Original Research Article

Phytochemical characterization and evaluation of the antiradical potential of fruit extracts of *Tetrapleura tetraptera* (Schumach. & Thonn.)Taub., a medicinal plant used in the North of Côte d'Ivoire

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ABSTRACT :

Aims : *Tetrapleura tetraptera* is a medicinal plant with multiple virtues, used in traditional medicine to treat various pathologies. The aim of this study was to determine the phytochemical profile and antiradical activity of aqueous and 70% hydroethanolic extracts of the fruits of *Tetrapleura tetraptera*, a medicinal plant used in northern Côte d'Ivoire.

Methodology : Phytochemical sorting of the extracts was carried out using the tube staining and precipitation technique, while the phenolic compound content was determined by UV spectrophotometry. The antioxidant capacity of the extracts was then determined using the 2,2-azino-bis 3-ethylbenzothiazoline-6 sulphonic acid (ABTS) reduction method, with Trolox as the reference molecule.

Results : The main chemical groups revealed were alkaloids, total polyphenols, anthocyanins, leucoanthocyanins, saponins and tannins. Determination of the phenolic compounds in the extracts showed that the hydroethanol extract had the highest levels of total polyphenols (18.66 ± 0.01 mg EAG/g dry extract); total flavonoids (22.33 ± 0.00 mg EQ/g dry extract); total tannins (36633 ± 1.69 mg EAT/g dry extract). This extract also showed the best anti-free radical activity, with an antioxidant capacity of 1.38 ± 0.001 µmolTE/L compared with 1.19 ± 0.001 µmolTE/L for the aqueous extract.

Conclusion : The fruits of *T. tetraptera* could be a source of bioactive molecules that could be used in the treatment of various infectious and metabolic pathologies.

Keywords : *Tetrapleura tetratera*,Tri phytochemical, Phenolic compounds, Antioxidant power

1-INTRODUCTION

Plants with pharmacological value are an integral part of traditional healing systems used by different ethnic communities in Africa. According to the World Health Organization (WHO), about 80% of the African population rely on traditional medicine to maintain good health **[1].** Côte d'Ivoire is no exception to this statement because traditional medicine occupies a very important place in primary health care

for the vast majority of its populations **[2].** Indeed, medicinal plants produce 70% of medicines, of which approximately 170,000 bioactive molecules have been identified **[3]**. Among these compounds, there are to a large extent secondary metabolites with various biological activities such as antioxidant, antidiabetic **[4]** and microbiological **[5]** activities. This is the case of *Tetrapleura tetraptera*, (Fabaceae-Mimosoideae), a species widespread in the West African sub-region, particularly in Ghana, Nigeria, Cameroon and Côte d'Ivoire **[6].** It is mainly used for its medicinal, food, cosmetic and economic virtues by the populations of these different countries. Thus, in ethnomedicine, the dried fruits of *T. tetraptera* are commonly used for the treatment of conditions such as hypertension, convulsions, leprosy, rheumatic pain, diabetes and arthritis **[7].** In addition, the fruit has molluscidal, antimicrobial, antiseptic, anticonvulsant, anti-inflammatory and insecticidal properties **[8, 9].** Despite these scientific data, the search for natural substances from a chemical and pharmacological point of view in the fruit of *T. tetraptera* remains to be explored in Côte d'Ivoire. It is in this sense that the present work is included, which aims to determine the phytochemical composition, the contents of phenolic compounds and the antiradical activity of the aqueous and 70% hydroethanolic extracts of the fruits of *T. tetraptera* with a view to their valorization.

2- MATERIAL AND METHODS

2.1. Plant material

The plant material consists of *T. tetraptera* fruits that were purchased at the large market in Korhogo (Northern Ivory Coast) in June 2024. Their authentication was done at the Department of Plant Biology of the Training and Research Unit (UFR) of Biological Sciences of the Peleforo GON COULIBALY University of Korhogo. These fruits were carefully cleaned with tap water to remove all impurities and then crushed into small pieces using a mortar to separate the seeds. The remaining fruits were dried in the dark at room temperature (27 ± 2 °C) for two weeks. At the end of drying, they were pulverized using an electric grinder (RETSCH, Type AS 200) to obtain a fine powder. This plant powder was stored in sterile jars and used for the preparation of extracts.

2.2. Preparation of *T. tetraptera* fruit extracts

In this study, two types of extraction were carried out: aqueous and 70% hydro-ethanolic. The extracts were prepared according to the method described by **[10]** with some modifications. Indeed, 100 g of each plant powder were macerated in one liter (1L) of distilled water or a 70% hydro-ethanolic mixture (70/30; V/V) under magnetic stirring for 24 hours. After maceration, the mixture obtained was first drained in a square of white cloth, then double filtered on hydrophilic cotton. The filtrates were concentrated in an oven at 50 ° C until complete evaporation of the solvent to obtain the aqueous and hydroethanolic dry extracts 70% of the fruits of *T. tetraptera*. These extracts were weighed and the extraction yield (R) of each extract was calculated using the following formula:

R **(%) = (m /M) ×100** with **M:** mass of the powder (g); **m**: mass of the crude extract (g)

2.2. Phytochemical analysis of the extracts

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The phytochemical study consisted of screening the molecules of the different extracts (aqueous and hydrol-ethanolic) of T. tetraptera by staining and in vitro precipitation tests in tubes as described by **[11].** The groups of molecules sought are: alkaloids, total polyphenols, flavonoids, tannins (catechuic and gallic), anthocyanins, leucoanthocyanins, saponins, terpenes and sterols. The phytochemical tests and characteristic reactions are summarized in **table I.**

Table I : Phytochemical tests and characteristic reactions

Secondary metabolites	**Tests and reagents**	**Positive reaction**
Alkaloids	Burchard and Dragendorff	Reddish brown black
Anthocyanins	Diluted sulfuric acid + 5 mL of ammonia	Blue-purple coloration in basic medium
Flavonoids	Diluted ammonia + aluminum chloride	Yellow coloration
Leucoanthocyanins	Cyanidine	Cherry red or purplish coloration
Polyphenols	Ferric chloride 1%	Dark blue blackish colour
Saponins	Vigorous agitation	Foam index > 100
Tannins	Sulfuric vanillin	Précipitate which turns blue black
Terpens et sterols	Liebermann,Chloroforme, concentred sulfuric acid	Red-brown ring at the interface

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2.3. Determination of phenolic constituents of extracts

2.3.1. Total polyphenols

The determination of total polyphenols was carried out according to the method described by **[12].** To a volume of 30 µL of extract, 2.5 mL of Folin-Ciocalteu reagent diluted 1/10 were added. The mixture obtained was kept for 2 min in the dark at room temperature and then 2 mL of sodium carbonate solution at 75 g/L were added. The solution obtained was then incubated at 50 °C for 15 min. The absorbance reading was carried out using a spectrophotometer at a wavelength of 760 nm against a blank. Gallic acid was used as a reference standard for establishing the calibration curve and for quantifying total polyphenol contents expressed in mg of gallic acid equivalent per gram of dry extract (mg GAE/g of extract).

2.3.2 Flavonoids

Flavonoids were quantified according to the method described by **[13].** Indeed, 0.75 mL of 5% (m/v) sodium nitrite and 0.75 mL of 10% (m/v) aluminum chloride were added to 2.5 mL of a methanolic solution of extract diluted to 1/500 (m/v). After 5 min of incubation, the mixture was brought into contact with 5 mL of a 1 M sodium hydroxide solution. The volume obtained was adjusted to 25 mL then shaken vigorously and the absorbance was measured at 510 nm against a blank. Quercetin was used as a reference standard and the total flavonoid contents were expressed in milligrams of quercetin equivalent per gram of dry extract (mg EQ/g of extract).

2.3.4. Tannins

The total tannin content was determined according to the method described by **[14].** A quantity of 50 mL of each extract was added to 1500 µL of the 4% solution of vanillin in methanol. The resulting mixture was vigorously shaken and 750 µL of concentrated hydrochloric acid was added. The resulting mixture was left to stand at room temperature for 20 min and the absorbance was measured at 550 nm against a blank. Tannic acid was used as a reference standard and the tannin contents were expressed as milligram equivalent of tannic acid per gram of dry extract (mg EAT/g of extract).

2.4. Evaluation of the anti-radical activity of the extracts

The anti-radical activity was evaluated by the $ABTS^{\circ+}$ cation radical decolorization test according to the technique used by **[15]** with some modifications. The $ABTS^{\circ+}$ cation radical was produced by reaction of an 8 mM ABTS solution and 3 mM potassium persulfate in a ratio of 1:1 (v/v). The mixture was then incubated in the dark at room temperature for 12 to 16 hours. This $ABTS^{\circ+}$ solution was diluted with methanol to obtain a solution whose absorbance was 0.7±0.02 at 734 nm. Thus, 3.9 mL of this diluted $ABTS^{\circ+}$ solution was added to 100 µL of each extract. After shaking, the mixture was incubated for 16 hours in the dark and the residual absorbance of the $ABTS^{+\circ}$ radical was then measured at 734 nm using a spectrophotometer and should be between 20% and 80% of the blank absorbance. The results were expressed in µmol Trolox equivalent per liter of extract (µmol TE/L). A calibration line was performed with the following concentrations: 0; 3.75; 5; 6.25; 10; 11.25; 12.5; 13.75; 15 of Trolox and the inhibition rate I (%) of $ABTS^{\circ+}$ was expressed as follows:

$$I\ (\%) = [(A0 - Absextract) / A0]$$

A0: diluted ABTS absorbance and Absextract: diluted ABTS absorbance + sample.

This line allowed us to express the antioxidant activity of the different extracts as follows:

$$C = (I \times Fd) / (4.99 \times 10)$$

C: Antioxidant capacity and Fd: Dilution factor and (I%): inhibition rate.

2.5. Statistical analysis of data

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The results were analyzed using Graph Pad Prism 8.0 software (Microsoft U.S.A) for multiple variances (ANOVA). The differences between the means were determined according to Duncan's test at the 5% level ($P < 0.05$ is considered significant). The graphical representation of the data was carried out using Excel software. The tests were carried out in triplicate for each sample and the results were expressed as the mean accompanied by the standard error of the mean.

3- RESULTS and DISCUSSION

3.1. Extraction yields

The yields obtained after extractions are shown in **table II.** They varied depending on the solvent used for extraction. Indeed, the best yield was obtained with the hydroethanolic extract (26.50%) compared to the aqueous extract (15.70%). This could be explained by the strong capacity of the ethanolic solvent to extract a large quantity of chemical constituents from plant organs such as the fruits of *T. tetraptera* **[16].**

Table II: Yield of the different extractions

***T. tetraptera* Extracts**	**Aqueous**	**Hydroethanolic 70 %**
Yields (%)	15,70 ± 0,06	26,5 ± 0,08

3.2. Phytochemical composition of *T. tetraptera* fruit extracts

The **table III** shows the main chemical constituents found in both extracts. These extracts contain the same chemical groups: alkaloids, total polyphenols, anthocyanins, leucoanthocyanins, catechic tannins and saponins. However, they lack flavonoids, gallic tannins, terpenes and sterols.

These phytochemical groups are already known in the literature to have various pharmacological properties including, antimicrobial, antidiarrheal anti-inflammatory, antioxidant etc **[17**]. Indeed, several studies have reported that flavonoids, tannins and saponins [18, 19] are responsible for the anti-diarrhoeal properties of certain plants such as *Alchornea cordifolia*. Similarly, according to [20], alkaloids inhibit the protein synthesis of micro-organisms by intercalating in their DNA.

Table III : Secondary metabolites of various *T. tetraptera* fruit extracts

Secondary metabolites	**Aqueous Extract**	**70% Hydrethanolic Extract**
Alcaloids	+	+
Anthocyanins	+	+
Flavonoids	–	–
Leucoanthocyanins	+	+
Polyphenols	+	+
Saponins	+	+

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Catechic tannins	+	+
Gallic tannins		_
Terpens et sterols	_	_

+ : Presence et - : Absence

3..3. Phenolic compound content of extracts

From the calibration curves for gallic acid (y = 0.6484x - 0.0025; R2 = 0.995), quercetin (y = 0.6455x - 0.0075; R2 = 0.9983) and tannic acid (y = 0.0009x + 0.0129; R2 = 0.9994), respectively, the contents of total polyphenols, flavonoids and tannins were determined and presented in **table IV.**

Analysis of the results shows that the hydroethanol extract had the highest levels of phenolic compound compared to the aqueous extract. The content of total polyphenols in the hydroethanol extract was 18.66 ± 0.00 mg EAG/g, significantly higher than that of the aqueous extract (13.16 ± 0.00 mg EAG/g). The same was true for flavonoids and tannins, whose levels in the hydro-ethanolic extract, 22.33 ± 0.00 mg EQ/g and 36633 ± 1.69 mg EAT/g respectively, were the highest, with a significant difference compared to the aqueous extract.. The results obtained showed variable levels of the compounds sought depending on the extraction solvents, i.e. water and 70% ethanol. This is in line with the results of [**21]** who concluded that the ethanol-water mixture (70%) was the best solvent for extracting phenolic compounds. The high extraction capacity of these molecules by ethanol is thought to be related to the high solubility of phenolic compounds in polar solvents **[22].** In addition, the hydro-ethanolic extract presented with a significant difference the best contents of phenolic compounds compared to the aqueous extract. These data are in line with the work of **[23]** on the same extracts (aqueous and hydroethanolic) obtained from *Kaya senegalensis* bark. The richness of *T. tetraptera* fruits in phenolic compounds could justify the traditional use of this plant to treat various pathologies such as leprosy and haemorrhoids **[24].**

Table IV : Phenolic compound content of *T. tetraptera* fruit extracts

Extracts	Phenolic compounds measured		
	Total Polyphenols to (mg EAG/g)	Flavonoids (mg EQ/g)	Tannins (mg EAT/g)
Aqueous	13,16 ± 0,00	13,5± 0,01[a]	31204,33 ± 2,92[c]
Hydro-éthanolic	18,66 ± 0,00*	22,33 ± 0,00[b]	36633 ± 1,69[d]

4. Antioxidant activity of extracts

The Trolox calibration line (y = 4.9901x; R2=0.9961) was used to determine the antioxidant capacity of the extracts as a function of the inhibition rate. The $ABTS^{\circ+}$ radical inhibition rate of the

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extracts ranged from 29.01 ± 0.87% to 69.08 ± 0.15%. **Figure 1** show the antioxidant capacity of the extracts. The hydroethanol extract showed the highest antioxidant capacity (1.38 ± 0.001 µmol/Leq Trolox), followed by the aqueous extract (1.19 ± 0.001 µmol/Leq Trolox). However, there was no significant difference between these values. These results are in line with those of the ABTS radical scavenging test already carried out by **[25]** on the same *Kaya sengalensis* stem bark extracts. However, the Trolox equivalent antioxidant capacity (TEAC) of the hydro-ethanolic extract (1.38 ± 0.02 µmol TE/L extract) was slightly higher than that of the aqueous extract (1.19 ± 0.01 µmol TE/L extract) but with no significant difference. The antioxidant activity of the extracts could be linked, on the one hand, to the presence of phytochemical constituents revealed by the phytochemical screening and, on the other hand, to the high phenolic compound content of the plant fruit extracts. The strong antioxidant power of these secondary metabolites has already been demonstrated by several authors **[26]**. They effectively combat oxidative stress, the main initial cause of a number of diseases such as diabetes, Alzheimer's disease and cardiovascular disease **[27]**, cancer and accelerated ageing **[28].**

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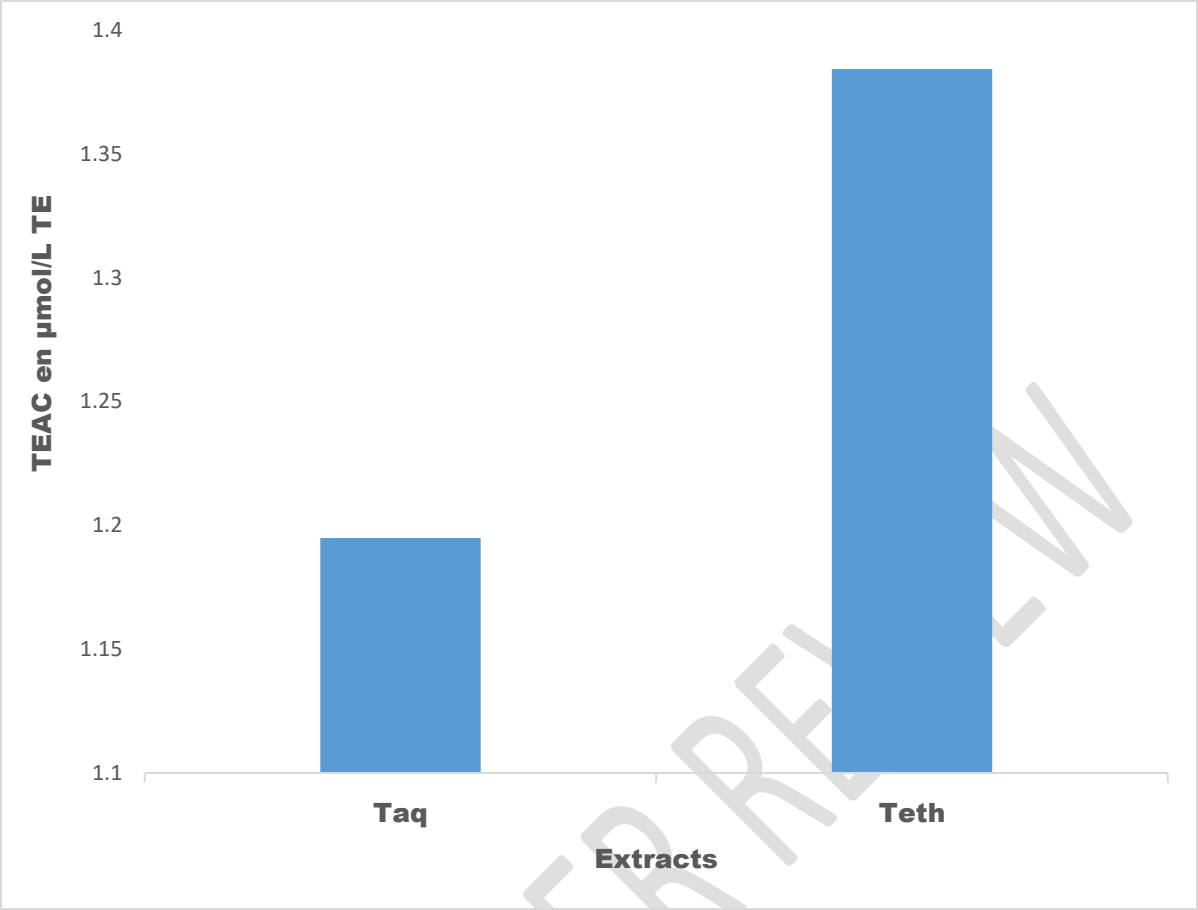

Figure 1 : ABTS+ antioxidant capacity of the two *T. tetraptera* extracts Taq: (aqueous extract) and Teth: (70% hydroethanolic extract)

4 CONCLUSION

This research is a contribution to the development of Ivorian medicinal plants with a view to their use in medicine. Phytochemical studies of T. tetraptera fruit extracts have shown that the hydroalcoholic extract is rich in secondary metabolites of interest with antioxidant activity, justifying its many traditional uses. These results could be used to develop Traditionally Improved Medicines (TIMs) to combat a wide range of diseases.

Consent

It is not applicable.

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