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Effect of harvest date on the yield and chemical composition of *Croton gratissimus* leaf essential oil

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Abstract
Essential oils are sensitive and complex mixtures which are composed of volatile secondary metabolites. The chemical profile of essential oils harvested from a particular plant vary with different parameters such as climate, soil type, methods of extraction, geographical origin, plant part and harvest conditions. As such, it is of paramount necessity to profile the essential oils extracted under different conditions as this can determine their potential applications. This study explored for the first time, the chemical composition of *Croton gratissimus* essential oil extracted by hydrodistillation from fresh leaves harvested on different dates. There was no difference in the essential oil yields obtained from the leaves harvested on different dates. The chemical profiling using gas chromatography mass spectrometry showed that the most dominant component in the essential oils extracted from the leaves harvested on different dates was cis-muurola-4(15),5-diene although its percentage abundance varied significantly. The essential oil extracted from the leaves harvested on different days also showed significant variation in the percentage composition of other major compounds such as camphor, germacrene D, β-himachalene, caryophyllene, α-muurolol and aromadendrene. The most abundant class of secondary metabolites for the essential oils harvested on all dates studied was the sesquiterpenes which also varied significantly in its percentage composition. These results point to a significant variation in the chemical profile of essential oils harvested from the same plant on different dates. Consequently, it is important to determine the chemical profile of the leaves of *Croton gratissimus* as it can vary depending on the conditions.

Keywords: Croton gratissimus, harvest date, essential oil, Croton zambesicus, chemical profile

INTRODUCTION

Essential oils are heterogenous mixtures of numerous volatile secondary plant metabolites which are mostly present in different concentrations depending on various factors [1]–[7]. These secondary metabolites are produced for the purposes of adaptation and mediation of interactions between plants and biotic environment[8], [9]. It has been widely reported that the chemical profiles of the essential oils are influenced by the plant species, climate, seasonal variation, growth conditions, agricultural methods, altitude, harvesting time, plant part used, sample size, processing methods and chemotype[8]. The chemotypes vary due to endogenous and exogenous factors and this may lead to differences in the oil quality of the same species [10][1]. Chemical variability and chemotype determination of the essential oils from a species, help in determining their pharmacological activities and toxicity levels [1]. Furthermore, the activities of essential oils may result from a synergistic action of all its chemical components[1] [11]. However, intraspecific variability of chemical constituents of essential oils of an individual plant has been reported[10]. This is mainly caused by the environmental factors[10]. *Croton gratissimus* essential oil is evidently receiving research attention due to its attractive yield and biological activity as well as the ethnomedicinal use of the plant[12]–[17]. Essential oils extracted from the leaves, bark, and roots of *Croton gratissimus* plant exhibit antimicrobial, vasorelaxant and antioxidant activities [ref]. Nevertheless, *Croton gratissimus* essential oils from different parts of the world have demonstrated significant variability in their chemical compositions[6], [12], [18]–[24]. The variation has even been reported in essential oils extracted from plants within the same

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geographical origin and region[5], [8], [10], [19]. It is a challenge to establish the cause of chemical variability in essential oils due to many varied parameters during harvesting of the plant part, extraction, processing, and analysis of essential oils[19], [24]–[26]. Therefore, this study explored for the first time, the effect of harvest date on the chemical composition of *Croton gratissimus* essential oil extracted by hydrodistillation from fresh leaves harvested on different dates. To reduce interferences caused by many variables, the essential oils were extracted from the leaves of a single plant which were harvested on the same time of the day, and the oil was extracted and analyzed under similar conditions.

MATERIALS AND METHODS

Plant Collection and Authentication

The *Croton gratissimus* plant harvested at the Botswana International University of Science and Technology (BIUST), in Palapye, Botswana was authenticated by a botanist in the department of biological sciences in BIUST. The leaves of *Croton gratissimus* were harvested on three different dates in October 2018 [Table 1] from a single plant between 0940 and 1010 hours.

Sample Preparation

The fresh leaves of *Croton gratissimus* plant were crushed immediately after harvesting and the oil was extracted on the same day of harvest.

Extraction of Essential Oils by Hydrodistillation

The fresh leaves of *Croton gratissimus* plant were crushed immediately after harvesting and the oil was extracted on the same day of harvest. Extraction of essential oils from fresh leaves of *Croton gratissimus* was carried out by hydrodistillation using Clevenger-type apparatus. The crushed leaves (50.00 g) were placed in a 2.0 L conical flask after which 1200 mL of cold distilled water was added. The mixture was distilled for 3 hours after collecting the first drop of the distillate. After cooling, the oil was separated from the distillate using liquid-liquid extraction with pentane. The oil obtained was dried on anhydrous sodium sulphate, weighed, placed in an amber bottle, and stored in a refrigerator at 4 °C until further analysis.

Determination of Chemical Components of essential oils by Gas Chromatography Mass Spectrometry

The chemical composition of essential oils obtained from the leaves of *Croton gratissimus* were determined using gas chromatography interfaced with mass spectrometry (GC-MS). One microliter samples of essential oils diluted in the ratio of 1:100 (v/v) in hexane were injected on an Agilent Technologies 7890 gas chromatograph system coupled to an Agilent 5977A MSD. Separation of components was done on HP-5MS column (30 m × 0.25 mm id × 0.25 µm film thickness) with helium being the carrier gas. The flow rate of helium was set at 1 mL/ minute. The injector was set at 250 °C with a split ratio of 25:1. The column oven was initially set at 60 °C for 2 minutes, and then ramped to 125 °C at 1 °C/ minute, held there for 2 minutes, and finally ramped to 220 °C at 2 °C/ minute and held at 220 °C for 2 minutes. The mass spectra data was acquired in the electron impact mode (70 eV) in the m/z range of 40–400 a.m.u and scan time of 1.5 s. Normal alkanes C_7-C_{30} standard was also run under the same conditions and method. Identification of oil components was achieved through the analysis of their retention indices calculated from the C_7-C_{30} n-alkane standard as well as comparing their mass spectral fragmentation patterns with those reported in the literature and stored on the MS library and the NIST Chemistry Webbook.

Statistical Analysis

Two-way ANOVA was performed on the compositional data. The p-value was found to be less than 0.05 and the F critical was greater than F. This shows that the observed differences in chemical compositions are statistically significant.

RESULTS AND DISCUSSION

The effect of harvest date on the chemical composition of *Croton gratissimus* essential oil extracted by hydrodistillation from fresh leaves harvested on 03/10/18, 19/10/18 and 23/10/18 was investigated using

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gas chromatography mass spectrometry. There was no difference on the essential oil yields obtained from the leaves harvested on different dates (0.12 -0.13%). However, the yield was lower than the literature values[12], [21]–[25]. The chromatograms of all sample essential oils obtained from GC-MS displayed more than 40 peaks [Figure 1 and 2]. Percentage compositions of compounds were calculated from the peak areas. The oils include cis-muurola-4(15),5-diene, β-himachalene and caryophyllene. Additionally, cis-muurola-4(15),5-diene was the most dominant compound in all sample oils. Its relative abundance ranged from 12.5% to 24.9%, the highest being from the oil extracted from the leaves harvested on 23/10/18. There was a noticeable difference in the percentage compositions of other major compounds such as germacrene D, aromadendrene, α-muurolol, γ-muurolene and camphor in the sample oils [Table 1 and Figure 3]. Germacrene D, caryophyllene and 1,8 cineole have been reported to be amongst the most abundant compound in *Croton gratissimus* essential oils[12], [25], [26]. The most dominant compound in the major classes of compounds include cis-muurola-4(15),5-diene sesquiterpene, camphor monoterpene and α-muurolol sesquiterpenoid [Figure 5]. The most abundant class of secondary metabolites for the sample essential oils was sesquiterpenes which ranged from 35.3 to 58. 5 % and being highest in sample

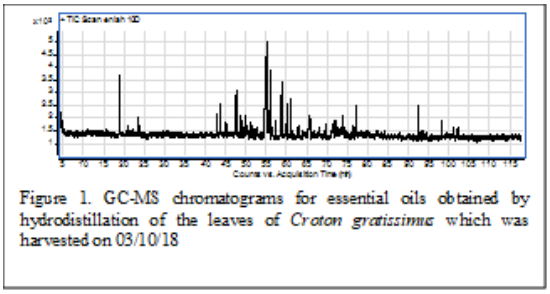

Figure 1. GC-MS chromatograms for essential oils obtained by hydrodistillation of the leaves of *Croton gratissimus* which was harvested on 03/10/18

results showed that all sample oils contained similar chemical compounds which varied in percentage compositions as depicted in Table 1. The most abundant compounds in each sample essential oil were identified and their percentage compositions were compared. The top three common major compounds identified in all sample essential oil from 23/10/18, as Figure 4 illustrates. The monoterpenoids 1,8 cineole and camphor were the most predominant in their class in all oils but the oil from 23/10/18 contained trace amount of 1,8 cineole. These results point to a significant variation in the percentage compositions of secondary metabolites found in essential oils harvested from the same plant on different dates. Intraspecific variability of chemical constituents of essential oils of an individual plant has been reported[10]. This has been attributed to environmental factors[10]. As such the variation in the percentage composition of the compounds in the sample essential oils in this study is thought to have been caused by environmental factors such as the amount of light, humidity or water, temperature, and weather conditions which the plant was subject to prior to harvesting. In the process of adapting to the environmental changes, the plant may have increased or decreased its production of some of the secondary metabolites.

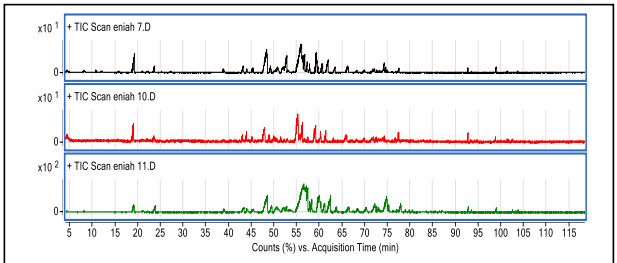

Figure 1. GC-MS chromatograms for essential oils obtained by hydrodistillation of the leaves of *Croton gratissimus* which were harvested at different dates: 7. D= 19/10/18, 10. D= 03/10/18, 11. D= 23/10/18, Zoomed chromatogram for purposes of clear illustration.

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Table 1. Percentage composition of *Croton gratissimus* essential oils obtained by hydrodistillation of the leaves *Croton gratissimus* which were harvested at different dates.

KI	Class	Compound Identity	3/10/18	19/10/18	23/10/18	SD
1032	OM	1,8 Cineole	0.4	0.8	tr	0.4
1142	OM	Camphor	5.4	5.9	1.8	2.3
1189	OM	α-Terpineol	1.3	1.3	1.6	0.2
1373	S	Bourbonene	1.8	0.9	0.8	0.6
1416	S	Caryophyllene	5.3	9.1	8.6	2.1
1451	S	Aromadendrene	0.3	6.1	1.3	3.1
1472	S	cis-Muurola-4(15),5-diene	12.5	21.9	24.9	6.5
1475	S	γ-Muurolene	1.8	2.9	5.8	2.1
1480	S	Germacrene D	5.8	3.4	4.1	1.2
1503	S	β-Himachalene	5.4	5.8	7.3	1.0
1535	S	α-Cadinene	2.4	2.4	5.7	1.9
1647	OS	α-Muurolol	0.7	3.2	5.9	2.6
		Class				
		Monoterpenoids	7.1	8.0	3.4	
		Sesquiterpenes	35.3	52.5	58.5	
		Sesquiterpenoids	0.7	3.2	5.9	

Key OM = Monoterpenoids, S = Sesquiterpenes and OS =Sesquiterpenoids KI=Kovats retention index. GC-MS= Gas chromatography mass spectrometry, HP-5MS column, tr=trace Percentage composition of each class reported in this table was derived from the compounds in this table only.

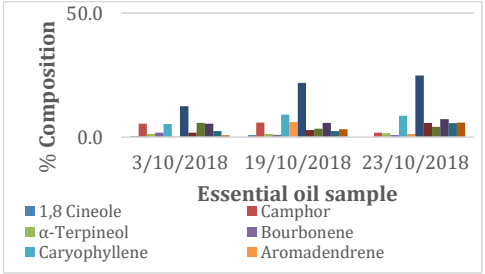

Figure 3. Major components of *Croton gratissimus* essential oils from the leaves harvested at different dates.

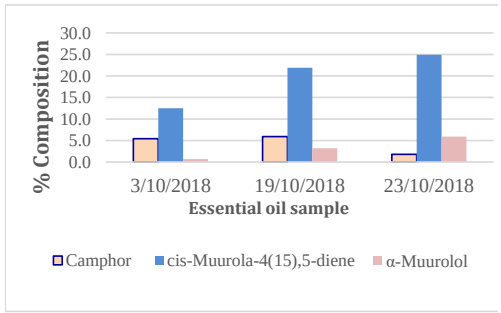

Figure 2. Comparison of selected major chemical components in *Croton gratissimus* essential oils harvested at different dates.

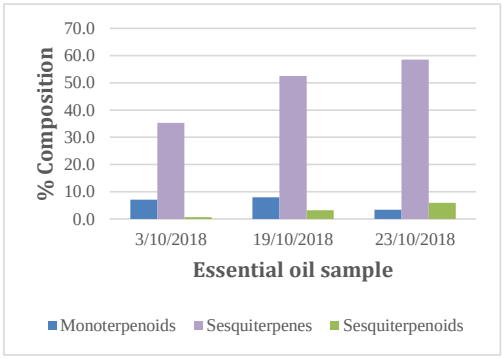

Figure 4. Major chemical classes of essential oils obtained by hydrodistillation of the *Croton gratissimus* leaves harvested at different dates.

CONCLUSIONS

The results of this study showed that all sample oils contained similar chemical compounds. However, there

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was a significant variation in the percentage proportions of secondary metabolites in the essential oils obtained from the leaves of *Croton gratissimus* harvested on different days. Consequently, for a reproducible chemical profile to be obtained, the leaves of *Croton gratissimus* need to be collected on the same day and time then be processed under similar conditions.

REFERENCES

[1] N. Zouari, "Essential Oils Chemotypes: A Less Known Side," *Med. Aromat. Plants*, vol. 02, no. 02, 2013, doi: 10.4172/2167-0412.1000e145.

[2] N. Sadgrove and G. Jones, "A Contemporary Introduction to Essential Oils: Chemistry, Bioactivity and Prospects for Australian Agriculture," *Agriculture*, vol. 5, no. 1, pp. 48–102, 2015, doi: 10.3390/agriculture5010048.

[3] M. A. Hanif, S. Nisar, Z. Mushtaq, and M. Zubair, "Essential Oil Research," *Essent. Oil Res.*, no. June, 2019, doi: 10.1007/978-3-030-16546-8.

[4] S. Roohinejad *et al.*, "Extraction Methods of Essential Oils From Herbs and Spices," *Essent. Oils Food Process. Chem. Saf. Appl.*, pp. 21–55, 2017, doi: 10.1002/9781119149392.ch2.

[5] C. Turek and F. C. Stintzing, "Stability of essential oils: A review," *Compr. Rev. Food Sci. Food Saf.*, vol. 12, no. 1, pp. 40–53, 2013, doi: 10.1111/1541-4337.12006.

[6] N. J. Sadgrove, G. F. Padilla-González, and M. Phumthum, "Fundamental Chemistry of Essential Oils and Volatile Organic Compounds, Methods of Analysis and Authentication," *Plants*, vol. 11, no. 6, 2022, doi: 10.3390/plants11060789.

[7] C. Franz and J. Novak, *Sources of Essential Oils*. 2015.

[8] M. Moghaddam and L. Mehdizadeh, *Chemistry of Essential Oils and Factors Influencing Their Constituents*, no. October. 2017.

[9] K. D. Magwilu, J. M. Nguta, I. Mapenay, and D. Matara, "Phylogeny, Phytomedicines, Phytochemistry, Pharmacological Properties, and Toxicity of Croton gratissimus Burch (Euphorbiaceae)," *Adv. Pharmacol. Pharm. Sci.*, vol. 2022, 2022, doi: 10.1155/2022/1238270.

[10] A. Barra, "Factors affecting chemical variability of essential oils: A review of recent developments," *Nat. Prod. Commun.*, vol. 4, no. 8, pp. 1147–1154, 2009, doi: 10.1177/1934578x0900400827.

[11] S. F. van Vuuren and A. M. Viljoen, "In vitro evidence of phyto-synergy for plant part combinations of Croton gratissimus (Euphorbiaceae) used in African traditional healing," *J. Ethnopharmacol.*, vol. 119, no. 3, pp. 700–704, 2008, doi: 10.1016/j.jep.2008.06.031.

[12] O. A. Lawal, I. A. Ogunwande, F. O. Osunsanmi, A. R. Opoku, and A. O. Oyedeji, "Croton gratissimus Leaf Essential Oil Composition, Antibacterial, Antiplatelet Aggregation, and Cytotoxic Activities," *J. Herbs, Spices Med. Plants*, vol. 23, no. 1, pp. 77–87, 2017, doi: 10.1080/10496475.2016.1270245.

[13] S. Block, G. Flamini, D. Brkic, I. Morelli, and J. Quetin-Leclercq, "Analysis of the essential oil from leaves of Croton zambesicus Muell. Arg. growing in Benin," *Flavour Fragr. J.*, vol. 21, no. 2, pp. 222–224, 2006, doi: 10.1002/ffj.1558.

[14] O. A. Ajayi and I. E. Omomagiowawi, "Antimicrobial Activity of Croton zambesicus on Staphylococcus aureus and Streptococcus species," no. November, 2018.

[15] A. Salatino, M. L. F. Salatino, and G. Negri, "Traditional uses, chemistry and pharmacology of Croton species (Euphorbiaceae)," *J. Braz. Chem. Soc.*, vol. 18, no. 1, pp. 11–33, 2007, doi: 10.1590/S0103-50532007000100002.

[16] C. Baccelli, A. Martinsen, N. Morel, and J. Quetin-Leclercq, "Vasorelaxant activity of essential oils from croton zambesicus and some of their constituents," *Planta Med.*, vol. 76, no. 14, pp. 1506–1511, 2010, doi: 10.1055/s-0030-1249820.

[17] C. Baccelli, I. Navarro, S. Block, A. Abad, N. Morel, and J. Quetin-Leclercq, "Vasorelaxant Activity of Diterpenes from Croton zambesicus and Synthetic Trachylobanes and Their Structure−Activity Relationships," *J. Nat. Prod.*, vol. 70, no. 6, pp. 910–917, Jun. 2007, doi: 10.1021/np0603976.

[18] J. L. L. Cucho-Medrano, S. W. Mendoza-Beingolea, C. M. Fuertes-Ruitón, M. E. Salazar-Salvatierra, and O. Herrera-Calderon, "Chemical profile of the volatile constituents and antimicrobial activity of the essential oils from croton adipatus, croton thurifer, and croton collinus," *Antibiotics*, vol. 10, no. 11, 2021, doi: 10.3390/antibiotics10111387.

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ISSN: 2521-2293

[19] S. van Vuuren, "The Antimicrobial Activity and Essential Oil Composition of Medicinal Aromatic Plants Used in African Traditional Healing," pp. 1–305, 2007.

[20] M. S. Owolabi, L. A. Oladipupo, L. Lajide, R. M. Hauser, and W. N. Setzer, "Leaf oil composition of Croton zambesicus Muell . Arg . growing in southwestern Nigeria : essential oil chemotypes of C . zambesicus," *Am. J. Essent. Oils Nat. Prod.*, vol. 1, no. 1, pp. 14–17, 2013.

[21] S. Yagi, R. Babiker, T. Tzanova, and H. Schohn, "Chemical composition, antiproliferative, antioxidant and antibacterial activities of essential oils from aromatic plants growing in Sudan," *Asian Pac. J. Trop. Med.*, vol. 9, no. 8, pp. 763–770, 2016, doi: 10.1016/j.apjtm.2016.06.009.

[22] F. F. Boyom *et al.*, "Essential oils from Croton zambesicus Muell. Arg. growing in Cameroon," *Flavour Fragr. J.*, vol. 17, no. 3, pp. 215–217, 2002, doi: 10.1002/ffj.1081.

[23] C. Menut *et al.*, "Aromatic plants of tropical Central Africa. XXII. volatile constituents of Croton aubrevillei J. Léonard and C. Zambesicus Muell. Arg.," *J. Essent. Oil Res.*, vol. 7, no. 4, pp. 419–422, 1995, doi: 10.1080/10412905.1995.9698552.

[24] M. P. Moremi, F. Makolo, A. M. Viljoen, and G. P. Kamatou, "A review of biological activities and phytochemistry of six ethnomedicinally important South African Croton species," *J. Ethnopharmacol.*, vol. 280, no. May, p. 114416, 2021, doi: 10.1016/j.jep.2021.114416.

[25] N. J. Sadgrove, L. G. Madeley, and B. E. Van Wyk, "Volatiles from African species of Croton (Euphorbiaceae), including new diterpenes in essential oil from Croton gratissimus," *Heliyon*, vol. 5, no. 10, p. e02677, 2019, doi: 10.1016/j.heliyon.2019.e02677.

[26] A. Ogundajo, I. Ogunwande, H. Gbadamosi, R. Giwa, and G. Flamini, "Chemical Composition of the Leaf Essential Oils of Croton zambesicus Müll.-Arg. Grown in Lagos, South-West Nigeria," *European J. Med. Plants*, vol. 4, no. 12, pp. 1524–1533, 2014, doi: 10.9734/ejmp/2014/7099.