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# Antibacterial Activity and Identification of Phenolic Compounds of Ethanolic Extracts from *Pseudocedrela kotschyi* Scheinf Harms

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## Abstract

*Pseudocedrela kotchsyi's* roots and trunk bark are overused in Togolese traditional medicine to cure various illnesses. Nevertheless, there is currently no scientific proof to support the claim that the leaves of this plant are less useful in herbal medicine than the root and trunk barks. This study aimed to identify the phenolic compounds and compare the antibacterial activities of extracts from the leaf, trunk bark, and root bark of the plant. Each part of the plant was macerated successively in three solvents of increasing polarity: hexane, dichloromethane, and ethanol. Usual methods of characterization were adopted to determine the phytochemical composition of each ethanolic extract. The antibacterial activity of the extracts was evaluated using the diffusion method in solid medium and by microdilution assay in liquid medium, to determine their MIC and MBC, respectively. The phenolic compounds in the extracts were quantified using HPLC-DAD analysis. Then, the identification of compounds in the leaf and root bark extracts was achieved using UHPLC-HRMS analysis. According to the results, the trunk bark yielded the best extraction (12.33 ± 1.43%). Phytochemical screening showed the presence of alkaloids, saponins, phenolic compounds, flavonoids, and tannins in all extracts. The trunk bark showed the most interesting antibacterial activity against three bacterial strains, including *Escherichia coli* ATCC 22925, *Pseudomonas aeruginosa*, and *Salmonella typhimurium*, with MICs ranging from 6.25 to 25 mg/mL and MBCs ranging from 6.25 to 50 mg/mL. Most of the compounds identified by HPLC-DAD and UHPLC-HRMS analyses could be responsible for the antibacterial activities exhibited by the plant extracts. These identified

compounds justify the frequent use in traditional medicine of this plant for the treatment of various diseases.

## Keywords

*Pseudocedrela kotschyi*, Phytochemicals, HPLC-DAD Analysis, UHPLC-HRMS Analysis, Antibacterial Activity

## 1. Introduction

Antibiotics are products often used against bacteria. Their discovery since the 1950s has led to progress in the treatment of infectious diseases of bacterial origin. However, their misuse and sometimes inappropriate use have led to the proliferation of resistant bacterial strains [1] and an increase in the rate of therapeutic failure. The search for new and more effective drugs against these multidrug-resistant bacterial strains is a major challenge that requires urgent attention from researchers [2]. To meet this challenge, many scientific studies are focusing on medicinal plants. In fact, medicinal plants represent a primary source of secondary metabolites, whose isolation has led to the development of several effective pharmaceutical agents. Moreover, it is estimated that about 25% of modern medicines available worldwide are derived from plants [3]. Polyphenols are a class of secondary metabolites known for their antibacterial activity [4]. Notably, polyphenols are widely applied in the food industry, cosmetics, and in folk medicine for their antimicrobial properties [4].

Furthermore, [5] and [6] reported that polyphenols, owing to their structural diversity, have antibacterial activity [7] [8] against gram-positive and gram-negative bacterial pathogens [9]. According to their action, polyphenols exert their antibacterial activity through multiple mechanisms. They can disrupt the bacterial cell membrane and cell wall integrity, while also acting internally by inhibiting enzymes, toxins, and biofilm formation [10].

Phenolic compounds have been reported to act on bacterial DNA and RNA, as well as protein synthesis in bacterial cells [11] [12]. The antibacterial activity of these compounds is linked to the number of hydroxyl groups on their aromatic rings, with evidence indicating that greater hydroxylation results in increased microbial toxicity [2] [13]. Studies have shown that oxidation of phenolic compounds enhances their inhibitory effect on microorganisms [14] [15], as the resulting quinones and reactive intermediates disrupt microbial cellular functions.

According to observations on the antibacterial efficacy of phenolic compounds, flavonoids and tannins showed stronger antimicrobial activity compared to other classes of phenols [10]. Therefore, they are used in the treatment of bacterial infections, including respiratory diseases (angina, bronchitis, tuberculosis, meningitis), gastrointestinal infections (gastroenteritis, salmonellosis, diarrhea, cholera, food poisoning such as botulism), urinary tract infections, and skin infections

(paronychia, abscesses).

Currently, in Togo, 60% - 80% of the population, mainly those of rural origin, use medicinal plants as the most accessible method for treating illnesses [16].

*P. kotschyi*, a monotypic plant of the genus Pseudocedrela, belonging to the Meliaceae family, is widely used in several African countries by traditional practitioners to treat various diseases such as ulcers, leprosy, diarrhea, dental caries, itching, high blood pressure, hemorrhoids, epilepsy, malaria, asthma, fever, yaws, diabetes, and many others [17]-[21]. Several scientific studies have shown that this plant possesses multiple biological properties, notably impressive antioxidant [22] [23], anticancer [24], anti-diarrheal [25] [26], antidiabetic [27], antimicrobial [28] [29], hepatoprotective, antimalarial, anti-inflammatory, analgesic, antibacterial, and antipyretic [30] activities. Furthermore, numerous qualitative phytochemical studies on *P. kotschyi* have shown that its organs are abundant in bioactive compounds, including alkaloids, triterpenes, anthraquinones, anthocyanins, flavonoids, tannins, and saponosides.

In summary, *P. kotschyi* has been the subject of numerous studies on its therapeutic properties. However, few studies have investigated the phenolic compounds present in different parts of the plant, including the leaf, trunk bark, and root bark. Therefore, it is important to conduct comprehensive research on the chemical composition of this plant and its therapeutic potential in Togo. In this context, the present study aims to identify the phenolic compounds in the three organs (leaf, trunk bark, and root bark) and to compare their antibacterial activities.

## 2. Materials and Methods

### 2.1. Plant Material

*P. kotschyi* was harvested in September 2021 at Wahala in Haho prefecture (Plateaux Region), Togo. The plant was identified and authenticated under the number "TOGO15976" by the specialists of the Laboratory of Botany and Plant Ecology of the Faculty of Sciences, University of Lomé, Togo. The leaves, trunk bark, and root bark were separated, air-dried at room temperature (20˚C - 25˚C), ground, and stored under conditions protected from light and moisture until further analysis.

### 2.2. Biological Material

Four bacterial strains were used to evaluate the antibacterial activity of the ethanolic extracts of the leaf, trunk bark, and root bark. Three clinical strains, isolated from patients with bacterial infection, were used as test strains: *Escherichia coli, Salmonella typhimurium,* and *Pseudomonas aeruginosa.* The reference strain was *Escherichia coli* ATCC 25922. All bacterial strains were obtained from the Bacteriology Laboratory of the University Hospital Center of Sylvanus Olympio (CHU Sylvanus Olympio), Togo.

Characterization of clinical strains:

***Escherichia coli***: Strain No. 711/2024 PWG isolated from urine. It is sensitive to all beta-lactams (no penicillinase), to aminoglycosides (Gentamicin, Tobramycin, Amikacin, and Netilmicin), to Tigecycline, and to Nitrofurans. It is resistant to Doxycycline, Quinolones, and Fluoroquinolones.

***Salmonella typhimurium***: Strain No. 430/2024 PWP isolated from stool. Low-level penicillinase with resistance to aminopenicillins, carboxypenicillins, and ureidopenicillins; resistance is overcome by beta-lactamase inhibitors.

***Pseudomonas aeruginosa***: Strain No. 493/2024 PWG. Isolated from pus, it is sensitive to ureidopenicillins, aminoglycosides, and shows sensitivity at high doses to carboxypenicillins, imipenem, ceftazidime, cefepime, monobactams (aztreonam), and ciprofloxacin.

## 3. Methods

### 3.1. Preparation of Total Extracts

A mass of 20 g of each powdered (leaf, trunk bark, and root bark) of *P. kotschyi* was macerated in 200 mL of solvents of increasing polarity: hexane, dichloromethane, and 96% ethanol. The mixtures were incubated at room temperature with continuous agitation for 72 hours. After maceration, the extracts were filtered, and the ethanolic filtrates were concentrated using a rotary evaporator at 45˚C. For each of the three parts of the plant (leaf, trunk bark, and root bark), the extraction process was repeated three times, and the mean obtained from three trials ± the standard deviation (SD) was considered the final result. The resulting ethanolic extracts were stored at −23˚C for subsequent analyses.

### 3.2. Qualitative Phytochemical Analyses of the Ethanolic Extracts

The presence of different groups of phytoconstituents in the ethanolic extracts of the leaf, trunk bark, and root bark of *P. kotschyi* was assessed using colorimetric and/or precipitation tests. Alkaloids were detected with Dragendorff's reagent [31]; polyphenols and tannins were identified using the Stiasny and $FeCl_3$ reagents, respectively [32]; flavonoids were revealed by the cyanidin reaction [32], while saponosides were confirmed by the foam test [33]. Finally, triterpenes were detected with the Liebermann-Bürchard reagent test [33].

### 3.3. Antibacterial Activity Evaluation of the Ethanolic Extracts

#### 3.3.1. Bacterial Broth Preparation

The four bacterial strains were suspended in Mueller-Hinton broth to obtain a turbidity corresponding to approximately $1 \times 10^8$ CFU/mL, which is equivalent to the 0.5 MacFarland standard [34]. This suspension was then diluted 1:100 to achieve the working inoculum.

#### 3.3.2. Determination of Minimum Inhibitory Concentration and Minimum Bactericidal Concentration

The four strains were suspended in the Mueller-Hinton broth to obtain a turbidity equivalent to $1 \times 10^8$ CFU/mL (0.5 MacFarland standard). The antibacterial tests

were performed using the broth microdilution method in 96-well Mueller-Hinton plates, coupled with agar spreading on control plates without extract [35].

The extracts were sterilized by filtering through a 0.45 µm Millipore membrane filter. The stock solutions of extracts were prepared at 100 mg/mL in physiological saline (NaCl 0.9%). For each sample, 50 µL of Mueller-Hinton broth was added to the first wells and 100 µL to the remaining wells. Then, 150 µL of the extract stock solution was dispensed into the first wells. After homogenization, serial two-fold dilutions were carried out by taking 100 µL of the solution from the first well to the next, to obtain final concentrations ranging from 50 to 6.25 mg/mL. For each extract concentration, the wells were prepared in duplicate. Subsequently, 50 µL of the bacterial inoculum was added to all the wells, and the plates were incubated at 37˚C for 18 to 24 hours.

The minimum inhibitory concentration (**MIC**) is the lowest concentration of the extract at which no visible bacterial growth is observed. An aliquot (10 µL) from all wells was streaked onto the surface of agar plates over a 5 cm line to assess bacterial growth. After 24 hours of incubation at 37˚C, the colony density on the agar plates was compared with that of the inoculum control (prepared on the first day from the initial strain) to estimate the percentage of surviving bacteria.

The minimum bactericidal concentration (**MBC**) is defined as the lowest concentration of extract that reduces the bacterial density to 0.01% of the initial inoculum. The antibiotics used as references were gentamicin and ceftriaxone. Their solutions were prepared by immersing the corresponding antibiotic disks in 5 mL of Mueller-Hinton broth to obtain a final concentration of 30 µg/mL.

The **MBC/MIC** ratio was used to determine the mode of action of the extract or fraction against the bacterial strains [36]. If this is less than or equal to 2, the substance is considered bactericidal. By contrast, if it is greater than 4, the substance is considered bacteriostatic [37]. This test was conducted at the Microbiology and Food Quality Control Laboratory (LAMICODA/ESTBA), University of Lomé, Togo.

## 3.4. Identification of Compounds in the Ethanolic Extracts

### 3.4.1. Identification and Quantification of Compounds Contained in the Ethanolic Extracts by HPLC–DAD Analysis

Phenolic compounds in the ethanolic extracts of the leaf, trunk bark, and root bark of *P. kotschyi* were identified and quantified by high-performance liquid chromatography coupled with a diode array detector (HPLC-DAD). For this analysis, 25 mL of each extract to be analyzed (EF, ET, and ER) was transferred into a separatory funnel, followed by the addition of 50 mL of water. The mixture was successively extracted three times with 15 mL of ethyl acetate. Each of the ethyl acetate fractions was collected and washed three times with 50 mL of water, dried over anhydrous sodium sulfate ($Na_2SO_4$), filtered, and evaporated to dryness at 45˚C under reduced pressure. Each residue was dissolved in methanol and adjusted to a final volume of 10 mL. These solutions were then used for the identification and quantification of flavonoid compounds present in the samples by

HPLC analysis.

This analysis was performed using a VARIAN HPLC system equipped with a Model 9012 ternary pump, a Model 9065 UV diode array detector, and a reverse-phase C18 column (250 × 4.6 mm, 5 µm particle size). The mobile phase consisted of two solvents: acetic acid 5% (A) and acetonitrile (B). The elution was programmed as follows: isocratic elution with 70% A from 0 to 10 min; elution with a gradient to 40% B from 10 to 20 min; and finally, isocratic elution from 20 to 30 min with 40% A. The flow rate of the mobile phase was 1 mL/min and the temperature was set at 30˚C. The elution was monitored in the UV range, and the acquisition of data for the analysis was carried out at 254 nm, 280 nm, and 367 nm. The method of external calibration was applied for the quantification of the concentrations of the samples in phenolic compounds.

The equations of the calibration curves are summarized in **Table 1.** All standards were of high purity at 97.50% (Extrasynthese, Lyon, France).

**Table 1.** Equations of the calibration curves.

| Phenolic compound | Equation | Correlation $R^2$ |
|---|---|---|
| gallic Acid | Y = 9039.9x − 523.69 | 0.9974 |
| Acid, coumaric acid | Y = 15685x + 7.9388 | 0.9934 |
| ferulic Acid | Y = 20566x − 208.37 | 0.9979 |
| caffeic Acid | Y = 1909.8x − 210.27 | 0.9879 |
| Catechin | Y = 19608x − 27.217 | 0.9982 |
| Epicatechin | Y = 8745.2x − 1533.1 | 0.9945 |
| Quercetin | Y = 28347x − 510.570 | 0.9957 |
| Myricetin | Y = 199490x − 96.5 | 0.9988 |
| Kaempferol | Y = 2856.3x − 99.791 | 0.9976 |
| Rutin | Y = 38854x − 178.4 | 0.9929 |
| Arbutin | Y = 805.7x − 38.5 | 0.9970 |

### 3.4.2. Identification of Compounds by UHPLC-HRMS

For the preservation of the species, non-volatile compounds in ethanolic leaf and root extracts of *P. kotschyi* were identified by UHPLC-HRMS. Indeed, although the trunk is also a vital organ of the plant, harvesting the root causes more fatal destruction of the species than that of the trunk by preventing its regeneration. Previous studies have already focused on the stem or trunk bark of this plant, as in the case of Sinan *et al.* (2021). Therefore, a study on the identification of phenolic compounds in the root bark of the species has been carried out in the current work.

In practice, two microliters (2 µL) of the plant extracts (1 mg/mL) were injected into the column. The ultra high performance liquid chromatography system (Vanquish model H, Thermo Scientific), equipped with a reverse phase and a Phenomenex Luna Omega Polar C18 column (1.6 µm, 150 × 2.1 mm) as the stationary

phase, was used at a temperature of 40˚C. This liquid chromatography system was coupled with a high-resolution mass spectrometer HRMS (*Thermo Scientific Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer*). Thus, the combination of these two systems (UHPLC-HESI-QExactive Plus) has allowed us to perform this analysis.

The samples were infused in ionization *electrospray,* positive and negative. The scanning range of the mass/charge ratio (m/z) was determined between 100 and 1500 Da. The fragmentation mode DDA was performed with a ramp collision energy of 20 to 60 eV for the four (4) ions in each scan. All acquisitions and analyses of the data were performed by the software Xcalibur.

For the solvents used as the mobile phase, a mixture of water + 0.05% formic acid (A) and acetonitrile + 0.05% formic acid (B) was used. A gradient system was used with a flow rate of 0.4 mL/min, as shown in Table 2.

Table 2. Proportions of the solvents used for the analysis.

| Time (min) | % A | % B |
|---|---|---|
| 0 | 98 | 2 |
| 0.5 | 98 | 2 |
| 8 | 30 | 70 |
| 9 | 2 | 98 |
| 12 | 2 | 98 |
| 12.1 | 98 | 2 |
| 14 | 92 | 8 |

### 3.5. Data Processing

The profiling of the chromatograms and mass spectra of LC-MS was processed using the software MZmine v4.5 [38]. The treatment of the profiles includes several successive steps to obtain a list of all peaks (m/z × RT) detected in the samples, as well as their respective areas. The peaks were annotated, based on their exact masses (Δppm = 5) and their MS/MS fragmentation patterns, under the following conditions:

- Annotation level 1: Databases of experimental spectra acquired on the standard analytical shelf-AgromiX;
- Annotation levels 2a and 2b: Databases of experimental spectra using the database FragHub [39];
- Annotation level 3: Database of spectra *in silico* internal tray AgromiX, including 1,000,000 compounds with a prioritization of compounds or families of chemical classes found in the genus, the family (level 3a), followed by those belonging to the databases of generic plants (KnapSack, UNPD, CheBi, HMDB: level 3b). This annotation is based on a classification of the structure, more likely as a function of its exact mass and fragmentation footprint HRMS/MS (Δppm = 15) compared to a theoretical candidate product footprint calculated *in silico*

using the software Sirius v 6.1 [40].

A final annotation score was generated between −2 and 11. A high score has been considered as a very probable annotation that was matched in the database of level 1, 2a, or gender.

## 4. Results and Discussion

### 4.1. Yields of the Extraction

The yield of ethanolic extracts of leaf, trunk bark, and root bark of *P. kotschyi* after the realization of the extraction with the three solvents, such as hexane, dichloromethane, and ethanol (96˚), with increasing polarity, is shown in Table 3.

Table 3. Extraction yields of the leaf, trunk bark, and root bark of *P. kotschyi.*

| Types of extract | Yield of extraction |
|---|---|
| EF | 4.11 ± 1.81 |
| ET | 12.33 ± 1.43 |
| ER | 11.68 ± 2.04 |

EF: Ethanolic extract of the leaf; ET: Ethanolic extract of the trunk bark; ER: Ethanolic extract of the root.

The analysis of the values shown in Table 3, at first sight, shows that the performance varies according to the type of body part that was used to make the solid-liquid extraction.

### 4.2. Phytochemical Profiling of the Three Ethanolic Extracts

The results shown in Table 4 revealed the different phytochemical groups present in the three ethanolic extracts of *P. kotschyi.*

Table 4. Phytochemical groups found in the three organs of *P. kotschyi.*

| Chemical group | EF | ET | ER |
|---|---|---|---|
| Alkaloids | + | + | + |
| Phenolic compounds | + | + | + |
| Flavonoids | + | + | + |
| Tannins | + | + | + |
| Saponins | + | + | + |
| Triterpenes | − | − | − |

− = Negative reaction; + = positive reaction; EF: ethanolic extract of the leaf; ET: ethanolic extract of the trunk bark; ER: ethanolic extract of the root bark.

In fact, the best performance was obtained from trunk bark with 12.33 ± 1.43%, while the lowest one was recorded at the level of the leaf, with 4.11 ± 1.81%. Concerning the root's bark, the extraction yield was 11.68 ± 2.04%, which is not significantly different from that of the trunk bark.

As shown in Table 4, alkaloids, phenolic compounds, flavonoids, tannins, and saponosides were revealed in the three organs of the plant *P. kotschyi.* However, the search for triterpenes was unsuccessful in all three organs of the plant.

### 4.3. Antibacterial Activity of the Ethanolic Extracts

The antibacterial activity of ethanolic extracts of the leaf, trunk bark, and root bark of *P. kotschyi* was investigated to determine the minimum inhibitory concentration (**MIC**) and minimum bactericidal concentration (**MBC**) of the tested strains (*E. coli, S. typhimurium*, *P. aeruginosa,* and the reference strain of *E. coli* ATCC 22925).

In this study, the results of the antibacterial effects of ethanolic extracts are shown in Table 5, while the values of MIC and MBC are recorded in Table 6.

Based on these results, the ethanolic extract of the trunk bark was active against the isolated strain *P. aeruginosa.* Indeed, with this strain, the MBC and MIC values of the ethanolic extract of the trunk bark were each equal to 6.25 mg/mL. The ethanolic extract of the trunk bark was also active, with MIC and MBC values of 25 mg/mL and 50 mg/mL, respectively, against the reference strain *E. coli* ATCC 22925; and then 6.25 mg/mL and 25 mg/mL, against the isolated strain *S. typhimurium.* Regarding the root bark, the ethanolic extract was not active against P. aeruginosa, with an MIC value of 50 mg/mL and an MBC value of 50 mg/mL. Therefore, the crude ethanolic extract of the leaf was active against P. aeruginosa, with an MIC of 50 mg/mL and an MBC of 50 mg/mL. This crude extract was also active against the isolated strain of *E. coli*, with an MIC of 50 mg/mL and an MBC of 50 mg/mL.

Table 5. Qualitative results of the antibacterial activity of ethanolic extracts.

| *Types of extract* | *P. Aeruginosa* | *E. coli* (Strain isolated) | *E. coli* ATCC 22925 (Strain ide reference) | *S. Typhimurium* |
|---|---|---|---|---|
| **ET** | Active | - | Active | Active |
| **ER** | Active | - | - | - |
| **EF** | Active | Active | - | - |
| **GM** | Active | Active | Active | - |
| **CRO** | Active | - | Active | - |

Strategically, the two antibiotics used as references, namely gentamicin (**GM**) and ceftriaxone (**CRO**), known for their powerful antibacterial effects against a wide range of bacterial strains, were also studied to compare the results of the samples investigated with them, in order to ensure the effectiveness of the antibacterial activity of the ethanolic extracts of *P. kotschyi.* The results indicated that GM was active against all bacterial strains tested, except the isolated strain of *E. coli*, which survived the harmful effects of GM, with a MIC of 7.5 µg/mL and an MBC of 15 µg/mL against *P. aeruginosa,* with a MIC of 1.87 µg/mL and an MBC of 1.87 µg/mL against the *E. coli* reference, and lastly with a MIC of 1.87 µg/mL

and an MBC of 3.75 µg/mL against the isolated strain of *E. coli.* However, CRO was only effective against *P. aeruginosa* and against the reference strain of *E. coli* ATCC 2292, with a MIC of 15.0 µg/mL and an MBC of 15.0 µg/mL against *P. aeruginosa,* and finally with a MIC of 0.23 µg/mL and an MBC of 0.23 µg/mL against the reference strain of *E. coli.*

**Table 6.** MIC and MBC of the ethanolic extracts (EF, ET, and ER) on the bacterial strains tested.

| | ER | | | | ET | | | | EF | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **Strains** | **MIC (mg/mL)** | **MBC (mg/mL)** | **MBC/MIC** | **Effect** | **MIC (mg/mL)** | **MBC (mg/mL)** | **MBC/MIC** | **Effect** | **MIC (mg/mL)** | **MBC (mg/mL)** | **MBC/MIC** | **Effect** |
| ***P. aeruginosa*** | 50 | 50 | 1 | BE | 6.25 | 6.25 | 1 | BE | 50 | 50 | 1 | BE |
| ***E. coli* ATCC 22925** | 50 | 50 | ND | ND | 25 | 50 | 2 | BE | 50 | 50 | 1 | ND |
| ***E. coli*** | 50 | 50 | ND | ND | 50 | 50 | ND | ND | 50 | 50 | 1 | BE |
| ***S. typhimurium*** | 50 | 50 | ND | ND | 6.25 | 25 | 4 | BE | 50 | 50 | ND | ND |

EF: ethanolic extract of the leaf; ET: ethanolic extract of trunk bark; ER: ethanolic extract of the root bark; GM: Gentamicin; CRO: Ceftriaxone; ND: Not determined; Bactericidal effect.

## 4.4. Identification of Phenolic Compounds

### 4.4.1. Identification and Quantification of Phenolic Compounds by HPLC-DAD

The analysis of the plant samples (EF, ET, and ER) by HPLC-DAD equipment was carried out to identify the phytoconstituents present in the ethanolic extracts. In this analysis, the method of external calibration was applied for the quantification of the phenolic compound contents in the samples. The calibration curves were established using the required standards.

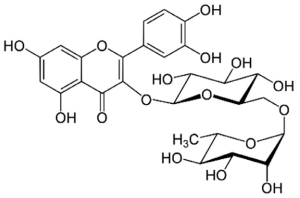
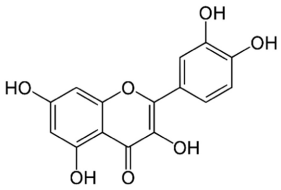
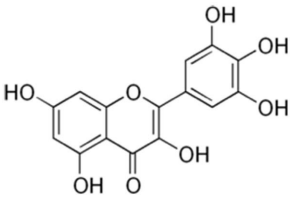
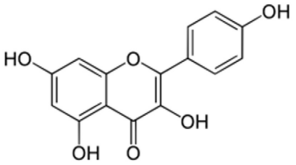
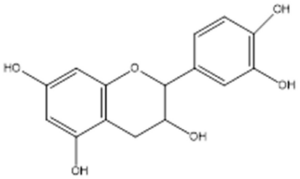
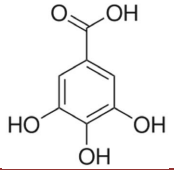
**Table 7.** List of the eleven (11) phenolic compounds detected with their contents quantified by HPLC-DAD analysis in the three (3) ethanolic extracts of *P. kotschyi.*

| | Content (mg/g) | | |
|---|---|---|---|
| **Identified Compounds** | **EF** | ET | ER |
| Gallic Acid | 12.571 | 14.007 | 13.617 |
| Coumaric Acid | 3.714 | 4.874 | 4.367 |
| Ferulic Acid | 0.401 | 0.748 | 1.556 |
| Caffeic Acid | 3.057 | 4.002 | 3.335 |
| Quercetin | 0.204 | 0.094 | 0.046 |
| Myricetin | 0.079 | 0.117 | 0.039 |
| Kaempferol | 0.036 | 0.224 | 0.387 |
| Catechin | 7.121 | 8.078 | 10.007 |
| Epicatechin | 0.034 | 0.067 | 0.031 |
| Rutin | 0.545 | 0.941 | 0.027 |
| Arbutin | 0.055 | 0.367 | 0.135 |

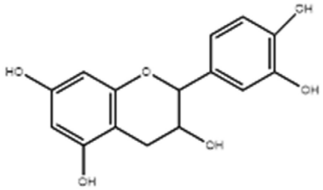
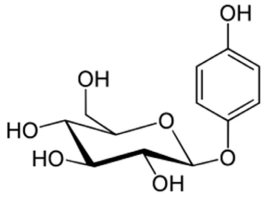
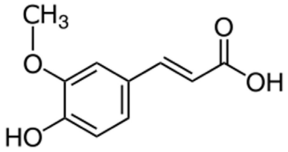
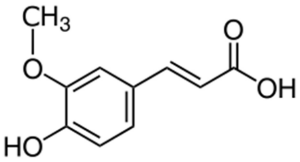
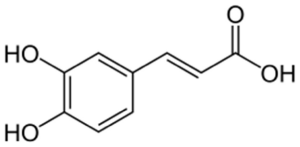
EF: Ethanolic extract of the leaf; ET: Ethanolic extract of the trunk; ER: Ethanolic extract of the root.

The results of analysis by HPLC-DAD revealed that eleven (11) phenolic compounds were detected in the saplings, and their contents are presented in Table 7. Among these 11 compounds found, four (04) are mainly distributed in the three plant samples. They are: acid, gallic acid, coumaric acid, caffeic acid, and catechin, but with variable grades. Among the eleven biomolecules present were: four (4) phenolic acids (such as: acid, gallic acid, coumaric acid, ferulic acid, and caffeic acid) and seven (7) flavonols (which are quercetin, myricetin, kaempferol, catechin, epicatechin, rutin, and arbutin), but in different proportions (Table 7). The structures and the corresponding names of these compounds identified in the ethanolic extracts are represented in Table 8.

Table 8. Names and molecular structures of the phenolic compounds detected by HPLC-DAD analysis in the ethanolic extracts of *P. kotschyi.*

| N° | Names of the Compounds | Molecular Structures |
|---|---|---|
| **1** | Rutin |  |
| **2** | Quercetin |  |
| **3** | Myricetin |  |
| **4** | Kaempferol |  |
| **5** | Epicatechin |  |
| **6** | Gallic Acid |  |

**Continued**

| | | |
|---|---|---|
| **7** | Catechin |  |
| **8** | Arbutin |  |
| **9** | Ferulic Acid |  |
| **10** | Coumaric acid |  |
| **11** | Caffeic Acid |  |

### 4.4.2. Identification of Compounds by UHPLC-HRMS Analysis

The analysis of the two ethanolic extracts (EF and ER) of *P. kotschyi* by UHPLC-HRMS equipment (*Thermo Scientific Q Exactive Hybrid Quadrupole-Orbitrap Mass Spectrometer*) was undertaken to identify the biomolecules present in the ethanolic extracts. The three databases previously mentioned in the section on data processing were exploited for the identification of the compounds suspected in the extracts. Due to poor fragmentation of the compounds analyzed with the instrument, those with masses greater than 700 Da are very often marked as unknown. The peaks marked between 0 and 1.5 (or 2) min correspond to the injection. These annotations are therefore to be considered with caution.

In this analysis, a number has been assigned to each detected peak, corresponding to the method of identification. This number (or final annotation score) expresses the probability of the identification of a correct mass spectrum, taking into account various factors such as the presence of specific ions, their relative abundances, and the potential adducts. It allows for the assessment of the reliability of the identification and annotation of the molecules analyzed. The highest degree of reliability of the identification was the final annotation score, so in the present study, this score ranged between −2 and 11.

*Chromatogram profiling of the compounds identified in the ethanolic extracts of P. kotschyi*

All these criteria of selection adopted in the current work allowed the identification of 26 and 23 compounds in the ethanolic extract of leaf (EF) and the ethanolic extract of the root bark (ER), with good precision as the results are shown in Table 9 and Table 10.

The results illustrated in Figures 1-4 showed that the ethanolic extract of the leaf studied revealed more than 2261 peaks with 677 unknown peaks after data integration in positive mode and negative mode. However, the root bark showed more than 2618 peaks with 855 unknown peaks after data integration in positive mode and negative mode. In order to make the identification of compounds in these samples much more reliable, the peaks whose scores were at least 5 and whose retention time was more than 2 min were favored. The compounds which were detected by ULHP-HRMS analysis in both of the two extracts are nine (9), including Gallic acid, Catechol, (+)-Procyanidin B2, Catechin-7-Methyl Ether, taxifolin, Epigallocatechin-3-O-(3,5-Di-O-Méthylgallate), epicatechin, 1-[2,4-Dihydroxy-6-[3,4,5-trihydroxy-6-(hydroxyméthyl)oxan-2-yl]oxyphenyl]-3-(4-hydroxyphényl)propan-1-one, and 2-{2-[3,7-dihydroxy-2-(3,4,5-trihydroxy phényl)-3,4-dihydro-2H-1-benzopyran-4-yl]-3,4,5-trihydroxyphényl}-3,7-dihydroxy-3,4-dihydro-2H-1-benzopyran-4-one.

The characteristics of these compounds detected in the two ethanolic extracts of *P. kotschyi* by ULHP-HRMS analysis are presented in Table 11 (for EF) and Table 12 (for ER).

Table 9. Characteristics of the compounds identified in the ethanolic extract of the leaf of *P. kotschyi.*

| N° | RT (min) | Names of the probable metabolites | Ionization mode | HRMS (m/z) | Raw formulas | Peak area | Final score |
|---|---|---|---|---|---|---|---|
| 1 | 2.6941 | Gallic Acid | [M-H]- | 169.01362 | $C_7H_6O_5$ | 36.53691 | 10.50 |
| 2 | 4.3807 | Epicatechin | [M-H]- | 290.07790 | $C_{15}H_{14}O_6$ | 32.65813 | 10.20 |
| 3 | 3.5807 | Catechol | [M]- | 109.02895 | $C_6H_6O_2$ | 2.22474 | 5.00 |
| 4 | 4.2786 | (+)-Procyanidin B2 | [M+FA]- | 577.13466 | $C_{30}H_{26}O_{12}$ | 1.34828 | 5.00 |
| 5 | 6.1431 | Catechin-7-Methyl Ether | [M+$CH_2O_2$-H]- | 349.09239 | $C_{16}H_{16}O_6$ | 1.23481 | 6.00 |
| 6 | 6.3404 | 1-[2,4-Dihydroxy-6-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyphenyl]-3-(4-hydroxyphenyl)propan-1-one | [M-H]- | 435.12935 | $C_{21}H_{24}O_{10}$ | 1.07827 | 9.50 |
| 7 | 6.3849 | Epigallocatechin-3-O-(3,5-Di-O-Méthylgallate) | [M-H]- | 485.10859 | $C_{24}H_{22}O_{11}$ | 0.99001 | 5.00 |
| 8 | 4.7934 | 2-{2-[3,7-dihydroxy-2-(3,4,5-trihydroxy phenyl)-3,4-dihydro-2H-1-benzopyran-4-yl] -3,4,5-trihydroxyphenyl}-3,7-dihydroxy-3,4 -dihydro-2H-1-benzopyran-4-one | [M-H]- | 591.11415 | $C_{30}H_{24}O_{13}$ | 1.01198 | 5.50 |
| 9 | 5.0843 | Taxifolin | Ind. | 305.06616 | $C_{15}H12O_7$ | 0.19547 | 8.50 |
| 10F | 6.1534 | Quercitrin | [M-H]- | 448.10056 | $C_{21}H_{20}O_{11}$ | 30.08997 | 8.80 |
| 11F | 5.6105 | Myricétine | Ind. | 318.03757 | $C_{15}H_{10}O_8$ | 17.51817 | 9.00 |

**Continued**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **12F** | 6.1437 | Cimicifugic Acid A | [M+H]+ | 449.10867 | $C_{21}H_{20}O_{11}$ | 15.88603 | 8.40 |
| **13F** | 3.6817 | 2,3-Dihydroxybenzoic Acid | [M-H]- | 153.01876 | $C_7H_6O_4$ | 6.48472 | 10.30 |
| **14F** | 6.6146 | Afzelin | [M-H]- | 431.09788 | $C_{21}H_{20}O_{10}$ | 4.29889 | 10.50 |
| **15F** | 4,2242 | 3-p-coumaroylquinic Acid | [M-H]- | 337.09272 | $C_{16}H_{18}O_8$ | 3.066382 | 9.00 |
| **16F** | 6.6031 | Kaempferol | Ind. | 287.05561 | $C_{15}H_{10}O_6$ | 1.90919 | 10.00 |
| **17F** | 4.0956 | 1,6-Digalloyl-beta-D-glucopyranose | [M+FA]- | 483.07777 | $C_{20}H_{20}O_{14}$ | 1.84554 | 5.00 |
| **18F** | 5.5839 | Fragransol-B | [M+H]+ | 331.15448 | $C_{19}H_{22}O_5$ | 1.21865 | 6.00 |
| **19F** | 5.7004 | Isoquercetin | [M+Na]+ | 487.08541 | $C_{21}H_{20}O_{12}$ | 1.15208 | 5.00 |
| **20F** | 3.0357 | 2-[2-hydroxy-4-(3-hydroxyprop-1-èn-1-yl)-6-méthoxyphenoxy]-6-(hydroxyméthyl) oxane-3,4,5-triol | [M-$H_2O$+H]+ | 341.12137 | $C_{16}H_{22}O_9$ | 1.02347 | 5.40 |
| **21F** | 2.8556 | 3,4,5-trihydroxybenzoate de [3,4,5-trihydroxy-6-(hydroxyethyl)oxan-2-yl] | [M-$H_2O$+H]+ | 315.07162 | $C_{13}H_{16}O_{10}$ | 1.02260 | 5.00 |
| **22F** | 5.9451 | Kaempférol 3-(6"-Galloylgalactoside) | [M-H]- | 599.10403 | $C_{28}H_{24}O_{15}$ | 0.87229 | 7.50 |
| **23F** | 6.4198 | 2-{[5,7-dihydroxy-2-(4-hydroxyphényl)-4-oxo-4H-chromen-3-yl] oxy}-3,5-dihydroxy-6-méthyloxan-4-yl 3,4,5-trihydroxybenzoate | [M-H]- | 583.10899 | $C_{28}H_{24}O_{14}$ | 0.81695 | 5.00 |
| **24F** | 6.2703 | Procyanidin A2 | [M+H]+ | 577.13484 | $C_{30}H_{24}O_{12}$ | 0.76958 | 5.10 |
| **25F** | 6.3709 | Gallocatéchines 3'-O-Gallate | [M-$H_2O$+H]+ | 441.08271 | $C_{22}H_{18}O_{11}$ | 0.39593 | 9.00 |
| **26F** | 3.4204 | 3-Méthoxy-4-[3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]oxybenzoic Acid | [M-H]- | 329.08757 | $C_{14}H_{18}O_9$ | 0.37153 | 9.30 |

**Table 10.** Characteristics of the compounds identified in the ethanolic extract of the root of *P. kotschyi.*

| N° | RT (min) | Names of the probable metabolites | Ionization mode | HRMS (m/z) | Chemical formulas | Peak area | Final score |
|---|---|---|---|---|---|---|---|
| **1** | 2.6928 | Gallic Acid | [M-H]- | 169.0136 | $C_7H_6O_5$ | 12.88387 | 10.50 |
| **2** | 4.3807 | Epicatechin | [M-H]- | 290.07790 | $C_{15}H_{14}O_6$ | 0.05255 | 10.20 |
| **3** | 3.5731 | Catechol | [M]- | 109.02889 | $C_6H_6O_2$ | 13.10725 | 5.00 |
| **4** | 4.0657 | (+)-Procyanidin B2 | [M-H]- | 577.1345 | $C_{30}H_{26}O_{12}$ | 9.44275 | 5.00 |
| **5** | 4.6239 | Catechin-7-Methylether | [M+$CH_2O_2$-H]- | 304.09469 | $C_{16}H_{16}O_6$ | 0.12440 | 5.20 |
| **6** | 6.3404 | 1-[2,4-Dihydroxy-6-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyphenyl]-3-(4-hydroxyphényl)propan-1-one | [M-H]- | 435.12935 | $C_{21}H_{24}O_{10}$ | 0.04743 | 9.50 |
| **7** | 6.3849 | Epigallocatechin-3-O-(3,5-Di-O-Methylgallate) | [M-H]- | 485.10859 | $C_{24}H_{22}O_{11}$ | 0.02429 | 6.00 |
| **8** | 4.7934 | 2-{2-[3,7-dihydroxy-2-(3,4,5-trihydroxy phenyl)-3,4-dihydro-2H-1-benzopyran-4-yl] -3,4,5-trihydroxyphenyl}-3,7-dihydroxy-3,4-dihydro-2H-1-benzopyran-4-one | [M-H]- | 591.11415 | $C_{30}H_{24}O_{13}$ | 0.05297 | 7.00 |
| **9** | 5.2798 | Taxifolin | [M+H]+ | 305.06619 | $C_{15}H_{12}O_7$ | 0.25639 | 9.40 |
| **10R** | 12.0849 | Bonaspectin C 4"-Beta-Glucoside | [M+H]+ | 775.35428 | $C_{40}H_{54}O_{15}$ | 7.27170 | 5.00 |
| **11R** | 4.0816 | Epigallocatechin | Ind | 307.08172 | $C_{15}H_{14}O_7$ | 4.20314 | 10.00 |
| **12R** | 10.3625 | Silvestrol | [M+H]+ | 655.23905 | $C_{34}H_{38}O_{13}$ | 3.26510 | 6.50 |
| **13R** | 5.6685 | (−)-Epicatechin gallate | [M-H]- | 441.0823 | $C_{22}H_{18}O_{10}$ | 1.44262 | 10.20 |
| **14R** | 5.4626 | L-(-) 3-Phenyllactic Acid | [M-H]- | 165.05508 | $C_9H_{10}O_3$ | 1.34958 | 10.50 |

**Continued**

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| **15R** | 5.2804 | 5,8-dihydroxy-3-methoxy-1-{[3,4,5-trihy droxy-6-(hydroxymethyl) oxan-2-yl] oxy}-9H-xanthen-9-one | [M-H]- | 435.09279 | $C_{20}H_{20}O_{11}$ | 0.83316 | 6.00 |
| **16R** | 3.7102 | 3-(3-hydroxyphenyl) prop-2-enoate de 6-{[2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl] oxy}-4,5-dihydroxy-2-(hydroxymethyl) oxan-3-yle | [M-H]- | 609.12448 | $C_{30}H_{26}O_{14}$ | 0.80146 | 5.00 |
| **17R** | 4.8605 | 7-[3,4-dihydroxy-5-(hydroxymethyl) oxolan-2-yl] oxy-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5-diol | [M-H]- | 421.11364 | $C_{20}H_{22}O_{10}$ | 0.75332 | 6.50 |
| **18R** | 3.8418 | Procyanidin | [M-H]- | 593.12959 | $C_{30}H_{26}O_{13}$ | 0.70378 | 5.00 |
| **19R** | 4.0003 | Catechin-(4Alpha-8)-7-O-Beta-Xylopyranosyl-Catéchine | [M+H]+ | 711.19332 | $C_{35}H_{34}O_{16}$ | 0.65290 | 7.00 |
| **20R** | 6.3403 | Phlorizin | [M-H]- | 435.12908 | $C_{21}H_{24}O_{10}$ | 0.62165 | 9.50 |
| **21R** | 6.5308 | 4-hydroxy-3,5-dimethoxybenzoate de [3,4,5-trihydroxy-6-(3,4,5-trimethoxyphenoxy) oxan-2-yl] methyl | [M-H]- | 526.16864 | $C_{24}H_{30}O_{13}$ | 0.23792 | 5.10 |
| **22R** | 4.8391 | 3-méthoxy-4-({3,4,5-trihydroxy-6-[(4-hydroxy-3-méthoxybenzoyloxy)methyl] oxan-2-yl}oxy)benzoic Acid | [M+$CH_2O_2$-H]- | 480.12677 | $C_{22}H_{24}O_{12}$ | 0.16534 | 6.20 |
| **23R** | 5.6921 | 4-hydroxy-3-méthoxybenzoate de [(2R,3S,4S,5R,6S)-3,4,5-trihydroxy-6-(4-hydroxy-3,5-dimethoxyphenoxy) oxan-2-yl]methyl | [M-H]- | 482.14242 | $C_{22}H_{26}O_{12}$ | 0.14379 | 5.00 |

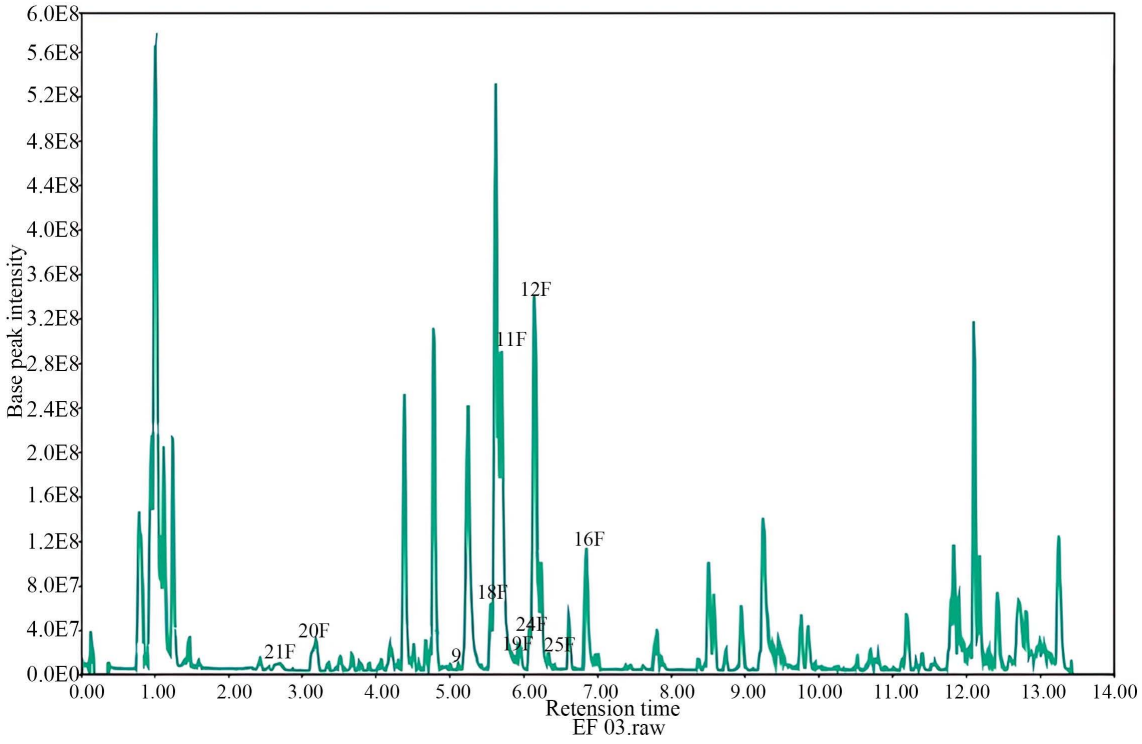



**Figure 1.** Chromatogram profiling of the ethanolic leaf extract (EF) by UHPLC-HRMS analysis in positive ESI mode.

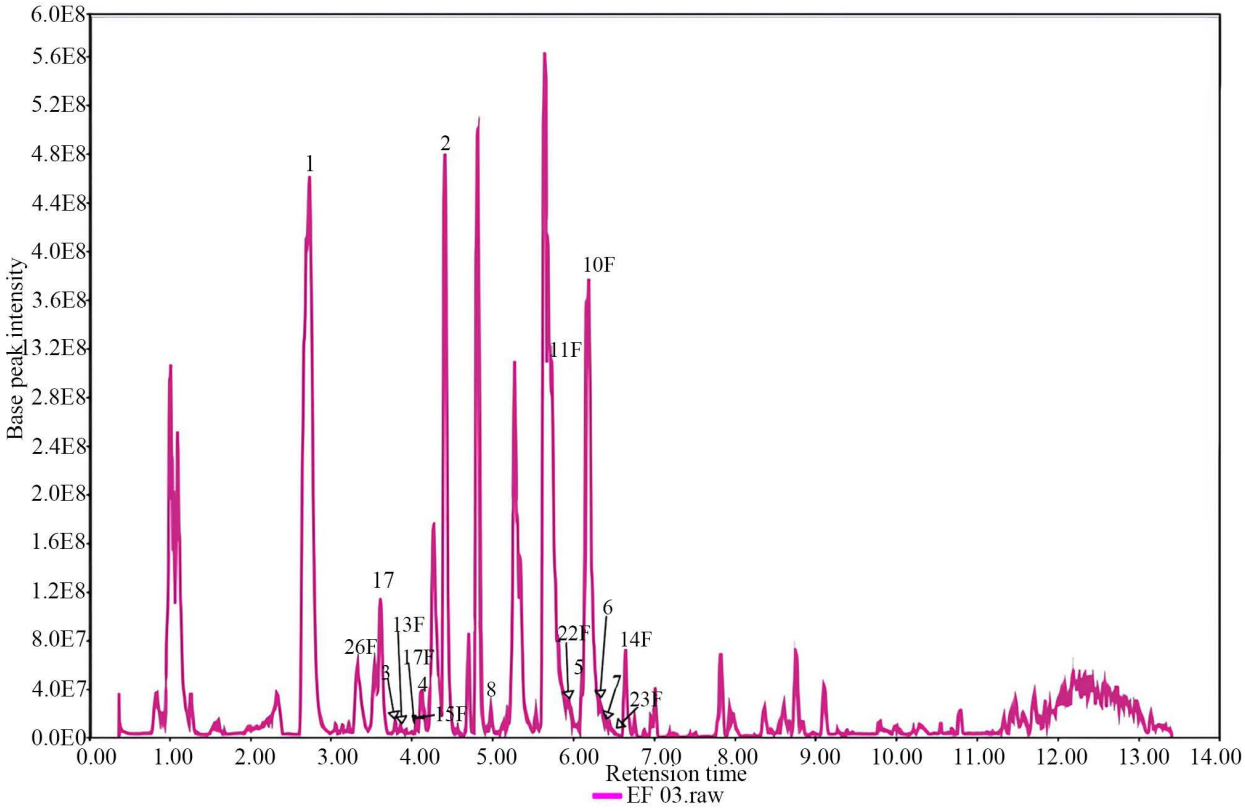



**Figure 2.** Chromatogram profile of the ethanolic leaf extract (EF) by UHPLC-HRMS analysis in negative ESI mode.

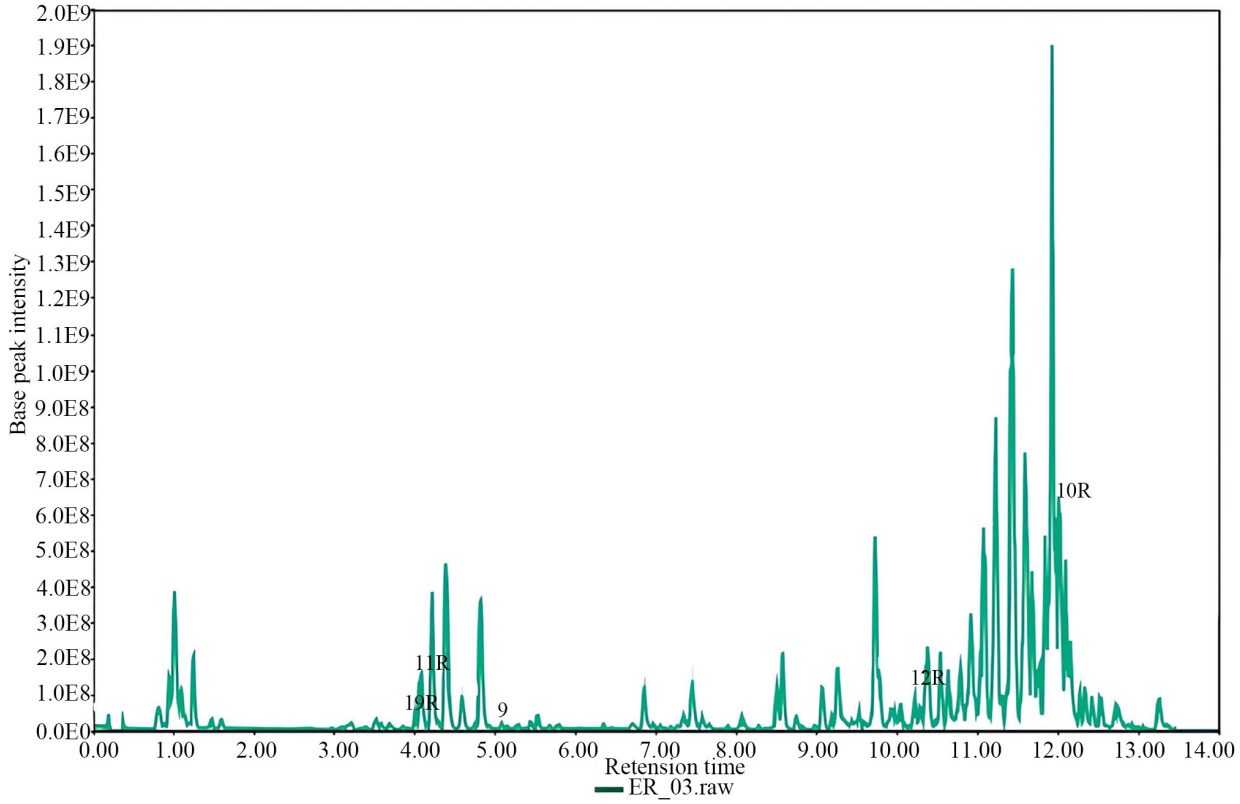



**Figure 3.** Chromatogram profiling of the ethanolic root extract (ER) by UHPLC-HRMS analysis in positive ESI mode.

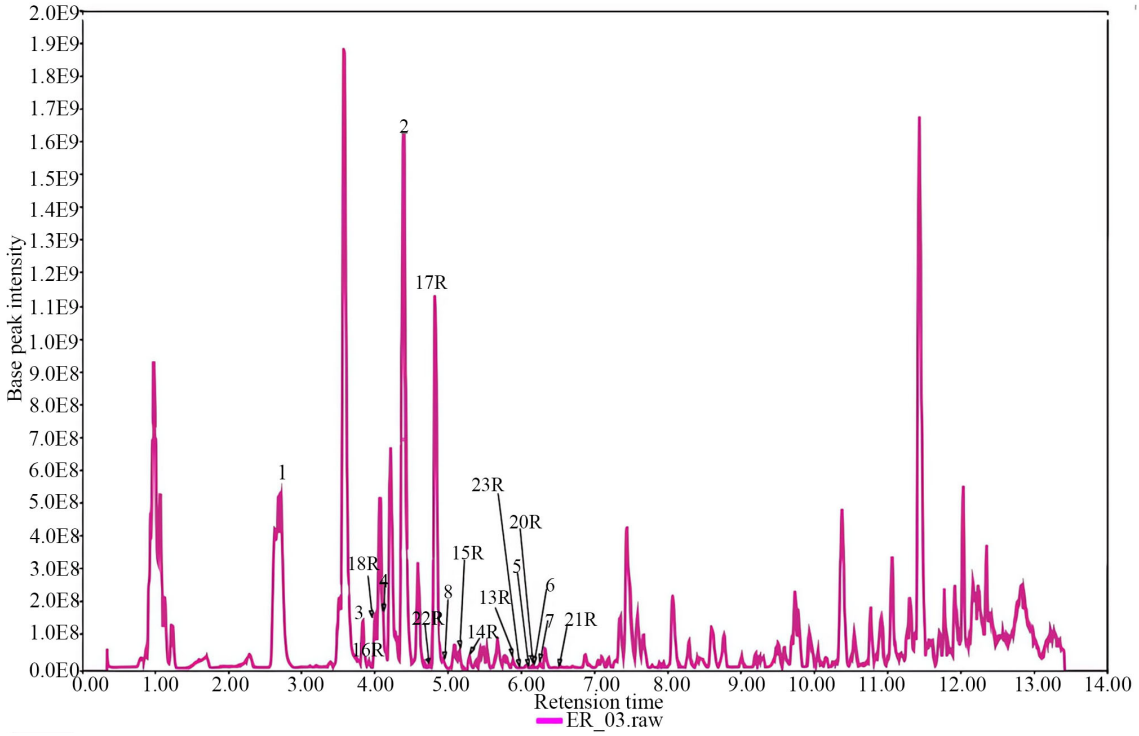
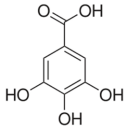
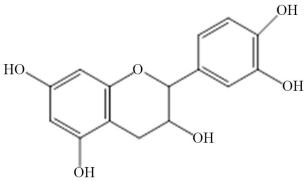
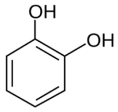
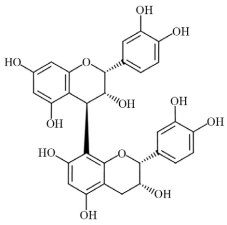
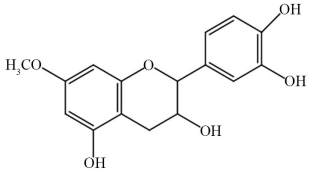
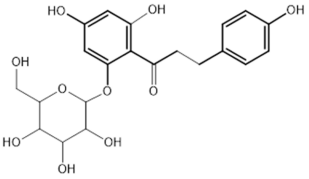
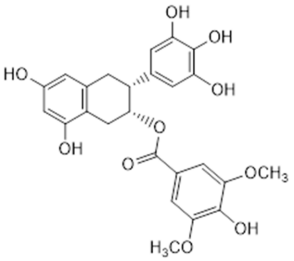
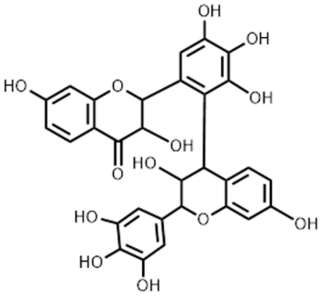
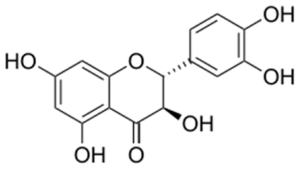
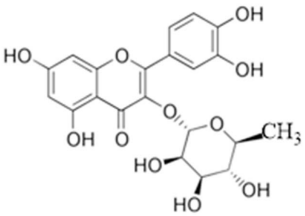



**Figure 4.** Chromatogram profiling of the ethanolic root extract (ER) by UHPLC-HRMS analysis in negative ESI mode.

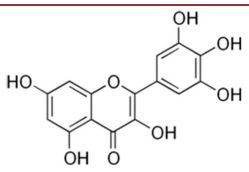
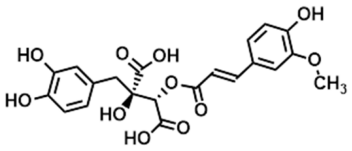
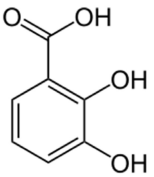
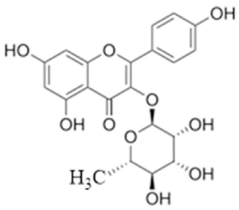
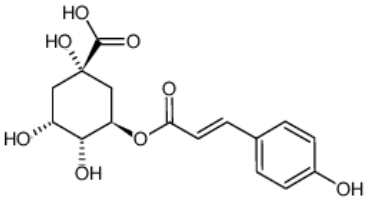
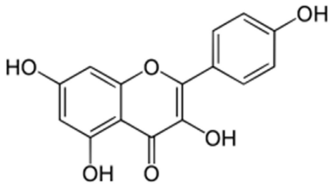
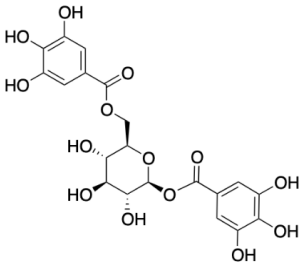
**Table 11.** Structure of the compounds detected in the ethanolic extract of *P. kotschyi* leaf by ULHP-HRMS analysis.

| N° | Molecular Structures | Chemical Families |
|---|---|---|
| **1** |  Gallic Acid | Phenolic Acids |
| **2** |  Epicatéchine | Flavonoids |
| **3** |  Catéchol | Polyphenols |
| **4** |  (+)-Procyanidine B2 | Tannins |

**Continued**

| | | |
|---|---|---|
| **5** |  <br>Catéchin-7-Méthyl Ether | Flavonoids |
| **6** |  <br>1-[2,4-Dihydroxy-6-[3,4,5-trihydroxy-6-(hydroxyethyl) oxan-2-yl] oxyphenyl]-3-(4-hydroxyphényl)propan-1-one | Flavonoids |
| **7** |  <br>Epigallocatechin 3-O-(3,5-Di-O-Methylgallate) | Tannins |
| **8** |  <br>2-{2-[3,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4 -dihydro-2H-1-benzopyran-4-yl]-3,4,5-trihydroxyphenyl} -3,7-dihydroxy-3,4-dihydro-2H-1-benzopyran-4-one | Tannins |
| **9** |  <br>Dihydroquercetin or taxifolin | Flavonoids |
| **10F** |  <br>Quercitrin | Flavonoids |

**Continued**

| | | |
|---|---|---|
| **11F** |  Myricetin | Flavonoids |
| **12F** |  Cimicifugic Acid A | Cinnamic Acids and Derivatives |
| **13F** |  2,3-dihydroxybenzoic Acid | Phenolic Acids |
| **14F** |  Afzelin | Flavonoids |
| **15F** |  3-p-Coumaroylquinic Acid | Polyols |
| **16F** |  Kaempferol | Flavonoids |
| **17F** |  1,6-Digalloyl-beta-D-glucopyranose | Tannins |

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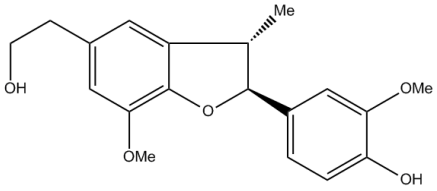
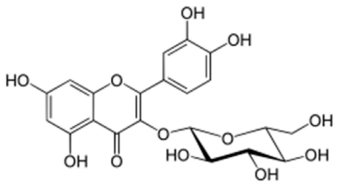
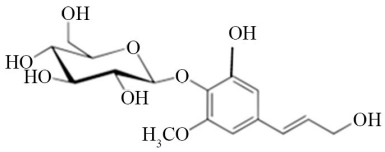
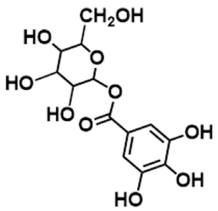
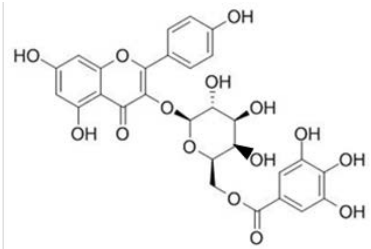
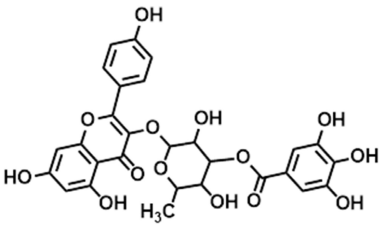
| | | |
|---|---|---|
| **18F** |  <br>Fragransol-B | Flavonoids |
| **19F** |  <br>Isoquercetin | Flavonoids |
| **20F** |  <br>2-[2-hydroxy-4-(3-hydroxyprop-1-én-1-yl)-6-méthoxyphénoxy]-6-(hydroxymethyl) oxane-3,4,5-triol | Polyphenols |
| **21F** |  <br>3,4,5-trihydroxybenzoate de 3,4,5-trihydroxy-6-(hydroxyethyl) oxan-2-yl | Tannins |
| **22F** |  <br>Kaempferol 3-(6"-Galloylgalactoside) | Flavonoids |
| **23F** |  <br>2-{[5,7-dihydroxy-2-(4-hydroxyphenyl)-4-oxo-4H-chromen-3-yl] oxy}-3,5-dihydroxy-6-methyloxan-4-yl 3,4,5-trihydroxybenzoate | Flavonoids |

**Continued**

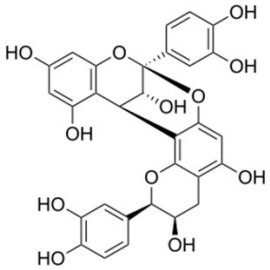
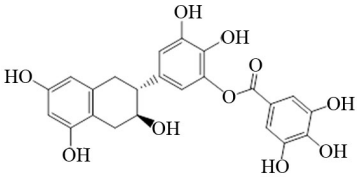
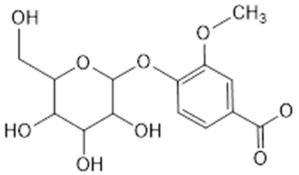
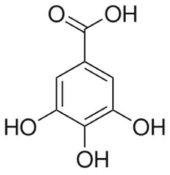
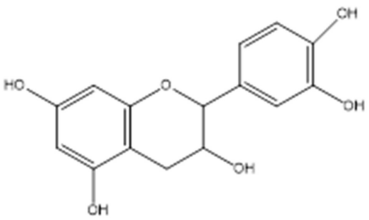
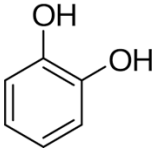
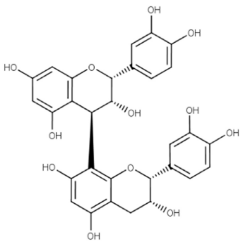
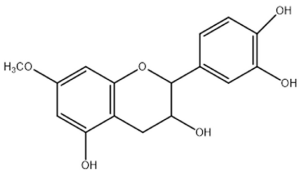
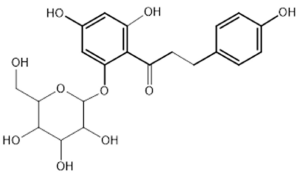
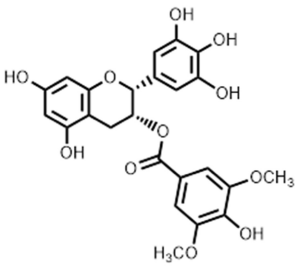
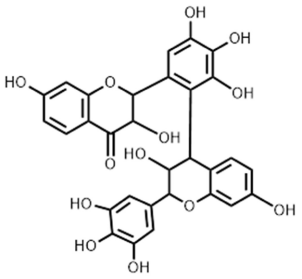
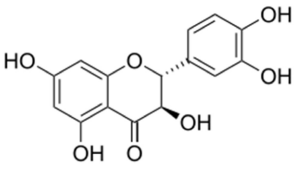
| | | |
|---|---|---|
| **24F** |  Procyanidin A2 | Tannins |
| **25F** |  Gallocatechin 3'-O-Gallate | Phenolic Acids |
| **26F** |  3-Methoxy-4-[3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]oxybenzoic Acid | Phenolic Acids |

**Table 12.** Structures of the compounds detected in the ethanolic extract of *P. kotschyi* root by UHPLC-HRMS analysis.

| N° | Molecular Structures | Chemical Families |
|---|---|---|
| **1** |  Gallic Acid | Phenolic Acids |
| **2** |  Epicatechin | Flavonoids |
| **3** |  Catechol | Polyphenols |

**Continued**

| | | |
|---|---|---|
| **4** |  <br>(+)-Procyanidin B2 | Tannins |
| **5** |  <br>Catechin-7-Methyl Ether | Flavonoids |
| **6** |  <br>1-[2,4-Dihydroxy-6-[3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl]oxyphenyl]-3-(4-hydroxyphenyl)propan-1-one | Flavonoids |
| **7** |  <br>Epigallocatechin 3-O-(3,5-Di-O-Methylgallate) | Tannins |
| **8** |  <br>2-{2-[3,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-yl]-3,4,5-trihydroxyphenyl} -3,7-dihydroxy-3,4-dihydro-2H-1-benzopyran-4-one | Tannins |
| **9** |  <br>Dihydroquinone or taxifolin | flavonoids |

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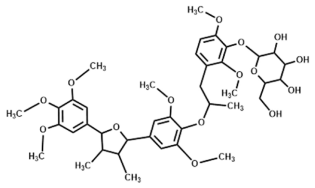
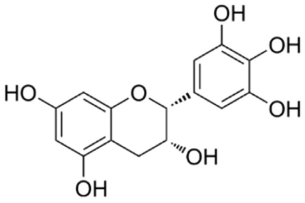
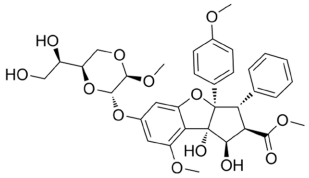
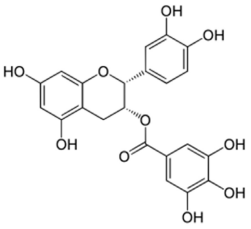
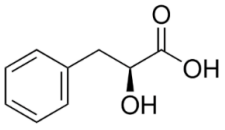
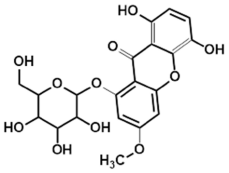
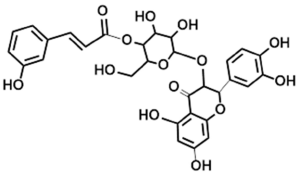
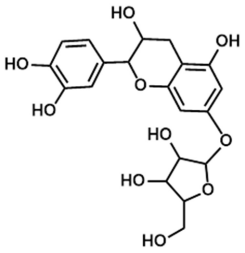
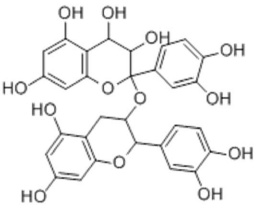
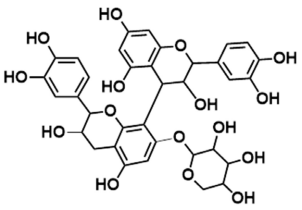
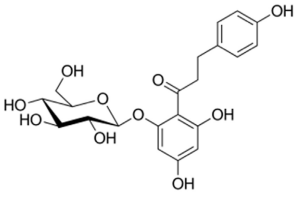
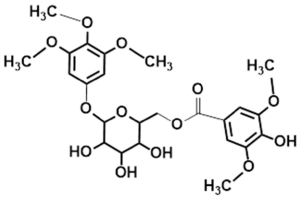
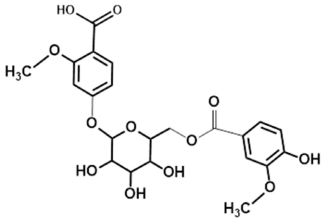
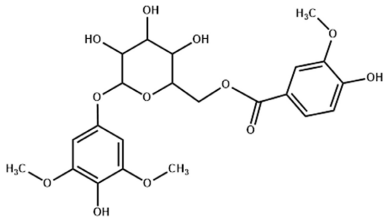
| | | |
|---|---|---|
| **10R** |  <br>Bonaspectin C 4''-Beta-Glucoside | Lignanes |
| **11R** |  <br>Epigallocatechin | Flavonoids |
| **12R** |  <br>Silvestrol | Lignanes |
| **13R** |  <br>(−)-Epicatechin gallate | Tannins |
| **14R** |  <br>L-(-) 3-Phénylactic Acid | Aromatic Acid |
| **15R** |  <br>5,8-dihydroxy-3-methoxy-1-{[3,4,5-trihydroxy-6-(hydroxymethyl) oxan-2-yl] oxy}-9H-xanthen-9-one | Xanthones |
| **16R** |  <br>3-(3-hydroxyphenyl) prop-2-enoate of 6-{[2-(3,4-dihydroxyphenyl)-5,7-dihydroxy-4-oxo-4H-chromen-3-yl] oxy}-4,5-dihydroxy-2-(hydroxymethyl) oxan-3-yl | Tannins |

**Continued**

| | | |
|---|---|---|
| **17R** |  <br>7-[3,4-dihydroxy-5-(hydroxymethyl) oxolan-2-yl] oxy-2-(3,4-dihydroxyphenyl)-3,4-dihydro-2H-chromene-3,5-diol | flavonoids |
| **18R** |  <br>Procyanidin | Tannins |
| **19R** |  <br>Catechin-(4-alpha-8)-7-O-Beta-Xylopyranosyl-Catechine | Tannins |
| **20R** |  <br>Phlorizin | flavonoids |
| **21R** |  <br>4-hydroxy-3,5-dimethoxybenzoate [3,4,5-trihydroxy-6-(3,4,5-trimethoxyphenoxy) oxan-2-yl] methyl | Tannins |
| **22R** |  <br>3-methoxy-4-({3,4,5-trihydroxy-6-[(4-hydroxy-3-methoxybenzoyloxy)methyl]oxan-2-yl}oxy)benzoic acid | Tannins |

**Continued**

| | | |
|---|---|---|
| **23R** |  | flavonoids |

4-hydroxy-3-methoxybenzoate de [(2R,3S,4S,5R,6S) -3,4,5-trihydroxy-6-(4-hydroxy-3,5-dimethoxyphenoxy) oxan-2-yl] methyl

## 5. Discussion

The results of solid-liquid extraction by maceration (Table 3) with increasing polarity of the three solvents used (hexane-dichloromethane-ethanol) showed that the ethanolic extract of the leaf was obtained with the lowest yield (4.11 ± 1.81%) compared to ethanolic extracts of the trunk bark (12.33 ± 1.43%) and root bark (11.68 ± 2.04%). Many reasons could explain the difference between the extraction yields of the three parts of this plant. Among these reasons include: the duration of exposure of the body in nature, the capacity of the plant body to store the biosynthesized compounds, the physiological state of the plant species to spread the biomolecules which are synthesized in its various bodies, the period of harvest of bodies, and the nature of the soil (pH, salinity, etc.) [41].

In the current work, the yield of the ethanolic extraction obtained for the trunk bark was low compared to the yields of 23.6% and 17.6% reported in the literature by [42], respectively, obtained from the extraction with the solvent hydroéthanolique (50/50) and with water. Regarding the extraction of the root bark of this plant, [43] obtained yields of 0.6%, 1.5%, 2.85%, and 30.22%, by maceration using respectively the following solvents: ethyl ether, dichloromethane, methanol, and water. As can be seen, the extraction yields of the parts of a plant depend not only on the type of solvent used and the extraction method adopted but also on the nature of the plant parts extracted. However, other factors affecting the extraction yield could also be considered. These factors include: the nature of the solvent, the harvest period, the environment where the plant grows, and the age of the plant [44].

In the present study, the selective extraction carried out by increasing the solvent polarity certainly had an effect on the extraction yield, since some compounds already extracted in the first two phases (hexane-dichloromethane) were not available in the residue to be re-extracted with ethanol used as an extraction solvent in this work.

The phytochemical tests carried out on the three ethanolic extracts revealed the presence of phenolic compounds, tannins, flavonoids, saponins, and alkaloids in all of the extracts (leaf, trunk bark and root bark) of *P. kotschyi*. However, triterpenes were not identified in any of the extracts analyzed. These findings are similar to those reported for the leaf [26] [45], the trunk bark [46] [47], and the root bark [48] [49]. Furthermore, triterpenes were not revealed in this study. This var-

iability in the composition of plant secondary metabolites could be related to the period of harvest of the plant body part, the chemical composition of the soil, the structure of the soil, climatic factors, or the stage of the plant's development [41] [50] [51]. It could also be due not only to the method of extraction, but also to the solvents used [41]. These increasingly recurring diseases, which are very dangerous for humans, are one of the main causes of morbidity and mortality, especially in developing countries. Indeed, these pathogenic strains develop resistance mechanisms to drugs currently sold on the market. Therefore, researchers have the difficult task of finding irreversible solutions to definitively eliminate these multi-resistant pathogenic strains causing new diseases. Finding effective drugs against these strains is a major challenge that must be met conscientiously by knowledgeable researchers. It is in this dynamic research and development of the community that the antibacterial activity of the secondary metabolites present in this medicinal plant has its full meaning and caught the author's attention in this study.

In the present work, the leaf, trunk bark, and root bark of *P. kotschyi* tested presented very interesting antibacterial activity. Indeed, the results of the antibacterial test showed that, in general, *P. kotschyi* has significant antibacterial properties. Indeed, the various parts of the plant showed an antibacterial effect on one or more of the tested germs. Moreover, the ratio of MBC/MIC was evaluated for each germ and, according to the indications provided by [37], showed that this ratio is less than or equal to four (4) when the extract has a bactericidal effect, whereas a ratio greater than four (4) indicates that the extract has a bacteriostatic effect on the germ.

Regarding the antibacterial activities of different ethanolic extracts of *P. kotschyi* on the reference strain (*E. coli* ATCC 22925), the extract of the trunk bark showed an MBC/MIC ratio of antibacterial activity equal to 2, evidencing therefore its bactericidal effect on the germ. This result was similar to those obtained by [42] using the reference strain (*E. coli* ATCC 0157H7).

Regarding the reference strain (*E. coli* ATCC 22925), the antibacterial molecules used as references were gentamicin (GM) and ceftriaxone (CRO), which are more active with a ratio of activity equal to 1. In relation to the clinical strain of *E. coli*, the leaf ethanolic extract presented a bactericidal effect comparable to that obtained by [29]. The antimicrobial activity of various extracts was also tested by these authors on *P. aeruginosa.* All samples tested showed a bactericidal effect on this bacterial strain. The results indicated a bactericidal effect, identical to that of the reference molecule on *Salmonella typhimurium*, on which the ethanolic extract of the trunk bark had a bactericidal activity with an activity ratio equal to 4.

The antimicrobial activity of this plant would be related to the presence of many compounds which are pharmacologically bioactive, such as flavonoids, tannins, anthraquinones, alkaloids, and other phenolic compounds involved in the antibacterial activities of many plants [52]. Indeed, the phytochemical screening carried out on the ethanolic extract of different parts of *P. kotschyi* has shown the presence of alkaloids, flavonoids, tannins, and phenolic compounds in the plant

extracts. The antibacterial activity of the plant could then be attributed to the presence of these pharmacologically important compounds identified in these extracts.

The analysis of the ethanolic extracts of leaf, trunk bark, and root bark by the HPLC-DAD system helped to identify and quantify 11 phenolic compounds, including four phenolic acids and seven flavonols. The four phenolic acids identified in the extracts of the plant were: gallic acid, coumaric acid, ferulic acid, and caffeic acid; while the seven flavonols were: quercetin, myricetin, kaempferol, catechin, epicatechin, rutin, and arbutin.

In addition, the analysis by UHPLC-HRMS (UHPLC-HESI-Q-Exactive Plus) was performed in order to identify the molecules in the crude ethanolic extract from the leaf compared with the crude ethanolic extract obtained from the root bark of *P. kotschyi*. According to the findings of this analysis, 26 compounds were identified in the crude ethanolic extract of the leaf (**Table 11**) and 23 compounds in the ethanolic extract of the root (**Table 12**). Among these compounds identified, nine (9) of them were found in both the extracts of the leaf and of the root bark of this plant. These phytochemicals were: gallic acid, catechol, (+)-procyanidin B2, catechin-7-way ether, epigallocatechin-3-O-(3,5-di-O-methylgallate), epicatechin; 1-[2,4-dihydroxy-6-[3,4,5-trihydroxy-6-(hydroxymethyl)oxan-2-yl]oxyphenyl]-3-(4-hydroxyphenyl)propan-1-one; 2-{2-[3,7-dihydroxy-2-(3,4,5-trihydroxyphenyl)-3,4-dihydro-2H-1-benzopyran-4-yl]-3,4,5-trihydroxyphenyl}-3,7-dihydroxy-3,4-dihydro-2H-1-benzopyran-4-one. Many of these molecules, which were revealed in the plant extract, are truly endowed with several biological activities by referring to the previous work done in the literature [29] [52]. Indeed, gallic acid and catechin derivatives act on gram-negative bacteria by disrupting the integrity of their cell membranes, leading to leaks of essential intracellular compounds and compromising the survival of these bacteria [53].

Antibiotics are substances or molecules of natural or synthetic chemicals, which have specific actions against microbes. The phenolic compounds identified in different extracts of *P. kotschyi* such as 1,6-Digalloyl-beta-D-glucopyranose, epigallocatechin, catechin, arbutin, epigallocatechin, dihydroquercétine, quercetin, myricetin, afzeline, kaempferol and isoquercétine, acid, caffeic acid, and coumaric acid, are known as excellent active principles for the formulation of antimicrobial drug products [54]-[56]. They target the processes fundamental to the life of bacteria. They have the capacity to act by inhibiting the synthesis of the cell wall in microbes, which protects them, causing the burst of the microbes. Consequently, they prevent microbial growth by interfering with the replication of their DNA. Some antimicrobials have the particularity of destroying the bacterial ribosome, which is the site of protein synthesis in microbial cells, while others block the production of the elements necessary for microbial cells by inhibiting their metabolic pathways.

Most of the molecules identified in the ethanolic extracts of *P. kotschyi* have specific biological properties. However, the molecules identified in this work are

not the only ones that constitute the full chemical composition of the extracts. The presence of all these compounds in different body parts of *P. kotschyi* would be the source of its continually expanding use in phytotherapy in Togo.

## 6. Conclusions

The completion of this work indicates that the best yield of extraction was obtained from the trunk bark. The three organs (leaf, trunk, and root) of *P. kotschyi* contain the same secondary metabolites. The study of *in vitro* antibacterial activity of ethanolic extracts of leaf, trunk bark, and root bark of *P. kotschyi* has shown that this plant possesses proven antibacterial properties. The analysis of the three ethanolic extracts by HPLC-DAD helped to identify and quantify 11 phenolic compounds. Moreover, the UHPLC-HRMS analysis facilitated the identification of 26 and 23 phenolic compounds in the ethanolic extract of leaf and in the ethanolic extract of the root bark, respectively. The richness of this plant in these biomolecules would explain its increasing use in herbal medicine.

Future research could focus on isolating bioactive compounds contained in the body parts of this plant in order to test their biological activities separately.

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## Conflicts of Interest

The authors declared that there are no conflicts of interest regarding this paper's publication.

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