Tanzania Journal of Science 51(2): 301-309, 2025
ISSN 0856-1761, e-ISSN 2507-7961

Comparative Analysis of Chemical Constituents and Antioxidant Properties of Fresh and Air-Dried Leaves' Essential Oils of *Rauwolfia vomitoria* (Afzel)

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Received 6 Feb 2025, Revised 13 May 2025, Accepted 29 May, Published 30 June 2025

https://dx.doi.org/10.4314/tjs.v51i2.4

Abstract

To enhance the essential oil information of *Rauwolfia vomitoria* (Afzel), we compared the essential oil constituents and antioxidant activities of the fresh and air-dried leaves. The leaves are traditionally used to treat infections, fevers, snake bites, rheumatism, and to calm people with anxiety or epilepsy. The GC, GC/MS results showed the fresh leaves' oil contained thirty-three compounds while the air-dried leaves' oil contained twenty-eight. The fresh and air-dried leaves had β-citral (31.32% and 33.68%), α-citral (26.04% and 38.09%), Isogeranial (9.99% and 1.33%), and isoneral (4.22% and 1.00%), respectively. Neric acid (2.27%), geranic acid (2.52%), geranyl acetate (2.33%), palmitic acid (2.17%), and organosilicon compounds (4.94%) were present in either of the oils. At 400 µg/mL, the fresh and air-dried leaves' oils gave 30.55% and 25.34% mild antioxidant activity compared to ascorbic acid's 96.50%. The natural antibiotic agents (Citral and its stereoisomers) present in the oils could contribute to the traditional use of the plant in treating various diseases, particularly those that are microbial. The mild antioxidant activity observed is important in health issues because the risk of adverse reactions or interactions is thus reduced compared to when stronger antioxidants are used. This work has improved the scarce information on the plant's essential oil constituents and activities.

Keywords: *Rauwolfia vomitoria*; α-citral; β-citral; DPPH assay; Essential oil

INTRODUCTION

Rauwolfia vomitoria is traditionally used to treat many diseases, including diabetes, bacterial, and fungal infections (Zirihi et al. 2005, Pesewu et al. 2008, Campbell-Tofte et al. 2011). Ethanolic extract (80%) of *Rauwolfia vomitoria* was found at 20 mg/kg - 50 mg/kg to significantly reduce by 36% - 66% the tumor growth in mice (Yu et al. 2013). Reports also highlight its anticonvulsant, sedative, and analgesic properties (Olatokunboh et al. 2009, Asoro et al. 2020).

The leaf extract of *Rauwolfia vomitoria* was found to be extremely good in treating diseases related to *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Aspergillus niger*, and for the treatment of cancer, benign prostate hyperplasia, and fibromyalgia (Chinonye et al. 2021). The limited information on the essential oil composition and activity is the main reason for this research work, and also to see if there would be any marked differences in the constituents and antioxidant activities of the fresh and air-dried leaves' oils.

MATERIALS AND METHODS

Plant Collection and Preparation

The fresh leaves of *Rauwolfia vomitoria* collected from the main campus of Olabisi Onabanjo University in Ago-Iwoye, Ogun State, Nigeria, were identified and authenticated at the Department of Botany, University of Ibadan, Nigeria, where a voucher specimen UIH 23132 was deposited. The fresh leaves were cleaned with distilled water and then divided into two portions: one portion was extracted immediately, while the other was air-dried in the laboratory at room temperature for fourteen days, pulverized, and then subjected to the extraction process.

Hydrodistillation of the Essential Oils

Essential oils were extracted via hydrodistillation using an all-glass Clevenger-type apparatus. *Rauwolfia vomitoria* fresh and air-dried leaves (1050 g each) were hydrodistilled separately according to established procedure (Yu et al. 2004), n-hexane was used to trap the extracted oils, which were later dried over anhydrous Na_2SO_4. The dried oils were put in well-labeled vial bottles and kept in a refrigerator at 4 ℃ until ready for analysis.

Gas Chromatography (GC) and Gas Chromatography-Mass Spectrometry (GC-MS)

Both oils were subjected to GC and GC-MS analyses performed on an Agilent Technology 7890 model, splitter mode HP-5973 at 70 eV for the GC, and an Agilent Technology with a split injector operated between 5973 and 7683 for GC-MS determination.

Identification of the Essential Oil Constituents

Identifying the individual constituents of both essential oils was based on their retention indices (RI) determined by co-injection with reference to a homologous series of n-alkanes, under matching experimental settings. Further identification was performed by comparing their mass spectra with those from the National Institute of Standards and Technology NIST (Database 69/chemstation data system) and the home-made MS library assembled from pure substances and components of previously identified essential oils, and also by comparison of their retention indices with existing literature values (Adams 2007).

Determination of Antioxidant Activities

The DPPH free radical scavenging activity was used to determine the antioxidant activities of the essential oils. This is because many essential oils contain compounds that can scavenge free radicals, which can be easily assessed by the DPPH assay using ascorbic acid as a standard. The antioxidant analysis of the essential oils from the fresh and air-dried leaves of *Rauwolfia vomitoria* was carried out according to the method used by Lee et al. (2006), in which methanol was used to prepare a 0.1 mM solution of DPPH. Then, 1 mL of the DPPH solution was added to 1 mL of the oil at different concentrations (25 to 400 µg/mL) to determine whether the antioxidant activity would be concentration-dependent. The mixture of DPPH and the oil sample was shaken vigorously and allowed to react for 30 min at room temperature in a cupboard to facilitate the reaction. The optical density of each concentration was then measured at 517 nm with the aid of a Buck model 752N UV-visible spectrophotometer. The experiment was done in triplicate. Percentage inhibition was calculated as follows:

$$I(\%) = 100 \times \frac{A_{blank} - A_{sample}}{A_{blank}}$$

where A_{blank} is the optical density of the control (all reagents minus the sample),
A_{sample} is the optical density of the test sample.

RESULTS AND DISCUSSION

Results

Both oils were light yellow and had a characteristic herbal lemon aroma attributable to the presence of their citral components. The yields were 0.25% (w/w) for the fresh leaves and 0.20% (w/w) for the air-dried leaves.

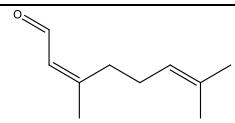
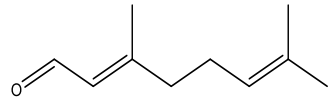
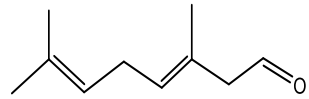
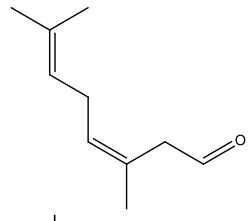
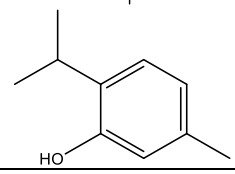
Table 1: Similar Chemical Constituents of the Essential Oils from the Fresh and Air-dried Leaves of *Rauwolfia vomitoria*

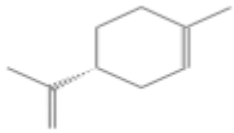
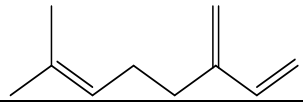
S/N	Compounds	Chemical Formula	Retention Time (Min)		% Composition	
			F. L	A. L	F. L	A. L
1.	β-Myrcene	$C_{10}H_{16}$	3.175	6.397	0.50	0.97
2.	D-Limonene	$C_{10}H_{16}$	3.673	3.730	0.91	0.86
3.	Isoneral	$C_{10}H_{16}O$	5.489	5.500	4.22	1.00
4.	Isogeranial	$C_{10}H_{16}O$	5.749	5.744	9.99	1.33
5.	β-Citral	$C_{10}H_{16}O$	6.745	6.599	31.32	33.68
6.	α-Citral	$C_{10}H_{16}O$	7.341	7.025	26.04	38.09
7.	Thymol	$C_{10}H_{14}O$	7.606	7.300	1.65	1.44
	Total				**74.63**	**77.37**

Key: F. L. = Fresh Leaves, A. L. = Air-dried Leaves

Table 1 highlights similarities in the constituents of the oils, despite variations in their percentage composition. Fifty-five compounds were identified: thirty-three represent 100% of the fresh leaves' essential oil, while 28 represent 99.99% of the air-dried leaves' oil, probably due to some components in the fresh leaves' oil degrading due to air-drying. Seven of these compounds were common to both oils as seen in Table 1: The acyclic monoterpene aldehydes - α-Citral (26.04% and 38.09%) and β-Citral (31.32% and 36.38%) were the major compounds, while Isogeranial (9.99% and 1.33%) and Isoneral (4.22% and 1.00%) were in lesser quantities in the fresh and air-dried leaves' oils respectively. Other classes in trace amounts were alkanes, terpenes, aromatic acids, and sesquiterpenoids.

Table 2: The structures of compounds common to both the fresh and air-dried leaves' essential oils of *Rauwolfia vomitoria*

S/N	Compounds	% Composition (F. L. Oil)	% Composition (A. L. Oil)	Structure
1.	β-Citral	31.32	33.68	
2.	α-Citral	26.04	38.09	
3.	Isogeranial	9.99	1.33	
4.	Isoneral	4.22	1.00	
5.	Thymol	1.65	1.44	

6.	D-Limonene	0.91	0.86	
7.	β-Myrcene	0.50	0.97	

Key: F. L. = Fresh leaves, A. L. = Air-dried leaves

Table 2 shows the structures of the common constituents of the fresh and air-dried leaves' essential oils of *Rauwolfia vomitoria.* Both samples contain Citral and its stereoisomers in various quantities, with the air-dried leaf oil samples having more of both β-Citral and α-Citral. It seemed that air-drying the leaves increased the amount of citral content. However, the fresh leaf oil contained more isogeranial and isoneral than the air-dried leaf oil.

Table 3: Antioxidant Activities of Essential Oils of Fresh and Air-dried Leaves *Rauwolfia vomitoria using* DPPH

Conc. (µg/mL)	Fresh Leaves (%) Inhibition	Air dried Leaves (%) Inhibition	Ascorbic acid (%) Inhibition
400	30.55 ± 0.29	25.34 ± 0.04	96.50 ± 0.02
200	26.76 ± 0.07	24.70 ± 0.1	95.81 ± 0.15
100	24.16 ± 0.03	22.36 ± 0.12	95.22 ± 0.04
50	22.32 ± 0.09	21.24 ± 0.00	95.02 ± 0.03
25	19.88 ± 0.21	18.90 ± 0.1	94.87 ± 0.03

Table 3 demonstrates mild DPPH scavenging activity for both oils, significantly lower than for ascorbic acid. Comparing the antioxidant profile of both samples, they were found to increase in a dose-dependent manner from 19.88 - 30.55% for the fresh leaf sample and 18.90 - 25.34% for the air-dried leaf sample, as ascorbic acid's, 94.87 - 96.50%.

DISCUSSION

The essential oils obtained from the fresh (0.25% w/w) and air-dried (0.20% w/w) leaves of *Rauwolfia vomitoria* were yellowish with a bit of lemon smell. Table 1 highlights similarities in the major constituents of the oils, despite variations in their percentage composition. Several other compounds were present that were not common to both oils, probably due to the air-dried leaves having lost water, undergone oxidation, and having reduced metabolism, amongst other factors. The oils mostly contained acyclic monoterpene aldehydes, including α-citral, β-citral, isoneral, and isogeranial. Other compounds, such as monoterpenes, monoterpenoids, sesquiterpenoids, and hydrocarbons, were found in trace amounts in either one or both oils. The high concentration of α-citral (26.04%- fresh leaf, and 38.09% -air-dried leaf) and β-citral (31.32 % - fresh leaf, and 33.68%- air-dried leaf) in both oils could account for the little bit of lemon odour observed and probably contribute to why *Rauwolfia vomitoria* is being used for the management of diseases like malaria, diabetes, bacterial and fungal infections, hypertension, impotence, dysentery, insomnia, worm infections, diarrhea, gastrointestinal diseases, and scabies (Pesewu et al. 2008, Campbell-Tofte et al. 2011). Several medicinal and therapeutic activities are associated with citral, such as antimicrobial, anticancer, antioxidant, anti-inflammatory, and anti-diabetic potentials (Sharma et al. 2021). According to Gao et al. (2020), Sharma et al. (2021), and Viktorová et al. (2020), citral has demonstrated effectiveness against Gram-positive and Gram-negative bacteria, fungi, and parasites. The compound has also been shown to be sedative, antipyretic,

antispasmodic, anti-inflammatory, analgesic, and diuretic (Saddiq and Khayyat 2010). The antiproliferative effect against human and murine cancer cell lines, anti-inflammatory, antidiabetic, and antihypertensive properties of citral have also been investigated (Santoro et al. 2007, Ruiz-Bustos et al. 2009, Sharma et al. 2021).

Table 3 demonstrates mild DPPH scavenging activity for both oils, significantly lower than that of ascorbic acid. The observed antioxidant activities of 19.88% - 30.55% (fresh leaves' essential oil) and 18.90%-25.34 % (air-dried leaves' essential oil) were dose-dependent (25 µg/ mL to 400 µg/mL) compared with ascorbic acid's 94.87%-96.50 % within the same concentration range. As expected, the fresh leaves' oil had a slightly higher antioxidant activity (Olubomehin et al., 2024), which could be due to the higher isogeranial and isoneral content. The mild antioxidant activities of both oils could be beneficial for health issues, where stronger antioxidants could cause adverse effects or interactions. Previous work on the DPPH free radical scavenging of the methanol extract of *Rauwolfia vomitoria* at between 7 µ/mL and 1000 µ/mL compared to ascorbic acid within the same concentration range showed a dose-dependent increment from 19.64% - 88.04% for the plant extract and 29.46% - 99.32% for ascorbic acid (Oraekei et al. 2024). The methanol plant extract seemed to possess stronger antioxidant activities, which may be due to the extract's constituents possessing more antioxidant activity than the constituents of the essential oils under study. The observed antioxidant activity may be due to the presence of citral and its stereoisomers in the oils, since citral has been shown to have antioxidant activity and protect IEC-6 cells against aspirin-induced oxidative stress (Bouzenna et al, 2017).

CONCLUSION

This study compared the constituents of the essential oils obtained from the fresh and air-dried leaves of *Rauwolfia vomitoria* and their antioxidant activity through DPPH free radical scavenging properties. Citral and its stereoisomers, as major components, may underlie the antioxidant and therapeutic properties of the plant. The higher citral content in the air-dried leaves' oil suggests its potential for enhanced antibacterial properties in treating health conditions like inflammation, managing high blood pressure, as a diuretic, sedative, anticonvulsant, and an antidiabetic agent. This study has helped improve the available data on *Rauwolfia vomitoria*, particularly its essential oil information. Future studies are recommended for testing the oils against specific bacterial strains and exploring the impact of different drying methods on oil yield and bioactivity.

Declaration of Interest

The authors declare no conflict of interest.

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Supplementary Data
Appendix 1

Table 1: Chemical Constituents of the Essential Oils from the Fresh and Air-dried Leaves of *Rauwolfia vomitoria*

S/N	Compounds	Chemical Formula	Retention Time (Min)		% Composition	
			Fresh Leaves	Air dried Leaves	Fresh Leaves	Air dried Leaves
1.	β-Myrcene	$C_{10}H_{16}$	3.175	-	0.50	-
2.	*o*-Cymene	$C_{10}H_{14}$	3.616	-	1.05	-
3.	*p*-Cymene	$C_{10}H_{14}$	-	3.678	-	0.38
4.	D-Limonene	$C_{10}H_{16}$	3.673	3.730	0.91	0.86
5.	Linalool	$C_{10}H_{18}O$	4.586	-	0.78	-
6.	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	-	4.617	-	2.51
7.	Cyclopentasiloxane, decamethyl	$C_{10}H_{30}O_5Si_5$	-	5.328	-	1.28
8.	(2E,6E,10E)-3,7,11,15-Tetramethylhexadeca-2,6,10,14-tetraen-1-yl formate	$C_{10}H_{18}O$	5.328	-	1.98	-
9.	Isoneral	$C_{10}H_{16}O$	5.489	5.500	4.22	1.00
10.	Isogeranial	$C_{10}H_{16}O$	5.749	5.744	9.99	1.33
11.	β-Myrcene	$C_{10}H_{16}$	-	6.397	-	0.97
12.	β-Citral	$C_{10}H_{16}O$	6.745	6.599	31.32	33.68
13.	α-Citral	$C_{10}H_{16}O$	7.341	7.025	26.04	38.09
14.	Thymol	$C_{10}H_{14}O$	7.606	7.300	1.65	1.44
15.	Cyclohexa silixonae, dodecamethyl	$C_{12}H_{36}O_6Si_6$	-	7.694	-	1.15
16.	α-Limonene diepoxide	$C_{10}H_{16}O_2$	-	7.969	-	0.57
17.	Geranic acid	$C_{10}H_{16}O_2$	-	8.286	-	2.52
18.	L-Histidine, methyl ester	$C_7H_{12}N_3O_2$	8.094	-	1.67	-
19.	7-oxabicyclo (4.1.0) heptane, 2-methylene	$C_{10}H_{16}O$	8.410		0.42	
20.	Geranyl acetate	$C_{12}H_{20}O_2$	-	8.457	-	2.33
21.	2,6-Octadien- 1-ol, 3,7-dimethyl-,	$C_{12}H_{20}O_2$	8.602	-	4.81	-

	acetate					
22.	Neric acid	$C_{10}H_{16}O_2$	8.727		2.27	-
23.	(E, Z). α-farnesene	$C_{15}H_{24}$	-	9.868	-	1.92
24.	Ethanone, 1-cyclohexyl	$C_8H_{14}O$	-	9.178	-	0.44
25.	Bicyclo (7.2.0) undec-4-ene, 4,11,11-trimethyl-8-methylene-,[IR -(IR*, 4Z, 9S*)]	$C_{15}H_{24}$	8.934	-	0.31	-
26.	3,5-Heptadienal,2–ethylidene-6-methyl	$C_{10}H_{14}O$	9.069	-	0.62	-
27.	Trans-α-Bergamotene	$C_{15}H_{24}$	9.235	-	0.78	-
28.	β-Selinene	$C_{15}H_{24}$	9.920	-	1.70	-
29.	α-Selinene	$C_{15}H_{24}$	10.008	-	0.37	-
30.	3-Tridecanol	$C_{13}H_{28}O$	10.117	-	0.33	-
31.	Carbonic acid, octadecyl Prop-1-ene-2-yl ester		-	10.424	-	0.67
32.	1-Propyne, 1-(ethenylthio)-	C_5H_8S	10.507		0.72	
33.	Caryophyllene oxide	$C_{15}H_{24}O$	11.088	-	0.63	-
34.	β-Maaliene	$C_{15}H_{24}$	-	11.456	-	0.82
35.	Selin-6-en-4α-ol	$C_{15}H_{26}O$	11.508	-	1.19	-
36.	2-(1-cyclopentanyl) furan	$C_9H_{10}O$	11.892	-	0.43	-
37.	2,5-Dihydro benzoic acid, 3TMS derivative	$C_{16}H_{30}O_4Si_3$	-	11.892	-	0.46
38.	(-) – Globulol		11.965	-	0.27	-
39.	Dodecane	$C_{12}H_{26}$	-	12.359	-	0.42
40.	Octadecane	$C_{18}H_{38}$	-	13.314	-	0.24
41.	2-pentadecanone, 6,10,14 – trimethyl	$C_{18}H_{36}O$	13.822	-	0.27	-
42.	3-Hexadecene, (Z)	$C_{16}H_{32}$	-	14.159	-	0.39
43.	Cyclotetradecane	$C_{14}H_{28}$	14.170	-	0.37	-
44.	Dotriacontane,1-iodo	$C_{32}H_{65}I$	-	14.592	-	0.97
45.	Palmitic acid	$C_{16}H_{32}O_2$	-	15.010	-	2.17

46.	4-n- pentylthiane, S, S-dioxide		15.872	-	0.51	-
47.	1,2-Epoxyundeane	$C_{12}H_{24}O$	-	16.416	-	1.47
48.	6-methyl-4,6- bis (4-methyl pent-3-en-1yl)cyclohexa-1,3-dienecarbaldehyde	$C_{20}H_{30}O$	16.484	-	1.95	-
49.	Tridecanedial	$C_{13}H_{24}O$	-	16.712	-	1.24
50.	5-Bromo-n-pentanol, cyclohexyl ether	$C_{11}H_{21}BrO$	16.748	-	0.95	-
51.	4-hexen-l-ol, 2- ethenyl-2, 5-dim ethyl	$C_{10}H_{18}O$	16.821	-	0.37	-
52.	β-Bisabolene	$C_{15}H_{24}$	-	16.935	-	0.20
53.	2,6-octadiene-l-ol, 3,7-dimethyl-, formate, (Z)	$C_{11}H_{18}O_2$	16.966	-	0.37	-
54.	2- isopropenyl-5-methyl hex-4-enal	$C_{10}H_{18}O$	17.454	-	0.25	-
55.	Mono (2-ethylhexyl) phthalate	$C_{16}H_{21}O_4$	-	20.832	-	0.47
	Total				**100**	**99.99**