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Chemical composition and antioxidant activity of the hexane fraction from leaf extracts of *Odontonema strictum*

Lokadi Pierre Luhata, Moena Hirao, Natsuki Mori and Toyonobu Usuki

Abstract
Plants of the genus *Odontonema* are used in traditional medicine to treat open wounds, inflammation, hypertension, and to induce labor. The objective of this study was to determine the chemical composition of the hexane fraction from *O. strictum* aerial parts and evaluate its ability to scavenge DPPH radicals under laboratory conditions. The chemical composition of the hexane fraction was determined by GC-MS. Essential oils exhibiting >95% search similarity was identified. Analysis of GC-MS spectra revealed the presence of neophytadiene, hexadecanoic acid, hexahydrofarnesyl acetone, phytol, tetratetracontane, squalene, methyl stearate or octadecanoic acid, 2-methyloctacosane, and tritetracontane. The hexane fraction demonstrated moderate antioxidant activity (IC_{50} = 1 mg/mL). This is the first report describing the chemical composition and antioxidant activity of extracts of fatty leaves of *O. strictum*. The results support the use of this tropical plant in folk medicine to heal open wounds and as an anti-inflammatory agent.

Keywords *Odontonema strictum*, Essential oils, GC-MS analysis, Antioxidant activity

1. Introduction
Plants of the genus *Odontonema* belong to the family Acanthaceae and are mainly found in tropical regions. Interesting pharmacological activities have been reported for three species. For example, a concoction of ground leaves and stems of *O. callistachyum* is used to heal wounds in Sierra Mazateca (Mexico) [1]. Kuna, Ngöbe-Buglé, and Teribe Indians use the leaves of *O. tubiforme* (Bertol) Kuntze for their anti-inflammatory and labor-inducing activities [2]. Traditional healers in Burkina Faso (western Africa) use aqueous extracts of *O. strictum* (Figure 1) to treat hypertension [3]. Biologically active phytochemicals such as stigmasterol, β-sitosterol, verbascoside, isoverbascoside, and α, β-unsaturated δ–lactone terpenes (umuravumbolide, deacetylumuravumbolie, dideacetylboronolide, and deacetylboronolide) have been isolated from leaf extracts of *O. strictum* [4-6].

Fig 1: *Odontonema strictum*

In our previous works, we isolated and characterized secondary metabolites from ethyl acetate and dichloromethane fractions of *O. strictum* [4-6]. This is the first report describing the chemical composition of the *n*-hexane fraction of leaves determined using gas chromatography

coupled with mass spectrometry (GC-MS) analysis and characterization of its antioxidant property using 1,1-diphenyl-2-picrylhydrazyl (DPPH).

2. Materials and methods

2.1. Chemicals

n-Hexane, dichloromethane (DCM), and methanol (MeOH) were purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). DPPH was purchased from TCI (Tokyo, Japan).

2.2. Plant collection and taxonomy

Plant specimens (leaves) were collected in Kinshasa (Democratic Republic of the Congo) in June-July 2021 and dried in the shade at room temperature for 2 weeks. The collected plant materials were compared to a previous voucher specimen identified by Dr. Chuba, PLL3, and deposited in the Herbarium of the Department of Biological Sciences at the University of Zambia and later sent to the Usuki Laboratory at Sophia University.

2.3. Preparation and extraction of the crude extract

Dried samples (365 g of leaves) were extracted three times with a mixture of 1 L of MeOH and 1 L of DCM at room temperature for 72 h (3×, 2L). The MeOH-DCM solution was filtered through a cotton hood and evaporated under vacuum at <40 °C to afford 47.45 g of crude extract (13% yield).

The MeOH-DCM extract was dissolved in 300 mL of H_2O (20% MeOH) and later fractionated with hexane (3×, 200 mL), which afforded 10 g of hexane crude extract. The dried fatty fraction (6 g) was subjected to normal-phase column chromatography with hexane elution (300 mL). One fraction was collected, and the solvent was evaporated under vacuum at <40 °C to afford 850 mg of material, which was then prepared for GC-MS analysis.

2.4. GC-MS analysis

A total of 1 mg of the hexane fraction of the leaves of *O. strictum* was dissolved in 2 mL of DCM and subsequently analyzed using GC-MS. The volume of sample to be injected was 3 µL. Analysis of this fraction was performed using a Shimadzu (Kyoto, Japan) GC-MS instrument (GCMS-QP2010 SE) with DB-WAX capillary column (30 m length, 0.25 mm internal diameter, 0.25 □m thickness, Agilent, Santa Clara). The temperature program was set as follows: 45 °C for 10 min, followed by an increase to 220 °C at a rate of 5 °C/min. The injector and detector temperatures were kept stable at 220 °C and 250 °C, respectively. The total GC-MS run time was 45 min, and helium was used as the carrier gas (3.0 mL/min). The volume of sample to be injected was 3 µL of DCM solution. Phytochemicals were identified by comparing the retention times with those of known compounds recorded in the instrument similarity search.

2.5. DPPH assay

The method described by Alhage and co-workers was used, with a few modifications [7]. Samples were prepared in MeOH at concentrations of 0.001, 0.01, 0.1, 0.5, and 1 mg/mL. To 600 µL of each sample was added 600 µL of DPPH (0.16 mM in MeOH), and the samples were kept in the dark for 30 min, after which the absorbance was read at 517 nm. The DPPH radical–scavenging activity was calculated using equation (1):

$$\% \text{ Inhibition} = \frac{(A\ control - A\ sample)}{A\ control} \times 100$$

Where, A control refers to the absorbance of the control (DPPH solution without sample), and A sample refers to the absorbance of the test sample (DPPH solution with test sample) at 517 nm. Triplicate measurements were carried out. Vitamin C was used as a positive control. The detailed data are shown in Tables S1 and S2 and Figures S1 and S2.

2.6. Statistical analyses

Analyses were conducted using Excel software (2018). The significance of differences between comparisons was determined using one-way analysis of variance. Results exhibiting a 5% level of confidence were regarded as statistically significant.

3. Results & Discussion

GC-MS analysis of the hexane fraction of leaves of *O. strictum* revealed nine major compounds (based on searches indicating >95% similarity), as shown in the ion chromatogram (Figure 2) and listed in Table 1. The compounds were identified using the internal library of the instrument, which contained mass spectra of pure substances and components of known oils [8]. The following essential oils were detected: neophytadiene (a), hexadecanoic acid (b), hexahydrofarnesyl acetone (c), tetratetracontane (d), methyl stearate or octadecanoid acid (e), phytol (f), 2-methyloctacosane (g), tritetracontane (h), and squalene (i) (Figure 3). Many other peaks were observed in the GC-MS spectrum. However, these compounds exhibited similarity values of <90%.

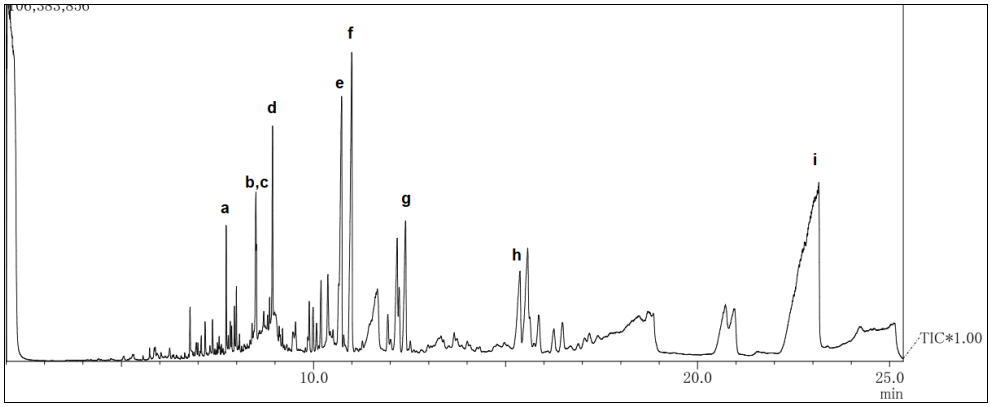

Fig 2: Total ion chromatogram of *n*-hexane extract of *O. strictum* leaves (GC-MS, 70 eV)

Table 1: List of major compounds identified in the hexane fraction by GC-MS analysis

Compound	RT (min)	PA (%)	Name of compound	MF
a	7.73	0.068	Neophytadiene	$C_{20}H_{38}$
b	8.50	0.074	Hexadecanoic acid	$C_{17}H_{34}O_2$
c	8.53	0.058	Hexahydrofarnesyl acetone	$C_{18}H_{36}O_1$
d	8.89	0.057	Tetratetracontane	$C_{44}H_{90}$
e	10.19	0.075	Octadecanoic acid	$C_{19}H_{38}O_2$
f	10.73	0.068	Phytol	$C_{22}H_{42}O_2$
g	13.12	0.057	2-methyloctacosane	$C_{29}H_{60}$
h	14.85	0.057	Tritetracontane	$C_{43}H_{88}$
i	22.78	0.069	Squalene	$C_{30}H_{50}$

RT: retention time; PA: peak area; MF: molecular formula

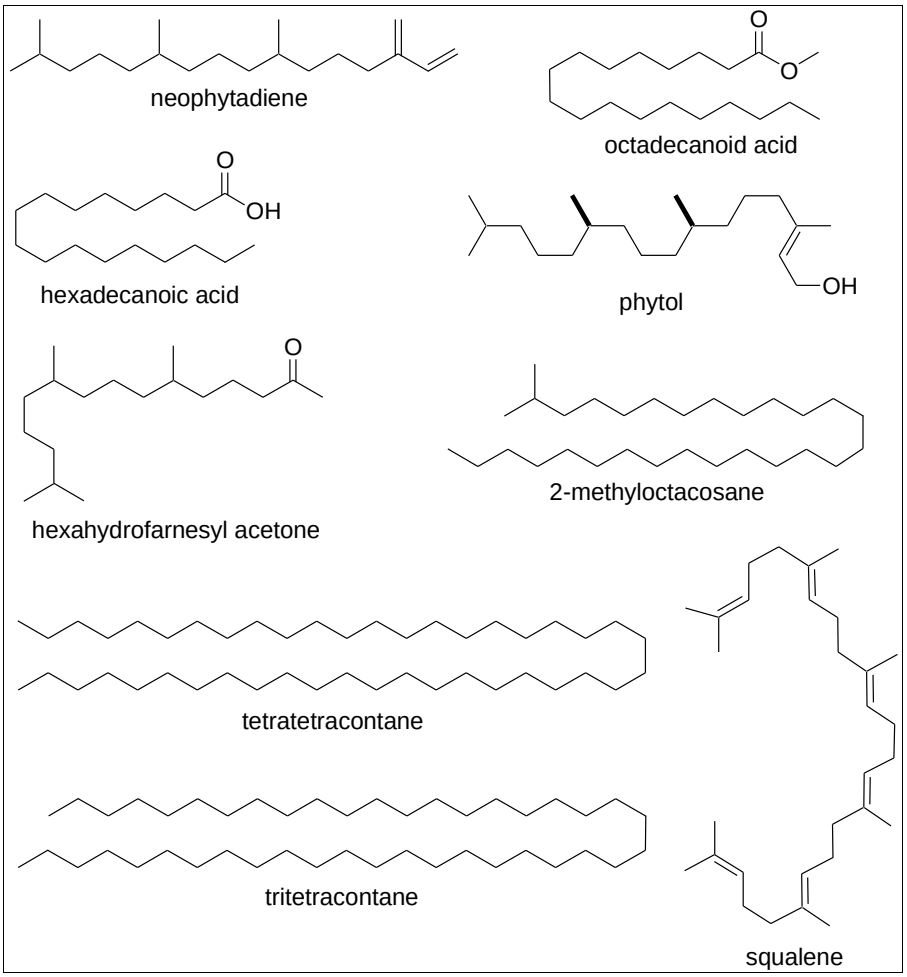

Fig 3: Chemical composition of the major volatile oils in the *n*-hexane fraction of *O. strictum* leaves

Neophytadiene (a) is a diterpene known for its anti-inflammatory and antimicrobial properties. According to Bhardwaj and co-workers, this diterpene exhibits cytotoxic activity against RAW 264.7 cells, with an IC_{50} of 50 µM [9]. *n*-Hexadecanoic acid (b) inhibits phospholipase A2 and is therefore used as an anti-inflammatory compound in folk medicine for the management of rheumatism. As shown in Table 1, after octadecanoid acid, *n*-hexadecanoic acid is one of the most abundant essential oils in the *n*-hexane fraction of *O. strictum* leaves. Thus, essential oils from *O. strictum* could potentially be used as anti-inflammatory agents [10]. Antibacterial, antinociceptive, and anti-inflammatory activities have been reported for the sesquiterpene hexahydrofarnesyl acetone (6, 10, 14-trimethyl-2-pentadecanone) (c) [11]. Potent anticancer and antimicrobial properties were reported for tetratetracontane (d) [12-13]. According to Conconi and co-workers, octadecanoid acid (e) plays an important role in the defense response to certain wavelengths of ultraviolet radiation [14]. The acyclic hydrogenated diterpene alcohol also known as phytol (f) exhibits a broad spectrum of pharmaceutical activities, including antinociceptive, anxiolytic, cytotoxic, antioxidant, anti-inflammatory, immune-modulating, autophagy- and apoptosis-inducing, and antimicrobial [15]. The essential oil tritetracontane (h) is used for its antibacterial properties [16]. The triterpene squalene (i) is the main component of skin surface polyunsaturated lipids, and it is abundant in olive oil, palm oil, wheat-germ oil, amaranth oil, and rice bran oil. This compound has some beneficial effects on the skin due to its emollient and antioxidant activities, and it also exhibits hydration and antitumor activities [17]. 2-Methyloctacosane (g) is a long alkane hydrocarbon chain. No specific pharmacological activity has been reported for this compound to date.

The present report is the first description of these active terpenes in extracts of leaves of *O. strictum*, and the above results support the use of plants of the genus *Odontonema* in traditional medicine to promote the healing of open wounds

and as an anti-inflammatory agent. The possibility of using essential oils from *O. strictum* to treat skin problems needs to be investigated further.

The ability of compounds in the *n*-hexane fraction of *O. strictum* leaves to scavenge DPPH free radicals was evaluated under laboratory conditions using ascorbic acid as a positive control. The *n*-hexane fraction exhibited moderate hydrogen-donating ability, as the concentration necessary to scavenge 50% of DPPH free radicals (IC_{50}) was 1 mg/mL. By comparison, the IC_{50} concentration of the positive control (ascorbic acid) was 0.31 µg/mL (Figure 4).

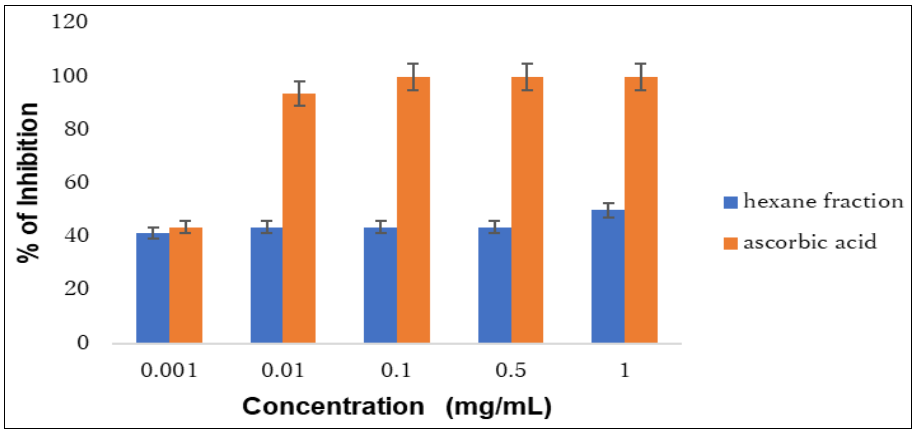

Fig 4: DPPH free radical–scavenging activity of hexane fraction of the leaves of *O. strictum.*

Zhang and co-workers investigated the antioxidant properties of essential oils isolated from the aerial parts of *Artemisia ordosica*, a plant used in China for its medicinal effects [18]. The IC_{50} values for DPPH and ABTS free radicals were approximately 5.9 mg/mL and 5.7 mg/mL, respectively. Essential oils from *Satureja hortensis* L., an aromatic plant used in Turkey as a tea or an additive in commercial spice mixtures for many foods to offer aroma and flavor, gave an IC_{50} value of 350.00 ± 5 µg/mL for scavenging DPPH free radicals [19]. The hexane fraction of *Nepeta flavida* leaf extracts exhibited moderate radical-scavenging activity (IC_{50} = 162 ± 1.73 µg/mL) against DPPH radicals [20]. The above results reported in the literature indicate that the antioxidant activities of essential oils depend essentially on their phytochemical constituents. In general, it is acceptable to acknowledge that essential oils exhibit moderate antioxidant activity against DPPH free radicals under laboratory conditions.

4. Conclusions

GC-MS analysis of the hexane fraction of extracts of *O. strictum* aerial parts revealed the presence of nine major essential oils known for their antimicrobial, anti-inflammatory, antinociceptive, emollient, and antioxidant activities. The presence of *n*-hexadecanoic acid in good quantity indicates that this fatty fraction could be used as a phytomedicine for the management of rheumatism. Other active molecules, such as squalene and phytol, are useful for curing rashes, skin problems, and other dermatologic conditions. Despite the moderate hydrogen-donating ability to scavenge DPPH free radicals, the fraction contains interesting bioactive molecules that could be useful in the cosmetic industry for promoting human health. This is the first report of the chemical composition of essential oils from leaf extracts of *O. strictum*, and the above results support the use of plants of the genus *Odontonema* in traditional medicine to promote the healing of open wounds and as anti-inflammatory agent. The possibility of using essential oils from *O. strictum* in cosmetic products needs to be investigated further.

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