American Journal of Essential Oils and Natural Products
Available online at www.essencejournal.com
AJEONP American Journal of Essential Oils and Natural Products

ISSN: 2321 9114
AJEONP 2014; 1 (4): 1-5
© 2014 AkiNik Publications
Received 13-03-2014
Accepted: 17-05-2014

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Extraction and Characterization of Essential Oils from *Tithonia diversifolia* (Hemsl.) A. Gray

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Abstract
The essential oils associated with the flowers of *Tithonia diversifolia* (Hemsl.) A. Gray were extracted and characterized via hydrodistillation and Gas Chromatography. Hydrodistillation was done in an all-glass Clevenger-type apparatus. The extracts from the hydrodistillation were subjected to gas chromatography where the components of the essential oils were separated based on their molecular weights, identified and characterized. The study characterized forty-five components (essential oils) and these include: Cymene, Cinnamic aldehyde, Tricyclene, α-Pinene, β-Pinene, Sabinene, Camphene, Limonene, Benzyl alcohol, *Cis*-ocimene, Myrcene, *Allo*-ocimene, Pinene-2-ol, α-Thujene, Thymol, Carvacrol, 3-Methoxyacetophenone, γ-Terpinene, Citronellal, Neral, Geranial, Borneol, Citronellol, Linalool, 1,8-Cineole, α-Terpineol, Terpinen-4-ol, Germacrene D, Thymyl Methl Ether, Linalyl acetate, Borneol acetate, Geranyl acetate, α-Cubebene, β-Caryophyllene, γ-Cardinene, β-Elemene, Germacrene B, α-Caryophyllene, α-Copane, Acetyleugenol, α-Selinene, γ-Muurolene, Elemicin, β-Selinene and Caryophyllene oxide. Out of these, α-pinene, β-caryophyllene, β-pinene, Germacrene D and 1,8-Cineole were found to be the major constituents with 34.42%, 22.34%, 11.14%, 11.13% and 8.76% respectively.

Keywords: *Tithonia diversifolia,* Hydrodistillation, Pinene, β-caryophyllene, Germacrene D, 1, 8-Cineole.

1. Introduction

Essential oils are natural volatile substances obtained from a variety of plants. Commercially, essential oils have many uses and have found applications in pharmaceuticals, flavour in many food products, odorants in fragrances and as insecticides [1]. Also, particular emphasis has been placed on the antibacterial, antifungal and insecticidal activities of essential oïl from plants [2, 3]. *Tithonia diversifolia* (Hemsl.) A. Gray is a member of the family Asteraceae. It is native to Mexico and Central America [4]. According to Akobundu and Agyakwa [5], it was probably introduced into West Africa as an ornamental plant and has become naturalized in many tropical countries. In Nigeria, it has become significant agronomic and economic factor to optimum and arable crop production especially in the Southern Guinea savannah zone [6]. Otherwise known as the Mexican Sunflower, it is an aggressive weed with high invasive capacity and the ability to compete successfully with agricultural crops [7].

Essential oils are volatile oils that are found in plants. They are very complex, natural mixtures and can contain about 20 to 60 compounds at different concentrations, characterized by two or three major components at fairly high concentrations (20 to 70%) compared to other components present in trace amounts [8]. Generally, these major components determine the biological properties of the essential oils. The components include two groups of distinct biosynthetic components [8]. The main group is composed of terpenes and terpenoids and the other of aromatic and aliphatic constituents, all characterized by low molecular weight [9]. Essential oils are found in leaves, rinds of fruit, seeds, bark, heartwood of trees, flowers and any other part of a plant. *T. diversifolia* is known to contain many compounds that are beneficial to man as antioxidants, antibacterial, pest controllers, as part of medicine [10-13] and for many other purposes in industries.

This study was carried out to identify bioactive chemicals associated with *T. diversifolia*, which can aid in developing a wide array of natural products such as cosmetics, pharmaceuticals, and biocides. This in turn can help in reducing our reliance on synthetic chemicals that are not affordable and not environmentally friendly.

2. Materials and Methods

2.1 Collection of Plant Materials

Fresh flowers of *Tithonia diversifolia* were collected within the University of Ibadan campus on

22 October 2012, identified and authenticated at the University of Ibadan Herbarium (UIH) with the Herbarium Voucher number UIH – 22388.

2.2 Extraction procedure

960 g of fresh flowers were subjected to hydro-distillation for 3 hours using a Clevenger-type apparatus. The distillate was collected in a glass vial and stored at cold temperature prior to characterization. The solvent Hexane (1 ml) was added to the experimental column to trap the oil above hot water.

2.3 Characterization: Gas chromatography was carried out using a HP gas chromatograph model 6890 powered with HP Chem. Station Rev. A. 09.01 [1206] software equipped with flame ionization detector (FID) and identified with HP-5MS capillary column (30 m x 0.25 mm, film thickness 0.25 µm). Inlet temperature was 150 ^{0}C. Column oven temperature was programmed from 40 to 220 ^{0}C at the rate of 5 ^{0}C/min and final temperature was held for 2 minutes. Hydrogen was used as a carrier gas with a flow Rate of 1.0 ml/min. A sample of 1.0 µL was injected, using slit mode (split ratio, 20:1). The results (composition) were reported as a relative percentage of the total peak area and grouped as: 0.001 – 0.063 (Trace), 0.064 – 1.563 (Low) and 1.564 – 39.063 (High).

Plate 1: *Tithonia diversifolia* growing along a road side in the University of Ibadan Campus.

Plate 2: Fresh flowers of *Tithonia diversifolia* used in the study.

3. Results and Discussion

Analysis of the essential oils associated with the flowers of *T. diversifolia using* Gas Chromatography identified and characterized 45 essential oil components with their retention time (Table 1).

Table 1: Concentration of essential oils in the flower of *T. diversifolia*

S/No	Name of Essential Oil	Normality (%)	Retention time (min)	Status
1	p-Cymene	0.001	6.53	Trace
2	Cinnamic aldehyde	0.001	7.19	Trace
3	Tricyclene	0.001	7.53	Trace
4	α-Pinene	34.416	7.92	High
5	β-Pinene	11.136	8.46	High
6	Sabinene	0.001	9.22	Trace
7	Camphene	0.001	9.91	Trace
8	Limonene	0.001	10.32	Trace
9	Benzyl alcohol	0.003	11.36	Trace
10	*Cis*-ocimene	0.001	12.10	Trace
11	Myrcene	0.123	12.95	Low
12	*Allo*-ocimene	0.014	13.29	Trace
13	Pinene-2-ol,	0.002	13.74	Trace
14	α-Thujene	1.118	14.16	Low
15	Thymol	0.001	14.25	Trace
16	Carvacrol	0.001	14.52	Trace
17	3-Methoxyacetophenone	0.001	14.70	Trace
18	γ-Terpinene	0.943	14.93	Low
19	Citronellal	0.001	15.04	Trace
20	Neral	0.002	15.32	Trace
21	Geranial	0.087	15.41	Low
22	Borneol	1.707	16.05	High
23	Citronellol	0.001	17.10	Trace
24	Linalool	0.248	17.79	Low
25	1,8 –Cineole	8.762	18.07	High
26	α-Terpineol	1.226	18.68	Low
27	Terpinen-4-ol	0.001	18.86	Trace
28	Germacrene D	11.126	19.54	High
29	ThymylMethl Ether	0.001	19.97	Trace
30	Linalyl acetate	0.002	20.80	Trace
31	Borneol acetate	0.003	21.69	Trace
32	Geranyl acetate	0.209	21.84	Low
33	α- Cubebene	2.607	21.92	High
34	β- Caryophyllene	22.341	22.62	High
35	γ-Cardinene	1.693	23.01	High
36	β-Elemene	2.201	23.24	High
37	Germacrene B	0.002	24.11	Trace
38	α-Caryophyllene	0.004	24.73	Trace
39	α- Copane	0.003	25.01	Trace
40	Acetyleugenol	0.001	25.87	Trace
41	α-Selinene	0.001	26.36	Trace
42	γ-Muurolene	0.001	27.44	Trace
43	Elemicin	0.001	28.31	Trace
44	β-Selinene	0.001	28.47	Trace
45	Caryophyllene oxide	0.001	29.09	Trace

The chemical analysis of these essential oils revealed that they were mainly Monoterpenes. However, 87.78% of the essential oils were contributed by α- Pinene, β- Caryophyllene, β- Pinene, Germacrene D and 1,8-Cineole in the proportion 34.41%, 22.34%, 11.14%, 11.13% and 8.76% respectively. Moronkola *et al*. [14] in their work on *T. diversifolia* obtained 72 essential oil components from the flowers while 45 essential oil components were recorded and characterized in this study. This may be attributed to the

environment and experimental conditions under which these studies were conducted. Akobundu and Agyakwa [5] had argued that environment and climate affect the physiology and nutrient contents of *T. diversifolia.* Moronkola *et al*. [14] reported 22.4% of Germacrene D in their work on the volatile compounds in the leaf and flower of *T. diversifolia* which is twice the quantity obtained in this study (11.13%). This study did not detect bicyclogermacrene, Naphtalene, Methyl salicylate and decanol but were among the components identified in their study. These differences may be as a result of environmental and genetic differences as highlighted earlier. However, the report of this study agrees with the findings of Perry *et al*. [15] who opined that environmental differences affect the biochemical yield of essential oils in plants.
In this study, a high oil yield of 0.45%w/w was obtained. This is similar to the findings of Hădărugă *et al* [16] who reported yields up to 0.5%w/w for many plants in the family - Asteraceae. On the contrary Moronkola *et al*. [14] reported a lower yield of 0.1%w/w from the flower of this plant. This low yield may be due to the fact that they air dried their plant materials contrary to the fresh flower samples used in this study. This suggests that for higher essential oil yields, fresh plant materials are preferred. The essential oils produced from members of this family (Asteraceae) are low compared to that of members of Lamiaceae family. The Lamiaceae family has been reported as one of the top essential oil producers with higher yields (up to 3%) as compared to the Asteraceae [17, 18].

3.1 Classification of the essential oils from *Tithonia diversifolia* (flowers) into component groups

The essential oils in *T. diversifolia* flowers were grouped based on the number of carbon atoms as monoterpenes, oxygenated monoterpenes, sesquiterpenes and oxygenated sesquiterpenes (Table 2). The essential oil components that could not be grouped in any of the four aromatic groups were grouped as 'Others'. The principal components of the essential oils belong to Monoterpenes (oxygenated monoterpenes inclusive) with percentage of 44.44% while Sesquiterpenes essential oils were 26.67% and others were 28.89%.

Table 2: Hydrocarbon classification of essential oil components in the flower of *Tithonia diversifolia*

S/NO	Hydrocarbons	Essential Oil Components	Number
1	Monoterpenes	p-Cymene, α-Pinene, β-Pinene, Sabinene, Camphene, Limonene, *Cis*-ocimene, Myrcene, γ-Terpinene, Citronellol, Linalool, α-Terpineol, Terpinen-4-ol	13
2	Oxygenated Monoterpenes	Thymol, Borneol, 1,8-Cineole, Linalyl Acetate, Borneol Acetate, Geranyl acetate, Acetyleugenol	7
3	Sesquiterpenes	Neral, Germacrene D, α-Cubebene, β-Caryophyllene, γ-Cardinene, β-Elemene, Germacrene B, α-Caryophyllene, α-Copane, γ-Muurolene	10
4	Oxygenated Sesquiterpenes	α-Thujene, Caryophyllene Oxide	2
5	Others	Cinnamic Aldehyde, Tricyclene, Benzyl Alcohol, *Allo*-ocimene, Pinene-2-ol, Carvacrol, 3-Methoxyacetophenone, Citronellal, Geranial, ThymylMethyl ether, α-Selinene, Elemicin, β- Selinene	13
Total			45

Forty five compounds were identified as the essential oil components of *T. diversifolia* representing 100% of the oil extracted from the flowers (Table 1). The major constituents (≥ 0.01%) in the *T. diversifolia* essential oil components were determined to be *Allo*-ocimene (0.01%), Geranial (0.09%), Myrcene (0.12%), Geranyl acetate (0.21%), Linalool (0.25%), γ-Terpinene (0.94%), α- Thujene (1.12%), α- Terpineol (1.23%), γ-Cardinene (1.69%), Borneol (1.71%), β- Elemene (2.20%), α-Cubebene (2.61%), 1,8-Cineole (8.76%), Germacrene D (11.13%), β- Pinene (11.14%), β- Caryophyllene (22.34%) and α- Pinene (34.42%). In addition, the tested *Tithonia* essential oil components also contained substantial amounts of various minor constituents. The essential oil components in *Tithonia diversifolia* flower were mainly Monoterpenes (oxygenated monoterpenes inclusive) and Sesquiterpenes (oxygenated sesquiterpenes inclusive) constituting 44.44% and 26.67% respectively. α-Pinene, β-Pinene, Myrcene, γ-Terpinene, Borneol, 1, 8-Cineole, α-Terpineol and Geranyl acetate were the principal monoterpenes in the essential oil components in the flowers. The sesquiterpene hydrocarbons were substantial and were majorly α-Thujene, β-Elemene, α-Cubebene, β-Caryophyllene, γ-Cardinene, Germacrene B and Germacrene D. Several compounds, mostly sesquiterpenes, diterpenes, monoterpenes and alicyclic compounds have been isolated from the leaves, stem and flowers of *T. diversifolia* [19] and this was not different with the findings of this study. Monoterpenoid compounds were higher than sesquiterpenoid compounds in the flower of *Tithonia diversifolia* in this study. In general, terpenes constituted the largest amount of essential oils (71.10%) and this agreed with Zule *et al.* [20] who reported similar findings. Other ingredients of essential oil components reported in this study were organic substances which include aldehydes, esters and alcohols. They are often used as raw materials in chemical, pharmaceutical, cosmetic and food producing industries [20, 21].
It can be concluded that *T. diversifolia* can be exploited for its chemical constituents which can be used in manufacturing industries since the plant adorns most temperate and tropical environments. Hence the plant offers a promising future to the dwindling global economy due to its potentials.

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