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Chemical study using FT-IR spectroscopy, antioxidant activity, and quantify phytochemical compounds in aqueous and hydroethanolic extracts of two traditional antidiabetic medicines (FM and PMM) sold in Korhogo

AKOUBET-OUAYOGODE Aminata [1], KABLAN Richmond Jean-François [2], N'GUESSAN Ocho Esther [2] and KABLAN Ahmont Landry Claude [2,*]

[1] *Laboratory of Pharmaceutical Sciences, Analytical Sciences, and Public Health, Department of Pharmaceutical Sciences, UFR SPB, Félix HOUPHOUËT-BOIGNY University, Cocody, Ivory Coast.*
[2] *UPR Chemistry, Department of Mathematics, Physics, and Chemistry, UFR Biological Sciences, Peleforo GON COULIBALY University, BP 1328 Korhogo, Ivory Coast.*

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Abstract

Diabetes is a public health problem in Côte d'Ivoire. This condition can lead to blindness, kidney failure, heart attack, stroke, and lower limb amputation. The high cost of treatment in low-income countries, who have limited access to modern medications, contributes to patients turning to traditional remedies. In this context, the WHO recommends intensified research into alternative therapeutic approaches, including those that utilize traditional treatments based on medicinal plants. Several traditional antidiabetic medicines are sold in Korhogo. However, little scientific evidence exists to confirm their chemical composition or efficacy. The present study aims to quantify phytochemical compounds and evaluate the antioxidant activity of the traditional antidiabetic medicines FM and PMM sold in Korhogo. The levels of total polyphenols, total flavonoids, and total tannins were determined. Subsequently, the presence of sterols, tannins, polyphenols, and coumarins in the extracts was confirmed by infrared (FT-IR) absorption bands. Finally, antioxidant activity was evaluated using the DPPH and ABTS methods. The study revealed significant antiradical activity (IC_{50} = 0.021 mg/mL for PMMm, 0.03 mg/mL for PMMe, 1.57 mg/mL for FMe, and 0.089 mg/mL for FMm) compared to vitamin C (IC_{50} = 0.04 mg/mL). The traditional medicine FMm inhibited the ABTS cation radical by 46.11% at a concentration of 0.92 µM Trolox, and PMMe inhibited the ABTS cation radical by 36.6% at a concentration of 0.92 µM Trolox. The results of this study may support their use in the city of Korhogo.

Keywords: Diabetes; Herbal Medicines; Phytochemical Content; Antioxidant Activity

1. Introduction

Diabetes is a multifactorial disease involving genetic and environmental factors and several genes, meaning that its onset is caused by the interaction of abnormalities in multiple genes that are activated only when exposed to specific environmental factors. Projections regarding the epidemic indicate that by 2030, the prevalence of diabetes in sub-Saharan Africa could increase by 98% (Diabetes Atlas, 4th ed., Brussels: International Diabetes Federation, 2009). This situation has led World Health Organization (WHO) to classify diabetes as a major public health issue. Côte d'Ivoire has a prevalence of diabetes ranging from 3% to 7% (Djédjé, 2002; Koffi et *al.*, 2009).

Diabetes care represents a considerable financial burden for people in developing nations. These high costs, which are unaffordable for those living in poverty, lead them to seek traditional solutions. Consequently, the World Health

* Corresponding author: KABLAN Ahmont Landry Claude

Organization calls for intensified research, including studies on methods involving traditional treatments with medicinal plants.

Although significant progress has been made in managing this disease, research into new treatments for diabetes remains necessary. Among the recommended options is herbal medicine for diabetes. Currently, this method represents a promising alternative thanks to the growing discovery of plant extracts that have been shown to be effective in the treatment of type 2 diabetes (Zhang et *al*., 2000; Katemo et *al.*, 2012). Many treatments for diabetes are available in Korhogo, in northern Côte d'Ivoire. Traditional medicines such as FM and PMM are sold to treat this condition. However, there is little scientific information to validate their chemical composition and antioxidant activity. Given the prevalence of diabetes, the objective of this study was to estimate the content of polyphenols, flavonoids, and total tannins, and to evaluate the antioxidant capacity of two traditional herbal medicines from Korhogo, which are used in the traditional management of this condition.

2. Materials and Methods

2.1. Plant Material

According to the traditional medicine practitioner, the traditional medicine PMM is composed of root bark from Cinnamomum verum and Vitellaria paradoxa. It was purchased from a traditional healer in the city of Korhogo in northern Côte d'Ivoire in December 2023.

The dried leaves of Paraclinanthus laxiflorus constitute the plant material FM, according to the traditional medicine practitioner. These leaves were purchased in the city of Korhogo, located in northern Côte d'Ivoire, in December 2023.

2.2. Methods

2.2.1. Preparation of FMe, FMm, PMMe, and PMMm powders

The traditional medicine PMM is in powder form. It was weighed and placed in a container for various analyses.

The traditional medicine FM consists of Paraclinanthus laxiflorus leaves. These leaves were dried at room temperature for about two weeks. After drying, they were ground into powder using a Binatone Blender -BLG-450P MK2-1.5L electric blender. The powder was then weighed and carefully placed into jars. Our chemical and biological analyses were conducted using this powder.

2.2.2. Extraction of FMe, FMm, PMMe, and PMMm

Extractions were performed. Consequently, for (FMe and PMMe), 100 g of powder was dissolved in 1 L of distilled water (Coulibaly et *al.*, 2011), while for FMm and PMMm, 0.7 L of ethanol and 0.3 L of distilled water (70:30) were used (Yohanna Muhammad et *al*., 2022; Amine Mohamed et *al*., 2017; Able et *al*., 2025). Homogeneity of the mixture was ensured by magnetic stirring for three hours at room temperature, followed by maceration at the same temperature for 24 hours prior to further processing. After another 5 minutes of stirring and sieving, the supernatant was then filtered using cotton placed over a funnel. The resulting filtrate is centrifuged at 10,000 rpm (using a Jouan TH 12 centrifuge) for 15 minutes at room temperature. The recovered solution is then placed in an oven at 50 °C. These four operations, carried out separately, yielded the crude extracts FMe, FMm, PMMe, and PMMm.

2.2.3. Determination of phytochemicals compounds

Determination of total polyphenols

The method proposed by Wood et *al.* (2002) and N'guessan et *al.* (2025a) was used to determine total polyphenols. A mixture was prepared by combining 2.5 mL of diluted Folin-Ciocalteu reagent (1:10) with 30 µL of extract at a concentration of 2 mg/mL. According to Cicco et al. (2009), this mixture was kept in the dark at room temperature for two minutes. Next, 2 mL of a sodium carbonate solution at a concentration of 75 g/L was added. The mixture was then placed in a water bath at 50°C for 15 minutes before being rapidly cooled. Absorbance was measured at a wavelength of 760 nm, using distilled water as a blank. A calibration curve was established using gallic acid at different concentrations. The analyses were performed in triplicate, and the polyphenol concentration was expressed in mg GAE/g.

Total flavonoid assay

The method developed by Dirar et *al.* (2019), adopted by Djamilatou et *al.* (2021), Marinova et *al.* (2005) and N'guessan et *al.* (2025b), was used to quantify total flavonoids. This involved adding 0.75 mL of 5% (w/v) sodium nitrite ($NaNO_2$) to a 25-mL flask containing 2.5 mL of extract at a concentration of 2 mg/mL. Subsequently, 0.75 mL of 10% (w/v) aluminum chloride ($AlCl_3$) was added to the mixture, which was then incubated in the dark for 6 minutes. After incubation, 5 mL of sodium hydroxide was added. (1 N NaOH), and the total volume was then adjusted to 25 mL. The mixture was thoroughly shaken before measurement with a UV-visible spectrophotometer. The reading was taken at 510 nm. The experiments were conducted in triplicate. The flavonoid content was expressed in grams per liter of extract as quercetin equivalent. (Bahorun et *al.*, 1996).

Total Tannin Assay

The method described by Kouamé et *al.* (2021) was used to determine the content of condensed tannins. A 50 mL volume of each extract at a concentration of 2 mg/mL was added to 1500 µL of a 4% vanillin solution dissolved in methanol. This mixture was vigorously stirred, 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 proceed. Subsequently, the absorbance was measured at 550 nm using a blank prepared from the 4% vanillin solution in methanol. Each sample was analyzed three times. A tannic acid reference solution was used to establish the calibration curve and quantify the condensed tannin contents, expressed in milligrams of tannic acid equivalents per gram of dry matter (mg TAE/g dry matter). The analyses were performed three times for each sample. The concentrations of condensed tannins in the extracts were evaluated using the calibration curve $Y = 0.1165X + 0.00003$ with $R^2 = 0.9996$; this curve was plotted using a standard solution of tannic acid.

2.2.4. Antioxidant Activity

DPPH Assay

To determine the antioxidant potential of the extracts, the method proposed by Coulibaly et *al.* (2025) was employed. DPPH was mixed with 100% ethanol to create a solution with a concentration of 0.3 mg/mL. 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 pure ethanol. Place 2.5 mL of plant extract and 1 mL of DPPH solution in sterile, dry tubes. After shaking the tubes, place them in a dark place for 30 minutes. Next, the absorbance of the mixture is measured at 517 nm, using 2.5 mL of pure ethanol and 1 mL of the DPPH solution as a blank. Ascorbic acid (vitamin C) serves as the positive control. The following formula is used to calculate the DPPH inhibition percentages:

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

I: Percentage of inhibition; A_b: Absorbance of the blank; A_e: Absorbance of the sample

The concentrations required to achieve 50 % inhibition (IC_{50}) of DPPH are determined from graphs showing the rate of DPPH inhibition relative to the concentrations of extracts or vitamin C.

ABTS Method: the $ABTS^+$ cation (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid)

This method is based on the ability of substances to reduce the $ABTS^+$ cation radical (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid). The experiment was conducted following the procedure described by Choong et *al.* (2007). The $ABTS^+$ cation radical was generated by the reaction of 8 mM ABTS (87.7 mg dissolved in 20 mL of distilled water) and 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 stored in the dark at room temperature for 12 to 16 hours. This $ABTS^+$ solution was subsequently diluted with methanol to obtain a solution with an absorbance of 0.7±0.02 at 734 nm. Specifically, 3.9 mL of the diluted $ABTS^+$ solution was mixed with 100 µL of the sample to be analyzed. After shaking, this mixture was incubated in the dark for 6 minutes (T = 30 ± 2°C). The residual absorbance of the $ABTS^{+\circ}$ radical was then measured at 734 nm using a UV-visible spectrophotometer and was maintained between 20% and 80% of the control absorbance. Measurements were performed in triplicate, and the results were expressed in µmol of Trolox equivalent per liter of extract (µmol TE/L). Using the following concentrations of Trolox: 0, 3.75, 5, 6.25, 10, 11.25, 12.5, 13.75, and 15, a calibration curve was constructed. The inhibition rate (%I) of $ABTS^+$ is expressed by the following formula:

$$\% I = [(A_0 - A_{bextract}) / A_0] \times 100$$

A_0 = absorbance of diluted ABTS,

$A_{bextract}$ = absorbance of diluted ABTS + sample

The resulting curve allowed the antioxidant activity of the different extracts to be expressed as follows:

Concentration or antioxidant activity (µM Trolox-eq) = (%I × fd) / (4.99 × 10)

fd: dilution factor

Extract concentration before dilution = 10 mg/mL

2.2.5. Infrared (IR) Spectroscopy

The principle of infrared spectroscopy is based on the interaction of light with most molecules in the infrared region of the electromagnetic spectrum, resulting in the conversion of this interaction into molecular vibrations. This method allows for the identification of the nature of chemical bonds and the various functional groups present within a molecule. Infrared (IR) spectra were obtained using a Perkin Elmer Spectrum 2 instrument (Figure 1). The 2 mg samples were first dissolved in methanol, and then the solvent was removed to create a uniform film on the cell (mold). The data were recorded over a spectrum ranging from 400 cm^{-1} to 4000 cm^{-1} using Spectrum 2 software (Johnny et *al.*, 2021).

Figure 1 Perkin Elmer Spectrum 2

3. Results

3.1. Extraction Yield

The yield is calculated based on the dry matter mass of the extract obtained by maceration. The results show that the binary mixture of distilled water and ethanol allows for better extraction with a yield of 22.40±0.954 for the PMMm hydroethanolic extract. However, the FMe aqueous extract obtained a low extraction yield with a value of 6.75±0.05 (Table 1).

Table 1 Yields of aqueous and hydroethanolic extracts of FM and PMM

Extracts	**FMe**	**FMm**	**PMMe**	**PMMm**
Yield (%)	6.75±0.05[a]	12.50±0.64[abc]	9.53±1.002[abc]	22.40±0.954[abc]

FMe: Aqueous extract of FM; FMm: Hydroethanolic extract of FM; PMMe: Aqueous extract of PMM; PMMm: Hydroethanolic extract of PMM

3.2. Determination of phytochemicals in aqueous and hydroethanolic extracts of FM and PMM

3.2.1. Spectrophotometric determination of total polyphenols

Total polyphenol content varies from one extract to another. The results of this study show that hydroethanolic extracts are rich in total polyphenols. In particular, the PMMm hydroethanolic extract has a value of 28.5 ± 0.50 mg EAG/g.

3.2.2. Spectrophotometric determination of total flavonoids

The PMMm hydroethanolic extract is exceptionally rich in flavonoids (60.83 ± 0.28bc mg QE/g). All extracts studied contain flavonoids.

3.2.3. Spectrophotometric determination of total tannins

The results of this study reveal the presence of tannins in all extracts studied. The tannin contents are 2.05 mg EAT/g for the FMe extract; 11 mg EAT/g for the FMm extract; 15.23 mg EAT/g for the PMMe extract; and 22.28 mg EAT/g for the PMMm extract. Indeed, the PMMm and FMm extracts are rich in tannins. In contrast, the FMe extract has a low tannin content.

This study shows that hydroethanolic extracts are richer in polyphenols, flavonoids, and tannins than aqueous extracts. In particular, the phytochemical content of the PMMm extract is higher than that of the other extracts (Table II).

Table 2 Content of phytochemicals, including polyphenols, flavonoids and total tannins

Extracts	Polyphenols (mg QE/g)	Flavonoids (mg EQ/G)	Tannins (mg EAT/g)
FMe	7.00± 0.76^{ab}	4.16± 0.00^{abc}	2.05± 0.008^{abc}
FMm	11.00± 0.28^{abc}	6.33± 0.00^{abc}	3.92± 0.016^{abc}
PMMe	ND	ND	ND
PMMm	60.83± 0.28^{bc}	28.5± 0.50^{bc}	22.8± 0.013^{bc}

ND: Not determined

3.3. Results of Antioxidant Activity

3.3.1. DPPH Assay

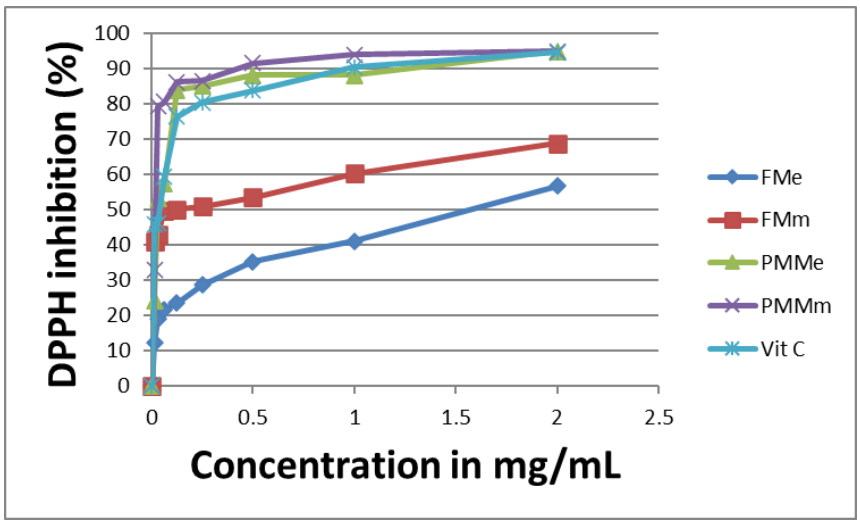

Figure 2 Percentage inhibition of the DPPH radical by FMe, FMm, PMMe, and PMMm extracts

The IC_{50} value for each extract represents the concentration required to reduce 50% of the DPPH in solution.

The results of the evaluation of the antioxidant activity of the FMe, FMm, PMMe, and PMMm extracts, as determined by the DPPH assay, are expressed as the percentage inhibition (I%) of the DPPH radical by the extracts and are presented graphically (I% as a function of concentration) (Figure 2). Ascorbic acid (Vitamin C) is used as a reference antioxidant. The concentration required to inhibit 50% of the DPPH radical (IC_{50}) is 0.04±0.00b mg/mL for ascorbic acid. The lower the IC_{50} value, the greater the antioxidant activity of the extract.

The study revealed significant antiradical activity in all extracts (IC_{50}: 1.57 ± 0.246 mg/mL for FMe; 0.089 ± 0.01 mg/mL for the FMm extract, 0.03 ± 0.01 mg/mL for PMMe, and 0.021 ± 0.001 mg/mL for PMMm).

3.3.2. Antioxidant activity by the ABTS method

The results obtained confirm antioxidant activity in all extracts. This activity is highest for the FMm hydroethanolic extract, which inhibited the ABTS radical by 46.11% at a low concentration (0.92 µM Trolox-eq) (Figure 3).

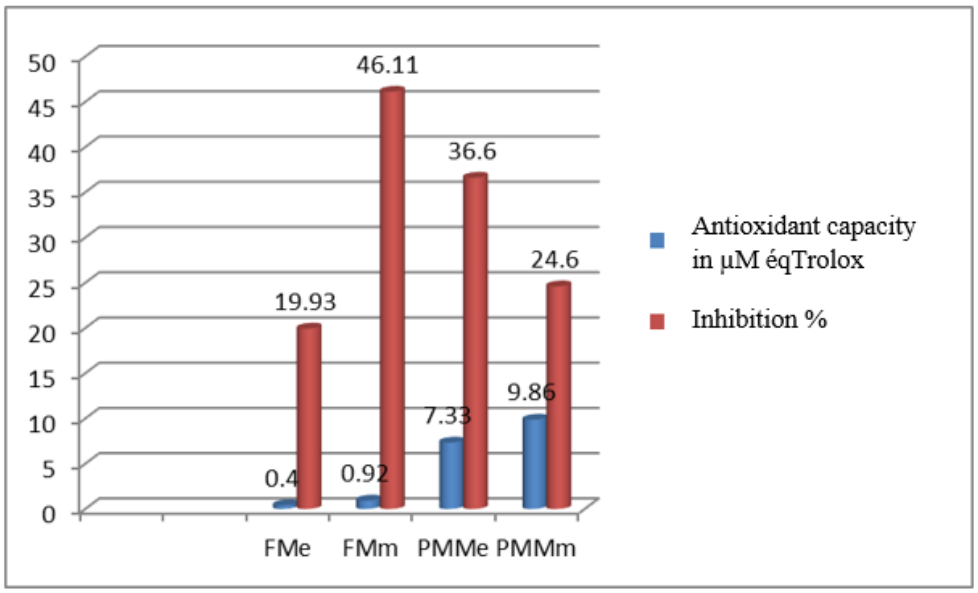

Figure 3 Inhibition Percentage and Antioxidant Capacity of the Extracts as Determined by the ABTS Assay

These two methods yield the same results. Indeed, all extracts exhibit significant antioxidant activity. The PMMm extract demonstrates the highest significant antioxidant activity.

3.4. Infrared Spectroscopy of the Tested Extracts

In flavonoids, the C-O-C bond of the pyran ring (C-ring) exhibits characteristic asymmetric stretching bands between 1030 cm^{-1} and 1150 cm^{-1}.

For flavonoids and tannins, the C-O bands appear mainly in the fingerprint region between 1000 cm^{-1} and 1300 cm^{-1} (Figure 7).

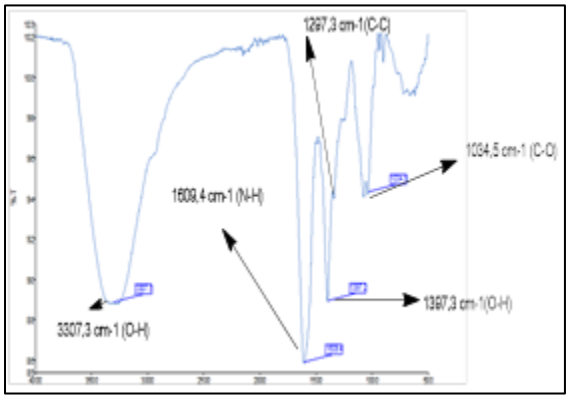

Figure 4 IR spectrum of FMe

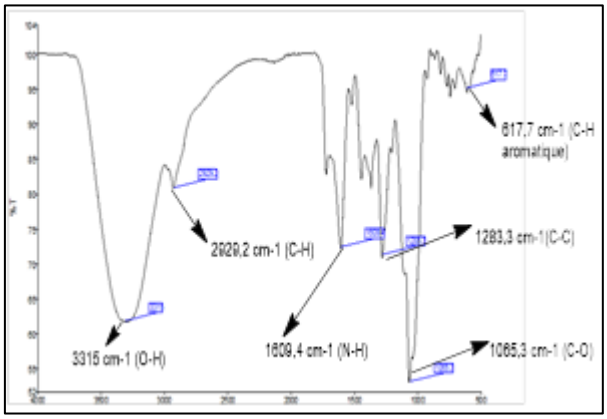

Figure 5 IR spectrum of FMm

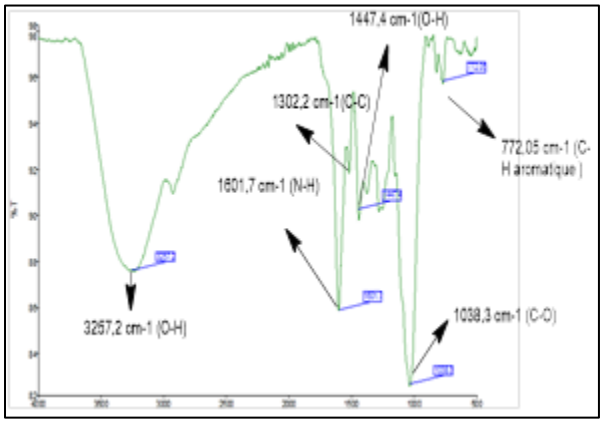

Figure 6 IR spectrum of PMMe

Figure 7 IR spectrum of PMMm

4. Discussion

To evaluate the best method for extracting polyphenols, aqueous and hydroethanolic extractions were performed, yielding the extracts FMe, FMm, PMMe, and PMMm.

Of the two extraction methods, hydroethanolic extraction yielded better results compared to aqueous extraction. However, the highest extraction yield was recorded for the hydroethanolic extract PMMm, with a value of 22.4%.

This variation in yield is influenced by various factors. Factors related to pre-extraction (type of plant part used, origin, particle size, moisture content, drying method, processing level, etc.) as well as those associated with extraction (extraction technique used, type of solvent selected, solvent-to-sample ratio, pH, solvent temperature, and extraction duration) (Azwinda, 2015; Tiwari et *al*., 2011; Su et *al.*, 2006), as well as the location and timing of sample collection, could account for these variations. In this study, the hydroethanolic extract may have a higher yield than the aqueous extract due to the nature of the solvent used for extraction and the composition of the sample.

The combined use of water and an organic solvent such as ethanol can facilitate the extraction of chemical substances that dissolve either in water or in this solvent (Do et *al*., 2014).

In fact, the extraction method that combines water and ethanol tends to yield extracts containing higher levels of polyphenols and flavonoids compared to extraction using water alone, due to the more efficient dissolution of these substances in the mixture. Although water extraction is simpler and less expensive, it may prove less effective for certain compounds that do not dissolve in water (Naima et *al*., 2015; Mohammedi and Atik, 2011; Trabelsi et *al*., 2010). The substances that show a preference for the water-ethanol mixture are those present in lower concentrations.

The polyphenol content was estimated at 7.00 ± 0.76 mg EAG/g in the aqueous FMe extract and at 11.00 ± 0.28 mg EAG/g for the hydroethanolic FMm extract; which was calculated at 60.83 ± 0.28 mg EAG/g for the PMMm hydroethanolic extract.

Quantitative data on flavonoids show that their content was measured at 28.50 ± 0.50 mg EQ/g in the PMMm hydroethanolic extract.

Polyphenols play a protective role, attributed to their antioxidant properties, which are capable of preventing oxidative damage at the molecular and cellular levels, thereby preventing various diseases (such as certain cancers or type 2 diabetes) (Amiot et *al*., 2009).

The positive impact of polyphenols on heart health is partly due to their direct action on blood vessels, particularly on the inner lining of blood vessels (Martin and Andriantsitohaina, 2002; Dina et *al.*, 2019).

As for flavonoids, which are found in various fruits, vegetables, medicinal plants, and other types of foods, these compounds may contribute to the prevention and management of type 2 diabetes (Al-Ishaq et *al*., 2019). Furthermore,

they may enhance insulin sensitivity, promote insulin production, and reduce inflammation—all essential for preventing and managing diabetes.

Chronic treatment with flavonoids (baicalein, flavone, and quercetin) protects endothelial vascular function in animals with hypertension through various potential mechanisms, such as increasing the production and availability of endothelial nitric oxide and lowering blood pressure (Macha and Mustafa, 2005)

Recent studies (Sun et *al*., 2020; Song et *al*., 2018) have revealed that, in general, tannins can influence glucose levels. Indeed, they reduce the risk of diabetes by facilitating glucose absorption and thereby lowering blood sugar levels (Sahakyan et *al.*, 2022). They therefore possess tissue-regenerative properties. These tannins, which are a type of polyphenol found in many plants, may have a positive effect on blood sugar levels, particularly in rats with type 1 diabetes (Omar et *al.*, 2022). They can lower glucose levels while promoting weight gain in rats with insulin deficiency. However, their effectiveness may be less pronounced in rats with type 2 diabetes.

Extracts rich in phenolic compounds possess various biological characteristics, including antioxidant and antidiabetic properties.

The presence of phytochemicals is confirmed by absorption bands visible in infrared spectra: NH for alkaloids, C-O for tannins, and OH for polyphenols and tannins.

Alkaloids may play a crucial role in diabetes management, particularly by stimulating the β-cells of the islets of Langerhans to release an adequate amount of insulin and by increasing the cells' responsiveness to this hormone. This contributes to proper blood glucose regulation (Ajebli et *al.*, 2021)

Tannins, which are polyphenolic compounds found in many plants, were studied by Omar et *al.* (2022) in rats with type 1 diabetes. Their research highlighted the positive effects of tannins on blood sugar levels. These compounds can lower blood glucose levels while promoting weight gain in rats with insulin deficiency. However, their impact appears to be less pronounced in rats with type 2 diabetes.

The search for new sources of natural antioxidants is of interest to many researchers because they are generally considered to have fewer side effects (Moein, 2010).

The extracts studied exhibit significant antioxidant activity. In fact, the PMMm hydroethanolic extract (0.021±0.001) and the PMMe extract (0.03±0.01) outperform vitamin C (0.04±0.00), the reference compound.

5. Conclusion

Traditional medicine has long been widely practiced throughout Côte d'Ivoire, particularly in Korhogo. Traditional medicine practitioners often recommend herbal preparations to treat various diseases, such as diabetes.

The main objective of our study was to evaluate the antioxidant potential and quantify the phytochemicals in two traditional herbal medicines used for the treatment of diabetes mellitus in Korhogo.

The phenolic compounds (polyphenols, flavonoids, and tannins) contained in these traditional medicines constitute a major group of compounds that act as primary antioxidants against free radicals (Ayoola et *al.*, 2008). This confirms the antioxidant potential of these traditional medicines.

The above observations suggest that the widespread use of these traditional medicines in the traditional management of diabetes is likely linked to their relatively high content of polyphenolic compounds.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest to be disclosed.

Author contributions Conceptualisation and literature acquisition: [Author 1]. Original draft preparation: [Author 1, Author 2]. Critical revision: [Author 3]. Supervision and final approval: [Author 4].

All authors approved the submitted version and agree to be accountable for all aspects of the work.

Data availability statement No new datasets were generated or analysed during the preparation of this review. All data cited are available in the published literature referenced herein.

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Editorial note: The manuscript was revised for scientific English, consistency of units, formatting of tables and figures, and bibliographic harmonization.