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Seasonal Variation in Yield and Major Essential Oil Constituents of Wild *Artemisia afra* in the Eastern Coastal Belt of the Eastern Cape Province, South Africa

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Abstract. **The variation in essential oil yield and composition of *Artemisia afra* across different geographical regions and harvesting seasons remains poorly understood, despite its significance for commercial and medicinal uses. This study aims to address this gap by investigating the impact of harvesting season on the yield and chemical composition of *A. afra* essential oil in the eastern coastal belt of the Eastern Cape, South Africa. Fresh shoot samples were collected from the Bizana, Ngqeleni, and Centane regions during summer, autumn, winter, and spring. The essential oils were extracted via steam distillation, and the chemical components were analyzed using gas chromatography-mass spectrometry (GC-MS). The highest oil yield (0.92% on a fresh mass basis) was observed in Centane, with a significantly higher concentration of 1.8-cineole (18.66%) in Ngqeleni. The content of Artemisia ketone was significantly higher in Centane (24.51%) during summer, whereas camphor peaked at 34.10% in Ngqeleni during winter. The *cis*- and *trans*-thujone isomers were most abundant in Bizana (40.37% and 10.39%, respectively). The results highlight significant variation in oil yield and composition influenced by location, season, and their interaction. These findings provide valuable insights into optimizing the production of *A. afra* essential oil based on geographical and seasonal factors.**

Medicinal plants play a crucial role in the health care systems of many African countries, where ∼27 million people rely on traditional medicine for treating ailments such as sore throat, poor appetite, diabetes, fever, and wounds. Various parts of medicinal plants, including roots, stems, and leaves are commonly used for these purposes (Fennell et al. 2014; Roberts 1990; Van Wyk 2008). Among these medicinal plants, *A. afra* is widely used in South Africa as an herbal remedy for respiratory conditions, coughs, colds, influenza, malaria, and gastrointestinal disorders (Du Toit and Van der Kooy 2019).

Furthermore, *Artemisia* is recognized as the largest and most widely distributed genus within the Asteraceae plant family, comprising more than 400 species (Koul et al. 2017). Some species of *Artemisia* exhibit significant medicinal properties due to their rich phytochemical and pharmacological profiles, which are primarily attributed to their essential oils (Mamatova et al. 2019). These essential oils possess antimicrobial, antifungal, and anti-inflammatory properties. However, the yield and composition of these oils vary depending on the geographical location where the plants are cultivated. For instance, Kilenga et al. (2022) reported that in *A. afra*, the major oil constituents in plants from Kenya were borneol (5.1%), 1,8-cineole (6.74%), and terpinene-4-ol (6.5%), whereas in plants from Ethiopia, the predominant constituents were artemisyl acetate and yomogi alcohol, both at 13.5%.

A study conducted by Asekum et al. (2017) did not detect artemisia ketone, artemisia alcohol, or artemisyl acetate, whereas previous studies by Mwangi et al. (1995) and Viljoen et al. (2006) reported different percentage compositions of constituents such as camphor, artemisia ketone, and 1.8 cineole compared with findings by Liu et al. (2009). Oyedeji et al. (2009), Liu et al. (2019), and Abad et al. (2012) attributed these variations in oil yield and composition to factors such as geographical location, harvesting season, soil type, chemotype or subspecies, genotype, and drying methods. In addition, the monoterpene content of certain *Artemisia* species, including camphor, chrysanthemum, and 1,8-cineole, varies seasonally, with the highest concentrations often occurring in the winter months. Other studies have identified α- and β-thujone, camphor, borneol, and 1,8-cineole as the primary constituents of *A. afra* essential oil (Graven et al. 1992; Mwangi et al. 1995). Essential oil biosynthesis is also influenced by climatic conditions (Worku and Rubiolo 1996).

Understanding the environmental factors affecting the yield and quality of medicinal plants and their by-products is essential for their commercialization. However, there is limited research on the seasonal variation of essential oil yield and composition of *A. afra* in the eastern coastal belt of the Eastern Cape Province, South Africa. Existing studies predominantly focus on the cultivation and oil composition of *A. afra* in other African countries, with insufficient information regarding the optimal harvesting period to maximize oil yield and constituent concentrations in South Africa (Mofokeng et al. 2024). Addressing this knowledge gap is crucial for identifying the best harvesting season, ensuring consistent and maximum oil yield as well as composition, thereby optimizing the therapeutic efficacy of *A. afra*.

Therefore, this study aimed to investigate the seasonal variation in the yield and major essential oil constituents of wild *A. afra* in the eastern coastal belt of the Eastern Cape Province, South Africa. The findings of this research will be valuable to traditional healers, commercial herbal medicine manufacturers, and conservationists. Identifying the optimal harvesting season will contribute to the consistency and efficacy of *A. afra*–based herbal remedies. In addition, the study will support sustainable harvesting practices, ensuring the long-term availability of this valuable medicinal plant.

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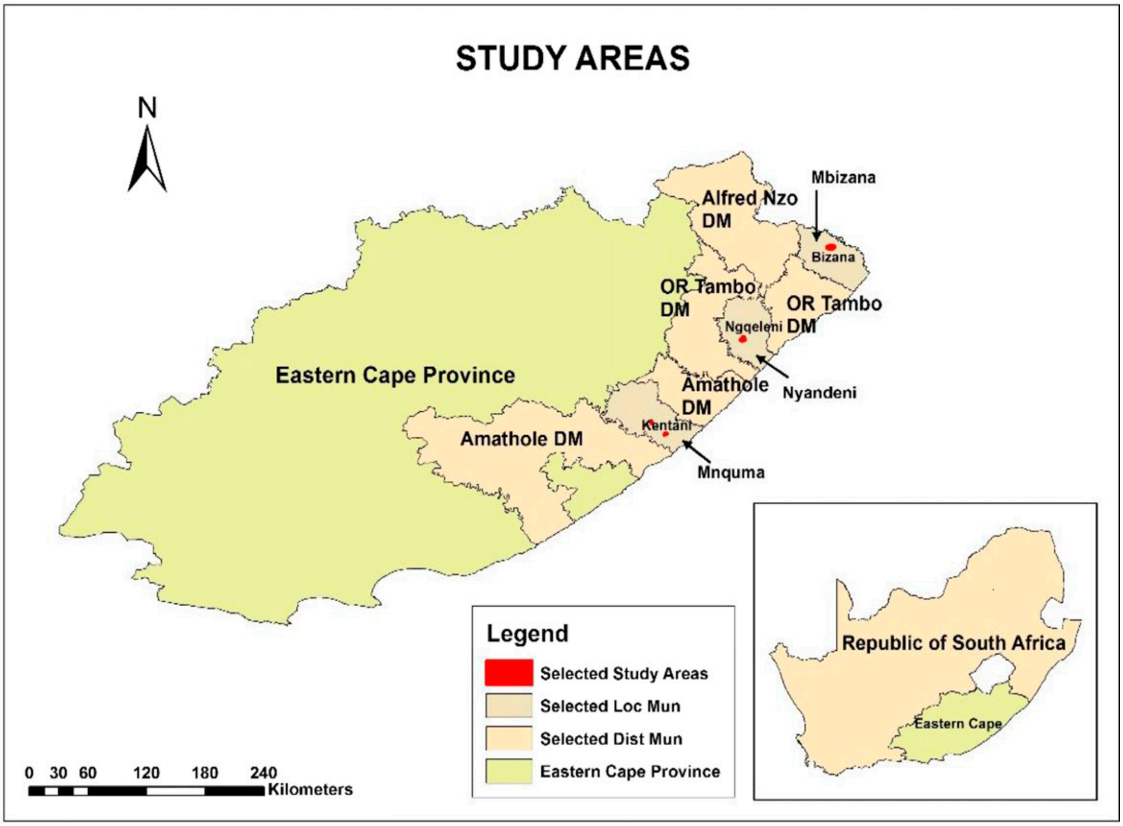

Fig. 1. Study areas in the Alfred Nzo, OR Tambo, and Amathole District Municipalities. (Designed by Mr. L. Meyer of the GIS Section at the Dohne Agricultural Development Institute.)

Materials and Methods

Study area description and sampling procedure

Fresh shoot samples from wild populations of *A. afra* were collected from the Bizana (S30°43′16.366″; E29°48′00.434″), Ngqeleni (S31°41.132′; E29°07.064′) and Centane (S32°22.375′; E28°11.939′) locations in the eastern coastal belt of the Eastern Cape Province, South Africa (Fig. 1). The altitude above sea level was measured at 726.45 m in Bizana, 580 m in Ngqeleni, and 690 m in Centane. Sampling was conducted during autumn, winter, spring, and summer seasons over 2 years (2017 to 2018). In each location, shoot samples of up to 3 kg each were collected from randomly selected plants. All plant shoots were harvested, leaving ≈15 cm of the stem above the ground to ensure plant re-growth. Immediately after harvesting, the samples were put in plastic bags and packed into cardboard boxes to reduce water and essential oil losses during transportation to the Dohne Agricultural Development Institute for analysis. Due to the distance between the areas of sampling and the Dohne Agricultural Development Institute, distillation was done over 2 d, after harvesting. During this time, the samples were stored under room temperature in the laboratory to reduce loss of essential oil due to temperature variations. Climatic data for the study areas were obtained from Agricultural Research Council of South Africa. Soil samples were also collected to determine the fertility status of the soil where the plants were growing (Table 1).

Rainfall and temperature distribution

Rainfall. Rainfall distribution differed among the seasons and locations (Fig. 2). The Bizana location recorded the highest rainfall both in the summer and spring of 2017 and 2018. In all locations, rainfall was low in 2017 compared with 2018. The summer season received a higher amount of rainfall, with the lowest rainfall recorded in winter in all locations for 2018. A similar trend was observed in 2017 when the lowest rainfall was recorded in winter for all locations. However, Ngqeleni and Centane had the higher rainfall in spring.

Temperature. Average minimum temperatures in winter of 2017 ranged from 8.19 °C (Ngqeleni) to 10.73 °C (Bizana). Temperatures were lower in 2017 than in 2018, as presented in Table 2. The spring season in 2017 recorded the highest temperature of 29.82 °C in the Centane location. The locations appear to have similar minimum and maximum temperatures, especially in summer and winter. However, the minimum temperature in Bizana was higher (18.68 °C) than Ngqeleni (11.32 °C) and Centane (9.77 °C) in autumn of 2017. There were differences in the minimum and maximum temperatures recorded across the locations in 2017 and 2018.

Sampling design

During sampling, the area was divided into three portions whereby fresh shoots from healthy *A. afra* plants were randomly harvested in summer, autumn, winter, and spring. Samples from each portion were packed together representing an experimental unit.

Data collection

Essential oil extraction and determination of essential oil content. Upon arrival to the laboratory at Dohne Agricultural Development Institute, each shoot sample was weighed before distillation to determine fresh mass. From the fresh shoot samples, essential oils were extracted using the steam distillation technique. Thereafter, the essential oil was weighed to determine the mass of the oil per

Table 1. Mineral analysis of soils from Bizana, Centane, and Ngqeleni.

Location	Phosphorus	Potassium	Calcium	Magnesium	Zinc	pH
	Mg/L					KCl
Bizana	10	122	3,303	982	2.9	5.13
Centane	4	205	2,397	422	2.7	4.78
Ngqeleni	7	102	2,675	462	2.1	4.76

Table 2. Average seasonal temperatures (°C) from different regions and seasons.

	Ngqeleni				Bizana				Centane			
	2017		2018		2017		2018		2017		2018	
Seasons	Min. T^0	Max. T^0	Min. T^0	Max. T^0	Min. T^0	Max. T^0	Min. T^0	Max. T^0	Min. T^0	Max. T^0	Min. T^0	Max. T^0
Summer	15.8	26.72	13.17	22.65	16.36	26.77	15.42	25.69	16.42	28.06	15.70	26.86
Autumn	11.32	24.84	11.23	21.71	18.68	24.14	11.66	23.63	9.77	25.07	10.24	24.52
Winter	8.19	22.31	9.54	20.62	10.73	21.99	9.97	22.21	8.76	22.81	8.36	22.64
Spring	16.20	26.74	10.98	21.86	14.49	23.65	13.29	23.66	17.59	29.82	11.89	24.19

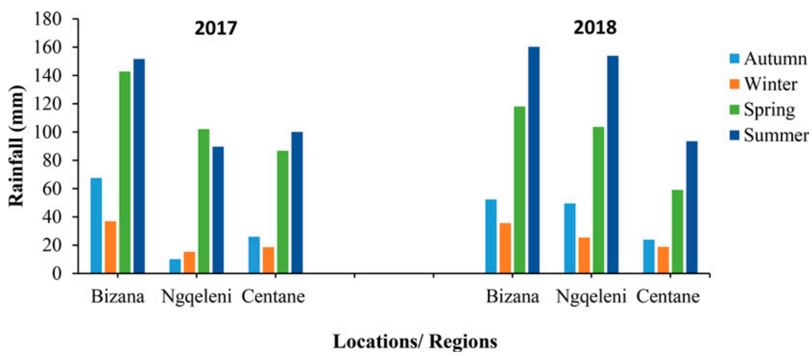

Fig. 2. Seasonal distribution of rainfall in different locations for 2017 and 2018.

shoot sample. Essential oil content (%) was determined using Eq. [1]:

$$\text{Oil content } (\%) = \frac{Essential\ oil\ mass\ (g)}{Fresh\ shoot\ mass\ (g)} \times 100 \quad [1]$$

Essential oil analysis. GC with MS was used for the separation of the chemical constituents of the essential oils of the *A. afra* samples. The oil constituents were quantified using a flame ionization detector. The initial GC temperature was set at 60 °C. Thereafter, it was progressively increased at a temperature ramp of 3 °C/min to 245 °C. Oil component identification was done using the National Institute of Standards and Technology Mass Spectra Library of the United States and the confirmation by retention index was done using the extensive essential oil identification dictionary (Adams 2007). Cross referencing with other oils containing the same compounds was also conducted.

Statistical analysis

Using three samplings per season per location, a combined analysis of variance was used to test differences caused by seasons, locations, and the interaction between season and location, using the statistical program GenStat 15. Years are considered as a random variable; therefore, it was not included as a source of variation in the analysis. The means were compared using Fisher's protected least significant difference test at the 5% level of significance. As the samples were collected in the veld, more variation is expected compared with field trials, where, at least to a certain extent, external sources of variation are controlled.

Results and Discussion

The oil yield percentage of *A. afra* from different regions and seasons

The two-way interaction of location × season was highly significant ($P < 0.001$) with respect to oil yield percentage (Table 3). The main factors of location and year greatly influenced ($P < 0.0001$) oil yield for 2017 and 2018, whereas on the other hand the location was significant at $P < 0.001$ with respect to oil yield. As presented in Table 3, oil yield percentage from Ngqeleni is significantly different from Bizana and Centane for 2017 in summer. Whereas in 2018, all the regions are significantly different from each other. In winter for both 2017 and 2018, Centane is significantly different from Bizana and Ngqeleni. Furthermore, significant differences among all the regions are observed in autumn of 2018.

The highest oil yield was recorded for summer in Centane (0.70%, 0.92%) for both 2017 and 2018, respectively. Summer in Bizana had the second-highest percentage of 0.83% in both years, followed by summer in Ngqeleni (0.61%, 0.59%) in both 2017 and 2018, respectively. Centane experienced the highest maximum summer temperatures in both years, followed by Bizana and Ngqeleni (Table 3). This suggests that *A. afra* will produce more oil at higher temperatures, supporting findings by El-Sherei et al. (2014), who reported the highest essential oil yield (0.75%) for *Solidago canadensis* plants obtained in flowers collected during spring to summer in Egypt. However, Shanjani et al. (2010) obtained the highest (0.85%) essential oil yield percentage from foliage and berries of *Juniperus excelsa* at Iran in autumn and the lowest (0.5%) in summer. The maximum temperatures in autumn for Iran (Iran climate, 1991 to 2020) are averaged at 18.7 °C compared with temperatures in Eastern Cape, signifying that the tested plant oil yield is influenced by low temperatures.

In addition, the essential oil content recorded for *Nectandra grandiflora* and *Nectandra lanceolate* harvested in Brazil were higher in spring and lowest in winter (Ferras et al. 2018). Summer harvest of *Cymbopogon citratus* species yielded higher yield of 0.25%, whereas lowest yield of 0.16% was recorded in leaves harvested in autumn

Table 3. The oil yield percentage of *Artemisia afra* across different regions and seasons.

		Oil yield (%)	
Location	Seasons	2017	2018
Bizana	Autumn	0.44 cd[i]	0.48 e
	Winter	0.58 bcd	0.52 de
	Spring	0.40 de	0.48 e
	Summer	0.83 a	0.83 b
Ngqeleni	Autumn	0.52 cd	0.66 c
	Winter	0.25 e	0.5 e
	Spring	0.47 cd	0.44 e
	Summer	0.61 bc	0.59 cd
Centane	Autumn	0.54 bcd	0.33 f
	Winter	0.49 cd	0.25 f
	Spring	0.51 cd	0.75 b
	Summer	0.70 ab	0.92 a
ANOVA significance			
	Location	***[ii]	**
	Season	***	***
	Season × location	***	***
	CV (%)	12.80	4.57

[i] Means within the same column followed by the same letter are not significantly different according to Tukey's test range.

[ii] **, *** Significant at $P < 0.01$ and 0.001, respectively.

ANOVA = analysis of variance; CV = coefficient of variance.

Table 4. The interaction effect of the major *Artemisia afra* oil constituents from different regions and seasons.

Treatments			Constituents (%)					
Year	Location	Seasons	1.8 Cineole	Ketone	*Cis*-Thujone	*Trans*-Thujone	Camphor	Camphene
	Bizana	Autumn	15.99 abc[i]	1.47 cd	40.37 a	10.23 a	15.02 ef	2.22 de
		Winter	15.04 bcd	0.41 d	38.65 a	8.94 a	14.88 ef	2.83 cd
		Spring	14.93 bcd	0.24 d	39.84 a	9.48 a	14.56 ef	2.05 e
		Summer	12.89 de	0.51 d	35.99 a	9.03 a	15.70 e	2.97 c
	Ngqeleni	Autumn	15.42 bcd	0.34 d	18.63 d	4.47 bc	29.43 b	5.53 a
		Winter	18.66 a	0.31 d	14.75 e	3.86 c	32.06 a	4.52 b
2017		Spring	12.81 de	0.32 d	20.78 d	5.66 bc	25.30 c	4.37 b
		Summer	13.26 cede	3.59 c	13.50 e	3.80 c	29.35 b	5.09 ab
	Centane	Autumn	16.45 ab	19.03 b	23.85 c	6.08 b	15.02 ef	2.97 c
		Winter	16.58 ab	20.74 b	18.95 d	4.15 bc	15.06 ef	2.41 cde
		Spring	13.97 bcd	23.18 a	19.22 d	4.41 bc	13.51 fg	2.03 e
		Summer	10.83 e	24.51 a	18.40 d	4.63 bc	12.67 g	2.92 cd
ANOVA significance								
		Location	NS[ii]	***	***	***	***	***
		Season	***	***	***	**	***	***
		Season × location	***	***	***	***	***	***
		CV (%)	7.42	11.69	3.73	12.59	3.77	8.90
	Bizana	Autumn	14.55 cde	1.36 cd	40.19 a	10.39 a	15.30 de	2.85 b
		Winter	14.55 de	0.39 d	38.22 ab	8.21 b	14.63 def	2.59 b
		Spring	13.38 de	0.27 d	39.81 a	9.78 a	14.29 ef	2.44 b
		Summer	13.88 de	0.55 cd	35.20 b	9.75 ab	15.23 de	2.875 b
	Ngqeleni	Autumn	14.89 cd	0.39 d	16.88 def	4.23 d	28.32 bc	5.28 a
		Winter	18.62 a	0.33 d	15.23 efg	3.91 d	34.10 a	4.28 a
2018		Spring	12.93 e	0.38 d	19.24 d	5.23 cd	26.14 c	4.77 a
		Summer	13.19 de	2.58 c	13.97 fg	4.19 d	29.26 b	5.262 a
	Centane	Autumn	16.46 bc	19.28 b	23.33 c	5.82 c	14.87 def	2.61 b
		Winter	17.22 ab	20.40 b	18.22 de	4.275 cd	17.22 d	2.28 b
		Spring	14.20 de	23.88 a	12.88 g	4.895 cd	13.87 ef	2.98 b
		Summer	10.26 f	24.58 a	18.20 de	4.805 cd	12.23 f	2.91 b
ANOVA significance								
		Location	*	***	***	***	***	***
		Season	***	***	***	***	***	**
		Season × location	***	***	***	*	***	ns
		CV (%)	5.18	10.70	5.56	9.76	5.58	13.06

[i] Means within the same column followed by the same letter are not significantly different according to Tukey's test range.

[ii] NS, *, **, *** Nonsignificant or significant at $P < 0.05$, 0.01, and 0.001, respectively.

ANOVA = analysis of variance; CV = coefficient of variance.

(Jardinetti et al. 2016). These results correspond with findings for the present study, in which the lowest oil yield recorded was 0.25%, observed during winter in Ngqeleni and Centane for 2017 and 2018, respectively. Rainfall could be a contributing factor, because augmented rainfall can lead to higher oil yields in *A. afra*, as it promotes healthy plant growth and development (Desrosiers et al. 2019). As evident in Fig. 2, in winter, rainfall for Bizana is considerably higher than that of Centane and Ngqeleni. Furthermore, Graven et al. (1992) argued that high oil yield on *A. afra* can be obtained when the plants are harvested during flowering and early seed as opposed to earlier or later in the reproductive period. This can be a result for the present study because the flowering was observed from late February to May across different areas.

Average essential oil constituents of *A. afra*

The results of this study indicated that the two-way interaction of location × season was highly significant ($P < 0.0001$) with respect to oil constituents' percentage (%) (Table 4). Regarding the constituent %, the location was significant at $P < 0.001$, whereas the year and season had a substantial impact ($P < 0.0001$) on the constituent percentage for 2017 and 2018.

1.8-cineole. It was observed that 1.8-cineole content was higher during cooler seasons (autumn and winter) than in warmer seasons (summer and spring). The constituent was highest in winter at Ngqeleni (18.66%, 18.62%) followed by Centane in winter (16.58%, 17.22%) and last in autumn at Centane (16.45%, 16.46%) for both 2017 and 2018, respectively. The lowest, however, was recorded in the summer months of 2017 and 2018 in Centane (10.83%, 10.26%). Bizana samples had the second-lowest percentage (12.89%) in 2017, followed by Ngqeleni samples (12.81%). In 2018, Ngqeleni samples had the second-lowest percentage in the spring (12.93%) and the third-lowest percentage in the summer (13.19%). Makunga and Mothapo (2017) alluded that the essential oil of *A. afra* had the highest 1.8-cineole on plants harvested during the winter months (19.78%) supporting findings of the present study.

Season had a major impact on the limonene, which has an aroma comparable to 1.8-cineole (Lola et al. 2002), according to contradicting findings reported by Sgarbossa et al. (2019) with *Aloysi triphylla* essential oil. In the summer (28.04%), when there was less evapotranspiration, limonene was found in greater amounts (Sgarbossa et al. 2019). The results of the current study, however, might be the consequence of less rainfall in winter, which in turn led to decreased evapotranspiration on the plants. Furthermore, as Ranjbar et al. (2020) described, plants collected at different developmental phases may have different oil profiles, which could account for differences in 1.8-cineole. According to Mohammadreza (2008), the main component of *Artemisia annua* L. during its flowering stage was 1.8-cineole. In addition, the plants' geographic location and altitude may have an impact on their oil content.

Artemisia ketone. Artemisia ketone was significantly higher in Centane for both years (2017 and 2018) compared with Ngqeleni and Bizana for all seasons. It was observed that during warmer seasons, Artemisia ketone percentage was higher than that produced in cooler seasons at Centane. These findings support findings of Mothapo and Makunga (2016), who suggested that Artemisa ketone percentage on *A. afra* increased greatly in summer. They further stated that the increase might be due to temperature-induced changes in enzyme activity, increased water stress, as well as the changes in plant growth and development. According to Mossa and El-Feraly (1990), production of ketones in the essential oil is a defense mechanism to water stress because water stress triggers the activation of enzymes involved in the biosynthesis of ketones.

Cis- and trans-*thujone.* In both 2017 and 2018, the Bizana area had the highest levels of *cis*-thujone (40.37%; 40.19%) and *trans*-thujone (10.23%; 10.39%) during autumn. For the Ngqeleni location, the summer months of 2017 were found to have the lowest levels of *cis*- (13.50%) and *trans*- (13.50%) thujone. Last, in 2018, Ngqeleni had the lowest *trans*-thujone (3.91%) and Centane had the lowest *cis*-thujone (12.88%). The result of the study confirms findings of Libbey and Sturtz (1989), who indicated that in South Africa, *cis*-thujone is a major component for essential oils of many plant species. Graven et al. (1992) specified that *trans*-thujone is a major oil constituent of *A. afra* in Zimbabwe. Furthermore, these findings correspond with findings of Lubbe and Verpoorte (2011), who alluded that on the composition of *A. afra*, *cis*- and *trans*-thujone content was found in plants grown in areas with high temperatures, low relative humidity, and high rainfall.

Camphor. Seasonally, camphor tended to be lower in the summer and higher in the winter, as shown in Table 4. In comparison with Centane and Bizana, Ngqeleni comparatively had the highest camphor for every season. Winter in Ngqeleni had the highest percentages at 32.06% and 34.06%, followed by autumn at 29.35% and 28.32%, and summer at 29.35% and 29.26% in 2017 and 2018, respectively. It was also noted that the cooler seasons had the highest percentage of camphor in all locations. Centane has the lowest camphor in 2017 and 2018, with summer season percentages of 12.67% and 12.23%, respectively. The present study contradicts findings of Makunga and Mothapho (2017), who explained that the camphor content of *A. afra* is highest during its flowering stages and summer. However, there is a possibility that the plants were harvested at their flowering stage because for the current study, the plants' developmental stages were not recorded. Kamatou and Viljoen (2010) argued that the camphor content in *A. afra* essential oil is highest from plants grown in well-drained soils having low nutrient levels.

According to Asekum et al. (2007), drying methods affect the relative quantities of the oil components, as a result camphor for *A. afra* when distilled fresh was 14.2% and when sun dried 8.2%. The composition of camphor decreased in air-dried (0.12%) samples compared with fresh material (0.27%) (Ashafa and Pitso 2014). This could have affected the camphor of the current study because some samples were kept at room temperature for ≈48 h before distilling.

Camphene. Winter at Centane had the greatest camphene in 2017, with autumn coming in second at 15.02% for both Centane and Bizana. Summer in Centane had the lowest rates, with 12.67% and 13.51%, respectively. Season had no effect on the camphene of all the plants' oil from the different locations for 2018. Nonetheless, Ngqeleni had the highest, followed by Bizana and Centane. Ferras et al. (2018) explained that the camphene can vary depending on the selected plants' developmental stages, which can be during flowering or fruiting. For this present study, it can be explained that even though the plants were harvested during the same seasons, the possibility of them being in different stages of growth was possible.

Conclusions

In conclusion, the essential oil yield of *A. afra* was significantly influenced by both location and season, as well as the interaction between these two factors. The study identified Artemisia ketone, 1.8-cineole, camphor, camphene, and *cis*- and *trans*-thujone as the major constituents of *A. afra* essential oil. These key compounds were found to be present in higher quantities in specific locations, indicating that environmental factors play a crucial role in the composition and yield of the essential oil. Understanding the effect of location and season on essential oil production can help optimize the cultivation and extraction processes of *A. afra* for various commercial applications such as pharmaceuticals and cosmetics industries.

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