Original Article

Natural Product Communications
June 2019: 1–5

DOI: 10.1177/1934578X19857022
journals.sagepub.com/home/npx

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Chemical Composition and Antimicrobial Activity of the Essential Oil From the Bark of *Xylopia hypolampra*

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Abstract

Hydrodistillation of *Xylopia hypolampra* Mildbr. stem bark afforded 39 mg (dry weight basis) of a pale yellow fragrant essential oil; gas chromatography-flame ionization detector and gas chromatography-mass spectrometry analyses allowed the identification of 28 compounds (90.5%, of the total oil composition). The major constituent was found to be verbenone (20.2%) followed by borneol (7.8%), eucalyptol (5.9%), nopinone (5.5%), *trans*-pinocarveol (4.9%), α-terpineol (4.4%), *para*-cymen-8-ol (3.5%), terpinen-4-ol (3.1%), cyperotundone (2.7%), and myrtenal co-eluted with myrtenol (6.8%). The antimicrobial activity was evaluated against *Streptococcus pyogenes*, *Staphylococcus aureus*, and *Escherichia coli* based on the minimum inhibitory concentration by the micro- and macrodilution methods.

Keywords

Xylopia hypolampra, essential oil, verbenone, antimicrobial activity

Received: January 27th, 2019; Accepted: March 29th, 2019.

Xylopia L. (Annonaceae) is a genus of pantropical distribution comprising of 130 genera and more than 2000 species. Numerous members of the Annonaceae family are odorous: the presence of essential oils, mainly containing terpene compounds, is responsible for the fragrance.[1-3] A large number of species are traditionally used as component of food and/or herbal remedies. A decoction of root of *Xylopia parviflora* is used in Tanzania by Nyamwezi people for stomach disorders, women's barrenness, and headache relief, while its bark as an analgesic and antispasmodic remedy.[4] Fruits and leaves of *Xylopia quintasii* are used for stomach and respiratory diseases[5] ; fresh fruits of *Xylopia laevigata* have been found to possess cytotoxic activity.[6] Fruits of *Xylopia aromatica* are commonly used in Venezuela as a substitute spice for *Piper nigrum* and in Nigeria as a component of herbal remedies with antidiabetic effects.[7] *Xylopia* species are traditionally employed also for their content in essential oils: *Xylopia langsdorfiana* leaves produce an essential oil with potential spasmolytic activity particularly against guinea pig ileum[8]; fruits of *X. parviflora*, used in Cameroon as flavoring ingredients, contain an essential oil with promising anticancer, anti-inflammatory, and antimicrobial activities[9]; the essential oil obtained from leaves of *X. laevigata* and *Xylopia frutescens*, plants commonly used in the Brazilian folk medicine, showed in vitro and in vivo significant anticancer[10] and anti-*trypanosoma cruzi* activities.[11] Notwithstanding all the abovementioned activities and although several studies on *Xylopia* species have been reported, to our knowledge, no studies have been carried out so far on *Xylopia hypolampra* Mildbr., a plant widely distributed in Cameroon, north republic of Congo and Gabon. Here, we report for the first time the chemical composition and the antimicrobial activity evaluation of essential oil obtained by hydrodistillation of *X. hypolampra* Mildbr. stem bark.

Hydrodistillation of *X. hypolampra* stem bark afforded 39 mg of pale yellow essential oil (XHEO) on dry vegetable material. Quantitative and qualitative analysis of components, achieved by gas chromatography-flame ionization detector (GC-FID) and gas chromatography-mass spectrometry (GC-MS), revealed the presence of 28 compounds (90.3% of the total oil) listed in Table 1 according to their elution order on the HP-5 capillary column and

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Table 1. Volatile Composition of Bark of *Xylopia hypolampra*.

#	Compounds	RIs[a]	RIs[b]	%[c]
1	*p*-Cyimene	1022	1022	0.3 ± 0.02
2	Eucalyptol	1026	1028	5.9 ± 0.50
3	Phenyl acetaldehyde	1042	1041	0.4 ± 0.02
4	*cis*-Linalool oxide (furanoic)	1072	1071	1.7 ± 0.04
5	*trans*-Linalool oxide (furanoic)	1084	1087	1.8 ± 0.09
6	Fenchol	1114	1110	1.6 ± 0.08
7	Sabina ketone	1117	1116	1.2 ± 0.06
8	Nopinone	1135	1134	5.5 ± 0.30
9	*trans*-Pinocarveol	1135	1136	4.9 ± 0.20
10	*trans*-Verbenol	1140	1142	1.2 ± 0.04
11	Camphene hydrate	1145	1144	2.0 ± 0.08
12	Pinocarvone	1160	1159	1.8 ± 0.07
13	Borneol	1166	11 665	7.8 ± 0.30
14	Terpinen-4-ol	1174	1175	3.1 ± 0.06
15	3′-Methylacetophenone	1179	1181	0.8 ± 0.10
16	*p*-Cyimen-8-ol	1186	1186	3.4 ± 0.03
17	α-terpineol	1189	1190	4.4 ± 0.10
18	Myrtenal	1195	1193	6.8 ± 1.10
19	Myrtenol	1195	1196	
20	Verbenone	1204	1208	20.2 ± 0.30
21	*trans*-Carveol	1215	1217	1.7 ± 0.03
22	2-Methyl-3-phenylpropanal	1216	1237	1.4 ± 0.01
23	Carvone	1239	1241	1.7 ± 0.01
24	*p*-Cyimen-7-ol	1289	1288	1.7 ± 0.02
25	Perillyl alcohol	1295	1296	1.1 ± 0.04
26	δ-Elemene	1337	1337	2.6 ± 0.05
27	Oxo-α-ylangene	1675	1678	2.0 ± 0.30
28	Cyperotundone	1695	1694	2.7 ± 0.30
	Oxygenated terpenes	73.9		
	Sesquiterpenes	7.3		
	Terpenes	5.2		
	Ketones	2.0		
	Aldehydes	1.9		
	Total	90,3		

RIs, retention indices.
[a]Retention indices relative to C8-C22 *n*-alkanes on a HP-5MS column.
[b]Retention indices from the literature data (Adams, 2007).
[c]Contents are based on the area percentages of the obtained compounds by gas chromatography-flame ionization detector and are means of 3 determinations ± standard deviation.

reported as percentage of the total oil composition. Oxygenate terpenes were found to be the main bulk of constituents (73.9%); verbenone (**20**) was found to be the most abundant compound (20.2%) followed by borneol (**13**) (7.8%), myrtenal, and myrtenol (**18, 19**) (6.80%), efficiently separated and identified in GC-MS but co-eluted in GC-FID. Other compounds belonging to this class, but present in a lower percentage, were found to be eucalyptol (**2**) (5.9%), nopinone (**8**) (5.5%), *trans*-pinocarveol (**9**) (4.9%), α-terpineol (**17**) (4.4%), *p*-cymen-8-ol (**16**) (3.4%), and terpinen-4-ol (**14**) (3.1%). Verbenone, an oxidation product of *trans*-verbenol, is one of the most ubiquitous oxygenated monoterpenes in Angiosperms. It was detected in the stem bark essential oil of other Xylopia species like *X. frutescens* (2.7%) and *X. aromatica* (5.9%), but in lower percentage compared to XHEO.[12-14] The

presence of this compound is strongly correlated to the allelochemical-like action and is a beetle-produced anti-aggregation pheromone found in Pinacee species, effective in limiting damage produced by bark beetles.[15,16] Also, the presence of a mixture of oxygenated monoterpenes like fencol, *trans*-verbenol, verbenone, myrtenal, and myrtenol reduces bark beetle *Dendroctonus rhizophagus* attraction.[17] As reported for other Cameroonian plants, like *Huga gabonii,* eucalyptol/*trans*-pinocarveol and α-terpineol/terpinen-4-ol impart fresh-camphoraceous and lilac-like notes to the essential oil, respectively.[18]

Other major constituents of the volatile fractions were found to be sesquiterpenes, accounting for 7.3% of the total oil composition; cyperotundone (**28**) (2.7%) is the most abundant compound, followed by δ-elemene (**26**) (2.6%) and oxo-α-ylangene (**27**) (2.0%), respectively.

Cyperotundone, a patchoulane-type sesquiterpene present in different plants, including *Cyperus* species, was found to act as allelochemicals on the surrounding plants, inhibiting the growth of shoots and roots.[19-21] δ-Elemene and other sesquiterpenes-related compounds are known to exert inhibitory action on insect oviposition.[22] Terpenes are present at the concentration of 5.2%. The most abundant compound of this class was found to be camphene hydrate (**11**) (2.0%) followed by fenchol (**6**) (1.6%) and perillyl alcohol (**25**) (1.1%).

The antimicrobial activity was evaluated based on the minimum inhibitory concentration (MIC) by micro- and macrodilution methods. *Xylopia hypolampra* stem bark afforded 39 mg of pale yellow essential oil was tested against the available bacteria and the results are reported in Table 2. Although essential oils are well known to possess antimicrobial activity,[23-25] a weak inhibition against the selected microorganism is shown.

To the best of our knowledge, this is the first study providing qualitative-quantitative data on volatile composition of *X. hypolampra* stem bark essential oil. Oxygenate terpenes and sesquiterpenes represent the major constituents being crucial for *X. hypolampra* defense mechanisms, acting as semiochemicals and allelopathic agents. Further investigations are needed to better understand the involvement of these compounds in the defense vs predator and pathogens.

Table 2. Antimicrobial Activity of the Essential Oil of *Xylopia hypolampra*.

Microorganism	MIC (μg/mL)[a]	MIC (μg/mL)[b]
Staphylococcus aureus ATCC 6538	>500	0.5
Streptococcus pyogenes ATCC 10708	>500	0.02
Escherichia coli ATCC 10536	>500	5

MIC, minimum inhibitory concentration.
[a]Minimum inhibitory concentration of the stem bark essential oil of *Xylopia hypolampra.*
[b]Minimum inhibitory concentration of ampicillin used as positive control.

Experimental

Isolation of Essential Oil

Triplicate samples (100 g) of stem bark powder were subjected to hydrodistillation for 3 hours using a Clevenger-type apparatus, followed by exhaustive extraction (3 × 50 mL) of the distillate with dichloromethane. The organic layer was dried over anhydrous sodium sulfate and concentrated firstly under *vacuum* by rotary evaporator and then by gentle stream of nitrogen for successive GC-FID and GC-MS analyses.

Gas Chromatography-Flame Ionization Detector Analyses

Gas chromatography-flame ionization detector analyses were performed on a gas chromatograph HP 5890A Series II (Agilent Technologies, CA, United States) equipped with an autosampler, a flame ionization detector (FID), and a HP-5MS capillary column (30 m, 0.25 mm I.D., 0.25 mm film thickness, Hewlett Packard). Helium (He) was used as carrier gas at flow rate of 1.2 mL/min. A temperature program was set as follows: isotherm at 40°C for 5 minutes, ramp from 40°C to 260°C at 4 °C/min, isotherm at 260°C for 10 minutes. A sample volume of 1 μL was injected. The injector operated at 250°C in the split mode (split ratio 27:1) with pressure of 22.5 psi. The detector temperature was set at 260°C with He as make-up gas at the flow rate of about 35 mL/min. Peak identification was assessed by comparison of the retention times with those obtained analyzing the same sample in GC-MS and the relative amount (Area %) of each component was calculated on the basis of the corresponding FID peak area without response factor correction.

Gas Chromatography-Mass Spectrometry Analysis

The analyses were carried out using a GC Model 6890N, coupled to a bench top MS Agilent 5973 Network, equipped with the same capillary column and following the same chromatographic conditions used for the GC-FID analyses. The carrier gas was He at constant flow of 1.0 mL/min. The essential oils were diluted prior to analysis (1 mg/10 mL in *n*-hexane), and 1.0 μL of the diluted solution was manually injected into the GC system with a split ratio of 30:1. The ion source temperature was set at 200°C, while the transfer line was at 300°C. The acquisition range was 40–500 amu in

electron-impact positive ionization mode using an ionization voltage of 70 eV.

Compounds Identification

The components were identified by comparing their mass spectra with NIST 98 and Wiley 5 MS Libraries as well as by comparing their retention indices, relative to a C_8-C_{22} homologous series of *n*-alkanes and calculated according to Van Den Dool.[26,27]

Antibacterial Activities

The essential oil was evaluated for antibacterial activity against the following strains: *Staphylococcus aureus* ATCC 6538, *Streptococcus pyogenes* ATCC 25175, and *Escherichia coli* ATCC 10536. Bacteria were cultured in Tryptone Soya Broth (TSB, Oxoid, Basingstoke, United Kingdom) at 37°C.

Evaluation of MIC

The antibacterial activity of volatile fractions was evaluated by 2-fold serial broth dilution method in Iso-Sensitest broth (ISB, Oxoid, Basingstoke, United Kingdom) according to Clinical and Laboratory Standards Institute procedures.[28,29] All the extracts were dissolved in 10% dimethyl sulfoxide aqueous solution. The MIC was the lowest concentration of extracts and of volatile fractions inhibiting observable microbial growth against the reference strains. The starting inoculum was 1.0×10^7 CFU/mL. Solvent blanks were included. All experiments were performed in triplicate. Stock standard solution of ampicillin was used as a positive control.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed no financial support for the research, authorship, and/or publication of this article.

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