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Research Article

Characterization of *Paullinia pinnata* (Sapindaceae) Root Extracts by GC-MS and HPLC-ESI-QTOF-MS: An Ivorian Medicinal Plant at the Service of Cardiovascular Diseases

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Abstract

The study of chemical constituents is an essential element in the valorization of medicinal plants, which can be used to treat a number of pathologies. This work aims to contribute to a better understanding of the chemical composition of *Paullinia pinnata* (Sapindaceae), a medicinal plant used in C ôte d'Ivoire for the traditional treatment of cardiovascular diseases. This study explores the relationship between the compounds identified in this plant and its use in cardiovascular disease treatment. Extraction methods (solid/liquid and liquid/liquid) and identification by GC-MS and HPLC-ESI-QTOF-MS analyses were used. The GC-MS analysis of the various extracts (hexane, dichloromethane and ethyl acetate) identified 32 compounds consisting of steroids, triterpenes, fatty acids, alkaloids and phenolic compounds. HPLC-ESI-QTOF-MS analysis of these extracted memes identified 21 phenolic compounds. In total 53 compounds were identified with the two analytical methods used. These molecules identified are: palmitic acid, 9-(E)-octadecenoic acid, stigmasterol, β-Sitosterol, umbelliferone, epicatechin quinone, clemiscosin A, caffeic acid, catechin, epicatechin and O-β-D-glucopyranosyloxy-4-methyl-2 (5 H)-furanone, cinnamtannin B-2; cinnamtannin B1, procyanidin B2, epicatechin quinone, clemiscosin C, clemiscosin A, kaempferide and epicatechin. These compounds have been identified for their antioxidant, anti-free radical, cardiotonic, neuroprotective and anti-inflammatory properties. The presence of these compounds could justify the use of *Paullinia pinnata* in the traditional treatment of cardiovascular diseases.

Keywords

Phytochemical Study, *Paullinia pinnata*, GC-MS, HPLC-ESI-QTOF-MS

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1. Introduction

Cardiovascular disease, a group of disorders affecting the heart and blood vessels, is one of the world's leading causes of death. They affect vital organs such as the heart, brain, lungs and kidneys, and can also be a source of disability or chronic morbidity [1]. According to a study published by the World Health Organization (WHO), the number of deaths attributable to cardiovascular disease is estimated at 17.7 million, or 31% of global mortality. The direct cost of treating these diseases is very high [2]. In Africa, particularly in Côte d'Ivoire, despite government efforts to bring improved care services closer to the population, the morbidity rate is rising sharply due to a lack of financial resources, care materials and the high cost of medicines [3].

Which means that today cardiovascular diseases have become a real public health problem throughout the African region. To address this problem, WHO encourages African countries to develop regional strategies taking into account traditional medicine, to undertake research on medicinal plants and promote their optimal uses in health delivery systems [4]. What's more, the World Health Organization considers traditional medicine to be a vehicle for access to low-cost healthcare, since it uses mainly plant-based therapies. Plants have always been an important source of medicines for mankind. [5, 6]. The pharmacological properties of plants are justified by the active compounds they contain. In this context, a recent study carried out on plants with cardiovascular properties in Côte d'Ivoire showed that Paullinia pinnata possessed good antioxidant activity and a high total polyphenol content. [7]. In view of *Paullinia pinnata's* many therapeutic virtues (anticancer, antimicrobial and antioxidant) [8-11], it is important to carry out further phytochemical studies. The present study aims to extract and identify biomolecules by GC-MS and HPLC-ESI-QTOF-MS analysis, in order to justify its use in traditional medicine.

2. Materiel and methods

2.1. Materiel

The plant material consists of *Paullinia pinnata* root barks. They were collected in Adiapodoumé(southern Côte d'Ivoire) in November 2019. They were identified and authenticated by Mr. TEHE in com-parison with herbariums available (N085 DOUGOUNE 2006) at the National Center for Agronomic Research located in Adiapodoumé (NCAR-Côte d'Ivoire). The barks were dried at room temperature for 3 weeks in the laboratory, sheltered from the light. The dried bark was crushed using a grinder and sorted. The crushed material was stored at 10 ℃ until further use.

2.2. Methods

2.2.1. Sample Preparation

500 g of *Paullinia pinnata* root powder are macerated in 1500 mL of methanol (MeOH) with permanent mechanical stirring for 24 h. The operation is repeated 3 times with the same substrates. After filtration, the extracts obtained are combined, then concentrated at 40 ℃ on a rotary evaporator. The residue obtained is then taken up in aqueous methanol (10% MeOH). The solution obtained is successively extracted with hexane (3 ×100 mL), dichloromethane (3 ×100 mL) and ethyl acetate (3 ×100 mL). The different organic fractions are recovered and concentrated in a rotary evaporator under reduced pressure, then stored in the refrigerator for later use.

2.2.2. GC-MS Analysis

Volatile compounds were analyzed by GC-MS. This consisted of an Agilent LC-MS system combined with a Shimadzu HPLC system, model QP2010SE. For this purpose, derivatization was carried out using three (3) mg of each extract (hexane, dichloromethane and ethyl acetate) obtained after extraction, added to 0.5 ml of distilled CH_2Cl_2 and 0.2 ml of MSTFA (N-methyl-N-(trimethylsilyl) trifluoroacetamide).

After 12 h incubation at room temperature, the CH_2Cl_2 -MSTFA mixture was condensed on a rotary evaporator. The dry residue was taken up with 1 ml CH_2Cl_2. The volume of extract injected using a pillbox was 1 µL. The initial oven temperature was 50 ℃ for 2.5 min, and the gradual temperature increase was programmed at 22 ℃/min up to 250 ℃, and maintained at this temperature for 30 min. Injector and detector temperatures were set at 200 ℃ and 280 ℃ respectively. Injection was performed in Scan mode. The mass spectrometer parameters for the electron impact mode were 280 ℃ for temperature, 70 eV for electron energy, 50 scans/s for scan speed and 10,000 u.m.a/s for acquisition speed. Compounds were identified by comparing the recorded spectra with those in the database (NIST08.LIB), and by referring to data in the literature.

2.2.3. HPLC-ESI-QTOF-MS Analysis

The analysis of thermosensitive compounds or very large and/or polar molecular masses was carried out by HPLC-ESI-QTOF-MS. It is composed of an Agilent LC-MS system associated with an Agilent 1260 Infinity HPLC system coupled to an Agilent 6530 Q-TOF-MS equipped with a positive ESI source.

Samples were prepared with one (1) mg of each extract (hexane, dichloromethane and ethyl acetate) dissolved in one (1) ml of HPLC grade methanol. The sample injection volume was set at 5 µL. A Sunfire C18 analytical column (150 × 2.1 mm; 3.5 µm, Waters) was used, with a flow rate of 250 µL/min and a linear two-solvent gradient: solvent A (95-0% H_2O + 0.1% formic acid), solvent B (5-100% B (ACN), organic solvent) for 30 minutes. ESI conditions were defined

with a capillary temperature of 320 ℃, a source voltage of 3.5 kV and a gas flow rate of 10 L/min. In the positive ion mode, purine $C_5H_4N_4$ (ion at m/z 121.050873 g/mol) and phosphazene $C_{18}H_{18}F_{24}N_3O_6P_3$ (ion at m/z 922.009 798 g/mol) were used as internal locking masses. Full scans were acquired at a resolution of 11,000 (at m/z 922). The chromatographic characteristics of the main compounds detected on the various chromatograms were determined using UV spectrometry at 254 and 280 nm and high-performance liquid chromatography-mass spectrometry. These data were compared with those in the literature3.

3. Results and Discussion

3.1. Liquid / Liquid Extraction

The yield of the liquid-liquid extraction of *Paullinia pinnata's* extract is shown in Table 1.

Table 1. *Liquid-liquid extraction yield of the methanol extract of Paullinia pinnata.*

Extracts	Extracts weight (g)	Yields (%)
Paullinia pinnata		
Hexanic	4.650	0.93
Dichloroethane	0.350	0.07
Ethyl acetate	3	0.6

The liquid-liquid extraction yield of the methanol extract of *Paullinia pinnata* made it possible to notice a variation in yield from one extract to another. The hexane and ethyl acetate extracts of *Paullinia pinnata* showed better yields compared to that of di-chloromethane. The highest yields are obtained with the hexane (0.93%) and ethyl acetate (0.6%) extracts. These two different extracts would contain more mass-extractable phytocompounds than in dichloromethane.

3.2. Characterization of Paullinia Pinnata Extracts by GC-MS

Gas chromatography coupled with mass spectroscopy (GC-MS) was carried out with the aim of identifying the different compounds contained in the root fractions of Paullinia pinnata. The identification of the different characteristic peaks was carried out by comparing the mass fragmentations with those available in the NIST 98 and Wiley 275 libraries and with those published in the literature. Figures 1, 2 and 3 present the GC chromatograms of the different extracts.

3.3. Characterization by HPLC-ESI-QTOF-MS of the Various Extracts from *Paullinia pinnata*

The different molecules detected were characterized by the study of their fragmentations obtained by ESI-QTOF-MS in positive mode, in comparison with data obtained from the literature on botanical species or in the genus. The chromatograms obtained in positive mode (ESI-QTOF-MS) of the different extracts of Paullinia pinnata were observable under UV 254nm. Figures 4, 5 and 6 present the HPLC chromatograms of the different extracts.

The GC-MS chromatographic profile of the hexane extract (Figure 1) of *Paullinia pinnata* identified 7 compound peaks, consisting of 2 (two) steroids, 1 (one) alcohol and 1 (one) alkaloid and 3 (three) fatty acids.

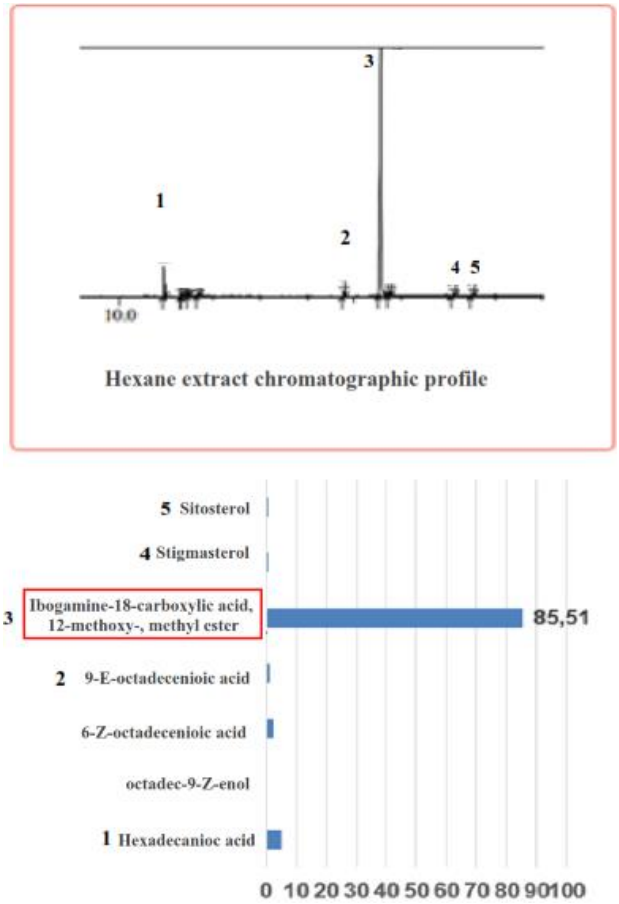

Figure 1. *GC-MS analysis of the Paullinia pinnata hexane extract.*

Retention times range from 10.94 min to 17.51 min. The main components are: hexadecenoic acid (tr=10.94; 4.95%), octadec-9-Z enol (tr=11.31; 0.55%), 6-Z-octadecenoic acid (tr=11.38; 2.4%), 9-Eoctadecenoic acid (tr= 11.68; 1.07%), acetate-3-β stigmasta-5,22-dien-3-ol (tr= 17.10; 0.97%), sitosterol (tr= 17.51; 0.92%) and Ibogamine-18-carboxylic acid, 12-methoxy-, methyl ester (tr= 15.56; 86.51%), as the

majority peak.

The GC-MS chromatographic profile of the dichloromethane extract (*Figure 2*) of *Paullinia pinnata* made it possible to identify 11 peaks of compounds consisting of 3 (three) steroids, 4 (four) alcohols, 1 (one) triterpene and 3 (three) fatty acids. The retention time ranged from 10.17 min to 17.90 min. The main components are: nonadecanol (tr=10.17; 3.5%), oct-tadecanoic acid (tr= 10.53; 10.94%), 9-octadecene-1-ol (tr= 11; 11.35%), heneicosan-1-ol (tr= 11.10; 5.55%), 9-Z-hexadecenal (tr= 11.32; 11.1%), octadecanoic acid (tr= 11.41; 3.35%), eicosane (tr= 12.36; 2.34%), 3-β-ergost-5- ѐn-3-ol (tr= 16.84; 3.57%), stigmasterol (tr= 17; 3.84%), lup-20(29)- ѐn-3-one (tr=17.90; 2.91%) and sitosterol (tr=17.41; 15.84%) as the majority peak.

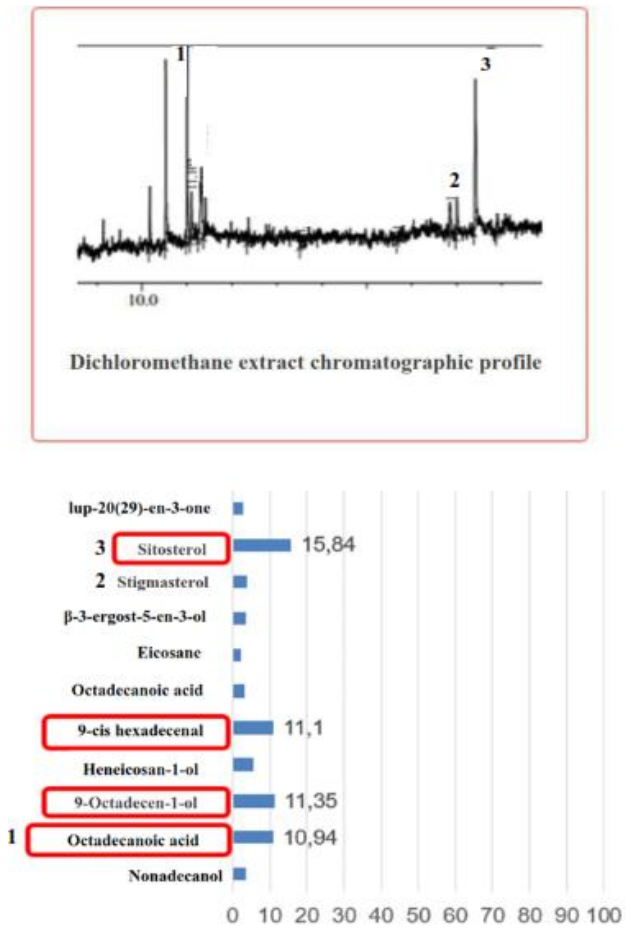

Figure 2. *GC-MS analysis of the Paullinia pinnata dichloromethane extract.*

The GC-MS chromatographic profile of the ethyl acetate extract (Figure 3) of Paullinia pinnata identified 14 compound peaks, grouped into 3 (three) steroids, 1 (one) alcohol, 1 (one) alkaloid, 1 (one) glycoside, 3 (three) polyphenols and 5 (five) fatty acids.

Retention times ranged from 5.93 min to 17.51 min. The main components identified were: phenol (tr= 5.93; 0.38%), glycerol (tr= 6.74; 4.78%), nonanoic acid (tr= 7.38; 0.28%), catechin (tr= 9.97; 0.83%), oleanitrile (tr= 11.15; 4.71%), octadec-9-Z-enol (tr= 11.30; 1.82), heptadecanoic acid (tr= 11.36; 0.62%), 9-E-octadecenoic acid (tr= 11.67; 3.72%), scopoline (tr= 12.18; 0.29%), β-D-glucopyranosyl thymol (tr= 12.54; 0.19%), cholesterol (tr= 16.34; 0.29%), stigmasterol (tr= 17.10; 1.83%), sitosterol (tr= 17.51; 4.84%) and palmitic acid (tr= 10.94; 8.61%) as the major peak. In summary, GC-MS analysis of various Paullinia pinnata root extracts identified twenty-nine (29) compounds, including palmitic acid, 9-(E)-octadecenoic acid, stigmasterol, sitosterol and catechin, recognized for their antioxidant, antiradical, cardiotonic, neuroprotective, hypocholesterolemic, im-munostimulant and inflammatory properties [12-16].

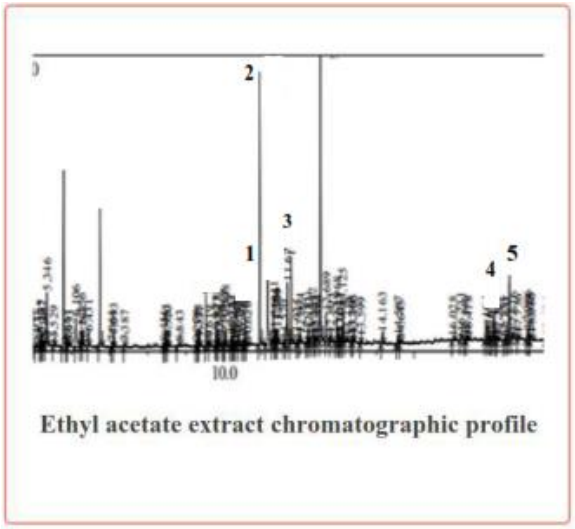

Figure 3. *GC-MS analysis of the Paullinia pinnata dichloromethane extract.*

Figure 4 shows the HPLC-ESI-QTOF-MS chromatogram of the hexane extract of *Paullinia pinnata*. It identified seven (7) peaks of polyphenol-type compounds. These are umbelliferone (tr= 21min), epicatechin quinone (tr= 22.13 min), clemiscosin A (tr= 23 min), caffeic acid (tr= 24.87 min),

catechin (tr= 32.26 min), O-β-D-glucopyranosyloxy -4-methyl-2 (5 H)- furanone (tr= 35.18 min) and epicatechin (tr= 33.35 min) as the major peak.

Figure 5 shows the HPLC-ESI-QTOF-MS chromatogram of the dichloromethane extract of Paullinia pinnata, identifying five (5) compound peaks grouped into five (5) polyphenols. Scopoletin (tr= 19.24 min), umbelliferone (tr= 20.94 min), clemiscosin C (tr= 22.13 min), kaempferide (tr= 28.66 min) and O-β-D-glucopyranosyloxy -4-methyl2 (5 H)-furanone (tr= 33.15 min) as majority peak.

Figure 6 shows the HPLC-ESI-QTOF-MS chromatographic profile of the ethyl acetate extract, identifying nine (9) peaks of compounds grouped into nine (9) polyphenols. O-β-D-glucopyranosyloxy -4-methyl-2 (5 H)-furanone (tr= 2.05 min), cinnamtannin B-2 (tr= 12.67 min), procyanidin B-2(tr= 15.84 min), epicatechin quinone (tr= 16.16 min), clemiscosin C (tr= 22.79 min), clemiscosin A (tr= 23.33 min), kaempferide (tr= 32.70 min), epicatechin (tr= 33.10 min) and cinnamtannin B-1(tr= 15.59 min) as the major peak.

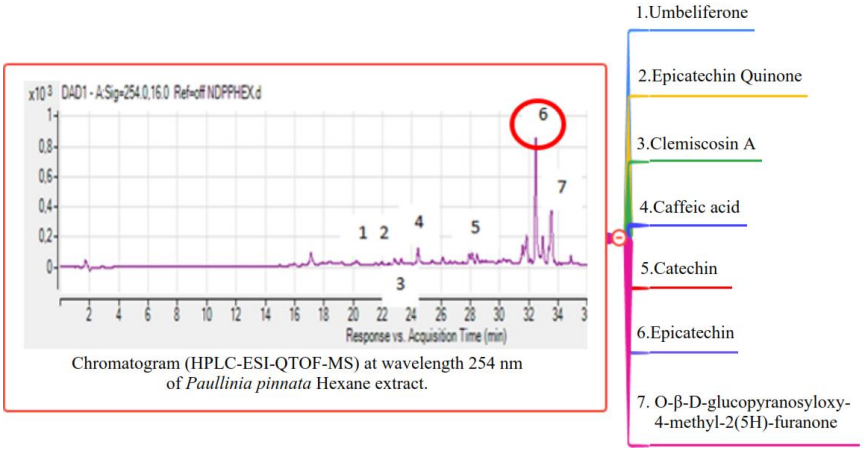

Figure 4. *HPLC-ESI-QTOF-MS analysis of the Paullinia pinnata hexane extract.*

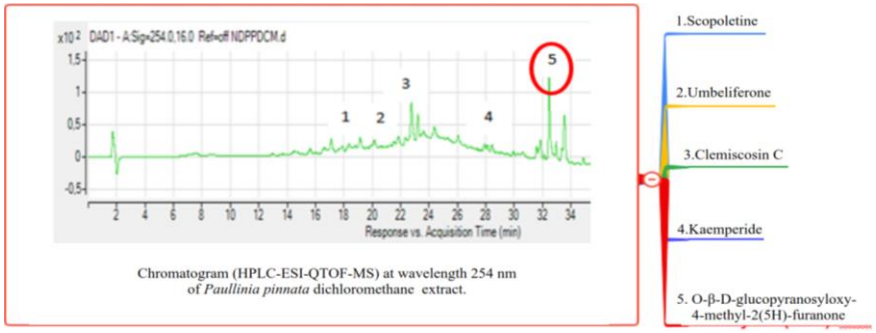

Figure 5. *HPLC-ESI-QTOF-MS analysis of the Paullinia pinnata dichloromethane extract.*

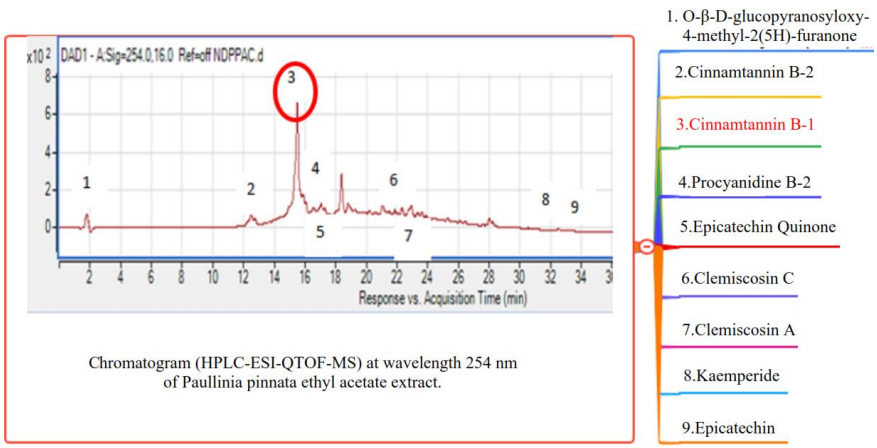

Figure 6. *HPLC-ESI-QTOF-MS analysis of the Paullinia pinnata ethyl acetate extract.*

HPLC-ESI-QTOF-MS analyses of various *Paullinia pinnata* root extracts identified fourteen (14) compounds, including umbeliferone, epicatechin quinone, clemiscosin A, caffeic acid, catechin, epicatechin and O-β-D-glucopyranosyloxy -4-methyl-2 (5 H)-furanone, cinnamtannin B-2, cinnamtannin B-1, procyanidin C-1, procyanidin B-2, scopoletin, clemiscosin C and kaempferol (figure 7) are well-known for their antioxidant, antiradical, cardiotonic and hypocholesterolemic neuroprotective properties; Immunostimulant and inflammatory [17-22].

The present work has highlighted the various compounds contained in Paullinia pinnata. The biomolecules identified by CPG-MS analysis are in line with the work carried out by Ouattara et al., 2019. Indeed, according to the studies carried out by this team, the molecules identified consist of fatty acids, polyphenols and carbohydrates [23]. Our results obtained after HPLC-ESI-QTOF analysis confirm those carried out by Kassi et al. Their studies revealed that Paullinia pinnata possesses significant antioxidant properties due to the presence of polyphenols (flavonoids) and alkaloids in extracts from this plant [7]. These bioactive metabolites present in Paullinia pinnata are associated with beneficial effects on heart health. These compounds may help reduce inflammation and improve blood circulation [7]. This is crucial for the prevention of cardiovascular disease.

The literature shows that plants rich in polyphenols are associated with a reduced risk of cardiovascular disease. These studies validate that treatment of cells with polyphenols counteracts the oxidative stress burden and influences signaling pathways to reduce risk associated with cardiovascular disease, and confirms their therapeutic efficacy [24, 25].

Its antihypertensive and antioxidant properties, as well as the cardioprotective effects of the molecules present in this plant, make it a preferred choice in traditional medicine.

In short, Paullinia pinnata is valued in traditional medicine for its potential effects on cardiovascular health, underlining the importance of scientific research to better understand and validate these traditional uses.

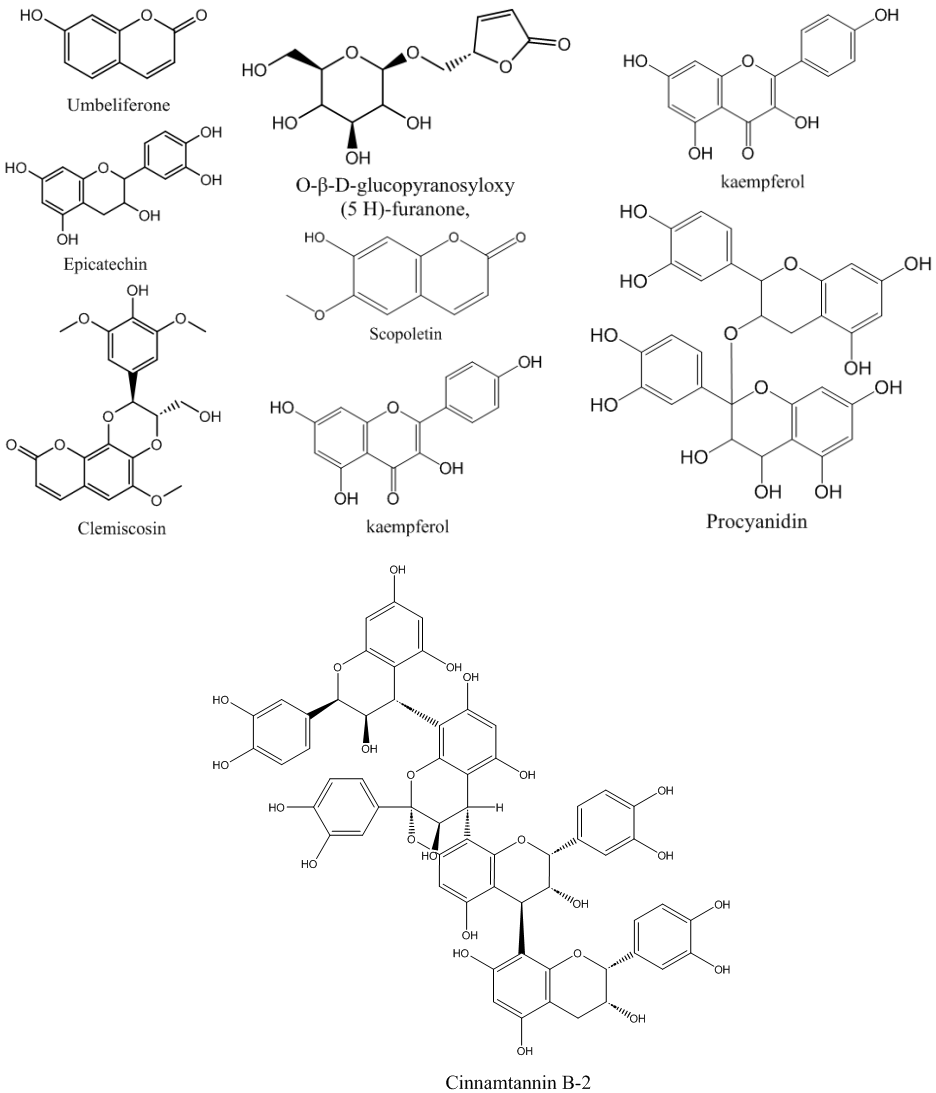

Figure 7. *Structures of compounds identified in different extracts from pp roots.*

4. Conclusion

The completion of this work is part of a better knowledge of the chemical composition of a medicinal plant (Paullinia pinnata) from Ivory Coast, used in the traditional treatment of cardiovascular diseases. This, through the discovery of biomolecules with antioxidant properties in order to justify its use in traditional medicine. To do this, two chromatography methods coupled with mass spectrometry were used. Namely gas chromatography coupled with mass spectroscopy (GC-MS) and high-performance liquid chromatography coupled with mass spectrometry and quadrupole-Time-of (HPLC-ESI-QTOF-MS). GC-MS analyzes of different extracts (hexane, dichloromethane and ethyl acetate) of Paullinia pinnata roots made it possible to identify twenty-nine (29) compounds including palmitic acid, 9-acid (E) octadecenoic acid, stigmasterol, sitosterol, catechin are recognized for their antioxidant, antiradical, cardiotonic, neuroprotective, hypocholesterolemic; immunostimulating and inflammatory. The HPLC-ESI-QTOF-MS analysis of the different extracts (hexane, dichloromethane and ethyl acetate) of Paullinia pinnata made it possible to identify fourteen (14) compounds including umbelliferone, epicatechin quinone, clemiscosin A, caffeic acid, catechin, epicatechin and O-β-D-glucopyranosyloxy -4-methyl-2(5H)-furanone, cinnamtannin B-2, cinnamtannin B-1, procyanidin C-1, procyanidin B-2, scopoletin, clemiscosin C, kaempferide are recognized for their antioxidant, anti-radical, cardiotonic, hypocholesterolemic neuroprotective properties; immunostimulating and inflammatory. These results obtained show that Paullinia pinnata root extracts are made up of molecules with antioxidant, neuroprotective and inflammatory properties, which would justify the use of this plant in the treatment of cardiovascular diseases.

Paullinia pinnata is a key component of the traditional pharmacopoeia for cardiovascular disease. Its hypotensive, antioxidant and circulatory properties make it a valuable plant for cardiac well-being. However, its use must be regulated to ensure its effectiveness and avoid risks.

Abbreviations

HPLC	High Performance Liquid Chromatography
MS	Mass Spectrometry
GC	Gas Chromatography
UV	Ultra-Violet
ESI	Electrospray Ionization
QTOF	Quadrupole Time-of-Flight

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Author Contributions

Jeanne Bervis Adjadi: Formal analysis, Writing – original draft, Writing – review & editing

Amian Brise Benjamin Kassi: Conceptualization**,** Supervision, Validation, Funding acquisition

Daouda Ballo: Conceptualization**,** Supervision, Funding acquisition

Nikiema Diakaridja: Formal analysis, Writing – review & editing

Virieux David: Supervision

Jean-Luc Pirat: Supervision

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Conflicts of Interest

The authors declare no conflicts of interest.

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