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Characterization of volatile compounds and polyphenols in leaves and stem barks of *Croton sylvaticus Hochst* (*Euphorbiaceae*) from Democratic Republic of the Congo

[Caracterisation des composes volatils et des polyphenols dans les feuilles et écorce des tiges de *Croton sylvaticus Hochst* (*Euphorbiaceae*) provenant de la République Démocratique du Congo]

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Résumé

L'objectif de cette étude était d'identifier les composés phytochimiques volatils et de déterminer quantitativement la teneur des polyphénols et flavonoïdes totaux de feuilles et écorces de tronc de Croton sylvaticus Hochst récoltées en République Démocratique du Congo. L'analyse par chromatographie en phase gazeuse couplée à la spectrométrie de masse (GC-MS) des extraits a permis d'identifier 35 composés volatils dans les feuilles et 18 dans les écorces de tronc. Les principaux composés des feuilles incluent limonène (11,93%), guaiadiène (8,37%), α- Copaène (7,92 %), valencène (7,57 %), germacrène (6,42 %), caryophyllène (6,06 %) et humulène (3,03 %), tandis que les écorces de tronc contiennent principalement α-pinène (9,05 %), guaïadiène (4,17 %), β-pinène (3,21 %) et cyperène. (3,06%). Les composés identifiés sont en majorité des monoterpènes et sesquiterpènes. Les teneurs en polyphénols et en flavonoïdes ont été déterminées respectivement par les méthodes de Folin Ciocalteu et de trichlorure d'aluminium. Les extraits dichlorométhanique, méthanolique et aqueux présentent des teneurs moyennes en polyphénols de 57.98 ± 0.15 mg d'équivalent d'acide gallique par gramme de matière sèche et en flavonoïdes de 32.7 ± 0.2 mg d'équivalent de quercétine par gramme. Ces résultats démontrent le potentiel chimique C. sylvaticus comme source des métabolites secondaires bioactifs.

Mots-clés : Croton sylvaticus, Composés volatils, Analyse phytochimique, GC-MS.

Abstract

The aim of this study was to identify the volatile phytochemical compounds and to quantitatively determine the total polyphenol and flavonoid content in the leaves and stem barks of Croton sylvaticus Hochst collected in the Democratic Republic of Congo. Gas chromatography coupled with mass spectrometry (GC-MS) analysis of the extracts led to the identification of 35 volatile compounds in the leaves and 18 in the stem barks. The main compounds found in the leaves included limonene (11.93%), guaiadiene (8.37%), α-copaene (7.92%), valencene (7.57%), germacrene (6.42%), caryophyllene (6.06%), and humulene (3.03%), whereas the stem barks primarily contained α-pinene (9.05%), guaiadiene (4.17%), β-pinene (3.21%), and cyperene (3.06%). The identified compounds were mainly mono- and sesquiterpenes. The polyphenol and flavonoid contents were determined using the Folin-Ciocalteu and aluminum chloride methods, respectively. Dichloromethane, methanolic, and aqueous extracts showed average polyphenol contents of 57.98 ± 0.15 mg gallic acid equivalent per gram of dry matter and flavonoid contents of 32.7 ± 0.2 mg quercetin equivalent per gram. These results demonstrated the chemical potential of C. sylvaticus as a source of bioactive secondary metabolites.

Keywords: Croton sylvaticus, Volatile compounds, phytochemical analysis, GC-MS.

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1. Introduction

Croton sylvaticus (Euphorbiaceae) is a semi-deciduous shrub or tree that can reach 20 m in height, with a trunk of 10-50 (sometimes up to 125 cm in diameter), without shoulders. The aromatic bark has a black pepper odor is smooth and gray in color. The fruits are orange and pointed. The flowers have a pedicel 4-8 mm long, sepals 2-3 mm long and 1-1.5 mm wide. The leaves have linear stipules, 2-8 mm long, deciduous, petiole 1-8 (13) cm long (Léonard, 1962; Latham & Konda, 2007). The species grows mainly in secondary forests in Angola, Mozambique, Cameroon, Central African Republic, Republic of Congo, Ethiopia, Gabon, Guinea, Ivory Coast, Kenya, Liberia, Uganda, Malawi, Nigeria, South Africa, Sudan, Swaziland, Tanzania, Zimbabwe, Zambia, and the Democratic Republic of the Congo (DRC) (Kongo-Central, Kasai, Katanga, Tshopo) (Van der Veken, 1960; Léonard, 1962).

For centuries, different species of the genus *Croton* have been used in traditional medicine in Africa, South Asia, and South America to treat various diseases, including malaria, fever, dysentery, diabetes, cancer, digestive disorders, wounds, inflammation, pain, and ulcers (Xu & Liu, 2018; Pimentel et al., 2020; Moremi and 2021). These traditional uses have generated great interest in exploration and characterization of their phytochemical composition and pharmacological properties.

Phytochemical studies of C. sylvaticus have indicated the presence of alkaloids, anthraquinones, essential oils, flavonoids, lignans, phenolic acids, sterols, tannins and terpenoids (Kapingu et al., 2006; 2012, Maroyi, 2017). Mwangi et al. (1998) identified β-caryophyllene oxide (35.1%) and α-humulene-1,2-epoxide (12%) as the main constituents of essential oils obtained by hydrodistillation of leaves. Hardwickiic acid, β-sitosterol, and stigmasterol have been isolated from the petroleum ether extract of the stem bark (Mwangi et al., 1998). The 1:1 methanol-dichloromethane extract of the root bark allowed the isolation of a diterpenoid, 15-formate-ent-3,13E-clerodadiene (Ndunda et al., 2015). Julocrotine, 2-[N-(2-methylbutanoyl)]-N-phenylethylglutarimide, lupeol, lup-20(29)-en-3β-ol, and penduliflaworosine have also been isolated from the leaves (Kapingu et al., 2006).

Several of biological activities have been reported, antibacterial (Selowa et al., 2010), antifungal (Araújo et al., 2020; De Almeida et al., 2013), anti-inflammatory, antioxidant (Da Costa et al., 2022; Luu-Dam et al., 2023), neuroprotective (Stafford et al., 2005; Aderogba et al., 2013), larvicidal (Kihampa et al., 2009), and mutagenic (Aderogba et al., 2013) activities. As part of our efforts to better document the phytochemistry of Croton species from DRC, this investigation aimed to identify volatile organic compounds present in the leaves and stem barks of C. sylvaticus and to quantify the total polyphenol and flavonoid contents in dichloromethane, methanolic, and aqueous extracts. This study constitutes a step in further research on the C. sylvaticus species present in the DRC.

2. Material and methods

2.1. Preparation of the vegetal material

The leaves and trunk barks of *C. sylvaticus* were collected in the Luki reserve in the Kongo Central province in DRC. The specimen was identified (voucher number P. Compere 1647 and R. Devred 392) at the Herbarium of the National Institute of Agronomic Studies and Research (INERA) of the Faculty of Science and Technology, University of Kinshasa. The samples were dried in the dark in the Laboratory of Organic Analysis and Synthesis, University of Kinshasa (LASORG-K) for 1 month. They were pulverized using a Blinder B-592 electric grinder, then sieved to obtain a fine powder. The powders were used for the various analyses.

2.2. GC-MS analysis

2.2.1. Sample preparation

Volatile organic compounds (VOCs) from plant material were collected by headspace sampling according to the method of Tamiru et al. (2011). A quantity of 10 g of each plant material was placed in airtight glass jars equipped with an air inlet and outlet. Purified air, filtered through activated carbon, was pumped through the inlet at a flow rate of 600 mL/min. The adsorbent, Porapak Q (0.05 g, 60/80 mesh; Supelco), was placed at the outlet, where air was drawn in at a flow rate of 300 mL/min. A slow flow rate at the outlet allowed sufficient contact time and pressure for the Porapak Q to effectively adsorb the emitted VOCs. After 24 hours of enrichment, volatile

compounds were eluted from Porapak Q using 0.5 mLof dichloromethane, into 2 ml sample vials, and then stored in a freezer at −20 °C until chemical analysis.

2.2.2. Analysis of volatile compounds

Aliquots of 2 µL of the extracts were analyzed using an Agilent 7890A instrument (Agilent Technologies, Palo Alto, USA) coupled to an MSD 5975C mass spectrometer (electron ionization mode). The instrument was equipped with a nonpolar HP5-MSI capillary column (30 m × 0.25 mm × 0.25 µm) (J & W Scientific, Folsom, USA). The mass spectrometer included a monolithic hyperbolic quadrupole filter with a mass range of 1.6–1050 atomic mass units (amu), an ion source set between 150 and 350 °C, and a triple-axis detector with a long-life electron multiplier. Compound identification was performed using HP Chemstation software by comparing spectra with three libraries: Adams2.L, Chememol.L, and NIST05a.L.

2.3. Determination of polyphenols

2.3.1. Extraction

Leaves and stem barks powders (300 g) were separately and each macerated in 3 L of dichloromethane (DCM) for 48 h at room temperature. The mixture obtained was then filtered through Whatman No. 1 filter paper and the solvent evaporated under reduced pressure at 40°C using a Büchi RE 120 rotary evaporator.

The residual mark from the first extraction was macerated in 2 L of methanol (MeOH) for 48 h. After filtration, the solvent was evaporated under reduced pressure. The residual mark obtained from methanol extraction was then used to produce the aqueous (AQ) extract under similar conditions.

2.3.2. Determination of total polyphenols

The estimation of the total phenol content of the extracts is carried out by spectrophotometer dosage, according to the modified Folin-Ciocalteu method (Timoléon, 2020).

Each extract (0.2 mL) was mixed with 1.5 mL of Folin-Ciocalteu reagent (10%). The solution was incubated for 5 minutes. Then, 1.5 mL of sodium carbonate solution (6%) was added. The entire mixture was finally incubated at room temperature in the dark for 5 minutes, and the absorbance was measured at 760 nm using a visible spectrophotometer (Zuzi manual scanning).

The total polyphenol content was expressed in milligrams of gallic acid equivalents per gram of dry extract (mg GAE/g). Standard solutions of gallic acid (5-50 mg/mL) were used to plot the calibration curve. The standard equation of the curve was $Y = 0.0041x + 0.0696$ ($R^2 = 0.998$).

2.3.2. Determination of total flavonoids

The estimation of the total flavonoid content of the extracts was carried out by spectrophotometer determination, according to the modified aluminum trichloride ($AlCl_3$) method (Timoléon, 2020).

Each extract (100 µL) was mixed with 4 mL distilled water and then with 0.3 mL 5% ($NaNO_2$). After 5 min, 0.02 mL of a 10% of aluminum chloride ($AlCl_3$) solution was added. 2 mL of 1M Na_2CO_3 solution were added to the mixture and the whole mixture diluted in 10 mL of double-distilled water after 5 minutes of standing. The mixture was then stirred using a vortex mixer (Heidolph No. 54119). Finally, absorbance was measured at 510 nm against the blank using a visible spectrophotometer (Zuzi manual scanning). Total flavonoid content in mg was expressed as Quecertin equivalents (QE) per gram of dry powder. Use standard solutions (Quecertin: 5-50 mg/ mL) to plot the calibration curve. The standard equation of the curve is $Y = 0.0204x + 0.0193$; ($R^2 = 0.9998$).

3. Résultats

3.1. Results

3.1.1. Extraction yield

The extraction yield results are presented in the table I.

Table 1. Percentage extraction of Croton sylvaticus extracts

Extracts	Leaves (%)	Stem barks (%)
EDCM	7.3	1.73
EME	6.7	3.43
EAQ	9.0	2.76

Legend:

EDCM : Dichloromethan extract;

EME: Methanol extract;

EAQ : Aqueous extract

3.1.2. Volatile organic compounds

The identified volatile organic compounds are listed in tables II (leaves) and III (stem barks). Figure 1 showed the chemical structures of some of the identified compounds and their reported biological activities. These were the major compounds identified in these organs (≥ 3%).

Table II. Volatile compounds identified in the leaves of C. sylvaticus

N°	RT	Compounds	N° CAS	Molecular formula	MW	Surface pic
1	9.0774	α-Phellandrene	99-83-2	$C_{10}H_{16}$	136.23	0.26
2	9.1827	α-Pinene	80-56-8	$C_{10}H_{16}$	136.23	1.56
3	9.5044	Camphene	79-92-5	$C_{10}H_{16}$	136.23	0.31
4	10.1128	β-Pinene	127-91-3	$C_{10}H_{16}$	136.23	3.19
5	10.4989	Myrcene	123-35-3	$C_{10}H_{16}$	136.23	0.34
6	10.8207	δ-3-Carene	13466-78-9	$C_{10}H_{16}$	136.23	0.13
7	10.9201	1,4-Cineole	470-67-7	$C_{10}H_{18}O$	154.25	0.23
8	11.1951	Limonene	138-86-3	$C_{10}H_{16}$	136.24	11.93
9	11.8269	γ-Terpinene	99-85-4	$C_{10}H_{16}$	136.23	0.83
10	12.0667	*Cis*-Linalool oxide (furanoid)	5989-33-3	$C_{10}H_{18}O_2$	170.25	0.16
11	12.3475	*Iso*-Sylvestrene	499-03-6	$C_{10}H_{16}$	136.24	0.26
12	12.5581	Tricyclene	508-32-7	$C_{10}H_{16}$	136.23	2.89
13	13.2309	Z-Tagetone	3588-18-9	$C_{10}H_{16}O$	152.23	0.16
14	13.6228	Isoborneol	124-76-5	$C_{10}H_{18}O$	154.25	0.26
15	14.1727	*Trans*-Isolimonene	5113-87-1	$C_{10}H_{16}$	136.23	0.54
16	16.1207	δ-Elemene	20307-84-0	$C_{15}H_{24}$	204.35	0.32
17	16.2845	α-Cubebene	17699-14-8	$C_{15}H_{24}$	204.35	0.47
18	16.5419	α-Amorphene	20085-19-2	$C_{15}H_{24}$	204.35	0.52
19	16.6648	α-Copaene	3856-25-5	$C_{15}H_{24}$	204.35	7.92
20	16.7876	β-Bourbonene	5208-59-3	$C_{15}H_{24}$	204.35	0.90
21	16.8637	Valencene	4630-07-3	$C_{15}H_{24}$	204.35	7.56
22	17.0041	Cyperene	2387-78-2	$C_{15}H_{24}$	204.35	0.40
23	17.1094	α-Gurjunene	489-40-7	$C_{15}H_{24}$	204.35	0.25
24	17.2615	*E*-Caryophyllene	87-44-5	$C_{15}H_{24}$	204.35	6.06
25	17.3726	β-Copaene	18252-44-3	$C_{15}H_{24}$	204.35	0.80
26	17.5423	6,9-Guaiadiene	37839-64-8	$C_{15}H_{24}$	204.35	8.37
27	17.7002	α - Humulene	6753-98-6	$C_{15}H_{24}$	204.35	3.03
28	17.7997	γ - Cadinene	39029-41-9	$C_{15}H_{24}$	204.35	2.32
29	17.9693	γ-Muurolene	30021-74-0	$C_{15}H_{24}$	204.35	0.89
30	18.0512	Germacrene D	23986-74-5	$C_{15}H_{24}$	204.35	6.42
31	18.2326	α-Selinene	473-13-2	$C_{15}H_{24}$	204.35	2.37
32	18.3554	β - Element	515-13-9	$C_{15}H_{24}$	204.35	1.35
33	18.5660	δ - Cadinene	483-76-1	$C_{15}H_{24}$	204.35	2.70
34	19.0165	Germacrene B	15423-57-1	$C_{15}H_{24}$	204.35	0.49
35	19.3558	*Hello* - Aromadendrene epoxide	855760-81-2	$C_{15}H_{24}O$	220.35	1.68

Table III. Volatile compounds identified in the stem barks of C. sylvaticus

N°	RT	Compounds	N° CAS	Molecular formula	MW	Peak surface
1	9.0771	δ -3-Carene	13466-78-9	$C_{10}H_{16}$	136.234	0.30
2	9.1883,	α-Pinene	80-56-8	$C_{10}H_{16}$	136.234	9.05
3	9.5159	Camphene	79-92-5	$C_{10}H_{16}$	136.234	0.96
4	10.1418	β-Pinene	127-91-3	$C_{10}H_{16}$	136.234	3.21
5	11.3820	Limonene	138-86-3	$C_{10}H_{16}$	136.24	0.52
6	13.7805	Borneol	507-70-0	$C_{10}H_{18}O$	154,249	0.48
7	14.1959	γ - Terpinene	99-85-4	$C_{10}H_{16}$	136.23	0.33
8	14.6287	8-Cedren-13-ol	18319-35-2	$C_{15}H_{24}O$	220.35	0.23
9	16.6294	Cyclosativene	22469-52-9	$C_{15}H_{24}$	204.351	0.66
10	16.7523	α-Copaene	3856-25-5	$C_{15}H_{24}$	204.35	2.44
11	16.9570	β-Elemene	515-13-9	$C_{15}H_{24}$	204.35	0.85
12	17.0448	Cyperene	2387-78-2	$C_{15}H_{24}$	204.35	3.06
13	17.3197	*E*-Caryophyllene	87-44-5	$C_{15}H_{24}$	204.35	2.34
14	17.6122	6,9-Guaiadiene	37839-64-18	$C_{15}H_{24}$	204.35	4.17
15	17.8345	*Allo* - Aromadendrene	25246-27-9	$C_{15}H_{24}$	204.35	2.58
16	18.0861	γ - Muurolene	30021-74-0	$C_{15}H_{24}$	204.35	0.74
17	18.7179	δ - Cadinene	483-76-1	$C_{15}H_{24}$	204.35	1.54
18	19.0864	α-Calacorene	21391-99-1	$C_{3:20}PM$	200.32	0.99

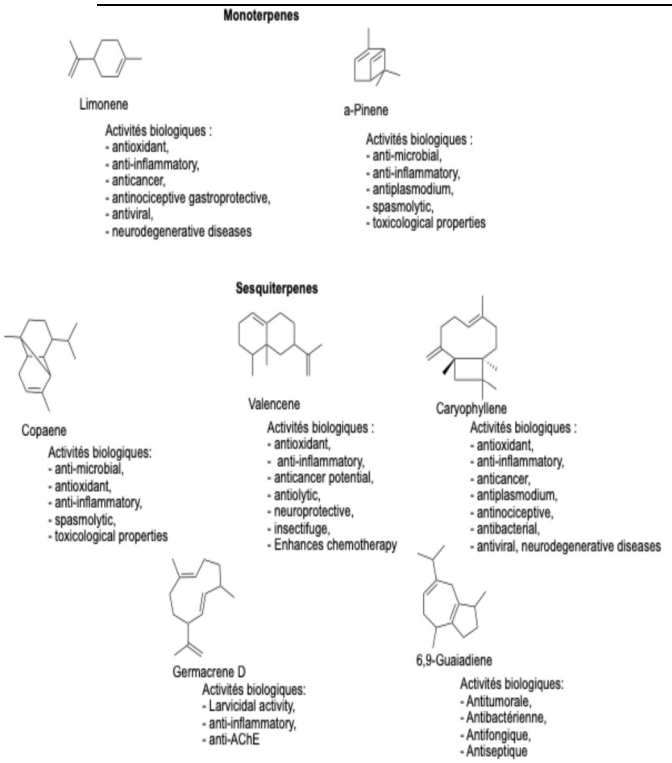

Figure 1. Some compounds identified in C. sylvaticus and their biological activities (Lujain et al., 2021; Wenzhuo et al., 2021; Albuquerque et al., 2022; Haoran et al., 2024; Master et al., 2024)

3.1.3. Estimation of total polyphenols and flavonoids

Table IV shows the polyphenol and flavonoid contents of the extracts of the leaves and trunk bark of *C. sylvaticus* (CS).

Table IV. Polyphenol and flavonoid contents of C. sylvaticus leaves and stem barks extracts

Parts	Polyphenol in mg GAE/g			Flavonoids in mg QE/g		
	EDCM	EME	EAQ	EDCM	EME	EAQ
Leaves	54.20±0.10	57.70±0.15	68.61±0.25	9.80±0.20	36.70±0.10	79.60±0.30
Barks	32.30±0.20	65.60±0.10	59.70±0.10	5.10±0.25	24.80±0.15	40.80±0.10
Mean	57.98±0.15			32.7±0.20		

3.2. Discussion

3.2.1. Extraction yield

Table III.I shows the extraction yield of plant leaf and barks extracts of *Croton sylvaticus*.The dichloromethane (EDCM) extraction yielded 7.3 % of crude material from the leaves and 1.73 % from the stem barks while methanol extraction (EME) gave 6.7 % of material from the leaves and 3.43 % from the stem barks, and the final aqueous (EAQ) extraction produced 9.0 % from the leaves and 2.76 % from the stem barks. Variability in yield was observed in the sequence EAQ > EDCM > EME for leaves and EME > EAQ > DCM for stem barks. It was found that aqueous extracts yield was higher than dichloromethane and methanol organic extracts. These variations could be attributed, on the one hand, to the nature of the plant, the harvesting period, and the age of the plant material. On the other hand, they could be attributed to environmental factors that influence the expression of genes governing the synthesis of secondary metabolites by affecting their quality and/or quantity (Falleh et al., 2008).

The use of different solvents with increasing polarity made it possible to separate the compounds according to their degree of solubility. This extraction method, carried out under continuous agitation and for a short period of time, allows for the extraction of the maximum amount of bioactive components and prevents their denaturation or probable modification (Hagermann et al., 2000).

3.2.2. Volatile organic compounds (VOC)

C. sylvaticus leaves and stem barks were analyzed by GC-MS. In the leaves extract 35 compounds were detected and identified. These compounds are mainly composed of monoterpenes (C_{10}) and sesquiterpenes (C_{15}), reflecting the diversity of secondary metabolites typical of essential oils. The most important (having a concentration greater than 3%) are limonene (11.94%), 6,9-Guaiadiene (8.37%), α-copaene (7.92%), valencene (7.57%), germacrene D (6.42%), *E*-caryophyllene (6.06%), *β*-pinene (3.20%), humulene (3.03%), and other compounds in very low quantities (table II).

The stem barks extracts analyzed using the same method yielded 18 volatile compounds, the most abundant of which were α-Pinene (9.05%), 6.9-Guaiadene (4.17%), *β*-pinene (3.21%), cyperene (3.06%), and the other terpenes were presents in low percentage (table III). However, it has been noted that the composition of the leaves extract is different from that of stem barks extracts except for 13 common compounds such as *δ*-3-Carene, *α*-and *β*-pinene, 6.9-guaiadene, *α*-copaene, *E*-caryophyllene, Limonene, Camphene, *γ*-Terpinene, *β*-elemene, cyperene, *γ*-muurolene and δ-cadinene.

These compounds appeared in both organs of the plant when we considered the entire list produced by the equipment used. The major compounds identified in *C. sylvaticus* were monoterpenes (42.86%) in the leaves and 38.89% in the bark extracts, and sesquiterpenes (57.14%) in the leaves extract and 61.11% in the stem barks extracts, with other compounds in low proportions.

Caryophyllene and humulene, previously isolated from the plant in oxidized form (Maangi et al., 1998, Renan et al., 2021) were found in the unoxidized state and at low percentages, including 6.06% for caryophyllene and 3.03% for humulene in the volatile extract of the leaves. Caryophyllene does not seem to be an important compound in the genus *Croton*, because it has been identified in several other species of the genus *Croton* at various percentages such as *C. ferrugineus* 20.47% (Eduardo et al., 2021), *C. glandulosus* 53.24% (Leticia et al., 2020), *C. hirtus* 31.75% (Touré et al., 2014), *C. piauhiensis* hull 43.58% (Jean et al., 2021), *C. pulegiodorus* 20.96%, *C. dybowskü* 16.21% (Tshiba et al., 2019), and *C. ceanothifolius* Bail (Araújo et al., 2020).

Important terpene compounds isolated from *C. sylvaticus*, including *α*- and *β*-pinene, limonene, guaiadiene, α-copaene, germacrene, and valencene have been reported in other species of the genus *Croton* (Touré et al., 2014). *α*-pinene and *β*-pinene isolated from *C. sylvaticus* bark extract at 9.05% and 3.21% were identified in *C. linearis* at 11. 05% and 1.18%, respectively (Jesus et al., 2020), guaiadiene 8.37% in *C. sylvaticus* was identified in *C. dybowskii* at 8.1% (Tshiba et al., 2019), while copaene (7.92%, leaves and 2.44%, stem barks) in *C. sylvaticus* was identified at 2.16% in *C. hirtus*.

These results corroborate the findings of this study. Germacrene 6.42% in *C. sylvaticus* was identified in several other species, including 22.57% in *C. hirtus* (Touré et al., 2014), 3.5% in *C. grossypiifolius* (Alirica et al., 2010), in *C. ferrugineus* 7.6% (Eduardo et al., 2021), and 5.56% in *C. piauhensis* (Jean et al., 2021). Limonene, mainly in the extract of the leaves of *C. sylvaticus* (11.93%) was found in *C. piauhensis* (Jean et al., 2021), and 6.9-guaiadiene (Tables I and II) was found among the major products in the leaves and stem barks of *C. sylvaticus*.

Pierre et al. (2025) in their study of the leaves, roots and stem barks of *C. sylvaticus* from Cameroon, identified several compounds, of which seven: (E)-*β*-caryophyllene, α-copaene, *α*-humulene, *δ*-cadinene, *β*-elemene, germacrene D and cyperene are common to our study, although their concentrations vary. Notably, germacrene D (6.37% vs. 6.42%) and cyperene (3.03% vs. 3.06%) displayed almost identical proportions in both the studies, underscoring their chemical stability. However, examinations of Tables 1 and 2 highlighted the presence of limonene, valencene, 6.9-Guaiadiene and *Allo*-aromadendrene epoxide, absent from the study by Pierre et al. (2025). This difference could result from factors impacting the biosynthesis of secondary metabolites, such as environment and harvest period, which could influence the recovery yield (Nea et al., 2019 ; Pierre et al., 2025).

In addition, Mvingu et al., (2025), in their recent study on the rapid dereplication of *C. sylvaticus* stem barks by MixONat were able to identify caryophyllene, camphene, copaene, pinene, cendrene, guaine and aromandendrene in dichloromethane (DCM) extract, among others. This may indicate that the DCM extract is richer in high-molecular-weight terpene structures, such as diterpenes and triterpenes, rather than volatile compounds such as monoterpenes and sesquiterpenes. GC-MS analysis of the leaves and stem barks of *C. sylvaticus* revealed that monoterpene and sesquiterpene hydrocarbons were the major constituents.

These compounds (figure 1) are known for their numerous biological activities, including larvicidal (Kihampa et al., 2009), antiparasitic (McMaster et al., 2024), antimicrobial, and antioxidant effects (Lujain et al., 2021). They also exhibit hepatoprotective, anxiolytic, antidepressant, and neuroprotective properties (Haoran et al., 2024; Pierre et al., 2025).

The diversity and complexity of the terpene compounds identified in this study highlight the bioactive potential of this species, reinforcing its interest for possible ethnopharmacological applications (Maroyi, 2017, 2019; Pierre, et al., 2025).

3.2.3. Polyphenol content and flavonoids

In both parts of the plant studied (Table 4), various polyphenol contents were observed in all the extracts. The aqueous extract (EAQ) revealed a higher content (68.61 ± 0.25 mg GAE/g), followed by the methanol extract EME (57.70 ± 0.15 mg GAE/g) and finally the dichloromethane extract EDCM (54.20 ± 0.10 mg GAE/g) for the leaves and EME (65.60 ± 0.10 mg GAE/g), followed by EAQ (59.70 ± 0.10 mg GAE/g) and EDCM (32.30 ± 0.20 mg GAE/g) for the stem barks. The Folin-Ciocalteu reagent used is extremely sensitive to the reduction of all hydroxyl groups, not only those of phenolic compounds, but also of certain sugars and proteins (Xu & Liu, 2018).

As for flavonoids, the results obtained show a difference of flavonoid contents as follow: EAQ (79.60 ± 0.30 mg EQ/g), EME (36.70 ± 0.30 mg EQ/g), and EDCM (9.80 ± 0.20 mg EQ/g) for leaves and EAQ (40.80 ± 0.15 mg EQ/g), EME (24.80 ± 0.15 mg EQ/g), EDCM (5.10 ± 0.25 mg EQ/g) for bark. The yellowish coloration formed in all extracts of *C. sylvaticus* after the addition of aluminum chloride solution ($AlCl_3$ 10%), revealing the presence of flavonoids in the analyzed extracts. In view of these results, it was found that flavonoids are more concentrated in the leaves and roots of plants.

Indeed, the part with the highest content being the leaves extracts for all the extracts in polyphenols (EAQ: 68.61 ± 0.25 mg GAE/g; EME: 57.70 ± 0.15 mg GAE/g; EDCM: 54.20 ± 0.10 mg GAE/g) and in flavonoids (EAQ: 79.60 ± 0.30 mg EQ/g; EME: 36.70 ± 0.10 mg EQ/g; EDCM: 9.80 ± 0.20 mg EQ/g) than the stem barks extracts (Table 3). These differences could be attributed to the environmental factors to which the leaves and stem barks of the species are exposed (Falleh et al., 2008 ; Nea et al, 2019).

4. Conclusion

C. sylvaticus leaves and stem barks extracts revealed the presence of mono- and sesquiterpenes as major compounds, a phytochemical composition typical of essential oils of the genus *Croton*. This study showed that the leaves have greater diversity and higher concentrations of volatile compounds than the stem bark. In addition, the extracts used in this study showed interesting contents of polyphenols (57.98 ± 0.15 mg GAE/g) and flavonoids (32.70 ± 0.20 mg EQ/g), with overall higher levels in leaves. These results indicated the chemical potential of *C. sylvaticus* as a source of bioactive secondary metabolites. Biological and pharmacological studies are currently underway to assess the therapeutic potential of these extracts and essential oil from the Congolese species.

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Conflicts of Interest

None declared.

Ethical Considerations

The study used publicly available data and did not involve any experiments with human or animal subjects. Therefore, ethical approval was not required.

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M.K.B, M.K.C, N.M.C, and M.D. participated in data collection and performed chemical analyses.

M.K.B, T.M.D, B.B.M, and M.M.B contributed to the interpretation of results and critically reviewed the manuscript.

M.K.B, M.P, and E.N.D conducted the literature review and contributed to formatting the document.

M.K.B, M.P, K.J.S, and M.M.B validated the data, contributed to the discussion, and approved the final version for submission.

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