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Research Article

Preliminary Phytochemical Screening, Quantitative Analysis and GC/MS Profiling of *Piliostigma thonningii*, *Euphorbia lactea* and *Richardia brasiliensis*

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Abstract

Background and Objective: Traditional medicinal plants are extensively used in Southern Benin, yet the phytochemical profiles of several commonly used species remain insufficiently characterized. This study characterizes the phytochemical composition of *Euphorbia lactea*, *Richardia brasiliensis* and *Piliostigma thonningii*, three plants traditionally used in Southern Benin. **Materials and Methods:** Differential precipitation staining was used for qualitative screening and total flavonoids, polyphenols and hydrolyzable tannins were quantified by spectrophotometric and Mole-Waterman methods. GC/MS analysis of extracts from the three plants was conducted using an Agilent Technologies 7890A GC system coupled to an Agilent 7000 Triple Quadrupole Mass Spectrometer (GCMS TQ). Data were analyzed using ANOVA with Tukey's or Dunnett's *post hoc* tests ($p < 0.05$). **Results:** Ethanolic extraction generally yielded higher recoveries than aqueous extraction, particularly in *P. thonningii*. Screening indicated that *E. lactea* contained abundant gallic tannins, flavonoids and Saponins, while *P. thonningii* was rich in alkaloids, flavonoids, anthocyanins and mucilages. *Richardia brasiliensis*, on the other hand, contained alkaloids, catechic tannins, gallic tannins, flavonoids, anthocyanins, mucilage and triterpenes. The results reveal that for quantitative assays, the ethanolic extract of *P. thonningii* had the highest levels of flavonoids, polyphenols and condensed tannins, whereas *R. brasiliensis* had the highest hydrolyzable tannin content ($p < 0.0001$). The GC-MS analysis identified 50 compounds in *E. lactea* (19 of which were not previously reported), 35 in *R. brasiliensis* (11 of which were not previously reported) and 44 in *P. thonningii* (12 of which were not previously reported), belonging to chemical families including alcohols, esters, fatty acids, terpenes, steroids and phenolics. **Conclusion:** These findings provide a detailed chemical basis for further investigation and suggest the presence of potentially novel metabolites. While consistent with traditional use, the findings do not establish efficacy but highlight opportunities for future pharmacological exploration.

Key words: *Euphorbia lactea*, *Richardia brasiliensis*, *Piliostigma thonningii*, GC-MS analysis, bioactive compounds

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

People have used plants as medicine for a long time to treat a wide range of illnesses and they are still considered the best source¹. Part of the reason for this is that it is cheap for people with low incomes, especially in rural regions and it is said to be safer than synthetic medications². Without a doubt, scientists and researchers are constantly interested in medicinal plants and herbal remedies as possible sources of new therapeutic molecules². Over 85% of Africa's population receives traditional medicine from traditional medicinal practitioners as their first-line treatment³. As a result, scientists have become interested in African medicinal plants, leading to the identification of bioactive chemicals². Among the many species exploited in Africa are *Piliostigma thonningii*, *Richardia brasiliensis* and *Euphorbia lactea*, which are recognized to treat a variety of ailments and are particularly popular in the Beninese pharmacopoeia. *Piliostigma thonningii* is a leguminous plant from the Caesalpiniaceae family, which includes trees, shrubs and rare scramblers⁴. For example, the root and twig have been used to treat diarrhea, fever, infections, respiratory problems, snake bites, hookworm and skin diseases⁴. Complementing the bioactivities reported for *Piliostigma thonningii*, *Richardia brasiliensis* has likewise demonstrated significant pharmacological effects, particularly in the management of diabetes, hemorrhoids and skin diseases⁵. Alongside *Richardia brasiliensis*, *Euphorbia lactea* has also attracted attention for its wide ethnomedicinal applications. It has been shown to cure vomiting, constipation, digestive issues, respiratory and skin infections, migraine, gonorrhoea, intestinal and parasite infections and cancer⁶.

Although these plants have been employed for centuries, their pharmacological activity is hindered by the lack of scientific data on their chemical composition, particularly for the stem of *Euphorbia lactea*, the aerial parts of *Richardia brasiliensis* and the leaves of *Piliostigma thonningii*.

Modern phytochemistry combines traditional usage with active molecular composition. Gas Chromatography-Mass Spectrometry (GC-MS) is ideal for this task since it allows for the simultaneous detection of known substances as well as possibly new metabolites. This study aimed to determine the phytochemical content of *Euphorbia lactea*, *Richardia brasiliensis* and *Piliostigma thonningii* using qualitative screening and quantitative spectrophotometric assays and generate comprehensive GC-MS profiles to provide a chemical framework for future pharmacological investigations.

MATERIALS AND METHODS

Materials

Plant material: The leaves of *Piliostigma thonningii* (Pt), the aerial parts of *Richardia brasiliensis* (Rb) and the stem of *Euphorbia lactea* (El) were collected in November 2024 in Bembèrèkè, University of Abomey-Calavi and Dassa, respectively. They were then identified and certified by the botany team at the Benin National Herbarium (Pt: YH 668/HNB, Rb: YH 610/HNB) and dried at laboratory temperature (16°C). The dried collected plants were ground into powder using a Retsch grinder type SM 2000/1430/Upm/Smf and stored in the laboratory for extraction.

Methods

Extraction: Two types of extracts were prepared: Ethanolic and aqueous extracts. The choice of these extraction methods was based on the traditional use of the plant and the type of secondary metabolites targeted for extraction.

Aqueous extract preparation: The total aqueous extracts were obtained using the method developed by Chabi-Sika *et al.*⁵. For this purpose, 50 g of dry plant powder was macerated in 500 mL of distilled water on a mechanical shaker for 72 hrs at room temperature. The resulting homogenate was filtered twice, first through absorbent cotton and then through Whatman paper. This extraction process was repeated after the initial 72 hrs maceration to ensure exhaustive recovery of soluble compounds. The filtrates were then combined, dried in an oven at 50°C and the powder obtained constituted the total aqueous extract, ready for use.

Ethanolic extract preparation: Similarly, 50 g of dry plant powder was macerated in 500 mL of 96% ethanol under continuous stirring for 72 hrs, following two successive filtrations through absorbent cotton and Whatman paper. After the initial 72 hrs maceration, the extraction procedure was repeated to maximize the recovery of soluble phytoconstituents. The successive filtrates were combined and concentrated under reduced pressure using a rotary evaporator at 50°C. The extract powders obtained were either used immediately for biological tests or stored at 4°C.

The ethanolic and aqueous extracts were used to quantify flavonoids, polyphenols and tannins.

Screening phytochemical: The preliminary phytochemical screening was carried out directly on the powder from the different plant organs. The method used was⁷, adapted to the laboratory conditions. It was a qualitative analysis based on differential color reactions and precipitation.

Extraction yield: The yield of the extraction is expressed as the ratio of the dry extract obtained to the mass of plant material treated⁸. It was obtained using the following formula:

$$R (\%) = \frac{Me}{Mv} \times 100$$

R (%) = Yield in %

Me = Mass of dry extract

Mv = Mass of plant material used

Total flavonoid quantification: The total flavonoid content of our extracts was estimated using a spectrophotometric method⁸ and adapted to the laboratory conditions. The reaction mixture contained 1 mL of the methanolic solution (80%) of each extract at a concentration of 25 mg/mL and 1 mL of 2% AlCl₃ (dissolved in methanol) and the mixture was well stirred. After one hour of incubation at room temperature and protected from light, absorbances were measured using a spectrophotometer at 415 nm. The mixtures were prepared in triplicate for each analysis and the average value was recorded.

For the blank preparation, 1 mL of methanol (80%) and 1 mL of extract were used. The calibration curve for quercetin was established using standard solutions ranging from 100 to 1000 µg/mL. The flavonoid content of the extracts was expressed in mg quercetin equivalent (QE)/g of dry extract, using the equation obtained from the calibration curve:

$$y = 0.0542x - 0.0367; R^2 = 0.9874$$

where, x represents the absorbance and y is the quercetin equivalent (mg/g). The calibration curve for total flavonoid quantification was constructed at the end.

Total polyphenol quantification: The total polyphenol content of our extracts was determined using the Folin-Ciocalteu method⁹.

Briefly, 10 mg/mL of each extract was diluted in 80% methanol to obtain a 1 mg/mL solution for each extract. Then, a reaction mixture was prepared for each extract, consisting of 0.5 mL of extract, 5.0 mL of distilled water and 0.5 mL of the

Folin-Ciocalteu reagent. After three minutes, 1.0 mL of a saturated 20% Na₂CO₃ solution was added. The prepared mixtures were stirred and incubated at room temperature, protected from light, for one hour. Absorbances were measured using a spectrophotometer at 725 nm.

Each quantification was repeated three times. The calibration curve for gallic acid was established using standard solutions ranging from 100 to 1000 µg/mL and the total polyphenol content was expressed in mg gallic acid equivalents (GAE)/g of dry extract using the following equation:

$$y = 0.0037x + 0.0218; R^2 = 0.9899$$

where, x represents the absorbance and y is the gallic acid equivalent (mg/g). The calibration curve for total polyphenol quantification was constructed at the end.

Determination of total condensed tannins in the extracts:

A stock solution of 10 mg/mL was prepared and then diluted 10-fold to obtain a 1 mg/mL working solution¹⁰. Briefly, 1 mL of the working solution was mixed with 2 mL of 1% vanillin solution (1 g of vanillin in 100 mL of 70% sulfuric acid) and incubated for 15 min in a water bath at 20°C. The absorbance of the mixture was measured using a UV-visible spectrophotometer (BioMATE 3S, Thermo) at 500 nm.

The condensed tannin content of the samples was determined in triplicate and the results were converted into micrograms of catechin equivalents (µg CE) per milligram of extract using the catechin calibration curve ($y = 0.0003x + 0.0062$; $R^2 = 0.99$), which was established using catechin at concentrations ranging from 100 to 1000 µg/mL.

The total condensed tannin (TCT) content was determined using the following formula:

$$TCT = \frac{X \times Ve}{me}$$

Where:

X = Concentration of the extract solution in total condensed tannins (µg CE/mL)

Ve = Volume of the extract used

me = Total mass of dry extract used to prepare the original stock solution (mg)

Determination of total hydrolyzable tannins in the extracts:

Total hydrolyzable tannins were quantified¹⁰. A stock solution of 10 mg/mL was prepared and then diluted 10-fold to obtain a 1 mg/mL working solution as well. Briefly, 1 mL of the

working solution was mixed with 3.5 mL of FeCl₃ reagent in 10⁻³ M hydrochloric acid (HCl). The absorbance was measured using a UV-visible spectrophotometer (BioMATE 3S, Shimadzu) at 660 nm after 15 min of incubation. Standard calibration curve was prepared using gallic acid solutions at concentrations ranging from 50 to 500 µg/mL ($y = 0.0023x + 0.015$; $R^2 = 0.998$).

The hydrolyzable tannin content of the samples was determined in triplicate and the results were expressed in mg of gallic acid equivalent (GAE) per gram of dry extract. The total hydrolyzable tannin (THT) content was determined using the following formula:

$$\text{THT} = \frac{A \times \text{MW} \times \text{Ve}}{\epsilon \text{mole} \times \text{me}}$$

Where:

A = Absorbance

MW = Molecular weight of gallic acid (170.12 g/mol)

Ve = Volume of the extract

εmole = 2169L/mol/cm (molar extinction coefficient of gallic acid)

me = Total mass (mg) of dry extract used to prepare the original stock solution (mg)

Gas Chromatography-Mass Spectrometry (GC/MS) analysis and identification of components:

The GC/MS analysis of extracts from the three plants was conducted using an Agilent Technologies 7890A GC system coupled to an Agilent 7000 Triple Quadrupole Mass Spectrometer (GCMS TQ). The system was operated with Mass Hunter Workstation Software (Version B.04.00). A PAL autosampler was used for sample injection with a syringe volume of 10 µL. Each sample (2.5 µL) was injected in split mode (split ratio 5:1) at an injection temperature of 250°C. The injection speed was set to 50 µL/sec. The chromatographic separation was achieved on a Zebtron ZB-5MS capillary column (30×0.25 mm i.d., 0.25 µm film thickness). The initial oven temperature was set to 50°C and held for 5 min, followed by a ramp of 7°C/min to 200°C (held for 15 min) and then increased at the same rate to 300°C (held for 15 min), with a total run time of approximately 70.7 min. The helium carrier gas was set to a constant flow of 1.2 mL/min. The mass spectrometer transfer line was maintained at 260°C.

Mass spectrometric detection was performed in electron ionization (EI) mode at 70 eV. The scan range was set according to the standard configuration of the MassHunter software. Data acquisition and analysis were conducted using the NIST/EPA/NIH Mass Spectral Library (Version 2.0f) and

compound identification was based on mass spectral matching with library entries, comparison of retention times and analysis of characteristic fragmentation patterns.

Statistical analysis: The data collected from our experiments were organized using Microsoft Excel 2016. Statistical analyses and graphing were performed using GraphPad Prism 8. Mean values of phenolic content were subjected to Analysis of Variance (ANOVA), followed by Tukey's or Dunnett's *post hoc* tests to identify significant differences between means, with significance set at $p < 0.05$.

RESULTS

Extraction yield: Analysis of the extraction yields with the two solvents (Fig. 1) showed that *Piliostigma thonningii* had the highest yield with ethanol (18.2%) compared to the ethanolic extracts of the other two plants, while *Richardia brasiliensis* had the highest aqueous extraction yield (29%), followed by *Euphorbia lactea* (15.17%), both of which were higher than the aqueous extract yield of *Piliostigma thonningii*.

Table 1 presents the results of the phytochemical screening performed on the powders of the studied plants. A strong presence of gallic tannins, flavonoids, Saponins, reducing compounds, triterpenes, coumarins and mucilages was observed in *Euphorbia lactea*. However, this plant did not contain leucoanthocyanins, anthocyanins, quinonic derivatives, cyanogenic derivatives, O-heterosides, reduced genin O-heterosides, C-heterosides, free anthracenes, alkaloids, or catechic tannins.

In contrast, the powders of *Piliostigma thonningii* showed a strong presence of alkaloids, gallic tannins, flavonoids, leucoanthocyanins, anthocyanins, coumarins, free anthracenes and mucilages. However, this plant lacked quinonic derivatives, cyanogenic derivatives, O-heterosides, reduced genin O-heterosides, C-heterosides, Saponins, reducing compounds, triterpenes and catechic tannins. *Richardia brasiliensis*, on the other hand, contains alkaloids, catechic tannins, gallic tannins, flavonoids, anthocyanins, mucilage and triterpenes. However, it lacks leucoanthocyanins, Saponins, reducing compounds and free anthracenes.

Flavonoid, polyphenolic, hydrolyzable and condensed tannin contents:

The contents of flavonoids, polyphenols, hydrolyzable tannins and condensed tannins are presented in Fig. 2a-d. These results indicate that ethanolic extracts generally contain higher concentrations of these secondary

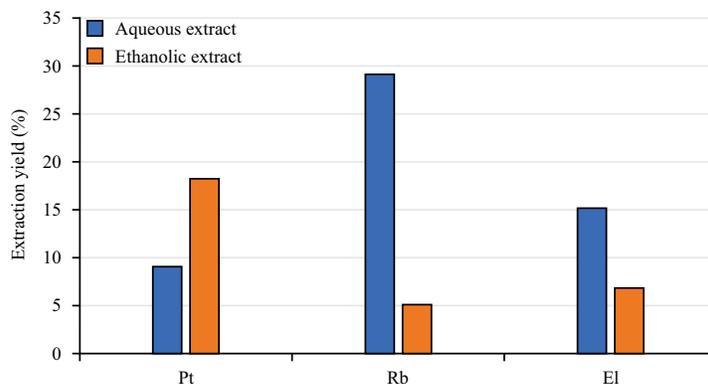


Fig. 1: Yield of the prepared extracts

Pt: *Ptilostigma thonningii*, Rb: *Richardia brasiliensis*, El: *Euphorbia lactea*

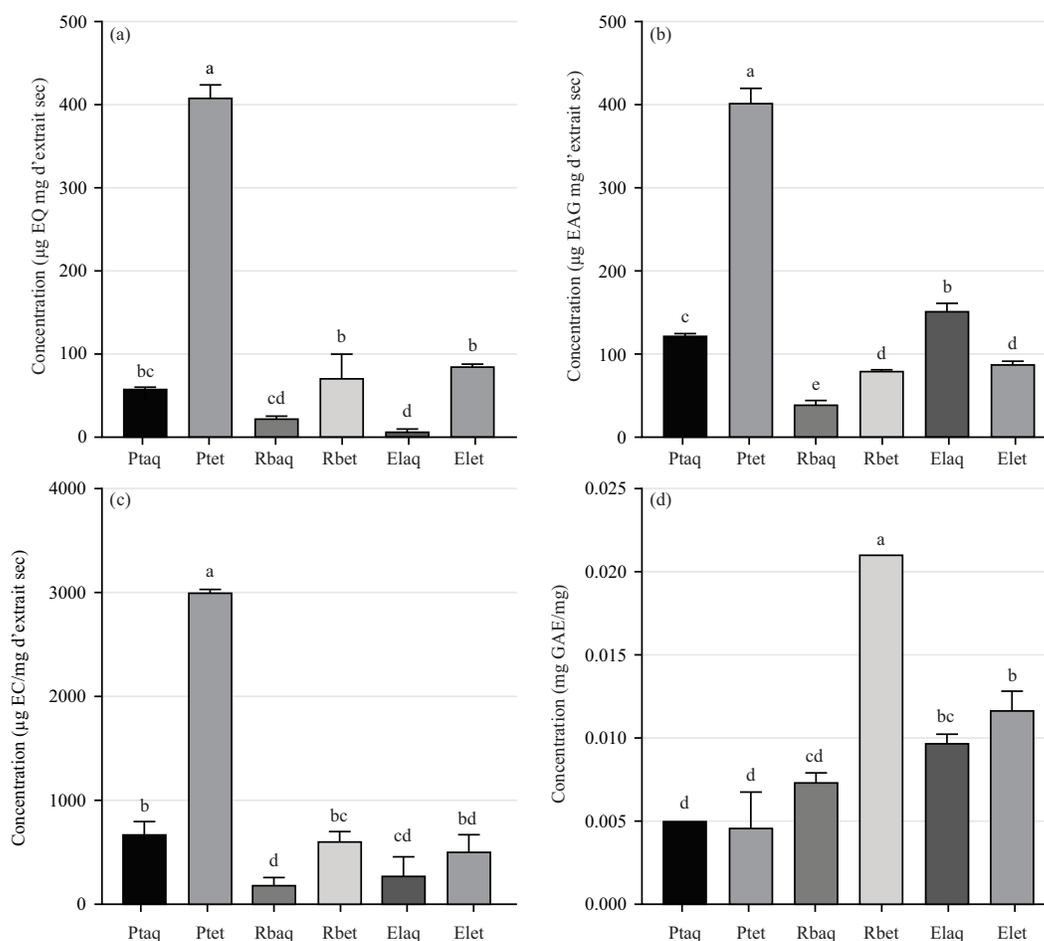


Fig.2(a-d): Quantification of the different compounds, (a) Flavonoids, (b) Total polyphenols, (c) Condensed tannins and (d) Hydrolyzable tannins

Ptaq aqueous extract of *Ptilostigma thonningii*, Ptet ethanolic extract of *Ptilostigma thonningii*, Rbaq aqueous extract of *Richardia brasiliensis*, Rbet ethanolic extract of *Richardia brasiliensis*, Elaq aqueous extract of *Euphorbia lactea*, Elet ethanolic extract of *Euphorbia lactea*
 Bars with different letters differ significantly ($p < 0.0001$) by Tukey's HSD test

metabolites compared to aqueous extracts. These figures show that the ethanolic extract of *Ptilostigma thonningii* had the highest concentrations of flavonoids (408.027 µg QE/mg),

polyphenols (401.75 µg GAE/mg) and condensed tannins (3013.6 µg CE/mg). However, the ethanolic extract of *Richardia brasiliensis* exhibited the highest level of hydrolysable tannins

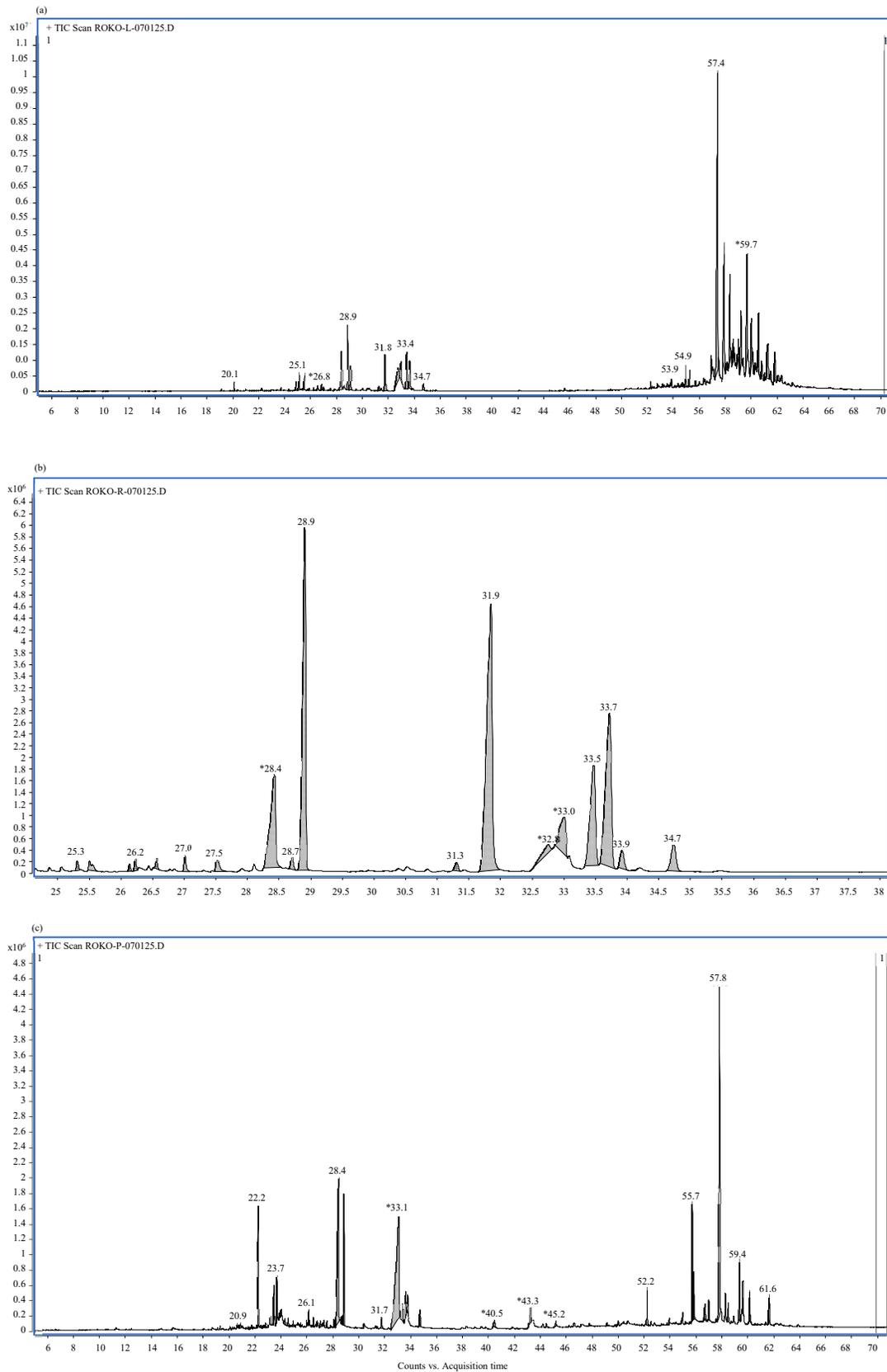


Fig. 3(a-c): Chromatograms and retention times of (a) *Euphorbia lactea*, (b) *Richardia brasiliensis* and (c) *Piliostigma thonningii*

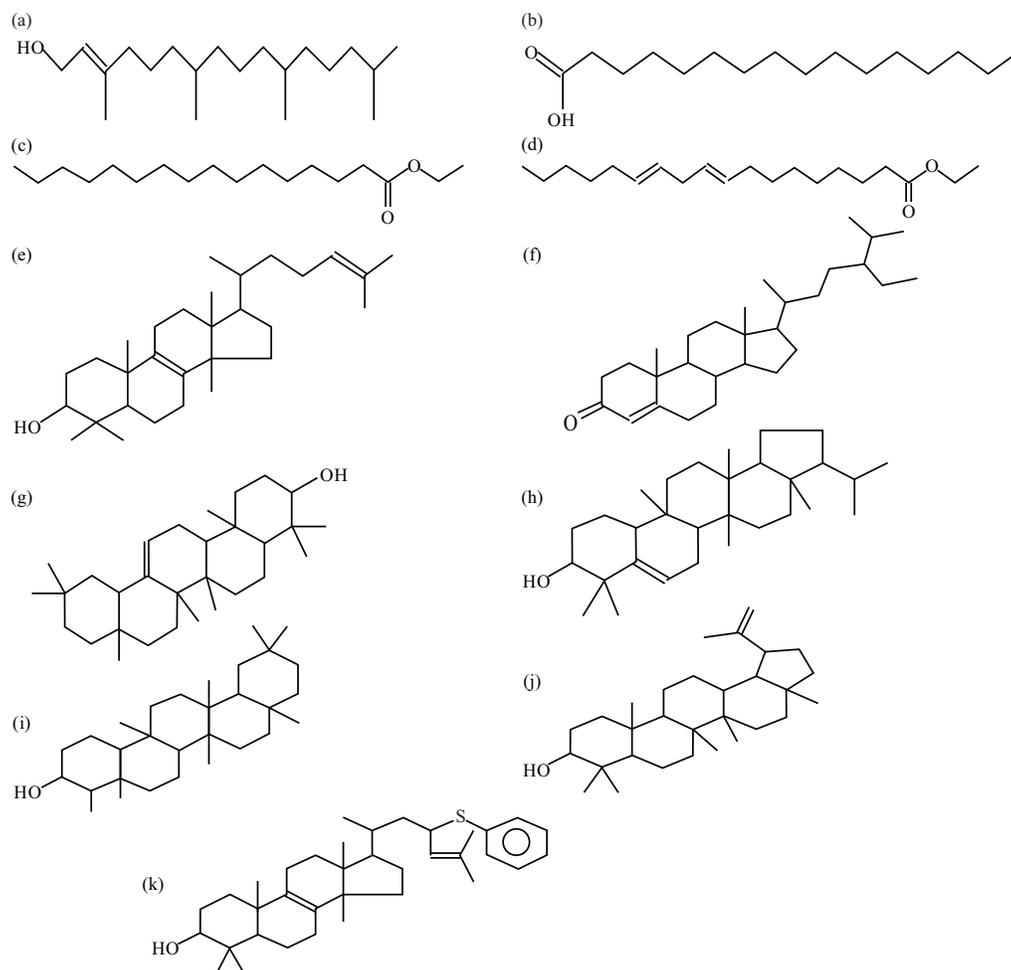


Fig. 4(a-k): Molecular structures of the major compounds identified in *Euphorbia lactea* by GC-MS

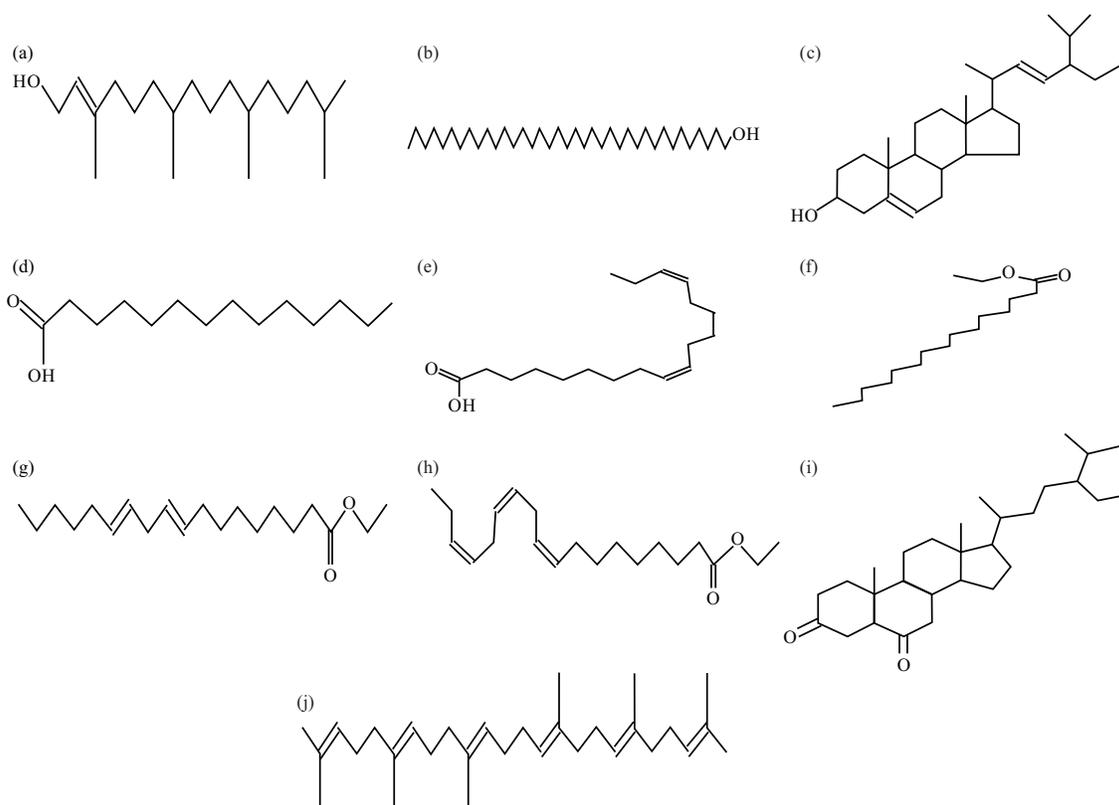
Molecular structures of the major compounds identified in *Euphorbia lactea* by GC-MS (NIST/EPA/NIH Mass Spectral Library through the Agilent MassHunter Workstation Software)

The numbers correspond to the order numbers of the compounds in Table 2

Table 1: Phytochemical screening of the powder from the different parts studied of each plant

Secondary metabolites	<i>Euphorbia lactea</i>	<i>Piliostigma thonningii</i>	<i>Richardia brasiliensis</i>
Alkaloids	-	++	++
Catechic tannins	-	-	+++
Gallic tannins	+++	++	+++
Flavonoids	++	+	+++
Anthocyanins	-	+	++
Leuco-anthocyanins	-	+	-
Saponins	++	-	-
Reducing compounds	+++	-	-
Free anthracenes	-	+	-
Mucilage	+++	+	++
Triterpenes	++	-	++
O-heterosides	-	-	-
O-heterosides a genie reduit	-	-	-
C-heterosides	-	-	-
Coumarins	++	+	-
Cyanogenic derivatives	-	-	-
Quinonic derivatives	-	-	-

+++ : Strong presence, ++ : Average presence; + : Low presence; - : absence of secondary metabolites

Fig. 5(a-j): Molecular structures of the major compounds identified in *Richardia brasiliensis* by GC-MS

Molecular structures of the major compounds identified in *Richardia brasiliensis* by GC-MS (NIST/EPA/NIH Mass Spectral Library through the Agilent MassHunter Workstation Software)

The numbers correspond to the order numbers of the compounds in Table 3

Table 2: Compounds identified by GC-MS in *Euphorbia lactea* with peak area percentages greater than 10%

N ^o	Compound name	RT (min)	Area (%)	Family of secondary metabolites
1	Phytol	31.8	10.56	Terpenoids
2	n-Hexadecanoic acid	28.4	11.4	Lipids
3	Hexadecanoic acid, ethyl ester	28.9	10.97	Esters
4	9,12-Octadecadienoic acid, ethyl ester	33.4	11.44	Esters
5	Lanosterol	57.4	100	Steroids
6	Lanosterol	57.9	37.13	Steroids
7	Stigmast-4-en-3-one	59.7	32.26	Steroids
8	β-Amyrin	58.4	22.28	Triterpenes
9	D:B-Friedo-B':A'-neogammacer-5-en-3-ol (3β)	59.2	13.59	Triterpenes
10	Lupeol	60.6	18.53	Triterpenes
11	17-(1,5-Dimethyl-3-phenylthiohex-4-enyl)-4,4,10,13,14-pentamethyl-2,3,4,5,6			

Table 3: Compounds identified by GC-MS in *Richardia brasiliensis* with peak area percentages greater than 10%

N ^o	Compound name	RT (min)	Area (%)	Family of secondary metabolites
1	Phytol	31.9	100	Terpenoids
2	1-Heptatriacontanol	53.5	0.76	Fatty alcohols
3	Stigmasterol	57.0	11.34	Phytosterols
4	n-Hexadecanoic acid	28.4	35.27	Lipids
5	9,12,15-Octadecatrienoic acid (Z,Z,Z)-	33.0	14.86	Lipids
6	Hexadecanoic acid, ethyl ester	28.9	73.54	Esters
7	9,12-Octadecadienoic acid, ethyl ester	33.5	37.3	Esters
8	9,12,15-Octadecatrienoic acid, ethyl ester (Z,Z,Z)-	33.7	61.29	Esters
9	Stigmastane-3,6-dione, (5α)-	62.3	12.93	Steroids
10	Squalene	52.3	14.44	Terpenes

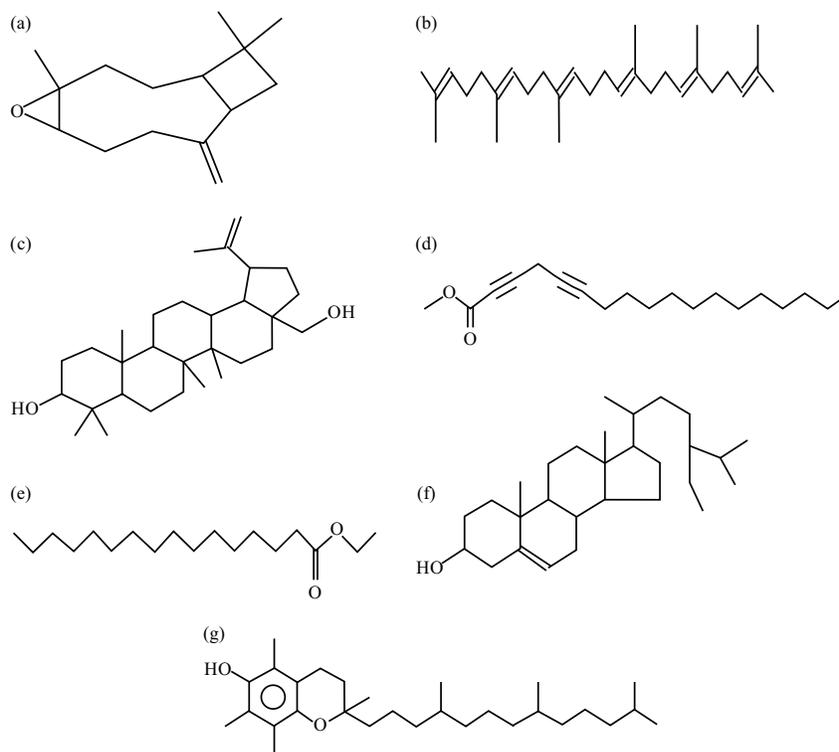


Fig. 6(a-g): Molecular structures of the major compounds identified in *Piliostigma thonningii* by GC-MS

Molecular structures of the major compounds identified in *Piliostigma thonningii* by GC-MS (NIST/EPA/NIH Mass Spectral Library through the Agilent MassHunter Workstation Software)

The numbers correspond to the order numbers of the compounds in Table 4

Table 4: Compounds identified by GC-MS in *Piliostigma thonningii* with peak area percentages greater than 10%

N°	Compound name	RT (min)	Area (%)	Family of secondary metabolites
1	Caryophyllene oxide	22.2	14.65	Terpenes
2	24-Methylenecycloartan-3-one	59.4	13.3	Triterpenes
3	n-Hexadecanoic acid	28.4	59.19	Fatty acids
4	9,12,15-Octadecatrienoic acid (Z,Z,Z)-	33.1	100	Fatty acids
5	Hexadecanoic acid, ethyl ester	28.9	21.22	Esters
6	γ-Sitosterol	57.8	81.55	Steroids
7	Vitamin E	55.7	18.29	Tocopherols

(0.021 mg GAE/mg). *Euphorbia lactea*, for its part, follows *Piliostigma thonningii*, whose aqueous and ethanolic extracts show higher polyphenol contents (150.9 and 89.3 μg GAE/mg, respectively) than those of *Richardia brasiliensis*.

GC-MS analysis of extracts: The chromatograms of the extracts from the three plants are presented in Fig. 3: *Euphorbia lactea* (A), *Richardia brasiliensis* (B) and *Piliostigma thonningii* (C). The number of detected peaks was 50 for *E. lactea*, 44 for *P. thonningii* and 35 for *R. brasiliensis*.

The names of the ten most abundant compounds (peak area >10%) present in the extracts of these plants are highlighted in Table 2- 4, together with their similarity index values (\geq identification threshold), retention times (RT), peak

area percentages and corresponding secondary metabolites. The chemical structures of these compounds are shown in Fig. 4-6.

The GC-MS analysis of *Euphorbia lactea* revealed the presence of 50 molecules, of which 31 are known and have already been referenced in existing literature, meaning 19 compounds have not been previously referenced in this plant. The identified compounds are classified into eight chemical groups: Benzenes, alcohols, phenols, fatty acids, esters, ethers, steroids and triterpenes (Table 2).

The GC-MS analysis of *Richardia brasiliensis* revealed 35 molecules, among which 24 are known and referenced, while 11 are not described, suggesting the identification of 11 compounds not previously reported in this plant. These

compounds belong to six chemical groups: Alcohols, carboxylic acids, esters, ketones, terpenes and phenolic compounds (Table 3).

The GC-MS analysis of *Piliostigma thonningii* revealed 44 compounds, 32 of which are known and referenced, leaving 12 as compounds that have not been previously referenced in this plant. These compounds are classified into five major chemical groups: Monosaccharides, sesquiterpenes and terpenoids, fatty acids and derivatives, esters and derivatives and steroidal steroids and triterpenes (Table 4).

DISCUSSION

The present study provides a comprehensive analysis of the phytochemical profiles and secondary metabolite contents of *Piliostigma thonningii*, *Euphorbia lactea* and *Richardia brasiliensis*. The growing interest in medicinal plants as sources for novel therapeutics has led to ethnobotanical surveys, which remain among the most reliable approaches. In Benin,¹¹ reported 193 plant species from 63 botanical families used in the management of gastric ulcers and hypertension. Similarly, a study by Dangnon *et al.*¹² identified 71 species in Southern Benin, of which 12 were frequently cited for ulcer treatment. Based on these ethnobotanical investigations and a thorough literature review, we selected three of the most frequently reported plants in the Beninese pharmacopeia: *Richardia brasiliensis*, *Euphorbia lactea* and *Piliostigma thonningii*.

The selection of plant organs was guided by existing phytochemical evidence, with preference given to underexplored parts. For *Piliostigma thonningii*, most studies have focused on the stem bark and roots, revealing a wide range of metabolites including flavonoids, alkaloids, tannins and sterols^{13,14}. In *Euphorbia lactea*, investigations have mainly targeted latex and stem extracts, highlighting triterpenoids such as tirucallol with anti-inflammatory activity^{15,16}. Similarly, for *Richardia brasiliensis*, aerial parts (leaves and stems) have demonstrated phytochemical richness and relevant biological properties, notably antimicrobial and antiproliferative effects^{5,17}. In this study, the leaves of *P. thonningii* were chosen not only for their potential accumulation of photosynthesis-related secondary metabolites but also for their sustainable harvest compared to roots. For *E. lactea*, the stem was selected due to its morphological features and limited prior phytochemical profiling. For *R. brasiliensis*, aerial parts were retained, given its herbaceous nature and previously reported bioactivity. Thus, by targeting leaves, stems and aerial parts respectively, we aimed to expand the phytochemical landscape of these species and address existing knowledge gaps in organ-specific metabolite distribution.

The extraction yields varied notably among the plants and solvents used. These variations may be attributed to the differential solubility of plant constituents in polar versus non-polar solvents. Ethanol, being less polar than water, is more efficient in extracting medium to low polarity compounds, whereas water favors the extraction of highly polar substances. This aligns with the findings of previous study by Usin and Daramola¹⁸, who reported higher yields in ethanolic extracts of *P. thonningii* leaves, suggesting the presence of diverse phytochemicals soluble in ethanol. These results are also consistent with previous studies reporting that ethanol, due to its intermediate polarity, can solubilize a broader spectrum of bioactive compounds compared to water¹⁹.

The qualitative and quantitative phytochemical composition of each plant varied significantly depending on the solvent employed for extraction, highlighting the solvent-dependent nature of metabolite recovery. The statistical significance of the differences ($p < 0.0001$) underscores the importance of solvent choice in optimizing the recovery of specific phytochemicals. Both *Piliostigma thonningii* and *Euphorbia lactea* exhibited notably high concentrations of flavonoids and tannins. Such variations in secondary metabolite composition reflect the biochemical specificity of each plant species. This aligns with findings of a study by Famurewa²⁰, reported that *Moringa oleifera* seeds' ethanolic extracts contained significant levels of flavonoids and polyphenols, suggesting that ethanol is an effective solvent for extracting these compounds. The prominence of flavonoids and polyphenols in *P. thonningii* corroborates the work⁴, who reported significant levels of these compounds in the seeds of the plant. Interestingly, the ethanolic extract of *Richardia brasiliensis* exhibited the highest concentration of hydrolyzable tannins.

The GC-MS analysis provided a detailed chemical fingerprint of the studied plants. Analysis reveals significant variation in chemical composition among the powders of distinct plant parts, highlighting differential metabolite accumulation. These molecules belong to various classes of secondary metabolites. For instance, a study by Russo and Marcu²¹ identified several pharmacologically active compounds in the essential oil of *Piliostigma thonningii*, including β -pinene, β -limonene and α -murolene. Comparative studies have shown that plants with similar phytochemical profiles such as *Vernonia amygdalina*, *Ficus exasperate* and *Ocimum gratissimum* exhibit significant antiulcer activity due to their rich content in flavonoids, tannins and terpenoids²²⁻²⁴. The similarity in metabolite classes across these species reinforces the therapeutic potential of *P. thonningii*, *E. lactea* and *R. brasiliensis* and underscores the need for further biological investigations.

Previous GC-MS studies of *Piliostigma thonningii*²⁵ and *Euphorbia lactea*²⁶ reported only a limited set of metabolites, including sugars, organic acids and a few lipophilic constituents, or a small number of bioactive compounds identified through fractionation. In contrast, our analyses revealed a considerably broader spectrum of chemical classes for both species, highlighting diverse metabolites that were not previously documented. These differences likely reflect variations in extraction methods, analytical depth and plant material, which can strongly influence metabolite detection. Collectively, these findings suggest that earlier studies may have underestimated the phytochemical diversity of these plants, underscoring the need for comprehensive profiling approaches to fully capture their chemical composition and pharmacological potential.

Although phytochemical studies on *Richardia brasiliensis* have been reported, most of them relied on classical screening methods, targeted isolation, or UHPLC-MS/MS analyses, without providing a comprehensive GC-MS profile^{5,27}. In this context, our study represents, to our knowledge, one of the first attempts to generate a detailed GC-MS profile of the aerial parts of *R. brasiliensis*. This broader characterization highlights a chemical diversity that complements previous reports and contributes to filling the existing gap in the literature. Nevertheless, as GC-MS is based toward volatile and semi-volatile compounds, further analyses integrating LC-MS/MS or NMR would be valuable to achieve a more exhaustive coverage of the phytochemical space of this species.

CONCLUSION

This study highlights the diverse phytochemical composition of *Piliostigma thonningii*, *Euphorbia lactea* and *Richardia brasiliensis*, characterized by significant levels of secondary metabolites. The detection of compounds not previously reported in the literature through GC-MS analysis provides a chemical basis for further investigation into their pharmacological potential. While these results are consistent with the traditional use of these plants, they do not in themselves confirm therapeutic efficacy, but rather offer a foundation for future pharmacological validation and potential integration into modern medicine.

SIGNIFICANCE STATEMENT

This study discovered the diverse phytochemicals and potentially novel metabolites in *Euphorbia lactea*, *Richardia brasiliensis* and *Piliostigma thonningii* that can be beneficial for the development of natural therapeutic agents and nutraceuticals. By providing detailed chemical profiles, this

study will help researchers uncover the critical areas of plant-based bioactive compounds that many researchers were not able to explore. Thus, a new theory on the pharmacological potential of traditionally used Southern Benin medicinal plants may be arrived at.

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