ISSN: 0975-833X

Available online at http://www.journalcra.com

International Journal of Current Research
Vol. 14, Issue, 10, pp.22536-22543, October, 2022
DOI: https://doi.org/10.24941/ijcr.44109.10.2022

INTERNATIONAL JOURNAL OF CURRENT RESEARCH

RESEARCH ARTICLE

PHYTOCHEMICAL STUDY AND ANTIOXIDANT ACTIVITY OF HYDROETHANOLIC EXTRACTS OF *DESMODIUM RAMOSISSIMUM* G. DON (1832) (FABACEAE)

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ARTICLE INFO

Article History:
Received 20th July, 2022
Received in revised form
17th August, 2022
Accepted 19th September, 2022
Published online 30th October, 2022

Key words:

Desmodium Ramosissimum, Phytochemical Screening, Antioxidant Activity.

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ABSTRACT

Desmodium ramosissimum is a medicinal plants used in rural areas in Togo. The aim of this study is to contribute to a better knowledge of the biological activities of *D. ramosissimum*, with a particular focus on the antioxidant activity of its different parts. Different organs such as leaves and roots were collected to extract active compounds by maceration. Antioxidant activity was evaluated using three different methods: DPPH radical scavenging, molybdenum ion reduction and iron reduction (FRAP method). Results recorded with all these methods showed that the leaves had a better antioxidant activity. The phytochemical screening revealed the presence of major chemical groups such as total polyphenols, tannins, alkaloids and saponosides. The leaves having shown the best antioxidant activity, they should be favored in the interest of preserving biodiversity.

Citation: ***DOSSOU Bayi Reine, MADJALANI Hèzouwè, SOUHO Tiatou, KANABIYA Essodjolon et al.*** **2022.** "Phytochemical study and antioxidant activity of hydroethanolic extracts of *Desmodium ramosissimum* G. Don (1832) (Fabaceae)*". International Journal of Current Research*, 14, (10), 22536-22543.

INTRODUCTION

Free radicals are formed as a result of the oxidation of carbohydrates, the non-enzymatic glycation of proteins and their subsequent degradation. They are compounds characterized by an unbalanced electronic structure which gives them a great reactivity on organic constituents and cellular structures. They are an integral part of the proper functioning of the organism, but their excess can be harmful and would be at the origin of various pathologies (Aurousseau, 2002) linked to oxidative stress. Oxidative stress is indeed an imbalance between free radicals and antioxidant defenses resulting in the degradation of cellular components (Khial, 2017) such as lipids, proteins, carbohydrates and deoxyribonucleic acid (DNA). Recent works (Khial, 2017) have shown that oxidative stress is involved in many pathologies such as obesity, type 2 diabetes, atherosclerosis, cancers, infectious, bacterial and viral diseases. Indeed, all living organisms that consume oxygen produce free radicals that are physiologically eliminated by antioxidants. An antioxidant prevents or slows down oxidation by neutralizing free radicals.

On the other hand, synthetic antioxidant molecules on the market are currently controversial because of the potential toxicological risks they present (Bougandoura, 2013). Nowadays, natural products constitute an important new plant source of natural antioxidants active against many diseases (Hakim, 2014). Indeed, polyphenols are natural compounds widely distributed in the plant kingdom that have beneficial effects on health (Bougandoura, 2013).

Their role as natural antioxidants is of increasing interest for the prevention and treatment of cancer, inflammatory and cardiovascular diseases (Madjalani, 2021). Scientific research has been developed for the extraction, identification and quantification of these compounds from different sources such as medicinal plants. Among the plants used in therapy, we can mention Desmodium ramosissimum, an erect plant of the Fabaceae family whose merits have been well vented (Farid, 2018). Our study aims to evaluate the antioxidant activity of the hydro-ethanolic extracts of D. ramosissimum according to the DPPH free radical scavenging method, the FRAP iron reduction method and the molybdenum ion method.

MATERIAL AND METHODS

Plant material and preparation of the extract: The plant material consisted of *D. ramosissimum* plants collected in the prefecture of Agoè-Nyivé, 15 km from Lome (Togo). The plant was identified and deposited under the number TOGO15658 in the herbarium of the Faculty of Sciences of the University of Lomé. The leaves, stem bark and roots were then collected, dried, crushed and stored in jars protected from light and humidity. Fifty grams (50 g) of plant powder of the different organs of *D. ramosissimum* were macerated in 500 ml of an Ethanol/Water mixture (50/50, 70/30, v/v). The preparation was macerated at room temperature for 72 hours in the dark. After filtration on Whatman N°1 filter paper, the macerates were evaporated under vacuum to dryness using a rotary evaporator and then dried in the oven at 40 °C for 24 h. The dry extracts obtained were weighed and stored in tubes at a temperature of 4 °C and protected from light until use.

Pharmacological tools and experimental setup: Ethanol, DPPH, gallic acid, sulfuric acid, sodium hydrogen phosphate, ammonium molybdate, ascorbic acid. The absorbance was read using a UV-Visible spectrophotometer (METASH, UV-5200 PC) equipped with MetaSpec Pro data acquisition software. Standard methods were used, with applications involving a milligram balance, UV lamp, thermometer, vortex, micropipettes, beakers, flasks, volumetric flasks, test tubes, racks and a Bunsen burner.

Methods

Phytochemical screening of D. ramosissimum extracts

Qualitative phytochemical analysis of *D. ramosissimum:* The hydro-ethanolic extract of *D. ramosissimum* was dissolved in distilled water and filtered. The filtrate is used for the investigation of major chemical groups such as flavonoids, tannins, alkaloids, reducing compounds and saponins.

Determination of flavonoids: The 1:10 NaOH solution and dilute hydrochloric acid were used for the determination of flavonoids. Table I shows the protocol for flavonoid identification (Kim, 2003).

Table I. Identification protocol of flavonoids

REAGENTS	PREPARATIONS	REACTIONS
NaOH 1%	2 ml de filtrate + few drops of reagent	Yellow staining + HCl Colorless

Determination of alkaloids: The presence of alkaloids was determined by three different tests (Hakim, 2014). Table II shows the different tests for identification of alkaloids.

Table II: Protocol for the identification of alkaloids

REAGENTS	PREPARATIONS	REACTIONS
Dragendorff (Bismuth basic nitrate + acetic acid + water) (Bisaublimated iodine + potassium iodide + water)	Reagent + 2 ml of extract	Red precipitate
Bouchardart (Bisaublimated iodine + potassium iodide + water)	Reagent + 2 ml of extract	Yellow coloration
Wagner (Iodine + potassium iodide)	Reagent + 2 ml of extract	Reddish brown precipitate

Tannins: Three reagents were used for the identification of tannins. The protocol used is recorded in table III.

Research of saponins: The presence of saponosides was determined by the test of mousse (Kim, 2003). According to the experimental protocol, 0.5 g of extract was diluted in 2 ml of distilled water contained in a graduated test tube, total volume 15 ml. After shaking the solution for a period of 15 seconds, the solution was left to stand for 15 minutes. The formation of a persistent foam layer at least 1 cm thick shows the presence of saponosides in the samples studied.

Table III: Identification protocol of tannins

REAGENTS	PREPARATIONS	REACTIONS
Ferric chloride 1 %	2 ml filtrate + a few drops of reagent	- Blue, blue-black or black coloring => gallic tannins - Green or dark green color => catechic tannins
Lead acetate 10 %	2 ml filtrate + a few drops of reagent	Whitish or brownish precipitate
Copper sulfate	2 ml filtrate + a few drops of reagent + 2 drops of ammonia	Blue-green precipitate

Research of reducing compounds

Fehling test: The extracts were each dissolved in 5 ml of distilled water, then hydrolyzed with dilute hydrochloric acid (HCl) and neutralised with an alkaline solution. After addition of Fehling's liquor (reagent A: copper sulfate and reagent B: potassium hydroxide), the mixture was stirred well and then heated in a test tube with a Bunsen burner flame until a red precipitate appeared indicating the presence of reducing compounds (Mahesh, 2017).

Research of phytosterols or triterpenes: The extracts were treated with a few drops of sulfuric acid (1 M), then shaken and left to rest. The appearance of a golden-yellow color indicates the presence of triterpenes (Himour, 2016).

Research of coumarins: A volume of 2 ml of the solution of each extract was introduced into a test tube and to which was added 0.5 ml of a NaOH solution (10%). The mixture was heated in a water bath until boiling. After cooling, the tubes containing the heated solutions were observed under UV lamp at 365 nm. A fluorescent yellow coloration means that coumarins are present in the samples.

Identification of total carbohydrates: To 1 ml of aqueous extract was added 500 µL of Molisch reagent (α-naphthol dissolved in ethanol). After mixing, 1 ml of concentrated sulfuric acid is slowly added to the wall of the inclined test tube without mixing, to form a layer. A positive reaction is indicated by the appearance of a purple ring at the interface between the acid and the sugar solution (Elzagheid, 2018).

Research of cardiac glycosides: A volume of 2 mL of chloroform is added 1 mL of the aqueous solution of each plant powder, the appearance of a reddish-brown coloration after the addition of sulfuric acid (H_2SO_2) indicates the presence of cardiac glycosides.

Quantitative phytochemical analysis of *D. ramosissimum*

Determination of total phenols

Principle: Total phenols were determined according to the method described by Singleton et al. (1999). This method is based on a redox reaction in which, the hydroxyl group (-OH) of phenols is oxidized while the Folin Ciocalteu Reagent (FCR) is reduced, resulting in a decrease in its colorimetric properties. The content of total phenolics was determined by extrapolation on a calibration curve obtained from a gallic acid solution (Figure 1-A). The equation of the calibration curve obtained from successive dilutions of the gallic acid stock solution (200 mg/L) leads to the result corresponding to the content of total phenolic compounds, expressed in mg Gallic Acid Equivalent (GAE)/g dry extract.

•***Assay procedure and development of the calibration curve:*** To each test tube were added 200 µL of the sample to be assayed (gallic acid or 1 mg/mL extract) and 500 µL of Folin Ciocalteu reagent (diluted ½ in distilled water). After 5 minutes of reaction, 500 µL of sodium carbonate (20 g/L) was added. The volume of the previous mixture was made up to 4 mL with distilled water.

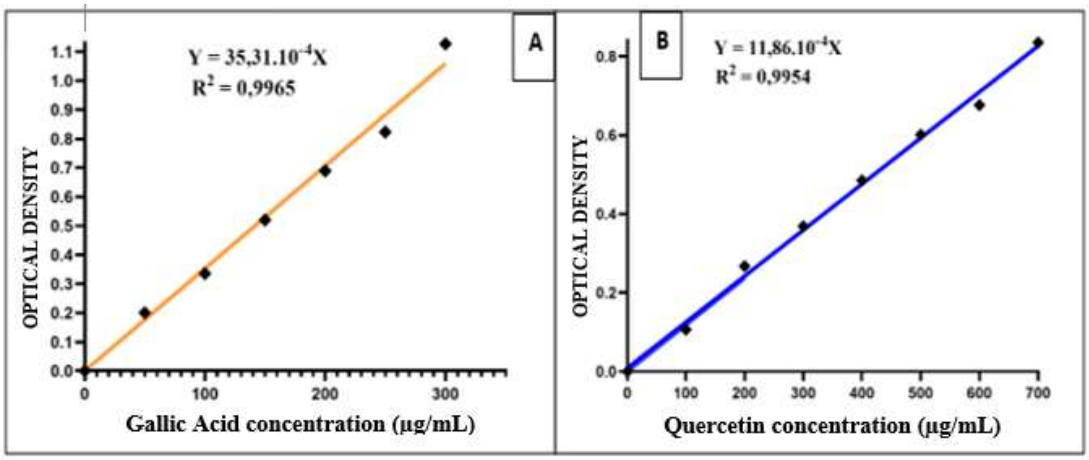

Figure 1. Standard curves of gallic acid (A) for the determination of total phenols and quercetin (B) for the determination of total flavonoids

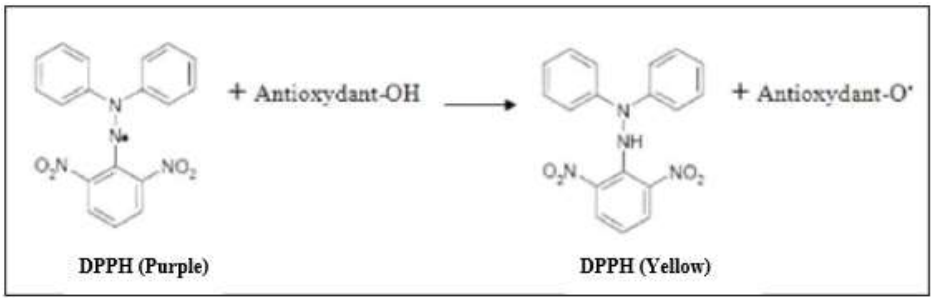

Figure 2. Chemical structure of the DPPH free radical

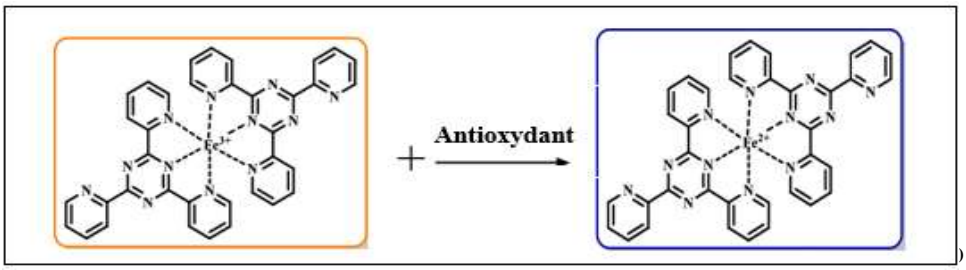

Figure 3. Mechanism of the transformation of the ferric tripyridyltriazine Fe (III)-TPTZ complex by an antioxidant (AH) in the FRAP test

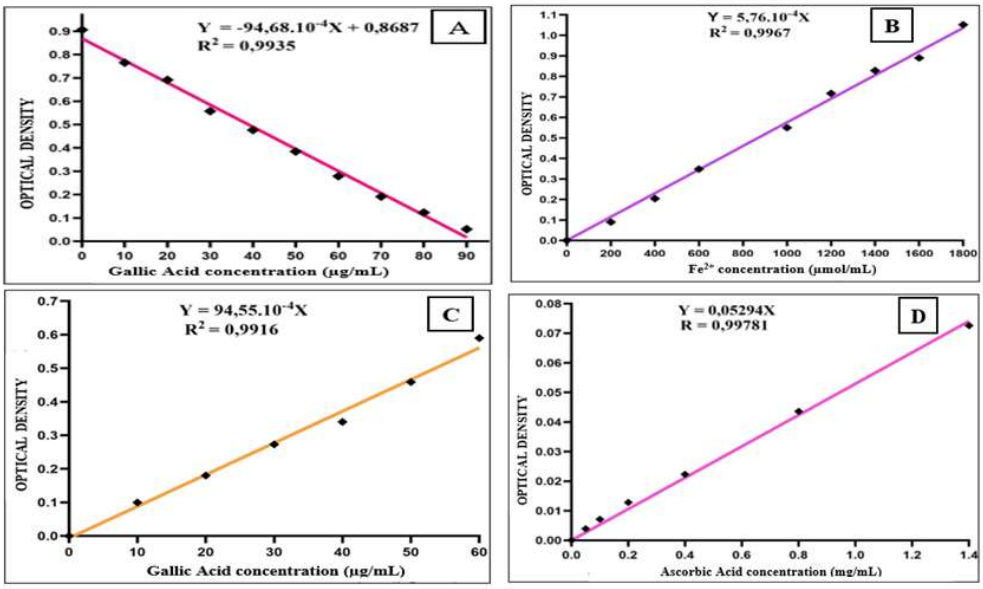

Figure 4. Standard curves for gallic acid (DPPH test) (A), ferrous ion (FRAP test) (B), gallic acid (RFP test) (C), Ascorbic acid (PM test) (D)

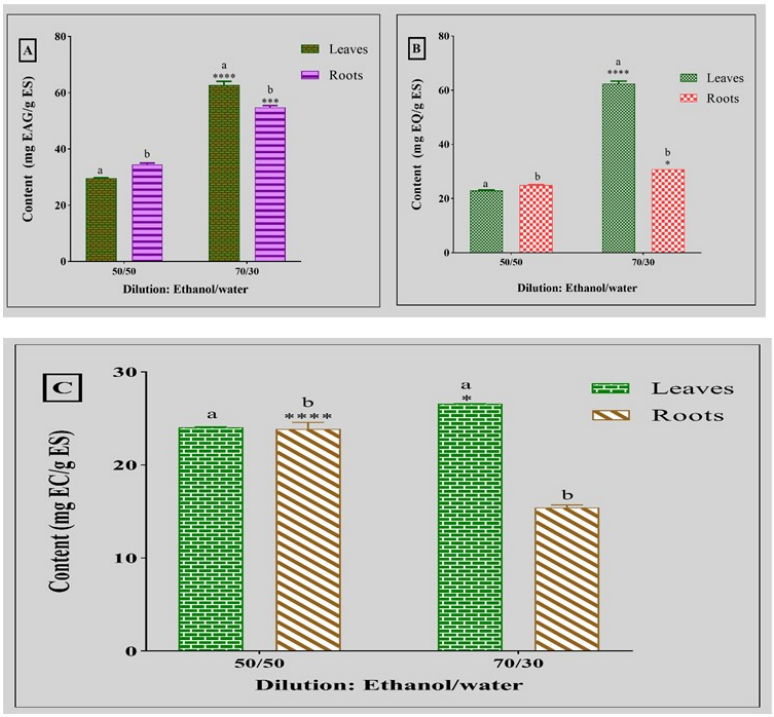

Figure 5: The contents of total phenols (A), total flavonoids (B), tannins (C) of leaf and root extracts of *D. ramosissimum*. The results are presented as the mean ± MSE of 4 values. Plots with the same letter, represent significantly different values. * p < 0.05; **** p < 0.0001

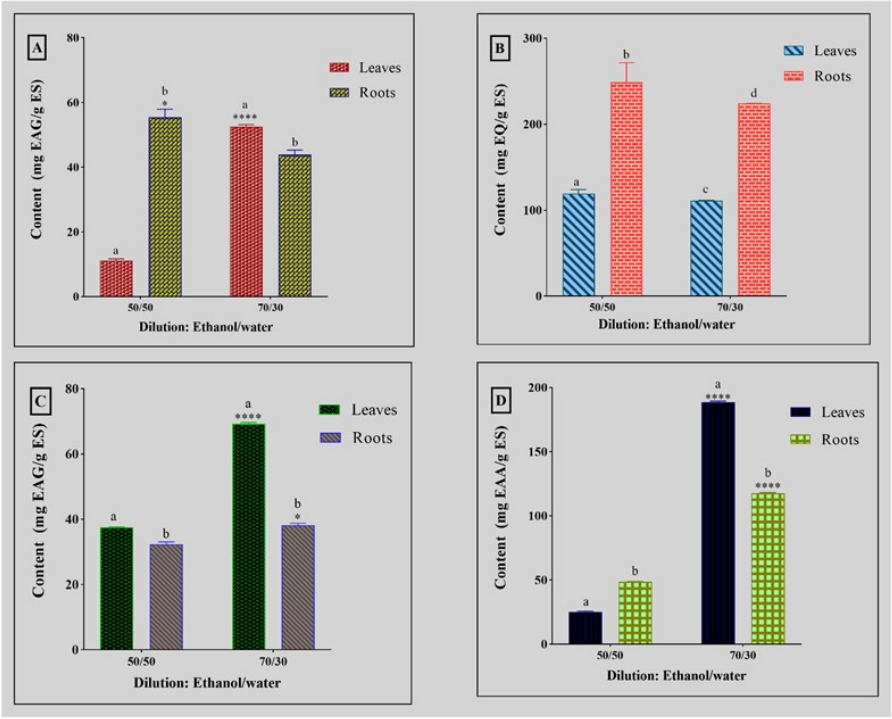

Figure 6. Content of anti-free radical compounds: DPPH(A), FRAP (B), RFP (C) and PM (D). Results are presented as the mean ± MSE of 4 values. Plots with the same letter, represent significantly different values. * p < 0.05; **** p < 0.0001

After shaking, the different solutions were incubated for 30 minutes at laboratory temperature in the dark. The optical density was read at 760 nm using a METASH UV-Visible spectrophotometer (UV-5200 PC) equipped with MetaSpec Pro data acquisition software against a blank obtained by mixing 500 µL of RFC, 500 µL of sodium carbonate, and 200 µL of distilled water.

The determination of proanthocyanidins or condensed tannins

The principle used: The determination of proanthocyanidins was performed by the butanol/hydrochloric acid (BuOH/HCl) method described by Makkar (1999). The assay reaction was performed in two steps. In the first step, flavan-3-ol polymers are hydrolyzed in a BuOH/HCl mixture. The hydrolysis is complete and releases catechin and epicatechin monomers. In the second step, these monomers are oxidized under the action of Fe^{3+} ion. The oxidation products give anthocyanidins or anthocyanidols which are quantifiable with a spectrophotometer at 540 nm. This is a colorimetric reaction in which the intensity of the red color is a function of the concentration of proanthocyanidins.

Table IV. Results of phytochemical screening of the different organs of *D. ramosissimum*

Chemical groups	Reagents	Leaves	Roots
Saponosides	Foam test	+	+
Flavonoids	NaOH : 1%	-	+
Total Carbohydrates	Molisch reagent + sulfuric acid	+	+
Reducing sugars	HCL + Fehling's liquor	+	+
Cardiac Glucosides	Chloroform + Sulfuric acid	+	+
Triterpenes	Sulfuric acid: 1 M	+	+
Coumarins	NaOH: 10 %	+	+
Alkaloids	Dragendorff	+	+
	Mayer	-	-
	Wagner	-	+
Tannins	Ferric chloride 1 %	+	+
	Lead acetate 10 %	+	+
	Copper sulfate	+	+

+: Presence of tested compounds; -: Absence of tested compounds

- ***Détermination procedure:*** In a test tube, were introduced 0.2 ml of a solution of ammoniacal iron sulfate ($NH_4Fe\ (SO_4)_2$: 20 g/L), then 7 ml of BuOH/HCl (95% - 5% : v/v) and 0.2 ml of extract (30 mg/ml) were added. The mixture was incubated in a water bath at 95°C for 40 minutes. A pink or red coloration was obtained. The proanthocyanidin concentrations of the samples were obtained by reading the optical density (OD) at a wavelength of λ = 550 nm using the METASH brand UV-Visible spectrophotometer (UV-5200 PC). To deduce the proanthocyanidin (TP) contents of the samples, expressed as mg Catechin Equivalent (CE)/g extract, the following formula established by Aksamit-Stachurska et al. (2008) was used to make the calculations:

$TP = \frac{OD}{0,280}$ with OD = 0.280, is equivalent to 1% catechin. Catechin was thus used as a standard molecule.

Determination of total flavonoids

- ***The principle:*** The determination of flavonoids was performed by the aluminium trichloride method described by Zhishen et al. (1999) with some modifications. This method is based on a complexation reaction during which aluminium trichloride forms a yellow complex with flavonoids and then the addition of sodium hydroxide forms a pink complex which absorbs in the visible range at a wavelength of 510 nm.

- ***Procedure for the determination and development of the calibration curve:*** To a glass hemolysis tube, containing 800 µL of extract, or standard, or distilled water for control, were added 240 µL of 5% $NaNO_2$. After 6 minutes of incubation at room temperature, 240 µL of 10% aluminum trichloride hexahydrate ($AlCl_3$, $6H_2O$) was added. After 6 minutes, l.6 L of 1 M sodium hydroxide NaOH was added. The absorbance was read immediately at 510 nm against the control. A calibration curve was performed with quercetin at different concentrations between 0 and 250 µg/mL under the same conditions as the samples (Figure 1-B).

The results obtained were expressed as mg Quercetin Equivalent (QE)/g dry extract.

Evaluation of the antiradical activity of the studied *D. ramosissimum* organs

Measurement of the antiradical activity by the DPPH radical method

- ***Principle:*** The 2,2-diphenyl-1-picrylhydrazyl (DPPH) which is an unstable free radical, by accepting an electron or a hydrogen radical, becomes a stable molecule. The effect of antioxidants on this radical is reflected by their ability to give it a hydrogen radical. This reduction capacity is determined by the decrease in absorbance at 517 nm, which is induced by the antioxidant. This is visualized by the color change from purple to yellow (Popovici et al., 2010).
- ***Procedure:*** In this test, antioxidants reduce DPPH with a purple color to a yellow compound, whose color intensity is inversely proportional to the ability of antioxidants present in the medium to donate protons (Talbi, 2015). The determination of the anti-free radical activity by DPPH assay was performed using the method described by Molyneux (Molyneux, 2003). A methanoic solution of DPPH˙ was prepared by dissolving 4 mg of it in 100 ml methanol. Then, to 250 µl of extract at a given concentration is added 3ml of the DPPH solution. The extracts of the different organs of *D. ramosissimum* as well as the reference (gallic acid) are tested at different concentrations ranging from 0 to 90 µg/ml, and then the absorbances were measured at 517 nm after 30 minutes of incubation in the dark. The results, determined from the calibration curve equation, are expressed as mg Gallic Acid Equivalent (GAE/g) of dry extract.

The FRAP test

Principle: It is based on the reduction of the ferric tripyridyltriazine complex $((F_e(III)TPTZ)_2)^{3+}$ to the ferrous tripyridyltriazine complex $((F_e(II)TPTZ)_2)^{2+}$ by an electron donating antioxidant (Figure 8). This reaction can also be monitored by measuring the change in absorption at 593 nm (Benzie, 1999).

Assay procedure and development of the calibration curve: The protocol used is that described by Nair et al.[20]. To test tubes containing 3 mL of freshly prepared FRAP solution (25 mL of acetate buffer + 2.5 mL of 10 mM Fe^{3+}-TPTZ prepared in 40 mM HCl solution + 2.5 mL of 20 mM $FeCl_{3\text{-}6H2O}$), 10 µL of sample was added. The reading was taken at 593 nm using the UV-Visible spectrophotometer, brand METASH (UV-5200 PC), against the blank after 10 min of incubation. A calibration curve was made from a concentration range (0 - 1800 µM) of an Iron sulfate solution ($FeSO_4$, $7H_2O$) dissolved in methanol (Figure 1-B). The result was expressed as µmol of $FeSO_4$ Equivalent/mg of dry extract.

Ferric ion reduction test of $K_3Fe\ (CN)_6$ (RFP test)

- ***Principle:*** This technique was developed to measure the ability of extracts to reduce ferric ions (Fe^{3+}) present in the $K_3Fe\ (CN)_6$ complex to Fe^{2+} (RFP test). The positive reaction is revealed by the yellow color change of ferric iron (Fe^{3+}) to blue-green color of ferrous iron (Fe^{2+}) (Jalal et al., 2019). An increase in absorbance corresponds to an increase in the reducing power of the extracts.

- ***Assay procedure and development of the calibration curve:*** The protocol used is the one described by karogözler et al. (2008) an 500 µL of the samples at different concentrations were mixed with 1.25 mL of a 0.2 M phosphate buffer solution (pH = 6.6) and 1.25 mL of a 1% potassium ferricyanide $K_3Fe\ (CN)_6$solution. This was incubated in a water bath at 50ºC for 20 min. Then, 1.25 mL of 10% TCA was added, and the tubes were centrifuged at 3000 rpm for 10 min. A volume of 1.25 mL of the supernatant was added to 1.25 mL of distilled water, and then 250 µL of a 0.1% $FeCl_3$ solution was added to the mixture as well. Absorbance readings were taken at 700 nm wavelength using a METASH UV-Visible spectrophotometer (UV-5200 PC) against a similarly prepared control by replacing the extract with ethanol. The results determined from the calibration curve equation (Figure 2-D), were expressed as mg Gallic Acid Equivalent (GAE/g) of dry extract.

Evaluation of the anti-free radical activity by the molybdenum ion method

- ***Principle:*** It is based on the reduction of the molybdate ion Mo^{6+} to molybdate ion Mo^{5+} by the antioxidant compounds contained in the extracts leading to the formation of a green complex of phosphomolybdate ($PMo_{12}O_{40}^{3-}$) which presents an absorption

maximum at the wavelength of 695 nm. The antioxidant capacity of the recipe constituted by the mixture was evaluated by the phosphomolybdate reduction method described by Prieto et al. (1999).

- ***Procedure:*** The phosphomolybdate reagent was prepared from a mixture of 90 mL of sulfuric acid (H_2SO_4:0.6 M), 5 mL of Sodium hydrogen phosphate (Na_2HPO_4: 0.1%) and 5 mL of ammonium molybdate (1%). To perform the test, 1 mL of extract (30 mg/ml) was added to 9 mL of phosphomolybdate reagent.

The solution was heated in a water bath at 95°C for 90 minutes and then allowed to cool to room temperature. Absorbances were read at a wavelength of 695 nm using a METASH UV-Visible spectrophotometer (UV-5200 PC) equipped with MetaSpec Pro data acquisition software. Ascorbic acid was used as standard. The equation of the calibration curve obtained from the successive dilutions of ascorbic acid allowed to determine the results in mg Ascorbic Acid Equivalent (EAA)/g of dry extract.

Statistical analysis of the data: The results were presented as the mean with the standard error of the mean (M ± SEM). They were processed using Graph Pad Prism 8 software (Graph Pad Software Inc. California) which was also used to construct the curves and histograms. Analysis of variance (ANOVA) followed by Dunnett's test was used to compare the means of the data from the treated spleens with those from the controls. Differences between results were considered significant at the 5% level ($p < 0.05$).

RESULTS

Phytochemical screening of D. ramosissimum organs used: Table IV presents the results of the qualitative phytochemical tests of the organs of *D. ramosissimum.* These tests revealed that the hydroalcoholic extract of the leaves of *D. ramosissimum* does not contain flavonoids contrary to the roots which contain them.
On the other hand these different organs contain tannins, saponosides and alkaloids.

Quantitative phytochemical study of the extracts of the different organs of D. ramosissimum used

Determination of total phenols: The total phenol contents of the hydro-ethanolic extracts of the different organs of *D. ramosissimum* (leaves and roots) were determined from the linear regression line (Y = 35.31 10-4 X; R2 = 0.9965) of the calibration curve of gallic acid. Thus, the total phenol contents obtained for a 50/50 Ethanol/Water mixture extraction in leaves and roots are 29.65±0.19 and 34.47±0.570 mg EAG/g dry extract, respectively. As for the extraction of the 70/30 Ethanol/Water mixture in leaves and roots, the contents are 62.72±1.310 and 54.80±0.6550 mg EAG/g dry extract, respectively. The comparative total phenol content of leaves and roots is shown in Figure 3-A.

Determination of flavonoids: The total flavonoid contents of the hydro-ethanolic extracts of the different organs of D. ramosissimum (leaves and roots) were determined from the linear regression line (Y = 11.86 10-4 X; R2 = 0.9954) of the calibration curve of quercetin. Thus, the total flavonoid contents obtained for the extractions of a 50/50 Ethanol/Water mixture are 23.05±0.1476 and 24.91±0.1815 mg EQ/g dry extract, respectively, in leaves and roots. As for the extraction of 70/30 Ethanol/Water mixture in leaves and roots, the contents are 62.45±0.9229 and 30.90±0.00 mg EQ/g dry extract respectively. The comparative total flavonoid content of the organs is shown in Figure 3-B.

Proanthocyanidin assay(PT): The condensed tannin contents of the hydro-ethanolic extracts of the different organs of D. ramosissimum (leaves and roots) expressed in mg Catechin Equivalent (CE)/g extract according to the following formula: $TP = \frac{OD}{0,280}$ with OD = 0.280, equivalent to 1% catechin. Thus, the proanthocyanidin contents obtained for the extractions of a 50/50 Ethanol/Water mixture in leaves and roots are respectively 24.07±0.0415 and 23.88±0.7085 mg EC/g dry extract. As for the extraction of the 70/30 Ethanol/Water mixture in leaves and roots, the contents are 26.59±0.0135 and 15.44±0.2615mg EC/g dry extract respectively. The comparative proanthocyanidin content of the organs is shown in figure 3-C.

Evaluation of the anti-free radical and antioxidant activity of D. ramosissimum leaf and root extracts: The antioxidant capacities of hydroethanolic extracts of leaves and roots of *D. ramosissimum*, determined by the DPPH● radical inhibition test and the molybdenum ion reduction test (PM) for the antiradical activity on the one hand, and on the other hand, by the $((Fe(III)TPTZ)_2)^{3+}$ ferric ion reduction test (FRAP) and the ferric ion reduction test present in the $K_3Fe(CN)^6$ complex (FRP) for the antioxidant activity, are represented by figure 6. The standard curves obtained for these four tests (Figure 2) indicate the accuracy of each test. The free radical scavenging activity of the DPPH● assay was 52.43 ± 0.7238; 11.16 ± 0.5454; 43.83 ± 1.459; 55.37 ± 2.573 mg EAG/g dry extract for the hydroethanolic extracts (70/30; 50/50: v/v) of the leaves and hydroethanolic extracts (70/30; 50/50: v/v) of the roots, respectively. With the PM test, the antiradical activity was 118.8 ± 0.615; 25.20 ± 0.500; 117.60 ± 0.500; 48.41 ± 0.450 mg EAA/g dry extract respectively for the hydroethanolic extracts (70/30; 50/50: v/v) of the leaves and hydroethanolic extracts (70/30; 50/50: v/v) of the roots. The evaluation of antioxidant power by the reduction of ferric ions of the $((Fe(III)TPTZ)_2)^{3+}$ complex resulted in antioxidant compound contents of 111.1 ± 0.3730; 119.3 ± 4.801; 224.3 ± 0.1472; 248.9 ± 22.69 mg EQ/g dry extract for the hydroethanolic extracts (70/30; 50/50: v/v) of the leaves and hydroethanolic extracts (70/30; 50/50: v/v) of the roots, respectively. With the method of reduction of ferric ions present in the $K_3Fe(CN)^6$ complex, the contents were 69.27 ± 0.420; 37.48 ± 0.220; 38.20 ± 0.585; 32.33 ± 0.715 mg EAG/g dry extract respectively for the hydroethanolic extracts (70/30; 50/50: v/v) of the leaves and hydroethanolic extracts (70/30; 50/50: v/v) of the roots.

DISCUSSION

The results of the phytochemical screening presented in Table I show that leaves and roots of *D. ramosissimum* contain the same phytochemical groups such as alkaloids; reducing compounds; tannins; free quinones, triterpenes, saponins, total carbohydrates and coumarins except for flavonoids, which are absent in leaves. This could be explained by the same type of solvent used. These results are in agreement with those obtained by Djoumbissie et al. [24]who, in their phytochemical study carried out on the methanolic extract of *D. ramosissimum*, isolated nine secondary metabolites including polyphenols; tannins; triterpenes, saponins, coumarins, sterols [5].Recent studies have shown that plants rich in phenolic compounds such as tannins, flavonoids and saponosides are known for their antioxidant, antimicrobial, antiviral, hypocholesterolemia and hepato-protective properties (Lee, 1995). The presence of its different chemical groups in the leaves of *D. ramosissimum* is then a major asset and justifying the therapeutic use indeed, *D. ramosissimum* is a plant intervening in traditional remedies in order to relieve or cure pathologies such as convulsions, diarrhea, malaria and gastrointestinal infections (Rabearivony, 2015). It is known to have hepatoprotective and immunostimulant properties. In herbal medicine, the aerial parts, stems and leaves are used more. According to figure 3, the contents of total phenols, flavonoids and condensed tannins of the four different hydroethanolic extracts obtained varied in relation to the different organs used and the proportion of the constituents of the hydroethanolic solvent. It should be noted that the content of total phenols, flavonoids and condensed tannins of the hydro-ethanolic extracts in the proportions (70/30; v/v) are significantly different ($p<0.001$) compared to the hydro-ethanolic extracts in the proportions (50/50; v/v). The overall results show that the leaf extracts in the proportions (70/30; v/v) have higher contents of polyphenols, flavonoids and condensed tannins compared to the root extracts in the same proportions of hydroethanolic solvent (70/30; v/v). This could

be explained by the high solubility of phenolic compounds of the leaves in the solvent used. The efficiency of the four hydroethanolic extracts of D. ramosissimum leaves and roots to reduce the different free radicals used depended on both the proportion of the hydroethanolic solvent constituents used and different organs of D. ramosissimum. Moreover, the contents of anti-free radical compounds in hydro-ethanolic extracts in the proportions (70/30; v/v) are significantly different ($p<0.001$) compared to hydro-ethanolic extracts in the proportions (50/50; v/v), either by DPPH test or PM, FRAP and FRP tests (Figure 6). This could be due to the physicochemical properties of the compounds contained in these extracts [17]. These interesting antioxidant activities of D. ramosissimum leaves and roots would probably be due to the presence of total phenols, flavonoids, tannins and pigmented constituents known for their antioxidant activity[25]. According to Kumaran and Karunakaran (Kumaran, 2007), the reducing power of a compound can serve as a significant indicator of its potential antioxidant activity. According to the work carried out by Hinneburg et al. (2006), who highlighted that the reduction of the DPPH● radical, molybdenum ions and ferric ions is correlated by the content of phenolic compounds, of the tested material.

These anti-free radical activities are probably due to the phenolic constituents. It has been shown that phenolic compounds, which are generally flavonoids, stilbenes, phenolic acids and tannins, have remarkable antioxidant properties. The chemical structure, number and distribution of hydroxyl groups of these phenolic compounds can scavenge, neutralize free radicals and inhibit enzymes responsible for free radical formation (Verma, 2009). According to Manach et al.[25].phenolic compounds are able to modulate the activity of certain enzymes and modify the behavior of several cellular systems, suggesting that they could exert a multitude of biological activities, including significant cardioprotective, antioxidant, anti-hepatotoxic, anti-allergic, anti-inflammatory, anti-ulcer and even anti-tumor properties. Indeed, leaves rich in anti-free radical and antioxidant compounds are promoters of cardiovascular, anticancer and reproductive health.

CONCLUSION

The study of the antioxidant activity of extracts from *D. ramosissimum* species, according to the iron reduction method, the DPPH free radical scavenging method and the molybdenum ion method showed that the four hydro-ethanolic extracts have a significant antioxidant activity and antiradical capacity, concomitantly with their content of phytochemical compounds. The principal component analysis showed that this activity is more correlated to phenolic compounds.

The use of synthetic antioxidant molecules is currently questioned because of potential toxicological risks. From now on, new plant sources of natural antioxidants are sought with beneficial properties on health. These extracts could therefore constitute an alternative to certain synthetic additives but they are raw extracts containing a large number of different compounds. It is therefore very likely that they contain compounds which, once purified, may have a greater activity. Further research is needed to identify, isolate and purify these constituents.

Sources of Support : None

Declaration of Competing Interest : The authors report no declarations of interest.

Author Contributions

Author Contributions: Conceived and designed the experiments: DOSSOU Bayi Reine, MADJALANI Hèzouwè and SOUHO Tiatou; Analyzed the data: MADJALANI Hèzouwè, KANABIYA Essodjolon and PAKOUSSI Tcha; Wrote the paper: DOSSOU Bayi Reine, MADJALANI Hèzouwè, SOUHO Tiatou, KANABIYA Essodjolon and PAKOUSSI Tcha; Contributed reagents/materials/analysis tools: PAKOUSSI Tcha, NABEDE Aklesso and METOWOGO Kossi

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