Review Article

Phytochemical study and evaluation of the antioxidant activity of extracts of the roots of *Chrysopogonnigritanus*

Abstract

In this work, we were interested in a medicinal plant named *Chrysopogonnigritanus*, belonging to the Poaceae family.In order to carry out this study, we were interested first in the phytochemical screening test, after having carried out the extraction with two solvents (water and isopropanol), to characterize the different families of metabolites present in the extracts.

Then, the assays of polyphenols and flavonoids were carried out in order to quantify the contents of these two compounds in each extract.

Finally, ~~the evaluation of~~ the antioxidant activity of the extracts was evaluated by three methods: the CUPRAC method, the trapping of the 2,2-diphenyl-1-picryl-hydrazyl radical ($DPPH^{\bullet}$) and the trapping of the $ABTS^{\bullet+}$ radical-cation (2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)).

The best antioxidant activities were~~a~~re obtained with the $ABTS^{\bullet+}$ radical scavenging with IC_{50} of 0.13 mg/mL for the aqueous extract and 0.19~~4~~ mg/mL for the isopropanol extract.

Keywords: Phytochemical screening, assay, antioxidant activity, CUPRAC, DPPH, ABTS, IC_{50}.

1. Introduction

In a broader sense, a medicinal plant is a plant endowed with a therapeutic effect on the organism, without being toxic at normal dose, as an antioxidant, antibacterial, anti-inflammatory, enzymatic inhibitor due to its~~by~~ active molecules and compounds (polyphenols, flavonoids, alkaloids, terpenes, etc.). Today, many works carried out in the field of ethnopharmacology~~,~~ show that the plants used in traditional medicine and which were tested are often on one hand, effective plants in the pharmacological model and on other hand would be deprived of toxicities at certain doses. Among the recognized biological potentialities of plants, antioxidant activity is of increasing interest because of the important roles that antioxidant compounds, found in plants, play in the treatment and prevention of oxidative stress-mediated diseases [1].

In this context, our choice is based on a medicinal plant which is named *Chrysopogonnigritanus*, belonging to the Poaceae family. *C.~~hrysopogon~~nigritanus* is a

Style Definition: Heading 3

Style Definition: No Spacing

Comment [DAP1]: Please do not use paragraphs in the abstract.

Formatted: Subscript

Comment [DAP2]: Please indicate some of the remaining results of the study and the main conclusions.

Comment [DAP3]: Consider deleting the keyword "assay".

Formatted: Subscript

Comment [DAP4]: What is the normal dose? Please clarify it.

Comment [DAP5]: The sentence is confusing. Please revise it.

Comment [DAP6]: After writing "*Chrysopogon nigritanus*" using full name, only use the abbreviated name "*C. nigritanus*".

medicinal plant widely used because of its considerable therapeutic virtues. ~~*Chrysopogon nigritanus* is one of the species of the Poaceae family.~~ The Poaceaes or grasses, are a family of monocotyledonous plants of the order Poales, which includes about 12,000 species grouped in 780 genera, with cosmopolitan distribution.

They are generally herbaceous plants, more rarely woody (bamboos), which share morphological characteristics that clearly distinguish them from other plant families.

C. ~~hrysopogon~~nigritanus is a large, erect perennial herb in cespitose clumps, entirely glabrous, reaching 3 m in height. The leaves are rough, linear, tip-terminated, often folded, with scabrous margins. The sheath is compressed, carinated, the ligule reduced to a line of hairs. The inflorescence is a large pyramidal panicle composed of numerous purple racemes arranged in whorls. The spikelets are paired, one sessile, hermaphrodite, the other pedicellate and only male [2].

Figur~~Image~~ 1: ~~The~~ Representation of *C. ~~hrysopogon~~nigritanus* plant.

C. ~~hrysopogon~~ nigritanus also called vetiver in French is a plant generally used in India, Pakistan, Malaysia and in many African countries for its numerous therapeutic virtues especially in traditional medicine. In India and Pakistan, vetiver is used to treat mouth ulcers, boils, burns, epilepsy, snake bites, fever, headaches, ~~and~~ infections, ~~...~~etc. In Malaysia, the wet powder is applied to the abdomen after the delivery of women. In Senegal, vetiver roots are used ~~as is~~ to purify water. Young girls use the infusion of roots to relieve cramps caused by menstruation or for intimate cleansing. The macerati~~o~~ne or infusion of the root is also used to calm stress and as an aphrodisiac. The root of the plant is given to new brides as an infusion or mixed with rice porridge during the seven days of her stay in the bridal chamber. In Casamance, the root powder is used to accelerate the wound healing ~~of wounds and heal open wounds~~[3].

Vetiver is grown for many purposes. The plant helps to stabilize the soil and protects it from erosion, but it can also protect fields from pests and weeds. Vetiver has favorable qualities for animal feed. Vetiver leaves are a tasty fodder easily eaten by cattle, goats and sheep.In the

Comment [DAP7]: Indicate some of the therapeutic uses of this medicinal plant.

Comment [DAP8]: This information is repeated in a previous sentence.

Comment [DAP9]: Please consider replacing "Image" by "Figure".

Formatted: English (United States)

Formatted: Font: Italic, English (United States)

Formatted: Font: Italic, English (United States)

Formatted: English (United States)

Formatted: Font: Not Bold

Formatted: Space Before: 12 pt

East, the root has been used since ancient times in the manufacture of baskets and brooms. Rural communities in Thailand, Indonesia, the Philippines, Latin America, and Africa use vetiver leaves to produce a high-quality craft, an important income-generating activity.

From the roots, oil is extracted and used for cosmetics, aromatherapy, herbal skin care and Ayurvedic soap. Vetiver is also used as a flavoring agent, usually in the form of khus syrup.

Vetiver is primarily grown for the fragrant essential oil distilled from its roots. It is a more common ingredient in men's fragrances.

The objectives are to explore the phytochemical compositionstudy and the evaluation of the antioxidant activity of *C.hrysopogonnigritanus* root extracts, in order to valorize and rationalize its traditional use and to identify potential compounds of therapeutic interest.

2. Material and Methods

2.1. Plant material

The roots of *C.hrysopogonnigritanus* were collected in Thiès, more precisely in Nguint (latitude: 14.8035° north and longitude: 16.92586° west). They were dried at the Organic Chemistry and Therapeutics Research Team (ECOT) in Ngoundiane. After drying, the roots were crushed and recovered in powder form.

FigurImage 2: Dry and crushed rootsof *C.nigritanus.*

2.2. Extraction

Extraction is a process that involves the removal of chemicals from a given organ. There are several extraction methods. In our study, we proceeded by solid-liquid extraction, which is one of the most used processes in phytochemistry by maceration.

Solid-liquid extraction by maceration

Maceration is an infusion in a cold solvent. The operation, although generally long and withoften poor yield, is the only method that can be used in the case of extraction of a set of fragile molecules.

Formatted: Space Before: 12 pt

Comment [DAP10]: What was the pore size? And how was the plant material stored? Please indicate it briefly.

Comment [DAP11]: Consider replacing "removal" by "recovery".

Formatted: Left

We put down twice 15 grams (15g) of ~~powder of the~~powdered roots of *C.~~hrysopogon~~nigritanus* to maketwo extractions with two solvents (water and isopropanol). The two 15g of the obtained powder were each immersed in a 250 mL flask with 150 mL of distilled water in one and 150 mL of isopropanol in the other. After shaking, the mixtures were put under cover and left to macerate for 24 h with a 50 mL renewal of solvents.

2.3. Phytochemicals screening

After extraction, we were interested in the phytochemical study of the extracts. This study is based on staining and precipitation reactions in order to characterize the different families of secondary metabolites present in the two extracts [4, 5].

2.4. Dosage

~~Determination of polyphenols~~

The polyphenols content is determined using the Folin-Ciocalteu~~reagent~~ procedure[6, 8].A volume of 40µL of the isopropanol extract was taken and evaporated to dryness, after which 200 µL of distilled water was added. For the aqueous extract, 200 µL of extract was taken. Then, 150 µL of Folin-Ciocalteu reagent, 600µL of 20% Na_2CO_3 solution and 2.32 mL of distilled water were added successively. In parallel, a gallic acid range is prepared from a stock solution of 0.1 mg/mL. After 30 minutes of incubation in the dark, the absorbance is read at 760 nm using a UV-visible spectrophotometer. The amount of polyphenols is deduced from the equation of the calibration line and results are~~is~~ expressed as mg of gallic acid equivalents(GAE)/g of plant material.

~~Determination of flavonoids~~

The determination of flavonoids in extracts is based on the formation of a complex between aluminum trichloride ($AlCl_3$) and flavonoids [8].The aluminum trichloride ($AlCl_3$) method is used to quantify flavonoids in our two extracts. 1.25 mL of extract is taken and made up to 2.5 mL with the extraction solvents. A volume of 2.5 mL of a 2% ethanolic solution of $AlCl_3$ is added. In parallel, a quercetin line is prepared. After 60 min of incubation, the absorbance is read at 425 nm. The quantity of flavonoids is deduced from the calibration range established with quercetin and results are~~is~~ expressed in milligram of quercetin equivalents (QE)per gram of dry matter (mg QE~~EQ~~/g of Ms).

2.5. Study of the antioxidant activity of the extracts

The antioxidant activity was determined with three ~~(3)~~ methods: DPPH$^{\bullet}$ free radical scavenging, CUPRAC method and ABTS$^{\bullet+}$ scavengingassays [9-11].

Comment [DAP12]: This subsection should provide more details about the procedures followed to explore the presence of each phytochemicals class in the extracts.

Comment [DAP13]: Please consider rewrite "2.4. Determination of polyphenols and flavonoids contents" and delete below.

Comment [DAP14]: Why was the isopropanol extract dried and the aqueous extract used as liquid extract? Please explain.

Comment [DAP15]: Indicate the range of gallic acid concentrations tested please.

Comment [DAP16]: Define the abbreviation "GAE".

Comment [DAP17]: Indicate the range of quercetin concentrations tested please.

Comment [DAP18]: Consider replacing "EQ" by "QE" considering the full name of the abbreviation.

Determination of antioxidant activity by the CUPRAC method

The CUPRAC method (cupric ion reducing antioxidant capacity) is based on the monitoring of the decrease of the increased absorption of the Neocuproin complex (NC). Indeed, in the presence of an antioxidant ~~agent~~compound, the copper-neocuproin complex is reduced and this reduction is quantified spectrophotometrically at a wavelength of 450 nm.

For the determination of the antioxidant capacity with the CUPRAC method, three solutions are prepared, namely a solution of copper chloride $CuCl_2$ at 10^{-2}M, a solution of ammonium acetate at pH~~=~~7 and a solution of neocuproin at 7.5 mM.

For this purpose, a volume of 1.25mL of extract was taken and supplemented to 2.5mL with distilled water. Then, 1mL of each of the three prepared solutions is added into the 2.5mL. A range of Trolox is prepared in parallel. The whole is incubated for one hour in the dark (60 min) and the absorbance is read at 450 nm. The result is determined thanks to the equation of the Trolox calibration line.

Determination of antioxidant activity using DPPH$^{\bullet}$

The principle of this method is the reduction of the purple DPPH (2,2-diphenyl-1-picrylhydrazyl) to the yellow 2,2 diphenyl-1-picrylhydrazine. DPPH absorbs at 517 nm, but upon reduction by an antioxidant its absorption decreases.

To determine the antioxidant capacity, 40µL of each extract was taken and completed to 200µL with distilled water. Then, 3.8mL of a 0.1014 mM DPPH$^{\bullet}$ solution is added into the diluted extracts. A Trolox line is prepared in parallel. After 30 minutes of incubation in the dark, the absorbance is read at 517nm.

After the determination of the antioxidant capacity, we were interested in the reaction kinetics of the DPPH$^{\bullet}$ radical. The study was done over 180 minutes to see the evolution of the antioxidant activity of our extracts over time.

To do this, 200µL of each extract was taken and 3.8mL of the DPPH solution was added. The absorbance is read every 5 minutes at 517nm. The inhibition is calculated with the following formula:

$$\%\mathbf{Inhibition} = \frac{\mathbf{Abs\ control - Abs\ sample}}{\mathbf{Abs\ control}} \times \mathbf{100} \quad (1)$$

Determination of antioxidant activity with ABTS$^{\bullet+}$

The antioxidant activity with the ABTS$^{\bullet+}$ radical is determined by the method of Re et al., ~~1999~~. The total activity of a molecule is deduced by its ability to inhibit the ABTS$^{\bullet+}$ radical.

Formatted: Font: Not Bold, Superscript

Comment [DAP19]: Indicate the range of Trolox concentrations tested please.

Comment [DAP20]: Consider adding "...and presented as Trolox equivalents (TE) per gram of dry matter (mg TE/g of Ms)."

Comment [DAP21]: Indicate the range of Trolox concentrations tested please.

Comment [DAP22]: Please indicate the meaning of the variables used in the equation.

Comment [DAP23]: Please add the reference.

Thus, by reacting with potassium persulfate ($K_2S_2O_8$), ABTS forms the turquoise blue radical $ABTS^{\bullet+}$, the addition of an antioxidant ~~will~~ reduces this radical and causes the discoloration of the mixture.

The antioxidant capacity is determined with the $ABTS^{\bullet+}$ radical by the method of Thaipong et *al.* with some modifications.

For this purpose, a stock solution is prepared by mixing a 2mM solution of potassium persulfate and a 7mM solution of $ABTS^{\bullet+}$ in equal volume. The mixture is incubated for 16 hours before use. A working solution (WS) is prepared by diluting the stock solution to the 25th with phosphate buffer (0.2M; pH 7.4; 150mM NaCl) to have a maximum absorbance of 0.750 at 734nm.

For the determination of antioxidant capacity, a volume of 25µL is taken for each extract and supplemented to 100µL with distilled water. Then, 2.9mL of the ST solution of ABTS is added to the extracts completed to 100µL with distilled water. A Trolox~~ROLOX~~ line is prepared in parallel. After 30 minutes of incubation in the dark, the absorbance is read at 734nm.

The IC_{50} is determined by making six ~~(6)~~ dilutions and determining the percentage of inhibition using equation (1).

3. Results and discussion

3.1. Yields of extracts

To calculate the yield, it is first necessary to determine the mass of the macerates. The mass of each macerate is determined by the difference in the mass of the tube containing the extract and the mass of the same tube under vacuum. Once these different masses were obtained, the yield was determined.

The yield of each extract is obtained by making the ratio of the dry extract (macerate) to the mass of the plant material (root powder), all multiplied by one hundred (100).

$$\text{Extraction yield } \%\ = \frac{\text{Mass of dry extract}}{\text{Mass of plant powder}} \times 100 \quad (2)$$

Table 1: E~~The~~ xtraction yields obtained for aqueous and isopropanol extracts of *C.nigritanus* roots.

Extracts	Aqueous extracts	Isopropanol extract
Yield %	3,16	2,21

Comment [DAP24]: What is the meaning of "25th"? Please clarify it.

Comment [DAP25]: Please indicate the range of Trolox concentrations tested.

Formatted: Font: Not Bold, Subscript

Comment [DAP26]: In this section, the authors should also compare the results obtained with the literature available on the subject under study.

Formatted: Space Before: 12 pt

Comment [DAP27]: Please consider replacing "R" by "Extraction yield".

Comment [DAP28]: Use points instead of commas in the results presented. Why is the standard deviation not presented? Please include it.

Table 1 reveals that the aqueous extract has the highest yield compared to that of the isopropanol extract. These results can be explained by the polarity of the extraction solvents. Indeed, water being more polar than isopropanol, it tends to extract more polar molecules. More polar molecules are extracted with the aqueous extract than with isopropanol.

Comment [DAP30]: Inlcude references to support these statements.

Formatted: Space Before: 12 pt

3.2. Phytochemicals screening

Phytochemicals screening of secondary metabolites in the extracts was performed using the methods mentioned above and according to the availability of reagents. Table 2 presents the results obtained.

Table 2: Results of phytochemical tests.

Compounds	Aqueous extract	Isopropanol extract
Terpenes	+	+
Polyphenols	+	+
Flavonoids	+	+
AlkaloidswithDragendorft	+	–
Alkaloidswith Wagner	+	+
Saponins	–	–
Condensed tannins	+	–
Gallic tannins	+	–
Coumarins	+	+

+: presence; –: Absence.

Comment [DAP31]: Or gallotannins?

Formatted: Centered

Phytochemical tests performed on both extracts revealed:

- The presence of terpenes, polyphenols, flavonoids and coumarins in both extracts,
- The presence of alkaloids, condensed and gallotannins in the aqueous extract,
- The absence of saponins in both extracts,
- The absence of condensed and gallotannins in the isopropanol extract.

From these results, we can say that the aqueous extract is richer in secondary metabolites than the isopropanol extract. Due to the high polarity of water, it is easier to extract polar molecules from the plant than isopropanol. Therefore, it is more likely that the ~~water~~ aqueous extract contains more polar compounds than the isopropanol extract.

3.3. ~~Dosages~~Determination of polyphenols and flavonoids contents

P~~Dosage of p~~olyphenols content

The polyphenols content is determined using the Folin-Ciocalteu reagent. Gallic acid is used as a standard, the result obtained is presented in a calibration curve (Appendix 1) whose equation: **Y = 0,0343x + 0,0762** with **R^2 = 0,9988**

Table 3: Polyphenols content of extracts.

Extracts	Polyphenols content (mg GAE~~EAG~~/g of~~de~~ Ms)
Isopropanol	1,171 ± 0,0108
Aqueous	1,170 ± 0,0082

According to the results of T~~t~~able 3, we notice that the two extracts have almost the same contents of polyphenols.~~:~~ These results are in correlation with the results of the phytochemicals screening concerning the polyphenols which showed the same aspect in the two extracts.

Determination of flavonoids

The determination of flavonoids in extracts is based on the formation of a complex between $AlCl_3$ and flavonoids. The absorbance is read at 425 nm.

Quercetin was used as standard, the calibration curve (Appendix 2) having the equation:

Y= 0,~~-~~0203x + 0,~~-~~0732 with **R^2 = 0,9953**

Table4: Flavonoids contents of extracts.

Extracts	Flavonoids content (mg QE~~EQ~~/g of~~de~~ Ms)
Isopropanol	0,0966 ± 0,00035
Aqueous	0,1584 ± 0,00093

The results of the flavonoid assay show that the aqueous extract has the highest amount of flavonoids with 0.1584 ~~E~~QE/g of Ms, while that of the isopropanol extract is 0.0966 ~~E~~QE/g of Ms.

According to these different results obtained, it has been disputed that the quantity of polyphenols is not proportional with that of flavonoids in both extracts.

3.4. Evaluation of the antioxidant activity of the extracts

The antioxidant activity of the extracts was determined by ~~the~~ three methods, namely~~: the~~ CUPRAC ~~method~~, $DPPH^{\bullet}$ and ~~the~~ $ABTS^{\bullet+}$ methods.

Determination of the antioxidant capacity by the CUPRAC method

Formatted: Space Before: 12 pt

Comment [DAP32]: Missing the statistical analysis between extracts.

Comment [DAP33]: Missing the statistical analysis between extracts. In addition, consider using the same number of decimal cases in all the results presented along the manuscript.

Formatted: Centered, Indent: First line: 0"

The CUPRAC method is based on the capacity of antioxidants to reduce Cu^{2+} ions to Cu^{+}. Therefore, neocuproin is used as a chelating agent. The Cu^{+} ions will therefore form acomplex with neocuproin.

This method thus allows the mobilization of antioxidants, having one or more thiol groups and lipophilic and hydrophilic antioxidants. ~~A range of~~ Trolox was used as standard and~~,~~ the calibration line of Trolox (Appendix 3) has the equation:

Y = 0.~~,~~0116 + 0.~~,~~2745with **R^2 = 0.~~,~~9917**

Table 5 summarizes the antioxidant capacities obtained with the CUPRAC method of extracts.

Table 5: Antioxidant capacity of extracts by the CUPRAC method.

Extracts	Antioxidant capacity (mg TE~~ET~~r/g of Ms)
Isopropanol	0.~~,~~925 ± 0.~~,~~0139
Aqueous	2.~~,~~914± 0.~~,~~0116

The results with the CUPRAC method revealed that the aqueous extract has the highest antioxidant capacity with 2.914 mg TE~~ET~~r/g Ms, while that of the isopropanol extract is 0.925 mg TE~~ET~~r/g Ms.

Comparing these results, it appears that even if the isopropanol extract is a little rich in polyphenols than the aqueous extract, the antioxidant capacity is better in the aqueous extract. It appears that the two extracts do not contain the same types of polyphenols or the same proportions for polyphenols of the same category.

Determination of antioxidant activity by the DPPH• method

Table 8 summarizes the results of the antioxidant capacity of each of the extracts determined using the Trolox equation line (Appendix 4) with the DPPH method.

Table 6: Antioxidant capacity of extracts by the DPPH•method.

Extracts	Antioxidant capacity (mg TE~~ET~~r/g extract)
Aqueous	0.~~,~~5945±0.~~,~~0019
Isopropanol	0.~~,~~5964±0.~~,~~0071

According to Table 6, the two extracts show a small difference in antioxidant capacity. Indeed, the isopropanol extract presents an antioxidant capacity of 0.5964 mg TE~~ET~~r/g of Ms,

Formatted: Font: 12 pt, Not Italic, Superscript

Comment [DAP34]: Missing the statistical analysis in all Tables presented. Please perform the statistical analyses for all data between the two extracts.

Comment [DAP35]: Missing the statistical analysis between extracts.

while that of the aqueous extract is 0.5945 mg TE~~ET~~/g of Ms. These results are in correlation with the contents of polyphenols in the two extracts which presented the same tendencies.

Reaction kinetics

After having determined the antioxidant capacity for the DPPH radical, we were interested in the reaction kinetics. The study was done over 180 minutes to see the evolution of the antioxidant activity of our extracts over time. The antioxidant capacity is measured every 5 minutes over the 180 minutes. Figure 1 shows the evolution of the antioxidant activity of our two extracts.

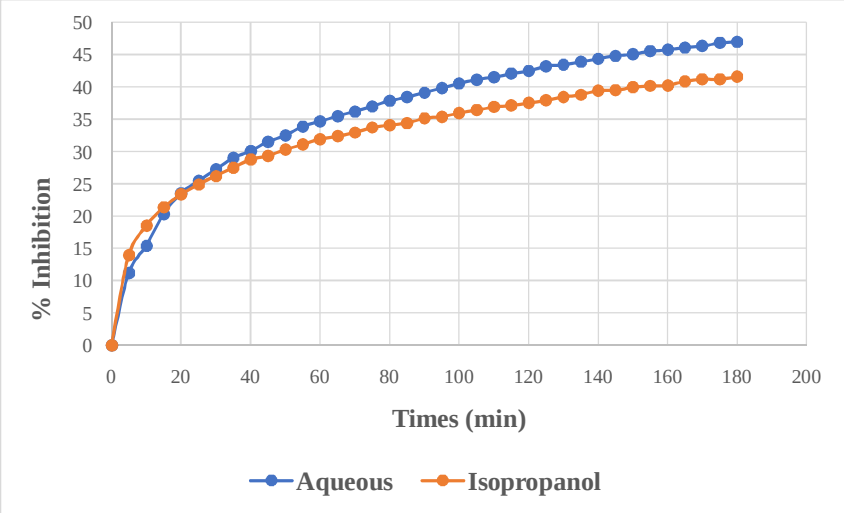

Figure 1: Evolution of the antioxidant activity of the two extracts.

Comment [DAP36]: Please provide the error bars for each timepoint.

Figure 1 shows that during the first 15 minutes, the antioxidant activity of the isopropanol extract is slightly higher than that of the aqueous extract, with an inhibition of 21.36% for the isopropanol extract against 20.33% for the aqueous extract after 15 minutes.

After 20 minutes until the end of the reaction, a progressive evolution of the oxidative activity of the two extracts is noticed. In this part of the evolution, the inhibitions of the aqueous extract are more important than those of the isopropanol extract with a maximum inhibition of 46.99% for the aqueous extract and 41.59% for the isopropanol extract. This makes a difference in inhibition of 5.4% in favor of the aqueous extract.

Evaluation of the antioxidant activity by the $ABTS^{\bullet+}$ method

The antioxidant capacity of each of the extracts is deduced from the equation line of Trolox (appendix 5) which has the equation: **Y=- 0,50347 + 0,56428 with R^2=0,59901** and the results are given in Table 7.

Table 7: Antioxidant capacity by $ABTS^{\bullet+}$ method.

Extracts	Antioxidant capacity (mg TE~~ET~~/g of~~de~~Ms)
Aqueous	6,324±0,068
Isopropanol	6,975±0,031

The results confined in Table 7 show that the antioxidant capacity of the isopropanol extract is a little higher than that of the aqueous extract. The antioxidant capacity of the two extracts ~~are~~is6.975±0.031 mg/g for the isopropanol extract and 6.324±0.068 mg/g for the aqueous extract respectively. These results can be correlated with those of the polyphenol contents: 1.171 mg EAG/g of Ms for the isopropanol extract and 1.170 mg EAG/g of Ms for the aqueous extract. So, we can say that it is partly due to the difference of the polyphenol content in the extracts, since there are other compounds playing the role of antioxidant.

Determination of the IC_{50} of extracts

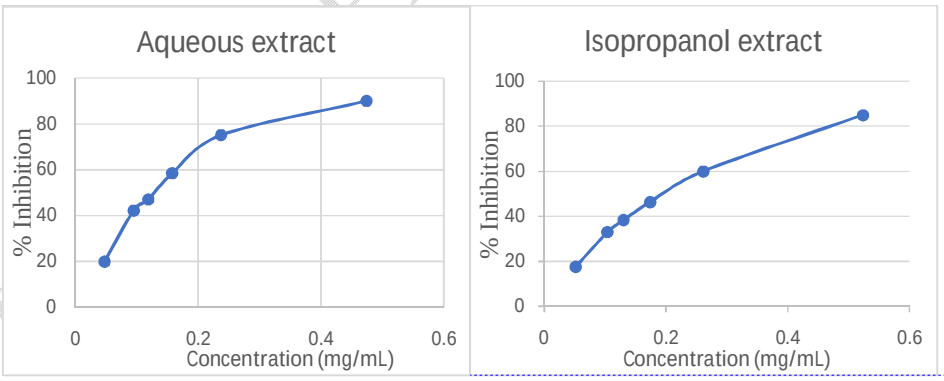

Figure 2: Curves of % inhibitions as a function of different concentrations.

After determining the antioxidant capacity, the evolution of the antioxidant activity of different concentrations of extract was evaluated. From figure 2, it can be seen that the antioxidant activity increases with the concentration. ~~It can be seen that both~~Both extracts are

Comment [DAP37]: Missing the statistical analysis between extracts.

Formatted: Font: 12 pt, Not Italic, Font color: Auto, Subscript

Comment [DAP38]: Use points in the numbers of axis x. Also, where are the error bars? Please provide them.

able to inhibit at least 50% of the radical activity induced by $ABTS^{\bullet+}$. We also notice that the aqueous extract is able to inhibit 90% of this activity with a concentration of 0.474mg/mL; for the isopropanol extract, we need a higher concentration of 0.523mg/mL to inhibit about 84.96% of this activity by the $ABTS^{\bullet+}$ radical.

Thanks to this experiment, we can say that the antiradical capacity is dependent on the dose and the nature of the compounds contained in the extract. We will then determine the concentration causing 50% of the inhibition of each extract (Table 8).

Table 8: IC_{50} of extracts by $ABTS^{\bullet+}$ radicals scavenging assay.

Extracts	IC_{50} (mg/mL)
Aqueous	0,13
Isopropanol	0,194

Table 8 shows that the aqueous extract has the best IC_{50} which is 0.13 mg/mL, while that of the isopropanol extract is 0.194 mg/mL. These results are not comparable with the polyphenol contents in the extracts. This can be explained by the presence of other compounds in the extracts acting as antioxidants.

According to the three methods to test the antioxidant activity, the test with $ABTS^{\bullet+}$ shows the best activity. It can be said that the activity also depends on the type of radical used to measure the activity.

Finally, we can say that the study of the antioxidant activity of *C.~~hrysopogon~~nigritanus* showed the potential of the free radicals of the roots of this plant which could be considered as a source of natural antioxidants. The observed antioxidant activity would be due to their content of polyphenols and flavonoids which are the main components of plant with antioxidant activity.

1. Conclusion

In the present work, we were interested in the phytochemicals screening, the determination of polyphenols and flavonoids and the evaluation of the antioxidant activity of ~~our~~ two different extracts of the roots of ~~the*Chrysopogon*~~ *C.nigritanus* plant.

From the phytochemical point of view, we performed the solid-liquid extraction by maceration. The phytochemicals screening showed the richness of our plant in secondary metabolites in the aqueous extract (the presence of alkaloids, steroids, polyphenols, flavonoids, condensed and gallic tannins and coumarin).

Formatted: Font: 12 pt, Not Italic, Font color: Auto, Subscript

Formatted: Font: 12 pt, Not Italic, Font color: Auto, Subscript

Comment [DAP39]: Please add the standard deviation to each result provided. Also, the statistical analysis is missing between extracts.

Formatted: Font: 12 pt, Not Italic, Font color: Auto, Subscript

Comment [DAP40]: What kind of compounds? Please discuss in more detail. Also, provide at least one reference to support this statement.

It appears from the study of the antioxidant activity evaluated by three different methods, namely the CUPRAC method, the DPPH method and the $ABTS^{\bullet+}$ method, that all the extracts of the plant exhibit antioxidant activity. However, the ABTS method gives the best activity with IC_{50} for each extract.

We can say that these results are encouraging and demonstrate the richness of this plant which is known for its therapeutic virtues.

4. References

[1]. fr.m.wekipéda.org (poaceae, famille de plante monocotylédones de l'ordre des Poales)

[2]. https://publish.planet.project.org(Riceweedsfr-poaceae-chrysopogonnigritanus (Benth.)) Veldkamp

[3]. Singleton V.L and Rossi J.A.J. (1965). Colorimetry of total phenolics with phosphomolybdic phosphotungstic acid reagents. *Amer. J. Enol. Viticult. Vol (16), p144-58.*

[4]. Berrani, A., Lrhorfi, L. A., Dahmani, Fkhir, H. and Bengueddour, R. (2015). Screening of two phytochemical medicinal plants: vitex agnus castus and anabasis aretioides. *ScienceLib Editions Mersenne*, *7*, 151201.

[5]. Kustiati, U., Wihadmadyatami, H; andKusindarta, D. L. (2022). Dataset of Phytochemical and secondary metabolite profiling of holy basil leaf (*Ocimum sanctum* Linn) ethanolic extract using spectrophotometry, thin layer chromatography, Fourier transform infrared spectroscopy, and nuclear magnetic resonance. *Data in Brief*, *40*, 107774. https://doi.org/10.1016/j.dib.2021.107774

[6]. Singleton, V. L. and Rossi, J. R. (1965). Colorimetry of total phenolics with phosphomolybdic –phosphothungstic acid. *Am. J. Enol. Vitic.*, *16*, 144–158.

[7]. Alara, O. R., Abdurahman, N. H. and Olalere, O. A. (2020). Ethanolic extraction of flavonoids, phenolics and antioxidants from *Vernonia amygdalina* leaf using two-level factorial design. *Journal of King Saud University - Science*, *32*(1), 7–16. https://doi.org/10.1016/j.jksus.2017.08.001

[8]. Benariba, N., Djaziri, R., Bellakhdar, W., Belkacem, N., Kadiata, M., Malaisse, W. J. and Sener, A. (2013). Phytochemical screening and free radical scavenging activity of *Citrullus colocynthis* seeds extracts. *Asian Pacific Journal of Tropical Biomedicine*,

Formatted: Font: 12 pt, Not Italic, Font color: Auto, Subscript

Comment [DAP41]: If possible, consider to discuss briefly the future perspectives on this study.

Comment [DAP42]: More references are needed to support the findings and statements made along the manuscript.

Comment [DAP43]: Revise these references from websites according to the journal guidelines.

Formatted: Font: 12 pt, Font color: Auto

Formatted: Font: 12 pt, Font color: Auto

Comment [DAP44]: Some journals names are presented as full name, while others are presented in the abbreviated name. Please revise them.

Formatted: Font: 12 pt, Font color: Auto

3(1), 35–40. https://doi.org/10.1016/S2221-1691(13)60020-9

[9]. Malika, B. and Imegrave, L. (2012). Antioxidant activity of the essential oil from the flowers of *Lavandula stoechas*. *Journal of Pharmacognosy and Phytotherapy*, *4*(7), 96–101. https://doi.org/10.5897/JPP12.030

[10]. Tanoh, E. A., Nea, F., Kenne Kemene, T., Genva, M., Saive, M., Tonzibo, F. Z. and Fauconnier, M.-L. (2019). Antioxidant and Lipoxygenase Inhibitory Activities of Essential Oils from Endemic Plants of Côte d'Ivoire: Zanthoxylum mezoneurispinosum Ake Assi and Zanthoxylum psammophilum Ake Assi. *Molecules*, *24*(13), 2445. https://doi.org/10.3390/molecules24132445

[11]. Souda, S., George, S., Mannathoko, N., Goercke, I. and Chabaesele, K. (2018). Antioxidant and Antibacterial Activity of Methanol Extract of Momordica Balsamina. *IRA-International Journal of Applied Sciences (ISSN 2455-4499)*, *10*(2), 7–17. https://doi.org/10.21013/jas.v10.n2.p1

Formatted: Font: 12 pt, Font color: Auto