Research Article

Evaluation of the Antioxidant Activity of Aqueous and Methanolic Extracts of *Tetrapleura tetraptera* Leaves (Schum. & Thonn.) Taub. (Fabaceae)

Yeo Sounta Oumar[1,2]*, Konan N'dri Seraphin[1], Mawa Traoré[1], Silué Kalamourou[1], Doumbia Idrissa[1,2], Djaman Allico Joseph[1, 3], Coulibaly Adama[1, 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.

3. Biochemical Laboratory of Pasteur Institute of Côte d'Ivoire, PO Box 490, Abidjan 01, Côte d'Ivoire.

***Corresponding author's E-mail:** yeosounta@yahoo.fr

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ABSTRACT

The objective of this study was to perform the determination of polyphenol and antioxidant activity evaluation of aqueous and methanolic extracts of *Tetrapleura tetraptera* (Fabaceae) leaves, a plant that is used in the traditional treatment of diabetes in Western Côte d'Ivoire. The quantitative estimation of total phenol contents by the colorimetric method showed that Methanolic Extract (ME) of leaves at 170 ± 0.88 mg GAE/g of extracts while Aqueous Extract (AqE) contains 140 ± 6.9 mg GAE/g of extracts. For flavonoids, the amount determined for ME is 87.33 ± 0.45 mg EQ/g of extracts, while AqE of leaves gave 54.66 ± 5.9 mg EQ/g. The results obtained indicate that ME of leaves from T. tetraptera contains more polyphenol compounds than AqE of leaves. The evaluation of the antioxidant activity of the various extracts was carried out according to two methods: the trapping of free radicals by the DPPH and the measurement of reducing power (FRAP). The antiradicalar power of ME of leaves gave an CI50 = 03.80 ± 0.97 µg/mL close to vitamin C (CI50 = 01.25 0.02 µg/mL) and higher than that of AqE of leaves with an CI50 = 08.60 ± 0.5 µg/mL. Similarly, for reducing power, ME of leaves with an CI50 = 04.24 ± 0.27 µg/mL, is similarly close to vitamin C (CI50 = 04.08 ± 0.01 µg/mL) and more effective than AqE of leaves (CI50 = 24.80 ± 0.37). So, this antioxidant activity could be an additional asset for the use of this plant in the traditional treatment of diabetes and other pathologies related to oxidative stress.

Keywords: *Tetrapleura tetraptera*, Antioxidant, Triphytochemistry, Polyphenols.

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INTRODUCTION

Throughout the centuries, human traditions have developed the knowledge and use of medicinal plants to overcome suffering and improve human health [1]. Free radicals are molecules produced mainly by our bodies in the process of converting nutrients into energy [2]. The vast majority of free radicals are involved in the maintenance and functioning of the body, but the excessive production of these molecules is the cause of several alterations in cellular components, which in turn cause several diseases such as cardiovascular disease and cancer. Faced with these attacks, our body is equipped with an endogenous antioxidant defence system, an imbalance between antioxidants and oxidants promotes oxidative stress. In order to maintain this balance, another exogenous antioxidant system is provided by the diet. Fruits, vegetables, spices and herbs have a protective effect, not only against cancer, but also against other chronic diseases, such as cardiovascular disease. Plants contain several varieties of free radical scavenging molecules: phenolic compounds, nitrogen compounds, vitamins, terpenoids, which have strong antioxidant activity [3].

Tetrapleura tetraptera, belongs to the Fabaceae family (formerly Leguminosae: Mimosoideae) and is a plant that is used as a vitamin-rich supplemental diet [4]. The leaves, bark, root and fruit of *Tetrapleura tetraptera* are used for medicinal purposes [5]. It is generally found in lowland forest in many tropical African countries. It is a robust, single-stemmed perennial tree with dark green leaves with a thick, woody base and spreading branches. Its fruits consist of fleshy pulp and small brownish-black seeds, with a characteristic fragrant and pungent aromatic odour [6]. It is used as a popular seasoning spice in southern and eastern Nigeria and its fruits are used in the management of convulsions, leprosy, inflammation, rheumatism, flatulence, jaundice and fevers, as well as in the management and control of type 2 diabetes mellitus in adults [7]. Previous studies have shown that the methanolic extract of the leaf of this plant has an antioxidant effect on carbon tetrachloride-induced hepatotoxicity [8]. While the aqueous extract of fruit showed hypoglycaemic properties[9].

The objective of this study is to evaluate the antioxidant properties (free radical scavenging activity and iron ion chelating capacity) of the aqueous and methanolic extracts of *Tetrapleura tetraptera* leaves.

MATERIALS AND METHODS

Plant material

The plant material consists of *Tetrapleura tetraptera* leaves collected in October 2018 in Kassiapleu, a village located 7 km from the city of Man on the Man-Danané axis, facing the University of Man (Côte d'Ivoire).

Technical equipment

The technical equipment includes a mechanical grinder type IKAMAG, a magnetic stirrer RCT type IKAMAG, a rotating evaporator Heidolph Type; a UV-Vis spectrophotometer (biomérieux), a P-type oven SELECTA and a precision balance (Denver Instrument).

Reagents

The reagents used are mainly the Folin-Ciocalteu reagent, sodium carbonate, methanol, aluminum trichloride, potassium acetate, 2,2-diphenylpicrylhydrazyl (DPPH), phosphate buffer, ethanol, hydrochloric acid, ferrous chloride, trichloroacetic acid, the ferrocene, potassium ferricyanide, quercetin and gallic acid provided by RYCA-PHARMA and CLE (Chemical Laboratory Equipment).

Preparation of plant extracts

Preparation of aqueous extract

100g powder of the leaves of *T. tetraptera* were macerated for 24 hours in 1L of distilled water [10]. The macerate has been wrung into a square of sterile tissue, filtered successively on cotton wool and one-fold on filter paper (Whatman paper® 2mm). 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 [11].

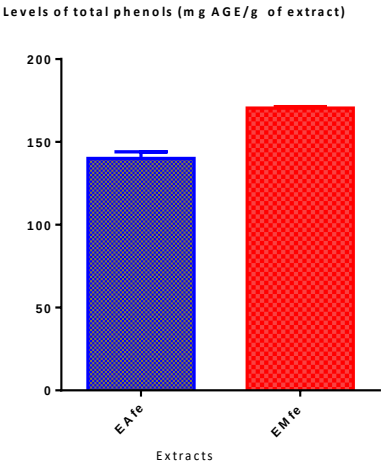
Preparation of methanolic extract

It was carried out using modified method [10]. A mass of 20g of plant powder was added in 100ml of methanol and subjected to maceration for 72 hours. The macerate was treated according to the same procedure like the aqueous extract.

Doses of polyphenols

Determination of total phenols

The total phenolic contents of two extracts of *Tetrapleura tetraptera* were determined by the Folin-Ciocalteu method [12]. To 0.5 mL of each plant extract of concentration 0.1 mg / mL respectively were added 5 mL of Folin-ciocalteu diluted 1/10 in distilled water and 4 mL of sodium carbonate (1M). The whole is incubated at room temperature for 15 minutes. The optical densities (OD) are then read in a spectrophotometer at 765 nm against a blank. Gallic acid was used as standard and prepared under the same conditions as above with a solvent mixture of methanol / water (50:50, V / V) at concentrations ranging from 0 to 0.5 mg / mL. The total phenolic contents of the extracts are expressed in milligrams of gallic acid equivalents per gram of extract (mg GAE/g extract). (Graph 1)

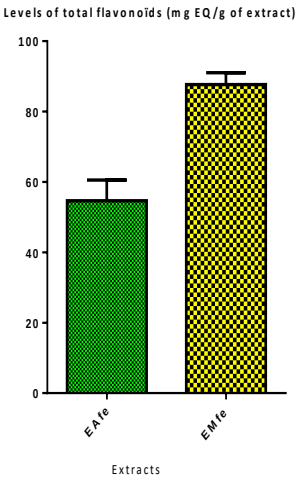


EAfe: Aqueous extract of leaves **EMfe**: Methanolic extract of leaves

GRAPH 1: LEVELS OF TOTAL PHENOLS OF AQUEOUS AND METHANOLIC EXTRACTS OF *TETRAPLEURA TETRAPTERA* LEAVES (MG/AGE/G OF EXTRACT) (MEAN + SD OF TREE TRIAL)

Determination of total flavonoids

The technique used for the determination of the levels of total flavonoids extracted from *Tetrapleura tetraptera* is the colorimetric method of aluminum trichloride described by [13]. Thus, 0.1 mL of 5 mg / mL of each extract plant are collected, to which are successively added 1.5 mL of methanol, 0.1 mL of 10 % aluminum trichloride, 0.1 mL of potassium acetate (1M) and 2.5 mL of distilled water. After incubation at room temperature for 30 minutes, the optical densities were measured in a spectrophotometer at 415 nm. A methanolic solution of quercetin with concentrations ranging from 0 to 100 mg / mL is used as a standard. The contents of flavonoids extracts are expressed in milligrams of quercetin equivalent per gram of extract (mg QE / g extract). (Graph 2)



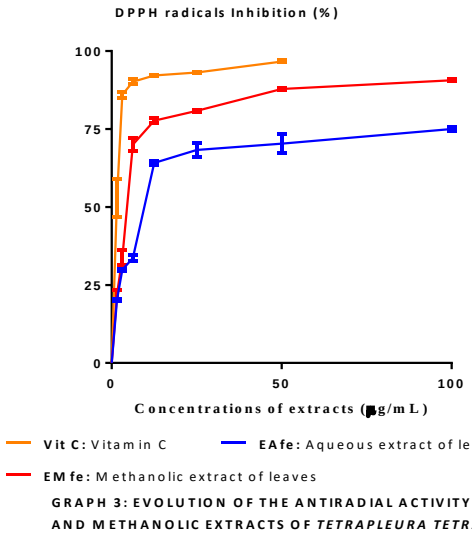
EAfe: Aqueous extract of leaves **EMfe**: Methanolic extract of leaves

GRAPH 2: LEVELS OF TOTAL FLAVONOÏDS OF AQUEOUS AND METHANOLIC EXTRACTS OF *TETRAPLEURA TETRAPTERA* LEAVES (MG/QE/G OF EXTRACT) (MEAN + SD OF TREE TRIAL)

In vitro evaluation of the antioxidant activity

Measurement of anti-radical power

The measurement of the antiradical activity of plant extracts was performed by testing the 2, 2- diphenyl-1-picrylhydrazyl (DPPH) according to the method of [14]. From a stock solution of each plant extract to 0.1 mg / mL, a concentration range is prepared by successive doubling dilution of 1.56 mg / mL to 100 mg / mL. Then, to each extract concentration, the same volume of a methanolic solution of DPPH is added. After 30 minutes of incubation at room temperature (37°C) and protected from light, the absorbance is read in a spectrophotometer at 517 nm against a blank sample (0 mg / mL of extract). Vitamin C (100 mg / mL) which is the reference material is prepared in the same conditions. (Graph 3)



GRAPH 3: EVOLUTION OF THE ANTIRADIAL ACTIVITY OF THE AQUEOUS AND METHANOLIC EXTRACTS OF *TETRAPLEURA TETRAPTERA* LEAVES

The percentage inhibition of DPPH radicals are calculated by the following formula:

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

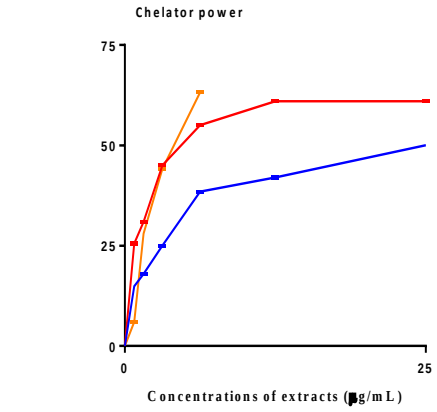
NB: **Inhibition (%)**: the percentage of inhibition of DPPH radical

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

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

Chelating power measurement

The colorimetric method of based on the determination of the complex formed by the ferrous ion (Fe^{2+}) and which was used to measure ferrocene the chelating power of plant extracts [15]. Thus, 3.7 mL of methanol, 0.1 mL of iron II chloride (2 mM) and 0.2 mL of ferrocene (5 mM) were added successively to 1 mL of each sample at various concentrations achieved by dilution of order of 2 100 to 0.78 mg / ml to initiate the reaction. After vigorous stirring and then incubated at room temperature for 10 minutes, absorbance is read at the spectrophotometer at 562 nm against a blank. Vitamin C is used at different concentrations as compared to the reference solution of the chelating activity of the extracts (Graph 4).



GRAPH 4: CHELATING POWER OF AQUEOUS AND METHANOLIC EXTRACTS OF *TETRAPLEURA TETRAPTERA* LEAVES

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 leave extracts of the plant and vitamin C.

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.

RESULTS AND DISCUSSION

Contents of total phenols and flavonoids leaves extracts of *Tetrapleura tetraptera*

The levels of total phenols and total flavonoids of leaves extracts of *Tetrapleura tetraptera* 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

Table 1: Levels of total phenols and total flavonoids of leaves extracts of *tetrapleura tetraptera* (mean ± SD of three trials).

Extracts	total phenols mg GAE / g of extract	total flavonoids mg EQ / g of extract
AqE of leaves	140 ± 6,9[a]	54,67±5,89[a]
ME of leaves	170 ± 0,88[a]	87 ±3,40[b]

Medium extracts the same column with different letters (a, b) with superscript are significantly different from the smallest to the largest average at P ≤ 0.05.

Total phenol and flavonoid content

The determination of total phenols and flavonoids in the aqueous and methanolic extract of *Tetrapleura tetraptera* leaves was done using separately the colorimetric methods (Folin-Ciocalteux and aluminium trichloride (AlCl3). The total phenol content estimated by the Folin-Ciocalteu method for each extract was reported in mg gallic acid equivalent/g dry plant material. The results show that the methanolic leaf extract has a high total phenol content (ME of leaves = 170 ± 0.88 mg GAE /g extract) compared to that of the aqueous extract (AqE of leaves = 140 ± 4 mg GAE /g extract) (Table 1). The flavonoid content determined by the aluminium trichloride method for each extract was reported in mg quercetin equivalent/g extract. The results show that the two extracts have different contents (Table 1). The results showed that ME of leaves with 87 ±3.40 mg EQ/g extract was richer in flavonoids than AqE of leaves = 54.67±5.89 mg EQ/g extract.

The ME of leaves of *Tetrapleura tetraptera* represents the highest contents of total phenols and flavonoids. This result confirms the high richness of the leaves in phenolic compounds. The results obtained confirm that the different extraction solvents used differ in their ability to extract phenolic compounds from *Tetrapleura tetraptera.*

Anti-radical activity and chelating power of *T. tetraptera* leaf extracts

The anti-free radical activity obtained reveals that the aqueous and methanolic extracts of the leaves of *Tetrapleura tetraptera* possess a dose-dependent activity. All the extracts have a good anti-free radical activity. The comparison between these extracts reveals that ME of leaves represents the most active extract with an IC50 of 3.80 ± 0.97 µg/mL and AqE of leaves with an CI50 of the order of 8.60 ± 0.50 µg/mL represents the lowest anti-free radical activity.

Table 2: Anti-radical and chelating properties of *tetrapleura tetraptera* leaf extracts and vitamin C resulting in 50% reduction of DPPH radicals and chelation of the ferrous ion.

Extracts and Compounds	Anti-radical Anti-free radical activity IC50 (µg/mL)	Chelating chelating power IC50 (µg/mL)
AqE of leaves	08,60 ± 0,50^{b}	24,8 ± 0,37^{c}
ME of leaves	03,80 ± 0, 97^{a}	04,25 ± 0,27^{a}
Vit C	01,25 ± 0.02^{a}	04,08 ± 0,01^{a}

The chelating activity revealed that ME of leaves with IC50 = 04.25 ± 0.27 µg/mL was the most active while AqE of leaves with IC50 = 24.8 ± 0.37 µg/mL was the least active. Furthermore, vitamin C used as a standard molecule with an CI50 of 04.08 ± 0.01 µg/mL was more active than ME of leaves.

For comparative purposes, we used vitamin C as the standard reference antioxidant molecule. It showed an interesting anti-free radical activity with an IC50 in the range of 01.25 ± 0.02 µg/mL. Comparing it with ME of leaves tested with an IC50 of about 3.80 ± 0.97 µg/mL, ME of leaves proved to be very active similar to the latter. The good anti-free radical activity could be linked to the high content of total phenols. Polyphenols are considered a major group of compounds that contribute to the antioxidant activities of plants as free radical scavengers due to their hydroxyl groups [16].

Phenolic compounds are widely distributed in plant tissues and include many free radical scavenging and antioxidant molecules. Furthermore, have shown the existence of a correlation between total phenol content and antiradical activity [17, 18, 19].

Our results are in agreement with the work [20]. According to the authors, plants with good antioxidant activity contain high levels of phenolic groups.

Several factors can influence the content of phenolic compounds. Different studies have shown that external factors (geographical and climatic factors), genetic factors, but also the degree of ripening of the plant and the duration of storage have a strong influence on the content of polyphenols [21, 22, 23, 24].

These results are consistent with those reported by other authors who have demonstrated a positive correlation between total phenolic content and antioxidant activity [25, 26, 27, 28]. The level of correlation between phenolic content and antioxidant activity is an aspect that should not be overlooked, as phenolic compounds respond differently in the analysis, depending on the number of phenolic groups, and total phenolic compounds do not necessarily incorporate all antioxidants that may be present in an extract [29].

Similarly, previous studies show that methanol is the most used solvent for high recovery of phenolic compounds and obtaining better antioxidant activity [30, 31].

Furthermore, the chelating power of the different extracts shows that ME of leaves has a significant activity with an IC50 of 04.25 ± 0.27 µg/mL which is close to that of vitamin C (IC50 = 04.08 ± 0.01 µg/mL). The chelating activity of ME of leaves can be explained by a synergistic effect between the constituents of this extract which would contain polyphenolic compounds such as the presence of phytocompounds observed in this work would justify the antioxidant activity of the tested plant species. Polyphenols are considered a major group of compounds that contribute to the antioxidant activities of plants as free radical scavengers due to their hydroxyl groups [16].

CONCLUSION

The study of the antioxidant activity of extracts from the species Tetrapleura tetraptera according to the DPPH free radical scavenging method and the ferrous ion chelation method showed that the methanolic extract of the leaves has a good antioxidant activity than the aqueous extract of the leaves. These extracts could therefore constitute an alternative to certain synthetic additives. However, the

activity is still much lower than that of ascorbic acid, but these are crude extracts containing a large number of different compounds. It is therefore very likely that they contain compounds which, once purified, may have activity comparable to that of ascorbic acid. Further research is needed to identify, isolate and purify these constituents.

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