E-ISSN: 2278-3229

IJGHC, September 2023 –November 2023, Sec. B; Vol.12, No.4, 324-334.
DOI: 10.24214/IJGHC/HC/12/4/32434.

International Journal of Green and Herbal Chemistry

An International Peer Review E-3 Journal of Sciences

Available online at www.ijghc.com

Section B: Herbal Chemistry

Research Article

CODEN (USA): IJGHAY

Evaluation of the antioxidant activity of the extract of young unopened leaves of *Piliostigma thonningii*

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Received: 06 August 2023; **Revised:** 26 September 2023; **Accepted:** 10 October 2023

Abstract: *Piliostigma thonningii* is a medicinal plant of the Fabaceae family. The young unopened leaves of *Piliostigma thonningii* are used for the treatment of various diseases. This study aims to evaluate the antioxidant activity of the extract and fractions of young unopened leaves of *Piliostigma thonningii*. Successive fractionation of the hydroalcoholic extract yielded the hexane, dichloromethane, ethyl acetate, ethanol and aqueous fraction. The antioxidant capacity of the hydroalcoholic extract and fractions was carried out using the DPPH and electrochemical (DPV) method. For the DPPH method, the ethyl acetate fraction (IC_{50} = 0.07 ± 0.02 mg/mL) has a better antioxidant activity with DPPH followed by the hydroalcoholic extract (IC_{50} = 0.08 ± 0.02 mg/mL) and dichloromethane fraction (IC_{50} = 0.50 ± 0.20 mg/mL). Concerning the electrochemical method, the ethyl acetate fraction has good antioxidant activity (IC_{50} = 0.273 ± 0.002 mg/mL) followed by the

hydroalcoholic extract (IC_{50} = 0.275 ± 0.001 mg/mL) and the dichloromethane fraction (IC_{50} = 0.314 ± 0.004 mg/mL).

Key words: *Piliostigma thonningii*, DPPH, DPV, antioxidant activity

1. INTRODUCTION

Medicinal plants play a very important role for making, as they can biosynthesise a large number of organic molecules with biological activities. They constitute a plant reservoir that provides humans with healing through a therapeutic gesture [1]. Indeed, the population of the world, and particularly those of Africa, are increasingly faced with the resurgence of certain diseases linked to oxidative stress, such as cardiovascular and neurodegenerative diseases, metabolic syndrome and digestive diseases [2]. Lack of antioxidants in the diet [3,4] or even exposure to prooxidant factors such as tobacco, alcohol, certain medicines and pesticides [4,5] are everyday situations that lead to the production of free radicals responsible for oxidative stress. Unfortunately, the synthetic antioxidants generally used are being called into question for reasons of potential health risks linked to their toxicity [6,7]. Faced with this situation, research is underway to find new sources of antioxidants in medical plants [7,8]. Plants are used to treat a number of illnesses by around 80% of the population in developing countries [9].

Piliostigma thonningii is a plant of the fabaceae family used in pharmacopoeia for its many biological properties. Its fresh leaves are used to treat ulcers [10] and malaria [11]. They have anti-inflammatory, antibacterial [12], antifungal [13] and antioxidant [14] properties. Traditional practitioners, in Ivory Coast to treat certain illnesses use the young unopened leaves of *Piliostigma thonningii*.The aim of this study was to evaluate the antioxidant activity of young unopened leaves of *Piliostigma thonningii* acclimatized in Ivory Coast, using chemical (DPPH) and electrochemical (DPV) methods.

2. MATERIAL AND METHODS

2.1. Material:

2.1.1. Vegetable material: The young unopened leaves of *Piliostigma thonningii* were collected before sunrise in May 2018 in Bouaké (6047'18.762" North and 5015'25.9992" west) in central Ivory Coast and identified by Mr Amani N'guessan, botanist at Félix HOUPHOUËT-BOIGNY National Polytechnic Institute of Yamoussoukro. The leaves were dried in the shade at room temperature in the laboratory (26 to 30 °C) for 14 days and then ground. The resulting powders were sieved and stored in a dark, dry place until further use.

2.1.2. Technical material: Reagents (sodium acetate, hydrochloric acid, sulphuric acid, acetic anhydride, ferric chloride, mercuric chloride, chloroform, magnesium chips, soda, Folin-ciocalteu reagent, vanillin, aluminium chloride, sodium nitrate, sodium carbonate, tannic acid, DPPH and ascorbic acid) and analytical or technical solvents (petroleum ether, ethyl acetate, ethanol dichloromethane, water, hexane and methanol) from Merck and Across. The technical solvents were purified before use. A Jasco instruments V-530 UV-visible spectrophotometer was used for qualitative and quantitative analysis. A BUCHI 461 rotary evaporator was used for solvent removal. The chemical reagents used to assess antioxidant activity were DPPH and ascorbic acid (vitamin C). Three electrodes were used for the electrochemical method: a glassy

carbon working electrode with a diameter of 3 mm, a platinum counter-electrode and a saturated silver chloride reference electrode (Ag/AgCl/KCl sat) connected to a Metrohm potentiostat (910 PSTAT mini). A computer with PSTAT controls this instrument

2.2. Method:

2.2.1. Preparation of vegetable material: The young, dry, unopened leaves of *Piliostigma thonningii* were pulverised using an electric grinder. The obtained was sieved using 0.40 mm sieve. The powder was then stored in polyethylene bags at a temperature of 4°C in a refrigerator, protected from light and moisture, until further use.

2.2.2.Extraction of vegetable material: A mass of 50g of the powder of young unopened leaves of *Piliostigma thonningii* was macerated with stirring in 500 mL of an ethanol/ water mixture (70/30) for 24 hours. After filtration, the filtrate was evaporated using a rotavapor. The mass obtained was dissolved in 250 mL of hot water at 60°C. The aqueous solution obtained is extracted successively with 500 mL dichloromethane and 500 mL ethyl acetate. The resulting residue was dried in an oven followed by 500 mL ethanol and the aqueous residue was dried in an oven. Evaporation of the extraction solvents gave the following fractions: hexanolic, dichloromethane, ethyl acetate, ethanolic and aqueous.

2.2.3. Anti-free radical activity test using the DPPH method

2.2.3.1. Principle of the antioxidant activity test using the 2,2 diphenyl-1-picry hydrazyl (DPPH) method: 2,2 diphenyl-1-picryl hydrazine (DPPH) is a stable free radical, soluble in methanol (or ethanol). In the presence of free radical scavengers, the violet-color DPPH in solution is reduced to yellow-color 2,2 diphenyl-1-picryl hydrazine. The DPPH antioxidant activity test was carried out according to the method described [15].

2.2.3.2. Preparation of the various stock solutions and the DPPH solution: A mass of 0.20 g of the hydroalcoholic extract, the ethyl acetate fraction and the dichloromethane fraction are respectively dissolved in 10 mL of a mixture of ethanol/ water (70/30) (v/v), ethyl acetate and dichloromethane to give a concentration C = 2 mg/mL (i.e 2000 µg/mL). The different solutions obtained are diluted to make new solution of concentration (50-100-250-500-1000 ug/mL). A stock solution of ascorbic acid was prepared and diluted under the same conditions as the fractions and the hydroalcoholic extract. DPPH (2.5mg) was solubilised in 100 mL methanol to obtain a solution with a concentration of $6.34.10^{-5}$ M. The solution obtained was protected from light (in the dark).

2.2.3.3. Determination of antioxidant potential: The antioxidant potential was detremined using the method described by Benhammou *et al*.[16], slightly modified as follows. A volume of 50 µL of the hydroalcoholic extract, fraction and ascorbic acid was added to 1950 µL of the DPPH methanol solution. The mixture was homogenized and incubated at room temperature (27°C) in the dark. After 30 mn incubation, the absorbance of the different solutions were read at 517 nm using a UV-visible spectrophotometer against a blank containing only the methanol solution of DPPH. Ascorbic acid was used as a standard and its absorbanc was measured under the same conditions as the samples. For each concentration, the test repeated three (3) times. The anti-free radical activity of the hydroalcoholic extract and fraction tested corresponds to the percentage inhibition (PI) of the DPPH radical and is calculated according to the following fromula :

DOI: 10.24214/IJGHC/HC/12/4/32434.

$$PI(\%) = \frac{(Ao - A) \times 100}{Ao}$$

A= absorbance of hydroalcoholic extract or fractions; Ao = absorbance of white

2.2.3.4. Evaluation of antioxidant capacity: The antioxidant capacity of the hydroalcoholic extract and fractions is determined from the inhibition concentration 50 (IC_{50}) corresponding to the sample concentration required to reduce 50 % of the DPPH radical. The IC_{50} are determined graphically by the percentage of inhibition as a function of the different concentrations of the extract and the fractions tested [17]. For the entire experiment, each test was carried out in triplicate and the antioxidant capacity was calculated as the average of the three tests.

2.2.4. Antioxidant activity test using the differential pulse Voltammetry Method (DPV)

2.2.4.1.Principle of the test: The general principle of voltammetry is to obtain a rapid response of information on the current redox processes of a system subjected to a disturbance (potential) responsible for the desired electrochemical reaction. From the graph obtained, it is then possible to determine the nature and concentration of Ox and Red, and also to evaluate electrchemical kinetics parameter in the event of chemical reactions coupled to electron transfer [18].

2.2.4.2. Experimental setup: The experimental setup for carrying out all the electrochemical (voltammetric) measurements requires the presence in the electrochemical cell an electrolytic solution containing the sample to be analysed and the three electrodes connected to the Metrohm potentiostat (910 PSTAT mini). A computer using PSTAT software controls the interface. The electrode system consists of a glassy carbon-working electrode with a diameter of 3mm on which the electrochemical reactions of ions or molecules are observed, a platinum counter-electrode and a saturated silver chloride reference electrode (Ag/AgCl/KClsat) [19].

2.2.4.3. Methodology for assessing antioxidant activity in the presence of oxygen using DPV: The tests were carried out using Le Bourvellec *et al.*[19], slightly modified protocol in the potential rang [-200 mV ; -1400 mV]. The experiments were carried out in 15mL of a solution of N, N-dimethylformamide **(**DMF**)** and the salt (support) used was tetrabutylammonium tetrafluoroborate (TBABF4) at a concentration of 0.1M.

For each test, oxygen is bubbled through the electrolyte solution for 10 min before the reduction voltammograms are recorded. The sweep speed was set at 100 mV/s, the pulse amplitude at 17 mV and the recording time at 30 ms. A mass of 50 mg of the hydroalcoholic extract, ethyl acetate and dichloromethane fraction is diluted in 10mL of N, N-diméthylformamide (DMF) solution. To perform the analyses, a range of increasing electrolyte concentrations (0,07 g/L ; 0,14 g/L ; 0,25 g/L ; 0,30 g/L ; 0,38 g/L ; 0,42 g/L) of the hydroalcoholic extract and fraction was prepared.

The intensities Ipa0 and Ipas are determined and the anti-free activity of the extract and fraction tested corresponding to the percentage inhibition **(**PI) is calculated according to the equation

$$PI = \frac{I_{pa0} - I_{pas}}{I_{pa0}}$$

Ipa0 and Ipas are the respective intensities of the oxygen reduction current before and after the addition of the antioxidant.

DOI: 10.24214/IJGHC/HC/12/4/32434.

3. RESULTS AND DISCUSSION

3.1. DPPH method: Assessment of the antioxidant activity of the hydroalcoholic extract and the dichloromethane and ethyl acetate fractions using the DPPH method is presented in **Table 1**. IC_{50} values ranged from 0.07 ± 0.02 mg/mL to 0.50 ± 0.20 mg/mL. The ethyl acetate fraction, the dichloromethane fraction and the hydroalcoholic extract have the following values respectively 0.07 ± 0.02 mg/mL, 0.50 ± 0.20 mg/mL and 0.08 ± 0.02 mg/mL.

Table 1: The IC_{50} values for the DPPH method

Extract and fractions	**IC_{50} value (mg/mL)**
Hydroalcoholic extract	0.08 ± 0.02
Dichloromethane fraction	0.50 ± 0.20
Ethyl acetate fraction	0.07 ± 0.02
Ascorbic acid	0.06 ± 0.02

The ascorbic acid used as a reference has an IC_{50} equal to 0.06 ± 0.02 mg/mL. IC_{50} were determined graphically from the graphs shown in **Figure 1**. The results show that the ethyl acetate fraction has a very high antioxidant activity with an IC_{50} = 0.07 ± 0.02 mg/mL.

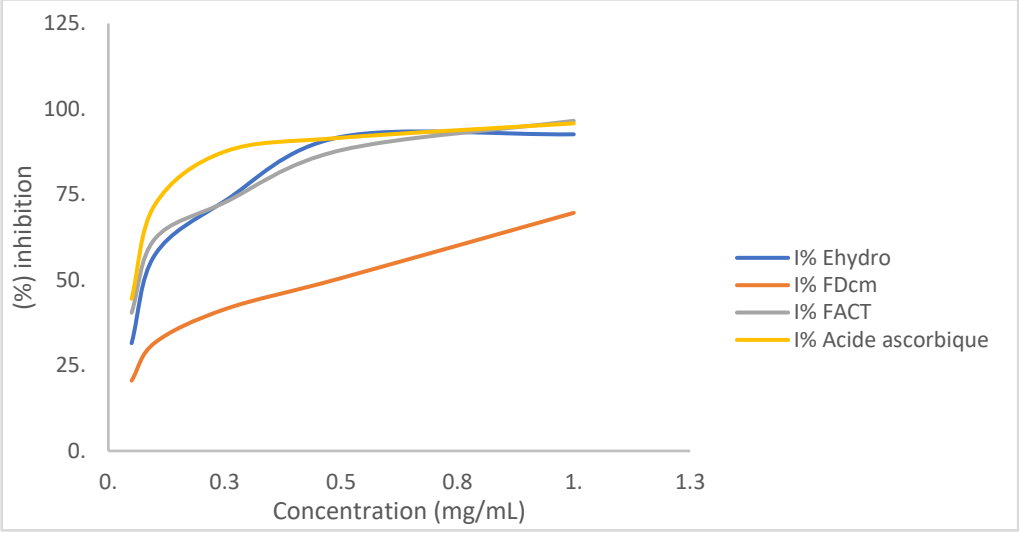

Figure 1: Determination graphs for the antioxidant activity of extract and fraction of young unopened leaves of *Piliostigma thonningii*

DOI: 10.24214/IJGHC/HC/12/4/32434.

This high antioxidant activity could be explained by their high flavonoid content, which is generally known for antioxidant properties [20-22], phenolic compounds also known for their antioxidant activity [23-26].

Our antioxidant activity are better than those obtained on mature leaves of the plant harvested in Senegal, which are 13.89 ± 0.20 mg/mL and 12.90 ± 0.15 mg/mL with hydroalcoholic extract [14,27].

Emmanuel and colleagues obtained 0.74 mg/mL and 0.40 mg/mL respectively for dichloromethane and ethyl acetate extracts in leaves of the Nigeria species [28]. These activities are much lower than our results obtained with young unopened leaves of *Piliostigma thonningii*.

However, other studies on mature leaves of Nigeria species revealed excellent antioxidant activities with IC_{50} values of 0.051 ± 0.003 mg/mL, 0.018 ± 0.003 mg/mL and 0.024 ± 0.003 mg/mL for the hydroalcoholic extract, the ethyl acetate fraction and the dichloromethane fraction [29,30].

Dieng *et al.,*[31] obtained an IC_{50} value of 0.052 ± 0.003 mg/mL for the ethyl acetate fraction of the leaves of the Senegal species. These results allow us to say that polar compounds have a good antioxidant activity with the DPPH method. These results could justify the richness of young unopened leaves of *Piliostigma thonningii*.

3.2. Electrochemical method (DPV): The electrochemical method (DPV) enabled us to obtain the voltammograms shown in **Figure 2** and to plot calibration graphs (**Figure 3**) for the hydroalcoholic extract and the fractions.

The evaluation of antioxidant activity by the electrochemical method of the hydroalcoholic extract and the dichloromethane and ethyl acetate fractions is presented in **Table 2**. IC_{50} values ranged from 0.273 ± 0.002 mg/mL to 0.314 ± 0.004 mg/mL. The ethyl acetate fraction, dichloromethane fraction and hydroalcoholic extract have IC_{50} values of 0.273 ± 0.002 mg/mL, 0.314 ± 0.004 mg/mL and 0.275 ± 0.001 mg/mL.

The results show that the ethyl acetate fraction has a slightly higher antioxidant activity (0.273 ± 0.002 mg/mL) than the hydroalcoholic extract (0.275 ± 0.001 mg/mL) and much higher than the dichloromethane fraction (0.314 ± 0.004 mg/mL). The ethyl acetate fraction was more active, followed by the hydroalcoholic extract. These results allow us to say that the polar compounds have a good antioxidant activity with the electrochemical method (DPV).

Table 2: The IC_{50} values for electrochemical method

Extract and fractions	**IC_{50} value (mg/mL)**
Hydroalcoholic extract	0.275 ± 0.001
Dichloromethane fraction	0.314 ± 0.004
Ethyl acetate fraction	0.273 ± 0.002

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The ethyl acetate fraction showed good antioxidant activity with both methods. We obtained a value of 0.066 ± 0.02 mg/mL for the DPPH method and 0.273 ± 0.002 mg/mL for the electrochemical method. The ethyl acetate fraction had a higher antioxidant activity with the DPPH method than with the electrochemical method. Both methods confirm that the ethyl acetate fraction has better antioxidant activity.

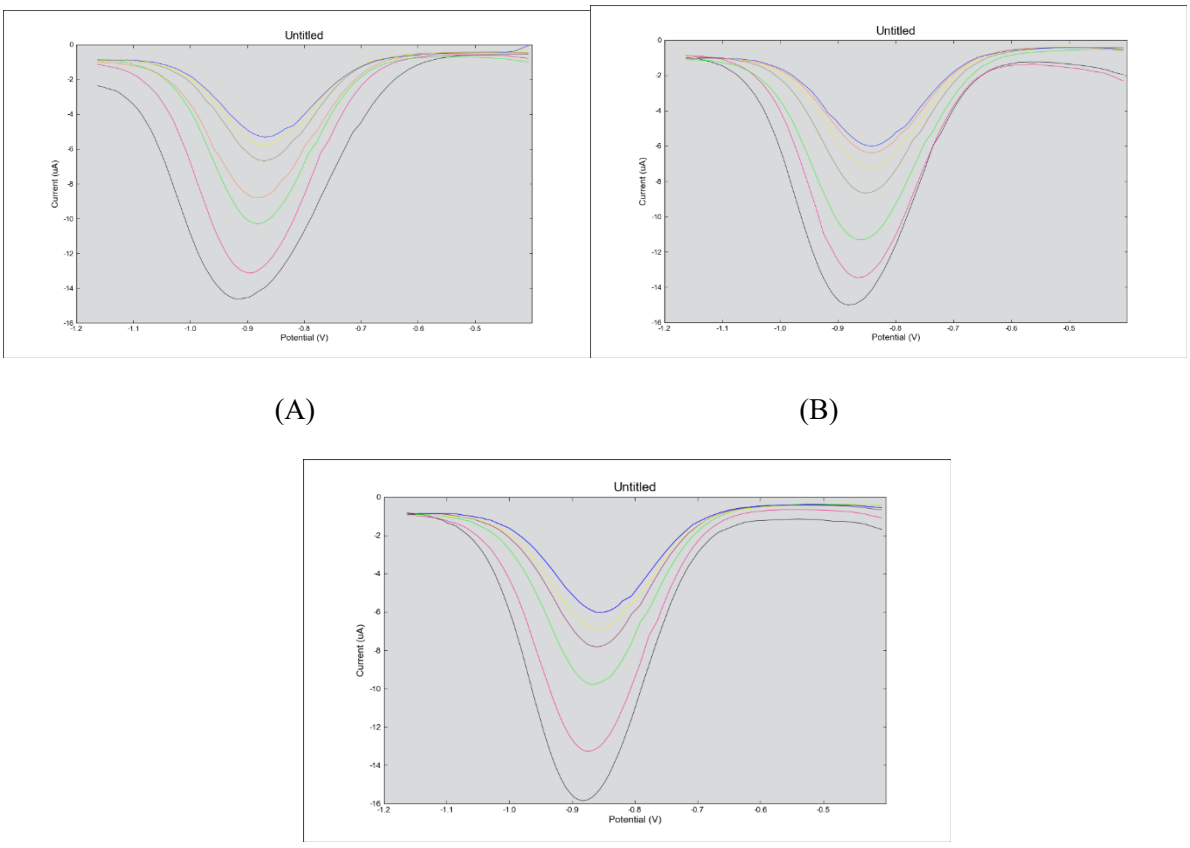
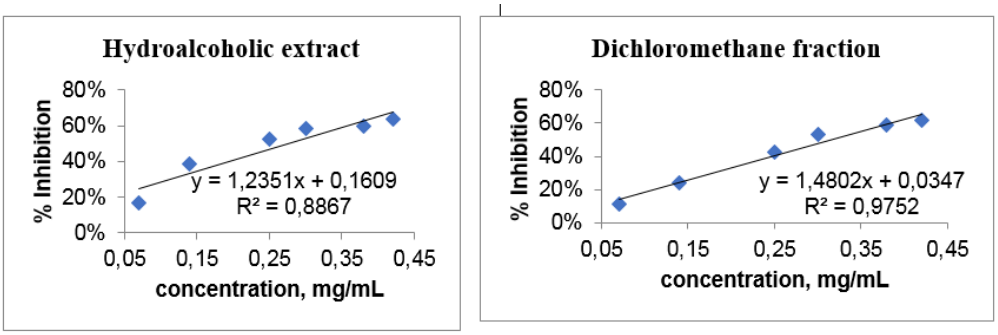


Figure 2: Oxygen reduction voltammograms for the ethyl acetate fraction (A), the hydroalcoholic extract (B) and the dichloromethane fraction (C)



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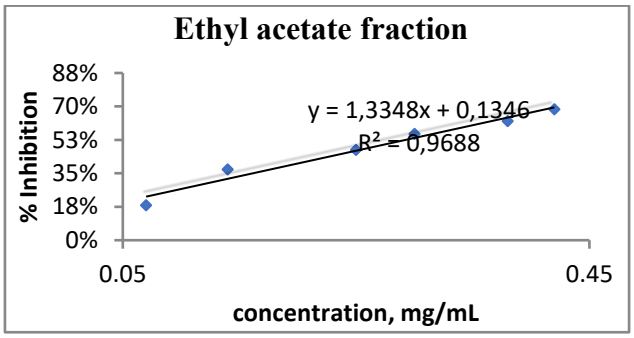

Figure 3: Calibration graphs for the hydroalcoholic extract, the ethyl acetate fraction and the dichloromethane fraction

The ethyl acetate fraction showed good antioxidant activity with both methods. We obtained a value of 0.066 ± 0.02 mg/mL for the DPPH method and 0.273 ± 0.002 mg/mL for the electrochemical method. The ethyl acetate fraction had a higher antioxidant activity with the DPPH method than with the electrochemical method. Both methods confirm that the ethyl acetate fraction has better antioxidant activity.

4. CONCLUSION

At the end of our study, we noted that the young unopened leaves of *Piliostigma thonningii* have good antioxidant activity. Assessment of antioxidant activity, using the DPPH and DPV methods, showed that the ethyl acetate fraction had good antioxidant activity. This study shows that extracts from the young unopened leaves of *Piliostigma thonningii* are a potential naturel source of antioxidants.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest between them or any other party in relation to this article.

AUTHOR'S CONTRIBUTIONS

We declare that the authors cited in this article carried out this work and any liability for claims relating to the content of this article will be borne by the authors. Thomas Konan KOUAMÉ harvested the leaves of the plant used, extracted and fractionated the plant material and performed the various methods. The work was supervised and the article written by Amian Brise Benjamin KASSI, Sorho SIAKA and Yaya SORO.

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Online publication Date: 12.10.2023