Available online at http://www.ifgdg.org

Int. J. Biol. Chem. Sci. 18(5): 2053-2065, October 2024

ISSN 1997-342X (Online), ISSN 1991-8631 (Print)

International Journal of Biological and Chemical Sciences

Original Paper http://ajol.info/index.php/ijbcs http://indexmedicus.afro.who.int

Evaluation of the antioxidant properties of the hydromethanolic extract and Triterpenes isolated from the roots of *Hallea ledermannii*

Kurouindé Viviane NEMLIN[1], Koffi Jean-Michel KOUAMÉ[2], Michel Boni BITCHI[4*], Zachée Louis Evariste AKISSI [3], Anderson Claver KIMOU[1], Philomène Akoua YAO-KOUASSI[4*] and Laurence Voutquenne-NAZABADIOKO[3]

[1]Laboratoire de Constitution et Réaction de la Matière, UFR Sciences des Structures de la Matière et de Technologie, Université Félix HOUPHOUËT-BOIGNY, 22 BP 582 Abidjan, Côte d'Ivoire.
[2]Laboratoire des Procédés Industriels de Synthèse, de l'Environnement et des Energies Nouvelles (LAPISEN), Institut National Polytechnique Félix HOUPHOUËT-BOIGNY de Yamoussoukro, BP 1093 Yamoussoukro
[3]UMR 7312, Institut de Chimie Moléculaire de Reims (ICMR), Chimie des Substances Naturelles, CNRS, Université de Reims Champagne-Ardenne, 51097 Reims, France [4]Université de San Pedro, BP V1800 San Pedro, Côte d'Ivoire.
[*] Corresponding author; E-mail: kouassiap@yahoo.fr; Tel: +2250757105617.

Received: 01-08-2024 Accepted: 25-10-2024 Published: 31-10-2024

ABSTRACT

Hallea ledermannii belongs to the Rubiaceae family. It is traditionally used to managed pathologies such as dysentery, fever, malaria, gonorrhea and other diseases. The present study aimed to report chemical composition and antioxidant properties of *Hallea ledermannii* root bark extracts. Antioxidant activity of the hydromethanolic extract was evaluated by the DPPH and ABTS methods. Phytochemical screening showed tannins, polyphenols, flavonoids, saponosides, leuco-anthocyanins, reducing compounds, polyterpenes and sterols. The total concentrations of polyphenols, flavonoids, tannins and anthocyanins contained in the extract were respectively estimated at 385.62 mg Eq acG/g, 287.715 mg Eq Q/g, 145.54 mg Eq acT/g and 0.42 mg Eq Cyanide glucoside/100g. Six known compounds: ursolic acid **(1)**, uncaric acid **(2)**, quinovic acid **(3),** 3-oxo-quinovic acid **(4),** quinovic acid 3-*O*-*β*-D-glucopyranoside **(5)**, quinovic acid-3-*O*-*β*-D-quinovopyranoside **(6)** were isolated from *Hallea ledermannii* roots extracts. The inhibition percentages of Trolox at a concentration of 10 mg/ml are 96.20% and 99.76% for DPPH and ABTS respectively.

Keywords: Secondary Metabolites, Triterpenes, Antioxidant, *Hallea ledermennii*.

INTRODUCTION

Known by the synonyms *Mitragyna* or *Fleroya*, *Hallea* is one of the most important genera of the Rubiaceae family. Plants of this genus are generally trees or shrubs with globose, opposite leaves (Raffa, 2015) and are traditional used to treat various ailments in Côte d'Ivoire. *Hallea ledermannii* is a plant species endemic to swamp forests. Its use extends to a lesser extent, in ethnomedicine, for the preparation of remedies against various pathologies (Mathouet et al., 2014; Djah et al., 2015). The aqueous decoction of the stem bark of *H. ledermannii* is reported been in the

DOI : https://dx.doi.org/10.4314/ijbcs.v18i5.33

treatment of infections, diarrhea, malaria, female infertility, painful menstruation, as a local anesthetic to lower blood pressure **(**Mba et al.,2017; Kouamé et al.,2022**)**. Antioxidant, anti-inflammatory, analgesic, antimicrobial activities studies of Hallea species have also been reported (Ahmad et al.,2022; Kaushik et al.,2009; Bidie et al., 2010). Chemically, various secondary metabolisms such as flavonoids, alkaloids, polyphenolic compounds, triterpenoids and triterpene-like saponosides have been isolated from the genus *Hallea* (Phongprueksapattana et al.,2008; Todd et al., 2020; Kouamé et al.,2022). In the continuous search for bioactive phytochemicals, the present work aimed at qualitatively and quantitatively screening the hydromethanolic root extracts of *H. ledermannii* and the antioxidant activities and characterization of isolated triterpenes.

MATERIALS AND METHODS

General experimental procedures

Extracts were first fractioned using vacuum liquid chromatography (VLC). Then, sub-fraction obtained were successively purified using both chromatography in open colonn and sephadex LH-20. Analytical TLC was performed on percolated silica-gel 60 F254 Merck and spots were observed under UV light at 254 and 365 nm or visualized by spraying the dried plates with sulfuric vanilin, followed by heating. Silica gel 60 (63-200 mesh, Merck) and RP-18 were used for column chromatography. NMR experiments were carried out in CD_3OD or DMSO-d_6 on Bruker Avance DRX III 500 instruments. HR-ESI-MS experiments were performed using a Micromass Q-TOF micro instrument.

Plant material

The roots of *Hallea ledermannii* were harvested at Adiopodoumé (long 8.484632, lat -3.135698) on 20 November 2019 and identified at the National Floristic Center (CNF) of yhe University FHB of Cocody (Abidjan) under the herbarium number UCJ 015307, in comparison with the herbarium specimen collected by AKE ASSI on 06/04/1966 in the Agnéby reserve (Côte d'Ivoire).

Quantitive Phytochemical Screening

The root extract of *H. ledermannii* was subjected to phytochemical screening in order to identify the presence of a number of chemical groups, including alkaloids, flavonoids, tannins, saponosides, polyphenols, leuco-anthocyanins, quinones, coumarins, cardiotonic heterosides, reducing compounds, polyterpenes and sterols. This was achieved through colorimetric tests described by Ali et al. (2014), Shaikh and Patil (2020).

Determination of total polyphenol content

The total polyphenols contents of crude hydromethanolic extract of the roots of *Hallea ledermannii* was estimated using the Folin-Ciocalteu Reagent. It was carried out using the slightly modified method of Wood et al. (2002). The diluted sample (0.50 mL) was added to a 1:10 diluted Folin-Ciocalteu reagent (2.5 mL). The solution obtained was incubated away from light for 15 min at 50°C and this absorbance was read at 765 nm after incubation. A calibration curve was established in parallel under the same operating conditions using gallic acid as a standard. Concentration of total polyphenol content in each sample was expressed as milligram Gallic acid equivalent in hundred grams of samples (mg GAE/100 g) on dry weight basis.

Determination of total flavonoid content

The determination of total flavonoid content was determined using the method describes by Marinova et al. (2005) where 0.75 mL of sample and 5% (m/v) of sodium nitrite solution were mixed. And then, 0.75 mL of 10% (m/v) aluminum chloride solution was

added to the mixture. The resulting mixture was added to 5 mL of a 1 M sodium hydroxide solution, adjusted to 25 mL, then shaken vigorously. Absorbance was measured at a wavelength of 510 nm against a blank consisting of 0.75 mL of 5% (m/v) sodium nitrate solution, 0.75 mL of chloride solution of 10% aluminum (m/v) and 5 mL of 1 M sodium hydroxide solution. Quercetin was used as a reference standard for the establishment of the calibration curve and for the quantification of the total flavonoid contents expressed in milligram of quercetin equivalent per gram of extract (mg Eq Q/g of extract). Triplicate measurement was done for the same extract.

Determination of total tannin content

Estimation of total tannin content was determined according to the vanillin method described by Julkunen-Titto **(**1985). About 50 µl of each extract was added to 1500 µl of 4% vanillin/methanol solution, then mixed vigorously. Then, 750 µl of concentrated hydrochloric acid (HCl) were added. The mixture obtained was left to react at room temperature for 20 min. Absorbance was measured at 550 nm against a blank. Different concentrations between 0 and 1 mg/ml prepared from a stock solution of catechin allowed the calibration curve to be drawn and results were expressed in mg Eq ac T/g.

Determination of total anthocyanin content

Estimation of total anthocyanin content was determined using the spectrophotometric differential pH method, by Giusti et al. (2001). It was based on determination of the absorbance of extractive solutions diluted with buffer solutions of pH = 1 and pH = 4.5. The colored form (oxonium) predominates at pH 1 and the colorless form (hemiacetal) at pH 4.5. Total anthocyanin content was measured in triplicate for the same extract and expressed in mg Eq cyanide glucoside/100 g.

Antioxidant activity

Antioxidant activity of extract was evaluated using the DPPH free radical scavenging method and free-radical-scavenging ABTS radical cation decolorization assay. For each concentration of the extract tested, the absorbance (Abs) was read 3 times (Thaipong et al., 2006). The results were expressed as percentage of inhibition (% Inhibition) as follows:

$$\%\ \text{Inhibition} = \left(1 - \frac{\text{Abs}_{\text{test}}}{\text{Abs}_{\text{control}}}\right) \times 100$$

Where **($Abs_{control}$)** is the absorbance control reaction and (**Abs_{test})** is the absorbance of the tested extract solution.

Extraction and Isolation

A total of 800 g of coarsely powdered *Hallea ledermannii* root was macerated with 6000 ml of methanol/water (80/20) for 24 hours. The crude hydromethanolic (124.4 g) was solubilized in 200 ml of distilled water and successively fractioned with dichloromethane, ethyl acetate, leading to the different sub-extracts: dichloromethane (10 g), ethyl acetate (8 g), and aqueous extract (32.73 g).

Ethyl acetate extract (8 g) was extracted by open column chromatography in normal phase using DCM / AcOEt / MeOH (9/ 1/0) to (0/0/1) (v /v /v) to give seven fractions (F1-F7).

Fraction F3 show colored precipitate and led to **compound 3** (43.6 mg) after successively washing, with hexane and DCM.

Fraction F4 was separated by open column chromatography, eluted with Hex/AcOEt (80/20 to 70/30; v/v). Twelve (12) sub-fractions (F4a to F4l) were obtained. Fraction F4h (38.9 mg) was subjected to preparative TLC with DCM/AcOEt system (9/1; v/v) to afford **compound 2** (12.1 mg).

Fractions (F6+F7) was fractionated on open column in normal phase following the gradient DCM/AcOEt/MeOH (90/10/0 to 0/90/10). Six (6) sub-fractions (F6a to F6f)

were obtained. Fraction F6d (40.15 mg) was purified on Sephadex LH-20 eluted with DCM/MeOH (2/1; v/v) to yield **compound 6** (9.6 mg).

The aqueous extract was separated into three (3) sub-fractions Aq1 (11.0247 g), Aq2 (9.2495 g) and Aq3 (0.6119 g) using VLC on RP-18 eluted with MeOH/H_2O (20/80 to 100 /0 ; v/v).

Aq3 (0.6119 g) was subjected to open column chromatography with normal silica gel eluted by the system Hexane/dichloromethane/AcOEt/methanol (30/70/0/0 to 0/0/90/10; v/v/v/v). This led ten fractions (A1 to A10) and the fraction A8 to yield **compound 1** (0.2 mg).

Aq2, on its part was subjected to open column in normal phase eluted with Dichloromethane/AcOEt (100/0 to 0/100; v/v). Seven (7) fractions B1 to B7 were obtained. Fraction B3 was purified on Sephadex LH-20 eluted with DCM/MeOH (2/1) to yield **compound 4** (1.5 mg).

Aq1 (11.0237 g) was separated on open column in normal phase eluted with Dichloromethane/AcOEt/MeOH (100/0/0 to 0/20/80; v/v/v), from which six fractions (C1 to C6) were obtained. Fraction C6 was subjected to successive purifications with hexane, DCM and ethyl acetate to afford **compound 5** (4.6 mg).

Acid hydrolysis of saponin mixture

The crude saponin mixture (100 mg) was refluxed with 300 ml of 2 N TFA for 3 h. The sapogenin was extracted with EtOAc (300 ml), and evaporated to dryness. The aqueous layer was freeze-dried. Seven sugars were identified with authentic samples by TLC as D-(glucose, fucose, quinovose, 6-deoxy-allofuranose, 6-deoxy-allopyranose and xylose) and L-rhamnose.

RESULTS

Phytochemical Screening

The main groups of phytochemicals found in the extract hydromethanolic of the bark of *H. ledermannii* as are tanins, polyphenols, saponins and reducing (Table 1).

Total phenolics, Flavonoides, Tannins and Anthocyanin contents

The total phenolic content of the hydromethanolic extract of *Hallea ledermannii* was determined using the Folin-Ciocalteau method. This extract contains a significant quantity of polyphenols, flavonoid and tannin with a content of 385.62 aG eq/g, 287.71 mg E Q /g, 145.54 mg ac T eq/g respectively (Table 2).

Structural elucidation

The NMR analysis spectra of isolated compounds (Table 4 and Table 5), as well as the acid hydrolysis allowed to attribute each sugar and their attachment to the identified genin. The known compounds (**1-6**) were identified as: Ursolic acid **(1)**; uncaric acid (**2)**; quinovic acid **(3)**; acid-3-oxo-quinovic **(4)**; quinovic acid 3-*O*-*β*-D-glucopyranoside **(5)** quinovic acid-3-*O*-*β*-D-quinovopyranoside **(6).** The structures of these compounds are shown in Figure 1**.**

Antioxidant Activity

The DPPH and ABTS radical scavenging activity, expressed as a percentage of inhibition of the extract, was evaluated and compared with the reference (Trolox). In addition, the different IC50 values (mg/L) obtained by the ABTS and DPPH methods for the extract and for Trolox were determined. As shown in Table 3, the inhibition percentages of hydromethanolic extract of *H. ledermannii* root at a concentration of 100 mg/ml are 80.89% and 95.99% for DPPH and ABTS respectively.

Table 1: Phytochemical charactization of *Hallea ledermannii*.

Species	Plant organ	Extract type	Family of compounds	Results
Hallea ledermannii	Root bark	Hydromethanolic extract	Tannins	++
			Polyphenols	+++
			flavonoids	++
			Quinones	-
			Coumarins	-
			Alkaloids	-
			Saponins	++
			Cardiotonic heterosides	-
			Leucoanthocyanins	+
			polyterpenes and sterols	+
			Reducing compounds	++

(+++): Test strongly positive; (++): Positive test; (+): Weak positive test; (-): Negative test

Table 2: Total phenolics, flavonoids, tannins and anthocyanin contents of *H. ledermannii* extract.

SECONDARY METABOLITES CONTENTS			
Total polyphenols (mg EqAcG/g extract)	**Total flavonoids (mg Eq Q/g extract)**	**Total tanins (mg Eq Ac T/g)**	**Total anthocyanes (mg Eq Cyan.gluc. / 100g)**
385.62	287.71	145.54	0.42

Table 3: Antioxidant characterization of *Hallea ledermannii.*

ANTIOXIDANT ACTIVITY						
Tests	**DPPH**			**ABTS**		
Samples	**Conc. (mg/ml)**	**%Inh.**	**IC_{50} (mg/ml)**	**Conc. (mg/ml)**	**%Inh.**	**IC_{50} (mg/mL)**
Hydromethanolic Extract	100	80.89	4.04	100	95.99	3.11
	50	79.83		50	94.10	
	25	75.61		25	93.51	
	12.5	63.76		12.5	92.57	
	6.25	49.81		6.25	33.05	
	3.12	39.04		3.12	90.60	

	1.56	31.31		1.56	78.77
	0.78	15.617		0.78	28.38
	10	96.20		10	99.76
	92.11	99.05		92.11	99.05
	89.84	98.23		89.84	98.23
	86.27	96.46		86.27	96.46
Trolox			2.40		2.95
	80.89	95.04		80.89	95.04
	76.34	93.75		76.34	93.75
	50.66	87.85		50.66	87.85
	5.23	49.88		5.23	49.88

Conc. : concentration ; **%IC :** inhibition percentages**; IC_{50} :** inhibition concentration

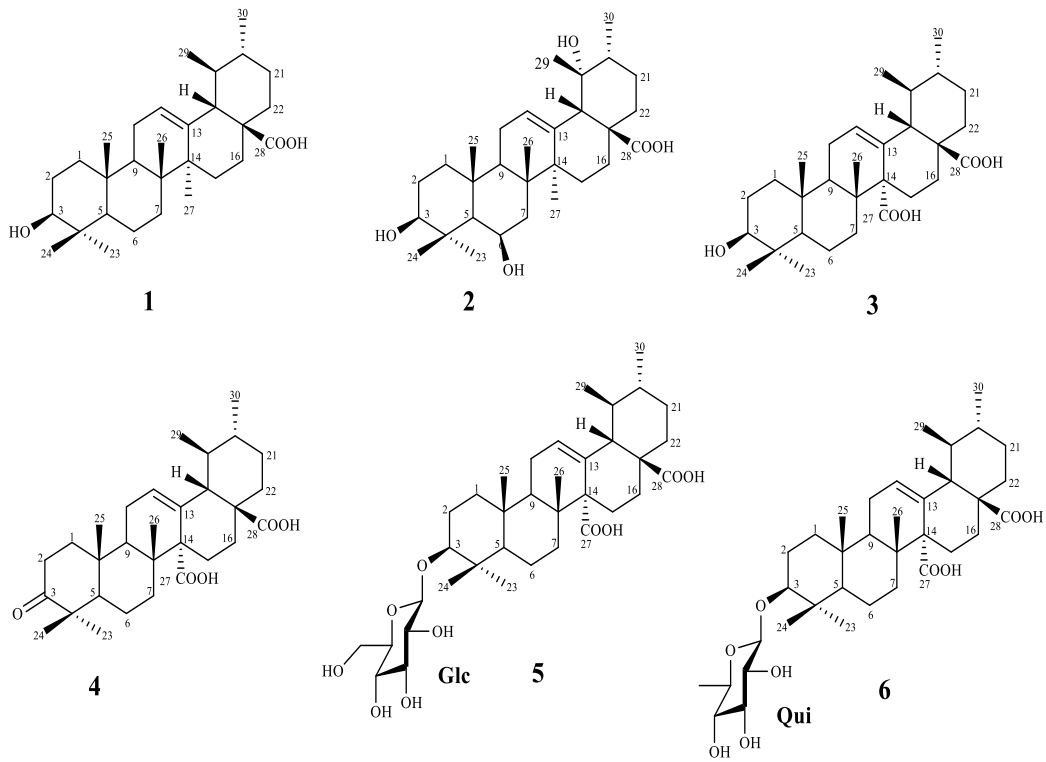

Figure 1: Isolated triterpenes from the roots of *H. ledermannii*.

Table 4: ^{1}H and ^{13}C NMR spectroscopic data of the aglycone moieties of compounds 1- 4 (in CD_3OD).

	1		2		3		4	
N°	**δ_H (J in Hz)**	**δ_C**	**δ_H (J in Hz)**	**δ_C**	**δ_H (J in Hz)**	**δ_C**	**δ_H (J in Hz)**	**δ_C**
1	1.52 m ; 0.92 m	38.7	1.44 m ;	20.8	1.57 m ;	38.9	1.84 m ; 1.36	39.3
2	1.45 m ; -	24.5	0.87 m	29.5	1.36 m	34.1	m	34.1
3	3.00 m	77.3	1.24 m ; -	77.9	2.47 m ;	77.4	2.47 m ; 2.37	216.8
4	-	38.8	2.92 dd	39.7	2.37 m	38.8	m	46.8
5	0.68 m	55.3	(10.5 ; 5.1)	55.8	2.97 dd	55.4	-	54.7
6	1.48 m ; 1.29 m	18.5	–	67.8	(10.6 ; 5.0)	18.5	-	19.2
7	1.45 m ; 1.26 m	33.2	0.62 m	41.1	-	36.7	1.23 m	35.9
8	-	39.8	4.33 m ;	38.9	1.22 m	39.3	1.43 m ; 1,34	39.2
9	1.45 m	47.5	1.56 m ;	47.8	1.44 m ;	46.7	m	45.6
10	-	37.0	1.42 m	36.6	1,24 m	37.0	1.59 m ; 1.15	36.6
11	1.8 m	23.3	-	27.7	1.56 m ;	22.9	m	23.1
12	5.13 t (3.43)	125.0	1.56 m	126.4	1.11 m	128.4	-	128.0
13	-	138.7	-	139.8	-	133.0	2.27 m	133.3
14	-	42.2	1.52 m ;	42.1	2.13 m	55.7	-	55.8
15	1.83 m ; 1.01 m	28.1	1.42 m	28.8	-	24.4	1.92 m ; 1.84	24.5
16	1.92 m ; 1.53 m	24.3	5.09 t	26.4	1.89 m ;	25.3	m	25.5
			(3.41)		1.78 m		5.5 m	
17	-	47.3	-	47.5	5.4 dd (5.0 ;	47.8	-	47.8
18	2.11 d (11.42)	52.9	-	54.5	2.4)	54.0	-	54.2
19	1.30 m	38.9	1.90 m ;	72.5	-	39.4	1.91 m ; 1.56	38.9
20	0.92 m	39.0	0.75 m	42.2	-	36.9	m	36.9
21	1.44 m ; 1.28 m	30.7	2.27 ddd	27.0	1.88 m ;	30.2	1.95 m ; 1.60	30.3
22	1.55 m	36.8	(13.9, 13.9,	38.3	1.54 m	36.3	m	36.4
23	0.95 s	28.7	4.3) ;1.39m	28.4	1.94 m ;1.60	28.8		27.2
24	0.68 s	16.5	-	17.8	m	16.6	-	21.5
25	0.87 s	15.7	2.50 s	16.9		16.1	2.16 d (10.1)	16.1
26	0.76 s	17.4	-	18.3	-	18.6	0.92 m	18.5
27	1.04 s	23.8	1.23 m	24.5	2.15 d	176.5	0.91 m	176.4
28	-	178.8	1.07 m	178.8	(10.2)	178.7	1.40 m ;1.16 m	178.8
29	0.81 d (6.52)	17.5	1.50 m	27.3	0.91 m	17.9	1.52 m	18.1
30	0.91d (10.38)	21.6	0.95 s	17.0	0.90 m	21.5	0.98 s	22.0
			1.06 s		1.40 m ;		0.94 s	
			1.22 s		1.15 m		0.95 s	
			0.99 s		1.52 m		0.82 s	
			1.23 s		0.87 s		-	
			-		0.79 s		-	
			1.06 s		0.95 s		0.83 d (5.42)	
			0.83 d		0.86 s		0.88 d (5.46)	
			(6.74)		-			
					-			
					0.81 d			
					(5.51)			
					0.86 d			
					(5.48)			

Table 5: ^{1}H and ^{13}C NMR spectroscopic data of compounds 5 and 6 (in CD_3OD).

	5		6	
N°	**δ_H (J en Hz)**	**δ_C**	**δ_H (J en Hz)**	**δ_C**
1	1.03 ; 1.68	39.9	0.96 m ; 1.66 m	39.9
2	1.70 m ; 1.93 m	27.1	1.68 m ; 1.87 m	27.0
3	3.10 dd (11.9; 4.8)	90.7	3.09 dd (11.6;4.6)	90.8
4	-	40.1	-	40.1
5	0.74 d (11.5)	56.9	0.71 d (12,4)	57.2
6	1.35 td (12.9; 4.1)	19.3	1.34 m	19.2
	1.50 dt (12.9; 3.5)		1.51 m	
7	1.21 td (12.8; 4.0)	38.0	1.21 m	38.7
	1.67 m		1.65 m	
8	-	40.6	-	40.7
9	2.24 dd (11.5; 4.0)	48.0	2.06 t (11.8)	48.3
10	-	37.8	-	37.9
11	1.90 m ; 1.98 m	23.8	1.90 m ; 1.95 m	24.0
12	5.61 dd (5.1; 2.5)	130.4	5.65 t (3.7)	127.9
13	-	133.9	-	137.3
14	-	57.3	-	57.1
15	1.68 m	26.5	1.65 m	25.1
	2.06 td (12.7; 3.5)		2.04 m	
16	1.73 td (14.2; 4.1); 2.08	25.7	1.73 m; 2.06 m	25.6
17	-	48.6	-	48.6
18	2.24 d (10.5)	55.5	2.91 dd (13.7; 3.5)	44.6
19	0.99 m	40.4	1.12 dt (11.0; 3.4)	44.6
20	1.01 m	38.3	1.01 m	38.3
21	1.29 m ; 1.51 m	31.2	1.19 m ; 1.32 m	34.7
22	1.69 m	37.6	1.55 td (12.1; 3.5) ; 1.75 m	32.7
23	1.01 s	28.5	1.01 s	28.5
24	0.83 s	17.1	0.82 s	17.0
25	0.97 s	16.9	0.97 s	16.9
26	0.89 s	19.1	0.88 s	17.4
27	-	179.0	-	179.8
28	-	181.6	-	177.9
29	0.92 d (5.6)	18.1	0.87 d (5.6)	33.6
30	0.91 d (5.7)	21.5	0.92 d (5.5)	24.0
	glc-en-3		**qui-in 3**	
1'	4.31 d (7.80)	106.7	4.26 d (7.82)	106.7
2'	3.18 t (8.0)	75.7	3.17 dd (9.3 ;7.7)	75.5
3'	3.33 t (9.2)	78.3	3.27 t (9.3)	77.9
4'	3.28 t (9.2)	71.6	2.97 t (9.3)	77.0
5'	3.25 ddd (8.5; 5.4; 2.4)	77.7	3.33 m	72.9
6'	3.66 dd (12.2; 5.4)	62.6	1.26 d (6.2)	18.2
	3,84 dd (12.2; 2.4)			

DISCUSSION

The phytochemical screening revealed the presence of a number of chemical compounds, including flavonoids, saponins, tannins, polyphenols, leucoanthocyanins, reducing compounds, polyterpenes and sterols. However, no alkaloids, coumarins, cardiotonic heterosides or quinones have been identified. In the literature, the root bark of this species is reported to contain alkaloids (Ogbunugafor et al., 2008). The divergence in the chemical composition of this plant can be attributed to a multiplicity of factors likely to influence it. Several studies have shown that biotic and abiotic factors, genetic predisposition, the degree of ripening of the plants, the length of storage and the extraction solvent have a significant influence on the content of secondary metabolites (Ngbolua, 2011a, 2011b).

Polyphenols are among the main plant constituents with antioxidant activity. This is confirmed by the phytochemical screening which revealed the presence of polyphenol such as flavonoids, tannins, and other secondary such as metabolites saponosides, triterpenes and sterols with high antioxidant activity (Dieng et al., 2017). After analysis of the assay results obtained in Table 2, it was estimated that the hydromethanolic extract contains a significant quantity of polyphenols with a content of 385.62 aG eq/g. The flavonoid content recorded in this extract (hydromethanolic extract) is 287.71 mg E Q /g. The tannin content was moderately low at 145.54 mg ac T eq/g. Finally, the anthocyanin content is very low at 0.422 mg cyanide glucoside/100 g. This content is found in very low quantities in the hydromethanolic extract. These results obtained could be linked to the nature of the extraction solvent. In general, hydroalcoholic extracts are the richest in secondary metabolites, particularly polyphenols, flavonoids and tannins (Kouamé et al., 2021).

The antioxydant activity of the extract was determined, as show in Table 3, we can see that the percentage of inhibition increases as the concentration used increases. Trolox used as a standard presented the highest inhibition percentages. The inhibition percentages of Trolox at a concentration of 10 mg/ml are 96.20% and 99.76% for DPPH and ABTS respectively. On the other hand, the inhibition percentage of hydromethanolic extract of *H. ledermannii* root at a concentration of 100 mg/ml are 80.89% and 95.99% for DPPH and ABTS respectively. The present results corroborate those reported by Ogbunugafor et al. (2008), on the percentage inhibition of DPPH by the hydroethanolic extract of *H. ledermannii* root bark. Comparison of these results shows that this extract has antioxidant power. It therefore exerts anti-radical activity. According to IC_{50}, the hydromethanolic extract (IC_{50} = 4.04 mg/ml DPPH and IC_{50} = 3.11 mg/ml ABTS) of the root extract of *H. ledermannii* is less active than Trolox (IC_{50} = 2.40 mg/ml DPPH and IC_{50} = 2.95 mg/ml ABTS). The results corroborate those reported by Bidié et al. (2005), who confirmed the antioxidant activity of *H. ledermannii* with an estimated IC_{50} of 10.5 ± 0.288 µg/ml. They concluded that the antioxidant activity of this plant is related to the presence of flavonoids and polyphenols, which are generally very good antioxidants. This result could be justified by the high content of polyphenols (385.62 Eq aG/g phenolic compounds), contained in the hydromethanolic extract.

In addition, six compounds were isolated and identified by monodimensional and bidimensional NMR, mass spectroscopy was also used. Compounds **1** and **2** were isolated as white amorphous powders. The ^{1}H-NMR spectrum of compound **1** showed seven methyl signals of 3H intensity each, including five forming singlets at 0.95 (H-23), 0.68 (H-24), 0.87 (H-25), 0.76 (H-26), 1.04 ppm (H-27) and two forming doublets at 0.81ppm (d, J = 6. 52Hz) (H-29) and 0.91ppm (d, J = 10.38Hz)

(H-30), nine methylene groups, a triplet at 5.13 ppm indicating an olefinic proton (H-12), a multiplet at 3.00 ppm attributable to the proton H-3 and a doublet at 2.11 ppm attributable to the proton H-18 (Table 4). These data together with characteristic peaks in the ^{13}C-NMR spectrum at δ_C 125.0 (C-12), 138.7 (C-13), 52.9 (C-18), 23.8 (C-27), 178.8 ppm (C-28) **(**Table 3), indicated that the compound **1** was pentacyclic triterpene acids. The presence of one carboxyl group indicated that compound **1** was ursolic acid (Mahmoud et al., 2019).

In compound **2**, H-18 appeared as singlet at 2.50 ppm and correlated in HMBC with carbon (C-19) at 72.5 ppm. H-5 (0.62 ppm) correlated in COSY with H-6 (4.33 ppm) (Table 4). The deshielded chemical shifts of carbons 6 and 19 at 67.8 and 72.5 ppm respectively, showed that these carbons were hydroxylated. Compound **2** was identified as uncaric acid (Sun et al., 2012).

Compounds **3, 4, 5** and **6** were isolated as white amorphous powders. The ^{1}H-NMR spectrum of compound **3** showed four 3H singlets and two 3H doublets of methyl groups, nine methylene groups, a doublet of doublets at 5.5 ppm indicating an olefinic proton (H-12), a doublet of doublets ranging from 2.97 ppm attributable to the H-3 proton (Table 4). These data together with characteristic peaks in the ^{13}C-NMR spectrum at δ_C 128.4 (C-12), 133.0 (C-13), 176.5 (C-27), 178.7 ppm (C-28), indicated that the compound **3** was pentacyclic triterpene acids (Jian et al., 2005). The presence of two carboxyl groups unambiguously indicated a quinovic acid (Njoya et al., 2023).

Compound **5** was assigned a molecular formula of $C_{36}H_{56}O_{10}$, as determined from their HRESIMS m/z: at m/z 603.3893 [M-H] ¯. The ^{1}H NMR and ^{13}C spectra of compounds **5** showed an anomeric proton at 4.31 ppm (d, J = 7.80 Hz) (Table 5). The analysis of their HSQC, and HMBC spectra and coupling constants led to identify 6-*β*-D-glucopyranose. On the HMBC spectrum, H-1' at 4.31 ppm correlated with C-3. Compound **5** was therefore assigned to quinovic acid 3-*O*-*β*-glucopyranoside (Kouamé et al., 2022).

The ^{1}H NMR and ^{13}C spectra of compound **4** are superimposable to that of compound **5**, unlike the presence of the ketone function in position 3 (δ_C 216.8 ppm) (Table 4). HMBC spectrum showed correlation between H-2, H-23 and C-3. Compound **4** was identified as acid-3-oxo-quinovic (Tao et al., 2012).

Compound **6** was assigned a molecular formula of $C_{36}H_{56}O_9$, as determined from their HRESIMS m/z: at m/z 587.3947 [M-H] ¯. The ^{1}H NMR and ^{13}C spectra of compounds **6** showed an anomeric proton at 4.26 ppm (J = 7.82 Hz) (Table 4). The analysis of their HSQC, and HMBC spectra and coupling constants led to identify 6-deoxy-*β*-D-glucopyranose (quinovose). HMBC spectrum showed a cross peak between the carbon at 90.8 ppm (C-3) (Table 5) and the anomeric proton at 4.26 ppm **(**Enas et al., 2023). Compound **6** was identified as quinovic acid-3-O-*β*-D-quinovopyranoside.

Conclusion

The chemical study that we conducted on *Hallea ledermannii* highlighted the nature and content of the chemical components contained in the root. In qualitative terms, several chemical groups were identified: tannins, polyphenols, flavonoids, saponosides, leuco-anthocyanins, reducing compounds, polyterpenes and sterols. In quantitative terms, high levels of total polyphenols, followed by total flavonoids and total tannins were revealed. The anthocyanins were found to be present in low concentration the extract. The different compounds elucidated were ursolic acid **(1)**, uncaric acide **(2)**, quinovic acid **(3)**, acid-3-oxo-quinovic **(4)**, quinovic acid 3-O-β-D-glucopyranoside **(5)** and quinovic acid-3-O-β-D-quinovopyranoside **(6)**. Evaluation of the antioxidant activity by method DPPH and ABTS showed a high scavenging potential of extract. These results could be correlated with

the high polyphenol and flavonoid contents found in the hydromethanolic extract. In future, study of other fractions with different chromatographic methods will be investigated to identify the exact nature of these polyphenols and flavonoids.

COMPETING INTERESTS

The authors declare that they have no competing interest.

AUTHORS' CONTRIBUTIONS

Design: KVN, KJMK, and MBB; Investigation, KVN, KJMK, ZLEA; Data analysis, PAYN, LVN, KJMK, KVN; Writing of the original version of the article: MBB, KVN, KJMK.

ACKNOWLEDGEMENTS

The authors are grateful to the technicians and engineers of "Chimie des Substances Naturelles" group at ICMRUMR7312 CNRS for their precious help, and University of Reims Champagne-Ardenne, and the members of the Laboratory of Constitution and Reaction of Matter of the University Felix Houphouet Boigny, Abidjan, Cocody (Côte d'Ivoire).

REFERENCES

Ahmad I, Prabowo WC, Arifuddin M, Fadraersada J, Indriyanti N, Herman H, Purwoko RY, Nainu F, Rahmadi A, Paramita S. 2022. Plants of the *Mitragyna* Species as Pharmacological Agents: From Abuse to Promising Pharmaceutical Products. *Life*, **12**: 193. DOI: http://dx.doi.org/10.3390/life12020193

Ali NS, Khan MR, Sattar S, Ahmad B, Mirza B. 2014. HPLC-DAD Analysis, Antioxidant Potential and anti-urease activity of *Asparagus gracilis* collected from District Islamabad. *BMC Complement. Altern. Med*., **14**(1): 1–12. DOI: http://www.biomedcentral.com/1472-6882/14/347

Bidie ADP, Koffi E, Yapi FH, Yémié AA, Djaman JA, Guede-guina F. 2010. Evaluation of the Toxicity of a Methanolic Total Extract of *Mitragyna ciliata* a Natural Anti-malaric *Int. J. Biol. Chem. Sci.,* **4**(5): 1509-1518. DOI: 10.4314/ijbcs.v4i5.65558

Bidie AP, N'guessan BB, Yapo AF, N'guessan JD, Djaman AJ. 2011. Activités Antioxydantes de Dix Plantes Médicinales de la Pharmacopée Ivoirienne. *Sci. Nat.* **8**(1): 1 – 11. URL : https://www.ajol.info/index.php/scinat/article/view/92838/82262

Dieng SIM, Fall A D, Diatta-Badji K, Sarr A, Sene M, Sene M, Mbaye A, Diatta W,

Bassene E. 2017. Evaluation de l'activité antioxydante des Extraits Hydro-ethanoliques des Feuilles et Ecorces de *Piliostigma thonningii* Schumach. *Int. J. Biol. Chem. Sci.,* **11**(2): 768-776. DOI : https://dx.doi.org/10.4314/ijbcs.v11i2.19

Djah FM, Danho FRN, Kouakou LK. 2015. Medicinal Plants and Traditional Healing Practices in Ehotile people, around the aby lagoon (eastern littoral of Côte d'Ivoire). *J. Ethnobiol. Ethnomed.*, **11**: 21. DOI : 10.1186/s13002-015-0004-8

Enas EE, Mohamed NS, Dina HM, Abdel-Kader SM, Abu-Elsaoud MA, Safwat AA, Amany IK, Jihan BM, Abdelhameed RFA. 2023. A New Saponin (Zygo-albuside D) from *Zygophyllum album* Roots Triggers Apoptosis in Non-Small Cell Lung Carcinoma (A549 Cells through CDK-2 Inhibition. *ACS Omega*, **8**: 30630-30639. DOI: https://doi.org/10.1021/acsomega.3c04314

Giusti MM, Wrolstad RE. 2001. Characterization and Measurement of Anthocyanins by UV-Visible Spectroscopy. *Curr. Prot. Food Anal. Chem.*, **0**(1): F1-2 DOI: https://doi.org/10.1002/0471142913.faf0102s00

Jian Z, Zhi-Hong C, Bo-Yang Y, Geoffrey AC, Qiu S. 2005. Novel Biotransformation of Pentacyclic Triterpenoid Acids by *Nocardia* sp. NRRL 5646. *Tetrahedron Lett.*, **46**: 2337–2340. DOI: https://doi.org/10.1016/j.tetlet.2005.01.155

Julkunen-Titto R. 1985. Phenolic constituents in the leaves of northern willows: Methods for the analysis of certain phenolics. *J. Agri. Food Chem.*, **33** (2): 213-217. DOI: https://doi.org/10.1021/jf00062a013

Kouamé Koffi JM, Yao-Kouassi PA, Magid A A, Akissi ZLE, Martinez A, Sayagh C, Nazabadioko LV. 2022. New Triterpenoid Saponins from the stem bark of *Hallea ledermannii*. *Tetrahedron Lett.*, 116: 154335. DOI: https://doi.org/10.1016/j.tetlet.2022

Kouamé TK, Siaka S, Kassi ABB, Soro Y. 2021. Détermination des Teneurs en Polyphénols Totaux, Flavonoïdes Totaux et Tanins de Jeunes Feuilles non Encore ouvertes de *Piliostigma thonningii* (Caesalpiniaceae). *Int. J. Biol. Chem. Sci.*, **15**(1): 97-105. DOI: https://dx.doi.org/10.4314/ijbcs.v15i1.9

Mba AR, Bikanga R, Ossibi Etou AW, Nkounkou LC and Ouamba JM. 2017. Phytochemical Screening, Acute Toxicity and Cardiovascular Effects of water extract of *Hallea ledermannii* (K. Krause) verdc (Rubiaceae), Medicinal Plant from Gabon. *Int. J. Adv. Res.*, **5**(3): 1055-1063. DOI: http://dx.doi.org/10.21474/IJAR01/3605

Mahmoud BK, Hamed AN, Samy MN, Mostafa EM, Wanas AS, Radwan MM, Elsohly MA, Kamel MS. 2019. Phytochemical Composition and Antimicrobial Properties of *Markhamia platycalyx* (Baker) Sprague leaf. *Trop. J. Pharm. Res.*, **18**: 2623-2631. DOI: http://dx.doi.org/10.4314/tjpr.v18i12.23

Marinova D, Ribarova F, Atanassova M. 2005. Total Phenolics and Total Flavonoids in bulgarian Fruits and Vegetables. *J. Univ. Chem. Technol. and Metallurgy.*, **40**: 255-260. URL : https://www.academia.edu/download/51329716/

Mathouet H, Aboughe AS, Mengome L, Eyele MMC, Rondi ML, Souza A, Lami DM. 2014. Etude Ethnobotanique des Plantes Utilisées en Médecine Traditionnelle pour des Affections Respiratoires au Gabon. *Science Lib Editions Mersenne*, **6**: 140905.

Ngbolua KN, Rakotoarimanana H, Rafatro H, Urverg RS, Mudogo V, Mpiana PT, Tshibangu DST. 2011a. Comparative Antimalarial and Cytotoxic Activities of two *Vernonia* species: *V. amygdalina* from the Democratic Republic of Congo and *V. cinerea* subsp vialis Endemic to Madagascar. *Int. J. Biol. Chem. Sci.*, **5**: 345-353. DOI: http://dx.doi.org/10.4314/ijbcs.v5i1.68111

Ngbolua KN, Rafatro H, Rakotoarimanana H, Urverg RS, Mudogo V, Mpiana PT, Tshibangu DST. 2011b. Pharmacological Screening of Some Traditionally-Used Antimalarial Plants from the Democratic Republic of Congo compared to its ecological taxonomic equivalence in Madagascar. *Int. J. Bio. Chem. Sci.*, **5**: 1797-1804. DOI: http://dx.doi.org/10.4314/ijbcs.v5i5.3

Njoya EM, Ndemangou B, Akinyelu J, Munvera, Chukwuma CI, Mkounga P, Mashele SS, Makhafola TJ, McGaw LJ. 2023. *In vitro* Antiproliferative, Antiinflammatory Effects and Molecular Docking Studies of Natural Compounds Isolated from *Sarcocephalus pobeguinii* (Hua ex Pobég). *Front Pharmacol.*, **14** : 1205414. DOI: https://doi.org/10.3389/fphar.2023.1205414

Ogbunugafor HA, Okpuzor VI, Odesanmi OS, Ebigwe FJ. 2008. The Butanol Extract of *Mitragyna ciliata* root: Potential as a Trypanocide. *Int. J. Pharmacol.*, **4**(2): 102-107. DOI: https://doi.org/10.3923/ijp.2008.102.107

Phongprueksapattana S, Putalun W, Keawpradub N, Wungsintaweekul J. 2008. *Mitragyna speciosa*: Hairy root Culture for Triterpenoid Production and High Yield of mitragynine by Regenerated Plants. *Zeitschrift für Naturforschung CZ. Naturforsch*., **63**: 691-698. DOI: https://doi.org/10.1515/znc-2008-9-1014

Raffa BR. 2015. *Kratom and Other Mitragynines: The Chemistry and Pharmacology of Opioids from a Non-Opium Source* (1st edn). CRC Press.

Shaikh JR, Patil MK. 2020. Qualitative Tests for Preliminary Phytochemical Screening: An overview. *Int. J. Chem. Stud*., **8**(2): 603-608 DOI: https://doi.org/10.22271/chemi.2020.v8.i2i.8834

Sun G, Zhang X, Xu X, Yang J, Zhong M, Yuan J. 2012. A new Triterpene from the plant of *Uncaria macrophylla*. *Molecules*, **17**(1): 504-510. DOI: https://doi.org/10.3390/molecules17010504

Tao JY, Dai SJ, Zhao F, Liu JF, Fang WS, Liu K. 2012. New Ursane-type Triterpene with NO Production Suppressing Activity from Nauclea officinalis. *J. Asian Nat. Prod. Res.*, **14**(2): 97-104. DOI: http://dx.doi.org/10.1080/10286020.2011.628839

Thaipong K, Boonprakob U, Crosby. 2006. Comparison of ABTS, DPPH, FRAP and ORAC Assays for Estimating Antioxidant Activity from Guava Fruit Extracts. *J. Food Composit. Anal.*, **19**: 669-75. DOI: https://doi.org/10.1016/j.jfca.2006.01.003

Todd DA, Kellogg JJ, Wallace ED, Khin M, Flores-Bocanegra L, Tanna RS, McIntosh S, Raja HA, Graf TN, Hemby SE, Paine MF, Oberlies NH, Cech NB. 2020. Chemical composition and biological effects of kratom *(Mitragyna speciosa*): In vitro studies with Implications for Efficacy and Drug Interactions. *Sci. Rep.*, **10**(1): 19158. DOI: https://doi.org/10.1038/s41598-020-76119-w

Wood JE, Senthilmohana ST, Peskinb AV. 2002. Antioxidant Activity of Procyanidin-Containing Plant Extracts at Different pHs. *Food Chem.*, **77**: 155-161. DOI : 10.1016/s0308-8146(01)00329-6