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EVALUATION OF ANTIOXIDANT ACTIVITY OF AQUEOUS AND ETHANOLIC EXTRACTS OF *MYRIANTHUS HOLSTII* (CECROPIACEAE) LEAVES, *IN VITRO*

Yeo Sounta Oumar[1,2]*, Konan N'dri Seraphin[1], Silué Kalamourou[1], Mawa Traoré[1], Doumbia Idrissa[1, 2] and Coulibaly Adama[2]

[1]University of Man, PO Box 20 Man, Côte d'Ivoire.
[2]Biochemical Pharmacodynamy Laboratory, Biosciences Department, Felix Houphouet-Boigny University, PO Box 582, Abidjan 22, Côte d'Ivoire.

***Corresponding Author: Yeo Sounta Oumar**
University of Man, PO Box 20 Man, Côte d'Ivoire.

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ABSTRAT
The purpose of this study is to evaluate the antioxidant activity of *Myrianthus holstii* (Cecropiaceae), a plant used in traditional medicine against gastroenteritis in western Côte d'Ivoire (Man). The extraction yields of the ethanolic extract (EE) and the aqueous extract are respectively 12.6% and 8.03%. Determination of the total phenol content by the colorimetric method gave the following results: EAfe (50.67 ± 2.08 mg GAE / g of extracts) and EEfe (136.7 ± 2.33 mg GAE / g of extracts). The flavonoid assay using the $AlCl_3$ aluminum chlorides method gave the following results: EAfe (71.33 ± 1.45 mg QE / g extracts) and EEfe (490 ± 17.32 mg QE / g of extracts). The evaluation of the antioxidant activity of extracts was carried out according two methods: the free radical scavenging by the DPPH and the measurement of the reducing power (FRAP). The results obtained indicate that ethanolic extract contains more polyphenolic compounds than the aqueous extract. Ethanolic extract (EAfe) antioxidant properties (EEfe, IC_{50} = 2.30 ± 0.12 µg / mL) are also greater than those of the aqueous extract (EAfe, IC_{50} = 4.40 ± 0.60 µg / mL). This antioxidant activity which remains close to that of vitamin C (01.25 ± 0.02) could represent an additional asset in the management of pathologies linked to oxidative stress.

KEYWORDS: Antioxidant Activity, *Myrianthus holstii*, ethanolic extract leaves (EE).

1-INTRODUCTION

For a long time, and despite scientific progress, traditional medicine has remained the primary means of treating people's health problems. According to the World Health Organization, about 80% of people depend on traditional medicine in primary care in daily life.[1] Currently, most diseases are caused by oxidative stress, which prompts research into new antioxidant remedies. It causes significant damage which are accelerations of cell aging. This aging leads to serious pathologies such as degenerative cardiovascular and neuro diseases, cancer, diabetes, metabolic syndrome and digestive diseases.[2] Indeed, plants represent an immense reservoir of potential compounds attributed to secondary metabolites which have the advantage of structural diversity and which possess a very wide range of biological activities. Thus, the evaluation of these activities remains a very interesting task that may be of interest to many studies.[3] Thus, a large number of scientific studies have been carried out to discover the functional properties of plant-based compounds, antioxidants or others, which could be effective for health.[4] However, the use of the available synthetic antioxidant molecules is currently being questioned due to the potential health risks and the toxicity they are capable of causing.[5,6] Therefore, the focus is increasingly on finding new sources of antioxidants including medicinal plants.[7,8] As a result, numerous studies have shown that these plants possess antioxidant properties.[9,10] Thus, our work is part of the research of natural antioxidants by assaying phenolic compounds and evaluating the antioxidant activity of the aqueous and ethanolic extracts of the leaves of *Myrianthus holstii*, a plant used in traditional medicine in western Côte d'Ivoire. To date, no scientific studies have been conducted on this part of the plant in this region. The results could allow the species to be valued in traditional medicine in Côte d'Ivoire.

2-MATERIAL AND METHODS

2.1-Plant Material

The leaves of *Myrianthus holstii* (Cecropiaceae), harvested in october 2018 in Kassiapleu near Man (western of Ivory Coast) have been identified by the National Center of Floristry at the University Felix Houphouet Boigny (Cocody-Abidjan). A specimen of the plant was deposited in the herbarium of this Center.

2.2-Preparation of Aqueous Extract

Myrianthus holstii leaves powder (100 g) were macerated for 48 hours in 1L of distilled water.[11] The macerate has been wrung into a square of sterile tissue, filtered successively on cotton wool and one fold on filter paper (Whatman paper® 2 mm). The filtrate was dried slowly in the stove at 50°C. The powder obtained was stored in a hermetically sealed jar and refrigerated at 4° C.[12]

2.3-Preparation of Ethanolic 70% Extract

It was carried out using modified method.[11] A mass of 20 g of plant powder was added in 100 mL of ethanol 70% and subjected to maceration for 72 hours. The macerate was treated according to the same procedure like the aqueous extract.

2.4-Total Phenolic Content

The total phenolic content of the extract was determined separately using the method of.[13,14] The calibration curve was prepared by mixing methanolic solution of gallic acid (1 mL; 0-100 g/mL) with 5 mL Folin-Ciocalteu reagent and sodium carbonate (4 mL, 1M). We measured absorbance at 765 nm and drew the calibration curve. 1 mL of extract (100 g/mL) was also mixed with the reagents above and after 15 min, the absorbance was measured to determine plant total phenolic contents. Experimentations were carried out in triplicate. The total phenol values are expressed in terms of gallic acid equivalent (mg GAE/g of extract), which is a common reference compound.

2.5-Flavonoids Content

The total flavonoids content was analyzed by aluminum chloride method.[15,16] Each plant extract (0.5 mL of 1 :100 µg/mL) was mixed with 1.5 mL methanol, 0.1 mL of $AlCl_3$ (10%), 0.1 mL of 1M potassium acetate and 2.8 mL of distilled water. The mixture was allowed to stand for 30 min at room temperature (25 °C) and absorbance was measured at 415 nm with a double beam Perkin Elmer UV/Visible spectrophotometer (USA). The calibration curve was prepared by preparing quercetin solutions at concentrations ranging from 0 to 100 µg/mL in methanol. Total flavonoids contents were expressed as mg of Quercetin equivalents (QE)/g of extract. Samples were analyzed in triplicates.

2.6-Antioxidant Activity

2.6.1-Chelating Ability

Chelating ability of Fe^{2+} was determined according to the method of.[17,18] Fe^{2+} was monitored by measuring the formation of ferrous iron-ferrozine complex at 562 nm. Different concentration of extract (1 mL) in 3.7 mL of methanol was mixed with $FeCl_2$ (0.1 mL, 2 mM) and ferrozine (0.2 mL, 5 mM). The resulting mixture was shaken and left to stand for 10 min at room temperature. EDTA was used as standard control. The absorbance of the resulting solution was measured at 562 nm. The capability to chelate the ferrous iron was calculated using the following equation.

Chelating Effect (%) = [(Ao - A1) / Ao] X 100; Ao was the absorbance of the control (containing all reagents except the test compound) and A1, the absorbance in presence of sample of extract and standard.

2.6.2-Free Radical Scavenging Activity

Hydrogen atom or electron donating abilities of the compounds were measured from the bleaching of the purple-coloured methanol solution of 2, 2-diphenyl-1-picryl hydrazyl (DPPH). This spectrophotometric assay uses the stable free radical, DPPH as a reagent.[19] Different concentrations of each extract were added, at an equal volume, to methanolic solution of DPPH (100 µL). After 30 min at room temperature, the absorbance was recorded at 517 nm. Test was repeated for three times. Vitamin C was used as standard control. The DPPH radical scavenging effect was calculated as inhibition of percentage (I %) using the following formula: I % = (A Blank-A Sample/A Blank); A blank is the absorbance of the control reaction (containing all reagents except the test compound) and A sample is the absorbance of the test compound. The values of inhibition were calculated for concentrations of the extract. IC_{50} values denote the concentration of sample, which is required to scavenge 50% of DPPH free radicals. Chemicals Reagents All chemicals used were of analytical grade. Methanol, aluminum chloride, potassium acetate, 2,2-Diphenyl-1-picrylhydrazyl (DPPH), ferrous chloride, ferrozine, potassium ferricyanide, Folinciocalteu reagent, standards such as Lascorbic acid, ethylenediamine tetraacetic acid (EDTA), gallic acid, quercetin all from Sigma Chemicals Co. (St. Louis, MO, USA).

2.7-Statistical Analysis

Statistical analysis was performed by Graph Pad Prism 6 statistical software. Results are expressed as mean ± SD and analyzed by ANOVA and Tukey tests with univariate rate determination of significance with $P \leq 0.05$ considered statistically significant.

3-RESULTS AND DISCUSSION

3.1-Contents of total phenols and flavonoids leaves extracts of *Myrianthus holstii*

The levels of total phenols and total flavonoids of bark extracts of *Myrianthus holstii* are determined from the calibration line $y = 0.004 x + 00$; $R^2 = 0.998$ and $y = 0.037 x + 00$; $R^2 = 0.997$ plotted using standard as gallic acid and quercetin, respectively.

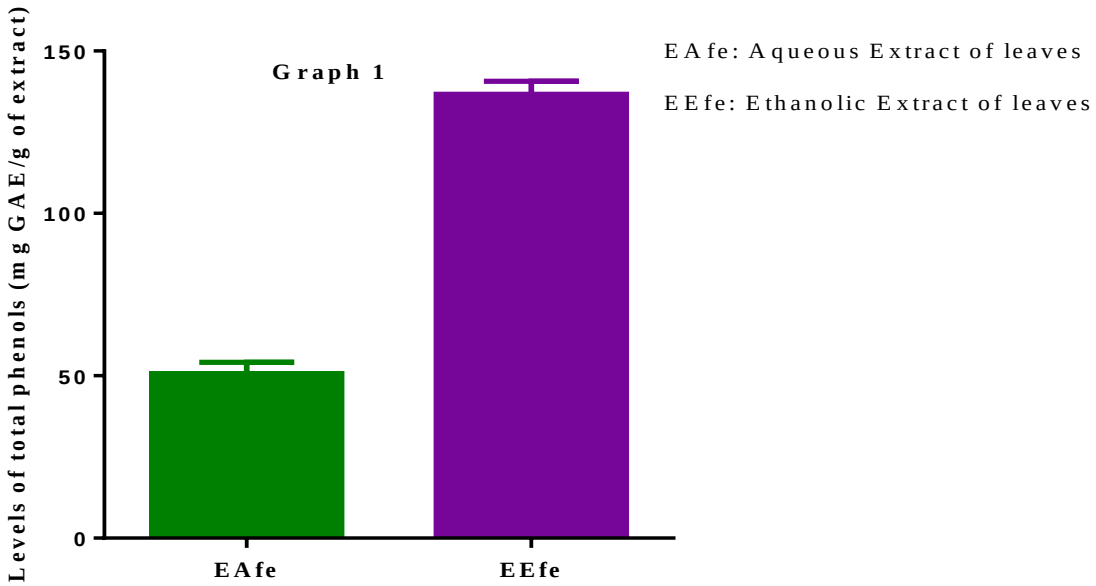
3.2-Total Phenol and Flavonoid Content

The total phenol content of *M. holstii* extracts was calculated from the calibration curve using gallic acid as the reference polyphenol. The results obtained are expressed in milligram gallic acid equivalent per gram of dry matter (mg GAE / g extract).

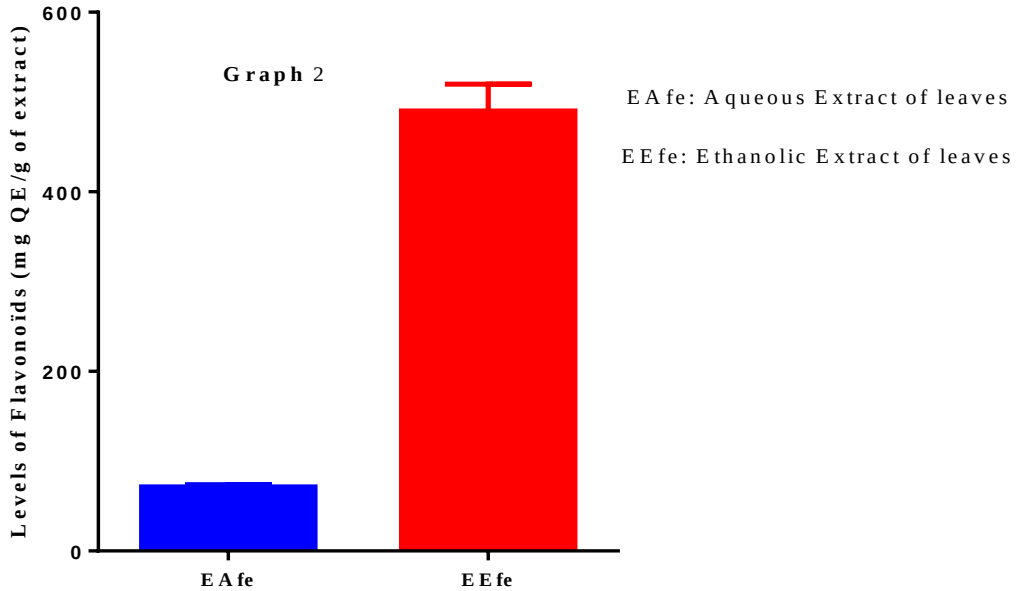
The Folin-ciocalteu method from the equation of the regression line ($y = 0.004 + 00$ $r^2 = 0.998$) of the calibration range of gallic acid (0-0.5mg / mL), shows

the content of the ethanol extract of the leaves (EEfe) of *M. holstii* with a value of 136.7 ± 2.33 mg GAE / g of extract is higher than the aqueous extract of leaves (EAfe) which has a value of 50.67 67 ± 2.08 mg GAE / g dry matter.

The values obtained are greater than those obtained in the leaves of *Myrtus communis* var. italica (33.67 mg GAE/g), as well as those of eleven medicinal plants including *Artemisia campestris* at the aerial level with the ethanolic extract 70%.[20, 21] On the other hand, those of the leaves of *Myrianthus holstii* are lower than the levels obtained in the leaves of *Cassia sieberiana* with 276.62 mg GAE/g.[22] The results of the determination of the total phenols showed that the ethanolic extract of the plant is rich in these compounds. This difference in levels can be explained by environmental conditions, climatic and collection period and also by genetic factors and experimental conditions.



Graph 1: Levels of total Phenols of Aqueous and Ethanolic Extracts of *Myranthus holstii* Leaves (mg GAE/g of extract) (Mean ± SD of tree trial)

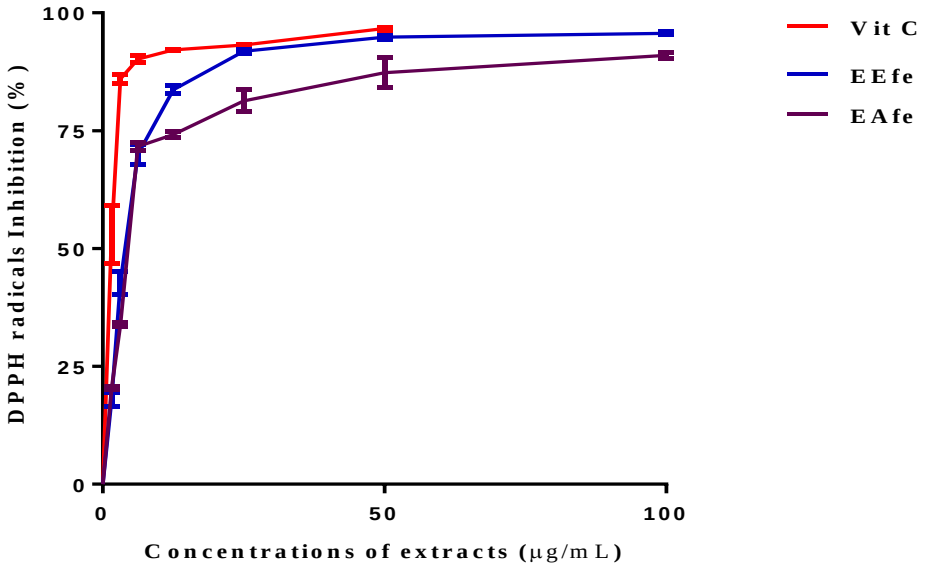


Graph-2: Levels of Flavonoids of Aqueous and Ethanolic Extracts of *Mynathus holstii* Leaves (mg QE/g of extract) (Mean ± SD of tree trial)

3.3-Antioxidant activity

For antioxidant activity, antioxidants reduce and decolorize the DPPH radical, to a yellow compound diphenyl picryl hydrazine, the extent of the reaction will depend on the ability of antioxidants to give the hydrogen.[23] The results are expressed as a percentage of the antiradical activity and expressed using the IC_{50} parameter, which is defined as the concentration of the substrate that causes a 50% loss of DPPH activity.[24] The measurements of the radical power of M. holstii reveal that the EEfe is more active with an IC_{50} of the order of 2.30 ± 0.12 µg/mL and the EAfe has the lowest anti-radical activity with an IC_{50} = 4.40 ± 0.60 µg / mL **(Graph 3).** The EEfe is close to the Vitamin C used as a reference molecule has an IC_{50} of the order of 01.25 ± 0.02 µg / mL.

Moreover, at 100 µg / mL, EAfe and EEfe extracts respectively have 91% and 95% inhibition of the DPPH radical. Our values are comparable to those of which worked on the roots of Cochlospermum planchonii and found a value of 1.83 ± 0.74 mg/mL with 96% ethanol.[25] Previous studies have confirmed that phenolic compound are the main antioxidant constituents in medicinal plants, vegetables, fruits and spices. Phenolic compounds can exhibit strong antioxidant power in vitro, they directly trap reactive oxygen species.[26] According to the study, polyphenols appear to be effective donors of hydrogen to the DPPH radical, due to their ideal structural chemistry.[27] Thus, the antioxidants in a mixture make the antioxidant activity depend not only on the concentration, but also on the structure and nature of the antioxidants.[28] It has been proved that phenolic compounds and more particularly flavonoids are mainly responsible for the scavenger effect of free radicals.[29,30]

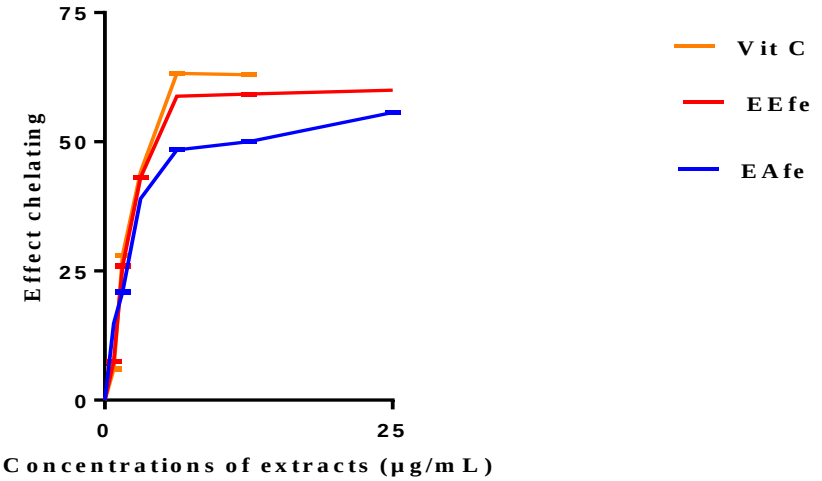


Graph 3: Evolution of the Antiradical of the Aqueous and Ethanolic Extracts of Myrianthus holstii Leaves

3.4-Power chelating

The chelating activity of the aqueous and ethanolic extracts of *M. holstii* leaves revealed that the EEfe with an IC_{50} = 4.50 ± 0.22 µg / mL is the most active and the EAfe has the lowest anti-radical activity with an IC_{50} = 12.50 ± 0.75 µg / mL (Graphe 4). Moreover, our EEfe is close to the vitamin C used as a standard molecule with an IC_{50} of 4.08 ± 0.01 µg / mL. The value of IC_{50} obtained with vitamin C is very low (04.08 ± 0.01 µg / mL), reflecting its strong chelating effect, this value is very close to the IC_{50} of 5.6 µg / mL reported by.[31] In a study conducted on extracts of fourteen barley varieties, highlighted the existing of a very low and insignificant correlation ($r = 0.041$, $p \leq 0.05$) between the chelating activity of these extracts and their contents of phenolic compounds.[32] In addition, the chelating capacity of a phenolic compound is dependent on the availability of a number of suitably oriented functional groups.[33]

So, some sample rich in phenolic compounds could not chelate the transition metals if its polyphenols do not have the functional groups necessary for the chelating activity.



Graph-4: Chelating Power of Aqeous Extracts and Ethanolic of Myrianthus holstii Leaves

CONCLUSION

The objective of this work was to determine the content of phenolic compounds and to evaluate the antioxidant power of the leaves of M. holstii, a medicinal plant used in traditiona medicine in western Côte d'Ivoire. Extracts from this study obtained by maceration with different solvents, namely ethanol and distilled water. On the basis of the results obtained, the determination of the phenolic compounds contained in the two extracts revealed a high content in the ethanolic extract. Similarly, the antioxidant activity of the ethanolic extract was greater than the aqueous extract and even close to vitamin C. This study shows that *Myrianthus holstii* leaves can be considered as a source of natural antioxidants for medicinal use.

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