SUPPLEMENTARY MATERIAL

Seasonal variation of volatile oil composition and antioxidant property of aerial parts of *Syzygium paniculatum* Gaertn. grown in the Eastern Cape, South Africa

Sunday O. Okoh [1, 2*], Omobola O. Okoh [3], & Anthony I. Okoh [1, 2]

Abstract

Syzygium paniculatum Gaertn of the family *Myrtaceae* is a medicinal and aromatic plant. The hydrodistilled volatile oil (VO) from the aerial parts was characterised by GC-MS and Kovat's index, while the antioxidant property was investigated using a spectrophotometric technique in summer and winter. Antioxidant capacities of the aerial parts VOs range from 0.12 – 0.93 mg/mL in scavenging 2, 2-diphenyl-1- picrylhydrazyl radicals (DPPH•). Overall, 75 and 67 compounds were identified from the summer and winter VOs respectively. The main compounds were α-pinene (33.13%), n-hexadecanoic acid (19.14%), limonene (14.26%), farnesol (14.21%), β-ocimene (13.04%), citronellol (12.67%), linoleic acid (11.50%), octahydro-1,4-dimethyl azulene (11.57%), citral (9.91%), phytol (5.07%), linoleic acid (4.85%) and thymol (2.23%). The bioavailability of citronellol, thymol, β-ocimene, and linoleic acid, used as bactericidal, fungicidal and antioxidant agents in cosmetics and perfumery, suggests *S. paniculatum* potential as a natural food flavouring and source of antibiotics in this era of emerging multi-drug-resistant pathogens.

Keywords: *S. paniculatum*; volatile oil; linoleic acid; citronellol; thymol; antioxidant

3. Experimental

The reagents were of analytical grade, bought from various suppliers in South Africa, and were used in the laboratory for the bioassays. *Syzygium paniculatum* fresh fruit, leaves and stem-bark were harvested in their natural habitat in subtropical soil, mainly clay soil from the University of Fort Hare, Alice premises, on Latitude: 32°46'59.99"S, Longitude: 26° 52' 59.99" E, during summer (February) and winter (July) in 2017 respectively. A taxonomist identified the plant at Albany Museum, Rhodes University, Grahamstown, South Africa and a specimen voucher was kept in Selmar Schonland Herbarium, Rhodes University, with specimen number SSOO5. Thereafter, the samples were washed with distilled water, then air-dried at room temperature for between five and seven days. The fruit was first pulverised and air-dried for seven days and stem-bark was cut into smaller pieces before air-drying for five days. The VO was separately extracted from the powder (250 g each) for 3h using modified Clevenger-type apparatus (Okoh et al. 2016). Anhydrous sodium sulphate was used to dry the extracted VO, thereafter dispensed into sample tinted vials and kept at 4°C. The yields of the VOs were calculated per gram (w/w %) of the plant material.

The VOs were analysed by high resolution GC/MS and Kovat's index, as described in a previous report (Okoh et al. 2016). Briefly, the GC-MS conditions were programmed in a Hewlett-Packed HP 5973 mass spectrometer interfaced with an HP 6890 gas chromatograph. Conditions of the temperature and column were; equilibration time 3.00 min, ramp 4 °C/min, initial temperature 70 °C, final temperature 240 °C; inlet: splitless, initial temperature 220 °C, pressure 8.27 psi, purge flow 30 mL/min, purge time 0.20 min, helium gas; column: capillary, 30 m × 0.25 mm, internal diameter 0.25 µm, film thickness 0.7 mL/min, average velocity 32 cm/sec; MS: EI method at 70 eV. Subsequently, identity of each constituent was carried out by agreement of their mass spectra data (MSD) with the reference held in the computer library (Wiley275, New York). Furthermore, the retention index (RI) of each constituent was matched with those in literature to confirm each compound identity, while total percentage composition of each constituent was its peak area.

The antioxidant property of the five VOs were evaluated on 2, 2-diphenyl-1-picrylhydrazyl (DPPH), lipid peroxyl (LP) and nitric oxide (NO) radicals and their scavenging activities investigated at different concentrations (0.05 - 0.50 mg/ mL). Thereafter, the IC_{50} value of each VO was determined by regression equations and the results were compared with two

positive controls, as described in the previous report (Okoh et al. 2016). Statistical analysis was performed using SPSS15.0 for windows (IBM SPSS Inc *OLRAC SPS*). Experimental results were expressed as means ± S.D, carried out in parallel triplicate. Percentage inhibition of radicals was concentration-dependent and linear regression equation generated from the standard curve for each antioxidant was used to calculate the IC_{50} value. *T*-Test correlation analysis was employed to test significant differences between the concentration and percentage inhibition. Significant difference was considered at a confidence level of $P < 0.05$.

Table S1: Profile of bioactive compounds from the aerial parts of *S. paniculatum* volatile oils during winter and summer

Compounds [a]	KI [b]	% compositions *S. paniculatum* VOs					Identification methods [c]	QA[d]	Molecular formula
		WLVO	WSBVO	SLVO	SFVO	SSBVO			
2,5-Dimethylresorcinol	901	-	0.08	-	-	1.01	(*) (#)	85	$C_8H_{10}O_2$
Cinnamaldehyde	907	6.80	0.20	2.30	-	5.64	(*) (#)	95	C_9H_8O
Ethinamate	915	-	0.84	-	-	-	(*)	91	$C_9H_{13}NO_2$
Azulene	921	3.40	2.20	0.23	0.04	1.26	(*) (#)	95	$C_{10}H_8$
p-Cymene	932	-	0.05	0.09	-	-	(*) (#)	96	$C_{10}H_{14}$
α-Pinene	938	4.20	0.90	33.13	7.36	0.05	(*) (#)	97	$C_{10}H_{16}$
β-Pinene	945	1.18	-	1.17	0.92	2.83	(*) (#)	90	$C_{10}H_{16}$
β-Myrcene	965	1.17	-	0.52	0.18	1.29	(*) (#)	99	$C_{10}H_{16}$
β-Phellandrene	976	0.13	-	-	0.07	-	(*) (#)	99	$C_{10}H_{16}$
Carene	982	1.06	0.05	0.36	7.38	-	(*) (#)	97	$C_{10}H_{16}$
Limonene	994	14.26	0.20	6.62	3.76	11.48	(*) (#)	98	$C_{10}H_{16}$
β-Ocimene	1012	13.04	-	8.13	0.10	1.03	(*) (#)	99	$C_{10}H_{16}$
Thymol	1022	-	2.23	-	-	0.20	(*) (#)	95	$C_{10}H_{14O}$
Pinocarvone	1070	-	-	0.07	0.04	-	(*) (#)	90	$C_{10}H_{14}O$
trans-Limonene oxide	1028	0.16	-	-	-	0.10	(*) (#)	97	$C_{10}H_{16O}$
Camphor	1031	0.40	0.05	-	-	0.36	(*) (#)	97	$C_{10}H_{16O}$
Linalool	1033	-	0.16	-	-	-	(*) (#)	96	$C_{10}H_{18O}$
Citral	1039	0.83	t	0.16	-	9.91	(*) (#)	99	$C_{10}H_{16}O$
Terpen-4-ol	1055	-	-	0.27	0.60	-	(*) (#)	95	$C_{10}H_{18}O$
α-Terpineol	1064	1.70	0.78	4.15	10.46	-	(*) (#)	99	$C_{10}H_{18}O$
Borneol	1120	-	2.06	-	1.25	3.07	(*) (#)	99	$C_{10}H_{18}O$
Y-Terpinene	1127	-	0.06	0.23	0.56	-	(*) (#)	98	$C_{10}H_{18}$

2-methyl -3-nonanone	1241	0.63	-	0.32	-	0.21	(*) (#)	95	$C_{10}H_{20}O$
fenchyl alcohol	1250	-	-	0.31	1.32	-	(*) (#)	99	$C_{10}H_{18}O$
Citronellol	1252	2.36	0.12	-	-	12.67	(*) (#)	99	$C_{10}H_{20}O$
2,6-dimethyl-4-octanol	1269	9.18	t	2.80	-	8.26	(*)(#)	99	$C_{10}H_{22}O$
3,4-dimethyl 2,4,6-octatriene	1477	0.23	-	0.74	-	-	(*) (#)	91	$C_{10}H_{16}$
5,6,7,8-tetra hydro-quinolone	1580	-	-	-	0.25	-	(*) (#)	89	$C_9H_{11}N$
2,4-diethyl-1-methyl benzene	1281	-	-	-	0.16	-	(*) (#)	89	$C_{11}H_{16}$
2 -napthyl methyl ketone	1286	0.08	-	-	-	-	(*)(#)	98	$C_{12}H_{10}O$
decanal	1283	-	0.07	-	-	-	(*)(#)	95	$C_{12}H_{10}O$
linalyl acetate	1288	2.65	0.16	-	-	3.38	(*) (#)	90	$C_{12}H_{20}O$
geranyl acetate	1291	0.16	0.14	-	-	0.40	(*) (#)	98	$C_{12}H_{20}O$
fenchyl acetate	1296	0.07	-	-	-	0.06	(*) (#)	99	$C_{12}H_{20}O$
trans-1-cinnamoylimidazole	1314	-	-	-	-	0.52	(*) (#)	87	$C_{12}H_{10}N_2O$
isopulegol acetate	1360	0.58	0.70	0.40	-	0.30	(*) (#)	89	$C_{12}H_{20}O_2$
acetic acid, 1,7,7-trimethyl-bicyclo [2.2.1] hept-2-yl ester	1369	-	0.13	-	-	-	(*) (#)	99	$C_{12}H_{20}O_2$
1,2,3,4-tetrahydro-1-naphthalene	1375	-	0.60	-	-	t	(*) (#)	90	$C_{13}H_{18}$
santolina alcohol	1381	0.17	-	0.22	-	-	(*)	89	$C_{10}H_{18}O$
bornyl acetate	1405	0.36	-	0.03	-	-	(*) (#)	90	$C_{12}H_{20}O_2$
α-ionone	1411	-	0.07	-	0.05	0.53	(*) (#)	90	$C_{13}H_{20}O$
Tridecane	1417	-	0.23	-	-	-	(*) (#)	80	$C_{13}H_{28}$
citronellyl butyrate	1426	-	0.45	-	-	t	(*) (#)	98	$C_{14}H_{26}O_2$
myrtenyl acetate	1432	-	-	-	0.20	-	(*) (#)	91	$C_{12}H_{18}O_2$
α –isomethyl ionone	1456	1.07	-	-	-		(*) (#)	90	$C_{14}H_{22}O$

1,4-dimethyl-1,2,3,3a,6,8a decahydro-azulene	1501	-	0.02	-	11.65	-	(*) (#)	97	$C_{15}H_{24}$
β-bourbonene	1508	-	-	0.96	0.17	-	(*) (#)	99	$C_{15}H_{24}$
himachalene	1517	-	0.38	-	0.19	t	(*) (#)	90	$C_{15}H_{24}$
Selinene	1525	-	-	1.36	1.63	-	(*) (#)	94	$C_{15}H_{24}$
α-calacorene	1531	-	0.21	0.33	0.66		(*) (#)	89	$C_{15}H_{20}$
cis-farnesene	1537	-	0.11	-	-		(*) (#)	96	$C_{15}H_{24}$
α-cubebene	1546	-	0.15	-			(*) (#)	97	$C_{15}H_{24}$
Humulene	1553	0.12	1.15	t			(*) (#)	99	$C_{15}H_{24}$
caryophyllene	1559	3.39	4.68	8.36		0.14	(*) (#)	99	$C_{15}H_{24}$
copaene	1567	-	0.71	0.13	0.91	-	(*) (#)	99	$C_{15}H_{24}$
Longifolene	1561	-	-	-	-	5.95	(*) (#)		
aromadendrene	1573	1.20	0.30	8.83	2.79	3.10	(*) (#)	90	$C_{15}H_{24}$
Ledol	1578	-	-	-	1.14	0.07	(*) (#)	91	$C_{15}H_{26}O$
α-maaliene	1586	-	0.87	-	0.21	2.33	(*) (#)	94	$C_{15}H_{24}$
4-methyl dodecanal	1591	-	-	0.22	-	-	(*) (#)	91	$C_{13}H_{26}O$
tetradecanoic acid	1768	-	2.89	-	-	3.04	(*) (#)	99	$C_{14}H_{28}O_2$
β -Bisabolene	1560	-	0.21	-	-	0.86	(*) (#)	95	$C_{15}H_{24}$
α -muurolene	1567	0.11	0.27	0.33	1.80	-	(*) (#)	95	$C_{15}H_{24}$
α-cuprenene	1570	-	0.19	-	-	0.31	(*) (#)	89	$C_{15}H_{24}$
γ- -muurolene	1573	-	0.21	0.43	0.48	-	(*) (#)	92	$C_{15}H_{24}$
α-Farnesene	-	-	-	-	-	6.60	(*) (#)	95	
Naphthalene, 1,2,3,4,4a, 5,6,8a Octahydro-7-methyl-4-methylene	1575	1.47	13.28	3.11	16.61	0.34	(*) (#)	92	$C_{15}H_{24}$
caryophyllene oxide	1579	-	4.44	0.42	2.05	t	(*) (#)	91	$C_{15}H_{24}O$
Guaiene	1583	-	-	0.74	7.82	-	(*) (#)	95	$C_{15}H_{24}$

sabinol isovalerate	1586	-	0.08	-	-	-	(*) (#)	90	$C_{15}H_{24}O_2$
caparratriene	1589	-	0.04	-	-	-	(*) (#)	87	$C_{15}H_{26}$
cubenol	1590	-	0.53	-	-	-	(*) (#)	95	$C_{15}H_{26}O$
α-cadinol	1592	-	0.91	-	1.20	-	(*) (#)	90	$C_{15}H_{26}O$
trans-farnesol	1596	14.21	11.50	7.22	-	t	(*) (#)	99	$C_{15}H_{26}O$
Nerolidol	1603	0.21	0.17	0.39	-	0.26	(*) (#)	98	$C_{15}H_{26}O$
Epiglobulol	1617	-	-	-	1.93	0.95	(*) (#)	90	$C_{15}H_{26}O$
isolongifolol, methyl ether	1624	-	0.19	-	-	0.24	(*) (#)	87	$C_{16}H_{28}O$
spathulenol	1630	-	-	2.25	-	-	(*) (#)	90	$C_{15}H_{24}O$
1,2-dimethyl-5-nitroadamantane	1638	-	0.51	-	-	-	(*) (#)	91	$C_{19}H_{31}NO_3$
hexadecanoic acid (palmitic acid)	1648	t	19.14	0.07	-	6.40	(*) (#)	99	$C_{16}H_{32}O_2$
Isopropyl myristate	1907	3.06	0.15	2.34	-	-	(*) (#)	89	$C_{17}H_{34}O_2$
9,12,15-Octadecatrienoic acid (linolenic acid)	1930	-	4.85	-	-	t	(*) (#)	98	$C_{18}H_{30}O_2$
9,12-octadecadienoic acid (linoleic acid)	1938	t	11.52	t	3.56	8.20	(*) (#)	99	$C_{18}H_{32}O_2$
1- nonadecanol	2148	-	0.28	-	-	0.14	(#)	95	$C_{19}H_{40}O_2$
neophytadiene	2010	-	0.62	-	-	-	(*) (#)	85	$C_{20}H_{38}$
Phytol	2045	5.07	3.05	0.05	0.48	1.35	(*) (#)	99	$C_{20}H_{40}O$
arachidic acid (eicosanoic acid)	2067	-	0.97	0.02	-	1.30	(*) (#)	95	$C_{20}H_{40}O_2$
docosanoic acid	2123	t	0.79	-	-	-	(*) (#)	98	$C_{22}H_{44}O_2$
Total (%)		94.88	96.73	97.94	97.21	98.66			
Total yield oil (%)		1.04	0.53	0.78	0.57	0.54			
Monoterpenes		38.67	4.06	51.22	20.37	17.94			
Oxygenated monoterpenes		20.40	6.99	8.73	14.28	40.83			
Sesquiterpenes		4.97	22.28	36.28	31.22	8.64			
Oxygenated sesquiterpenes		18.95	18.16	12.84	26.79	1.93			
Diterpoids		5.07	3.65	0.05	0.48	1.35			
Fatty acids		0.02	40.16	0.10	3.56	18.95			
Others		6.80	1.12	2.30	0.16	6.65			

[a] Compound listed in order of elution from HB-5 column; [b] KI: Retention index relative to C_{9}-- C_{23} n-alkanes on column; [c] =method of identification = retention index (*); mass spectra (#); t = < 0.01; WLVO = winter *S paniculatum* leaves VO; WSBVO = winter *S. paniculatum* stem-bark VO, SLVO = *summer S. paniculatum* leaves VO; SFVO = *summer S. paniculatum* fruit VO, SSBVO=summer stem-bark VO

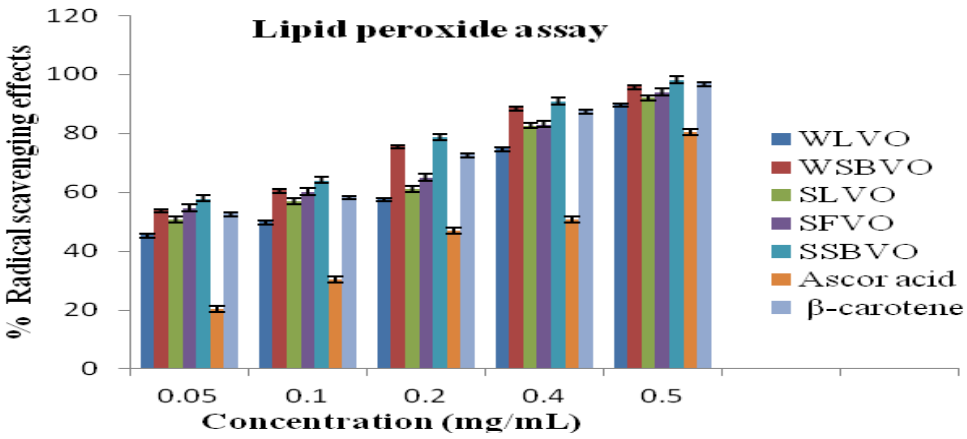

Figure S1: Radical scavenging effects of *S. paniculatum* volatile oils and reference compounds on lipid peroxide radicals during winter and summer.

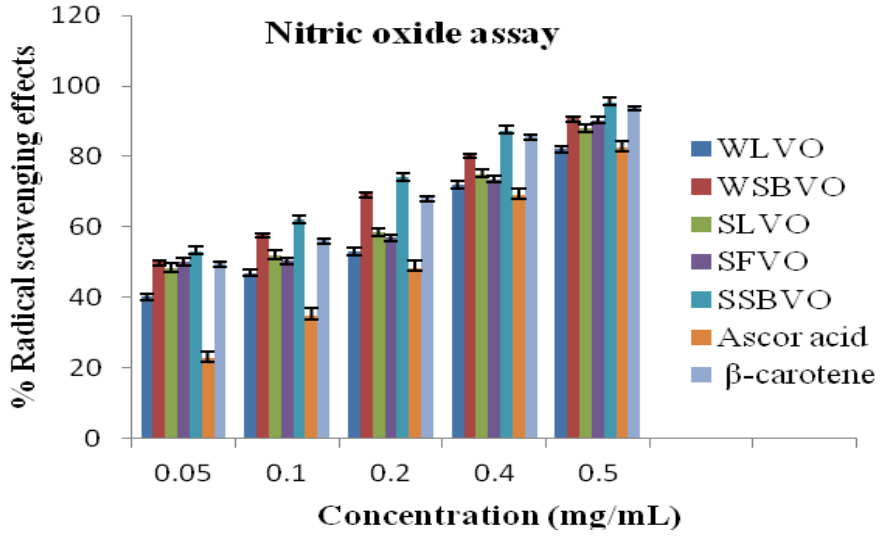

Figure S2: Radical scavenging effects of *S. paniculatum* volatile oils and reference compounds on nitric oxide radicals during winter and summer.