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

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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 diseases5; fresh fruits of Xylopia laevigata have been found to possess cytotoxic activity.6 Fruits of Xylopia aromaticum 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 langsdorffiana leaves produce an essential oil with potential spasmyloytic activity particularly against guinea pig ileum8; fruits of X. parviflora, used in Cameroon as flavoring ingredients, contain an essential oil with promising anticancer, anti-inflammatory, and antimicrobial activities9; 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 anticancer10 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...
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. \textsuperscript{12-14} The

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

| #  | Compounds                          | RIs\textsuperscript{a} | RIs\textsuperscript{b} | %\textsuperscript{c} |
|----|-----------------------------------|------------------------|------------------------|----------------------|
| 1  | p-Cymene                          | 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 (furanico)     | 1072                   | 1071                   | 1.7 ± 0.04           |
| 5  | trans-Linalool oxide (furanico)   | 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                   | 1165                   | 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-Cymen-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-Cymen-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.

\textsuperscript{a}Retention indices relative to C8-C22 n-alkanes on a HP-5MS column.

\textsuperscript{b}Retention indices from the literature data (Adams, 2007).

\textsuperscript{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.
presence of this compound is strongly correlated to the allelochemical-like action and is a beetle-produced anti-aggregation pheromone found in Pinaceae species, effective in limiting damage produced by bark beetles. Also, the presence of a mixture of oxygenated monoterpenes like fenchol, trans-verbenol, verbenone, myrtenal, and myrtenol reduces bark beetle *Dendroctonus rhizophagus* attraction. As reported for other Cameroonian plants, like *Huga gabonii*, eucalyptol/trans-pinocarveol and α-terpinene/terpinen-4-ol impart fresh-camphoraceous and liliaceous-like notes to the essential oil, respectively. 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 o xo-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. δ-Elemene and other sesquiterpenes-related compounds are known to exert inhibitory action on insect oviposition. 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, a weak inhibition against the selected microorganisms is shown.

| 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            |

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

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.

**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...
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<sub>12</sub>-C<sub>22</sub> homologous series of n-alkanes and calculated according to Van Den Dool.\textsuperscript{26,27}

Antibacterial Activities

The essential oil was evaluated for antibacterial activity against the following strains: \textit{Staphylococcus aureus} ATCC 6538, \textit{Streptococcus pyogenes} ATCC 25175, and \textit{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.\textsuperscript{28,29} All the extracts were dissolved in 10% dimethyl sulfoxide aqueous solution. The MIC was the lowest concentration of all the extracts were dissolved in 10% dimethyl sulfoxide (DMSO) and calculated according to Van Den Dool.\textsuperscript{26,27}

Declaration of Conflicting Interests

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References

1. Fournier G, Leboeuf M, Cavé A. Annonaceae essential oils: a review. \textit{J Essent Oil Res.} 1999;11(2):131-142.
2. Yapi TA, Ouattara ZA, Boti J-B, et al. Composition and chemical variability of Ivorian \textit{Xylopia rubescens} trunk bark oil. \textit{Nat Prod Commun.} 2018;13(6):761-762.
3. Yapi TA, Boti JB, Ahibo AC, et al. Composition and chemical variability of Ivoirian \textit{Xylopia staudtii} leaf oil. \textit{Nat Prod Commun.} 2015;10(6):1059-1062.
4. Nishiyama Y, Moriyasu M, Ichimaru M, et al. Antinociceptive effects of the extracts of \textit{Xylopia parviflora} bark and its alkaloidal components in experimental animals. \textit{J Nat Med.} 2010;64(1):9-15.
5. Bele MY, Foch DA, Egbe EA, Chuyong BG. Ethnobotanical survey of the uses of Annonaceae around Mount Cameroon. \textit{Afr J Plant Sci.} 2011;5(4):237-247.
6. Costa EV, Da Silva TB, Costa Cinara Oliveira D’Souza, Soares MBP, Bezerra DP. Chemical composition of the essential oil from the fresh fruits of \textit{Xylopia laeavigata} and its cytotoxic evaluation. \textit{Nat Prod Commun.} 2016;11(3):417-418.
7. Gomet SA, Ogugu VN, Odo CE, Joshua PE. Effects of some anti-diabetic plants on the hepatic marker enzymes of diabetic rats. \textit{Afr J Biotechnol.} 2014;13(7):905-909.
8. Correia ACdeC, Ferreira TF, Martins IRR, et al. Essential oil from the leaves of \textit{Xylopia langsdorfiana} (Annonaceae) as a possible spasmyloytic agent. \textit{Nat Prod Res.} 2015;29(10):980-984.
9. Woguem V, Fogang HPD, Maggi F, et al. Volatile oil from striped African pepper (\textit{Xylopia parviflora}, Annonaceae) possesses notable chemopreventive, anti-inflammatory and antimicrobial potential. \textit{Food Chem.} 2014;149:183-189.
10. Quintans JdeSS, Soares BM, Ferraz RPC, et al. Chemical constituents and anticancer effects of the essential oil from leaves of \textit{Xylopia laeavigata}. \textit{Planta Med.} 2013;79(2):123-130.
11. da Silva TB, Menezes LRA, Sampaio MFC, et al. Chemical composition and anti-\textit{Trypanosoma cruzi} activity of essential oils obtained from leaves of \textit{Xylopia frutescens} and \textit{X. laeavigata} (Annonaceae). \textit{Nat Prod Commun.} 2013;8(3):403-406.
12. Fournier G, Hadijakhoondi A, Leboeuf M, Cavé A, Charles B, Fourniat J. Volatile constituents of \textit{Xylopia frutescens}, \textit{X. pynaertii} and \textit{X. sericea}: chemical and biological study. \textit{Phytother Res.} 1994;8(3):166-169.
13. Birgersson G, Leufvén A. The influence of host tree response to \textit{Ips typographus} and fungal attack on production of semi-chemicals. \textit{Insect Biochem.} 1988;18(8):761-770.
14. Fournier G, Hadijakhoondi A, Charles B, Fourniat J, Leboeuf M, Cavé A. Chemical and biological studies of \textit{Xylopia aromatic}a stem bark and leaf oils. \textit{Planta Med.} 1994;60(3):283-284.
15. Kainulainen P, Holopainen JK. Concentrations of secondary compounds in Scots pine needles at different stages of decomposition. \textit{Soil Biol Biochem.} 2002;34(1):37-42.
16. Gillette NE, Stein JD, Owen DR, et al. Verbenone-releasing flakes protect individual \textit{Pinus contorta} trees from attack by \textit{Dendroctonus ponderosae} and \textit{Dendroctonus valens} (Coleoptera: curculionidae, Scolytinae). \textit{Agric For Entomol.} 2006;8(3):243-251.
17. Cano-Ramírez C, Armendáriz-Toledano F, Macías-Sámano JE, Sullivan BT, Zühiga G. Electrophysiological and behavioral responses of the bark beetle \textit{Dendroctonus rhizophagus} to volatiles from host pines and conspecifics. \textit{J Chem Ecol.} 2012;38(5):512-524.
18. Jirovetz L, Buchbauer G, Ngassoum M, Geissler M. Analysis of the headspace aroma compounds of the seeds of the Cameroonian "garlic plant" \textit{Hua gabonii} using SPME/GC/FID, SPME/GC/MS and olfactometry. \textit{Eur Food Res Technol.} 2002;214(3):212-215.
19. Morimoto M, Komai K. Plant growth inhibitors: Patchoulan- type sesquiterpenes from \textit{Cyperus rotundus} L. \textit{Weed Biol Manag.} 2005;5(4):203-209.
20. Jilani G, Mahmood S, Chaudhry AN, Hassan I, Akram M. Allelochemicals: sources, toxicity and microbial transformation in soil—a review. *Ann Microbiol*. 2008;58(3):351-357.

21. El Gendy AE-NG, Abd El-Gawad AM, Taher RF, El-Khrisy EE-DAM, Omer EA, Elshamy AI. Essential oils constituents of aerial parts of *Cyperus capitatus* L. and *Cyperus difformis* L. grown wild in Egypt. *J Essent Oil Bearing Plant*. 2017;20(6):1659-1665.

22. Anastasaki E, Drizou F, Milonas PG. Electrophysiological and oviposition responses of *Tuta absoluta* females to herbivore-induced volatiles in tomato plants. *J Chem Ecol*. 2018;44(3):288-298.

23. Edris AE. Pharmaceutical and therapeutic potentials of essential oils and their individual volatile constituents: a review. *Phytother Res*. 2007;21(4):308-323.

24. Bakkali F, Averbeck S, Averbeck D, Idaomar M. Biological effects of essential oils—a review. *Food Chem Toxicol*. 2008;46(2):446-475.

25. Properzi A, Angelini P, Bertuzzi G, Venanzoni R. Some biological activities of essential oils. *Med Aromatic Plant*. 2013;2(5):136-140.

26. Adams RP, Sparkman OD. Review of identification of essential oil components by gas chromatography/mass spectrometry. *J Am Soc Mass Spectrom*. 2007;18(4):803-806.

27. van Den Dool H, Dec. Kratz P. A generalization of the retention index system including linear temperature programmed gas—liquid partition chromatography. *J Chromatogr A*. 1963;11(C):463-471.

28. Barry AL, Craig WA, Nadler H, Reller LB, Sanders CC, Swenson JM. *Methods for Determining Bactericidal Activity of Antimicrobial Agents: Approved Guideline. NCCLS document M26-A*. Pennsylvania, USA: NCCLS; 1999:Volume 19: 18.

29. Jorgensen HJ. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard. In: *National Committee for Clinical Laboratory Standards Antimicrobial Susceptibility Testing, NCCLS-M7*. Pennsylvania, USA: NCCLS; 1993.