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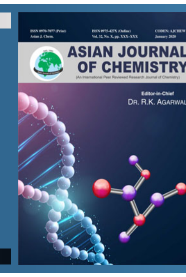

Effect of Seasonal Variation on the Secondary Metabolites and Antioxidant Activity of *Callistemon citrinus* (Curtis) Skeels (Myrtaceae) Grown in Eastern Cape of South Africa

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Callistemon citrinus belong to the kingdom plantae, family Myrtaceae and genus *Callistemon*. It grows in Australia but has been naturalized in South Africa. It is used as traditional herb to combat both gastro-intestinal and respiratory diseases, pain, diseases caused by bacteria and fungi infection. About 500 g of the air-dried leaves were pulverized each month and subjected to hydro-distillation using a modified Clevenger apparatus. Hydro-distillation of the fresh leaves of *C. citrinus* gave a pale yellow volatile oil with a strong scenty fragrance, about 97 components were identified in the 12 treatments analyzed each month. The key components were pinocarvone (1.25-6.17 %), pinocarveol (0.10-9.56 %), α-terpineol (5.24-9.94 %), α-pinene (7.45-22.75 %), limonene (24.08) and eucalyptol (14.69-72.35 %). The compositional profile of the leaves of *C. citrinus* varied between (January-December). Treatments under investigation revealed markedly qualitatively and quantitatively differences. Antioxidant capacity of the volatile oil of *C. citrinus* leaves evaluated also demonstrated significance influence of seasonal variation on its activity. The most exigent activity for DPPH free radical scavenging was recorded in the month of September (spring) with an IC_{50} of 0.50 ± 0.04 mg mL^{-1}, while the most paramount activity for the ABTS assay was 0.94 ± 0.02 mg mL^{-1} in January (summer).The lowest activity were observed in the June (winter) collection with IC_{50} of 1.45 ± 0.00 mg mL^{-1} and 2.19 ± 0.05 mg mL^{-1} for DPPH and ABTS assays, respectively. Results show that seasonal variations affected the chemical compositions, oil yield as well as antioxidant activities of the volatile oil of the leaves of (*C. citrinus*); therefore, it is important to consider such effects for industrial and therapeutic purposes.

Keywords: *Callistemon citrinus*, Seasonal variation, Volatile oil, GC-MS, DPPH, Antioxidant.

INTRODUCTION

Majority of consumers all over the world still depend on medicinal plants as a way out of the health problems militating against them [1]. In South Africa alone more than 60 % from the black communities still depends on herbal mixtures made by traditional health practitioners to cure their ailments in preference to the modern synthetic drugs [2]. *C. citrinus* (Curtis) Skeels, is frequently referred to as 'Red Bottle brush because of its flower that looks like a traditional brush used in rural settings. It is an evergreen tree or flowering shrub, of the family Myrtaceae. It has been naturalized in South Africa and it is found grown in almost every province of the country. The height is about 6-15 m and 1.3-1.5 m in girth with spiky piercing intermediate-green leaves [3]. Various parts of this herb have been engaged as common remedies for treatment of diarrhea, dysentery bronchitis, cough, inflammation, rheumatic pain and also as insecticides in traditional settings [4-6]. This plant possesses abundant of polyphenols in its essential oil and organic extracts [7]. Volatile oils obtained from aromatic plants have been employed by ancient Egyptians in embalming to prevent bacterial growth and thwart decay. Volatile oils have gained rising awareness among consumers over the years as alternative additives to food, cosmetic ingredients or medicinal product [8,9]. These oils are made up many lipophilic and extremely volatile constituents like terpenoids (mono, bi, tricyclic mono and sesquiterpenoids), small chain hydrocarbons and phenylpropanoids obtained from vast varieties of chemical group of compounds recognized to be predisposed to alteration and degradation. If the defensive compartmentation in the plant matrix is deprived, volatile components would be made prone to chemical transformation, oxidative damage and polymerization brought about by heat, light, air, or the developmental stage of the plant which could lead to disparity in the chemical constituents of the oil [10,11]. Due to structural organization common in the same chemical clusters, components of volatile oils have been recognized to simply change into each other by cyclization, isomerization, oxidation, dehydration and dehydrogenation brought about by chemical or enzymatic reactions. Constituents of volatile oil undergo chemical transformation during the course of growth or during oil distillation, thus probing the legitimacy of the genuineness of the oil. Aromatic and medicinal plants exhibit changes in their active components at different seasons of the year (autumn, spring, summer and winter) which could be as a result of the fluctuation of different environmental variables like temperatures and rainfall [12,13]. Research conducted on *Pelargonium graveolens* leaves gave the best yield and maximum geraniol content (29.87 %) in winter [14]. Similarly, spathulenol and caryophyllene oxide which are the major components of *Eugenia uniflora* leaves were found higher in the oil obtained from the leaves in dry seasons (April-September) as a result of the biotic pressure that is capable of altering the plant volatiles [15]. The volatile oil of *Melissa officinalis* exhibited disparity in percentage yield monthly possibly because of the influence of the sun and shade in the micro environment where the plants grow [16]. Higher percentages were obtained in the extraction of the volatile oils (2.5 and 1.95 %) from *E. camadulensis* and *E. cinera*, respectively during summer season. This may be linked with the physical and chemical strain encountered by plants during the period in which secretion to various defense components of the secondary metabolite particularly the terpenes/terpenoids compounds occurs [17]. As documented in a recent study *Mentha canadensis* yielded the maximum menthol content (5.3 %) in February and lowest in May (3.5 %) owing to the plant ontogeny, environmental regulation and seasonal fluctuation which affects the genetic expression of oil production [18,19], likewise thymol which is the major component of *Origanum syriacum* was the highest in summer (46.70 %) while *p*-cymene was the highest in early spring (62.18 %) [20]. This development was attributed to the long photoperiods which could be responsible for the increase in volatile oil of the foliage and phenolic monoterpenes in the oil [21,22]. In the same vein *Ocimum basilicum* exhibited highest volatile amount in winter and lowest in summer. The low yield in content might be due to high temperature and partial evaporation of some of its constituents [23].

To circumvent variations in oil components with respect to fluctuation in season, all collected leaves were obtained from fully established plants with no flowering activity and no visual damage. Although, a number of researchers have documented disparity in the chemical constituents of volatile oils with respect to their origin, environmental circumstances and the developmental phase of the collected plant materials, there is a dearth of information on the seasonal fluctuations of the chemical constituents, antioxidant capacity and percentage yield of oil of *C. citrinus*. Our aim was to ascertain if there is any relationship between the chemical disparity observed in the volatile oil constituents, antioxidant capacities, percentage yield of the oil of *C. citrinus* and the fluctuation in season taking place yearly.

EXPERIMENTAL

Fresh leaves of this plant were collected from their natural habitation in Alice, Eastern Cape of South Africa from January to December (2017) and the volatile oils were isolated through hydro-distillation for about 3 h using Clevenger apparatus as earlier [24].

Extraction and analysis: Freshly extracted oils were immediately analyzed with GC-MS to enable the examination of the impacts of variation in seasons on the components of the distilled oils during the study period (January-December). The volatile oils were pale yellow and liquid at ambient temperature, with a strong scenty fragrance.

GC-MS analysis: Investigation of the GC-MS analyses of the leaf oils of *C. citrinus* was performed on a Hewlett-Packed HP 5973 mass spectrometer connected with an HP-6890 gas chromatograph as previously described [25,26], functioning conditions for this analyses were as followed: column temperature (original temperature: 70 °C, maximum temperature: 325 °C), equilibration time: 3 min, ramp: 4 °C min^{-1}, finishing temperature: 240 °C; inlet mode: split less, original temperature: 220 °C, pressure: 8.27 psi, flush out flow: 30 mL/mL, flush out time: 0.20 min, gas brand: helium, column: capillary, 30 m × 0.25 mm i.d., coat width: 0.25 µm, original flow: 0.7 mL/min, linear speed: 32 cm/s; MS: EI method at 70 eV.

Determination of constituents: The recognition of the assorted components was based on comparison of their mass spectra with those of Nist Library mass spectra data base and mass spectra from literature. The height areas were employed to get total percentage composition of the volatile oil.

in vitro Antioxidant activity

DPPH assay: The antioxidant capacity and free radical scavenging potential of the volatile oils of the leaf of *C. citrinus* were tested against the free radical DPPH. Different concentrations (0.025-0.40 mg mL^{-1}) of the volatile oils were incubated with DMSO solution, thereafter 1 mL of DPPH was added, the mixture was thoroughly vortexed and left for about 0.5 h at room temperature in the dark, absorbance was read at 517 nm. The volatile oils capability to scavenge DPPH free radical was calculated based on the equation below:

$$\text{Inhibition (\%)} = \frac{A_{control} - A_{vo}}{A_{control}} \times 100$$

where $A_{control}$ is the absorbance of DPPH + DMSO; A_{vo} is the absorbance of DPPH + volatile oil. The dose response curve was plotted and the IC_{50} value of the typical antioxidant and volatile oil were calculated [7,27].

ABTS assay: Modified method of Witayapen *et al.* [28] was employed to assess the ABTS potential of *Callistemon citrinus*. Mixture of ratio 1:1 volume of ABTS 7.0 mM with 4.9 mM potassium persulfate was used to obtain the stock solution; the uniform solution obtained from the two mixture above was kept under ambient temperature for 12 h in a dark cupboard. Dimethyl sulfoxide was added to the ABTS radical so as to counter balance the absorbance to 0.705 ± 0.001 at 743 nm. The assay was engaged upon by mixing 500 µL of the volatile oil in DMSO (0.025-0.40 mg mL^{-1}) with 500 µL of ABTS radical cation ($ABT^{\bullet+}$) to obtain a final volume of 1 mL for each mixture, which is left to react for approximately 8-10 min. Absorbance was determined at 760 nm spectrophotometrically and the radical scavenging ability of the volatile oil was conveyed as % inhibition of ABTS using the equation earlier reported above for DPPH as [7,27].

Statistical analysis: Statistical analyses were carried out using Microsoft Excel 2007.

RESULTS AND DISCUSSION

Chemical composition of the volatile oil from *Callistemon citrinus*: Hydro-distillation of the fresh leaves of *Callistemon citrinus* gave a pale yellow volatile oil with a strong fragrance; 98 components were identified in the 12 treatments analyzed each month for a period of 1 year. The results show that the content of the essential oil varied throughout the months and seasons of the year (Table-1). The key components were pinocarvone (1.25-6.17 %), pinocarveol (0.10-9.56 %), α-terpineol (24-9.94 %), α-pinene (7.45-22.75 %), limonene

TABLE-1
FRACTIONAL COMPOSITION OF THE CONSTITUENTS OF THE LEAVES OF *Callistemon citrinus* (JANUARY-DECEMBER, 2017)

Month of harvest	RT (min)	Jan.	Feb.	Mar.	April	May	June	July	Aug.	Sep.	Oct.	Nov.	Dec.
Treatment		1	2	3	4	5	6	7	8	9	10	11	12
Yield (%)		0.27	0.31	0.23	0.25	0.22	0.18	0.20	0.19	0.24	0.25	0.25	0.30
Compounds		Percentage composition of various components											
3-Hexen-1-ol	3.301	–	–	0.03	0.11	–	0.10	0.12	0.03	–	0.06	0.02	0.20
Isoamyl acetate	3.436	1.72	0.17	0.71	1.46	0.91	0.66	0.01	0.05	0.51	1.01	0.58	2.38
Methyl 4-methyl, methyl valerate	3.551	–	–	0.04	0.06	0.05	0.03	–	–	–	0.06	0.03	0.12
Butyl isobutanoate	3.724	–	–	–	–	–	–	–	–	–	–	–	0.02
β-Thujene	3.908	–	–	0.36	0.06	0.04	–	0.46	3.29	–	–	–	0.03
α-Pinene	3.986	14.85	20.02	22.75	22.32	13.60	12.39	9.44	10.83	7.45	9.88	14.85	10.77
Camphene	4.177	–	0.63	0.18	0.30	0.63	0.39	–	0.16	0.20	0.30	0.48	0.69
Sabinene	4.316	–	–	0.11	–	–	–	–	0.47	–	0.16	–	–
β-Pinene	4.382	0.60	1.10	5.87	1.28	2.33	0.96	2.75	8.43	0.63	5.83	1.10	1.26
Heptane-3,4-dimethyl	4.523	–	0.81	–	–	–	–	–	–	–	–	–	–
α-Phellandrene	4.541	–	–	0.61	–	–	–	3.47	10.64	–	–	0.03	–
Terpinolene	4.645	–	–	0.34	–	–	–	–	–	–	–	–	–
4-Hexen-1-ol, acetate	4.674	–	–	–	0.09	–	–	–	–	–	–	–	–
o-Cymene	4.698	0.74	–	–	–	–	–	–	–	–	–	–	–
Limonene	4.789	–	–	–	–	–	–	–	24.08	–	–	–	–
Eucalyptol	4.795	63.72	48.98	37.17	51.92	27.25	26.43	38.77	–	72.35	14.69	40.89	39.99
α-Terpinene	–	–	–	–	–	–	–	–	–	–	–	–	–
γ-Terpinene	4.647	–	0.21	0.61	0.23	0.43	0.17	0.77	2.78	0.17	1.54	–	0.15
α-Terpinolene	–	–	–	–	–	–	0.18	–	–	–	–	–	–
3-Carene	5.217	–	0.17	–	–	1.49	–	–	–	–	–	–	1.22
4-Carene	5.265	–	0.76	2.61	–	–	–	0.12	3.06	–	2.84	0.93	–
Linalool	5.307	0.51	0.57	2.07	0.59	0.83	0.43	1.48	–	0.28	2.88	0.51	1.09
α-Pinene oxide	5.386	–	–	–	0.04	–	–	–	–	–	0.09	–	0.21
cis-p-Metha-1(7), 8-diene-2-ol	5.446	0.30	0.53	–	0.29	1.20	0.76	0.56	0.06	–	0.05	0.10	1.36
α-Fenchol	5.487	0.58	0.93	0.13	0.11	1.25	0.81	1.02	–	0.48	0.34	1.08	1.87
Campholenal	5.565	–	–	–	0.11	0.50	0.26	0.10	0.03	0.20	0.13	0.38	0.56
cis-p-2,8-Menthadien-1-ol	5.502	–	–	0.44	0.03	0.11	0.09	0.17	–	–	–	0.10	0.18
trans-p-2,8-Menthadien-1-ol	5.523	–	–	–	–	0.18	–	–	–	–	0.20	–	–
α-Fenchene	5.639	–	–	0.04	–	–	–	–	–	–	–	–	–
Pinocarveol	5.679	3.98	5.75	0.10	2.76	6.75	5.29	2.35	0.16	3.86	–	8.07	9.56
Pinocarvone	5.755	1.83	2.81	–	1.25	3.76	2.74	2.20	–	1.40	–	4.09	6.17
Camphenilanol	5.765	–	0.27	–	–	–	–	0.21	0.11	–	–	–	–
Terpinen-4-ol	5.976	0.63	0.79	1.31	0.58	1.33	0.72	4.23	3.17	–	2.45	0.76	0.86
Myrtenol	6.095	–	0.29	–	0.20	0.76	0.48	0.62	–	0.13	–	0.62	0.87
α-Terpineol	6.104	6.18	8.01	8.47	5.24	9.94	6.78	7.38	7.39	5.48	8.89	8.26	8.75
cis-Carveol	6.227	0.44	0.70	–	0.41	1.58	0.95	0.79	–	0.68	–	1.12	1.55
α-Bergamontene	6.383	–	–	1.29	–	–	–	–	–	–	–	–	–
Nerol	6.421	0.27	–	–	–	–	–	–	0.23	–	–	–	–
D-Carvone	6.429	–	–	–	–	–	–	–	–	–	–	–	0.92
Geraniol	6.479	–	0.40	1.60	0.38	0.90	0.46	0.99	2.20	–	3.12	0.55	–
Citral	6.588	–	–	–	–	–	–	–	0.02	–	0.11	–	–
Longifolene	6.574	–	–	0.05	–	–	–	–	–	–	–	–	–
Carvacrol	6.635	–	–	–	–	–	0.04	0.03	–	–	–	–	–
β-Guaiene	6.683	–	–	0.08	–	–	–	–	–	–	–	–	–
β-Bisabolene	6.753	–	–	0.05	–	–	–	–	–	–	–	–	–

Bornyl acetate	6.761	–	–	–	–	0.26	0.04	–	–	–	0.14	0.07	0.08
3,4-Xylenol	6.766	–	–	–	–	–	–	–	–	–	–	0.05	–
Thymol	6.782	–	–	–	–	0.08	–	0.33	–	–	–	–	–
Methyl nerolate	6.926	–	–	–	0.10	0.35	–	–	–	–	–	–	–
Methyl geranate	6.932	–	–	0.27	–	–	0.12	–	–	–	1.24	–	–
α-Sinensal	7.018	–	–	–	–	–	–	–	–	–	–	–	3.01
Adamantan-2-ol	7.022	–	–	–	–	3.53	–	–	–	–	–	–	–
2-Acetoxy-1,8-cineole	7.123	–	0.18	0.28	0.30	0.64	0.32	0.29	0.26	0.23	0.90	0.35	0.47
α-Farnesene	7.196	–	–	0.07	–	–	–	–	–	–	–	2.54	–
Neryl acetate	7.197	–	–	–	–	–	–	–	0.22	–	0.25	–	–
Eugenol	7.220	–	–	–	–	–	–	0.47	–	–	–	–	–
Geranyl acetate	7.320	–	–	0.47	0.07	0.08	0.03	0.35	1.85	–	1.41	0.07	0.14
α-Himachalene	7.373	–	–	0.03	–	–	–	–	–	–	–	–	–
Methyl cinnamate	7.418	–	–	0.18	0.04	0.11	0.04	–	–	–	0.56	0.06	0.08
Pivarose	7.458	–	–	–	–	0.08	–	–	–	–	–	0.06	–
β-Phenylethyl acetate	7.478	–	–	–	–	–	–	–	–	–	0.13	–	–
α-Santalol	7.640	–	–	–	–	0.10	–	–	–	–	0.05	–	–
Di-epi-α-cedrene	7.688	–	–	–	–	–	–	–	–	–	0.16	–	–
α-Gurjunene	7.689	–	–	0.06	–	–	–	–	–	–	–	–	–
Caryophyllene	7.765	–	–	0.54	–	–	–	0.04	0.41	–	1.61	–	–
Aromandendrene	7.893	–	–	0.2	–	0.11	–	0.04	–	–	0.65	–	–
Humulene	7.988	–	–	0.13	–	–	–	–	–	–	0.44	0.11	–
Alloaromandendrene	8.040	–	–	0.11	–	0.31	0.03	0.09	–	–	1.72	–	–
α-Elemene	8.077	–	–	–	–	–	–	–	–	–	0.20	–	–
Trifluoroacetyl-lavandulol	8.169	–	–	–	–	–	–	–	–	–	0.24	–	–
γ-Gurjenene	8.176	–	–	0.77	–	–	–	–	0.08	–	–	–	–
1,4-Dimethyl tetralin	8.178	–	–	–	–	–	–	–	–	–	–	0.06	–
Bicyclogermacrene	8.247	–	–	0.70	–	–	–	–	–	–	–	–	–
Germacrene	8.252	–	–	–	–	–	–	–	–	–	2.30	–	–
Durohydroquinone	8.322	–	–	0.14	0.05	0.07	0.02	0.11	–	–	–	0.03	0.05
δ-Cardinene	8.365	–	–	0.13	–	–	–	–	–	–	0.46	–	0.15
Epizonarene	8.399	–	–	–	–	–	–	–	–	–	0.14	–	–
Elemol	8.503	–	–	–	–	0.07	–	–	–	–	–	–	–
Epiglobulol	8.658	–	0.20	–	0.06	0.24	0.33	0.15	0.35	–	0.38	0.12	0.19
Valencene	8.704	–	–	–	–	–	–	–	0.54	–	–	–	–
Palustrol	8.721	–	–	–	–	0.24	–	–	–	–	0.65	0.11	0.12
Aromandendr-1-ene	8.723	–	–	0.17	–	–	–	0.16	–	–	–	–	–
Spathulenol	8.780	–	0.19	0.93	0.14	1.07	–	0.22	1.21	–	1.98	0.57	0.45
Globulol	8.831	–	0.39	0.71	0.30	1.56	0.67	0.82	1.81	0.43	2.18	0.72	0.71
Viridiflorol	8.882	–	–	0.08	0.14	0.71	–	0.46	1.20	–	1.62	0.38	0.36
Rosifoliol	8.902	–	–	–	–	0.90	–	–	1.59	–	–	0.43	–
Ledol	8.936	–	–	–	–	–	–	0.11	2.15	–	–	–	–
1-Acetyl-2-amino-3-cyano-7-isopropyl-4-methylazulene	8.996	–	–	–	–	4.70	2.42	–	–	–	7.48	–	–
β-Caryophylladienol	9.103	–	–	–	–	0.40	–	–	–	–	–	–	–
Viridiflorene	9.247	0.21	–	–	–	–	–	–	0.33	–	0.25	–	–
trans-Farnesol	9.440	–	–	–	–	–	–	0.04	0.34	–	0.39	–	–
Cedran-diol (8S,14)	9.475	–	–	–	–	0.02	–	–	–	–	–	–	–
Eudesma-4,11-dien-2-ol	9.549	–	–	–	–	–	–	–	0.12	–	–	–	–
Benzyl benzoate	9.969	–	–	–	–	0.04	–	–	0.02	–	–	0.01	0.04
Farnesol acetate	10.05	–	–	–	–	–	–	–	0.01	–	0.03	–	–
(*Z,Z*)-α-Farnesene	10.24	–	–	–	–	–	–	–	–	–	0.02	–	–
Phytol	11.40	–	–	–	–	–	–	–	–	–	0.31	–	0.08
Total (%)		96.56	94.86	92.99	91.02	91.49	65.14	81.72	89.68	94.48	82.52	90.29	96.71
Hydrocarbons monoterpene		16.19	22.89	33.48	24.19	18.52	14.09	17.01	63.74	8.45	20.55	17.39	14.12
Oxygenated monoterpenes		78.44	70.03	51.29	63.91	59.95	46.24	61.23	13.37	84.86	32.95	66.53	73.94
Sesquiterpene hydrocarbons		0.21	–	4.38	–	0.82	0.03	0.33	1.36	–	7.95	2.65	0.15
Oxygenated sesquiterpenes		–	0.78	–	0.64	4.89	1.00	1.80	8.77	0.43	7.25	2.33	1.83
Diterpene		–	–	–	–	–	–	–	–	–	0.31	–	0.08
Hydrocarbon			0.81	–	–	–	–	–	–	–		0.06	–
Esters		1.72	0.35	1.95	2.03	2.48	1.24	0.65	2.41	0.74	5.73	1.23	3.29
Alcohol		–	–	0.03	0.2	–	0.10	0.12	0.03	–	0.06	0.07	0.20
Aldehyde		–	–		–	–	–		–	–	–	–	3.01
Others		–	–	1.85	0.05	4.83	2.44	0.58	–	–	7.72	0.03	0.09

(24.08) and eucalyptol (14.69-72.35 %), the compositional profile of the leaves of *Callistemon citrinus* (January-December) treatments under investigation revealed marked qualitative and quantitative differences. The oils of the 12 treatments were rich in monoterpene hydrocarbon (8.45-63.74 %), oxygenated monoterpenes (13.37-84.86 %), sesquiterpene hydrocarbons (0.03-7.95 %), oxygenated sesquiterpenes (0.43-8.77 %), diterpenes (0.08-0.31 %), esters (0.35-5.73 %) and alcohols (0.03-0.20 %). The highest monoterpene hydrocarbon content (63.74 %) was recorded in August (winter period) while the highest oxygenated monoterpene (84.86 %) was obtained in September (spring). Ester content of the oil had the highest percentage of (5.73 %) in October (spring). Diterpenes are scarcely encountered in genuine volatile oils obtained through distillation due to their low volatility, but phytol ($C_{20}H_{40}O$), an acyclic diterpene alcohol was seen in trace amount (0.08-0.31 %) for the first time in the volatile oil of *Callistemon citrinus* grown in South Africa. Triterpenoids and higher terpenoids like sterols or carotenoids are only present in the non-volatile extracts such as plant resins or gums [29].

Effect of seasonal variation on percentage yield of the volatile oil: The oil yields of the 12 treatments generally ranged from 0.18-0.31 % with the highest percentage recorded in summer period (0.31 %) and the lowest in winter (0.18 %). The lower yield of the volatile oil in winter may be linked to the presence of moisture content in the leaves as a result of high relative humidity, which is known to reduce the volatile oil production as a result of surplus water [30]. The results in this study (Table-1) showed seasonal fluctuation in the yields and this corroborates the report of other researchers on volatile oil yields and chemical composition which are majorly influenced by factors such as light, rainfall, liming, time of harvest, soil, altitude and developmental phase of plant [31-35].

Effect of seasonal variation on volatile oil content: Effect of seasonal fluctuation was also established in the volatile oil contents of this study as it was revealed that maximum oil content was recorded in December-February (94.86-96.63 %) summer period in South Africa (Table-1), which gave credence to a previous study where it was reported that extraction of the volatile oil of *Thymus serpyllum* L produced highest percentage of oil content in summer season [35]. The lowest oil content from this study was recorded in June (58.72 %) (Table-1) when winter began in South Africa, this might be related to excess of water, which has the tendency to reduce volatile oil production [30]. Seasonal effects on volatile oil production usually make summer stands out as the season with the maximum volatile oil content this could be linked to the positive influence of higher temperature in this season coupled with precipitation which is capable of affecting the vegetative growth of the plants [36,37]. Eucalyptol, a cyclic monoterpenoid ether, with various degrees of pharmacological effects which also serves as a marker for medicinal essential oil classification [38] is the dominant component of the volatile oils. It compared favourably well with the volatile oil component from previous study of the same plant from South Africa [7], it reached its highest value (72.35 %) in spring (September) and the lowest value (48.98 %) in summer (February) (Table-1) substantiating the findings documented [17].

Sudden disappearance of eucalyptol in the month of August and the appearance of limonene (24.08 %), which could not be detected in any other month, may be due to the reversal of eucalyptol to α-terpineol and loss of water (dehydration) from α-terpineol leading back to α-terpinyl cation and further loss of a proton from this cation giving rise to limonene [39-41]. Formation of α-phellandrene as a result of the disappearance of eucalyptol in the month of August may also be due to the α-terpinyl cation following the 1,7-hydride shift pathway leading to the formation of phellandryl cation and further loss of a proton from one of the carbon of the phenyl ring to give α-phellandrene (Fig. 1).

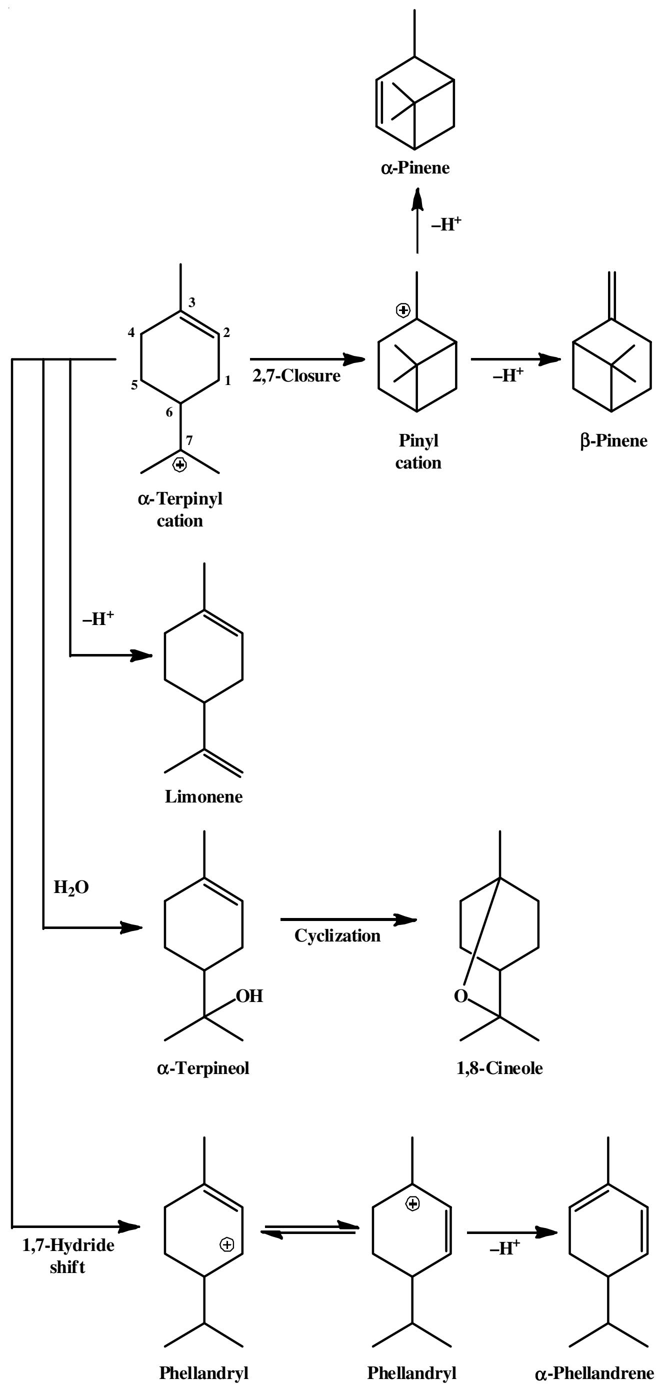

Fig. 1. Biosynthetic pathway of some of the components of *Callistemon citrinus* volatile oil

TABLE-2
EFFECT OF SEASON ON ANTIOXIDANT CAPACITY OF *Callistemon citrinus* VOLATILE OIL IC_{50} (mg mL^{-1})

Volatile oil	Summer leaf oil	Winter leaf oil	Spring leaf oil	Autumn leaf oil
$DPPH^{\bullet}$	0.88 ± 0.05	1.45 ± 0.00	0.50 ± 0.04	1.35 ± 0.04
ABTS	0.94 ± 0.02	2.15 ± 0.07	1.14 ± 0.05	2.19 ± 0.08

The next key component in the volatile oil of this plant was α-pinene, which gave a range of 7.45-22.75 %. It has maximum value in the month of March (autumn) 22.75 % and minimum value in the month of September (spring) 7.45 %. α-Terpineol an unsaturated volatile monocyclic alcohol is the third main component with utmost value in the month of May (9.94 %) and least value in the month of April (5.24 %). Its antihypernociceptive behaviour and anti-inflammatory activity has been documented [42]. It can be concluded from Table-1 that seasons affect the volatile oil components of this plant.

Effect of flowering phase on percentage yield and volatile oil content: Another significant aspect to be considered is the flowering phase of *C. citrinus*. According to the field surveillance of this investigation, the flowering of this plant occurs during spring and summer period and remains in autumn. Both volatile oil content and percentage yield suggest that they might be linked to the flowering period because the highest percentage yield of 0.31 % in February, 0.30 % in December, 0.25 % in November and 0.25 % in April, coupled with the highest oil content of 96.63 % in December, 96.56 % in January, 92.99 % in March and 90.29 % in November, fell between the summer, spring and autumn period of South African season. Comparable behaviour during the flowering phase has been recorded for *Lamiaceae* species [34,36,42].

Effect of seasonal variation on the antioxidant activity of *Callistemon citrinus*: Antioxidant capacity of the volatile oil of *C. citrinus* leaves also demonstrated significant influence of seasonal variation on its activity. The most prominent activity was recorded in the month of September (spring) with an IC_{50} of 0.50 mg mL^{-1} for DPPH, followed by January (summer) IC_{50} 0.88 mg mL^{-1}, April (autumn) IC_{50} 1.35 mg mL^{-1}. The least activity was observed in June (winter) collection with IC_{50} of 1.45 mg mL^{-1} (Table-2), while the most effective activity for ABTS was in the summer IC_{50} of 0.94 mg mL^{-1} followed by September (spring) with IC_{50} of 1.14 mg mL^{-1}. The least activity for the ABTS model was in April (autumn) with IC_{50} of 2.19 mg mL^{-1}. It has been documented that antioxidant activity of volatile oils is not only due to phenolic content of the oil but constituents like monoterpene alcohols, ketones, aldehydes, hydrocarbons and ethers, which also add to the free radical scavenging activity of some volatile oil [43]. The volatile oils of *Thymus caespititus, Thyme camphorates and Thyme mastichina* which have high contents of linalool and eucalyptol were shown to have high antioxidant activity, which were almost equal to that of α-tocopherol [11]. Similarly, the high scavenging activity of *M. aquatic* was also linked to eucalyptol in the volatile oil [44]. The volatile oil of the plant in this study also yielded high content of eucalyptol (cyclic monoterpenoid ether) in the month of September (72.35 %), January (63.72 %) and April (51.92 %), which were spring, summer and autumn seasons, respectively. This might contribute to its high antioxidant activity in addition to phenolic compound like terpenoids and pinocarveol. Similar behaviour have been reported for the antioxidant capacity of *Bellis perennis* flowers which exhibited highest antioxidant capacity for samples collected from spring to autumn. The discrepancy in the antioxidant action of this plant was reportedly due to the fluctuation in environmental factors such as day and night temperature, rainfalls, drought and the duration/intensity of sunshine [45]. The effect of seasonal fluctuation on the antioxidant activity in the present study revealed that the least activity recorded in the winter was in variance with that reported for *Ocimum basilicum* which exhibited it highest antioxidant capacity in winter season with an IC_{50} value of 4.8 μg mL^{-1} [23].

Conclusion

It was concluded from this investigation that season brings about chemical disparity in oil yield, antioxidant capacity and oil content of *C. citrinus* from South Africa. The dominance of eucalyptol, in the different treatments (January-December) (except for the month of August) of the leaf of this plant under investigation, makes it an excellent marker for *C. citrinus* species. Taking into account that summer, spring and autumn gave the highest yields of volatile oil, antioxidant capacity as well as maximum content of eucalyptol. It can be said that these seasons are the most suitable period to get a better quality of the oil of this plant in South Africa. More so the leaves of this plant possess volatile oil which differs in quantity and quality as a result of seasonal fluctuation. It is important for researchers to know the season with the highest quality of volatile oil as this tends to give the best biological activity.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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