**PHYTOCHEMICAL AND *in-vitro* ANTIOXIDANT ACTIVITY OF *Guierasenegalensis* (COMBRETACEAE) LEAF EXTRACTS**


## ABSTRACT

*Guierasenegalensis* is a plant of the Combretaceae family, 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 and collaborators 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. Carbohydrates, proteins and polyphenols had a higher concentration in the hydro-ehtanolic extract with concentrations of 45,38 ± 1,88, 1172,31 ± 17,59, 310,27 ± 11,83 µg/ml respectively. Flavonoids, flavonols and alkaloids in the Aqueous maceration extract with concentrations of 145,84 ± 8,34, 169,52 ± 10,13, 320,56 ± 67,52 µg/ml. and Tannins in decoction extract with a concentration of 338,55 ± 12,25 µg/ml.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. This study confirms the use of the plant in category 1 traditional medicine, and results show a promising potential for developing a category 2 phytomedicine. Future studies should explore the use of phytochemical compounds involved in this antioxidant activity as well as its mechanism



Key word:Guiera, leaf extracts, phytochemical screening, antioxidants activity, flavonoids,


## 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 [2]. 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,

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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].

*Guierasenegalensis* 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 liver affections, respiratory, antihypertensive, antipyretic and antimalarial conditions [6]. This plant appears in the form of a bush [6]. In this study, we will focus on the phytochemical and antioxidant property of *Guierasenegalensis* leaf extract.

## METHODOLOGY

### Treatment of plant material and preparation of extracts

The plant material consisted of *Guierasenegalensis*leaves, harvested at Guider, North Cameroon . Identification of the species was done by a taxonomist at 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. We should observe 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. We should observe the formation of a white precipitate.

**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 result 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 result in the appearance of a blue-green colour.

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

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- 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 is formed.

**Cardiac glycoside identification test**: The Keller-Killani test was used for the identification of cardiotonicglycoside :

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

**Mucilage identification test**: The mucilage was 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 is 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 is 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 appeare.

- 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 hydroxide was added; yellow fluorescence appeared

**Quantitative phytochemistry screening**

All the methods below was conducted by adding to a precise amount of extract a specific reagent with which it produce a colour. The absorbance of the colour was then meseare.

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- **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 maximum absorption of the colour produced (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 colour measured 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**: In a tube containing 100µL of extract, we add 1000µL of picric acid 13%, 1000µL of NaOH 4%.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**: In a tube with 1000µl of extract we add 2000 µl of Lowry reagent. 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**

Regarding the oxidation test, it consisted of adding specific reagent (Folin-ciocalteu for and phosphomolybdate) to a range of different concentration of extract. The absorbance of the colour produce was then read and compare to the Vitamin C absorbance.

**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].

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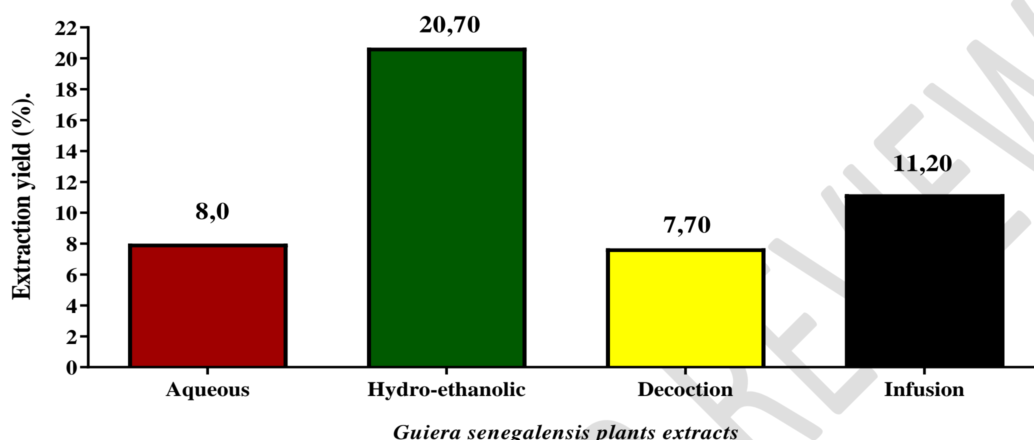
## RESULTS

### Extraction yield

The extraction of the powder from the leaves of *Guierasenegalensis* 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 ure 1 illustrates the different yields depending on the solvent and the method used




**ure 1. T**he 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:

***Table 1 : 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 | + | + | + | + |
| **Vitamin C** | $H_2SO_4$ +DNPH | + | + | + | + |
| **Carotenoïds** | HCl + $H_2SO_4$ | - | - | - | - |

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(+) Presence, (-) absence

**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:

***Table 2** : **Secondary metabolites presents in extracts***

| Metabolites | Reagents | Aqueous | Hydro-ethanolic | Decoction | Infusion |
|---|---|---|---|---|---|
| Polyphenols | $FeCl_3$ | + | + | + | + |
| | Lead acetate | + | + | + | + |
| Alkaloids | Wagner | + | + | + | + |
| | Hager | + | + | + | + |
| | Valse-Meyer | + | + | + | + |
| | Tannic acid | + | + | + | + |
| Flavonoids | NaOH + H2SO$_4$ | + | + | + | + |
| | $AlCl_3$ | + | + | + | + |
| 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 acid acétique | - | - | - | - |
| 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 extracts showed the hydro-ethanolic extract with the greatest concentration. Followed by the decoction extract with a significant

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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 ure 2.

**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.

**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. The decoction extract has the the lowest concentration with a significant difference and a p-value < 0.05 in comparison with the hydro-ethanolic extract.

**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.

**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.

**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.

**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

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maceration, the hydro-ethanolic extract, decoction extract with a non-significant difference with a p-value > 0.05.

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** | $22.84 \pm 0.40^{f,i}$ | $45.38 \pm 1.88$ | $29.97 \pm 0.83^{f}$ | $24.59 \pm 1.13^{f,h}$ |
| **Proteins** | $961.03 \pm 73.87^{e}$ | $1172.31 \pm 17.59$ | $1067.44 \pm 68.61$ | $981.28 \pm 23.55^{e}$ |
| **Polyphenols** | $306.80 \pm 13.22$ | $310.27 \pm 11.83$ | $298.27 \pm 13.03^{d}$ | $302.67 \pm 19.90$ |
| **Flavonoids** | $145.84 \pm 8.34$ | $140.94 \pm 9.52$ | $142.51 \pm 14.20$ | $122.61 \pm 5.35$ |
| **Flavonols** | $169.52 \pm 10.13$ | $163.57 \pm 11.56$ | $165.48 \pm 17.25$ | $141.31 \pm 6.49$ |
| **Total tannins** | $194.73 \pm 14.22^{e,i,l}$ | $254.86 \pm 18.04^{i,k}$ | $338.55 \pm 12.25$ | $312.07 \pm 9.77$ |
| **Alkaloids** | $320.56 \pm 67.52$ | $310.56 \pm 15.75$ | $295.00 \pm 60.83$ | $373.89 \pm 22.69$ |

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 Tukey 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.

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 aqueous maceration extract and infsuion extract, in comparison to vitamin C. table 4 present the results obtain after the antioxidant activity done by the Folin method

***Table 4: Folin test results of plant extracts.***

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| 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.

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. The table 5 below present the results obtain after the phosphomolybdenum test on plant extraxts.

***Table 5 : 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 |

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| 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. There is a wide variety of secondary metabolites distributed throughout the plant kingdom varying in quality and quantity [19]. They can be classified as alkaloids, which represent the most important class of secondary metabolites, polyphenolic compounds, and phytosterols representing enormous therapeutic potential [19].

The qualitative phytochemical screening in this study showed the presence of metabolite 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 [20]. Phytochemicals are subdivided into phenolic acid, flavonoids and stilbene [20]. 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 vitamin C. Which agrees with the results obtained by Ogunma et *al* in 2020 in their study on In vitro and in vivo antioxidant evaluation of Guiera senegalensis methanol leaves extract [6]. 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 [22].

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## CONCLUSION

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

## REFERENCES


1. Singh A, Kukreti R, Saso L, Kukreti S. Oxidative Stress: A Key Modulator in Neurodegenerative Diseases. Molecules. janv 2019;24(8):1583.

2. Zhang L, Wang X, Cueto R, Effi C, Zhang Y, Tan H, et al. Biochemical basis and metabolic interplay of redox regulation. Redox Biol. 1 sept 2019;26:101284.

3. Trist BG, Hare DJ, Double KL. Oxidative stress in the aging substantia nigra and the etiology of Parkinson's disease. Aging Cell. 2019;18(6):e13031.

4. Chiocchio I, Mandrone M, Tomasi P, Marincich L, Poli F. Plant Secondary Metabolites: An Opportunity for Circular Economy. Molecules. janv 2021;26(2):495.

5. Bhatti MZ, Ismail H, Kayani WK, Bhatti MZ, Ismail H, Kayani WK. Plant Secondary Metabolites: Therapeutic Potential and Pharmacological Properties. In: Secondary Metabolites - Trends and Reviews. IntechOpen; 2022

6. Gabriel BO, Otakhor KO, Obaseki EO. In vitro and in vivo antioxidant evaluation of Guiera senegalensis methanol leaves extract. J Basic Pharmacol Toxicol. 16 déc 2020;4(2):6‑ 12.

7. Fonmboh D, Ejoh A, Fokunang T, Bayaga H, Teke G, Rose N, et al. An Overview of Methods of Extraction, Isolation and Characterization of Natural Medicinal Plant Products in Improved Traditional Medicine Research. Asian J Res Med Pharm Sci. 20 nov 2020;31‑ 57.

8. Shaikh J, Patil M. Qualitative tests for preliminary phytochemical screening: An overview. 1 mars 2020;8:603‑ 8.

9. Singleton VL, Rossi JA. Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. Am J Enol Vitic. 1 janv 1965;16(3):144‑ 58.

10. Zhishen J, Mengcheng T, Jianming W. The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem. 1 mars 1999;64(4):555‑ 9.


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11. Miliauskas G, Venskutonis PR, van Beek TA. Screening of radical scavenging activity of some medicinal and aromatic plant extracts. Food Chem. 1 avr 2004;85(2):231‑ 7.

12. Thomas W, Dutcher RA. ACS Publications. American Chemical Society; 2002. The colorimetric determination of carbohydrates in plants by the picric acid reduction method the estimation of reducing sugars and sucrose.

13. Reagent FP. Protein measurement with the. J Biol Chem. 1951;193:265‑ 75.

14. Ali-Rachedi F, Meraghni S, Touaibia N, Mesbah S. Analyse quantitative des composés phénoliques d'une endémique algérienne Scabiosa Atropurpurea sub. Maritima L. Bull Société R Sci Liège. 1 janv 2018

15. Graham HD, Thomas LB. Color reaction of alkaloids with dichromate-sulfuric acid and its use for quantitative assay. J Pharm Sci. nov 1961;50:901‑ 4.

16. Prieto P, Pineda M, Aguilar M. Spectrophotometric Quantitation of Antioxidant Capacity through the Formation of a Phosphomolybdenum Complex: Specific Application to the Determination of Vitamin E. Anal Biochem. 1 mai 1999;269(2):337‑ 41.

17. Miaffo D, Ntchapda F, Kamgue OG, Mahamad AT, Kamanyi A. Glucose-lowering potential of Guiera senegalensis roots in a diabetic rat model. Avicenna J Phytomedicine. 2020;10(6):653‑ 63.

18. Ifijen I, Mamza A, Fasina K, Omoruyi J, Ikhuoria E. Phytochemical analysis of Guiera senegalensis jF Gmel extract and its anti-plasmodial properties on wister albino mice via oral route. Int J Pharmacol Phytochem Ethnomed. 2019;13:35‑ 44.

19. Jain C, Khatana S, Vijayvergia R. Bioactivity of secondary metabolites of various plants: A review. Int J Pharm Sci Res. 1 janv 2019;10:494.

20. Nisar A. Medicinal Plants and Phenolic Compounds. In: Phenolic Compounds - Chemistry, Synthesis, Diversity, Non-Conventional Industrial, Pharmaceutical and Therapeutic Applications [Internet]. IntechOpen; 2022

21. Mukhtar S, Xiaoxiong Z, Qamer S, Saad M, Mubarik MS, Mahmoud AH, et al. Hepatoprotective activity of silymarin encapsulation against hepatic damage in albino rats. Saudi J Biol Sci. 1 janv 2021;28(1):717‑ 23.

22. Kshirsagar A, Ingawale D, Ashok P, Vyawahare N. Silymarin: A Comprehensive Review. Pharmacogn Rev. 1 janv 2009;3:126‑ 34.


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