loam soils. The pH of all the samples was slightly acidic to neutral as they ranged from 5.3 to 6.86 on the pH scale. Significant differences in soil microbiology, especially the carbon source utilization, were recorded among the soil samples. The MaxEnt model showed that the Northern Cape Province was less suitable for *A. afra* growth. The long-term average annual rainfall, the highest long-term average monthly maximum temperature, and average soil depth were the environmental factors that contributed more to the MaxEnt model predictions. The areas with the highest probability of suitability for *A. afra* growth were mainly recorded

in the Eastern Cape, KwaZulu-Natal, and Mpumalanga provinces. The Free State, Gauteng, Limpopo, and Western Cape provinces had a moderate probability of suitability. The model predicted no major changes in the distribution of *A. afra* from 2021 to 2050, whereas changes are expected from 2051 to 2080.

The reflectance pattern in the visible and red edge (700-795 nm) showed no distinct within-species differences, while beyond these ranges, there were differences in the spectral patterns recorded for the sampled plants. The pattern of increase and decrease in reflectance percentage was the same for all nine *A. afra* measurements. A similar pattern to that observed in wild populations, with increases and decreases in reflectance percentages, was observed in cultivated *A. afra*. The *A. afra* spectral measurements were always higher than those of *M. oleifera*, except in the region between 723 nm and 901 nm, where the two were comparable. The spectral measurements further showed a distinct difference between *A. afra*, *Asparagus laricinus*, and a grass species. The normalized difference vegetation index (NDVI) was low for *A. afra* compared to *A. laricinus.* Using the Sentinel-2 Multispectral Imager, *A. afra* was mostly confused with grass. At least six *A. afra* plants were misidentified as grass because of the spectral mix-up between the two, followed by *A. laricinus* where 27 of them were misidentified as *A. afra.* This indicates the difficulty of mapping the species when co-occurring with *A. laricinus* and grass species.

After four weeks of *in vitro* culturing *A. afra* nodal explants, the highest shoot proliferation (average of 4 shoots per explant) occurred on growing media supplemented with 4 µM benzyladenine (BA, a cytokinin) with a high frequency of branching (average of 15 branches per shoot after 8 weeks). Stem cuttings of *A. afra* set in cocopeat as a growing medium with no rooting hormone had the highest survival percentage (40%), followed by Dynaroot-treated cuttings (22%). The lowest survival rate was observed when the rooting hormone was applied immediately at setting, when compared to applying the rooting hormone one week and five weeks after setting.

Plants harvested after 60 days with no terminal water stress (HP_2TWS_0) had significantly increased average fresh weight and number of branches per plant. Deficit irrigation had a significant effect, with maximum plant height recorded when the plants were exposed to 20-25% ADL, while the lowest plant height was recorded when the plants were exposed to 80-85% ADL. Similarly, the well-watered treatment significantly increased the total biomass yield compared to the water stress treatments. The chemical profiling of the plants exposed to different water regimes revealed differences in the number and identities of compounds tentatively identified. Phytochemical fingerprinting of samples collected from the three different provinces revealed similar tentatively identified compounds, albeit at different concentrations.

Overall, the study mapped out suitable areas in South Africa where *A. afra* can be cultivated; however, there is a need for further investigations into the crop pests and diseases. Annual rainfall seems to be the most significant variable affecting the distribution of *A. afra*, followed by maximum temperature and

soil depth. *Artemisia afra* seems to prefer a moderate to slightly acidic pH. Resource-poor farmers can be encouraged to propagate *A. afra* in cocopeat as a growing medium without the use of rooting hormone, thereby providing additional environmental benefits. Delaying the application of rooting hormones by one week could enhance survival of the cuttings; however, the cost implications and the environmental benefits of no hormone application may outweigh the enhancement. The different compounds tentatively identified include some interesting compounds that could substantiate the various uses of *A. afra* in traditional medicine.

The suitable climatic conditions, having been identified, present an opportunity for further scientific studies that may simulate the conditions and/or manipulate the environment for the synthesis of secondary metabolites. The irrigation studies have shown differences in compounds under different watering regimes; thus, further studies can be linked to specific compounds for specific uses. Water and nutrient management are always linked; thus, cultivation studies focusing on their combination are important. Plant population density is also critical for maximising the use of resources, such as land. Cultivation of *A. afra* under monoculture will always present challenges such as pests and diseases; thus, this aspect of agronomy will also need to be investigated. It is finally recommended that conservation agencies note the potential threats posed by human activities to the wild population of *A. afra* in the short and medium term and start placing greater emphasis on cultivation.

Capacity building, in a research project, is aimed at transferring skills. Two female MSc students and one PhD student were trained in the project. Furthermore, three communities across three provinces (Limpopo, Gauteng and Free State provinces) were trained in the project. The training, which was mainly practical and hands-on, included cultivation of *A. afra*, scouting for pests and diseases, and the use of chameleon sensors in monitoring soil water content as part of irrigation scheduling.

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CHAPTER 1
BACKGROUND

Cultivation of medicinal plants in general has been advocated for many years as a conservation strategy. However, information generation in that regard has received little to no attention. Farmers and growers may not be interested in cultivating medicinal plants if information such as how to grow them at a commercial scale, their nutrient and water needs, pests and diseases related to their cultivation, and the economic return on investment is not readily available.

The demand for medicinal plants is expanding, in parallel with the increase in human populations and the related increases in health-related challenges. COVID-19, a pandemic that was experienced worldwide in the early 2020s, and other health-related challenges have resulted in an increase in pressure exerted on wild populations of medicinal plants such as *Artemisia afra*. Wild harvesting of medicinal plants poses challenges such as misidentification of some species, adulteration and/or contamination of the harvested material, and inconsistencies in the quality of the harvested material. Cultivation, on the other hand, ensures a consistent and sustainable supply of good quality plant material. The project aimed to investigate the distribution and cultivation of *A. afra*, focusing on irrigation, as a case study for the conservation of medicinal plants.

1.1 INTRODUCTION

Plants have been associated with human health since the ancient period, and the use of plant products as the main source of medicines can be traced back to the beginning of human civilisation (Manzini, 2005). Plants remain one of the most significant sources of drugs in modern and traditional systems of medicine. To date, medicinal plants commonly used in traditional and modern medicine are considered highly economical (Mofokeng *et al*., 2022). Some recognised plant-derived medicines include morphine, aspirin, cocaine, guanine, codeine and atropine (Manzini, 2005).

Medicinal plants play an important role in primary healthcare through traditional medicine, which remains the most affordable and easily accessible source of treatment for most resource-poor communities around the world (Maroyi, 2013). In many areas of South Africa, herbal plants and their extracts are sold in informal markets and in the commercial sectors of the economy (Mofokeng *et al*., 2022). As more people turn to medicinal plants for primary healthcare, challenges such as overexploitation, extinction, and severe threats to biodiversity arise (Ndou *et al*., 2019). Although challenges and opportunities regarding medicinal plants have been documented, the cultivation of medicinal plants has been neglected for a long time due to several factors, including the perception that cultivating, gathering, preparing, and applying medicinal plants were associated with religious beliefs and witchcraft, in some cases (Kelatwang, 2015; Van Wyk and Prinsloo, 2018).

Rapid advances in chemistry and pharmacology, resulting in the isolation and establishment of the biological activities of phytochemical compounds, have contributed to scientifically valorising the utilisation of some medicinal plants. An increasing global population depends on or prefers healthcare and dietary supplements from herbal and natural products (Ekor, 2014), opening new opportunities for the medicinal plant industry. It was reported that more than 25% of the global pharmaceutical drugs are of plant origin (Mofokeng *et al*., 2022).

In traditional African societies, Complementary and Alternative Medicine (CAM) continues to play an important role in treating many diseases and ailments due to a lack of or poor access to modern health facilities (Sunmonu and Afolayan, 2012), amongst others. As the primary source of medicine for most of the rural population, medicinal plants significantly contribute to the healthcare systems of local African communities. Yet, the current production levels of medicinal plants by commercial and smallholder farmers, using different cultivation practices, remain insignificant, even as demand is on the rise.

The recent rise in demand for many medicinal plants has increased pressure on the sustainability of these resources while bringing greater opportunities for primary producers. *Artemisia afra* Jacq. Ex. Wild (family: Asteraceae) is one of the indigenous medicinal plants that has been harvested from the wild for various medicinal purposes, with little to no cultivation.

The role of medicinal plants as a source of income for rural and urban households has been established in many regions worldwide. The medicinal plant trade is extremely diverse, with species availability varying by country, region, or community. Several studies have demonstrated the economic potential of medicinal plants, and the global market for herbal medicine was not only large but also growing at a 15-20% per annum (Subrat, 2002; Van Wyk, 2008; Van Wyk, 2001; Mofokeng *et al*., 2022). This is because markets, particularly international markets for medicinal plants, have always been large and complex at times. The use and trade of medicinal plants are no longer limited to traditional healers in many parts of the world but have entered both the informal and formal entrepreneurial sectors of the economy. However, most local trade is conducted informally, and there is also significant cross-border trade of medicinal plants. It is estimated that 27 million customers participate in the traditional medicine market in South Africa, resulting in an annual value of R2.9 billion (Mofokeng *et al*., 2022). The cultivation and trade of *A. afra* have the potential to generate employment opportunities for a significant number of rural women.

1.2 PROBLEM STATEMENT

Artemisia afra is a medicinal plant traditionally used to treat colds. Several studies found that *A. afra* extracts could be used to treat and/or manage upper respiratory infections and cancers (Martini *et al*., 2020; Daddy *et al*., 2021; Nie *et al*., 2021; Spies *et al*., 2012). In some cases, the species has been reported as an invasive plant that can compete with food crops for limited natural resources (Dubula *et al*., 2016; Setshedi *et al*., 2022). With the previously experienced COVID-19 pandemic, many farmers and phytochemical industries have become interested in growing the plant. There is a pressing need to

further investigate its phytochemical profile, which includes approximately 133 volatile and 44 non-volatile secondary metabolites (Liu *et al*., 2009). The large number of secondary metabolites can be explained by the wide distribution of *A. afra,* which may result in variations in the phytochemical composition.

Plant-to-plant chemical variations within a population (Viljoen *et al*., 2006; Sotenjwa *et al*., 2020) have been documented, resulting in various chemotypes. Some *A. afra* samples had a high thujone content (Viljoen *et al*., 2006), one of the essential oils considered toxic at high concentrations (Oyedeji *et al*., 2009). Previous research has recommended *A. afra* plants with camphene (7%), 1.8-cineole (15%), camphor (49%), and no thujone for product development (Viljoen *et al*., 2006). However, the main issue is that harvesters and growers of *A. afra* may be unaware of the appropriate chemotype for therapeutic or medicinal purposes, raising the need for community awareness.

The wide range of medicinal uses of *A. afra* has increased harvesting pressure on wild populations and will have a long-term impact on its natural ecosystem. In addition, the plant's water use has received little attention, despite reports of the species' adaptation to wide environments. Light conditions (UV), irrigation, and other crop management factors can influence the synthesis of bioactive secondary metabolites (Kane *et al*., 2019; Koehorst *et al*., 2010; Nigam *et al*., 2019), which define the medicinal quality of *A. afra*.

1.3 PROJECT AIM AND OBJECTIVES

This project sought to contribute to the conservation of *A. afra* by developing a monitoring tool and to begin developing cultivation information. Given that information on the water requirements of *A. afra* for optimised biomass production and bioactive secondary metabolite production is limited or non-existent, the cultivation aspect focused primarily on irrigation management.

The specific objectives of the project were:

- To investigate the use of remote sensing technology to map the distribution of *A. afra* wild populations to monitor its population dynamics
- To identify the best chemotype and develop propagation and cultivation techniques for the identified chemotype in three provinces
- To document the growth performance and water requirement of *A. afra* for optimized biomass production and bioactive secondary metabolites

1.4 SCOPE AND LIMITATIONS

The project focused on three provinces of South Africa, while *A. afra* grows in other provinces as well. Information from other provinces could have contributed to the distribution mapping. Access to private and protected land limited access to other *A. afra* populations. One of the major limitations experienced was the disturbance of wild populations by human activities, such as land development for human

settlement or business opportunities. Population sizes of *A. afra* in the wild, which were mostly composed of individual plants widely spaced, affected the use of remote sensing for monitoring wild populations.

CHAPTER 2
LITERATURE REVIEW ON *ARTEMISIA AFRA* AND CROP PRODUCTION

2.1 INTRODUCTION

The genus *Artemisia* (family: Asteraceae) comprises approximately 500 species distributed across several continents worldwide (du Toit and van der Kooy, 2019; Ivănescu *et al*., 2021), with the highest diversity in the Asian region (Trendafilova *et al*., 2021). Several *Artemisia* spp. are found worldwide, including *A. santolinifolia* Trucz ex. Besser, *A. iwayomogi* Kitamura, *A. annua* Linnaeus (Figure 2.1.), *A. herba-alba* Asso (syn. *Artemisia maritima* L., *Artemisia brevifolia* Wall.), *A. absinthium* Linnaeus, *A. abrotanum* L., *A. aboresscens* L., *A. argyi* Levl. et Vant., *A. biennis* Willd, *A. campestris* L., *A. douglasiana* Besser., *A. dracunculus* L., *A. echegaray* Hieron, *A. fukudo* Makino, *A. haussknechtii* Boiss., *A. judaica* L., *A. nilagirica* (Clarke) Pamp, *A. princeps* Willd., *A. rubripes* Nakai, *A. scoparia* Waldst. & Kit., *A. spicigera* C. Koch, *A. tridentata* Nutt., *A. cana* Pursh., *A. frigida* Willd., *A. ludoviciana* Nutt., *A. argentia* L'Hér, *A. canariensis* Less., *A. gorgonum* Webb, *A. granatensis* Boiss., *A. magellanica* Sch. Bip., *A. mauiensis* (A. Gray) Skottsb., *A. molinieri* Quézel, Barbero & R. Loisel, *A. negrei* A. Ouyahya, *A. californica* Less., *A. tripartita* Rydb., *A. umbelliformis* Lam., and *A. vulgaris* L. (Gabuka, 2009; Abad *et al*., 2012; Vallés *et al*., 2011). *Artemisia* species are used for different purposes such as medicine, food, beverages, ornamentals, and land reclamation (Vallés *et al*., 2011).

Figure 2.1. *Artemisia absinthium* (A), *A. annua* (B) and *A. afra* (C) plants showing differences in leaf colour, shape and form. (Source: Agricultural Research Council).

2.2 *ARTEMISIA AFRA* AND ITS DISTRIBUTION

Interest in *A. afra* has recently increased significantly, as indicated by the rise in publications featuring this species, and this may be attributed to its widespread use (Liu *et al*., 2009). The plant is taxonomically classified as Kingdom: Plantae, Division: Magnoliophyta, Class: Magnoliopsida, Sub-class: Asteridae, Order: Asterales, Family: Asteraceae, Genus: *Artemisia* and Species: *afra* (Kriel,

2010). This soft aromatic shrub has multiple common names, such as Wild wormwood, African wormwood (English); Wilde-als (Afrikaans); Umhlonyane (isiXhosa); Mhlonyane (isiZulu); and Lengana (Tswana) (van der Walt, 2004; Asekun *et al*., 2007; Oyedeji *et al*., 2009; DAFF, 2012; Swart, 2022). It is an aromatic, multi-stemmed, erect plant with a shrubby outlook. This perennial plant grows in thick, bushy areas and can reach 2 m in height, with a leafy, hairy, ridged stem (Liu *et al*., 2009; Van der Walt, 2004; Swart, 2022). Its leaves are soft and finely divided, reaching 8 cm in length and 4 cm in width (More *et al*., 2012). The seeds are tiny (seed weight is approximately 0.03 g per 1000 and 1 mm in length), oblong, yellow-brownish with a lustrous surface marked by vertical furrows; the seed endosperm is creamy white in colour and fatty in content. The plant is wind-pollinated, and the seeds are unlikely to be wind-dispersed due to the absence of a pappus (Setshedi *et al*., 2022). *Artemisia afra* blooms from January to June, creating yellow, butter-coloured blossoms with inexhaustible bracts (www.plantzAfrica.com). The plant has an effortlessly identifiable fragrant odour, with an impactful and sweet smell after bruising (Setshedi *et al*., 2022). The branches quickly recover from the base after the winter period (Sunmonu and Afolayan, 2012).

Artemisia afra is distributed within the mountain regions of the African continent. It is widely distributed across eastern and southern African countries, including Kenya, Tanzania, Angola, South Africa, Namibia, Zambia, and Zimbabwe (Patil *et al*., 2011). *Artemisia afra* grows naturally in Lesotho, Swaziland, Kenya, Tanzania, Uganda and Ethiopia (Ketema *et al*., 2020; Liu *et al*., 2009). In South Africa, it grows in Gauteng, Limpopo, Western Cape, KwaZulu-Natal, Free State, and Eastern Cape provinces (Liu *et al*., 2009; Oyedeji *et al*., 2009) (Figure 2.2).

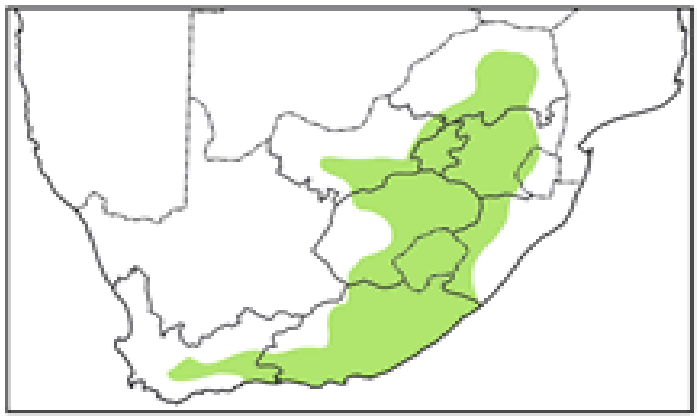

Figure 2.2. Distribution of *Artemisia afra* in South Africa (Department of Agriculture, Forestry and Fisheries, 2009).

Artemisia afra prefers sunny conditions and an average optimal growth temperature of 20-33 °C, with soil pH ranging from 5 to 7.5 (Koehorst *et al*., 2010). Plant growth is slow in cold seasons, and although it can withstand winter temperatures, it succumbs to temperatures below -7 °C. *Artemisia* spp. have shown a remarkable ability to grow and adapt across different ecosystems, from arid to wet regions, and thus have a wide distribution and a high potential for invasiveness (Trendafilova *et al*., 2021). For example, *A. princeps*, native to some Asian countries, is an invasive species in Belgium and the

Netherlands, and *A. verlotiorum* is an invasive alien species in Croatia (Trendafilova *et al*., 2021). *Artemisia afra* can be invasive where non-native with highly specialised physiological characteristics, such as the capacity to draw water and nutrients deeper in the soil profile (Dubula *et al*., 2016). Setshedi *et al*. (2022) reported that, despite efforts to remove *A. afra* plants in a nature reserve and during drought, it showed potential to regrow, with encroachment likely to continue. *Artemisia tridentata* demonstrated the ability to maintain nutrient uptake even under water stress, and this may be due to higher root length and extensive root hair development across all levels of water potential (Matzner and Richards, 1996). This mechanism, which may be applicable to *A. afra* plants, could increase their invasive potential, leading them to outcompete other plants under environmental stress.

2.3 MEDICINAL AND OTHER USES OF *ARTEMISIA AFRA*

In nature, essential oils and other secondary metabolites play an important role in protecting plants by acting as antibacterials, antivirals, antifungals, and herbivore deterrents, reducing the palatability of plants and repelling undesirable insects (Abad *et al*., 2012). *Artemisia afra*, which contains essential oils, is a widely used herb for treating various ailments, including haemorrhoids, inflammation, and menstrual pain (Rogers, 2008; Liu, 2011). Furthermore, its extracts have shown activity against *Trypanosoma brucei* (More *et al*., 2012), a parasitic protozoan that poses a significant health risk known as African sleeping sickness. The plant is used as a bitter tonic and appetite stimulant in the Cape region of South Africa (Thring and Weitz, 2006). The roots, stems, and leaves are used in many ways: as poultices, infusions, body washes, and lotions, and are smoked or taken as a hot beverage. Warmed leaves may be applied externally as a poultice to relieve inflammation and aqueous infusions applied as a lotion to treat haemorrhoids (Liu *et al*., 2009). Its potential in the management of tuberculosis and cancer has been reported previously (Spies *et al*., 2012; Martini *et al*., 2020; Daddy *et al*., 2021). Another study also showed the potential of *A. afra* to inhibit SARS-CoV-2 (Nie *et al*., 2021). *Artemisia afra* extracts were found to be effective against oral micro-organisms that cause tooth decay, gingivitis, and periodontitis (More *et al*., 2012). Variations in the use of *Artemisia* spp. can be due to geographical location and cultural differences among users.

2.4 EFFECTS OF AGRONOMIC PRACTICES ON GROWTH AND YIELD OF *ARTEMISIA* SPECIES

Due to various environmental factors, the essential oil industry faces several challenges, including inconsistent quality, supply shortages, and variability in active ingredients. This has encouraged end-users to rely on synthetic oils to eliminate the issues above. Because of growing public interest in "natural" products and health consciousness, as well as the perception that natural products are of higher quality, there is an opportunity to effectively market naturally grown essential oils if the issues above are addressed through cultivation practices. The harvesting season, fertiliser and soil pH, the drying methods and conditions, geographic location, and extraction method all influence the quality and yield of essential oils from *Artemisia* species (Abad *et al*., 2012). Identification of the correct chemotype, mass propagation of the chemotype for cultivation, and development of appropriate agronomic

practices and cultivation guidelines will support the industry and growers of *A. afra* plants. This will improve the chances of accessing opportunities and markets in the essential oil and medicinal plants industry.

2.4.1 Propagation of *Artemisia* species

The process of establishing new plants from existing plant material, such as seeds, cuttings, or other plant parts, is known as plant propagation. Additionally, the term "plant propagation" can refer to the dispersal of seeds, whether intentional or accidental. In most cases, the propagation process is an integral component of the overarching plant growth cycle (Hartmann *et al*., 1997). It happens after the seeds have matured and been dispersed, after vegetative parts have been detached or pruned, or when asexually reproducing plants develop a new plant from existing parts of the plant (LeBude and Blazich, 2018). Plant propagation occurs mainly through sexual reproduction (propagation by seeds) and vegetative or asexual reproduction (which includes layering and grafting). The two types of plant material mostly used for reproduction are seeds and cuttings (Bailey, 2020).

Artemisia afra seeds can be sown in spring or summer in well-drained soil with a slightly acidic to neutral pH (Koehorst *et al*., 2010). The soil should be kept moist until germination and seedling emergence, which usually takes 2 to 8 weeks. A study on *A. annua* found that seed-derived plants exhibit highly variable artemisinin content, thereby lowering artemisinin yield per hectare (Wetzstein *et al*., 2018). The inability to produce inbred lines due to self-incompatibility has stymied breeding efforts to produce improved F1 hybrids (Wetzstein *et al*., 2018). *Artemisia* spp. can also be propagated by stem cuttings that root easily in spring and summer, division in spring, and pruning back in spring to stimulate growth. Wetzstein *et al*. (2018) study indicated that tissue culture-regenerated plants and rooted cuttings of *A. annua* outperformed seed-derived plants in terms of uniformity, yield, and consistently high artemisinin content. A similar case can be true with the multiplication of *A. afra*. With the right chemotype, farmers can produce plants with uniform characteristics, increasing yield per cultivated area significantly and producing the desired material for the industry and market.

2.4.2 Cultivation of *Artemisia* species

Available information on the effects of cultivation practices on the growth and biological activity of many African medicinal plants is very scanty. According to Patil *et al*. (2011), several factors that can influence medicinal plant cultivation include proximity to plant source, time spent collecting the plant, number of ailments perceived to be healed, frequency of usage, retention of activity, acceptance of cultivated plants, ease of cultivation, availability of seeds or grafts, soil quality, vulnerability to pests, and water requirements. *Artemisia afra* prefers temperate and subtropical regions, and although it is actively growing in the summer months, it can tolerate cold temperatures (Kriel, 2010). It can grow on a wide variety of soils and seems to prefer well-drained sandy to sandy loam soils in high rainfall areas. At pH lower than 4.5 and higher than 8.5, *A. afra* yield was significantly reduced compared to when grown at

pH 5.5-7.5 (Koehorst *et al*., 2010). The lower branches of the plant become woody with age, and the plant may need to be replaced after 3 to 4 years.

Table 2.1. The advantages and disadvantages of wild resource versus cultivated medicinal plant species (Chen *et al*., 2016).

Characteristics	**Wild resource**	**Cultivated species**
Advantages	It is an open-access resource without investment	It relieves harvesting pressure on rare and threatened species
	It is a natural resource and free from pesticides	It can keep genotypes standardized or improved
	A wild resource is supposed to be more efficacious	It guarantees the continuing supply of quality raw medicinal materials Production volume and price can be stable for longer periods
Disadvantages	A wild resource is becoming scarce and threatened by overharvesting	It needs substantial investment before and during production
	There exists a risk of adulteration and resource exhaustion	It narrows genetic diversity in the gene pool of wild populations
	Uncontrolled harvesting leads to the extinction of ecotypes and species	Reintroduced plants can cause genetic pollution of wild resources
	There is a lack of resource inventories and related management practices	Cultivated species may have negative impacts on ecosystems
		There is a lack of successful cultivation techniques for some species

The principles of good agricultural management must be followed when cultivating *Artemisia spp.* Conservation agriculture can be used where appropriate, especially for organic matter accumulation (composting, mulching) and soil moisture conservation (mulching, sustainable irrigation). Farmers can implement practices that contribute to soil conservation and reduce erosion, such as creating buffer zones along watercourses and planting cover crops and green manure (to be incorporated into the soil when ploughed) (WHO, 2006).

Generally, plants synthesise and accumulate secondary metabolites in response to the pressure exerted by biotic and abiotic ecological factors in their immediate environment. If such interactions are disrupted, these plant species will likely yield only trace amounts of the compounds of interest (Bapela *et al*., 2008). Cultivation trials necessitate parallel studies, mainly aimed at elucidating factors such as exogenous application of elicitors, fertilisers and soil properties that have been shown to affect plant productivity and quality (Bapela *et al*., 2008). The medicinal attributes of cultivated plant material need to be evaluated using chemical profiling techniques and biological activity assays to ensure the quality and safety of extracted phytochemicals.

2.4.3 Soil fertility and crop production

Soil fertility is the soil's ability to provide nutrients in adequate amounts and in the proper balance to promote plant growth when other factors (such as light, moisture, temperature, and soil structure) are favourable (Gatiboni, 2018). When soil fertility is low, natural or man-made materials may be added to the soil to provide the nutrients plants require. These are known as fertilisers, though the term is typically reserved for materials that are primarily inorganic. Fertilisers contain the chemical components required to improve plant development and productivity. Fertilisers either increase the soil's natural fertility or replenish the chemical elements removed by plants.

Each nutrient significantly affects crop nutrition, which is critical to achieving a proper balance of macronutrients and micronutrients in plants. The plant's roots take up nutrients from the soil and translocate them to the stems and leaves (Oritani, 1995). Plant nutrient deficiency-related symptoms (Figure 2.3) are a common source of alarm for crop farmers (APFL, 2020). Most growers use a complete fertiliser to supply nitrogen (N), phosphorus (P), and potassium (K). These elements are known as macronutrients because large amounts are required for plant growth and development (Barak, 1999). Boron (B), chlorine (Cl), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), and zinc (Zn) are known as micronutrients because plants require them in much smaller amounts than macronutrients (Barak, 1999). It can be challenging to provide plants with adequate micronutrient levels. However, many soils contain micronutrients, but most last only for short periods, especially under irrigation, due to leaching. This means additional micronutrient sources must be provided to meet the plant's nutritional needs. When plants experience nutrient deficiencies, they show deficiency symptoms, while excess nutrients result in toxicity. The primary considerations for nutrient fertilisation are the source, the amount provided, and the availability or form.

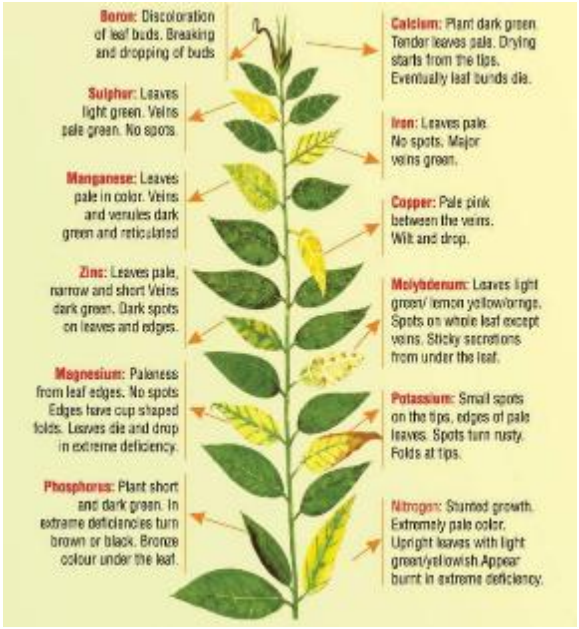

Figure 2.3. Nutrient deficiency symptoms in crops (APFL, 2020).

2.4.4 Response of *Artemisia* species to fertilizer application

Plant materials harvested from wild stocks cannot meet the escalating demand for natural products in the growing global medicinal and aromatic plants market (Bapela *et al*., 2008). Effective propagation and cultivation has become inevitable to guarantee a sustained supply of renewable raw plant materials to both local and global markets (Jäger and Van Staden, 2000). Introducing medicinal plants into cultivation will ensure a continued supply of phytochemicals and help conserve genetic resources from wild populations. This can alleviate their overexploitation and relieve pressure on wild plant species.

Like many other plants, *A. afra* requires the right combination of nutrients to sustain its growth and maximum yield. Studies suggest that applying a combination of nitrogen, phosphorus, and potassium (NPK) helps plant foliage grow efficiently, develop roots and flowers, and improve overall plant health (Sinha and Tandon, 2020). The combined application of NPK can reduce phosphorus deficiencies, which may appear somewhat like nitrogen deficiency when plants are still small. When no fertiliser was applied on *A. afra*, a relatively consistent yield of 7.5 t·ha^{-1} fresh mass (Figure 2.4) was achieved. On the other hand, all N treatments significantly increased fresh mass yield (up to 29 t/ha) compared with the control (no nitrogen application). *Artemisia afra* appears to have nitrogen fertiliser requirements comparable to those of vegetables, ranging from 180 to 360 kg N·ha^{-1} depending on the type of fertiliser used (Prinsloo *et al*., 2013). This could be because the economic yield for *A. afra* is the leaves, like in most vegetables.

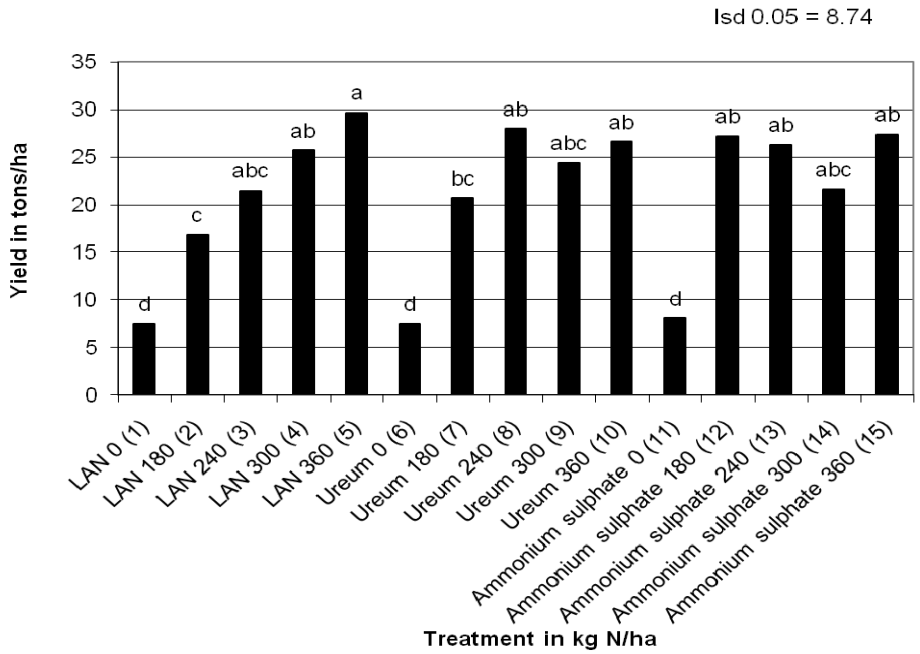

Figure 2.4. Comparison of total fresh biomass yield per season for *A. afra*, as influenced by fertiliser application (Prinsloo *et al*., 2013).

Optimal nutrient supply improves growth rate, yield, and quality in *A. annua* production (Strong, 1995). Only a few studies on *A. annua* fertilisation have been reported, and a low nitrogen level of 40 kg N·ha^{-1} had no effect on *A. annua* growth and yield, whereas 80 kg N·ha^{-1} or 120 kg N·ha^{-1} significantly increased both parameters (Rengel, 1998). Similarly, Nyoni *et al*. (2020) reported that 80 kg N·ha^{-1}, 120 kg N·ha^{-1}, and 160 kg N·ha^{-1} significantly improved the growth and yield of A. annua (Figure 2.5) and recommended 80 kg N·ha^{-1}, as there were no significant differences in growth and yield with higher application rates. *Artemisia annua* plants responded well to nitrogen, phosphate, and potash applications of 60 kg N·ha^{-1}, 60 kg P·ha^{-1}, and 50 kg K·ha^{-1} (Rengel, 1998). Organic compost at 3 - 5 t·ha^{-1} produced satisfactory yield results (Fabian and Germishuizen, 1997; Du Preez, 2005). Application of poultry manure at a rate of 4 t·ha^{-1} resulted in the highest leaf yield and total artemisinin content in *A. annua* (Yeboah *et al*., 2012).

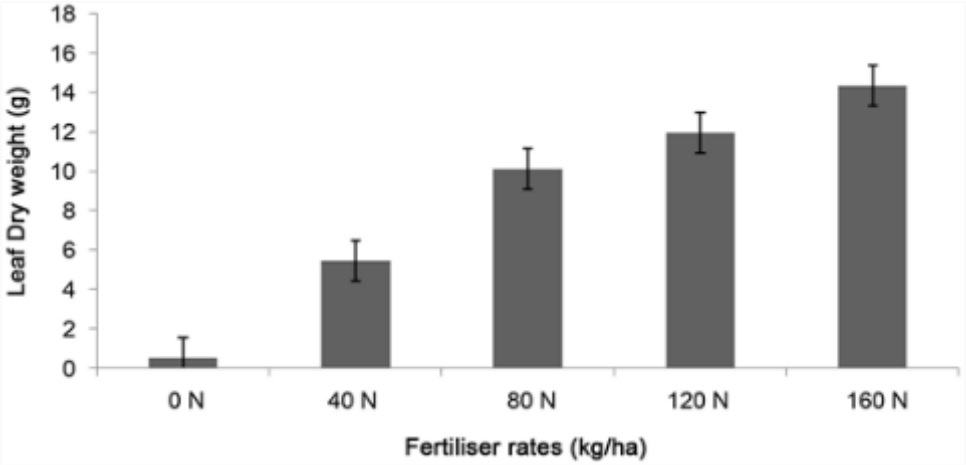

Figure 2.5. Effects of fertiliser rates on leaf biomass of *A. annua* (Nyoni *et al*., 2020).

The impact of cultivation on the secondary metabolite yield of *A. afra* is not well documented. Keeping medicinal plants in controlled environments may reduce their chemical effectiveness while improving agronomic qualities, whereas exposing them to environmental stress may enhance their chemical composition and effectiveness.

2.4.5 Irrigation in crop production and on *Artemisia* species

The current increase in human populations is driving rising food demand and the need to improve crop water-use efficiency to increase crop production. Rainfall cannot meet the total water needs of plants in semi-arid areas such as South Africa, and irrigation must supplement rainfall (Neal *et al*., 2011). Irrigation is the artificial supply of water to land for growing crops or other vegetation where rainfall cannot provide the water required for sustained growth and crop production (https://southafrica.co.za/irrigation.htm). Limited irrigation water availability limits plant biomass production in South Africa, as it does in many other parts of the world. As a result, agricultural production has shifted from the country's centre to higher rainfall areas such as the KwaZulu-Natal Midlands and the Cape Coastal regions (Dickinson *et al*. 2004). However, in most regions of South Africa, irrigation

water scarcity remains a major limiting factor for agricultural production, and encouraging farmers to adopt simple techniques that optimise production could help alleviate the problem. In addition to appropriate fertiliser application, irrigation is also considered a prominent agricultural practice that enhances plant growth. Water and nutrient management are important, as these factors can affect plant growth, biomass yield, and chemical composition (Mofokeng *et al*. 2015). Careful management of fertiliser and irrigation integration is necessary to minimise the risk of nutrient leaching. An inadequate (or excess) water supply to plants can slow seed germination and/or hinder plant growth, leading to decreased yields (Vandoorne *et al*., 2012). With increasing aridity due to climate change, fresh water will likely become even scarcer in the future (Chaves *et al*., 2003). Although irrigation systems are expensive to install and maintain, they can increase crop yields and support the sustainable cultivation of medicinal plants.

There are no reports on the water requirements, water use, or irrigation management of *A. afra*, except for a study by Koehorst *et al*. (2010), which focused on the plant's response to various pH levels in hydroponic cultivation. Thus, studies conducted on other *Artemisia* species will be discussed. A decrease in the potential yield of *Artemisia* species can be caused by moisture stress, which can occur at any point during the growing season. The seasonal consumption of *Artemisia* is approximately 250 to 420 millimetres, but this figure can vary depending on climate conditions, agronomy, and chemotype (http://maison-artemisia.org). During the first three months of establishing *A. afra* in the field, meeting its water requirements is of utmost importance (WHO, 2006). When *A. annua* plants were irrigated at 40, 80, and 120 mm soil moisture deficits, 150, 240, and 480 mm of water were applied per season (Scarcella *et al*., 2011). Once established, *A. afra* seems able to withstand prolonged periods of drought (Setshedi *et al*., 2022), and there may be a decrease in water needs (Patil *et al*., 2011). In Tanzania, *A. afra* is found in the wild in regions that receive rainfall that ranges from 900 to 2400 millimetres per year (FAO, 1986). On the other hand, the cultivation of *A. annua* demands a significant amount of water, since during the dry season each plant requires ample water both in the morning and evening (http://maison-artemisia.org). It is crucial to avoid excessive watering when cultivating both species. Excessive irrigation can cause nutrients to leach from the plant root zone or decrease the plant's root depth (Patil *et al*., 2011). In addition, *Artemisia* sp. does not do well in waterlogged conditions; thus, drainage channels or well-drained soils are required to cultivate them successfully (Anonymous, 2009; Liu *et al*., 2009).

2.4.6 Harvesting and economic potential of *Artemisia* species

Artemisia afra is a perennial plant, and the leaves can be harvested twice in one season, in midsummer and late summer or early autumn or picked as needed. The harvesting period is important, as there were differences in oil composition between *A. afra* samples harvested during the growing season and those harvested in winter (Chagonda *et al*., 1999). Harvesting occurs during the beginning to mid-flowering period (Barstow, 2012). With three to four cuts per year, one plant can produce 500 g - 1.5 kg of dry leaf material per year. *Artemisia afra*, like *A. annua*, develops branches and grows stronger after being cut. Pruning and coppicing increase biomass production by producing more branches. Plants can

be pruned very lightly for the first time once they reach 50 cm in height. Plants may reach this height in the first month after transplanting, but depending on growing conditions, it may take up to three months. The first harvest is small but encourages a bushier plant growth, resulting in much higher production later. Once established, this perennial plant is hardier and yields more (https://maison-artemisia.org).

2.4.7 Phytochemistry of *Artemisia afra* and other *Artemisia* species

Several compounds were identified in *A. afra*, including about 133 volatile secondary metabolites and 44 non-volatile secondary metabolites (Liu *et al*., 2009), in addition to other compounds identified in various *Artemisia* spp (Table 2.2). Often present are 1,8-cineole (eucalyptol), alpha-thujone, beta-thujone, camphor, borneol, and sesquiterpenoids such as chrysanthenyl acetate and devannone (Abad *et al*., 2012; Sainz *et al*., 2017; Sainz *et al*., 2017). Sesquiterpene lactones (guaianolides and glaucolides), which are mainly isolated from the aerial parts, are responsible for effects such as their antispasmodic and appetite-stimulating properties. Non-volatile constituents include triterpenes (amyrin and friedelin), alkanes (ceryl cerotinate and N-nonacosane), and flavonoids (methyl ethers of luteolin), coumarins (scopoletin) and polyacetylenes (Mukinda and Syce, 2007). Gabuka (2009) further identified sesquiterpenoids such as taurine, artesin, maritimin, artemin, norsantolinifolide, santoinifolide, and rynosin; coumarin scopoletin; acetophenones such as p-hydroxyacetophenone and 2,4-dihydroxy-6-methoxyacetophenone; and a flavonoid, 5-hydroxy-7,4’-dimethoxyflavone. In addition, More *et al*. (2012) isolated acetin, 12α, 4α-dihydroxybishopsolicepolide, α-amyrin, phytol, and the pentacyclic triterpenoid betulinic acid. Table 2.2 indicates the distribution and variation in major compounds of selected *Artemisia* spp. that are found in the literature.

Table 2.2. Selected *Artemisia* species and major compounds, their distribution and uses.

***Artemisia* species**	**Distribution**	**Uses/ailments treated**	**Major compounds, and plant part**	**Reference**
A. abrotanum L.	Europe, Asia, America	Repellent	borneol; leaves 1,8-cineole; aerial parts presil-phiperfolan-9α-ol	Abad *et al*., 2012 Sainz *et al*., 2017
A. absinthium L.	Europe, Siberia	Alcoholic beverage, anti-parasitic	camphor; aerial parts chamazulene; aerial parts ß-myrcene; aerial parts ß-pinene; aerial parts *trans*-sabinyl acetate; aerial parts ß-thujone; aerial parts	Vallés *et al*., 2011 Abad *et al*., 2012
A. abyssinica Schultz-Bip	Saudi Arabia	Anthelmintic, antirheumatic	camphor; aerial parts	Abad *et al*., 2012
A. afra Jacq. Ex Willd	Southern Africa	Anti-diabetic, asthma, bronchitis	camphor; leaves	Vallés *et al*., 2011 Abad *et al*., 2012
A. alba Turra	Mediterranean region		spathulenol, artemisia ketone, camphor, 1,8-cineole	Sainz *et al*., 2017
A. annua L.	Asia, Europe	Anti-malaria, fever	artemisia ketone; aerial parts camphor; aerial parts linalool; aerial parts	Vallés *et al*., 2011
A. arborescens (Vaill.) L.	Mediterranean region	Anti-inflammatory remedy	chamazulene; aerial parts ß-thujone; aerial parts	Abad *et al*., 2012 Sainz *et al*., 2017
A. argentea Buch				Vallés *et al*., 2011
A. argyi Levl. et Vant.	China, Japan	Liver, spleen and kidney complications	borneol; flowers bornyl acetate; flowers spathulenol; flower heads	Abad *et al*., 2012
A. armeniaca Lam	Iberian Peninsula, Russia, Iran		α-pinene; spathulenol; nonadecane; 6,10,14-trimethyl-2-pentadecanone; z-verbenol; davanone; 1,8-cineole	Sainz *et al*., 2017
A. aucheri Boiss.			geranyl acetate; aerial parts linalool; aerial parts	Abad *et al*., 2012
A. barrelieri Besser	Iberian Peninsula		α-thujone; 1,8-cineole; chrysanthenone; filifolone; camphor	Sainz *et al*., 2017
A. biennis Willd.	Europe and North America	Antiseptic, upper respiratory infections	farnesene; aerial parts *trans*-ocimene; aerial parts	Vallés *et al*., 2011 Abad *et al*., 2012

***Artemisia* species**	**Distribution**	**Uses/ailments treated**	**Major compounds, and plant part**	**Reference**
A. caerulescens L.	Iberian Peninsula, Mediterranean region			Sainz *et al*., 2017
A. cana Pursh		Culinary spice, antiseptic	camphor; aerial parts 1,8-cineole; aerial parts	Abad *et al*., 2012 Nigam *et al*., 2019
A. canariensis Less.	Canary Islands			Vallés *et al*., 2011
A. campestris L.	Tunisia, Europe, Asia	Antivenin, anti-inflammatory, anti-rheumatic	caryophyllene oxide; aerial parts germacrene D; aerial parts	Abad *et al*., 2012 Sainz *et al*., 2017
A. capillaris Thunb.			9,12,15-octadecatrienal; aerial parts phytol; aerial parts	Abad *et al*., 2012
A. chamaemelifolia Vill.	Europe, Asia	Antibacterial, antifungal	1,8-cineole; camphor; borneol; thymol; carvacrol; *p*-cymene	Sainz *et al*., 2017
A. douglasiana Besser.		Peptic ulcers, gastrointestinal disorders	artemisia ketone; leaves camphor; leaves	Abad *et al*., 2012
A. dracunculus Pursh	Asia, Europe, North America	Culinary spice, heart pulpitations	*trans*-anethole; aerial parts limonene; aerial parts methyl chavicol; aerial parts *trans*-ocimene; aerial parts	Vallés *et al*., 2011 Abad *et al*., 2012 Trendafilova *et al*., 2021
A. echegaray Hieron	Argentina	Food additive		Abad *et al*., 2012
A. fragans Willd.	Iran		camphene; roots camphor; roots, aerial parts chrysanthenone; aerial parts 1,8-cineole; leaves	Abad *et al*., 2012
A. frigida Willd.		Diabetes, coughs	borneol; leaves bornyl acetate; leaves camphor, aerial parts 1,8-cineole; aerial parts germacrene D; leaves α-thujone; leaves ß-thujone; leaves	Abad *et al*., 2012
A. fukudo Makino	South Korea, Japan, Taiwan	Cosmetics, antitumour, anti-inflammatory	α-thujone; leaves ß-thujone; leaves	Abad *et al*., 2012
A. gorgonum Webb	Cape Verde		camphor; aerial parts chrysanthenone; aerial parts	Vallés *et al*., 2011 Abad *et al*., 2012

***Artemisia* species**	**Distribution**	**Uses/ailments treated**	**Major compounds, and plant part**	**Reference**
A. granatensis Boiss.	Spain, Iberian Peninsula			Vallés *et al*., 2011 Sainz *et al*., 2017
A. haussknechtii Boiss.	Iran	Perfumes, pharmaceutical products	camphor; aerial parts 1,8-cineole; aerial parts	Abad *et al*., 2012
A. herba-alba Asso	Jordan, France, England, Morocco	Fever, menstrual and nervous problems	chrysanthenyl propionate; aerial parts elixene; aerial parts	Abad *et al*., 2012 Sainz *et al*., 2017
A. incana B. Keller			borneol; aerial parts camphor; aerial parts 1,8-cineole; aerial parts	Abad *et al*., 2012
A. iwayomogi Kitamura	Korea	Liver conditions, hepatitis	borneol; aerial parts camphor; aerial parts 1,8-cineole; aerial parts	Abad *et al*., 2012
A. judaica L.	North Africa, Middle East	Anthelmintic	camphor; aerial parts piperitone; aerial parts	Abad *et al*., 2012
A. kulbadica Boiss. & Buhse	Iran		ɣ-cadinene, aerial parts sabinene; aerial parts ß-thujone; aerial parts	Abad *et al*., 2012
A. lavandulaefolia Nakai			caryophyllene; aerial parts eucaliptol; aerial parts farnesene; aerial parts ß-thujone; aerial parts	Abad *et al*., 2012
A. longifolia Nutt.			camphor; aerial parts 1,8-cineole; aerial parts	
A. lucentica O. Bolós, Vallès & Vigo	Iberian peninsula			Sainz *et al*., 2017
A. ludoviciana Nutt.	Mexico	Antidiarrheal remedy	camphor; aerial parts 1,8-cineole; aerial parts davanone; aerial parts	Abad *et al*., 2012
A. magellanica Sch. Bip.	Argentina			Vallés *et al*., 2011 Abad *et al*., 2012
A. mauiensis Skottsb.	Hawaii			Vallés *et al*., 2011 Abad *et al*., 2012
A. melanolepsis Boiss.	Irania			Vallés *et al*., 2011 Abad *et al*., 2012
A. molinieri Quézel, Barbero & R.J. Loisel	France			Vallés *et al*., 2011 Abad *et al*., 2012

***Artemisia* species**	**Distribution**	**Uses/ailments treated**	**Major compounds, and plant part**	**Reference**
A. mongolica Fisch. ex Besser	China	Anti-inflammatory	α-pinene; aerial parts	Nigam *et al*., 2019
A. negrei A. Ouyahya	Morocco			Vallés *et al*., 2011 Abad *et al*., 2012
A. nilagirica (Clarke) Pamp	India	Insecticide	borneol; aerial parts caryophyllene oxide; aerial parts	Abad *et al*., 2012
A. ordosica Krasch	China, Mongolia	Nasal bleeding, rheumatoid arthritis, headache, carbuncle	α-cadinol, aerial parts epiglobulol; aerial parts *cis*-lanceol; aerial parts ß-bisabolol; aerial parts	Abad *et al*., 2012 Trendafilova *et al*., 2021
A. pontica Burm.f.			1,8-cineole; aerial parts α-thujone; aerial parts	Abad *et al*., 2012
A. princeps Willd.	Japan, China, Korea	Anti-inflammatory, antidiarrheal remedy, circulatory disorders		Abad *et al*., 2012 Trendafilova *et al*., 2021
A. reptans C. Sm.	Canary island, Morroco			Sainz *et al*., 2017
A. rubripes Nakai	Korea	Stomach-ache, antidiarrheal remedy, haemostatic agent	camphor; leaves caryophyllene; leaves eucaliptol; leaves	Abad *et al*., 2012
A. santonicum L.			camphor; aerial parts	Abad *et al*., 2012
A. scoparia Waldst. & Kit.	Asia, Europe	Insecticide, anti-cholesterol emic, anti-pyretic, hepatitis, jaundice	camphor; aerial parts 1,8-cineole; aerial parts *p*-cymene; leaves limonene; leaves ß-myrcene; leaves ß-pinene; aerial parts ɣ-terpinene; leaves, roots α-thujone; aerial parts ß-thujone; aerial parts	Abad *et al*., 2012
A. sieberi Besser	Middle East	Ulcers, anthelmintic	camphor; aerial parts α-thujone; aerial parts ß-thujone; aerial parts	Abad *et al*., 2012 Nigam *et al*., 2019

***Artemisia* species**	**Distribution**	**Uses/ailments treated**	**Major compounds, and plant part**	**Reference**
A. sieversiana Ehrh. Ex Willd.			1,8-cineole; aerial parts	Abad *et al*., 2012
A. spicigera C. Koch	Turkey	Skin diseases, ulcerative sores	camphor; aerial parts 1,8-cineole; aerial parts ß-thujone; aerial parts	Abad *et al*., 2012
A. thuscula Cav.	Canary islands	Spasmolitic activity	davanone; camphor; chrysanthenone; ß-thujone	Sainz *et al*., 2017
A. tournefortiana Rchb.	Europe, Asia, North America	Antibacterial	(z)-nerolidol; ß-caryophyllene; santolina triene	Sainz *et al*., 2017
A. tridentata Nutt.	North America	Food resource for many animals		Abad *et al*., 2012
A. umbelliformis Lam.	Iberian peninsula	Wound-healing, respiratory infections, dyspepsia	thujone; borneol; ß-pinene; α-pinene	Sainz *et al*., 2017
A. verlotiorum Lamotte	Europe, Africa, Asia, South America	Hypertension, stomach-ache	α- thujone; camphor	Sainz *et al*., 2017 Trendafilova *et al*., 2021
A. vulgaris L.	Asia, Europe, North America	Antihypertensive, antispasmodic, dysmenorrhoea	α- thujone; camphor; 1,8-cineole; γ-muurolene	Abad *et al*., 2012 Sainz *et al*., 2017

The analysis method and instrumentation appear to affect the chemical compounds or essential oils identified, mainly because some are volatile and others are non-volatile. For example, gas chromatography-mass spectrometry (GC-MS) analysis of *A. afra* leaves identified 86 essential oil components (Falowo *et al*., 2019), including thujone, artemiseole, camphene, and others. The hydrogen-nuclear magnetic resonance (^{1}H-NMR) analysis (Liu *et al*., 2010) identified 26 compounds (Table 2.3). However, it is worth noting that GC-MS is mainly used for volatile compounds, whereas nuclear magnetic resonance (NMR) is used for structural elucidation.

Table 2.3. Chemical composition of *Artemisia afra* as per various analytical instruments.

Compound	Analytic instrument	Reference
Acetic acid	^{1}H-NMR	Liu *et al*. 2010
Adenine	^{1}H-NMR	Liu *et al*. 2010
Alanine	^{1}H-NMR	Liu *et al*. 2010
Artemiseole	GC-MS	Falowo *et al*. 2019
Artemisia ketone	GC (FID)	Vagionas *et al*. 2007
Aromadendrene	GC-MS	Falowo *et al*. 2019
Aspartic acid	^{1}H-NMR	Liu *et al*. 2010
Benzaldehyde	GC (FID)	Vagionas *et al*. 2007
Benzaldehyde, o-(diethylboryl)oxim	GC-MS	Falowo *et al*. 2019
Benzene, 1,1'-(diazomethylene)bis	GC-MS	Falowo *et al*. 2019
Benzene, tert-butyl	GC-MS	Falowo *et al*. 2019
Benzene, 1-ethyl-2,3-dimethyl	GC-MS	Falowo *et al*. 2019
Benzene, 1-methyl-3-(1-methylethyl)	GC-MS	Falowo *et al*. 2019
Benzoic acid, nonadecyl ester	GC-MS	Falowo *et al*. 2019
Benzoic acid, 3-methoxy-, methyl ester	GC-MS	Falowo *et al*. 2019
Bergamotol, Z-α-trans-	GC-MS	Falowo *et al*. 2019
Bicycle [2.2.1] hept-2-ene, 1,7,7-trimethyl	GC-MS	Falowo *et al*. 2019
Bicycle [3.1.0] hexan-3-one, 4-methyl-1-(1-methylethyl)	GC-MS	Falowo *et al*. 2019
Bicycle [3.1.0] hexane, 4-methylene-1-(1-methylethyl)	GC-MS	Falowo *et al*. 2019
Bicyclo (3.1.0) hexane, 6-isopropylidene-1-methyl	GC-MS	Falowo *et al*. 2019
Bicyclogermacrene	GC-MS	Falowo *et al*. 2019
Borneol	GC (FID)	Vagionas *et al*. 2007
Bornyl acetate	GC (FID)	Vagionas *et al*. 2007
Butanoic acid, 3-hexenyl ester	GC-MS	Falowo *et al*. 2019
Caffeic acid	^{1}H-NMR	Liu *et al*. 2010
Camphene	GC-MS; GC (FID)	Falowo *et al*. 2019; Vagionas *et al*. 2007
Camphor	GC (FID)	Vagionas *et al*. 2007
Camphor	GC-MS	Falowo *et al*. 2019
Caryophyllene	GC-MS	Falowo *et al*. 2019
Caryophyllene oxide	GC-MS; GC (FID)	Falowo *et al*. 2019; Vagionas *et al*. 2007
Chamazulene	GC-MS	Falowo *et al*. 2019
Chlorogenic acid	^{1}H-NMR	Liu *et al*. 2010
Choline	^{1}H-NMR	Liu *et al*. 2010
Citric acid	^{1}H-NMR	Liu *et al*. 2010
cis-chrysanthenyl acetate	GC (FID)	Vagionas *et al*. 2007
cis- ß-farnesene	GC-MS	Falowo *et al*. 2019
cis-hexenyloctyne carbonate	GC-MS	Falowo *et al*. 2019
cis-muurola-3,5-diene	GC-MS	Falowo *et al*. 2019
cis-p-mentha-1(7),8-dien-2-ol	GC-MS	Falowo *et al*. 2019
Copaene	GC-MS	Falowo *et al*. 2019

Compound	Analytic instrument	Reference
Cuminaldeyde	GC (FID)	Vagionas *et al*. 2007
Cyclohexanol, 2-methyl-5-(1-methylethyl) - *cis*	GC-MS	Falowo *et al*. 2019
Cyclohexanol, 2-methyl-5-(1-methylethenyl)	GC-MS	Falowo *et al*. 2019
Cyclohexanone, 2-(1-mercapto-1-methylethyl)-5-methyl	GC-MS	Falowo *et al*. 2019
Cyclohexene, 3-methyl-6-(1-methylethylidene)	GC-MS	Falowo *et al*. 2019
Cyclooctene, 3-(1-methylethnyl)	GC-MS	Falowo *et al*. 2019
Cyclopropane, 1,1-dimethyl-2-(3-methyl-1,3-butadienyl)	GC-MS	Falowo *et al*. 2019
Cyclotrisiloxane, hexamethyl	GC-MS	Falowo *et al*. 2019
Dehydro-sabina ketone	GC (FID)	Vagionas *et al*. 2007
D-limonene	GC-MS	Falowo *et al*. 2019
Eucalyptol	GC-MS	Falowo *et al*. 2019
endo-borneol	GC-MS	Falowo *et al*. 2019
Epianastrephin	GC-MS	Falowo *et al*. 2019
(+)-epi-bicyclosesquiphellandrene	GC-MS	Falowo *et al*. 2019
Eugenol	GC-MS; GC (FID)	Falowo *et al*. 2019; Vagionas *et al*. 2007
Formic acid	^{1}H-NMR	Liu *et al*. 2010
Fumaric acid	^{1}H-NMR	Liu *et al*. 2010
Germacrene D	GC (FID)	Vagionas *et al*. 2007
Glutamic acid	^{1}H-NMR	Liu *et al*. 2010
Hexane, 1,6-dibromo	GC-MS	Falowo *et al*. 2019
Humulene	GC-MS	Falowo *et al*. 2019
Leucine	^{1}H-NMR	Liu *et al*. 2010
Malic acid	^{1}H-NMR	Liu *et al*. 2010
(-)-myrtenol	GC-MS	Falowo *et al*. 2019
Nerolidol 2	GC-MS	Falowo *et al*. 2019
Neryl (S)-2-methylbutanoate	GC-MS	Falowo *et al*. 2019
o-Cymene	GC-MS	Falowo *et al*. 2019
o-toluic acid, 3-chloropen-2-enylester	GC-MS	Falowo *et al*. 2019
p-cymene	GC-MS	Falowo *et al*. 2019
p-cymene-8-ol	GC (FID)	Vagionas *et al*. 2007
p-cymene-7-ol	GC (FID)	Vagionas *et al*. 2007
Phellandral	GC (FID)	Vagionas *et al*. 2007
Phosphine, dimenthoxy-menthyl	GC-MS	Falowo *et al*. 2019
Pinocarvone	GC (FID)	Vagionas *et al*. 2007
Proline	^{1}H-NMR	Liu *et al*. 2010
Phosphatidylcholine	^{1}H-NMR	Liu *et al*. 2010
p-hydroxy benzoic acid	^{1}H-NMR	Liu *et al*. 2010
Phytol	GC-MS	Falowo *et al*. 2019
p-menth-8-ene, 3-methylene	GC-MS	Falowo *et al*. 2019
Quercetin analogue	^{1}H-NMR	Liu *et al*. 2010
Quinolone, 4-propyl	GC-MS	Falowo *et al*. 2019
Rhamnose	^{1}H-NMR	Liu *et al*. 2010
Sabinene	GC (FID)	Vagionas *et al*. 2007
Succinic acid	^{1}H-NMR	Liu *et al*. 2010
Sucrose	^{1}H-NMR	Liu *et al*. 2010
Spathulenol	GC (FID)	Vagionas *et al*. 2007
Terpinen-4-ol	GC-MS; GC (FID)	Falowo *et al*. 2019; Vagionas *et al*. 2007
Threonine	^{1}H-NMR	Liu *et al*. 2010
Thujone	GC-MS	Falowo *et al*. 2019
trans-linalool oxide	GC (FID)	Vagionas *et al*. 2007
Tricycle [2.2.1.0(2,6)] heptane,1,3,3-trimethyl	GC-MS	Falowo *et al*. 2019
Tricyclene	GC (FID)	Vagionas *et al*. 2007
Undecanal	GC-MS	Falowo *et al*. 2019
Undecane, 4-cyclohexyl	GC-MS	Falowo *et al*. 2019

Compound	Analytic instrument	Reference
Valine	^{1}H-NMR	Liu *et al*. 2010
Yomogi alcohol	GC (FID)	Vagionas *et al*. 2007
1,2-bis(trimethylsilyl)benzene	GC-MS	Falowo *et al*. 2019
1,3-butadiene, 2-methyl	GC-MS	Falowo *et al*. 2019
1,8-cineole	GC (FID)	Vagionas *et al*. 2007
1,3-cyclohexadiene, 1-methyl-4-(1-methylethyl)	GC-MS	Falowo *et al*. 2019
1-decene-3-yne	GC-MS	Falowo *et al*. 2019
1-dimethyl(phenyl)silyloxypentane	GC-MS	Falowo *et al*. 2019
1-octen-3-ol	GC (FID)	Vagionas *et al*. 2007
1-*O*-ethyl-ß-D-glucoside	^{1}H-NMR	Liu *et al*. 2010
2-benzothiazolamine, 4-methyl	GC-MS	Falowo *et al*. 2019
2-carene	GC-MS	Falowo *et al*. 2019
2-ethyl-(E)-2-butenal	GC-MS	Falowo *et al*. 2019
2,15-hexadecanedione	GC-MS	Falowo *et al*. 2019
2-hydrazinopyridine	GC-MS	Falowo *et al*. 2019
2-methylbutanoic abhydride	GC-MS	Falowo *et al*. 2019
2,4,6-triphenyl-1,3-dioxane	GC-MS	Falowo *et al*. 2019
3,5-dimethylcyclopentene	GC-MS	Falowo *et al*. 2019
3,5-dicaffeoyl quinic acid	^{1}H-NMR	Liu *et al*. 2010
4-pyridinol	GC-MS	Falowo *et al*. 2019
5,9-hexacosadienoic acid, methyl e	GC-MS	Falowo *et al*. 2019
α-bourbonene	GC-MS	Falowo *et al*. 2019
α-cadinol or epiglobulol	GC-MS	Falowo *et al*. 2019
α-campholenal	GC-MS	Falowo *et al*. 2019
α-copaene	GC (FID)	Vagionas *et al*. 2007
α-phellandrene	GC-MS	Falowo *et al*. 2019
α-pinene	GC-MS; GC (FID)	Falowo *et al*. 2019; Vagionas *et al*. 2007
α-glucose	^{1}H-NMR	Liu *et al*. 2010
α-terpineol	GC-MS; GC (FID)	Falowo *et al*. 2019; Vagionas *et al*. 2007
α-thujone	GC (FID)	Vagionas *et al*. 2007
ß-caryophyllene	GC (FID)	Vagionas *et al*. 2007
ß-copaene	GC-MS	Falowo *et al*. 2019
ß-glucose	^{1}H-NMR	Liu *et al*. 2010
ß-myrcene	GC-MS	Falowo *et al*. 2019
ß-thujone	GC (FID)	Vagionas *et al*. 2007
p-cymene	GC-MS	Falowo *et al*. 2019
γ-terpinene	GC-MS; GC (FID)	Falowo *et al*. 2019; Vagionas *et al*. 2007
δ-cadinene	GC (FID)	Vagionas *et al*. 2007

^{1}H-NMR = Hydrogen-Nuclear Magnetic Resonance, GC (FID) = Gas Chrotomataphy (Flame Ionization Detection), GC-MS = Gas Chrotomataphy-Mass Spectrometry

Apart from instrumentation and methods, variations in chemical composition can be due to the plant's wide distribution and the range of altitudes at which it grows. The qualitative and quantitative differences among many *Artemisia* spp. may correlate with environmental conditions, UV radiation, geographic, climatic, genetic factors, plant age, soil, plant parts, and harvesting season (Nigam *et al*., 2019; Kane *et al*., 2019). Analysis of *A. afra* samples purchased from three vendors in the same market revealed variation in chemical compound content and quantities (Motshudi *et al*., 2021). The variation could mean that the vendors had different sources of the same plant material. Two chemotypes were identified previously, based on their essential oil composition and they are 1) the thujone chemotype with cineol,

camphor and thujone as the major components, and 2) the ketone chemotype with cineol, camphor and 'Artemisia' ketone as the major constituents (Gabuka, 2009). Altitude plays an important role in UV radiation and, consequently, in the essential oil composition of *Artemisia* spp. For example, the essential oil content of *A. nilagirica*, a species growing in the Himalachal Pradesh, India, varied with changes in altitude, where characteristic compounds at lower altitudes were 2-hexene-1-ol, *ß*-thujone, thujanol, myrtenol, and lynalyl acetate, while at higher altitudes α-pinene, ß-pinene, limonene, linalool, γ-gurijunene, germacrane D and farnesol were the major compounds (Abad *et al*., 2012). The essential oil content of *A. annua* plants also varied between 0.3 and 0.7%, at different growth stages (Abad *et al*., 2012).

2.5 POTENTIAL TOXICITY OF SOME *ARTEMISIA* SPECIES

Toxicity is defined as "the potential of a substance to exert a harmful effect on humans or animals. The volatile oil of *A. afra* is said to be as toxic as oil of Sabine, producing hemorrhagic nephritis, degenerative changes in the liver and pulmonary oedema (Mukinda and Syce, 2007). The volatile oil of *A. afra* caused hemorrhagic nephritis and sometimes abortion in pregnant animals (Dosoky and Setzer, 2021). Moreover, it has been reported that this volatile oil's toxic and hallucinogenic effects are attributed to thujone, and overdoses or continued use over long periods are potentially harmful (Mikunda, 2007). The effects of excessive ingestion include restlessness, vomiting, vertigo, tremor, convulsions, and liver degeneration.

Many *Artemisia* species are considered toxic due to their high thujone content, which can negatively affect brain, kidney, and liver cells if consumed regularly and in large quantities (Odeyeji *et al*., 2009). In their study, Lachenmeier *et al*. (2006) concluded that thujone plays no or a minor role in a mental disorder called absinthism. However, another study on *A. absinthium* showed that samples containing 45% sabinyl acetate, 12% thujone, 6% myrcene, and 5.3% 1.8-cineole were toxic, whereas samples lacking thujone were less toxic, even if they contained other compounds (Judzentiene *et al*., 2012). A thujone-free *A. absinthium* chemotype, with 45% (z)-epoxyocimene and 18% cis-chrysanthenol, lacked toxicity (Sainz *et al*., 2017). A study by Muto *et al*. (2003) found that a 2% *A. absinthium* extract had no toxic effects on rats. However, the test animals rejected drinking water infused with 5% extract of *A. absinthium*. The rejection could indicate that a higher percentage of the extract was toxic. An extract of *A. afra* was nontoxic at lower concentrations of 3.06-6.12 *μ*g/mL; however, at higher concentrations of 12.5-400 *μ*g/mL, toxicity effects were apparent (More *et al*., 2012). In 1997, a case of poisoning that resulted in acute kidney failure of a 31-year-old man was reported after he drank approximately 10 ml of an essential oil extract from *A. absinthium* (Weisbord *et al*., 1997). Turliuc *et al*. (2019) also discussed a case of absinthe toxicity-related death of a man who lived in France. A review by Dosoky and Setzer (2021) summarised the effects of thujone and camphor contained in *A. afra* (Liu *et al*., 2009) and concluded that both compounds should be avoided during pregnancy as they may cause abortions. Similarly, the abortifacient effect of *A. kopetdaghensis* was attributed to its high camphor content (Trendafilova *et al*., 2021). The various reports on the toxicity of *Artemisia* could be attributed to differences in the quantitative and qualitative composition of bioactive compounds, which are correlated

with environmental, geographic, and climatic conditions; plant age; harvesting period and methods; and the plant part harvested (Nigam *et al*., 2019). For example, Nigam *et al*. (2019) recommended *A. afra* collected in Burundi as a strong candidate for malaria treatment, as it showed no toxic effects, which could be due to variations in thujone content, as reported by Oyedeji *et al*. (2009) and Viljoen *et al*. (2006).

2.6 AGRO-PROCESSING OF *ARTEMISIA AFRA* AND PRODUCT DEVELOPMENT

Artemisia afra essential oils are mainly extracted by hydrodistillation, steam distillation, or organic solvent extraction (Adeogun *et al*., 2018). Steam distillation and solvent extraction result in the degradation and loss of some volatile compounds, whereas the hydrolytic process destabilises unsaturated oil components and reduces volatile compounds (Adeogun *et al*., 2018). The extraction method and pre-processing practices can influence the oil composition of *A. afra* leaves. For example, there were variations in a few compounds between solvent-free microwave extraction and hydrodistillation (Table 2.4).

Table 2.4. Chemical content of *Artemisia afra* as influenced by drying and extraction method. (SME = solvent-free microwave extraction; HD = hydrodistillation).

Chemical compounds	Essential oil group	Fresh leaves SME	Dried leaves SME	Fresh leaves HD	Dried leaves HD
			%		
Artemisia ketone	Monoterpene	4.24	0.99	3.29	2.90
Camphor	hydrocarbons	13.26		11.71	13.00
Camphene		1.73			1.57
Cis-piperitol		0.60	0.67	1.28	1.51
Cis-p-mentha-1 (7). 8- dien-2-ol					0.15
Cis-verbenone		0.27	2.76		
Eucalyptol		12.73	3.74	9.57	12.90
Eugenol		0.13			
Isothujol		0.22		0.33	
L-. α- terpineol		0.40	0.82	0.44	0.41
(-)-limonene		0.88			
Linalool					
Myrtenol				0.72	
Piperitol		0.62	0.39		0.72
Sabinene		0.65	0.23	0.56	0.51
Santolina triene		0.48		0.06	0.38
(-) – terpinen-4-ol		1.91	0.7	1.45	1.70
Thujone		**26.57**	**25.82**	**32.02**	**30.26**
Trans-pinocamphone			2.22		
Yomogi alcohol		0.71	0.11	0.38	0.61
α-phellandrene		0.12	0.41	0.08	0.19
α-pinene		1.10		0.05	
α-thujenal		0.28		0.27	
α-thujene					0.10
ß-myrcene		0.44	2.68	0.38	0.33
p-cymene		1.54	1.45	0.88	1.66
2- carene		0.83	0.40	0.86	
3-allylguaiacol			0.20	0.19	0.11
3-tert-butyphenol			2.47		
3-p-menthene				1.79	
(+) -4-carene		0.32		0.14	0.34
Borneal	Oxygenated	2.51	1.17	3.16	2.50
Chamazulene	monoterpenes	0.06			0.06

Chemical compounds	Essential oil group	Fresh leaves SME	Dried leaves SME	Fresh leaves HD	Dried leaves HD
		%			
Phytol		0.08			
Alloaromadendrene	Sesquiterpene		0.05		0.06
(-) -. B. Bourbonene	hydrocarbons		0.25		
Bicyclogermarcrene				0.82	0.89
Cadina – 1(2), 4 diene				0.10	0.20
Caryophyllene		0.35	0.89	0.24	0.28
Caryophyllene oxide			0.60		
Cubenol			0.11		
Germacerene D		1.89		1.96	1.67
Humulene		0.07	0.07	0.07	0.08
Ledene oxide- (II)			0.09		
Nerolidol		0.14	0.05		0.16
Shyobunone			0.06		
Spathulenol		0.47	0.42	0.51	0.63
Tau. –muurolol		1.28			
Viridiflorol		0.19			0.17
α-cadinol		0.50	0.44	1.04	1.21
α. - copaene			0.23	0.47	0.26
α-gurjunene					0.65
ß-cubebene			0.87		
ß- copaene		0.05	0.06		0.06
ß-gurjunene			0.13		
Y- muurolene		0.24			
ɣ-selinene			0.10		
δ- candinene		0.40	0.15	0.48	0.27
Acetic acid,1,7,7-trimethyl-bicyclo [2.2.1] hept-2-yl ester	Esters			0.97	
Butanoic acid, 2 methyl-, 2- methylbutyl ester					0.20
Cyclohexane, (2-nitro-2-propenyl)			0.46		
(E)-2-isopropyl -5- methylphenyl 2-methylbut-2-enoate			0.62		
Pentanoic acid, 2- methylbutyl ester		0.16			
3- methyl-2-butenoic acid, cyclobutyl ester			0.14		
3- methyl-2-butenoic acid, oct-3-en-yl ester		1.81			
3-methyl-2-butenoic acid, 2-methyl oct-5-yn-4-yl ester			0.08		

Chemical compounds	Essential oil group	Fresh leaves SME	Dried leaves SME	Fresh leaves HD	Dried leaves HD
			%		
3-methyl-2-butenoic acid, pent-2-en-4-ynyl ester			0.13		
3- methylbut-2-enoic acid, 2-methyl oct-5-yn-4-yl ester			0.53		
3- methybut-2-enoic acid, 4-isopropylphenyl ester			0.62		
3-methylbut-2-enoic acid, 4-nitrophenyl ester			0.14		
3- methylbut-2-enoic acid, oct-3-en-2-yl ester			1.25		
3-methylbut-2-enoic acid, 2,3,4,6- tetrachlorophenyl ester			0.23		
5- isopropyl-2-methylphenyl 2-mehtylbut-2-enoate			0.15		

(Adapted from Adeogun *et al*. 2018)

Asekun *et al*. (2007) reported that oven- and air-drying of *A. afra* resulted in lower oil contents of 0.88% and 1.54%, respectively, compared to sun-drying, which yielded an oil content of 1.83% (Table 2.5). The fresh leaves of *A. afra* had the lowest essential oil content compared to the dried leaves (Asekun *et al*. 2007). Similar results were reported *in A. judaica,* where dried samples, regardless of drying method, yielded higher essential oil than fresh leaves (Al-Qudah *et al*., 2021). The α- and ß-thujone contents were also high when the leaves of *A. afra* were sun-dried (Asekun *et al*., 2007). The artemisinin content of *A. annua* leaves dried in the sun or shade was also higher than in oven-dried leaves (Laughlin, 2002). Drying temperature significantly affected the essential oil content (composition and proportion) of *A. annua* where increasing the temperature from room temperature decreased the essential oil content as follows: room temperature (1.12%), 35°C (0.88%), 45°C (0.55%), 55°C (0.50%) and 65°C (0.37%) (Khangholi and Rezaeinodehi, 2008).

Table 2.5. The effect of drying method on the chemical composition of *Artemisia afra*.

Essential oil compound	**Fresh leaves**	**Sun-dried**	**Oven-dried**	**Air-dried**
		%		
Artemisia ketone	6.9	-	12.9*	-
Bicycloelemene	0.1	-	<0.05	-
Bicyclogermacrene	0.5	-	0.4	0.3
Borneol	7.8	6.1	3.4	2.7
(-)-bornyl acetate	1.6	0.4	1.0	0.4
Camphene	3.8	2.1	3.9	1.8
Camphor	14.2	12.4	14.4	8.2
Carvone	<0.05	-	0.1	<0.05
Caryophyllene oxide	-	-	0.2	0.2
Chrysanthenone	-	1.8	-	3.1
Chrysanthenyl acetate	-	0.1	0.3	0.3
E, E-α-farnesene	0.1	-	-	-
Eugenol	0.1	-	<0.05	-
Germacrene D	0.6	0.1	0.4	0.4
Myrtenol	0.4	0.3	0.3	0.4
Oil content	0.18	1.88	0.88	1.54
Pulegone	0.4	-	-	-
Spathulenol	0.2	-	0.2	0.2
Terpinen-4-ol	1.6	1.3	1.5	1.4
Total oil composition	98.9	99.7	93.2	86.3
trans (+)-carveol	0.1	0.3	0.1	0.1
trans-piperitol	0.2	-	0.1	-
T-muurolol	0.5	-	0.4	0.5
Viridiflorol	0.3	-	0.1	-
α-copaene	0.1	-	0.1	0.1
α-humulene	-	-	0.1	-
α- terpineol	0.3	0.4	0.2	0.3
α- terpinene	0.1	-	-	0.3
α- and ß-thujone	45.0	52.1	39.8	47.6
ß-caryophyllene	0.1	-	0.1	0.2
ɣ-gurjunene	-	-	-	0.3
ɣ-terpinene	-	0.5	0.2	-
δ- candinene	0.1	-	0.1	0.1
1,8-cineole	13.9	21.8	13.1	17.1

(Adapted from Asekun *et al*. 2007, *highest percentage for each compound)

Several *A. afra* products have been developed (Table 2.6), including packaging ground dried leaves as capsules (Van der Kooy *et al*., 2008). Komperlla (2004) developed herbal tablets, but the challenge was the tendency of the tablets to absorb moisture. Similarly, Sekhonyana-Khetsekile (2018) developed capsules from *A. afra* freeze-dried aqueous extracts; however, the moisture content was too high, posing a challenge to microbial contamination. Teabags are reported to be suitable for packaging dried *A. afra* leaves (Dube, 2006).

Table 2.6. A few commercially available *Artemisia afra* products.

Commercial products	Description	Supplier/manufacturer
Aromatic herb	Dried and cut roots, stems and leaves	www.soapoilsandherbs.co.za
Capsules	Powder in capsules	www.medicoherbs.co.za
Powder	Milled powder	Afrigetics Botanicals
Tincture	Herbal extract	www.eOil.co.za
Dried herb	Cut stems and leaves	www.takealot.com
Essential oil	Essential oil	www.essentiallynatural.co.za www.sundaynatural.de
Energy drink	Extract	www.drinkscore.co.za
Herbal tea	Loose tea	www.indiza.com www.nuleafhealthshop.co.za

2.7 REMOTE SENSING STUDIES ON *ARTEMISIA AFRA* AND VARIOUS MEDICINAL PLANTS

Remote sensing uses the interaction between electromagnetic radiation and objects on the Earth's surface to obtain information about an object without direct contact and has been used to discriminate between plant species (Dubula *et al*., 2016; Tesfamichael *et al*., 2018). Remote sensing was successfully used to map the distribution of water hyacinth in Rwanda (Mukarugwiro *et al*., 2019) and eucalyptus trees in Johannesburg (Abutaleb *et al*., 2020).

2.7.1 Vegetation mapping using remote sensing

Spatial information on vegetation distribution is important for monitoring the effects of environmental changes and human impacts on species distribution (Calviño-Cancela and Martín-Herrero, 2016). Mapping provides information on the spatial and temporal distribution of plant species for better management (Dubula *et al*., 2016), but it can also be a useful tool for predicting areas suitable for producing specific plant species. Remote sensing is a cost-effective and time-efficient technique used in mapping to cover large spatial areas. The major limiting factors to using remote sensing for vegetation monitoring and mapping include cloud cover, spatial resolution and detection accuracy (Dammer *et al*., 2012).

Hyperspectral imagery can separate plant species, which can be difficult in multispectral imagery (Dubula *et al*., 2016). This could be because, in hyperspectral sensors, which are suited for vegetation studies, reflectance/absorption spectral signatures from individual species and more complex mixed-

pixel communities can be better differentiated using much wider spectral bands (Xie *et al*., 2008). Remote sensing can also be used for other applications, such as yield estimation. Tshabalala *et al*. (2021) recommended hyperspectral data collected at the canopy level as a relatively effective non-destructive means to measure and extrapolate the yield of the medicinal tree *Moringa oleifera*. The ability to map the distribution of *A. tridentata* was improved by blending hyperspectral data with light detection and ranging (LiDAR) data to minimise spectral confusion introduced by partial-cover canopies and soil variability (Mundt *et al*., 2006). Phenology metrics derived from remote sensing data resulted in ecologically meaningful spatial patterns of production and vegetation growth across South Africa, with 76% accuracy (Wessels *et al*., 2011).

2.7.2 Vegetation indices used in vegetation mapping

Various sensors can be used in vegetation mapping and monitoring (Table 2.7). However, one challenge with remote sensing is the interpretation of measurements from sparse canopies, as vegetation indices are strongly controlled by changes in fractional vegetation cover rather than by changes in canopy optical thickness (Leprieur *et al*., 2000). Thus, mathematical combinations of different spectral bands and a variety of spectral reflectance values were used to develop various vegetation indices (VIs) (Nouri *et al*., 2014). Special spectral features, also called reflectance, emission regions, or spectral radiance, of various objects, including vegetation, can be used to identify objects in remote sensing imagery (Xie *et al*., 2008). Hyperspectral imagery is preferred over multispectral imagery because it includes hundreds of spectral bands, compared to a dozen in multispectral imagery (Calviño-Cancela and Martín-Herrero, 2016; Xie *et al*., 2008). In hyperspectral sensors suited for vegetation studies, reflectance/absorption spectral signatures from individual species and more complex mixed-pixel communities can be better differentiated from the much wider spectral bands (Xie *et al*., 2008). Thus, hyperspectral imagery can separate plant species, which can be difficult in multispectral imagery (Dubula *et al*., 2016).

The vegetation indices combine surface reflectance from two or more spectral bands to study different vegetation characteristics and allow inter-comparison (Ximena 2017). The most commonly used vegetation indices include the Normalised Difference Vegetation Index (NDVI), which is sensitive to soil brightness changes, the Soil-Adjusted Vegetation Index (SAVI), which was developed to minimise the soil influence on canopy reflectance, and the Global Environment Monitoring Index (GEMI), developed to correct for atmospheric contributions in some remotely sensed data (Leprieur *et al*., 200). The Transformed Difference Vegetation Index (TDVI) has the sensitivity of the SAVI to the optical properties of bare soil, while it does not saturate like NDVI (Bannari *et al*., 2002). Xue and Su (2017) reviewed more than 100 vegetation indices and included the Chlorophyll Absorption Ratio Index (CARI), the Renormalised Difference Vegetation Index (RDVI), the Triangular Vegetation Index (TVI), and the Three-band Gradient Difference Vegetation Index (TGDVI), which are mostly based on the separation of vegetation from soil and water (Nouri *et al*., 2014). It was concluded that each vegetation index (VI) has its own expression of green vegetation, its own suitability for specific uses, and its own limiting factors (Xue and Su, 2017).

Table 2.7. Some of the commonly used sensors in vegetation monitoring and mapping through remote sensing.

Sensors	Date: first launch	Description	Vegetation mapping applications	Advantages/disadvantages
ASTER	1999	Advanced Spaceborne Thermal Emission Reflection Radiometer is an image instrument on board Terra.	Regional to national scale mapping at species or community level.	Creates detailed maps of land surface temperature, reflectance, and elevation. Studies the interaction among geosphere, hydrosphere, cryosphere and the atmosphere.
AVHRR	1978	Carried on board the NOAA's polar-orbiting Environmental Satellite series.	National, continental and global scale mapping.	Widely used for studying and monitoring vegetation conditions. Low costs with a high probability of obtaining a cloud-free view of land surfaces. Useful for studying long-term vegetation changes. Suitable for mapping land cover types. Limitations are mainly in calibration, geometry, and orbital drift, limited and variable spectral coverage.
AVIRIS	1986	Airborne Visible /Infrared Imaging Spectrometry	Local-to-regional scale mapping at the community or species level.	Imagery carried out as one-time operations. Imagery data not always readily available as it obtained on a 'as needed' basis.
Hyperion	2000	A high-resolution hyperspectral imager with 220 unique spectral channels	Regional scale mapping at community or species level.	
IKONOS	1999	A commercial sun-synchronous earth observation satellite.	Local, regional scale vegetation mapping at species or community level.	Useful as a source of 'virtual' ground measurements for lower spatial resolutions. It can be used to map vegetation cover at a local scale, and to validate vegetation cover classified from other remote sensing images. It has a 3–5-day revisit interval.
MODIS	1999	Moderate-resolution imaging spectroradiometer (MODIS) is a key instrument on board the Terra (EOS AM) and Aqua (EOS PM). EOS = Earth Observation Satellites.	National, continental, and global-scale mapping.	Can view the earths' surface every 1-2 days. Suitable for mapping land cover types but not recommended for local or regional scale mapping. MODIS images can be integrated with other remote sensing images for improved mapping results.
QuickBird	2001	A commercial satellite with the highest publicly available resolution.	Local and regional-scale mapping at the species or community level.	Offers highly accurate and higher resolution imagery. It can also be used for validation purposes.

Sensors	Date: first launch	Description	Vegetation mapping applications	Advantages/disadvantages
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Hyperion	2000	A high-resolution hyperspectral imager with 220 unique spectral channels	Regional scale mapping at community or species level.	
IKONOS	1999	A commercial sun-synchronous earth observation satellite.	Local, regional scale vegetation mapping at species or community level.	Useful as a source of 'virtual' ground measurements for lower spatial resolutions. It can be used to map vegetation cover at a local scale, and to validate vegetation cover classified from other remote sensing images. It has a 3–5-day revisit interval. High costs with rigid technical parameters make applying in large areas impractical.
Worldview	2009	A commercial sun-synchronous satellite		

(Adapted from Eugenio *et al*., 2013; Leprieur *et al*., 2000; Xie *et al*., 2008; Xu and Su, 2017)

2.8 CONCLUSIONS

Artemisia afra is one of the medicinal plants used for cold-related ailments and has potential for managing tuberculosis, cancer, and SARS-CoV-2. In many cases, the plant has been reported as a potential invasive species that can compete with food crops for limited natural resources. However, during the COVID-19 pandemic, many farmers and phytochemical industries have become interested in growing the plant. The wide medicinal use of *A. afra* has increased harvesting pressure on wild populations and will impact on its natural ecosystem in the long term. Conservation and management strategies should balance the risk of over-harvesting and the invasive potential of the species. If successful, remote sensing can support the efforts to conserve *A. afra* wild populations by providing a tool for an effective, less costly and less tedious vegetation distribution monitoring process. It is important to develop distribution maps of medicinal plants, including *A. afra*, to monitor wild populations. There is an urgent need to develop monitoring tools for *A. afra* wild populations and to investigate the water requirements and other attributes related to its phytochemical content.

Linked to its wide distribution are variations in its chemical composition. The synthesis of bioactive secondary metabolites, which define the medicinal quality of *A. afra*, can be influenced by different environmental and management factors. Plant-to-plant chemical variation, within a population, has been reported in *A. afra*. A high thujone content, one of the essential oil components considered toxic at high levels, has been reported in some *A. afra* samples. *Artemisia afra* plants with camphene (7%), 1,8-cineole (15%), camphor (49%), and no thujone have been recommended in previous studies for product development. The major challenge is that harvesters and growers of *A. afra* may not be aware of the correct chemotype for therapeutic use. Despite reports that *A. afra* was identified as one of the species with the potential for commercial production, very little research on cultivation practices has been done. There are no studies on water requirements, water use, or irrigation management for *A. afra*. Propagation and cultivation of the correct *A. afra* chemotype will support conservation of the species, while providing opportunities for commercialisation through the supply of high-quality plant material.

CHAPTER 3

SUITABLE AGRO-CLIMATES FOR GROWING *ARTEMISIA AFRA*

3.1 INTRODUCTION

Apart from climate change, land-use reallocation to human settlements, habitat destruction, and overexploitation of medicinal plants due to growing demands have led to the extinction or threatened status of many medicinal plants. The ever-increasing demand for medicinal plants has been observed for several species. For example, an article in Nature News in 2010 indicated that farmers and scientists could not keep up with the demands for artemisinin derived from *Artemisia annua* (Van Noorden, 2010). Habitat restoration and cultivation of medicinal plants have been advocated as an ecological measure for the conservation of endangered medicinal plants (Zhang *et al*., 2016) or even as a solution for the sustainable exploitation of medicinal plants, which will ensure that the market needs are met without depletion of natural resources (Bariotakis *et al*., 2019). However, it is not desirable to introduce medicinal plant species indiscriminately to inappropriate cultivation regions (Zhang *et al*., 2016) as this may negatively affect productivity and quality (Bariotakis *et al*., 2019). The mentioned risks, coupled with the sometimes-low incentive to cultivate medicinal plants compared to wild harvesting, could discourage farmers from cultivating these plants.

One of the important questions in the conservation of medicinal plants or natural plant biodiversity in general is: “What controls the distribution of plant species?” or “What environmental factors mostly determine a plant community structure?” (Maharjan *et al*., 2022; Rahman *et al*., 2022). An answer to these questions will facilitate the development of effective strategies for conserving various medicinal plants and for understanding how environmental factors influence plant species distribution and diversity, which is a longstanding challenge in ecological studies (Chauvier *et al*., 2021). This is more urgent now that climate change is forcing species to ‘track’ suitable climatic conditions and shift their distribution ranges (Maharjan *et al*., 2022). The rapid changes in temperature and precipitation, already experienced in the African continent and other parts of the world, have affected ecosystems and biodiversity, with evident shifts in species ranges and changes in ecosystems (Randin *et al*., 2009; Tshwene-Mauchaza and Aguirre-Gutiérrez, 2019).

Environmental factors such as temperature, solar radiation, precipitation, and soil properties play an important role in the distribution of plant species (Table 3.1) (Zhang *et al*., 2016; Rahman *et al*., 2022). These environmental factors, among others, are major contributors to secondary metabolite production in medicinal plants (Ncube *et al*., 2012). While climate has a direct effect on plant growth and physiology, soils provide nutrients and water, and the soils’ physical as well as chemical properties influence plant distribution (Chauvier *et al*., 2021; Chen *et al*., 2015). Soils on steep slopes are shallower and have reduced opportunities for root development, water, and nutrient uptake (Maharjan *et al*., 2022). Shallow-rooted plant species may prefer such soils. Nutrient balance and availability in the soil influence

metabolic regulation in plants (Ncube *et al*., 2012). Temperature, which regulates plant metabolic rates vital to plant growth and reproduction, and soil microbial activity (which influences nutrient availability), decreases with increasing elevation (Maharjan *et al*., 2022). Temperature also affects the length of a growing season. Solar radiation is one of the important environmental factors required for plant growth and development (Ncube *et al*., 2012). Solar radiation increases with elevation as atmospheric turbidity decreases; however, other factors, such as clouds and fog, which also increase with elevation, may reduce the amount of solar radiation received by organisms (Maharjan *et al*., 2022). Solar radiation influences evapotranspiration, which in turn affects soil water availability and photosynthesis (Maharjan *et al*., 2022). Although climate exerted the strongest influence on species distribution in the European Alps, its influence declined with increasing altitude (Chauvier *et al*., 2021). Zhang *et al*. (2016) also reported that climate had a greater influence than soil factors on the habitat distribution of *Scutellaria baicalensis*. The above-mentioned factors play a critical role in the distribution and, thus, the need to identify suitable regions for the cultivation of medicinal plants.

Table 3.1. Environmental factors and their effects on the distribution of some plant species.

Environmental factors			**Effects on plants**
Category	**Sub-category**	**Indicators**	
Climatic	Radiation	Cloud cover seasonality	Solar radiation energy is required for plant growth and development, through its effect on photosynthetic carbon fixation and biomass accumulation
		Mean annual cloud frequency	
	Temperature	Mean annual temperature	Influences metabolic rates and physiological processes in plants. Controls length of growing season
		Mean diurnal temperature range (mean monthly max-min °C)	
		Annual temperature range (max warmest °C of warmest month – min °C of coldest month)	
		Isothermality (mean diurnal temperature range/annual temperature range) *100	
	Precipitation/Moisture	Mean annual rainfall	Affects soil water content, plant available water and plant moisture stress
		Rainfall pattern/distribution	
Soil/edaphic	Chemical	pH	Affects the availability and uptake of plant nutrients
		Organic matter content	
		Nutrient status/content	Soil nutrient availability affects plant growth, physiology, tissue chemistry, and stress tolerance. Plant nutrient balance influences secondary metabolite production and thus metabolic regulations in plants.
	Physical	Water holding capacity and available soil water (%)	Water limitations will reduce photosynthetic rates
		Clay content (%)	High clay content improves water availability and soil water holding capacity
		Silt content (%)	
		Coarse fragments (Sand) content (%)	Higher sand content results in lower water holding capacity, and drier soils, with a more acidic pH
Topographic		Aspect	
		Slope	Steep slopes have more runoff and erosion, thus shallow for shallow rooted plants only
		Altitude	At higher elevations, climate conditions become extreme, more geological barriers occur, there is a lack of well-developed soils

(Maharjan *et al*., 2022; Chauvier *et al*., 2021, Ncube *et al*., 2012)

The analysis of suitable regions for the cultivation of medicinal plants has been heavily dependent on traditional experience based on the observation of one or a few climatic factors (Wu *et al*., 2019). This can introduce risks to the productivity and quality of medicinal plants (Bariotakis *et al*., 2019; Zhang *et al*., 2016), and thus, there is a need to develop effective methods to analyse the suitability of a region for cultivation, which are based on the geographical distribution of wild populations of the medicinal plant species (Wu *et al*., 2019).

Ecological modelling to understand the distribution of suitable habitats for medicinal plants, and the environmental factors that determine that distribution and the formation of active ingredients in medicinal plants, are critical for selecting cultivation sites (Zhang *et al*., 2016). These methods or models can be effective if they are based on the geographical distribution of wild populations and consider climatic and soil factors influencing medicinal plant growth (Wu *et al*., 2019). The incorporation of modern technological tools into model development should also be considered (Bariotakis *et al*., 2019). Tshwene-Mauchaza and Aguirre-Gutiérrez (2019) used a Species Distribution Model (SDM). This technique relies on associating species occurrence data with climate and environmental predictors to predict variables suitable for species distribution. The results of their study suggested that rainfall and soil moisture play important roles in determining plant distribution. The Maxent modelling approach is sometimes preferred over the SDM as it is more powerful and can efficiently handle complex interactions between species presence records and environmental predictors, while providing accurate predictions with a small number of presence records (Maharjan *et al*., 2022). The mean annual temperature and soil clay content were found to be the most important environmental variables for predicting species distribution along the elevation gradient in the Himalayan tropical montane (Maharjan *et al*., 2022). Zhang *et al*. (2016) also used the Maxent model to predict environmental factors suitable for the cultivation of *Scutellaria baicalensis* in China. They reported that altitude, temperature, precipitation, and soil pH are factors influencing the species distribution. Using the canonical correspondence analysis (CCA) and variation partitioning tests to determine environmental variables that mostly determine plant species distribution in moist temperate zone, Rahman *et al*. (2022) indicated that altitude, slope, temperature, soil K and P, pH, electrical conductivity (EC), organic matter, and soil particle structure were among the environmental variables that drive plant distribution.

Soil microbiology is another important aspect, as soil microbes break down organic matter and thus contribute to the nutrient cycle (Figure 3.1) (Sullivan *et al*., 2017). Soil pH, nutrient availability, and toxicity are among the factors that influence soil microbiology.

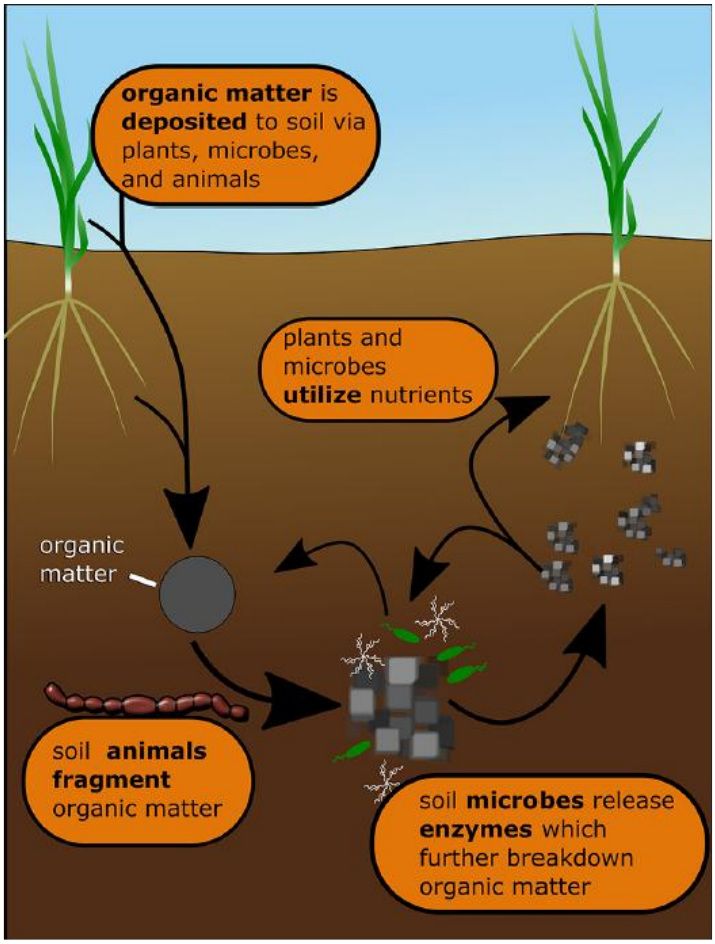

Figure 3.1. Decomposition of organic matter in the soil by microbes, and release of nutrients (Sullivan *et al*., 2017).

3.2 RESEARCH METHODOLOGIES

To meet the specific objectives of this chapter, plant and soil samples were collected from various populations for analysis as detailed below. The material and methods used are described below.

3.2.1. Soil sample collection

Soil samples were collected from three populations in the Maluti-A-Phofung local municipality in the Free State Province (Figure 3.2). Three samples were taken using a 150 mm Johnson bucket soil auger from each population, each soil sample with a plant sample. Top- (0-15 cm) and subsoil (15-30 cm) samples were taken for chemical, biological and physical analysis. A total of 18 samples were collected from the three populations for analysis (Ntalo *et al*., 2022). The samples were immediately stored in a cooler box and transported to the Agricultural Research Council's (ARC) laboratories for analysis.

The samples were labelled as follows:

FS1 SP1 Top – Free State population 1, sample 1 topsoil

FS1 SP1 Sub – Free State population 1, sample 1 subsoil

FS1 SP2 Top – Free State population 1, sample 2 topsoil

FS1 SP2 Sub – Free State population 1, sample 2 subsoil

FS1 SP3 Top – Free State population 1, sample 3 topsoil

FS1 SP3 Sub – Free State population 1, sample 3 subsoil

FS2 SP1 Top – Free State population 2, sample 1 topsoil

FS2 SP1 Sub – Free State population 2, sample 1 subsoil

FS2 SP2 Top – Free State population 2, sample 2 topsoil

FS2 SP2 Sub – Free State population 2, sample 2 subsoil

FS2 SP3 Top – Free State population 2, sample 3 topsoil

FS2 SP3 Sub – Free State population 2, sample 3 subsoil

FS3 SP1 Top – Free State population 3, sample 1 topsoil

FS3 SP1 Sub – Free State population 3, sample 1 subsoil

FS3 SP2 Top – Free State population 3, sample 2 topsoil

FS3 SP2 Sub – Free State population 3, sample 2 subsoil

FS3 SP3 Top – Free State population 3, sample 3 topsoil

FS3 SP3 Sub – Free State population 3, sample 3 subsoil

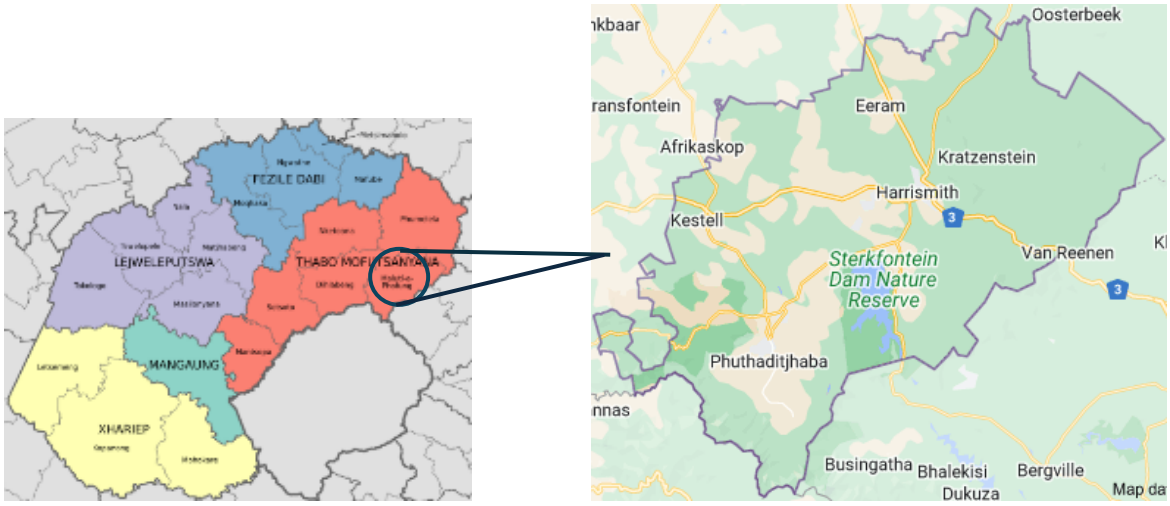

Figure 3.2. Map of Free State Province and the Maluti-A-Phofung- municipality, where soil and plant samples of *Artemisia afra* were collected.

3.2.2 Soil microbiology analysis

The soil samples were analysed at the Agricultural Research Council (ARC) - Plant Health and Protection campus (Roodeplaat, Pretoria) for carbon source utilization (microbial functional diversity), enzyme activities, and the total microbial count. The methods described by Mofokeng *et al*. (2020) were adapted for this study.

The carbon source utilisation was conducted on homogenised soil samples suspended in a saline buffer. Thereafter, 150 µl of the aqueous suspensions were pipetted into 96 well Biolog microplates, which were subsequently incubated at 26 °C for one week. The rate of colour change (pattern development) in each well was measured using Biolog MicroLogTM for optical density (OD) and recorded every 24 hours for 7 days or until a constant reading. Data from each plate reading were normalised by subtracting the control values from the values of each microplate well, and the microbial response in each well was quantified using average well colour development (AWCD) before statistical analysis. The determined specific utilization of carbon sources included group substrates such as carbohydrates, amino acids, polymers, and carboxylic acids. The optical density of 0.25 was used as a threshold for a positive response and the Shannon-Wiener index (H') value, the species richness and evenness were calculated. Species richness is the number of microbial species present in a sample, and evenness is the proportion of each species. The Shannon-Weaver Index is used to quantify the functional diversity of soil microbial populations based on the amount of different carbon sources utilized in the Biolog microplates.

Phosphatase activity was determined as the amount of *p*-nitrophenol (µl *p*-nitrophenol/g soil) produced after incubating soil samples with appropriate reagents and measuring the optical density of the culture filtrates. Similarly, β-glucosidase activity was determined using different reagents. The total microbial

count was determined by suspending soil samples in saline buffer, performing serial dilutions, and spreading 100 µl aliquots on nutrient agar. The plates were then incubated for 24-48 hours, and the resulting colonies were counted to determine colony-forming units (CFU) per gram of soil.

3.2.3 Soil physical and chemical analysis

The chemical properties of the soil samples were determined in a laboratory at the ARC – Soil, Climate and Water campus (Arcadia, Pretoria) using standard laboratory procedures for various macro- and microelements. Similarly, the particle size distribution was determined at the same laboratory for sand, silt, and clay content.

3.2.4 Mapping of *Artemisia afra* distribution and potential regions for cultivation

3.2.4.1 Natural populations of Artemisia afra

To identify the suitable climatic conditions for the natural areas of growth and distribution of *A. afra*, the South African National Botanical Institute (SANBI) database (Plants of southern Africa, POSA database, http://posa.sanbi.org/) was consulted for the global positioning system (GPS) locations of the natural populations. Using information from the SANBI database, literature searches, and personal communications, various provinces and regions were visited for the physical collection of GPS points. Sixty-five GPS coordinates were collected, while 185 GPS locations recorded in the SANBI database were used in the study.

3.2.4.2 Mapping of suitable regions

To identify suitable climatic conditions for the natural areas of growth and distribution of A. afra, the South African National Botanical Institute (SANBI) database (Plants of Southern Africa, POSA database, http://posa.sanbi.org/, access date: 15 August 2023) was consulted for the quarter-degree squares (QDSs) of the natural populations. Using information from the SANBI database, a literature search, and personal communications, GPS points were collected across different regions in three provinces. Sixty-five GPS coordinates (covering 17 QDSs) were collected, while 213 QDS locations recorded in the SANBI database were used in the study.

The maximum entropy model (MaxEnt model, version 3.4.4) was used to create maps of suitable areas for the growth and cultivation of *A. afra*. The following environmental variables were used for the predictions: the maximum and minimum temperatures of the coolest and warmest months (max and min tx; max and min tn), mean maximum and minimum humidity (hx and hn), rainfall, average soil clay %, and the average soil depth. To estimate the effect of climate change on climate suitability for the distribution of *A. afra*, monthly long-term average interpolated weather surfaces were derived from the ARC Weather Station Network for the period 1991–2020. The network consists of about 520 active automatic weather stations covering South Africa, and the data are recorded in the Agrometeorology Staff records of 2023. The averages of six different dynamically downscaled Coupled Global Climate Model (CGCM) projections of future climate change were used for the 2021–2050 and 2051–2080

periods. All CGCM simulations from the A2 Special Report on Emissions Scenarios (SRES) were downscaled for the period of 1961–2100 (Engelbrecht and Bopape, 2011). The CGCMs that were downscaled are as follows:

• GFDL-CM2.0 from the National Oceanic and Atmospheric Administration (NOAA);

• GFDL-CM2.1 of NOAA;

• ECHAM5/MPI-Ocean Model from Germany;

• UKMO-HadCM3 from the United Kingdom;

• MIROC3.2-medres from the Japanese Agency for Marine-Earth Science and Technology (JAMSTEC);

• CSIRO Mark3.5 from Australia.

Finally, the median values of changes from the current situation (1991–2020) to the periods 2021–2050 and 2051–2080 were applied to the long-term average surfaces derived from the ARC weather station network.

Average topsoil clay percentage and average soil depth were derived from the ARC Land Type Information System, which includes the land type maps and memoirs containing soil and terrain inventories in paper or electronic formats (Land Type Survey Staff records of the 1972–2006 period). Land types are areas with homogeneous terrain, macroclimate, and soil pattern and are delineated at a 1:250,000 scale. The percentage distribution of various soil attributes is linked to the following terrain units: crests, scarps, mid-slopes, foot-slopes, and valley bottoms.

The performance of the MaxEnt model was evaluated using the computed receiver operating characteristic (ROC) curve and the area under the curve (AUC) (Zhao *et al*., 2021). The advantage of the ROC analysis, which has been applied to a variety of classification problems in machine learning and the evaluation of species distribution models, is that the AUC provides a single measure of model performance independent of any choice of thresholds (Phillips *et al*., 2006). The MaxEnt model software has other functions that were used to evaluate the relative importance of environmental factors to species distribution (Yan *et al*., 2021). They include the permutation importance value, which is independent of the specific algorithm path of the software and only depends on the results of the model, and the percentage contribution degree of each environmental variable, which depends on random enumerated variable values on the training point (existence point and background value) and the resulting decrease in the training AUC value (Yan *et al*., 2021).

3.2.4.3. Data Analysis

Data on carbon source utilisation were subjected to nonparametric statistical analyses using STATISTICA software (StatSoft, Inc. ©, OK, USA). Soil microbial diversity and activity were analysed using principal component analysis (PCA) and hierarchical clustering (vertical tree plots). One-way analysis of variance (ANOVA) was used to determine significant differences between the different

samples. Homogenous grouping with Fisher's Least Significant Difference (LSD) was determined and calculated with the confidence level set at $p < 0.05$.

3.3 PROPERTIES OF SOIL COLLECTED AROUND *ARTEMISIA AFRA* WILD POPULATIONS

3.3.1 Soil physical properties

The soil collected from three sites where *A. afra* grows naturally in the eastern part of Free State Province showed variations in the clay, silt and sand content (Table 3.2). The clay content ranged from a low of 11% to a high of 38%, the silt content ranged from 19 to 30%, and the sand content ranged from 34 to 67%. The total sand percentage, calculated as the sum of the top- and subsoil contents, was highest in the FS1 SP2 soil collection.

Figure 3.3 presents the textural classification of the soil samples, showing that the soils ranged from loam to clay loam. However, 61% of the samples were classified as clay loam soils, 17% as sandy loam, 17% as sandy clay loam, and 5% as loam soils.

Table 3.2. Particle size distribution of soil samples collected from *Artemisia afra* populations in the eastern part of the Free State Province.

	Sand (%)				Silt (%)		Clay (%)
Sample ID	**Coarse 2-0.5 mm**	**Medium 0.5-0.25**	**Fine 0.25-0.106**	**Very Fine 0.106-0.05**	**Coarse 0.05-0.02**	**Fine 0.02-0.002**	**<0.002**
1. FS 1 SP 1 top	3.1	5.0	20.1	12.1	12.8	14.6	31.3
2. FS 1 SP 1 sub	0.6	2.9	19.0	11.9	11.4	14.9	38.1
3. FS 1 SP 2 top	16.1	12.6	22.7	15.1	9.4	10.0	13.2
4. FS 1 SP 2 sub	22.3	9.6	20.2	14.6	12.2	9.4	10.7
5. FS 1 SP 3 top	6.3	5.6	22.6	11.2	11.0	18.8	23.7
6. FS 1 SP 3 sub	6.4	3.2	17.1	10.3	10.2	20.8	30.7
7. FS 2 SP 1 top	11.6	5.3	11.9	12.7	13.5	14.9	28.6
8. FS 2 SP 1 sub	2.7	7.7	20.2	20.6	12.8	12.6	22.1
9. FS 2 SP 2 top	8.0	8.2	13.8	15.3	12.1	14.3	27.3
10. FS 2 SP 2 sub	5.6	6.6	12.6	16.0	13.1	16.4	28.4
11. FS 2 SP 3 top	22.0	10.2	17.7	13.4	12.3	9.3	13.9
12. FS 2 SP 3 sub	12.2	7.9	14.8	15.0	11.1	13.4	24.4
13. FS 3 SP 1 top	3.6	4.1	18.4	13.4	10.1	17.2	32.0
14. FS 3 SP 1 sub	3.9	3.0	17.7	12.8	11.2	19.9	30.5
15. FS 3 SP 2 top	4.6	6.1	21.2	14.5	11.6	14.4	26.6
16. FS 3 SP 2 sub	4.3	6.4	22.9	14.3	10.4	14.1	26.4
17. FS 3 SP 3 top	4.2	6.0	18.7	12.9	12.1	17.7	27.6
18. FS 3 SP 3 sub	4.6	6.6	19.0	13.1	10.1	15.2	30.3

FS 1 SP 1 top – Free State population 1, sample 1 topsoil; FS 1 SP 1 sub – Free State population 1, sample 1 subsoil; FS 1 SP 2 top – Free State population 1, sample 2 topsoil; FS 1 SP 2 sub – Free State population 1, sample 2 subsoil; FS 1 SP 3 top – Free State population 1, sample 3 topsoil; FS 1 SP 3 sub – Free State population 1, sample 3 subsoil; …

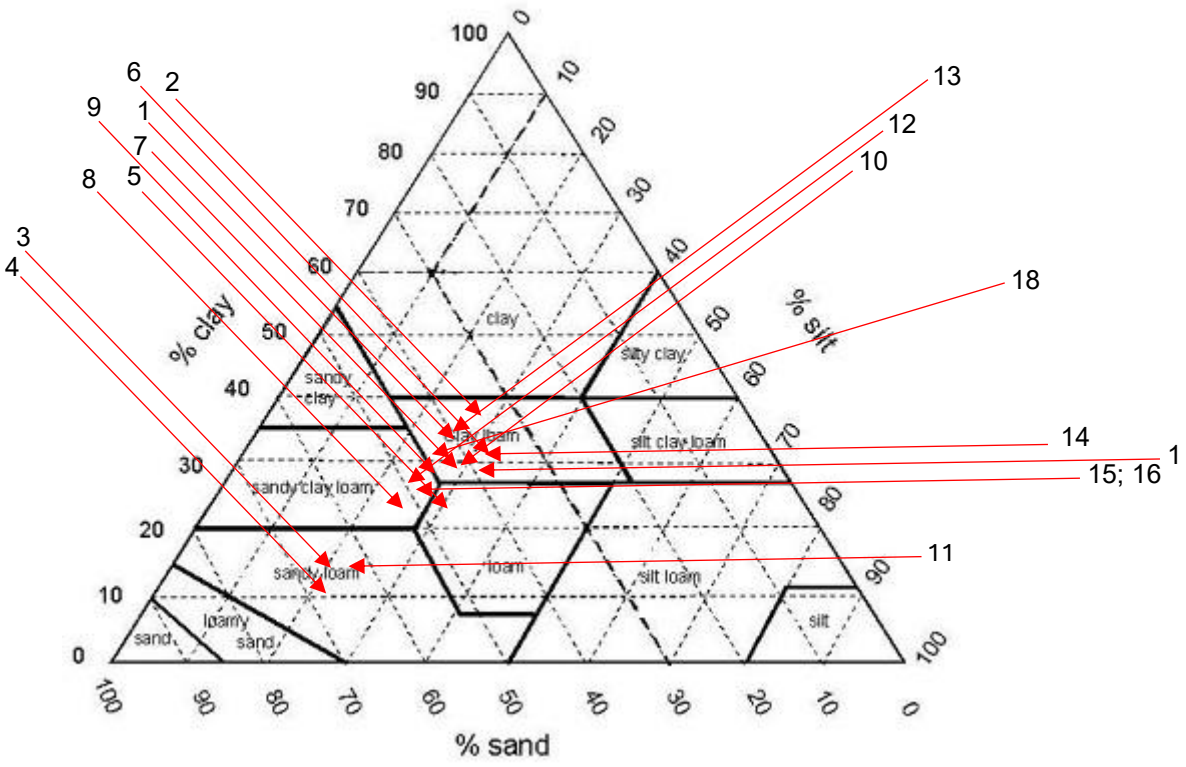

Figure 3.3. The textural classification (on the soil texture triangle) of soil samples collected from *Artemisia afra* populations in the eastern part of the Free State Province (1-18 are the numbers allocated as per Table 3.2).

3.3.2 Soil chemical properties

The pH of all samples was slightly acidic to neutral, ranging from 5.3 to 6.86 (Table 3.3). Only one sample had a pH of 5.3, and this was in the subsoil. There were slight increases or changes in the content of other chemical properties, except for ammonium content, which varied from 1.6 to 6.9 mg·kg^{-1} across the soil samples. The ammonium form of nitrogen was mostly higher than the nitrate form.

Table 3.3. The chemical properties of soils collected from natural populations of *Artemisia afra* in the eastern part of the Free State Province.

Sample ID	Total P	Total N	N-NO_3	N-NH_4	P	K	Na	Ca	Mg	CEC	pH	OM
	ppm	mg/kg								cmol(+)/kg	H_2O	%
FS 1 SP 1 top	4.67	0.131	0.70	6.67	1.79	277	15.6	2300	532	23.19	6.66	0.050
FS 1 SP 1 sub	4.44	0.120	0.71	5.07	0.42	210	44.4	2300	651	33.04	5.95	0.074
FS 1 SP 2 top	5.06	0.120	1.03	4.60	1.63	223	15.3	1720	502	25.35	5.3	1.264
FS 1 SP 2 sub	5.46	0.113	1.67	4.84	2.06	265	23.9	1560	428	21.02	5.91	0.098
FS 1 SP 3 top	5.08	0.162	0.59	3.75	4.02	458	7.92	2130	525	21.89	6.72	0.068
FS 1 SP 3 sub	3.79	0.096	0.83	3.36	1.28	263	14.6	2560	700	26.42	6.29	0.049
FS 2 SP 1 top	4.32	0.067	0.63	2.19	0.38	390	14.6	3340	1240	36.79	6.48	0.059
FS 2 SP 1 sub	3.74	0.067	1.18	6.91	0.06	140	19.3	2700	988	35.52	6.70	0.048
FS 2 SP 2 top	4.13	0.089	0.64	3.47	1.36	317	18.7	3060	1080	33.61	6.22	0.060
FS 2 SP 2 sub	3.94	0.071	0.73	5.11	0.70	251	22.2	3470	1110	34.86	6.59	0.057
FS 2 SP 3 top	5.43	0.090	1.60	8.25	1.29	196	44.9	2090	653	23.14	6.66	0.841
FS 2 SP 3 sub	3.92	0.067	0.84	5.20	0.13	147	25.4	2900	990	33.11	6.86	0.055
FS 3 SP 1 top	4.32	0.152	1.33	3.43	0.67	409	8.16	1300	648	23.35	6.15	0.062
FS 3 SP 1 sub	4.33	0.151	1.67	3.27	0.44	334	10.0	1310	660	17.99	6.04	0.058
FS 3 SP 2 top	4.32	0.115	1.71	1.60	0.79	284	20.9	1220	586	21.70	6.50	0.047
FS 3 SP 2 sub	4.02	0.096	1.47	2.61	0.49	190	22.4	982	529	20.39	6.24	0.042
FS 3 SP 3 top	4.31	0.155	2.25	5.40	1.09	405	7.8	1320	638	20.54	6.21	0.065
FS 3 SP 3 sub	3.96	0.121	0.74	4.94	0.26	189	12.3	1320	747	26.61	6.06	0.049

FS 1 SP 1 top – Free State population 1, sample 1 topsoil; FS 1 SP 1 sub – Free State population 1, sample 1 subsoil; FS 1 SP 2 top – Free State population 1, sample 2 topsoil; FS 1 SP 2 sub – Free State population 1, sample 2 subsoil; FS 1 SP 3 top – Free State population 1, sample 3 topsoil; FS 1 SP 3 sub – Free State population 1, sample 3 subsoil; …

3.3.3 Soil microbiology

The highest C-source utilisation by microbial communities, as indicated by average well colour development (AWCD), was observed in samples FS1 SP2 sub, FS1 SP2 top, and FS1 SP1 top (Table 3.4). The lowest C-source utilization was recorded for samples FS3 SP1 top, FS3 SP2 sub, FS2 SP2 top, and FS3 SP2 top. Samples FS3 SP1 sub, FS2 SP3 top, FS1 SP3 top, and FS2 SP1 sub showed no significant differences in C-source utilization when compared to FS1 SP2 top and FS1 SP1 top.

Significant differences were recorded in the Shannon Weaver Diversity Index (H'), where samples FS1 SP2 top, FS1 SP2 sub and FS1 SP1 top showed a significantly higher H' compared to FS3 SP2 sub, FS3 SP1 top, and FS2 SP2 top. When compared to all other samples, sample FS3 SP2 top recorded a significantly lower H'. Similarly, samples FS1 SP2 sub and FS1 SP2 top showed significantly higher species richness (S) than samples FS3 SP2 top and sub, which showed the lowest richness. Samples FS1 SP1 sub and FS3 SP3 sub were the only samples that recorded significantly lower evenness compared to sample FS3 SP2 sub.

Two enzyme activities of the soil microbial communities tested were β-glucosidase and phosphatase, to provide an indication of microbial activities in terms of carbon (C) and phosphorus (P) cycling, respectively. Although no significant differences were observed, variations in soil microbial communities across the samples were noted, and most samples exhibited high β-glucosidase activity (µg *p*–nitrophenol g^{-1} soil). The lowest β-glucosidase activity was recorded with samples FS1 SP3 sub, FS2 SP1 sub, FS2 SP3 sub and FS3 SP2 sub. In terms of phosphatase enzyme activity, microbial communities across all soil samples exhibited similar levels and showed high phosphatase activity (µg *p*–nitrophenol g^{-1} soil). However, FS2 S3 sub and FS3 S2 sub showed the lowest phosphatase activity.

Table 3.4. The functional diversity and enzyme activities of different soil samples collected from various *Artemisia afra* populations.

Sample ID	Carbon Source Utilization (Functional diversity)				Enzyme Activities	
	AWCD	Shannon Weaver Index (H')	Richness (S)	Evenness	β-glucosidase	Phosphatase
FS 1 SP 1 top	1.547^{abc}	3.36^{a}	29.33^{ab}	0.994^{abc}	19.51	92.13
FS 1 SP 1 sub	1.316^{cde}	3.33^{abc}	29.66^{ab}	0.982^{c}	16.79	99.56
FS 1 SP 2 top	1.614^{ab}	3.38^{a}	30.66^{a}	0.988^{bc}	36.58	93.46
FS 1 SP 2 sub	1.753^{a}	3.36^{a}	30.33^{a}	0.995^{abc}	30.35	99.86
FS 1 SP 3 top	1.453^{bcd}	3.34^{abc}	29.00^{ab}	0.993^{abc}	25.76	93.15
FS 1 SP 3 sub	1.279^{de}	3.35^{ab}	29.66^{ab}	0.991^{abc}	14.59	91.11
FS 2 SP 1 top	1.296^{de}	3.35^{ab}	29.66^{ab}	0.991^{abc}	19.99	106.20
FS 2 SP 1 sub	1.385^{b-e}	3.34^{abc}	29.00^{ab}	0.991^{abc}	13.44	102.81
FS 2 SP 2 top	0.941^{g}	3.26^{cd}	27.33^{abc}	0.988^{bc}	17.05	98.19
FS 2 SP 2 sub	1.355^{cde}	3.34^{abc}	29.33^{ab}	0.988^{bc}	25.31	96.94
FS 2 SP 3 top	1.444^{bcd}	3.33^{abc}	29.00^{ab}	0.984^{abc}	25.54	99.73
FS 2 SP 3 sub	1.284^{de}	3.29^{bcd}	28.00^{ab}	0.988^{abc}	11.97	87.55
FS 3 SP 1 top	0.841^{g}	3.25^{d}	26.00^{bc}	0.996^{abc}	21.70	101.82
FS 3 SP 1 sub	1.425^{bcd}	3.28^{bcd}	27.33^{abc}	0.992^{abc}	18.33	92.16
FS 3 SP 2 top	0.995^{fg}	3.05^{e}	23.66^{c}	0.989^{abc}	18.53	90.31
FS 3 SP 2 sub	0.847^{g}	3.02^{e}	19.33^{d}	1.004^{ab}	14.84	88.24
FS 3 SP 3 top	1.359^{cde}	3.33^{abc}	29.00^{ab}	0.988^{abc}	15.28	90.04
FS 3 SP 3 sub	1.186^{ef}	3.28^{bcd}	28.33^{ab}	0.982^{c}	17.36	95.81
$LSD_{0.05}$	0.236	0.081	3.69	0.019		

Values within a column with different letters indicate a significant difference ($p < 0.05$).
FS1 SP1 top – Free State population 1, sample 1 topsoil; FS1 SP1 sub – Free State population 1, sample 1 subsoil; FS1 SP2 top – Free State population 1, sample 2 topsoil; FS1 SP2 sub – Free State population 1, sample 2 subsoil; FS1 SP3 top – Free State population 1, sample 3 topsoil; FS1 SP3 sub – Free State population 1, sample 3 subsoil; …

Samples FS1 SP2 top and FS1 SP2 sub exhibited significantly higher utilization of amino acids, carbohydrates and carboxylic acids compared to samples FS2 SP2 top, FS3 SP1 top, FS3 SP2 top, and FS3 SP2 sub (Table 3.5). However, samples FS1 SP2 sub and FS2 SP3 top expressed significantly higher utilization of polymers compared to sample FS1 SP3 sub, FS2 SP2 top, FS3 SP 1 top, FS3 SP1 sub, FS3 SP2 top, FS3 SP2 sub, and FS3 SP3 sub. In the case of amines, only sample FS1 SP2 top exhibited significantly higher utilisation than samples FS2 SP2 top, FS2 SP3 sub, FS3 SP1 top, FS3 SP2 top, and FS3 SP3 sub. Based on the total bacterial count result as a microbial indicator of soil health, four soil samples, FS1 S2 sub, FS2 S1 sub, FS2 S2 sub, and FS3 S3 top, had the highest

bacterial count, indicated as the average number of colonies. The total microbial load (bacterial count), expressed as $cfu \cdot g^{-1}$, was higher in some samples than expected for a healthy soil. Four soil samples, FS1 S2 sub, FS2 S1 sub, FS2 S2 sub, and FS3 S3 top, had the highest bacterial count.

The principal component analysis explained 91.77% of the variations among the factors and grouped the soil samples that had similar C-source utilization patterns into two major groups in which soil samples FS3 S2 sub, FS2 S2 top, FS3 S1 top and FS3 S2 top with the lowest scores for the attributes measured were grouped together, as compared to the rest of the samples (Figure 3.4A). Similar groupings are also indicated on the hierarchical clustering dissimilarity chart in Figure 3.4B.

Table 3.5. The carbon source utilization and the total microbial load of different soil samples collected from various *Artemisia afra* populations.

Sample ID	Amino acids	Carbohydrates	Carboxylic acids	Polymers	Amines	Avg. No. of colonies	Colony forming units (CFU g^{-1})
FS 1 SP 1 top	1.628^{abc}	1.579^{a-d}	1.381^{abc}	1.689^{ab}	1.609^{ab}	17(10)^{6}	1.7 x 10^{8}
FS 1 SP 1 sub	1.502^{bcd}	1.299^{cde}	1.189^{c-f}	1.313^{b-e}	1.417^{a-c}	5(10)^{6}	5.0 x 10^{8}
FS 1 SP 2 top	1.778^{ab}	1.626^{ab}	1.521^{ab}	1.547^{a-d}	1.619^{a}	95(10)^{6}	9.5 x 10^{6}
FS 1 SP 2 sub	1.912^{a}	1.766^{a}	1.626^{a}	1.858^{a}	1.586^{ab}	19(10)^{6}	1.9 x 10^{8}
FS 1 SP 3 top	1.576^{a-d}	1.434^{def}	1.338^{a-d}	1.604^{ab}	1.402^{a-c}	13(10)^{6}	1.3 x 10^{8}
FS 1 SP 3 sub	1.445^{bcd}	1.249^{ef}	1.219^{b-e}	1.230^{b-e}	1.276^{a-c}	27(10)^{6}	2.7 x 10^{8}
FS 2 SP 1 top	1.385^{cd}	1.265^{def}	1.171^{c-f}	1.527^{a-d}	1.294^{a-c}	55(10)^{4}	5.5 x 10^{6}
FS 2 SP 1 sub	1.647^{abc}	1.347^{b-f}	1.176^{c-f}	1.566^{a-c}	1.372^{a-c}	300(10)^{7}	3 x 10^{10}
FS 2 SP 2 top	0.957^{ef}	0.888^{gh}	0.906^{fg}	1.211^{b-e}	0.768^{fg}	13(10)^{6}	1.3 x 10^{8}
FS 2 SP 2 sub	1.542^{bd}	1.461^{a-e}	1.063^{d-f}	1.526^{a-d}	1.240^{a-e}	39(10)^{7}	3.9 x 10^{9}
FS 2 SP 3 top	1.487^{bcd}	1.580^{a-d}	1.134^{c-f}	1.835^{a}	1.259^{a-d}	13(10)^{6}	1.3 x 10^{8}
FS 2 SP 3 sub	1.439^{bcd}	1.336^{b-f}	1.079^{c-f}	1.503^{a-d}	1.045^{c-f}	12(10)^{6}	1.2 x 10^{8}
FS 3 SP 1 top	0.981^{ef}	0.769^{hi}	0.721^{g}	1.093^{c-e}	0.805^{efg}	45(10)^{4}	4.5 x 10^{6}
FS 3 SP 1 sub	1.708^{abc}	1.616^{abc}	1.159^{c-f}	1.508^{de}	1.542^{ab}	300(10)^{7}	3.0 x 10^{10}
FS 3 SP 2 top	1.273^{de}	0.740^{hi}	0.975^{e-g}	0.859^{ef}	0.823^{d-g}	33(10)^{4}	3.3 x 10^{6}
FS 3 SP 2 sub	1.032^{e}	0.698^{hi}	0.948^{e-g}	0.840^{ef}	1.542^{ab}	24(10)^{4}	2.4 x 10^{6}
FS 3 SP 3 top	1.481^{bc}	1.444^{a-e}	1.161^{c-f}	1.498^{a-d}	1.191^{a-f}	14(10)^{7}	1.4 x 10^{9}
FS 3 SP 3 sub	1.560^{bcd}	1.110^{fg}	1.128^{c-f}	0.961^{e}	1.161^{b-f}	10(10)^{4}	1.0 x 10^{6}
$LSD_{0.05}$	0.339	0.342	0.308	0.500	0.451		

Values within a column with different letters indicate a significant difference ($p < 0.05$).

FS 1 SP 1 top – Free State population 1, sample 1 topsoil; FS 1 SP 1 sub – Free State population 1, sample 1 subsoil; FS 1 SP 2 top – Free State population 1, sample 2 topsoil; FS 1 SP 2 sub – Free State population 1, sample 2 subsoil; FS 1 SP 3 top – Free State population 1, sample 3 topsoil; FS 1 SP 3 sub – Free State population 1, sample 3 subsoil; …

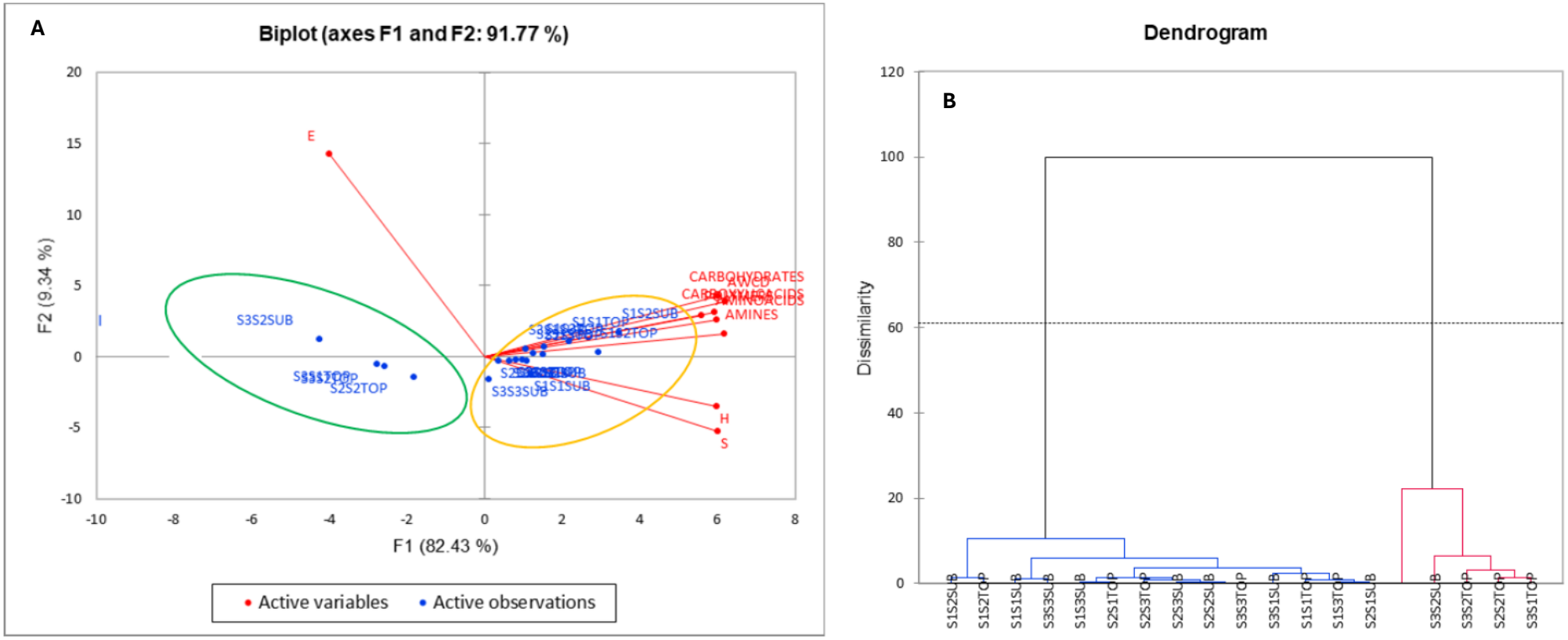

Figure 3.4. The principal component analysis (A) and the dendrogram (B) used to explain the variation between soil samples collected from different *Artemisia afra* populations.

3.3.4 Mapping of potentially suitable areas for *Artemisia afra* cultivation

Using occurrence records and environmental variables, the Maxent model was used to predict the distribution of *A. afra* and suitable areas for its cultivation. GPS coordinates from SANBI records and those collected physically, were used in the model.

3.3.4.1 Mapping using the Maxent model

Figure 3.5 shows the natural distribution of *A. afra*, as represented in the SANBI database by green circles, and the physically collected points, represented by red circles. The map shows a wide distribution of the species across South Africa, with very few populations recorded in the Northern Cape Province (near the border with the Free State Province). The MaxEnt model further indicates that the Northern Cape Province was less suitable (with a very low probability of suitability) for *A. afra* growth. The areas with the highest probability of suitability for the growth and cultivation of *A. afra*, which were optimal to suboptimal, were mainly recorded in the Eastern Cape, KwaZulu-Natal, and Mpumalanga provinces. The Free State, Gauteng, Limpopo, and Western Cape provinces had sub-optimal to moderate areas, which may represent a moderate probability of suitability for the growth and cultivation of *A. afra*.

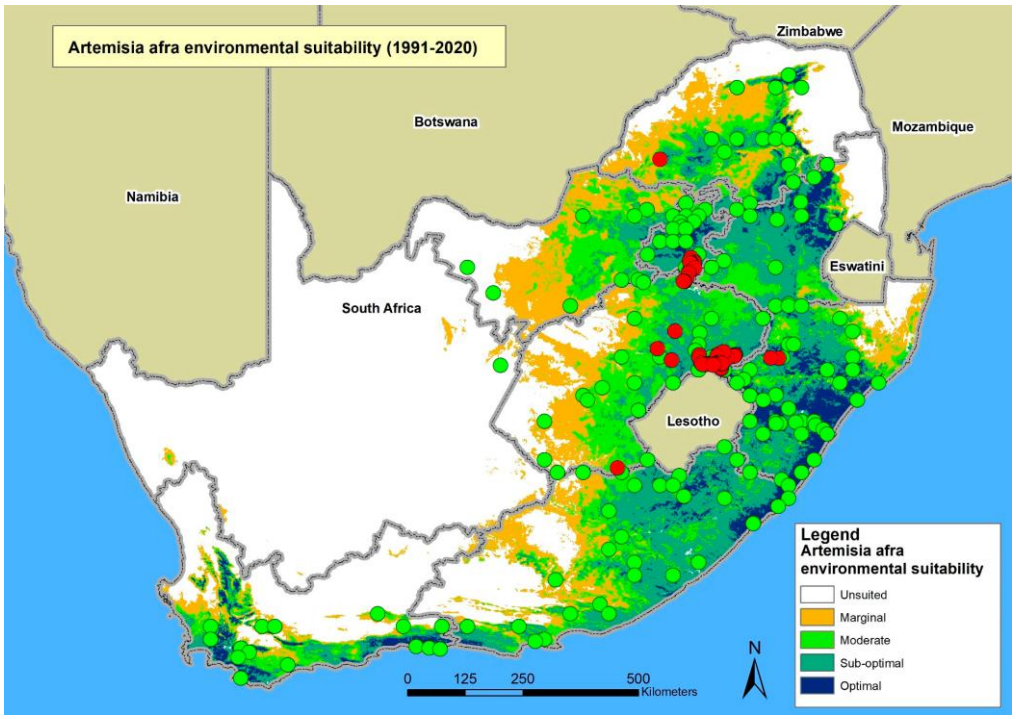

Figure 3.5. The natural distribution of *Artemisia afra*. The green circles indicate points from the SANBI database, and the red circles represent physically collected points.

The future predictions (Figure 3.6 A, B) indicate that there will be no major changes in the distribution of *A. afra* from 2021 to 2050; however, changes are expected from 2051 to 2080. Most areas that show optimal to suboptimal environmental suitability for the distribution of *A. afra* will change from suboptimal to moderate and from moderate to marginal during 2051–2080.

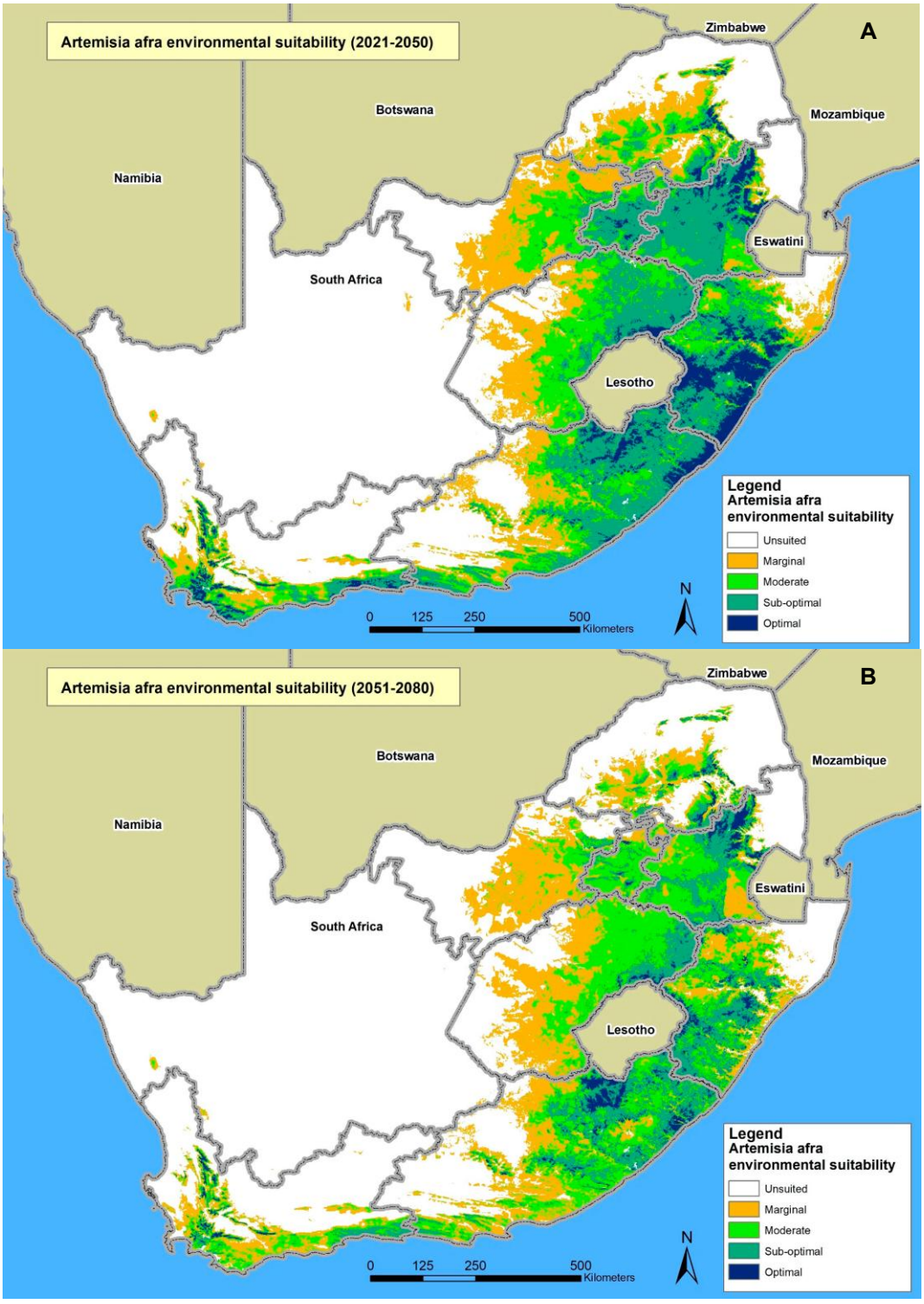

Figure 3.6. The predicted distribution of *Artemisia afra* from the year 2021 to 2050 (A) and 2051 to 2080.

The results of the model's performance evaluation are shown in Figure 3.7. The area under the curve (AUC) values of the training data, used to create the predictive model, and the test data, which assess the model's accuracy, were 0.836 and 0.903, indicating that MaxEnt performed excellent modelling as an AUC value of 0.75 is considered to be reliably accurate (Tshabalala *et al*., 2022).

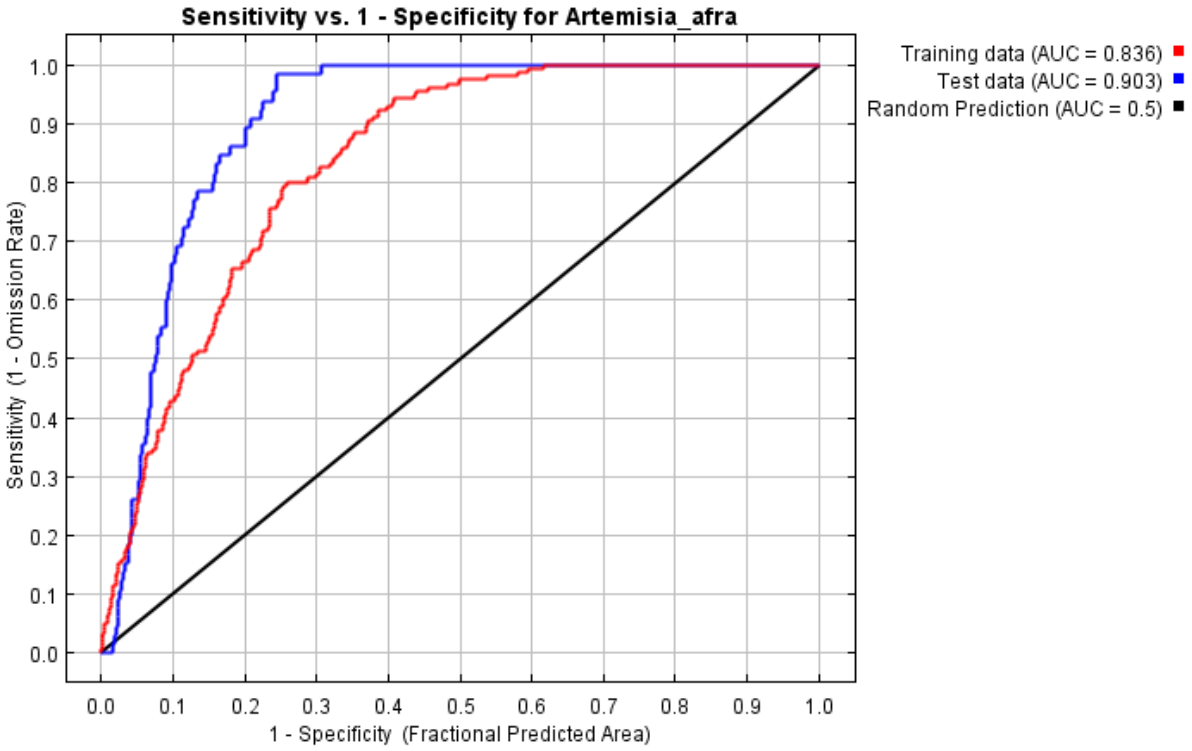

Figure 3.7. The performance of the MaxEnt model, as measured by the computed receiver operating characteristic (ROC) curve, with a specific focus on the area under the curve (AUC).

Table 3.5 present estimates of the relative contributions of each environmental variable to the MaxEnt model. The long-term average annual rainfall (62%), the highest long-term average monthly maximum temperature (21.5%), and the average soil depth (7.5%) were the environmental variables that significantly influenced the prediction. Similarly, permutation importance indicated that the long-term average annual rainfall, the highest long-term average monthly maximum temperature, and average soil depth are the environmental factors that contribute most to the MaxEnt model. It is important to note that because this is a predictive model, this estimate of importance does not imply directionality or causality.

Table 3.6. Relative percentage contribution of each environmental variable to MaxEnt model predictions of environmental suitability for growth of *Artemisia afra*.

Environmental Variable	Percentage contribution	Permutation importance
Long-term average rainfall	62.0	41.0
Highest long-term average monthly maximum temperature	21.5	26.1
Average soil depth	7.5	11.1
Lowest long-term average monthly maximum temperature	3.5	5.3
Long-term average monthly minimum relative humidity	2.0	4.2
Lowest long-term average monthly minimum temperature	1.1	1.9
Highest long-term average monthly minimum temperature	1.0	6.7
Average soil clay%	1.0	2.8
Long-term average monthly maximum relative humidity	0.4	0.9

3.4 DISCUSSION

Chemotypic variation has been reported among and within wild populations of *A. afra*. These differences occur across countries, within countries, and even within regions (Viljoen *et al*., 2006; Kane *et al*., 2019; Oyedeji *et al*., 2009), and they strongly influence the selection of favorable chemotypes for commercial development. Oyedeji *et al*. (2009) reported that environmental conditions play a significant role in the synthesis of essential oils in *A. afra*. However, the relationship between environmental factors and *A. afra* chemotypes has not been previously reported. Different medicinal plants have different optimal soil conditions for survival. For example, *Radix isatidis* grows well in slightly acidic to slightly alkaline, loose sandy loam or light sandy soils with 60-80% moisture content, whereas *Baphicacanthus cusia* prefers acidic to neutral sandy or loam soils with 22 – 33% soil moisture content (Chen *et al*., 2015).

Although the soil physical properties indicate that *A. afra* is adapted to a wide range of soils, most of the soil samples collected from the three *A. afra* populations were clay loam soils, containing 30-40% clay, 20-40% sand, and lower levels of silt. In the same vein, the mean annual temperature and soil clay content were found to be the most important environmental variables for predicting species distribution along the elevation gradient in the Himalayan tropical montane (Maharjan *et al*., 2022). Most samples showed no substantial differences in particle-size distribution between topsoil and subsoil.

Similarly, no substantial changes in organic matter content were recorded between the top- and subsoil samples. Organic matter is a major source of nutrients and microbial energy in the soil, and it holds water, promotes soil aggregation, and improves infiltration and water-use efficiency (Du Preez *et al*., 2011). Organic matter content ranged from 0.04 to 1.26%, indicating very low levels, which are characteristic of most South African soils (Zhou *et al*., 2019). The limited variation in organic matter content may reflect the similar soil texture of the samples, which was mostly clay loam. No study has been reported on *A. afra* growth in different soils, but *A. annua* plants cultivated in clay loam soils grew significantly taller, had significantly more branches, and had thicker stems than those grown in sandy loam soils (Omer *et al*., 2013). Fresh and dry biomass yields were also higher in plants grown in clay loam soils than in sandy loam soils (Omer *et al*., 2013). However, the artemisinin (%) and essential oil contents (%) were higher in *A. annua* plants grown in sandy loam soils than in clay loam soils, and this could have been due to nutrient and water stress in the sandier soil (Omer *et al*., 2013).

Soil pH also regulates soil nutrient availability, organic matter turnover, and many other processes in the soil (Zhou *et al*., 2019). The soil samples had a slightly acidic pH (5.5 to 6.5). All macro-nutrients (N, P, K, Ca, and Mg) are available for uptake within this range (Table 3.7), and this range should be suitable for most plants. At low pH, micro/trace elements are more soluble due to high desorption, whereas adsorption increases with increasing pH, making these elements less soluble and leading to low concentrations in the soil solution (Neina, 2019). The clay content of the soil samples could be an advantage, as it reduces the likelihood of leaching of nutrients, especially N, Ca, and Mg (Sukitprapanon *et al*., 2020; Zhou *et al*., 2019). Zhang *et al*. (2016) reported that soil pH is one of the environmental factors that affect the suitability for cultivation of *Scutellaria baicalensis* in China.

Artemisia afra plants grown in a hydroponics system at pH levels of 5.5, 6.5, and 7.5 recorded no significant differences in total dry weight, shoot dry weight, and root dry weight; however, these parameters were significantly decreased at pH levels of 4.5 and 8.5 (Koehorst *et al*., 2010). This could indicate that *A. afra* doesn't grow well in acidic or alkaline soils, whereas the soil pH of the collected samples was within the favourable pH range. Soils with high sand content (>85%) have a low pH (4.5-5.5) and low water-holding capacity and plant nutrient content (Sukitprapanon *et al*., 2020). Hansen-Quartey *et al*. (1998) compared soil samples from a patch where *A. afra* grows to the ones collected where *A. afra* does not grow, and they reported no significant differences in organic carbon, pH, Ca, Mg, K and Na.

Table 3.7. The effects of soil pH on nutrient availability.

Nutrient elements	pH												
	*a			b			c		d		e		
	4.0	4.5	5.0	5.5	6.0	6.5	7.0	7.5	8.0	8.5	9.0	9.5	10
N		**											
P													
K													
Ca & Mg													
S													
B													
Cu													
Zn													
Mo													
Fe & Mn													
Al													

*a - Strong acid, b – medium to slightly acid, c – neutral, d – slightly to medium alkaline, e – strongly alkaline. ** The green colour depicts nutrient availability. The lighter the colour the lower the availability and the darker the colour, the higher the availability.

Soil pH plays a critical role in shaping the structure and diversity of microbial communities and thus controls decomposition and nitrification (Zhou *et al*., 2019). Soil pH is also essential for proper enzymatic activity in the soil, and it secondarily regulates enzymes through its effect on the microbes that produce them (Neina, 2019); it also influences microbial diversity in the soil (Sinsabaugh *et al*., 2008). The lack of significant differences in β-glucosidase and phosphatase activities between the soil samples in the current study could have been due to the narrow pH range of 5.5 to 6.5, which may have been suitable for these activities. The optimum pH for β-glucosidase activity was found to be between 4 and 6 in tropical rain forests (Turner 2010), and between 4.5 and 6.5 for phosphatase activity (Sinsabaugh *et al*., 2008). The β-glucosidase activity was confined to an acidic pH range, with negligible activity in the alkaline range, whereas the phosphomonoesterase activity was found in the acid pH range (4-5) and in the alkaline pH range (9.5-11.5) (Turner 2010).

Among the variables examined by Fierer and Jackson (2006), soil pH was the best predictor of soil bacterial diversity and richness, and environmental factors such as temperature and evapotranspiration had only a minor effect on soil bacterial diversity. Similarly, soil pH was found to alter the composition and diversity of soil bacteria (Hartman *et al*., 2008). Soils with a pH of 6.8 recorded a bacterial richness 60% higher than soils with a pH of 5.1, and soils with a pH of 5.5 recorded a bacterial richness 26% higher than those with a pH of 4.1 (Fierer and Jackson, 2006). Will *et al*. (2010) reported no significant correlation between changes in the abundance of the dominant acidobacterial subgroups and other phylogenetic groups and soil pH.

One of the reasons advanced was that the pH values of all soil samples collected were near neutral. Rousk *et al*. (2010) also reported insignificant differences in bacterial community composition when soil pH values were above 6.8. The medium to slightly acidic pH range of the samples analyzed in the current work could explain the lack of significant difference in the AWCD, except for a few samples that recorded a significantly lower AWCD. Samples FS2 SP2 top, FS3 SP1 top, FS3 SP2 top, and FS3 SP2 sub recorded significantly lower AWCD, although the pH values were still within the 5.5 to 6.5 range. However, no significant differences were recorded for microbial species evenness, and for richness only sample FS3 SP2 sub recorded significantly lower values. For the Shannon Weaver Index, samples FS3 SP2 top and sub recorded significantly lower values. The results indicate that there were mostly the same number of microbial species, in similar proportions, and they were mostly all similarly active in all the samples.

The major group of carbon sources utilized, as indicated by the AWCD, showed significant differences in the utilization of five major carbon sources. The highest AWCD was detected for the utilization of amino acids and polymers by the microbial communities of sample FS1 S2 sub (amino acids 1.91 and polymers 1.85). This was followed by high rates of amino acid utilization for FS1 S2 top (1.78), FS3 S1 sub (1.71), FS1 S1 top (1.63), and FS2 S1 sub (1.65). Sample FS3 S2 sub had the lowest utilization rates across all five groups of carbon sources. Carbon source utilization, the Shannon-Weaver Index, and species richness were positively correlated with one group of soil samples, whereas species evenness was positively correlated with a second group that included samples FS3 S2 sub, FS2 S2 top, FS3 S1 top, and FS3 S2 top. Principal component analysis explained 91.77% of the variation, with evenness contributing 9.34% and CSU, H, and S contributing 82.43%.

The Maxent model was used to predict the probability of suitability for the growth and distribution of A. afra, after consulting the SANBI database and collecting GPS coordinates of natural populations of the species. The model showed that most areas with the highest probability of suitability are along the Drakensberg Great Escarpment, specifically in the Eastern Cape, Free State, Gauteng, Limpopo, and Mpumalanga provinces (Carbutt, 2019; Knight and Grab, 2015), with a few areas of lower suitability along the coast. The SANBI database indicated the natural occurrence of *A. afra* in eight of the nine South African provinces. The only population recorded in the Northern Cape province was near the Free State province border, which could have been the only marginally suitable area in the province. Based on the Maxent model prediction and the data from the SANBI database, *A. afra* was found in areas with a probability of suitability ranging from 20% to 100%. When the GPS coordinates from the physical collection were used in the model to predict the probability of suitability, areas with optimal, sub-optimal, and moderate suitability were recorded along part of the Drakensberg Great Escarpment, as described by Carbutt (2019) as the Drakensberg Mountain Centre (DMC) and further north (Figure 3.8a). The area is part of the Drakensberg Grassland Bioregion of the Grassland Biome (Figure 3.8b), characterized by moist grasslands with annual rainfall of 600 mm or more (Berruti, 2017). Setshedi *et al*. (2022) reported that *A. afra* populations tend to be denser where grass biomass is higher.

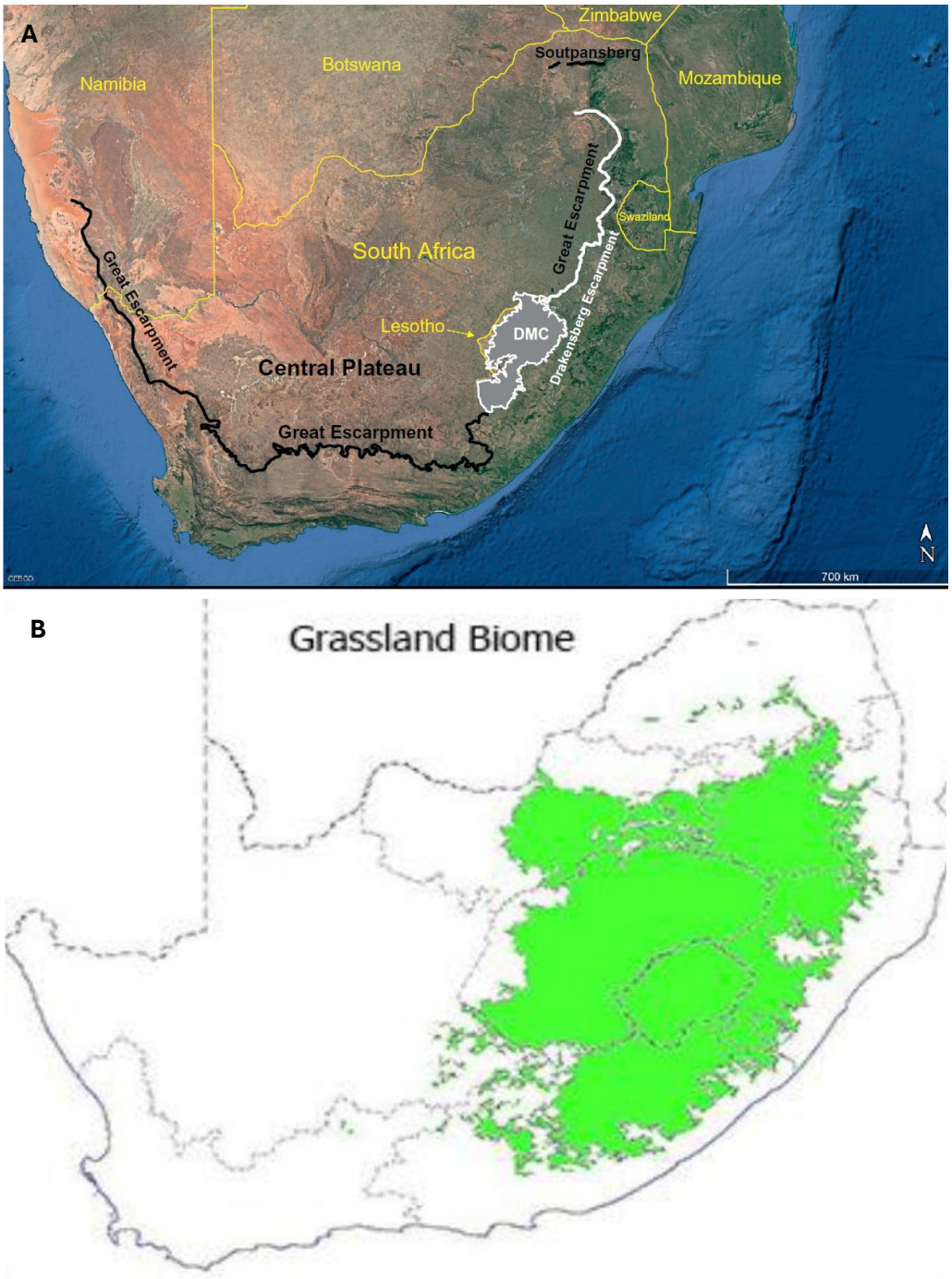

Figure 3.8. Maps showing the Drakensberg Mountain Centre (A) and the Grassland Biome (B).

The MaxEnt model identified areas with the highest probability of suitability for *A. afra* growth and cultivation, and these areas are mainly warm temperate regions with dry winters and hot to cool summers, as well as warm temperate regions that are fully humid with hot to cool summers, according to the Köppen–Geiger climate classification described in another study (Conradie, 2012). The SANBI database indicates the natural occurrence of *A. afra* in eight of the nine South African provinces. The only population recorded in the Northern Cape province was near the Free State province border, which may have been the only

marginally suitable area in the province. The MaxEnt model also showed that the Northern Cape province has very few areas with marginal suitability for *A. afra* growth. The annual rainfall in the Northern Cape province may not be suitable for *A. afra*. The MaxEnt model predicted average annual rainfall as the most important factor in *A. afra*'s distribution, followed by temperature and soil depth. According to the map of South Africa's annual rainfall across water management areas (Cole *et al*., 2018), the areas identified as suitable for *A. afra* in the MaxEnt model have average annual rainfall ranging from 500 mm to> 1500 mm. Although cultivation can be a conservation strategy, the risk of introducing *A. afra* into new cultivation areas should be assessed, especially since it can be invasive if not managed (Setshedi *et al*., 2022). Future predictions indicate that climate change will affect the distribution of *A. afra* only during the period 2051-2080. Tshabalala *et al*. (2022) reported that the habitat distribution of a number of medicinal plants was mainly affected by precipitation-based variables. Similarly, temperature and precipitation were reported to be the main factors affecting the MaxEnt prediction results for *Codonopsis pilosula* in China (Yan *et al*., 2021). Major factors expected to affect *A. afra* populations include habitat destruction due to agricultural development, human settlement, and environmental pollution. The AUC values were between 0.836 and 0.903 when the model was evaluated, indicating that it was very good at predicting the distribution of *A. afra*, as it was closer to 1 (Yan *et al*., 2021). The AUC values in the range of 0-0.5 indicate a very poor prediction, 0.6-0.7 indicate a poor prediction, 07-08 indicate a fair prediction, 0.8-0.9 indicate a good prediction, and 0.9-1 indicate an excellent prediction (Zhao *et al*., 2021).

3.5 CONCLUSIONS

The study's results suggest that annual rainfall is the most significant variable, followed by maximum temperature and soil depth. Some *A. afra* populations have been recorded outside the Drakensberg Grassland Bioregion, which appears to be the optimal area for the species' growth. These populations are growing in areas of marginal suitability. Soil pH also appears to be critical, as the soil samples had a pH in the range of moderately to slightly acidic. The most suitable areas recommended for cultivating *A. afra* are mainly in the Free State province, followed by Gauteng, KwaZulu-Natal, and Limpopo provinces.

The study successfully predicted suitable climatic and soil conditions for the species' distribution and growth and mapped potential areas for cultivating *A. afra*. Although the Maxent model was validated using AUC values, there remains uncertainty about the species' distribution because the model relies heavily on parameter settings and can yield biased estimates, incorrect inference, and poor performance. The study's results are critical for advising potential growers of the species who may be in marginal or unsuitable regions, as this can lead to crop failure or increased input costs.

CHAPTER 4

POTENTIAL TO USE REMOTE SENSING TECHNOLOGY IN MONITORING WILD POPULATIONS OF *ARTEMISIA AFRA*

4.1 INTRODUCTION

Climate change and other human factors, such as land-use reallocation for human settlement and agriculture, habitat destruction, and the over-exploitation of medicinal plants due to growing demand, have led to the extinction or threatened status of many medicinal plants. One direct impact of climate change is forcing species to 'track' suitable climatic conditions and shift their distribution ranges (Maharjan *et al*., 2022). It is therefore important to investigate the potential of using technology to monitor changes in species distributions. Traditional mapping methods, such as field surveys, are tedious, require considerable logistical arrangements, and are impractical for large spatial areas (Dubula *et al*., 2016). Remote sensing is a cost-effective and time-efficient technique for mapping large spatial areas. Major limiting factors in using remote sensing for vegetation monitoring and mapping include cloud cover, spatial resolution, and detection accuracy (Dammer *et al*., 2012).

Remote sensing technology uses the interaction between electromagnetic radiation to discriminate between plant species (Dubula *et al*., 2016; Tesfamichael *et al*., 2018). Two species, *Ambrosia artemisiifolia* and *Artemisia vulgaris* were successfully separated using spectral data from 550 to 680 nm Dammer *et al*. (2012). Dubula *et al*. (2016) found that the near-infrared region of the reflectance spectrum could be used to discriminate *A. afra* from adjacent land cover crops. Tesfamichael *et al*. (2018) concluded that narrow wavelength hyperspectral bands and broad bands could be used to separate narrow-leaved plants, such as *A. afra*, *Asparagus laricinus*, and *Seriphium plumosum*, from each other and from other morphologically similar cohabitant plants.

The quality and confidence of vegetation indices are affected by radiometric variations caused by various physical effects that affect the sensor signal. For example, chlorophyll and structural indices are sensitive to variations in light and the sun angle (Bannari *et al*., 2002; Ximena, 2017). One challenge with remote sensing is interpreting measurements from sparse canopies because vegetation indices are strongly controlled by changes in fractional vegetation cover rather than by changes in the optical thickness of the canopy (Leprieur *et al*., 2000).

4.2 RESEARCH METHODOLOGIES

4.2.1 Site selection for spectral signatures/reflectance measurements

To investigate the potential of using remote sensing technology to monitor wild populations of *A. afra*, the information recorded in Chapter 3 was used to determine the most suitable populations or areas where *A. afra* is distributed. The criteria for selecting areas were that they have more plants closer together within a 10 m radius for uniformity, to accommodate the potential extension of analysis using space-borne remote sensing techniques. The selected site was in the Free State, in the Denneysville area of Metsimaholo Municipality (Figure 4.1). Follow-up studies were conducted in the Gauteng and Free State provinces. A handheld Global Positioning System (GPS) receiver (Garmin eTrex 20 X) was used to collect a total of 294 points from Gauteng province, of which 220 points were collected from Klipriviersberg Nature Reserve, and the remaining 74 points were collected from Suikerbosrand Nature Reserve and around the Heidelberg area. Most of the *A. afra* population coordinates visited in the northeastern part of the Free State province, namely around Qwaqwa, Kestell, Clarens, Rouxville, and Golden Gate Nature Reserve, were located. The largest population was predominantly found in the latter.

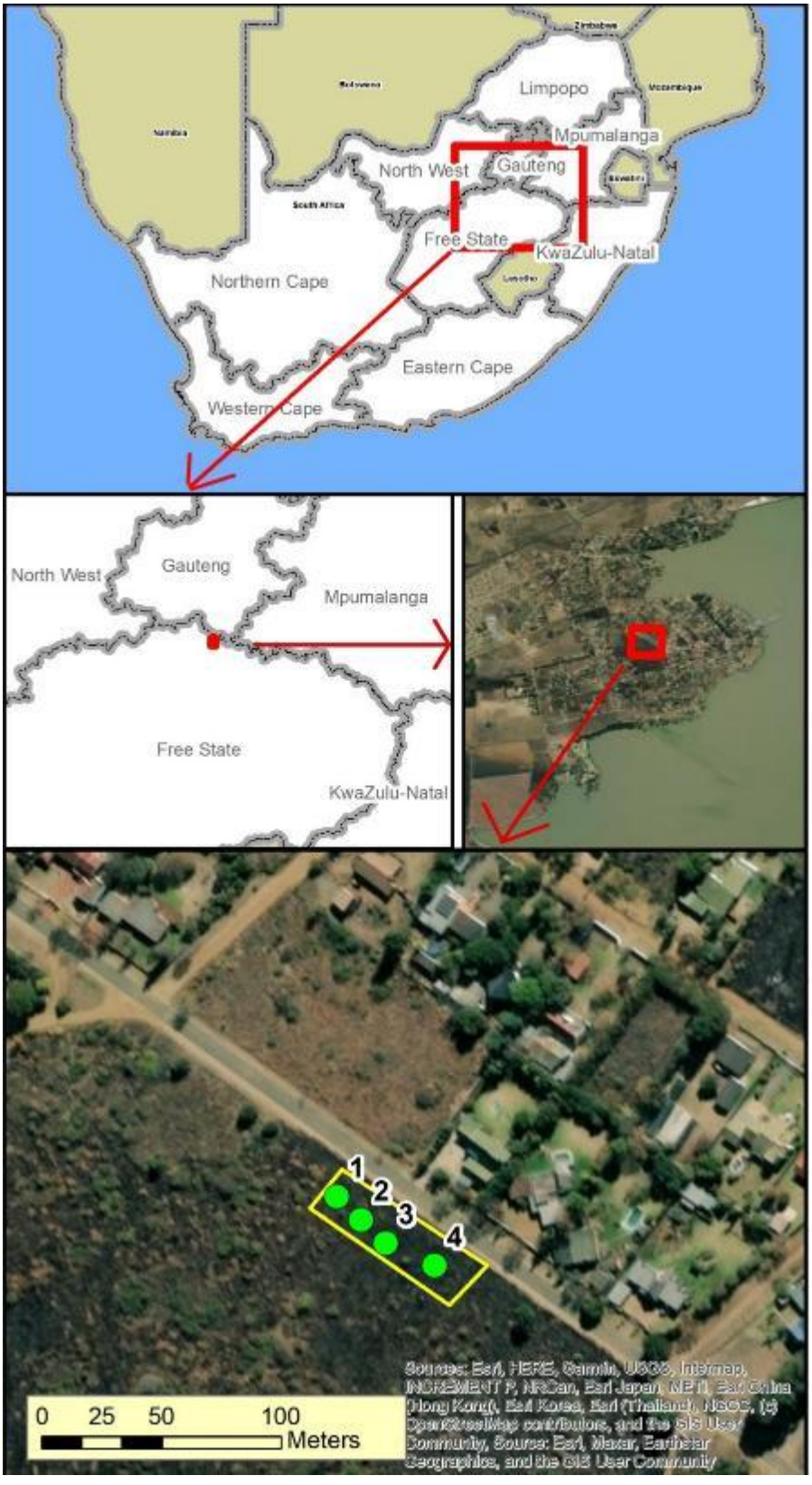

Figure 4.1. The location of the studied *Artemisia afra* population in Denneysville, Metsimaholo Municipality, Free State province, with the individual plants on which measurements were taken shown by the numbers 1 to 4.

4.2.2 Field spectral measurements

Spectral signatures of *A. afra* were collected with an Analytical Spectral Devices (ASD) FieldSpec 4 Hi-Res spectroradiometer (SciAps Inc., Colorado, USA) (Figure 4.2). This device has a 25° Field of View (FOV) via a permanent fiber-optic cable (Newete *et al*., 2014) and a narrow-band 1 nm sampling interval, and it acquires spectral data between 350 and 2500 nm. The ASD FieldSpec 4 Hi-Res spectroradiometer provides 3 nm spectral resolution in the visible near-infrared (VNIR) and 8 nm in the short-wave infrared (SWIR), delivering superior spectral performance across the full solar irradiance spectrum (350 – 2500 nm). Spectral measurements were calibrated against a white reference panel (barium sulfate coating) and recorded as percentage reflectance. A single spectral measurement was collected from each of the 40 plants per species, yielding a total of 200 spectral measurements per species, since the ASD collects five spectral measurements internally for every single measurement taken.

Figure 4.2. An ASD FieldSpec 4 Hi-Res spectroradiometer was used to collect spectral data on wild populations of *Artemisia afra*.

Ground-truthing data for the follow-up studies in Gauteng and the Free State provinces were collected at random from previously recorded coordinates. The ground truthing data were labelled or described in four categories as: individual plants (1 single plant), few plants (2-10 individuals), several individuals (50-100), and many individuals (> 100 plants) (Figure 4.3). Spectral measurements of *A. afra* and the other two plants, namely grass and *Asparagus laricinus* (Figure 4.4), were taken from the continuous plant canopy at a height of 40 cm above the top of each plant.

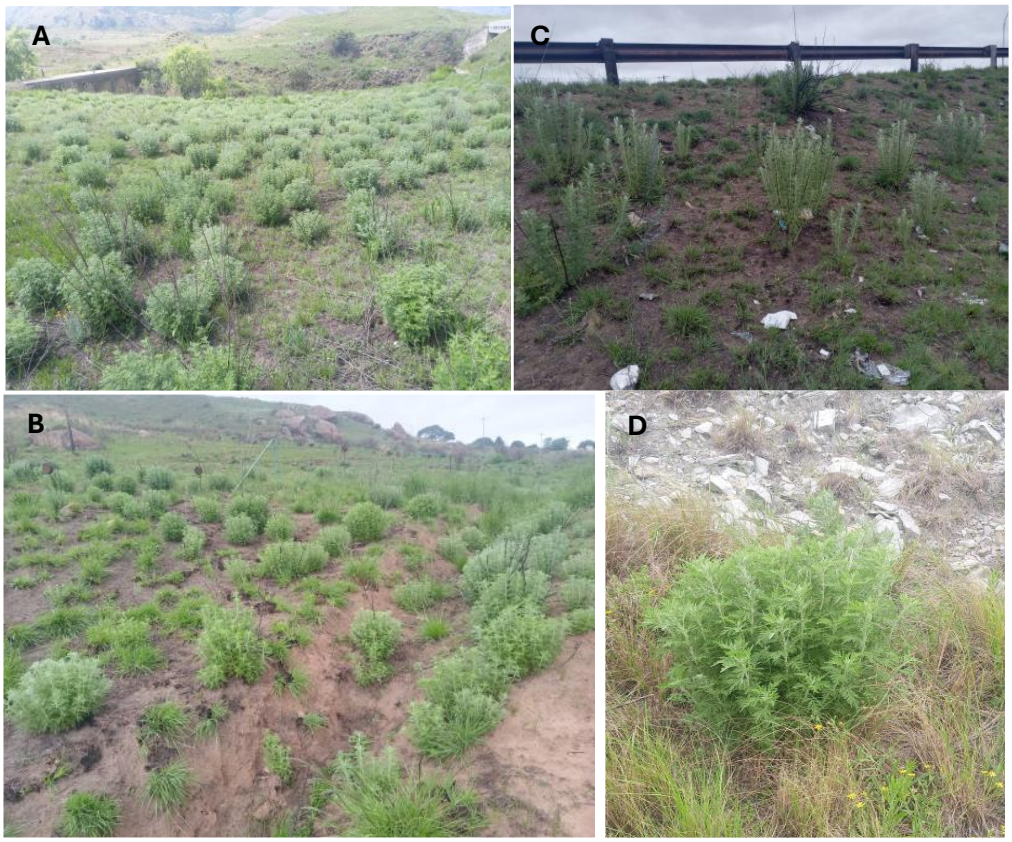

Figure 4.3. The population sizes of the *Artemisia afra* categorized as (a) many individuals (> 100 plants), (b) several individuals (50 - 100), (c) few plants (2-10 individuals), and (d) individual plant (1 single plant) in Gauteng and Free State provinces, South Africa.

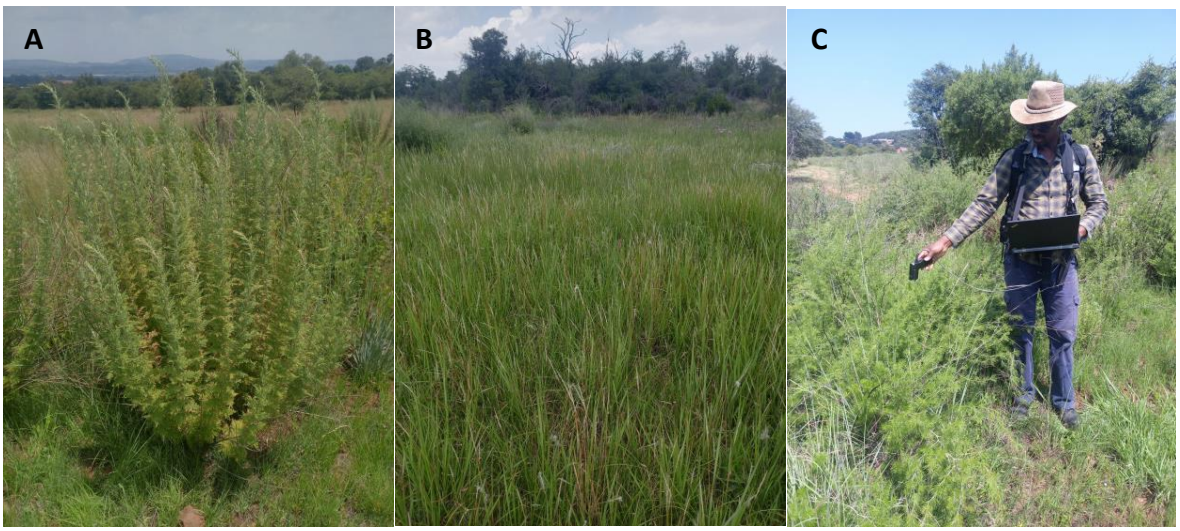

Figure 4.4. Spectral reflectance measurements taken from (a) *Artemisia afra*, (b) grass species and (c) *Asparaghus laricinus* in Klipriviersberg Nature Reserve, Gauteng province, South Africa using Analytical Spectral Device (ASD).

In the follow-up study, a feature selection method using all bands was applied to the spectral measurement data and sorted by the highest Mean Decrease Accuracy and Mean Decrease Gini, from which 50 important bands contributing to the decrease in Accuracy and Gini were selected. A Random Forest algorithm was then used to classify *A. afra*, *A. laricinus*, and the grass species based on the 50 bands, and the accuracy assessment was generated. The ground-truthing data were first randomly split into 70% training and 30% validation or test data sets, and the latter was used to compute a confusion matrix for the overall, producer's, and user's accuracies, and the kappa coefficient.

4.2.3 Classification of *Artemisia afra* using the Sentinel-2 Imager

The spectral signatures were compared with pixel values across the different bands of the Sentinel-2 Multispectral Imager. The Sentinel-2 Multispectral Imager has 12 bands at varying spectral and spatial resolutions, as illustrated in Table 4.1. Spatial resolution indicates the ground area measured per pixel, while spectral resolution (bandwidth) is defined as the instrument's ability to distinguish features in the electromagnetic spectrum.

Table 4.1. The spatial and spectral resolutions of the Sentinel-2 Multispectral Imager.

Band	Description	Resolution (m)	Wavelength (nm)	Central Wavelength (µm)	Bandwidth (nm)
B1	Coastal aerosol	60	433–453	0.443	20
B2	Blue	10	458–522	0.49	65
B3	Green	10	543–577	0.56	35
B4	Red	10	650–680	0.665	30
B5	Vegetation Red Edge	20	698–712	0.705	15
B6	Vegetation Red Edge	20	733–747	0.74	15
B7	Vegetation Red Edge	20	773–793	0.783	20
B8	NIR	10	784–899	0.842	115
B8A	Narrow NIR	20	855–875	0.865	20
B9	Water vapour	60	935–955	0.945	20
B10	SWIR Cirrus	60	1360–1390	1.375	20
B11	SWIR	20	1565–1655	1.61	90
B12	SWIR	20	2100–2280	2.19	180

Three spatial resolutions of the Sentinel-2 Multispectral Imager bands used in the study are shown in Figure 4.5. The yellow lines in the illustration indicate the location of the *A. afra* population in the Denneysville area.

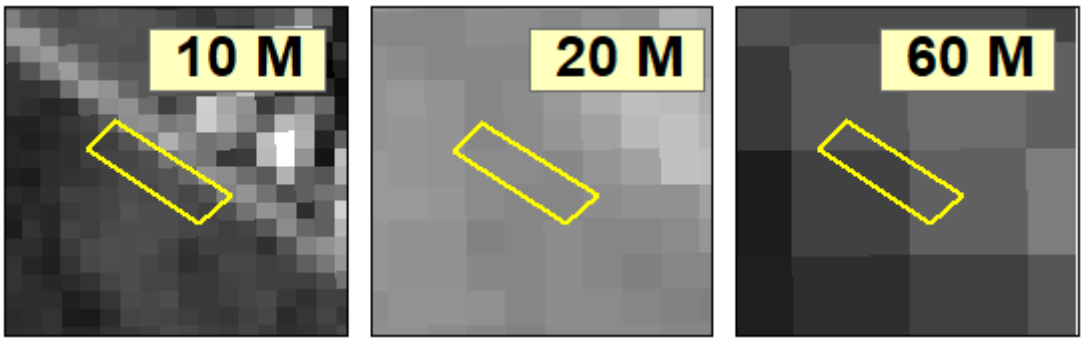

Figure 4.5. Illustration of different spatial resolutions of Sentinel-2 Multispectral Imager bands and the location of the *Artemisia afra* population demarcated in yellow lines.

The *A. afra* population in Figure 4.6 A, from the same population in Denneysville, was used as an example to illustrate Sentinel-2 Multispectral Imager band reflectance values in comparison with the spectral signature from the spectroradiometer. Additional measurements were also taken on cultivated *A. afra* at the ARC Roodeplaat farm in Pretoria, Gauteng (Figure 4.6 B). The spectral measurements of cultivated *A. afra* plants were compared with those of a nearby *Moringa oleifera* plantation (Figure 4.7).

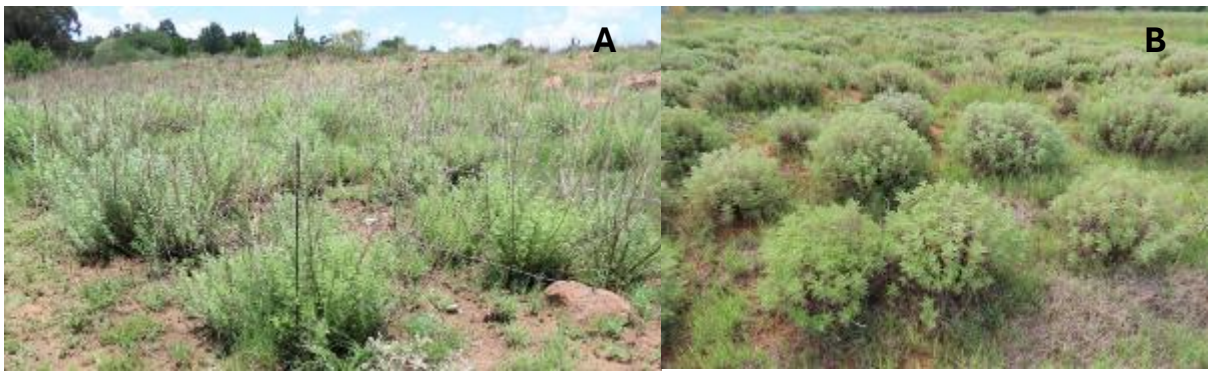

Figure 4.6. *Artemisia afra* wild population in Denneysville, Metsimaholo Municipality (A) and cultivated plants at the ARC-Roodeplaat (B) farm where spectral measurements were taken.

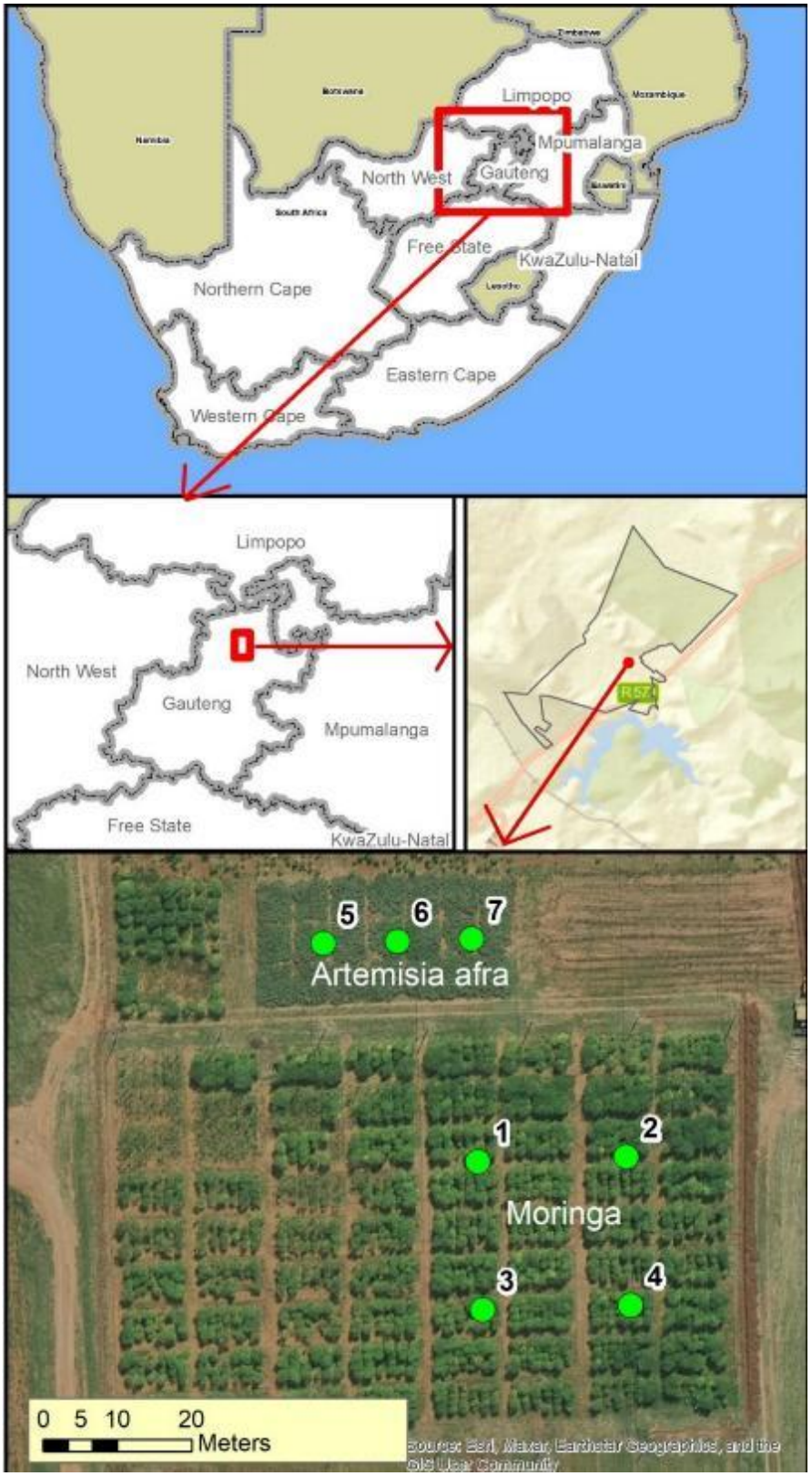

Figure 4.7. The location of the cultivated plants in the ARC Roodeplaat farm, Pretoria, Gauteng province, with the individual plants on which measurements were taken, shown by the numbers 1 to 4 for *Moringa oleifera* and 5 to 7 for *Artemisia afra*.

In follow-up studies, the Sentinel-2 Multispectral Imager was used to classify the distribution of *Artemisia afra* in the Klipriviersberg Nature Reserve (KNR). Training samples (n = 58) were collected from *A. afra* and six co-occurring land cover types, including *Asparaghus laricinus*, grass, tree/bush, bare land, built-up, and waterbodies. The Random Forest (Breiman, 2001) classification algorithm was applied to train on the 58 samples and the corresponding pixels in the Sentinel-2 imagery, and to further classify the imagery for the rest of the study area. The accuracy of the classified product was evaluated using 246 independent test samples representing the seven classes, including *A. afra*.

4.2.4 Geographic Information System and ArcMap tools to map *Artemisia afra* distribution

The ground-truthing survey of *A. afra*, particularly in the Free State Province, showed that the plants were sparsely distributed. Thus, mapping each individual plant would require a high spatial resolution satellite sensor, with each plant fitting within a single pixel. The Sentinel 2 satellite imagery, available free of charge for research use, has only 10 m or higher resolution, whereas the *A. afra* plant canopy size is about 1 m or less. Thus, a distribution map of *A. afra* for the two provinces, namely Gauteng and Free State, was produced from raster data using GIS and ArcMap tools, with four distinct categories to provide a coarse estimate of individual plants based on visual observations recorded at each coordinate where *A. afra* was located.

4.3 RESULTS AND DISCUSSION

A field spectroradiometer was used to collect spectral data from *A. afra* populations, and the data were compared with the pixel values of the Sentinel-2 Multispectral Imager band. Figure 4.8 shows the spectral measurements (*A. afra* 1-9) along the electromagnetic spectrum, recorded at a population in Denneysville using an ASD spectroradiometer, and the Sentinel-2 Multispectral Imager band values (pnt 1 – 4) taken on different dates. The nine spectral sample measurements exhibited a similar pattern of increases and decreases; however, the Sentinel-2 Multispectral Imager band measurements differed slightly from the spectral measurements. Because the plants were very sparse or widely spaced, the reflectance captured in the pixel values was a mixture of the reflectance from companion plants, soils, and other objects in the environment. When plants are found in a scattered pattern within a study area, the number of areas with a substantial concentration of the species is reduced, which is disadvantageous for pixel-based measurements (Dubula *et al*., 2016). The detection accuracy of aerial images depends on the present biomass of the specific plant species (Dammer *et al*., 2013). The reflectance patterns in the visible (400–700 nm) and red edge (700–795 nm) showed no distinct within-species differences, whereas beyond these ranges, spectral patterns differed among the sampled *A. afra* plants. However, the pattern of increase and decrease in reflectance percentage was the same for all nine *A. afra* measurements. Dammer *et al*. (2012) discriminated between *Artemisia vulgaris* and *Ambrosia artemisiifolia* using hyperspectral image analysis and reported that at least two wavelengths on the electromagnetic spectrum, 550 and 650 nm, were suitable for discriminating between the two species.

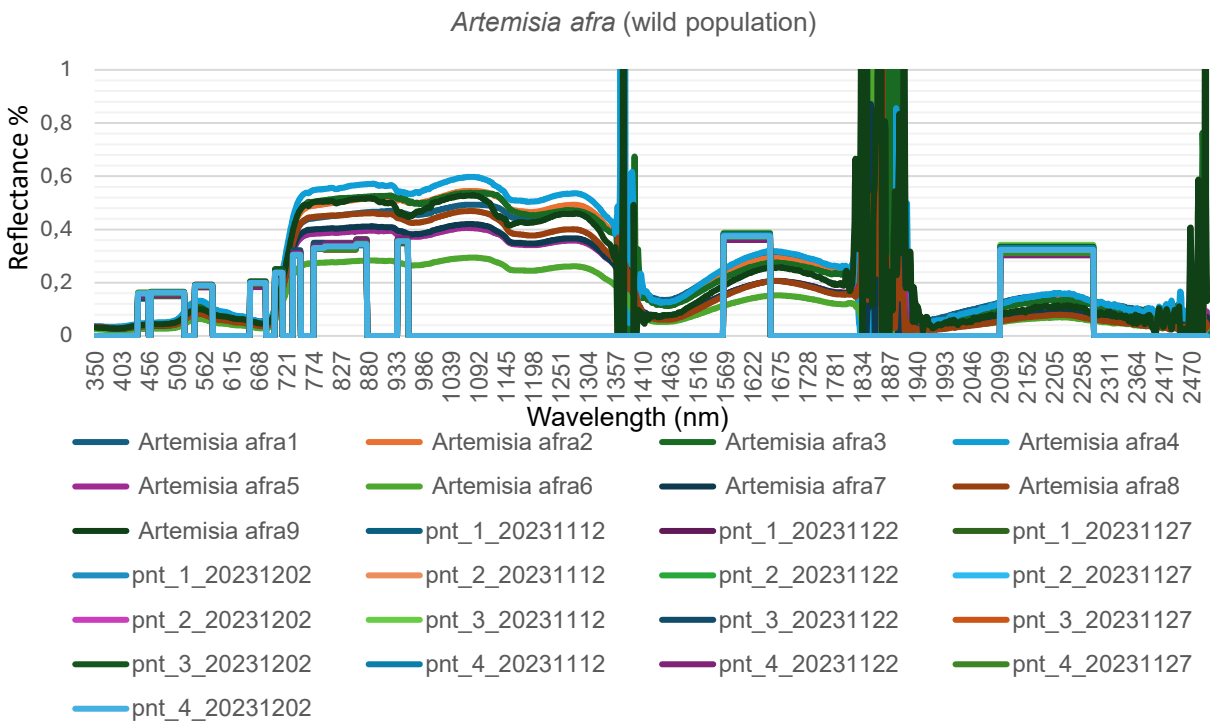

Figure 4.8. Spectral reflectance of wild populations of *Artemisia afra* (*Artemisia afra* 1 to 9) taken with a FieldSpec spectroradiometer and Sentinel 2 band values taken on different dates (pnt 1 to 4).

Other medicinal plants growing in the wild were considered to investigate whether there are any differences in their spectral reflectance compared with that of *A. afra*. Measurements were taken from a *Sutherlandia frutescens* population in the Free State province. Spectral measurements were obtained from green, actively growing, and brown, dry *S. frutescens* plants (Figure 4.9). The dry *S. frutescens* plants were separated from the green plants on the electromagnetic spectrum. This may be because chlorophyll, water, and dry matter content of plants influence reflectance across different plants (Tshabalala *et al*., 2021). The reflectance measurements from six green *S. frutescens* plants showed some variation; however, the pattern of increase and decrease in reflectance along the electromagnetic spectrum followed a similar pattern. The pattern also seems different from that in Figure 4.8 for *A. afra*.

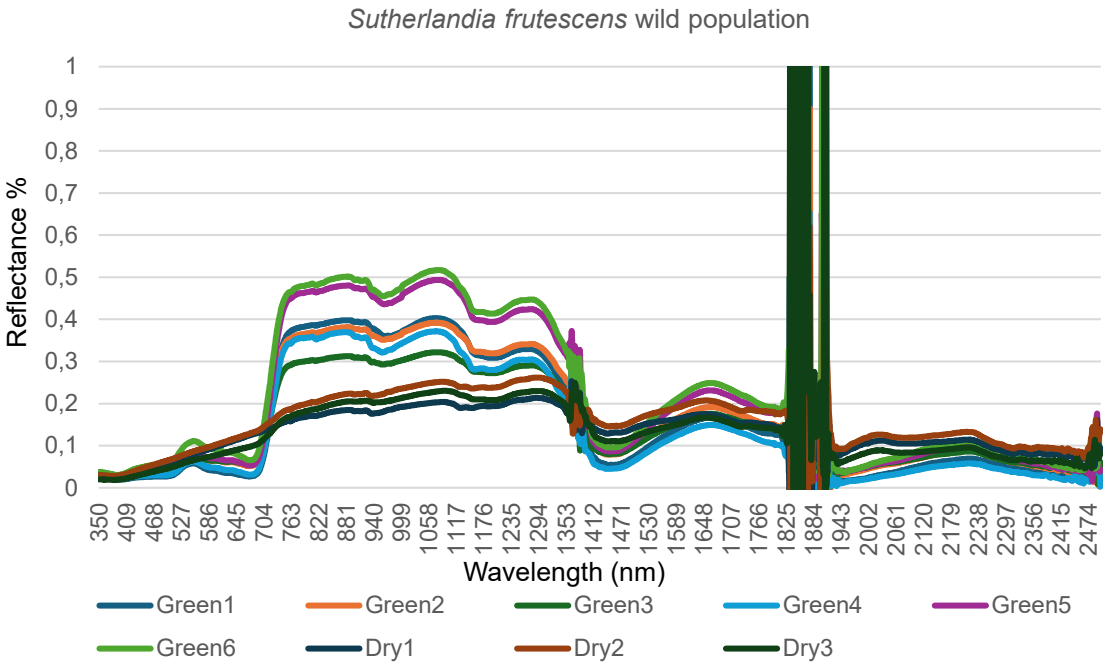

Figure 4.9. The spectral measurements taken from green actively growing (Green 1 to 6) and brown dry (Dry 1 to 3) *Sutherlandia frutescens* plants, using an ASD spectroradiometer.

The foliage density, canopy architecture, leaf size, shape, and orientation are important factors in plant reflectance (Tshabalala *et al*., 2021). To compare a sparse, widely spaced plant population with a denser, less scattered population, the exercise was repeated in cultivated fields at the ARC campus. Figure 4.10 shows a pattern similar to that of the wild populations (Figure 4.8) in the increase and decrease in reflectance percentages. Both the wild and cultivated *A. afra* followed a pattern reported by Tesfamichael *et al*. (2018) when comparing *A. afra* with other thin-leaved cohabitant plants using a Spectral Evolution® PSR-3500 Remote Sensing Portable Spectroradiometer.

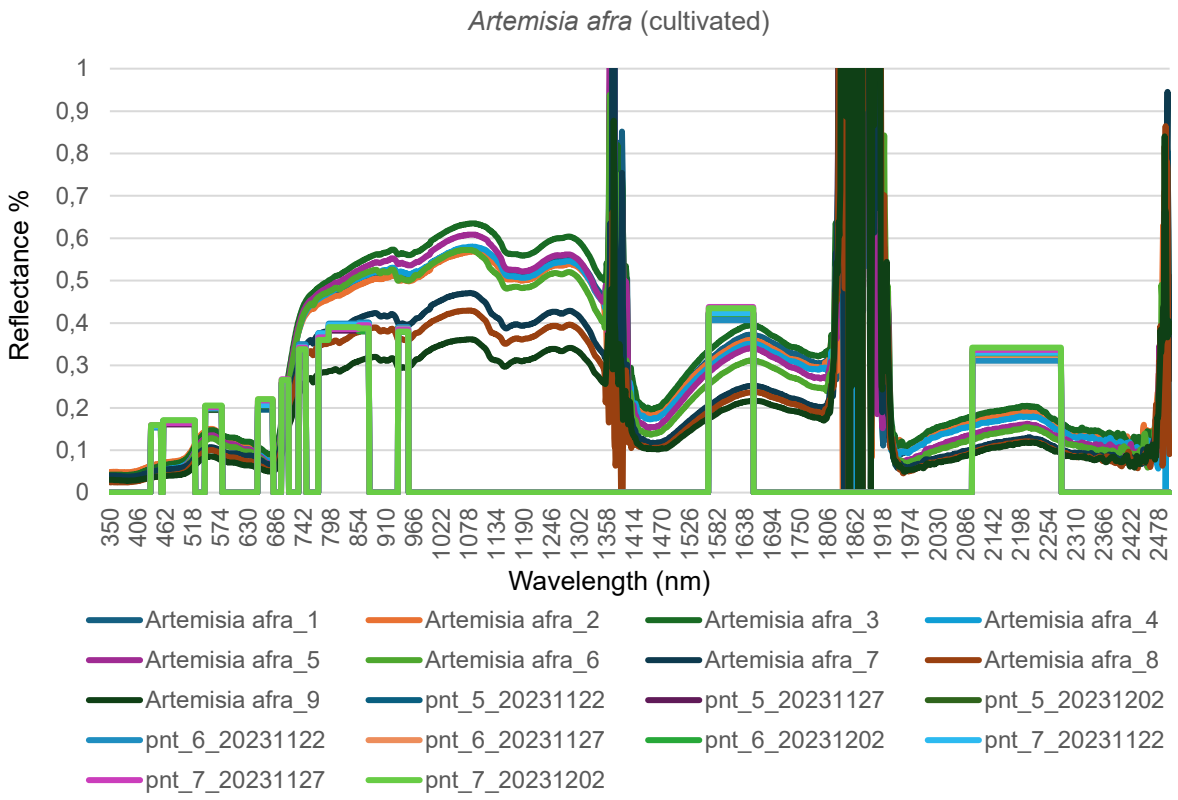

Figure 4.10. Spectral reflectance of cultivated *Artemisia afra* (*Artemisia afra* 1 to 9) taken with an ASD spectroradiometer and Sentinel-2 Multispectral Imager band values (pnt 5 to 7).

Furthermore, spectral measurements were also taken on a *Moringa oleifera* plantation near the cultivated *A. afra*. The advantage is that both are cultivated with limited occupation by other species, thereby improving the accuracy of pixel measurements. The cultivated *M. oleifera* spectral measurements showed an increase in reflectance percentage at about 680 nm and a major decrease at 1130 nm (Figure 4.11). The reflectance pattern followed that reported by Tshabalala *et al*. (2021) for various *M. oleifera* cultivars grown under controlled conditions.

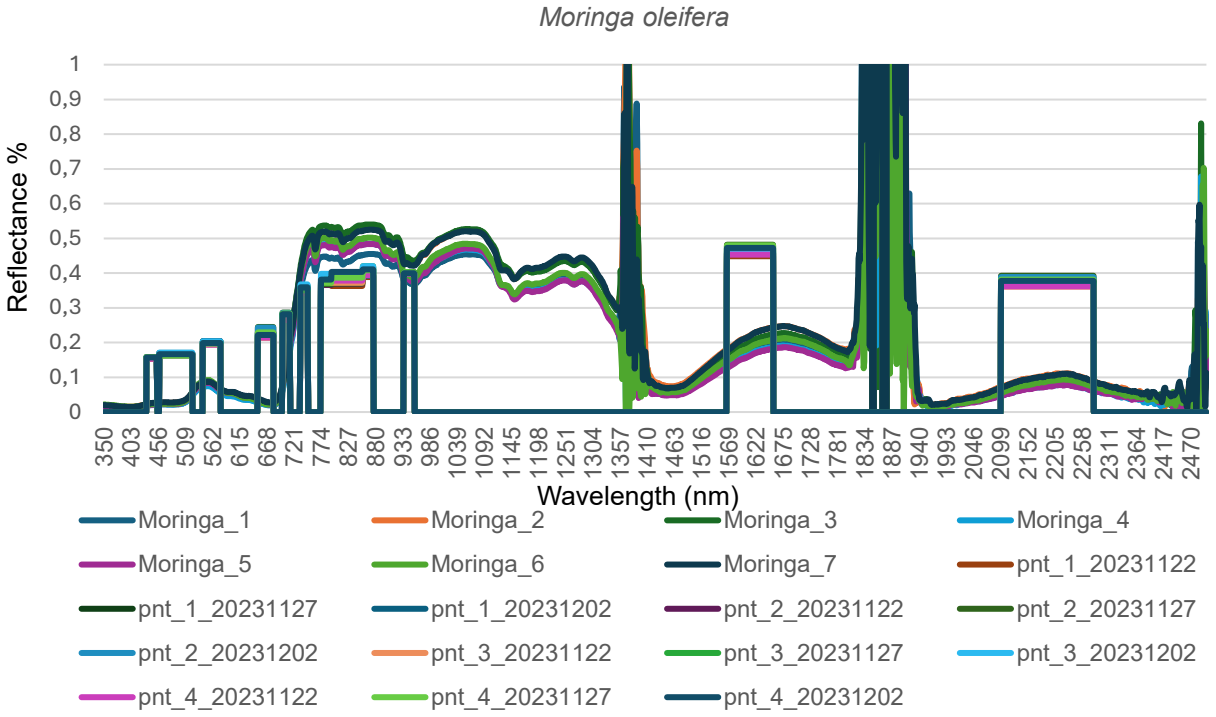

Figure 4.11. Spectral reflectance of cultivated *Moringa oleifera* (Moringa 1 to 7) taken with an ASD spectroradiometer and Sentinel-2 Multispectral Imager band values (pnt 1 to 4).

The spectral/reflectance percentages for cultivated *A. afra* and *M. oleifera* were averaged and compared (Figure 4.12). The figure shows that *A. afra* spectral measurements were always higher than those for *M. oleifera*, except in the region between wavelengths 723 nm and 901 nm.

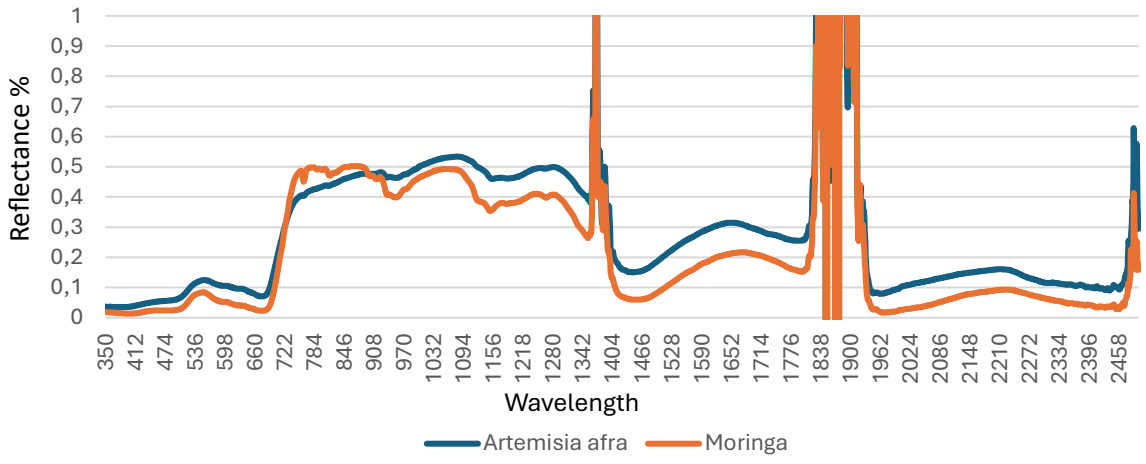

Figure 4.12. Comparison of average reflectance measurements for *Artemisia afra* and *Moringa oleifera*, taken using an ASD spectroradiometer.

The general colour of plant leaves, from which spectral measurements are taken, is green, with very similar spectral properties. Thus, leaf shape and texture are mostly used to discriminate plant species in agricultural fields at early growth stages when overlapping leaves can be excluded (Dammer *et al*., 2012). *Artemisia afra*, like most wild-growing medicinal plants, grows together with other plant species, making species overlap the most limiting factor for the use of leaf shape and/or texture in species discrimination. This could be the reason Sentinel-2 Multispectral Imager band values (reflectance values) look similar in all the figures, even though measurements from the ASD FieldSpec spectroradiometer showed some differences.

Similarly, spectral measurements using the ASD spectroradiometer showed distinct differences among the three plants, namely *A. afra*, *A. laricinus*, and grass species (Figure 4.13). The highest spectral reflectance (56%) was observed in *A. afra* at approximately 800 nm, followed by *A. laricinus* (43%), whereas grass showed the lowest spectral reflectance (38%). Conversely, the shallowest depth at a wavelength of about 700 nm was reported in *A. afra*, and the deepest in *A. laricinus*.

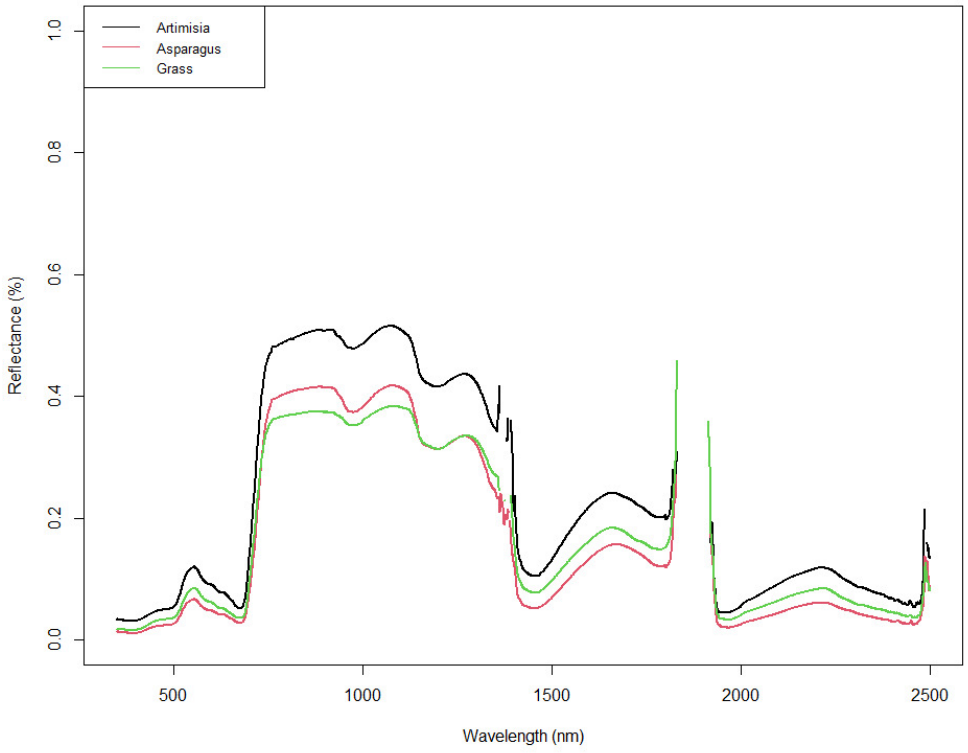

Figure 4.13. Spectral measurements of *Artemisia afra*, *Asparagus laricinus* and grass species taken using the ASD spectroradiometer.

The normalised difference vegetation index (NDVI) was computed for all three species (Figure 4.14). *Artemisia afra* showed the lowest NDVI, with a mean of only 0.8, and the highest was recorded in *A. laricinus* (0.87), following the pattern of spectral reflectance shown in Figure 4.13.

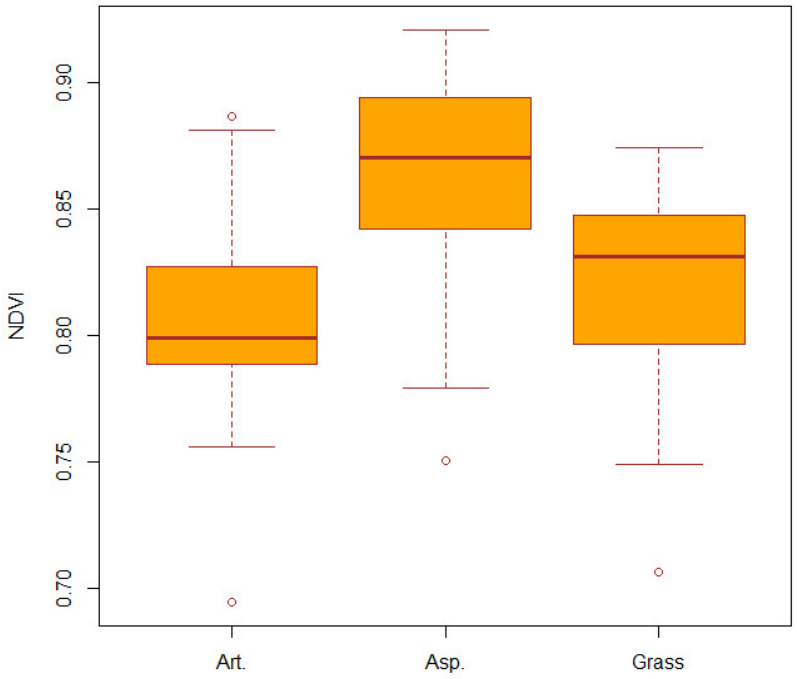

Figure 4.14. The normalized difference vegetation index (NDVI) was computed for the three species, namely *Artemisia afra, Asparagus laricinus* and grass species in the Klipriviersberg Nature Reserve.

A random forest classification was conducted on the 50 selected bands from the 70% training data using the feature selection Random Forest model, and a classification accuracy of 89% with a kappa value of 0.82 was obtained (Table 4.2). All *A. afra* plants were correctly identified, and a few *A. laricinus* plants were misidentified as *A. afra* (1 plant) and as grass (2 plants).

Table 4.2. Confusion Matrix and Statistics for Accuracy Assessment.

Classes	*A. afra*	***A. laricinus***	**Grass**	**Total**	***PA**
A. afra	**10	0	0	**10**	**89**
A. laricinus	1	16	2	**19**	**24**
Grass	1	0	6	**7**	**8**
Total	**12**	**16**	**8**	**26**	
UA (%)					**OA = 89%** **Kappa = 0.82**

*PA denotes producer's accuracy; UA: user's accuracy; OA: over accuracy.

**Shaded cells indicate accurately mapped numbers of samples.

The results of the spatial distribution map for Klipriviersberg Nature Reserve are shown in Figure 4.15, while the accuracy assessment is shown in Table 4.3. Although the overall accuracy (OA) of the classification was relatively low at 52%, *A. afra* had a high producer's accuracy (PA) of 89%. In contrast, the user's

accuracy for the species was much lower (53%). *Artemisia afra* was mostly confused with grass, with at least six *A. afra* plants misidentified as grass due to spectral overlap between the two *A. laricinus* was also misclassified, with 27 of them identified as *A. afra*, indicating the difficulty of mapping the species when co-occurring with these two land cover types.

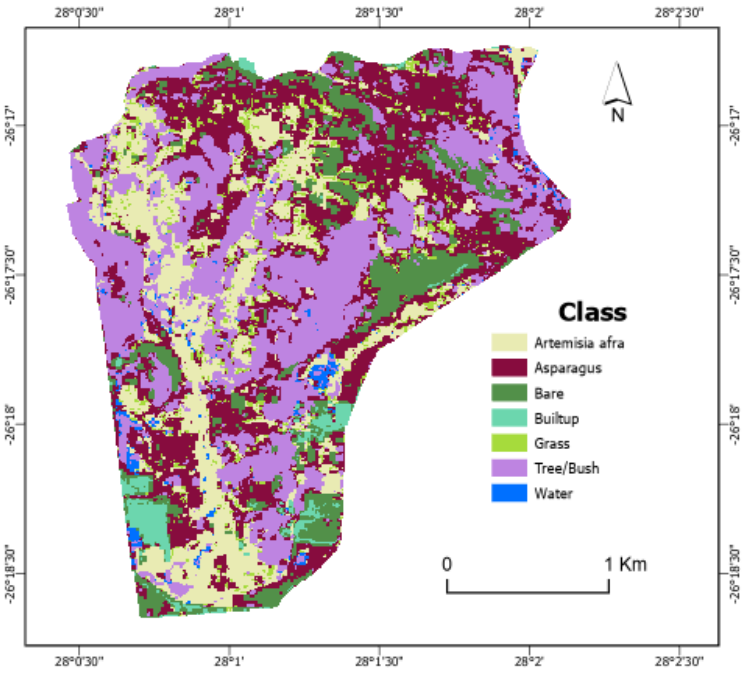

Figure 4.15. Distribution map of *Artemisia afra* and the co-occurring land cover types in the Klipriviersberg Nature Reserve.

Table 4.3. Confusion matrix of reference and classified *Artemisia afra* and other co-occurring land cover types.

	Classification								
Classes	***A. afra***	***A. laricinus***	**Bare land**	**Built up**	**Grass**	**Tree/Bush**	**Water**	**Total**	**PA**
A. afra	79	3	0	0	6	1	0	**89**	**89**
A. laricinus	**27**	**9**	0	0	**2**	**0**	**0**	**38**	**24**
Bare land	2	4	9	2	0	**0**	**0**	**17**	**53**
Built up	0	0	1	4	0	**1**	**0**	**6**	**67**
Grass	**38**	**18**	2	1	**5**	**0**	**2**	**66**	**8**
Tree/Bush	2	2	0	0	1	**20**	**0**	**25**	**80**
Water	2	0	0	1	1	**0**	**1**	**5**	**20**
Total	**150**	**36**	**12**	**8**	**15**	**22**	**3**	**246**	
UA (%)	**53**	**25**	**75**	**50**	**33**	**91**	**53**		**OA = 52%**

NB: PA denotes producer's accuracy, UA: user's accuracy, OA: overall accuracy. Shaded cells indicate accurately mapped numbers of samples.

The highest population of *A. afra* was recorded in Gauteng province, where over 15 points visited showed many individual plants. The largest such dense population was recorded in the Klipriviersberg Nature Reserve, with over 100 points visited showing a dense population (many individual *A. afra* plants) (Figure 4.16 A). Other indicators of *A. afra* distribution, including the size categories of 'several individuals' and 'few individuals,' were recorded highest in the Free State province, showing 28 and 32 points, respectively. The number of single individuals of *A. afra* recorded during the survey was higher in Gauteng than in the Free State province. Many of the *A. afra* plants located using the previously recorded coordinates and others encountered during the field survey were particularly concentrated in the southern parts of Gauteng and the northeastern part of the Free State province (Figure 4.16 B).

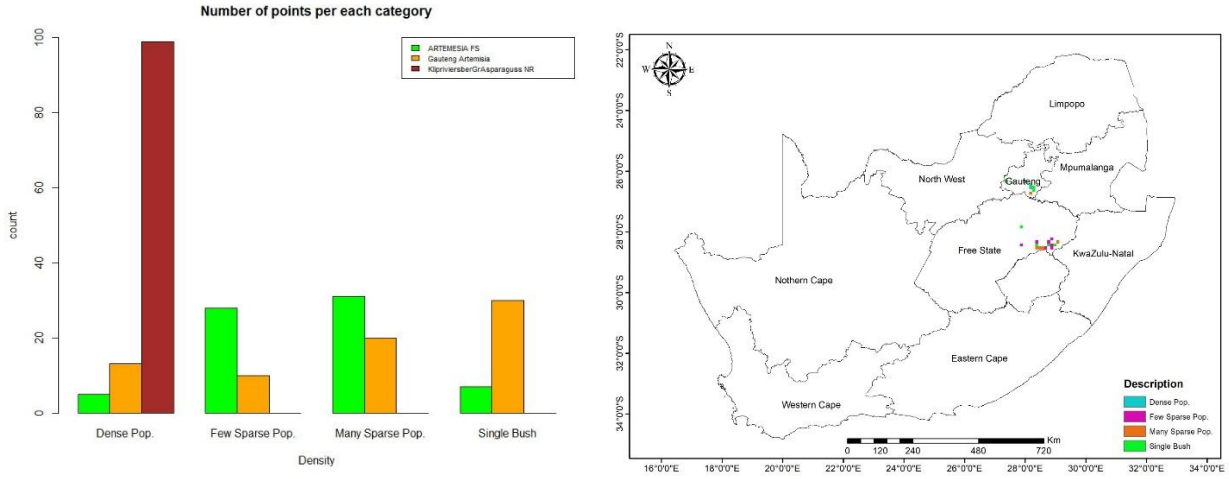

Figure 4.16. The number of *Artemisia afra* plants recorded per field survey points or coordinates visited in the Free State and Gauteng provinces, as well as the Klipriviersberg Nature Reserve within Gauteng (A) and the distribution map of *Artemisia afra* in Gauteng and Free State provinces (B) as categorized into groups of individual plants (1 single plant), few plants (2-10 individuals), several individuals (50 - 100), and many individuals (> 100 plants).

4.4 CONCLUSION

The major challenge identified in this study was that the plants in wild populations were mostly widely spaced, with other plants growing in between, thus affecting the aerial imagery. However, a follow-up study showed that *A. afra* could be accurately mapped using high-resolution satellite imagery. For instance, measurements with the Analytic Spectral Device (ASD) hyperspectral handheld field spectrometer revealed that *A. afra* had the highest spectral reflectance and a shallower absorption around the red edge between 690 and 720 than the other closely co-occurring species, namely *A. laricinus* and grass species. This is also evident in the computed NDVI results, which show a consistent pattern: *A. afra* exhibited low chlorophyll content relative to the other two species. The random forest classification model trained on the 50 most important bands selected from the 70% training data achieved an overall accuracy of 89% and a kappa coefficient of 0.82. This result indicates that *A. afra* can be effectively mapped using high-resolution satellite data, as the benchmark for effective classification is an overall accuracy of 85%.

A Sentinel 2 classification of *A. afra* and other land use types using the Random Forest algorithm was processed only for the Klipriviersberg Nature Reserve. Unlike the hyperspectral ASD data, the Sentinel 2 classification did not produce a map with high overall accuracy, achieving only 52%. *Artemisia afra* was misidentified as grass in 6% of cases, and 71% of *A. laricinus* was identified as *A. afra*, suggesting that *A. afra* could be effectively mapped only with significant accuracy using satellite imagery with high spatial and spectral resolution. Many of the *A. afra* populations in the Free State and Gauteng provinces, except for the Golden Gate and Klipriviersberg Nature Reserves, respectively, where at least some dense populations were present, were not closed enough for mapping unless a high spatial resolution satellite sensor is used, as indicated by the classification results for the latter reserve. Thus, mapping and classifying *A. afra* across the Gauteng and Free State provinces was not possible.

Thus, the *A. afra* distribution was mapped using GIS and ArcMap tools. To determine the population size of plants, in situ data on the number of individuals in a specific area are required. Unfortunately, the size of *A. afra* individuals at each coordinate visited in the two provinces was not counted, nor was the area where *A. afra* occurred at each point recorded. However, during field ground-truthing data collection, the size of *A. afra* individual plants was estimated from coarse visual observation and categorized into four groups as ‘individual plant’ (1 single plant), ‘few plants’ (2-10 individuals), ‘several individuals’ (50-100), and ‘many individuals’ (> 100 plants). The most dense populations were found in Klipriviersberg Nature Reserve in Gauteng, followed by the Golden Gate Reserve in Free State province.

CHAPTER 5
VEGETATIVE PROPAGATION OF *ARTEMISIA AFRA*

5.1 INTRODUCTION

The traditional health system, the natural products industry, and some sectors of the pharmaceutical industry depend on the availability and sustainability of various medicinal plant species (Fajinmi *et al*., 2023). Propagation and cultivation of medicinal plants have long been advocated as a suitable alternative to wild harvesting; however, plants must be produced at a reasonable cost and in large quantities (Van Wyk and Prinsloo, 2018). Thus, investigating affordable propagation techniques that make initial plant material available at reasonable costs is the first step. Propagation of medicinal plants also relieves pressure on wild populations and conserves highly utilized, vulnerable, scarce, and endangered species (Fajinmi *et al*., 2023). Propagation can be done vegetatively, using plant parts such as stems, leaves, and roots, or from seeds.

Artemisia afra seeds can be sown in spring or summer in well-drained soil with a slightly acidic to neutral pH (Koehorst *et al*., 2010). The soil should be kept moist until germination and seedling emergence, which usually takes 2 to 8 weeks. Letsoalo (2022) reported that soaking *A. afra* seeds in hot and cold water improved germination to 70%, compared with 20% achieved with physical scarification, acid treatment, and 10% in the control. A study on *A. annua* found that seed-derived plants are highly variable in artemisinin content, lowering artemisinin yield per hectare (Wetzstein *et al*., 2018). High variability in *A. afra* seedlings can be expected due to cross-pollination, which is a disadvantage where true-to-type plant material is required. *Artemisia* spp. plants can also be propagated by stem cuttings, which root easily in spring and summer, or by division in spring. Tissue culture-regenerated plants and rooted cuttings of *A. annua* outperformed seed-derived plants in terms of uniformity, yield, and consistency in artemisinin content (Wetzstein *et al*., 2018). A similar case can be true with the seed multiplication of *A. afra*, so vegetative propagation may be the most suitable alternative. With the right chemotype, farmers can produce plants with uniform characteristics, significantly increase yield per cultivated area, and produce the desired material for the industry and market.

5.2 RESEARCH METHODOLOGIES

5.2.1 Micropropagation

Nodal explants (1.5–2 cm long) from middle lateral branches were excised, washed three times with tap water and commercial liquid detergent, and then transferred to the laminar flow cabinet. The explants were immersed in 70% ethanol for 1 min, rinsed twice in sterile distilled water, and further surface sterilized with 10% v/v Jik (3.5% sodium hypochlorite solution) mixed with water for 10 min. Finally, the explants were rinsed thrice with sterile distilled water. Surface-decontaminated nodal explants were trimmed to remove all dead and chlorine-affected tissues before culturing on Murashige and Skoog (MS) medium fortified with different concentrations of benzyladenine (0, 2, 4, and 6 µM BA). In a follow-up study, the explants were

also cultured on 4 µM *meta*-Topolin (*m*T) to compare shoot multiplication efficiencies with the 4 µM BA-treated explants.

5.2.2 Stem cuttings

Stem cuttings (3-5 cm long) were collected from *A. afra* plants and set in different growing media after being dipped in two different plant growth substances. The growing media used were cocopeat, hygromix, and a mixture of hygromix and cocopeat (50/50, v/v). The plant growth substances used were *Moringa oleifera* leaf extracts (Moringa dip) and Dynaroot powder (No. 1). The Moringa leaf extract contains various phytochemicals (gibberellin, auxin, and cytokinin), and the extraction method affects their content (Yuniati *et al*., 2022). Aqueous extraction using a home blender is considered safer, less expensive, easier, and applicable to resource-poor farmers (Yuniati *et al*., 2022). The Moringa leaf extract was prepared by mixing 100 g of the leaf powder with 20 ml of water and blending for 10 minutes. The extract was then stored in a freezer for 2 days before being defrosted for use. The cuttings were dipped in the defrosted extract for five seconds and then set in seedling trays.

Dynaroot is a commercial rooting hormone powder that stimulates fast and prolific rooting of plant cuttings. The active ingredient in Dynaroot is an auxin, 4-indole-3yl butyric acid (IBA). The cuttings were dipped in the powder for five seconds, the excess powder shaken off and then the cuttings were set in seedling trays. The study was conducted in a glasshouse compartment with temperatures of 25-28°C.

A second experiment was laid out randomly with eight treatments and three replicates, with cocopeat as a growing medium. The eight treatments (T) consisted of control (no Dynaroot applied, T0), T1 (Dynaroot applied immediately at setting of the cuttings), T2 (Dynaroot applied one week after setting the cuttings), T3 (Dynaroot applied two weeks after setting the cuttings), T4 (Dynaroot applied 3 weeks after setting the cuttings), T5 (Dynaroot applied 4 weeks after setting the cuttings), T6 (Dynaroot applied 5 weeks after setting the cuttings), and T7 (Dynaroot applied 6 weeks after setting the cuttings). The rooting hormone was applied once during the specific week by carefully removing the cuttings and rinsing them with distilled water followed by dipping them in the Dynaroot powder and re-planting in the same seedling tray cavity.

5.2.3. Data collection and statistical analysis

The cuttings were assessed for root length, number of shoots, plant height, and survival rate. The survival percentage was calculated by dividing the total number of cuttings with shoot growth by the total number of cuttings planted for each treatment. The rooting parameters were measured by carefully removing the rooted cuttings from the seedling trays and washing the root zone with water. The results were then subjected to analysis of variance (ANOVA) using the Statistical Package for the Social Sciences (SPSS) for Windows.

5.3 RESULTS AND DISCUSSIONS

After four weeks of culturing *A. afra* nodal explants *in vitro*, the highest shoot proliferation occurred when MS medium was supplemented with 4 µM BA, which resulted in an average of 4 new shoots per explant. There were no significant differences in terms of shoot numbers per explant between *m*T and BA at 4 µM, except that BA was associated with high incidences of hyperhydricity, a physiological disorder characterized by thickened stems, short internodes, and twisted leaves. Each of the multiplied shoots (from 4 µM BA or *m*T treatment) had a high frequency of branching (average of 15 branches per shoot after 8 weeks) and a greater number of broader leaves, resulting in higher plant density than the control without plant growth regulators (Figures 5.1 and 5.2).

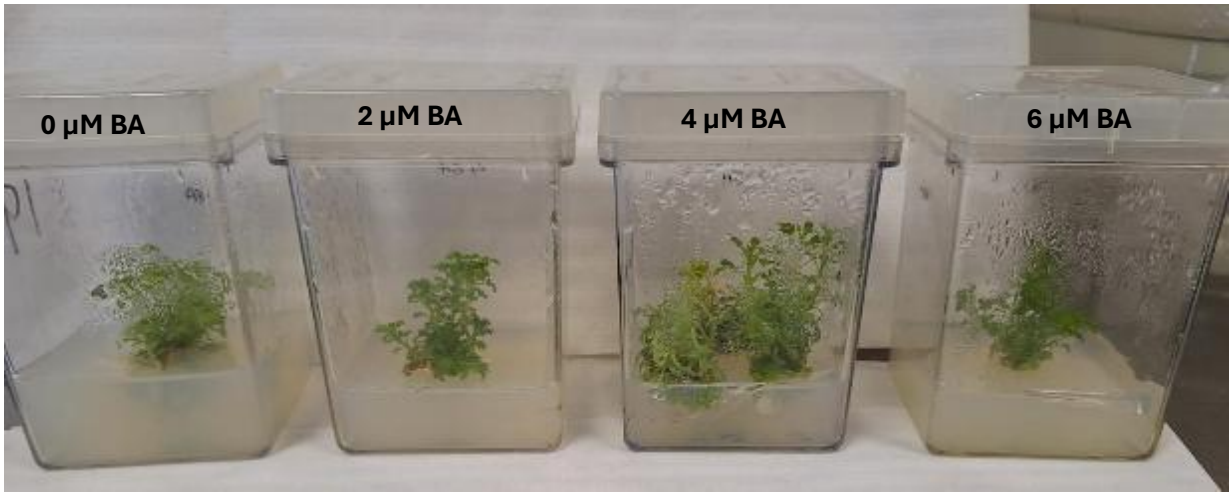

Figure 5.1. *Artemisia afra* regenerated *in vitro* from nodal explants cultured on medium with different BA concentrations.

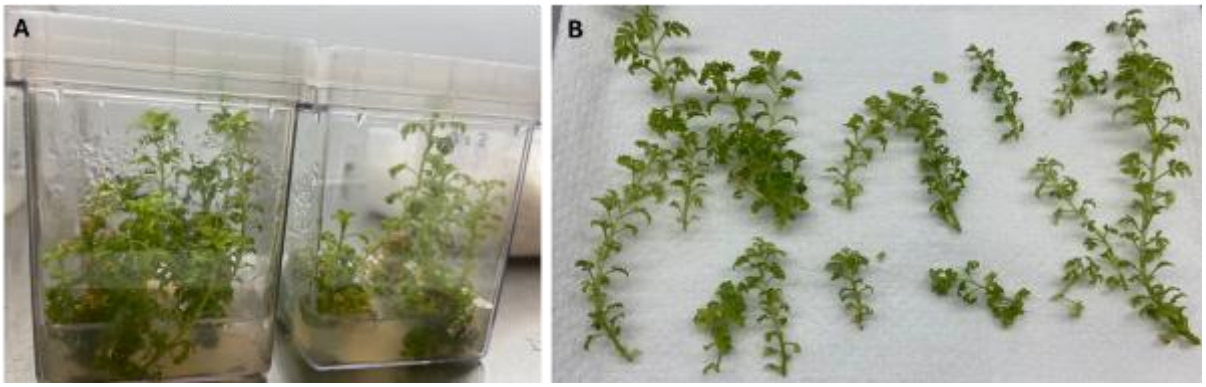

Figure 5.2. *In vitro* shoot multiplication of *Artemisia afra* on media supplemented with *4* µM *meta*-topolin. Plantlets inside magenta vessels 8 weeks after culturing (**A**) and individual shoots separated from one shoot cluster (**B**), each having the potential to become a complete rooted plant.

Different species respond differently to plant growth regulator treatments. *Artemisia annua* nodal explants produced the highest number of shoots (8.05 ± 0.66/explant) when cultured on MS + 1 mg/l BAP + 0.1 mg/l IBA (Hailu *et al*., 2013). Similarly, *A. nilagirica* cultured on MS medium supplemented with 2.5 µM 6-benzylaminopurine (BAP) + 7.5 µM 2-isopentenyl (2-iP) resulted in the highest regeneration frequency (83.3 %) with the highest number of shoots (10.16 ± 2.24) (Shinde *et al*., 2016). However, nodal explants of *A. japonica* cultured on MS medium augmented with 2.5-10 µM kinetin (Kn) or 2-iP produced a single shoot whereas those cultured MS medium with 2.5-10 µM BAP resulted in multiple (20 per explant) shoot regeneration (Shinde *et al*., 2017).

Stem cuttings of *A. afra* were removed after 60 days to assess rooting in different growing media, as shown in Figure 5.3. The pictures in Figure 5.3 suggest poor rooting in cuttings dipped in Dynaroot and set in Hygromix. *Artemisia vulgaris* cuttings that were not treated with any plant growth regulator produced the lowest fresh root biomass (Njualem *et al*., 2003), suggesting a need for treatment with a plant growth regulator.

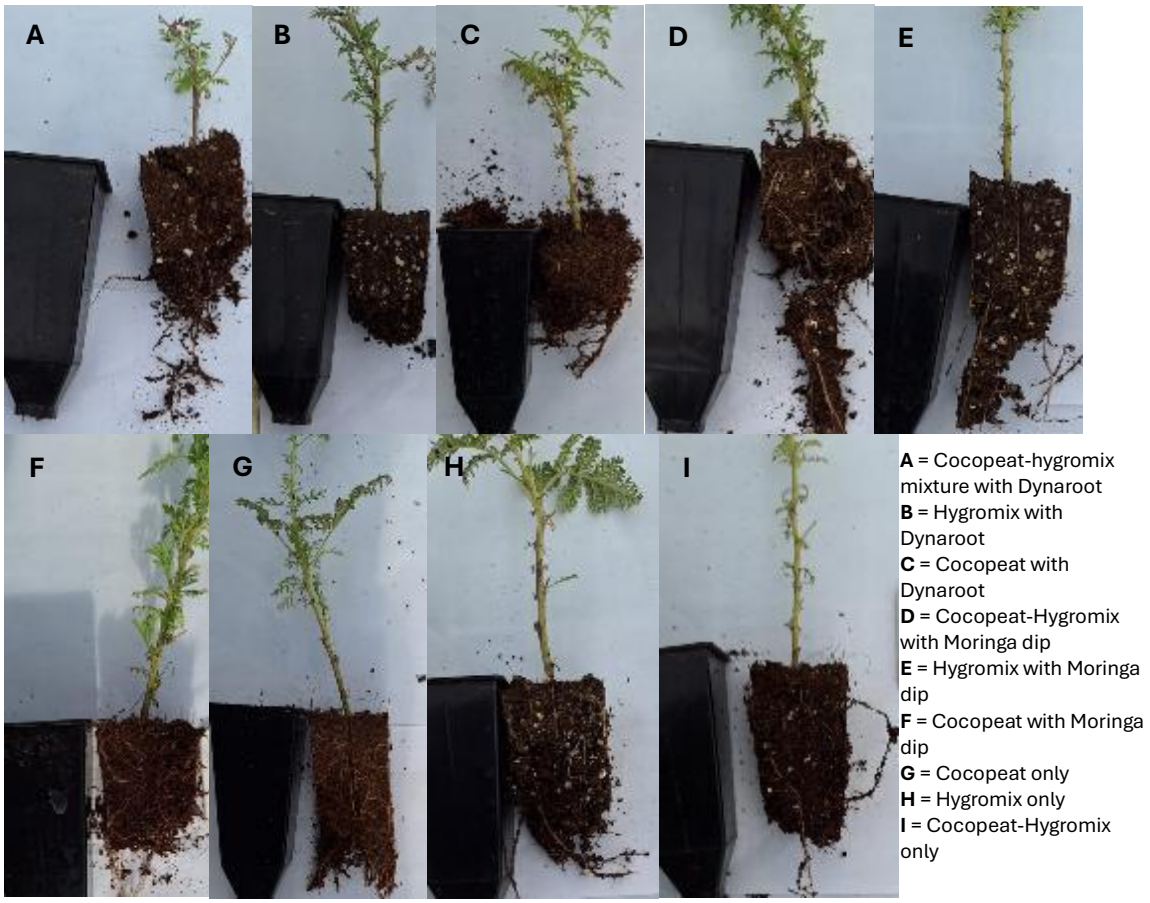

Figure 5.3. Cutting of *Artemisia afra* 60 days after treatment in various growing media and dipping in rooting stimulators.

Figure 5.4 shows that the control (no dipping in a plant growth regulator) with cocopeat as the growing medium recorded, on average, the highest survival percentage (40%) of *A. afra* cuttings, followed by Dynaroot-treated cuttings (22%) set in cocopeat. Nijualem *et al*. (2003) reported survival percentages ranging from 7.75 to 10% for *A. vulgaris* stem cuttings treated with IBA, natural rooting hormone, apple cider vinegar, coconut water, and *Aloe vera* gel. The time of harvesting cuttings also seems to affect cutting mortality, as cuttings of *Aloe aborescens* collected in February showed 44% mortality, 26% in April, and 5% in November (Fascella *et al*., 2012). This could be attributed to internal hormone distributions during different growth stages of the plants, since the exogenous application of the auxin NAA did not influence the percentage of cutting mortality or the number of rooted cuttings (Fascella *et al*., 2012).

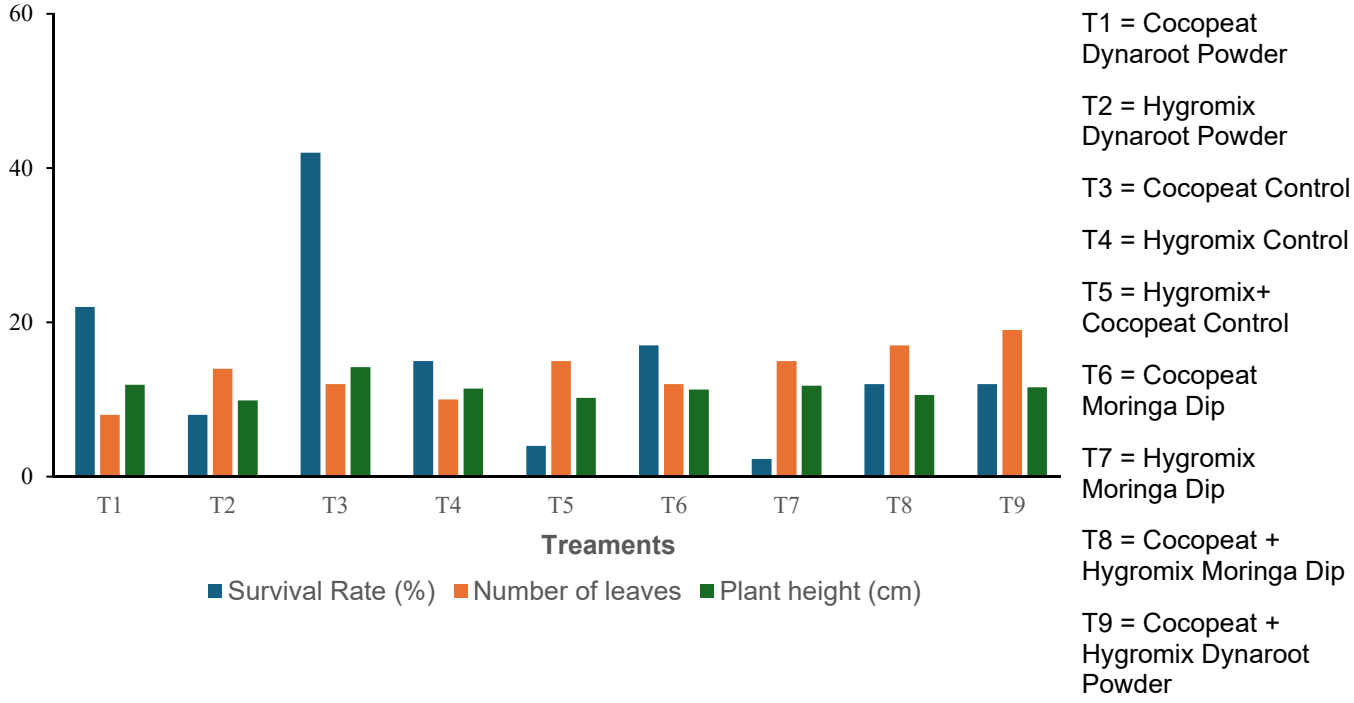

Figure 5.4. The survival rate (%), number of leaves, and plant height (cm) of *Artemisia afra* 60 days after treatment initiation, in response to different growing media and rooting stimulation.

The survival percentage and root length parameters, as influenced by the delayed application of growth hormones to *A. afra* stem cuttings, are presented in Figure 5.5. No significant difference was observed in the survival rate between T2 and T6; however, T2 had a significantly higher survival rate than the control. Treatments 1, 2 and 5 produced significantly longer roots than the control. The longest roots recorded were 4.9 cm in T1, followed by T2 with a root length of 4.2 cm. In contrast, the shortest shoot length of 0.1 cm was observed in T7.

Applying rooting hormone either immediately at setting or delaying its application had a significant effect on the survival rates of the cuttings. The lowest survival rate (3%) occurred when the rooting hormone was applied immediately at setting (T1), and this was not significantly different from applying at different weeks after setting of the cuttings (T3, T4, T5 and T7). Although the differences were not statistically significant, the maximum survival rate (68.3%) was observed at T2, followed by T6 (58.3%) (Figure 5.5 B). There is currently no published information on how delaying the application of rooting hormone affects the survival of *A. afra* cuttings.

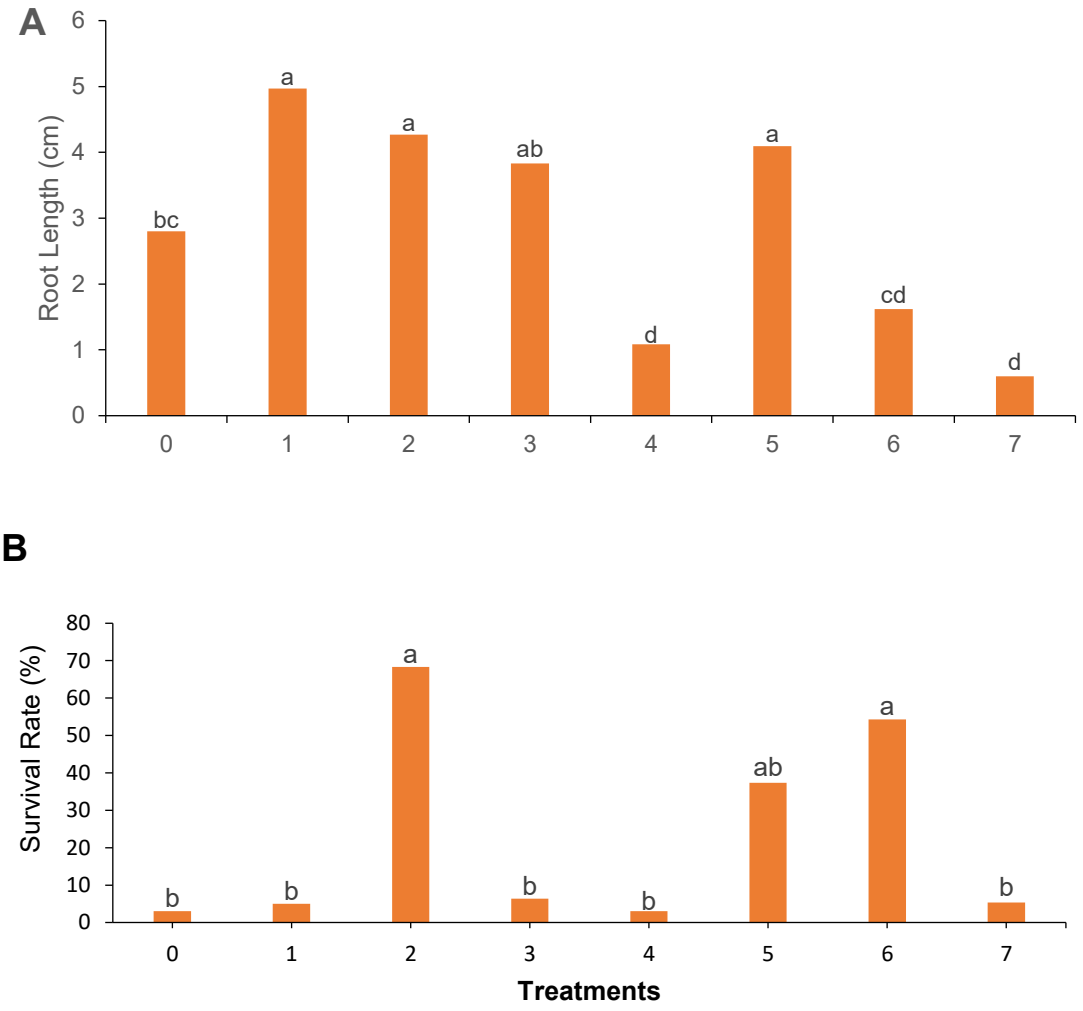

Figure 5.5. Root length (A) and seedlings survival rate (B) of *Artemisia afra* cuttings in response to delayed application of rooting hormone.

Delaying the application of rooting hormone by two weeks increased the survival rate of *A. afra*; however, the ability of cuttings to root can be influenced by both internal and external factors (Giancarlo *et al*., 2012). Internal factors include the plant's genotype, nutritional status, and phenological stage, which are closely related to the levels of plant growth regulators required for the rooting process (Lynch *et al*., 2012). External

factors include environmental conditions such as temperature and light intensity. Mngomba (2023) investigated the effects of delaying the application of rooting hormone and the timing of stem cutting collection on the rooting ability and root growth of *Flacourtia indica* and concluded that the delayed application of Seradix® No. 2 enhances root development. The results of the current study contrast with those reported by Lodama *et al*. (2016) in *Lobostemon* species, where treatment applied immediately at setting resulted in the highest survival percentages. Luckman and Menary (2000) noted factors that may affect the results, for example, differences in the amount of auxin that is absorbed or adheres to cuttings. Figure 5.6 shows roots observed on cuttings exposed to (a) the delayed application of rooting hormone by one week, (b) two weeks, and (c) six weeks.

Figure 5.6. Rooted *Artemisia afra* cuttings after delayed application of rooting hormone (A = control, B = 1 week, C = 2 weeks, D = 4 weeks, E = 6 weeks of delayed application).

5.4 CONCLUSIONS

Successful micropropagation starts with culture initiation, followed by shoot induction, shoot multiplication, shoot elongation, rooting, hardening off and planting out. However, the lack of facilities for resource-poor farmers and the costs of micropropagated plant material necessitate investigating other, less expensive vegetative propagation methods. Stem cuttings of *A. afra* can be used for species propagation without phytohormone treatment, using cocopeat as the growing medium. This is an advantage, as phytohormones are considered emerging pollutants in the environment. The study results showed that delaying the application of rooting hormones could enhance the survival of cuttings. However, less expensive and readily available growing media, such as sand, need to be investigated. It should be noted that variation in the physical and chemical properties of sand as a growing medium may affect the repeatability and consistency of the results.

CHAPTER 6

THE RESPONSE OF *ARTEMISIA AFRA* TO DIFFERENT WATERING REGIMES

6.1 INTRODUCTION

Highly valued medicinal plants are becoming increasingly inaccessible to the traditional medicine industry. There is a major concern that several species are becoming extinct in the wild due to over-harvesting, driven by high demand for medicinal plants used to treat or manage various ailments. This presents an opportunity to research and develop ways to domesticate, cultivate, and agro-process such highly demanded species (Van Wyk, 2011). *Artemisia afra* has been identified as one of the most popular species in South Africa, with commercial production potential because of its variety of medicinal uses (Van Wyk, 2008). Although studies suggest that its cultivation presents a financially viable opportunity in South Africa, sustained cultivation practices are scarce.

The main constituents of essential oils from *A. afra* are typically 1,8-cineole (eucalyptol), thujone, camphor, and borneol, as well as sesquiterpenoids (chrysanthenyl acetate) (Mukinda and Syce, 2007). Due to various environmental effects, the essential oil industry faces several challenges, including inconsistent quality, variable supplies, and variable active ingredient content. This has encouraged end-users to rely on synthetic oils to address these issues.

Although wild-harvested medicinal plant resources are sometimes regarded as more effective than cultivated resources, domestic cultivation is a widely used and accepted practice (Gepts, 2006). Cultivation enables the use of new techniques to address problems encountered in the production of medicinal plants, such as toxic components (e.g., heavy metals) in uncontrolled environments, low active-ingredient contents, and misidentification or contamination with unwanted plant material (Raina *et al*., 2011). Cultivation under controlled growth conditions can boost yields of active compounds, which are almost always secondary metabolites, and ensure production stability. To improve target-product yields, cultivation practices are designed to provide optimal levels of water, nutrients, optional additives, and environmental factors such as temperature, light, and humidity (Chen *et al*., 2016). Furthermore, increased cultivation contributes to a decrease in the volume of wild-harvested medicinal plants and benefits the recovery of their wild resources (Hamilton, 2004). With the increase in agricultural water use, there is a need to improve crop water use efficiency to increase crop production. Rainfall cannot meet plants' water needs in semi-arid areas such as South Africa and must be supplemented with irrigation (Neal *et al*., 2011). Irrigation is the artificial supply of water to land for growing crops or other vegetation where rainfall cannot provide the required water for sustained growth and crop production (https://southafrica.co.za/irrigation.htm). Agricultural production has shifted from the country's centre to high-rainfall areas such as the KwaZulu-Natal Midlands and the Cape Coastal regions (Dickinson *et al*., 2004). However, in most regions of South Africa, irrigation water scarcity is still a major limiting resource for agricultural production, and encouraging farmers to adopt simple techniques that optimise production could help alleviate the problem.

Water and nutrient management are important because these factors can affect plant growth, biomass yield, and chemical composition (Mofokeng *et al*. 2015). Careful integration of fertiliser and irrigation is necessary to minimise the risk of nutrient leaching. Water is commonly one of the most important environmental constraints to plant survival and crop productivity in agricultural systems. An inadequate (or excess) water supply to plants can slow seed germination and/or hinder plant growth, leading to decreased yields (Vandoorne *et al*., 2012). As aridity increases due to climate change, fresh water will likely become even scarcer in the future. Although irrigation systems are expensive to install and maintain, they can potentially increase crop yields and contribute to the sustainable cropping of medicinal plants. The primary objective of an irrigation system is to supply the plant with the optimal quantity and quality of water (appropriate application rate), at the appropriate time, with minimal losses, and as economically as possible (Reinders, 2011). Therefore, irrigation management's purpose is to ensure that the crop is always provided with access to adequate water.

There are no reports on the water requirement, water use or irrigation management of *A. afra*, except for a study by Koehorst *et al*. (2010), which focused on the plant's response to various pH levels under hydroponic cultivation. Moisture stress can reduce the potential yield of an irrigated *Artemisia* species at any point during the growing season. Seasonal water consumption for *Artemisia* is approximately 250 to 420 mm, but this can vary with climate, agronomy and chemotype (http://maison-artemisia.org). When *A. annua* plants were irrigated at 40, 80 and 120 mm soil moisture deficits, 150, 240 and 480 mm of water were applied per season (Scarcella *et al*., 2011). Once established, *A. afra* seems to be able to withstand more prolonged periods of drought (Setshedi *et al*., 2022). After establishment, the frequency of watering decreases (Patil *et al*., 2011). In Tanzania, wild *A. afra* populations are found in regions that receive rainfall ranging from 900 to 2400 mm per year (FAO, 1986). By contrast, the cultivation of *A. annua* demands a significant amount of water, as each plant requires plentiful water both in the morning and evening during the dry season (http://maison-artemisia.org). This requirement can be reduced in line with rainfall during the wet season. *Artemisia annua* can be cultivated during the dry season; however, the results may not be as satisfactory as they would be during the wet season. During the first three months of establishing *A. afra* in the field, meeting its water requirements is of utmost importance (WHO, 2006). It is crucial to avoid excessive water when cultivating both species, as it may cause nutrients to leach from the plant or reduce root depth (Patil *et al*., 2011). In addition, *Artemisia* sp. is susceptible to waterlogging. As a result, drainage channels are required to cultivate *Artemisia* sp. successfully (Anonymous, 2009; Liu *et al*., 2009).

Moisture stress applied to *Artemisia* at various growth stages significantly affected all recorded yield and yield components, including water use efficiency. Significantly higher yields, such as fresh leaf weight, fresh biomass, and essential oil yield, were recorded under higher irrigation without moisture stress at any growth stage, despite low water use efficiency (Meskelu *et al*., 2021). Exposing *A. annua* plants to 38 and 62 hours of no irrigation increased the leaf artemisinin content, with 38 hours of no irrigation significantly increasing both leaf and plant artemisinin yield (Marchese *et al*., 2010). Soni and Abdin (2017) also reported a significant reduction in yield of *A. annua* under water stress; however, there were slight increases in the artemisinin content under severe water stress. Maximum economic yields (fresh leaf mass and essential oil yield) of *A. annua* were obtained with irrigation to field capacity (100% ETc), whereas 50% ETc produced

the maximum water use efficiency (Tesfaye *et al*., 2021). This finding suggests that *Artemisia* plants can be grown under optimal conditions to increase total biomass yield, and then exposed to a brief stress period to increase the essential oil content.

6.2 RESEARCH METHODOLOGIES

The studies were conducted at the ARC - Vegetable, Industrial and Medicinal Plants campus at Roodeplaat, Pretoria. The weather data (Figure 6.1) during the experimental period was collected from a weather station (Campbell Scientifc, USA).

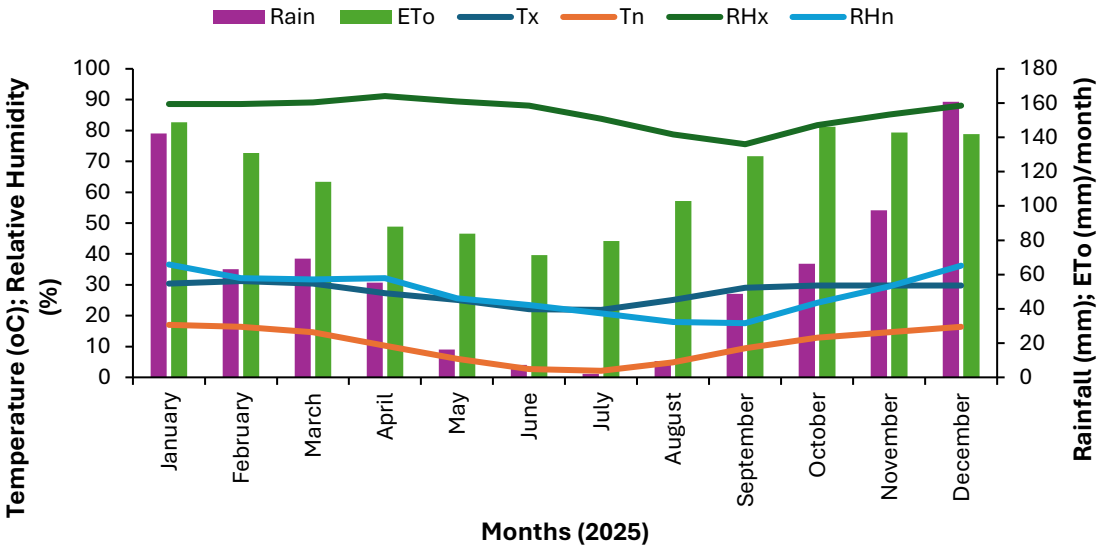

Figure 6.1. Weather conditions at the experimental site during the year 2025.

6.2.1 Terminal water stress on *Artemisia afra*

This experiment was a glasshouse study aimed at investigating the effects of different water-stress levels before harvesting on the yield and quality of *A. afra*. It was a 2x2 factorial study arranged in a randomised complete block design (RCBD), where six-month old *A. afra* plants, in 25-cm diameter pots filled with a mixture of topsoil and river sand (3:1, v/v) were established in a glasshouse. The study consisted of two factors *viz*. factor 1 = harvesting periods (HP) with two separate harvests (harvesting at 30 days and 60 days; HP_1 and HP_2) and factor 2 = terminal water stress (TWS) at two levels (no terminal water stress and one-week of terminal water stress; TWS_0 and TWS_1), with each treatment combination consisting of six replicates and each replicate had three data plants. The plants in treatment HP_1 were harvested 30 days after establishment in the glasshouse, and the HP_2-treated plants were harvested 60 days after establishment. In both harvesting treatments, the plants were exposed to terminal water stress by withholding water for 7 days. The treatment combinations were HP_1TWS_0 (T1), HP_1TWS_1 (T2), HP_2TWS_0 (T3) and HP_1TWS_1 (T4).

Soil moisture was monitored using a Hydro-sensor II soil moisture sensor (Campbell Scientific®, South Africa), which was pre-calibrated before use. The instrument uses volumetric water content at field capacity and at the permanent wilting point, determined during calibration, to automatically calculate soil moisture deficits after inserting 20 cm sensor rods into the soil or growing media.

6.2.2 Deficit irrigation effects on *Artemisia afra*

A randomised complete block design (RCBD) was used to set up the experiment with six replicates and four treatments, in a rainout shelter. The rainout shelter is designed to exclude rainfall from the experiment by automatically closing when raindrops fall onto a sensor. The physical and chemical properties of the soil in the experimental site are given in Table 6.1.

Table 6.1. Physical and chemical properties of the soil at the experimental site.

Depth (cm)	Sand (%)	Silt (%)	Clay (%)	Texture	PWP (%)	FC (%)	BD
0-20	78	6	16	sandy loam	10.3	19.9	1.59
20-40	70	8	22	sandy clay loam	12.9	25.5	1.56
40-60	64	8	28	sandy clay loam	15.2	25.3	1.45
60-80	62	8	30	sandy clay loam	14.7	28.0	1.38
80-100	60	8	32	sandy clay loam	15.1	28.1	1.35

	Fe	Mn	Cu	Zn	P	K	Ca	Mg	Na	Total N	pH
	mg/kg									%	
Top soil	13.74	44.10	9.24	14.00	80.90	134	980	298	24.7	0.20	7.26
Sub soil	9.74	28.50	5.64	7.43	60.40	94	1201	370	39.4	0.21	7.44

The four predetermined water-stress treatments were applied by allowing a specified percentage of plant-available water to be depleted before irrigating back to field capacity, and this was repeated throughout the growing season of *A. afra*. The allowable depletion levels (ADL) were applied as 20–25% ADL (T1, severe stress), 40–45% ADL (T2, moderate stress), 60-65% ADL (T3, mild stress), and 80–85% ADL (T4, well-watered). Soil moisture content was monitored daily using an Onset MX2304 HOBO weatherproof soil moisture logger. The HOBO sensors were installed at a depth of 25 cm in the centre of each treatment plot (Figure 6.1), and the logger recorded data every 2 h. The plants, which were spaced 1 m x 1 m, were cut back to 30 cm before treatment initiation and then allowed to grow under different water-stress conditions (Figure 6.2).

The allowable soil water deficits for the four water levels were calculated using the field capacity (FC), permanent wilting point (PWP), and bulk density (BD) at a depth of 20 cm.

PAW $_{(0\text{-}300mm)}$ = ((FC-PWP) x BD) x 300 mm

= ((0.199 – 0.103) x 1.59) x 300 mm

= 45.79 mm

20-25% deficit = 9.16-11.45 mm

40-45% deficit = 18.32-20.66 mm

60-65% deficit = 27.45-29.76 mm

80-85% deficit = 36.63-38.92 mm

Figure 6.2. *Artemisia afra* plants under drip irrigation to initiate water stress trials (A and B) and installation of water sensors (C).

Figure 6.3. *Artemisia afra* plants growing under different irrigation regimes.

6.2.3 Data collection and statistical analysis

Plant height was measured with a tape measure, and the number of branches was counted manually. Fresh plant biomass was weighed at harvest and then sun-dried to obtain dry plant biomass. Moisture content was determined using the AOAC 930.15 standard method, as described by Hasizah *et al*. (2022), as follows:

$$MC = m_w/m_{ds}\ x\ 100\% \quad (1)$$

$$m_w = m_s - m_{ds} \quad (2)$$

where M_C is moisture content, m_w is the weight of water and m_{ds} is the weight of dry solids; and m_s is the fresh weight of the sample.

Figure 6.4. Data collection on *Artemisia afra* plants.

Total leaf area was measured with a leaf area meter (LI-3300, Li-Cor Inc., Lincoln, USA) after harvesting. The relative water content (RWC) was calculated using the formula described by Soni and Abdin (2017).

$$RWC = [(FW - DW)/(\text{turgid weight} - DW)] \times 100 \quad (3)$$

where FW = fresh weight, DW = dry weight. Turgid weight was measured by floating a leaf sample in distilled water for four hours, then blotted dry and weighed.

Electrolyte leakage (EL) was calculated according to Abbaspour and Ehsanpour (2016). Leaf samples weighing 0.2 g were placed in test tubes containing 10 ml of double-distilled water and incubated on a shaker at 25 °C for 6 hours. Thereafter, the electrical conductivity (EC_1) was measured. The samples were further autoclaved at 120 °C for 20 minutes, allowed to cool at room temperature and the final electrical conductivity (EC_2) was measured.

$$EL = (EC_1/EC_2) \times 100 \quad (4)$$

where EL = electrolyte leakage, EC_1 = electrical conductivity of the leaf sample, EC_2 = electrical conductivity of the leaf sample after autoclaving.

Water use efficiency (WUE) was calculated following Hunt and Kirkegaard (2009), where the total amount of water applied was divided by the yield and expressed as $g \cdot mm^{-1}$, based on fresh yield.

Data were subjected to Analysis of Variance (ANOVA) and for the glasshouse study, a two-way ANOVA was employed using *GenStat* 64-bit (Version 24.2) by VSN International Ltd. Treatment means were separated using Fisher's protected *t*-test for least significant differences (LSD) at 5% level of significance.

6.2.4 Chemical analysis and profiling

Freeze dried powdered samples of *A. afra* were extracted using ultra-LC methanol, sonicated for 60 minutes, then centrifuged and dried. The plant extracts were prepared for analysis by reconstituting with 1:1 acetonitrile: ultra-LC water and filtered.

The UPLC-QTOF-MS (Waters Synapt XS QTOF with ACQUITY UPLC I-Class PLUS) was used for analysis, with a Waters Acquity UPLC BEH C18 column (100 mm, 2.1 mm, 1.7 mm particle size). The mobile phase consisted of 0.1% formic acid in water (Solvent A) and 0.1% formic acid in acetonitrile (Solvent B), with a flow rate of 0.45 mL/min. Profiling, following analysis in the positive and negative ion modes, was performed using MassLynx 4.1 and Waters Connect software, with the Waters UNIFI Scientific Information System as the database. Table 6.2 shows the gradient elution for the analysis of the samples.

Table 6.2. Gradient elution during the analysis of *Artemisia afra* samples.

Time (min)	% Solvent A	% Solvent B	Curve
Initial	98	2	Initial
0.50	98	2	6
11.00	70	30	6
15.30	60	40	6
15.60	0.0	100	6
18.00	0.0	100	6
20.00	98	2	1

Desolvation gas: Nitrogen (flow rate of 400 L/h); Capillary voltage: 2.3 kV; Sampling cone voltage: 30V; Desolvation temperature: 300 °C; Source temperature: 120 °C; Electro spray ionisation (ESI) modes: positive and negative

6.3 RESULTS AND DISCUSSIONS

6.3.1 The effect of terminal water stress on *Artemisia afra*

The interaction between harvesting frequencies and terminal water stress (HP x TWS) had a significant effect ($p \leq 0.01$) on both fresh weight and the number of branches. This interaction accounted for 10% and

78% of the total variation in these variables, respectively (Table 6.3). Terminal water stress alone (TWS) had a highly significant effect on fresh weight, plant height, and number of branches, accounting for 83%, 94%, and 18%, respectively, of the total variation. Harvesting period (HP) had a significant effect only on fresh weight and plant height.

Table 6.3. Responses of fresh weight, plant height and number of branches to harvesting time and terminal water stress interaction under glasshouse conditions.

Sources	DF	**Fresh weight (g · plant^{-1})**		**Plant height (cm)**		**No. of branches**	
		MS	TTV %	MS	TTV %	MS	TTV %
Replication	5	227.3	0	113.58	1	112.97	3
Harvesting Period (HP)	1	4753.1	6**	378.12	4*	0.06	0^{ns}
Terminal water stress (TWS)	1	69378.1	83**	9135.01	94**	722.00	18**
HP x TWS	1	8778.1	10**	6.12	0^{ns}	3173.39	78**
Error	63	538.5	1	77.83	1	42.25	1
Total	71	83675.1	100	17415.83	100	4050.67	100

DF = ; MS = mean separation; TTV = total treatment variation; ns = not significant

* = ; ** =

Treatment combination HP_2TWS_0 significantly increased the average fresh weight to 120 g·plant^{-1}, and the number of branches per plant was increased to 41.33 (Table 6.4). Terminal water stress had a significant effect on plant height, with TWS_0 having the highest value of plant height (74.19 cm) (Figure 6.4). Harvesting frequency (HP_2) resulted in a significantly higher plant height of 65.22 cm. Other studies have shown that moisture stress during different growth stages reduced plant height in crops, including *A. annua* (Soni & Abdin, 2017; Meskelu *et al*., 2021; Tesfaye *et al*., 2021). When exposed to pH levels ranging from 4.5 to 8.5 in a hydroponics system, the reported average fresh weight ranged from 43 to 100 g·plant^{-1} (Koehorst *et al*., 2010), which is comparable to the fresh weights reported in the current study. Water deficit significantly affected the growth and leaf dry mass of *Leonotus dysophylla* and other medicinal plants in a study by Netshiluvhi and Eloff (2016).

Table 6.4. Two-way matrix for fresh shoot mass and number of leaves as affected by the interaction of harvesting time and terminal water stress under glasshouse conditions.

Harvesting Frequencies (HP)	**Total Fresh Weight (g · plant^{-1})**		**Number of branches**	
	Terminal Water Stress (TWS)			
	TWS_0	TWS_1	TWS_0	TWS_1
HP_1	81.67^{b}	41.67^{c}	34.94^{b}	21.72^{d}
HP_2	120.00^{a}	35.83^{c}	41.33^{a}	28.00^{c}
LSD	15.458		4.329	

Different letters indicate a significant difference ($p<0.05$) between treatments

HP1 = harvesting at 30 days; HP2 = harvesting at 60 days; TWS0 = no terminal water stress; TWS1 = one-week of terminal water stress

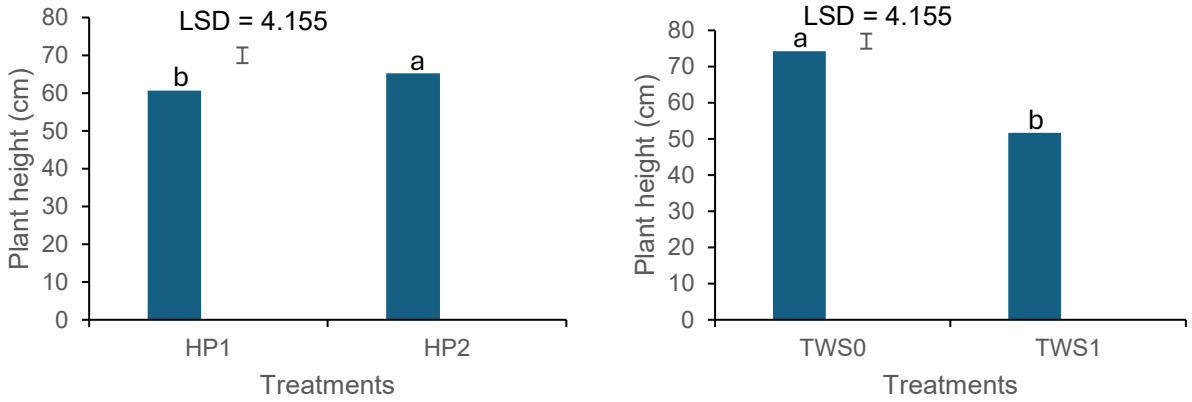

Figure 6.5. Plant height of *Artemisia afra* in response to harvesting frequency and terminal water stress. HP1 = harvesting at 30 days; HP2 = harvesting at 60 days; TWS0 = no terminal water stress; TWS1 = one-week of terminal water stress

The chemical profiles of the *A. afra* samples exposed to different harvesting periods and terminal water stress levels are shown in Figure 6.6 and Table 6.5 (ESI positive mode). The results show that combination treatment 1 (HP1TWS0) had 27 compounds detected, treatment 2 (HP1TWS1) had 30 compounds, treatment 3 (HP2TWS0) had 29 compounds, and treatment 4 (HP1TWS1) had 24 compounds. The extra compound in treatment 2 is Chlorogenic acid, which was not present in any of the other treatments. Chlorogenic acid is known to enhance plant resistance to adverse conditions (Kai *et al*., 2021). Celastrol, which was identified in treatment 4 only, was previously isolated from *Tripterygium wilfordii* and reported to alleviate stress induced cell damage in cucumber seedlings (Zhu *et al*., 2017). Isochlorogenic acid which was identified in all treatments, was also reported in *Artemisia sieberi* (Jamal *et al*., 2023).

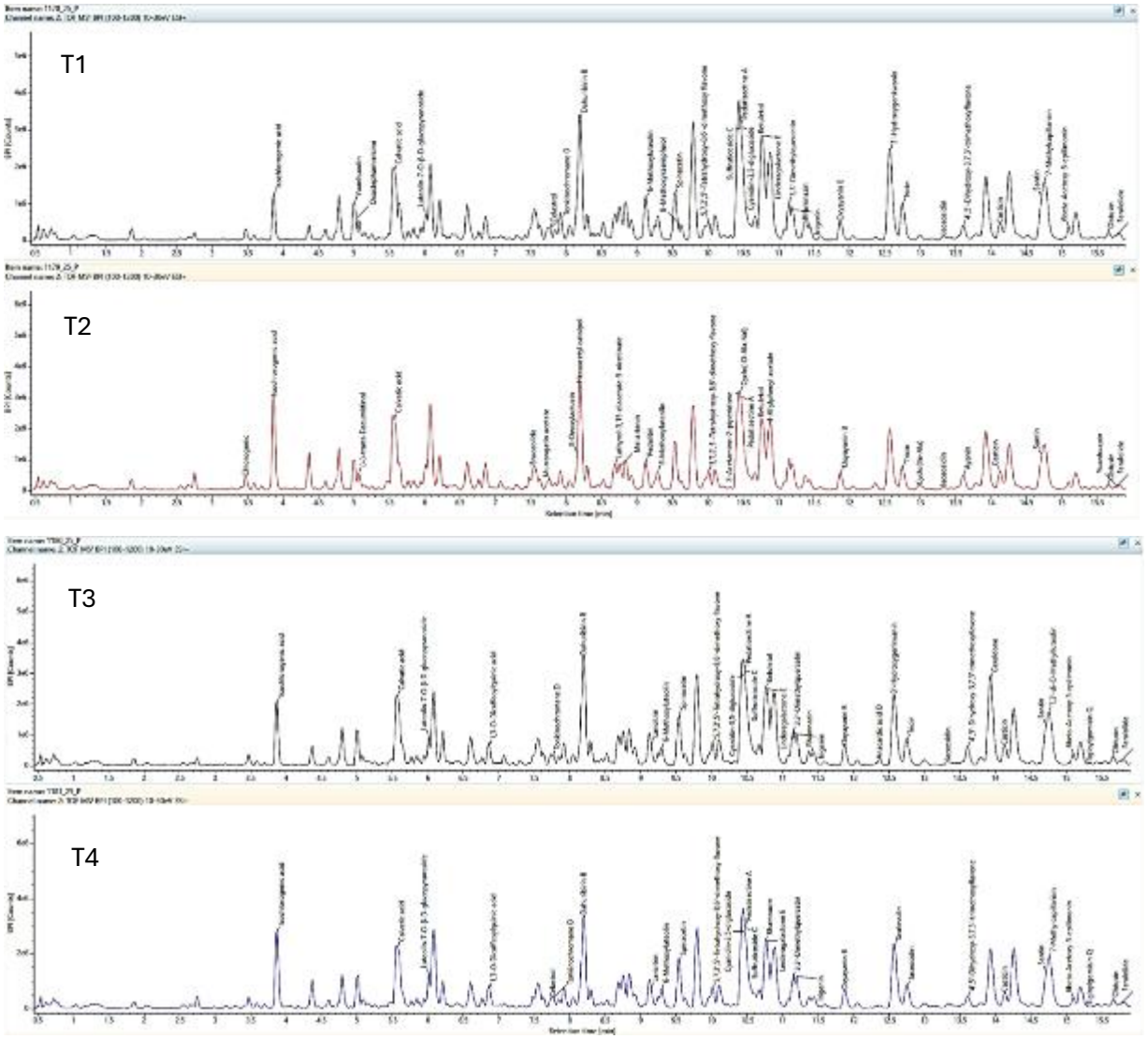

Figure 6.6. Chromatogram of *Artemisia afra* samples (ESI positive mode) exposed to different harvesting periods and terminal water stress levels (T1: HP_1TWS_0, T2: HP_1TWS_1, T3: HP_2TWS_0, T4: HP_1TWS_1).

Table 6.5. The number and names of compounds tentatively identified (ESI positive mode) in *Artemisia afra* sample exposed to different treatments.

*Trt	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30
1		X		X	X		X	X					X									X			X					X
2	X	X	X			X	X			X	X				X	X		X	X	X	X	X				X	X	X	X	X
3		X					X	X	X				X				X					X	X	X	X	X				X
4		X					X		X			X	X				X					X	X		X	X				X

31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	Total
X	X		X	X	X		X	X	X	X	X	X		X	X		X	X	X	X	X			27
			X			X						X	X	X		X	X	X			X	X	X	30
X	X		X	X			X	X	X	X	X	X		X	X		X	X		X	X	X		29
X	X		X					X	X					X	X		X	X	X	X	X	X		24

*TRT= Treatments - T1: HP_1TWS_0, T2: HP_1TWS_1, T3: HP_2TWS_0, T4: HP_1TWS_1

1	Chlorogenic	11	Bruceolide	21	Pedalitin	31	Cyanidin-3,5-diglucoside	41	Oxyayanin B	51	6beta-Acetoxy-5-epilimonin
2	Isochlorogenic acid	12	Celastrol	22	6-Methoxyluteolin	32	Suffruticoside C	42	3'-Hydroxygenkwanin	52	Obtusin
3	Syringetin-3-O-beta-D-galactopyranoside	13	Tonkinochromane D	23	Laricitrin	33	3'-Hydroxygenkwanin	43	Tricin	53	Feralolide
4	Yuanhuadin	14	Murrangatin acetate	24	Spinacetin	34	Betuletol	44	Cyclo(Ile-Ala)	54	Yuanhuapin
5	Oxostephamiersine	15	8-Deoxylactucin	25	8-Methoxykaempferol	35	Linderagalactone E	45	Jaceosidin		
6	(+)-trans-Decursidinol	16	Hexaacetyl catalpol	26	5,7,2',5'-Tetrahydroxy-8,6'-dimethoxy flavone	36	(+)-5,5'-Dimethoxy-9-O-beta-D-glucopyranosyl secoisolariciresinol	46	4',5'-Dihydroxy-3,7,3'-trimethoxyflavone		
7	Calvatic acid	17	Dahuribirin B	27	Cyclo(-D-Ala-Val)	37	4-Allylphenyl acetate	47	Ayanin		
8	Luteolin-7-O-β-D-glucopyranoside	18	Lathyrol-3,15-diacetate-5-nicotinate	28	3-Acetamino-2-piperidone	38	3,3'-Dimethylquercetin	48	Casticin		
9	1,3-O-Dicaffeoylquinic acid	19	Meliartenin	29	6-Hydroxy-indan-4-carbaldehyde	39	Irigenin	49	Santin		
10	1-[2-(3,4-Dihydroxyphenyl)-1-carboxy] ethoxycarbonyl-2-(3,4-dihydroxyphenyl)-7,8-dihydroxy-1,2-dihydronaphthalene-3-carboxylic acid	20	Caruilignan C	30	Pedatisectine A	40	Rhamnazin	50	7-Methylcapillarisin		

In the negative mode, treatment 2 (HP_1TWS_1) still showed the most compounds (31), followed by treatment 3 (HP_2TWS_0) with 29 compounds, treatment 4 (HP_1TWS_1) with 28 compounds and treatment 1 (HP_1TWS_0) with 21 compounds (Figure 6.7 and Table 6.6). Cachineside III and Tortoside A are additional compounds tentatively identified in treatment 2.

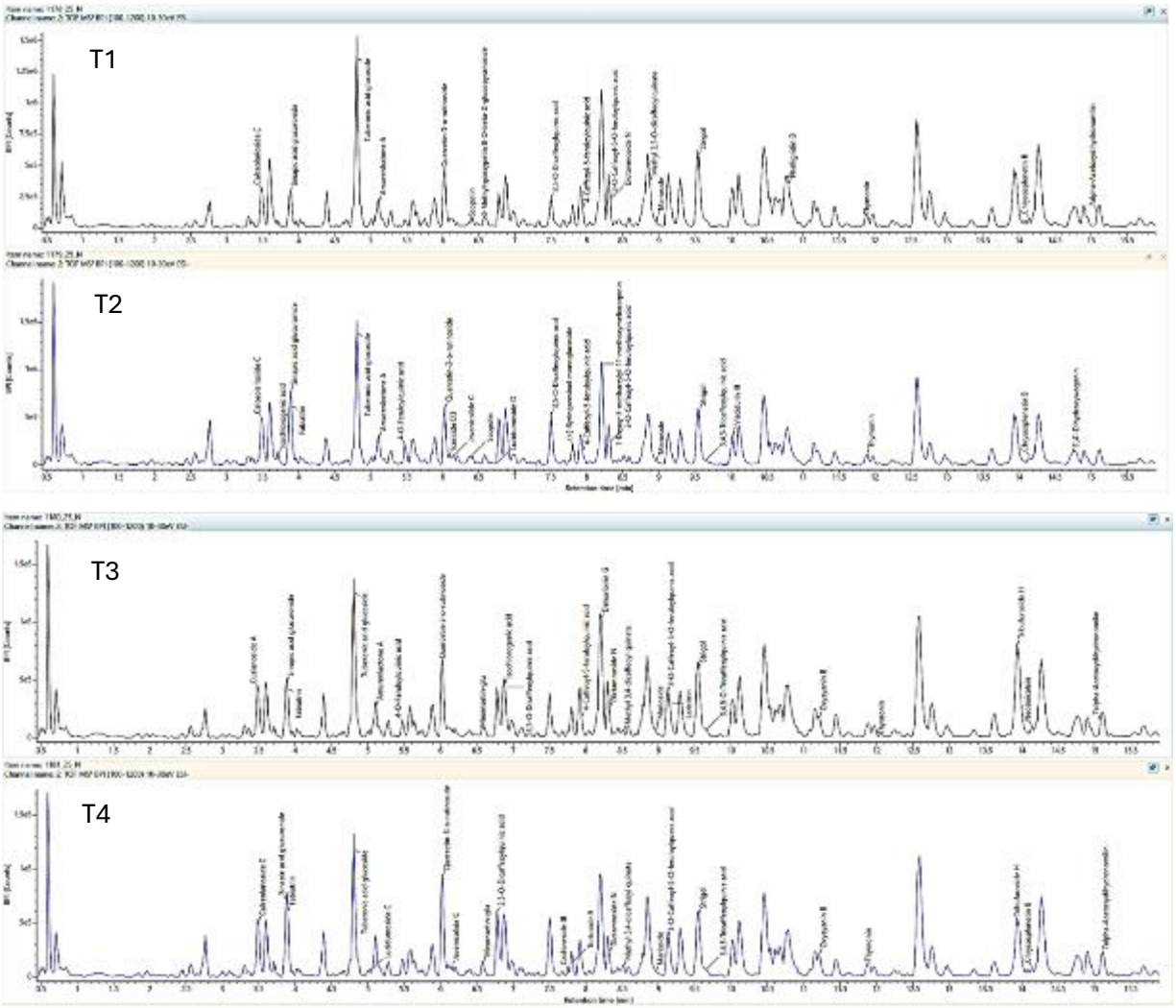

Figure 6.7. Chromatogram of *Artemisia afra* samples (ESI negative mode) exposed to different harvesting periods and terminal water stress levels (T1: HP_1TWS_0, T2: HP_1TWS_1, T3: HP_2TWS_0, T4: HP_1TWS_1).

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Table 6.6. The number and names of compounds tentatively identified (ESI negative mode) in *Artemisia afra* sample exposed to different treatments.

Trt	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
1	X		X	X			X	X	X		X	X			X	X			X			X		X
2	X		X	X	X	X	X	X	X	X	X			X		X	X	X	X		X	X	X	X
3	X	X		X	X	X	X	X	X	X			X		X	X			X	X		X	X	X
4	X		X	X		X	X		X	X						X	X		X		X	X	X	X

25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	Total
	X			X		X	X	X		X	X		X										21
X	X		X		X	X	X		X	X	X			X					X	X			31
	X	X	X			X	X			X		X	X	X	X	X	X						29
X	X		X		X	X	X			X	X		X	X	X	X					X	X	28

*TRT= Treatments - T1: HP_1TWS_0, T2: HP_1TWS_1, T3: HP_2TWS_0, T4: HP_1TWS_1

1	Apigenin 6-C-beta-xylopyranosyl-8-C-(6'''-O-(3-hydroxy-3-methylglutaroyl)-beta-glucopyranoside)	11	Scopolin	21	Javanicolide C	31	Marioside	41	Tribufuroside H
2	Cusianoside A	12	3-Methyl-gossypetin 8-O-beta-D-glucopyranoside	22	3-O-Caffeoyl-5-O-feruloylquinic acid	32	Strigol	42	Neobaicalein
3	Calceolarioside C	13	Mearnsetin-glu	23	Isorhamnetin 3-O-beta-D-glucopyranosyl-(1-2)-alpha-L-rhamnopyranoside	33	Phelligridin D	43	3,4,5-Tricaffeoylquinic acid
4	Sinapic acid glucuronide	14	19-O-[beta-D-Apiofuranosyl(1->2)-beta-D-glucopyranoyl]-3,14-dideoxyandrographolide	24	Dictamnoside N	34	(+)-Syringaresinol monoglucoside	44	Viscidulin III
5	Isochlorogenic acid	15	3,5-O-Dicaffeoylquinic acid	25	Mearnsetin-glu	35	Thymonin	45	3',4'-Dihydroxywogonin
6	Fabiatrin	16	4-Caffeoyl-5-feruloylquinic acid	26	Methyl 3,4-dicaffeoyl quinate	36	Chrysosplenetin B	46	Cachineside III
7	Tuberonic acid glucoside	17	3,5-O-Dicaffeoylquinic acid	27	Dahuribirin G	37	Laricitrin	47	Tortoside A
8	Amurenlactone A	18	Icariside D3	28	11-(6-O-trans-Sinapoylglucopyranosyl)-gardendiol	38	7alpha-Acetoxydihydronomilin		
9	Quercetin-3-o-rutinoside	19	1-Deoxy-3-methacrylyl-11-methoxymeliacarpinin	29	Methyl 3,5-O-dicaffeoylquinate	39	3,4,5-O-Tricaffeoylquinic acid		
10	4-O-Feruloylquinic acid	20	Pseudolaric acid C2	30	Lucidumoside D	40	Oxyayanin B		

6.3.2 The effects of deficit irrigation on *Artemisia afra*

The soil water deficits (in mm) before irrigation and the soil water content after irrigation are shown in Figure 6.8. The total amount of water applied over the treatment period was 248.03 mm (20-25%), 197.01 mm (40-45%), 166.6 mm (60-65%) and 152.02 mm (80-85%), for the respective treatments.

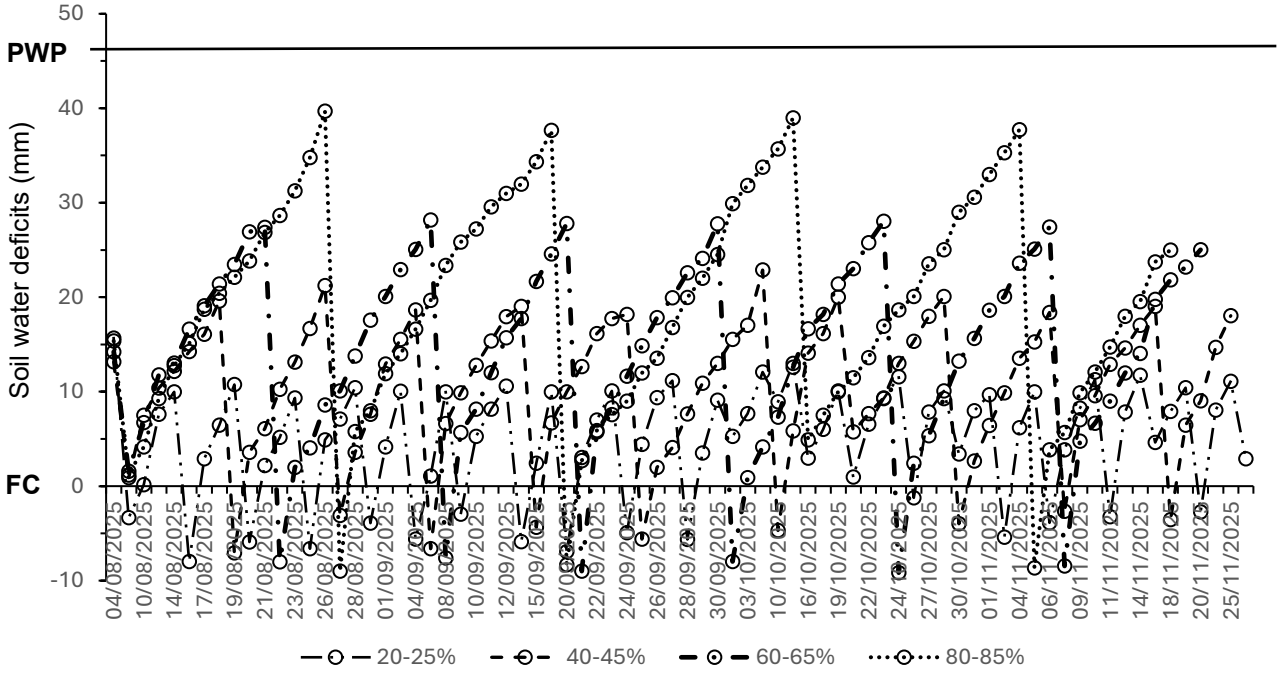


Figure 6.8. Fluctuations in soil water deficits for the top 300mm root zone, for the well-watered treatment (20-25%), mild water stress (40-45%), moderate stress (60-65%) and the severe stress treatment (80-85%).

Two harvests were conducted during the trial, with the first at 60 days after treatment application and the second at 120 days after treatment application. In both harvests, water stress had a significant effect on plant height in *A. afra*, with an inverse relationship observed (Figure 6.9). The maximum plant height (72.83 cm) was recorded when *A. afra* plants were exposed to 20-25% ADL, considered well-watered conditions, and the minimum plant height (63.21 cm) was recorded when plants were exposed to 80-85% ADL (severe water stress). No significant differences were observed amongst T2 (40-45%), T3 (60-65%) and T4 (80-85%) in both harvests.

Similar results, showing that increased water stress reduced plant height, were reported in various plants (Soni & Abdin, 2017). Mohamed & Abdu (2004) reported that limiting watering frequency caused water stress, which significantly reduced plant height. However, mild water stress may generally enhance growth in other plants (Shil & Dewanjee, 2022). Moisture stress at various growth stages can result in decreased plant height due to reduced photosynthesis (Jaleel *et al*., 2009). The height of *A. annua* plants, which ranged from 166.9 cm to 184.4 cm across irrigation levels, did not differ significantly (Scarcella *et al*., 2011). In the

current study, the maximum plant height was 72 cm, which was less than half the value reported by Scarcella *et al*. (2011). This difference may be due to the plant cutting back before treatment application.

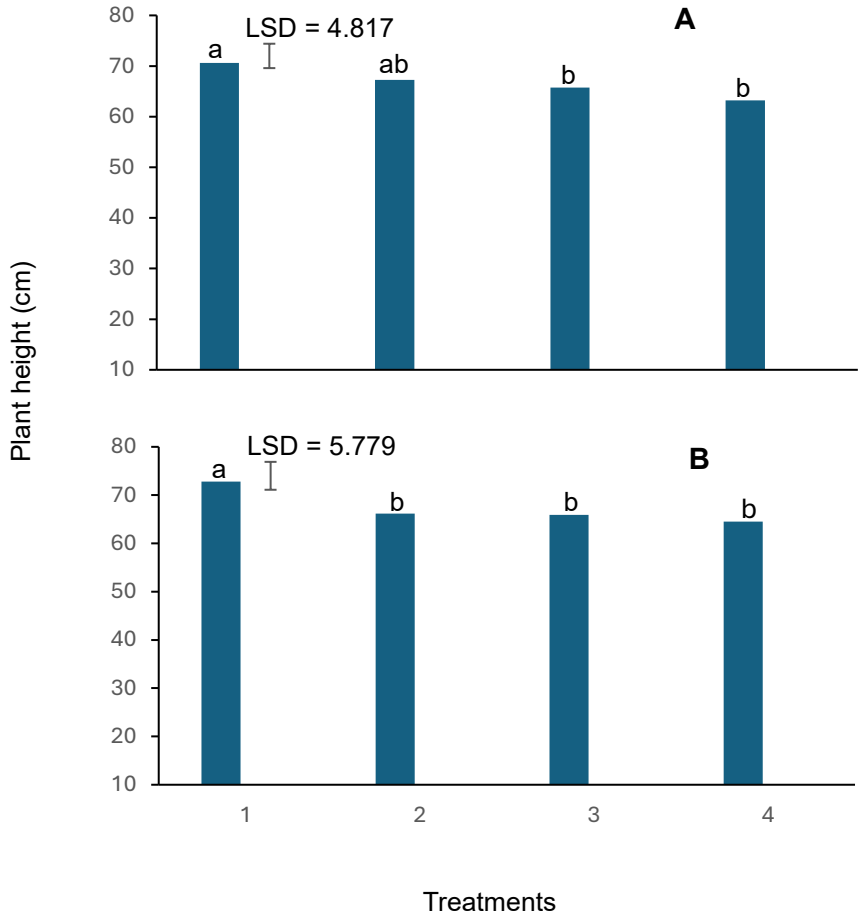


Figure 6.9. Plant height of *Artemisia afra* in the first harvest (A) and in the second harvest (B) under various water stress levels. Treatments = T1 20–25% allowable depletion level (ADL), T2 = 40–45% ADL, T3 = 60-65% ADL, T4 = 80–85% ADL.

There were no significant differences in fresh yield between the first and second harvests. However, there was a significant difference between the well-watered treatment and the water-stress treatments in total fresh yield of *A. afra* (Figure 6.10A). The well-watered treatment yielded 1168.8 g·plant^{-1}, whereas the water-stress treatments yielded 813.4 to 861.7 g·plant^{-1}. Based on their dry weight, the well-watered treatment had 74% moisture content, whereas the mild, moderate and severe water-stress treatments had 71%, 69% and 68% moisture content, respectively. There were no significant differences in dry weight across the different water-stress levels (Figure 6.10B). In the first harvest, the well-watered plants had significantly more branches than the water-stressed plants, with no significant difference between the water-stressed plants (Figure 6.10C). In the second harvest, there were no significant differences (Figure 6.10D).

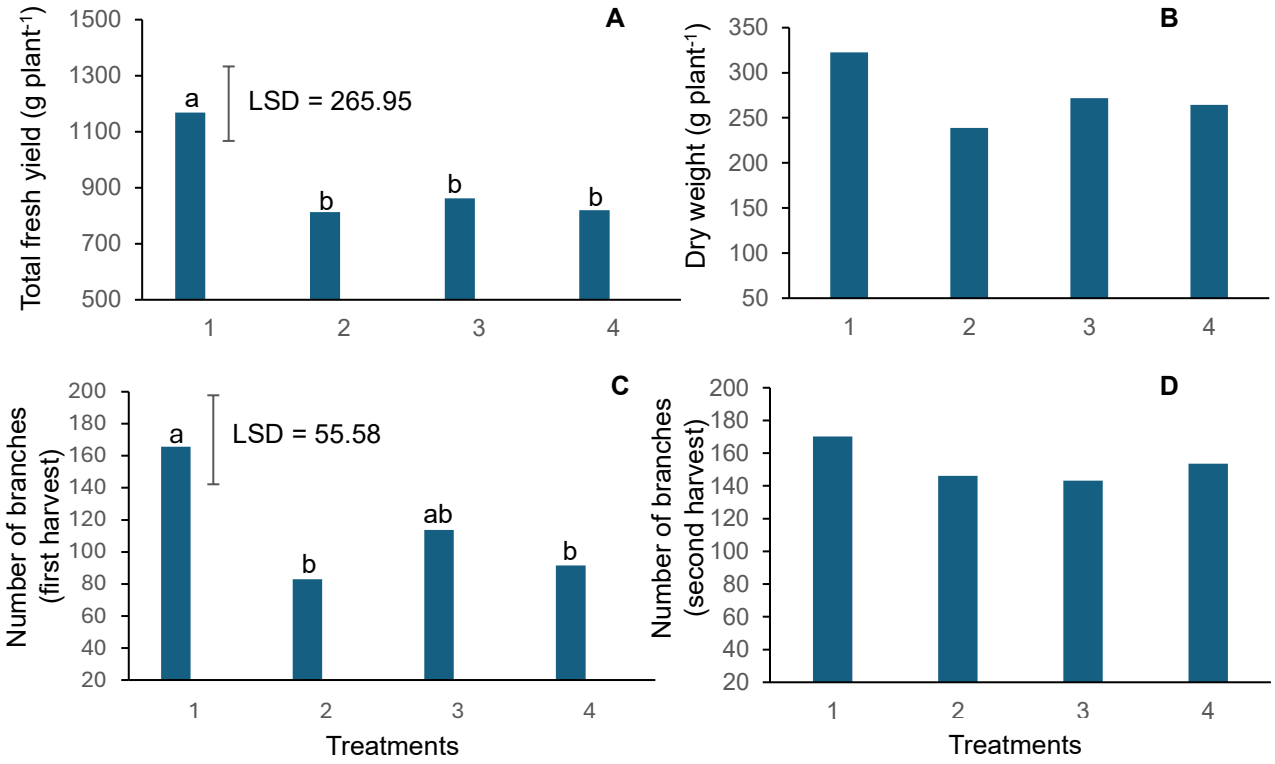


Figure 6.10. Total fresh yield (g·plant^{-1}) (A), dry weight (g·plant^{-1}) (B), number of branches in first harvest (C), and in second harvest (D) of *Artemisia afra* under different water stress treatments (T1 = 20-25% allowable depletion level (ADL), T2 = 40-45% ADL, T3 = 60-65% ADL, T4 = 80-85% ADL).

Compared with well-watered plants, the total fresh weight of *Ocimum basilicum* L decreased significantly under drought conditions, due to negative effects on both plant growth and productivity (Khalid, 2006). This may result from insufficient root biomass growth, low moisture availability around the roots, or reduced nutrient uptake (Staniszewska *et al*., 2003). Meskelu *et al*. (2021) reported that the fresh leaf weight of *A. annua* decreases as irrigation water levels decline, with a 49.4% reduction in fresh biomass under water stress. Maximum fresh leaf weight and biomass were achieved when *A. annua* plants were irrigated at 100% ETc (Tesfaye *et al*., 2021). Crop canopy and biomass reduction directly undermine production, resulting in significant decreases in both fresh and dry yields during severe water stress at 80–85% ADL (Yuan *et al*., 2003). The moisture content of *A. afra* plants exposed to different deficit irrigation levels in the current study ranged from 68 to 74%, with the well-watered treatment showing the highest moisture content.

No significant differences in leaf area were recorded between all treatments in both harvests (Figure 6.11A). However, leaf area in the first harvest tended to be higher than in the second, which could reflect plant adaptation to water stress. The water stress treatment had a significant effect on relative water content, which decreased with increasing water stress (Figure 6.11B). Similar results were reported by Soni and Abdin (2017) for *A. annua*. The relative leaf water content of *Melissa officinalis* was higher in the well-watered control than in the water stress treatment, although the two were not significantly different (Farahani

et al., 2025). This suggests an adaptive strategy in most plants when exposed to water stress, as seen in the current study, where, in the second harvest, there were no significant differences between the well-watered and the water stress treatments. Relative leaf water content is a critical indicator of plant water status, revealing the balance between leaf tissue water supply and transpiration rate (Farahani *et al*., 2025).

The electrolyte leakage was significantly high under severe water stress conditions in the first harvest only (Figure 6.11C). Electrolyte leakage of 50 to 60% was reported under water stress conditions, compared with 28 to 32% in well-watered conditions in two Kochia species (Masoumi *et al*., 2010). Again, the electrolyte leakage was higher in the water stress treatment than in the well-watered control in *Melissa officinalis*, although there were no significant differences. Electrolyte leakage can be related to several physiological and biochemical indicators of plant response to environmental conditions (Bajji *et al*., 2001).

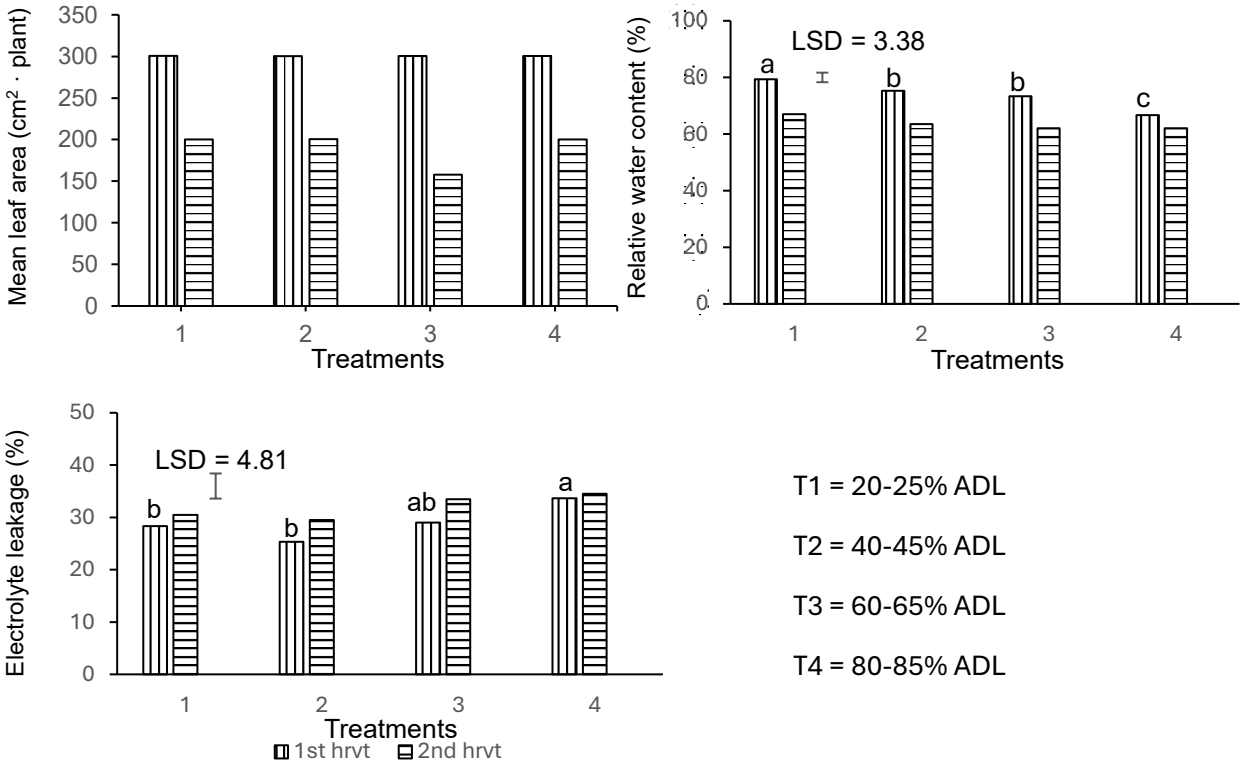


Figure 6.11. Mean leaf area (A), relative water content (B) and electrolyte leakage (C) of *Artemisia afra* under different water stress levels.

The water use efficiency increased with increasing water stress (Table 6.7). The results show that under well-watered conditions, there is high loss through evapotranspiration, but dry-matter production did not increase proportionally. Similar results were reported by Eiasu *et al*. (2009), who found that the water use efficiency of rose-scented geranium increased with increasing water stress.

Table 6.7. Water-use efficiency of *Artemisia afra* in response to various water-stress treatments.

Treatment (%)	Water use (mm)	Average fresh yield ($g \cdot plant^{-1}$)	Water use efficiency ($g \cdot mm^{-1}$)
20-25	248.03	1168.8	4.71
40-45	197.01	813.4	4.13
60-65	166.6	861.7	5.17
80-85	154.02	819.5	5.32

The chemical profiling of *Artemisia afra* plants exposed to different water deficit levels showed differences in the number and names of compounds tentatively identified in the positive mode (Figure 6.12 and Table 6.8). In total, 60 compounds were identified, of which 44 were identified in treatment 1 (well-watered), 43 in the severely stressed treatment (T4), 39 in the moderate stress treatment, and 35 in the mild stress treatment (Table 6.8). Treatment 1 had some compounds that were not identified in other treatments, including Skimmin, Celastrol, Dahuribirin B, Pedalitin, Viscidulin III and 6beta-Acetoxy-5-epilimonin. Skimmin is a major pharmacological compound in *Hydrangea paniculata,* a Chinese traditional medicinal herb with anti-inflammatory properties (Zhang *et al*., 2013). Celastrol, a triterpene extracted from *Tripterygium wilfordii*, has been reported to have anti-neurogenerative disease properties and potential therapeutic effects on lipid-related diseases such as diabetes, cancer, and obesity (Lei *et al*., 2025; Gu *et al*., 2023; Benmouna *et al*., 2025).

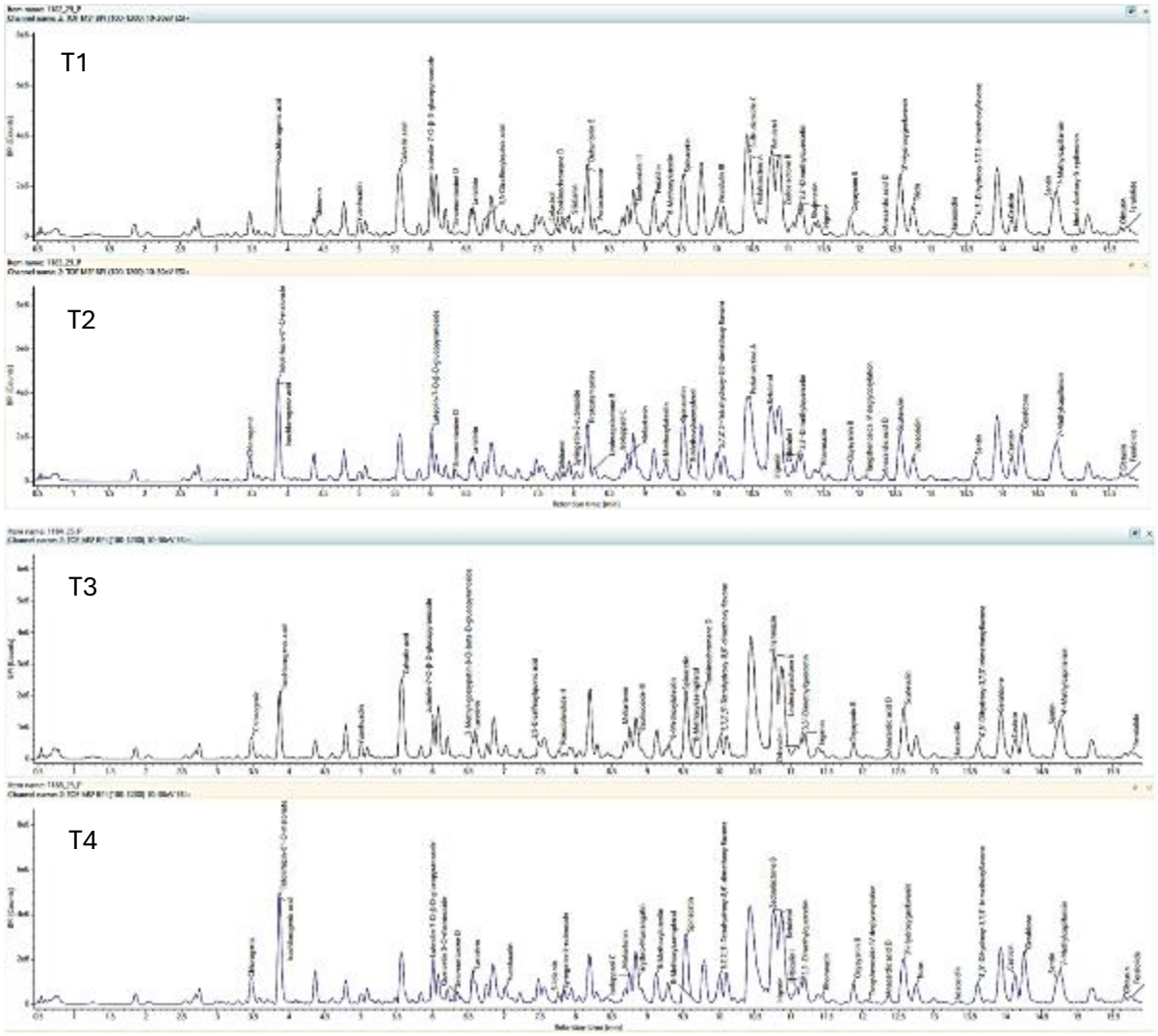


Figure 6.12. Chromatogram of *Artemisia afra* samples (ESI positive mode) under different water-deficit levels. T1: 20-25% (well-watered), T2: 40-45% (mild stress), T3: 60-65% (moderate stress), T4: 80-85% severe stress.

Table 6.8. The number and names of compounds from *Artemisia afra* samples (ESI positive mode) exposed to different water deficit levels.

Trt	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33
1		X	X	X		X	X	X	X			X	X		X	X	X				X		X	X	X		X			X	X	X	
2	X	X			X		X	X		X		X	X	X	X	X		X	X			X		X			X				X	X	
3	X	X		X		X	X	X		X	X			X	X	X	X		X				X			X			X			X	X
4	X	X		X	X		X	X		X		X	X	X	X	X			X	X		X		X			X	X	X		X		

34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	Total
X		X	X	X	X	X	X	X		X	X	X			X	X	X	X	X	X	X	X		X	X	X	X	X		44
	X	X	X	X		X		X	X	X		X		X	X	X	X	X			X		X	X	X		X	X	X	39
	X	X	X	X		X						X	X	X	X	X	X	X			X	X	X	X				X		35
	X	X	X	X					X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X		X	X	X	43

TRT = Treatments - T1: 20-25% (well watered), T2: 40-45% (mild stress), T3: 60-65% (moderate stress), T4: 80-85% severe stress

1	Chlorogenic	11	Pseudolarolide H	21	Celastrol	31	Protostemonine	41	Suffruticoside C	51	Oxyayanin B
2	Isochlorogenic acid	12	Sinomontanine D	22	Isolappaol C	32	Baohuoside III	42	Pedatisectine A	52	Anacardic acid D
3	Skimmin	13	Quercetin 3-O-rhamnoside	23	Tonkinochromane D	33	Linderagalactone E	43	Tangshenoside IV deglycosylation	53	3'-Hydroxygenkwanin
4	Yuanhuadin	14	Meliartenin	24	Silidianin	34	Pedalitin	44	Betuletol	54	Tricin
5	2alpha-Hydroxyneoanisatin (3-deoxy-2alpha-hydroxyanisatin	15	Laricitrin	25	Chrysin-7-O-β-D-glucuronide	35	Orbiculin I	45	Zedoalactone B	55	Jaceosidin
6	Calvatic acid	16	3-Methyl-gossypetin 8-O-beta-D-glucopyranoside	26	(+)-5,5'-Dimethoxy-9-O-beta-D-glucopyranosyl secoisolariciresinol	36	6-Methoxyluteolin	46	3,3'-Dimethylquercetin	56	4',5'-Dihydroxy-3,7,3'-trimethoxyflavone
7	(2R)-Eriodictyol-7-O-beta-D-glucopyranosiduronic acid	17	3,5-Dicaffeoylquinic acid	27	Syringetin-3-rutinoside	37	Spinacetin	47	4'-Methylcapillarisin	57	Casticin
8	Luteolin-7-O-β-D-glucopyranoside	18	Linderagalactone B	28	Tutin	38	8-Methoxykaempferol	48	Geraldone	58	Santin
9	3,4-Di-O-caffeoyl-5-O-(3-hydroxy-3-methyl) glutaroyl quinic acid	19	5,7,2',5'-Tetrahydroxy-8,6'-dimethoxy flavone	29	Tectorigenin	39	Viscidulin III	49	Irigenin	59	7-Methylcapillarisin
10	1-Deoxy-3-methacrylyl-11-methoxymeliacarpinin	20	erythro-Murrangatin	30	Dahuribirin B	40	Scutevulin	50	Rhamnazin	60	6beta-Acetoxy-5-epilimonin

61	Obtusin
62	Feralolide
63	Trifolirhizin-6''-O-malonate

The negative ionisation mode also showed variation in chemical profiling of the *A. afra* samples, with a total of 86 compounds (Figure 6.13 and Table 6.9). Treatment 4 (severely stressed) had 67 compounds detected, treatment 1 (well-watered) 65 compounds, treatment 2 (mild stressed) had 54 compounds and treatment 3 (moderately stress) showed 34 compounds. The compounds 3',4'-Dihydroxywogonin, Hedyotoside, Chrysosplenetin B, Yadanzioside N deglycosylation, Saponarin, Scopolin and Chavicol beta-D-glucoside were only identified in treatment 4. Nuezhengalaside, Quercetin 3-(2''-acetylgalactoside), 2-O-Cinnamoyl-glucogallin, Gynostemoside A, 11-Homohydroxyldidrovaltrate and Perilloside B were identified in treatment 1 only. Scopolin and saponarin are some natural antioxidants found in leaves of several plants (Karunarathna *et al*., 2024; Min *et al*., 2021).

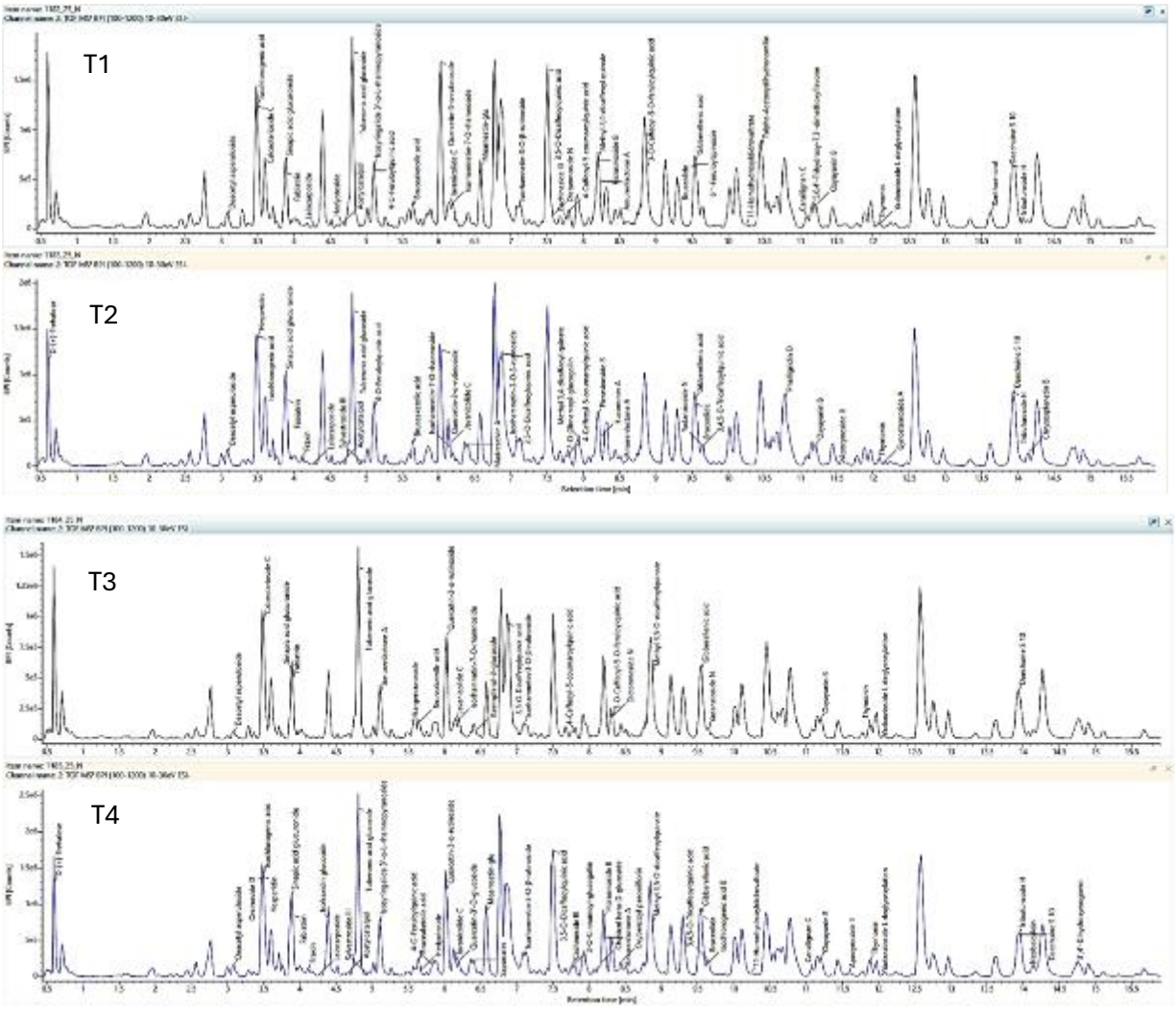


Figure 6.13. Chromatogram of *Artemisia afra* samples (ESI negative mode) exposed to different water deficit levels. T1: 20-25% (well-watered), T2: 40-45% (mild stress), T3: 60-65% (moderate stress), T4: 80-85% severe stress.

Table 6.9. The number and names of compounds from *Artemisia afra* samples (ESI negative mode) exposed to different water deficit levels.

Trt	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43
1	X	X	X	X	X	X	X					X		X	X	X		X	X	X	X		X	X		X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X
2	X	X			X	X	X	X			X	X		X	X	X	X	X	X		X	X	X	X	X	X			X	X	X				X	X	X		X	X		X	X
3	X	X	X		X	X			X					X					X	X	X		X	X		X			X		X			X	X		X		X				
4	X	X			X	X	X	X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			X			X	X	X	X	X	X	X	X	X	X	X		X	X

44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	Total
X	X	X		X	X	X	X		X	X		X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X		X	X	X						X		65
X		X	X	X	X	X	X	X	X	X	X	X		X	X					X	X							X	X	X	X		X	X	X						X		54
		X	X		X		X		X	X			X				X	X			X							X	X	X	X		X										34
X		X	X	X	X	X	X		X	X	X	X	X	X			X	X		X		X	X			X		X	X	X	X		X	X		X	X	X	X	X	X	X	67

1	Deacetyl asperuloside	11	Orcinoside D	21	19-O-[beta-D-Apiofuranosyl(1->2)-beta-D-glucopyranoyl]-3,14-dideoxyandrographolide	31	Madreselvin A	41	Quercetin 3-(2''-acetylgalactoside)	51	Eucommin A
2	Apigenin 6-C-beta-xylopyranosyl-8-C-(6'''-O-(3-hydroxy-3-methylglutaroyl)-beta-glucopyranoside)	12	Hesperidin	22	Quercetin-3-o-rutinoside	32	Kaempferol-3-glucoside	42	Tortoside A	52	3-O-Caffeoyl-5-O-feruloylquinic acid
3	Isofraxidin glucoside	13	Hedyotoside	23	Secologanoside	33	Mearnsetin-glu	43	Methyl 3,4-dicaffeoyl quinate	53	Phelligridin D
4	Calceolarioside C	14	Isofraxidin glucoside	24	Kaempferol 3-O-beta-D-glucopyranoside-7-O-alpha-L-arabinofuranoside	34	11-(6-O-trans-Sinapoylglucopyranosyl)-gardendiol	44	11-O-Vanilloylbergenin	54	2,3,5,4'-Tetrahydroxystilbene 2-O-Glucoside
5	regaloside F	15	Tuberonic acid glucoside	25	Javanicolide C	35	Isorhamnetin-3-O-β-rutinoside	45	2-O-Cinnamoyl-glucogallin	55	Malonyldaidzin
6	Sinapic acid glucuronide	16	Sylvestroside III	26	Kaempferol 3-O-Rhamnoside-7-O-Glucoside	36	3,5-O-Dicaffeoylquinic acid	46	Methyl 3,4-dicaffeoyl quinate	56	Apocynoside II
7	Fabiatrin	17	Isosyringalide 3'-α-L-rhamnopyranoside	27	Isorhamnetin-7-O-rhamnoside	37	Isochlorogenic acid	47	1-Deoxy-3-methacrylyl-11-methoxymeliacarpinin	57	Oxybenzoyl paeoniflorin
8	Leiocarposide	18	D-(+)-Trehalose	28	Nuezhengalaside	38	Dictamnoside N	48	Methyl 3,5-O-dicaffeoylquinate	58	Isochlorogenic acid B
9	Fraxin	19	4-O-Feruloylquinic acid	29	Safflor yellow (A)	39	Cachineside III	49	Picrasinoside B	59	Licorice glycoside A
10	Sargencuneside	20	Bruceaketolic acid	30	3-Methyl-gossypetin 8-O-beta-D-glucopyranoside	40	4-Caffeoyl-5-coumaroylquinic acid	50	Amurenlactone A	60	Gynostemoside A

61	Methyl 3,4-dicaffeoyl quinate	71	Caruilignan C	81	Yadanzioside N deglycosylation
62	Neobaicalein	72	5,6,4'-Trihydroxy-7,3'-dimethoxyflavone	82	Saponarin
63	Gibberellenic acid	73	Oxyayanin B	83	Scopolin
64	6'''-Feruloylspinosin	74	1-Acetoxy-6-hydroxy2-methylanthraquinone-3-O-α-rhamnosyl(1→4)-α-glucoside	84	Chavicol beta-D-glucoside
65	Bruceolide	75	Thymonin	85	2''-O-Rhamnosyl ikarisoside A
66	Yadanzioside N	76	Yadanzioside L deglycosylation	86	3',4'-Dihydroxywogonin
67	3,4,5-O-Tricaffeoylquinic acid	77	Xanthomicrol		
68	11-Homohydroxyldidrovaltrate	78	Daechuine S 10		
69	Perilloside B	79	Tribufuroside H		
70	7alpha-Acetoxydihydronomilin	80	Chrysosplenetin B		

A summary of the compound distribution according to the water depletion levels is given in Table 6.10. The well watered treatment and the severe water stress treatment had the highest number of compounds.

Table 6.10. Summary of the compound distribution according to the water deficit levels in *Artemisia afra*.

Stress Level	Water Deficit %	Positive Mode Count	Negative Mode Count
Well Watered	20–25%	44	65
Mild Stress	40–45%	35	54
Moderate Stress	60–65%	39	34
Severe Stress	80–85%	43	67

6.4 CONCLUSION

The results of this study showed that, in general, water stress reduced plant height, the number of branches, and fresh yield in *A. afra*. The harvest included branches, which were cut back. Including the soft branches in the yield could have increased it, but when the leaf area was measured without the branches, no significant differences were observed. The moisture in the branches from the well-watered treatment could also have contributed to the higher fresh yield. This suggests that under well-watered conditions, *A. afra* plants grow taller and increase the number of branches, but under drought conditions the plants are shorter with fewer branches, while the leaf area does not change. The leaves of *A. afra* are thinner, thus adapted to drought conditions, but the observation in the trial was that under water stress they tend to be more greyish, which could be an indication of leaf hairs to reduce water loss.

Based on the tentative chemical profiling of *A. afra*, water stress (water deficit) significantly affects the plant's chemical composition, altering the presence of specific bioactive compounds in both positive and negative ionisation modes (Table 6.10). The positive mode analysis revealed that across all treatments, 60 compounds were identified, with the number varying with the severity of the stress. The highest number of compounds (44) was found in well-watered plants (T1), followed by severely stressed plants (T4) with 43. Several compounds were highly sensitive to water deficit, detected only under well-watered conditions (T1) and disappearing even under mild stress. These sensitive compounds include:

- Skimmin: Known for anti-inflammatory properties.
- Celastrol: Reported to have anti-neurodegenerative properties.
- Others: Dahuribirin B, Pedalitin, Viscidulin III, and 6-beta-Acetoxy-5-epilimonin.

The negative mode showed higher overall diversity, with 86 total compounds, and demonstrated that severe stress can stimulate the production of certain metabolites. Under severe stress, unlike the positive mode, the highest number of compounds (67) was identified in the severely stressed treatment (T4), compared

with 65 in the well-watered control. The following compounds were identified only under severe stress (T4), suggesting they are prevalent biomarkers for terminal water deficit:

- Antioxidants: Scopolin and Saponarin.
- Others: 3',4'-Dihydroxywogonin, Hedyotoside, Chrysosplenetin B, Yadanzioside N deglycosylation, and Chavicol beta-D-glucoside.
- Compounds Sensitive to Stress: Conversely, certain compounds were unique to well-watered plants (T1) and were lost during stress, including Nuezhengalaside, Quercetin 3-(2''-acetylgalactoside), and Perilloside B.

Under terminal water stress, the chemical profiling of *A. afra* acts as a metabolic "switch," significantly altering the plant's chemical fingerprint. The stress response is nonlinear and varies markedly between positive and negative ionisation modes. In the positive ionisation mode, well-watered plants (T1) exhibited the highest diversity. Stress appears to suppress the synthesis of several major pharmacological compounds. In the negative ionisation mode, severe stress (T4) increased chemical diversity, reaching a total of 67 compounds. This suggests that severe water deficit triggers the production of specific defensive metabolites. Table 6.11 details the specific compounds that are either "sensitive" (lost during stress) or "prevalent" (induced/triggered by stress).

Table 6.11. Comparative metabolite sensitivity and prevalence of *Artemisia afra*, as influenced by terminal water stress.

Mode Analysis	Status	Compounds Identified	Biological/Pharmacological Note
Positive ionization	**Sensitive** (Lost during stress)	Skimmin, Celastrol, Dahuribirin B, Pedalitin, Viscidulin III, 6beta-Acetoxy-5-epilimonin	Skimmin has anti-inflammatory properties; Celastrol targets neurodegenerative diseases.
	Resilient (Present in severe stress)	43 compounds identified in T4	Indicates a high level of metabolic stability despite a severe deficit.
Negative ionization	**Prevalent** (Triggered by severe stress)	3',4'-Dihydroxywogonin, Hedyotoside, Chrysosplenetin B, Yadanzioside N deglycosylation, Saponarin, Scopolin, Chavicol beta-D-glucoside	Scopolin and Saponarin are potent natural antioxidants.
	Sensitive (Lost during stress)	Nuezhengalaside, Quercetin 3-(2''-acetylgalactoside), 2-O-Cinnamoyl-glucogallin, Gynostemoside A, 11-Homohydroxyldidrovaltrate, Perilloside B	These are unique markers of well-watered *A. afra* (T1).

The study found that terminal water stress does not result in a simple decline in all metabolites; rather, it facilitates a qualitative shift in the plant's profile. Under terminal stress (T4), 43 compounds were detected, compared with 44 in T1. While the count is similar, the loss of Skimmin and Celastrol signifies a reduction

in specific anti-inflammatory and neuroprotective potential. Similarly, severe stress (80–85% deficit) acts as a powerful elicitor. It produced the highest compound count (67) across all treatments in this mode. On the other hand, Unique Markers such as Scopolin and Saponarin were present only under severe stress, indicating that T4 plants may have higher antioxidant capacity than well-watered plants. Terminal water stress exerts a profound and significant effect on the chemical profile of *A. afra*. At the same time, it causes the loss of specific "stress-sensitive" compounds like Skimmin and Celastrol. Severe water deficit serves as a necessary elicitor for "stress-prevalent" bioactives like Yuanhuapin, Scopolin, and Saponarin.

The plant exhibits remarkable metabolic plasticity: severe stress (T4) results in a more diverse chemical profile in the negative mode (67 compounds) than under well-watered conditions (65 compounds). Consequently, water management is not merely a matter of plant survival, but a powerful tool for "metabolic engineering" to enhance the medicinal value of *A. afra*. Therefore, it is recommended that the industry distinguish between "irrigated" *A. afra* (high in Skimmin/Celastrol) and "stress-conditioned" *A. afra* (high in Scopolin/Yuanhuapin) to meet specific pharmaceutical needs.

The study results suggest that the cultivation conditions of *A. afra* can be manipulated to enhance the synthesis of specific metabolites for specific pharmaceutical applications.

CHAPTER 7

GROWTH AND PERFORMANCE OF *ARTEMISIA AFRA* IN THREE DIFFERENT LOCATIONS

7.1 INTRODUCTION

Cultivation of *Artemisia* species such as *A. annua* and *A. afra* offers rural communities both a source of affordable medicine and a source of income (Räth *et al*., 2004). Artemisinin-based malaria treatment is currently in short supply, despite farmers growing *A. annua* (Noorden, 2010; Kindermans, 2007). This scarcity can be attributed to poor crop management, which results in low leaf yield (Rengel, 1998). Optimal nutrient supply improves growth rate, yield, and quality in *A. annua* production (Strong, 1995). This indicates a need for participatory research with communities to facilitate technology transfer and promote the cultivation of medicinal plants.

Artemisia spp. are able to grow and adapt across various ecosystems, resulting in wide distribution (Trendafilova *et al*., 2021) and potentially altering chemical composition in response to environmental factors. *Artemisia afra* plants collected from three provinces of South Africa showed variation in chemical composition. Samples collected from Phillipolis, in the Free State province, showed over 70% α-thujone content, which decreased to 60-65% in samples from Keiskammahoek (Eastern Cape province), 44% in samples from the University of Fort Hare (Eastern Cape province), 10.5-12.2% in samples from University of Zululand (KwaZulu-Natal province), 2.3-7.9% in samples from Empangeni (KwaZulu-Natal province) and 1.6-5.1% in samples from Gqumahshe (Eastern Cape province) (Oyedeji *et al*., 2009). The *A. afra* plant samples from Gqumahshe showed the highest concentration of 1, 8-cineole at 47-49.5%, while the Hogsback and University of Fort Hare samples were the only specimens that had artemisia ketone and alcohol (Oyedeji *et al*., 2009). Similarly, *A. afra* plant sample collected from Qwaqwa in the Free State contained santolina alcohol and santolinyl acetate, which were not detected in samples collected from Giant's Castle (KwaZulu-Natal) and Klipriversberg (Gauteng) (Viljoen *et al*., 2006). Using the LC/MS and LC-UV methods, Avula *et al*. (2009) reported variations in flavonoid content of *A. afra* samples collected in various places in the Western Cape and KwaZulu-Natal provinces of South Africa. For example, tamarixetin and genkwanin were either not detected or below the limits of quantification, in some *A. afra* samples (Avula *et al*., 2009). Seeds of *A. afra* collected from two different sites (Nyanga and Marondera, Zimbabwe) for cultivation, were classified as chemotypes 1) dominated by artemisia ketone, α-copaene/camphor, 1.8-cineole, and 2) dominated by 1.8-cineole, α-copaene/camphor, and borneol (Chagonda *et al*., 1999).

Plant-to-plant variation in chemical composition has also been reported in *A. afra*. Viljoen *et al*. (2006) reported that *A. afra* sample C, collected from Setibeng in Lesotho, contained 17% and 78% α- and β-thujone, respectively, whereas these compounds were absent in another sample (sample A) collected from the same area. Artemisia ketone at 15%, yomogi alcohol at 4% and *Artemisia* alcohol at 9% were detected in sample A collected from Giant Castle (KwaZulu-Natal Province), but not in samples B and C from the same population (Viljoen *et al*., 2006). However, *A. afra* plant sample B (Giant's Castle) had 58% ß-thujone

while sample A had 8%, and sample B from Klipriversberg had 1% ß-thujone while sample A and C only had α-thujone at 0.1 and 6% respectively (Viljoen *et al*., 2006). Similarly, Sotenjwa *et al*. (2020) reported six compounds that were consistently detected across 12 *A. afra* samples collected from different provinces of South Africa (Eastern Cape, Free State, Gauteng, KwaZulu-Natal, and Mpumalanga), but chemical fingerprinting revealed inter-population variation in other constituent compounds.

7.2 CULTIVATION AND GROWTH OF *ARTEMISIA AFRA* IN THREE PROVINCES

7.2.1 Site preparations

The sites were first prepared for the installation of shadenet structures and irrigation. This aims to compare *A. afra* grown under a protected structure with that grown in the field. Figure 7.1 shows the ARC team erecting the shadenet structure and installing irrigation with the beneficiary as part of skill transfer, which is important for the structure's maintenance.

Figure 7.1. Erecting a shade net structure in Lebowakgomo, with irrigation installation.

The second shadenet structure was erected in Mamelodi (Figure 7.2). The ARC team again erected the structure with the beneficiary. The shade net used for the structures was 40% white.

Figure 7.2. Erecting a shade net structure and installing irrigation pipes in Mamelodi.

7.2.2 Planting of *Artemisia afra*

After the structures were completed and irrigation was installed, planting was carried out (Figure 7.3). The structures accommodated 150 *A. afra* plants, with another 150 planted outdoors in the open field. The survival rate of the plantlets was above 90%.

Figure 7.3. Planting of *Artemisia afra* inside the shade net structures.

The major challenge observed so far is pest infestation, mainly the black aphids as shown in Figure 7.4. The infestation was severe inside the shadenet structures, with the aphids more on the shoot tips. To control

the pest, a contact insecticide for the control of aphids was applied. Care was taken to discuss the chemical with the farmer to ensure that there is no harvest within the waiting period.

Figure 7.4. Black aphids infestation on *Artemisia afra* plants and spraying with an insecticide.

7.2.3 Technology transfer

The project also supports communities by introducing technologies that will help the farm improve. Irrigation scheduling may sound too scientific to some community members; however, recently developed technologies have made it simpler for resource-poor farmers to understand soil moisture content and irrigate accordingly. The chameleon card system (Virtual Irrigation Academy, VIA) (Figure 7.5) uses a colour code to indicate if the soil is wet (blue, no irrigation), moist (green, irrigate) or dry (red, irrigate urgently).

The communities were supplied with chameleon cards (Figure 7.6); they were part of the installation of the sensors, and a demonstration was part of the package to ensure that they could use the chameleon cards properly (Figure 7.6). The sensors were installed as per the instructions in the VIA Chameleon card manual (Figure 7.7).

Figure 7.5. Chameleon card and sensors that were supplied to the communities (A) and the installation instruction manual (B).

Figure 7.6. Handing over (A), demonstration (B, C) and installation (D) of the chameleon sensors in the field.

Figure 7.7. Chameleon card sensors installed in one of the community projects.

7.3 QUALITY OF *ARTEMISIA AFRA* AS INFLUENCED BY DIFFERENT CLIMATIC ZONES

7.3.1 Research Methodologies

7.3.1.1 Plant collection and extraction

Artemisia afra samples were collected from seven wild populations in three different provinces. Three samples were collected from the Free State, two from Gauteng, and two from Limpopo provinces. The samples were coded as shown in Table 7.1.

Artemisia afra samples were extracted with DCM/MeOH (1:1), followed by extraction using 100% MeOH. After filtration, the combined organic layers were dried at 35 °C using a Genevac HT-6 to generate the dry extracts. Thereafter, approximately five (5) mg of the dried extracts were dissolved in MeOH/H_2O (1:1), and the solution was then filtered through a 0.22 µm nylon syringe filter to remove particulate matter in preparation for UPLC-QTOF-MS analysis.

Table 7.1. *Artemisia afra* plant samples collected from different provinces and their extraction yields.

Sample code	Mass of sample extracted (g)	Mass of extract (mg)
WJE-124-1A (FS)	1.0283	78.6
WJE-124-1B (FS)	1.2370	61.4
WJE-124-1C (FS)	1.0105	50.3
WJE-124-1D (GP)	1.0103	55
WJE-124-1E (GP)	1.0288	49.1
WJE-124-1F (LP)	1.1361	42.4
WJE-124-1G (LP)	1.0361	44.2

Provinces - FS: Free State, GP: Gauteng, LP: Limpopo

7.3.1.2 UPLC-QTOF-MS analysis of samples

UPLC-HRMS analysis was performed using a Waters Acquity UPLC system (Waters Corp., MA, USA), equipped with a binary solvent delivery system and an autosampler. Separation was achieved on an ACQUITY UPLC® BEH (2.1 × 100 mm, 1.7 µm) column (Waters Inc., Milford, MA, USA). A gradient elution method was used, with H_2O (0.1% formic acid) and MeOH (0.1% formic acid) (Romil-SpS™, Microsep, South Africa) as solvents A and B, respectively. The method was optimised as follows: 97% solvent A, held for 0.1 min, followed by a linear gradient to 100% solvent B at 14 min. A 3-min wash hold was used (14–17 min) before reconditioning the column with the starting conditions (17.5–20 min). The column temperature was held constant at 40 °C to ensure repeatable results, with a fixed flow rate of 0.3 mL/min and an injection volume of 5 µL.

The system was setup with a Waters ACQUITY UPLC® hyphenated to a quadrupole mass filter with a high-resolution time-of-flight (TOF) mass analyser. A Waters® Xevo G2 high-definition mass spectrometer (HDMS) system (Waters Inc., Milford, MA, USA) was used for compound separation and detection. The instrument was operated using MassLynx™ v. 4.1 (Waters Inc., Milford, MA, USA) software. Sodium iodide clusters were used to calibrate the MS using the Intellistart software function over a mass range of 50–1200 Da. The MS source parameters were optimised for ESI negative mode and were set as follows: source temperature of 120°C, extraction cone voltage of 4.0 V, sampling cone of 30.0 V, cone gas flow of 20.0 L/h, desolvation temperature of 350°C, desolvation gas flow of 600.0 L/h, and a capillary voltage of 2.0 kV for the negative mode. An internal lock mass control standard comprised a 2 mg/µL solution of leucine enkephalin (m/z 555.2693). To account for experimental mass drift, a lock mass solution was infused directly into the source at 3 µL/min. The lock mass infusion was performed intermittently at 10-second intervals. The Waters UNIFI Scientific Information System was used for further data processing and analysis. It is a software platform designed to integrate and manage data from liquid chromatography (LC) and high-performance mass spectrometry (MS) systems. It merges both quadrupole and time-of-flight MS data into

a single solution, encompassing data acquisition, processing, visualization, reporting, and configurable compliance tools within a networked laboratory environment.

7.4. RESULTS AND DISCUSSIONS

The chromatograms generated from the UPLC MS analysis in the positive ionization mode (ESI+ ion, BPI generated) for one sample WJE-I24-1A and with labelled peaks are shown in Figure 7.8. The tentative identification of the compounds is in Table 7.2.

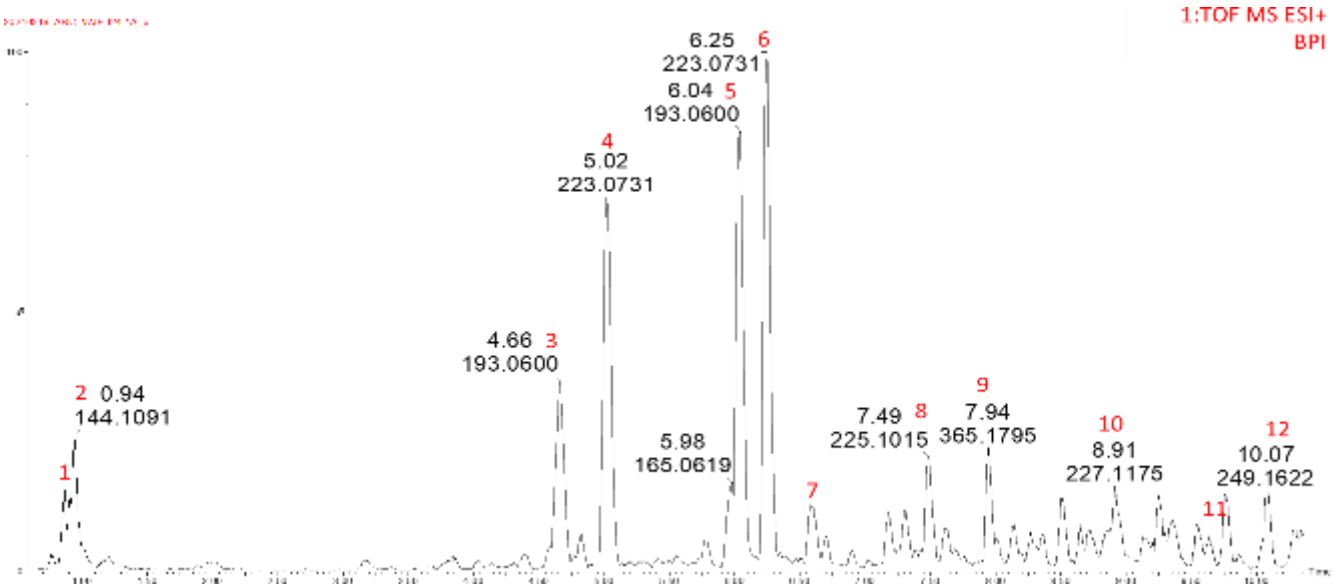
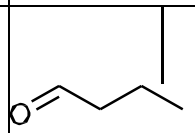
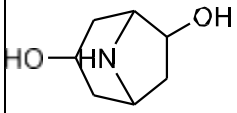
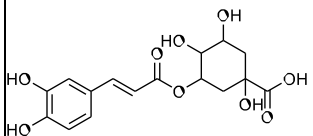
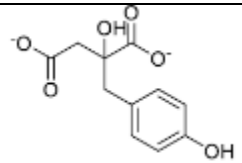
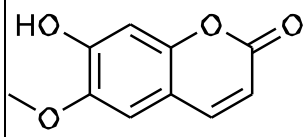
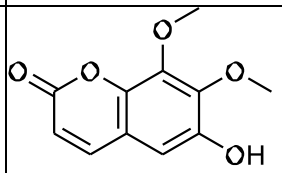
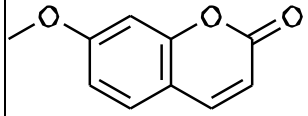
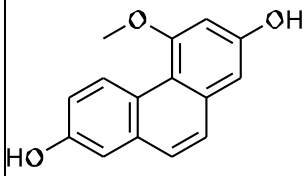
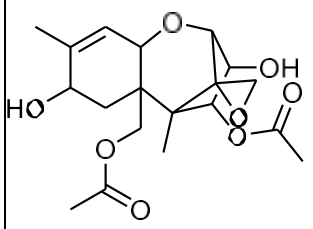
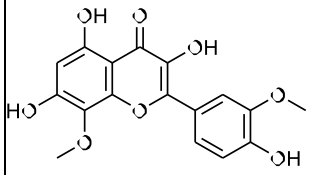
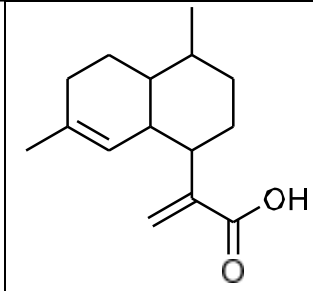
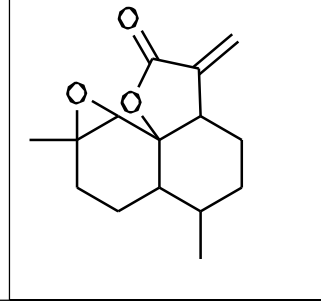


Figure 7.8. Chromatogram of *Artemisia afra* sample collected from the Free State province. Peaks were labelled based on ESI positive (+ve ion), and a total of 12 peaks were selected for identification purposes.

Table 7.2. Tentative identification of *Artemisia afra* compounds (positive mode) using the Waters UNIFI Scientific Information System and their references in the chromatogram.

Peak number	Retenti on time (min)	Acquired $[M+H]^+$ m/z	Adduct	Mass error (ppm)	Formula of possible compound.	Calculated accurate mass.	Possible compound	Possible Structure	References
1	0.95	104.1123	[M+NH4]+	-0.8	$C_5H_{10}O$	86.073165	Isovaleraldehyde		Li *et al.*, 2012
2	1.02	144.1091	$[M+H]^+$	-1.1	$C_7H_{13}NO_2$	143.094629	3β,6exo- Dihydroxy nortropane		Unidentified
3	4.66	355.1013	$[M+H]^+$	-3.1	$C_{16}H_{18}O_9$	354	Chlorogenic acid		Zhao *et al.*, 2015

4	5.02	223.0731	[M- H2O]$^{+}$	-1.1	$C_{11}H_{10}O_6^{-2}$	238.047740	2-(4-Hydroxybenzyl) malic acid		Unidentified
5	6.04	193.0484	[M+H]$^{+}$	-5.7	$C_{10}H_8O_4$	192.04	6-Methoxyl-7-hydroxycoumarin (Scopoletin)		Brown *et al*. 2003; More *et al*., 2012
6	6.25	223.0731	[M-H, Na] +	-4.8	$C_{11}H_{10}O_5$	222.0510	Fraxidin		Deng *et al*., 2016
7	6.58	177.0641	[M+H]+	-1.1	$C_{10}H_8O_3$	176.047345	7-Methoxycoumarin		Taleghani *et al*., 202
8	7.49	225.1015	[M-H2O, +H, -COOH]+	-0.8	$C_{15}H_{12}O_3$	240.078645	Flavanthrinin		You *et al*., 2014

9	7.94	365.1795	[M- H2O]+	-0.6	$C_{19}H_{26}O_8$	382.162770	Neosolaniol		El-Shazly *et al.*, 2022
10	8.91	347.0919	[M+H]+	-1.3	$C_{17}H_{14}O_8$	346	Limocitrin		Yang *et al.*, 1995
11	9.76	235.180	[M+H, -H_2O]$^+$	-0.7	$C_{15}H_{22}O$	234.161980	Artemisinic		Messaili *et al.*, 2020
12	10.07	249.162	[M+H]$^+$	-0.7	$C_{15}H_{20}O$	248.141245	Arteannuin		Messaili *et al.*, 2020

A total of 6 peaks were labelled on UPLC MS chromatogram of *Artemisia afra extract on* negative ionization mode (ESI), Figure 7.9. Tentative identification of selected peaks is shown in Table 7.3.

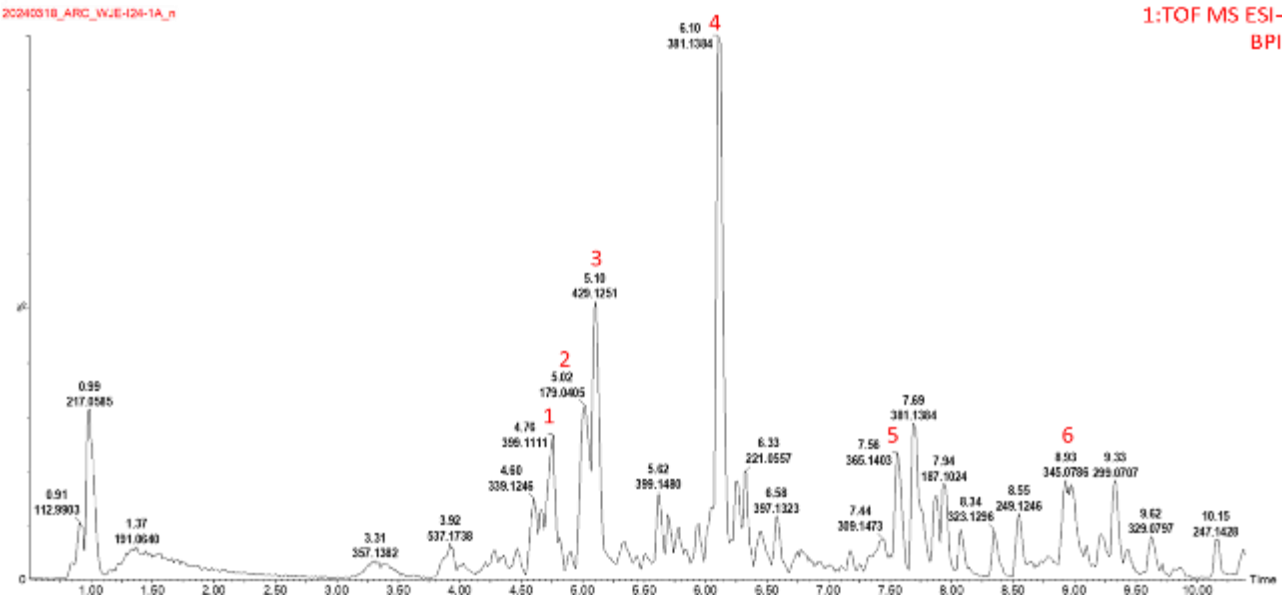
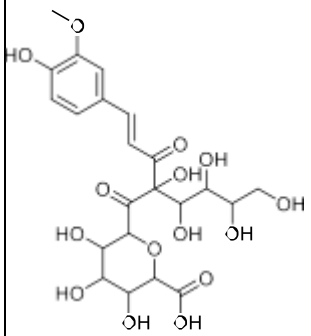
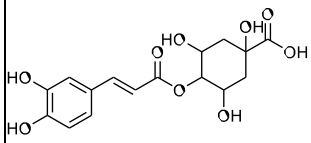
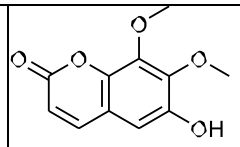
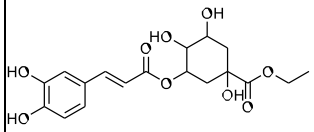
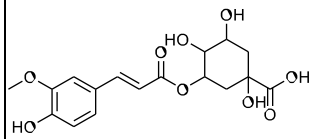
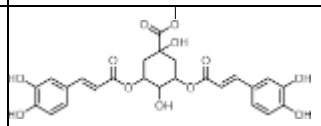
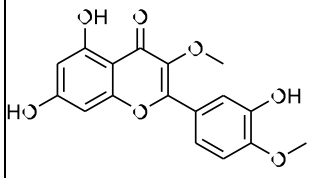


Figure 7.9. Chromatogram of *Artemisia afra* sample collected from the Free State province. Peaks were labelled based on negative ionization mode (-ve ion) mode, a total of 6 peaks were selected for identification purposes.

Table 7.3. Tentative identification of *Artemisia afra* compounds (negative mode) using the Waters UNIFI Scientific Information System and their references in the chromatogram.

Peak number	Retentio n time(mi n)	Acquired [M-H]⁻ m/z	Adduct	Mass error (ppm)	Formula of possible compound.	Calculate d accurate mass.	Possible compound	Possible Structure	References
1	4.76	399.1111	[M-H]⁻	-1.3	$C_{22}H_{28}O_{15}$	532.1428	Feruloylglucuronosyl glucose		Lotus (Q105134970)
2	5.02	179.0405	[M-H]⁻	-1.4	$C_{16}H_{18}O_9$	353.0865	4-O-Caffeoylquinic acid		Olennikov *et al*., 2019
3	5.10	221.0556	[M-H]⁻	-8.4	$C_{11}H_{10}O_5$	222.0510	Fraxidin		Deng *et al*., 2016

									
4	6.10	381.1175	$[M-H]^-$	-1.6	$C_{18}H_{22}O_9$	382.1264	Ningposide A		Ibekwe *et al*., 2018
		367.1021	[M-H]-	-1.3	$C_{17}H_{20}O_9$	368.1107	3-Feruloylquinic acid		Ibekwe *et al*., 2018
5	7.56	529.1344	[M-H]-	-0.8	$C_{26}H_{26}O_{12}$	530.1424	Macranthion G		Anaya-Eugenio *et al*., 2014
6	8.93	345.0599	[M-H]-	-1.7	$C_{17}H_{14}O_8$	346.0689	Quercetagetin-3,4'-dimethyl ether		Nikolova *et al*., 2004

A comparison of the sample extracts was done by overlaying the positive ionization mode chromatograms as shown in Figure 7.3. The overlays indicate that the chromatograms are substantially similar, indicating that the compounds tentatively identified in Table 7.2 (12 compounds) are all present in the extracts. It may be noted that compounds 8-12 appear to be at a lower concentration in other samples compared to sample WJE-124-1A. However, as this was not a quantitative study, the findings are not conclusive.

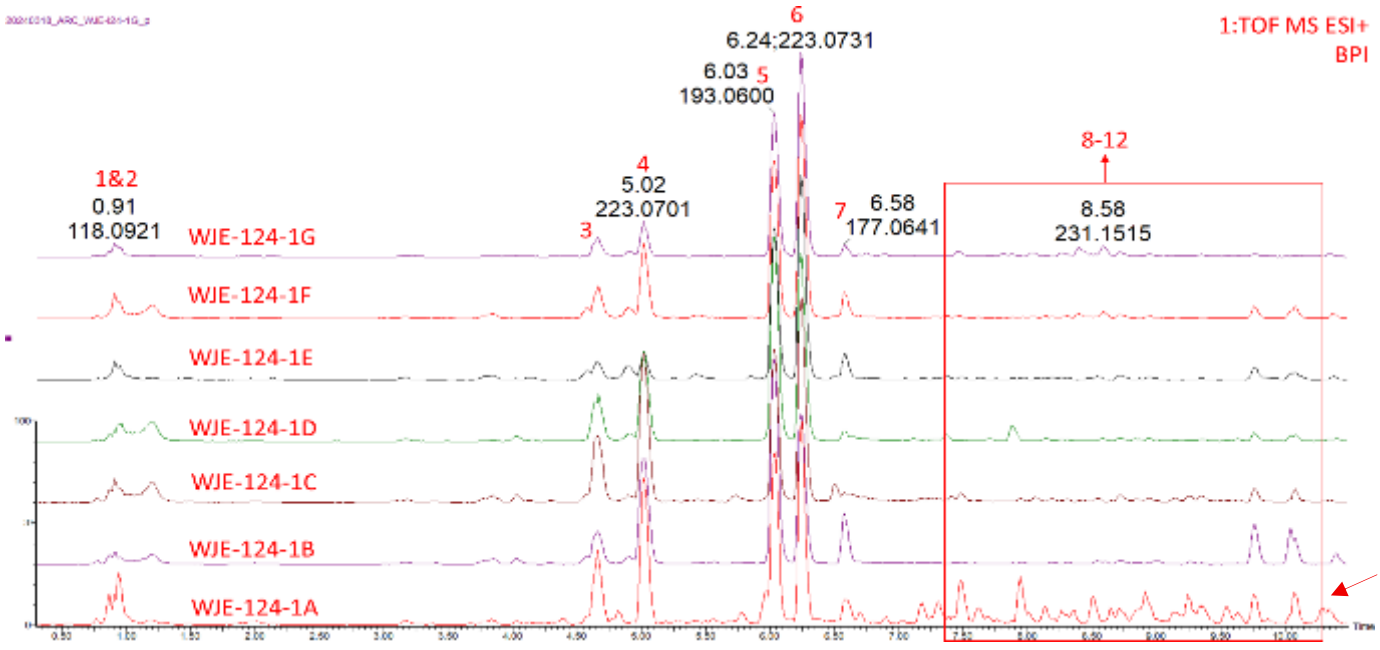


Figure 7.10. Overlayed chromatograms in positive ionization mode (ESI ion), to compare the different *Artemisia afra* samples.

A comparison of the sample extracts was done by overlaying the negative ionization mode chromatograms as shown in Figure 7.4. The overlays indicate that the chromatograms are substantially similar, indicating that the compounds tentatively identified in Table 7.3 (6 compounds) are all present in the extracts. It may be noted that compound 6 appears to be at a lower concentration in other samples compared to sample WJE-124-1A.

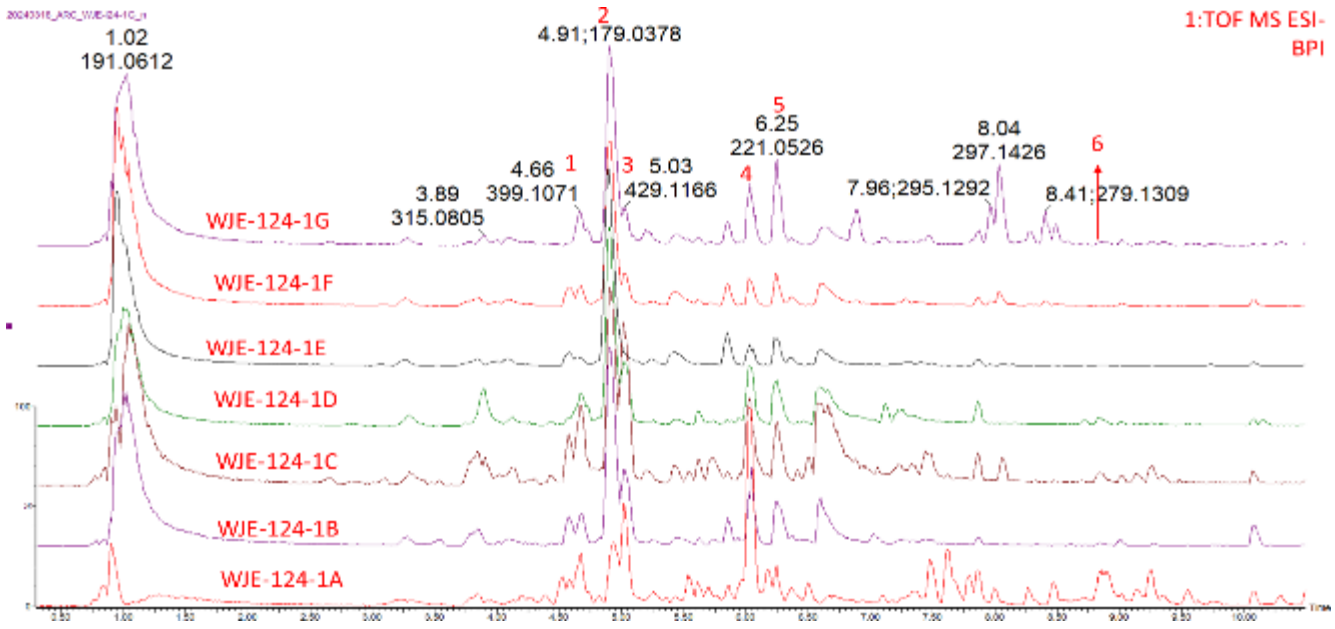


Figure 7.11. Overlayed chromatograms in negative ionization mode (ESI ion), to compare the different *Artemisia afra* samples.

7.5 CONCLUSION

Based on the chemical profiling of the seven extracts of *A. afra*, it is evident that all extracts have substantially similar chemical constituents. This consistency in the chemical composition of these different extracts suggests a high degree of uniformity in the plant material and the extraction procedure. Some of the identified compounds have been reported in *Artemisia* plant extracts, including scopoletin, artemisinic acid, arteannuin B, and 7-methoxycoumarin. The uniformity suggests that *A. afra* is adapted to a wide range of environmental conditions and could be successfully cultivated in the three provinces.

Technology transfer to the three communities and their acceptance of the technology could be a good indication of the communities' willingness to adopt advanced water-management technologies. It also indicated a willingness to begin cultivating medicinal plants and a need for more information.

CHAPTER 8
GENERAL CONCLUSIONS AND RECOMMENDATIONS

The cultivation of medicinal plants has been promoted for many years. However, the number of growers and hectares planted has been low. One of the major reasons has been a lack of cultivation information on environmental requirements (including soil, temperature, and related factors), agronomic practices for optimal production, and return on investment. Information on *A. afra*, a commercially important medicinal plant in South Africa, is lacking. The project aimed to address information gaps regarding *A. afra*, as growers and farmers cannot be expected to begin cultivating the plant if such information is unavailable.

The project was divided into several studies that addressed potential areas for species growth, propagation, and responses to different watering regimes. Mapping potential areas for the distribution and/or growth of *A. afra* will help guide prospective growers. This will also ensure that production failures are avoided, production costs are minimised, and profits are increased by avoiding suboptimal regions. The mapping conducted in this project confirms the wide distribution potential of *A. afra* and indicates limiting environmental factors, including rainfall, temperature, and soil parameters.

The development of a simpler vegetative propagation protocol could benefit both resource-poor and commercial farmers. The micropropagation protocol could be advantageous for nurseries with tissue culture facilities that aim to produce disease-free planting material. Ensuring that planting material of *A. afra* is available to farmers at reasonable costs will promote cultivation. Thus, the propagation by cuttings provides an alternative for resource-poor farmers who cannot afford advanced facilities for micropropagation but are interested in growing *A. afra*.

Exposing plant species to different environmental stresses has been reported to influence their chemical composition. *Artemisia afra* plants grown under varying water-stress conditions exhibited variation in chemical composition. If further confirmed, some of the compounds could help explain the many uses of *A. afra*, but others warrant further investigation for potential new uses of the species and the development of niche products. The project also included a community development component, in which three communities across three provinces were selected to transfer technology for simpler, affordable water management techniques and to encourage community-led species cultivation as a conservation strategy.

Introducing the cultivation of medicinal plants to communities, harvesters and users such as traditional health practitioners will ensure easy access to a sustainable supply of plant material and assurance quality of the material. The adoption of some technologies, such as the chameleon water sensors as done in the project, will assist with the advancement of cultivation in communities using technology to monitor inputs such as irrigation water. Basic, farm-gate processing of the plant material can also add

value to the production, improve the preservation of the material for use when needed, and increase opportunities for income generation.

The project has started to close the research gaps and although there are some success stories to tell, there are also recommendations for future research. The identified suitable climatic conditions for *A. afra* growth present an opportunity for further scientific studies on how to simulate and/or manipulate environmental conditions to synthesise selected secondary metabolites. While irrigation studies have shown significant effects on yield and on various compounds across different watering regimes, further studies that incorporate nutrient management and planting densities are recommended.

Artemisia afra seems to be one of the species that can survive drought and wildfires. The species is also known to grow well along water streams, while the environmental suitability studies suggest that it is favoured by higher rainfall. This suggests that an adaptive strategy during drought could include a deeper root system to access water deeper in the soil profile. Thus, the effect of *A. afra* on water as a natural resource, water streamflow and underground water should also be considered.

Studies on potential yields under various agronomic practices, including pest and disease management, with the intention of optimising the economic returns, should also be considered. Follow-up research on the use of remote sensing, as technology advances, is also recommended. The tentatively identified compounds can be further investigated for potential medicinal use and for how plant stress may contribute to their synthesis. It is also recommended that conservation agencies note the potential threats posed by human activities to the wild populations of *A. afra* and advocate for greater cultivation.

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Appendix A

Propagation of *Artemisia afra*

Propagation and cultivation of medicinal plants can reduce pressure on wild populations and contribute to the conservation of highly utilized, vulnerable, scarce, and endangered species. Propagation can be vegetative, using plant parts such as stems, leaves, and roots, or from seeds.

Artemisia afra seeds can be sown in spring or summer in well-drained soil after soaking in water to improve germination. High variability in *A. afra* seedlings can be expected due to cross-pollination. To propagate vegetatively, soft to semi-hardwood cuttings of 3-5 cm long can be collected from healthy *A. afra* mother plants. Cuttings can be set in cocopeat as a growing medium with rooting hormone (active ingredient - IBA, 0.1 %) applied after a week of setting (**Figure 1**). Cuttings can also root easily with no rooting hormone.

Figure 1. Rooted cuttings of *Artemisia afra*.

Micropropagation of *A. afra* through tissue culture can be achieved by excising 1.5–2 cm long nodal explants from middle lateral branches. Surface decontamination of the nodal explants could be done by immersing in 70% ethanol for 1 min, rinsing in sterile distilled water, and further surface-sterilizing with 10% v/v Jik (3.5% sodium hypochlorite solution) mixed with water for 10 min and rinsing again with sterile distilled water. All dead and/or chlorine-affected tissue must be trimmed off before culturing on Murashige and Skoog (MS) media fortified with 4 µM BA. After four weeks of aseptic culturing *A. afra* nodal explants, shoot proliferation can be observed. An individual or a plantlet can have a high frequency of branching, with an average of 15 branches per shoot after 8 weeks (**Figure 2**). This approach is particularly beneficial for large-scale propagation in a small space all year round.

Figure 2. *In vitro* shoot multiplication of *Artemisia afra* and individual shoots separated from one shoot cluster.

Nonetheless, the lack of facilities and required expertise for resource-poor farmers and the costs of micropropagated plant materials make the less expensive vegetative propagation methods through cuttings a critical component. Tissue culture protocols can be used to generate disease-free, high-quality planting material, and cuttings can be used to produce relatively less costly planting material.

Appendix B

Cultivating *Artemisia afra*

Artemisia afra is a perennial plant, and its leaves can be harvested twice or more in a single season. Harvesting can be done in midsummer and late summer or in early autumn, or picked as needed. Artemisia afra develops more branches and grows stronger after each harvest, which can be done by pruning or cutting back (Figure 1). Plants can be pruned very lightly for the first time once they reach 50 cm in height, which can be in the first month after transplanting, but depending on growing conditions, it may take up to three months. The first harvest is small but encourages a bushier plant growth, resulting in much higher production later.

Figure 1. Bushier *Artemisia afra* plants after cutting back.

It requires the right combination of nutrients and water management to sustain its growth and maximize yield. Applying a combination of nitrogen, phosphorus, and potassium (NPK) helps plant foliage grow efficiently, develop roots and flowers, and improve overall plant health. When no fertiliser is applied on A. afra, a relatively consistent yield of 7.5 t/ha fresh mass can be achieved. Nitrogen application, in the form of ammonium sulphate at a rate of 180 kg/ha, significantly increases the fresh yield of up to 29 t/ha. *Artemisia afra* appears to have a high nitrogen fertiliser requirement, which could be due to its economic yield being the leaves with multiple harvests. Irrigation increases fresh biomass to yields of up to 11.5 t/ha compared to rainfed production, when 20 mm/week is applied with two harvests, at a plant population of 10, 000 plants/ha. Reducing water application to 12 mm/week reduces the fresh biomass yield to 8.5 t/ha. Drip irrigation is preferable as it saves water by delivering to the plant root zone (**Figure 2**).

Figure 2. *Artemisia afra* plant with a drip irrigation system installed to supply water.

Appendix C

Distribution map of *Artemisia afra* in South Africa

Environmental factors such as temperature, solar radiation, precipitation, and soil properties play an important role in the distribution of plant species. While climate has a direct effect on plant growth and physiology, soils provide nutrients and water, and the soils' physical as well as chemical properties influence plant distribution.

Climate change is forcing species distribution to follow suitable climatic conditions and shift their distribution ranges. With the rapid changes in temperature and precipitation already experienced in many parts of the world, ecosystems and biodiversity are affected resulting in evident shift in species distribution ranges. *Artemisia afra* grows well on sandy loam to clay loam soils with a slightly to moderately acidic pH (**Table 1**).

Table 1. General soil properties of places where *Artemisia afra* grows naturally.

Soil properties	Description of effects	Range
pH	Availability and uptake of plant nutrients	5.5 to 6.5
Soil type	Water availability, drainage and aeration	Sandy loam to clay loam

Rainfall, temperature and humidity are the main climatic variables affecting the distribution of *A. afra* (**Table 2**). *Artemisia afra* prefers warm temperate regions with dry winters and hot to cool summers, as well as warm temperate regions that are fully humid with hot to cool summers. The areas identified as suitable for *A. afra* have average annual rainfall ranging from 500 mm to >1500 mm.

The current distribution of *A. afra* and the future predictions until 2080, shows that the distribution suitability will only be slightly affected in the long-term and this could mainly be due to climate change (**Figure 1**). The major distribution is in KZN, Free State, Gauteng, North-West, Eastern Cape, Limpopo, Mpumalanga and Western Cape.

Table 2. Climate factors that affect the distribution of *Artemisia afra*.

Climate variable	Description
Rainfall	Long-term average annual rainfall
Temperature	Highest long-term average monthly maximum
	Lowest long-term average monthly maximum
Humidity	Long-term average monthly minimum relative humidity
	Long-term average monthly maximum relative humidity

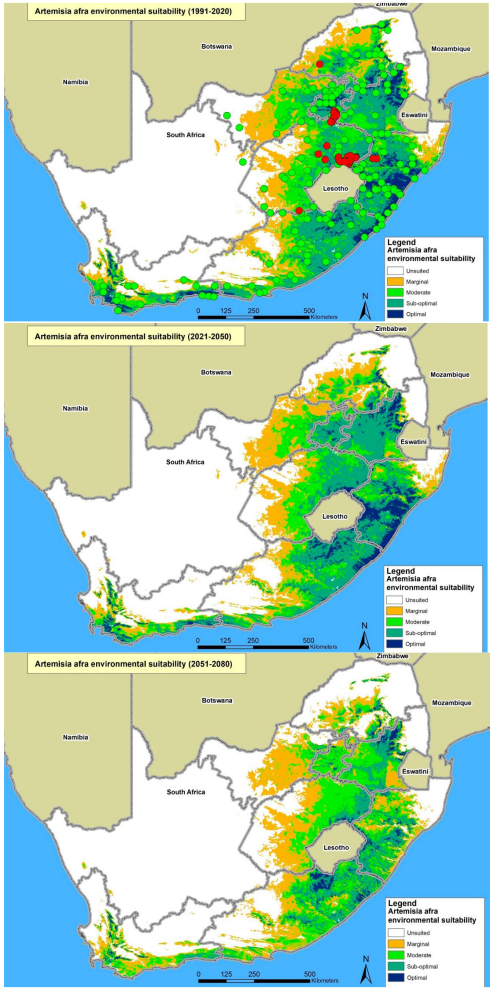

Figure 1. The distribution of *Artemisia afra* and future predictions for distribution suitability.