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DETERMINATION OF POLYPHENOL CONTENT AND EVALUATION OF ANTIOXIDANT ACTIVITY IN EXTRACTS FROM THE *CHRYSOPOGON NIGRITANUS* PLANT

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Abstract

Chrysopogon nigritanus is a plant traditionally used for various purposes, particularly in traditional medicine due to its various properties of pharmaceutical and cosmetic properties. From a scientific point of view, this plant has not been widely studied, particularly in the field of pharmacology. This study aims to evaluate the antioxidant activity of *C. nigritanus* roots using the DPPH and FRAP methods as well as to determine its polyphenolic compound content, which is directly related to this activity. Four extracts obtained by successive maceration according to a polarity gradient (cyclohexane, ethyl acetate, ethanol, and water) were studied. The results of the DPPH assay revealed significant antioxidant activity for all extracts, with respective IC_{50} values of 2.445; 0.555; 0.052 and 0.115 mg/mL, compared to 0.002 mg/mL for ascorbic acid used as a reference. The FRAP assay showed an increase in reducing power proportional to the concentration of the extracts, thus confirming the results obtained with the DPPH assay. These results suggest that *C. nigritanus* roots are a potential source of natural antioxidant compounds.

Keywords: *Chrysopogon nigritanus, polyphenols, flavonoids, antioxidant, DPPH, and FRAP.*

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Introduction

Natural antioxidants of plant origin are of major interest due to their central role in preventing oxidative stress and associated diseases. Beyond their contribution to health promotion, , these compounds also play a role in product storage, as endogenous antioxidant constituents help to extend the shelf life of foods [1]. Phenolic compounds, particularly polyphenols and flavonoids, represent one of the main classes of secondary metabolites responsible for the antioxidant activity of plants. They play an essential role in human health because they are able to neutralize free radicals and inhibit or delay harmful oxidation reactions. Their mechanisms of action are include on scavenging free radicals, donating electrons or hydrogen atoms, complexing metal ions, inhibiting oxidizing enzymes, or inhibiting lipid auto-oxidation. They protect the body against the effects of oxidative stress and react synergistically with other antioxidants to regenerate themselves. Antioxidants are effective at low concentrations and protect against many chronic diseases, including inflammatory, neurodegenerative, cardiovascular, and cancerous conditions, as well as respiratory infections caused by soot or carbon. [2-7].

In this context, the scientific study of medicinal plants used in traditional African pharmacopoeia is a relevant approach for identifying new sources of natural antioxidants [8]. It is in this vein that we have we focused on the species *Chrysopogon nigritanus*, a plant widely used for medicinal and agri-food purposes. The latter remains largely unexplored in pharmacological terms, with existing work focusing mainly on its essential oil. This study therefore aims to fill this gap by systematically evaluating the antioxidant activity and phytochemical composition of root extracts from this species.

Presentation of the plant

The plant *Chrysopogon nigritanus* (Benth), better known by its taxonomic synonym Vétiveria nigritana, is a perennial grass species of the Poaceae family, a monocotyledon that grows along waterways and floodplains. Vetiver has neither stolons nor rhizomes, and its well-structured, massive root system can grow very quickly, with a root depth of up to 3 to 4 m in the first year under certain conditions [9-11]. Traditionally, *C. nigritanus* is used in African and Ayurvedic medicine, among others, for the treatment of various conditions, particularly thanks to its antioxidant, antimicrobial, antifungal, anti-inflammatory, and healing properties. In addition, the plant is used in cosmetics, ecology, and perfumery, which highlights the

value of its scientific exploitation [12, 14].

Fig 01: An image of the Chrysopogon Nigritanus plant

Materials and Methods

The roots of *Chrysopogon nigritanus* were harvested in October 2023 in the Dakar region, specifically in Hann Bel-Air. This locality is home to the Hann Forest and Geological Park, located between 14° 43′ 34″ north and 17° 26′ 02″ west, covering approximately 60 hectares. This park is a haven of nature, with its ethnobotanical garden teeming with hundreds of identified plant species of diverse origins.

After botanical identification, the samples were washed, dried away from light at room temperature, and then finely ground. The resulting plant powder was stored in airtight glass containers until use.

1. Extraction Method

The extraction of our four extracts, namely cyclohexane, ethyl acetate, ethanol, and aqueous, was performed using a gradient based on the increasing polarity of the extraction solvents from 30 g of *Chrysopogon Nigritanus* powder. First, the powder was macerated in 250 mL of cyclohexane for 24 hours to obtain the cyclohexane extract, and the dried residue was used with 250 mL of ethyl acetate for maceration for 24 hours to obtain the latter extract. The dry residue was then macerated with 250 mL of ethanol for 24 hours as well, and our ethanolic extract was obtained after filtration. Finally, the dry residue was macerated in 250 mL of distilled water for 24 hours to obtain the aqueous extract after filtration. The extracts were evaporated to dryness in an oven.

The extraction percentage was calculated using formula (1):

$$\mathbf{EP}\ (\%) = \frac{\mathrm{M}}{M_0} \times 100 \quad (1)$$

EP: extraction percentage. M: mass in grams of the resulting extract, M_0: mass in grams of the plant material used.

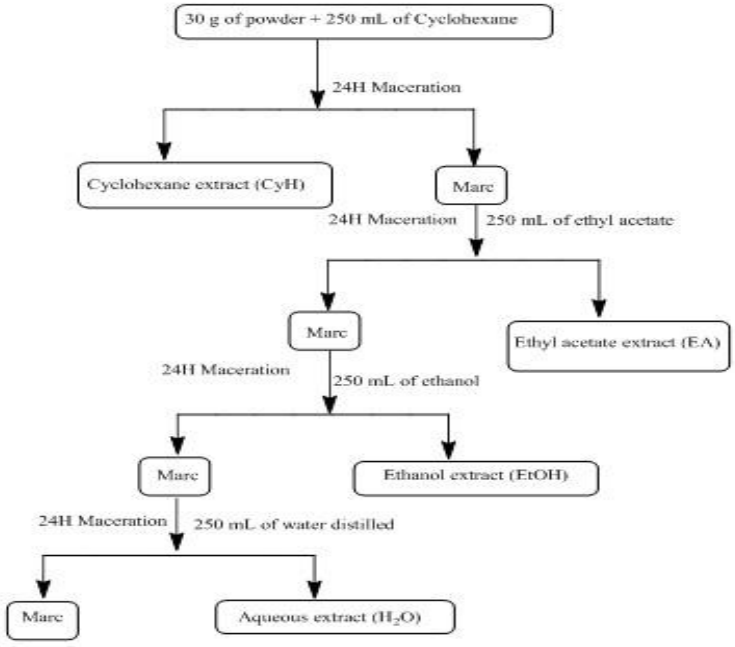

Figure 01: Extraction diagram

1. Chemical Screening

Phytochemical screening is a method used to identify certain families of natural products contained in a plant sample. Specific reagents are used to detect the presence or absence of certain molecules or groups of molecules in a given sample. These assays are based on reactions involving coloration, turbidity, foaming, fluorescence, or precipitation, and the results obtained confirm the presence or absence of these molecules but do not allow their concentration to be quantified. There are several types of assays that reveal molecular families of natural products, which are listed in the table above:

Table 01: Phytochemical screening assays

Chemical groups	assay titles	Preparations	Positive results	References
Alkaloids	Dragendorff and Mayer	2 mL of extract + 5 drops of Dragendorff's or Mayer's reagent	Formation of brick-red colored precipitates	[14]
Polyphenols	Iron chloride	2 mL of extract + 5 drops of 2% $FeCl_3$	Appearance of a bluish-black or greenish coloration, more or less dark.	[8]
Tannins	Ferric chloride	2 mL of extract + 1 to 2 drops of 1% $FeCl_3$	Greenish or blue-green coloration	[15]
Flavonoids	Shinoda or cyanidin	2 mL of extract + 3 to 4 magnesium fragments + 3 to 4 drops of HCl	Cherry red or yellow coloring	[14]
Anthocyanins	Demonstration of anthocyanins	5 ml of the extract + 5 ml concentrated sulfuric acid + 5 ml of NH_4OH	Appearance of a red coloration in acidic medium and a bluish-purple coloration in basic medium	[15]
Saponins	Foam assay	2 mL of extract + 3 mL H_2O + stirring	Persistent foam forms after 15 minutes	[16]
Coumarins	NH_4OH Confirmation assay	1 mL of extract + A few drops of ammonia	Appearance of a Blue fluorescence under UV	[16]
Terpenes	Liebermann Burchard	Dissolve the dry residue of the extract in 1 mL of acetic anhydride + 0.5 mL of H_2SO_4 using a hot solution.	Appearance at the interface of a purple or violet ring turning green	[8]

2. Dosages

▪ The total phenolic content (TPC)

The dosage of phenolic compounds allows for a quantitative assessment of the richness of these compounds in the extracts of the *C. nigritanus* roots studied. The total phenolic compound content of the extracts was determined using the Folin–Ciocalteu colorimetric method, according to the procedure described by Alara et al. (2015) [17]. The results were expressed in milligrams of gallic acid equivalent per gram of dry extract (mg GAE/g DE).

For each analysis, 100 µL of extract solution (1 mg/mL) or gallic acid standard solution at different concentrations were mixed with 500 µL of Folin–Ciocalteu reagent diluted to one-tenth. After 2 minutes of reaction, 2 mL of 20% sodium carbonate (Na_2CO_3) was added to alkalize the medium. The mixtures were incubated in the dark for 30 minutes at room temperature, then the absorbance was measured at 760 nm against a reagent blank. All measurements were performed in triplicate. The total polyphenol content was calculated in mg gallic acid equivalent (GAE)/g dry extract.

▪ Total flavonoid content (TFC)

The flavonoid content allows for a quantitative assessment of the richness of these compounds in the extracts of the C. nigritanus roots studied. The total flavonoid content was determined using the aluminum chloride method described by Zhishen et al. (1999) [18], using catechin as the standard. The results were expressed in milligrams of catechin equivalent per gram of dry extract (mg CE/g dry extract).

3. In an assay tube, 250 µL of extract solution (1 mg/mL) or catechin standard solution was mixed with 1 mL of distilled water, followed by the addition of 75 µL of $NaNO_2$ (15%). After incubation for 6 minutes at room temperature, 75 µL of aluminum chloride ($AlCl_3$, 10%) was added, followed by 1 mL of NaOH (1 M). The final volume was adjusted to 2.5 mL with distilled water. After incubation for 15 minutes, the absorbance was measured at 510 nm. The analyses were performed in triplicate.

▪ Antioxidant Activity Assays *Dpph Assay*

The DPPH• radical is a compound that is initially purple in color. It discolors in the presence of an electron donor to stabilize into DPPHH, which is yellow-white in color, depending on the concentration of the extract that is the electron donor. This reaction is monitored using a spectrophotometer at 517 nm. The intensity of the yellow coloration and the decrease in violet absorbance are proportional to the antioxidant's ability to trap free radicals independently of any enzymatic activity [19, 20]. The assay was performed according to the procedure described by Barakat et al. (2012) [21].

A methanolic solution of DPPH• (0.04 mg/mL) was prepared and stored away from light. The extracts were assayed at different concentrations, prepared by dissolving them in their respective solvents.

In each tube, 1.5 mL of extract solution was mixed with 1.5 mL of DPPH• solution and then incubated in the dark for 30 minutes. The absorbance was measured at 517 nm using a UV-visible spectrophotometer. Ascorbic acid was used as a positive control. The percentage inhibition was calculated using equation (4), and the 50% radical inhibitory concentration (IC50) was determined using the linear regression equation of IPs as a function of concentration.

▪ FRAP assay

The ferrous reduction antioxidant power (FRAP) assay is a widely used method for evaluating the antioxidant capacity of a product. The objective of the reaction is to reduce Fe^{3+}-TPTZ (iron [III]-2,4,6-tripyridyl-S-triazine) to Fe^{2+}-TPTZ, resulting in

an intense blue color at the end of the reaction. The absorbance is proportional to the reducing power [22-24]. The assay is performed according to the experimental procedure described by Lamia et al. (2020) [25].

The extract solutions were prepared at different concentrations. For each assay, 1 mL of extract was mixed with 1 mL of phosphate buffer (0.2 M; pH 6.6) and 1 mL of potassium ferricyanide (1%). The mixture was incubated in a water bath at 50°C for 20 minutes.

After incubation, 1 mL of trichloroacetic acid (10%) was added, followed by centrifugation at 3000 g for 10 minutes. The supernatant was collected and mixed with 1.5 mL of distilled water and 150 µL of ferric chloride (0.1%). The absorbance was measured at 700 nm. The increase in absorbance as a function of concentration was interpreted as an increase in antioxidant capacity. The activity was evaluated by comparing the absorbance of the sample to that of the standard (AA).

Results and Discussion

Results

The results of the extraction, phytochemical screening, assays, and DPPH and FRAP assays of the four extracts from *C. nigritanus* roots are given in tables and represented graphically for better understanding and readability of the differences.

Extraction results

Table 02: Extraction Results

Extract	% extraction
CyH	2 .58
EA	1.69
EtOH	4.86
H_2O	2.5

Screening Results

Table 01: Results of phytochemical screening

Chemical families	CyH extract	EA extract	EtOH extract	H_2O extract
Dragendorff Alkaloids	+	+	-	-
Wagner Alkaloids	+	+	-	-
Polyphenols	+	+	+	+
Tannins	+	+	+	+
Flavonoids	+	+	+	+
Anthocyanins	+	+	+	+
Saponins	-	-	+	+
Coumarins	+	+	-	+
Terpenes	+	+	-	-

Legend: + presence and – absence

Total polyphenol content

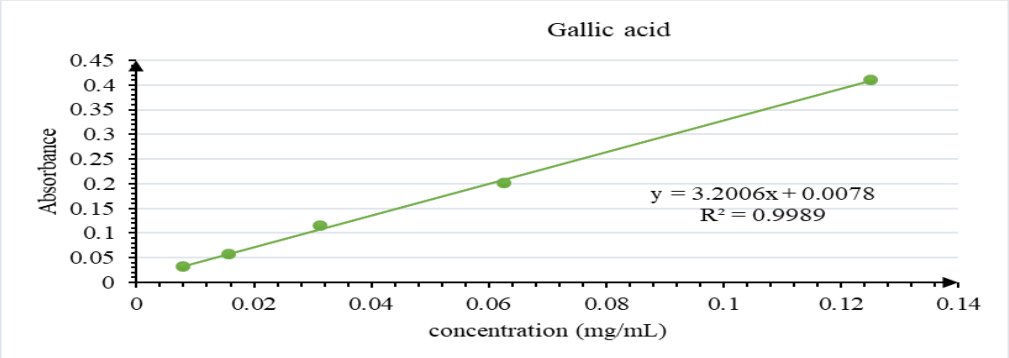

Fig 02: Gallic acid calibration curve

Table 02: Polyphenol content per mg GAE/g of dry extract

Extract	Average TCP (mg GAE/g)
CyH	12.466 ± 1.010
EA	41.513 ± 8.159
EtOH	61.905 ± 4.033
H_2O	26.131 ± 0.806

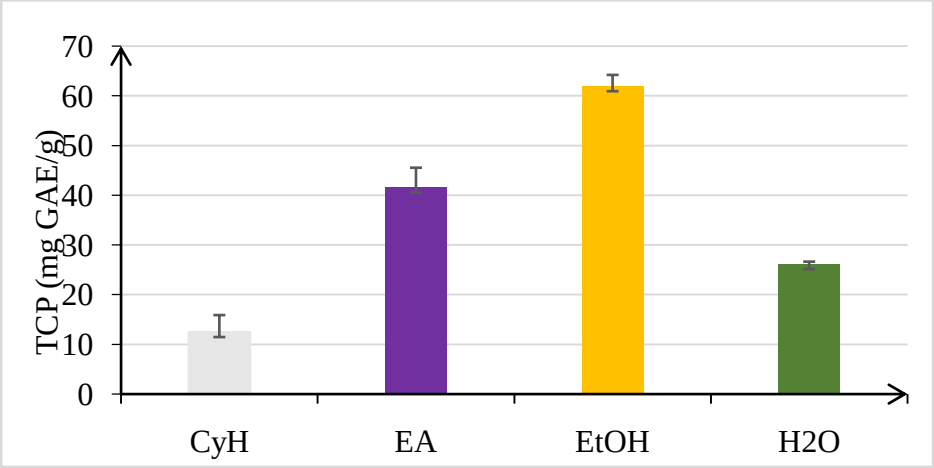

Fig 03: TPC in mg EAG/g of dry extract

Results of flavonoid analysis

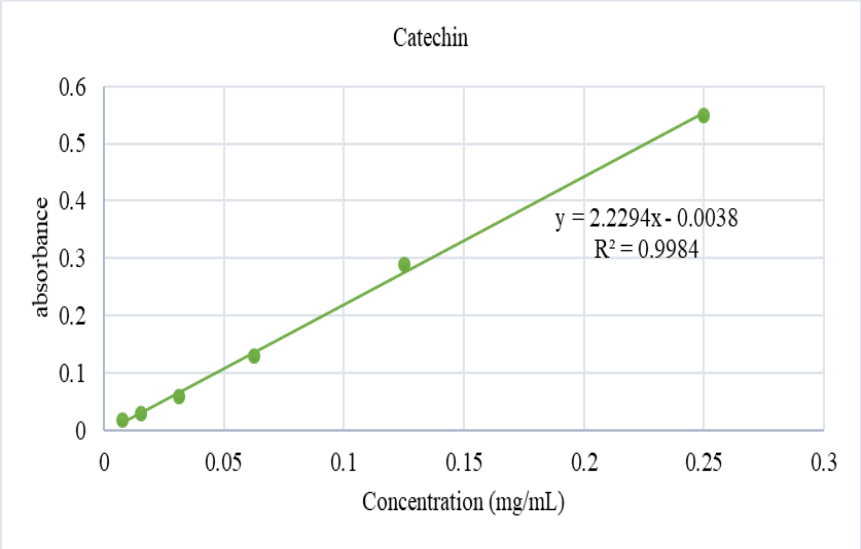

Fig 04: Catechin calibration curve

Table 03: Flavonoid content in mg EC/g of dry extract

Extract	Average TCF (mg EC/g)
CyH	44.257 ± 4.354
EA	34.074 ± 1.643
EtOH	27.629 ± 2.062
H_2O	14.263 ± 1.992

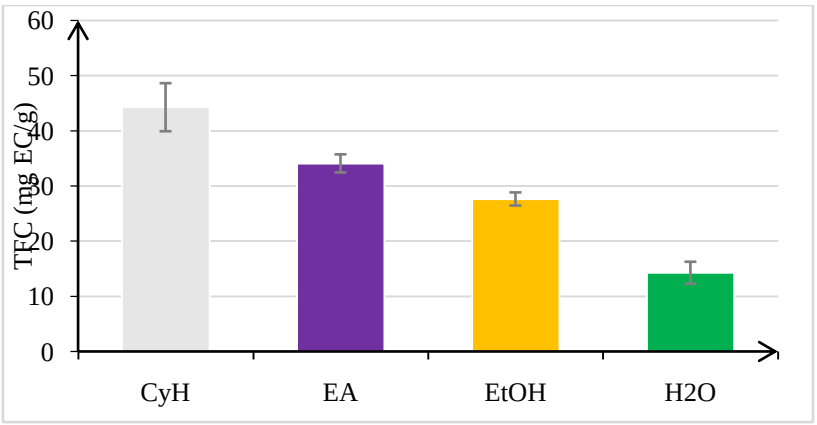

Fig 05: Representative diagram of the total fat content in mg EC/g of dry product

The results of the DPPH assay

The results of the DPPH method assay for antioxidant activity in C. nigritanus root extracts are shown in the table below:

Table 04: DPPH assay results

		Concentration (mg/mL)				
Extracts	**PI/repetition**	**4**	**2**	**1**	**0.5**	**Average CI_{50}**
CyH	PI N1	39.812	35.387	28.842	24.298	2.445 ± 0.532
	PI N2	48.715	35.175	27.305	23.37	
	PI N3	48.079	36.977	26.961	24.059	
EA		2	1.5	1	0.5	
	PI N1	73.278	64.043	47.033	27.515	0.555 ± 0.018
	PI N2	69.057	65.725	52.809	31.926	
	PI N3	72.771	64.551	50.428	29.419	
EtOH		0.25	0.125	0.0625		
	PI N1	65.366	54.718	47.174		0.052 ± 0.008
	PI N2	64.666	55.058	41.601		
	PI N3	63.586	55.058	41.601		
H_2O		0.5	0.25	0.125	0.0625	
	PI N1	65.422	62.464	40.581	28,551	0.115 ± 0.006
	PI N2	72.254	60.13	38.304	31.202	
	PI N3	69.599	60.889	41.125	33.037	
AA		0.008	0.0016	0.00032		
	PI N1	83.309	54.003	20.275		0.002 ± 0.0
	PI N2	79.48	52.791	19.896		
	PI N3	82.62	53.232	18.817		

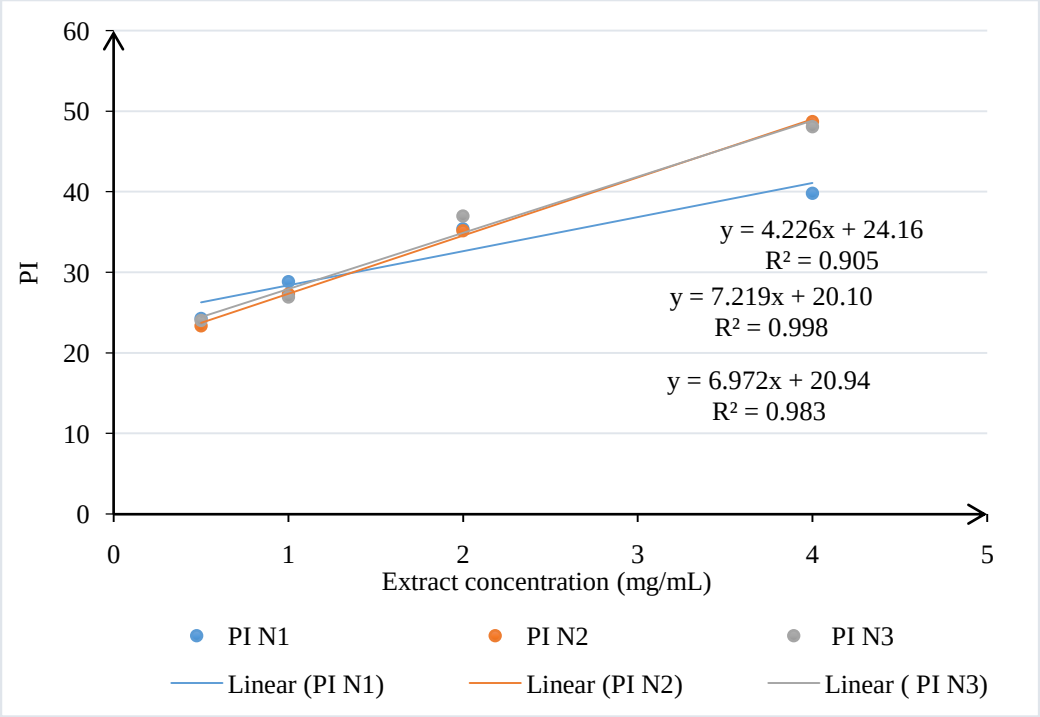

Fig 06: Evolution of PI as a function of CyH extract concentration

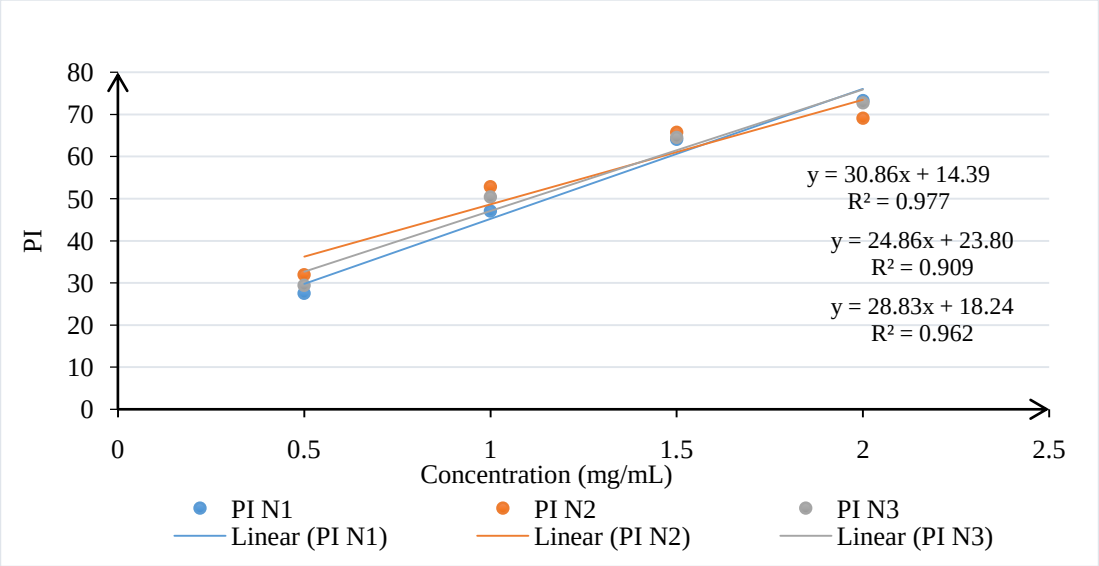

Figure 7: Evolution of PI as a function of EA extract concentration

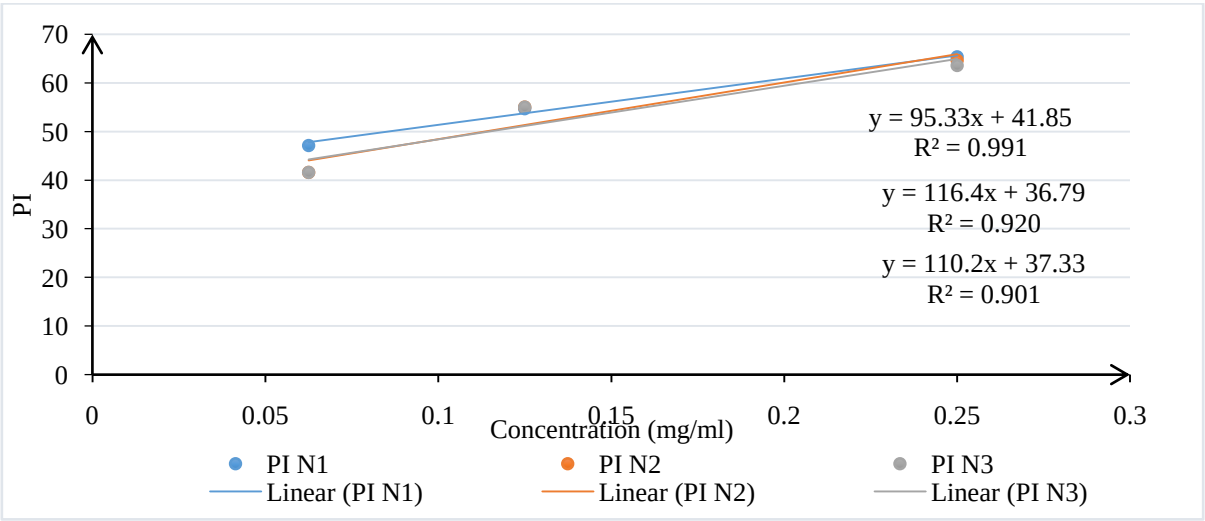

Figure 8: Evolution of PI as a function of EtOH extract concentration

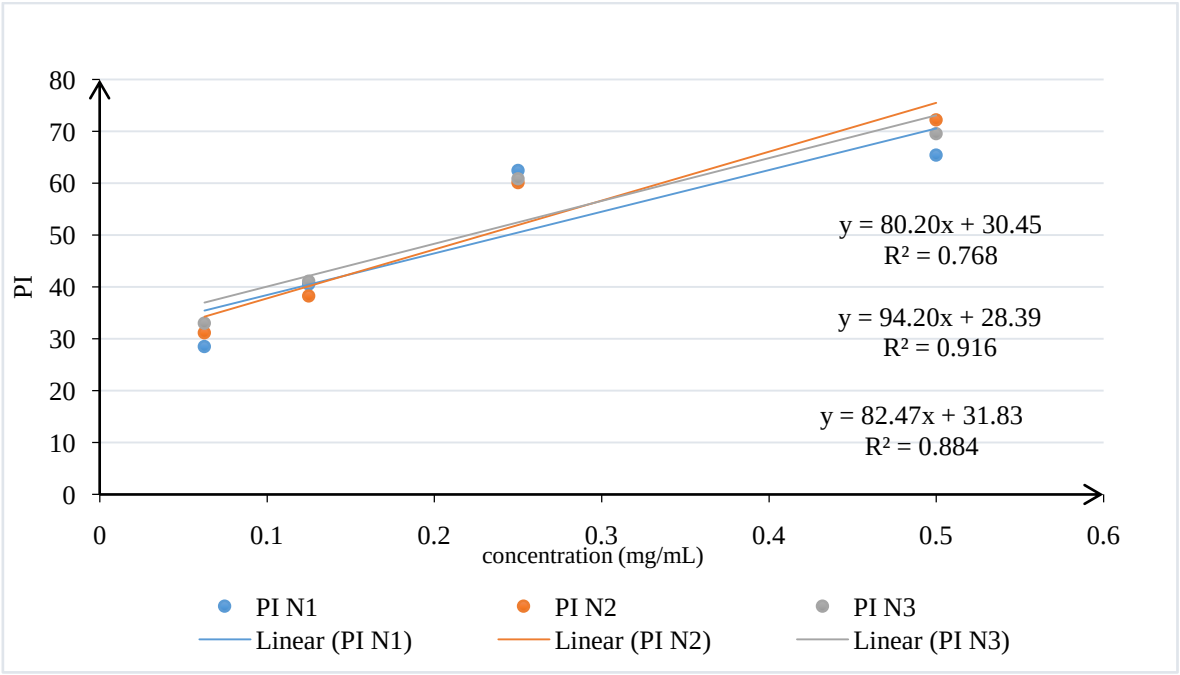

Fig 09: Evolution of PI as a function of H_2O extract concentration

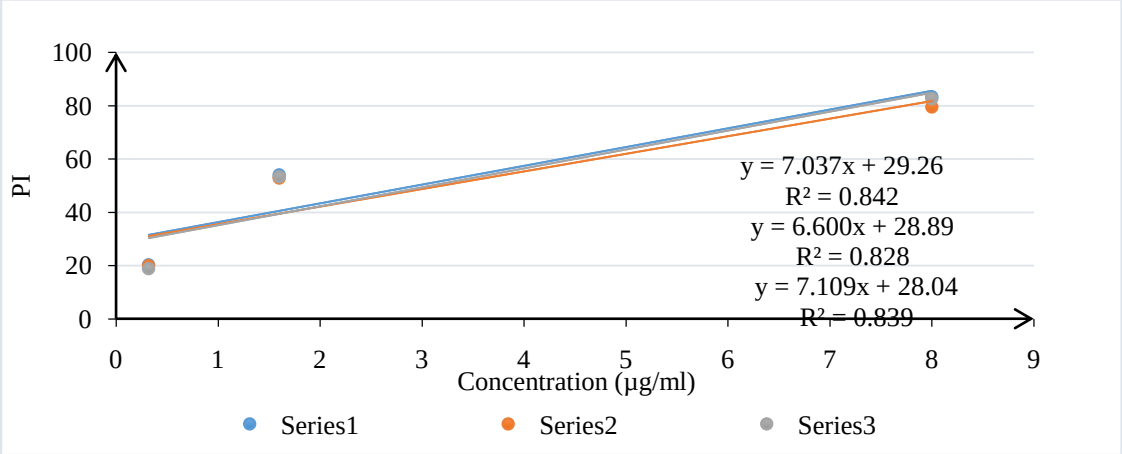

Figure 10: Evolution of PI as a function of AA extract concentration

The average CI_{50} for each extract and for the standard is calculated and shown in the diagram below:

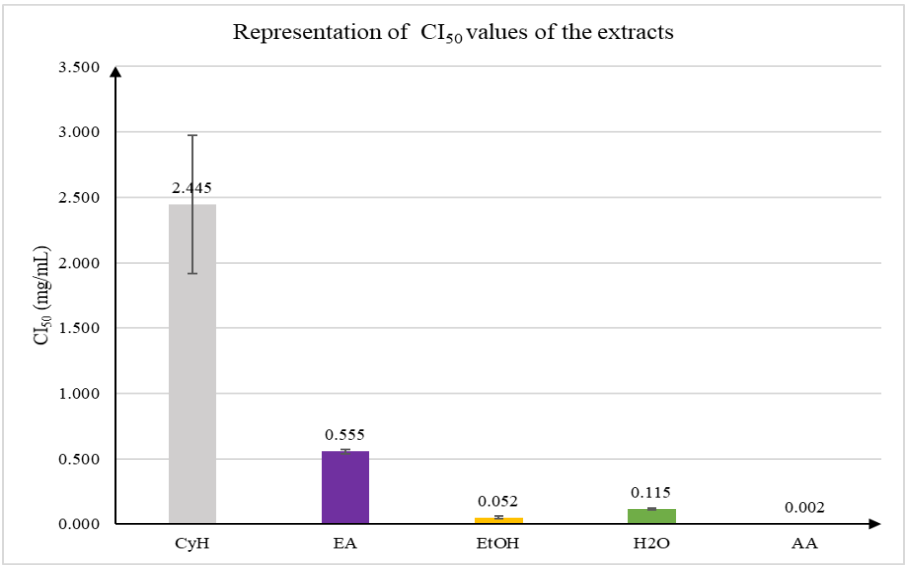

Fig 11: Representative diagram of the IC50 values of the different extracts and ascorbic acid

FRAP assay results

The assay results are in the table below:

Table 5: Reducing power FRAP

	Average absorption / Concentration (mg/mL)			
Extract	2	1.5	1	0.5
CyH	0.259	0.207	0.160	0.056
EA	0.255	0.220	0.164	0.075
EtOH	0.300	0.267	0.198	0.131
H_2O	0.301	0.253	0.193	0.111
AA	0.1	0.25	0.5	1
	1.30	1.35	1.35	1.37

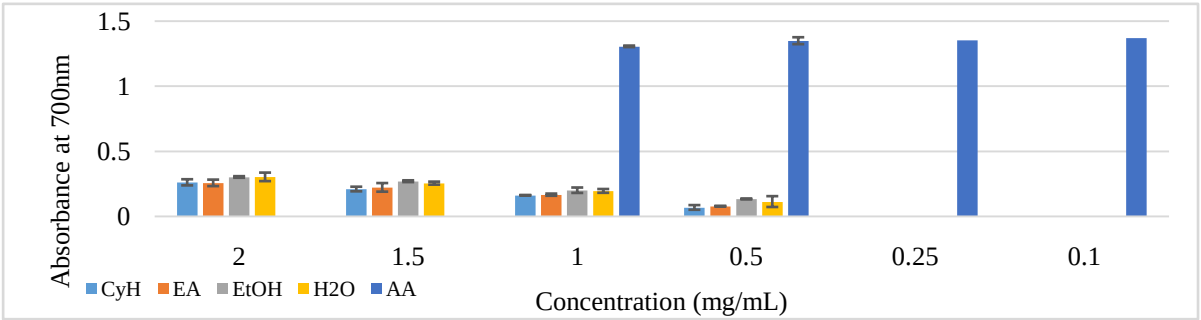

Fig 12: Diagram representing the absorbances of the extracts and ascorbic acid as a function of the extract concentrations

DISCUSSION

The extraction results favor EtOH extract with an extraction percentage of 4.86, followed by CyH and H_2O with 2.58 and 2.5% respectively, with EA coming in last with an extraction percentage of 1.69. These results suggest that the most compounds soluble in polar solvents would be removed during their passage through EtOH before their turn in the most polar solvent (H_2O) for the depletion of the remaining fraction. Phytochemical screening allows us to identify the families of secondary metabolites present in each extract: polyphenols, tannins, flavonoids, and anthocyanins are present in all four extracts; alkaloids and terpenes are depleted in the first two extractions (CyH and EA) and saponins in the last two (EtOH and H_2O); the absence of coumarins was noted only in the EtOH extract.

The results of the total polyphenol assay show that the EtOH extract has the highest content with a value of 61.905 ± 4.033 mg EAG/g (ES), followed by the AE extract with 41.513 ± 8.158 mg EAG/g, then the H_2O extract with 26.130 ± 0.805, and finally the CyH extract with a polyphenol content of 12.466 ± 1.010 mg EAG/g. The flavonoid content of the different root extracts was calculated on an equivalence basis in mg of catechin content per gram of dry extract and with respectively: 44.257 ± 4.354; 34.074 ± 1.643; 27.629 ± 2.062 and 14.263 ± 1.992 mg EC/g (ES) respectively for the CyH, EA, EtOH and H2O extracts.

These results on polyphenol and flavonoid content differ significantly from previous studies [14, 26], which found lower values: 5.7 ± 0.47; 1.171 ± 0.0108 and 1.170 ± 0.0082 mg EAG/g respectively for the MeOH, H_2O, and isopropanol extracts of C. nigritanus roots. A flavonoid content of 0.1584 and 0.0966 mg EQ/g respectively for the H_2O and isopropanol extracts.

The results of the antioxidant activity assessment of the roots always vary depending on the extracts studied. For the DPPH assay, we see that the EtOH extract has the lowest IC50, equal to 0.052 mg/mL, followed by the H_2O extract with an IC_{50} of 0. 115 mg/mL, then the EA extract with an IC_{50} of 0.555 mg/mL, and finally the CyH extract with the highest IC_{50} value of 2.445 mg/mL. For the FRAP assay, the EtOH extract has higher absorbances than the other three extracts for the different concentrations used, except at a concentration of 2 mg/mL, where its absorbance is equal to that of the H_2O extract. It is followed by the latter; there is almost equality between the CyH extract and the EA extract, except at a concentration of 0.5 mg/mL, where the AE extract has a higher absorbance. In this assay too, AA has a higher reducing power with higher absorbance values that are almost standard from the lowest concentration of 0.1 mg/mL.

In the DPPH assay, we see that the IP increases with the concentration of the extract and we have low but moderate IC_{50} values compared to the standard. The FRAP results show us that the absorbance of the samples increases with the concentration of the C. nigritanus root extract. From these results, we can deduce that C. nigritanus roots have satisfactory but moderate antioxidant power compared to the standard, which is ascorbic acid.

The results obtained are comparable to those of Mbodj et al. (2023) [14], who studied the antioxidant activity of the aqueous and isopropanolic extracts of C. nigritanus roots, with a CI_{50} of 0.13 mg/mL for the aqueous extract and 0.19 mg/mL for the isopropanolic extract using the ABTS assay. It should be noted that despite the difference in extraction method (direct extraction and fractionation), there is a slight difference in IC_{50} in favor of our extract.

By correlating the results of the assays with those of the antioxidant activity assays, we see that the EA extract has a higher polyphenol content than the H_2O extract, while the latter has a lower IC_{50}. The phenolic composition content and antioxidant assay results of the different extracts are not comparable, which could suggest the presence of other molecules acting as antioxidants.

This study has enabled us to scientifically confirm the antioxidant properties of C. nigritanus roots, in line with their use in traditional medicine. It has also enabled us to decipher their chemical potential by showing us the heterogeneity of their chemical groups (polyphenols, alkaloids, flavonoids, coumarins, etc.).

CONCLUSION

The results of this study highlight the significant antioxidant activity of Chrysopogon nigritanus root extracts, although this remains lower than that of ascorbic acid, which was used as a standard. The ethanolic extract proved to be the most active, followed by the aqueous, ethyl acetate, and cyclohexane extracts. This ranking suggests a major involvement of polar phenolic compounds in the antioxidant activity observed.

These results provide scientific validation for the traditional use of C. nigritanus and open up research prospects for the isolation and characterization of its active ingredients, as well as for the evaluation of other biological activities such as antibacterial, anti-inflammatory, and antifungal activities.

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CONFLICT OF INTEREST

The authors declare no conflict of interest

INFORMED CONSENT

All authors have read and agreed to the published version of the manuscript

ETHICAL STATEMENT

The authors state that this study was carried out in compliance with prevailing ethical principles

AUTHOR CONTRIBUTION

All authors made substantial contributions to the conception, execution, and drafting of this manuscript

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