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Seasonal variation in essential oil composition, oil toxicity and the biological activity of solvent extracts of three South African *Salvia* species

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Abstract

Aromatic plants contain both volatile and non-volatile fractions and the chemical composition of these two fractions may be influenced by seasonal changes. The essential oil and solvent extracts of *S. africana-caerulea*, *S. africana-lutea* and *S. lanceolata*, collected at the same locality throughout the 2005 growing season, were compared in terms of essential oil composition, yields and biological activities. Mostly quantitative, rather than qualitative variation was observed in the oil composition of each species. Major fluctuations in the composition of *S. africana-caerulea* oil included limonene (2–33%) and viridiflorol (2–24%). Levels of α-pinene (1–12%), myrcene (2–12%) and α-eusdesmol (trace-13%) fluctuated seasonally in the *S. africana-lutea* oil. In *S. lanceolata*, considerable changes were noted for β-caryophyllene (1–19%), β-caryophyllene oxide (1–21%) and ledol (3–12%). The extract prepared from *S. lanceolata* harvested in winter was more active against Gram-positive bacteria. The *S. africana-caerulea* extract exhibited the most favourable anti-plasmodial activity when harvested in winter (IC_{50} value: 12 μg ml^{-1}), which contrasts with the lowest anti-plasmodial activity of *S. lanceolata* obtained at the same period (IC_{50} value: 43 μg ml^{-1}). The anti-oxidant activity of the solvent extracts also displayed variation over seasons with the winter collection of *S. africana-lutea* yielding the most favourable anti-oxidant activity (IC_{50} value: 10 μg ml^{-1}). All the solvent extracts prepared from the winter collection exhibited the lowest toxicity ($20 < IC_{50}$ values < 60 μg ml^{-1}), while the three essential oils obtained from autumn collection were more toxic ($0.03 < IC_{50}$ values < 0.4 μg ml^{-1}).

Keywords: Essential oil composition; *Salvia africana-caerulea*; *S. africana-lutea*; *S. lanceolata*; Biological activities; Seasonal variation

1. Introduction

Biological activities are correlated to the presence of chemical compounds, particularly secondary metabolites. The presence of these compounds may assist in predicting some traditional uses of medicinal plants (Rasoanaivo and Ratsimamanga-Urverg, 1993). The season and even the number of hours plants receive sunlight may influence the phytochemistry of the plant since some compounds may be accumulated at a particular period to respond to environmental changes (Koenen, 2001). Plant material collected at different times of the year may contain other possible novel compounds with other bio-activities (Eloff, 1999). *Salvia* species are used in traditional medicine locally to treat various ailments including malaria, wounds, microbial infections and symptoms associated with cancer (Watt and Breyer-Brandwijk, 1962). We previously demonstrated that indigenous *Salvia* species exhibited various *in vitro* pharmacological properties (Kamatou et al., 2005, 2006). This prompted an investigation to determine which season of the year would provide the *Salvia* extracts with the most promising biological activity. Furthermore, there has been local interest to develop these species for the commercial production of essential oil. Information on seasonal variation and the impact thereof on oil composition are crucial to optimize harvesting protocols.

In this study, we determined the seasonal variation in the essential oil composition of three *Salvia* species, evaluated the biological activities of the solvent extracts, investigated the

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toxicity profile of the essential oils and solvent extracts over the seasons and recommended the best time of the year for harvesting plants in terms of essential oil yields.

2. Materials and methods

2.1. Plant material

Three *Salvia* species, namely *Salvia africana-caerulea*, *S. africana-lutea* and *S. lanceolata*, all collected from the South Western Cape (South Africa) were investigated. The aerial parts of each species were harvested for 12 consecutive months at the same location during the year 2005. The identification of the three plants was confirmed by the South African Biodiversity Institute (Tshwane) and voucher specimens are housed at the School of Pharmacy, Tshwane University of Technology.

2.2. Isolation and analysis of the essential oils

The essential oils were isolated from fresh plant material by hydrodistillation using a Clevenger type apparatus for 3 h. The aerial parts of each species were air dried at room temperature, powdered in a grinder and extracted with methanol:chloroform (1:1) for 4 h at 37 °C. The extracts were concentrated and dried under vacuum.

GC analyses were performed using a Perkin Elmer 8700 gas chromatograph equipped with two FID's, a data handling system and a vaporizing injector port into which two columns of different polarities were installed: a DB-1 fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm; J & W Scientific Inc., Rancho Cordova, CA, USA) and a DB-17HT fused-silica column (30 m × 0.25 mm i.d., film thickness 0.15 μm; J & W Scientific Inc.). Oven temperature was programmed, 45–175 °C, at 3 °C min^{-1}, subsequently at 15 °C min^{-1} up to 300 °C, and then held isothermal for 10 min; injector and detector temperatures, 280 °C and 290 °C, respectively; carrier gas, hydrogen, adjusted to a linear velocity of 30 cm s^{-1}. Samples were injected using the split sampling technique, ratio 1:50. The percentage composition of the oils was computed by the normalization method from the GC peak areas, which were calculated as mean values of two injections of each oil sample, without using response factors.

The GC-MS unit consisted of a Perkin Elmer Autosystem XL gas chromatograph equipped with DB-1 fused-silica column (30 m × 0.25 mm i.d., film thickness 0.25 μm; J & W Scientific Inc.), and interfaced with Perkin Elmer Turbomass mass spectrometer (software version 4.1). Oven temperature was programmed, 45–175 °C, at 3 °C min^{-1}, subsequently at 15 °C min^{-1} up to 300 °C, and then held isothermal for 10 min; injector and detector temperatures, 280 °C and 290 °C, respectively; transfer line temperature, 280 C; ion trap temperature, 220 C; carrier gas, helium, adjusted to a linear velocity of 30 cm s^{-1}; split ratio, 1:40; ionization energy, 70 eV; ionization current, 60 μA; scan range, 40–300 μ; scan time, 1 s.

The identity of the components was assigned by comparison of their relative retention indices, relative to C_8–C_{17} *n*-alkane indices and GC-MS spectra from a home-made library, construction based on the analyses of reference oils, laboratory-synthesised components and commercially available standards.

2.3. Evaluation of the biological activities

The biological activities were determined at the end of each season of the year as follows: November (spring), February (summer), May (autumn) and August (winter).

2.3.1. The anti-bacterial activity

The anti-bacterial activity of the solvent extracts was performed on *Staphylococcus aureus* (ATCC 25923), *Bacillus cereus* (ATCC 11778), *Escherichia coli* (ATCC 8739) and *Klebsiella pneumoniae* (NCTC 9633) strains using the microwell dilution assay (Eloff, 1998). Stock solutions of the solvent extracts (64 mg ml^{-1}) in acetone were serially diluted with sterile water in a 96-well plate. Bacterial suspensions were then added to the wells prior to incubation for 24 h at 37 °C. *p*-Iodonitrotetrazolium chloride [40 μl of 0.04% (w/v); Sigma] was added directly to the culture medium of each well and the minimum inhibitory concentration (MIC) determined after 6 h. The assay was performed in duplicate and repeated at least twice. Ciprofloxacin (Merck) was used as the positive control. The final concentration of acetone in the well had no effect on bacterial growth.

2.3.2. The anti-plasmodial activity

The anti-plasmodial activity was assessed using the [^{3}H]-radiometric method (Desjardins et al., 1979; Van Zyl et al., 2006) over a single growth cycle (48 h) against the *Plasmodium falciparum* chloroquine-resistant FCR-3 strain. Dilutions of the solvent extracts were plated out in triplicate in a 96-well plate with control wells before parasitised red blood cells (1% haematocrit and 0.5% parasitaemia) were added to test samples and incubated for 24 h in a candle jar at 37 °C. The [^{3}H]-hypoxanthine radio-isotope (Amersham) was added to the plate and incubated for a further 24 h. The amount of [^{3}H]-hypoxanthine radio-isotope incorporated into the parasite was determined with a Beta scintillation counter (Wallac®). The inhibitory concentration which killed 50% of parasite growth (IC_{50} value) was determined. Chloroquine diphosphate (Sigma) was used as the positive control.

2.3.3. The anti-oxidant activity

The scavenging capacity of the solvent extracts was measured using 2,2-diphenyl-1-picrylhydrazyl (DPPH). The DPPH method uses a relatively stable free radical 2,2-diphenyl-1-picrylhydrazyl (Fluka) as a reagent (Mambro et al., 2003). Dilutions of the samples in DMSO were plated out in triplicate in a 96-well plate. The $DPPH^{\cdot}$ radical (96 μM) was added to the test samples and HPLC grade methanol to the controls and the plate incubated in the dark for 30 min at room temperature before measuring the absorbance at 550 nm. Percentage decolourisation of the DPPH was calculated with the methanol treated controls. The IC_{50} values were determined as the concentration of test sample required to scavenge 50% of the DPPH radical. Vitamin C (Saarchem) was used as a positive control. Essential oils are known to have negligible anti-oxidant activity

and due to the low yields of oil in the volatiles they were not included in this assay.

2.3.4. The toxicity profile

The 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT; USB®) colourimetric assay was performed on transformed human kidney epithelial cells (Graham) at 37 °C in 5% CO_2 (Mosmann, 1983). Various dilutions of the essential oils and solvent extracts were plated out in triplicate in a 96-well plate containing approximately 0.25 million cell ml^{-1} with appropriate control wells and incubated for 44 h. The MTT solution (0.05 g ml^{-1}) was then added to all the wells and the

Table 1
Percentage of the components identified in the essential oil of *Salvia africana-caerulea* over a one year period

Components	RRI	*Salvia africana-caerulea*											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
α-thujene	924	t	0.1	1.6	0.1	0.1	t	1.2	0.3	0.1	0.4	0.1	0.1
benzaldehyde	927	t	t	t	t	t	t	t	t	t	t	t	t
α-pinene	930	1.7	4.2	t	3.4	4.0	2.6	t	3.9	3.7	8.2	4.1	4.7
camphene	938	0.7	0.1	0.1	0.1	0.1	t	0.1	0.1	0.1	0.1	0.1	0.1
sabinene	958	0.2	0.2	t	0.3	0.4	0.2	t	0.2	0.3	0.1	t	0.4
1-octen-3-ol	961	0.2	0.2	t	0.3	0.4	0.2	0.2	0.2	t	0.1	t	0.4
β-pinene	963	t	0.3	t	t	t	t	0.2	0.3	1.0	2.9	1.3	t
myrcene	975	0.6	0.8	0.6	0.7	0.6	0.6	t	0.8	0.6	10.7	0.2	0.8
α-phellandrene	995	0.6	0.5	0.4	0.5	0.5	0.3	0.3	0.3	0.2	0.8	t	0.6
p-cymene	1003	t	0.1	0.1	0.1	t	0.2	0.1	0.6	0.2	0.2	1.5	0.2
o-cymene	1000	0.2	0.4	0.7	0.4	t	0.2	0.1	0.3	0.2	0.2		0.6
1,8-cineole	1005	t	t	t	t	t	t	t	t	t	t	0.5	t
β-phellandrene	1005	0.4	0.8	t	0.4	1.0	0.4	t	0.1	1.2	7.8		0.8
limonene	1009	24.9	31.4	23.4	27.3	28.5	21.4	10.2	29.2	31.5	5.8	1.5	32.6
cis-β-ocimene	1017	0.1	0.1	0.1	0.2	t	0.1	t	0.3	0.2	3.1	t	0.2
trans-β-ocimene	1027	0.3	t	t	0.1	t	t	t	0.1	0.2	1.0	t	t
γ-terpinene	1035	t	0.1	t	0.1	0.1	0.1	t	0.1	0.1	0.2		0.1
trans-sabinene hydrate	1037	t	0.1	t	0.1	0.1	0.1	t	0.1	0.2	0.2	t	0.1
cis-sabinene hydrate	1066	t	t	t	t	t	t	t	t	t	t	0.1	t
linalol	1074	1.5	0.4	1.6	0.6	0.4	0.3	0.4	0.4	0.4	0.3	0.3	0.4
trans-p-2-menthen-1-ol	1074	t	t	t	t	t	t	t	t	t	t	0.6	t
camphor	1095	0.4	0.2	0.1	0.3	0.2	0.3	0.5	t	t	0.3	t	0.2
δ-terpineol	1134											t	
terpinen-4-ol	1148	8.3	1.4	0.3	0.4	3.2	2.2	7.3	1.7	1.1	1.1	0.5	1.1
α-terpineol	1159	1.2	0.4	1.5	1.1	0.4	0.2	1.4	0.2	0.1	0.5	0.7	0.4
trans-carveol	1189	t	t	t	t	t	t	t	t	t	t	t	t
carvone	1206	t	t	t	t	t	t	t	t	t	t	0.3	t
cis-jasmone	1372	t	t	t	t	t	t	t	t	t	t	t	t
α-gurjunene	1400	t	t	t	t	t	t	t	t	t	t	t	t
β-caryophyllene	1414	0.7	0.4	0.5	0.4	0.5	0.2	0.7	0.3	0.4	0.3	1.9	0.4
β-gurjunene*	1426	6.1	1.4	1.3	2.3	3.8	3.9	6.7	2.0	1.3	4.3	0.7	1.0
aromadendrene	1428	t	0.5	0.7	t	t	t	2.2	t	t	t	0.9	0.6
eudesmadiene*	1435	t	t	t	t	t	t	t	t	t	t	0.8	t
α-humulene	1447	t	0.3	0.6	1.4	t	0.9	t	1.3	0.5	1.8	1.2	0.4
allo-aromadendrene	1456	1.5	0.9	0.7	0.1	2.5	0.7	1.3	0.2	1.4	0.5	t	0.9
viridiflorene	1487	0.1	1.1	2.2	1.3	1.0	1.8	2.3	1.0	1.5	1.1	0.2	1.2
α-muurolene	1494	1.0	0.4	1.4	0.6	2.2	2.8	1.1	0.4	0.5	1.1	t	0.4
γ-cadinene	1500	0.6	1.0	0.9	1.2	0.9	0.9	t	2.4	1.3	0.3	1.5	2.0
trans-calamenene	1505	0.2	0.8	2.8	3.4	4.4	8.9	t	0.7	0.3	3.4	0.3	1.0
trans-nerolidol	1549	1.2	1.9	2.2	2.3	2.5	2.4	2.0	2.4	2.3	0.7	1.4	1.9
β-caryophyllene alcohol	1550	1.8	2.3	2.9	3.0	2.6	3.1	1.6	4.0	3.6	1.4	1.4	2.2
spathulenol	1551	t	t	t	t	t	t	t	t	t	t	29.1	t
β-caryophyllene oxide	1561	t	t	t	t	t	t	t	t	t	t	14.6	t
globulol	1566											t	
viridiflorol	1569	11.1	21.0	24.4	22.8	18.9	20.1	14.2	22.8	21.2	1.6	6.5	20.6
ledol	1580	6.4	2.6	3.7	3.7	3.7	5.6	7.5	3.4	3.1	1.9	t	2.2
epi-cubenol	1600	1.3	0.8	1.3	1.3	1.4	2.0	1.1	1.2	1.2	0.8	t	0.8
T-cadinol	1616	0.7	3.4	2.5	3.8	3.4	3.4	2.5	3.9	3.5	1.3	3.0	3.3
β-eudesmol	1620	0.4	0.8	3.2	1.0	0.8	0.7	0.7	0.5	0.6	3.8	0.7	0.8
α-eudesmol	1634	1.4	1.4	3.0	2.3	2.2	2.3	2.7	1.5	1.4	7.7	2.0	1.5
% Identification		75.8	82.8	84.8	87.4	90.8	89.1	68.6	87.2	85.5	76.0	78.0	85.0
Yield (w/f.w.)		0.15	0.13	0.15	0.18	0.16	0.16	0.07	0.19	0.11	0.13	0.15	0.16

RRI: Relative retention indices calculated against *n*-alkanes, t: trace (<0.05%); *determined by mass spectra only.

plate incubated for further 4 h before the absorbance was measured at the test wavelength of 540 nm and the reference wavelength of 690 nm using a spectrophotometer (Labsystems) connected to the Ascent® version 2.4 software. The IC_{50} values (concentration at which 50% of cells were killed) were calculated using the appropriate controls. The toxicity of the essential oils and solvent extracts was compared arbitrary to the anti-cancer drug, 5′-fluorouracil (Merck).

2.3.5. Data analysis

All the IC_{50} values were determined from the log sigmoid-dose response curve generated using the Enzfitter® version 1.05 software. Comparison of biological activities between seasons was determined using ANOVA. The IC_{50} values are given as a mean ± S.D. of at least three replicate experiments. Pearson's correlation coefficient was used to determine any correlation between the toxicity profile and major components of each essential oil.

Table 2
Percentage of the components identified in the essential oil of *Salvia africana-lutea* over a one year period

Components	RRI	*Salvia africana-lutea*											
		Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sept	Oct	Nov	Dec
α-thujene	924	0.5	0.1	0.4	0.4	0.3	0.3	0.5	0.4	0.2	0.2	0.6	0.1
α-pinene	930	11.0	0.5	7.1	8.6	11.6	5.0	11.9	9.8	4.6	3.2	6.0	1.1
camphene	938	t	0.1	t	t	0.1	t	0.1	0.1	0.1	0.1	t	0.1
sabinene	958	t	0.3	1.4	1.9	3.9	2.7	0.9	0.1	0.1	0.7	0.9	0.1
1-octen-3-ol	961	t	0.3	1.4	1.9	3.8	2.7	0.9	0.1	0.1	0.7	0.9	0.1
β-pinene	963	3.4	0.2	t	t	t	1.8	2.9	3.8	1.9	t	0.2	0.9
myrcene	975	10.7	3.7	5.0	2.6	4.3	5.9	5.3	8.4	2.7	5.4	11.5	2.2
α-phellandrene	995	0.6	0.2	0.8	0.4	0.9	1.1	1.0	0.8	0.8	0.7	t	0.6
δ-3-carene	1000	1.9	3.9	1.1	7.8	1.5	8.3	8.0	3.5	4.4	0.1	t	6.8
o-cymene	1000	10.3	0.4	8.1	3.0	7.1	0.6	3.4	0.7	0.5	0.3	t	1.1
p-cymene	1003	2.6	0.2	1.3	0.7	0.4	0.3	0.4	0.2	0.2	0.1	7.6	0.3
1,8-cineole	1005	5.3	0.5	3.2	2.6	2.4	4.0	4.9	3.5	2.4	2.5	1.9	3.3
β-phellandrene	1005	5.3	0.5	3.2	2.6	2.04	4.0	4.9	3.5	2.4	2.5	t	3.3
limonene	1009	t	3.4	t	2.8	t	6.0	3.7	5.4	3.7	3.3	1.6	5.3
cis-β-ocimene	1017	1.4	3.0	1.3	t	0.5	0.5	0.5	1.7	0.2	2.1	5.4	0.2
trans-β-ocimene	1027	0.5	1.1	0.6	t	0.1	0.2	0.1	0.6	0.2	0.7	1.8	0.1
γ-terpinene	1035	t	0.4	0.1	t	0.1	0.2	0.2	0.1	0.2	0.1	t	0.1
trans-sabinene hydrate	1037	t	0.4	0.1	t	0.1	0.2	0.2	0.1	0.2	0.1	t	4.7
linalol	1074	0.6	0.2	0.3	0.2	t	t	t	0.4	0.7	0.4	0.8	0.8
camphor	1095	0.2	0.4	0.5	0.4	t	t	t	0.3	0.2	0.4	0.2	0.3
terpinen-4-ol	1148	1.3	0.1	0.5	0.7	t	t	t	0.2	0.2	t	0.6	1.6
α-terpineol	1159	t	0.3	0.1	t	t	t	t	t	0.2	t	0.8	0.3
1-decanol	1259	0.8	t	0.4	0.4	0.4	0.6	0.6	0.8	0.5	0.5	t	1.4
bornyl acetate	1265	0.5	t	t	0.6	t	t	t	t	0.1	t	t	0.2
α-gurjunene	1400	0.5	0.6	4.7	5.0	4.6	4.3	2.5	0.7	0.8	0.4	1.1	0.9
β-caryophyllene	1414	2.7	2.0	2.4	1.5	1.1	0.8	0.9	2.1	4.3	2.6	3.4	3.4
β-gurjunene*	1426	1.0	3.3	t	t	0.2	t	0.2	2.8	1.5	1.0	0.3	1.2
aromadendrene	1428	1.0	1.1	1.2	0.7	1.4	0.6	0.8	1.4	1.4	0.9	2.7	0.6
α-humulene	1447	1.1	1.1	2.4	2.4	1.3	1.8	1.8	1.1	1.9	1.5	1.9	1.7
trans-β-farnesene	1455	t	0.4	t	t	0.7	t	0.4	0.9	0.2	0.4	t	0.1
allo-aromadendrene	1456	t	0.4	t	t	0.7	t	0.4	0.9	0.2	0.4	1.3	0.1
germacrene-D	1474	0.5	0.9	1.4	0.5	0.4	0.3	0.7	0.5	0.6	1.0	t	0.8
bicyclogermacrene	1487	2.2	2.3	1.0	0.8	0.4	0.5	0.2	0.1	0.2	0.5		0.4
viridiflorene	1487		t	t	t	t						1.3	
β-bisabolene	1495	0.5	1.8	0.9	0.5	0.5	0.4	0.5	0.5	0.8	3.4	1.3	0.8
δ-cadinene	1505	0.4	2.3	0.6	0.5	0.5	0.4	0.4	0.5	0.6	0.4	4.7	0.5
trans-calamenene	1505	t	t	t	t	t	t	t	t	t	t	0.9	t
elemol	1530	1.6	0.9	1.2	1.3	0.6	0.7	0.8	0.7	1.4	1.8	3.5	2.2
trans-nerolidol	1549	0.2	0.7	0.7	0.3	0.5	0.2	0.5	0.7	0.6	0.6	2.0	0.3
β-caryophyllene	1550	0.2	0.7	0.4	0.3	0.5	0.2	0.5	0.7	0.6	0.6	2.0	0.3
spathulenol	1551	3.3	2.8	6.3	5.6	10.9	2.3	4.3	6.8	6.7	3.8	1.9	3.1
β-caryophyllene	1561	2.3	2.7	4.1	2.8	2.4	1.9	1.9	2.8	3.5	3.9	0.8	2.6
humulene epoxide*	1579	0.5	2.6	0.8	0.9	0.4	0.7	0.7		t	1.3	3.3	0.7
epi-cubenol	1600	t	t	t	t	t	t	t	t	t	t	1.8	t
γ-eudesmol	1609	0.9	4.0	2.4	1.5	2.6	2.9	2.8	2.4	2.2	3.3	t	3.6
T-cadinol	1616	1.3	2.2	1.7	2.7	2.3	3.2	1.8	1.5	2.3	2.2	1.9	1.9
β-eudesmol	1620	3.2	8.9	2.1	4.6	4.7	1.4	5.3	1.8	9.9	2.1	1.7	12.7
α-eudesmol	1634	5.0	10.6	8.1	9.5	10.4	10.9	9.3	8.5	12.6	9.1	t	12.9
% Identification		85.3	72.5	79.3	79.0	87.0	77.9	87.1	81.9	79.1	65.3	78.6	85.8
Yield (w/f.w.)		0.12	0.15	0.14	0.12	0.17	0.11	0.09	0.11	0.18	0.20	0.12	0.10

RRI: Relative retention indices calculated against *n*-alkanes, t: trace (<0.05%); *determined by mass spectra only.

3. Results and discussion

3.1. Variation in yield and essential oil composition

The essential oil yield [w/w, fresh weight (f.w.)] of each species varied with the respective seasons. The highest yield was obtained from plants collected in late winter for *S. africana-caerulea* (August), spring for *S. africana-lutea* and *S. lanceolata* (October and September, respectively) (Tables 1, 2 and 3). All three species demonstrated the lowest essential oil yield in early or mid-winter (July for *S. africana-caerulea* and *S. africana-lutea* and June for *S. lanceolata*). A continuous increase in the yield was noted for *S. africana-lutea* and *S. lanceolata*. Pitarevic et al. (1984) recorded a variation in essential oil yield

Table 3
Percentage of the components identified in the essential oil of *Salvia lanceolata* over a one year period

Components	RRI	*Salvia lanceolata*										
		Jan	Mar	Apr	May	Jun	July	Aug	Sep	Oct	Nov	Dec
α-thujene	924	0.4	0.5	0.5	0.4	0.1	0.1	0.2	0.4	0.4	0.5	0.2
α-pinene	930	3.8	t	t	0.3	1.2	0.8	1.4	0.5	2.7	2.6	0.3
camphene	938	0.1	t	t	t	t	1.1	t	t	0.1	t	0.1
sabinene	958	1.9	0.3	0.3	0.2	0.3	0.7	0.8	0.1	1.9	1.4	0.1
1-octen-3-ol	961	1.9	0.3	0.3	0.2	0.3	0.7	0.8	0.1	1.9	1.4	0.1
β-pinene	963	1.8	t	t	0.6	0.8	0.8	2.2	0.7	1.3	1.0	0.1
myrcene	975	3.9	0.7	0.1	1.6	1.0	3.0	1.5	2.0	6.1	0.6	0.1
α-phellandrene	995	0.1	t	0.1	t	0.2	0.3	0.2	t	0.1	t	0.1
δ-3-carene	1000	0.1	t	t	0.1	0.2	0.1	0.5	0.1	0.1	t	0.4
o-cymene	1000	0.9	t	t	t	0.5	0.8	0.3	t	1.0	t	t
p-cymene	1003	1.1	1.0	0.4	0.9	0.5	0.2	0.7	1.4	0.5	1.1	0.2
1,8-cineole	1005	0.7	1.0	0.5	1.3	1.2	1.0	0.5	1.0	1.0	1.8	0.6
β-phellandrene	1005	0.7	1.0	0.5	0.7	1.2	1.0	1.4	1.0	1.0	t	0.6
limonene	1009	3.6	t	t	t	t	1.6	2.1	t	1.9	1.8	t
cis-β-ocimene	1017	0.8	0.1	t	1.1	1.5	1.9	3.1	1.6	2.5	t	t
trans-β-ocimene	1027	0.4	t	t	0.7	1.0	1.3	1.6	1.5	1.9	t	t
γ-terpinene	1035	t	t	t	t	0.6	t	0.1	0.1	0.1	t	t
trans-sabinene hydrate	1037	t	t	t	t	0.4	t	0.2	0.1	0.1	t	t
linalol	1074	0.6	t	0.3	0.8	0.7	0.3	0.2	t	1.1	t	0.2
camphor	1095	0.4	t	0.1	0.4	0.7	0.3	0.2	0.3	t	t	0.4
terpinen-4-ol	1148	0.2	1.0	0.7	0.5	0.6	0.6	0.8	0.7	0.8	1.0	0.4
α-terpineol	1159	t	0.2	0.5	0.1	0.4	0.5	0.9	0.1	0.2	1.3	0.3
bornyl acetate	1265	t	t	1.1	t	t	0.5	t	t	t	t	t
β-bourbonene	1373	0.3	1.5	1.1	0.8	2.1	1.2	1.2	1.0	1.3	t	0.8
β-elemene	1388	0.7	2.0	1.3	2.2	1.8	1.0	1.1	2.6	0.9	t	0.6
α-gurjunene	1400	0.3	0.4	0.9	0.2	0.3	t	t	0.2	0.3	0.2	0.9
β-caryophyllene	1414	12.9	13.1	3.1	19.2	18.6	17.8	17.0	18.4	12.7	5.7	0.7
β-gurjunene*	1426	1.6	1.8	1.1	1.5	1.2	2.3	1.4	1.5	0.7	0.1	1.8
aromadendrene	1428		2.9	0.5	3.2	0.2			1.0	0.4	0.8	0.6
γ-elemene*	1430	0.5	2.0	0.9	0.6	2.0	1.0	1.3	1.2	3.4		0.6
geranyl acetate	1434	0.4	0.8	1.9	0.6	2.0	t	0.9	5.2	5.2	t	
α-humulene	1447	5.9	8.4	2.7	t	9.7	8.5	8.6	8.5	5.5	4.7	1.6
allo-aromadendrene	1456	0.9	0.5	0.5	0.8	0.9	1.0	1.1	0.9	0.6	2.4	0.6
germacrene-D	1474	2.2	1.4	0.3	2.7	2.2	1.9	4.3	1.2	3.0	t	1.2
bicyclogermacrene	1487	4.8	1.3	1.0	3.4	6.0	5.4	7.9	2.5	4.7	t	1.8
β-bisabolene	1495	0.9	1.3	0.3	0.9	0.8	1.0	0.9	0.9	0.8	t	0.6
γ-cadinene	1500	1.1	0.7	0.6	1.0	0.8	1.0	1.1	0.6	0.8	2.5	0.3
trans-nerolidol	1549	0.7	0.9	0.8	0.8	0.7	3.1	0.9	4.6	0.8	2.2	0.8
spathulenol	1551	6.5	4.3	10.6	3.1	2.6	2.8	4.0	1.9	2.4	18.3	0.7
β-caryophyllene oxide	1561	3.4	7.1	21.2	3.3	2.2	2.0	1.8	2.4	1.4	14.3	10.3
globulol	1566	t	t	t	t	t	t	t	t	t	0.6	t
viridiflorol	1569	t	t	t	t	t	t	t	t	t	0.9	t
ledol	1580		4.1	2.9	11.7	4.5	3.6	3.7	4.1	4.3	5.2	t
humulene epoxide*	1579	2.8	6.7	3.6	4.6	4.5	4.4	4.0	4.3	6.5	t	10.8
epi-cubenol	1600	0.8	1.4	0.4	0.9	0.7	0.8	t	1.0	t	0.6	0.8
T-cadinol	1616	0.7	0.8	1.3	0.6	0.7	0.9	1.6	0.2	t	1.9	1.4
β-eudesmol	1620	0.9	1.3	t	1.1	1.0	0.8	3.4	0.2	3.9	1.3	1.7
α-eudesmol	1634	1.2	3.8	1.3	3.3	3.4	2.7	t	3.3	t	0.4	1.0
% Identification		72.9	74.6	63.7	76.4	82.3	80.8	85.9	79.4	86.3	81.1	43.8
Yield (v/f.w.)		0.07	0.04	0.04	0.03	0.03	0.06	0.04	0.11	0.08	0.10	0.05

RRI: Relative retention indices calculated against *n*-alkanes, t: trace (<0.05 %), *determined by mass spectra only. NB: Sample from February collection was not done due to the lack of plant material.

for *S. officinalis* collected in Yugoslavia over various seasons, with July (flowering period) giving the highest yield. The oil yields varied from 0.07% (July) to 0.19% (August) for *S. africana-caerulea*. In *S. africana-lutea*, the highest yield (0.20%) was obtained in October, while July gave the lowest yield (0.09%). In *S. lanceolata*, the lowest yield was obtained in May and June (0.03%), with September giving the highest yield (0.11%). The higher essential oil yield obtained in early spring, may be explained by the fact the plants remain in flower from August to January, a period of full vegetation. During this period, plants may produce substantial amounts of essential oils in order to attract more pollinators (Palá-Paúl et al., 2001).

The components found in the essential oil of each species at the different seasons are reported in Tables 1, 2 and 3. Mostly quantitative variation was noted. Major fluctuations in oil components of *S. africana-caerulea* include α-pinene (trace-8%), limonene (2–33%), terpinen-4-ol (0.3–8%), *trans*-calamenene (0.2–9%) and viridiflorol (2–24%) (Table 1). These components represent up to 60% of the total oil in January and only 20% of the total oil in December. In the November sample of *S. africana-caerulea*, spathulenol and β-caryophyllene oxide were present in high amounts, but were present only in trace amounts during the rest of the year.

The major variation in *S. africana-lutea* oil (Table 2) includes α-pinene (1–12%), myrcene (2–12%), *o*-cymene (0.3–10%), spathulenol (2–11%) and α-eudesmol (trace-13%). These five components of *S. africana-lutea* represent up to 45% of the total oil in May, but only 20% in November.

The major components in *S. lanceolata* showing considerable changes include β-caryophyllene (trace-19%), α-humulene (trace-10%), spathulenol (1–18%), β-caryophyllene oxide (1–21%) and ledol (3–12%) (Table 3). These components represent close to 50% of the total oil in April and only 15% in December.

Although no pattern in the fluctuation of the components of an individual oil component was observed, the most conspicuous variation was recorded for β-caryophyllene and β-caryophyllene oxide in *S. lanceolata*. The variation of these two compounds was inversely related: a rise in the β-caryophyllene oxide content was accompanied by a decrease in the content of β-caryophyllene (Table 3). The content of β-caryophyllene oxide increased from January and peaked in April when the content of β-caryophyllene was at the lowest.

Researchers have reported seasonal variation in essential oil yields and composition for many species. Pitarevic et al. (1984) also noted variation in essential oil composition of *S. officinalis* collected at various seasons of the year. Those components that significantly varied included 1,8-cineole (6–13%), α-thujene (11–23%), β-thujene (25–40%) and camphor (7–16%). The qualitative variation in essential oil composition of *S. libanotica* was also demonstrated (Farhat et al., 2001), where the major components of the oil including 1,8-cineole (48–57%) and camphor (8–12%) fluctuated from one season to another. This study has demonstrated that the essential oil yield of *Salvia* and change in the amount of each component varied from one season to another. It is known that many factors affect the essential oil yield and composition such as season, herbivory, temperature and reproductive stage (Putievsky et al., 1986; Figueiredo et al., 1997).

3.2. Seasonal variation in biological activities

3.2.1. The anti-bacterial activity of the solvent extracts

Most of the solvent extracts of the three species studied exhibited anti-bacterial activity (MIC values ≤ 8 mg ml^{-1}). The MIC values of each plant for the seasons are given in Table 4. The best activity against Gram-negative bacteria was obtained from the spring sample (November), with the exception of *S. africana-caerulea* against *K. pneumoniae*. For *S. africana-lutea*, the Gram-positive bacteria exhibiting the best activity was *B. cereus* (0.8 mg ml^{-1}) from the sample harvested in spring and *S. aureus* (0.30 mg ml^{-1}) from the collection done in autumn. The solvent extract of the winter collection of *S. lanceolata* was more active against *S. aureus* (1.0 mg ml^{-1}) and summer/winter

Table 4
The biological activities of the solvents extracts and the toxicity profile (essential oils and solvent extracts) over seasons of three indigenous *Salvia* species: values followed by ± are the standard deviation (S.D.) of the mean

Species	Seasons	Biological activities (n=3)							
		Anti-bacterial (MIC values in mg ml^{-1})				Anti-plasmodial IC_{50} in μg ml^{-1}	Anti-oxidant IC_{50} in μg ml^{-1}	Toxicity profile (IC_{50} in μg ml^{-1})	
		Ec	Kp	Bc	Sa			EO	SE
S. africana-caerulea	Summer	8.0	8.0	1.0	0.1	16.6±1.8	62.0±4.5	1.6±0.7	18.5±1.1
	Autumn	8.0	4.0	2.0	1.0	26.4±3.2	39.1±1.9	0.5±0.1	23.0±3.9
	Winter	8.0	4.0	0.5	2.0	11.6±4.5	42.3±1.9	1.0±0.3	29.8±3.8
	Spring	4.0	4.7	6.0	3.0	22.7±2.8	47.6±2.6	1.9±0.5	14.4±3.4
S. africana-lutea	Summer	8.0	8.0	2.0	1.0	27.0±5.2	25.7±1.1	1.6±0.3	21.4±2.1
	Autumn	8.0	8.0	8.0	0.3	27.1±1.0	12.6±1.4	0.1±0.0	27.4±2.7
	Winter	4.0	4.0	4.0	4.0	32.9±2.0	10.1±0.5	2.2±0.7	60.3±8.5
	Spring	3.0	3.0	0.8	0.8	15.9±5.0	33.4±3.7	7.2±0.7	25.0±1.9
S. lanceolata	Summer	16.0	8.0	2.0	4.0	26.7±1.4	29.2±3.5	4.3±1.0	15.2±0.9
	Autumn	16.0	8.0	4.0	2.0	24.1±4.2	38.2±3.0	0.04±0.01	39.6±4.0
	Winter	8.0	4.0	2.0	1.0	42.6±6.3	34.6±1.2	1.8±0.7	52.3±6.3
	Spring	4.0	2.0	3.0	2.0	26.0±3.0	68.1±3.7	3.7±0.9	26.7±6.4
Controls		4.0×10^{-5}[a]	1.6×10^{-4}[a]	4.1×10^{-5}[a]	3.1×10^{-4}[a]	0.06±0.01[b]	4.7±0.1[c]	136.1±16.6[d]	

[a]Ciprofloxacin, [b]Chloroquine diphosphate, [c]Vitamin C, [d]5-Fluorouracil, Ec: *E. coli* (ATCC 8739); Kp: *K. pneumoniae* (NCTT 9633); Bc: *B. cereus* (ATCC 11778); Sa: *S. aureus* (ATCC 25923); EO: Essential oil; SE: Solvent extract.

collection against *B. cereus* (2 mg ml^{-1}). *Salvia africana-caerulea* exhibited its best activity against *B. cereus* in winter (0.5 mg ml^{-1}) and *S. aureus* in summer (0.1 mg ml^{-1}) (Table 4).

Many studies have reported differences in the biological activity of the same plant collected in different seasons. McGaw et al. (2002) investigated the variation in anti-bacterial activity of *Schotia* species and noted slight fluctuations which corresponded to small variations in the chromatograms obtained from one season to another. Maréchal et al. (2004) also demonstrated seasonal variation in anti-bacterial activity of solvent extracts of *Bifurcaria bifurcata* (Cystoseiraceae) with the highest level of activity obtained from spring to summer. In the current study, the variation obtained in the anti-bacterial activity depended on both the plant species and the test organism involved.

3.2.2. The anti-plasmodial activity of the solvent extracts

The anti-plasmodial activity of the three species as influenced by the seasons varied between and within species (Table 4). The sample of *S. africana-caerulea* collected during winter was the most active (IC_{50} value: 12±5 μg ml^{-1}), while the winter collection of *S. lanceolata* (IC_{50} value: 43±6 μg ml^{-1}) was the least active (Table 4). An ANOVA analysis indicated a significant variation between the anti-plasmodial activities of samples collected at the different seasons ($p<0.05$). The multiple comparison tests (Tukey test) showed that the activity of *S. africana-caerulea* from the sample collected in winter was the most favourable (IC_{50} value: 12±5 μg ml^{-1}; $p<0.05$), whilst the autumn sample exhibited the lowest activity (IC_{50} value: 26±3 μg ml^{-1}; $p<0.05$) (Table 4). The activity of *S. africana-lutea* also varied over seasons with the spring sample giving the most favourable activity (IC_{50} value: 16±5 μg ml^{-1}) and winter the lowest activity (IC_{50} value: 33±2 μg ml^{-1}). In contrast to *S. africana-caerulea,* which exhibited its best activity in winter, the winter sample of *S. lanceolata* yielded its lowest activity (IC_{50} value: 43± 6 μg ml^{-1}), while no variation was observed for anti-plasmodial activity of *S. lanceolata* in spring, summer and autumn ($p>0.05$).

3.2.3. The anti-oxidant activity of the solvent extracts

The anti-oxidant activity of the solvent extracts against the $DPPH^{\cdot}$ radical of the three species over four seasons is presented in Table 4. An ANOVA showed significant variation in the anti-oxidant activity of each species across seasons ($p<0.05$). The multiple comparison tests showed that the activity of *S. africana-caerulea* obtained in autumn was statistically higher compared to the activity obtained in other seasons ($p<0.05$). *S. africana-lutea* exhibited its best activity with the extract harvested in winter (IC_{50} value: 10±1 μg ml^{-1}). In contrast, the best activity of *S. lanceolata* (IC_{50} value: 29±4 μg ml^{-1}) was obtained in summer ($p<0.05$), with the spring extract yielding the lowest activity.

3.2.4. Seasonal variation in the toxicity profile of the solvent extracts and the essential oils

The toxicity of the essential oils and solvent extracts was evaluated against human kidney epithelial cells. The analysis of variance showed variation in the toxicity over the four seasons, both for the solvent extracts and essential oils ($p<0.05$) (Table 4). The solvent extracts exhibited the lowest toxicity for all three species with samples harvested in winter (higher IC_{50} values) (Table 4), while the highest toxicity profile (lower IC_{50} values) was obtained with spring sample for *S. africana-caerulea* (IC_{50} value: 14±3 μg ml^{-1}) and summer samples for *S. africana-lutea* and *S. lanceolata* (IC_{50} values: 21±2 μg ml^{-1}, 15±1 μg ml^{-1}, respectively).

The oil samples of the three species collected during autumn were more toxic compared to the other seasons ($p<0.05$). The lowest toxicity for the essential oil was obtained with samples collected during spring for *S. africana-caerulea* and *S. africana-lutea* (IC_{50} values: 2±1 μg.ml^{-1}; 7±1 μg ml^{-1}, respectively) and sample harvested in summer for *S. lanceolata* (IC_{50} value: 4±1 μg ml^{-1}).

We previously demonstrated that the solvent extracts and essential oils of indigenous *Salvia* species exhibited a range of biological activities including anti-plasmodial, anti-oxidant, anti-inflammatory and anti-bacterial activities (Kamatou et al., 2005, 2006) and the essential oils were found to be more toxic than the solvent extracts (Kamatou et al., 2005) which is consistent with the current study.

The biological activities of the same plant collected at different periods of the year showed significant variation, which could be attributed to changes in the amounts of the active compounds present in each plant at different seasons. Changes in chemical composition could be explained in terms of a thermoregulatory action of the hydrophobic compounds which could protect the plant from desiccation. Furthermore, polar solids (triterpenes, flavonoids, diterpene acids) present in the plants might act as a physical barrier to prevent water permeation and dehydration (Harbone et al., 1975).

The toxicity of the solvent extracts of all three species was low during winter (Table 4), while the three essential oils were significantly (4 to 142 times) more toxic during autumn (Table 4). An attempt to find a relationship between the amount of major components of each essential oil and the toxicity profile was unsuccessful. To account for this, two possibilities can be proposed: either the toxicity profile was due to other minor components or there were synergistic or antagonistic effect occurring between the major components of the essential oils and other molecules. A negative correlation was observed between the toxicity and the major components [Pearson's correlation coefficient $r=-0.98$, $p<0.05$, (*S. africana-lutea)*; $r=-0.97$, $p<0.05$, (*S. lanceolata*), while *S. africana-caerulea* showed no significant correlation ($r=0.45$, $p>0.05$) between the toxicity of the oil and its major components. This suggests that both major and minor components of the essential oil may interact to contribute to the toxicity of the oil.

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