Scientific Research Publishing

American Journal of Plant Sciences, 2025, 16(12), 1225-1243
https://www.scirp.org/journal/ajps
ISSN Online: 2158-2750
ISSN Print: 2158-2742

Phenolic Contents, Antioxidant and Anti-Inflammatory Properties of *Desmodium ramosissimum* Extract from Benin

Adjoavi Esse Agossou[1], Farid Toyigbénan Badé[1], Durand Dah-Nouvlessounon[1], Haziz Sina[1], Adolphe Adjanohoun[2], Aly Savadogo[3], Lamine Baba-Moussa[2*]

[1]Laboratory of Biology and Molecular Typing in Microbiology, Faculty of Science and Technology, University of Abomey-Calavi, Abomey-Calavi, Benin

[2]South Agricultural Research Centre, National Agricultural Research Institute of Benin, Attogon, Benin

[3]Laboratory of Biochemistry and Applied (LABIA), Faculty of Life and Earth Sciences, Doctoral School of Sciences and Technologies, Ouaga I Pr-Joseph KI-ZERBO University, Ouagadougou, Burkina Faso

Email: *laminesaid@yahoo.fr

How to cite this paper: Agossou, A.E., Badé, F.T., Dah-Nouvlessounon, D., Sina, H., Adjanohoun, A., Savadogo, A. and Baba-Moussa, L. (2025) Phenolic Contents, Antioxidant and Anti-Inflammatory Properties of *Desmodium ramosissimum* Extract from Benin. *American Journal of Plant Sciences*, **16**, 1225-1243.
https://doi.org/10.4236/ajps.2025.1612081

Received: August 23, 2025
Accepted: December 13, 2025
Published: December 16, 2025

Copyright © 2025 by author(s) and Scientific Research Publishing Inc.
This work is licensed under the Creative Commons Attribution International License (CC BY 4.0).
http://creativecommons.org/licenses/by/4.0/

Open Access

Abstract

Species of the genus *Desmodium* are generally used in the traditional Beninese pharmacopoeia for the treatment of many diseases, including infectious diseases. The objective of this work is to evaluate the antioxidant, anti-inflammatory activities and cytotoxicity of *Desmodium ramosissimum* herb extract. Identification of phenolic compounds was achieved using HPLC. Ferric reduction/antioxidant power (FRAP) and the free radical DPPH essay were used to evaluate *D. ramosissimum* antioxidant activity. The anti-inflammatory activity was evaluated *in vitro* by the albumin denaturation method using Diclofenac as a reference. The cytotoxicity of the extracts was evaluated with *Artemia salina* larvae. All the extracts have a remarkable antioxidant activity. However, the lowest IC_{50} values were obtained with the methanol (2.45 ± 0.14 µg/ml) and ethanol (2.5 ± 0.7 µg/ml) extracts. Furthermore, the hydroethanol extract showed the strongest anti-inflammatory activity at a rate of 92.99% ± 0.04% inhibition of albumin denaturation. Cytotoxic tests showed that no toxicity towards shrimp larvae was noticed. *D. ramosissimum* occupies an important place in the Beninese therapeutic arsenal. These results confirm the traditional use of these plants in Benin. Furthermore, *D. ramosissimum* opens up prospects for the search for active ingredients of plant origin with antimicrobial, anti-inflammatory and antioxidant properties.

Keywords

Medicinal Plant, Phytochemistry, Biological Activity, *Desmodium ramosissimum*, Benin

1. Introduction

About 50% of Beninese population resides in rural areas [1]. The non-availability of recent healthcare facilities in these rural communities in Benin has made people to rely upon medicinal plants in treating lots of degenerative diseases since ancient time. The efficacy of those plants lies on their numerous bioactive compounds [2]. Ancestral knowledge of medicinal plants has always guided the seek for new cures. Despite the arrival of contemporary high-throughput drug discovery and organic chemistry, traditional medical systems have given clues to the invention of valuable drugs [3]. Traditional medicinal plants are often cheaper, locally available and simply consumable, raw or as simple medicinal preparations [2]. Nowadays, traditional medicinal practices form a part of holistic or alternative medicines. Although their efficacy and mechanism of action have not been tested scientifically in most cases, these simple medicinal preparations often mediate beneficial responses thanks to their active chemical constituents [4]. Free radicals, produced thanks to normal biochemical reactions within the body, are implicated in numerous diseases. They play a task as toxic compounds, since they will be harmful to the body. Within the body, an overload of free radicals that cannot be destroyed gradually generates a phenomenon called oxidative stress [5]. This process plays a significant part in the development of chronic and degenerative illnesses. Cancer, autoimmune disorders, aging, cataract, autoimmune disease, cardiovascular and neurodegenerative diseases are most the times the consequences due to their accumulation in the body [5]. Apart from our innate defence mechanisms to counter free radicals, plants are used as food supplement to reinforce our body defence. Phenolic compounds present in plants possess strong biological properties that are beneficial to human body.

There have been several studies on the antioxidant and anti-inflammatory activities of various herbs/plants with medicinal values. The present study aims to investigate the free radical scavenging and anti-inflammatory activities, the quantification of phenolic compounds by high-performance liquid chromatography and *in vitro* cytotoxic activity of *D. ramosissimum*, a commonly used medicinal plant in South Benin.

2. Material and Methods

2.1. Collection of Plant Material

The leaves and stems of *D. ramossisimum* were collected in March 2018 in the town of Porto-Novo. At the laboratory, the leaves and stems were washed with distilled water, then dried at room temperature 25˚C ± 3˚C for 16 days. They were then

ground into a uniform powder, using a Retsch mill type SM 2000/1430/Upm/Smfet. The obtained powder was used for the preparation of different extracts.

2.2. Preparation of Plant Extracts

2.2.1. Methanol Extract and Water-Ethanol Extract

The extraction method described by Dah-Nouvlessounon *et al.* [6] was used. Briefly, 50 g of powder were macerated in 500 ml of solvent: methanol and water-ethanol (30:70, v/v) under continuous stirring for 48 hours. The mixture was then filtered thrice through cotton wool and once with the filter paper Whatman N˚1. The extracts were concentrated to dryness using a rotary evaporator (RE 300). The resulting residues were dried at 40˚C in oven. The resulting powders constitute the methanol dry extract and water-ethanol dry extract ready for use. Dry extracts were stored at 4˚C until analysis.

2.2.2. Successive Extraction with Hexane, Ethanol, and Ethyl Acetate

The extraction technique is an adaptation of the protocol used by N'Guessan *et al.* [7]. Briefly, 100 g of powder was macerated in 1 L of ethanol 96˚ under continuous stirring for 72 hours. The mixture was filtered thrice. Then, 50 ml of distilled water and 50 ml of hexane were added to it. The mixture obtained was then poured in a separatory funnel. After decantation, two phases are observed: a lower aqueous phase and an upper organic phase. The organic layer was collected and then dried to obtain the hexane dry extract. The first half of the lower layer was dried at 40˚C to obtain the ethanol dry extract. For the second half of aqueous phase, 200 ml of distilled water and 100 ml of ethyl acetate were added to it. The resulting mixture was left decanted to obtain two phases. The upper layer was recovered and dried to obtain the dry extract of ethyl acetate. The lower phase was dried to obtain the residual ethanol dry extract. The extracts obtained were stored in sterile bottles for further use. The extraction yield was determined by the ratio between the mass of dry extract obtained after evaporation and the mass of plant material.

2.3. Identification and Quantification of Phenolic Compounds

The phenolic compounds were quantified using high-performance liquid chromatography (HPLC) in reverse mode equipped with 600 E pump associated with Waters 486 UV tunable absorbance detector and 20 µl injection and a reversed-phase column (Inertsil ODS RP C18 Column × 150 mm; size, 5 µm) with a flow rate of 1 ml/min. The compound content was determined using a five-point calibration curve of each standard (R^2 = 0.99) in the linearity range of 10 - 100 µg/ml. The analyses were carried out at a wavelength of 320 nm. The identification and quantification of the phenolic compounds contained in the extracts was done by comparing the retention time of the surfaces of the standards used with those obtained by analyzing the extracts. The gradient used is constituted of three mobile phases:

- Solvent A: 50 mM of phosphate ammonium at 2.6 pH adjusted ($NH_4H_2PO_4$) with orthophosphoric acid (H_3PO_4).
- Solvent B: composition 80:20 (v/v) of acetonitrile/solvent A.

- Solvent C: composition 200 mM orthophosphoric acid (H_3PO_4) at pH 1.5 adjusted with NaOH).

The solvents were filtered through filter paper, Whatman N˚1 and disposed in ultrasound apparatus for 25 min. The profile of the gradient used is resumed in Table 1.

Table 1. Profile of the gradient for HPLC analyses.

Time (minutes)	Nature of solvents and composition (%)		
	A	B	C
0	100	0	0
4	92	8	0
10	0	14	86
22.5	0	16	84
27.5	0	25	75
50	0	80	20
55	100	0	0
60	100	0	0

2.4. Antioxidant Activities

2.4.1. DPPH Radical Scavenging Test

The antioxidant activity of *D. ramosissimum* extracts, on the basis of the scavenging activity of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical, was determined by the method described by Lamien-Médard *et al.* [8]. A methanol solution of 100 µg/ml of DPPH was mixed with extracts of *Desmodium ramosissimum* prepared from a stock solution of 400 µg/ml (400; 200; 100; 50; 25; 12.5; 6.25; 3.125; 1.56; 0.78 µg/ml). Briefly, 1 ml of the DPPH methanol solution was added to 1 ml of each extract, the mixture was vigorously stirred, and then the tubes were incubated at room temperature and in the dark for 30 minutes. Absorbance was read at 517 nm against a blank consisting of DPPH and methanol. Ascorbic acid, quercetin, BHA and BHT were used as positive control. Each test was carried out in triplicates. The antiradical activity is estimated according to the equation below:

$$\%\text{Inh} = \frac{\text{Blank's absorbance} - \text{Sample's absorbance}}{\text{Blank's absorbance}} \times 100$$

The IC_{50} (Concentration providing 50% inhibition) was calculated graphically using a calibration curve in the linear range by plotting the extract concentration and the corresponding scavenging effect. Antioxidant activity index (AAI) was calculated according to the formula used by Scherer and Godoy [9].

2.4.2. Ferric Reducing/Antioxidant Power (FRAP)

FRAP essay was conducted according to the protocol described by Dieng *et al.* [10].

Thus, 0.5 ml of sample at different concentrations was mixed with 1 ml of phosphate buffer (0.2 M; pH = 6.6) and 1 ml of 1% potassium hexacyanoferrate [$K_3Fe(CN)_6$]. After incubating the mixture at 50˚C for 30 minutes, 1 ml of 10% trichloroacetic acid was added to stop the reaction, and then the tubes were centrifuged at 3000 rpm for 10 minutes. Then, 1 ml of the supernatant from each tube was mixed with 0.2 ml of a 0.1% $FeCl_3$ solution and left to stand in the dark for 30 minutes before measuring optical densities at 700 nm. The antioxidant activity related to the reducing power of the extracts is expressed in reducing power (RP) using the following formula:

$$\%RP = \frac{\text{Sample's's absorbance} - \text{Blank's absorbance}}{\text{Sample's absorbance}} \times 100$$

IC_{50} (Concentration that inhibits the 50% of DPPH or reduces the 50% of Fe^{3+}) was determined by the probit method.

2.5. *In Vitro* Anti-Inflammatory Activity

The *in vitro* anti-inflammatory activity has been evaluated using the method of inhibition of albumin denaturation described by Ghumre [11]. Briefly, 5 ml of the reaction mixture consisted of 0.2 ml of egg albumin, 2.8 ml of phosphate-buffered saline (PBS, pH 6.4) and 2 ml of variable concentration of extracts (1000; 500; 250; 125; 62.5; 31.5 µg/ml). A similar volume of distilled water was used as a control. The mixture was then incubated at 37˚C for about 15 minutes and then heated to 70˚C for 5 minutes. After cooling, absorbances were measured at 660 nm. Diclofenac sodium inj.Pb./Drugs/1804-B was used as standard at a concentration of 6.25 to 25 mg/ml under the same conditions as the extracts. The inhibition's percentage of protein denaturation was calculated by the formula mentioned below:

$$\%Inh = \frac{\text{sabsorbance control} - \text{absorbance sample}}{\text{absorbance control}} \times 100$$

2.6. Cytotoxicity Essay

The cytotoxic effect of the extracts was evaluated based on the method described by Kawsar *et al.* [12]. Larvae were obtained by hatching 10 mg of freeze-dried eggs of *Artemia salina* (ARTEMIO JBL GmbH D-67141 Neuhofem) under continuous agitation in 1 L of seawater for 72 h. From a concentration of 20 mg/ml, a range of 10 concentrations is achieved with sterile distilled water following dilutions of 1/2 in test tubes numbered T1 to T10. Then, 1 ml of sea water containing 16 alive larvae was added to all tubes. The number of surviving larvae was counted after 24 h incubation. For each extract, the lethal concentration 50 (LC_{50}) was determined from the regression line obtained from the curve representing the number of surviving larvae as a function of extract concentration (the curves are appended to this manuscript). The results were interpreted using the correlation grid associating the degree of toxicity to LC_{50} proposed by Moshi *et al.* [13].

3. Results

The extractions yields are summarised in Table 2. They are expressed as a percentage of the mass of the initial powder. The extraction yields vary from one type of extract to another. The ethanol extract yielded the best result (16%), while the hexane extract showed the lowest yield (2.24%).

Table 2. Extracts yields.

Extract	Mass of powder (g)	Mass of extract (g)	Yield (%)
Ethanol	50	8	16
Water-ethanol	50	6.7	13.4
Methanol	50	7.39	14.78
Ethyl acetate	50	2.6	5.2
Hexane	50	1.12	2.24
Ethanol residual	50	2.11	4.22

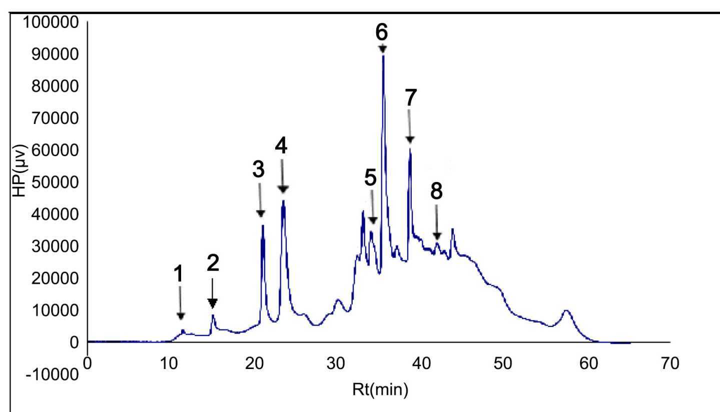
3.1. HPLC Analysis

Table 3 presents the different phenolic compounds identified in the hydro-ethanol and methanol extracts of *D. ramossissimum*. The analysis of Table 3 shows that 63% of the compounds were identified in the hydro-ethanol extract, while 50% were found in the methanol extract. Among the quantified compounds, the highest levels of phenolic compounds were obtained with glucose quercetin (0.45 ± 0.03 mg/ml) and quercetin dihydrate (2.12 ± 0.16 mg/ml) for the hydro-ethanol and methanol extracts, respectively. Furthermore, among the quantified compounds, quercetin had the lowest level (0.05 ± 0.04 mg/ml) with the hydro-ethanol extract, while cinnamic acid had the lowest level (0.78 ± 0.07 mg/ml) with the methanol extract (Figures 1-3).

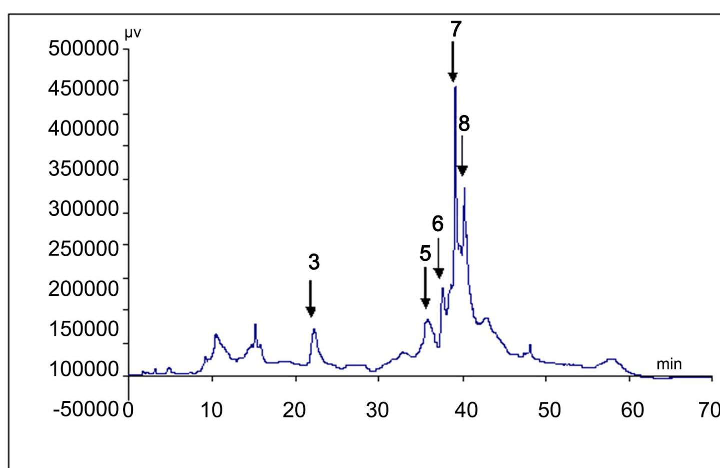
Table 3. Concentration of phenolic compounds contained in hydro-ethanol and methanol extracts.

Rt (min)	Ref.	Compounds	H-Eth (mg/ml)	MEth (mg/ml)
11.98 ± 0.22	1	Gallic acid	nd	nd
14.78 ± 0.92	2	Protocatechuic acid	nd	nd
23.96 ± 0.23	3	Catechin	0.05 ± 0.04	nd
280 ± 0.23	4	Chlorogenic acid	nd	nd
37.02 ± 0.36	5	Rutin	0.23 ± 0.01	0.29 ± 0.04
37.71 ± 0.18	6	Glucose-containing quercetin	0.45 ± 0.03	0.18 ± 0.01
39.47 ± 0.19	7	Quercetin dihydrate	0.16 ± 0.02	2.12 ± 0.16
41.68 ± 0.65	8	Cinnamic acid	0.13 ± 0.01	0.78 ± 0.07

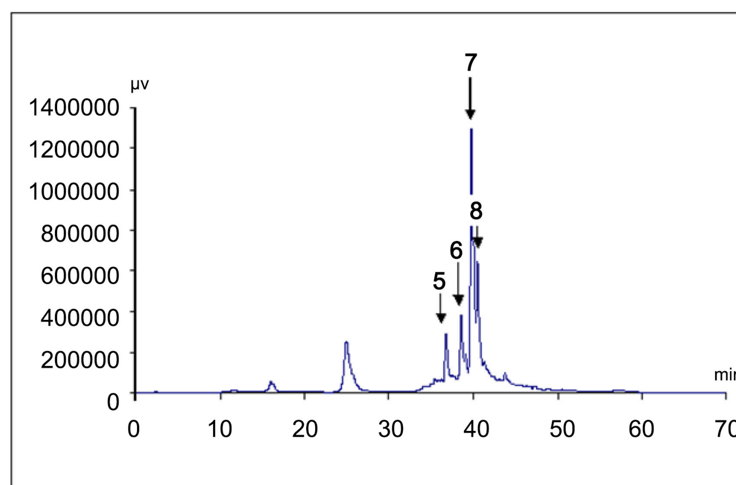
H-Eth: hydro-ethanol; MEth: methanol extract; Ref.: reference; Rt: retention time; nd: not detected.



(a)



(b)



(c)

1: gallic acid; 2: protocatechuic acid; 3: catechin; 4: chlorogenic acid; 5: rutin; 6: glucose quercetin; 7: quercetin dihydrate; 8: cinnamic acid.

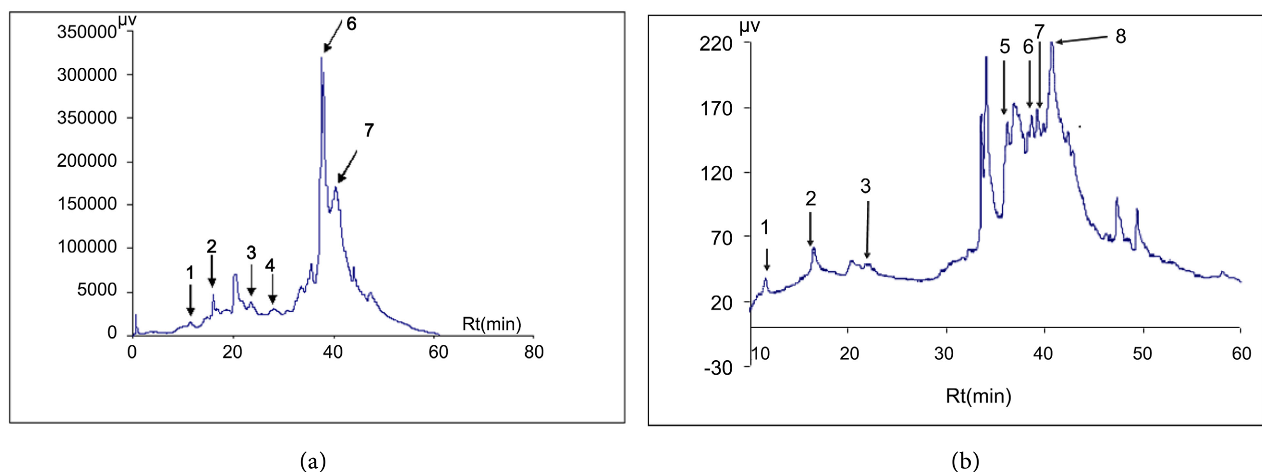
Figure 1. Chromatographic profile of the standard mixture (a), hydro-ethanol (b) and methanol (c) extract.

Table 4 presents the different phenolic compounds identified in the ethanol and residual ethanol extracts of *D. ramossissimum*. The analysis of Table 4 reveals that 87.5% of the compounds were identified in ethanol extract against 75% in residual ethanol extract. The minority compounds extracted with the ethanol were catechin (40.10 ± 0.04 mg/ml) and gallic acid (50.11 ± 0.01 mg/ml), chlorogenic acid was not determined. However, in the residual ethanol extract, quercetin dihydrate (50.20 ± 0.02 mg/ml) and glucose quercetin (39.39 ± 0.03 mg/ml) are predominantly represented. Rutin and cinnamic acid were not determined in the residual ethanol extract.

Table 4. Concentration of phenolic compounds contained in residual ethanol and ethanol extracts.

Rt (min)	Ref.	Compounds	Ethr (mg/ml)	Eth (mg/ml)
11.98 ± 0.22	1	Gallic acid	10.10 ± 0.01	50.11 ± 0.01
14.78 ± 0.92	2	Protocatechuic acid	19.9 ± 0.02	80.10 ± 0.02
23.96 ± 0.23	3	Catechin	15.08 ± 0.04	40.10 ± 0.04
25.80 ± 0.23	4	Chlorogenic acid	25.05± 0.01	nd
37.02 ± 0.36	5	Rutin	nd	60.20 ± 0.01
37.71 ± 0.18	6	Glucose-containing quercetin	39.39± 0.03	85.40 ± 0.03
39.47 ± 0.19	7	Quercetin dihydrate	50.20 ± 0.02	70.20 ± 0.02
41.68 ± 0.65	8	Cinnamic acid	nd	95.18 ± 0.01

Eth: ethanol extract; Ethr: residual ethanol extract; Ref.: reference; Rt: retention time; nd: not detected.



(a) (b)

1: gallic acid; 2: protocatechuic acid; 3: catechin; 4: chlorogenic acid; 5: rutin; 6: glucose quercetin; 7: quercetin dihydrate; 8: cinnamic acid.

Figure 2. Chromatographic profile of the residual ethanol (a) and ethanol extract (b).

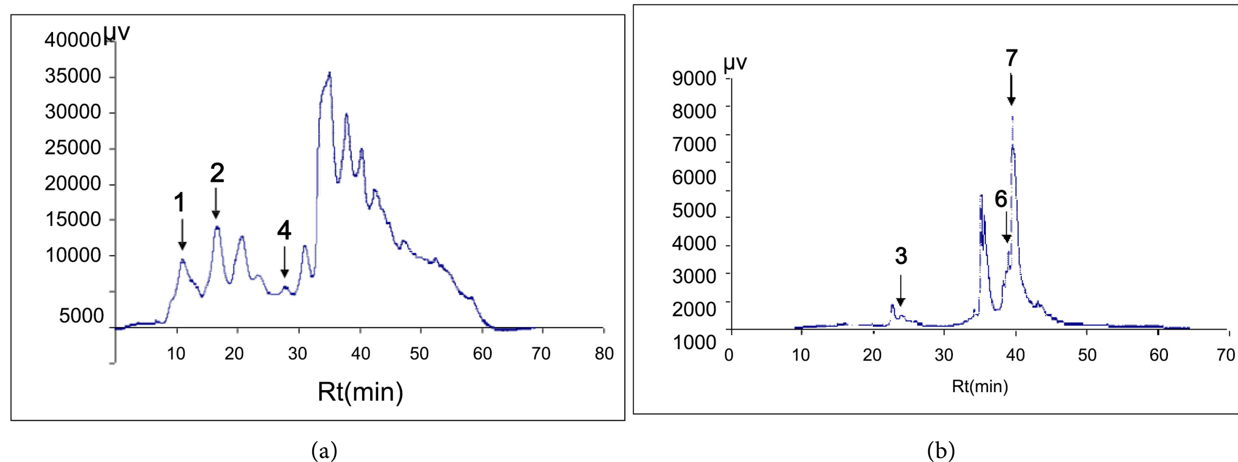
Table 5 presents the different phenolic compounds identified in the hexane and ethyl acetate extracts of *D. ramossissimum*. Table 5 reveals that the hexane extract and the ethyl acetate extract have the same proportion of compound (50%). Among

the quantified compounds, chlorogenic acid is predominantly represented (17.10 ± 0.01 mg/ml) in the hexane extract, followed by hydrated quercetin (13.07 ± 0.02 mg/ml) in the ethyl acetate extract. The lowest level of compound recorded in the quantified compounds was obtained with protocatechuic acid (10.05 ± 0.02 mg/ml) for the hexane extract, while catechin showed the lowest level (8.08 ± 0.04 mg/ml) with the ethyl acetate extract.

Table 5. Concentration of phenolic compounds contained in the hexane and ethyl acetate extracts.

Rt (min)	Ref.	Name of compounds	Hex (mg/ml)	Eth-ace (mg/ml)
11.98 ± 0.22	1	Gallic acid	15.08 ± 0.01	nd
14.78 ± 0.92	2	Protocatechuic acid	10.05 ± 0.02	nd
23.96 ± 0.23	3	Catechin	nd	8.08 ± 0.04
25.80 ± 0.23	4	Chlorogenic acid	17.10 ± 0.01	nd
37.02 ± 0.36	5	Rutin	nd	nd
37.71 ± 0.18	6	Glucose-containing quercetin	nd	10.05 ± 0.03
39.47 ± 0.19	7	Quercetin dihydrate	nd	13.07 ± 0.02
41.68 ± 0.65	8	Cinnamic acid	nd	nd

Hex: hexane extract; Eth-ace: ethyl acetate extract, Ref.: reference, Rt: retention time, nd: not detected.



(a) (b)

1: gallic acid; 2: protocatechuic acid; 3: catechin; 4: chlorogenic acid; 6: glucose quercetin; 7: quercetin dihydrate.

Figure 3. Chromatographic profile of the hexane (a) and ethyl acetate (b) extract.

3.2. Antioxidant Activities

The results of the antiradical activity of our extracts were expressed in millimole equivalent of ascorbic acid per gram of extract (Table 6) in reference to a standard curve ($y = 0.0522x + 0.056$, $R^2 = 0.9934$) and in inhibition's percentage of the free radical DPPH-. Therefore, the concentrations that inhibit 50% of the free radicals (IC_{50}) as well as the antioxidant activity index (AAI) are respectively consigned to

Table 6.

The ethanolic extract presented the highest concentration (37 ± 0.66 mmol EAA/g of sample) in ascorbic acid equivalent, with a low IC_{50} (2.5 ± 0.7 µg/ml), thus showing its strongest antioxidant power. Furthermore, although the hexanic extract presented the lowest concentration (7 ± 0.03 mmol EAA/g of sample), it is the hydroethanolic extract that recorded the highest IC_{50} (37.5 ± 0.06 µg/ml), thus showing its weak antioxidant potential.

Table 6. Equivalent content of ascorbic acid and IC_{50} of *D. ramosissimum* extracts.

Extraits	Content in ascorbic acid equivalent (mmol EAA/g of sample)	IC_{50} (µg/ml)	AAI
Hydro-ethanol	10.97 ± 0.02	37.5 ± 0.06	1.33 ± 0.09
Methanol	26 ±0.3	2.45 ± 0.14	20.40 ± 0.5
Hexane	7 ± 0.03	6 ± 0.08	8.33 ± 0.04
Ethyl acetate	11.75 ± 0.55	10.5 ± 0.15	4.76 ± 0.45
Ethanol residual	13.8 ± 0.05	4 ± 0.17	12.5 ± 0.1
Ethanol	37 ± 0.66	2.5 ± 0.7	20 ± 0.09

3.2.1. DPPH Radical Scavenging Test

The inhibition percentages vary depending on the extracts. Indeed, the highest percentage was obtained with the hydroethanolic extract, followed by the residual ethanolic extract, the methanolic extract and finally the ethanolic extract (Figure 4).

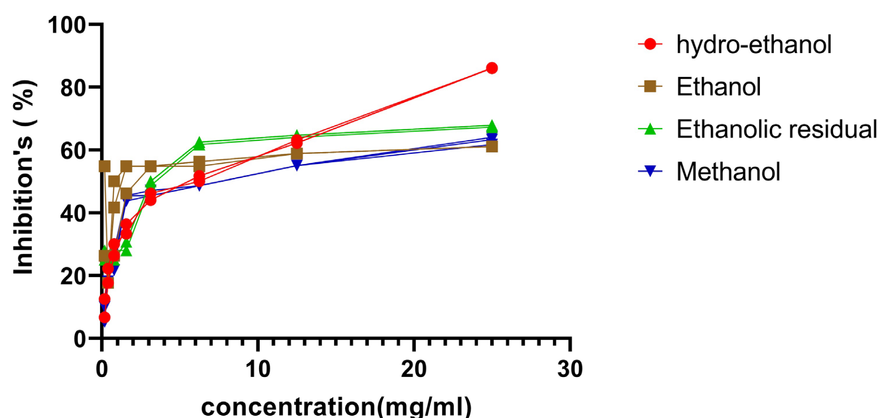


Figure 4. Variation in the percentage of inhibition in function of extract concentration.

3.2.2. Ferric Reducing/Antioxidant Power (FRAP)

The ferric ion reduction power was shown in Table 7. The results show that four out of six extracts tested show antioxidant activity using the FRAP method. The IC_{50} ranges from 1.1 mg/ml (ethanolic extract) to 7 mg/ml (methanolic extract), which shows that the ethanolic extract has a higher ferric ion reduction power than the other extracts. The analysis of variance shows that the interaction between the inhibitory power of extracts varies according to their concentrations. Indeed, the

percentage of inhibition of extracts varies significantly ($p = 0.0001$).

Table 7. Percentage of inhibition of *D. ramosissimum* extracts and IC_{50}.

	Percentage of inhibition (%)						
Extracts	BHT	Hydro-E	Eth	Eth R	Meth	Ethyl acetate	Hexane
25	78.16	86	61.11	67.27	61.70	-	-
12.5	63.39	62.162	58.82	64	55	-	-
6.25	57.29	51.72	56.25	61.70	48.57	-	-
3.125	56.55	44	54.83	48.57	45.45	-	-
1.56	44.23	36.36	54.84	28	45.45	-	-
0.78	36.79	26.34	41.66	28	21.74	-	-
0.39	15.56	17.65	17.64	18.18	25	-	-
0.18	12.14	6.67	26.32	25	5.26	-	-
IC_{50} (mg/ml)	**2.12**	**5.5**	**1.1**	**3.4**	**7**	-	-

3.3. *In Vitro* Anti-Inflammatory Activity

The *in vitro* anti-inflammatory effect of *D. ramosissimum* extracts is summarised in Table 8. Indeed, the highest inhibition percentages obtained were for hydro-ethanolic 92.99% ± 0.04%, ethanolic 92.55% ± 0% and residual ethanolic 92.85% ± 0.04% extracts. In addition, the analysis of variance showed a significant difference ($p < 0.05$) between the concentrations at 15.75 µg/ml. However, the hydro-ethanol extract was more active than the ethanol extract. This was confirmed by comparing their IC_{50} values. Indeed, the hydro-ethanol extract shows the strongest inhibition of protein denaturation (egg albumin). Diclofenac sodium (in the concentration range of 6.25 to 25 mg/ml) was used as the reference drug, which also showed a concentration-dependent inhibition of protein denaturation. The results showed that the high activity (94.55% ± 0.12%) of Diclofenac is obtained at 25 mg/ml.

Table 8. Inhibition percentage of Diclofenac and *Desmodium ramosissi mum* extracts.

Concentration		Inhibition percentage (%)						
Extraits (µg/ml)	Diclofenac (mg/ml)	Hydro-Ethanol	Ethanol	Ethanol R	Methanol	Ethyle acetate	Hexane	Diclofenac
1000	25000	92.99 ± 0.04	92.55 ± 0	92.85 ± 0.04	78.94 ± 0.06	86.54 ± 0.04	75.89 ± 0.08	94.55 ± 0.12
500	12500	91.10 ± 0.07	91.94 ± 0.04	91.79 ± 0.02	77.87 ± 0.04	85.48 ± 0.02	74.83 ± 0.02	89.10 ± 0.25
250	6250	94.54 ± 0.02	91.60 ± 0.04	90.72 ± 0.02	76.81 ± 0.21	84.41 ± 0.02	73.38 ± 0	87.23 ± 0.37
125	-	89.55 ± 0.04	87.64 ± 0.02	89.66 ± 0.04	75.74 ± 0.04	83.36 ± 0.02	72.32 ± 0.02	-
62.5	-	86.21 ± 0.06	87.45 ± 0.05	88.59 ± 0.04	74.68 ± 0.04	82.28 ± 0.02	71.63 ± 0.02	-
31.25	-	84.92 ± 0.04	87.11 ± 0.04	87.53 ± 0.2	73.61 ± 0.04	81.22 ± 0.04	71.33 ± 0.02	-
15.75	-	82.31 ± 0.07	86.35 ± 0.04	86.46 ± 0.6	72.55 ± 0.02	80.15 ± 0.02	70.27 ± 0.02	-
IC_{50} (µg/ml)		**<15.75**	**<15.75**	**<15.75**	**<15.75**	**<15.75**	**<15.75**	

From this table, it can be seen that the greatest inhibition percentages (92.99% ± 0.04%) obtained are the one of hydro-ethanol extract, ethanol extract (92.55% ± 0%) and residual ethanol extract (92.85% ± 0.04%).

3.4. Cytotoxicity Essay

The cytotoxicity of *D.ramosissimum* extracts has been evaluated on *Artemia salina*. After 24-hour of exposure to the tested extracts, larval mortality was investigated using a regression curve obtained from the number of surviving larvae in each tube according to the extract's concentrations. Figure 5 shows the number of surviving larvae with each extract after 24 hours of incubation.

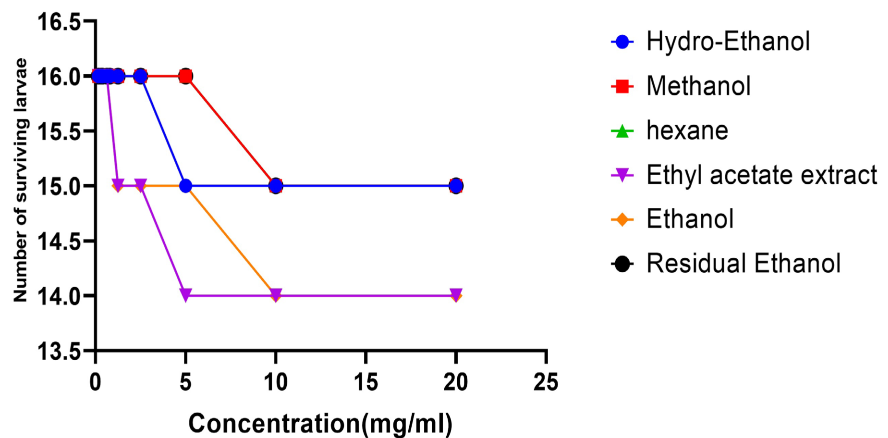


Figure 5. Variation in larval mortality of *Artemia salina* according to *D. ramosissimum* extracts.

4. Discussion

4.1. Extraction Efficiency of the Different Solvents Used

The yields of extracts obtained from *D. ramosissimum* are in decreasing order: 16% for the ethanol extract, 14.78% for the methanol extract, 13.4% for the hydro-ethanol extract, 5.2% for the acetic extract followed by 4.22% for the residual ethanol extract and 2.24% for the hexane extract. These results reveal that they would contain bioactive molecules that were extracted at various degrees depending on the type of solvent. These results also show that ethanol is the most suitable solvent for the extraction. Alcoholic solvents are capable of increasing the permeability of cell walls by facilitating the extraction of a greater number of polar's molecules of medium and low polarity [14]. Moreover, the maceration process, under stirring for a spread-out time (72 hours) and at room temperature, allows, respectively, the exhaustion of the solvent in active compounds and the prevention of their alteration or modification probably due to the high temperature. According to Wu *et al.* [15], the extraction yield could be explained by several factors: the chemical structure of the compounds, the maceration time, the temperature, the nature of the solvent and the storage conditions. For example, Dah-Nouvlessounon *et al.* [6] showed that the variability in extraction yields could be explained by the extraction

capacity of each solvent, which depends on the affinity of the solvent for the phytomolecules on one hand and the polarity of the solvent on the other hand. The drying of the plant was carried out in an open air in a shady environment in order to preserve the active ingredients from degradation by the hydrolases present in the fresh plant material. Drying the plant in the dark prevents chemical transformations such as isomerisation and degradation caused by ultraviolet radiation from sunlight [16]. Grinding and sieving have been carried out to recover as much fine powder as possible with a diameter smaller than 63 µm, because according to the work of Chen *et al.* [17], this diameter increases the contact surface of the solvent with the powder for better yield. Also, we used several solvents during extraction to ensure separation between polar and non-polar compounds [18]. In addition, extraction with several solvents allows better separation of compounds with antioxidant activity [19]. These extraction yields that we obtained are not consistent with the results of Maddi *et al.* [20], who found 26.37% for the ethanol extract and 33.32% for the acetate extract. These discrepancies could be due to several reasons, including the stage of maturity of the leaves collected [21], the extraction process, the geographical origin of the plant drug [22], the drying conditions of the leaves [23], the harvesting season and the quality of the solvents used. However, our results corroborate with those of Onzo *et al.* [24] who reported that the hexane extract has the lowest extraction yield, as in the present study.

4.2. Antioxidant Property of *Desmodium ramosissimum* Extracts

The antioxidant activity of the extracts is evaluated by two techniques, iron reduction (Ferric reducing antioxidant power or FRAP) and the scavenging of the free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH). The results of the DPPH test show that *D. ramosissimum* extracts have interesting antiradical properties that vary from one type of extract to another. The results showed that the equivalent ascorbic acid content of the ethanol (37 ± 0.66 mmol EAA/g extract) and methanol (26 ± 0.3 mmol EAA/g extract) extracts is quite high. This result could be explained by the fact that these extracts have a high reducing power and therefore contain molecules with a stronger electron-donating reducing potential than the other extracts. As a result, the reducing electron-donating power is dependent on the bioactive molecules. These results corroborate with those of Nounagnon *et al.* [25] who found the best ascorbic acid equivalent contents with the ethanol and methanol extracts of *Combretum adenogonium*. The results of the DPPH test show that at a concentration of 200 µg/ml, the ethanol, methanol and residual ethanol extracts of *D. ramosissimum* have respectively a scavenging power of the DPPH free radical at 84.79%; 78.95% and 80.05%, while at the same concentration, ascorbic acid, quercetin, BHA and BHT respectively inhibit 91.73%;74.8%; 65.95% and 72.54% of this free radical. These results show that *D. ramosissimum* extracts have low IC_{50} values, which means that they would be excellent antiradical agents. Comparing these values to those of standard antioxidants shows that these extracts are more active than BHT and BHA. This antioxidant property of *D. ramosis-*

simum would be linked to the presence of polyphenols such as flavonoids, tannins, and coumarins. These compounds are identified by Hooper *et al.* [26] in the leaves and roots of *D. ramosissimum*. The results of the qualitative and quantitative analyses carried out by HPLC have revealed that our extracts are rich in phenolic compounds, including gallic acid, protocatechic acid, catechin, chlorogenic acid, rutin, glucosed quercetin, quercetin dihydrate, and cinnamic acid. These results would explain the strong antioxidant activity observed in the plant.

The high levels of **quercetin dihydrate** and **chlorogenic acid** identified by HPLC provide a clear biochemical basis for the strong antioxidant and anti-inflammatory activities of *D. ramosissimum*. **Quercetin** is a potent flavonoid known for its free-radical-scavenging capacity and its ability to inhibit key inflammatory pathways such as NF-κB and COX-2 (Boots *et al.*, 2008; Li *et al.*, 2016) [27] [28]. Likewise, **chlorogenic acid** exhibits strong antioxidant effects through ROS neutralization and reduces inflammation by suppressing oxidative-stress-mediated signaling (Naveed *et al.*, 2018) [29]. The presence of these well-characterized compounds therefore supports the biological activities observed and strengthens the therapeutic relevance of this species.

Furthermore, considering the results of the antioxidant activity indexes (AAI) according to Scherer and Goddoy [9], we can conclude that apart from the hydroethanol extract, all the other extracts of *D. ramosissimum* have a very high DPPH radical scavenging power (AAI > 2). The action of these antioxidants is assumed to be due to their ability to donate hydrogen atoms or electrons derived mainly from the A-ring hydroxyl of flavonoids [30]. In addition, Albano and Miguel [31] reported that flavonoids and phenolic acids are more effective in scavenging free radicals after extraction by moderate or hydrophilic solvents. The effectiveness of the antioxidant (AH) increases if the strength of the A-H bond is low and the resulting (A) radical must be as stable as possible, which is the case for phenolic and flavonoid compounds, which are better electron or hydrogen donors [32]. Our results regarding inhibition percentages are comparable to those of Kabran *et al.* [33], who obtained 95% with *D. adscendens* extracts. *D. ramosissimum* extracts can protect the organism against the oxidative stress. Stress is a major contributor to the appearance of many pathologies such as cancers, cardiovascular diseases and degenerative pathologies of the nervous system [34]. Faced with this situation, scientific work has shown that natural antioxidants are more protective than synthetic antioxidants [35].

With regard to the determination of antioxidant activity by the FRAP method, the increase in iron reduction is proportional to the concentrations used. Except for the ethanol extract, all our extracts have higher IC_{50} than BHT. FRAP is based on the capacity of the extracts to reduce ferric iron Fe^{3+} to ferrous iron Fe^{2+}. The high activity of *D. ramosissimum* extracts might be due to their high content of polyphenolic compounds. The reducing power of these extracts would be due to the hydroxyl group in the phenolic compounds that can serve as electron donors. Therefore, antioxidants can be considered as reducing and inactivating oxidants

[36]. Some previous studies have also shown that the reducing power of a compound can serve as a significant indicator of its potential antioxidant activity [37]. Alcohol-based extracts are the most active, this is surely due to the composition of these extracts in polyphenolic substances and the synergy between them for a better antioxidant activity [38]. Several studies have shown that the hydroxyl groups in phenolic and flavonoid compounds are responsible for their antioxidant power [39]. These results are consistent with the results obtained in terms of HPLC, which revealed that our extracts are rich in phenolic compounds.

4.3. Anti-Inflammatory Activity

The extracts showed concentration-dependent inhibition of protein denaturation (egg albumin) over the entire concentration range from 15.75 to 1000 μg/ml. These results showed that the extracts have a good inhibition of protein denaturation compared to the reference molecule (Diclofenac sodium).

4.4. Larval Cytotoxicity

Referring to the toxicity scale established by Moshi *et al.* [13], the LD_{50} of extracts are above 0.1 mg/ml. At this value, the extract is considered to be non-toxic. Indeed, the extracts tested on *Artemia salina* were found to be non-toxic at the tested doses. However, the mortality of brine shrimp (*A. salina*) would increase by increasing the concentration of our extracts. Mayer *et al.* [40] came to the same conclusion in their study of several species of the genus *Euphorbia* with the *Artemia salina* larvae. The same results were found by Bapna *et al.* (2014) [41] with different extracts of *Citrus aurantifolia*.

This toxicity test showed that at a dose of 20 mg/ml, the extracts showed no toxicity on the cells of shrimp larvae. By definition, a substance is toxic if it causes alterations or disturbances in the functions of the living organism, leading to harmful effects. The most serious harm is the death of this organism [42] (Ramade, 1979). Also, according to Cowan *et al.* [43], the mechanism of toxicity could be linked to the inhibition of hydrolytic enzymes (proteases and carbohydrolase) or other interactions to inactivate microbial adhesins, transport and cell envelope proteins. The *Artemia salina* model has been suggested for the evaluation of cytotoxicity in pharmacology because cytotoxicity in shrimp larvae is correlated with 9 PS and 9 KB cells [44]; A-549 cells in lung carcinoma and HT-29 cells in colon carcinoma [45]. It is a test that is much more widely used in underdeveloped countries where traditional medicine plays a key role. The evaluation of this activity with *D. ramosissimum* can therefore serve as an indication in the choice of therapeutic doses.

5. Conclusion

Phytotherapy occupies a prominent place in developing countries despite the spectacular development of modern medicine. The development of this heritage involves the production of traditionally improved medicines, *i.e.*, effective medicines with proven safety and precise dosages. In this work, some biological activities of the

different extracts of *D. ramosissimum* were evaluated in order to highlight the potential of this plant. At the end of this study, it appears that *D. ramosissimum* has important antiradical activity. The assessment of the antioxidant power of the different extracts (ethanol, methanol, ethyl acetate, hexane, hydro-ethanol and residual ethanol) of *D. ramosissimum* using the DPPH free radical scavenging method showed that the ethanol extract is the most active, with an IC_{50} value of 2.5 ± 0.7 µg/ml, comparable to that of quercetin (IC_{50} = 2.05 ± 07 µg/ml). Furthermore, it should be noted that the extracts of this plant act at low doses and have low toxicity. With regard to these results, *Desmodium ramosissimum* is a promising source of antioxidant, anti-inflammatory compounds and has a preliminary safety profile. Further studies, including *in vivo* confirmation of its antioxidant capacity and the evaluation of additional biological activities such as hepatoprotective, antiviral, and anti-inflammatory effects, are necessary. It would also be essential to elucidate the specific molecular targets and receptors involved in its mechanisms of action in order to support the development of traditionally improved medicines at lower cost in Benin. Taken together, the marked bioactivity and minimal toxicity provide compelling evidence for the plant's relevance in developing standardized and reliable phytomedicines and simultaneously affirm the scientific validity of traditional healing knowledge.

Conflicts of Interest

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

References

[1] World Bank (2023) Urban Population (% of Total Population). Rural Population (% of Total Population)—Benin|Data.

[2] Toyigbénan, B.F., Durand, D., Haziz, S., Chimène, N., Wassiyath, M., Aklesso, N., *et al.* (2020) Phytochemical Screening and Antimicrobial Activity of *Desmodium ramosissimum*. *American Journal of Plant Sciences*, **11**, 51-63. https://doi.org/10.4236/ajps.2020.111005

[3] Buenz, E., Schnepple, D., Bauer, B., Elkin, P., Riddle, J. and Motley, T. (2004) Techniques: Bioprospecting Historical Herbal Texts by Hunting for New Leads in Old Tomes. *Trends in Pharmacological Sciences*, **25**, 494-498. https://doi.org/10.1016/j.tips.2004.07.003

[4] Jung Park, E. and Pezzuto, J.M. (2002) Botanicals in Cancer Chemoprevention. *Cancer and Metastasis Reviews*, **21**, 231-255. https://doi.org/10.1023/a:1021254725842

[5] Lien, A.P.H., Hua, H. and Chuong, P.H. (2008) Free Radicals, Antioxidants in Disease and Health. *International Journal of Biomedical Science*, **4**, 89-96.

[6] Dah-Nouvlessounon, D., Adoukonou-Sagbadja, H., Diarrassouba, N., Sina, H., Adjanohoun, A., Inoussa, M., *et al.* (2015) Phytochemical Analysis and Biological Activities of *Cola nitida* Bark. *Biochemistry Research International*, **2015**, Article ID: 493879. https://doi.org/10.1155/2015/493879

[7] N'Guessan, K., Kadja, B., Zirihi, G., Traoré, D. and Aké-Assi, L. (2009) Screening phytochimique de quelques plantes médicinales ivoiriennes utilisées en pays Krobou (Agboville, Côte-d'Ivoire). *Sciences & Nature*, **6**, 1-15. https://doi.org/10.4314/scinat.v6i1.48575

[8] Lamien-Meda, A., Lamien, C.E., Compaoré, M.M.Y., Meda, R.N.T., Kiendrebeogo, M., Zeba, B., *et al.* (2008) Polyphenol Content and Antioxidant Activity of Fourteen Wild Edible Fruits from Burkina Faso. *Molecules*, **13**, 581-594. https://doi.org/10.3390/molecules13030581

[9] Scherer, R. and Godoy, H.T. (2009) Antioxidant Activity Index (AAI) by the 2,2-Diphenyl-1-Picrylhydrazyl Method. *Food Chemistry*, **112**, 654-658. https://doi.org/10.1016/j.foodchem.2008.06.026

[10] Dieng, S.I.M., Fall, A.D., Diatta-Badji, K., Sarr, A., Sene, M., Sene, M., *et al.* (2017) Evaluation de l'activité antioxydante des extraits hydro-ethanoliques des feuilles et écorces de *Piliostigma thonningii* Schumach. *International Journal of Biological and Chemical Sciences*, **11**, 768-776. https://doi.org/10.4314/ijbcs.v11i2.19

[11] Ghumre, S.V. (2017) Assessment of *In- Vitro* Anti-Inflammatory Activity of *Cynodon dactylon* and Acyclovir Showing Synergistic Effect by Albumin Denaturation and Membrane Stabilization Assay. *Modern Approaches in Drug Designing*, **1**, 1-5. https://doi.org/10.31031/madd.2017.01.000506

[12] Kawsar, S.M.A., Huq, E. and Nahar, N. (2008) Cytotoxicity Assessment of the Aerial Parts of Macrotyloma Uniflorum Linn. *International Journal of Pharmacology*, **4**, 297-300. https://doi.org/10.3923/ijp.2008.297.300

[13] Moshi, M.J., Cosam, J.C., Mbwambo, Z.H., Kapingu, M. and Nkunya, M.H.H. (2004) Testing Beyond Ethnomedical Claims: Brine Shrimp Lethality of Some Tanzanian Plants. *Pharmaceutical Biology*, **42**, 547-551. https://doi.org/10.3109/13880200490897920

[14] Seidel, V. (2006) Initial and Bulk Extraction. In: Sarker, S.D., Latif, Z. and Gray, A.I., Eds., *Natural Products Isolation*, Humana Press, 27-46. https://doi.org/10.1385/1-59259-955-9:27

[15] Wu, G., Xiao, M., Yang, C. and Yu, Y. (2010) U2 snRNA Is Inducibly Pseudouridylated at Novel Sites by Pus7p and snR81 RNP. *The EMBO Journal*, **30**, 79-89. https://doi.org/10.1038/emboj.2010.316

[16] Jones, W.P. and Kinghorn, A.D. (2006) Extraction of Plant Secondary Metabolites. In: Sarker, S.D., Latif, Z. and Gray, A.I., Eds., *Natural Products Isolation*, Humana Press, 323-351. https://doi.org/10.1385/1-59259-955-9:323

[17] Chen, B., McClements, D.J., Gray, D.A. and Decker, E.A. (2012) Physical and Oxidative Stability of Pre-Emulsified Oil Bodies Extracted from Soybeans. *Food Chemistry*, **132**, 1514-1520. https://doi.org/10.1016/j.foodchem.2011.11.144

[18] Atmani, D., *et al.* (2011) Antioxidant Potential, Cytotoxic Activity and Phenolic Content of *Clematis flammula* Leaf Extracts. *Journal of Medicinal Plants Research*, **5**, 589-598.

[19] Spigno, G. and De Faveri, D.M. (2007) Antioxidants from Grape Stalks and Marc: Influence of Extraction Procedure on Yield, Purity and Antioxidant Power of the Extracts. *Journal of Food Engineering*, **78**, 793-801. https://doi.org/10.1016/j.jfoodeng.2005.11.020

[20] Maddi, A., Dettman, A., Fu, C., Seiler, S. and Free, S.J. (2012) WSC-1 and HAM-7 Are MAK-1 MAP Kinase Pathway Sensors Required for Cell Wall Integrity and Hyphal Fusion in Neurospora Crassa. *PLOS ONE*, **7**, e42374. https://doi.org/10.1371/journal.pone.0042374

[21] Sell, S. and Leffert, H.L. (2008) Liver Cancer Stem Cells. *Journal of Clinical Oncology*, **26**, 2800-2805. https://doi.org/10.1200/jco.2007.15.5945

[22] Kpadonou, D., Kpadonou-Kpoviessi, B., Glinma, B., Orou, A.S., Agbani, P., Gbaguidi, F., *et al.* (2022) Effects of the Chemical Composition of Essential Oils from Seven

Plants Used in Traditional Medicine in Benin on the Growth of Eleven Pathogenic Bacteria in Antimicrobial Control. *Journal of Pharmacognosy and Phytochemistry*, **11**, 23-31. https://doi.org/10.22271/phyto.2022.v11.i1a.14317

[23] Adeyemi, S.B., Ogundele, K.O. and Animasaun, M.A. (2014) Influence of Drying Methods on the Proximate Andphytochemical Composition of *Moringa oleifera* Lam. *Advances in Natural and Applied Sciences*, **2**, 1-5.

[24] Onzo, C.F., Azokpota, P., Dah-Nouvlessounon, D., Lehmane, T.H., Adjatin, A. and Baba-Moussa, L. (2016) Évaluation de l'activité antimicrobienne de quatre feuilles utilisées comme emballages dans l'artisanat agroalimentaire au Bénin. *Journal of Applied Biosciences*, **95**, 9015-9027. https://doi.org/10.4314/jab.v95i1.11

[25] Nounagnon, S.M., *et al.* (2016) Antimicrobial Activities of *Combretum micranthum* Extracts on *Staphylococcus aureus* Strains Isolated from Skin Infections and Some Reference Strains. *Asian Journal of Plant Science and Research*, **6**, 40-47.

[26] Hooper, A.M., Caulfield, J.C., Hao, B., Pickett, J.A., Midega, C.A.O. and Khan, Z.R. (2015) Isolation and Identification of Desmodium Root Exudates from Drought Tolerant Species Used as Intercrops against Striga Hermonthica. *Phytochemistry*, **117**, 380-387. https://doi.org/10.1016/j.phytochem.2015.06.026

[27] Boots, A.W., Haenen, G.R.M.M. and Bast, A. (2008) Health Effects of Quercetin: From Antioxidant to Nutraceutical. *European Journal of Pharmacology*, **585**, 325-337. https://doi.org/10.1016/j.ejphar.2008.03.008

[28] Li, Y., Yao, J., Han, C., Yang, J., Chaudhry, M., Wang, S., *et al.* (2016) Quercetin, Inflammation and Immunity. *Nutrients*, **8**, Article 167. https://doi.org/10.3390/nu8030167

[29] Naveed, M., Hejazi, V., Abbas, M., Kamboh, A.A., Khan, G.J., Shumzaid, M., *et al.* (2018) Chlorogenic Acid (CGA): A Pharmacological Review and Call for Further Research. *Biomedicine & Pharmacotherapy*, **97**, 67-74. https://doi.org/10.1016/j.biopha.2017.10.064

[30] Lee, E., Moon, B., Park, Y., Hong, S., Lee, S., Lee, Y. and Lim, Y. (2008) Effects of Hydroxy and Methoxy Substituents on NMR Data in Flavonols. *Bulletin of the Korean Chemical Society*, **29**, 507-510.

[31] Albano, S.M. and Miguel, M.G. (2011) Biological Activities of Extracts of Plants Grown in Portugal. *Industrial Crops and Products*, **33**, 338-343. https://doi.org/10.1016/j.indcrop.2010.11.012

[32] Naczk, M. and Shahidi, F. (2004) Extraction and Analysis of Phenolics in Food. *Journal of Chromatography A*, **1054**, 95-111. https://doi.org/10.1016/s0021-9673(04)01409-8

[33] Kabran, F.A., Okpekon, T.A., Roblot, F., Séon-Méniel, B., Leblanc, K., Bories, C., *et al.* (2015) Bioactive Phloroglucinols from Mallotus Oppositifolius. *Fitoterapia*, **107**, 100-104. https://doi.org/10.1016/j.fitote.2015.09.015

[34] Aruoma, O.I. (1999) Free Radicals, Antioxidants and International Nutrition. *Asia Pacific Journal of Clinical Nutrition*, **8**, 53-63. https://doi.org/10.1046/j.1440-6047.1999.00036.x

[35] Zhu, Y.Z., Huang, S.H., Tan, B.K.H., Sun, J., Whiteman, M. and Zhu, Y. (2004) Antioxidants in Chinese Herbal Medicines: A Biochemical Perspective. *Natural Product Reports*, **21**, 478-489. https://doi.org/10.1039/b304821g

[36] Siddhuraju, P. and Becker, K. (2007) The Antioxidant and Free Radical Scavenging Activities of Processed Cowpea (*Vigna unguiculata* (L.) Walp.) Seed Extracts. *Food Chemistry*, **101**, 10-19. https://doi.org/10.1016/j.foodchem.2006.01.004

[37] Jeong, H. (2004) RNase H2 of Saccharomyces Cerevisiae Is a Complex of Three Proteins. *Nucleic Acids Research*, **32**, 407-414. https://doi.org/10.1093/nar/gkh209

[38] Vermerris, W. and Nicholson, R. (2006) Phenolic Compound Biochemistry. Springer.

[39] Heim, K.E., Tagliaferro, A.R. and Bobilya, D.J. (2002) Flavonoid Antioxidants: Chemistry, Metabolism and Structure-Activity Relationships. *The Journal of Nutritional Biochemistry*, **13**, 572-584. https://doi.org/10.1016/s0955-2863(02)00208-5

[40] Meyer, B., Ferrigni, N., Putnam, J., Jacobsen, L., Nichols, D. and McLaughlin, J. (1982) Brine Shrimp: A Convenient General Bioassay for Active Plant Constituents. *Planta Medica*, **45**, 31-34. https://doi.org/10.1055/s-2007-971236

[41] Bapna, S., Ramaiya, M. and Chowdhary, A. (2014) Brine Shrimp Toxicity and *Invitro* Antimalarial Activity of Citrus Aurantifolia (Christm.) Swingle against Plasmodium Falciparum 3D7. *IOSR Journal of Pharmacy and Biological Sciences*, **9**, 24-27. https://doi.org/10.9790/3008-09512427

[42] Ramade, F. (1979) Ecotoxicologie. 2nd Edition, Masson.

[43] Cowan, M.M. (1999) Plant Products as Antimicrobial Agents. *Clinical Microbiology Reviews*, **12**, 564-582. https://doi.org/10.1128/cmr.12.4.564

[44] Pelka, M., Danzl, C., Distler, W. and Petschelt, A. (2000) A New Screening Test for Toxicity Testing of Dental Materials. *Journal of Dentistry*, **28**, 341-345. https://doi.org/10.1016/s0300-5712(00)00007-5

[45] Carballo, J.L., Hernández-Inda, Z.L., Pérez, P. and García-Grávalos, M.D. (2002) A Comparison between Two Brine Shrimp Assays to Detect *in Vitro* Cytotoxicity in Marine Natural Products. *BMC Biotechnology*, **2**, Article No. 17. https://doi.org/10.1186/1472-6750-2-17