

Research Article

Phenolic Compounds of *Mareya Micrantha* (Benth.) Mull. Arg. and Their Antioxidant Activity

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Abstract

Mareya Micrantha is a West African medicinal plant endowed with numerous therapeutic properties. It was characterized by a high content of steroidal derivatives such as cucurbitacins (C-30) and nor-cucurbitacins (C-29). A phytochemical study was conducted on its phenolic composition and the antioxidant activity of these compounds. To carry out this investigation, solid-liquid extraction, liquid-liquid fractionation, chromatographic column isolations, and compound characterization were performed. The phenolic compounds were identified using HPLC-UV-DAD method, and their concentrations were determined using colorimetric assays and HPLC-UV method. Isolated compounds structures were determined by spectroscopic analysis (UV, IR, MS, and NMR). Antioxidant activities were evaluated using two different tests (DPPH and CUPRAC). This study led to the isolation of amentoflavone and astragalin two known flavonoids and two original phenolic compounds including an isocoumarin named micranthin acid and a hydrolysable tannin named mareyanin. This is the first descriptive study of phenolic compounds from *Mareya micrantha*. Both original compounds showed greater antioxidant activity than Trolox. These compounds are present in high concentrations in extracts from the leaves of this plant. They could be considered as the most potent antioxidant and major phenolic compounds of *Mareya micrantha*. The isolated phenolic compounds exhibited greater activity than the previously isolated steroidal derivatives.

Keywords

Mareya micrantha, Phenolic compounds, Flavonoids, Tannins, Isocoumarins, Antioxidant

1. Introduction

Identification of new antioxidant agents is still a big challenge worldwide for their multiple application in food industry, cosmetic and pharmacy as oxidative processes are in-

involved in many human pathologies [1]. Actually, under physiological or psychological stress, the human body produces reactive oxygen species such as superoxide anions, hydroxyl radicals, and hydrogen peroxide which can contribute

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to the pathogenesis of severe diseases, including cancer [2]. The production of these harmful compounds can be limited by antioxidants compounds derived from medicinal plants [3]. Numerous plants are renowned for their antioxidant properties and their therapeutic efficacy against various diseases. A prime example is *Mareya micrantha* (Benth.) Müll. Arg, a species recognized for its antioxidant activity, which has nonetheless received limited phytochemical investigation. This plant is widely utilized in traditional West African medicine. In a preliminary study, phytochemical screening led to the isolation of steroid terpenoid derivatives, specifically cucurbitacins and nor-cucurbitacins [4]. These compounds have demonstrated significant antioxidant potential as well as anti-proliferative activity against hepatocellular carcinoma [5]. Among plant metabolites, Phenolic compounds belong to a chemical class whose antioxidant properties are well documented in the literature [6]. Therefore, a study was conducted to investigate on phenolic compounds derived from *M. micrantha* and their antioxidant activity. This study led to identify two original phenolic compounds, whose both chemical and biological characteristics are described here.

2. Materials and Methods

2.1. Material

2.1.1. Plant Material

Leaves of *Mareya micrantha* were collected in December 2023 from Guezem (6°15'31.68"N, 6°11'35.99"W), Ivory Coast. Botanical identification and authentication were performed by Prof. Joseph Ipou Ipou at the Herbarium of the National Floristic Center, Félix Houphouët-Boigny University (Ivory Coast), by comparison with the historical voucher specimen (No. 8.482, collected on January 23, 1966, by L. Aké Assi) deposited in the facility. The collected leaves were shade-dried in the laboratory at 25 °C for four weeks and subsequently pulverized to a particle size of 250 µm using an electric ball grinder.

2.1.2. Experimental Material

Column chromatography (CC) was performed using silica gel (Merck, 70-230 mesh) and Sephadex® LH-20 (GE Healthcare). FT-IR (ATR) spectra were recorded on a Perkin Elmer Spectrum 65 FT-IR spectrometer. 1D and 2D NMR spectra were acquired in CD₃OD at 500 MHz for ¹H and 125 MHz for ¹³C using a Bruker Avance 500 spectrometer equipped with a 5 mm TCI cryoprobe. Chemical shifts δ are reported in ppm and coupling constants *J* are given in Hz.

LC-UV (DAD) profiles were obtained on a Thermo Fisher Ultimate 3000 system equipped with a photodiode array detector (DAD) using a Phenomenex Luna C18 3µ PFP (2) 100 Å (150 × 3.0 mm) column. LC-HRMS analyses were performed on a UHPLC-DAD-QTOF instrument consisting of an

ACQUITY UPLC system coupled to a Xevo G2 QToF mass spectrometer (Waters, MA, USA) equipped with an electrospray ionization (ESI) source operating in negative mode. Chromatographic separation was achieved on a UPLC BEH C18 column (100 × 2.1 mm, 1.7 µm, Waters).

2.2. Methods

2.2.1. Extract and Fractions Preparation

The leaf powder (1,000 g) was subjected to maceration extraction (m/v, 1/5) three times (3 × 24h) in the solvent mixture ethanol/water (70: 30, v/v, 15 L). After filtration on WAT-MANN papers, the extracts were concentrated under reduced pressure using a Rotary evaporator at 40 °C to yield the crude hydroethanolic extracts, designated as FM₀. The resulting extract was dissolved in 500 mL of distilled water and subsequently subjected to liquid-liquid extraction using hexane (3 × 500 mL) followed by ethyl acetate (4 × 400 mL). Upon evaporation to dryness, the hexane fraction (FM₁) and the ethyl acetate fraction (FM₂) were obtained. The residual aqueous phase was lyophilized to yield the aqueous extract (FM₃).

2.2.2. Total Polyphenols Content

The total polyphenols content was measured according by Folin-Ciocalteu method with some modifications [7]. To 100 µL of extract (1000 mg/L, in MeOH) in a tube were added successively 900 µL of demineralized water, 900 µL of Folin-Ciocalteu reagent (1N), then 200 µL of sodium carbonate (Na₂CO₃ at 20%). The tubes containing the mixtures are incubated (25 °C, 30 min) in the dark. A calibration curve was obtained based on Gallic acid (0-100 µg/mL) as standard, prepared under the same conditions described above. The absorbance of sample was measured with a spectrophotometer (Shimadzu UV-1800) at 760 nm against a methanol used as a blank. The total phenolic content was expressed in milligrams of Gallic acid equivalent per gram of extract (mg. GAE/g dry extract).

2.2.3. Total Flavonoids Content

The total flavonoids content was measured according to the method of Zhishen with slight modifications [8]. To 250 µL of extract (1000 mg/L, in MeOH) in a tube, was added 1 mL of demineralized water. Then, at t₀ (0 min), 75 µL of NaNO₂ solution (5%) was added; at t₁ (5 min), 75 µL of AlCl₃ solution (10%); at t₂ (10 min), 500 µL of NaOH (1N). Finally, the mixture was completed with 2.5 mL of demineralized water and stirred vigorously. Calibration curve was obtained based on Rutin (0-100 µg/mL) as standard, prepared under the same conditions described above. The absorbance of the sample was measured at 510 nm against a methanol used as a blank. The total flavonoid content was expressed in milligram Rutin equivalent per gram of extract (mg. RE / g dry extract).

2.2.4. Total Hydrolysable Tannins

The total hydrolysable tannins content was measured according to Mole method with some modifications [9]. Separately 5 mL of extract (1000 mg/L, H₂O) were introduced into two different test tubes (T1 and T2). Two drops of NaOH (4M) are added to test tube T1. This tube is placed in a boiling water bath for 2 h. It was then cooled and the content was neutralized by adding two drops of HCl (4M). At t_0 (0 min), to 500 μ L of each tube (T1 and T2), were added 3.5 mL of the ferric chloride solution (1.62 g of FeCl₃ dissolved in 0.001 M of HCl to give a volume of 1 L). Calibration curve was obtained based on Tannic acid (0-1.2 μ g/mL) as standard, prepared under the same conditions described above. Test tubes were shaken and their absorbance were read at 660 nm, against a blank consisting only of the reagents. The total hydrolysable tannin content was expressed in milligram Tannic acid equivalent per gram of extract (mg, TAE / g dry extract).

2.2.5. Isolation of Compounds

Only the ethyl acetate fraction FM₂ was fractionated. To be done 9 g of FM₂ were dissolved in a hexane/ethyl acetate mixture (3: 7, v/v, 30 mL) under heat (80 °C) and then left to stand (25 °C) during three hours. A precipitate and a supernatant were formed, then separated into two parts: a liquid phase and an amorphous solid phase. Fraction FM₂-1 (5 g) was obtained by evaporating the solvent from the liquid phase, whereas FM₂-2 (3 g) was isolated after drying the amorphous phase. A portion of FM₂-1 (4 g) was then subjected to silica gel column chromatography (220 g, 27 $\times$ 5.1 cm). Elution was performed using a gradient system of solvent C (ethyl acetate) and solvent D (acetone/ethanol/water, 6: 3: 1, v/v/v), variation from 100: 0 to 60: 40 (v/v) at a flow rate of 20 mL/min, to yield sub-fractions FM₂-1.1 (60 mL: Tubes 5-8) and FM₂-1.2 (20 mL: Tubes 9-10). A sample of FM₂-1.1 (0.75 g) was purified by silica gel column chromatography (100 g; column dimensions: 17.5 $\times$ 3.5 cm), using gradient system C/D ranging from 90: 10 to 70: 30 (v/v) at a flow rate of 10 mL/min. The resulting fractions were further purified by gel filtration chromatography on a Sephadex® LH20 column (35 g; column dimensions: 12 $\times$ 3 cm), eluting with a mixture of methanol and methylene chloride (70: 30, v/v) at a flow rate of 5 mL/min. This led to purified compounds 1 (17 mg) and 2 (20 mg). A sample of subfraction FM₂-1.2 (2.5 g) was chromatographed under the same conditions as FM₂-1.1, yielding purified compound 3 (8 mg). As for FM₂-2, 500 mg of this fraction was washed with ethyl acetate to remove impurities and then purified on Sephadex® LH20 column (35 g; column dimensions: 12 $\times$ 3 cm) to give compound 4 (80 mg).

Compound 1: Yellow amorphous powder, $[\alpha]_D^{30,7} = +47$ (C: 0.45, MeOH), UV $\lambda_{\max}$: 209, 268 and 336 nm, FT-IR (ATR): $\nu_{\max}$ 3200, 1643, 1600, 1564 cm^{-1} . For ¹H NMR (CD₃OD, 500 MHz) and ¹³C NMR (CD₃OD, 125 MHz) see spectral data, HR-ESI-MS 1: $[M - H]^-$ m/z 537.0822 (Calc. for C₃₀H₁₈O₁₀, 538.0900).

Compound 2: Yellow amorphous powder, $[\alpha]_D^{30,4} = +29$ (C: 0.4, MeOH), UV $\lambda_{\max}$: 203, 264 and 347 nm, FT-IR (ATR): $\nu_{\max}$ 3356, 1655 and 1598 cm^{-1} . For ¹H NMR ((CD₃)₂CO, 300 MHz) and ¹³C NMR ((CD₃)₂CO, 75 MHz) see spectral data, HR-ESI-MS 2: $[M - H]^-$ m/z 447.0927 (Calc. for C₂₁H₂₀O₁₁, 448.1005).

Compound 3: Yellow-orange amorphous powder, $[\alpha]_D^{29,8} = +50$ (C: 0.4, MeOH), UV $\lambda_{\max}$: 270-287 and 357 nm, FT-IR (ATR): $\nu_{\max}$ 3250, 1666, 1584 cm^{-1} . For ¹H NMR (CD₃OD, 500 MHz) and ¹³C NMR (CD₃OD, 125 MHz) spectral data, see Table 2, HR-ESI-MS 3: $[M - H]^-$ m/z 291,0141 (Calc. for C₁₃H₈O₈, 292.0219).

Compound 4: Yellow amorphous powder, $[\alpha]_D^{29,3} = +48$ (C: 0.06, MeOH), UV $\lambda_{\max}$: 225, 268 nm; FT-IR (ATR): $\nu_{\max}$ 3270, 1607 and 1513 cm^{-1} . For ¹H NMR (CD₃OD, 500 MHz) and ¹³C NMR (CD₃OD, 125 MHz) spectral data, see Table 3, HR-ESI-MS 4: $[M - H]^-$ m/z 799.0630 (Calc. for C₃₄H₂₄O₂₃, 800.0708).

2.3. Antioxidant Activity

2.3.1. Copper Ion Reduction Assay

CUPRAC-BCS method was performed according to Campos method with some modifications [10]. The reaction mixture consisted of 40 μ L of samples, 160 μ L of bathocuproinedisulfonic acid BCS solution (4.2 mg in 30 mL of distilled water) and 50 μ L of CuSO₄ solution (18.7 mg in 30 mL of distilled water). Fifteen minutes after mixing the reagents, 50 μ L of EDTA (43.8 mg in 30 mL of distilled water) was added to stop the reaction. The blank was obtained from ethanol processed under the same conditions described above. The absorbances were measured at 490 nm using a microplate reader (BMG Labtech spectrometer). The curves showing the percentage reduction of copper were plotted using the following equation (1):

$$\% \text{ Reduction } (Cu^{2+}) = (1 - A_0/A) \times 100 \quad (1)$$

A: Absorbance of the reaction mixture; A₀: Absorbance of the blank.

2.3.2. DPPH Radical Reduction Assay

DPPH method was performed according to Parejo method with moderate changes [11]. The reaction mixture consisted of 150 μ L of samples and 150 μ L of a DPPH solution (0.238 mM, 4.7 mg in 50 mL EtOH). These mixtures were incubated in the dark for 45 min. Then, their absorbances were read at 25 °C, in 96-well plates using a microplate reader (BMG Labtech spectrometer). The curves describing the reduction percentage of DPPH were plotted using the following equation (2):

$$\% \text{ Réduction } (DPPH) = (1 - A/A_0) \times 100 \quad (2)$$

A: Absorbance of the reaction mixture; A_0 : Absorbance of the blank.

The concentrations to reduce 50% (IC_{50}) of DPPH radical and copper ion (Cu^{2+}) were determined based on 3 independent experiments using the GraphPad Prism software Inc., USA. The IC_{50} ($\mu g/mL$) are expressed as the mean $\pm$ standard deviation (SD).

2.3.3. Isolated Compounds Content

Crude extract FM_0 and ethyl acetate fraction FM_2 solutions were respectively prepared at 10 mg/mL and 5 mg/mL. Solutions of compound 1 and 2 (5, 10, 15, 20, 25 and 50 $\mu g/mL$) as well as 3 and 4 (25, 50, 100, 150 and 200 $\mu g/mL$) were also prepared. All samples were submitted to HPLC analysis in reverse phase at controlled temperature (40 °C). The amount of sample injected is 20 μL . The mobile phase was composed of solvents A (water with 0.1% formic acid) and B (methanol with 0.1% formic acid). The elution rate was set at 0.3 mL/min, with a gradient of: 5% (B) in 5 min, 5 to 95% (B) in 45 min, 95% (B) in 5 min, 95 to 5% (B) in 5 min, and initialization to 5% (B) in 5 min. Chromatograms are recorded using the Chromeleon Thermo Scientific, France software. Calibration curves were obtained from the isolated compounds.

2.4. Statistical Analysis

All analyses were conducted in triplicate, and the data are

expressed as mean $\pm$ standard deviation (SD). Statistical significance was determined using a one-way analysis of variance (ANOVA), followed by Dunnett's post hoc test for multiple comparisons. Differences were considered statistically significant at $p < 0.05$. All statistical analyses were performed using GraphPad Prism.

3. Results and Discussion

3.1. Extraction and Compounds Identification

Hydroethanolic extraction yielded the crude extract FM_0 . The yield was 12.5%. This extract was fractionated and yielding aqueous fraction FM_3 , acetate fraction FM_2 and hexane fraction FM_1 . The yields were 8%, 3%, and 0.5% respectively. It was observed that the polar fractions (FM_2 and FM_3) had higher yields than the nonpolar fraction (FM_1). It can be suggested that *M. micrantha* compounds are predominantly polar. Other phytochemical studies report the isolation of nor-cucurbitacin derivatives and glycosylated flavonoid [4, 5, 12]. These compounds are polar and were isolated using polar solvents, such as methanol, ethanol, acetone, and water [13, 14].

HPLC-UV (DAD) was used to identify the compounds of crude extract FM_0 . Figure 1 shows the resulting chromatogram. Fractionated compounds were detected at 254 nm. A total of 42 compounds were detected (Table 1).

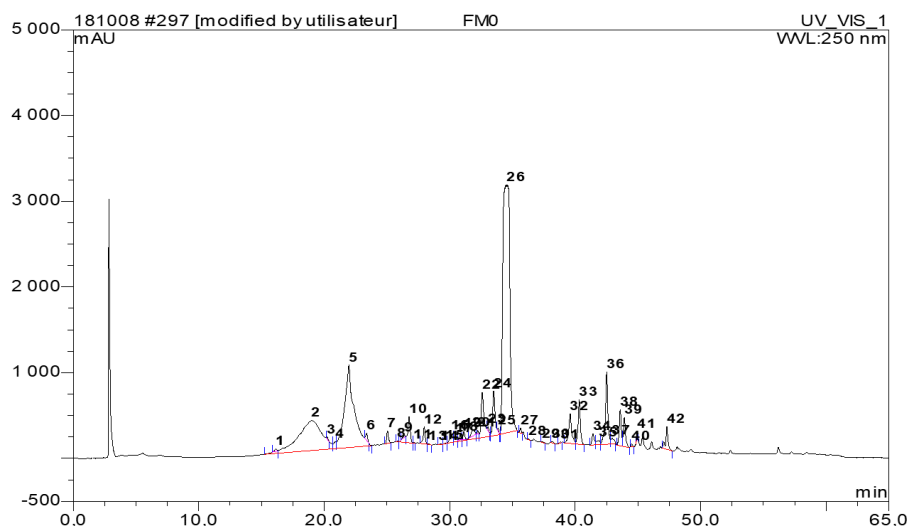


Figure 1. Chromatogram of crude extract FM_0 from *M. micrantha*.

Table 1. Chemical profile of crude extract FM_0 from *M. micrantha*.

Pic	Rt (min)	Abs%	UV λ_{max} (nm)	Chemical family
1	16,15	0,10	221.8 274.6	THs
2	19,01	16,09	221.8 265.3	THs
3	20,24	0,07	229.0 268.3	THs

Pic	Rt (min)	Abs%	UV $\lambda_{\max}$ (nm)				Chemical family
4	20,88	0,38	221.3	280			THs
5	21,97	18,09	227.6	267.0			THs
6	23,38	0,29	217.3	273.7			THs
7	25,04	0,51	226.1	270			THs
8	25,85	0,02	216.1	238.1	300.7		P.acid
9	26,40	0,10	225.2	276.0	362.4		P. ID
10	26,77	1,84	216	277.4	355.6		P. ID
11	27,15	0,02	223.4	260.0	303.1		P.acid
12	27,97	0,75	216.9	241.0	290	328.2	P.acid
13	28,34	0,03	270.0	374.2			P. ID
14	29,27	0,03	264.9	348.0			Flavon
15	29,70	0,03	276.7	361.0			P. ID
16	30,11	0,53	213.1	267.4	348.3		Flavon
17	30,46	0,30	214.0	275.6	358.4		P. ID
18	30,83	0,17	203.3	264.1	347.5		Flavon
19	31,13	0,28	204.3	264.8	343.7		Flavon
20	31,82	0,46	234.0	322.9			P.acid
21	32,25	0,10	214.0	277.3	355.3		P. ID
22	32,60	4,17	224.9	260.4	377.1		P. ID
23	33,05	0,11	252.7	343.6			Flavon
24	33,51	2,61	227.7	280.3	386.2		P. ID
25	33,85	0,07	260.6				Cub
26	34,57	38,45	255				Cub
27	35,65	0,15	205.7	264.4	346.1		Flavon
28	36,27	0,04	229.0	260			Cub
29	37,36	0,05	245.9				Cub
30	38,16	0,07	252				Cub
31	38,85	0,04	208.7	232.2	280	325.2	P.acid
32	39,61	1,93	244.7				Cub
33	40,33	2,28	226.3	281.4			A. Cub
34	41,42	0,58	236.9				Cub
35	41,90	0,33	203	240			Cub
36	42,52	4,07	243.7				Cub
37	42,94	0,41	233.4	276.8			A. Cub
38	43,59	1,64	228.0	285.5			A. Cub
39	43,93	1,41	240.9				Cub
40	44,52	0,09	227.6	283.4			A. Cub
41	44,95	0,11	240.5				Cub

Pic	Rt (min)	Abs%	UV $\lambda_{\max}$ (nm)	Chemical family
42	47,31	1,19	243.2	Cub

Rt: retention time, Abs: absorbance, THs: tannin, P: phenolic, ID: indefinite, A: aromatic, Cub: nor-cucurbitacin.

The detected compounds were classified into six families based on the similarity of their UV spectra (Figure 2). Each chemical family was identified by comparing UV absorption spectrum with those of known molecules isolated in Euphorbiaceae.

The spectrum Figure 2a shows two maxima at $\lambda_{\max}$ 227 and 269 nm indicating hydrolysable tannins [15]. The spectrum Figure 2b with maxima at $\lambda_{\max}$ 216, 240, 296 and 328 nm,

would be characteristic of phenolic acids [16]. The spectrum Figure 2c with $\lambda_{\max}$ 277 and 365 nm corresponds to phenolics compounds. The spectrum Figure 2d with maxima $\lambda_{\max}$ 204, 264 and 345 nm indicates flavones [17]. Finally, the spectra Figure 2e and Figure 2f with absorptions $\lambda_{\max}$ at 245 nm and 226, 281 nm respectively reveal nor-cucurbitacin derivatives already isolated from *M. micrantha* [4].

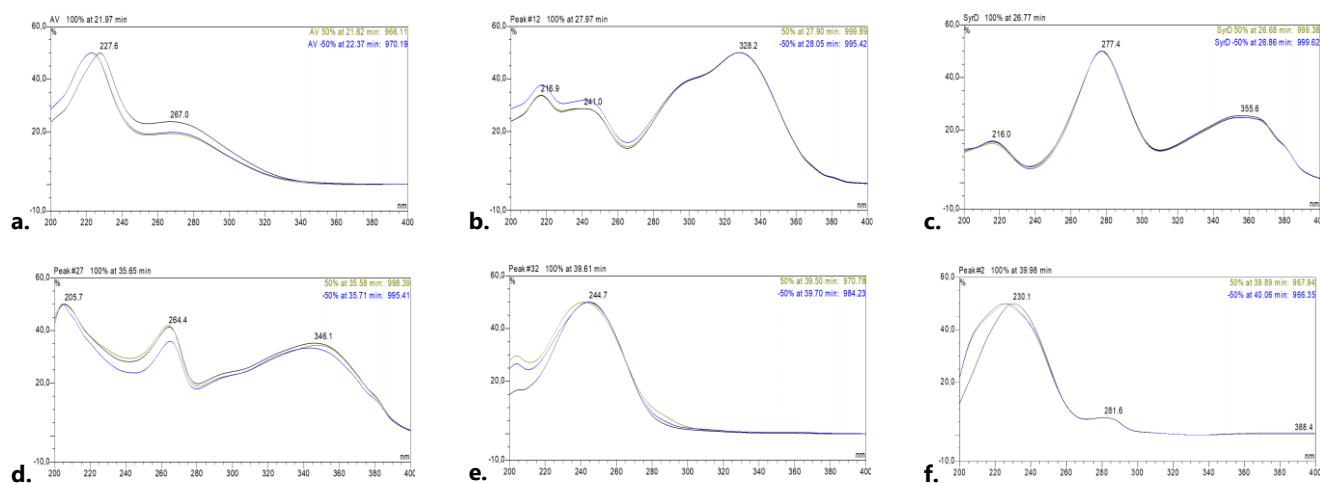


Figure 2. UV spectra of compounds families of *M. micrantha* (a: Hydrolysable tannins, b: Phenolic acids, c: Undefined phenolic compounds, d: Flavones, e: Nor-cucurbitacins, f: Aromatic nor-cucurbitacins).

These six chemical families were classified into two groups based on their structural core. The diagram in Figure 3 shows the percentage of compounds in each family.

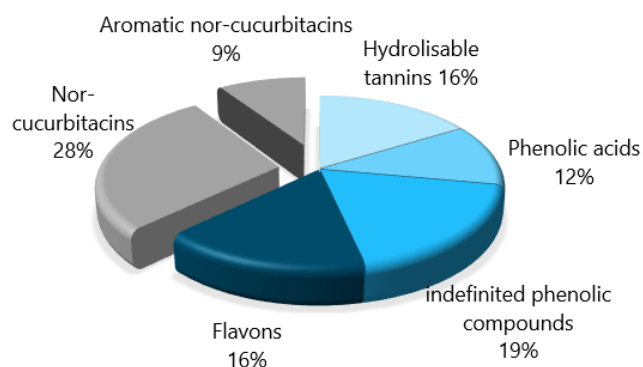


Figure 3. Chemical families identified from *M. micrantha*.

The first group comprises phenolic compounds, including hydrolysable tannins, phenolic acids, and flavonoids. Among these phenolic compounds, one chemical family remained unidentified. Phenolic group accounts 63%. The second group consists of aromatic and non-aromatic nor-cucurbitacin derivatives. This group of compounds belongs to the steroids. It accounts 37%. Phenolic group contains a larger number of compounds than the steroid group. This result would suggest that phenolic compounds have the greatest structural diversity.

Structural diversity alone cannot explain abundance of compounds. Therefore, the total phenolic compounds contents were determined.

3.2. Phenolic Compounds Content

The total phenolic compounds (TPC), total flavonoid compounds (TFC), and total hydrolysable tannins (HTs) contents were determined using colorimetric assays. The results are expressed in mg/g of extract (Table 2).

Table 2. Total contents of Phenolic compounds.

Extract/Fractions	TPC (mg GAE/g)	TFC (mg RE/g)	THs (mg TAE/g)
Crude extract (FM ₀)	382.63 ± 19.52 ^b	95.84 ± 5.82 ^b	210.53 ± 3.25 ^c
Hexane (FM ₁)	68.12 ± 27.03 ^d	9.05 ± 1.57 ^d	11.87 ± 0.61 ^d
Ethyl acetate (FM ₂)	797.91 ± 3.52 ^a	171.99 ± 1.65 ^a	636.76 ± 20.49 ^a
Aqueous (FM ₃)	256.32 ± 11.33 ^c	51.16 ± 2.53 ^c	230.41 ± 6.76 ^b

Total contents of Polyphenol TPC, Flavonoids TFC and Hydrolysable tannins THs.

The results are presented as mean ± SD (n = 3) of three replicates, within each column, values carrying different superscript letters indicate statistically significant differences based on Dunnett's T3 multiple comparisons test (p < 0.05), GAE, RE and TAE (Gallic acid, Rutin and Tannic acid equivalent, respectively).

The TPC ranged from 68.12 - 797.91 mg GAE/g. The maximum value was obtained in the acetate fraction FM₂ and the lowest in the hexane fraction FM₁. Significant differences were observed. The TPC of the crude extract FM₀ (382.63 ± 19.52 mg GAE/g) is greater than of the values (293-225 mg GAE/g) obtained for *E. humifusa*, *E. hirta*, *E. umbellata* and *E. spendia* which like *M. micrantha* belong to the Euphorbiaceae family [18-21]. The result shows that *M. micrantha* phenolic compounds in the crude extract are concentrated in the acetate fraction FM₂ due to its high observed TPC value.

Analysis of the phenolic compound classes revealed that TFC and HTs ranged from 9.05 to 171.99 mg RE/g and 11.87 to 636.76 mg TAE/g, respectively. Significant differences were observed. The maximum values of TFC and HTs were obtained in the acetate fraction FM₂ and the lowest in hexane fraction FM₁. All HTs values were higher than TFC values in the various fractions. This result shows that the acetate fraction was concentrated in flavonoids and hydrolyzable tannins than the other fractions. Hydrolysable tannins are relatively less abundant in *M. micrantha*. Naturally, there are two main groups of hydrolysable tannins ellagitannin and gallotannin [22, 23].

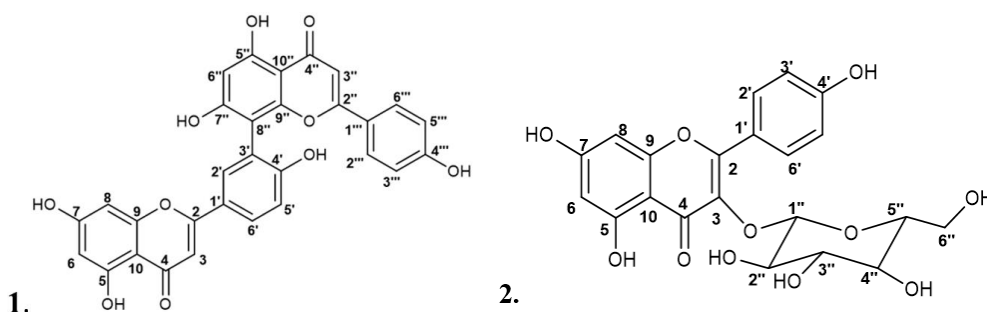
Hydrolyzable tannins, flavonoids, and phenolic acids are

phenolic compounds that are known to have antioxidant properties [24]. These compounds may be responsible of *M. micrantha* antioxidant activity.

3.3. Structure Elucidation of Compounds

Four pure phenolic compounds (1-4) (Figure 4) were isolated from ethyl acetate fraction FM₂. This fraction was selected because it was found to contain a high concentration of polyphenols.

Compounds 1-2 were purified as yellow amorphous powders and showed UV spectra (Appendix: Figures S1.3 and S2.3) with absorption (λ_{max} 209, 268 and 336 nm; λ_{max} : 203, 264 and 347 nm, respectively) similar to those of flavones. 1 and 2 were soluble in dichloromethane and methanol respectively making 2 is more polar than 1. Their HR-ESI-MS analysis yielded molecular ions $[M - H]^- m/z$ 537.0822 (Calc. for C₃₀H₁₈O₁₀, 538.0900) and $[M - H]^- m/z$ 447.0927 (Calc. for C₂₁H₂₀O₁₁, 448.1005) characteristic of biflavone and glycosylated flavone respectively. Their structures were determined to be known compounds by comparing their 1D (¹H, ¹³C) and 2D (COSY, HSQC and HMBC) NMR spectral data (Appendix, Figures S1.7-11 and S2.7-11) with literature values. Compounds 1 and 2 were identified as *Amentoflavone* and *Astragalin*, respectively (Figure 4) [25]. *Amentoflavone* was previously isolated from many species which belong to several families including Euphorbiaceae [26]. *Astragalin* was isolated and reported in the *Euphorbia* sp. and *Phyllanthus* sp. These genera belong to Euphorbiaceae family [27].



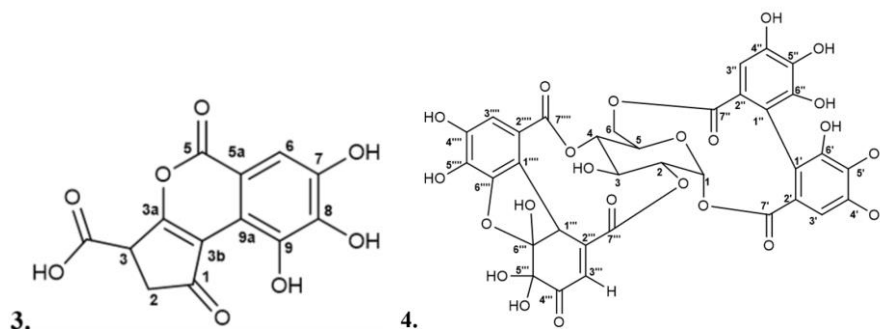


Figure 4. Isolated compounds: 1-Amentoflavone, 2-Astragalin, 3-Micranthin acid and 4-Mareyanin.

Compound 3 was obtained as a yellow-orange powder soluble in methanol. Its UV spectrum (Appendix: Figure S3.3) exhibited absorptions at $\lambda_{\max}$ 280 and 357 nm similar to those of the unidentified chemical family. The IR spectrum (Appendix: Figure S3.4) of 3 indicates hydroxyl group (OH) at $\nu_{\max}$ 3200 cm^{-1} and unsaturated α , β carbonyl group (C=O) at $\nu_{\max}$ 1686 cm^{-1} . The HR-ESI-MS analysis of 3 exhibited a quasi-molecular ion peak $[M - H]^- m/z$ 291.0140 (Calc. for $\text{C}_{13}\text{H}_8\text{O}_8$, 292.0219) corresponding to ten degrees of unsaturation. This molecular formula establishes a C13 framework, thereby excluding a C15 flavonoid structure. The ^1H NMR spectral data of 3 (Table 3) displayed aromatic proton at δ_{H} 7.38 (s) assigned to H-6, and alicyclic proton at δ_{H} 4.52 (dd, $J = 6.7, 1.2$ Hz) assigned to H-3 (CH) and methylenic protons at δ_{H} 2.79 (dd, $J = 18.4, 6.7$ Hz) and 2.94 (dd, $J = 18.4, 1.2$ Hz) ascribed to $\text{H}_{\text{a}}-2$ and $\text{H}_{\text{b}}-2$ respectively. The ^{13}C NMR (J -mod) spectral data (Table 3) of 3 revealed the presence of sp^3 methinic carbon and methylene at δ_{C} 43.9 (C-3) and 38.9 (C-2) respectively. In addition, the ^{13}C NMR (J -mod) spectrum also revealed the presence of sp^2 carbons. These included two sp^2 carboxydes at δ_{C} 163 (C-5) and 177.3 (3-COOH), one sp^2 carbonyl at δ_{C} 197.3 (C-1) and eight sp^2 aromatic carbons (δ_{C} 110.3, 115.1, 117.5, 142.2, 143.2, 145.2, 146.4 and 150.6). Based on the unsaturated carbons and the molecular formula (Calc. for $\text{C}_{13}\text{H}_7\text{O}_8$, 292.0219, with ten degrees of unsaturation) it was suggested three rings in the structure of 3.

The complete structure of 3 was established using the HSQC and HMBC spectrum data, on which $^1J_{\text{C-H}}$, $^2J_{\text{C-H}}$, $^3J_{\text{C-H}}$ and $^4J_{\text{C-H}}$ carbon-proton correlations were observed. Proton at δ_{H} 4.52 correlates with carbons at δ_{C} 38.9 (C-2), 142.2 (C-3b), 146.4 (C-3a), 177.3 (3-COOH) and 197.3 (C-1). Geminal protons at δ_{H} 2.79 and 2.94 correlate with carbons at δ_{C} 43.9 (C-

3), 142.2 (C-3b), 146.4 (C-3a), 177.3 (3-COOH) and 197.3 (C-1). These correlations suggested the formation of a five-membered carbon ring. Concerning proton at δ_{H} 7.38, it was observed a $^1J_{\text{C-H}}$ correlation with carbon at δ_{C} 110.3 (C-6), $^2J_{\text{C-H}}$ correlation with carbons at δ_{C} 115.1 (C-5a), and 145.2 (C-7), $^3J_{\text{C-H}}$ with carbons at δ_{C} 117.5 (C-9a), 143.2 (C-8), and 163.0 (C-5), but also $^4J_{\text{C-H}}$ correlation with carbons at δ_{C} 142.2 (C-3b), and 150.6 (C-9). This allows for the six-membered aromatic ring. The connection between the five-membered ring and the aromatic ring was possible through the $^4J_{\text{C-H}}$ correlation of aromatic proton at δ_{H} 7.38 and carbon at δ_{C} 142.2 (C-3b) and between alicyclic proton at δ_{H} 4.52 and carbon δ_{C} 117.5 (C-9a) (Figure 5). The third ring of 3 was obtained through an ester bond between carbons C-5 and C-3a. A comparison of our spectral data with the literature suggests that Compound 3 is an analogue of *Brevifolin acid*. The difference between them lies at carbon atoms C1 and C3. *Brevifolin acid* has a carboxyl group at position C1 and a ketone group at position C3. Compound 3 is an isomer of *Broccchlin acid* which was isolated from *Chrozophora broccchiana* (Euphorbiaceae) and described only once [28].

Compound 3 is an original compound derived from *M. micrantha*, it was named “*Micranthin acid*.” It is an isocoumarin.

Compound 4 was obtained as a yellow powder soluble in methanol. Its UV spectrum (Appendix: Figure S4.3) showed two absorptions at $\lambda_{\max}$ 221 nm, and 269 nm similar to hydrolysable tannins.

The IR spectrum (Appendix: Figure S4.4) showed absorption bands for hydroxyl groups (OH) at $\nu_{\max}$ 3270.6 cm^{-1} , and carbonyl groups at $\nu_{\max}$ 1698 cm^{-1} (C=O, ester) and $\nu_{\max}$ 1607.8 cm^{-1} (C=O, ketone).

Table 3. ^1H -NMR and ^{13}C -NMR spectra data of compound 3.

Position	^{13}C -NMR δ (ppm)	^1H -NMR δ_{H} (ppm)
1	197.3	C
2	38.9	CH_2 2.94 (dd, 18.4, 1.2), 2.79 (dd, 18.4, 6.7)
3	43.9	CH 4.52 (dd, 6.7, 1, 2)

Position	$^{13}\text{C-NMR } \delta$ (ppm)	$^1\text{H-NMR } \delta_{\text{H}}$ (ppm)
3a	146.4	C
3b	142.2	C
5	163	C
5a	115.1	C
6	110.3	CH 7,38 (s)
7	145.2	C
8	143.2	C
9	150.6	C
9a	117.5	C
3-COOH	177.3	C

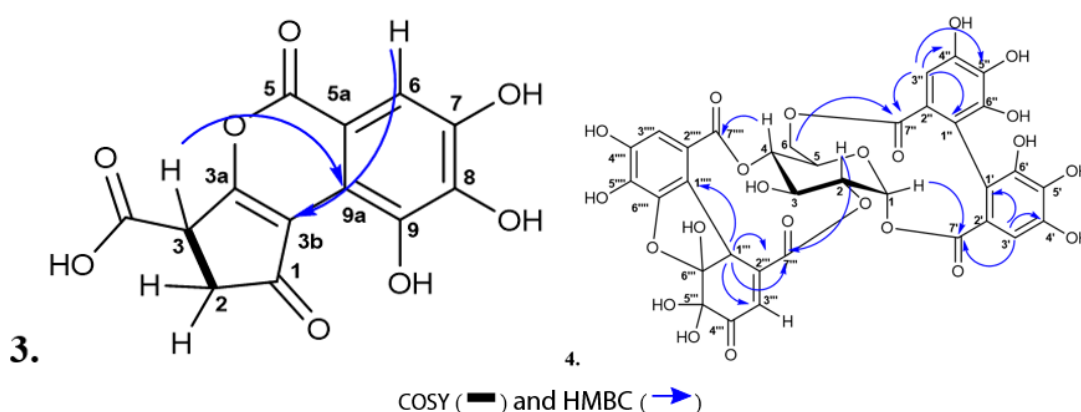


Figure 5. COSY and HMBC correlations of compounds 3-4.

Compound 4 molecular formula was established as $\text{C}_{34}\text{H}_{24}\text{O}_{23}$ by HR-ESI-MS (Appendix: Figure S4.5-6), which showed a deprotonated molecular ion at $[\text{M} - \text{H}]^- m/z$ 799.0642 (calcd for $\text{C}_{34}\text{H}_{23}\text{O}_{23}^-$, 800.0708). The MS spectrum displayed a characteristic fragment ion at m/z 301, indicative of an ellagitannin core [16]. The ^1H NMR spectrum data of 4 (Table 4) revealed aromatic proton signals at δ_{H} 7.28 (s), 6.89 (s), 6.76 (s), 6.57 (d, $J = 0.5$ Hz) and 5.55 (d, $J = 0.5$ Hz) assigned to H-5''', H-5', H-5'', H-5''' and H-1''' respectively. The signals between δ_{H} 4.08 and 5.17, suggested sugar unit. On the ^{13}C NMR (J -mod) spectrum of 4 the shielded sp^3 carbons at δ_{C} 90.9 (C-1), 71.1 (C-2), 62.2 (C-3), 69.8 (C-4), 61.2 (C-5), and 64.7 (C-6) characteristic of oxygenated carbons of glucose engaged in ester linkages suggested the presence of hydrolysable tannin [29]. The assignment of sp^2 carbon signals allowed identification of two carboxydes ($\text{C}=\text{O}$, ester) at δ_{C} 167.7 (C-7') and 170.1 (C-7'') and twelve aromatic carbons at δ_{C} 116.3 (C-1'), 145.3 (C-2'), 145.7 (C-3'), 138.3 (C-4'), 110.5 (C-5'), 117.9 (C-6'), 116.3 (C-1''), 145.3 (C-2''), 145.7 (C-3''), 137.2 (C-4''), 108.7 (C-5'') and 116.3 (C-6''), all characteristic of the hexahydroxydiphenyl (HHDP) ring of ellagic tannins [30]. Also two carboxydes ($\text{C}=\text{O}$, ester) were observed at δ_{C}

166.8 (C-7''') and 165.8 (C-7'''), one carbonyl ($\text{C}=\text{O}$, ketone) at δ_{C} 192.3 (C-4''') and carbons at δ_{C} 41.6 (C-1'''), 95.8 (C-2'''), 96.3 (C-3'''), 129.9 (C-5'''), 153.4 (C-6'''), 119.6 (C-1'''), 143.2 (C-2'''), 146.3 (C-3'''), 139.5 (C-4'''), 114.3 (C-5''') and 115.4 (C-6'''), all characteristic of the dehydrohexahydroxydiphenyl (DHHDP) another ring of ellagic tannins [30].

Table 4. ^1H -NMR and ^{13}C -NMR spectra data of compound 4.

Position	$^{13}\text{C-NMR } \delta$ (ppm)	$^1\text{H-NMR } \delta_{\text{H}}$ (ppm)
Glucose		
1	90.9 CH	6.09 (d, 1.3)
2	71.1 CH	5.02 (dt, 2.9, 1.3)
3	62.2 CH	4.50 (dt, 2.9, 1.4)
4	69.8 CH	5.17 (m)
5	61.2 CH	4.62 (m)
6	64.7 CH ₂	5.13 (dd, 12.8, 1.4), 4.09 (dd, 12.8,

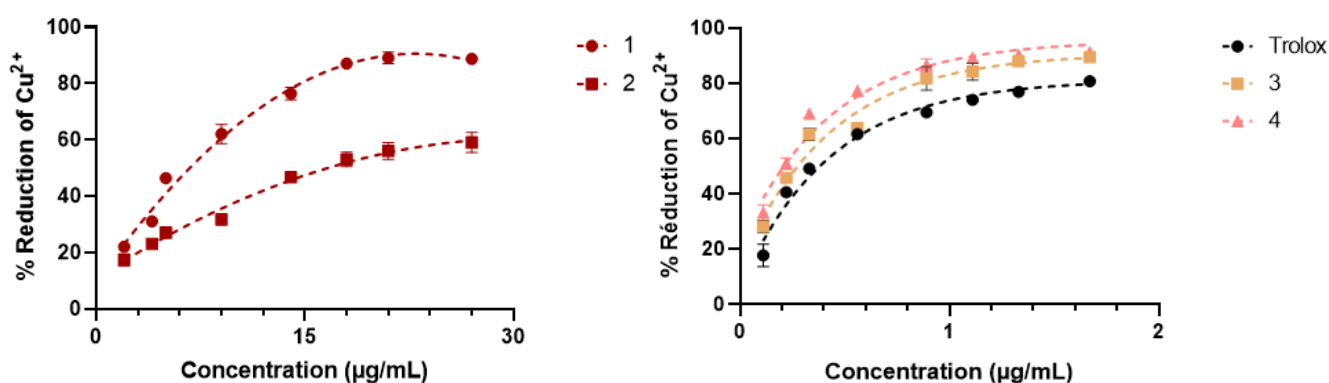
Position	$^{13}\text{C-NMR } \delta$ (ppm)	$^1\text{H-NMR } \delta_{\text{H}}$ (ppm)
Hexahydroxydiphenyl (HHDP)		
1'	116.3	C
2'	117.9	C
3'	110.5	CH 6.89 (s)
4'	138.3	C
5'	145.7	C
6'	145.3	C
7'	167.7	C
1''	116.3	C
2''	116.3	C
3''	108.7	CH 6.76 (s)
4''	137.2	C
5''	145.7	C
6''	145.3	C
7''	170.1	C
Dehydrohexahydroxydiphenyl (DHHDP)		
1'''	41.6	CH 5.55 (d, 0.5)
2'''	153.4	C
3'''	129.9	CH 6.57 (d, 0.5)
4'''	192.3	C
5'''	96.3	C
6'''	95.8	C
7'''	165.8	C
1''''	119.6	C
2''''	115.4	C
3''''	114.3	CH 7.28 (s)
4''''	139.5	C

Position	$^{13}\text{C-NMR } \delta$ (ppm)	$^1\text{H-NMR } \delta_{\text{H}}$ (ppm)
5'''	146.2	C
6'''	143.2	C
7'''	166.8	C

The HMBC spectrum data allowed ester linkages between glucose and the HHDP ring at carbons C-1 and C-6 and the DHHDP ring at carbons C-2 and C-4. It was demonstrated by correlations observed between proton at δ_{H} 6.09 (H-1) and carbon at 167.7 (C-7'), protons at δ_{H} 4.09/5.13 (H_a-6/H_b-6) and carbon at 170.1 (C-7'') and correlations between proton at δ_{H} 5.02 (H-2) and carbon at 165.8 (C-7''') and proton at δ_{H} 5.17 (H-4) and carbon at 166.8 (C-7''') (Figure 5). The anomeric proton at δ_{H} 6.09 (d, $J = 1.0$ Hz) assigned to H-1 indicated α -D configuration of glucose. The combination of all spectral data allowed the structural characterization of 4 as (1, 6)-HHDP-(2, 4)-DHHDP- α -D-glucose (Figure 5). Comparing these spectral data with the literature, this compound has been identified as an original isomeric compound of *Granatin A* isolated from *Punica granatum* (Lythraceae) [31]. Compound 4 has an α -D-glucose configuration, while *Granatin A* has a β -D-glucose configuration. They would be anomers. *Mareyanin* was proposed as the trivial name for compound 4 because it is derived from *Mareya micrantha*.

3.4. Antioxidant Activity of Isolated Compound

The antioxidant activities of compounds 1-4 were determined using DPPH and CUPRAC methods. The first method is based on DPPH radical reduction. The second method is based on the ion Cu^{2+} reduction. The results (Figure 6) showed that the isolated compounds exhibit antioxidant activities with both methods. These activities are dose dependent. The inhibition concentration to reduce 50% (IC_{50}) of DPPH radical or Cu^{2+} ion were determined to compare compound activities (Table 5). The best activity is determined by the lowest IC_{50} .



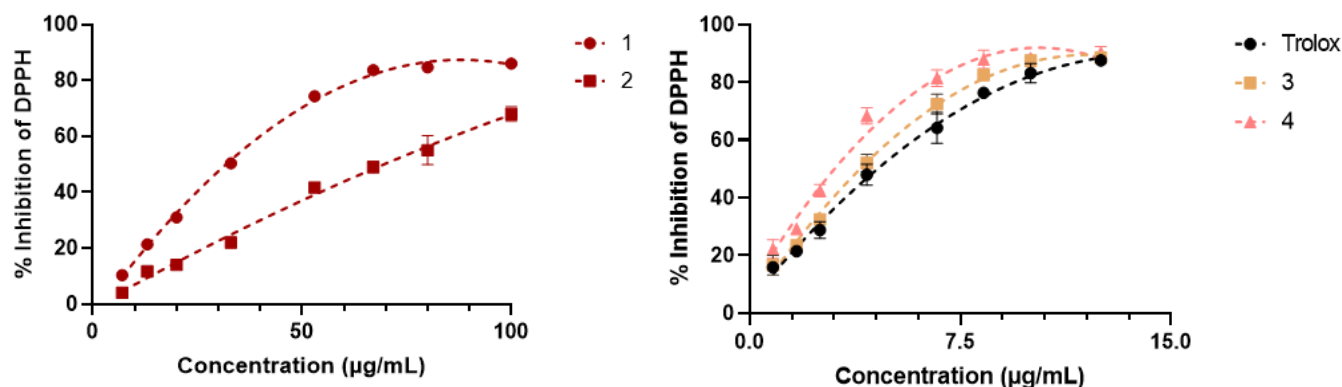


Figure 6. Cu^{2+} reduction and DPPH inhibition curves of isolated compounds.

Table 5. Antioxidant activity and quantification of isolated compounds.

Compounds	$\text{IC}_{50}/\text{Cu}^{2+}$ (µg/mL)	$\text{IC}_{50}/\text{DPPH}$ (µg/mL)	Extract FM_0 (mg/g of dry extract)	Fraction FM_2
1	6.74 ± 0.24^c	34.46 ± 0.50^c	< 5	6.01 ± 0.10
2	16.84 ± 0.67^d	68.88 ± 1.87^d	7.58 ± 0.03	23.93 ± 0.26
3	0.31 ± 0.01^b	4.24 ± 0.14^b	77.73 ± 0.33	81.86 ± 0.27
4	0.22 ± 0.01^a	3.26 ± 0.01^a	196.76 ± 2.67	> 200
Trolox	0.39 ± 0.01^b	4.86 ± 0.30^b	-	-

Results are presented as mean $\pm$ SD ($n = 3$) of three replicates, within each column, values carrying different superscript letters indicate statistically significant differences based on Dunnett's T3 multiple comparisons test ($p < 0.05$), Trolox was positive control use as reference antioxidant compound.

Compounds 3 and 4 exhibited in vitro antioxidant activity superior to that of Trolox (Vitamin E analog), which was used as a reference standard (Table 5). However, this superiority achieved statistical significance only for compound 4. According to both assays (DPPH and CUPRAC), the antioxidant potency followed the decreasing order of $4 > 3 > 1 > 2$. Isolated phenolic compounds 1-4 are more active than nor-cucurbitacins derivatives already isolated from *M. micrantha* previously [4].

The antioxidant activities of these phenolic compounds would be due to electrons delocalization observed on double bonds and conjugated such as aromatic rings [32], and on α , β -unsaturated carbonyl groups [33]. The activity is also due to protons of hydroxyl groups [34] and proton of acidic group [35]. These functional groups are observed in the isolated compounds.

Compound concentrations were determined in the crude extract FM_0 and the polyphenol enriched fraction FM_2 using HPLC-UV method. Among the isolated compounds, compound 4 exhibited the highest concentration in both the crude

extract and the ethyl acetate fraction. The compounds followed a decreasing order of concentration: $4 > 3 > 2 > 1$ (Table 5). This quantitative profile likely reflects their natural abundance in the plant, which may contribute significantly to its overall antioxidant activity. Notably, this is the first study to characterize the polyphenolic profile of *M. micrantha* and evaluate its antioxidant potential.

4. Conclusion

Analysis of the polyphenolic compounds in *Mareya micrantha* leaf extract demonstrated that the plant contains highly structurally diverse constituents. These compounds belong to five distinct classes: flavonoids, isocoumarins, hydrolyzable tannins, phenolic acids, and aromatic nor-cucurbitacins. This plant exhibits a high total polyphenol content. Fractionation and purification of these phytoconstituents led to the isolation of four major compounds: two known flavonoids, *Amentoflavone* and *Astragalin*, and two novel compounds, a hydrolyzable tannin named *Mareyanin* and an isocoumarin designated as *Micranthin acid*. Overall, the isolated polyphenols demonstrated antioxidant activity superior to that of the nor-cucurbitacins previously described in this species. The antioxidant capacity of *Mareya micrantha* is considered to be driven chiefly by phenolic compounds, with steroidal

components contributing to a lesser degree. *Mareya micrantha* undoubtedly deserves its reputation as a medicinal plant in West Africa. Further studies will help to identify additional natural compounds with unique chemical structures and antioxidant properties.

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Author Contributions

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Yapo Severin Katou: Formal Analysis, Investigation

Aka Faustin Kabran: Supervision, Validation, Visualization, Writing – review & editing

Barthelemy Koffi Attioua: Project administration, Supervision

Conflicts of Interest

The authors declare no conflicts of interest.

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