



CORRELATION BETWEEN PHENOLIC PROFILE AND ANTIOXIDANT ACTIVITY OF PHYLLANTHUS NIRURI EXTRACT

**Konan Dibi Jacques^{*1}, Nikiema Diakaridja², Konan Kouassi Pierre Alain¹, Belemlilga Bonewendé Mohamed³,
Mamyrbekova-Bekro Janat Akhanovna¹ and Bekro Yves-Alain¹**

¹Laboratoire De Chimie Bio-Organique Et De Substances Naturelles, UFR-SFA, Université Nangui Abrogoua, 02 BP 801 Abidjan 02, Côte d'Ivoire.

²Laboratoire De Constitution Et Réaction De La Matière, UFR Sciences Des Structures De La Matière Et Technologie, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire.

³Laboratoire De Recherche-Développement De Phytomédicaments Et Médicaments, Institut De Recherche En Sciences De La Santé, Centre National De La Recherche Scientifique Et Technologique, BP 7047 Ouagadougou 03, Burkina, Faso.



***Corresponding Author: Konan Dibi Jacques**

Laboratoire De Chimie Bio-Organique Et De Substances Naturelles, UFR-SFA, Université Nangui Abrogoua, 02 BP 801 Abidjan 02, Côte d'Ivoire.

Article Received on 20/09/2024

Article Revised on 10/10/2024

Article Accepted on 30/10/2024

ABSTRACT

This study explores the relationship between the content of phenolic compounds and the antioxidant activity of ethanolic extracts of *Phyllanthus niruri*. Phenolic compounds, such as polyphenols, flavonoids and tannins, are known for their antioxidant properties, and their quantification was carried out using spectrophotometric methods (notably the Folin-Ciocalteu method for total polyphenols). Antioxidant activity was measured by standard tests such as DPPH and FRAP, expressing the ability of the extract to neutralize free radicals. The phytochemical screening carried out on the whole plant extract studied revealed the presence of polyphenols, flavonoids and tannins. The total polyphenol content of ethanolic extract (in mg EAG/g) is 515.62 ± 6.75 . That of flavonoids (in mgEQ/gE) is 26.32 ± 1.21 ; the tannin content (in mgEC/gE) is 11.48 ± 1.35 . The ethanolic extract studied has appreciable antioxidant power. The DPPH method gives a value of 0.013 mg/ml. As for the FRAP test, it gives the following values 146 ± 0.85 mMET/g DM (millimoles of Trolox equivalents per gram of dry matter) and 17.75 ± 0.1 mMEV/g DM (millimoles of vitamin C equivalents per gram of dry matter). Phenolic compounds are well known for their ability to scavenge free radicals, which explains the high antioxidant activity in the Trolox-based assay. The study demonstrates that the phenolic compounds of *Phyllanthus niruri* largely contribute to its antioxidant potential. These results confirm the importance of this plant in the prevention and treatment of diseases linked to oxidative stress and open perspectives for its use in herbal medicine as a natural antioxidant agent.

KEYWORDS: *Phyllanthus niruri*; phytochemical composition; total phenols; antioxidant activity.

INTRODUCTION

Phyllanthus niruri, commonly known as "chanca piedra", is a medicinal plant widely used in traditional medicine in many cultures, including South America and Asia.

In Côte d'Ivoire, traditional medicine occupies a central place in health practices, particularly in rural areas where access to modern care may be limited. Among the most popular medicinal plants is *Phyllanthus niruri*, locally known by various names, however, "a thousand wonders" is the most used name. This plant is renowned for its varied therapeutic properties.^[1] This plant, widely used in the African pharmacopoeia, is attracting growing interest for its numerous benefits, particularly in the treatment of kidney disorders, liver infections, notably

hepatitis, and various infectious and inflammatory conditions.^[2, 3, 4, 5] Its virtues are attributed to the richness of its secondary metabolites, such as flavonoids and alkaloids, saponins and tannins which give it antiviral, antimicrobial, antioxidant and anti-inflammatory properties.^[6, 7] These secondary metabolites play a crucial role in these pharmacological effects, acting as bioactive compounds that contribute to its medicinal benefits. The study of the dosages of these secondary metabolites is crucial to precisely identify and quantify these compounds, and thus scientifically validate the traditional uses of the plant. In Côte d'Ivoire, where herbal medicine plays an essential role in health care, the standardization of methods for measuring metabolites in *Phyllanthus niruri* constitutes a major challenge to guarantee the effectiveness and safety of herbal

treatments. The popularity of *Phyllanthus niruri* in Côte d'Ivoire demonstrates the strong reliance on natural remedies to prevent and treat diseases, while emphasizing the importance of preserving traditional knowledge about medicinal plants.

The study of secondary metabolites is essential to understand the mechanisms of action of this plant and to identify its active compounds. Modern analytical approaches, such as high-performance liquid chromatography (HPLC) and nuclear magnetic resonance (NMR), offer powerful tools for the precise characterization of these metabolites. Using these techniques, it is possible to identify and quantify the various compounds present in the plant, explore their interactions and evaluate their bioactive potential. This research work therefore aims to explore this correlation in order to provide a better understanding of the role of different phenols in the biological activity of the plant. It will also contribute to the standardization of *Phyllanthus niruri* extracts for safer and more effective use in herbal medicine.

MATERIAL AND METHODS

MATERIAL

Plant material

The plant material consisted of the whole *Phyllanthus niruri* plant. The harvest was carried out in the “petit yapo” forest (2024), a site located in the south-east of the Ivory Coast. The plant was identified at the National Floristic Center (CNF) of Félix HOUPOUËT-BOIGNY University (Cocody / Abidjan).

The harvested plants were washed then dried by exposure under a ventilated shelter for two weeks before being reduced to powder. The plants were pulverized using an electric grinder (nutri blitzer GS 158V1457). The powder obtained was cleaned and then dried for a week away from light in a ventilated room at room temperature. The raw material is then stored in conservation bags in the laboratory.

Laboratory equipment used for the work

For processing the raw material, the equipment consists of glassware, an oven, a dryer and an electric grinder (Nutri Blitzer GS 158-V1457). Regarding the phytochemical study of the extracts obtained a brand rotary evaporator (BÜCHI type EL-131), a UV-254/366 nm lamp (VL-6.LC), an electronic balance (XY6002C), a hood, a refrigerator and chromatate plates (silica gel 60 F254, aluminum rack, 20 × 20) (Merck) were used. For the dosage methods, a UV-visible spectrophotometer (Spectro AL 800) was used for the analyzes.

Cold extraction in ethanol

A mass of 5 g of vegetable powder was placed in a 250 ml beaker with 100 ml of 80% ethanol. The mixture remained under mechanical agitation at room temperature for 1 h, then centrifuged for 15 min at 10,000 x g. The supernatant was decanted and the

organic phase was collected. This process was repeated three times. All collected organic phases were combined and filtered through a funnel containing fiberglass filter paper. The mixture was concentrated using a rotary evaporator at 50 °C, under vacuum, at 200 millibars. The extract obtained is stored at 4°C.

Total Phenols content

The total polyphenol contents were determined using the Folin-Ciocalteu colorimetric method (Singleton et al., 1999).^[10] To 1 ml of extract is added 1.5 ml of Na₂CO₃ (17%, m/v) and 0.5 ml of Folin-Ciocalteu reagent (0.5N). The whole is incubated at 37°C for 30 min; the absorbance is read at 760 nm against a blank without extract taken as a reference. The quantification of total polyphenols is carried out according to a linear calibration line ($y = ax + b$) carried out by a standard extract of gallic acid at different concentrations (0 to 0,025g/ml) under the same conditions. Than the sample. The results are expressed in milligrams of gallic acid equivalent per gram of sample (mg GAE/gE) of the powdered plant.

Condensed tannins content

The determination of condensed tannins in the different extracts was carried out according to the method described by Heimler et al. (2006).^[11] For 400 µL of the sample or standard, 3 ml of a 4% methanolic vanillin solution and 1.5 ml of concentrated hydrochloric acid are added. The mixture is incubated for 15 min and the absorbance is read at 500nm. The concentrations of condensed tannins are deduced from the calibration ranges established with catechin (0-0,17µg/ml), and are expressed in mg of catechin equivalent per g of sample (mgCE/gE).

Total flavonoids content

This assay was carried out according to the method used by Arvouet-Grand et al (1994).^[12] 500 µL of 2% aluminum chloride (AlCl₃) in methanol were added to an equal volume of extract. After 10 minutes' incubation, the reading was taken with a spectrophotometer at 415 nm. Quercetin (0-0, 13 mg/L), used as a standard, was used to establish the calibration curve. Three (3) tests were carried out for each extract, and the result given is an average of the three readings. Results are expressed in milligrams of Quercetin Equivalent (QE) per gram of sample (mg EQ / gE).

Evaluation of antioxidant power by spectrophotometry

The method used is that of Blois (1958) with slight modifications.^[13] DPPH is solubilized in 70% ethanol to obtain a solution with a concentration of 0.03 mg/ml. Different concentration ranges of each extract are prepared with 70% ethanol. 1 mL of extract solution to be analyzed and 2 mL of DPPH solution are introduced into dry and sterile tubes.

After shaking, the tubes are placed away from light for 30 min. The absorbance of the mixture is measured at 517 nm against a blank consisting of (2 ml of DPPH solution + 1 ml of 70% Et OH). The positive reference control used is ascorbic acid (vitamin C) prepared under the same conditions as the samples. The percentage of DPPH reductions calculated according to the formula.

$$PR = [1 - (Ae/Ab)] \times 100$$

PR: percentage of DPPH reduction

Ae: sample absorbance

Ab: absorbance of white

The effectiveness of the samples against DPPH was assessed by graphical determination of the CR50 (concentration that reduces 50% of DPPH).^[14]

FRAP test

The methodology described by Hinneburg et al., 2006 and by Benzie and Strain, 1996 was used to determine the in vitro reducing power of *Phyllanthus niruri* extracts.^[15,16,17] The FRAP reagent which was used for the analysis is a mixture of three solutions: sodium

acetate buffer (300 mM, pH = 3.6), TPTZ solution (2, 4, 6-Tri (2-pyridyl) -s-triazine) (10 mM of this solution prepared in 40 mM hydrochloric acid HCl); 20 mM FeCl₃ in a volume ratio 10:1:1. The freshly prepared FRAP reagent is preheated to 37°C in a water bath. 100 µl of Trolox (0.187 mM; 0.375 mM; 0.75 mM and 1.5 mM) and a 0,01 g sample of the plant extract are added to 3 ml of the FRAP reagent. Vitamin C and Trolox were also used as a reference. The absorbance of the complex (TPTZ-Fe³⁺) is read at 593 nm after 4 min with the UV-visible spectrophotometer. The FRAP antioxidant activity (expressed in micromole Trolox Equivalent per gram of dry matter (µM TE/g of DM and micromole Vitamin C Equivalent per gram of dry matter.

RESULTS ET DISCUSSION

RESULTS

Phenolic compounds content

Table 1 summarizes the different values of the phenolic compound contents of the ethanolic extract of *Phyllanthus niruri*. These results show that *Phyllanthus niruri* is mainly composed of polyphenols.

Table 1: Phenolic content of the ethanolic extract.

Extract	polyphenols (a)	Flavonoids (b)	Tannins condense (c)
Ethanolic extract	515,62 ± 6,75	26,32 ± 1,21	11,48 ± 1,35

a) mg of gallic acid equivalent per milligram of extract

b) mg of quercetin equivalent per milligram of extract

c) mg of catechin equivalent per milligram of extract

The values reported in table 1 represent the average of 3 measurements ± SD.

Total polyphenol content

The quantitative estimation of the content of *Phyllanthus niruri* in total polyphenols revealed that this plant contains (515.62 ± 6.75 mg of gallic acid equivalent per gram of dry extract (mg GAE/gE). This value has been obtained from the gallic acid calibration curve ($y=17.173x+0.0192$ with an $R^2=0.9905$) *Figure1*.

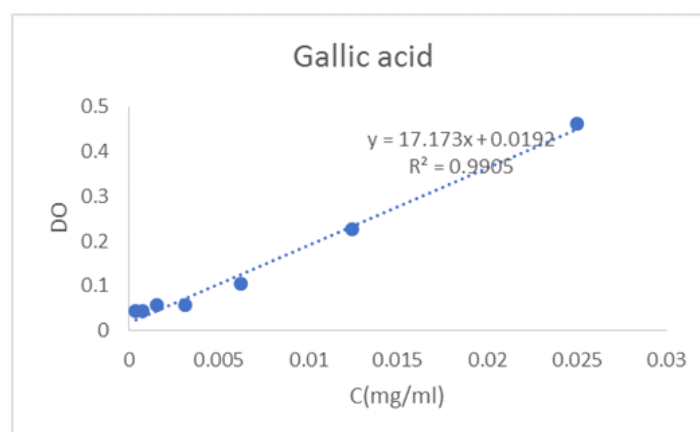


Figure 1: Gallic acid calibration curve.

The value of total polyphenols is approximately twice that reported by Amin et al.,^[18] Indeed, their study revealed that the ethanolic extract obtained after 3 days of maceration contains 270 ± 0.003 mg GAE/g of dry *Phyllanthus niruri* extract).

Total flavonoid content

The results show that the whole *Phyllanthus niruri* plant contains 26.32 ± 1.21 mg EQ/gE of total flavonoids in the ethanolic extract. The level of flavonoids in the extract was determined from the calibration curve, plotted using quercetin as a standard ($y=5.4217x-0.0457$; $R^2=0.9943$) (**Figure 2**).

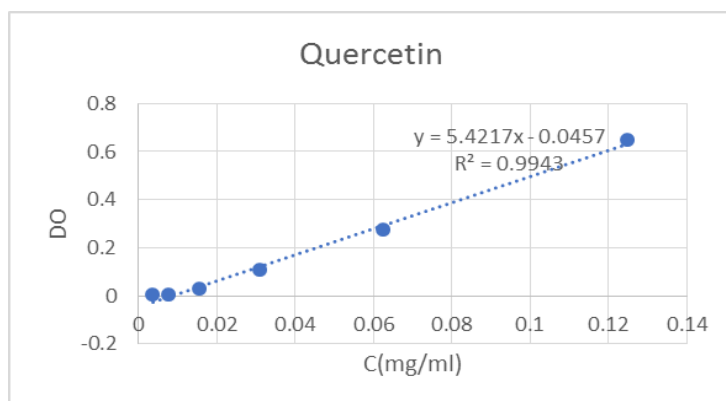


Figure 2: Quercetin calibration curve.

Condensed tannin content

Regarding the determination of the value of the condensed tannin content of the *Phyllanthus niruri* extract, the catechin calibration curve was used

($y = 3.0898x + 0.0112$; $R^2 = 0.9982$). The tannin content determined is 11.48 ± 1.35 mg EC/gE in the ethanolic extract studied.

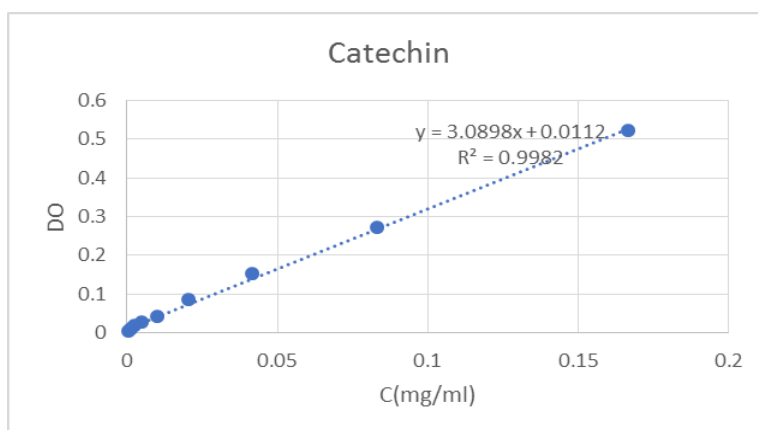


Figure 3: Catechin calibration curve.

Antioxidant activity DPPH test

At all concentrations tested, ethanolic extracts of *Phyllanthus niruri* significantly inhibited the DPPH radical in a dose-dependent manner. Indeed, the

Phyllanthus niruri extract almost reaches its maximum activity at 0.03125 mg/ml with an reduction percentage (PR) of $92.22 \pm 0.25\%$ (Figure 4).

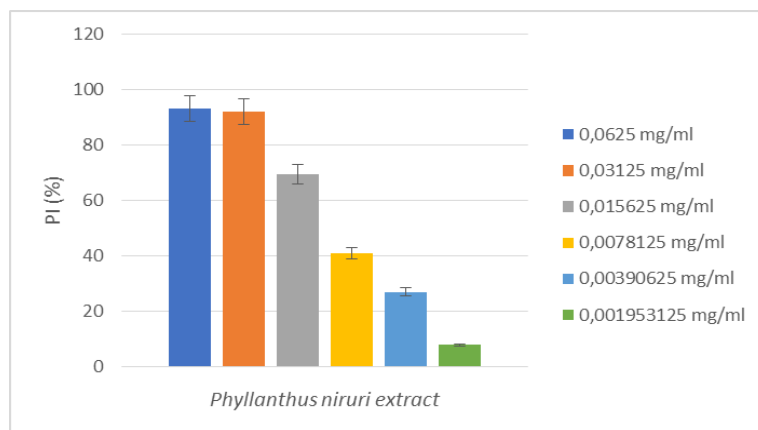


Figure 4: Percentage of radical inhibition DPPH of *Phyllanthus niruri* extract.

To better assess the activity of the plant extract tested (*Phyllanthus niruri*), the CR_{50} was determined. Thus, *Phyllanthus niruri* extract has an CR_{50} of 0.013 mg/ml.

Ascorbic acid (Vitamin C) presented an CR_{50} equal to 0.0071 mg/ml as shown in table 2.

Table 2: Values of IC₅₀ for the antioxidant activity of the ethanolic extract of *Phyllanthus niruri* by dosage of 2, 2-diphenyl-1-picrylhydrazyl (DPPH). Data are expressed as a triple average.

Samples	CR ₅₀ (mg/mL)
Ascorbic acid	0,0071
Ethanolic extract of <i>Phyllanthus niruri</i>	0,013

Antioxidant activity FRAP test

The evaluation of the reducing power of the *Phyllanthus niruri* extract gave a value of 17.75 ± 0.1 mMTE/g DM (table 3). This value of the reducing power of the extract

was determined from the calibration curve, plotted using Trolox as a standard ($y=0.2879x-0.0134$; $R^2=0.9956$) (Figure 5), as well as the acid ascorbic ($y = 2.3726x + 0.0152$; $R^2 = 0.9933$) Figure 6.

Table 3: Values of the antioxidant activity of the ethanolic extract of *Phyllanthus niruri* by determination of the iron reducing antioxidant power (FRAP). Data are expressed as a triple average.

Standards	Antioxidant activity of the ethanolic extract of <i>Phyllanthus niruri</i>
Trolox	$146 \pm 0,85$ mMTE/ g DM
Ascorbic acid	$17,75 \pm 0,1$ mMVE/ g DM

mMTE/ g DM: millimolar Trolox equivalent per gram of dry matter

mMVE/ g DM: millimolar vitamin C equivalent per gram of dry matter

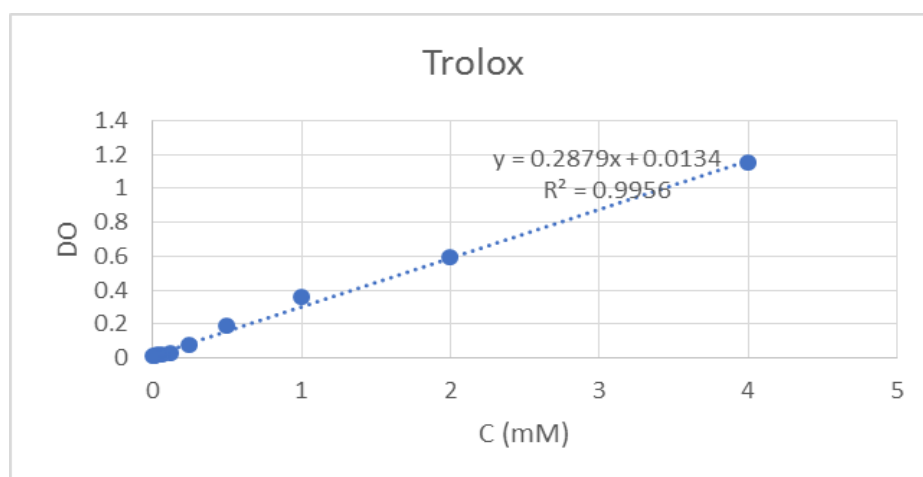


Figure 5: Trolox calibration curve.

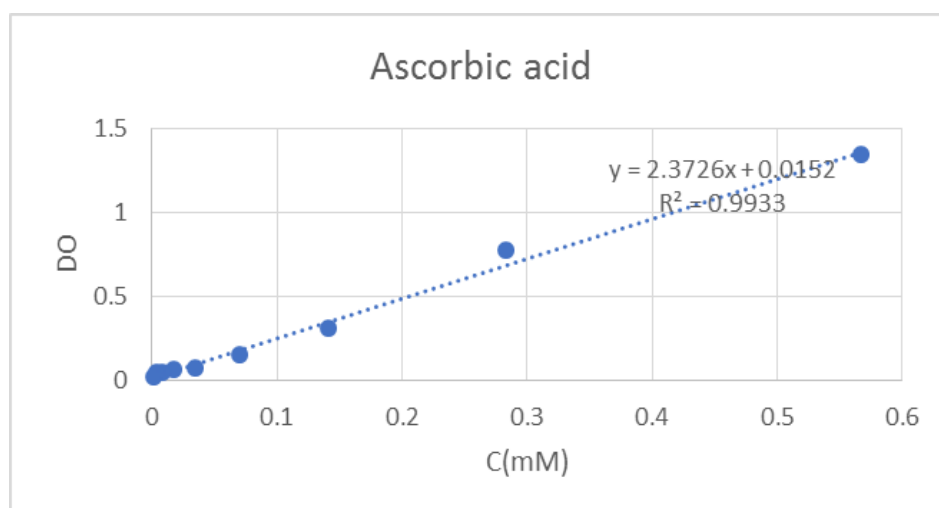


Figure 6: Ascorbic acid (vitamin C) calibration curve.

DISCUSSION

The results obtained in the study of the correlation between the phenolic profile and the antioxidant activity of *Phyllanthus niruri* highlight the importance of phenolic compounds in the biological properties of this

plant. By analyzing the polyphenols, flavonoids and tannins present in *Phyllanthus niruri* extract, we see a strong relationship between the content of phenolic compounds and antioxidant activity, measured through tests such as DPPH and FRAP (figure 7).

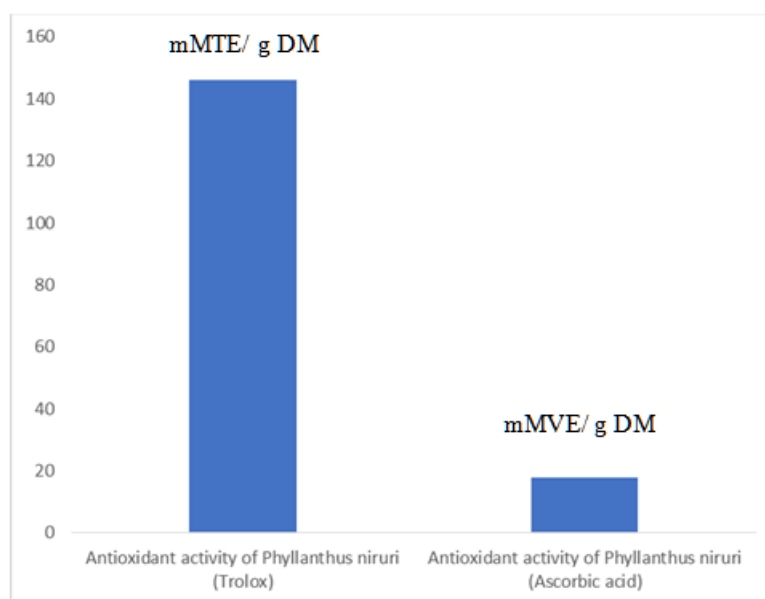


Figure 7: Correlation between phenolic profile and antioxidant activity (FRAP).

These results obtained are similar to those reported in the literature by [Nelli et al., (2014); Hidanah et al., (2022)].^[19,20] The use of an alcoholic solvent (80% ethanol) made it possible to extract, from the entire *Phyllanthus niruri* plant, polar compounds such as polyphenols which are among the main components of plants with antioxidant activity. This notion of polarity seems to be confirmed by phytochemical screening which is illustrated by the presence of flavonoids.^[21,22]

The results on phenolic profile show that *Phyllanthus niruri* contains a significant quantity of total polyphenols and an average concentration of flavonoids and tannins (table1). These molecules are known for their ability to neutralize free radicals.

The work of Amin et al., 2012 also showed that the ethanolic extract of *Phyllanthus niruri* from Malaysia contains a high level of flavonoid content (123.9 ± 0.002 mg g⁻¹) and also a high content of polyphenol (376 ± 0.02 mg g⁻¹). Our results are different from those of Amin et al., 2012. Contrary to our results, we observe that the Malaysian extract is richer in flavonoids. Several factors can influence the content of phenolic compounds. Different studies have shown that external factors such as geographical and climatic factors, as well as genetic factors, but also the degree of maturation of the plant and the duration of storage have a strong influence on the content of polyphenols and flavonoids.^[23,24,25,26]

The Folin-Ciocalteu method revealed a relatively high content of total polyphenols, confirming the crucial role of these compounds in the bioactivity of the *Phyllanthus niruri* plant.

The richness of the ethanolic extract of *Phyllanthus niruri* in polyphenols could explain the multiple use of this in traditional medicine. Indeed, polyphenols, through

their multiple biological properties, are essential natural compounds for human health. Their antioxidant, anti-inflammatory, antimicrobial and protective role makes them agents of choice in the prevention of numerous chronic and degenerative diseases.^[1,19]

The CR₅₀ values (50% reduction concentration) obtained (Figure 4) make it possible to compare the DPPH radical trapping capacity of the extract tested in relation to Vitamin C. The antioxidant power being inversely proportional to the CR₅₀ value. Vitamin C with the lowest CR₅₀ 0.0071 mg/mL, then has the greatest anti-radical activity than the ethanolic extract of *Phyllanthus niruri* tested (0.013 mg/ml).

The FRAP test of the ethanolic extract of *Phyllanthus niruri* gives the following results, 146 ± 0.85 mMTE/ g DM with Trolox and 17.75 ± 0.1 mMVE/ g DM with ascorbic acid (Vitamin C).

These two values 146 ± 0.85 mMTE/g DM (millimoles Trolox equivalents per gram of dry matter) and 17.75 ± 0.1 mMVE/g DM (millimoles Vitamin C equivalents per gram of dry matter) highlight the differences between the antioxidant activity measured through distinct references. The results clearly show that the value of 146 mMTE/g DM is much higher than 17.75 mMVE/g DM, indicating that the antioxidant activity measured in Trolox equivalents is much greater than that measured in vitamin C equivalents. This difference of antioxidant activity could be explained by the nature of the antioxidant compounds present in the *Phyllanthus niruri* extract. Some phenolic compounds and flavonoids may be more comparable to Trolox in terms of antioxidant effectiveness, while others may be more effective in reference to vitamin C.

The high value of 146 mMTE/g DM suggests that the studied extract contains a high concentration of phenolic compounds capable of reacting effectively with free radicals, such as polyphenols, flavonoids and tannins. These compounds are well known for their ability to scavenge free radicals in aqueous media, which explains the high antioxidant activity in the Trolox-based assay.

In contrast, the value of 17.75 mMEV/g DM in vitamin C equivalents could indicate lower antioxidant activity in an environment or a relatively lower concentration of compounds compared to those measured in Trolox equivalents.

This result reflects not only the differences in the chemical nature of the antioxidants present in *Phyllanthus niruri* extract, but also the impact of the choice of standard used for the measurement. This analysis highlights the importance of selecting testing methods adapted to the type of environment in which the extract will be used.

CONCLUSIONS

Methods for determining total polyphenols, total flavonoids and tannins are key tools to evaluate the bioactive composition of *Phyllanthus niruri* extracts. They make it possible to quantify the main secondary metabolites responsible for the beneficial pharmacological effects of this plant, in particular its antioxidant activity. These methods, coupled with the evaluation of the antioxidant activity of the extract, are crucial to scientifically validate its therapeutic use.

Methods for measuring total polyphenols, total flavonoids and tannins, combined with antioxidant activity tests, are essential to evaluate the therapeutic potential of *Phyllanthus niruri*. These analyzes confirm that the plant is a rich source of bioactive compounds with antioxidant, anti-inflammatory and protective properties against various diseases. This reinforces interest in the traditional use of the plant and its development as a standardized phytotherapeutic product, promising for the prevention and treatment of diseases linked to oxidative stress.

ACKNOWLEDGEMENTS

We would like to express our deep gratitude and appreciation to everyone who contributed to the successful completion of this research project.

RÉFÉRENCES

1. V. V. Paithankar, K. S. Raut, R. M. Charde, et J. V. Vyas, (*Phyllanthus niruri*: A magic herb), *Research in Pharmacy*, 2015; 1(4).
2. D. A. Awomukwu, B. L. Nyananyo, N. D. Onukwube, C. J. Uka, C. U. Okeke, et A. I. Ikpeama, (Comparative phytochemical constituents and pharmacognostic importance of the vegetative organs of some *Phyllanthus* species in South Eastern Nigeria), *Int J Modern Bot*; 2014; 4(2): 29–39.
3. MF Souza Silva, F de Cássia E de Oliveira, L dos Santos, M Claudia, GJ Zocolo, C Pessas (Biochemical and Pharmaceutic Properties of Genus *Phyllanthus* spp.: Focus on *P. amarus* Schum. & Thonn. And *P. niruri* L.) *Current Traditional Medicine*, 2021; 7(2): 203-221, (19).
4. N. Kaur, B. Kaur, ET G. Sirhindi, (Phytochemistry and Pharmacology of *Phyllanthus niruri* L.: A Review), *Phytotherapy Research*, 2017; 31(7): 980–1004.
5. K. S. Kumara, S. Shishupala, ET H. S. Prakash, (The Genus *Phyllanthus*: A Rich Source of Pharmacologically Active Compounds Useful in Traditional and Modern Medicines), in *Ethnic Knowledge and Perspectives of Medicinal Plants*, Apple Academic Press, 2023; 245–273.
6. A. Mediani *et al.*, (Characterization of metabolite profile in *Phyllanthus niruri* and correlation with bioactivity elucidated by nuclear magnetic resonance based metabolomics), *Molecules*, 2017; 22(6): 902.
7. MI Ezzat, MM Okba, SH Ahmed, HA El-Banna, A Prince, SO Mohamed, SM Ezzat (In-depth hepatoprotective mechanistic study of *Phyllanthus niruri*: In vitro and in vivo studies and its chemical characterization), *PloS one*, 2020; 15(1): e0226185.
8. G. S.-P. Bohui, S. Beourou, D. Sanogo, J. D. N'guessan, K. B. Attioua, et A. A. Adima, (Phytochemical Screening and Evaluation of the Antioxidant and Antiplasmodial Activities of "Ahoutou", a Recipe Used in the Treatment of Malaria in Ivory Coast), *Science Journal of Chemistry*, 2024; 8(1): 109–116.
9. N. Dohou, K. Yamni, N. Gmira, et L. M. I. Hassani, (Etude de polyphénols des feuilles d'une endémique ibéro marocaine, *Thymelaea lythroides*.), *Acta botánica malacitana*, 2004; 29: 233–239.
10. VL Singleton, R Orthofer, RM Lamuela-Raventós (Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent), *Methods in Enzymology*, 1999; 299: 152–178.
11. D. Heimler, P. Vignolini, M. G. Dini, F. F. Vincieri, et A. Romani, (Antiradical activity and polyphenol composition of local Brassicaceae edible varieties), *Food chemistry*, 2006; 99(3): 464–469.
12. A. Arvouet-Grand, B. Vennat, A. Pourrat, et P. Legret, (Standardization of propolis extract and identification of principal constituents), *Journal de pharmacie de Belgique*, 1994; 49(6): 462–468.
13. M. S. Blois, (Antioxidant determinations by the use of a stable free radical), *Nature*, 1958; 181(4617): 1199–1200.
14. Tanoh SK, N'gaman-Kouassi CC, Boa D, Mamyrbekova-Békro AJ, Békro YA. Activité antioxydante des extraits bruts hydroéthanoliques et hydroacétoniques des organes de quatre plantes de Côte d'Ivoire médicinales. *Nature & Technol. French*, 2019; 22: 1-7.

15. I. Hinneburg, H. D. Dorman, et R. Hiltunen, (Antioxidant activities of extracts from selected culinary herbs and spices), *Food chemistry*, 2006; 97(1): 122–129.
16. R. Amarowicz, R. B. Pegg, P. Rahimi-Moghaddam, B. Barl, et J. A. Weil, (Free-radical scavenging capacity and antioxidant activity of selected plant species from the Canadian prairies), *Food chemistry*, 2004; 84(4): 551–562.
17. I. F. Benzie ET J. J. Strain, (The ferric reducing ability of plasma (FRAP) as a measure of “antioxidant power”: the FRAP assay), *Analytical biochemistry*, 1996; 239(1): 70–76.
18. Z. A. Amin, M. Bilgen, M. A. Alshawsh, H. M. Ali, A. H. A. Hadi, et M. A. Abdulla, (Protective Role of Phyllanthus niruri Extract against Thioacetamide-Induced Liver Cirrhosis in Rat Model), *Evidence-Based Complementary and Alternative Medicine*, 2012; 2012(1): 241583.
19. N. Giribabu, P. V. Rao, K. P. Kumar, S. Muniandy, S. Swapna Rekha, et N. Salleh, (Aqueous Extract of Phyllanthus niruri Leaves Displays In Vitro Antioxidant Activity and Prevents the Elevation of Oxidative Stress in the Kidney of Streptozotocin-Induced Diabetic Male Rats), *Evidence-Based Complementary and Alternative Medicine*, 2014; 1: 834815.
20. S. Hidanah (The activity of Meniran (Phyllanthus niruri Linn.) extract on Salmonella pullorum infected broilers), *Veterinary World*, 2022; 15(5): 1373.
21. S. O. Sarr (Etude de l'activité antioxydante des extraits des feuilles de Vitex doniana (Verbenacea)), *International Journal of Biological and Chemical Sciences*, 2015; 9(3): 1263–1269.
22. O. O. Akanni, S. E. Owumi, et O. A. Adaramoye, (In vitro studies to assess the antioxidative, radical scavenging and arginase inhibitory potentials of extracts from Atocarpus altilis, Ficus exasperate and Kigelia africana), *Asian Pacific journal of tropical biomedicine*, 2014; 4: S492–S499.
23. A. A. Aganga et K. W. Mosase, (Tannin content, nutritive value and dry matter digestibility of Lonchocarpus capassa, Zizyphus mucronata, Sclerocarya birrea, Kirkia acuminata and Rhus lancea seeds), *Animal Feed Science and Technology*, 2001; 91(1–2): 107–113.
24. K. S. Evenamede, K. Kpegba, O. Simalou, P. Boyode, A. Agbonon, et M. Gbeassor, (Etude comparative des activités antioxydantes d'extraits éthanoliques de feuilles, d'écorces et de racines de Cassia sieberiana), *International Journal of Biological and Chemical Sciences*, 2017; 11(6): 2924–2935.
25. A. Khouchlaa et al. (Détermination des composés phénoliques et évaluation de l'activité litholytique in vitro sur la lithiase urinaire d'extrait de Zizyphus lotus L. d'origine marocaine), *Phytothérapie*, 2017; 16(1): 14–19.
26. F. Manolaraki, « Propriétés anthelminthiques du sainfoin (Onobrychis viciifoliae): Analyse des facteurs de variations et du rôle des composés phénoliques impliqués », PhD Thesis, Institut National Polytechnique de Toulouse-INPT, 2011.