

Effect of Time of Harvest on the Chemical Composition and Antioxidant Potential of Leaf Essential Oil of *Syzygium guineense* Growing in North Central Nigeria (Willd.) Dc. Var.

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Received January 29th, 2023; Accepted May 17th, 2023.

DOI: <http://dx.doi.org/10.29356/jmcs.v68i2.2035>

Abstract. The use of synthetic antioxidants to ameliorate oxidative stress goes with side effects. Some plants are known to be sources of natural antioxidants and, hence, could be used as alternatives to synthetic antioxidants without side effects. Meanwhile, the presence of the phytochemicals that exhibit antioxidant activity in plants depends on environmental conditions that vary with the time of harvest of plant materials. This study, therefore, investigated the effect of time of harvest on the chemical composition and antioxidant potential of leaf essential oil of *Syzygium guineense* native to North central Nigeria. To accomplish these, pulverized (500 g) leaves of *S. guineense* harvested in the morning and afternoon were separately hydrodistilled and yielded 0.25 ± 0.002 % (w/w) and 0.27 ± 0.003 % (w/w) of essential oils. Characterization of the oils using GC-MS revealed the presence of twenty-two and twenty-three compounds in the oils from morning and afternoon harvests. The most abundant compound in the oils was β -bergamotene (30.1 % and 27.3 %). D-limonene (2.9 % and 5.6 %), β -ocimene (4.2 % and 10.2 %), α -santalene (7.4 % and 7.7 %), α -cedrene (8.6 % and 9.0 %), β -farnesene (9.1 % and 10.2 %) and calamenene (7.1 % and 5.2 %) were detected in significant quantities in the oils. DPPH radial scavenging assay was used to evaluate the antioxidant activity of the oils with butylated hydroxyl toluene (BHT) as standard. The oils exhibited antioxidant activity with IC_{50} of 41.92 μ g/mL and 33.12 μ g/mL for the oils from morning and afternoon harvests. Although the oils exhibited lower antioxidant activity than the standard (IC_{50} of 28.63 μ g/mL), but the oils could be used to ameliorate oxidative stress after clinical trials.

Keywords: *Syzygium guineense*; antioxidant; α -bergamotene; β -farnesene.

Resumen. El uso de antioxidantes sintéticos para mejorar el estrés oxidativo conlleva efectos secundarios. Se sabe que algunas plantas son fuentes de antioxidantes naturales y, por lo tanto, podrían usarse como alternativas a los antioxidantes sintéticos sin efectos secundarios. La presencia de fitoquímicos que exhiben la actividad antioxidante en las plantas depende de las condiciones ambientales que varían con el momento de la cosecha de los materiales vegetales. Por lo tanto, en este estudio se investigó el efecto del momento de la cosecha sobre la composición química y el potencial antioxidante del aceite esencial de hoja de *Syzygium guineense*, originario del centro norte de Nigeria. Para lograr esto, se hidrodestilaron por separado hojas pulverizadas (500 g) de *S. guineense* cosechadas en la mañana y en la tarde que produjeron 0.25 ± 0.002 % (p/p) y 0.27 ± 0.003 % (p/p) de aceites esenciales. La caracterización de los aceites mediante GC-MS reveló la presencia de veintidós y veintitrés compuestos en los aceites de las cosechas de la mañana y la tarde. Los compuestos más abundantes en los aceites fueron el β -bergamoteno (30.1 % y 27.3 %), D-limoneno (2.9 % y 5.6 %), β -ocimeno (4.2 % y 10.2 %), α -santaleno (7.4 % y 7.7 %), α -cedreno (8.6 % y 9.0 %), β -farneseno (9.1 % y 10.2 %) y calameneno (7.1 % y 5.2 %). Se utilizó el ensayo de eliminación radial DPPH para evaluar la actividad antioxidante de los aceites con hidroxil tolueno butilado (BHT) como estándar. Los aceites exhibieron actividad antioxidante con

IC50 de 41.92 µg/mL y 33.12 µg/mL para los aceites de las cosechas de la mañana y la tarde. Si bien los aceites exhibieron una actividad antioxidante menor que el estándar (IC50 de 28.63 µg/mL), los aceites podrían usarse para mejorar el estrés oxidativo después de los ensayos clínicos.

Palabras clave: *Syzygium guineense*; antioxidante; α -bergamoteno; β -farneseno.

Introduction

Syzygium guineense (Willd.) DC. var. belongs to the family Myrtaceae. It is widely grown in the forests and savannah regions of Africa [1]. The plant is commonly called “malmoo” by the Hausas, “sumsum” by the Fulanis and “adere or igi-aro” by the Yorubas in Nigeria [2,3]. Traditionally, the fruit of the plant is used to treat dysentery while the leaf decoction is used to cure stomach-ache, diarrhea, diabetes, ulcers, rheumatism, malaria and inflammations. The bark is used for the treatment of hypertension [4,5]. The crude extracts from the plant have been reported to possess antibacterial, antidiabetes, analgesic, anti-inflammatory, antihypertensive, antioxidant and antimalarial activities [6-8]. Hence, justify its use in traditional medicine. Reports on the phytochemical analysis of the plant extracts revealed the presence of flavonoids, tannins, saponin, alkaloids and cardiac glycosides [9-11]. The phytochemicals detected in its various extracts, were responsible for the reported biological and biochemical activities.

Previous work on the leaf essential oil of *S. guineense* from Natitingou, Peperkou, Tchaourou and Terou locations in Republic of Benin revealed that the oils were of β -caryophyllene, *cis*-calamene-10-ol, viridiflorol and α -humullene chemotypes respectively. Other major constituents in the oils were humullene-1,2-epoxide, epi- α -muurol, α -cadinol and caryophyllene oxide [9]. The leaf essential oil of the plant native to south-western Nigeria was earlier characterized [12]. The oil was found to be of aromadendrene chemotype. Germacrene D, *tau*-cadinol, caryophyllene oxide and α -cadinol were the other principal constituents in the oil. The chemotypic variation of the oils is attributable to differences in environmental factors at various location of the plant. The oil of the south-western Nigerian grown plant was reported to exhibit antioxidant activity. The activity of the oil was linked to abundance of oxygenated terpenoids in the oil. The presence of these terpenoids in an essential oil may vary with time of harvest of plant materials that bear the oil. It is on this basis that this work investigated the effect of time of harvest on the chemical composition and antioxidant potential of essential oils from leaves of *S. guineense* native to North central, Nigeria.

Experimental

Sample collection

Leaves of *Syzygium guineense* (1500 g) were separately harvested in the morning (7.00 a.m.) and afternoon (1.00 p.m.) at Oke Odo, Tanke, Ilorin-South LGA, Kwara State, Nigeria. The plant was identified by Mr. Bolu at the Herbarium of Plant Biology Department, University of Ilorin, where voucher specimens were deposited [UILH/002/0673].

Extraction of essential oil

Pulverized leaves (500 g) of *S. guineense* from morning and afternoon harvests were separately hydrodistilled for 3 hours in a Clevenger setup based on British Pharmacopoeia specification [13]. The oils were collected, preserved in a sealed sample tube, and stored under refrigeration at 4 °C until the analyses were carried out.

Gas Chromatography-Mass Spectrometry (GC/MS) analysis of the oils

An Agilent 19091S gas chromatograph coupled with a quadruple focusing mass spectrometer 433HP-5 mass detector was used. Helium was used as the carrier gas at a flow rate of 1.5 mL/min; all analyses were performed at constant flow. The GC was fitted with a 30 m by 0.25 mm fused silica capillary column coated with phenyl methyl siloxane at a split ratio 1:50. The film thickness was 0.25 µm. Oven temperature was initially kept

at 100 °C for 5 min. and then 150 °C at a rate of 4 °C/min. for 8 min. and to 250 °C at a rate of 20 °C/min. Mass detector conditions were as follows: Transfer line temperature at 300 °C, ionization mode electron impact at 70 eV. The percentage composition of the oil was computed in each case from GC peak areas.

Identification of constituents in the oils

The identification of the constituents in the oils was based on (i) comparison of their retention indices (RI), calculated using a homologous series of n-alkanes (C₇–C₃₀, Supelco Bellefonte, PA, USA) under identical experimental conditions, co-injection with standards and compared with those data from Wiley 275 and NIST 08 libraries (ii) comparison of fragmentation pattern in the mass spectra of each constituent with those data from Wiley 275 and NIST 08 libraries [14–17]. The relative quantity of each constituent was calculated based on the peak area of the GC (FID response) without using a correction factor.

DPPH antioxidant assay of the oils

The antioxidant potential of the oils was measured in terms of its hydrogen-donating or radical scavenging ability against DPPH, using the method reported by Ilhami [18]. In the method, 2,2-diphenyl-1-picryl-hydrazil, DPPH, solution (1.5 ml of 10⁻⁴M, in 95 % ethanol) (Meyer, Mexico) was separately mixed with the oil (1.5) at various concentrations (12.5–200 µg/mL). Each of the mixtures was shaken thoroughly and incubated in the dark for 30 minutes at ambient temperature. The control was prepared using the same procedure without the oil. The absorbance of the solution was measured at 517 nm using UV-spectrophotometer. The assay was carried out in triplicate and the results were expressed as mean values ± standard deviation. The concentration of the oil that gave 50 % inhibition (IC₅₀) was calculated from the graph of percentage inhibition against the oil concentration. Butylatedhydroxy toluene (BHT) was used as standard. The percentage inhibitions were calculated using the equation:

$$\% \text{ Inhibition} = \frac{A_0 - A_T}{A_0} \times 100$$

where A₀ is the absorbance of the control sample (containing all reagents except the test compound) and A_T is the absorbance of the test samples. Butylated hydroxytoluene (BHT, 1.0 mg/mL) (Sigma-Aldrich, Mexico) was used as a positive control.

Statistical analysis

Tests were carried out in triplicates. The mean values were calculated from the three values. The data for various biochemical parameters was expressed as mean ± SD (n = 3) and compared using one way analysis of variance (ANOVA) test, followed by Dunnett multiple comparison test with an equal sample size test. Values were considered statistically significant at p < 0.05. The IC₅₀ values were calculated by non-linear regression analysis from the mean values. Statistics were done using SPSS for Windows version 10.

Results and discussion

Hydrodistilled leaves of *S. guineense* harvested in the morning and afternoon afforded essential oils in the yields of 0.25 ± 0.002 % (w/w) and 0.27 ± 0.003 % (w/w) respectively. The yields were lower than the yield from the leaves of the plants growing in South western Nigeria, but higher than the yields of oils from the leaves of the plant harvested from four different locations in the Republic of Benin [12,19]. The variations in the oil yields could be linked to differences in the number of secretory organs in the leaves of the plant at their respective locations. Meanwhile, the afternoon harvest yielded more oil than the morning harvest. This is attributable to environmental conditions that favoured the formation of more oil in the afternoon than in the morning. The identities, retention indices and percentage composition of constituents of leaf essential oils of the plant harvested in the morning and afternoon are presented in Table 1.

Table 1. Chemical Composition (%) of Essential Oils from Leaves of *S. guneense*.

S/N	Compound	RI ^a	RI ^b	% Composition		Mass spectra data
				Morning	Afternoon	
1	2-Carene	1001	1001	1.4	-	150,121, 93 ,67,53
2	β -Pinene	980	982	3.2	-	68 ,79,93,107,121
3	β -Ocimine	1050	1053	4.2	10.2	68 ,79,93,107,121
4	γ -Terpinene	1062	1057	-	0.5	136,105, 93 ,77,65
5	α -Terpinolene	1088	1089	-	1.4	27,53, 93 ,121,136
6	Limonene	1031	1027	2.9	5.6	136, 107, 93, 68 ,53
7	α -Terpineol	1189	1189	0.7	1.2	43, 59 ,81,93,107
8	Thymol	1290	1291	1.5	0.7	51,77,91,121, 135
9	α -Santalene	1420	1422	7.4	7.7	41,69, 94 ,121,161
10	α -Cubebene	1345	1351	0.5	0.4	105,119, 161 ,189,204
11	α -Copaene	1378	1376	4.0	3.5	105,119, 161 ,189,204
12	α -Cedrene	1409	1410	8.6	9.0	55,93, 119 ,136,161
13	β -Santalene	1462	1462	1.3	1.0	41,69, 94 ,107,148
14	α-Bergamotene	1436	1435	30.1	27.3	55, 79, 93 , 119, 161
15	β -Farnesene	1458	1455	9.1	10.2	93, 119 ,161,134,147
16	α -Muuroolene	1499	1499	1.2	0.8	204,134, 105 ,93,81
17	Muurolo-3,5-diene	1450	1454	-	0.4	133,145, 161 ,175,189
18	Calamenene	1521	1498	7.1	5.2	133,147, 161 ,179,189
19	β -Bisabolene	1509	1513	1.5	-	69 ,79,93,109,119
20	Sesquieneole	1541	1541	1.3	1.3	139 ,140,161,176,189
21	δ -Cadinene	1524	1524	2.8	2.4	119,134, 161 ,189,204
22	Cadina-1,4-diene	1532	1532	2.1	2.8	91,105, 119 ,161,204
23	β -Cadin-4-en-10-ol	1580	1581	1.6	0.6	135,149, 161 ,179,189
24	Nerolidol	1564	1564	-	0.6	41, 69 ,93,123,161
25	β -Bisabolol	1668	1662	2.9	2.8	69, 82 ,93,111,119
26	α -Bisabolol	1683	1685	2.1	1.1	69, 109 ,119,134,147

	% Monoterpenoids			13.9	19.6	
	% Hydrocarbon Sesquiterpenoids			77.0	72.0	
	% Oxygenated Sesquiterpenoids			6.6	5.1	
	Total (%)			97.5	96.7	

Compounds are listed in order of elution from fused silica capillary column coated on CP-Sil 5; RI^a = Literature Retention Indices, RI^b = Calculated Retention Indices; Bolded name = Chemotype

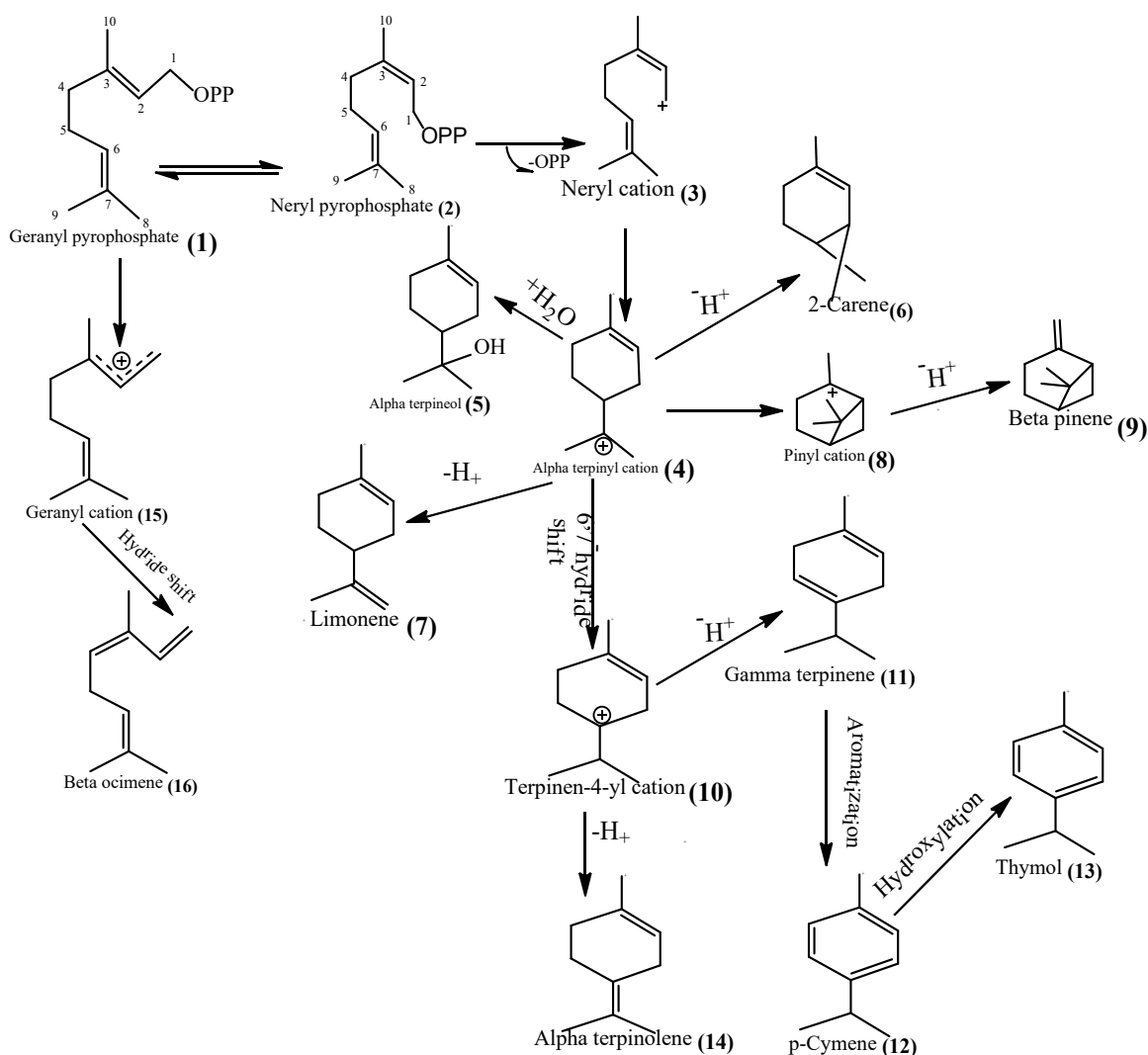
In the Table, twenty-two and twenty-three compounds that constituted 97.1 % and 96.7 % of the oils from morning and afternoon harvests were identified from their mass spectra. The percentage composition of monoterpenoids in the oils from morning and afternoon harvests were 13.9 % and 19.6 %. Sesquiterpenoids constituted 91.6 % and 76.8 % of the oils from morning and afternoon harvests respectively. The major constituents of the oils were; β -ocimene (4.2 % and 10.2 %), D-limonene (2.9 % and 5.6 %), α -santalene (7.4 % and 7.7 %), α -copaene (4.0 % and 3.5 %), α -cedrene (8.6 % and 9.0 %), α -bergamotene (30.1 % and 27.3 %), β -farnesene (9.1 % and 10.2 %), calamenene (7.1 % and 5.2 %), δ -cadinene (2.8 % and 2.4 %), cadina-1,4-diene (2.1 % and 2.8 %), α -bisabolol (2.9 % and 2.8 %) and β -bisabolol (2.1 % and 1.1 %). Other principal constituents in the oil from morning harvest were β -pinene (3.2 %) and β -ocimene (4.2 %). Terpenoids that were detected in significant quantities in the oils from the two harvests were α -terpineole (0.7 % and 1.2 %), thymol (1.7 % and 0.7 %), β -santalene (1.3 % and 1.0 %), α -muurolene (1.2 % and 0.8 %), and β -cadin-4-en-10-ol (1.6 % and 0.6 %). Sesquieneole constituted 1.3% of each of the oils. 2-Carene (1.4 %) and β -bisabolene (1.5 %) that were present in appreciable amounts in the oil from morning harvest were not detected in the oil from afternoon harvest. Meanwhile, the oil from the afternoon harvest contained appreciable quantities of γ -terpinene (0.5%), α -terpinolene (1.4 %) and nerolidol (0.6 %) but the compounds were not found in the oil of the morning harvest. α -Cubebene (0.5 % and 0.4 %) was detected as a minor constituent in the oils. Irrespective of time of harvest of the leaves, α -bergamotene was the most prominent compound in the oils. This revealed that the oils were of α -bergamotene chemotypes. Aromadendrene and α -hunnmullene chemotypes were earlier reported for the leaf essential oils of the plant native to Republic of Benin and south-western Nigeria [12,19]. The chemotypic differences in the oils could be attributed to differences in climatic conditions of the three geographical regions.

It has been found that the syntheses of the most abundant mono- and sesquiterpenoids in an essential oil usually facilitate the biosynthesis of all terpenoids in plants [20-22]. The activities of the syntheses are influenced by environmental factors that subsequently determine the quantity and the type of compounds in the oil. The predominance of β -ocimene and α -bergamotene in the oils of morning and afternoon harvests implied that their syntheses facilitated the transformation of their precursors, (geranyl, neryl and farnesyl pyrophosphates) to all mono- and sesquiterpenoids in the oils. β -Ocimene synthase aided the formation of 2-carene [6] and β -pinene [9] in the oil of morning harvest but the environmental conditions did not favour the activity of the synthase to facilitate the biosynthesis of the compounds in the oil from afternoon harvest. Meanwhile, the environmental conditions that favour the formation of γ -terpinene [11] and α -terpinolene [14] that constituted the oil from afternoon harvest did not favour their formation in the oil of the morning harvest. The α -bergamotene synthase facilitated the formation of β -bisabolene [21] in the oil of the morning harvest but the environmental condition was not conducive for its activity to mediate the formation of the compound in the oil from afternoon harvest. In contrast, the environmental condition was conducive for the activity of the synthase to aid the biosynthesis of muurola-3,5-diene and nerolidol in the oil of the afternoon harvest but did not favour their formation in the oil of the morning harvest.

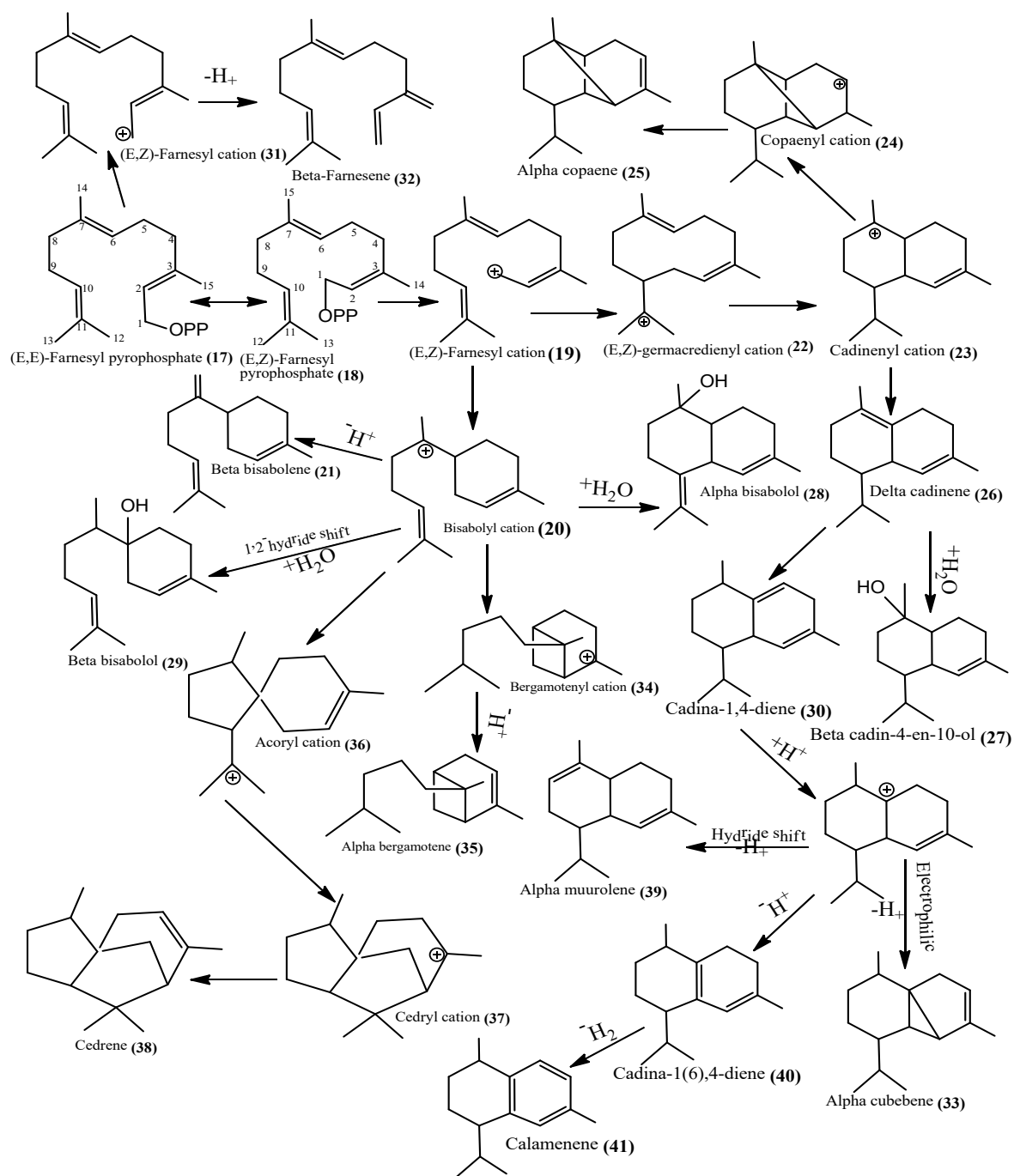
β -Ocimene synthase catalyzed the biosynthesis of β -ocimene [16], limonene [7], α -terpineol [5] and thymol [13] in the oils. However, the compounds were of greater quantities in the oil of the afternoon harvest than that of the morning harvest except thymol. Similarly, both oils contained α -santalene, α -cubebene, α -copaene [25], α -cedrene [37], β -santalene, α -bergamotene [35], β -farnesene [32], α -muurolene [39],

calamenene [41], α -sesquieneole, δ -cadinene [26], cadina-1,4-diene, β -Cadin-4-en-10-ol, α -bisabolol [28] and β -bisabolol [29]. Their formations in the oils were aided by α -bergamotene synthase. Meanwhile, α -cubebene [33], α -copaene [25], α -bergamotene [35], β -santalene, α -muurolene [39], calamenene [41], δ -cadinene [26], β -cadin-4-en-10-ol [27], α -bisabolol [28] and β -bisabolol [29] were of higher quantities in the oil of morning harvest than the oil of afternoon harvest. On the contrary, the oil from afternoon harvest was richer in α -santalene, α -cedrene [38], β -farnesene [32] and cadina-1,4-diene [30] than the oil from morning harvest. The lower quantity of some compounds in the oils could be attributed to subjection of their reactive intermediates to premature termination [23]. The mechanisms of biogenesis of some of the mono- and sesquiterpenoids in the essential oils from both during their formation harvests are presented in Reaction Schemes 1 and 2.

The DPPH radical scavenging of essential oils from leaves of *S. guineense* harvested in the morning and afternoon is shown in Fig. 1.



Scheme 1. Mechanism of biogenesis of monoterpenoids in the essential oils from leaves of *S. guineense*.



Scheme 2. Mechanisms of biogenesis of sesquiterpenoids in the essential oils from leaves of *S. guineense*.

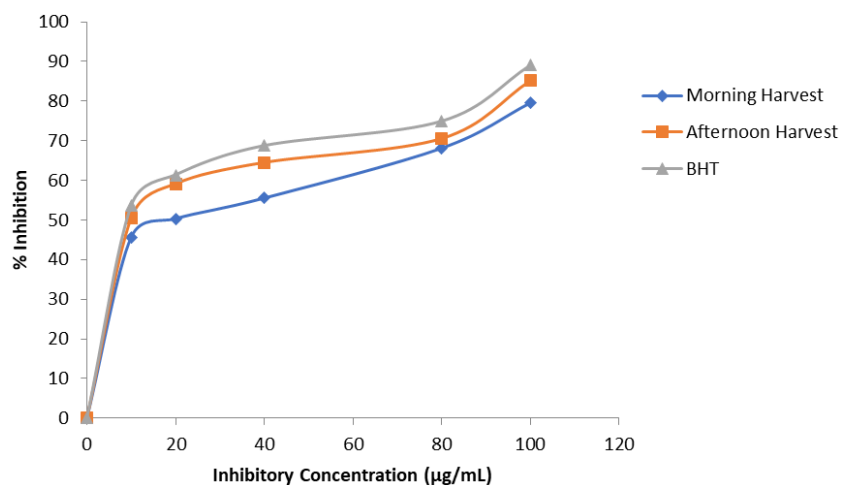


Fig. 1. DPPH Radical Scavenging Activity of Essential Oils from Leaves of *S. guineense* Harvested in the Morning (7.00 a.m.) and Afternoon (1.00 p.m.).

The figure revealed that the antioxidant activity of the oils was concentration dependent. The oils scavenged DPPH radical with IC_{50} of 41.92 $\mu\text{g/mL}$ and 33.12 $\mu\text{g/mL}$ for the oils from morning and afternoon harvests respectively. The oils were less active when compared to the activity of butylated hydroxytoluene, BHT, ($IC_{50} = 28.63 \mu\text{g/mL}$) that was used as standard. Irrespective of the time of harvest, the oils showed better antioxidant activity than the oil from the leaves of the plant earlier reported from the northern part of Nigeria (12). Interestingly, the oil from afternoon harvest showed higher activity than the oil from morning harvest. The oil was richer in α -terpineol and limonene and also contained γ -terpinene, α -terpinolene and nerolidol which were not found in the oil from morning harvest. It has been established that essential oils containing greater amounts of oxygenated compounds usually possess higher antioxidant activity [24,25]. For instance, the DPPH radical scavenging activity of leaf essential oils of *Ocimum basilicum* and *Scutia buxifolia* was linked to the predominant of linalool, carvacrol, 1,8-cineole and spathulenol in the oils [26,27]. The oils also contained terpinolene and γ -terpinene which were separately reported to show stronger DPPH radical-scavenging activity than trolox which was used as a reference. The DPPH radical scavenging activity of limonene and α -terpineol has also been documented [28,29]. Thus, the stronger DPPH radical scavenging activity of the oil from afternoon harvest could be attributed to the presence and higher quantities of the listed compounds above as compared to the oil from morning harvest.

Conclusions

The yields of essential oils obtained from *S. guineense* harvested in the morning and afternoon differ significantly. More oil was obtained from the afternoon harvest which was linked to a higher number of secretory cells in the afternoon than in the morning. The phytochemical profile of the oils also varied with times of harvest which sample harvested in the subsequently affected plant harvested in their antioxidant activity. Irrespective of the time of harvest of the leaves, the oils scavenged DPPH radicals with the oil from afternoon harvest showing greater antioxidant activity. Hence, the oils could be used as alternative antioxidants to ameliorate oxidative stress after clinical trials.

Acknowledgements

The authors wish to acknowledge Mr. Bolu, of the department of Plant Biology, University of Ilorin, Ilorin, Nigeria who assisted in the identification of the plant.

References

1. Bankefa, E.; Onileke, F.; Dada, E. *J. Sci. Res.* **2014**, 2, 21-252.
2. Agwu, C. O. C.; Okeke, G. I. *J. Bot.* **1997**, 9-10, 25–36.
3. Iwu, M. M. in: *Handbook of African Medicinal Plants*, CRP Press, BOC Raton, Florida. **1993**, 12 – 78.
4. Burkill, H. M. in: *The useful plants of west tropical families M-R*, Vol. 4, 2nd Edition, Kew: Royal Botanical Gardens. **1997**, 253–254.
5. Seyoum, G.; Abebe, M.; Asres, K.; Bekuretsion, Y.; Woldkidan, S.; Debebe, E. *Evid.-based Complement. Altern. Med.* **2021**, 5, 1 – 10.
6. Wubneh, Z. B., Tadesse, S. A. *BMC Complement. Altern. Med.* **2017**, 17, 21- 28.
7. Abera, B.; Adane, L.; Mamo, F. *J. Pharmacogn. Phytochem.* **2018**, 7, 3104 – 3111.
8. Ezenyi, I. C.; Mbamalu, O. N.; Balogun, L.; Omorogbe, L.; Ameh, F. S.; Salawu, O. A. *J. Phytopharmacol.* **2016**, 5, 150-156.
9. Kristanti, A. N.; Aung, E. A.; Aminah, N. S.; Takaya, Y.; Ramadhan, R. *Open Chem. J.* **2020**, 18, 1256–1281.
10. Pieme, C. A.; Ngoupayo, J.; Nkoulou, C. H.; Moukette, B. M.; Nono, B. L.; Moor, V. J. *Antioxidants.* **2014**, 3, 618–635.
11. Tadesse, A. S.; Wubneh, Z. B. *BMC Complement. Altern. Med.* **2017**, 17, 1–7.
12. Okhale, S. E.; Buba, C. I.; Oladosu, P.; Ugbabe, G. E.; Ibrahim, J. A.; Egharevba, H. O.; Kunle, O. F. *IJPPR.* **2018**, 10, 341-349.
13. British pharmacopoeia II. HM, stationary office, London, **1980**, 109.
14. Adams, R. P. in *Identification of essential oil components by gas chromatography and mass spectrometry*. Allured publ. corp., Carol Stream, IL. USA. **2012**, 78 – 109.
15. Jennings, W.; Shibamoto, T. in: *Qualitative analysis of flavour volatiles by gas capillary chromatography*. Academic press, New York, **1980**; 68–109.
16. Joulain, D.; Koenig, W. A. in: *The atlas of spectra data of sesquiterpene hydrocarbon*. E. B. Verlay Hamburg, Germany, **1998**, 112–153.
17. Usman, L. A.; Agboola, T. A.; AbdulWaheed, J. O.; Ismaeel, R. O.; Ogundele, V. A.; Ibrahim, A. *NJPAS.* **2016**, 29, 2968–2976.
18. Ilhami, G. *Chem-Biol. Interact.* **2009**, 179, 71-80.
19. Chalchat, J.; Noudogbessi, J.; Sohounloue, D. C. K.; Yedomonhan, P.; Figueredo, G. *Rec. Nat. Prod.* **2008**, 2, 33-38.
20. Trapp, S. C.; Croteau, R. B. *Genetics.* **2001**, 158, 811–832.
21. Degenhardt, J.; Koöllner, T. G.; Gershenson, J. *Phytochem.* **2009**, 70, 1621–1637.
22. Usman, L. A.; Watti, O. I.; Ismaeel, R. O.; Ojumoola, A. O. *JOTCSA.* **2016**, 3, 1–18.
23. Iijima, Y.; Davidovich-Rikanati, R.; Fridman, E.; Gang, D. R. *Plant Physiol.* **2004**, 136, 3724–3736.
24. Mkaddem, M. G.; Romdhane, M.; Ibrahim, H.; Enn-ajar, M.; Lebrihi, A.; Mathieu, F.; Bouajila, J. J. *Med. Food.* **2010**, 13, 1500–1504.
25. Paolini, J.; El Ouariachi, E.; Bouyanza, A.; Tomi, P.; Hammouti, B.; Salghi, R.; Majidi, L.; Costa J. J. *Med. Plants Res.* **2011**, 5, 5773–5778.
26. Hussein, A. I.; Anwar, F.; Sherazi, S. T. H.; Pryzbylski, R. *Food Chem.* **2008**, 108, 986-995.
27. Boligon, A. A.; Piana, M.; Brum, T. F.; Froeder, A. L. F.; Belke, B. V.; Schwanz, T. G.; Mario, D. N.; Alves, S. H. Athayde, M. L. *An. Acad. Bras. Cienc.* **2014**, 86, 1463-1469.
28. Mehta, A. A.; Shah, B. B. *Asian J. Pharm. Pharmacol.* **2018**, 4, 883–887.
29. Hou, C.; Chen, Y.; Wang, C. *Int. J. Food Prop.* **2019**, 22, 230–238.