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Volatile Constituents, Antioxidant and Insecticidal Activities of Essential Oil from the Leaves of *Thaumatococcus Danielli* (Benn.) Benth. From Nigeria

Anthony B. Ojekale [1 *], Oladipupo A. Lawal [2], Adeola A. Segun[1], Folorunso O. Samuel[1], Azeez I. Ismaila [1], and Andy R. Opoku [3]

[1]Department of Biochemistry, [2]Department of Chemistry, Lagos State University, PMB 001, LASU Post Office, Ojo, Lagos, Nigeria ,[3]Department of Biochemistry and Microbiology, University of Zululand, KwaDlangezwa 3886, South Africa

Abstract: *The volatile constituents from the leaf oil of Thaumatococcus danielli (Benn.) Benth. growing in Nigeria was studied by GC and GC-MS. Twenty-one constituents were characterized representing 95.8% of the total oil. The major components of the oil were octadecamethyl cyclononasiloxane (48.3%), phytol (12.9%) and 6,10,14-trimethyl-2-pentadecanone (9.3%). The antioxidant activity of the oil (10–50 mg/mL) was evaluated by measurement of 1,1-diphenylpicryl-hydrazyl (DPPH) radical, metal chelating and nitric oxide radical methods. The essential oil showed no metal chelating activity, but, displayed significant antioxidant activity against 1,1-diphenyl-2-picrylhydrazyl (DPPH) and nitric oxide radicals with IC_{50} values of 16.32 and 24.68 mg/mL, respectively, compared to synthetic antioxidants: butylated hydroxyanisole, butylated hydroxytoluene, ascorbic acid and α-tocopherol with ($IC_{50} \leq 17.96$ mg/ mL). The insecticidal activity was assayed against Sitophilus zeamais and the result showed LC_{50} value of 345.2 mg/mL after 72 H.*

Keywords: *Thaumatococcus danielli, Marantaceae, essential oil, antioxidant, insecticidal,.*

I. INTRODUCTION

Thaumatococcus danielli (Benn.) Benth. (syn: *Phrynium daniellii*) is a multipurpose rhizomatous, perennial and monocotyledonous plant propagated by itself through the rhizomes[1]. It belongs to the family Marantaceae and native to West Africa, particularly Ghana, Cote d'Ivoire and Nigeria. It also exist in the Princes Islands, Angola, the Central African Republic, Uganda and Indonesia[2]. The leaves (*ca* 45 cm x 30 cm) are ovate-elliptic rounded, truncate at the base and shortly acuminate at the apex, and of different sizes depending on maturity of the plant[3-4]. The inflorescences are simple with spikes and bracts of about (8 to 10 cm) wide and (3 to 4 cm) long, respectively. The flowers most prolific from July until late October, and ripening from January until mid-April are purple pinkish with short spikes and long bracts at the base of the swollen petiole[1]. The fruit furnishes the protein sweetener which is widely used in beverage, confectionary and pharmaceuticals industries. In addition, the stalks in some cases are used to line utensils in which food is prepared particularly in Southwestern, Nigeria[5]. Furthermore, *T. danielli* has contributed to the rural economy of some West African natives, who have been using the various part of the plant for centuries in wrapping food materials, making thatching roots, weaving baskets, mats and as taste modifier [5].

A survey of literature on *T. danielli* shows numerous investigations and the presence of alkaloids, flavonoids, tannins, saponins, anthraquinones and cardiac glycosides[3-4]. In addition, mineral composition, biological and chemical evaluations of *T. danielli* waste, and as well as, antimicrobial, autolytic and proteolytic activities[6-9]. Previous phytochemical analyses of *T. daniellii* led to the isolation of carbohydrates, trypsin inhibitors, proteases, thaumatins I and II[10].

In Southwest Nigeria, *T. daniellii* have been reported used in traditional primary health care delivery [11-12] and also, a common feature at homes and parties, where the leaves are used in packaging and presentation of a variety of food items[13]. Although, the essential oil from the leaves of this plant was proposed as the source of flavor associated with foods wrapped in them [4]. However, to the best of our knowledge, no previous information concerning the chemical composition of essential oil of *T. daniellii* from Nigeria has been reported and no previous information on the constituents from the essential oil of *T. daniellii* is reported anywhere. In continuation of our studies, on the chemical composition and biological activity of the essential oils from aromatic and medicinal plants of Nigeria origin [14-16], the present investigation reports for the first time the

volatile constituents, antioxidant and insecticidal activities of essential oil from the leaves of *T. daniellii* growing wild in Lusada town, Ogun State, Southwestern part of Nigeria.

II. MATERIALS AND METHODS

2.1 Chemicals

Folin-Ciocalteau reagent, 2,2-Diphenyl-1-picryl-hydrazyl (DPPH), 4,4- [3-(2-pyridinyl)-1,2,4-triazine-5,6-dryl] bisbenzene sulphonic acid (ferrozine), naphthylethylenediamine dihydrochloride, sodium nitroprusside, sulphanilic acid, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), gallic acid, ascorbic acid (AA), α-tocopherol and Dimethylsulfoxide (DMSO) were obtained from Sigma-Aldrich Co., Ltd (Steinheim, Germany). All other chemicals and solvents were of analytical grade.

2.2 Plant Material

Fresh leaves materials of *T. daniellii* were collected from Lusada town, Ado-Odo/Ota Local Government Area, Ogun State, Nigeria. Identification of the plant material was done at the Department of Botany, University of Lagos, Akoka-Yaba, Lagos, by Mr. T. K Odewo. A voucher specimen (LUH 5246) was deposited in the Herbarium of the University.

2.3 Isolation of essential oils

Air-dry and crushed leaves (250 g) of *T. daniellii* were subjected to hydrodistillation using Clevenger-type apparatus for 8 h in accordance with the British Pharmacopoeia specification[17]. The distillate isolated was preserved in a sealed sample tube and stored under refrigeration until analysis.

2.4 GC analyses

GC analysis was carried out on a Hewlett Packard HP 6820 Gas Chromatograph equipped with a FID detector and HP-5MS column (60 m x 0.25 mm id), film thickness was 0.25 µm and the split ratio was 1:25. The oven temperature was programmed from 50 °C (after 2 min) to 240 °C at 5 °C/min and the final temperature was held for 10 min. Injection and detector temperatures were 200 °C and 240 °C, respectively. Hydrogen was the carrier gas. An aliquot (0.5 µL of the diluted oil) was injected into the GC. Peaks were measured by electronic integration. A homologous series of *n*-alkanes were run under the same conditions for determination of retention indices.

2.5 GC-MS analyses

GC-MS analysis of the oil was performed on a Hewlett Packard Gas Chromatography HP 6890 interfaced with a Hewlett Packard 5973 mass spectrometer system equipped with a HP-5MS capillary column (30 m x 0.25 mm id, film thickness 0.25 µm). The oven temperature was programmed from 70-240^{o}C at the rate of 5^{o}C/min. The ion source was set at 240^{o}C and electron ionization at 70eV. Helium was used as the carrier gas at a flow rate of 1mL/min. The scanning range was 35 to 425 amu. Diluted oil in *n*-hexane (1.0 µL) was injected into the GC/MS.

2.6 Identification of compounds

The components of the oil were identified base on the comparison of their retention indices and mass spectra with those standards, Wiley library mass spectra database of the GC/MS system and published data [18-20].

2.7 Antioxidant activity

2.7.1 1,1-diphenylpicryl-hydrazyl radical scavenging activity

The free radical scavenging ability of the essential oils was evaluated as described[21] and modified [22]. 0.2 mL of different concentrations (10 - 250 µg/mL) of *T. daniellii* essential oils in methanol was mixed with 2.7 mL of 1.0 x 10^{-4} M methanol solution of DPPH. The absorbance at 517 nm was measured using UV-Visible Genesys 20 spectrophotometer; after the solution had been allowed to stand in the dark for 60 min. The absorbance of the samples, the control and the blank were measured in comparison with methanol. Lower absorbance of the reaction mixture indicates higher DPPH scavenging activity. BHT, BHA, ascorbic acid and α-tocopherol were used as standards.

DPPH scavenging activity was calculated using the following formula:

$$\text{DPPH scavenging activity (\%)} = \{1- (S-SB)/(C-CB)\} \text{ X } 100\%$$

Where S, SB, C and CB were the absorbances of the sample, the blank sample (2.0 mL of methanol plus 0.2 mL of sample at different concentrations), the control (2.0 mL of DPPH solution plus 0.2 mL of methanol), and the blank control (methanol) respectively. The concentration providing 50% inhibition (IC_{50}) was calculated from the graph of percentage inhibition against oil concentrations.

2.7.2 Metal chelating activity

The Fe^{2+} chelating effect of the oil was measured[23]. To 0.5 mL of various concentrations (0-50 mg/L) of T. daniellii essential oil in methanol, 1.6mL of deionized water and 0.05 mL of $FeCl_2$ (2 mM) were added. After 30 s, the reaction was initiated by the addition of 5 Mm ferrozine (0.1 mL). Then, the mixture was shaken and left at room temperature for 10 min. Absorbance of the mixture was measured spectrophotometrically at 562 nm. Ascorbic acid and α-tocopherol were used as standard.

The inhibitory effect of *T. daniellii* essential oil was calculated as:

$$\% \text{ Inhibition} = \{(A_0 - A_1)/A_0 \times 100\}$$

Where, A_0 is the absorbance value of the fully oxidized control and A_1 is the absorbance of the oil. The inhibitory concentration providing 50% inhibition (IC_{50}) was calculated from the graph of percentage inhibition against *T. daniellii* essential oil concentrations.

2.7.3 Nitric Oxide Radical (NO·) scavenging activity

The scavenger activity of *T. daniellii* essential oil to competes with oxygen and reduces the production nitric oxide was determined using Griess Illosvoy reaction Garrat[24]. The reaction mixture (3 mL) containing 2 mL of 10 mM sodium nitroprusside, 0.5 mL of phosphate buffer saline (pH 7.4, 0.01 M) and 0.5 mL of different concentrations of essential oil were incubated at 25 °C for 150 min. Thereafter, 0.5 mL of the reaction mixture containing nitrite was pipette and mixed with 1 mL of sulphanilic acid reagent (0.33% in 20% glacial acetic acid) and allowed to stand for 5 min for completing diazotisation. Then, 1 mL of naphthylethylenediamine dihydrochloride (0.1%) was added, and allowed to stand for 30 min in diffused light. The absorbance of the pink coloured chromophore was measured at 540 nm against the corresponding blank solution. BHT, BHA, ascorbic acid and α-tocopherol were used as standards.

The inhibitory effect of *T. daniellii* essential oil was calculated by:

$$\% \text{ Inhibition} = \{(A_0 - A_1)/A_0 \times 100\}$$

Where A_0 is the absorbance of the control and A_1 is the absorbance in the presence of the essential oils. The concentration providing 50% inhibition (IC_{50}) was calculated from the graph of percentage inhibition against oil concentrations.

2.8 Insecticidal activity

The fumigant toxicity of *T. daniellii* essential oil was assayed[25]. Adult insects of mixed sex, 7-14 days old of *Sitophilus zeamais* reared on maize and at 25 ± 1 °C and 65% ± 5% relative humidity (R.H.) was used for the bioassay. Filter paper (Whatman No. 1, cut into 2-cm diameter pieces) was impregnated with *T. daniellii* oil at doses calculated to give equivalent fumigant concentrations of 5, 10, 20, 50, 100 and 250 mg/L air. The impregnated filter paper was then attached to the undersurface of the Petri dishes (90 mm) containing 10 adults each of *A. obtectus*, *S. zeamais* and *T. castaneum* to different concentrations of the oil. Each concentration and the control were replicated three times. Mortality was determined after 24, 48 and 72 h from the commencement of exposure. When no leg movement was observed, insects were considered dead. The percentage insect mortality was calculated using Abbott's formula for natural mortality in untreated controls[26]. Probit analysis was used to estimate LC_{50} value.

2.9 Statistics

The mean and standard deviation of three experiments were determined. Statistical analysis of the differences between mean values obtained for experimental groups were calculated as means ± standard deviation (SD) of three independent measurement using Microsoft excel program, 2003 and Origin 6.0 for IC_{50}. Data were subjected to one way analysis of variance (ANOVA). *P* values ≤ 0.05 were regarded as significant and *P* values ≤ 0.01 as very significant.

III. RESULTS

Table I: Volatile constituents of essential oil of *Thaumatococcus danielli*[a]

Compound	MF	Mw	RT[b]	% Area
3,7-dimethylnonane	$C_{11}H_{24}$	156.4	3.95	1.1
3,6-dimethyl undecane	$C_{13}H_{28}$	184.3	4.2	1.7
Dodecamethyl cyclohexasiloxane	$C_{12}H_{36}O_6Si_6$	444.9	4.59	0.8
2,3,5-trimethyl decane	$C_{13}H_{28}$	184.3	5.96	1.3
Tetradecamethyl cycloheptasiloxane	$C_{14}H_{42}O_7Si_7$	519	7.38	0.6
Decamethyl cycloheptasiloxane	$C_{10}H_{30}O_5Si_5$	370	9.95	1.6

6,10,14-trimethyl-2-pentadecanone	$C_{18}H_{36}O$	268.4	13.04	9.3
Methyl pentadecyl ether	$C_{16}H_{34}O$	242.4	13.22	1.1
3,7,11-trimethyl-2,6,10-dodecatrien-1-ol	$C_{15}H_{26}O$	222.3	14.3	4.5
n-Hexadecanoic acid	$C_{16}H_{32}O$	256.4	15.61	2.8
(E)-3-Eicosene	$C_{20}H_{40}$	280.5	16.1	3.1
Phytol	$C_{20}H_{40}O$	296.5	16.34	12.9
Geranylgeraniol	$C_{20}H_{34}O$	290.4	16.88	2
Octadecamethyl cyclononasiloxane	$C_{18}H_{54}O_9Si_9$	667.3	21.49	43.8
Squalene	$C_{30}H_{50}$	410.7	23.02	0.6
Tetratetracontane	$C_{44}H_{90}$	619.1	23.31	0.8
Tetradecanal	$C_{14}H_{28}O$	212.3	27.73	1.3
13-Tetradecen-1-ol acetate	$C_{16}H_{30}O_2$	254.4	28.58	1.9
Oleic acid	$C_{18}H_{34}O_2$	282.4	29.37	0.1
9-Hexadecenoic acid, octadecyl ester	$C_{34}H_{66}O_2$	506.8	29.91	2.2
2- Chloropropionic acid, hexadecyl ester	$C_{19}H_{37}ClO_2$	332.9	30.78	2.3
Total identified				95.8

[a] Compounds are listed in order of elution.

[b] RI = RI (retention index) calculated from retention times relative to that of *n*-alkanes (C_6-C_{24}) on the non-polar HP-5MS column.

Table II: Antioxidant and insecticidal activities of *T. danielli* essential oil [a]

	Antioxidant activity [a]		**Insecticidal activity (LC_{50}) @ 72 H**
	DPPH	**Nitric oxide**	
T. danielli [b,c]	26.3	19.6	345.2
BHT (IC_{50})	35.9	-	-
BHA (IC_{50})	36.1	-	-
Ascorbic acid (IC_{50})	36.8	25.2	-
α-Tocopherol (IC_{50})	41.7	37.9	-
Allethrin (LC_{50})	-	-	7.45
Permethrin (LC_{50})	-	-	11.1

[a](n= 3, X ± SEM; [b](IC_{50}) - value determined to be the inhibitory concentrations at which DPPH and nitric oxide radicals were scavenged by 50% respectively.

[c]LC_{50} - Lethal concentrations with 50 % mortality.

IV. DISCUSSION

Hydrodistillation of the leaves of *T. daniellii* gave 0.09 % yield of pale yellow oil. Analysis of the oil shows the presence of 21 constituents, accounting for 95.8% of the total oil. Table I shows the volatile components of the oil sample, where compounds are listed in order of their elution from the DB-5 column. A classification of the constituents of the oil showed the presence of a wide range of compounds, including polysiloxanes, terpenoids, aromatics, long-chain hydrocarbons, polysteroids, alcohols, aldehydes, ketones, fatty acids and their esters. The polysiloxane compounds were predominant constituents, with octadecamethyl cyclononasiloxane (48.3%) being most dominate. Other major constituents were phytol (12.9%), 6,10,14-trimethyl-2-pentadecanone (9.3%), 4-methyl-2-octadiene (4.5%), (E)-3-eicosene (3.1%) and hexadecanoic acid (2.8%). Interestingly, octadecamethyl cyclononasiloxane, which was found to be a major component in our study, has not been reported as the main constituent in any genus of the Marantaceae family. Though, it has been reported as a constituent of some plant extracts[12, 27].

The ability of *T. daniellii* essential oil to scavenge DPPH radical, metal chelating and nitric oxide radical scavenging activities was evaluated and the result is shown in Table II. The result shows that the *T. daniellii* essential oil, did not exhibit any activity for metal chelating, but, significantly and dose dependently reduced DPPH radicals. At a concentration of 50 mg/L, *T. daniellii* essential oil scavenged over 80% of DPPH radicals with IC_{50} value of 26.3 mg/L which was greater than the synthetic antioxidant, BHA (36.1mg/L), BHT (35.9mg/L), ascorbic acid (36.8 mg/L) and α-tocopherol (41.7 mg/L), respectively. The oil was also found to be very effective scavenger of nitric oxide radical and the activity increased in a concentration dependent manner (Table 11). At 50 mg/L, the oil exerted the highest nitric oxide scavenging activity of 78. 5% (IC_{50} of 19.6 mg/L), whereas ascorbic acid and α-tocopherol exhibited lower activities. From the results, it can be observed that the essential oil of *T. daniellii* is a good free radical scavenger, and it could effectively act as a primary antioxidant against free radicals and can be regarded as a good source of natural antioxidant in preventing lipid peroxidation and protection from oxidative stress caused by excess nitric oxide radical generation such as inflammation, cancer and other physical or mental disorders that are harmful to human health[28]. The insecticidal

activity of *T. daniellii* essential oil was determined against *Sitophilus zeamais.* Table II shows the result of the toxicity of *T. daniellii* essential oil and the control (permethrin and allethrin) after 72 h was found to be directly proportional to the different concentrations of the oil, with lethal concentration (LC_{50}) of 345.2 mg/100 ml, compared to the standard permethrin (LC_{50} = 7.45 and 11.13 mg/100 ml). It is apparent that *T. daniellii* essential oil has a weak toxic activity. However, the findings of this study are in agreement with other previous reports on insecticidal activities of some essential oils against storage-product beetles[29-30]. Although, the effectiveness of octadecamethyl cyclononasiloxane as a constituent of essential oil has not yet been known. However, it is perceptible that the antioxidant activity and the relatively weak toxic effect of *T. daniellii* may have contributed to its uses in food and pharmaceuticals industries, as well as in traditional medicine of some West Africa countries.

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