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Evaluation of the antioxidant activity of the aqueous and methanolic extracts of *Tetrapleura tetraptera* (Schum. & Thonn.) Taub., 1891, (Mimosaceae) Barks, *in vitro*

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Abstract
This study aimed to evaluate the antioxidant activity of aqueous and methanolic extracts of of *Tetrapleura tetraptera* barks. It's a food plant that is also used by people in western Côte d'Ivoire, to treat various ailments. The quantitative estimate of the total phenolic contents of the aqueous extract by the colorimetric method showed that the aqueous extract (E. Aec) contains 78.66 ± 0.12 mg GAE/g of extracts while the methanolic extract (E. Mec) have 464.66 ± 0.22 mg GAE/g of extracts. As for total flavonoid, the quantity determined for the aqueous extract (E. Aec) is 17.33 ± 0.97 mg QE/g when the methanolic extract (E. Mec) gave 147.33 ± 0.45 mg QE/g of extracts. The evaluation of the antioxidant activity of extracts was carried out according to two methods: the free radical scavenging by the DPPH and the measurement of the reducing power (FRAP). The results obtained indicate that the methanolic extract contains more polyphenolic Compounds than the aqueous extract. The methanolic extract (E. Aec) antioxidant properties (E. Mec, IC_{50} = 02.57 ± 0.16 mg/mL) are also greater than those of the aqueous extract (E. Aec, IC_{50} = 07.50 ± 0.60 mg/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: *T. tetraptera*, aqueous, methanolic extracts, antioxidant

1. Introduction
The use of antioxidant molecules of synthetic origin is currently questioned because of the potential toxicological risks they may constitute. It is for this reason that new sources of natural antioxidants are actively sought [1, 2]. Eyes then turn more and more to the medicinal plants supplying bioactive substances. Medicinal plants have been used since ancient times to relieve and cure human diseases. In fact, their therapeutic properties are due to the presence of hundreds or even thousands of bioactive natural compounds called: secondary metabolites [3]. These compounds (anthocyanins and phenolic compounds) are potent antioxidants and can provide protection against inflammation, atherosclerosis, cancer, diabetes and neurodegenerative diseases [4].
Many studies have shown that they have antioxidant properties [5-7]. Among these plants, *Tetrapleura tetraptera* belonging to the family Fabaceae-Mimosaceae, is a medicinal, food and economic species widespread throughout the sub-region of West Africa [8].
The tree known as the vernacular of Aridan in western Nigeria or Prekesse in Akan of Ghana and Zan in Yacouba in Côte d'Ivoire has molluscidal, antimicrobial, antiseptic, anticonvulsant, anti-inflammatory and insecticidal properties [9, 10]. In ethnomedicine, dried fruits of *T. tetraptera* are commonly used for the treatment of conditions such as hypertension, seizures, leprosy, rheumatic pain, diabetes, arthritis [11]. Also, in eastern Nigeria, *T. tetraptera* fruits are used for the production of therapeutic soups for nannies to prevent postpartum contractions [8].
However, very little work has been done on the leaves and bark of this plant. The main objective of this work is to evaluate the antioxidant activity of the aqueous and methanolic extracts of *Tetrapleura tetraptera* bark.

2. Material and Methods
2.1 Plant material
The barks of *Tetrapleura tetraptera* (Schum. & Thonn.) Taub., 1891, (Mimosaceae), harvested in october 2018 in Kassiapleu near Man (western of Côte d'Ivoire) 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 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)

2.3 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 ferrosine, potassium ferricyanide, quercetin and gallic acid provided by RYCA-PHARMA and CLE (Chemical Laboratory Equipment).

2.4 Preparation of plant extracts

2.4.1 Preparation of aqueous extract

T. tetraptera barks powder (100 g) were macerated for 24 hours in 1L of distilled water [12]. 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 [13].

2.4.2 Preparation of methanolic extract

It was carried out using modified Olakunle *et al*. [12] method . A mass of 20g of plant powder was added in 100ml of methanolic solvant and subjected to maceration for 72 hours . The macerate was treated according to the same procedure like the aqueous extract.

2.5 Doses of polyphenols

2.5.1 Determination of total phenols

The total phenolic contents of four extracts of *Tetrapleura tetraptera* were determined by the Folin-Ciocalteu method [14]. 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).

2.5.2 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 [15]**.** 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).

2.6 *In vitro* evaluation of the antioxidant activity

2.6.1 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 [16]. 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, 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).

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 root extracts of the plant and vitamin C.

2.6.2 Chelating power measurement

The colorimetric method of **Le *et al*., (2007)** [17] based on the determination of the complex formed by the ferrous ion (Fe^{2+}) 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 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 of the chelating activity of the extracts (Graph 4). 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.

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 bark extracts of *Tetrapleura tetraptera*

The levels of total phenolic contents and total flavonoids of

bark 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 bark extracts of *Tetrapleura tetraptera* (Mean ± SD of three trials).

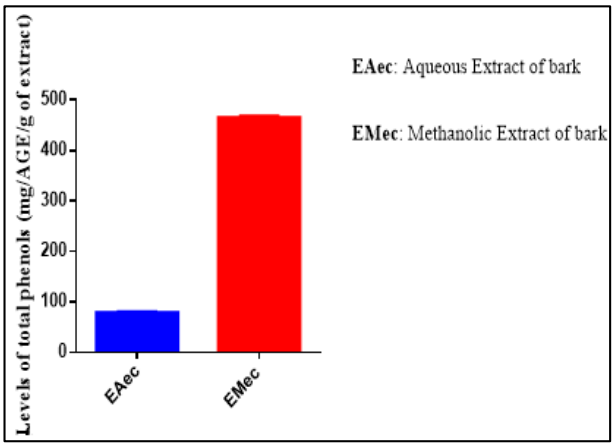
Extracts	Total phenolic contents mg GAE / g of extract	Total flavonoids mg QE / g of extract
E Aec	78.66 ± 0.12[a]	17.33 ± 0.97[a]
E Mec	464.66 ± 0.22[b]	147.33 ± 0.45[b]

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

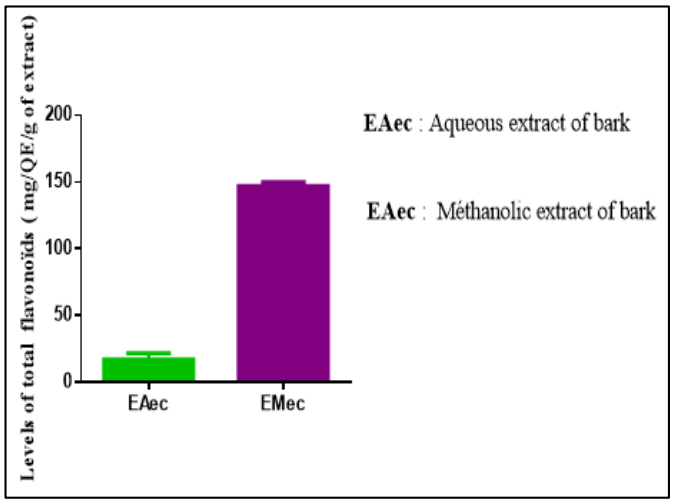
3.2 Total phenol and total flavonoid contents

Determination of the total phenol and total flavonoid contents in the aqueous and methanolic extract of the *Tetrapleura tetraptera* bark was made using separately the colorimetric methods (Folin-Ciocalteu and aluminum trichloride (AlCl3).) Total phenol contents estimated by the Folin-Ciocalteu method for each extract was reported as mg gallic acid / g dry plant material. The results show that the methanolic extract has a high total phenol content (464.66 ± 0.22 mg EAG / g of extract) compared to that of the aqueous extract (78.66 ± 0.12 mg EAG / g extract) (Table 1). The flavonoid content determined by the aluminum trichloride method for each extract was reported in mg equivalent of quercetin / g of extract The results reveal that both extracts have moderate contents (Table 2). It is found that the flavonoids represent 147.33 ± 0.45 mg EQ / g of extract in the extract.

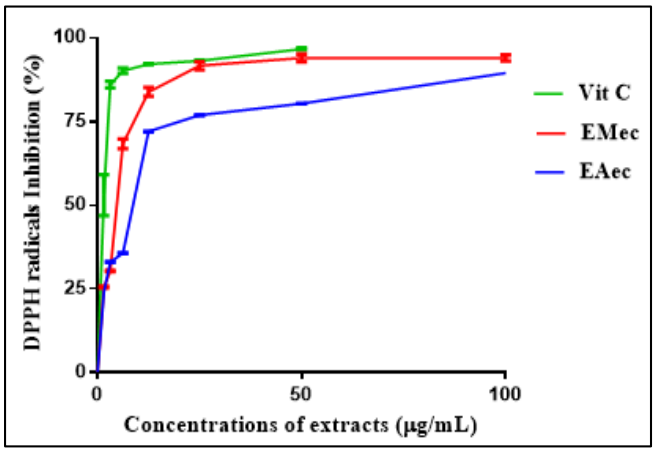
Tetrapleura tetraptera methanolic extract E. Mec represent the highest levels of total phenols and flavonoids. This result confirms the great richness of barks in phenolic compounds. The results obtained confirm that the different extraction solvents used have differences in their ability to extract the phenolic compounds from *Tetrapleura tetraptera.*



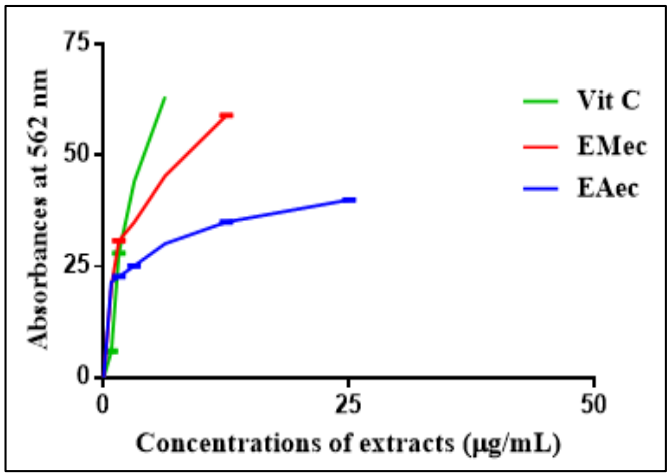
Graph 1: Levels of total phenols of aqueous and Methanolic extracts of T.Tetraptera bark (mg/AGE/g of extract) (Mean + SD of tree trial)



Graph 2: Levels of total flavonoids of aqueous and methanolic extracts of T. tetraptera bark (mg/QE/g of extract) (Mean + SD of tree trial)



Graph 3: Evolution of the antiradial activity of the aqueous and mathanolic extracts of T. tetraptera bark



Graph 4: Chelating power of aqueous and methanolc extracts of T. tetraptera bark

Table 2: Anti-free radical powers and chelating of bark extracts of *T. tetraptera* and vitamin C resulting in 50% reduction of the DPPH radicals and ferrous ion chelation.

Extracts and compounds	Antiradical activity CI50 (µg/mL)	Chelator power CI50 (µg/mL)
E Ace	07, 50 ± 0,60[b]	Nd[a]
E. Mec	02, 57 ± 0,16[a]	07, 83 ± 0.43[b]
Vit C	01, 25 ± 0.02[a]	04, 08 ± 0,01[a]

Not determined : Nd

3.3 Anti-free radical powers and chelating power of bark extracts of *T. tetraptera*

The anti-radical activity obtained reveals that the aqueous and methanolic extracts of *T. tetraptera* bark have a dose-dependent activity. All extracts have good anti-radical activity. Because the comparison between these extracts, reveals that the E. Mec represents the most active extract with an IC_{50} of 02.57 ± 0.16 µg / mL and E. Aec with an IC_{50} of the order of 07.50 ± 0.60 µg / mL represents the lowest anti-radical activity.

For comparative purposes, we used vitamin C as the standard antioxidant reference molecule. It showed interesting anti-radical activity with an IC_{50} of the order of 01.25 ± 0.02 µg / mL. Compared with the E. Mec tested with an IC_{50} of the order of 02.57 ± 0.16 µg / mL, the E. Mec are very active similar to the latter. The good anti-radical activity would be linked to the high content of total phenols. Polyphenols are considered to be a major group of compounds that contribute to the antioxidant activities of plants as free radical scavengers due to their hydroxyl groups [18].

Phenolic compounds are widely distributed in plant tissues, among which are numerous anti-free radical and antioxidant molecules. In addition, **(**Hatano *et al*., 1989, Duh *et al*., 1999 and N'guessan *et al*., (2007) [19-21] have shown the existence of a correlation between the total phenol contents and the antiradical activity.

Our results are in agreement with the work (from Adedapo *et al*., 2008) [22]. According to the authors, the plants which have a good antioxidant activity contain high levels of phenolic groups.

Several factors can affect the phenol content. Various studies have shown that external factors (geographical and climatic factors), genetic factors, but also the degree of maturation of the plant and the duration of storage have a strong influence on the polyphenol content [23-26].

These results are consistent with those reported by other authors who have demonstrated a positive correlation between the total content of phenolic compounds and antioxidant activity [27-30]. The correlation level between the phenolic content and the antioxidant activity is an aspect not to be neglected, because it must be considered that the phenolic compounds respond differently in the analysis, according to the number of phenolic groups and that the total phenol compounds not necessarily incorporate all the antioxidants that may be present in an extract [31].

Similarly, previous studies show that methanol is the most used solvent for high recovery of phenolic compounds and improved antioxidant activity [32, 33].

In addition, the chelating power of the various extracts shows that E. Mec has a high activity with an IC_{50} of 07.83 ± 0.43 µg / mL which is close to that of vitamin C (IC_{50} = 04.08 ± 0.01 µg / mL). The chelating activity of the E. Mec surely finds its explanation, in a synergistic effect between the constituents of this extract which would contain polyphenolic compounds such that the presence of phyto-compounds observed in this work would justify the antioxidant activity of the plant species tested. Polyphenols are considered to be a major group of compounds that contribute to the antioxidant activities of plants as free radical scavengers due to their hydroxyl groups [18].

4. Conclusion

The study of the antioxidant activity of the Tetrapleura tetraptera extracts using the DPPH free radical scavenging method and the ferrous ion chelation method showed that the aqueous and methanol extracts both possess antioxidant activity with higher activity for the latter. These extracts could therefore be an alternative to certain synthetic additives. This activity is nevertheless 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, can exhibit activity comparable to that of ascorbic acid. Further research is needed to identify, isolate and purify these constituents.

5. Conflict of interests

The authors claim that there is no conflict of interest.

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