PHYTOCHEMICAL CHARACTERIZATION AND *in-vitro* ANTIOXIDANT ACTIVITY OF *Guiera senegalensis* (COMBRETACEAE) LEAF EXTRACTS

ABSTRACT

Guiera senegalensis is a plant of the Combretaceaefamily, found in West Africa and Central Africa. It has many properties such as antibacterial, antifungal, anticancer, antioxidants, which are the subject of this study. The objective of this study was to realize a qualitative and quantitative phytochemical screening, evaluation of the antioxidant activity of the plant leaf extracts. Plant extractions was done in accordance with the methods described by Fonmboh andcollaborators on the leaves of the plant; a qualitative phytochemical screening according to the methods described by Shaik and*al* quantitative phytochemical screening consisting of colorimetric dosage and finally the antioxidant activity was explored *in-vitro* by the Folin-Ciocalteu method and by the phosphomolybdenum method.

This study revealed the presence of primary metabolites such as carbohydrates and proteins but also secondary metabolites such as alkaloids, flavonoids and coumarins. Quantification analysis demonstrated a significant concentration of these metabolites depending on the extract. The *in-vitro* antioxidant activity demonstrated an inhibition percentage of the order of 70.96 ± 0.58% in the extract obtained by hydro-ethanolic maceration.

INTRODUCTION

Oxidative stress is a condition caused by an imbalance between oxidizing agents and antioxidants in an organism either by an increase in the quantity of oxidant or by a deficit in the functioning of the antioxidant system [1]. Oxygen is a substance involved in many different functions of organisms but can be the cause of oxidative stress. Among the reactive oxygen species (ROS) we find radicals (reactive oxygen derivatives) such as $NO^{\bullet}$, $RO^{\bullet}/ROO^{\bullet}$, $O_2^{\bullet}$, $HO^{\bullet}$, $NO_2^{\bullet}$, $CO_3^{\bullet}$. And non-radical substances such as H_2O_2, ONOO-, ONOOH, HOCl, O_2. These substances are not always harmful; in some cases, they can be protective. This is the case of the NO radical. It can react with lipid free radicals LO. (Lipid oxy) and LOO. (peroxy radical) terminating the lipid peroxidation reaction [2]. This would mean that free radicals are not fundamentally pathological but rather constitute signalling molecules and it would be pathological when it accumulates in the organism and interact with lipids, proteins, nucleic acids and could cause damages at the neurological or psychological level or on other biological systems [3] hence the interest in the search for exogenous antioxidant substances.

Secondary metabolites, unlike primary metabolites, are substances produced by plants contributing directly to various processes such as survival, defence against predators, attraction of pollinator's agents [4]. Among these secondary metabolites, we find alkaloids, flavonoids, tannins and stilbenes. This has demonstrated numerous pharmacological properties such as antibacterial, antifungal, anticancer and antioxidants [5].

Guiera senegalensis is a plant of the Combretaceae family found in the tropical regions of West and Central Africa such as Senegal, Mali, and Cameroon. It is use in traditional medicine to treat liveraffections, respiratory, antihypertensive, antipyretic and antimalarial conditions. This plant appears in the form of a bush [6]. In this study, we will focus on the antioxidant property of *Guiera senegalensis* leaf extract.

METHODOLOGY

Treatment of plant material and preparation of extracts

The plant material consisted of *Guiera senegalensis* leaves. Identification of the species was done by a taxonomistat the National Herbarium of Cameroon in comparison with herbarium specimen No. 14902 /SRF.cam. The leaves *of G. senegalensis* were treated as follows:

Drying of the leaves: the leaves were dried at room temperature away from sunlight to prevent any likely photodecomposition effect of the metabolites it contained. The grinding of the dried leaves into powder was done with an electric robot grinding machine. The preparation of the crude leaf extracts was done in accordance with the methods described by Fonmboh et al in 2020 [7]

Phytochemical screening

Qualitative phytochemical screening

The qualitative phytochemical screening consisted of the precipitation and colorimetric methods described by Shaikh et al [8]

Identification tests for phenolic compounds: The identification of phenolic compounds consisted of carrying out two separate identification tests, the ferric chloride test and the lead acetate test:

- Ferric chloride test: Aqueous solution of plant extract + a few drops of 5% ferric chloride solution. There was appearance of a greenish black/bluish black colouring

- Lead acetate test: dissolution of plant extract in 5mL of distilled water + 3mL of a 10% lead acetate solution. A white precipitate was formed.

Tannin identification test: The different tannin identification tests, namely the gelatine test; the Braymer test; the 10% NaOH test; the bromine water test; the lead acetate test; the phenazone test; The Mitchell test was performed as follows:

- Gelatine test: dissolution of plant extract in 5mL of distilled water + 1% gelatine solution + 10% NaCl. The positive reaction resulted in the appearance of a white precipitate

- Braymer test: 1mL of a filtrate of 3mg of extract powder boiled in 50mL of distilled water for 3 minutes + 3mL of distilled water + 3 drops of 10% ferric chloride solution. The positive reaction resulted in the appearance of a blue-green colour.

- 10% NaOH test: 0.4mL of plant extract + 4mL of 10% NaOH + shake. There wasformation of an emulsion (Hydrolysable tannins)

- Bromine water test 10 ml of bromine water + 0.5 mg of plant extract. The positive reaction results in a discoloration of the bromine (reddish yellow)

- Lead acetate test 1mL of a filtrate of a small quantity of extract boiled in 5mL of 45% ethanol for 5 min and cooled + 3 drops of lead acetate solution. A creamy gelatinous precipitate was formed.

Cardiac glycoside identification test: The cardiotonic glycoside identification test used was that of Keller-Killani:

- Keller-Killani test: 1mL filtrate of the extract + 1.5mL of glacial acetic acid + 1 drops of ferric chloride + concentrated H_2SO_4. We observed a blue colouredsolution.

Mucilage identification test: The mucilagewas identified by carrying out the alcohol test:

- Alcohol test: Dissolve 100mg extract in 10mL of distilled water + 25mL of absolute alcohol (while stirring). Formation of a white or flaky precipitate.

Quinone identification test: The test used for the identification of quinones was that with concentrated HCl:

- Concentrated HCl test: plant extract + concentrated HCl. Observation of a green colour

Terpenoid identification test: the terpenoid identification test, their procedure and the observed result:

- 2ml chloroform + 5mL of plant (evaporated in a water bath) + 3mL concentrated H2SO4 (boiled then evaporated in a water bath) + 3mL concentrated H_2SO_4 (boiled in a water bath). The observed result was the appearance of a grey coloured solution

Alkaloid identification test: Four identifications tests were carried out, the Hager test; the Mayer test; the Wagner test; the tannic acid test:

- Hager test: Some mL of 50 mg of extract (free of solvent) is mixed with a few mL of diluted and filtered HCl + 1-2 mL of Hager reagent. The reaction was positive when a creamy white precipitate appeared.

- Mayer/Bertrand/Valser test: Some mL of 50mg of extract (free of solvent) is mixed with a few mL of diluted and filtered HCl + 1-2 drops of Mayer's reagent. The reaction was positive when a creamy white/yellow precipitate appeared.

- Wagner test: Some mL of 50mg of extract (free of solvent) was mixed with a few mL of diluted and filtered HCl + 1-2 drops of Wagner reagent. The reaction was positive when a red/brown precipitate appeared.

- Tannic acid test: Acidified extract + 10% tannic acid solution the reaction was positive when a buff-coloured precipitate appeared.

Flavonoid identification test: identification using the alkaline reagent test:

- Alkaline reagent: 1mL of extract + 2mL of a 2% NaOH solution (+ a few drops of diluted HCl). An intense yellow colour which disappeared when diluted acid/plant extract + 10% ammonium hydroxidewas added; yellow fluorescence appeared

Quantitative phytochemistry screening

- **Quantitative analysis of total polyphenols**: Folin-ciocalteu reagent described in1965 by Singleton and Rossi. The reagent consisted of a mixture of phosphotungstic acid (H3PW12OU4O) and phosphomolybdic acid (H3PMo12O4O). It was reduced during the oxidation of phenols to a mixture of blue oxides of tungsten (W8O23) and molybdenum (Mo8O23). The colouring produced, the maximum absorption of which was between 725 and 760 nm, was proportional to the quantity of polyphenols present in the plant extracts [9].

- **Quantitative analysis of total flavonoids**: Aluminium chloride forms stable acid complexes with the C-4 ketone group and with the C-3 or C-5 carbon hydroxyl group of flavones and flavonols. Additionally, aluminium chloride forms labile acid complexes with

ortho-dihydroxyl groups in the A or B ring of flavonoids, resulting in the formation of a pink colourmeasured at 510 nm [10].

- **Quantitative analysis of total flavonols**: The sample containing flavonols results in the formation of green colour when reacted with aluminium chloride and sodium acetate, measurement at 440 nm in a spectrophotometer UV-Vis [11].

- **Quantitative analysis of carbohydrates**: the reaction of picric acid with glucose producing Picramic Acid of orange or brown colour. The wavelength was measured at 570 nm made it possible to quantify the carbohydrates in the extracts [12]

- **Quantitative analysis of total proteins**: using the Lowry method complementary to that of Biuret . Indeed, the protein first reacts with an alkaline cupric reagent (Gornall reagent of the biuret method) then a second reagent, called phosphotungstomolybdic reagent (Folin-Cioccalteu reagent), is added. It is composed of a mixture of sodium tungstate and sodium molybdate dissolved in phosphoric acid and hydrochloric acid. This reagent allows the reduction of aromatic amino acids (tyrosine and tryptophan) leading to the formation of a dark blue coloured complex whose absorbance will be measured between 650 and 750 nm [13].

- **Quantitative analysis of total tannins**: The technique for measuring condensed tannins by the Folin-Ciocalteu method is based on the reduction of phosphomolybdic and tungstic acid in an alkaline medium, in the presence of tannins to give a blue colour whose intensity was measured between 640 and 760 nm [14].

- **Quantitative analysis of total alkaloids**: The alkaloid, in contact with concentrated sulfuric acid and potassium dichromate, develops a violet line which turned blue then green, so the maximum absorption was proportional to the intensity of the colour developed was of 650nm [15].

Evaluation of antioxidant activity

Evaluation by the Foli-Ciocalteu method: this method is based on the transfer of electrons in an alkaline medium from phenolic compounds and all other reducing species to molybdenum, forming blue complexes which can be detected by spectrophotometry at 750-765nm [9]

Total Antioxidant Capacity using Phosphomolybdate: by dosing Phosphomolybdate. This method was based on the reduction of Mo(VI) to Mo(V) by extracts forming a green Mo(V) phosphate complex under acidic conditions [16].

RESULTS

Extraction yield

The extraction of the powder from the leaves of *Guiera senegalensis*showed maximum extraction with the hydro-ethanolic extract, followed by the aqueous extract obtained by infusion, the aqueous extract obtained by maceration, the aqueous extract obtained by decoction showing the lowest yield. The figure 1 illustrates the different yields depending on the solvent and the method used

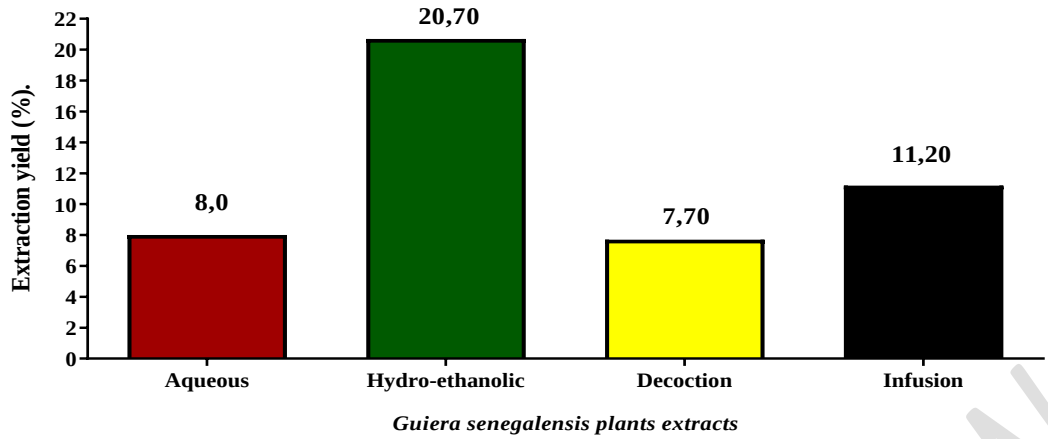

Figure 1. The different yields depending on the solvent and the method used

Qualitative phytochemical screening

The qualitative phytochemical evaluation allowed the identification of numerous compounds in the plant extracts such as proteins, carbohydrates, vitamin C and the absence of lipids and carotenoids as primary metabolites. Table 1 illustrates the primary metabolites identified in plant extracts:

Tablel : Primary metabolites in the extracts

Metabolites	Reagents	Aqueous	Hydro-ethanolic	Decoction	Infusion
Lipids	Ethanol + H_2O	-	-	-	-
	$CuSO_4$ + NaOH	-	-	-	-
Proteins	Biuret	+	+	+	+
	Folin	+	+	+	+
carbohydrates	Picric acid + NaOH	+	+	+	+
	Fehling's solution	+	+	+	+
Vitamine C	H_2SO_4 +DNPH	+	+	+	+
Carotenoïds	HCl + H_2SO_4	-	-	-	-

Evaluation of secondary metabolites

The evaluation of secondary metabolites indicated the presence of polyphenols, alkaloids, gallic and catechic tannins, flavonoids, saponosides, coumarins, chalcones, betacyanes, cardiac glycosides, flavonols and flavones (table 2). We observed the absence of resins, oxalates, anthraquinones, anthracyanins, phlobotannins, anthocyanins, steroids and quinones as secondary metabolites. Table 2 illustrates the secondary metabolites identified in the plant extracts:

***Table2** : Secondary metabolites presents in extracts*

Metabolites	Reagents	Aqueous	Hydro-ethanolic	Decoction	Infusion
	$FeCl_3$	+	+	+	+
Polyphenols	Lead acetate	+	+	+	+
	Wagner	+	+	+	+
	Hager	+	+	+	+
Alkaloids	Valse-Meyer	+	+	+	+
	Tannic acid	+	+	+	+
	$NaOH + H2SO_4$	+	+	+	+
Flavonoids	$AlCl_3$	+	+	+	+
Total Tannins	NaOH + Copper	+	+	+	+
Cathechic Tannins	Stiasny reagent	+	+	+	+
Gallic Tannins	sodium acetate + $FeCl_3$	+	+	+	+
Mucilage	Ethanol	+	-	+	+
Saponosides	Foam	+	+	+	+
Coumarin	$FeCl_3 + HNO_3$	+	+	+	+
Oxalates	Acetic acidacétique	-	-	-	-
Resins	Acetic acid + H_2SO_4	-	-	-	-
Chalcone	Ammonia	+	+	+	+
Anthraquinone	Ammonia	-	-	-	-
Anthracyanine	NaOH + heat 5 min	-	-	-	-
Phlobotanins	HCl + heat	-	-	-	-
Betacyanes	NaOH heat	+	+	+	+
Anthocyanes	$NH_4OH + H_2SO_4$	-	-	-	-
Glucosides cardiotonique	Keller-killani	+	+	+	+
Stéroïdes	Liberman	-	-	-	-
Quinones	H_2SO_4	-	-	-	-
Flavonols et flavones	$AlCl_3$	+	+	+	+

(+) presence, (-) Absence

Quantitative phytochemical screening

Quantitative estimation of carbohydrates

The distribution of carbohydrates in the different plant extractsshowed the hydro-ethanolic extract with the greatest concentration.Followed by the decoction extract with a significant decrease with a p-value < 0.001, the infusion with a p-value < 0.001 and a reduction in the concentration of the infusion compared to the decoction with a p-value < 0.01. Aqueous extract obtained by maceration, presenting the lowest concentration compared to the hydro-ethanolic extracts and the decoction with a p-value < 0.001 as indicated in figure 2.

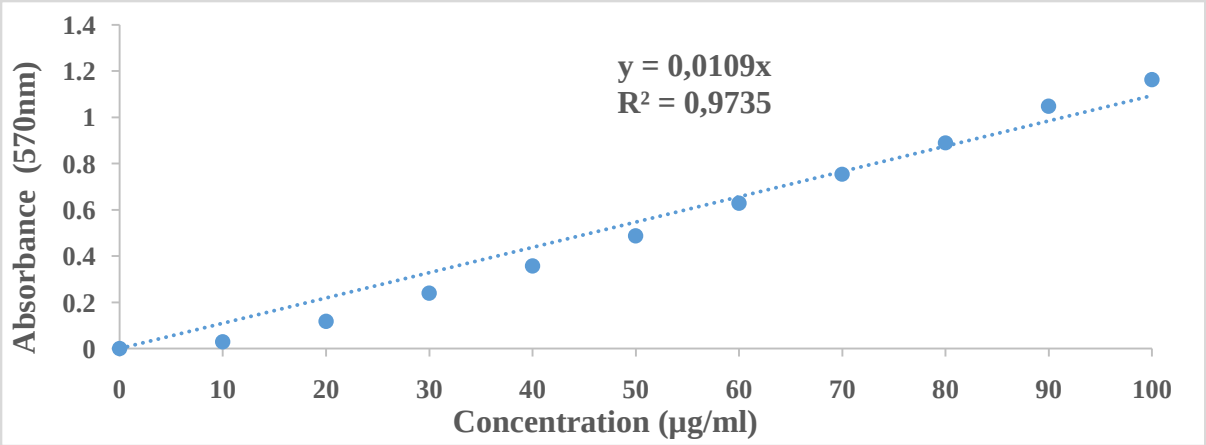

Figure 2 *: Calibration curve of glucose.*

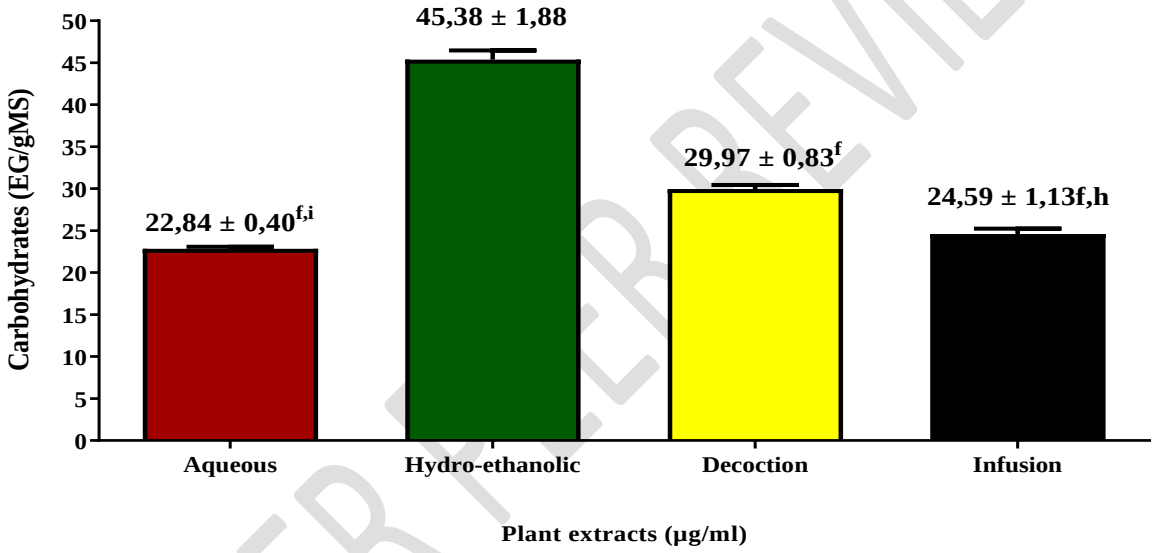

Figure 3 *:Concentration of carbohydrates in plant extracts*

Quantitative estimation of total proteins

The distribution of proteins in the different plant extracts shows that the hydro-ethanolic extract presents the greatest concentration. Followed by the decoction extract with a non-significant difference and a p-value > 0.05, the infusion extract with a significant difference and a p-value < 0.01, the aqueous extract obtained by maceration having the lowest concentration with a p-value < 0.01 in comparison with the hydro-ethanolic extract.

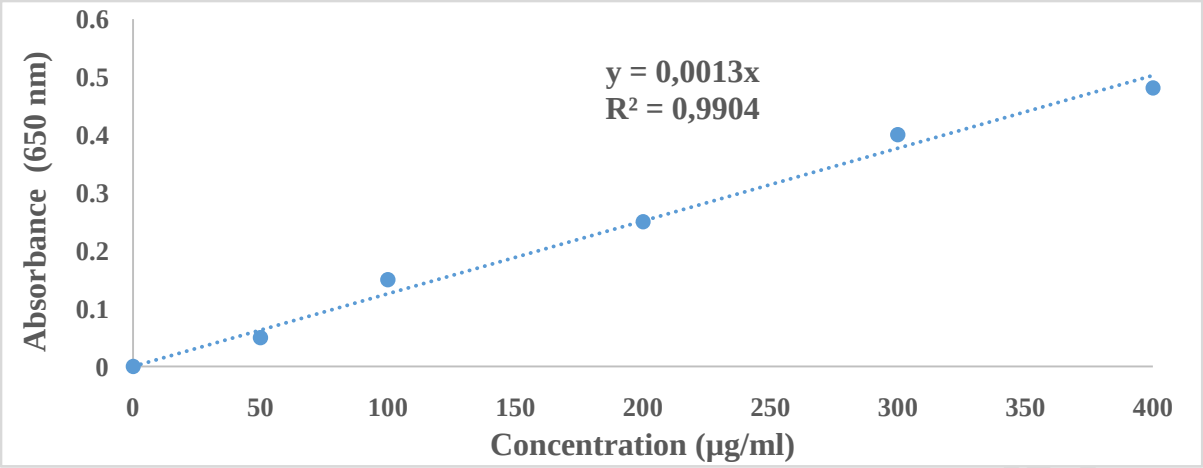

Figure 4 *: Calibration curve of bovinalbumine*

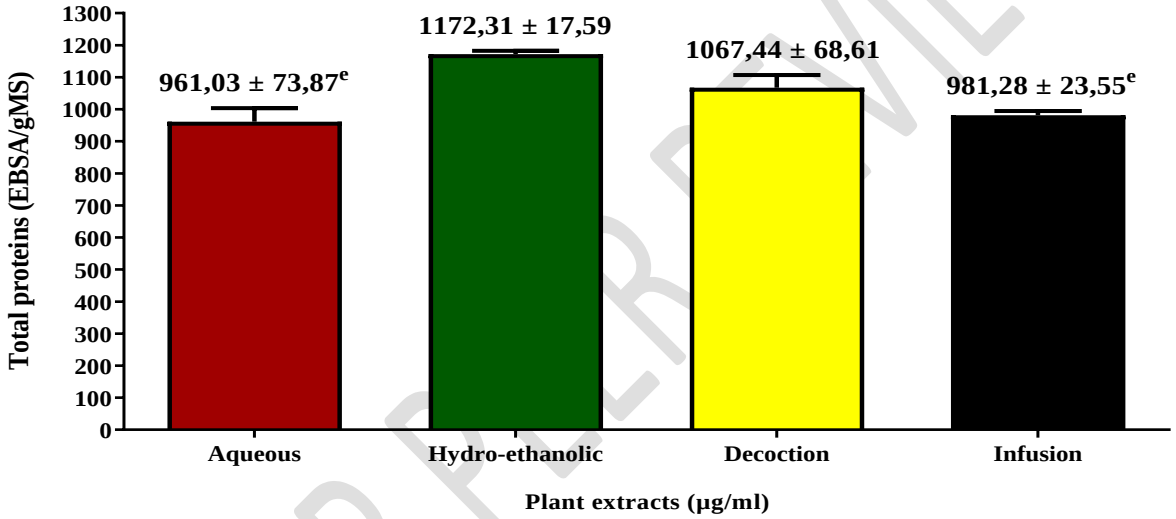

Figure 5 :Concentration of proteins in plant extracts

Quantitative estimation of total polyphenols

The distribution of total polyphenols in the different extracts of the plant shows that the hydro-ethanolic extract presents the greatest concentration, followed by the infusion extract, and the aqueous extract obtained by maceration with a non-significant difference. In addition, a p-value > 0.05, the decoctiondecoction having the lowest concentration with a significant difference and a p-value < 0.05 in comparison with the hydro-ethanolic extract.

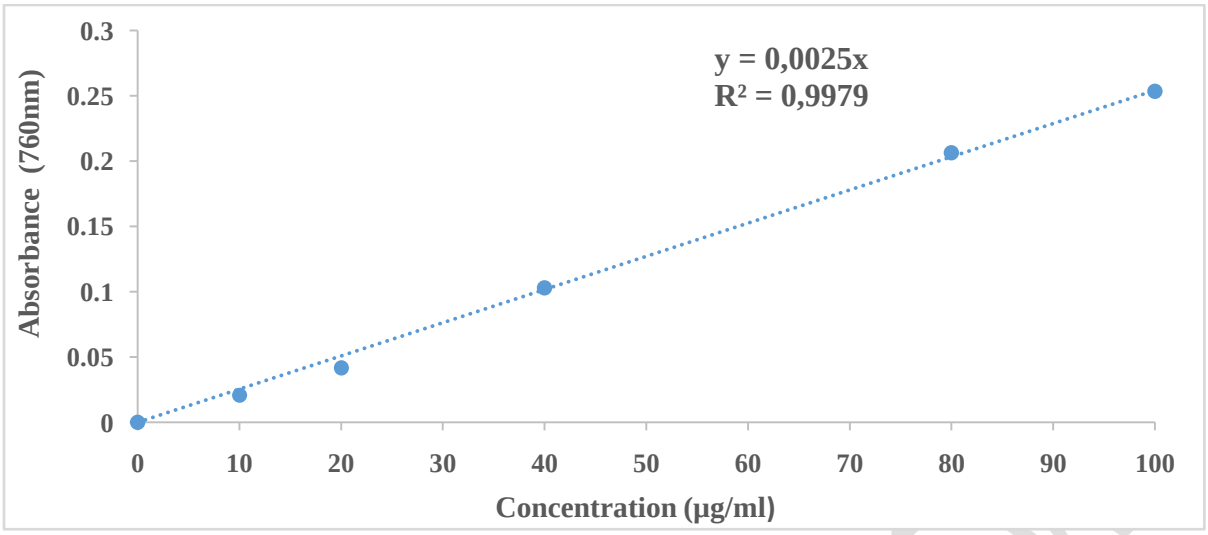

Figure 6 *: Calibration of Gallic acid*

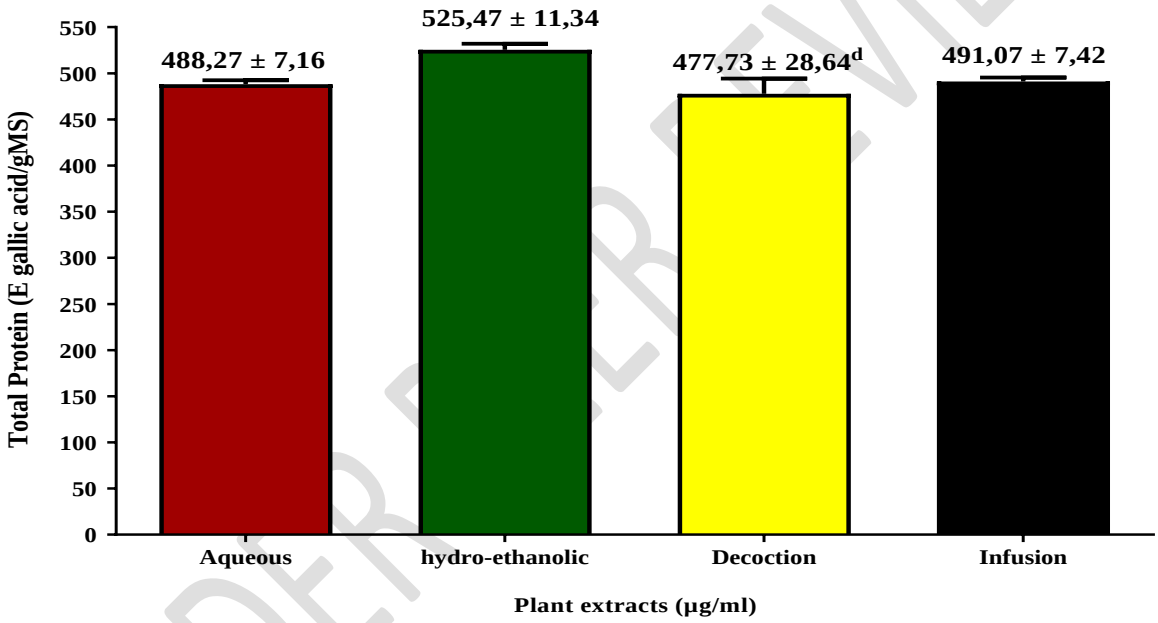

Figure 7 *:Concentration of polyphenols in plant extracts*

Quantitative estimation of total flavonoids

The distribution of total flavonoids in the different extracts of the plant shows that the aqueous extract obtained by maceration has the greatest concentration, followed by decoction extract, the hydro-ethanolic extract, the infusion extract, presenting the lowest concentration. We observes No significant difference between the different extracts with a p-value > 0.05.

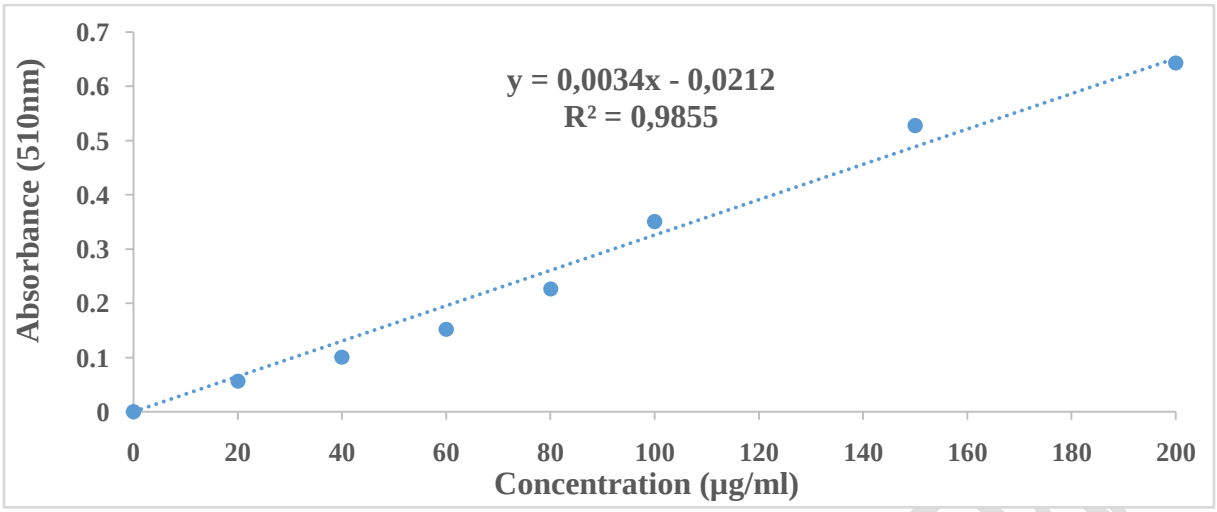

Figure 8 *:Calibation curve of quercetin*

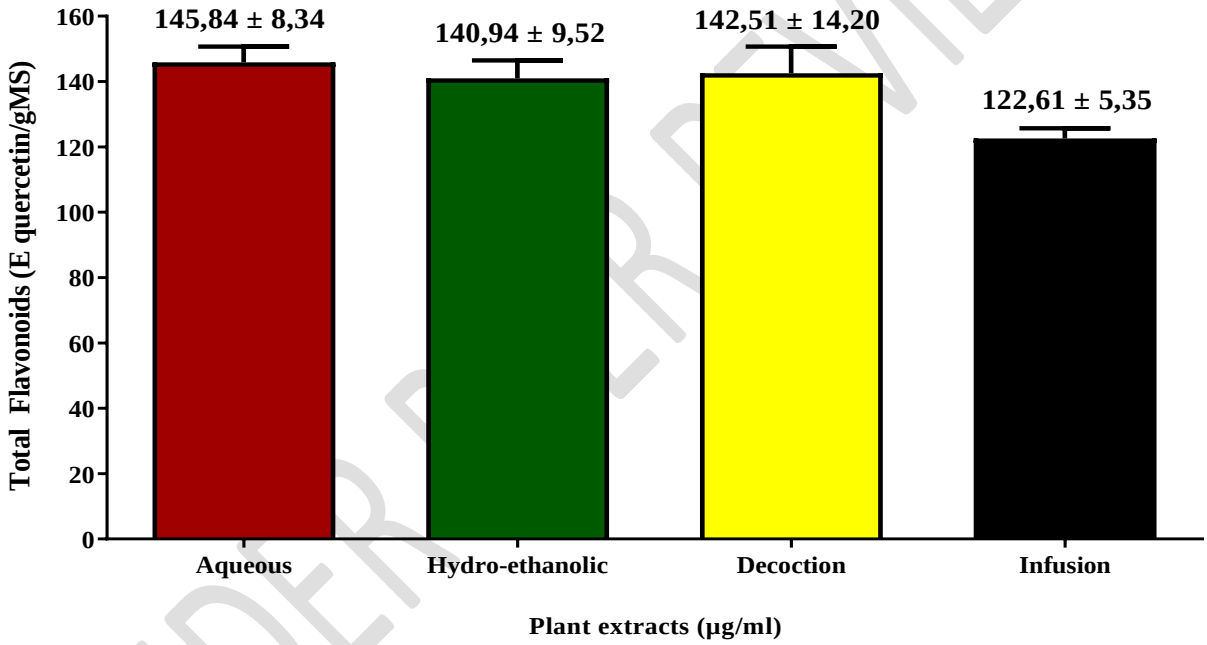

Figure 9: Concentration of Flavonoids in plant extracts

Quantitative estimation of total flavonols

The distribution of total flavonols in the different extracts of the plant shows that the aqueous extract obtained by maceration has the greatest concentration, followed by decoction extract, the hydro-ethanolic extract, the infusion extract, presenting the lowest concentration. We observed no significant difference between the different extracts with a p-value > 0.05.

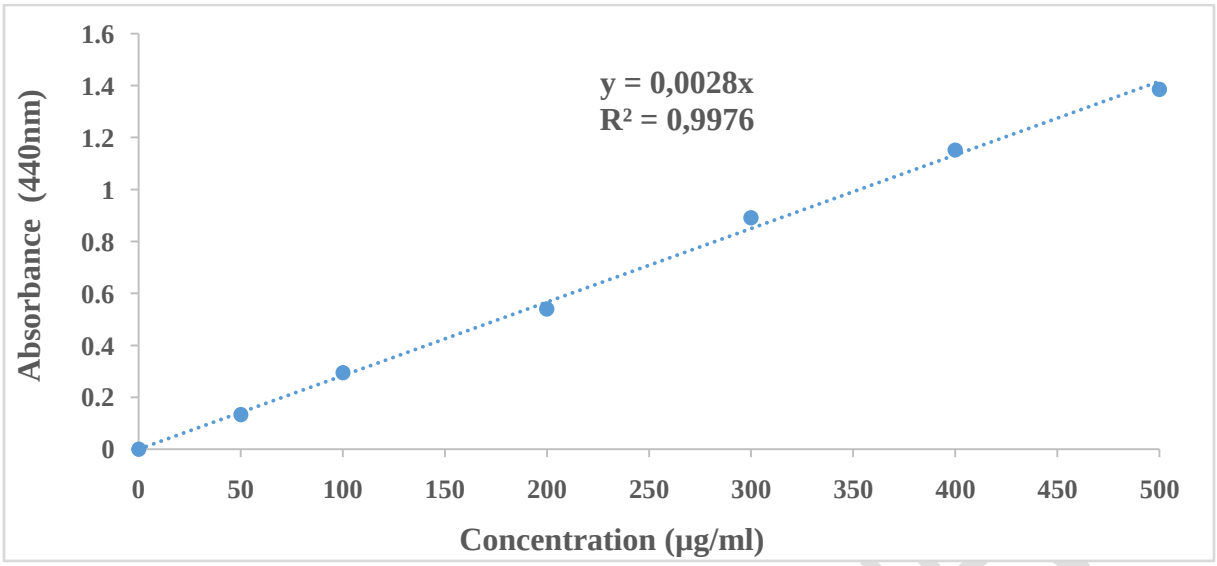

Figure 10 *:Calibration cureve of quercetin*

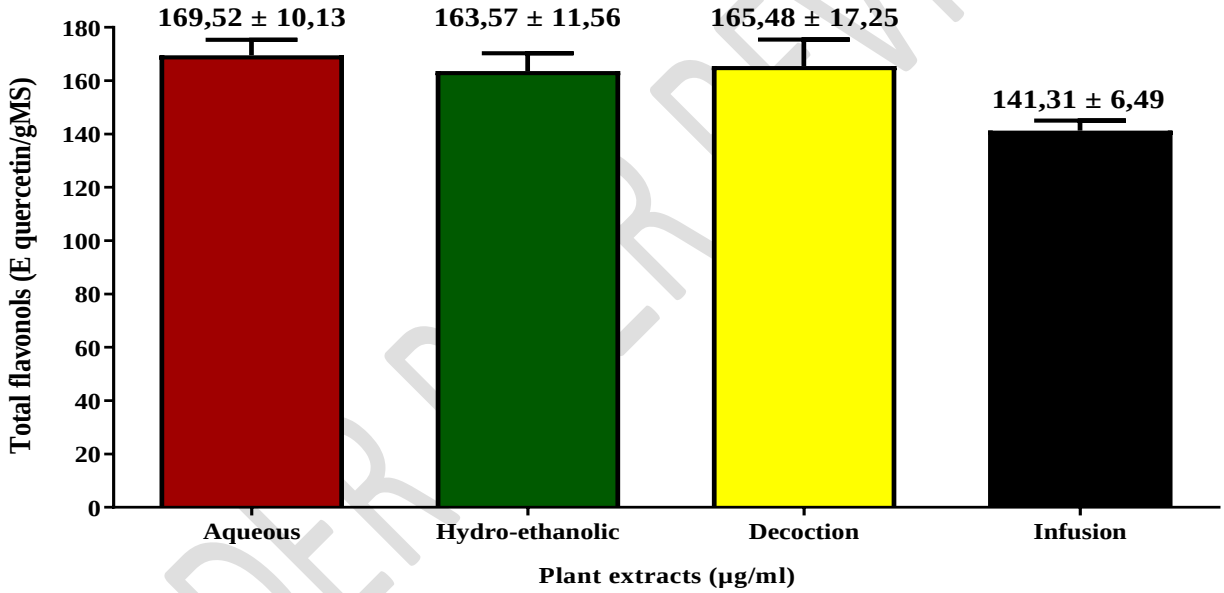

Figure 11 : Concentration offlavonols in plant extracts

Quantitative estimation of total tannins

The concentrations of total tannins obtained from the calibration curve show that the Concentration of tannins is preponderant in the decoction extract. Followed by infusion extract with a non-significant difference with a p-value > 0.05. The hydro-ethanolic extract with a significant difference with the infusion extract with a p-value < 0.01 and in comparison with the decoction extract with a p -value < 0.001. The aqueous extract obtained by maceration at room temperature having the lowest concentration with a significant difference with the hydro-ethanolic extract with a p-value < 0.01, decoction and by infusion with a p-value < 0.001.

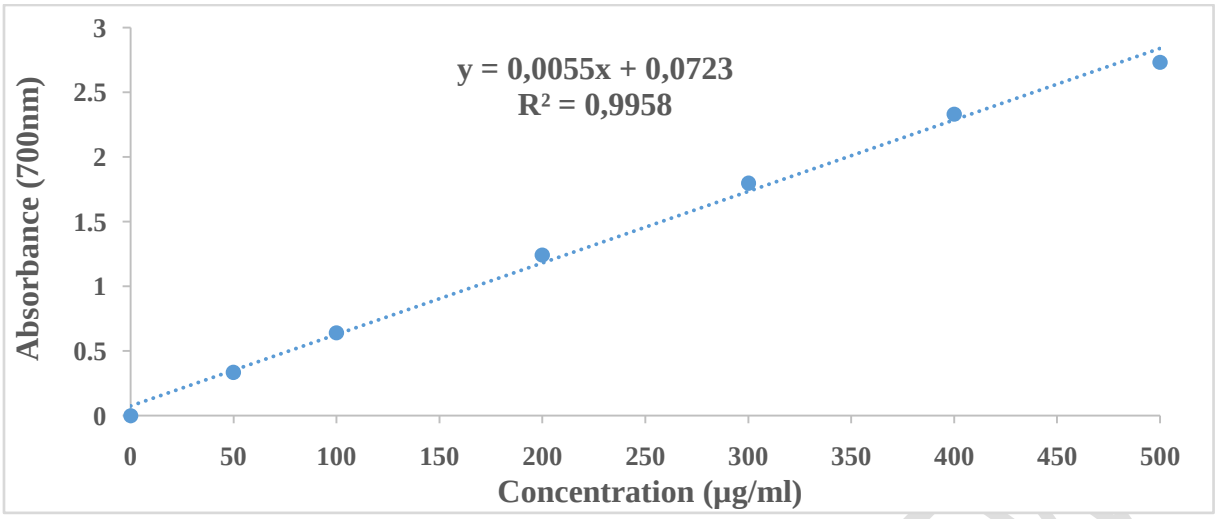

Figure 12 :Calibration curve of Tannic acid

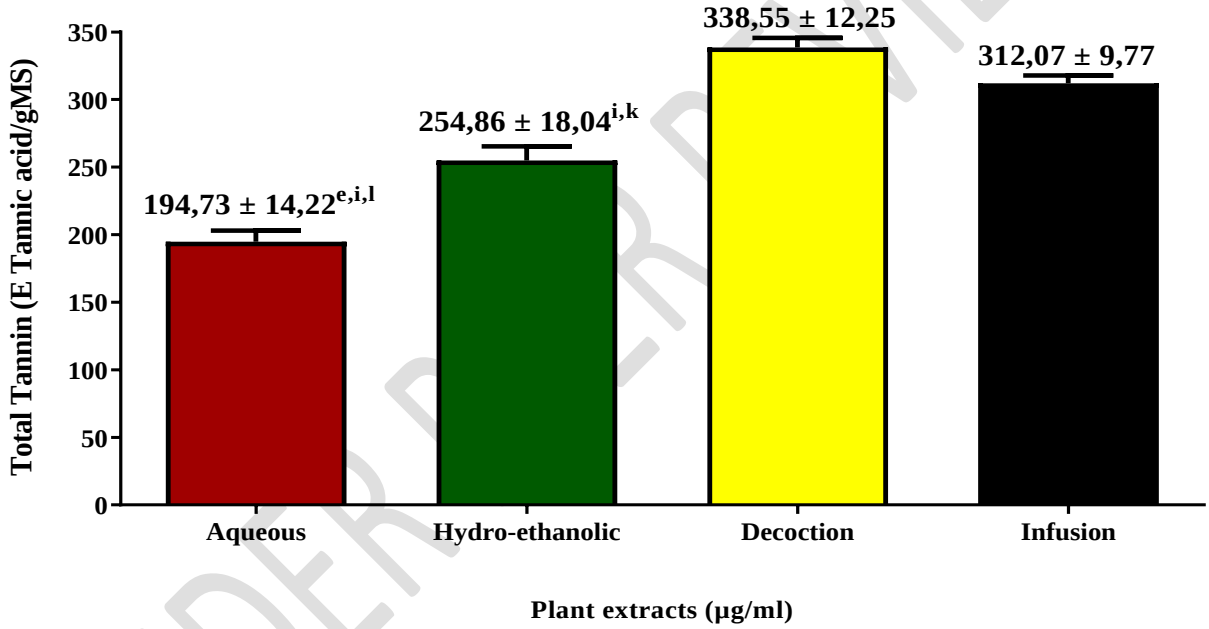

Figure 13 : Concentration of Tannin in plant extracts

Quantitative Estimation of Total Alkaloids

The distribution of total alkaloids in the different extracts of the plant shows that the infusion extract has the greatest concentration, followed by the aqueous extract obtained by maceration, the hydro-ethanolic extract, decoction extract with a non-significant difference with a p-value > 0.05.

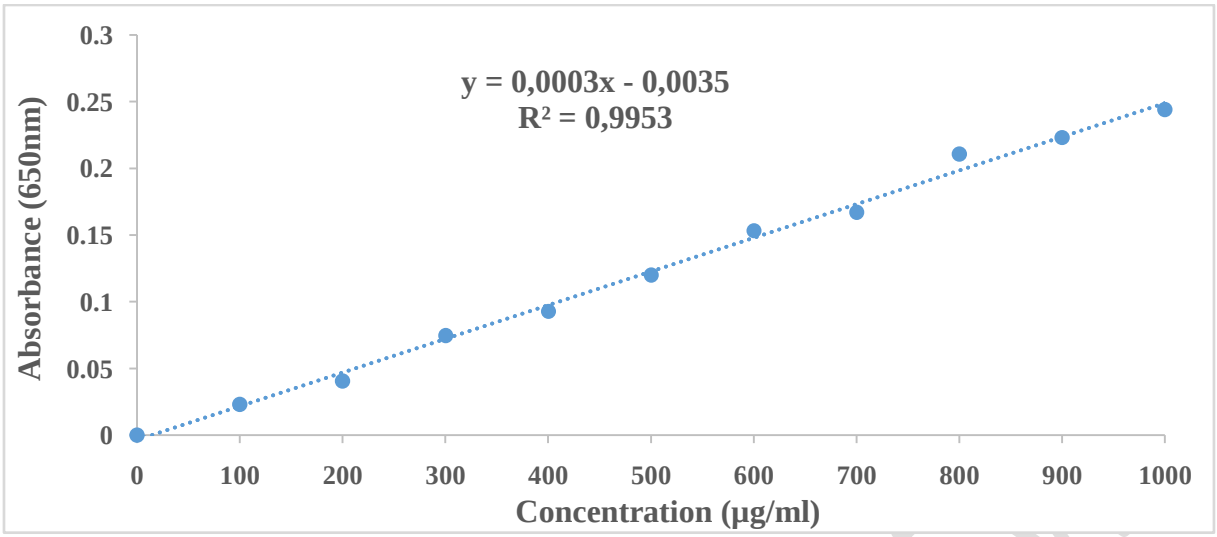

***Figure 14**: Calibration curve of quinine*

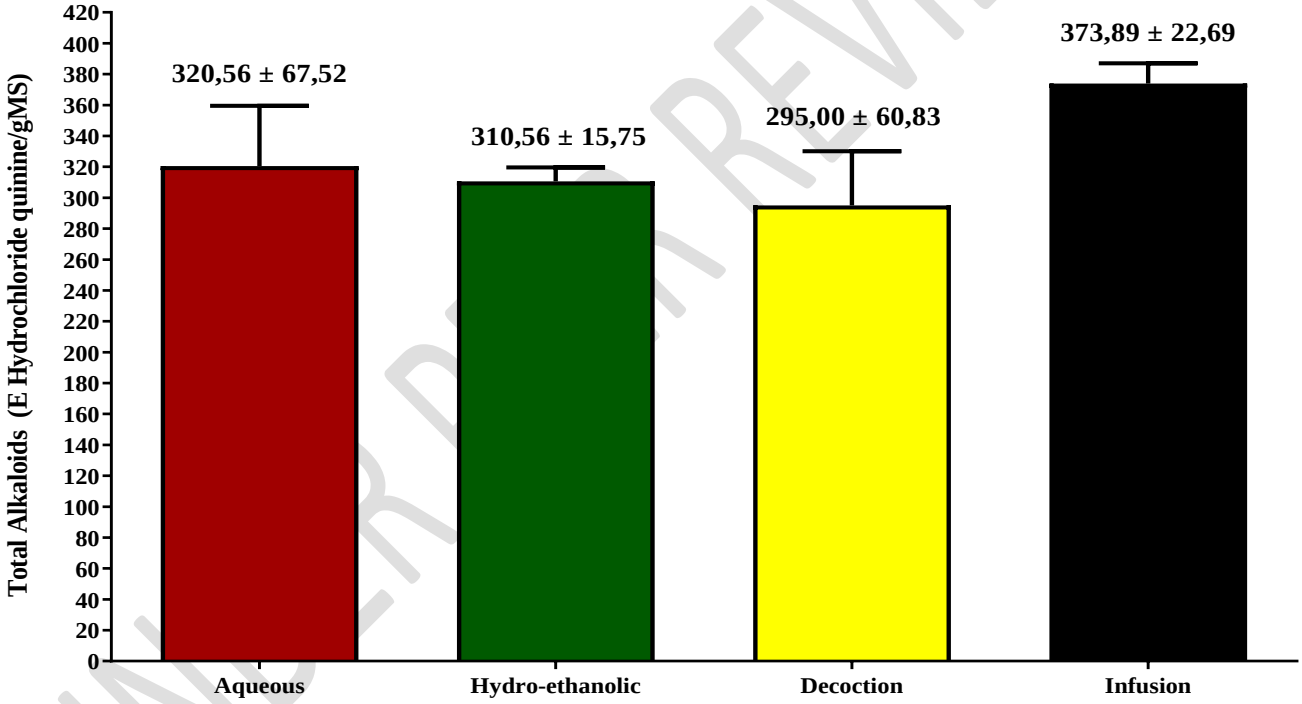

***Figure 15**: Alkaloids concentration in plant extracts*

Quantitative phytochemical screening helps to estimate the concentration of secondary metabolites in plant extracts. The table below summarizes the concentrations obtained according to the different plant leaf extracts:

***Table 3** : Concentration of metabolites in plant extracts*

Metabolites	Aqueous	Hydro-ethanolic	Decoction	Infusion
Carbohydrates	22,84 ± 0,40^{f,i}	45,38 ± 1,88	29,97 ± 0,83^{f}	24,59 ± 1,13^{f,h}
Proteins	961,03 ± 73,87^{e}	1172,31 ± 17,59	1067,44 ± 68,61	981,28 ± 23,55^{e}
Polyphenols	306,80 ± 13,22	310,27 ± 11,83	298,27 ± 13,03^{d}	302,67 ± 19,90
Flavonoids	145,84 ± 8,34	140,94 ± 9,52	142,51 ± 14,20	122,61 ± 5,35
Flavonols	169,52 ± 10,13	163,57 ± 11,56	165,48 ± 17,25	141,31 ± 6,49
	194,73 ± 14,22^{e,i,l}	254,86 ± 18,04^{i,k}	338,55 ± 12,25	312,07 ± 9,77

Alkaloids	320,56 ± 67,52	310,56 ± 15,75	295,00 ± 60,83	373,89 ± 22,69

Values are expressed in terms of mean ± standard deviation, (n = 3). The comparison between groups is made using the variance test (ANOVA) followed by the Turkey Kramer post hoc test. The difference is significant with a < 0.05; b < 0.01; c < 0.001 for the aqueous extract obtained by maceration at room temperature; d <0.05; e < 0.01; f < 0.001 for the hydro-ethanolic extract; g < 0.05; h < 0.01; i < 0.001 for the aqueous extract obtained by decoction; j < 0.05; k < 0.01; l < 0.001 for the aqueous extract obtained by infusion.

Evaluation of the in-vitro antioxidant activity of leaf extracts

Evaluation of total antioxidant activity by the Folin test

The total antioxidant capacity by the Folin test of the different extracts of the plant given to us from the calibration curve obtained from vitamin C the straight line of equation Y = 0.013x + 0.0059 and a correlation coefficient R2 = 0.9981. We note that the reference molecule, which is vitamin C, shows the highest kinetics, followed by the hydro-ethanolic extract, the aqueous extract obtained by decoction, the aqueous extract obtained by maceration at room temperature, the aqueous extract obtained by infusion.

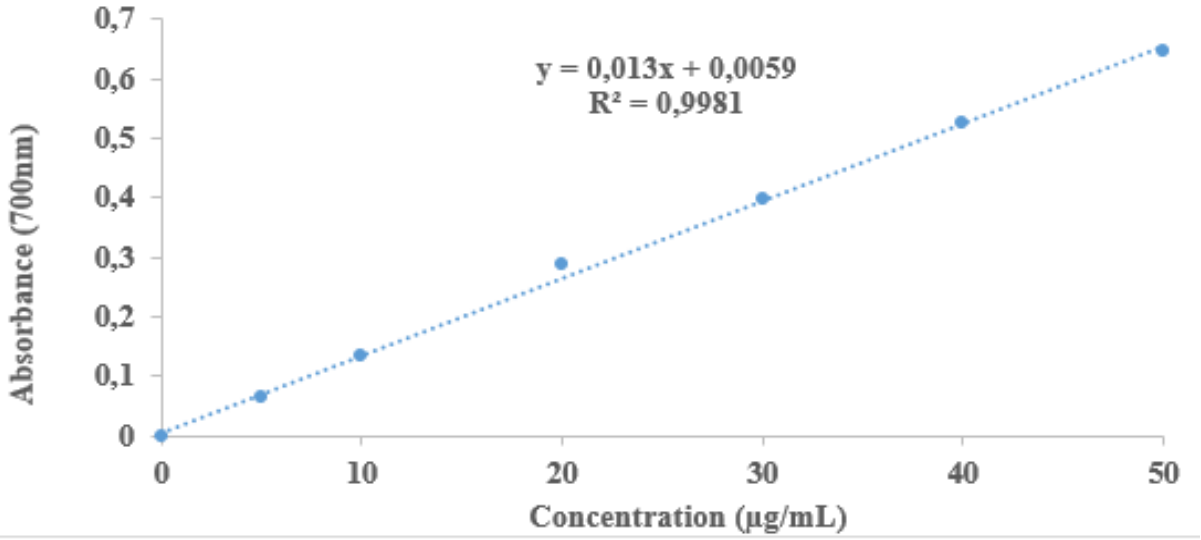

Figure 16 *: Calibration curve of vitamin C*

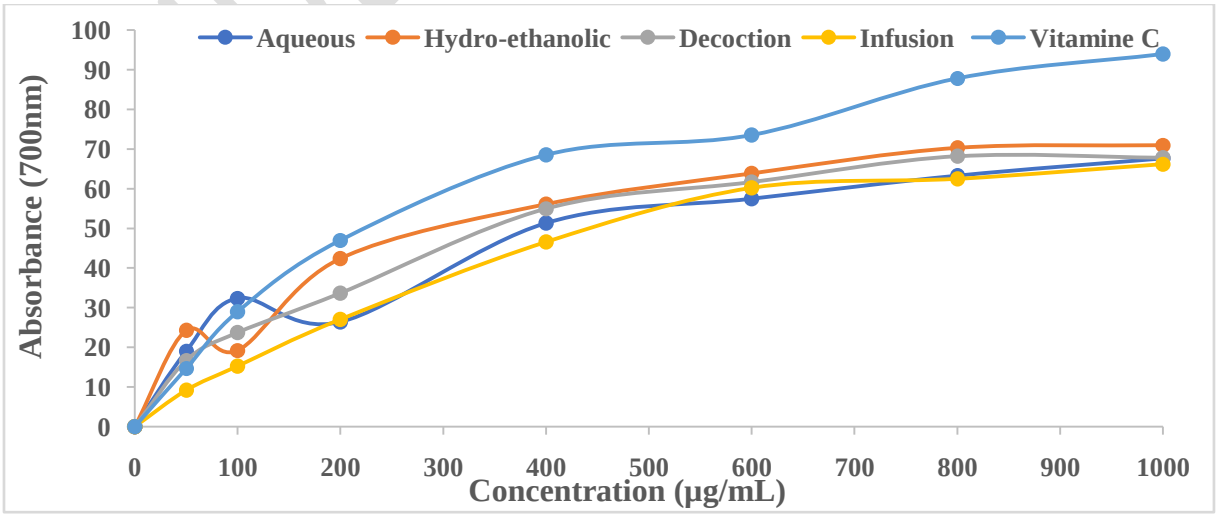

Figure 17 *:Folin test of antioxydant activity of plant extracts.*

The reference molecule, vitamin C, presents the highest inhibition percentage of the order of (93.93 ± 5.58) % and an IC50 (373.37 ± 23.72) µg/mL.Followed by of the hydro-ethanolic extract with an IC50 value of (499.93 ± 5.14) µg/mL and an inhibitory concentration at a dose of 1000µg/mL of the order of (70.96 ± 0.58) %. The aqueous extract obtained by decoction with an IC50 of (535.66 ± 6.59)µg/mL and an inhibitory concentration at a dose of 1000µg/mL of the order of (67.80 ± 0. 59)%. The aqueous extract obtained by aqueous maceration at room temperature with an IC50 of (568.02 ± 22.39)µg/mL and an inhibitory concentration at a dose of 1000µg/mL of the order of (67. 60± 3.42)%. The aqueous extract obtained by infusion with an IC50 of (603.90 ± 3.34) µg/mL and an inhibitory concentration at a dose of 1000 µg/mL of the order of (66.21 ± 1.35)%. We can therefore conclude in view of the results obtained, that among the four extracts of the plant, the hydro-ethanolic extract presents the best total antioxidant activity, followed by decoction extract, the maceration extract and decoctionextract, in comparison to vitamin C.

Table4: *:Folin test of plant extracts*. You should cite your table in the text

Conc (µg/mL)	**Plant extracts**				
	Aqueous	**Hydro-ethanolic**	**Decoction**	**Infusion**	**Vitamin C**
50	19,08 ± 1,42	24,29 ± 9,37	16,75 ± 5,51	9,19 ± 2,28	14,73 ± 3,37
100	32,42 ± 6,24	19,26 ± 1,51	23,75 ± 2,47	15,26 ± 2,560	28,96 ± 0,82
200	26,44 ± 2,46	42,39 ± 3,75	33,65 ± 4,04	27,03 ± 1,50	47,01 ± 2,62
400	51,34 ± 4,27	56,11 ± 4,83	55,01 ± 2,23	46,57 ± 1,36	68,52 ± 9,29
600	57,44 ± 2,28	63,83 ± 2,71	61,67 ± 3,67	60,19 ± 1,16	73,49 ± 3,13
800	63,29 ± 1,66	70,26 ± 1,05	68,16 ± 0,73	62,47 ± 0,89	87,83 ± 2,23
1000	67,60 ± 3,42	70,96 ± 0,58	67,80 ± 0,59	66,21 ± 1,35	93,93 ± 5,58
IC50	568,02 ± 22,39	499,93 ± 5,14	535,66 ± 6,59	603,90 ± 3,34	373,37 ± 23,72

Values are expressed in terms of mean ± standard deviation, (n = 3). The comparison between groups are carried out using the variance test (ANOVA) followed by Dunnet's post hoc test.

Total Antioxidant Capacity by Phosphomolybdate (CAT)

After analysis, we note that the hydro-ethanolic extract presents the highest kinetics depending on the concentration of the extract, followed by the aqueous extract obtained by decoction, the aqueous extract obtained by infusion, the aqueous extract obtained by cold maceration, presenting the lowest kinetics.

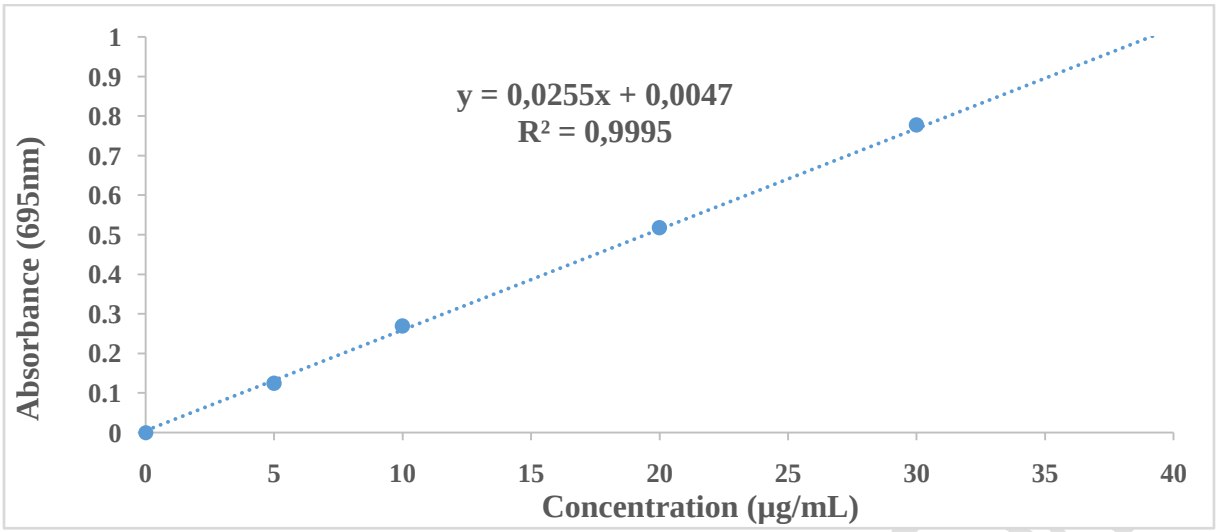

Figure 18 *:Calibration curve of Vitamin C*

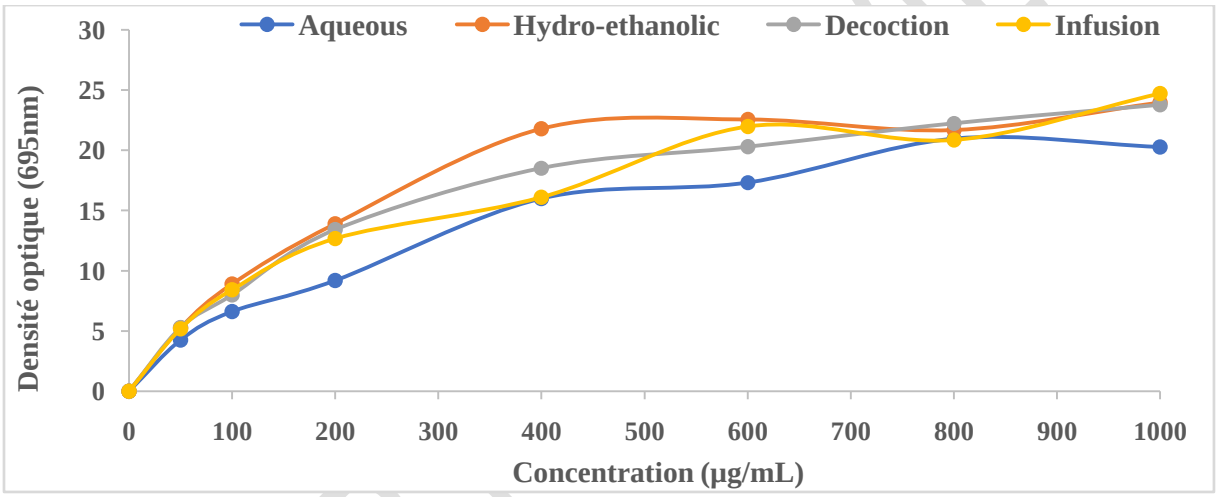

Figure 19 *:Phosphomolybdenum test on plant extracts*

The hydro-ethanolic extract presents the greatest antioxidant activity with an IC50 value of (2031.76 ± 135.38)µg/mL and an inhibitory concentration at a dose of 1000µg/mL of the order of (23.97 ± 0.36)%. Followed by infusion extract with an IC50 of (2041.157 ± 107.98)µg/mL and an inhibitory concentration at a dose of 1000µg/mL of the order of (24.72 ± 0.16)%. Decoction extract with an IC50 of (2062.65 ± 24.88) µg/mL and an inhibitory concentration at a dose of 1000 µg/mL of the order of (23.79 ± 1.24)%.Maceration extract at room temperature with an IC50 of (2315.78 ± 61.91)µg/mL and an inhibitory concentration at a dose of 1000µg/ mL of the order of (20.29 ± 0.78)%. We can therefore conclude in view of the results that among the four extracts of the plant, the hydro-ethanolic extract presents the best total antioxidant activity by the phosphomolybdenum test, followed by the aqueous extract obtained by infusion, the aqueous extract obtained by decoction and in the last position of the aqueous extract obtained by maceration at room temperature. The Table below presents the total antioxidant capacity of Guiera senegalensis extracts according to the method using phosphomolybdenum

Table5 *:Phosphomolybdenum test on plant extracts*

Concentration (µg/mL)	Plant extracts			
	Aqueous	**Hydro-ethanolic**	**Decoction**	**Infusion**
50	4,25 ± 0,73	5,27 ± 0,30	5,29 ± 0,70	5,19 ± 0,58
100	6,61 ± 0,64	8,90 ± 0,91	8,01 ± 0,73	8,27 ± 0,76
200	9,20 ± 0,68	13,91 ± 0,85	13,42 ± 0,78	12,68 ± 1,30
400	16,01 ± 0,75	21,79 ± 0,92	18,53 ± 0,51	16,10 ± 0,82
600	17,35 ± 1,19	22,56 ± 2,08	20,30 ± 0,88	21,99 ± 1,40
800	20,98 ± 0,24	21,70 ± 2,19	22,26 ± 0,14	20,86 ± 1,00
1000	20,29 ± 0,78	23,97 ± 0,36	23,79 ± 1,24	24,72 ± 0,16
IC50	2315,78 ± 61,91	2031,76 ± 135,38	2062,65 ± 24,88	2041,157 ± 107,98

Values are expressed in terms of mean ± standard deviation, (n = 3). The comparison between groups is carried out using the variance test (ANOVA) followed by Dunnet's post hoc test.

DISCUSSION

G.senegalensis is a plant of the Combretaceae family found in the Sudano-Sahelian zone and present in Chad, Sudan, Senegal and Cameroon [17]. It has many pharmacological properties including antidiabetic [17], antimalarial [18], antibacterial, antifungal [6]. Secondary metabolites represent a class of molecules produced by plants involved in various biological processes such as cellular structuring, defence, interaction with various other organisms [4]; these are in opposition to the primary metabolites. There is a wide variety of secondary metabolites distributed throughout the plant kingdom varying in quality and quantity [19]. There are several classes of secondary metabolites including alkaloids, which represent the most important class of secondary metabolites, polyphenolic compounds, phytosterols representing enormous therapeutic potential [19].

The qualitative phytochemical screening made it possible to highlight metabolites such as alkaloids, flavonoids, coumarins, tannins, saponosides but also polyphenolic compounds which is in agreement with the results obtained by Ifijen et al 2019 in their study on the phytochemical analysis of *G. senegale* leaf extract as well as its anti-plasmodial properties [18]. Polyphenolic compounds represent a class of secondary metabolites present in most plants of the plant kingdom and responsible for various biological processes such as defence against external attacks. Phytochemicals are subdivided into phenolic acid, flavonoids and stilbene. This class has demonstrated important pharmacological properties including hypoglycemic, anticancer, antioxidant and antihypertensive properties [20]. We also note the presence of cardiac glycoside in the four extracts which is in contradiction with the results obtained by Ifijen et *al* 2019 in their study on the phytochemical analysis of G. senegalensis leaf extract as well as its anti-plasmodial properties. [18]. this difference could be explained by a different alcohol level used in this study. The presence of polyphenolic compounds could justify the therapeutic use of *G. senegalensis* in hypertensive and hepatic conditions. The quantitative evaluation made it possible to show a concentration of the order of 310.27 ± 11.83 mgGAE/gMS of total polyphenols in the extract obtained by hydro-ethanolic maceration, a higher concentration compared to that obtained in the different extracts. This concentration is much higher than that obtained by Ifijen et al 2019 [18]. This difference in concentration could be explained by geographical and climatic conditions favoring a high concentration of polyphenolic compounds in the extract obtained by hydroethanolic maceration, compounds that could be responsible for the hepatoprotective property of Guiera senegalensis leaf extracts as demonstrated by polyphenolic molecules used therapeutically

as hepatoprotectors, including Silymarin [21]. The in-vitro antioxidant activity was evaluated during this study via two tests, namely the Folin test and Total Antioxidant Capacity by Phosphomolybdate. Regarding the Folin test, we observe an IC50 for the extract obtained by hydro-ethanolic maceration higher than that of the other extracts of the order of 499.93 ± 5.14 µg/mL, which represents 70.96 ± 0 .58% inhibition, quite close to that of viamin C. Which agrees with the results obtained by Ogunmaet *al* in 2020 in their study on In vitro and in vivo antioxidant evaluation of Guiera senegalensis methanol leaves extract [6]. The Folin-Ciocalteu method is a method based on electron transfer, which measures the reduction capacity of an antioxidant. This method correlates quite well with other methods for evaluating the antioxidant activity of a compound such as the DDPH method [22]. One of the mechanisms involved in hepatoprotection is the antioxidant capacity as described for silymarin which is a polyphenolic compound extracted from plants, this will act by inhibiting lipid peroxidant and the action of pro-oxidative metabolites produced during oxidative stress condition [23].

CONCLUSION

This study on *Guiera senegalensis* leaf extracts consisted of a qualitative and quantitative phytochemical screening, which made it possible to identify alkaloids, flavonoids, coumarins, tannins, saponosides but also polyphenolic compounds. The quantitative evaluation made it possible to show the presence of total polyphenols in the extract obtained by hydro-ethanolic maceration, a higher concentration compared to that obtained in the different extracts. As for the antioxidant activity, we noted a significant antioxidant activity of the extract obtained from hydroethanolic maceration. Future studies should explore the phytochemical compounds involved in this antioxidant activity as well as its mechanism.This study confirms the use of the plant in category 1 traditional medicine, and results show a promising potentila for developing a category 2 phytomedicine.

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