

## Chemical study using HPLC-ESI-QTOF and FT-IR, antioxidant activities, and quantification of phytochemicals in aqueous and hydroethanolic extracts of *Albizia zygia*

Akoubet-Ouayogode Aminata<sup>1</sup>, N'guessan Ocho Esther<sup>2</sup>, Kablan Richmond Jean-François<sup>2</sup>, Kanga Yao<sup>3</sup>, Konan Dibi Jacques<sup>4</sup>, Kablan Ahmont Landry Claude<sup>2\*</sup>

<sup>1</sup> Department of Analytical, Faculty of Pharmaceutical and Biological Sciences, Inorganic and General Chemistry, Food Technology, Félix Houphouët-Boigny University, Abidjan, Ivory Coast

<sup>2</sup> Department of Mathematics-Physics-Chemistry, Chemistry Unit, Faculty of Biological Sciences, Peleforo Gon Coulibaly University, Korhogo, Ivory Coast

<sup>3</sup> Department of Plant Biology, Faculty of Biological Sciences, Peleforo Gon Coulibaly University, Korhogo, Ivory Coast

<sup>4</sup> Laboratory of Bio-Organic Chemistry and Natural Substances (LCBOSN), UFR-SFA, Nangui Abrogoua University, Abidjan, Ivory Coast

**Corresponding Author:** Kablan Ahmont Landry Claude

### Abstract

*Albizia zygia* is a plant widely used in West Africa in the traditional treatment of several pathologies, including diabetes. The objective of this work is to identify the structures of the molecules, to quantify the phytochemicals, and to evaluate the antioxidant activity of aqueous and hydroethanolic extracts of *Albizia zygia*. Compound identification was performed using FT-IR spectroscopy and HPLC-ESI-QTOF-MS/MS mass spectrometry. Antioxidant activity was assessed using Abts and DPPH methods. Analysis of the HPLC-ESI-QTOF-MS/MS and IR spectra revealed the presence of alizarin and 2,6-di-tert-butyl-4-hydroxymethylphenol. Phytochemical quantification showed that total polyphenol content, expressed as gallic acid equivalent (GAE), ranged from  $9.78 \pm 0.001$  to  $32.83 \pm 0.28$  mg GAE /g for the two *Albizia zygia* extracts. The hydroethanolic extract of *Albizia zygia* stem bark has the highest total flavonoid content, with a value of  $16.00 \pm 0.50$  mg QE/g, and total tannin content, with a value of  $10.78 \pm 0.009$  mg QE/g. The hydroethanolic extract of *Albizia zygia* leaves exhibits good antiradical activity compared to vitamin C. The results of this study could confirm its use as a traditional medicine in Korhogo.

**Keywords:** *Albizia zygia*, HPLC-ESI-QTOF-MS/MS, FT-IR, antioxidant activity, assay

### Introduction

People in developing countries rely on plants from traditional medicine for their health. Indeed, this global growth in the use of medicinal plants for treating metabolic diseases (WHO, 2010) is of interest to both the population and scientists, particularly in ethnobotanical and ethnomedical surveys conducted by several researchers (N'Guessan, 2008 ; Tra Bi *et al.*, 2008) [21, 34]. These authors highlight several plants used in traditional medicine in Côte d'Ivoire. Medicinal plants possess diverse biological and pharmacological activities. They are sources of an excellent variety of specific metabolites such as alkaloids, phenolic compounds, and terpenoids (Zerargui *et al.*, 2015) [39].

Diabetes is a major public health problem worldwide, and particularly in Côte d'Ivoire. Over time, this disease leads to serious damage to many organ systems, especially the nerves and blood vessels (WHO, 2024). Despite the efforts made by various countries, this condition remains a problem for populations in Africa. In 2014, 8.5% of the adult population (18 years and older) had diabetes. In 2015, diabetes was the direct cause of 1.6 million deaths, and in 2012, hyperglycemia caused an additional 2.2 million deaths. However, managing diabetes with conventional medication is a lifelong treatment and remains expensive. This leads many patients to turn to traditional medicine (Deteix, 2005 [8]), which provides a form of primary care. However, non-toxic products with proven efficacy and quality must be used for all treatments. Antioxidants are crucial in managing diabetes because they combat oxidative

stress, an imbalance that worsens the disease, by protecting cells from free radicals. A diet rich in antioxidants (fruits, vegetables, tea, herbs) reduces the risk of type 2 diabetes by improving insulin sensitivity and secretion, while also decreasing complications. They work by neutralizing free radicals, thus reducing the cellular and vascular damage associated with diabetes. Polyphenols play a protective role against type 2 diabetes by improving insulin sensitivity, reducing inflammation and oxidative stress, and modulating glucose and lipid metabolism, thus contributing to better glycemic control and the prevention of complications. They act by protecting pancreatic beta cells, regulating digestive enzymes (such as  $\alpha$ -amylase), and improving insulin signaling, which can lead to a decrease in fasting blood glucose and glycated hemoglobin. Given the beneficial effects of polyphenols and the prevalence of diabetes, this study aimed to identify some of the molecules present, evaluate the total polyphenol content, and assess the antioxidant capacity of aqueous and hydroethanolic extracts of *Albizia zygia*, a species in the Ivorian pharmacopoeia used in the traditional management of diabetes in Korhogo.

### Materials and methods

#### 1. Plant material

The leaves and trunk bark of *Albizia zygia* were collected in March 2023 [29] at the botanical garden of Peleforo Gon Coulibaly University. The stem bark was harvested using a knife. The samples were identified by Dr. Kanga Yao, botanist at Peleforo Gon Coulibaly University.

## 2. Methods

### AMETe, AMETm, JFe and JFm powder

The various parts of *Albizia zygia* were dried at room temperature for two weeks for the leaves and four weeks for the trunk bark. They were then ground into a powder using a Binatone Blender-BLG-450P MK2-1.5L. The resulting powder was then stored in containers for our various analyses.

### Extraction of AMETe, AMETm, JFe and JFm

#### - Obtaining AMETe, AMETm, JFe and JFm powder

The method used was that applied by (Yohanna *et al.*, 2022; Amine, Mohamed *et al.*, 2017; Able *et al.*, 2025<sup>[1, 4, 38]</sup>). After drying and pulverizing the plant material, extractions were performed. Thus, 250 g of powder from each organ were dissolved in 2.5 L of distilled water for AMETe and JFe, and in 1.75 L of ethanol and 0.75 L of distilled water (70/30) for AMETm and JFm. The mixture was homogenized by magnetic stirring for three hours at room temperature, before being macerated at the same temperature for 24 hours for further processing. The supernatant is then filtered using cotton placed on a funnel after being stirred again for 5 minutes and sieved. The resulting filtrate is centrifuged at 10,000 rpm (Jouan centrifuge, TH 12) for 15 minutes at room temperature. The recovered solution is then evaporated in an oven at 50°C. These four separate operations yield the crude extracts AMETe, AMETm, JFe, and JFm.

AMETe: aqueous extract of *Albizia zygia* trunk bar ;  
AMETm: hydroethanolic extract of *Albizia zygia* trunk bark;  
JFe: aqueous extract of *Albizia zygia* leaves; JFm: hydroethanolic extract of *Albizia zygia* leaves

## 3. Dosage of phytochemicals

### Total polyphenol content

The method of Wood *et al.*, (2002); N'Guessan *et al.*, (2025a)<sup>[6, 36]</sup> has been applied for the determination of total polyphenols. A 2.5 mL sample of diluted (1/10) Folin-Ciocalteu reagent was mixed with 30 µL of extract at a concentration of 2 mg/ mL. This mixture was stored protected from light at room temperature for 2 minutes, then 2 mL A 75 g / L sodium carbonate solution was incorporated. The mixture was then placed in a water bath at 50°C for 15 minutes, and then rapidly cooled. Absorbance was evaluated at a wavelength of 760 nm, with distilled water serving as a blank. A calibration curve was established using gallic acid at different concentrations. The analyses were performed in three replicates, and the polyphenol concentration was expressed in mg quercetin equivalent per gram of dry extract (mg QE/g).

### Total flavonoid content assay

The technique developed by Marinova *et al.* (2005); N'Guessan *et al.*, (2025b)<sup>[6, 18]</sup> was used for the determination of total flavonoids. In a 25 mL volumetric flask, 0.75 mL of 5% (w/v) sodium nitrite (NaNO<sub>2</sub>) was added to 2.5 mL of an extract at a concentration of 2 mg/ mL. Then, 0.75 mL of 10% (w/v) aluminum chloride (AlCl<sub>3</sub>) was added to the mixture, which was subsequently incubated for 6 minutes in the dark. After incubation, 5 mL of 1N sodium hydroxide (NaOH) was added, and the volume was brought up to 25 mL. The mixture was thoroughly stirred before measurement with a UV-visible spectrophotometer. The reading was taken at 510 nm. The

assays were performed in triplicate. The flavonoid content was expressed in grams per liter of quercetin equivalent extract.

### Total tannin content

The condensed tannin content was established according to the method presented by Kouamé *et al.*, (2021)<sup>[15]</sup>. A quantity of 50 mL of each extract at a concentration of 2 mg/ mL was added to 1500 µL of the 4% vanillin in methanol solution. The mixture was stirred vigorously, and then 750 µL of concentrated hydrochloric acid was added. The resulting mixture was left at room temperature for 20 minutes to allow the reaction to occur. The absorbance was then measured at 550 nm, using a blank consisting of the 4% vanillin solution in methanol. Each sample was tested three times. A stock solution of tannic acid served as a standard reference for establishing the calibration curve and quantifying condensed tannin content, expressed as milligram equivalent of tannic acid per gram of dry matter (mg EAT/g dry matter). Tests were performed three times for each sample. Condensed tannin content in the extracts was determined from the calibration curve  $Y = 0.1165X + 0.00003$  with  $R^2 = 0.9996$ , plotted using the stock solution of tannic acid as the standard.

## 4. Antioxidant activities

### DPPH Method

To evaluate the antioxidant potential of the extracts, the method described by Coulibaly *et al.* (2025)<sup>[6]</sup> The following procedure was used: DPPH was dissolved in absolute EtOH to obtain a solution with a concentration of 0.3 mg/ mL. The various concentrations (2 mg/ mL, 1 mg/ mL, 0.5 mg/ mL, 0.25 mg/ mL, 0.125 mg/ mL, and 0.0625 mg/ mL) of the extract were prepared in absolute EtOH. A volume of 2.5 mL of plant extract and 1 mL of DPPH ethanolic solution were placed in dry, sterile tubes. After shaking, the tubes were left in a dark place for half an hour. The absorbance of the mixture was then measured at 517 nm using a blank consisting of 2.5 mL of pure EtOH and 1 mL of DPPH solution. Ascorbic acid (vitamin C) was used as the positive control. The following formula is used to calculate the percentages of the DPPH:

$$I (\%) = (A_b - A_e) / A_b \times 100$$

% : Percentage of inhibition ; A<sub>b</sub> : Absorbance of the blank ; A<sub>e</sub> : Absorbance of the sample

I: Percentage of inhibition; A<sub>b</sub> : Absorbance of the blank; A<sub>e</sub> : Absorbance of the sample

The concentrations to inhibit 50% (IC<sub>50</sub>) of DPPH are determined from the graphs illustrating the percentage of DPPH inhibition as a function of extract or vitamin C concentrations.

### ABTS Method: the Abts radical-cation<sup>+</sup> (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid)

This technique relies on the ability of the compounds to decrease the Abts<sup>+</sup> radical cation (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid). The test was performed following the technique described by Choong *et al.*, (2007)<sup>[5]</sup>. The ABTS<sup>+</sup> radical cation was produced by reacting 8 mM ABTS (87.7 mg dissolved in 20 mL of distilled water) with 3 mM potassium persulfate (0.0162 g dissolved in 20 mL of distilled water) in a 1: 1 (v/v) ratio. The mixture was then incubated in the dark at room temperature for 12–16 hours. This ABTS<sup>+</sup> solution was

diluted with methanol to obtain a solution with an absorbance of  $0.7 \pm 0.02$  at 734 nm. A 3.9 mL sample of this diluted ABTS<sup>+</sup> solution was added to 100  $\mu$ L of the test compound. After stirring, the mixture was incubated in the dark for 6 minutes ( $T = 30 \pm 2^\circ\text{C}$ ). The residual absorbance of the ABTS<sup>+</sup> radical was subsequently measured at 734 nm using a UV-visible spectrophotometer and was expected to be between 20% and 80% of the blank absorbance. Measurements were performed in triplicate, and the results were quantified in  $\mu\text{mol}$  of Trolox equivalent per liter of extract ( $\mu\text{mol TE/L}$ ). Using the following Trolox concentrations: 0, 3.75, 5, 6.25, 10, 11.25, 12.5, 13.75, and 15, a calibration curve was developed. The expression for the inhibition percentage (%I) of ABTS<sup>+</sup> is given by the following formula:

$\% I = [(A_0 - \text{Extracted Abs}) / A_0] \times 100$ ;  $A_0$  = diluted ABTS absorbance,  $\text{Extracted Abs}$  = diluted ABTS absorbance + sample

The resulting straight line allowed us to express the antioxidant activity of the different extracts as follows:

Concentration or antioxidant activity ( $\mu\text{M eq Trolox}$ ) =  $(\%I \times \text{fd}) / (4.99 \times 10)$ ; Fd : dilution factor; Extract concentration before dilution = 10 mg/mL  $4.99 \times 10$  (slope of the Trolox curve).

## 5. Infrared (IR) Spectroscopy

The principle of infrared spectroscopy is based on the absorption of light by most molecules in the infrared region of the electromagnetic spectrum and the conversion of this absorption into molecular vibrations. This technique allows the determination of the nature of chemical bonds and the various functional groups within a molecule. Infrared (IR) spectra were recorded on a Perkin Elmer Spectrum 2 instrument (Figure 1). The samples (2 mg) were deposited in a methanol solution, and the solvent was then evaporated to allow the formation of a uniform film on the cell (mold). Data were collected over a spectrum ranging from  $400 \text{ cm}^{-1}$  to  $4000 \text{ cm}^{-1}$  using Spectrum 2 software (Johnny *et al.*, 2021).



Fig 1: Perkin Elmer spectrum 2.

## 6. Mass Spectrometry (HPLC-ESI-QTOF-MS/MS)

The recently developed HPLC-ESI-Q-TOF-MS/MS coupling has established itself as a valuable tool for the analysis of complex chemical compound systems (Han *et al.*, 2012)<sup>[12]</sup>. It is a device that combines various analytical techniques:

- HPLC offers a fast and efficient method for analyzing samples and separating their components.
- ESI spectrometry: This is a soft ionization technique that can produce both protonated and deprotonated molecules. It typically provides the peak of the ionic peak. It is a very useful technique for determining the mass of large molecules.
- The Q-TOF-MS method provides high-resolution mass and allows validation of the elemental composition of parent ions and ionic fragments (Quirantes *et al.*, 2013).

## Statistical Analysis

The statistical differences between these average values at 95% confidence level are indicated on the same line by the different letters a and b in superscript.

Statistical analysis of the results was performed using one-way analysis of variance (ANOVA). Differences between means were determined using Dunnett's test with a significance level of 5%. A p-value < 0.05 indicates a significant difference; otherwise, it is not significant.

## Results and discussion

### 1. Results

#### Extraction yield

The results show that the best yields were obtained by extraction with the binary solvent mixture of distilled water and ethanol. Furthermore, the JFm extract showed a higher yield compared to the other extracts (Table 1).

**Table 1:** Yield of aqueous and hydroethanolic extracts of *Albizia zygia*

Extracts	AMETe (g)	AMETm (g)	JFe (g)	JFm (g)
Yields	13.33 ± 0.265 <sup>b</sup>	13.50 ± 0.2 <sup>b</sup>	17.06 ± 0.058 <sup>a</sup>	23.00 ± 2.272 <sup>a</sup>

### Determination of phytochemicals from aqueous and hydroethanolic extracts of *Albizia zygia*

hydroethanolic extracts are richer in total polyphenols, total flavonoids, and total tannins than aqueous extracts. In

particular, the phytochemical content of the AMETm extract is higher than that of the other extracts ( Table 2 ). The study also revealed a higher concentration of these compounds in the plant's trunk bark compared to the leaves.

**Table 2:** Contents of phytochemicals in polyphenols, flavonoids and total tannins

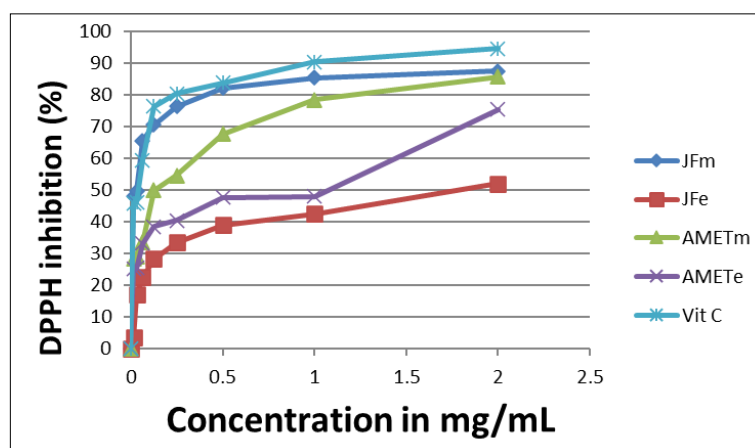
Excerpts	Total polyphenols (mg GAE/g)	Total flavonoids ( mg EQ/g)	Total tannins ( mg EAT/g)
AMETe	16.83 ± 0.28	8.16 ± 0.28	5.17 ± 0.022
AMETm	32.83 ± 0.28	16.00 ± 0.50	10.78 ± 0.009
JFe	9.78 ± 0.001	5.58 ± 0.00	3.83 ± 0.00
JFm	17.5 ± 0.00	7.54 ± 0.00	4.42 ± 0.03

### Result of the antiradical potential

#### DPPH test

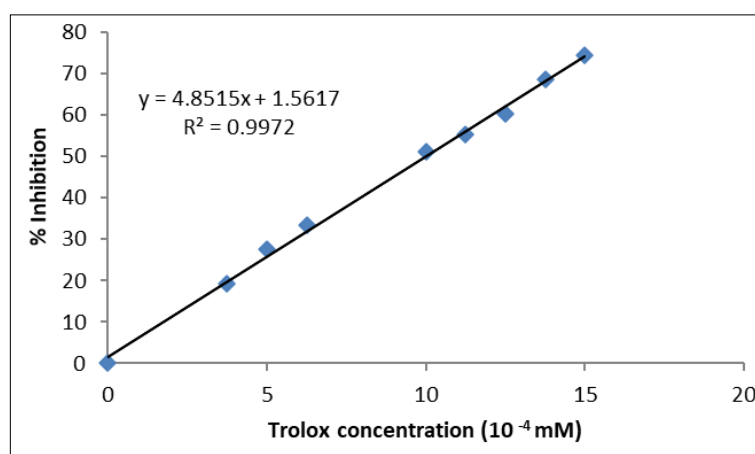
The results of the evaluation of the antiradical activity of the extracts AMETe, AMETm, JFe, and JFm, studied using the DPPH assay, are expressed as the percentage of inhibition (I%) of the DPPH radical by the extracts and are represented graphically (I% as a function of concentration) ( Figure 2). Ascorbic acid (Vitamin C) is used as the reference antioxidant. The inhibitory concentration scavenging 50%

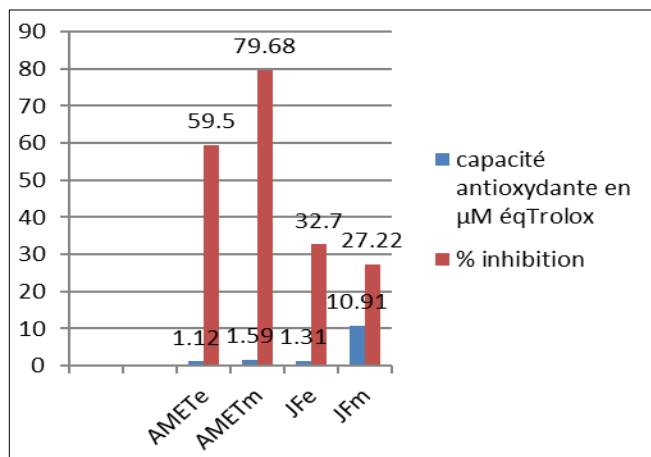
of the DPPH radical ( $IC_{50}$ ) is  $0.04 \pm 0.00$  mg / mL for ascorbic acid. The lower the  $IC_{50}$  value, the greater the antioxidant activity of the extract. The study revealed significant free radical scavenging activity ( $0.03 \pm 0.0$  mg / mL) for the hydroethanolic extract of *Albizia zygia* leaves ( JFm ). The study showed significant free radical scavenging activity for AMETe ( $IC_{50}$ :  $1.07 \pm 0.026$  mg/mL), AMETm ( $IC_{50}$ :  $0.127 \pm 0.020$  mg/mL) and JFe ( $IC_{50}$ :  $1.78 \pm 0.13$  mg/mL).

**Fig 2:** Percentage of DPPH radical inhibition by AMETe, AMETm, JFe and JFm extracts

### Antioxidant activity by the Abts method

Trolox calibration curve (Figure 3) allowed us to determine the percentage of inhibition on the Abts radical and the concentration.

**Fig 3:** Trolox calibration curve



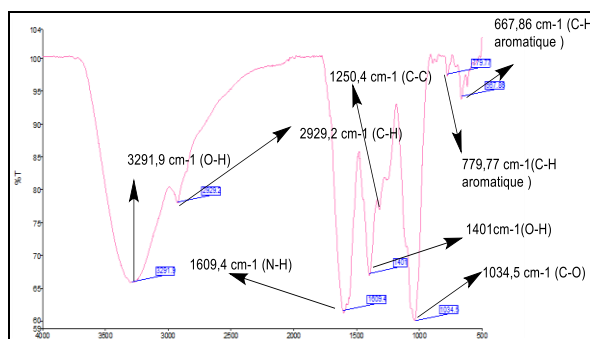
**Fig 4:** Percentage of inhibition and antioxidant capacity of extracts by the Abts test

The results obtained for the trunk bark confirm that the activity obtained with the hydroethanolic extract is better

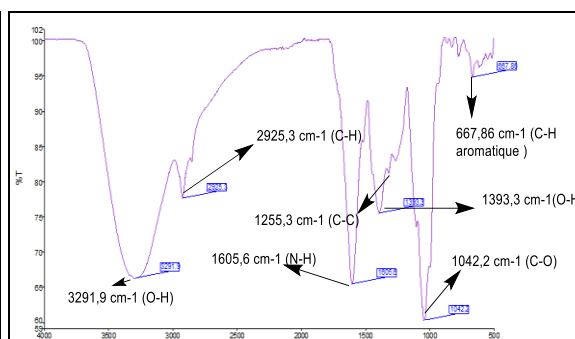
than that of the aqueous extract. Indeed, the inhibitory power obtained is high for the AMETm extract. These two methods yield the same result. Indeed, the AMETm extract exhibits significant antioxidant activity.

### Infrared Spectroscopy of the tested extracts

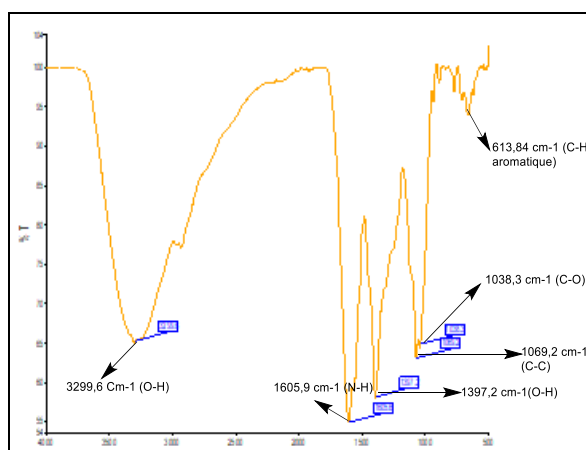
The presence of polyphenols could be confirmed by OH absorption bands. The different values observed reveal the presence of alkaloid ( $\text{NH}_4^+$ ) absorption bands at  $1609.4 \text{ cm}^{-1}$  and polyphenol ( $\text{OH}^-$ ) absorption bands at  $3291.9 \text{ cm}^{-1}$  for the AMETe extract ( Figure 5 ). Regarding the AMETm extract, the spectrum could reveal the presence of alkaloid ( $\text{NH}_4^+$ ) absorption bands at  $1605.6 \text{ cm}^{-1}$  and tannin ( $\text{OH}^-$  and  $\text{CO}_2$ ) absorption bands at  $3291.9 \text{ cm}^{-1}$  and  $1042.2 \text{ cm}^{-1}$ , respectively ( Figure 6 ). The presence of alkaloid ( $\text{NH}_4^+$ ) absorption bands at  $1605.9 \text{ cm}^{-1}$  and tannin ( $\text{OH}^-$  and  $\text{CO}_2$ ) absorption bands at  $3299.6 \text{ cm}^{-1}$  and  $1038.3 \text{ cm}^{-1}$  is visible for the JFe extract ( Figure 7 ). Finally, for extract JFm, the presence of alkaloids (NH) at  $1609.1 \text{ cm}^{-1}$  and polyphenols (OH) at  $3264.9 \text{ cm}^{-1}$  ( Figure 8 ) are observable.



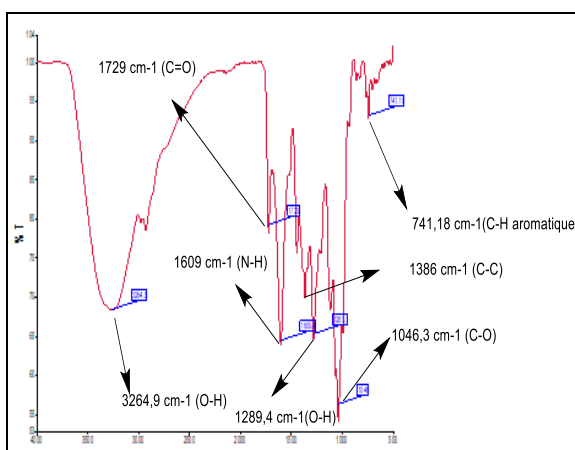
**Fig 5:** IR spectrum of AMETe



**Fig 6:** Spectrum of AMETm



**Fig 7:** IR spectrum of Jfe

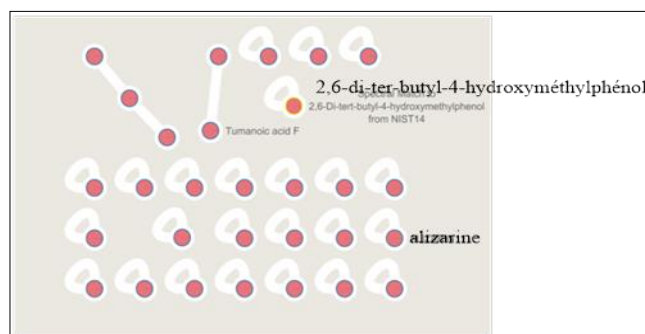


**Fig 8:** IR spectrum of JFm

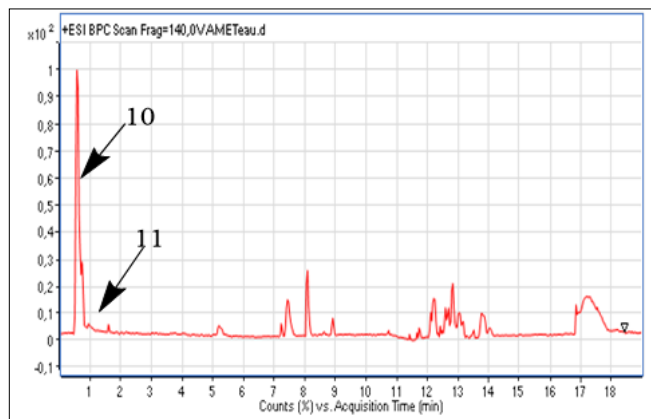
### Mass Spectrometry (HPLC-ESI-QTOF-MS/MS)

The analyzed extracts possess good antioxidant properties. In order to identify the major compounds present in the extracts, a dereplicative approach based on the coupling of High Performance Liquid Chromatography (HPLC) with Quadripolar Time-of-Flight (Q/TOF) tandem mass spectrometry was implemented.

Analysis of this spectrum revealed the presence in the AMETe extract of two compounds, 2,6-di-ter-butyl-4-hydroxymethylphenol ( 10, retention time: 0.684 min ) and alizarin ( 11; retention time: 1.205 min) ( Figures 9 and 10 ).



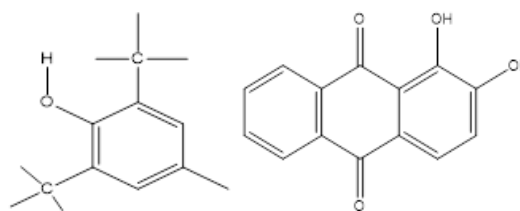
**Fig 9:** Network of the AMETe extract



**Fig 10:** Chromatogram of analysis +ESI BPC of the AMETe extract

2,6-di-tert-butyl-4-methylphenol, also known as BHT (butylated hydroxytoluene), is a potent synthetic antioxidant used as a food additive (E321) and in cosmetics. This compound is an antioxidant that prevents the oxidation of fats and oils in food and cosmetic products. According to the National Center for Biotechnology Information (2025), 2,6-di-tert-butyl-4-hydroxymethylphenol (Figure 11) is a phenolic compound, a potent antioxidant, a ferroptose inhibitor, a food additive, and a geroprotectant ([https://pubchem.ncbi.nlm.nih.gov/compound/2\\_6-Di-tert-butyl-4-methylphenol](https://pubchem.ncbi.nlm.nih.gov/compound/2_6-Di-tert-butyl-4-methylphenol)). In a study conducted on diabetic

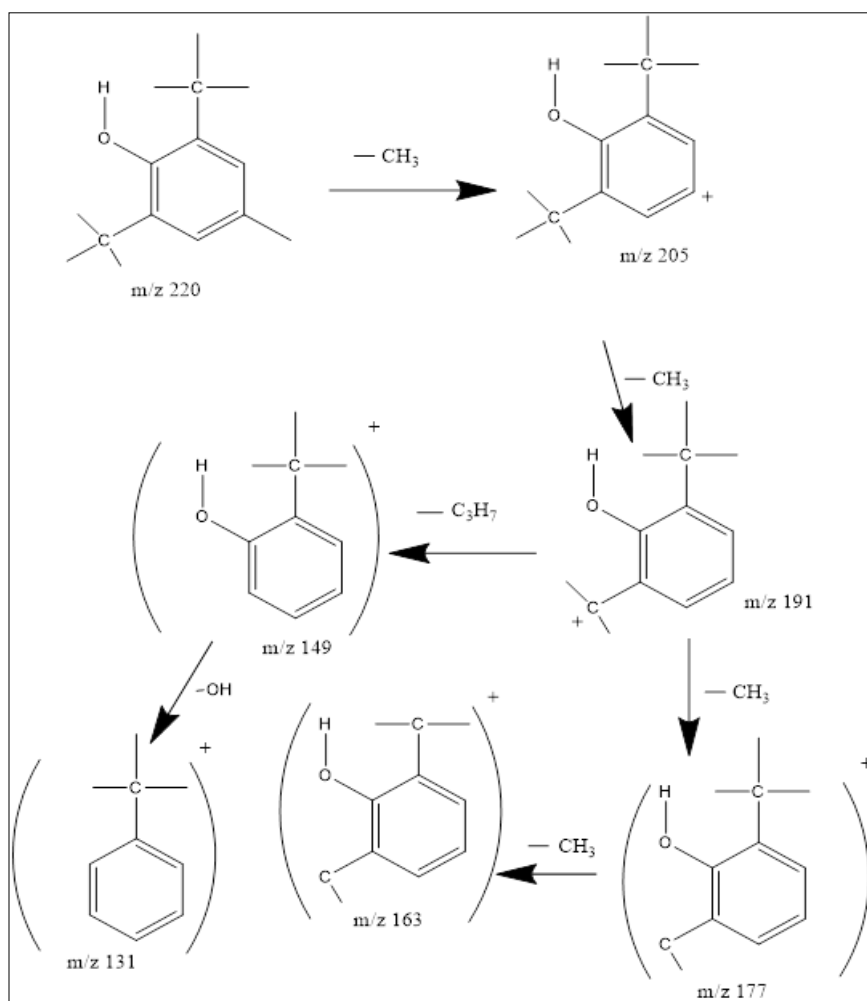
mice, authors demonstrated that alizarin (11), an anthraquinone dye, could facilitate glucose uptake by adipocytes in mice made obese by diet (Sadasivan *et al.*, 2014<sup>[31]</sup>; Xu *et al.*, 2019). Work carried out by Cuoco, (2009)<sup>[7]</sup> revealed the isolation anthraquinones such as alizarin in madder, a plant used as an antidiabetic



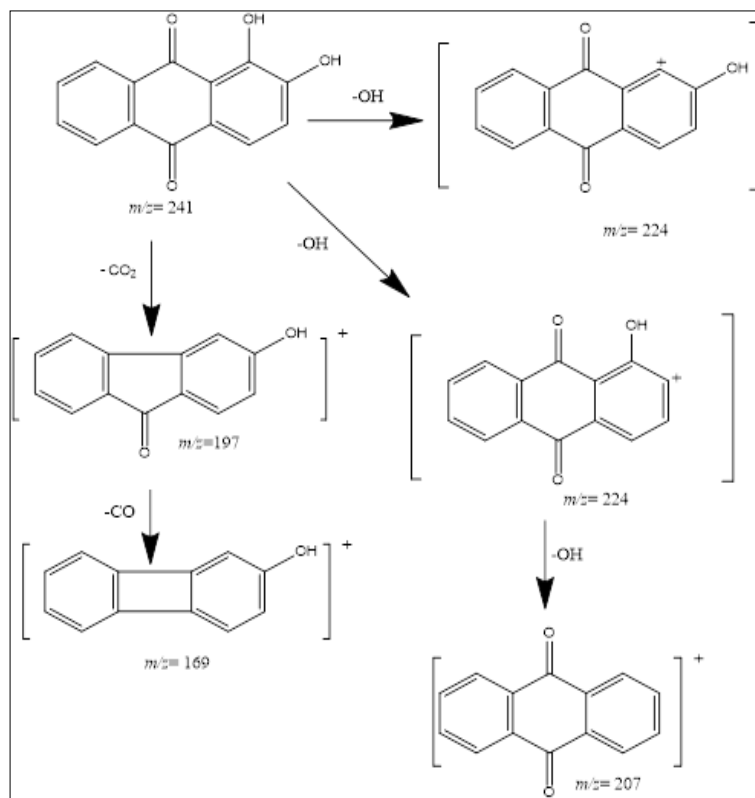
2,6-di-tert-butyl-4-hydroxymethylphenol, alizarin

**Fig 11:** 2,6-di-tert-butyl-4-hydroxymethylphenol and alizarin

The ESI-MS/MS mass spectrum analysis of mass losses yields characteristic fragments. A fragmentation scheme has been proposed. Thus, structures have been proposed for fragments with  $m/z$  : 205 [M-15] (loss of  $\text{CH}_3$ ),  $m/z$  191 [M-29] (loss of  $\text{C}_2\text{H}_5$ ),  $m/z$  : 177 [M-43] (loss of  $\text{C}_2\text{H}_3\text{O}$ ),  $m/z$  : 163 [M-57] (loss of  $\text{C}_4\text{H}_9$ ),  $m/z$  : 149 [M-71] (loss of  $\text{C}_5\text{H}_{11}$ ) and  $m/z$  : 131 [M-89] (loss of  $\text{C}_5\text{H}_{13}\text{O}$ ) for the 2,6-di-tert-butyl-4-hydroxymethylphenol molecule (Figure 10) and  $m/z$  : 224 [M+H-17] (loss of OH),  $m/z$  : 207 [M+H-34] (loss of 2OH),  $m/z$  : 197 [M+H-44] ( $\text{CO}_2$  loss) for alizarin (Figure 11).



**Fig 12:** Fragmentation mode of 2,6-di-tert-butyl-4-hydroxymethylphenol



**Fig 13:** Proposed fragmentation of alizarin

## Discussion

The extraction methods yielded aqueous and hydroethanolic extracts. However, the yields obtained varied from one extract to another. Indeed, the hydroethanolic extract JFm of the leaves revealed the best yield. Yield is generally influenced by various factors such as the solvent used, pH, temperature, extraction time, and sample composition (Do *et al.*, 2014). In this study, the results obtained could be due to the nature of the extraction solvent and the composition of the different extracts.

The simultaneous use of water and an organic solvent such as ethanol can facilitate the extraction of chemicals soluble in either water or the organic solvent (Do *et al.*, 2014). Indeed, extraction using both a water and ethanol binary mixture tends to produce extracts with higher concentrations of polyphenols and flavonoids compared to extraction with water alone, due to the better dissolution of these compounds in the ethanol-water mixture. Water extraction, although easier and less expensive, may prove less efficient for certain compounds that do not dissolve in water (Naima *et al.*, 2015; Mohammadi and Atik, 2011; Trabelsi *et al.*, 2010) [19, 22, 35]. According to the study conducted by Poutoum *et al.*, 2023, analyses of aqueous and hydroethanolic extracts of *Albizia zygia* leaves and trunk bark revealed a higher extraction yield for the aqueous extract of the stem bark and a lower yield for the hydroethanolic extract of the leaves. They recorded higher extraction rates (26.71% for the hydroethanolic extract and 33.2% for the aqueous extract) for the trunk bark compared to the aqueous and hydroethanolic extracts of leaves from the same plant. These results are inconsistent with those obtained in our study. This could be due to the harvesting location and extraction time. Compounds with an affinity for the water-ethanol binary mixture were the least abundant hydroethanolic extracts, compared to aqueous extracts, have the highest concentrations of total polyphenols, total flavonoids, and total tannins. Furthermore, the study

indicates that the highest concentrations are found in the trunk bark compared to the leaves. This result is consistent with that of Habibou *et al.* (2018) [11] in a study conducted on *Detarium. microcarpum* Guill. and Perr., a plant species used in the treatment of parasitic diseases in Niger. Indeed, the authors observed a high content of polyphenols, flavonoids, and tannins in the trunk bark of *Detarium. microcarpum* Guill and Perr. The results obtained also show that total flavonoids are more concentrated in hydroethanolic extracts compared to aqueous extracts. These results are consistent with those obtained by other scientists. Indeed, according to Moungang (2021 [20]), hydroethanolic extracts contain a greater quantity of polyphenols compared to aqueous extracts. This could be due to the efficiency of the extraction. In fact, as Mohammadi and Atik (2011 [19]) point out, the use of mixed solvents offers a considerable opportunity to enrich extracts with polyphenols. The affinity of polyphenols for the binary hydroalcoholic mixture This would be due to the increased solubility of phenolic compounds in extracts obtained with mixed solvents compared to those obtained with pure solvents (Trabelsi *et al.*, 2010) [35]. Therefore, to obtain polyphenol-rich fractions, it is preferable to use mixtures of appropriate organic solvents with water ( Mahmoudi *et al.*, 2013 [17]).

The presence of polyphenols could be confirmed by absorption bands visible on the infrared spectra, CO for tannins, OH for polyphenols and tannins.

Indeed, studies conducted by Olukanni (2020) [25] revealed the presence of alcohols, phenols, alkanes, an aromatic ring, alkyl halides, ether bonds and alkynes in the methanoic extract of *Albizia zygia* leaves.

Tannins are polyphenols found in many plants. Work by Omar *et al.* (2022) [26] on type 1 diabetic rats showed the beneficial effect of tannins on blood glucose levels. They can reduce blood glucose while improving body weight in

rats with insulin deficiency. However, their effect may be less pronounced in type 2 diabetic rats.

As for flavonoids, found in many fruits, vegetables, medicinal plants, and other foods, they are compounds that could help prevent and manage type 2 diabetes (Al-Ishaq *et al.*, 2019<sup>[3]</sup>). They could also improve insulin sensitivity, stimulate insulin secretion, and reduce inflammation—all important factors in the prevention and control of diabetes.

According to other research conducted by Poutoum *et al.*, (2023<sup>[29]</sup>), the hydroethanolic extract of the trunk bark of *Albizia zygia* is rich in flavonoids, alkaloids, polyphenols, tannins, anthocyanins, saponins, anthraquinones, sterols, and triterpenes. Furthermore, saponins, alkaloids, flavonoids, and terpenes are present in some species of the genus *Albizia* (Odeyemi) *et al.*, 2014<sup>[24]</sup>). The results obtained during this study are consistent with those in the literature. The work carried out by some authors highlighted the presence of flavonoids, anthocyanins, tannins, anthraquinones and saponins in the leaves of *Albizia zygia*.

Regarding the evaluation of antioxidant activity, the results obtained are in agreement with those of Olukanni *et al.*, (2020)<sup>[25]</sup> showing significant antioxidant activity of the methanolic extract *Albizia zygia* leaves by DPPH trapping, with an estimated IC<sub>50</sub> of 152 µg/ mL versus 34 µg/ mL for the reference, standard ascorbic acid.

The IC<sub>50</sub> value for JFm (0.03 ± 0.0 mg/mL) is reported to be lower than that for vitamin C (0.04 ± 0.00 mg/mL), which means that the extract is more active than vitamin C, the reference compound. The antioxidant activity of the plant showed that all extracts possessed antioxidant activity. This could be due to the presence of phenolic compounds, which were found in all the extracts studied. The two methods (DPPH and ABTS) have shown that the extracts possess good antioxidant activity, which varies from one extract to another. These variations may be associated with the composition of the extracts in antioxidant molecules (Lahouazi, H. (2020)<sup>[16]</sup>). Therefore, several studies revealed that flavonoids and anthocyanins exhibit high antioxidant activity. In our study, there may be a link between antioxidant activity and the nature of the phenolic compounds. Inhibition concentrations vary depending on the method used. This variation could be due to the existence of compounds that display absorption bands at the same wavelength as the DPPH radical, thus causing an increase in absorbance (Sarr. *et al.*, 2015)<sup>[33]</sup>. In this context, the study conducted on *Acanthospermum hispidum* Dc. and *Ximenia americana* L., two plants with antihypertensive properties, show that the antioxidant activity of the extracts could be due to the high content of polyphenols, flavonoids, and tannins in the extracts (Djamilatou *et al* 2021)<sup>[9]</sup>.

Indeed, numerous studies indicate that antioxidants, particularly those found in our diet and medicinal plants, could have a beneficial effect in preventing and managing type 2 diabetes. By reducing oxidative stress and optimizing various metabolic functions, they could help lower the likelihood of developing this disease and treat its complications. There is a link between antioxidants and antidiabetic activities due to their ability to combat oxidative stress, which is a determining factor in the onset and progression of diabetes. Antioxidants work by neutralizing free radicals, which reduces the damage caused by oxidation to cells and tissues. Many compounds known for their antioxidant properties also show beneficial effects against diabetes, by improving glucose metabolism, insulin

sensitivity and reducing the risk of complications ( Sarian *et al.*, 2017; Ochuko *et al.*, 2023<sup>[23, 32]</sup> ).

The molecules identified through HPLC-ESI-QTOF-MS/MS in the studied extracts are all important in this study. Indeed, they are involved in the treatment of diabetes. In a study conducted on diabetic mice, researchers demonstrated that alizarin, a dye of origin anthraquinone, could improve glucose uptake by fat cells in mice that have become obese due to their diet (Sadasivan *et al.*, 2014; Xu *et al.*, 2019). Cuoco, ( 2009<sup>[3, 7, 31]</sup> ) extracted anthraquinones such as alizarin from madder, a plant used for its antidiabetic properties. 2,6-Di-tert-butyl-4-hydroxymethylphenol has been identified in the AMETe extract. According to the National Center for Biotechnology Information (2025), 2,6-di-tert-butyl-4-hydroxymethylphenol is a phenol. It is a potent antioxidant, a ferroptose inhibitor, a food additive, and a geroprotectant ([https://pubchem.ncbi.nlm.nih.gov/compound/2\\_6-Di-tert-butyl-4-methylphenol](https://pubchem.ncbi.nlm.nih.gov/compound/2_6-Di-tert-butyl-4-methylphenol)).

**Acknowledgments:** Thanks to the University Peleforo GON COULIBALY (Côte d'Ivoire), which served as the basis for carrying out this project.

**Conflicts of Interest:** The authors declare no conflicts of interest.

## Conclusion

Analysis of the aqueous and hydroethanolic extracts by HPLC-ESI-QTOF-MS/MS allowed for the identification of the structures of two molecules in the different extracts. Alizarin and 2,6-di-tert-butyl-4-hydroxymethylphenol were identified in the AMETe extract. Quantification of total polyphenolic compounds, total flavonoids, and total tannins revealed that the highest total polyphenol content was measured in the hydroethanolic extract of the trunk bark (32.83 ± 0.28 mg GAE/g). However, the aqueous extract of the leaves (9.78 ± 0.001 mg GAE/g) yielded the lowest content. The antiradical activity of the hydroethanolic extract of the leaves (IC<sub>50</sub> : 0.03±0.0<sup>ab</sup> mg/ mL ) is better than that of vitamin C (CI<sub>50</sub> : 0.04 ± 0.00 mg/ mL ), the reference molecule. Observations suggest that the recurring use of this plant in the traditional management of diabetes may be linked to its relative richness in polyphenolic compounds. This plant could be beneficial for the treatment of diabetes.

## References

1. Able MCG, Kone M, Kablan ALC, Kouamé ANB. Effectiveness of Plant Extracts Against *Podagrica decolorata* and *Amrasca biguttulain Abelmoschus esculentus* (L.) Cultivation. Journal of Agricultural Science,2025:17(1):102-114. URL: <https://doi.org/10.5539/jas.v17n1p102>
2. Ajebli M, Khan H, Eddouks M. Natural Alkaloids and Diabetes Mellitus: A Review. Endocrine Metabolic & Immune Disorders-Drug Targets,2021:21(1):111-130. DOI: 10.2174/1871530320666200821124817. PMID: 32955004.
3. Al-Ishaq RK, Abotaleb M, Kubatka P, Kajo K, Büsselberg D. Flavonoids and Their Anti-Diabetic Effects: Cellular Mechanisms and Effects to Improve Blood Sugar Levels. Biomolecules,2019:9(9):430.
4. Amine D, Mohamed B, Zoubida H, Jamal I, Laila N. Antifungal Activity of Aqueous Extracts of *Calendula Officinalis* L, *Urginea Maritima* (L.) Baker

- and *Chenopodium Ambrosioides* L. European Scientific Journal,2017;3(24):483. <https://doi.org/10.19044/esj.2017.v13n24p48>
5. Choong C, Van-Den TT, Roger F. Antioxidant Activities, Phenolic and Beta-Carotene Contents of Sweet Potato Genotypes with Varying Flesh Colours. Food Chemistry,2007;103:829-838.
  6. Coulibaly SA, Touré A, Kablan RJF, N'Guessan OE, Yéo ND, Kablan ALC. Phytochemical and antioxidant properties of mangoes produced in Korhogo and Sinématiali departments, north of Côte d'Ivoire. Journal of Pharmacognosy and Phytochemistry,2025;14(2):408-415.
  7. Cuoco G. Chemical study and characterization of coloring principles historically used in the printing of printed cotton fabrics in Provence. Thesis Avignon, 2009. <http://www.theses.fr/2009AVIG0232/document>
  8. Deteix, 2005. P. <http://www.airg-france.org/textes/traitements/hypertension-artérielle-contenu.htm>.
  9. Djamilatou ZS, Djibo AK, Seini BSSH. Phytochemical screening, polyphenol quantification and determination of antioxidant activity of two antihypertensive plants from Niger. European Scientific Journal ESJ,2021;17(17):335-349.
  10. Do QD, Angkawijaya AE, Tran-Nguyen PL, Huynh LH, Soetaredjo FE, Ismadji S, *et al.* Effect of extraction solvent on total phenol content, total flavonoid content, and antioxidant activity of *Limnophila aromatica*. Journal of food and drug analysis,2014;22(3):296-302.
  11. Habibou HH, Moutari SK, Lawaly MM, Idrissa M, Rabani A, Khalid I. Phytochemical screening and determination of polyphenols in *Detarium microcarpum* (Guill. and Perr.) used in the treatment of parasitic diseases in Niger. Africa Science,2018;14(5):390-399.
  12. Han L, Boakye-Yiadom M, Liu E, Zhang Y, Li W, Song X, *et al.* Structural characterization and identification of phenylethanoid glycosides from *Cistanches deserticola* YC Ma by UHPLC/ESI-QTOF-MS/MS. Phytochemical Analysis,2012;23:668-676.
  13. Hokayem M, Bisbal C, Lambert K, Avignon A. What role for antioxidants in the prevention of type 2 diabetes? Medicine of Metabolic Diseases,2012;6(4):327-331.
  14. Beaugrand J, Goudenhooff C, Alvarado C, Devaux MF, Rivard C, Durand S, *et al.* Evolution of the flax cell wall composition during development and after gravitropism by synchrotron fluorescence imaging. Industrial Crops and Products,2022;175:114256. <https://doi.org/10.1016/j.indcrop.2021.114256>.
  15. Kouamé TK, Siaka S, Kassi ABB, Soro Y. Determination of the contents of total polyphenols, total flavonoids and tannins in young, unopened leaves of *Piliostigma thonningii* (Caesalpinaceae). International Journal of Biological and Chemical Sciences,2021;15:97-105.
  16. Lahouazi H. Influence of solvent, genus and altitude on the yield of total polyphenols and essential oils of *Pistacia* leaves *lentiscus* L. from the Zekri forest (w. Tizi-Ouzou) (Doctoral dissertation, Mouloud Mammeri University), 2020.
  17. Mahmoudi R, Tajik H, Ehsani ALI, Farshid AA, Zare P, Hadian M. Effects of *Mentha* essential oil *longifolia* L. on the viability and cellular ultrastructure of *Lactobacillus casei* during the ripening of probiotic feta. International Journal of Dairy Technology,2013;66(1):77-82.
  18. Marinova D, Ribarova F, Atanassova M. Total phenolics and flavonoids in Bulgarian fruits and vegetables. Journal of the University of Chemical Technology and Metallurgy,2005;40(3):255-260.
  19. Mohammedi Z, Atik F. Impact of solvent extraction type on total polyphenols content and biological activity from *Tamarix aphylla* (L.) Karst. International Journal of Pharma and Bio Sciences,2011;2:609-615.
  20. Mounang LM. Phytochemical screening and *in vitro* evaluation of the antibacterial activity of organic extracts of *Cussonia* root bark *arborea* (Araliaceae) (Doctoral thesis, Faculty of Medicine and Biomedical Sciences, University of Yaoundé I, Cameroon), 2021.
  21. N'Guessan K. Medicinal plants and traditional medical practices among the Abbey and Krobo peoples of the Agboville Department (Côte d'Ivoire). Doctoral thesis in Natural Sciences. University of Cocody- Abidjan, UFR Biosciences, Laboratory of Botany, 2008, 235.
  22. Naima R, Oumam M, Hannache H, Sesbou A, Charrier B, Pizzi A, *et al.* Comparison of the impact of different extraction methods on polyphenols yields and tannins extracted from Moroccan *Acacia mollissima* barks. Industrial Crops and Products,2015;70:245-252.
  23. Ochuko L, Erukainure KP, Otukile KR, Harejane VF, Salau AA, Chika I, *et al.* Computational insights into the antioxidant and antidiabetic mechanisms of cannabidiol: An *in vitro* and *in silico* study. Arabian Journal of Chemistry,2023;16(7):104842.
  24. Odeyemi O, Oluduro AO, David OM. Evaluation of Antimicrobial Activities of *Albizia zygia* DC leaf extracts against some clinically important pathogens. Journal of Natural Sciences Research,2014;4(9):98-105.
  25. Olukanni OD, Lugard E, Emmanuel E, Olukanni AT, Ayoade F, Durugbo EU. Antioxidant and *in vitro* anti-inflammatory activities of *Albizia zygia* (DC) JF Mebr and the evaluation of its phytochemical constituents. Journal of Medicinal Plants,2020;8(4):317-23.
  26. Omar N, Ismail CAN, Long I. Tannins in the Treatment of Diabetic Neuropathic Pain: Research Progress and Future Challenges. Frontiers in Pharmacology,2022;10(12):805-854. DOI: 10.3389/fphar.2021.805854.
  27. Resilience Toolkit : A WHO global public health good supporting the sustainable development and strengthening of health system resilience in diverse country contexts. In Resilience Toolkit : A WHO global public health good supporting the sustainable development and strengthening of health system resilience in diverse country contexts.
  28. WHO. WHO Policy Perspectives on Medicines: Continuity and change implementing the third WHO Medicines Strategy, 2010. 2008-2013. WHO/EMP/2010.2 <http://apps.who.int/medicinedocs/d/ocuments/s16880e/s16880e.pdf>, 6,
  29. Poutoum YY, Mounang LM, Kweyang BPT, Fonkui TY, Meva'a GRZ, Njeunkam AD, *et al.* Evaluation of the Efficacy of *Albizia zygia* Extracts on Bacterial

- Inhibition in Aquatic Microcosm. Journal of Geoscience and Environment Protection,2023;11(7):227-241.
30. Quirantes-Piné R, Lozano-Sánchez J, Herrero M, Ibáñez E, Segura-Carretero A, Fernández Gutiérrez A. HPLC-ESI-QTOF-MS as a powerful analytical tool for characterizing phenolic compounds in olive leaf extracts. P hytochemical Analysis,2013;24:213-223.
  31. Sadasivan SK, Vasamsetti B, Singh J, Siddaraju N, Khan KM, Oommen AM, *et al.* Modulation of de novo purine biosynthesis leads to AMPK activation and improves glucose handling and insulin sensitivity. Journal of Diabetes & Metabolic Disorders,2014;13:1-9.
  32. Sarian MN, Ahmed QU, Mat So'ad SZ, Alhassan AM, Murugesu S, Perumal V, *et al.* Antioxidant and antidiabetic effects of flavonoids: A structure-activity relationship based study. BioMed research international,2017;(1):Article ID 8386065, 14 pages, <https://doi.org/10.1155/2017/8386065>.
  33. Sarr SO, Fall AD, Gueye R, Diop A, Diatta K, Diop N, *et al.* Study of the antioxidant activity of extracts from the leaves of *Vitex doniana* (*Verbenacea*). International Journal of Biological and Chemical Sciences,2015;9(3):1263-1269.
  34. Tra Bi FH, Irié GM, N'Gaman KCC, Mohou CHB. Studies of some therapeutic plants used in the treatment of arterial hypertension and diabetes: two emerging diseases in Côte d'Ivoire. Sci. Nat.,2008;5(1):39-48.
  35. Trabelsi N, Megdiche W, Ksouri R, Falleh H, Oueslati S, Soumaya B, *et al.* Solvent Effects on Phenolic Contents and Biological Activities of the Halophyte *Limoniastrum monopetalum* Leaves. LWT-Food Science and Technology,2010;43:632-639.
  36. Wood JE, Senthilmohana ST, Peskinb AV. Antioxidant activity of procyanidin-containing plant extracts at different pHs. Food Chemistry,2002;77(2):155-161.
  37. Xu L, Xing M, Xu F. Medicinal Chemistry,2019;11(5):395-406. doi: 10.4155/fmc-2018-0515.
  38. Yohanna B, Muhammad L, Mailafiya DM. Effects of some botanical extracts on the control of major insect pests of okra in the Sudan Savannah Agro -Ecological Zone of Nigeria. Agrosearch,2022;21(1-2):80-88.
  39. Zerargui F, Boumerfeg S, Charef N, Baghiani A, Djarmouni M, Khennouf S, *et al.* Antioxidant potential and xanthine oxidase inhibitory effect of two furanocoumarins isolated from *Tamus communis* L. Medicinal Chemistry,2015;11(5):506-513.