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Characterization of volatile metabolites of *Tithonia diversifolia* (Hemsley) A. gray leaves and flowers

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Abstract
The leaf and floral essential oils of *Tithonia diversifolia* (Hemsley) A. Gray (Asteraceae) were analyzed for composition by means of gas chromatography-mass spectrometry (GC-MS). Forty-two constituents were identified in the leaf and floral oils, accounting for 97.2% and 95.3% of the total composition, respectively. Germacrene D (46.0%), β-caryophyllene (12.2%), and methyl carvacrol (9.8%) were the prominent components of the leaf oil, while the main constituents of the floral oil consisted of germacrene D (32.5%), α-pinene (7.5%), and β-caryophyllene (6.6%).

Keywords: asteraceae, *Tithonia diversifolia*, essential oil composition, germacrene D, β-caryophyllene

1. Introduction
Tithonia diversifolia (Hemsl) A. Gray (Mexican sunflower), is a member of the Asteraceae family. *Tithonia diversifolia* is a prolific plant, which occurs along roadsides, farmlands and lawns in association with a vast array of weeds. It is employed in ethno-medicine for the treatment of malaria, diabetes mellitus, sore throat and liver pains [1]. Non-volatile constituents (sesquiterpene lactones, diversifolol, thithoniaquinone A, and tithoniamide B) from leaves of *T. diversifolia* have been isolated and characterized [2-5]. *Tithonia diversifolia* specimens from some geographical locations of the world have been studied for their essential oil composition [6-11]. Lamaty *et al*. [6] and Menut *et al*. [7] reported the high content of (*Z*)-β-ocimene, α-pinene, and limonene in the leaf and floral oil of *T. diversifolia*; also, α-pinene and β-pinene [8], and α-pinene, (*E,E*)-α-farnesene, and β- caryophyllene are reported in the composition of the leaf oils [9]. In this communication, we present the characterization of the leaf and floral essential oils extracted from *T. diversifolia* harvested from a different geographic region of Nigeria (South-South), in furtherance of a previous study on *T. diversifolia* oil from Nigeria [12].

2. Materials and Methods
2.1 Plant materials
The leaves and flowers of *T. diversifolia* were collected within the vicinity of the University of Uyo campus, in January 2017. The plants were identified by a taxonomist in the Department of Botany and Ecological Studies, University of Uyo, where the voucher specimen (UU 11395) were prepared and deposited in the herbarium.

2.2 Isolation of the essential oils
The volatile oils were obtained by hydro-distillation (4 h) of the fresh plant parts using a Clevenger-type apparatus in accordance with the British Pharmacopoeia [13]. The oils were dried over sodium sulfate (Sigma-Aldrich, St. Louis, MO, USA) and stored in a refrigerator (4 °C) after the estimation of percentage yield.

2.3 GC-EIMS analyses
GC-EIMS analyses were performed with a Varian CP-3800 gas chromatograph, equipped with a DB-5 capillary column (30 m × 0.25 mm; coating thickness, 0.25 μm) and a Varian Saturn 2000 ion trap mass detector. Analytical conditions: injector and transfer line temperatures 220 and 240 °C, respectively; oven temperature programmed from 60 to 240°C at 3°C/min; carrier gas helium at 1 mL/min; injection of 0.2 μL (10 % hexane solution); split ratio of 1:30. Constituents identification was based on comparison of retention times with those of authentic samples; this implied comparing their LRIs with the series of *n*-hydrocarbons and using computer matching against commercial (NIST 2014 and ADAMS) and home-made library

mass spectra (built up from pure substances and components of known oils and mass spectra literature data [14, 15].

3. Results & Discussion

The volatile constituents of *T. diversifolia* essential oils are presented in Table 1. The pale yellow oils from the leaf and floral part of *T. diversifolia* afforded yields of 0.05% and 1.1% (w/w), respectively. A total of forty-two constituents in the leaf and floral oils accounted for 97.2% and 95.3% of the oils composition, respectively. Twenty-eight constituents were identified in the leaf oil and thirty-five in the floral oil, though twenty-one compounds were common to both oils. Generally, each oil displayed a unique chemical profile, which differed both quantitatively and qualitatively. Germacrene D (46.0%), β-caryophyllene (12.2%), and methyl carvacrol (9.8%) were the prominent components of the leaf oil, while the main constituents of the floral oil consisted of germacrene D (32.5%), α-pinene (7.5%), and β-caryophyllene (6.6%). The percentage compositions of classes of compounds in *T. diversifolia* leaf and floral oils, respectively, were indicated as such: monoterpene hydrocarbon (5.1 and 33.3%), oxygenated monoterpenes (2.2 and 6.4%), sesquiterpene hydrocarbons (74.3 and 48.6%), oxygenated sesquiterpenes (5.8 and 7.0%), and phenylpropanoids (9.8 and 0.0%). The hydrocarbon constituents of the leaf oil (79.4%) and floral oil (81.9%) showed dominance over the oxygenated components. The abundance of germacrene D and β-caryophyllene in the leaf oil in this study makes the oil similar to previously reported Nigerian sample [12], though it differs in its relatively much lower content of α-pinene, β-pinene, and 1,8-cineole. Likewise, the floral oils were similar in the dominance of germacrene D and β-caryophyllene, but varied significantly in the content of bicyclogermacrene and the absence of aliphatic acids. Notably, Moronkola and co-workers [12] collected *T. diversifolia* in a different geographic location during the day, and hydrodistilled air-dried samples; whereas, samples in this study were obtained in the early morning hours and fresh samples were hydrodistilled. Apparently, the main components [(*Z*)-β-ocimene, α-pinene, β-pinene, and limonene] of *T. diversifolia* leaf, floral, aerial part, and stem essential oils from other regions of the world [6-11] were identified as minor components of the leaf and floral volatile oils from Nigeria.

Table 1: Composition of *Tithonia diversifolia* essential oils

Constituents	LRI	Relative abundance (%)		Constituents	LRI	Relative abundance (%)	
		Leaf	Flower			Leaf	Flower
α-Thujene	933	-	1.0	γ-Muurolene	1478	-	0.4
α-Pinene	941	2.3	7.5	Germacrene D	1482	46.0	32.5
Sabinene	977	0.5	3.2	Bicyclogermacrene	1496	1.2	0.9
β-Pinene	982	0.9	3.7	α-Bulnesene	1507	1.6	1.0
Myrcene	993	0.2	1.5	*(E,E)*-α-Farnesene	1508	1.1	0.5
α-Phellandrene	1006	-	4.0	*trans*-γ-Cadinene	1514	0.2	-
δ-3-Carene	1013	0.2	-	Cubebol	1515	0.4	
α-Terpinene	1020	-	0.8	δ-Cadinene	1524	1.2	1.5
p-Cymene	1028	0.3	5.4	Germacrene B	1557	2.2	1.1
Limonene	1032	0.7	2.6	*(E)*-Nerolidol	1564	0.6	0.7
1,8-Cineole	1034	1.0	-	Spathulenol	1577	-	0.5
(E)-β-Ocimene	1052	-	0.5	Caryophyllene oxide	1582	2.3	0.6
γ-Terpinene	1063	-	3.1	Guaiol	1596	1.3	1.3
4-Terpineol	1179	-	0.9	Rosifoliol	1606		0.4
Methyl chavicol	1196	9.8	-	Humulene epoxide II	1607	0.7	-
Methyl thymol	1234	1.2	3.7	1-*Epi*-cubenol	1629	-	0.3
Carvacrol	1301		1.8	γ-Eudesmol	1632	-	0.4
β-Bourbonene	1385	0.8		T-Cadinol	1641	-	0.9
β-Cubebene	1391	0.4	0.3	α-Cadinol	1652	0.5	1.4
β-Elemene	1392	0.9	0.6	Bulnesol	1667	-	0.5
β-Caryophyllene	1419	12.2	6.6	Total identified		97.2	95.3
α-Humulene	1455	6.5	3.2				

Note: LRI: Linear retention index on a DB5 column; - : Not detected

4. Conclusions

The leaf and floral volatile constituents of *T. diversifolia* have been analyzed and identified. The oils obtained are predominantly endowed with germacrene D and β-caryophyllene.

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