A multi-analytical approach for the chemical characterization of copal Mbaka from *Guibourtia demeusei* (Harms) J.Leonard

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Abstract :
Copal from the Mbembe forest in the north of the Republic of Congo, known locally as "Mbaka", is traditionally used fresh as an adhesive and to light repellent fires. This copal, produced by *Guibourtia demeusei* (Harms) J.Leonard, has physical characteristics similar to those of other copals exported and studied in Europe, but has never been studied chemically. The main aim of this work is therefore to chemically characterize this copal using a multi-analytical approach. Infrared analyses confirmed that this copal is a terpenoid resinous exudate. Analysis of the volatile fraction, obtained either by hydrodistillation (HD) or headspace solid-phase microextraction (HS-SPME), revealed the presence of monoterpenes, sesquiterpenes and diterpenes. The essential oil of this copal consists mainly of sesquiterpenes with a majority of β-caryophyllene (40%). Using two-dimensional gas chromatography (GCxGC), 19 additional minor compounds were identified compared to the 1D GC results. Non-volatile organic compounds were extracted using Soxhlet, then processed before analysis by high-temperature gas chromatography with mass spectrometric detection (HT-GC-MS). They are labdane-type diterpenic acids. The presence of cativic acid distinguishes this copal from other Congolese copals, and its non-volatile fraction shows similarities to copal samples from Latin America, particularly those from the genus *Hymenaea.*

Keywords : Copal, *Guibourtia demeusei*, Terpenoid resin, HS-SPME, GCxGC-MS, HT-GC-MS.

Caractérisation chimique du copal Mbaka produit par *Guibourtia demeusei* (Harms) J.Leonard grâce à une approche multi-analytique

Résumé:
Le copal provenant de la forêt de Mbembe dans le nord de la République du Congo, connu localement sous le nom de « Mbaka » est traditionnellement utilisé frais comme adhésif ainsi que pour allumer des feux répulsifs. Ce copal, produit par *Guibourtia demeusei* (Harms) J. Leonard, a des caractéristiques physiques proches de celles d'autres copals exportés et étudiés en Europe mais il n'a jamais été étudié chimiquement. Ainsi l'objectif principal de ce travail est de caractériser chimiquement ce copal grâce à une approche multi-analytique. Les analyses infrarouges ont confirmé que ce copal est un exsudat résineux terpénoïde. L'analyse de la fraction volatile, obtenue soit par hydrodistillation (HD) ou par microextraction sur phase solide en espace de tête (HS-SPME), a révélé la présence de monoterpènes, de sesquiterpènes et de diterpènes. L'huile essentielle de ce copal est principalement constituée de sesquiterpènes et majoritairement de β-caryophyllène (40 %). En utilisant la chromatographie gazeuse bidimensionnelle (GCxGC), 19 composés mineurs supplémentaires ont été identifiés par rapport aux résultats de la GC 1D. Les composés organiques non volatils ont été extraits grâce au Soxhlet, puis traités avant d'être analysés par chromatographie gazeuse à haute température avec détection spectrométrique de masse (HT-GC-MS). Il s'agit d'acides diterpéniques de type labdane. La présence d'acide cativique distingue ce copal des autres copals congolais, et sa fraction non volatile présente des similitudes avec les copals d'Amérique latine, en particulier du genre *Hymenaea*, tandis que la forme solide s'explique par la tendance de l'acide ozique à se polymériser.

Mots-clés: Copal, *Guibourtia demeusei*, Résine terpénoïde, HS-SPME, GCxGC-MS, HT-GC-MS.

Introduction

Desmodium adscendens The Republic of Congo, located in Central Africa, is a country well-known for its vast floral diversity. This richness is partly due to its climate, which promotes the growth of different plant species. Among these species, some produce exudates. These complex substances, can be divided into four groups: latex, gums, resinous exudates, and resinous exudates obtained in the form of mixtures such as gum resins (Bruneton, 2002; Langenheim, 2003).

These substances have long been used by Congolese populations. In traditional medicine, exudates are well-known to treat various ailments, including abdominal pain, genitourinary diseases, fungal infections, and skin wounds. Resinous exudates are applied to wounds after slight epidermal scarification, while gums are used as hemostatics and to promote healing (Bouquet, 1969; Elouma Ndinga, 2010; Veiga and Pinto, 2002). Moreover, the medicinal use of exudates is not limited to Central Africa. Oleoresin-type exudates are also widely used in the Brazilian Amazon, where they are employed for their anti-inflammatory and contraceptive properties (Trindade et al., 2018). Within the class of terpenoid resinous exudates, there are copals. The term "copal" is highly

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controversial in the literature due to its varied use to designate different types of resinous exudates. It has been applied to several exudates, notably those from species of the Burseraceae family, particularly those from the genera *Protium* and *Bursera* (Abad-Fitz et al., 2020; Case et al., 2003; Langenheim, 2003). However, only copals produced by woody species, either African or American, from the *Fabaceae* family will be considered true copals (Léonard, 1950).

The genus *Guibourtia* (family Fabaceae, subfamily Detarioideae) includes two South American and thirteen African species, among which *Guibourtia demeusei* (Harms) J. Léonard is found (Tosso et al., 2018). The chemical properties and uses of the African *Guibourtia* species remain poorly documented. This lack of information contributes to significant confusion regarding the botanical identity of copals from the Congo Basin, particularly in the Democratic Republic of Congo (DRC) and the Republic of Congo (RC). In these regions, these resinous exudates are often generically referred to as "Congo copals," which obscures their true botanical diversity and hinders the understanding of their characteristics and potential industrial or artisanal applications.

Several studies have attempted to characterize samples of so-called “Congo copals,” although their botanical origin has not always been clearly certified. Bevan et al. (1968) analyzed a sample from the genus *Daniellia*, revealing the presence of polyozoic acid, ozol, and enantio-biformene. Van den Berg et al. (1999), using a different sample, identified free acids such as eperuic, copalic, and kovalenoic acids. Steigenberger (2013) observed a dominance of ent-labd-8(20)-en-15-oic acid and its derivatives, while Hugel et al. (1996), based on another sample, detected various oxygenated labdane compounds.

However, these studies focused mainly on the non-volatile fraction, leaving the volatile composition largely unexplored. Moreover, the frequent lack of reliable botanical identification limits the taxonomic and chemical relevance of the results.

To address these gaps, this study focuses on a copal collected in the Mbembe forest, in northern Republic of Congo (Owando and Likouala-Mossaka regions). Locally known as “Mbaka” or “Mpaka,” this resin is traditionally used by local communities as an adhesive, firestarter, and insect repellent. Its botanical origin has been formally identified as *Guibourtia demeusei* (Harms) J. Léonard, based on anatomical and botanical criteria established by a botanist from the National Herbarium of the Republic of Congo.

This study aims to thoroughly characterize both the volatile and non-volatile fractions of this botanically certified copal using infrared spectroscopy and advanced chromatographic techniques. The objective is to establish a solid scientific foundation for the valorization of Congolese resinous exudates and to enhance the understanding of their chemical properties and potential applications.

Materials and Methods

1. *Materials*

1.1. Copal samples

Copal samples were collected during ethnobotanical surveys conducted in January 2010 in the Mbembe forest, near Owando (the administrative center of the Cuvette Department, located 550 km from Brazzaville, Republic of the Congo). The resin, locally known as Copal Mbaka, is generally translucent to transparent, with a colour ranging from golden yellow to pale yellow. Upon exposure to air, it gradually hardens.

These samples were obtained from the trunk bark of *G. demeusei* (Harms) J. Leonard. The plant material was authenticated at the National Herbarium of the Republic of the Congo, where a voucher specimen was deposited under the reference GD 79 E CG..

1.2. Solvents and reagents

Solvents (acetonitrile, diethyl ether, hexane) were all of analytical grade and obtained from Merck (Darmstadt, Germany). N,O-bis (trimethyl)silyltrifluoro-acetamide (BSTFA) containing 1% trimethylchlorosilane and (Trimethylsilyl) diazomethane solution (2 M in hexane) were purchased from Sigma-Aldrich (Milan, Italy).

2. *Methods*

2.1. Sample preparation

2.1.1. Hydrodistillation

La The crude copals were manually released from fragments of bark, when it was necessary, and pulverized with an agate mortar. Two hundred grams (200 g) of the obtained powder were submitted to hydrodistillation in a Clevenger-type apparatus at 100 °C for 4 h. The essential oil obtained was extracted with diethyl ether and dried over anhydrous sodium sulfate (Elouma Ndinga, 2010). After filtration, the yield of essential oil was 0.04% (w/w). Then, the solution of essential oil 1% (v/v) in diethyl ether was analyzed by GC-MS and GCxGC-MS.

2.1.2. Headspace Solid-Phase Micro-Extraction (HS-SPME)

The volatile compounds were extracted in the headspace mode by a 65 µm (Stable Flex) PDMS/DVB (Polydimethylsiloxane/divinylbenzene) fiber supplied by Supelco (Bellefonte, PA, USA). Before every extraction, the fiber was conditioned at 250 °C for 10 min. All extractions were performed manually in 2 mL glass vials equipped with screw caps and PTFE/silicone septa. 7.5 mg of copal powder (obtained by pulverizing the sample with an agate mortar) was introduced in a vial placed in an oil bath at 80 °C. The SPME fiber was inserted into the sample vial, through the septum and was allowed to equilibrate with the headspace volatiles for 30 min. After this extraction procedure inspired by previous work (Hamm et al., 2003), the analytes were thermally desorbed for 5 min at 250 °C in the injector of the gas chromatograph.

2.1.3. Liquid extraction

Copal powder (20 g) was subjected to extraction with a Soxhlet extractor. The solvent used was petroleum ether. The extraction was conducted at 80 °C for 4 hours. Then, extract was concentrated on a rotary evaporator and dried.

2.1.4. Derivatization procedures

2.1.4.1. Methylation by treatment with TMS-diazomethane

1 mg of petroleum ether extract was placed in a vial with 350 µL of benzene and 100 µL of methanol. 10 µL of TMS-diazomethane (2.0 M in hexane) were added. The mixture was continuously stirred for 30 min at room temperature. The vial remains open to allow the evacuation of nitrogen formed during the reaction. After thirty minutes, 1 µL of the solution was directly injected into the injector of the chromatograph (Scalarone et al., 2002).

2.1.4.2. Silylation with BSTFA/pyridine

1 mg of each extract was trimethylsilylated with 200 µL of a solution of BSTFA/pyridine (50/50, v/v). The reaction was carried out at room temperature for 30 minutes, then the solution was evaporated to dryness under a nitrogen flow. The residue was solubilized in 1 mL of isooctane and then injected into the chromatograph (Bleton et al., 1996).

2.2. Analytical procedures and instrumentation

2.2.1. Infrared analysis (FTIR)

Infrared analysis (FTIR) analysis was performed on a Thermo Scientific Nicolet IS10 FT-IR spectrometer (resolution: 4 cm-1, number of scans: 64) equipped with a DTGS KBr detector operated with OMNIC Spectra 2.0 acquisition software. Analyses were performed using both Attenuated Total Reflectance and KBr pellet methods. For the first method, solid samples were coarsely crushed and placed on the ATR crystal. The excellent mechanical properties of diamond make it an ideal material for ATR, particularly when studying robust solids. For the second one, solid samples were ground homogeneously in an agate mortar with 150 times their weight of anhydrous potassium bromide (SPECAC, Kent). The powder is then compressed under a pressure of 10 tons.cm-2 to form very fine translucent pellets (Guiliano et al., 2006; Guiliano et al., 2007; Regert et al., 2008).

The assignment of absorption bands was made based on Larkin's reference book (Larkin, 2011) and other literature data (Blancas et al., 2022; Delclòs et al., 2020; Garcia-Valles et al., 2023; Guiliano et al., 2007; Scalarone et al., 2003).

2.2.2. Gas chromatography-mass spectrometry analysis

An HP 6890 gas chromatograph system, coupled with an HP MD5973 quadrupole mass spectrometer was used. The extracted compounds were separated on a RTX-5ms capillary column (30 m x 0.25 mm x 0.25 µm film thickness). Volatiles obtained by both HS-SPME and HD techniques were injected using the split mode with a split ratio of 50:1. The carrier gas was helium at a flow rate of 1.3 mL/min. The temperature program was 40 °C for 1 min, 10 °C/min increase rate up to 130 °C, followed by a 3 °C/min increase rate up to 250 °C. The injector temperature and the ion source temperature were at 250 °C and 150 °C, respectively. The ionizing energy was 70 eV. All data were obtained by collecting the full-scan mass spectra with the mass range 29–300 amu.

2.2.3. Two-dimensional gas chromatography-mass spectrometry analysis

The essential oil of copal Mbaka was also analyzed using a Trace GCxGC system, coupled with a Trace DSQ II quadrupole mass spectrometer from Thermo-Electron Corporation. It was fitted with a double jets carbon dioxide cryogenic modulator, and a split/splitless injector. The first column was a non-polar DB-5ms, (30 m x 0.25 µm x 0.25 µm), Agilent Technologies J&W, and the second column was a TR-Wax MS (1 m x 0.1 µm x 0.1 µm), Thermo Electron. The columns were connected using a press-fit connector. Helium was used in constant pressure mode as carrier gas. The inlet pressure was 70 KPa. The oven temperature program started at 60 °C, then an increase at 3 °C/min was applied up to 250 °C, then held for 5 min. The injector was operated at 250 °C with a split flow of 30 mL/min. The MS transfer line temperature was 200 °C, and the source temperature was 200 °C. The modulation time was 6 s. The DSQ II mass spectrometer was operated in electron impact ionization (EI) using positive mode. The electron energy was 70 eV. Mass spectra were collected with the scan range m/z 30 - 400. Excalibur software was used for acquisition. Then, data were imported into Hyperchrom S/W software for the visualization of 2D and 3D chromatograms.

2.2.4. *High-temperature gas chromatography-mass spectrometry analysis (HT-GC-MS)*

Methylated and silylated extracts of copal Mbaka were analyzed in HT-GC-MS (Trace GC Ultra Thermo Electron corporation/ DSQ II) equipped with an Optima 5-HT (30 m x 0.32 mm x 0.10 µm) capillary column. One microliter of derivatives was injected in on-column mode. Helium was used as a carrier gas at a constant flow of 2.0 mL/min through the column. The oven temperature of the gas chromatograph was programmed from 80 °C to 390 °C at a rate of 30 °C/min and was finally held for 8 min. The temperatures of the transfer line and electron impact (EI) ion source were set to 350 °C and 250 °C respectively. The electron energy was 70 eV and mass spectra were collected in the full scan mode (m/z 35-1000) (Cartoni et al., 2004; Scalarone et al., 2002).

2.2.5. *Components identification*

The compounds were identified by comparison of their mass spectra with those of standards available in commercial mass spectral libraries like the NIST'05 database. The identification of most of the molecules was confirmed by comparison of their experimental GC retention Kovats index (KI) with those provided by Adams'book. The retention times of n-alkanes required to calculate the retention index were obtained with a commercial mixture of C8-C20 in n-hexane. Relative amounts of individual components were calculated based on their GC peak areas (Adams, 2001).

Results

1. *FTIR spectra*

Figure 1 shows infrared spectra of the Mbaka sample and the attributions are reported in the Table I.

2. *Analysis of the volatile fraction of copal Mbaka using GC-MS*

Volatile fraction of copal Mbaka was collected by HS-SPME and HD and then analyzed by GC-MS. Figure 2 shows the chromatographic profile of volatile fraction obtained using HS-SPME sample preparation.

Table II shows the fifty-one compounds detected, including forty-eight identified. The volatile fraction is composed mainly of sesquiterpenes.

3. *GC×GC–MS analysis of essential oils*

The essential oil is a mixture of terpenoid compounds, thus it is often difficult to separate all sample constituents by conventional techniques (GC-FID or GC-MS). In particular, minor constituents that can have interesting pharmacological properties may be hidden by co-elution with other components. To overcome this problem, the essential oil of Mbaka exudate was analyzed using two-dimensional gas chromatography coupled with mass spectrometry (GCxGC-MS). Figure 3 shows the GCxGC-MS chromatogram of the essential oil distilled from copal Mbaka. It highlights that this essential oil contains many sesquiterpenes. Sixty-three compounds were separated using the GCxGC-MS method. Table III shows that compared with the GC-MS technique, eighteen new compounds have been detected with the GCxGC-MS technique.

4. *HT- GC-MS analysis*

Extract of copal Mbaka (petroleum ether) was derivatized to ease the analysis of compounds and the HT-GC-MS technique was chosen to reach a wider range of compound volatility. In order to optimize the detection and identification of exudate components, methylated and silylated derivatives were formed. As the identification is based on the comparison of mass spectra with literature and library data (Doménech-Carbo et al., 2009; Popova et al., 2010; Scalarone et al., 2003) and NIST MS Library, 2005, analysis of both methylated and silylated compounds allowed reliable determination of extract composition. Figure 4 shows the chromatogram of the petroleum ether extract after methylation and labels for the peaks are given in Table IV.

Discussion

4.1. Interpretation of FTIR spectra

The analyzed "Mbaka" copal are translucent, cream-colored like the Congo copal specimens analyzed by Garcia-Valles et al. (2023). The FTIR spectrum of the "Mbaka" resin sample reveals key vibrational features consistent with a diterpenoid resinous material.

A strong absorption band at 1697 cm^{-1} is attributed to the carbonyl stretching of carboxylic acid functions. In addition, absorption bands in the 1270–1110 cm^{-1} region correspond to C–O stretching vibrations, confirming the presence of carboxylic acid groups. A broad, medium-intensity band centered at 3432 cm^{-1} is assigned to the O–H stretching of hydrogen-bonded carboxylic acids.

This O–H band falls within the 3411–3432 cm^{-1} range described by Delclòs et al. (2020) as characteristic of East African copals, suggesting a possible geographic and botanical affinity.

These resins are typically produced by species of the genus Hymenaea, known for generating labdane-type diterpenes.

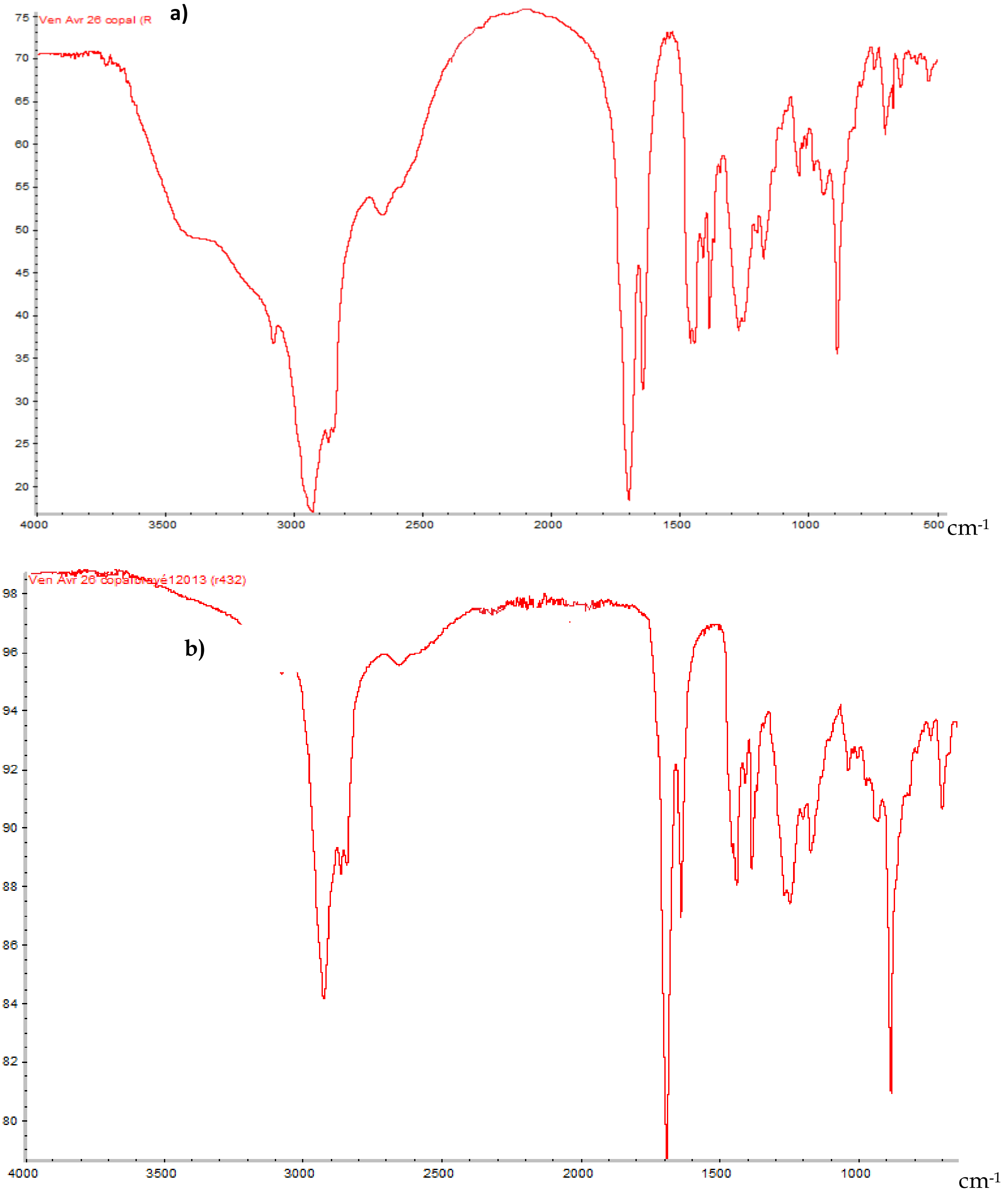

Figure 1 : Mid-infrared specra of « Mbaka » exudate a) prepared in a KBr pellet b) with an ATR accessory.

Table I: Attributions of main bands from « Mbaka » FTIR spectrum

Wavenumber (cm^{-1})	**Attributions**
3432	ν OH
3085	ν H-C= (out-of-phase stretch)
2934	ν_{as} (C-H) CH2, CH3
2868, 2848	ν_{s} (C-H), CH2, CH3 (in-phase stretch)
1697	ν(C=O) carboxylic acid
1643	ν(C=C)
1444, 1440	δ(C-H), CH2, CH3
1384	δ(C-H) CH3
1270	ν(C-O) carboxylic acid
888	γ(C-H) exomethylene

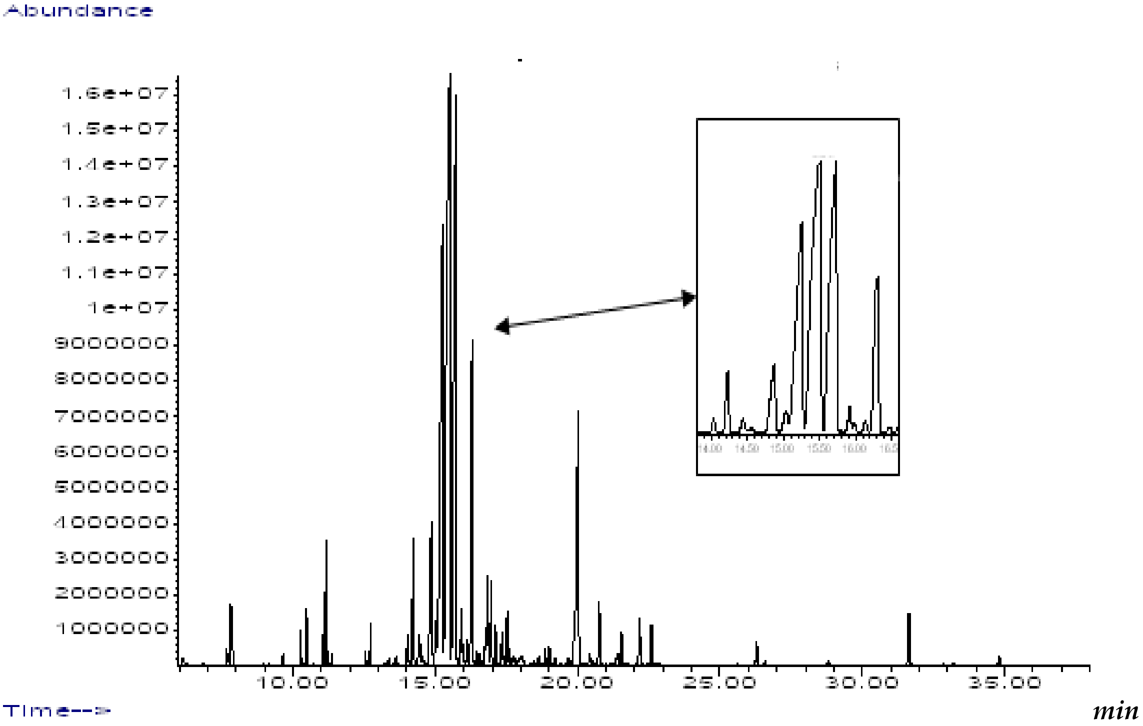

Figure 2: Chromatographic profile of the volatile fraction of copal Mbaka obtained by Headspace microextraction in solid phase.

Three diagnostic bands at 3082, 1643, and 888 cm^{-1} are clearly visible in the Mbaka spectrum and correspond to vibrational modes of exocyclic methylene groups (v=C–H and δ=CH$_2$), characteristic of labdane-structured diterpenoid acids. Delclòs et al. (2020) demonstrated that these bands—especially the one at 888 cm^{-1}—are strong in recent East African copals and greatly diminished or absent in older ambers. The relative intensity of the 888 cm^{-1} band in the Mbaka spectrum therefore suggests a low degree of polymerization, consistent with a subfossil resin (copal) rather than true amber. Strong CH stretching bands between 2929–2937 cm^{-1} and 2844–2874 cm^{-1}, attributed to asymmetric and symmetric stretching of methyl and methylene groups, are observed in all terpenoid resins. While not exclusive to copals, their presence aligns with the molecular structure of diterpenoid resins. Notably, a low-intensity absorption band at 3079–3082 cm^{-1}, corresponding to =C–H stretching and absent in triterpenoid resins, serves as a diterpenoid marker, as reported by Regert et al. (2008) and Delclòs et al. (2020).

A minor band at 2655 cm^{-1}, which may indicate the presence of carboxylic acid dimers (O–H stretching), is also observed and has been documented in certain African copals (Derrick et al., 1999). The presence of bands at 1644, 1445–1448, 1386, and 888 cm^{-1}—associated with varying degrees of polymerization in resinous materials (Scalarone et al., 2003)—further supports the classification of this sample as a relatively young, weakly polymerized resin.

Overall, the spectroscopic characteristics of the Mbaka sample closely match the FTIR profiles of East African copals derived from *Hymenaea* species, as reported in recent studies (Delclòs et al., 2020; Regert et al., 2008; Guiliano et al., 2006). The combination of a strong 888 cm^{-1} band, the visible presence of exocyclic methylene groups, a band near 3085 cm^{-1}, and a broad hydroxyl band around 3432 cm^{-1} is diagnostic of a subfossil copal, ruling out fossilization processes associated with true amber.

4.2. Analysis of the volatile fraction of copal Mbaka using GC-MS

The chromatographic profile in Figure 2 is similar to that obtained with HD preparation; the same qualitative composition could be deduced from the two chromatographic fingerprints. HS-SPME extraction can be considered an effective alternative technique for isolating volatiles from tree exudates. HS-SPME is a simpler and more rapid procedure for the extraction of the volatile fraction from plants (Belliardo et al., 2006; Lo et al., 2021; Souza Silva et al., 2017) in comparison with hydrodistillation, which is time-consuming and needs a large amount of sample. HS-SPME analysis determines the volatile fraction composition using a small quantity of material. PDMS/DVB fiber seems to be suitable for the study of these volatile compounds as it has been shown for the characterization of archaeological balms present in ancient Egypt objects (Hamm et al., 2003).

Table 2 summarizes the chemical compositions of headspace and hydrodistillation fractions from copal Mbaka in the elution order obtained on the RTX5-MS column. The quantitative but not qualitative differences observed between the volatile fractions extracted using hydrodistillation and HS-SPME, respectively, can be explained by the fact that the first technique is based on the liquid quasi-total extraction of plant volatiles and the latter technique is controlled by a solid/gas equilibrium step. This was also reported by Benyelles (2011) when studying the composition of essential oil extracted from *Rhaponticum acaule* L. roots depending on the extraction method. The main sesquiterpenes found in copal Mbaka are: β-caryophyllene, trans-α-bergamotene, cis-α-bergamotene, α-humulene and caryophyllene oxide. Chemically, the composition of the volatile fraction of copal Mbaka is close to Copaiba oil exuding from the trunk of the genus *Copaifera*. However, the chemical composition of the Copaiba oil differs between species and within the same species, depending on environmental conditions. Chromatographic studies showed that Copaiba oils obtained from *Copaifera multijuga* Hayne, *C. cearensis* Huber ex Ducke and *C. reticulata* Ducke species are mainly composed of β-Caryophyllene (57.5%, 19.7% and 40.9%, respectively), followed by α-humulene, α-copaene, α-bergamotene, δ-cadinene, with different amounts in each oleoresin (Veiga Junior et al., 2007).

More recent studies have confirmed that β-caryophyllene remains the major component in various *Copaifera* species (Rodrigues et al., 2020; Trindade et al., 2017). Although β-caryophyllene is present in numerous plant species, it is usually the major constituent and a chemical marker of these oleoresins (Cascon and Gilbert, 2000).

The repellent activity attributed to the copal Mbaka by populations of Likouala when burned can be justified by the presence of β-Caryophyllene and its oxide in the volatile fraction. Recent studies have confirmed that β-Caryophyllene possesses strong insecticidal properties. For instance, encapsulated β-Caryophyllene nanoliposomes demonstrated effective insecticidal activity against Bemisia tabaci, with a median lethal concentration (LC$_{50}$) of 1.51 g/L, while being non-toxic to tomato plants (Gao et al., 2024). In addition, β-Caryophyllene induced dose-dependent larvicidal, genotoxic and cytotoxic effects on Spodoptera litura, significantly reducing larval survival and growth (Mahajan et al., 2022). Another study showed that β-Caryophyllene altered gene expression and reduced survival in Myzus persicae, affecting their reproductive and digestive systems (Chohan et al., 2023). Furthermore, β-Caryophyllene oxide combined with vetiver oil exhibited repellent and knockdown effects against *Aedes aegypti* and *Anopheles minimus*, supporting its potential as a natural mosquito repellent (Nararak et al., 2023).

These findings reinforce the traditional use of copal Mbaka as a natural repellent and confirm β-Caryophyllene's potential as a bioactive compound in eco-friendly pest management strategies. Recent literature also reports that several monoterpenes found in essential oils exhibit repellent or insecticidal properties.

Table II: Compounds identified in volatile fraction of Mbaka copal and the obtained relatives percents. IKcalc: Kovats retention indexes determined in GC–MS analysis on apolar RTX-5 MS column.

- Base peak in bold.
- Molecular mass in bold and underlined.
- Molecular absent on the spectrum between parenthesis, in bold and underlined.

n.	Compound	EIMS Fragmentation	IK ^{Calc}	%area HD	%area SPME
	α-thujene	41 ; 53 ; 65 ; 77 ; **93** ; 105 ; **136**	927	0.02	0.10
2	α-pinene	53; 67; 77; 91; **93** ; 105; 121; **136**	934	0.16	0.30
3	sabinene	41; 53; 65; 69; 77; 79; **93**; 121; **136**	975	0.01	0. 03
4	β-pinene	41; 53; 69; 79; **93**; 107; 121; **136**	980	0.03	0.10
5	α-methylstyrene	51 ; 78 ; 91 ; 103 ; 115 ; 117 ; **118**	985	0.02	0.03
6	myrcene	**41**;53;55; 67; 69;93; **136**	991	0.03	0.10
7	α-phellandrene	39;65;77;**93**;119;**136**	1007	0.08	0.39
8	p-cymene	39 ; 51 ; 65 ; 77 ; 91 ; 103 ; **119** ; **134**	1024	1.17	1.26
9	D-limonene	53; **68**;79; 96; 107; 121; **136**	1030	0.30	0.28
10	1,8-cineol	**43** ; 55 ; 71 ; 81 ; 93 ; 108 ; 125 ; 139 ; **154**	1034	2.03	1.27
11	γ-terpinene	51 ; 65; 77 ; **93** ; 105 ; 121; **136**	1061	0.03	0.04
12	terpinen-4-ol	43 ; 55; 67; **71**; 77; 86 ; 93; 111; 136; **154**	1180	0.40	0.42
13	naphtalene	51 ; 63 ; 74 ; 77 ; 102; **128**	1187	0.02	0.03
14	p-cymen-8-ol	**43** ; 51 ; 65 ; 77 ; 91 ; 135 ; **150**	1190	0.02	0.06
15	α-terpineol	43; 55; **59**; 77; 81; 93; 121 ; 136 ; (**154**)	1195	0.77	0.59
16	thymol, methyl ether	65 ; 77 ; 91 ; 105 ; 119 ; 134 ; **149** ; **164**	1230	0.78	0.50
17	carvacrol, methyl ether	65 ; 77 ; 91 ; 105 ; 119 ; 134 ; **149** ; **164**	1268	1.16	0.70
18	2-isopropyl-1-methoxy-4-methylbenzene	65 ; 77 ; 91 ; 105 ; 119 ; 134 ; **149** ; **164**	1273	0.07	0.10
19	isopropylbenzoate	43 ; 51 ; 59 ; 77 ; **105** ; 123; 149; **164**	1309	0.11	0.13
20	unidentified sesquiterpene 1	67 ;77 ;91 ;105 ;**119** ;131 ;145 ;159 ;174 ;187 ;**204**	1345	0.02	0.06
21	cyclosativene	41 ; 55 ; 79 ; 94 ; **105** ; 120 ; 133 ; 161 ; 189 ; **204**	1365	0.29	0.32
22	α-Copaene	41 ; 55 ; 69 ; 81 ; 93 ; 105 ; 119 ; **161** ; 189 ; **204**	1376	1.10	1.00
23	β-Elemene	41; 53 ; 67 ; 81 ; **93** ; 107; 121; 133; 147; 161; 189; **204**	1396	0.26	0.31

24	cyperene	91 ; 105 ; 119 ; 133 ; 147 ; 161 ; 175 ; 189 ; **204**	1402	0.11	0.18
25	italicene	41 ;55 ;69 ;79 ;93 ;105 ;**119** ;134 ;148 ;161 ; 189 ;**204**	1409	1.99	1.49
26	α-cedrene	55 ; 69 ; 77 ; 93 ; 105 ; **119** ; 147 ; 161 ; 189 ; **204**	1416	0.80	0.93
27	cis-α-bergamotene	69 ; 79 ; **93** ; 107 ; 119 ; 133 ; 147 ; 161 ; 189 ; **204**	1419	10.39	11.11
28	β-caryophyllene	69 ; 79 ; **93** ; 105 ; 120 ; 133 ; 147 ; 161 ; 189 ; **204**	1423	40.00	40.91
29	β-ylangene	55 ; 67 ; 79 ; 91 ; 105 ; 119 ; 147 ; 161 ; 189 ; **204**	1433	0.18	0.10
30	α- trans-bergamotene	69 ; 79 ; **93** ; 107 ; 119 ; 133 ; 147 ; 161 ; 189 ; **204**	1439	15.41	14.43
31	cis-β-farnesene	**41** ; 55 ; 69 ; 79 ; 93 ; 105 ; 120 ; 133 ; 147 ; 161; **204**	1449	0.33	1.15
32	epi-β-santalene	41;69 ;79 ;109 ;121 ;135 ;149 ;161 ;177 ;187 ;205	1453	0.12	0.41
33	α-humulene	41; 55 ; 67 ; 79 ; **93** ; 107 ; 122 ; 161; **204**	1457	6.91	7.08
34	sesquisabinene	41;55;**69**;77;93;105;120;133;161;175;189;**204**	1463	0.91	0.76
35	unidentified sesquiterpene 2		1469	0.18	0.14
36	ar-curcumene	41 ; 55 ; 83 ; 91 ; 105 ; **119** ; 132 ; 145 ; 187 ; **202**	1484	0.16	0.26
37	γ-curcumene	41;55;69;77;93;105;**119**;134; 147 ; 161; 189;**204**	1487	1.34	0.83
38	unidentified sesquiterpene 3		1498	0.40	0.34
39	β-bisabolene	**41** ; 53 ; 69 ; 79 ; 93 ; 107 ; 119 ; 134 ; 161 ; 189 ; **204**	1509	0.18	0.25
40	β-curcumene	41 ; 55 ; 69 ; 77 ; 93 ; 105 ; **119** ; 134 ; 161 ; **204**	1516	0.34	0.30
41	sesquicineole	**43**;55 ; 69 ;81 ; 95;109 ;119 ;**139** ;161 ; 204 ; (**222)**	1518	0.20	0.27
42	δ-cadinene	41 ; 55 ; 69 ; 81 ; 91 ;105 ;119 ;134 ;145 ;**161** ; 189;**204**	1528	0.09	0.06
43	unidentified sesquiterpene 4	**79** ;93 ;107 ; 119 ;138 ;149 ;161 ; 177 ; 189 ; 204 ; (**220**)	1557	0.28	0.25
44	caryophyllene oxide	**43** ;55 ;69 ;79 ;93 ;109 ; 121 ;135 ;149 ;161 ; 177 ; (**220)**	1584	5.56	5.49
45	humulene epoxide II	**43** ;55 ;67 ;79 ;96 ;109 ; 123 ;138 ;177 ;189 ; 205 ; **220**	1614	0.84	0.79
46	α-acorenol	59 ; 67 ; 79 ;93 ;105 ;**119** ;134 ;147 ;161;189 ;204;(**220)**	1636	0.18	0.09
47	caryophylla-4(14), 8(15)-dien-5-ol	55 ; 69 ; 79 ; 91 ;105 ;117 ;123 ; **136** ;149 ;159 ; (**220)**	1643	0.50	0.36
48	14-hydroxy-9-epi-cis-caryophyllene	**41** ; 55 ; 69 ; 79 ; **91** ;105 ;121 ; 131 ; 187;205 ; (**220)**	1676	1.27	0.88
49	unidentified diterpene 1	109 ; 123 ; 136 ; 151 ; 166 ; 177 ; 190 ; 229 ; 247 ; **262**	1791	0.13	0.03
50	unidentified diterpene 2	107 ;123 ;137 ;147 ;159 ;177 ;189 ;204 ;229 ;244 ;**262**	1957	0.60	0.49
51	Manool	81 ; 95 ; 107 ; 123 ; **137** ; 161 ; 177 ; 204 ; 229 ; 244 ; 257; 272 ; **290**	2060	1.26	0.20

Table III: Compounds detected in 2D-chromatograms. Bold: compounds undetected in 1D-GC-MS.

- Base peak in bold.
- Molecular mass in bold and underlined.
- Molecular absent on the spectrum between parenthesis, in bold and underlined.

RT_1	Compound	EIMS Fragmentation
10.57	α-Thujene	
10.82	α-Pinene	
11.47	**Camphene**	41 ; 53 ; 67 ; 79 ; **93** ; 107 ; 121 ; **136**
12.22	Sabinene	
12.42	β-Pinene	
12.47	Myrcene	
12.87	**2,3-Dehydro-1,8-cineole**	43;67; 79;94;**109**;124;**152**
13.12	**Unidentified monoterpene 1**	55;69;79;**83**;95;105;111;126;**138**
13.47	α-Phellandrene	
13.97	**α-Terpinene**	43; 51; 77; 93;105;**121**;**136**
14.27	p-Cymene	
14.47	Limonene	
14.68	1,8-Cineole	
15.72	γ-Terpinene	
15.82	**Pinocamphone**	41 ; 55 ; 69 ; **83** ; 95 ; 110 ; **152**
17.07	**Allo-Ocimene**	41 ; 53 ; 65 ; 79 ; 91 ; 105 ; **121** ; **136**
17.12	**3-Iso-Thujanol**	43 ; 55 ; 67 ; 77 ; 81 ; **93** ; 107 ; 121 ; 136 ; **154**
17.53	**Linalool**	43; 55; 67 ; **71**;80;93;121;136; **154**
17.58	**p-Menth-1-en-8-ol**	43 ; **59**; 67; 81; 93; 107; 121; 136; **154**
21.33	Terpinen-4-ol	
21.54	p-Cymen-8-ol	
21.73	Naphtalene	
21.93	α-Terpineol	
22.53	Isopropyl benzoate	
23.82	Thymol, methyl ether	
23.87	Carvacrol, methyl ether	
24.28	2-Isopropyl-1-methoxy-4-methylbenzene	
26.27	**Isobornyl acetate**	43 ; 55 ; 80 ; **95** ; 108 ; 121 ; 136 ; 154 ; **196**
26.92	**Sec-butyl benzoate**	56 ; 77 ; **105** ; 123 ; 154 ; **177**
28.27	Unidentified Sesquiterpene 1	67 ;77 ;91 ;105 ;**119** ;131 ;145 ;159 ;174 ;187 ;**204**
28.67	Unidentified Sesquiterpene 2	41;57;79;**91**;107;119;133;148;161;189;**204**
28.78	**Exo-2-hydroxy-1,8-cineole acetate**	**43**;71;82;93;126;137;**212**
28.97	Unidentified Sesquiterpene 3	41 ; 53 ; 65 ; 79 ; 91 ; 107 ; **122** ; 147 ; 162 ; **204**
29.27	**α-cubebene**	41;55;69;81;91;**105**;119;133;161; **204**
30.17	Cyclosativene	
30.22	Cyperene	
30.28	Italicene	
30.62	α-Copaene	
30.78	α-Ylangene	
30.87	β-Elemene	
31.92	α-Cedrene	
32.63	cis-α-Bergamotene	
33.23	β-Caryophyllene	
33.62	trans-α-Bergamotene	
34.62	α-Humulene	
35.22	Ar-Curcumene	
35.42	Sesquisabinene	
36.22	β-Bisabolene	
36.56	**Caryophyllenyl alcohol**	55 ; 69 ; 81 ; 95 ; **111** ; 123 ; 161 ; 179 ; 189 ; 204 ; **222**
36.88	δ-Cadinene	

37.72	**epoxide, cis-Farnesene**	41 ; 59 ; 79 ; 105 ; 119 ; 134 ; 159 ; 220
38.13	**trans Sesquisabinene hydrate**	69; 82; 93;109 ; 119; 137; 161; 179; 189; 204 ; 207 ; **222**
39.83	Caryophyllene oxide	
40.08	Unidentified Sesquiterpene 4	69 ;**79** ;93 ;107 ;119 ;138 ;149 ;161 ;177 ;189 ; 204 ;**220**
40.59	Humulene epoxide II	
41.33	α-Acorenol	
41.74	Caryophylla-4(14),8(15)-dien-5-ol	
42.54	Unidentified Sesquiterpene 5	
42.83	**Epi-β-bisabolol**	41 ; 55 ; 69 ; **82** ; 93 ; 111 ; 119 ; 161 ; 189 ; 204 ; **222**
43.04	14-hydroxy-9-epi-(E)-caryophyllene	
47.43	Unidentified Diterpene 1	
53.14	Unidentified Diterpene 2	
56.56	Manool	

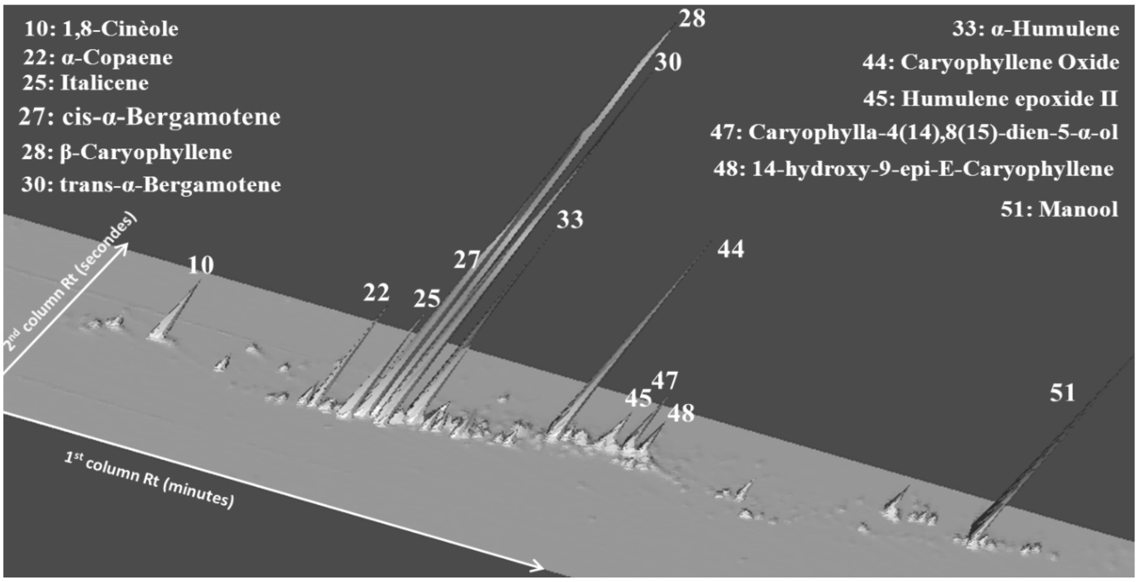

Figure 3: 3D view of GC×GC chromatogram of Mbaka exudate essential oil with some terpenic compounds'identification

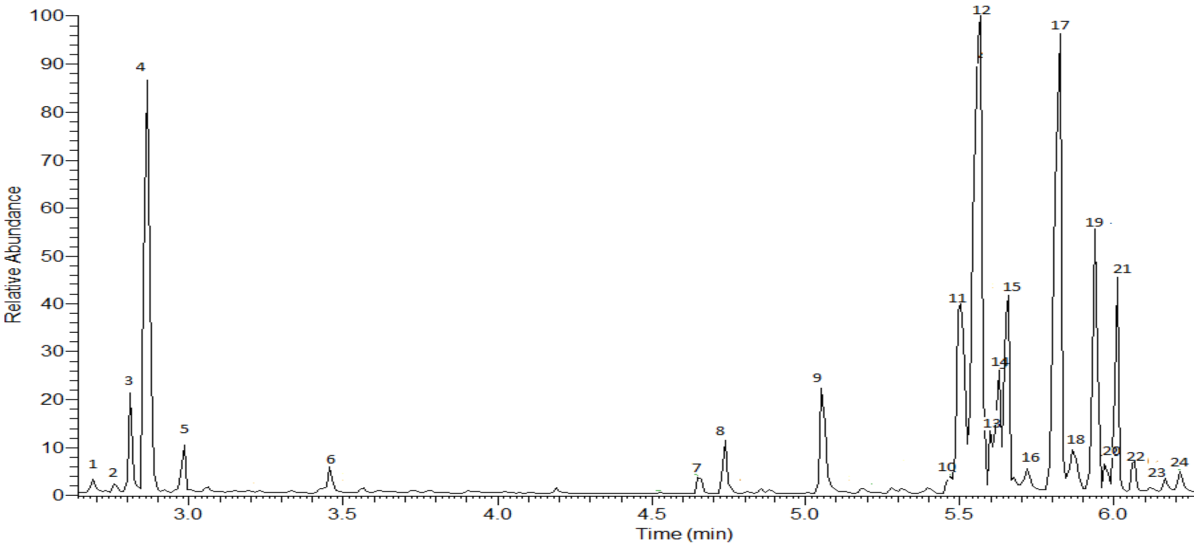

Figure 4: Chromatogram of the petroleum ether extract of copal Mbaka after derivatisation with TMS-diazomethane. The identification of the numbered peaks is listed in Table 4.

Table IV: Terpenoids identified in the petroleum ether extract of copal by HT-GC-MS.

- Base peak in bold
- Molecular mass in bold and underlined
- Molecular absent on the spectrum between parenthesis, in bold and underlined

n.	Compound	Rt	m/z values of characteristic fragment
1	α-Copaene	2.69	55, 69, 81, 91, 105, 119, 133, 147, **161**, 189, **204**
2	α-Cedrene	2.76	55,69,77,93,105,**119**,136,147,161,175,189,**204**
3	α-Bergamotene	2.81	79, 91, 93, 107, **119**, 120, 133,147, 161, 189, **204**
4	E-Caryophyllene	2.87	79,91 93, 105, 119, **133**, 147, 161, 175, 189, **204**
5	α-Humulene	2.99	79, 80, 91, **93**, 107, 121, 147, 148, 189, **204**
6	Caryophylene oxide	3.46	**79**, 91, 93, 109, 121, 123, 135, 149, 161, 177, 187, 205
7	Labda-8(20), 12,14-triene (enantio-Biformene)	4.65	81, 90, 109, 123,137, 149, 161, 175, 187, 203, 216, 229, 243, **257**, **272**
8	Unidentified diterpene	4.74	79,81,91,93,95,107,123,**137**,147,159,173,177,189,191,204, 229, 244, 262, **272**
9	Epi-Manool	5.05	81, 95, 123, **137**, 257, 272, (**290**)
10	Labd-8-en-15-oic acid (iso-Eperuic acid), methyl ester	5.47	93, 95, 109,121, 136, **191**, 238, 277, 305, **320**
11	Ozic acid, methyl ester	5.50	79, 93, 107, **121**, 163,187, 241, 257, 269, 301, **316**
12	Labd-8(20)-en-15-oic acid (Eperuic acid), methyl ester	5.57	55, 69, 81, 95, 123, **137**, 177, 191, 223, 277, 289, 305, **320**
13	Unidentified	5.60	81, 93, 107, 121, 147, 161, 191, **257**, 273
14	Isomer of (10)	5.63	95, 109, 121, 136, 163, **191**, 223, 238, 251, 277, 305, **320**
15	Labd-7-en-15-oic acid (Cativic acid), methyl ester	5.66	95, 107, 109, 122, **191**, 196, 277, 289, 305, **320**
16	Unidentified	5.72	73, 137, 156, 187,229, 244, **257**, 272, 277,347
17	Labd-8(20),13-dien-15-oic acid (Copalic acid), methyl ester	5.82	55, 79, 81, 95, **114**, 123, 137, 205, 244, 271, 287, 303, **318**
18	Labd-7,13-dien-15-oic acid, methyl ester	5.87	95,107,121,135,**189**,191,286,303,**318**
19	Unidentified labdane	5.94	81, 109, 135, 161, 175, 189,204, 243, 253, 271, 287, **318**
20	Methyl labdanolate	5.97	71, 101, 125, **144**, 177, 185, 235,267, 291,305, 320, 338
21	Cleroda-3,13-dien-15-oic acid (kolavenic acid), methyl ester	6.01	93, 95, 107, 109, 121, 123, 149, 175, **189**,190, 191, 243, 271, 303, **318**
22	Unidentified	6.07	82, 137, 156, 172, 197, 229, **244**, 258, 286, 361,**376**
23	Unidentified	6.21	71, 95, 109, 121, 135, 149, 163, **191**, 204, 220, 257, 272, 361
24	Unidentified	6.29	82, 95, 109, 114, 123, 138, 163, 177, 191, 205, 243, **266**, 276, 289, 304, 377, **393**

α-Terpineol, for instance, has been shown to be insecticidal against *Plutella xylostella*, along with terpinen-4-ol, both identified as active components in *Artemisia lavandulaefolia* essential oil (Huang et al., 2022). α-Pinene demonstrated significant toxicity against Sitophilus zeamais, a major pest of stored maize (Langsi et al., 2020). Limonene, a major constituent of citrus peels, was identified as responsible for the insecticidal activity against *Callosobruchus chinensis* (Gupta et al., 2025). Finally, 1,8-cineole, the main component of Eucalyptus cinerea essential oil, showed insecticidal effects against *Ectomyelois ceratoniae* and *Ephestia kuehniella* (Achouri et al., 2024).

4.3. GC×GC–MS analysis of essential oils

Thanks to GC×GC–MS analysis, traces of several minor compounds were identified, including camphene, pinocamphone, α-terpinene, 2,3-dehydro-1,8-cineole, allo-ocimene, 3-isothujanol, linalool, p-ment-1-en-8-ol, isobornyl acetate, sec-butylbenzoate, exo-2-hydroxy-1,8-cineole acetate, trans-sesquisabinene hydrate, cis-farnesene epoxide, caryophyllenyl alcohol, α-cubebene, and epi-β-bisabolol. Some of these compounds have been reported to exhibit significant biological activities, including antimicrobial and insecticidal effects.

Recent studies have confirmed the insecticidal potential of several of these minor constituents. For example, camphene has demonstrated significant toxicity against *Helicoverpa armigera*, suggesting its possible application as a botanical insecticide (Benelli et al., 2017). Likewise, epi-β-bisabolol, along with other components of *Matricaria chamomilla* essential oil such as (E)-β-farnesene and germacrene D, exhibited strong insecticidal activity against Helicoverpa armigera and Aedes vittatus (Achouri et al., 2023).

These findings support the hypothesis that the insecticidal effect of the volatile fraction of this exudate is likely not due solely to the major compounds, but also to synergistic interactions involving the minor constituents.

4.4. HT- GC-MS analysis

The presence of labdane diterpenoids was confirmed. Labd-7-en-15-oic acid (cativic acid) and Labd-8(20)-en-15-oic acid (eperuic acid) methyl esters were found in copal Mbaka. The corresponding mass spectra show a molecular peak at m/z 320. The peak at m/z 191 corresponds to the loss of the side chain after allylic cleavage of the C(9)-C(11) bond. The presence of a six-membered ring bearing a double bond in cativic acid involves immediate retro Diels-Alder fragmentation giving the ion at m/z 124, which even after loss of a methyl group led to the ion m/z 109. A characteristic fragmentation reaction of labdanoid having a C(8)-C(20) double bond is the cleavage of the C(9)-C(10) bond accompanied by hydrogen transfer, giving an ion comprising ring A. Then an abundant ion is obtained at m/z 137 for eperuic acid. The HT-GC-MS analysis of Mbaka copal revealed the presence of approximately twenty-four compounds, including sesquiterpenes (eluted between 2 and 3 minutes), diterpenes (eluted between 4.6 and 5.1 minutes), and diterpenic acids (eluted between 5.5 and 6.5 minutes). This copal is distinguished by a specific profile of diterpenic acids, characterized by a significant amount of eperuic, copalic, cativic, ozic, and kolavenic acids.

Compared to other copals described in the literature, it is evident that diterpenic resins can be grouped into two main categories. The first group includes resins derived from conifers, belonging to families such as *Pinaceae, Cupressaceae*, and *Araucariaceae*. The second group comprises trees from the subfamily *Caesalpinioideae*, which belong to the large Fabaceae family (Cartoni et al., 2004). This latter group is relevant for Mbaka copal, which comes from the species *G. demeusei* within this family.

The results obtained for Mbaka copal corroborate the work of van den Berg et al. (1999), who reported the presence of free acids in other Congolese copals, including eperuic, cativic, and copalic acids. In contrast, samples of copals from the Congo are generally dominated by acids such as ent-labd-8(20)-en-15-oic, isomers of ent-labdan-8-ol-15-oic acid, and kovalene acids (Steigenberger, 2013). While these compounds are also present in Mbaka copal, the latter stands out due to its high concentration of specific acids, such as cativic acid, which is not always predominant in other copals.

The work of Hugel et al. (1966) also highlighted the presence of large quantities of copalic acid, as well as other diterpenic acids. However, Mbaka copal appears to exhibit a diversity of specific diterpenes and acids that have not been as thoroughly documented in other sources of Congo copal, such as cativic acid.

Other studies, such as those by Mills and White (1994), show that the copal from Zanzibar, derived from *Hymenaea verrucosa*, also contains ozic acid, the isomer of iso-ozic acid, as well as eperuic and copalic acids, reinforcing the idea of a chemical relationship between these resins.

Moreover, the similarity in the chemical composition of the non-volatile fraction of Mbaka copal with that of Jatobá resin or Brazilian copal (Hymenaea) is striking. Brazilian copals are primarily known for their high content of enantio-labdanoid diterpenes (Langenheim, 2003). The results reported by Doménech-Carbo on the resin of Hymenaea stigonocarpa also show a profile close to that of Mbaka copal, although some compounds, such as iso-ozic acid and epi-pinifolic acid, seem to be absent in the studied sample. The observed differences may result from distinct sample preparation techniques, particularly pyrolysis.

Conclusions

The Mbaka copal, used by the people of Likouala for lighting at night, is reputed for its insect-repellent properties. Infrared analyses have revealed that it is a terpenoid resinous exudate of copal type. The study of its volatile fraction showed that the Mbaka exudate contains mono, sesquiterpenes, and diterpenes. A comparison between hydrodistillation (HD) and headspace solid-phase microextraction (HS-SPME) methods revealed slight quantitative differences but no qualitative differences in the analysis of the volatile fraction.

The essential oil of copal is primarily composed of sesquiterpenes, with the main compounds being β-caryophyllene (40.0%), trans-α-bergamotene (15.4%), cis-α-bergamotene (10.4%), α-humulene (6.9%), and caryophyllene oxide (5.6%). The predominance of β-caryophyllene could explain its use as a repellent. Additionally, the analysis using the recent GCxGC-MS technique allowed the detection of additional compounds, such as linalool, which may also contribute to this repellent effect. This study also identified the main components of the non-volatile fraction present in the exudate.

Eperuic, copalic, ozic, and cativic acids are the primary components of this copal fraction. Other compounds, such as iso-eperuic and kolavenic acids, were identified as minor components. The presence of cativic acid distinguishes this copal from other Congolese copals described in the literature. Furthermore, the non-volatile fraction of this copal shows notable similarities to copal samples from Latin America, particularly those from the genus Hymenaea (Fabaceae).

Finally, the solid appearance of the copal sample can be explained by the presence of ozic acid (an enantiomer of communic acid), which has a strong tendency to polymerize.

It is the first time that a study reports in detail the chemical composition of a Congolese copal with certified botanical origin, based on advanced analytical techniques such as GC×GC-MS and HT-GC-MS. Then, this study represents a major contribution to the chemical characterization of resins from Central Africa.

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