

The Journal of Phytopharmacology

(Pharmacognosy and phytomedicine Research)

Research Article

ISSN 2320-480X

JPHYTO 2021; 10(6): 443-449

November- December

Received: 03-10-2021

Accepted: 15-11-2021

©2021, All rights reserved

doi: 10.31254/phyto.2021.10604

MONYN Ebalah Delphine

Department of Agronomic, Forestry and Environmental Engineering of the University of Man, Man, Ivory Coast

YEO Sounta Oumar

Department of Agronomic, Forestry and Environmental Engineering of the University of Man, Man, Ivory Coast

KOUAME Bosson Antoine

Laboratory of Bio-organic Chemistry and Natural Substances, Nangui Abrogoua University, Abidjan, Ivory Coast

KONE Mamidou Witabouna

Swiss Center for Scientific Research, Abidjan, Ivory Coast

Correspondence:

Dr. MONYN Ebalah Delphine

Department of Agronomic, Forestry and Environmental Engineering of the University of Man, University of Man, Man, Ivory Coast

+2250707590823/PO Box 20 Man (Côte d'Ivoire)

Email: ebalah.monyn@univ-man.edu.ci

Antioxidant activity and chemical composition of extracts from the leaves of *Hydrocotyle bonariensis* comm. Ex Lam

MONYN Ebalah Delphine, YEO Sounta Oumar, KOUAME Bosson Antoine, KONE Mamidou Witabouna

ABSTRACT

Several studies revealed that oxidative stress was involved in a great number of diseases as a triggering factor or associated with evolutionary complications. Due to the side effects of synthetic molecules, medicinal plants always remained the reliable source of active substances for their therapeutic properties. In effect, this study of antioxidant activity and chemical composition was carried on *Hydrocotyle bonariensis*, a species frequently used for medical applications in Ivory Coast, but still very under researched. Different extracts obtained with solvents of increasing polarity were tested by the ABTS radical scavenging method and compared to a reference antioxidant, namely vitamin C. Qualitative phytochemical screening was performed according to standard procedures. The results revealed that all extracts significantly and dose-dependently inhibited the ABTS- radical. Ethylacetate and methanolic extracts were most active with IC₅₀ values of 58.8±0.30 and 86.4±0.51 µg/mL, respectively, relative to vitamin C (IC₅₀ = 15.7±0.06 µg/mL). Antioxidant-associated phytochemicals such as flavonoids and coumarins were detected in ethylacetate and methanolic extracts. These compounds were responsible for the ABTS radical reduction reported. This study indicated the presence of substances in *Hydrocotyle bonariensis*'s leaves, which, in generally are excellent antioxidants and can contribute to prevent various diseases as cardiovascular diseases among others.

Keywords: *Hydrocotyle bonariensis*, Antioxidant Activity, Chemical composition, Ivory Coast.

INTRODUCTION

Nearly half of the drugs we currently use are plant-based, and a quarter contain plant extracts or active molecules derived directly from plants [1]. Research on antioxidants has increased over the years and appears to be the health emergency. Several studies have demonstrated that oxidative stress involved in the many chronic non-transmissible diseases such as diabetes [2], cancer [3], neurodegenerative diseases [4], and cardiovascular diseases [5]. In cases where natural mechanisms are overpowered by the attack of free radicals, the organism requires exogenous antioxidants from foods or therapeutic agents that are recognized for their benefit to health. Thus, plants represent an important source of bioactive molecules. As a result, various natural antioxidants are already commercially exploited and their probable implications in the prevention of pathologies related to oxidative stress were proven [6-8]. In fact, wherever in Africa, as in Côte d'Ivoire, the healthcare system needs to find new drugs able to provide some solutions in the fight against serious diseases such as the prevention of cardiovascular diseases. Therefore, this study was designed to investigate the antioxidant activity of an indigenous species, *Hydrocotyle bonariensis*, used in the Ivorian pharmacopoeia. Although some authors have undertaken out limited studies on this species [9-11], few have concerned on the pharmacological properties of its leaves. This study was to evaluate the reduction capacity of free radicals responsible of oxidative stress and to determine the chemical composition of the extracts of the leaves of this plant species.

MATERIALS AND METHODS

Plant collection, identification and processing

The material plant was essentially composed of *H. bonariensis* leaves collected at Nangui Abrogoua University of Abidjan. The identification was made in comparison to herbarium specimens of the Swiss Research Center in Ivory Coast, previously identified by Guillaumet J-L. (No 174), Adjanohoun E. (No 5099) and Aké-Assi L. (No 10628). After washing, the leaves were dried under permanent air-conditioning at a temperature of 18°C for two weeks at the Botany and African Pharmacopoeia Research Center of the Nangui Abrogoua University. They were pulverized with an electric grinder (RETSCH brand, type SM 100). The powder obtained was stored in plastic container awaiting extraction.

Extraction procedures

Different types of extracts were prepared according to the method [12] using solvents of increasing polarity. In order to proceed, 500 g of the powder obtained were macerated in 500 mL of analytical-grade hexane in a 2-liter conical flask and covered with aluminium foil. The mixture was stirred for 24 h, then decanted and filtered through Whatman Filter Paper (No.1). The recovered pomace was taken up with 500 mL using the same procedure. The filtrate obtained (1 L) was concentrated in vacuo using a rotary evaporator. The resulting extract was transferred to a glass dish and then dried in a hot air oven at 35° C. This marc was dried and then reworked successively using the same process with chloroform, ethyl acetate and methanol. As a result, four (4) different extracts were obtained to perform the tests: hexanic, chloroformic, ethylacetatic and methanolic extracts. The dried extracts were weighed, and their respective percentage yields were determined. All the extracts were stored in a refrigerator at 4°C awaiting testing.

Evaluation of antioxidant activity by the ABTS test

To determine the trapping power of the ABTS radical, the method of [13] was adopted. The radical cation ABTS was generated by mixing a solution of ABTS (7.0 mM) and potassium persulfate (2.6 mM). The mixture was left to stand for 12 h at room temperature, protected from light. The mixing ratio was 1: 1, v / v. Then, a volume of 1 mL of the obtained solution was mixed with methanol (1 to 4 mL) in order to obtain an absorbance value of between 1.0 and 1.5 at 734 nm. For each test, a fresh solution was prepared. A volume of 100 µL of each extract at different concentrations was incubated with 2500 µL of ABTS • + solution for 7 min in the dark and then the absorbances were measured with a spectrophotometer (Hach DR 2400) at 734 nm. The tests were done in triplicate. Ascorbic acid (vitamin C) was taken as a benchmark antioxidant. The percentages of inhibition were calculated according to the formula below:

$$\text{ABTS Inhibition} = \frac{A_0 - A_1}{A_0} \times 100$$

A₀ = absorbance of ABTS, A₁ = absorbance after adding products at a given concentration, at a given time.

The concentration of the sample required to neutralize 50 % of free radicals (IC₅₀) was determined using sratgraphics plus5.0 software.

Qualitative phytochemical screening

In this study, the standard methods for determining chemical compounds described in the research of Mamyrbékova-Bèkro *et al.* [14] and N'gaman *et al.* [15] were used. Phytochemical testing was performed by means of Thin-layer chromatography. The following systems were used as developers: cyclohexane / ethyl acetate (8: 2; v / v), chloroform / ethyl acetate / hexane (8: 2: 1; v / v / v), chloroform / ethyl acetate (6: 3; v / v), hexane / ethyl acetate / methanol (6: 4: 1; v / v / v) for the hexane, chloroform, ethyl acetate and methanolic extracts respectively. After development, the chromatograms were dried and visualized with or without developer either in visible or

under UV light at 366 nm. The colorations appearing were noted and frontal ratios calculated (formula 2):

$$\text{Frontal ratio} = \frac{\text{Distance traveled by a compound}}{\text{Distance traveled by solvent front}}$$

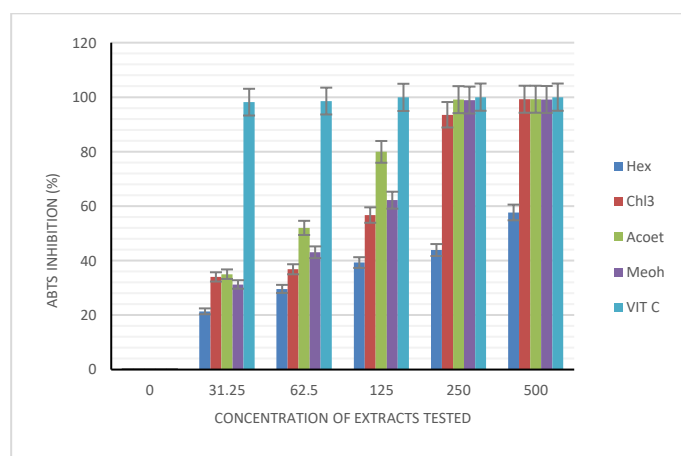
Data management and statistical analysis

For data analysis, STATISTICA 7.1 software was applied. For data entry, Microsoft Access 2007 was used to enter data. The results were then exported to Microsoft Excel 2007 for processing. One-criteria analysis of variance (ANOVA 1) was performed to determine significant differences among means followed by Tukey's post hoc test for pairwise comparisons and separations of means at α=0.05% [16]. The IC₅₀ values were determined graphically from the curves of percent inhibition versus concentrations.

RESULTS

Antioxidant activity of the studied extracts

The effects of the hexanic, chloroformic, ethylacetatic and methanolic extracts were tested against ABTS radical. All the extracts exhibited inhibition towards the ABTS radical. This inhibition was more significant as the concentration of the extract was increased, corresponding to a dose-dependent action. At concentrations of 31.25 to 125 µg / mL, an average activity of all extracts was observed in comparison that of vitamin C. From 250 µg / mL onwards, ABTS radical inhibition has strongly increased and was increasingly important at 500 µg / mL for all the extracts tested (Figure 1).



VIT C = Vitamin C. Hex = Hexane; Chl3 = Chloroforme; Acoet = Ethyl acetate; Meoh = Méthanol

Figure 1: Effects of different extracts from *Hydrocotyle bonariensis* on ABTS radical in comparison to vitamin C

In order to illustrate antioxidant activity, IC₅₀ values of different extracts tested and reference product (vitamin C) were determined. Thus, ethylacetatic and methanolic extracts exhibited higher inhibitions activities compared to other extracts. Their IC₅₀ values were 58.86 ±0.30 and 84.6 ±0.51 µg/mL, respectively. However, these IC₅₀ were inferior to vitamin C used as a reference at 15.7 ± 0.06 µg/mL. Among all extracts tested, the lowest free radical activity reduction was observed with hexanic extract whose IC₅₀ value of 358.8 ±1.16 µg/mL (Table 1).

Table 1: Inhibitory concentrations 50 (IC50) extracts tested in relative to vitamin C

Standard and extracts	Yield	IC50 (µg/mL)
Vitamine C	Nd	15.7± 0.06
Hexanic	1.024	358.8±1.16 ^c
Chloroformic	0.474	103.8± 0.46 ^b
Ethylacetate	0.206	58.8±0.30 ^a
Méthanolic	2.122	86.4±0.51 ^a
Statistical parameters of ANOVA		
	Dl	4
	F	44992.5
	P	< 0001

Nd = not determined

Values for the same items were not significantly different (P = 0.0001)

Table 2: Compounds identified in hexanic extract

Visible		UV 254 nm		UV 366 nm		Godin ¹		Liebermann-buchard ²		Possible compounds		
Rf	Color	Rf	Color	Rf	Orange	Rf	Color	Visible UV 366 nm	Rf	Color		
00	Green	00	Grey	00	Orange	00	Grey	00	Pale green	00	Orange	Trit ²
-	-	-	-	-	-	0.04	Violet	0.05	Pale green	-	Brown yellow	Ster ^{1,2}
0.09	Yellow	-	-	0.1	Orange yellow	0.06	Violet	-	-	-	-	Ster ¹
0.16	Yellow	0.16	Grey	0.17	Jaune	0.14	Violet	0.16	Light green	0.5	Orange	Ster ¹ /Trit ²
-	-	-	-	-	-	0.2	Violet	-	-	0.19	Purplish Blue	Ster ¹
-	-	-	-	0.21	Red	-	-	-	-	0.22	Orange	-
0.26	Yellow	-	-	0.27	Red	0.25	Light green	0.28	Light green	0.26	Light violet	Trit ¹
-	-	0.32	Grey	-	-	-	-	-	-	0.31	Orange	Tri ²
0.38	Light green	-	-	0.38	Orange yellow	0.35	Light violet	0.38	Light green	0.36	Light violet	Ster ¹
0.42	Yellow	-	-	0.42	Red	0.4	Violet	-	-	0.41	Orange	Ster ¹ /Trit ²
-	-	-	-	-	-	0.43	Violet	-	-	0.45	Light violet	Ster ¹
0.52	Green	0.51	Grey	0.51	Red	0.5	Violet	0.5	Green	0.51	Brown yellow	Ster ^{1,2}
-	-	-	-	-	-	0.56	Violet	-	-	0.58	Red	Ster ¹ /Ol&urs ²
-	-	0.65	Grey	0.62	Red	0.64	Violet	-	-	-	-	Ster ¹
0.69	Pale yellow	-	-	-	-	-	-	-	-	-	-	-
0.75	Pale yellow	-	-	0.76	Brown	-	-	-	-	-	-	-
0.81	Yellow	-	-	-	-	0.81	Yellow	-	-	-	-	-
-	-	-	-	-	-	0.85	Violet	0.8	Brown	0.82	Purplish Blue	Ster ¹
0.87	Yellow	0.87	Gris	0.88	Yellow	0.87	Green yellow	-	-	-	-	-
						0.93	Violet	0.94	Green yellow	0.95	Yellow	Ster ^{1,2}

Support: silicagel 60 F254/Eluent: Cyclohexane / Ethylacetate 8 : 2

Tri= Triterpenes; Ster= Sterols; Ol&urs= oleanane or ursane type triterpenes;

1 = Compounds revealed with Godin's reagent; 2 = Compounds revealed with Liebermann-Büchard's reagent

Phytocompounds detected in chloroform extract

Chromatograms revealed by Liebermann-Büchard reagents then visualized under UV at 366 nm showed different secondary metabolites (Table 3). The UV 366 nm red spots of Rf = 0.10; 0.39 and 0.71 correspond to triterpenes of oleanane or ursane type, while the orange-yellow spot (Rf = 0.91) at 366 nm indicated lupin type. At

Qualitative phytochemical analysis

Phytocompounds detected in hexanic extract

Several groups of chemical compounds were observed after the chromatograms were developed (Table 2). Sterols from 12 purple spots were observed in visible and at 366 nm with Godin's reagent. These spots were Rf= 0.04; 0.06; 0.14; 0.2; 0.35; 0.4; 0.43; 0.5; 0.56; 0.64; 0.85; and 0, 93 corresponding to saponins or iridoids. Three more spots appeared in green and yellow in the visible range with the Liebermann-Büchard reagent and intensified in yellow fluorescence at 366 nm with Rf= 0.05; 0.51 and 0.93. Terpenes fluoresced red (Rf =0.27) at 366 nm without revelation, but turned light green (Rf = 0.25) using Godin's reagent. They were classified as oleanane or ursane type triterpenes by Liebermann-Büchard reagent if the spots fluoresced red under UV / 366nm as observed at Rf = 0.58.

Rf = 0.04 the purple spot in visible range corresponds to a triterpene genine. Alkaloids appear orange in visible with Dragendorff's reagent (Rf = 0.05). Polyphenols appeared in the presence of iron trichloride (FeCl₃) in green at Rf = 0.1; 0.28; 0.44; 0.56 and 0.92. Grey spots in visible with FeCl₃ at Rf = 0.04; 0.10; 0.12; 0.19; 0.56; 0.75 and 0.92 were corresponding tannins.

Table 3: Compounds identified in chloroform extract

Before revelation		After revelation												Possibles Compounds
Visible	UV 254 nm	UV 366 nm	Liebermann-Buchard ¹		Dragendorff ²		FeCl ₃ ³							
Rf	Color	Rf	Color	Rf	Color	Rf	Color	Rf	Color	Rf	Color	Rf	Color	
00	Brown	00	Grey	00	Yellow	00	Brown	00	Brown	00	Orange	00	Grey	Alk ² /Tan ³
-	-	-	-	0.03	Orange yellow	0.04	Pale violet	-	-	0.05	Orange	0.04	Grey	Alk ² /Tan ³ /G.tri ¹
0.09	Dark green	0.1	Grey	0.1	Black red	0.1	Green	0.10	Red	-	-	0.1	Grey green	Ol&urs ¹ /Tan ³ /Poly ³
-	-	-	-	-	-	-	-	-	-	0.12	Green	0.12	Grey	Tan ³ /Poly ³
0.19	Light yellow	-	-	-	-	-	-	-	-	0.19	Pale green	0.19	Grey	Poly ³ /Tan ³
0.26	Light yellow	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	0.28	Yellow	0.31	Blue	-	-	-	-	0.28	Green	Poly ³
-	-	-	-	0.36	Pink	-	-	0.39	Pale red	-	-	-	-	Ol&urs ¹
0.42	Yellow	-	-	-	-	-	-	-	-	-	-	-	-	-
-	-	0.44	Grey	-	-	-	-	-	-	-	-	0.44	Pale green	Poly ³
0.5	Yellow	0.49	Grey	0.49	Brown yellow	0.48	Green	-	-	0.49	Light green	-	-	-
-	-	0.55	Grey	0.56	Pink	0.56	Green	0.55	Brown	0.55	Dark green	0.56	Grey green	Tan ³ /Poly ³
-	-	0.61	Grey	-	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	0.68	Light blue	-	-	-	-	-
-	-	-	-	-	-	-	-	0.71	Red	0.72	Orange brown	-	-	Ol&urs ¹
0.75	Green	0.75	Grey	0.75	Black red	0.74	Green yellow	-	-	-	-	0.75	Grey	Tan ³
-	-	0.81	Grey	0.82	Red	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	-	-	0.91	Red yellow	0.9	Brown	0.92	Grey green	trit ¹ /Tan ³ /Poly ³
0.91	Green	0.91	Grey	0.91	Black red	0.91	Green yellow	-	-	-	-	-	-	-
0.94	Yellow	-	-	-	-	-	-	-	-	-	-	-	-	-

Support: Silicagel 60 F254; Eluent: chloroform/Ethylacetate/hexane (8 : 2 : 1) Alk= Alkaloids; Tan= Tannins; Tri= Triterpenes; Poly= Polyphenols; G.tri= Triterpenic genins; 1 = Compounds revealed by Liebermann-Buchard; 2 = Compounds revealed by Dragendorff's reagent; 3 = Compounds revealed by FeCl₃

Phytochemicals detected in ethylacetate extract

The spots appearing under various colors (Table 4) corresponded to many groups of compounds. Previously, the yellow and green spots observed in visible range and showing yellow-orange, yellow-green and red under UV 366 nm are related to flavonoid. Godin's reagent revealed them in pink, orange and yellow with Rf= 0.46; 0.71 and 0.89. They are materialized by the yellow fluorescences with AlCl₃ in

visible, which intensified or diversified in blue to brown under UV 366 nm. It results from the spot on the baseline and the Rf= 0.10 and 0.66. A yellow or red spot in visible with KOH methanolic solution indicated coumarins. They were of anthrone type when their yellow coloration intensified or anthraquinone type when the coloration diversified or persists in red under UV at 366 nm. These compounds were observed at Rf = 00, 0.57; 0.62 and 0.45; 0.82; 0.90 respectively.

Table 4: Compounds identified in ethylacetatic extract

Before revelation		After revelation												
Visible		UV 254 nm		UV 366 nm		Godin ¹		KOH ²		Visible UV 254 nm		UV 366 nm		Possibles compounds
Rf	Color	Rf	Color	Rf	Color	Rf	Color	Rf	Color	Rf	Color	Rf	Color	
00	Pale yellow	00	Grey	00	Yellow	00	Violet	00	Pale yellow	00	Grey	00	Yellow	Ster ¹ /Coum ²
-	-	-	-	0.08	Orange yellow	-	-	-	-	-	-	-	-	-
0.12	Pale yellow	-	-	-	-	0.11	Violet	-	-	-	-	-	-	Ster ¹
-	-	0.18	Grey	0.18	Orange yellow	-	-	0.19	Pale yellow	-	-	0.19	Orange	-
-	-	-	-	-	-	0.25	Violet	-	-	-	-	-	-	Ster ¹
-	-	-	-	0.41	Red	-	-	-	-	-	-	-	-	-
-	-	0.44	Grey	-	-	0.46	Pale pink	0.46	Green	-	-	0.45	Black red	Flav ¹ / Coum ²
0.49	Green grey	-	-	-	-	0.5	Violet	-	-	0.48	Grey	-	-	Ster ¹
0.56	Pale yellow	-	-	-	-	-	-	0.57	Pale yellow	-	-	0.57	Pale yellow	Coum ²
0.6	Yellow	-	-	0.59	Green yellow	-	-	-	-	-	-	0.62	Green yellow	Coum ²
-	-	-	-	-	-	0.65	Purplish Blue	0.64	Yellow	-	-	-	-	Ster ¹
-	-	0.7	Grey	0.69	Pale red	0.71	Pink	-	-	-	-	-	-	Flav ¹
-	-	-	-	-	-	0.76	Violet	-	-	-	-	-	-	Ster ¹
0.82	Green yellow	-	-	0.81	Red	-	-	-	-	-	-	0.82	Red	Coum ²
-	-	-	-	-	-	-	-	0.85	Pale green	-	-	-	-	-
-	-	-	-	-	-	0.89	Green yellow	-	-	0.88	Grey	-	-	Flav ¹ /Trit ¹
0.91	Green yellow	0.9	Grey	0.91	Black red	0.92	Green	0.91	Brown yellow	-	-	0.90	Black red	Trit ¹ /Coum ²
-	-	-	-	-	-	0.96	Violet	-	-	-	-	-	-	Ster ¹

Support: Silicagel 60 F254; Eluent: (Cloroform/ethylacetate (6/3; V/V). Trit= Triterpenes; Ster= Sterols Flav= Flavonoids; Coum= Coumarins; 1 = Compounds revealed by Godin; 2 = Compounds revealed by KOH

Phytocompounds detected in methanolic extract

A majority spots visualized revealed different compounds (Table 5). The green spot (Rf= 0.96) with Godin's reagent corresponded to a triterpene. The methanolic KOH solution showed anthrones at

Rf=0.19; 0.82 and 0.86; anthraquinones corresponding to Rf= 0.54; 0.68; 0.94 and two other spots of Rf= 0.08 and 0.24 which also represented coumarins. Eight yellow, pink and orange spots of Rf = 0.10; 0.19; 0.24; 0.45; 0.58; 0.71; 0.84; 0.90 revealed with Godin's reagent attested to flavonoids.

Table 5: Compounds identified in methanolic extract

Before revelation		After revelation												
Visible		UV 254 nm		UV 366 nm		Godin ¹		KOH ²		Visible UV 366 nm		UV 366 nm		Possible compounds
Rf	Color	Rf	Color	Rf	Color	Rf	Color	Rf	Color	Rf	Color	Rf	Color	
00	Pale yellow	00	Gris	00	Green yellow	00	Pale yellow	00	Yellow	00	Grey	00	Brown yellow	Flav ¹ /Anthr ²
-	-	-	-	-	-	0.05	Violet	-	-	-	-	-	-	Stér ¹
0.09	Pale yellow	0.09	Grey	0.08	Brown	-	-	0.08	Yellow	0.08	Grey	0.08	Brown yellow	Coum ²
0.12	J Pale yellow	-	-	-	-	0.1	Yellow	-	-	-	-	-	-	Flav ¹
0.19	Pale yellow	-	-	-	-	0.19	Pale yellow	0.19	Pale yellow	-	-	0.19	Yellow	Flav ¹ /Anthr ²
0.24	Rose pâle	-	-	-	-	0.24	Pale pink	-	-	-	-	0.24	Brun	Flav ¹ /Coum ²
-	-	-	-	-	-	0.28	Violet	-	-	-	-	-	-	Stér ¹
-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

-	-	0.46	Grey	0.45	Orange	0.45	Orange	-	-	-	-	-	-	-	Flav ¹
0.48	Pale yellow	-	-	-	-	-	-	-	-	-	-	-	-	-	-
0.51	Pale green	0.52	Grey	0.51	Red	-	-	-	-	-	-	-	-	-	-
-	-	-	-	-	-	0.55	Violet	0.55	Pale yellow	-	-	0.54	Brown red	Stér ¹ / Anthrq ²	
0.58	Pale green	-	-	-	-	0.58	Orange	-	-	-	-	-	-	Flav ¹	
0.62	Pale green	-	-	0.62	Red	-	-	0.62	Pale yellow	-	-	0.64	Brown red.	Anthrq ²	
-	-	-	-	0.67	Red	-	-	-	-	-	-	0.68	Brown red	Anthrq ²	
-	-	-	-	0.69	Pale pink	0.71	Pale pink	-	-	-	-	-	-	Flav ¹	
0.82	Pale yellow	-	-	0.82	Orange	0.84	Yellow	-	-	-	-	0.82	Blue yellow	Flav ¹ /Anthr ²	
-	-	-	-	-	-	0.89	Violet	0.86	Pale yellow	-	-	0.86	Yellow	Stér ¹ /Anthr ²	
-	-	-	-	-	-	0.90	Yellow	-	-	0.90	Grey	-	-	Flav ¹	
-	-	-	-	-	-	0.96	Green	0.94	Vert	-	-	0.94	Red	Trit ¹ /Anthrq ²	

Support: silicagel 60 F254; Eluent: (hexane/Ethylacetate/methanol): 6/4/1; V/V/V). Coum= Coumarins; stér= Sterols;Flav= Flavonoids;Trit= Triterpenes; Antr= Anthrones; Anthrq= Antraquinones 1=Compounds revealed by Godin; 2=Compounds revealed by KOH.

DISCUSSION

This study of the effects of *H. bonariensis* leaves required extraction with various solvents of increasing polarity. The choice was based on the need to extract chemical compounds with antioxidant properties including flavonoids, tannins, saponins, triterpenoids and alkaloids but also to identify a more active extract. This option appeared to be appropriate because extraction method was a crucial factor for the optimal evaluation of the pharmacological effects of plant [17, 18]. The anti-radical capacity of the extracts compared to vitamin C could be explained by the antioxidant compounds present in the plant [19].

However, the high radical reducing capacity of ethylacetic and methanolic extracts would be caused by their abundance of radical reducing constituents [20]. In fact, ANOVA 1 and Turkey's multiple comparison test showed that the means of the inhibition percentages of the extracts were not significantly different from that of the control, which is vitamin C. The IC50 values of the different extracts tested and the reference product (Vitamin C) confirmed that ethylacetic and methanolic extracts were more active.

So the research of phytochemicals in extracts was carried out in order to investigate compounds responsible for the observed activity. Thin-layer chromatography was used because it appeared more reliable than characterization tests [21]. Indeed, it provides more detailed analysis of chemical composition of extracts to be investigated.

These results showed that leaves of *H. bonariensis* contained many phytochemical groups including anthraquinones, total polyphenols, sterols and polyterpenes, tannins, coumarins, flavonoids, and alkaloids. These secondary metabolites in this plant would have justified its various therapeutic values in the treatment of several pathologies. Indeed, it is admitted that these chemical groups are generally involved in biological activity of medicinal plants [22]. In addition, several authors have demonstrated a correlation between phenolic compounds and antioxidant activity of plant [23, 24]. They are indeed chemically structured to capture free radicals and according to studies, they appear to be involved in free radical scavenging activity [25, 26]. Based on results of the present study, it is suggested that antioxidant activity observed in this study could be related to polyphenolic compounds such as flavonoids. These compounds were only detected in ethylacetic and methanolic extracts that showed

high antioxidant activity. This statement can be supported by Bidié *et al.* [27] who demonstrated that antioxidant activities of many medicinal plant of Ivorian pharmacopoeia were associated to flavonoid activity. According to N'guessan *et al.* [28] and Zhi *et al.* [29] flavonoids were endowed with antioxidant capacity. These antioxidant components in extracts of *H. bonariensis* leaves justified its application in Ivorian pharmacopoeia and in many areas of west Africa.

CONCLUSION

This study evaluated antioxidant activity and performed phytochemical screening of *H. bonariensis* leaves extracts. The results showed that leaves extracts of this indigenous species were rich in antioxidants and various phytochemicals with pharmaceutical properties, which could justify its use according to traditional medicine. This plant could be recommended against pathologies caused by free radicals. Toxicological and safety studies of the studied extracts should therefore be undertaken to establish their safety.

Data availability

All data in this study are included within the manuscript; however, any additional information is available from authors upon request

Conflict of interest

The authors declare that they have no conflict of interest in this study.

Author's contributions

Monyn Ebalah Delphine conceived the research idea and performed the experiments under the close supervision of Koné Mamidou Witabouna. Kouamé Bosson Antoine guided the experiments, and assisted with data analysis and interpretation. Yeo Sounta Oumar contributed to antioxidant tests. All authors reviewed and approved the final manuscript for publication.

Funding

We did not receive any funding from any granting/funding agency/corporation in the public or private sector. This study was solely achieved through our personal finances.

Acknowledgements

We acknowledge the Departement of Botany and Chemistry Laboratory of the Swiss Research Center for availing the lab facility, reagents, solvents and equipment for this study.

REFERENCES

1. Newman DJ, Cragg GM. Natural products as sources of new drugs over the 30 years from 1981 to 2010. *J. Nat. Pro.* 2012; 75(3):311-335.
2. Baynes JW. Role of oxidative stress in development of complications in diabetes. *Diab.* 1991; 40:405-412.
3. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int. j. biochem. cell boil.* 2007; 39(1):44-84.
4. Kadenbach B, Ramzan R, Vogt S. Degenerative diseases, oxidative stress and cytochrome c oxidase function. *Trends Mol. Med.* 2009; 15:139-140.
5. Mladenka P, Zatloukalova L, Filipisky T, Hrdina R. Cardiovascular effects of flavonoids are not caused only by direct antioxidant activity. *Free Radic. Biol. Med.* 2010; 49 (6):963-975.
6. Laguerre M, Lecomte J, Villeneuve P. Evaluation of the ability of antioxidants to counteract lipid oxidation: Existing methods, new trends and challenges. *Prog. Lipid Res.* 2007; 46:244-282
7. Elzbieta S, Ewa C, Teresa L, Agnieszka F-F, Pawel M. P. The antioxidant activity of selected cruciferous vegetables subjected to aquathermal processing. *Food Chem.* 2008; 107:55-59.
8. Gülçin. Antioxidant activity of food constituents: an overview. *Arch. Toxicol.* 2012; 86(3):345-91.
9. Tempone AG, Sartorelli P, Teixeira D, Prado FO, ARL Calixto IARL, Harri LH, Melhem SCM. Brazilian flora extracts as source of novel antileishmanial and antifungal compounds. *Mem. Inst. Oswaldo Cruz* 2008; 103 (5):443-449.
10. Masoumian M, Arbakariya A, Syahida A, Maziah M. Flavonoids production in *Hydrocotyle bonariensis* callus tissues. *Res. J. Medicinal Plant.* 2011; 5 (9):1564-1574.
11. Florinsiah L, Farida Z MY, Nur SN, Norfazlina MN, Suziana ZCF, Rajab NF. Mutagenicity Effect of *Hydrocotyle Bonariensis* Extracts in Salmonella/Microsome Assay. *Int. J. Pharm. Sci. Rev. Res.* 2013; 20 (2):47-50.
12. Békro YA, Békro JAM, Boua BB, Tra BFH & Ehilé EE (2007) Etude ethnobotanique et screening phytochimique de *Caesalpinia benthamiana* (Baill.) Herend. et Zarucchi (Caesalpinaceae). *Sci.Nat.* 2007; 4(2):217-225
13. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med* 1999; 26 (9):1231-1237.
14. Mamyrbékova-Békro JA, Konan MK, Békro YA, Djié Bi MG, Zomi Bi TJ, Mambo V, Boua BB. Phytochemicals of the extracts of four medicinal plants of Côte d'Ivoire and assessment of their potential antioxidant by thin layer chromatography. *Eur. J. Med. Res.* 2008; 24 (2):219-228.
15. N'gaman KC, Békro YA, Mamyrbékova-Békro JA, Bénie A, Gooré BS. On the composition in secondary metabolites and antioxidant activity of crude extracts of *Gmelina arborea* Roxb. (Verbenaceae) from Côte d'Ivoire, West Africa: Analysis by Thin Layer Chromatography. *Eur. J. Med. Res.* 2009; 2 (36):161-171.
16. Dagnelie P. Théories et méthodes statistiques, Presse agronomique de Gembloux: 2e Ed. Gembloux: Belgique; 1999.
17. Sylvain B, Karim T, Karamoko O, André OT, Souleymane M, Ako A. Phytochemical screening of some medicinal plants used to treat malaria in Côte d'Ivoire (West Africa). *Int. J. Pharm. Pharm. Sci.* 2014; 2(6):919-925
18. Kamarudin NA, Markom M, Latip J. Effects of Solvents and Extraction Methods on Herbal Plants *Phyllanthus niruri*, *Orthosiphon stamineus* and *Labisia pumila*. *Indian J Sci Technol.* 2016; 9(21):1-5.
19. Pourmorad F, Hosseinimehr S, Shahabimajd. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. *Afr. J. Biotechnol* 2006; 5(11):1142-1145.
20. Athamena S, Chalghem I, Kassah-Laouar A, Laroui S, Khebri S. Activité anti-oxydante et antimicrobienne d'extraits de *Cuminum cyminum* L. *Leban Sci J.* 2010; 11(1):69-81.
21. Boua B, Békro Y-A, Mamyrbékova-Békro J, Wacothon K, Ehilé E. E. Assessment of sexual stimulant potential of total flavonoids extracted from leaves of *Palisota hirsuta* Thumb. K. Schum (Commenilaceae). *Eur. J. Med. Res.* 2008; 22 (4):533-538.
22. Olasunkanmi OO, Akinpelu DA, Adeniyi PO, Femi Ajayi O, Omololu-Aso J, Olorunmola FO. Investigations into Antibacterial, Phytochemical and Antioxidant Properties of *Vitellaria paradoxa* (Gaertn.) Stem Bark Extracts. *J. Pharm. Res. Int.* 2017; 20 (5):1-17.
23. Zongo C, Savadogo A, Ouattara L, Bassole IHN, Ouattara CAT, Ouattara AS, Barro N, Koudou J, Traore A.S. Polyphenols content, antioxidant and antibacterial activities of *Ampelocissus grantii* (Baker) Planch. (Vitaceae): a medicinal plant from Burkina Faso. *Int. J. Pharm.* 2010; 6(6):880-887.
24. Ouattara L, Koudou J, Zongo C, Barro N, Savadogo A, Bassole IHN, Ouattara AS, Traore A.S. Antioxidant and antibacterial activities of three species of *Lannea* from Burkina Faso. *J. Appl. Sci.* 2011; 11(1):157-162.
25. Babu PVA, Sabitha KS, Shyamaladevi C.S. Therapeutic effect of green tea extract on oxidative stress in aorta and heart of streptozotocin diabetic rats. *Chem. Biol. Interact.* 2006; 162(2):114-120.
26. Karou S.D., Tchacondo T., Ouattara L., Anani K., Savadogo A., Agbonon A., Ben Attaia M., De Souza C., Sakly M., Simporé J. Antimicrobial, antiplasmodial, haemolytic and antioxidant activities of crude extracts from three selected Togolese medicinal plants. *Asian Pac. J. Trop. Med.* 2011; 4(10):808-813.
27. Bidié A dit P, N'Guessan BB, Yapo AF, N'Guessan JD, Allico J D. Antioxidant activities of ten medicinal plants from the Ivorian pharmacopoeia. *Sci. Nat.* 2011; 1:1 – 11.
28. N'guessan JD, Zirihni GN, Kra AKM, KouakouK, Djaman AJ, Guede-Guina F. Free radical scavenging activity, flavonoid and phenolic contents of selected Ivoirian plants. *IJANS.* 2007; 4:425-429.
29. Zhi PR, Liang LZ, Yi ML. Evaluation of the Antioxidant Activity of *Syzygium cumini* Leaves. *Mol.* 13:2008; 2545-2556.

HOW TO CITE THIS ARTICLE

Delphine ME, Oumar YS, Antoine KB, Witabouna KM. Antioxidant activity and chemical composition of extracts from the leaves of *Hydrocotyle bonariensis* comm. *Ex Lam. J Phytopharmacol* 2021; 10(6):443-449. doi: 10.31254/phyto.2021.10604

Creative Commons (CC) License-

This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY 4.0) license. This license permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. (<http://creativecommons.org/licenses/by/4.0/>).