Scientific Research Publishing

Advances in Biological Chemistry, 2023, 13, 209-227
https://www.scirp.org/journal/abc
ISSN Online: 2162-2191
ISSN Print: 2162-2183

LC-MS Phytochemical Profiles of Phenolic Compounds and Antimicrobial, Antioxidant, and Antiplasmodial Activities of Ethanol Extracts of *Pycnostachys erici-rosenii* R.E.Fr and *Leucas martinicensis* (Jack.) R.Br (Lamiaceae)

Uwikunda Serondo Héritier[1,2,3,4], Nizigiyimana Libérata[2,4], Pius Tshimankinda Mpiana[5], Nyamangyoku Ishibwela Obedi[6], Ngezahayo Jérémie[2,4], Dan Cristian Vodnar[7], Daniela Hanganu[8], Ioana Matei[9], Sanda Andrei[3]

[1]Department of Chemistry, Faculty of Sciences, Institut Supérieur Pédagogique de Bukavu, Bukavu, Democratic Republic of Congo
[2]Centre de Recherche en Sciences Naturelles et de l'Environnement (CRSNE), Faculté des Sciences, Université du Burundi, Bujumbura, Burundi
[3]Department of Preclinical Sciences, Faculty of Veterinary Medicine, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania
[4]Ecole Doctorale de l'Université du Burundi, Bujumbura, Burundi
[5]Department of Chemistry, Faculty of Sciences, Université de Kinshasa, Kinshasa, Democratic Republic of Congo
[6]Faculty of Agriculture, Université Catholique de Bukavu, Bukavu, Democratic Republic of Congo
[7]Department of Food Science, Faculty of Food Science and Technology, University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca, Cluj-Napoca, Romania
[8]Department of Pharmacognosy, Faculty of Pharmacy, University of Medicine and Pharmacy "Iuliu Hatieganu", Cluj-Napoca, Romania
[9]Department of Microbiology, Mycology and Immunology, Faculty of Veterinary Medicine, University of Agricultural Science and Veterinary Medicine, Cluj-Napoca, Romania
Email: serondo1084@gmail.com

How to cite this paper: Héritier, U.S., Libérata, N., Mpiana, P.T., Obedi, N.I., Jérémie, N., Vodnar, D.C., Hanganu, D., Matei, I. and Andrei, S. (2023) LC-MS Phytochemical Profiles of Phenolic Compounds and Antimicrobial, Antioxidant, and Antiplasmodial Activities of Ethanol Extracts of *Pycnostachys erici-rosenii* R.E.Fr and *Leucas martinicensis* (Jack.) R.Br (Lamiaceae). *Advances in Biological Chemistry*, **13**, 209-227.
https://doi.org/10.4236/abc.2023.136015

Abstract

This study has been conducted to analyze the chemical composition and antimicrobial, antioxidant and antiplasmodial properties of ethanol extracts of *Pycnostachys erici-rosenii* R.E.Fr and *Leucas martinicensis* (Jack.) R.Br that are not yet well known. The HPLC-DAD-MS-ESI$^+$ method was used to investigate the chemical profile, the disc diffusion and microdilution method for the antimicrobial activities, the DPPH and FRAP assays for antioxidant property and SYBR Green I-based growth inhibition assay on *Pf* 3D7 (CQ-sensitive) strain for antiplasmodial properties. The results indicated significant amounts of hydroxybenzoic acid, flavonol and hydroxycinnamic

Received: August 28, 2023
Accepted: December 1, 2023
Published: December 4, 2023

acid in the two species. The total polyphenols analysis gave 209.0 ± 16.1 mg GAE/100g DW for *P. erici-rosenii* and 175.3 ± 1.6 mg GAE/100g DW for *L. martinicensis* while for total flavonoids analysis 33.4 ± 1.8 mg TE/100g DW was found for *P. erici-rosenii* and 39.6 ± 0.6 mg TE/100g DW for *L. martinicensis*. Moreover, antimicrobial tests revealed 0.125 mg/mL on *E. faecalis* ATCC25922 for ethanol extracts *P. erici-rosenii* and *L. martinicensis*. The antioxidant assays revealed IC_{50} 56.17 µg/mL and 78.43 µg/mL for DPPH assay, and 112.03 µM TE/100mL and 90.46 µM TE/100mL) for FRAP assay for ethanol extracts of *P. erici-rosenii* and *L. martinicensis* respectively. Finally, for Antiplasmodial activity, IC_{50} was 4.07 µg/mL for *P. erici-rosenii* and 12.29 µg/mL for *L. martinicensis*. Therefore, the antimicrobial, the antioxidant, and the antiplasmodial results suggest that the ethanolic extracts of the aerial parts of *P. erici-rosenii* and *L. martinicensis* contain compounds with antimicrobial, antioxidant, and antiplasmodial properties, which can be searched in new drug discovery for treatment of infectious diseases.

Keywords

Pycnostachys erici-rosenii, *Leucas martinicensis*, Phytochemical Profile, Antimicrobial Activity, Antioxidant Activity, Antiplasmodial Activity

1. Introduction

In African countries, plants are the main medicinal resources for public health care. Among these plants, is the Lamiceae (Labiatae) family, from the Latin (Labia: lip) which includes about 258 genera and 7000 species spread especially from the Mediterranean Basin to Central Asia. The Lamiaceae are herbaceous, annual or perennial and aromatic plants. This family is an important source of essential oils for aromatherapy, perfumery and the cosmetics industry [1] [2].

In the traditional African pharmacopoeia, plants of the Lamiaceae family are used as diuretics, anti-syphilitics, anti-diarrhoea, healing, antiseptic and in the treatment of many ailments such as intestinal problems or meteorism (bloating of the belly due to gas) [1]. *P. erici-rosenii* R.E.Fr and *L. martinicensis* are among the plants of the Lamiaceae family, which are the subject of the present study.

P. erici-rosenii and *L. martinicensis* are used in traditional medicine of Democratic Republic of Congo (DRC) to treat diseases of bacterial origin and those due to oxidatives stress, but also many others such as hypogastritis, cough, malaria, colibacillosis, asthma, tuberculosis, panaria, boils, food poisoning, snake bites, nervous disorders, liver disorders, diarrhoea, etc. *P. erici-rosenii* and *L. martinicensis* are frequently used in combination with other plants like *Crassocephalum montuosum* (Asteraceae), *Cinchona ledgeriana* (Rubiaceae), *Bridelia bridelifolia* (Phyllanthaceae), *Centella asiatica* (Apiaceae), *Psidium guajava* (Myrtaceae), *Leonotis naepetifolia* (Lamiaceae), *Ageratum conyzoides* (Asteraceae), *Phyllanthus nuriri* (Phyllanthaceae), *Solanum nigrum* (Solanaceae), etc.

Actually, the phenomenon of bacterial resistance concerns all families of antibiotics. Bacterial species develop different mechanisms according to their initial sensitivity and their capacity to express the various resistances. This bacterial resistance is increasing at an alarming rate and is responsible for more than 700,000 deaths each year worldwide [3] and if nothing is done, by 2050, it will cause 10 million deaths annually [4].

Regarding malaria, the number of malaria cases worldwide was estimated at 247 million in 2021 in 84 malaria-endemic countries, up from 245 million in 2020. Most of this increase occurs in the WHO African Region Countries. In 2015, the baseline year for the Global Technical Strategy for Malaria 2016-2030, there were an estimated 230 million malaria cases. The incidence of malaria cases fell from 82 in 2000 to 57 in 2019, before rising to 59 in 2020. The incidence of cases did not change between 2020 and 2021. The increase in 2020 was associated with the disruption of services during the Coronavirus Disease 2019 (COVID-19) pandemic. About 96% of global malaria deaths occurred in 29 countries, and four countries accounted for just over half of all global malaria deaths in 2021: Nigeria (31%), the Democratic Republic of the Congo (13%), Niger (4%) and the United Republic of Tanzania (4%) [5].

Thus, in view of the different uses of *P. erici-rosenii* and *L. martinicensis* in traditional medicine, the ethanol extracts from aerial parts of these two species were scripted for antimicrobial, antioxidant and antiplasmodial agents.

The investigation of the chemical composition of the aerial parts of *P. erici-rosenii* and *L. martinicensis* for the antimicrobial, antioxidant, and antiplasmodial properties of the ethanol extracts is useful in light of recent epidemiological studies. Those studies have suggested that certain plant extracts containing polyphenols may be helpful in preventing the development or slowing infectious diseases. Therefore, we investigated these two plants, which have not been investigated before for these purposes.

2. Materials and Methods

2.1. Plant Material

The aerial parts of *P. erici-rosenii* (synonym: *Coleus erici-rosenii* (R.E. Fr.) A.J. Paton) and *L. martinicensis* (Synonym: *Leonotis martinicensis* (Jacq.) J.C. Manning & Goldblatt) were collected in November 2020 in the territory of Kabare, South Kivu Province, in the East of the Democratic Republic of Congo. After the identification of plants in the Herbarium of the "Centre de Recherche en Sciences Naturelles de Lwiro (CRSN/Lwiro)", the aerial parts were collected, dried at room temperature and then crushed to obtain the plant powders. The powders were kept in sealed bags at room temperature for further analysis (**Figure 1**).

2.2. Preparation of Extracts

The ethanol extracts of *P. erici-rosenii* and *L. martinicensis* were prepared by

(a) (b)

Figure 1. Photos of aerial parts of *P. erici-rosenii* (a) and *L. martinicensis* (b) (Credit: AfricanPlant Data Base).

maceration using ethanol 96% v/v for 48 hours. After filtration, the different filtrates were concentrated by evaporation using rotavapor BUCHI Switzerland Heating Bath B-100 under reduced pressure (Vacuum Pump V-100). The extracts were carefully preserved for further phytochemical analysis and for antimicrobial, antioxidant and antiplasmodial tests.

2.3. Total Phenolic Content

The Total Phenolic Contents of ethanol extracts of *P. erici-rosenii* and *L. Martinicensis* were measured using the Folin-Ciocalteu method with a visible wavelength of 765 nm. A calibration curve with various concentrations of Gallic acid (1 mg/100mL; 0.5 mg/100mL; 0.25 mg/100mL; 0.125 mg/mL, and 0.0625 mg/mL) was established and had an equation $y = 2.364x + 0.0649$ with $R^2 = 0.9965$. Wells of 3 mL was used, containing 2.35 mL distilled water, 0.05 mL of the extract, 0.15 mL Folin-Ciocalteu reagent, and 0.45 mL Na_2CO_3 (7.5%). The samples were left for 2 hours in darkness before measuring the absorbance on the UV-Vis spectrometer at 765 nm to determine total polyphenols expressed in mg Gallic acid equivalents (GAE)/100g dry weight (DW) through the calibration curve. The analysis was completed three times and done using the SPECTROstar® Nano microplate spectrophotometer (from BMG Labtech, Ortenberg, Baden-Württemberg, Germany) [6] [7].

2.4. Total Flavonoid Content

The total flavonoid content was measured using the Plant Flavonoids Colorimetric Assay Kit, based on the reaction between flavonoids and aluminum ions, resulting in a red complex. The concentration of flavonoids in the sample was determined by recording absorbance at 510 nm. We prepared a standard curve

following the instructions in the kit, diluting a 1 mg/mL standard solution with double-distilled water for concentrations of 150, 120, 100, 80, 60 and 20 µg/mL. The calibration curve was established and had an equation $y = 0.0056x - 0.0126$ with $R^2 = 0.9936$. After that, samples have been prepared and their absorbances read against double-distilled water as a blank. The result obtained was expressed as mg Quercetin equivalent (QE)/100g DW and a spectrophotometer SPECTROstar® Nano (BMG Labtech, Ortenberg, Baden-Württemberg, Germany) was used [8].

2.5. Total Antioxidant Capacity

2.5.1. FRAP (Ferric Reducing Antioxidant Power) Assay

The FRAP method relies on the change in the color of a complex with Fe^{+3} ion of the 2,4,6-tri(2-pyridyl)-1,3,5-triazine (TPTZ) radical by the reduction of the ferric ion to the ferrous ion (Fe^{+2}) in this complex. To 2.5 mL of 10 mM TPTZ solution in 40 mM HCl, 2.5 mL 20 mM ferric chloride solution and 25 mL acetate buffer (pH = 3.6) were added. This mixture is the FRAP reagent. To 4.0 mL extract up to 0.8 mL water and 6.0 mL FRAP reagent were added. The blank solution was prepared in the same manner using water instead of a sample. Trolox was used as a reference. The color change was correlated with the antioxidant capacity by measuring absorbance at 450 nm. Using a calibration curve ($R^2 = 0.992$), the result was converted to µM Trolox equivalents/100mL extract [9] [10] [11].

2.5.2. DPPH Radical Scavenging Activity

DPPH (2,2-diphenyl-picrylhydrazil) radical scavenging assay is a spectrophotometric quantitative method based upon the reaction between the antioxidant compounds of the extract and $DPPH^{\bullet}$ reagent in an alcoholic solution. 2 mL (at different concentrations) of the extract was added to 2 mL of a 0.1 $g{\cdot}L^{-1}$ $DPPH^{\bullet}$ methanol solution. After 30 minutes in a thermostatic bath at 40˚C, the variation of the absorbance was measured at 517 nm ($R^2 = 0.997$). The percent of $DPPH^{\bullet}$ scavenging ability was calculated as follows: DPPH scavenging ability % = (A control − A sample/A control) × 100, where A control is the absorbance of $DPPH^{\bullet}$ radical + methanol (containing all reagents except the sample) and A sample is the absorbance of DPPH radical + sample extract. The DPPH radical scavenging activity of the extract was expressed as IC_{50} (µg/mL) [9] [10] [11].

2.6. Antimicrobial Properties Assay

The antimicrobial activities of ethanol extracts of *P. erici-rosenii* and *L. martinicensis* were evaluated on seven bacterial strains: *Staphylococcus aureus* 29213, *Listeria monocytogenes* ATCC 13932, *Enterococcus faecalis* ATCC25922, *Escherichia coli* ATCC51299, *Bacillus cereus* ACTT 11778, *Pseudomonas aeruginosa* QC76110 and *Candida albicans* ATCC 10231. The bacteria were replicated in 45 mL sterile nutrient broth (Oxoid Ltd., Basingstoke, Hampshire, UK) at 37˚C for 24 h, and *C. albicans* was grown at 30˚C. Purity of the inoculum was confirmed

by plating on appropriate media and viewing under a microscope (Optika microscope, B252, M.A.D; Apparecchiature Scientifiche, Milan, Italy). Plates were incubated for 24 h at 30˚C for *C. albicans*, and 37˚C for other strains. Stock cultures were kept at refrigeration temperatures to obtain freshly cultured suspensions, which were later used to assess antimicrobial activity via the disk-diffusion method. Several colonies from standard cultures cultivated on Mueller-Hinton agar (Oxoid Ltd., Basingstoke, Hampshire, UK) were transferred in saline solution (8.5 g/L) and then adjusted to match a McFarland 0.5 standard, before preparing a bacterial suspension of 1.5×10^6 CFU/mL. 15 mL Mueller-Hinton agar was poured into Petri dishes and 100 µL culture suspension was distributed over the plates using a Drigalski spatula [12] [13].

To create an inoculum, a 0.5 McFarland microbial suspension was diluted 1:150 to achieve 106 CFU/mL. This solution underwent a further dilution of 1:2, resulting in a final inoculum of 5×10^5 CFU/mL. Six test tubes received 0.1 mL of varying concentrations of the tested compound, ranging from 8 to 0.25 mg/mL; each was also given 0.4 mL of Mueller-Hinton broth with 2% - 5% lysed horse blood (for Streptococcus spp.), and 0.5 mL of the microorganism suspension at ~0.5×10^5 CFU/mL. After incubating at 37˚C for 24 hours, the minimum inhibitory concentration (MIC) was recorded (MIC is the lowest concentration without visible growth). In a control tube containing ethanol instead of a tested compound; 0.5 mL of microbial suspension and 0.4 mL Mueller-Hinton broth were added [13] [14].

To determine the minimum bactericidal concentration (MBC), 1 µL from tubes with no growth was taken and applied to Columbia agar with 5% blood. The minimum fungicidal concentration (MFC) was assessed by inoculating a Sabouraud with chloramphenicol medium both incubated at 37˚C for 24 hours afterwards [12] [13] [14].

2.7. Antiplasmodial Properties Assay

2.7.1. Culture Protocol for the *Plasmodium falciparum* Strain

The culture technique used was that of Trager and Jensen [15]. The chloroquine-sensitive *Plasmodium falciparum* strain Pf3D7 was grown in fresh human red blood cells, group O Rhesus positive at 4% haematocrit in complete RPMI medium [500 mL RPMI 1640 (Gibco, UK) supplemented with 25 mM HEPES (Gibco, UK), 0.50% Albumax I (Gibco, USA), 1X hypoxanthine (Gibco, USA) and 20 µg/mL gentamicin (Gibco, China)] and incubated at 37˚C in a humidified incubator consisting of 92% N_2, 5% CO_2 and 3% O_2. The medium was replaced daily with a complete RPMI medium to facilitate the growth of parasites in culture. Subsequently, fine blood smears were taken and stained with Giemsa and then observed under a microscope at 100× objective with immersion oil in order to follow all the steps of the cell cycle and to evaluate the parasitemia.

2.7.2. Synchronisation of Cultures

Before each test for antiplasmodial activity, parasitic cultures containing the

majority of ring stages (>80%) were synchronised to the same evolutionary stage (ring stage) by treatment with 5% (w/v) sorbitol for 10 min according to the protocol of [16]. The use of synchronised cultures at the same evolutionary stage compared to mixed stage cultures allows the evaluation of the plant extract's effect on all three evolutionary phases (rings, trophozoite, schizont) of the 48 hours life cycle of *P. falciparum* [17] [18].

2.7.3. Preparation of Plant Extracts and References (Artemisinin and Chloroquine)

Stock solutions were prepared in 10% DMSO at concentrations of 10 mg/mL and 1 mM for each plant extract, and artemisinin, and chloroquine references respectively. For this purpose, 10 mg of extract from each plant was dissolved in 100 µL of dimethylsulphoxide (DMSO) followed by vortexing for at least 15 minutes to get a homogeneous solution. After dissolution, the volume was made up to 1 mL to obtain a 10 mg/mL solution. The solution obtained was filtered and the filtrate was used for the anti-plasmodial activity tests [17] [18].

Intermediate concentrations of each sample were achieved by adding 10 µL of stock solution of each plant extract (10 mg/mL) and 20 µL of stock solution of each positive control (100 µM) into 190 µL and 180 µL of incomplete RPMI1640 medium respectively in a 96-well microplate followed by a geometric dilution of order 5. Intermediate concentrations ranged from 8 to 5000 µg/mL for the extracts of each plant and from 0.016 to 10 µM for the positive controls [17] [18].

2.7.4. *In Vitro* Test for *P. falciparum* Growth Inhibition Based on SYBR Green Fluorescence

The anti-plasmodial activity assay of the extracts from each plant and the positive controls were performed in 96-well flat-bottom microplates based on the fluorescence of SYBR green I [17]. The ability of SYBR green I to produce strong fluorescence only in the presence of DNA is the basis for its use in assessing cell proliferation. The lack of cell nuclei within human red blood cells in which the parasite proliferates allows specific monitoring of plasmodial growth using SYBR green I. Experimentally, 90 µL of the parasite suspension synchronised to the ring stage at 2% parasitaemia and 1% haematocrit was incubated with 10 µL of different concentrations of pre-diluted plant extracts, artemisinin and chloroquine. The plates were then incubated at 37˚C in a humidified incubator consisting of 92% N_2, 5% CO_2 and 3% O_2 for 72 h. The final concentrations in the test plates varied from 0.8 to 500 µg/mL (DMSO < 1%) for the extracts of each plant and from 0.0016 to 1 µM (DMSO 0.1%) for the positive controls in the final volume of 100 µL. The experiments were performed in duplicate [17] [18].

After 72 hours of incubation, 100 µL of SYBR Green I buffer 6 µL of 10,000 × SYBR Green I (Invitrogen) + 600 µL of red cell lysis buffer [Tris (25 mM; pH 7.5)] + 360 µL of EDTA (7.5 mM) + 19.2 µL of parasite lysis solution {saponin (0.012%; wt/vol)} and 28.8 µL of Triton X-100 (0.08%; vol/vol)]} were gently added to each well, followed by incubation at 37˚C for 1 hour in the dark. After 1 hour of incubation, fluorescence was measured using the Infinite M200 (Te-

can) Microplate reader at an excitation and emission wavelength of 485 and 538 nm, respectively. The fluorescence values obtained were used to calculate the percent inhibition using Microsoft Excel. Subsequently, the 50% inhibitory concentrations (IC_{50}) were determined from the concentration-response curves obtained by plotting the logarithm of the concentration versus the percentage inhibition using GraphPad Prism software [17] [18].

2.8. HPLC-DAD-MS-ESI$^+$ Analysis

2.8.1. Chemical Reagents and Materials

Acetonitrile, HPLC-gradient, was provided by Merck (Germany) and water was purified with a Direct-Q UV system by Millipore (USA). The pure standard of gallic, chlorogenic acid (purity 99% HPLC) and rutin (purity 99% HPLC) was purchased from Sigma (USA) [19] [20].

2.8.2. Chromatographic Condition

To evaluate polyphenolic compounds, the HP-1200 liquid chromatography was used, fitted with a quaternary pump, autosampler, DAD detector, and MS-6110 single quadrupole API-electrospray detector (Agilent Technologies, Santa Clara, CA, USA). Detection was done in the positive ionization mode. Different fragments or voltages were applied in the range of 50 - 100 V. Separation was achieved with an Eclipse XDB-C18 (5 μm; 4.5 × 150 mm i.d.) column (Agilent) and a mobile phase consisting of 0.1% acetic acid in water (A) and 0.1% acetic acid in acetonitrile (B). A multistep linear gradient was employed for elution from 5% to 90% B over 20 minutes, followed by a 4-minute hold at 90% B and a 6-minute return to 5% B keeping the flow rate at 0.5 mL/min and temperature at 25˚C ± 0.5˚C. Phenolic compounds in the extracts of *P. erici-rosenii* and *L. martinicensis* were identified by comparing retention times, UV visible spectra and mass spectra to the database. Data acquisition was conducted using the scan mode on a mass spectrometer set at 3500˚C, 7 L/min nitrogen flow, nebulizer pressure 35 psi, capillary voltage 3000 V, fragment or 100 V and m/z 120 - 1200 chromatograms recorded at λ = 280 and 340 nm were processed through Agilent ChemStation software [19] [20].

3. Results

3.1. Phytochemicals Content of *P. erici-rosenii* and *L. martinicensis*

Table 1 below presents the results of the total phenolic compounds content, and the total flavonoid content of the aerial parts of *P. erici-rosenii* and *L. martinicensis*.

3.2. Antioxidant Activities Assays of *P. erici-rosenii* and *L. martinicensis*

The *in vitro* antioxidant capacities of ethanol extracts of aerial parts of *P. erici-rosenii* and *L. martinicensis* were evaluated by two different methods: 2,2-diphenyl-picrylhydrazil (DPPH•) scavenging assay and ferric-reducing antioxidant power (FRAP) (Table 2).

3.3. Antimicrobial Activities of *P. erici-rosenii* and *L. martinicensis*

Table 3 presents the antimicrobial activities of the ethanol extracts of aerial parts of *P. erici-rosenii* and *L. martinicensis*. Two methods were used to assess these activities: the disk diffusion method, indicated by the diameter of the inhibition zone, and the dilution method, indicated by the MIC and the MBC/MFC.

3.4. Antiplasmodial Properties Assay

Table 4 presents the results obtained for antiplasmodial properties assay of the

Table 1. Phytochemicals Content of the aerial parts of *P. erici-rosenii* and *L. martinicensis.*

Assays	*P. erici-rosenii*		*L. martinicensis*	
	Total Polyphenols mg GAE/100g DW	Flavonoids mg QE/100g DW	Total Polyphenols mg GAE/100g DW	Flavonoids mg QE/100g DW
Assay 1	225.1	33.5	177.3	40.1
Assay 2	209.0	31.5	174.3	39.6
Assay 3	193.0	35.2	174.0	39.0
Average ± SD	209.0 ± 16.1	33.4 ± 1.8	175.3 ± 1.6	39.6 ± 0.6

SD: Standard Deviation, QE : Quercetin Equivalents, GAE: Gallic Acid Equivalents.

Table 2. Antioxidant activities assays of *P. erici-rosenii* and *L. martinicensis.*

Ethanol Extracts	DPPH (IC_{50} µg/mL)	FRAP (µM TE/100mL)
P. erici-rosenii	56.17	112.03
L. martinicensis	78.43	90.46

Table 3. The diameter of inhibition growth zone, the minimum inhibitory concentration, and minimum bactericidal concentration of *P. erici-rosenii* and *L. martinicensis.*

	P. erici-roseni			*L. martinicensis*		
	Disk diffusion method	Dilution method		Disk diffusion method	Dilution method	
	Inhibition zone diameter (mm)	MIC (mg/mL)	MFC MBC (mg/mL)	Inhibition zone diameter (mm)	MIC (mg/mL)	MFC MBC (mg/mL)
S. aureus 29213	12.2	0.250	0.250	10.0	0.250	0.250
L. monocytogenes ATCC 13932	12.5	0.125	0.125	10.6	0.500	0.500
E. faecalis ATCC25922	11.9	0.125	0.125	10.0	0.250	0.250
E. coli ATCC51299	10.7	0.250	-	10.0	0.500	-
B. cereus ACTT 11778	10.0	0.250	-	10.0	0.500	-
P. aeruginosa QC76110	10.0	0.250	0.250	10.0	0.500	-
C. albicans ATCC 10231	10.0	0.125	-	10.0	0.500	-

MIC: Minimum Inhibitory Concentration; MBC: Minimum Bactericidal Concentration; MFC: Minimum Fungicidal Concentration.

ethanol extracts of aerial parts of *P. erici-rosenii* and *L. martinicensis* on *Pf* 3D7 (CQ-sensitive) (Tested concentration range: 0.78125 - 100 µg/mL; Assay type: SYBR Green I-based growth inhibition assay; Activity: $IC_{50} \leq 20$ µg/mL).

3.5. HPLC-DAD-ESI$^+$ Analysis

Figures 2-5 and Table 5 present the results obtained for the HPLC-DAD-ESI$^+$ analysis of phenolic compounds found in the ethanol extracts of aerial parts of *P. erici-rosenii* and *L. martinicensis*.

4. Discussion

The Total Phenolic Contents of the aerial parts of *P. erici-rosenii* and *L. martinicensis* were 209.0 ± 16.1 mg GAE/100g DW for *P. erici-rosenii* and 175.3 ± 1.6 mg GAE/100g DW (Table 1). For the Total Flavonoids Contents of the aerial parts of *P. erici-rosenii* and *L. martinicensis*, the values were 33.4 ± 1.8 mg QE/100g DW and 39.6 ± 0.6 mg QE/100g DW respectively (Table 1). When comparing these obtained values to other values from some other Lamiaceae species (169 mg GAE/g DW of aqueous extract from *Salvia sclarea*, 170 mg GAE/g

Table 4. Antiplasmodial test results of ethanol extracts of *P. erici-rosenii* and *L. martinicensis*.

Samples	% inhibition	IC_{50} 3D7 (µg/mL)
Ethanol extract of *P. erici-rosenii*	89.04	4.07
Ethanol extract of *L. martinicensis*	70.00	12.29

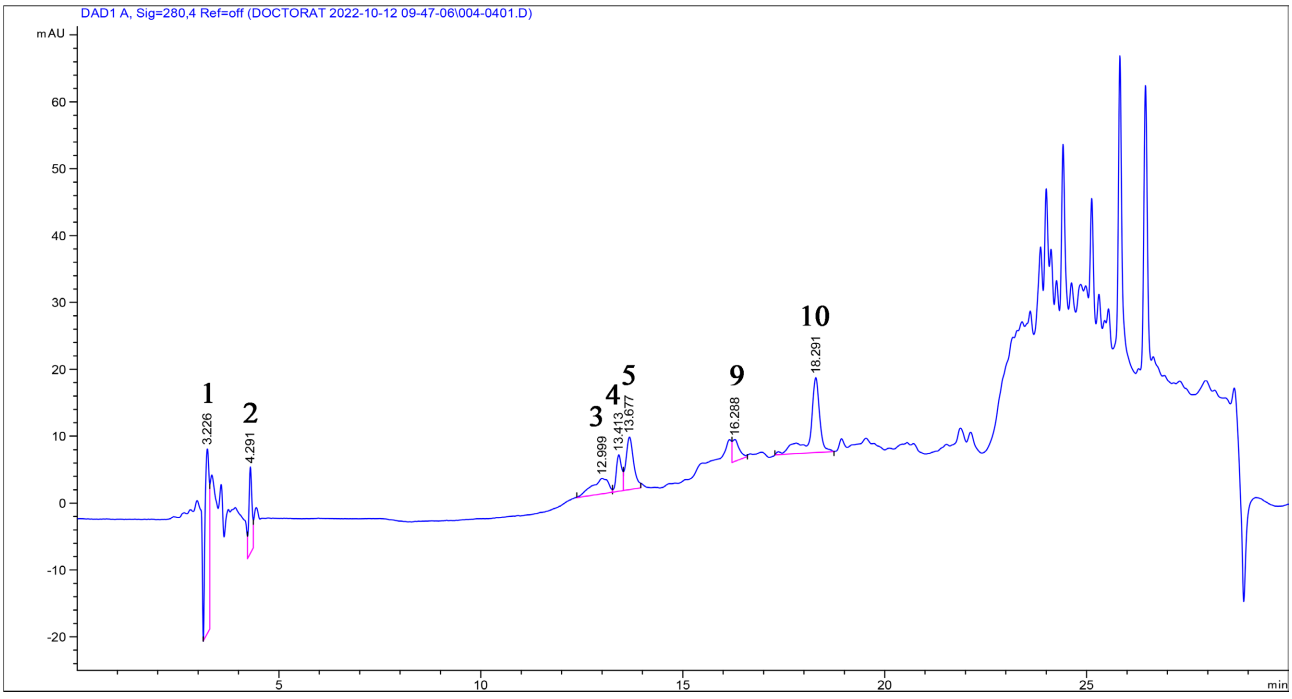

Figure 2. HPLC-DAD chromatogram of ethanol extract of *P. erici-rosenii* at 280 nm (1: 2-hydroxybenzoic acid; 2: gallic acid; 3: 4-hydroxybenzoic acid; 4: kaempferol-rutinoside; 5: vanilic acid; 9: quercetin-glucoside; 10: coumaroylquinic acid)

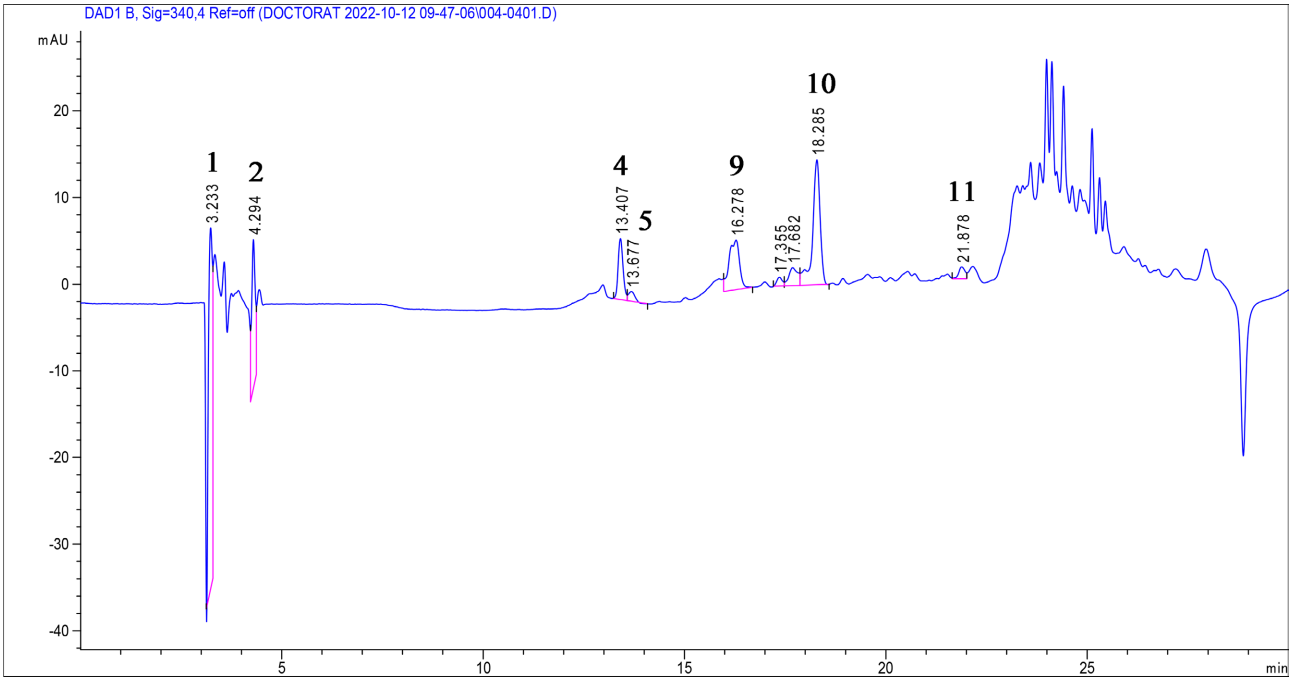

Figure 3. HPLC-DAD chromatogram of ethanol extract of *P. erici-rosenii* at 340 nm (1: 2-Hydroxybenzoic acid; 2: gallic acid; 4: kaempferol-rutinoside; 5: vanilic acid; 9: quercetin-glucoside; 11: quercetin)

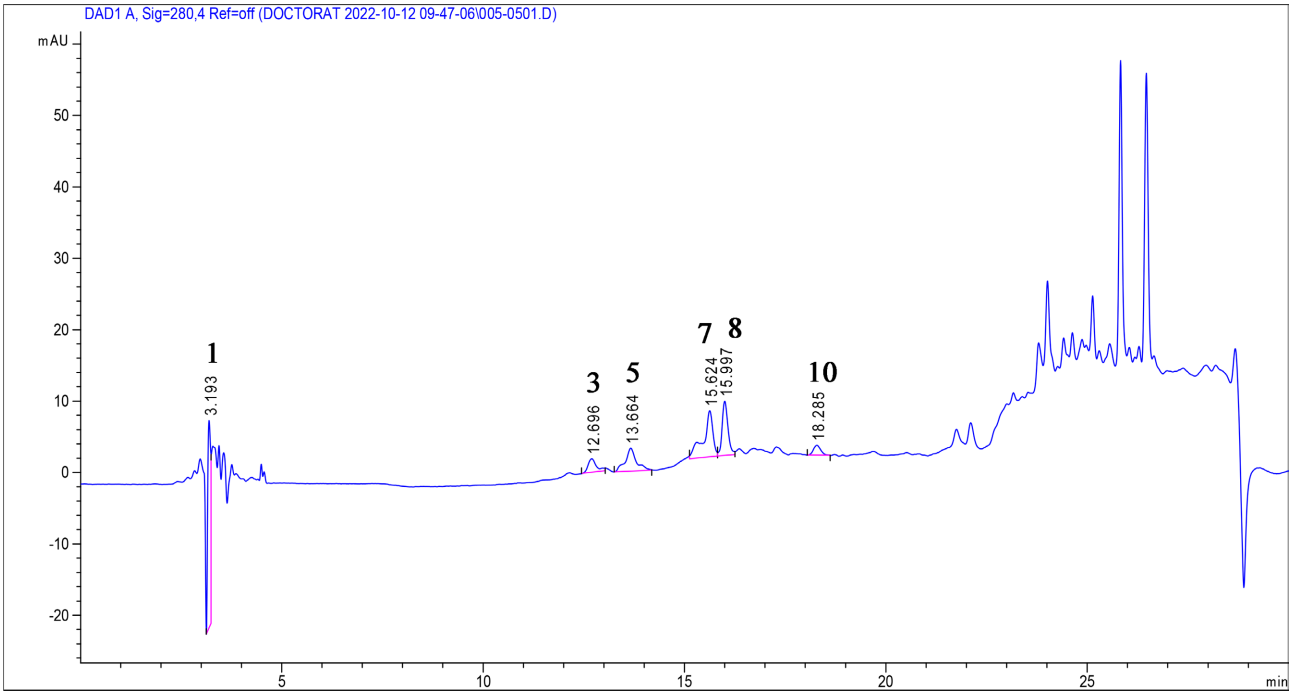

Figure 4. HPLC-DAD chromatogram of ethanol extract of *L. martinicensis* at 280 nm (1: 2-hydroxybenzoic acid; 3: 4-hydroxybenzoic acid; 5: vanilic acid; 7: ferulic acid; 8: feruloylquinic acid; 10: coumaroylquinic acid).

DW aqueous extract from *Salvia raeseri* ssp; 138 mg GAE/g DW of aqueous extract from *Mentha pulegium),* it can be noticed that ethanol extracts of these two species from the Eastern Democratic Republic of Congo were rich in phenolics and flavonoids content. This would explain the high antioxidant capacity

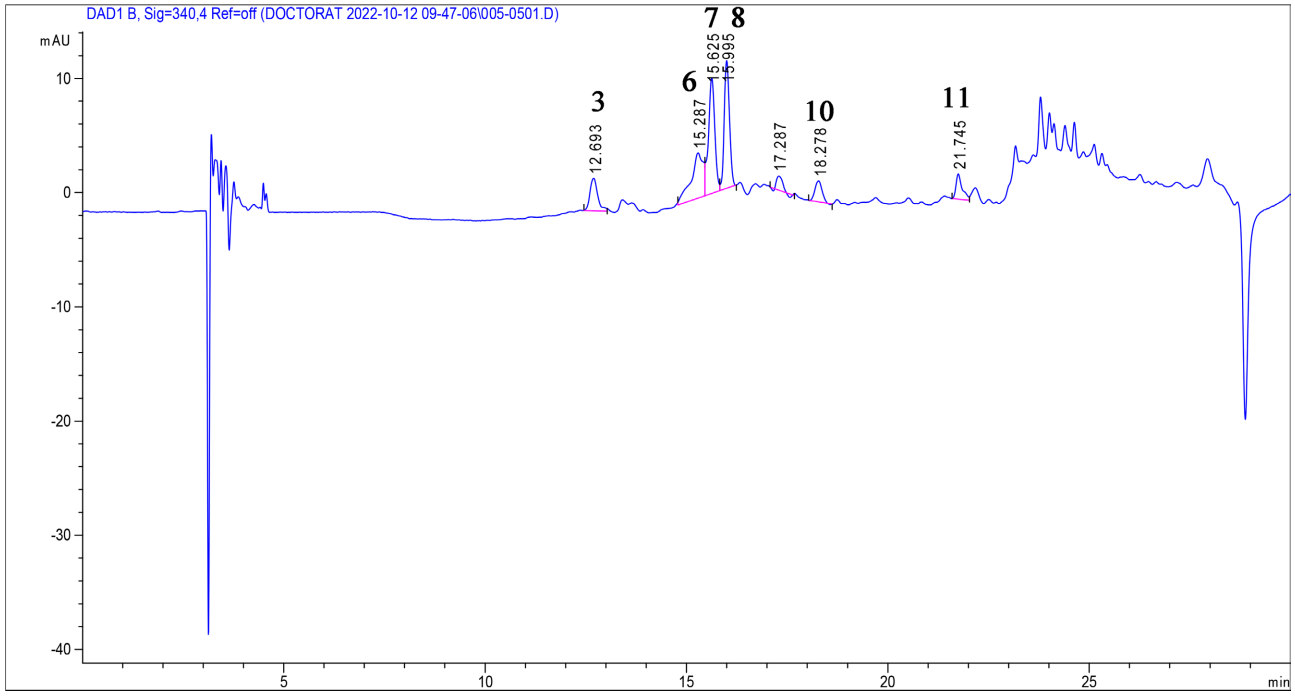

Figure 5. HPLC-DAD chromatogram of ethanol extract of *L. martinicensis* at 340 nm (3: 4-hydroxybenzoic acid; 6: *p*-coumaric acid; 7: ferulic acid; 8: feruloylquinic acid; 10: coumaroylquinic acid; 11: quercetin).

Table 5. Quantification and identification of phenolic compounds (µg/mL dry vegetal product) in the ethanol extracts of aerial parts of *P. erici-rosenii* and *L. martinicensis* by HPLC-DAD-MS-ESI$^+$ analysis.

Peak No.	Phenolic compounds	R_t (min)	UV λ_{max} (nm)	$[M+H]^+$ (m/z)	PL µg/mL	LM µg/mL
1	2-Hydroxybenzoic acid	3.24	270.000	139.000	4.976	3.608
2	Gallic acid	4.29	275.000	171.000	1.140	-
3	4-Hydroxybenzoic acid	12.69	270.000	139.000	1.080	0.010
4	Kaempferol-rutinoside	13.41	350.250	595.287	3.519	-
5	Vanilic acid	13.67	280.000	169.000	2.210	0.931
6	*p*-Coumaric acid	15.21	332.000	165.000	-	3.309
7	Ferulic acid	15.63	334.000	195.000	-	5.345
8	Feruloylquinic acid	15.99	334.000	369.000	-	6.630
9	Quercetin-glucoside	16.28	360.260	465.303	5.153	-
10	Coumaroylquinic acid	18.28	332.000	339.000	9.995	6.142
11	Quercetin	21.49	360.260	303.000	1.811	-
	Total phenolics				29.884	25.975

found in the ethanol extracts of these two species (Table 2). According to Boizot and Charpentier [21], polyphenols are secondary metabolites characterised by the presence of an aromatic ring with free hydroxyl groups or hydroxyl groups linked to a carbohydrate. Phenolic compounds are a class of antioxidant agents

that act as free radical scavengers and are responsible for antioxidant activity in medicinal plants. They are present in all parts of higher plants (roots, stems, leaves, flowers, pollen, fruits, seeds and wood) and are involved in non-enzymatic defense systems against free radicals. Flavonoids have biological and pharmaceutical properties such as anti-allergic [22], anti-inflammatory [23], antihypertensive [24], antifungal [25], antiviral [26], antiulcer [22], anti-free radical [27], and they are also recognised as having antimalarial activity [28].

For investigating the antioxidant capacity of the extracts, two methods were used, DPPH and FRAP (Ferric Reducing Antioxidant Power) methods, since more than one method of antioxidant capacity assessment needs to be performed to take into account the various modes of action of antioxidants [29]. Both DPPH and FRAP antioxidant assays showed that all the tested extracts possess antioxidant activities. For the DPPH assessment, the results showed the IC_{50} of 56.17 µg/mL and 78.43 µg/mL for the ethanol extract of the aerial parts of *P. erici-rosenii* and *L. martinicensis* respectively, while those values of IC_{50} were of 112.03 µM TE/ 100mL and 90.46 µM TE/100mL for FRAP assessment. Similar IC_{50} values have been reported in other studies for Lamiaceae species. Previous studies on extracts of different plant species show that the Lamiaceae family has the strongest antioxidant activities [30] [31] [32]. This would explain the encouraging results for the ethanol extracts of aerial parts of *P. erici-rosenii* and *L. martinicensis* in this study (**Table 2**).

In this study, the greatest inhibition zone diameters (12.5 mm, 12.2 mm, and 11.9 mm) were obtained for the ethanol extracts of *P. erici-rosenii* on the *L. monocytogenes* ATCC 13932, *S. aureus* 29213, and *E. faecalis* ATCC25922 strains respectively. Inhibition zone diameters for other strains were 10.0 mm-10.7 mm for the ethanol extract of *P. erici-rosenii*. Concerning the *L. martinicensis* ethanol extract, the greatest inhibition zone diameters (10.6 mm) were obtained on the *L. monocytogenes* ATCC 13932 strains and 10.0 mm for other strains.

According to the extract activity classification scale established by Kuete *et al.* [33] based on MICs (Significant: MIC < 100 µg/mL; Moderate: 100 µg/mL < MIC ≤ 625 µg/mL and Negligible: MIC > 625 µg/mL); it can be noted that: the ethanol extract of *P. erici-rosenii* showed moderate activity on all trains with 0.125 mg/mL on *L. monocytogenes* ATCC 13932, *E. faecalis* ATCC25922, and *C. albicans* ATCC 10231 as the best activity. The same observations have been made for ethanol extract of *L. martinicensis* with 0.250 mg/mL on *S. aureus* 29213 and *E. faecalis* ATCC25922 as the best activity. Overall, the results showed that the ethanol extract of the aerial parts of *P. erici-rosenii* and *L. martinicensis* weren't bactericides on *E. coli* ATCC51299, *B. cereus* ACTT 11778, *C. albicans* ATCC 10231 because no MBC/MFC was observed on these strains (**Table 3**).

These results are in agreement with the work carried out by Sarac and Ugur [34] which showed that several plants belonging to the Lamiaceae family were found to be potentially antimicrobial. Among those plants, *Phlomis fruticosa*, *Salvia verbenaca*, *Salvia argentea*, *Teucrium chamaedrys* ssp. lydium, *Teucrium*

divaricatum ssp. villosum, *Teucrium polium*, *Stachys cretica* ssp. smyrnaea, *Stachys annua* ssp. annua, *Stachys albiflora*, *Sideritis leptoclada*, *Melissa officinalis* ssp. officinalis, M*icromeria juliana* and *Prunella vulgaris* can be mentioned.

In the present study, two species (*P. erici-rosenii* and *L. martinicensis*) from the Lamiaceae family were evaluated for their antimalarial potencies. The ethanol extracts of these two species have been analyzed for the first time against *P. falciparum*. The ethanol extract of *P. erici-rosenii* was found to be more potent (IC_{50} 3D7: 4.07 µg/mL) than the ethanol extract of *L. martinicensis* (IC_{50} 3D7: 12.29 µg/mL). In the study of Murugan *et al.* [35], the leaf extracts of *Senna occidentalis* (Cassiae) and *Ocimum basilicum* (Lamiaceae) were evaluated for antimalarial activity against *P. falciparum* strains 3D7 and INDO. *Senna occidentalis* IC_{50} was 48.80 µg/ml (CQ-s) and 54.28 µg/mL (CQ-r), while *O. basilicum* IC_{50} was 68.14 µg/mL (CQ-s) and 67.27 µg/mL (CQ-r); IC_{50} of chloroquine was 85 µg/mL (CQ-s) and 91 µg/mL (CQ-r). However, it should be pointed out that the activity of these plant extracts can be affected by the region of origin and the growing conditions. For instance, the ethanol leaf extract of *S. occidentalis* from Congo showed a higher *in vitro* antimalarial activity against *P. falciparum* CQ-sensitive strains with an $IC_{50} < 3$ µg/mL [36]. The ethanol extracts of *P. erici-rosenii* and *L. martinicensis* could therefore be used against malaria, since they are effective at lower doses (**Table 4**) and could be advantageous sources of metabolites to build newer and safer antiplasmodial compounds.

The antimicrobial, antioxidant, and antiplasmodial results of the ethanol extracts of the aerial parts of *P. erici-rosenii* and *L. martinicensis* (Lamiaceae) have been reported for the first time in this study.

The HPLC-DAD-MS-ESI^+ analysis has clearly shown significant differences concerning the quantity of chemical compounds in the ethanol extracts of *P. erici-rosenii* and *L. martinicensis*. Eleven polyphenols have been identified and quantified in all analyzed extracts (**Table 5**). These eleven polyphenols were free or glycosylated chemical compounds belonging to various therapeutically important structural classes: hydroxybenzoic acid (2-hydroxybenzoic acid, gallic acid, 4-hydroxybenzoic acid and vanilic acid), flavonol (kaempferol-rutinoside, quercetin-glucoside and quercetin), and hydroxycinnamic acid s (*p*-coumaric acid, ferulic acid, feruloylquinic acid and foumaroylquinic acid). Ferulic acid, feruloylquinic acid and *p*-coumaric acid were only present in ethanol extract of *L. martinicensis* while gallic acid, kaempferol-rutinoside, quercetin-glucoside, and quercetin were only present in ethanol extract of *P. erici-rosenii* (**Table 5**).

Furthermore, significantly high amounts of certain identified compounds, namely 2-hydroxybenzoic acid (4.976 µg/mL), gallic acid (1.140 µg/mL), 4-hydroxybenzoic acid (1.080 µg/mL), kaempferol-rutinoside (3.519 µg/mL), vanilic acid (2.210 µg/mL), quercetin-glucoside (5.153 µg/mL), coumaroylquinic acid (9.995 µg/mL), and quercetin (1.811 µg/mL) were observed in the case of the ethanol extract of *P. erici-rosenii*. For the ethanol extract of *L. martinicensis*, 2-hydroxybenzoic acid (3.608 µg/mL), *p*-coumaric acid (3.309 µg/mL), vanilic

acid (0.931 μg/mL), ferulic acid (5.345 μg/mL), feruloylquinic acid (6.630 μg/mL), and coumaroylquinic acid (6.142 μg/mL) were observed.

These results are in agreement with the work carried out by Maria *et al.* [37], in Lamiaceae family (*Ocimum basilicum* L., *Origanum vulgare* L., and *Thymus vulgaris* L.). They found gallic acid, caftaric acid, chlorogenic acid, 4-OH phenylacetic acid, 4-OH benzoic acid, caffeic acid, isoquercetin, vanillic acid, syringic acid, chicoric acid, *p*-coumaric acid, ferulic acid, rutin, rosmarinic acid, *o*-coumaric acid, cinnamic acid and quercetin.

Although the literature does not give detailed information regarding the chemical profile of phenolic compounds from the ethanol extracts of *P. erici-rosenii* and *L. martinicensis*. Thus, this work is original because it provides for the first time the profile of polyphenolic compounds in the ethanol extract of these two species and their biological activities such as antimalarial activity.

Therefore, the antimicrobial, antioxidant, and antiplasmodial results may suggest that the ethanol extracts of the aerial parts of *P. erici-rosenii* and *L. martinicensis* (Lamiaceae) contain compounds with antimicrobial, antioxidant, and antiplasmodial properties, which can be searched in new drug discovery for treatment of infectious diseases.

5. Conclusion

This study looked at the phytochemical and biological activities of ethanolic extracts of the aerial parts of *P. erici-rosenii* and *L. martinicensis* (Lamiaceae) from Eastern of Democratic Republic of Congo. The extracts had a high concentration of polyphenols and flavonoids, and showed significant antimicrobial, antioxidant and antiplasmodial activities.

However, further studies are needed. Among these studies, some are to identify the pure compounds responsible for these biological activities, toxicity tests and obtaining more information regarding the practical effectiveness of the extracts in animal models.

Acknowledgements

The authors thank the Laboratories of the Yaounde-Bielefeld Bilateral Graduate School Natural Products with Antiparasitic and Antibacterial Activity (YaBiNaPA) in partnership with "Institut Pasteur de Yaoundé/Cameroon" for the antiplasmodial test (Project: 57316173/2021-2025) and The University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania, for antimicrobial, and antioxidant activity assessment and HPLC-DAD-MS-ESI$^+$ Analysis (Project: "Bourses "Eugen Ionescu" 2021-2022").

Author Contributions

Conceptualisation, plants and extracts preparation: U. S. H., N. L. and P. T. M. Biological tests and acquisition of HPLC-DAD-MS-ESI$^+$ data: U. S. H., D. C. V., D. H., I. M. and S. A. Writing original draft preparation: U. S. H., N. L. and P. T.

M. Writing and editing: U. S. H., N. L., P. T. M., N. I. O., N. J. and S. A. All authors read and agreed to the published version of the manuscript.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Naghibi, F., Mosaddegh, M., Mohammadi, M.S. and Ghorbani, A. (2005) Labiatae Family in Folk Medicine in Iran. *Iranian Journal of Pharmaceutical Research*, **2**, 63-79.

[2] Noura, S.D. and William, N.S. (2018) The Genus *Conradina* (Lamiaceae): A Review. *Plants*, **7**, Article 19. https://doi.org/10.3390/plants7010019

[3] O'Neill, J. (2016) Antimicrobial Resistance: Tackling a Crisis for the Health and Wealth of Nations. Review on Antimicrobial Resistance. https://amr-review.org/

[4] De Kraker, M.E.A., Stewardson, A.J. and Harbarth, S. (2016) Will 10 Million People Die a Year Due to Antimicrobial Resistance by 2050? *PLOS Medicine*, **13**, e1002184. https://doi.org/10.1371/journal.pmed.1002184

[5] WHO: World Health Organization (2022) World Malaria Report 2022. Geneva.

[6] Farah, H., Elbadrawy, E. and Al-Atoom, A.A. (2015) Evaluation of Antioxidant and Antimicrobial Activities of Ethanolic Extracts of Parsley (*Petroselinum erispum*) and Coriander (*Coriandrum sativum*) Plants Grown in Saudi Arabia. *International Journal of Advanced Research*, **3**, 1244-1255.

[7] Catunescu, G.M., Rotar, I., Vidican, R., Bunghez, F. and Rotar, A.M. (2017) γ Radiation Enhances the Bioactivity of Fresh Parsley (*Petroselinum crispum* (Mill.) Fuss Var. Neapolitanum). *Radiation Physics and Chemistry*, **132**, 22-29. https://doi.org/10.1016/j.radphyschem.2016.11.010

[8] Dumitras, D.A., Bunea, A., Vodnar, D.C., Hanganu, D., Pall, E., Cenariu, M., Gal, A.F. and Andrei, S. (2022) Phytochemical Characterization of *Taxus baccata* L. Aril with Emphasis on Evaluation of the Antiproliferative and Pro-Apoptotic Activity of *Rhodoxanthin*. *Antioxidants*, **11**, Article 1039. https://doi.org/10.3390/antiox11061039

[9] Mihai, B., Andrei, M., Laurian, V., Ovidiu, C., Irina, I., Ana-Maria, G., Dan-Cristian, V., Gianina, C. and Ramona P. (2018) Phytochemical Analysis, Antioxidant and Antimicrobial Activities of *Helichrysum arenarium* (L.) Moench. and *Antennaria dioica* (L.) Gaertn. Flowers. *Molecules*, **23**, Article 409. https://doi.org/10.3390/molecules23020409

[10] Benedec, D., Hanganu, D., Lorena, F., Oniga, I., Brindusa, T., Olah, N.K., Gheldiu, A.M., Raita, O. and Vlase, L. (2017) Chemical, Antioxidant and Antibacterial Studies of Romanian *Heracleum sphondylium*. *Farmacia*, **65**, 252-256. https://farmaciajournal.com/arhiva/201702/art-15-Benedec_Oniga_Vlase_252-256.pdf

[11] Mot, A., Damian, G., Sârbu, C. and Silaghi-Dumitrescu, R. (2009) Redox Reactivity in Propolis: Direct Detection of Free Radicals in Basic Medium and Interaction with Hemoglobin. *Redox Report*, **14**, 267-274. https://doi.org/10.1179/135100009X12525712409814

[12] Arendrup, M.C., Hope, W., Cuenca-Estrella, M. and Lass-Flörl, C. (2012) EUCAST Technical Note on the EUCAST Definitive Document EDef 7.2: Method for the Determination of Broth Dilution Minimum Inhibitory Concentrations of Antifungal Agents for Yeasts EDef 7.2 (EUCAST-AFST). *Clinical Microbiology and Infection*, **18**, 246-247. https://doi.org/10.1111/j.1469-0691.2012.03880.x

[13] Danciu, C., Muntean, D., Alexa, E., Watz, C., Oprean, C., Zupkó, I., Bor, A., Minda, D., Proks, M. and Buda, V. (2018) Phytochemical Characterization and Evaluation of the Antimicrobial, Antiproliferative and Pro-Apoptotic Potential of *Ephedra alata Decne*. Hydroalcoholic Extract against the MCF-7 Breast Cancer Cell Line. *Molecules*, **24**, Article 13. https://doi.org/10.3390/molecules24010013

[14] Muntean, D., Licker, M., Alexa, E., Popescu, I., Jianu, C., Buda, V., Dehelean, C.A., Ghiulai, R., Horhat, F.G. and Horhat, D. (2019) Evaluation of Essential Oil Obtained from *Mentha piperita* L. against Multidrug-Resistant Strains. *Infection and Drug Resistance*, **12**, 2905-2914. https://doi.org/10.2147/IDR.S218141

[15] Trager, W. and Jensen J.B. (1976) Human Malaria Parasites in Continuous Culture. *Science*, **193**, 673-675. https://doi.org/10.1126/science.781840

[16] Lambros, C. and Vanderberg, J.P. (1979) Synchronization of Plasmodium Falciparum Erythrocytic Stages in Culture. *The Journal of Parasitology*, **65**, 418-432. https://doi.org/10.2307/3280287

[17] Smilkstein, M., Sriwilaijaroen, N., Kelly, J.X., Wilairat, P. and Riscoe, M. (2004). Simple and Inexpensive Fluorescence-Based Technique for High-Throughput Antimalarial Drug Screening. *Antimicrobial Agents and Chemotherapy*, **48**, 1803-1806. https://doi.org/10.1128/AAC.48.5.1803-1806.2004

[18] Singh, N., Kaushik, N.K., Mohanakrishnan, D., Tiwari, S.K. and Sahal, D. (2015) Antiplasmodial Activity of Medicinal Plants from Chhotanagpur Plateau, Jharkhand, Indian. *Journal of Ethnopharmacology*, **165**, 152-162. https://doi.org/10.1016/j.jep.2015.02.038

[19] Bădălica-Petrescu, M., Drăgan, S., Ranga, F., Fetea, F. and Socaciu, C. (2014) Comparative HPLC-DAD-MS-ESI$^+$ Fingerprint and Quantification of Phenolic and Flavonoid Composition of Aqueous Leaf Extracts of *Cornus mas* and *Crataegus monogyna*, in Relation to Their Cardiotonic Potential. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, **42**, 9-18. https://doi.org/10.15835/nbha4219270

[20] Hanganu, D., Niculae, M., Ielciu, I., Olah, N.K., Munteanu, M., Burtescu, R.S., Tefan, R., Olar, L., Pall, E. and Andrei, S., (2021) Chemical Profile, Cytotoxic Activity and Oxidative Stress Reduction of Different *Syringa vulgaris* L. Extracts. *Molecules*, **26**, Article 3104. https://doi.org/10.3390/molecules26113104

[21] Boizot, N. and Charpentier, J.P. (2006) Méthode rapide d'évaluation du contenu en composés phénoliques des organes d'un arbre forestier. https://www.researchgate.net/profile/Nathalie-Boizot/publication/341819016_Methode_rapide_d'evaluation_du_contenu_en_composes_phenoliques_des_organes_d'un_arbre_forestier/links/60d1f22da6fdcce58ba7f0e3/Methode-rapide-devaluation-du-contenu-en-composes-phenoliques-des-organes-dun-arbre-forestier.pdf

[22] Di Carlo, G., Mascolo, N., Izzo, A.A. and Capasso, F. (1999) Flavonoids: Old and New Aspects of a Class of Natural Therapeutic Drugs. *Life Sciences*, **65**, 337-353. https://doi.org/10.1016/S0024-3205(99)00120-4

[23] Nakujima, T., Manishi, M.I., Yamamoto, K., Cyong, J.C. and Hirai, K. (2001) Inhibitory Effects of Baicalein, a Flavonoid in *Scutellaria* Root, on Eotaxin Production by Human Dermal Fibroblasts. *Planta Medica*, **67**, 132-135. https://doi.org/10.1055/s-2001-11532

[24] Eibi, G. and Wagner, H. (1991) A New Method for the *in vivo* Screening of Inhibitors of Angiotensin Converting Enzyme (ACE), Using the Chromophore Labeled Substrate Dansyltriglycine. *Planta Medica*, **57**, 137-141. https://doi.org/10.1055/s-2006-960050

[25] Elsohly, H.N., Joshi, A.S., Nimrod, A.C., Walker, L.A. and Clark, A.M. (2001) Antifungal Chalcones fom *Maclura tinctoria. Planta Medica*, **67**, 87-89. https://doi.org/10.1055/s-2001-10621

[26] Shahat, A.A., Cos, P., Bruyne, T.D., Apers, S., Hammouda, F.M., Ismail, S.I., Azzam, S., Claeys, M., Goovaerts, E., Pieters, L., Berghe, D.V. and Vlietinck, A.J. (2002) Antiviral and Antioxidant activity of Flavonoids and Proanthocyanidins from *Crataegus sinaica*. *Planta Medica*, **68**, 539-541. https://doi.org/10.1055/s-2002-32547

[27] Colette, N., Bernier, J.L., Henichart, J.P., Catteau, J.P., Gaydou, E.M. and Wallt, J.C. (1992) Scavanger and Antioxydant Properties of Ten Synthetic Flavones. *Free Radical Biology and Medicine*, **13**, 211-219. https://doi.org/10.1016/0891-5849(92)90017-B

[28] Murakami, N., Mostaqul, H.M., Tamura, S., Itagak, S. and Horii, T. (2001) A New Anti-Malarial Flavonol Glycoside from *Hydrangeae dulcis* Folium (*Hydrangea macrophylla*). *Bioorganic and Medicinal Chemistry Letters*, **11**, 2445-2447. https://doi.org/10.1016/S0960-894X(01)00467-X

[29] Barreira, J., Ferreira, I., Oliveira, M. and Pereira, J. (2008) Antioxidant Activities of the Extracts from Chestnut Flower, Leaf, Skins and Fruit. *Food Chemistry*, **107**, 1106-1113. https://doi.org/10.1016/j.foodchem.2007.09.030

[30] Lamaison, J.L. and Freytet, P.J.C. (1996) Medicinal Lamiaceae with Antioxidant Activity, Potential Sources of Rosmarinic Acid. *Pharmaceutica Acta Helvetiae*, **66**, 185-188.

[31] Zheng, W. and Wang, S.Y. (2001) Antioxidant Activity and Phenolic Compound in Selected Herbs. *Journal of Agricultural and Food Chemistry*, **49**, 5165-5170. https://doi.org/10.1021/jf010697n

[32] Shan, B., Cai, Y.Z., Sun, M. and Corke, H. (2005) Antioxidant Capacity of 26 Spice Extracts and Characterization of Their Phenolic Constituents. *Journal of Agricultural and Food Chemistry*, **53**, 7749-7759. https://doi.org/10.1021/jf051513y

[33] Kuete, V., Nana, F., Ngameni, B., Mbaveng, A.T., Keumedjio, F. and Nyadjui, B.T. (2009) Antimicrobial Activity of the Crude Extract, Fractions and Compounds from Stem of *Ficus ovata* (Moraceae). *Journal of Ethnopharmacology*, **124**, 556-561. https://doi.org/10.1016/j.jep.2009.05.003

[34] Sarac, N. and Ugur, A. (2007) Antimicrobial Activities and Usage in Folkloric Medicine of Some Lamiaceae Species Growing in Mugla, Turkey. *EurAsian Journal of BioSciences*, **4**, 28-37. http://www.ejobios.com/content/1/4/28-34

[35] Murugan, K., Aarthi, N. and Kovendan, K. (2015) Mosquitocidal and Antiplasmodial Activity of *Senna occidentalis* (Cassiae) and *Ocimum basilicum* (Lamiaceae) from Maruthamalai Hills against *Anopheles stephensi* and *Plasmodium falciparum*. *Parasitology Research*, **114**, 3657-3664. https://doi.org/10.1007/s00436-015-4593-x

[36] Tona, L., Cimanga, R.K., Mesia, K., Musuamba, C.T., De Bruyne, T., Apers, S., Hernans, N., Miert, S.V., Pieters, L., Totté, J. and Vlietinck, A.J. (2004) *In vitro* Antiplasmodial Activity of Extracts and Fractions from Seven Medicinal Plants Used in Democratic Republic of Congo. *Journal of Ethnopharmacology*, **93**, 27-32. https://doi.org/10.1016/j.jep.2004.02.022

[37] Maria, C.S., Marika, P., Manuel, S., Paola, P., Antonella, R. and Dario, C. (2018) Analysis of Polyphenols in the Lamiaceae Family by Matrix Solid-Phase Dispersion Extraction Followed by Ultra-High-Performance Liquid Chromatography—Tandem Mass Spectrometry Determination. *ACS Omega*, **3**, 17610-17616. https://doi.org/10.1021/acsomega.8b02239