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ANTIOXIDANT ACTIVITY OF WATER EXTRACT OF *MAREYA MICRANTHA* (BENTH.) MÜLL. ARG. (EUPHORBIACEAE)

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ABSTRACT
Mareya micrantha (Benth.), Syn. *Mareya spicata* (Baill), Müll. Arg. (Euphorbiaceae) is a shrub that is found in west and central Africa. The plant flourishes in tropical climate and it is commonly used in different parts of west and central Africa to treat diseases which require drastic action, such as tapeworm infections, gonorrhea and leprosy. Its extract used in this study was powdered form obtained from the maceration of the dried plant bark in water, followed by evaporation to dryness. In this study, the antioxidative and radical scavenging activities of the total aqueous extract were studied throughout six standard biological tests: DPPH reduction, ferric thiocyanate (FTC) lipidic peroxidation inhibition and thiobarbituric acid reacting substances (TBARS), reducing power, Chelating power and the FRAP method. Vitamin C was used as a reference. This study on the assessment of *in vitro* antioxidant activity of *Mareya micrantha* showed the presence of phenolic compounds including phenols, total flavonoids and flavonols with low antioxidant activities compared to vitamin C which is a reference molecule.

KEYWORDS: *Mareya micrantha*, oxidative stress, antioxidant activity, chelating power, anti-radical activity, reducing power.

INTRODUCTION
Plants have long been a very important source of drugs against several diseases including constipation. *Mareya micrantha* is well known in the traditional medical practice of the south of Côte d'Ivoire (West Africa) where the leaves of this plant is extracted with water and the extract is taken orally for the treatment of constipation. It is commonly called "oyia" in the local language of "Attié".[1] *Mareya micrantha* (Benth.), Syn. *Mareya spicata* (Baill), Müll. Arg. (Euphorbiaceae) is a shrub that is found in west and central Africa. The plant flourishes in tropical climate and it is commonly used in different parts of west and central Africa to treat diseases which require drastic action, such as tapeworm infections, gonorrhea and leprosy.[2] Previous studies showed that the aqueous leaf extracts of M. *micrantha* suppressed cardiac contractility of isolated frog and rat hearts in a concentration dependent way.[3,4] In another test, an aqueous leaves extracts elicited concentration-dependent contractions of the longitudinal muscle of isolated guinea-pig ileum.[5] Leaves extracts caused hypotension in dogs, and a root extract caused paralysis of the respiratory center in rats. The methanol and cold aqueous extracts of the leaves showed antibacterial activity against *Enterobacter aerogenes*, *Agrobacterium tumefaciens*, *Bacillus subtilis*, *Clostridium sporogenes*, *Escherichia coli* and *Staphylococcus aureus*.[2] Ethanolic leaves extracts showed low antiplasmodial activity against a chloroquine-resistant strain of *Plasmodium falciparum*.[6] Although quite a number of scientific investigations have been undertaken to validate the local use of this plant, there seems to be no report on the antioxidant activity of the leaves of the plant. The present study was planned to examine the antioxidant activity of the aqueous extract of *Mareya micrantha* leaves.

Based on the traditional claims surrounding and the lack of scientific studies of its potential pharmacological properties, the objective of this study was to evaluate the antioxidant activity through direct free radical scavenging methods and also elucidate total phenolic content (TPC) and polyphenolic flavonoids constituents.

MATERIAL AND METHODS

Plant material

The leaves, used for this study were collected from *Mareya micrantha* plants located at Akoupé (south of Côte d'Ivoire, West Africa) in June 2015. The plant was identified and authenticated by the Department of Botany, University of Cocody. After identification, a voucher specimen (N°18041) was deposited at the herbarium of "Centre National de Floristique" of the University of Cocody-Abidjan.

Extraction procedure

The harvested leaves were air-dried and then reduced to powder with mortar and pestles. 80 g of the powder was extracted at a temperature of 80°C (by the process of maceration) with 2 L of distilled water. The macerated mixture was filtered and the filtrate was evaporated in a carefully regulated water bath (maintained at temperature of 80°C) to yield 4.5 g of dark solid extract.[7] The extract was stored at a temperature of - 4°C pending the time for biological investigations.

Chemicals

Folin-Ciocalteu reagent, sodium carbonate, gallic acid, rutin, aluminium chloride, sodium nitrate, sodium hydroxide, 2,2-diphenyl-1-picrylhydrazyl (DPPH), trichloroacetic acid, potassium ferricyanide, sodium acetate buffer, neocuproine, deoxyribose, EDTA, potassium phosphate buffer, hydrogen peroxide, ascorbic acid, TBA, 2,4,6 tripyridyl-s-triazine (TPTZ), ferric chloride, HCl, ammonium molybdate, sodium phosphate, sulfuric acid, ammonium

thiocyanate, and all other chemicals used were of analytical grade. All the chemicals were obtained from E. Merck PROLABO.

Determination of total Phenolic

Total phenolic content was determined using the Folin-Ciocalteu reagent.[8] 0.1mL of extract was diluted with 1mL distilled water and added to solution of 0.5mL of Folin-Ciocalteu reagent and 1.5mL of 20% sodium carbonate solution. The reaction mixture was incubated for 2 hours, and, finally, the volume was raised to 10 mL, and the absorbance was read at 765 nm. Gallic acid (0–200 μg/mL) was used for calibration of standard curve. The total phenolic content was expressed as milligram gallic acid equivalent (mgGAE)/g dry weight of plant material.

Determination of the total flavonoids

Total flavonoids content was determined by using a method described.[9] Briefly, 0.25 ml of each fraction (1-5 mg/ml; dissolved in respective solvent) and rutin standard solution (15-250 µg/ml) was mixed with 1.25 ml of distilled water in a test tube, followed by addition of 75µl of a 5% (w/v) sodium nitrite solution. After 6 min, 150µl of 10% (w/v) aluminum chloride solution was added, and the mixture was allowed to stand for a further 5 min before 0.5 ml of 1 M NaOH was added. The mixture was made up to 2.5 ml with distilled water and mixed well. The absorbance was measured immediately at 510 nm. The results of samples were expressed as mg of rutin equivalents of total extractable compounds.

All fractions were run in triplicate.

Determination of total flavonols

Total flavonols in the plant extracts were estimated using the method of Kumaran and Karunakaran.[10] To 2 mL of sample (standard), 2 mL of 2% AlCl3 ethanol solution and 3 mL sodium acetate solution (50 g/l) were added. After 2.5 h at 20°C, the absorbance was read at 440 nm using spectrophotometer (SPECTRONIC GENESIS 5).Extract samples were evaluated at afinal concentration of 0.1 g/mL and quercetin solutions at concentrations 6.25 to 100µg/mL were used as a standard. Total flavonol contents were calculated as quercetin equivalents (mg QE/g extract).

DPPH radical scavenging activity assay

The free radical scavenging activity of the fractions was measured *in vitro* by 2,20- diphenyl-1-picrylhydrazyl (DPPH) assay according to the method described earlier.[11] The stock solution was prepared by dissolving 24 mg DPPH with 100 ml methanol and stored at 20°C until required. The working solution was obtained by diluting DPPH solution with methanol to attain an absorbance of about 0.98 ± 0.02 at 517 nm using the spectrophotometer. A 3 ml aliquot of this solution was mixed with 100 µl of the sample at various concentrations (10 - 500 µg/ml). The reaction mixture was shaken well and incubated in the dark for 15 min at room temperature. Then the absorbance was taken at 517 nm. The control was prepared as above without any sample. The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation:

Inhibition (%) = [(white ABS - ABS sample / white ABS)] x 100.

NB: **Inhibition (%)**: the percentage of inhibition of DPPH radical

White ABS: the absorbance of the blank. (No excerpt)

ABS sample: the absorbance of the root extracts of the plant and vitamin C.

Measurement of reducing power

The method described by Yildirin et *al.*, (2001)[12] was used for determining the reducing power of the plant extracts. It measures the ability of the extracts to reduce ferric ion (Fe3 +) to ferrous ion (Fe2 +). Thus, 1 mL of each extract and vitamin C at different concentrations are mixed separately with one mL of phosphate buffer (0.2 mM, pH 6.6) and 1 mL of 1% potassium ferricyanide. The mixture was incubated in a water bath at 50°C for 30 minutes. After adding 1 mL of 10% trichloroacetic, the reaction mixture was centrifuged at 3000revs/min for 10 minutes acid. Supernatant are added to 0.1mL of ferric chloride and 2% to 0.1mL of distilled water. After 10

minutes incubation at room temperature, the absorbance is measured with a spectrophotometer at 700 nm against a blank, prepared in the same conditions. The increase in absorbance of the sample indicates an increase in reducing power, according to the concentrations of the extracts and vitamin C. The concentrations inducing an absorbance of 0.5 nm (EC50) of the extracts were determined and compared to that of vitamin C.

Chelating power measurement

The colorimetric method of Le et *al*., (2007)[13] based on the determination of the complex formed by the ferrous ion (Fe2 +) and which was used to measure ferrosine the chelating power plant extracts. Thus, 3.7 mL of methanol , 0.1 mL of iron II chloride (2 mM) and 0.2 mL of ferrosine (5 mM) were added successively to 1 mL of each sample at various concentrations achieved by dilution of the order of from 2 100 to 0.78 mg / ml to initiate the reaction. After vigorous stirring and then incubated at room temperature for 10 minutes, absorbance are read at the spectrophotometer at 562 nm against a blank. Vitamin C is used at different concentrations as compared to the reference solution for the chelating activity of the extracts. Chelation samples can be determined using the following formula:

Power chelator (%) = [(white ABS - ABS sample) / ABS white] x 100

NB: Inhibition (%): the percentage of inhibition of DPPH radical

White ABS: the absorbance of the blank. (No excerpt)

ABS sample: the absorbance of the root extracts of the plant and vitamin C.

Anti-lipid peroxidation assay

The effect of extracts on lipid peroxidation inhibition was determined by the ammonium thiocyanate method[14] with a slight modification. The principle is based on the measure of the absorbance of a red color at 500 nm which decreases in the presence of antioxidants. Different concentrations (0.2-6 mg/mL) of each extract (0.5 mL) were mixed with 0.2 ml of diluted linoleic acid (25 mg/mL in 99% ethanol) and 0.4 ml of 50 mM phosphate buffer (pH 7.4). After 15 min of incubation at 40°C, an aliquot (0.1 mL) from the reaction mixture was mixed with reaction solution containing 3 mL of 70% ethanol, 0.1 mL of ammonium thiocyanate (30 mg/mL in distilled water) and 50 µL of ferrous chloride (2.45 mg/mL in 3.5% hydrochloric acid). The final reaction solution was mixed and incubated at room temperature for 3 min. The absorbance was then measured at 500 nm. The experiment was repeated for three times for each sample. Linoleic acid emulsion without extract served as control and vitamin C (0.2-1 mg/mL) was used as standard control. Inhibition of linoleic acid oxidation was calculated by using the following formula: Inhibition % = x 100

Where Abs control is the absorbance of linoleic acid emulsion without sample extract and Abs sample is the absorbance of linoleic acid emulsion with sample extract.

Statistical analysis

Data are expressed as mean ± SD from three separate observations. For in vitro antioxidant assays one way ANOVA test followed by Tukey's test ($P < 0.05$) was used to analyze the differences among EC50 of various fractions for different antioxidant assays. The EC50 values were determined using the Graph Pad Prism 5 software. Data on biochemical investigations of *in vivo* experiments were analyzed by one-way (ANOVA) and the group means were compared by Dunnet's Multiple Range Test. A probability of $P < 0.05$ was considered as significant.

RESULTS AND DISCUSSION

Polyphenol content of the plant

The polyphenol content of *Mareya micrantha* were determined by projecting the optical densities obtained on the calibration curves of gallic acid and quercetin (Table I; Figure 1, and 2).

Anti -radical and iron chelating activities of plant

The anti-radical capacity and chelating of total aqueous extract of *Mareya micrantha* are shown in Figures 3 and 4. The concentrations of the plant extract causing 50% reduction of DPPH radicals and chelating (IC50) are listed in Table II.

Reducing power

Reducing power measurement is used to evaluate the capacity of the plant extract and vitamin C to reduce ferric ion to ferrous ion by the increase in green chromophore coloration. The plots of absorbance against concentrations of the plant extract and vitamin C graph indicated a low reducing power of *Mareya micrantha* compared to vitamin C with the effective concentrations resulting in absorbance of 0.5 (EC50) 87.7 µg / mL for the plant extract and 3.12 µg /mL for Vitamin C (Table III).

Total antioxidant power

The results showed that the *Mareya micrantha* aqueous extract has a total antioxidant power of 434 µmol Fe II while Vitamin C exhibits a total antioxidant power of 1647 µmol Fe II (Table IV).

Lipid peroxidation inhibition power

The inhibition of peroxidation (absorbance) against number of days (Figure 6) showed that the curves of the plant extract have a similar appearance to that of Vitamin C and well below that of the negative control. This observation was confirmed by the TBARS method which showed no difference between the percentage of inhibition of lipid peroxidation of *Mareya micrantha* (68.67 ± 1.07%) and Vitamin C (75.17 ± 2.30%)(Table V).

Several techniques have been used to determine the *in vitro* antioxidant activity in order to allow rapid screening of substances, since substances that have low antioxidant activity *in vitro*, will probably show little

activity *in vivo*.[15] This study highlighted the antioxidant activity of *Mareya micrantha* starting from six biological tests which are the reduction of radicals DPPH, the ions (Fe3+), the chelating activity of the metal ions such as the ferro-ion (Fe2+), the inhibition of the lipidic peroxidation (FTC and TBARS) and the content of total phenols of the plant.

Indeed, this study showed that *Mareya micrantha*'s aqueous extract has a low antioxidant activity by the reducing activity on DPPH radicals and Ferric ions (Fe3+). This potential is due to its polyphenol content. It was reported that phenolic compounds were associated with antioxidant activity and that they play an important role in stabilizing lipid peroxidation[16]

Free radicals are known to play a definite role in a wide variety of pathological manifestations. Antioxidants fight against free radicals and protect us from various diseases. They exert their action either by scavenging the reactive oxygen species or protecting the antioxidant defense mechanisms.[17] The electron donation ability of natural products can be measured by 2,20-diphenyl-1-picrylhydrazyl radical (DPPH) purple-coloured solution bleaching.[15] The method is based on scavenging of DPPH through the addition of a radical species or antioxidant that decolourizes the DPPH solution. The degree of colour change is proportional to the concentration and potency of the antioxidants. A large decrease in the absorbance of the reaction mixture indicates significant free radical scavenging activity of the compound under test.[18] In the present study the extract showed significantly higher inhibition percentage and positively correlated with total phenolic content. Results of this study suggest that the plant extract contain phytochemical constituents that are capable of donating hydrogen to a free radical to scavenge the potential damage.

The antioxidant activity can be also evaluated by the measurement of the chelating activity of metal ions such as ferrous iron (Fe2+), indicates that the total aqueous extract of the plant species has a good chelating power, so a good antioxidant power.

Our results are in accordance with those of[19] who showed that the green tea polyphenols confers its good antioxidant activity to it. For this author, these polyphenols are able to inhibit the oxidative stress by chelating the metal ions to inert forms.

The measurement of the inhibition of lipidic peroxidation for the evaluation of the antioxidant power of the vegetal extract has shown that total aqueous extract of *Mareya micrantha* has a high inhibition of lipid peroxidation substantially similar to that of vitamin C, which is a reference antioxidant molecule. This inhibitory activity is due to its polyphenol content especially the presence of total flavonoids because there is a linear correlation between the lipid peroxidation inhibitory power of medicinal plants and their total flavonoids content. Thus it is possible to draw the conclusion from the presence of the good antioxidant activity. Indeed, according to N'khili[19], polyphenols in general and flavonoids in particular, are thermodynamically capable to reduce the peroxide radicals by electron transfer due to their low redox potential.

Table: I Levels of Phenolic Compounds of The Plant Extract Representing Average Of Three Experiments With Standard Deviations.

	Phenolic compound		
	Total phenols (µg GAE/g of extract)	**Total flavonoids (µg QE/g of extract)**	**Total flavonols (µg QE/g of extract)**
Mareya micrantha	119.3 ± 0.667	13.80 ± 1.039	50.13 ± 11.57

Table: II CONCENTRATIONS INDUCING 50% REDUCTION IN DPPH AND CHELATOR, IC_{50} OF THE PLANT EXTRACT.

	IC_{50} (µg/mL)	
	Anti-radical activity	**Chelating power**
Mareya micrantha	14.50 ± 1,423	143.0 ± 1.12
Vitamin C	1.95 ± 1,633	
EDTA		18.6 ± 0,93
Quercetin		452.0 ± 4.20

NB: Vitamin C was used as reference molecule for testing of the anti-radical activity while Quercetin and EDTA were used to test the chelating power.

Table: III EFFECTIVES CONCENTRATIONS (EC_{50}) OF VITAMIN C AND PLANTE EXTRACT

Effectives Concentrations (EC_{50}) µg/mL	
Vitamin C	***M. micrantha***
3.12 ± 0.0032	87.7 ± 0.0062

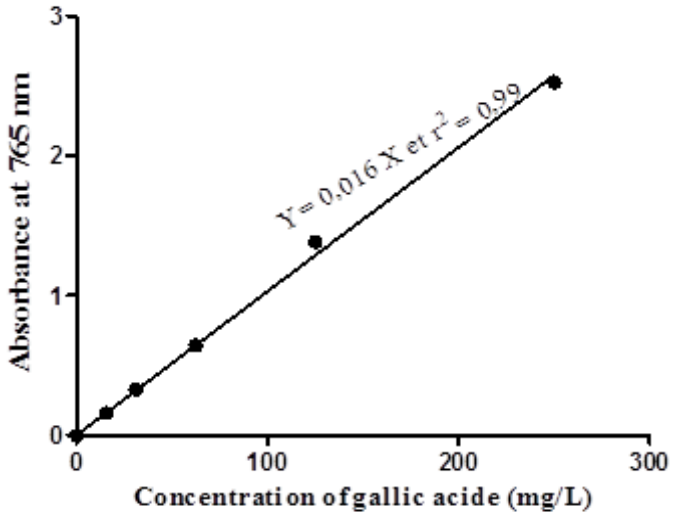

Fig 1: Calibration curve of gallic acid

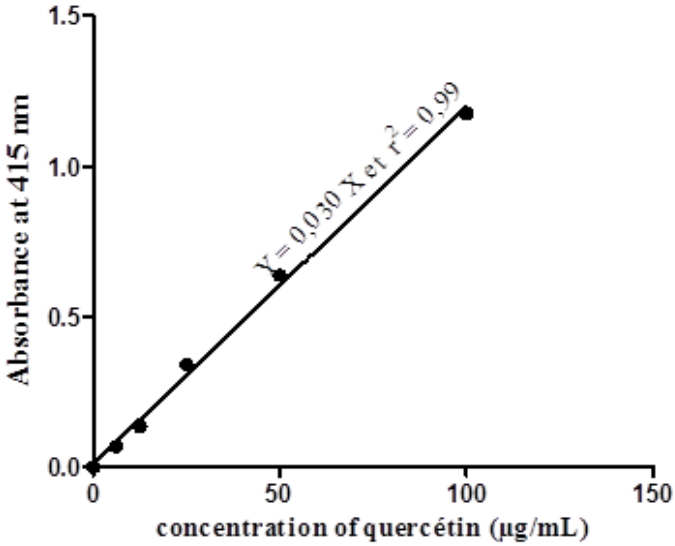

Fig 2: Calibration curve of quercetin

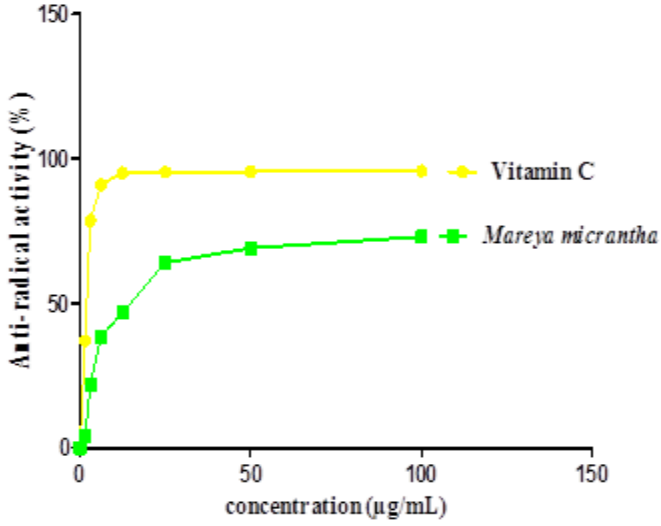

Fig 3:Anti-radical power of *Mareya micrantha* in comparison of vitamin C

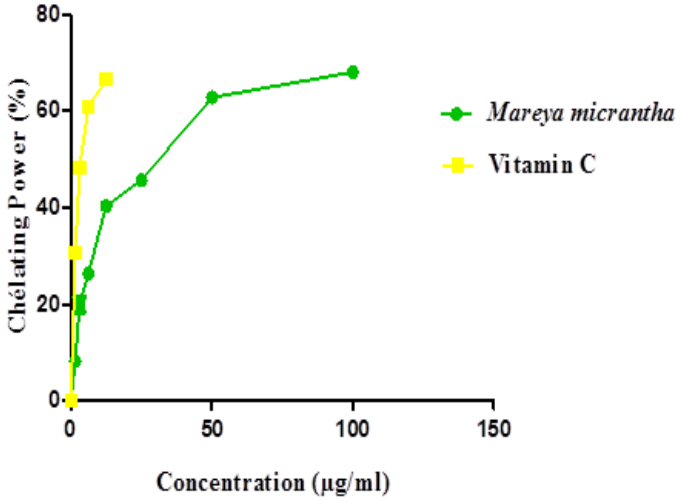

Fig 4: Chelating power of plant extract in comparison of those of vitamin C

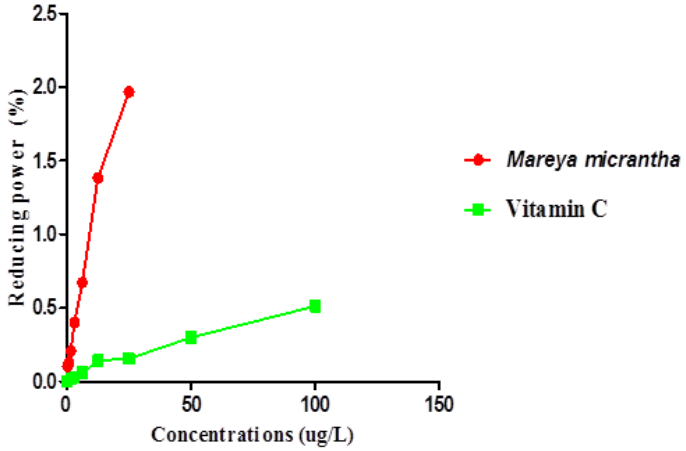

Fig 5: Reducing power of *Mareya micrantha* extract in comparison of vitamin C

Table Iv: Total Antioxidant Power Of *Mareya Micrantha* In Comparison Of Vitamin C

Total antioxidant power (µmol de Fer II)	
Vitamin C	***M. micrantha***
1647 ± 12.02	434 ± 29.26

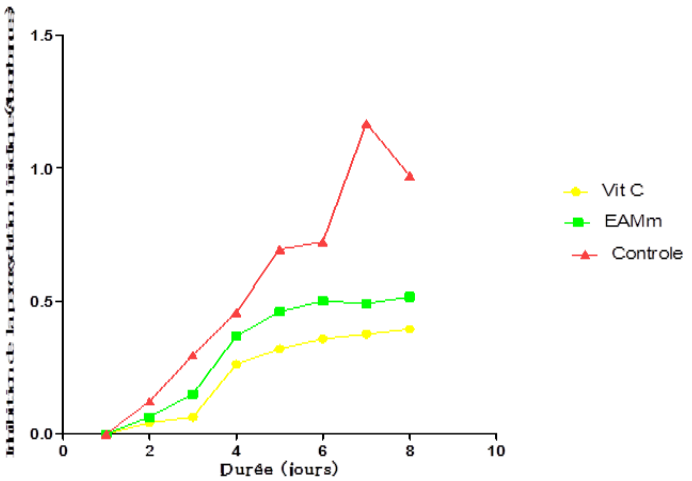

Fig 6: Comparison of inhibition of lipid peroxidation by the method of FTC

Table V: AVERAGE PERCENTAGES OF INHIBITION OF LIPID PEROXIDATION WITH STANDARD DEVIATIONS (TBARS METHOD)

Echantillons	Vit C	EAMm
Inhibition (%)	75.17 ± 2.30	68.67 ± 1.07

CONCLUSION

This study on the assessment of *in vitro* antioxidant activity of *Mareya micrantha* showed the presence of phenolic compounds including phenols, total flavonoids and flavonols with low antioxidant activities compared to vitamin C which is a reference molecule. This medicinal plant, whose total aqueous extract is studied here, could be a promising source of new natural antioxidants molecules needed to fight against metabolic diseases related to oxidative stress. In future studies we will test the extracts obtained from other solvents which could improve the antioxidant activity of *Mareya micrantha*.

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