GSC Biological and Pharmaceutical Sciences
eISSN: 2581-3250 CODEN (USA): GBPSC2
Cross Ref DOI: 10.30574/gscbps
Journal homepage: https://gsconlinepress.com/journals/gscbps/

(RESEARCH ARTICLE)

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# Phytochemical screening and acute toxicity of traditional drugs FM, MCHIM and MCHIM2

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GSC Biological and Pharmaceutical Sciences, 2026, 35(02), 266–276

Publication history: Received on 09 April 2026; revised on 22 May 2026; accepted on 25 May 2026

Article DOI: https://doi.org/10.30574/gscbps.2026.35.2.0191

## Abstract

Diabetes mellitus is a very common disease worldwide. Although pharmaceutical treatments are available in pharmacies in Côte d'Ivoire, many patients also turn to herbal remedies. In the city of Korhogo, located in northern Côte d'Ivoire, various traditional remedies are prepared from medicinal plants, whose chemical properties remain largely unknown. This research aims to identify the families of chemical compounds and to assess the non-toxicity of plant-based remedies. A phytochemical screening was conducted using methods such as thin-layer chromatography and tube tests, including staining and precipitation techniques. Acute toxicity assessment was performed using OECD Method 423. The results obtained through thin-layer chromatography reveal that traditional remedies contain polyphenols, sterols, flavonoids, tannins, polyterpenes, and alkaloids. These various categories of chemical compounds have already demonstrated efficacy against diabetes. All tested compounds are non-toxic at 2000 mg/kg body weight. The presence of these groups of compounds, particularly alkaloids, may explain the antidiabetic effect attributed to them by traditional medicine practitioners in the Korhogo region.

**Keywords:** Phytochemical Screening; Acute Toxicity; OECD 423; Korhogo; Traditional Drugs

## 1. Introduction

Diabetes is one of the oldest diseases known to humankind, and its destructive impact is growing daily, reaching epidemic proportions (Wild et *al*., 2004). This condition is linked to a dysfunction in the metabolism of carbohydrates, proteins, and lipids, resulting from a total or partial lack of insulin action (Rodier, 2001).

Several indicators suggest that the main risk factors driving the rise in diabetes mellitus include urbanization, lifestyle changes, lack of physical activity, obesity, and the psychosocial stress that affects people daily (Whiting et *al.*, 2011).

Diabetes is showing a very marked increase; according to data from the WHO and the IDF (2016), 422 million people worldwide have diabetes, a figure that could reach 552 million by 2030—one in ten people—and half of those with diabetes remain undiagnosed. Diabetes is also notable for the severity of the complications it causes. In fact, it is the leading cause of end-stage renal disease, blindness, and lower limb amputations; furthermore, it is the sixth leading cause of death (Hanae, 2018).

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In Côte d'Ivoire, the number of adults aged 20 to 79 with diabetes was 42,700 in 2000, 406,500 in 2011, and reached 529,200 in 2024.

Diabetes care is extremely costly for people in developing countries. These significant expenses, which people living in poverty struggle to afford, drive them toward traditional treatments. In fact, the World Health Organization encourages the advancement of research, including on methods that incorporate traditional care using medicinal plants (WHO, 1995). Recently, diabetologists have realized that incorporating treatments based on plant extracts is essential for improving diabetes management (June et *al*., 2015; Pareek et *al*., 2009).

The field of medicinal plant research has grown significantly in recent years, and interest in the use of natural remedies for treating diabetes is increasing worldwide. Existing research reveals that there are more than 400 plant species with antidiabetic properties (Grover et al., 2002). Although some of these species are widely recognized in traditional medicine, many have yet to be scientifically validated (Kolling et *al*., 2010). Scientific research on traditional herbal remedies for diabetes can provide important insights for the development of new drugs and alternative approaches (Pan et *al*., 2013).

Multiple herbal remedies for diabetes exist in Korhogo, a city located in northern Côte d'Ivoire, such as FM, PMM, PN, MChim, and MChim2. However, scientific data are lacking to confirm their chemical composition, efficacy, and safety. Given the prevalence of diabetes, this study aims to identify the families of compounds present in these remedies and to assess the acute toxicity of the herbal remedies from Korhogo, which are used in the traditional management of this condition.

## 2. Materials and Methods

### 2.1. Plant Material

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. The herbal medicine Mchim is a liquid remedy made from plant extracts, primarily *Chrysanthellum indicum*, dark green in color and coded as Mchim. It was purchased in Korhogo. The herbal medicine Mchim2 is a liquid remedy made from extracts of the leaves of *Cnestis ferruginea, Anogeissus leiocarpus*, and *Aloe vera*. It was purchased from a traditional medicine practitioner in January 2024 in the city of Korhogo.

### 2.2. Methods

#### *2.2.1. Preparation of FMe, FMm Powders*

The traditional FM remedy consists of leaves of *Paraclinanthus laxiflorus*. These leaves were dried at room temperature for approximately two weeks. Once dried, they were ground into powder using a Binatone Blender -BLG-450P MK2-1.5 L electric blender. The resulting powder was then weighed and carefully stored in jars for our various chemical and biological analyses. Mchim and Mchim2 were placed in an oven for 24 hours at a temperature of 50 °C. The extracts obtained were used to perform our various analyses.

#### *2.2.2. Extraction of FMe and FMm*

For FMe 100 g of powder were dissolved in 1 L of distilled water (Coulibaly et *al*., 2011), while for FMm, a mixture of 0.7 L of ethanol and 0.3 L of distilled water (70/30) was used (N'guessan et *al*., 2025; Able et *al*., 2025). The mixture was homogenized by magnetic stirring for three hours at room temperature, followed by a 24-hour maceration period at the same temperature for subsequent processing. After stirring for an additional 5 minutes and passing the mixture through a sieve, the supernatant was filtered using cotton placed over a funnel. The resulting filtrate was centrifuged at 10,000 rpm (using a Jouan TH 12 centrifuge) for 15 minutes at room temperature. The collected solution was then placed in an oven at 50 degrees Celsius. These four steps, performed separately, yield the crude extracts FMe and FMm.

#### *2.2.3. Phytochemical Screening*

The detection of specific metabolites has made it possible to identify chemical groups such as alkaloids, polyphenols, sterols, and triterpenes present in herbal remedies

### 2.3. Screening for alkaloids in test tubes

Alkaloids were identified using Dragendorff's reagent (potassium iodo-bismuthate reagent). A volume of six (6) mL of the solution was evaporated to dryness. This residue was dissolved in 6 mL of 60% ethanol. The addition of two drops of Dragendorff's reagent to this alcoholic solution produced a precipitate or an orange tint (Békro et *al*., 2007).

### 2.4. Detection of alkaloids using Dragendorff's reagent in TLC

The stationary phase of the chromatographic plate consists of silica gel 60 F 254, supported on an aluminum base. The solvent used for development (or mobile phase) is a mixture of ethyl acetate and methanol in a 90:10 (v/v) ratio . Using a capillary tube, a few drops of each specific extract are applied at points spaced one centimeter apart on the baseline, drawn one centimeter from the bottom of the plate. Once dry, the plates are placed in a tank containing a solvent mixture appropriate for each extract. After the phytochemicals have been extracted, the plate is dried. After being sprayed with Dragendorff's reagent, any orange or red stains that appear indicate the presence of alkaloids (Lagnika, 2005).

### 2.5. Screening for Sterols and Polyterpenes by TLC

Sterols and polyterpenes were detected using the Liebermann method. A volume of five (5) mL of the extract was completely evaporated using a sand bath. The resulting residue was dissolved at high temperature in 1 mL of acetic anhydride; then, 0.5 mL of concentrated sulfuric acid was added to the mixture. The appearance of a purple or violet ring at the interface, which then changed to blue and then to green, indicated a positive reaction (Békro, 2007).

### 2.6. Detection of polyphenols in a test tube

To detect polyphenols, 2 mL of each extract solution was added to a few drops of a 2% (w/v) aqueous ferric chloride solution. The appearance of a blue-black or green-black color indicates the presence of polyphenols (Ladyguina et al., 1983; Békro et *al.*, 2007).

### 2.7. Detection of polyphenols by TLC

The extracts were dissolved in diethyl ether and examined on a silica gel plate. The solvent used for migration was a mixture of acetic acid and chloroform in a 1:9 (V/V) ratio. Once the plate was developed, the spots were visible under UV lamps at 254 nm and 365 nm (Harbone, 1998). The presence of polyphenols is indicated by a blue-black or green-black color.

### 2.8. Determination of Saponins by Tube (Foam Index)

The method for detecting saponins involves dissolving 2 grams of powder in 100 milliliters of distilled water. The solution is then heated to boiling for 30 minutes. Once cooled, the solution is filtered, and the filtrate is adjusted to a volume of 100 milliliters with distilled water. The stock solution is divided into 10 test tubes (1 mL, 2 mL, 3 mL, 4 mL, 5 mL, 6 mL, 7 mL, 8 mL, 9 mL, and 10 mL). Each tube is topped up with distilled water to a total of 10 milliliters. The solution is vigorously shaken for 15 seconds, then allowed to stand for 15 minutes. Finally, the height of the foam in each tube is recorded. The presence of saponins is determined as follows:

- No foam = negative test

- Foam less than 1 cm = weakly positive test
- Foam 1–2 cm = positive test
- Foam more than 2 cm = strongly positive test (Trease and Evans, 1987).

The foam index (I) is calculated using the following formula: I = 1000/N, where N is the number of the tube in which the foam height is 1 cm. A foam index greater than 100 indicates the presence of saponins.

### 2.9. Screening for saponins (steroidal and triterpenic) by TLC

A qualitative method was performed using thin-layer chromatography (TLC) on aqueous and hydroalcoholic extracts of MS. The stationary phase used was Merck aluminum, Silica Gel 60 F 254, while the most effective mobile phase was a ternary mixture [$CH_2Cl_2$/AcOEt/acetic acid (7/2/1)]. A few drops of each specific extract are applied using a capillary tube at points spaced 1 cm apart on the starting line, which is drawn 1 cm from the bottom of the chromatography plate. Once dry, the plates are placed in a chamber filled with a solvent mixture suitable for each extract. After the phytochemicals have eluted, the plate is dried again. Development was performed using an ethanol solution containing 1% $SbCl_3$ (Bekro et *al*., 2007).

### 2.10. Testing for coumarins in a test tube

As part of the coumarin study, 2 mL of the 5% infusion is poured into a test tube, then 3 mL of NaOH (10%) is added. Upon thoroughly stirring the resulting solution, a yellowish tint appears, indicating the presence of coumarins (Diallo, 2000).

### 2.11. Detection of coumarins by thin-layer chromatography (TLC).

The presence of coumarins was detected using Godin's reagent. The chromatographic plates are treated successively with this reagent, followed by a 10% ethanol solution of $H_2SO_4$ (5.5 mL of sulfuric acid with a density of d = 1.84 mixed with 95 mL of ethanol). They are then heated to 100°C until blue-colored spots become visible (Lagnika, 2005).

### 2.12. Acute Toxicity

*2.12.1. Assessment of the acute toxicity of the extracts in mice*

This study was conducted due to the possible presence of contaminants or undesirable substances in the various batches. During the tests, we followed OECD Protocol 423 (OECD, 2001). Each phase involved three mice weighing between 19 and 26 grams. For the first dose, we selected values of 50, 300, and 2000 mg/kg body weight (Figure 1).

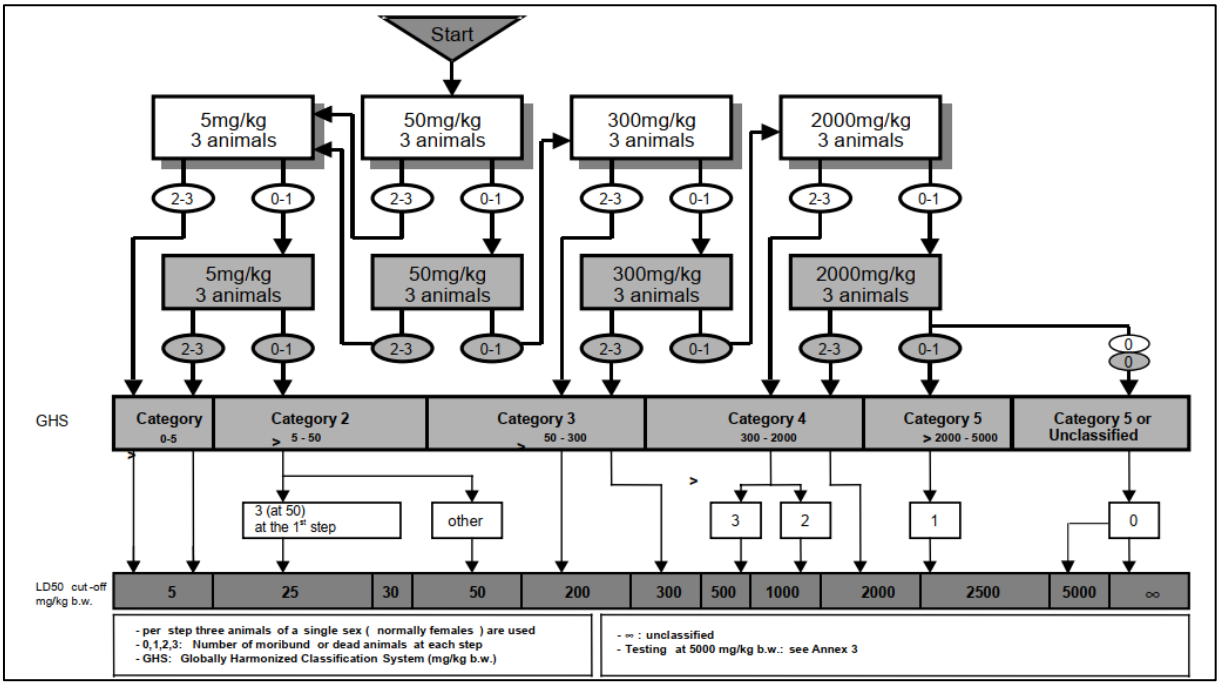


**Figure 1** Test design with an initial dose of 5 mg/kg body weight (OECD 423)

The toxicity level selected was the one expected to result in mortality in some of the treated animals. The selection of female animals is required by the relevant directive, due to the high sensitivity of mice. Each group of animals was force-fed with a defined observation interval (every 30 minutes). When the treated group showed no deaths or other signs of toxicity (such as aggression, mobility, alertness, stool consistency, vomiting, mortality, etc.), the next group was treated. The animals were monitored on the first day, then regularly every day for 14 days.

### 2.13. Method of Administration

After fasting the mice for 24 hours, they were fed via a rigid tube. This method is based on a basic concept. It involved holding the mouse firmly to prevent any movement during administration. Once the tube is inserted into the animal's throat, the extract is also administered slowly (Silué et al., 2018). The process of treating the mice for the trials was carried out as follows: the animals were divided into 5 groups of 3 mice that received the following treatment:

- Group I (control): the animals received distilled water.
- Group II (treated): the animals received 50 mg/kg body weight of extract
- Group III (treated): the animals received 300 mg/kg body weight of extract.

- Group IV (treated): the animals received 2000 mg/kg body weight of extract

After treatment, the mice were monitored individually every hour on the first day and daily for a period of two weeks (14 days). The mice's behavior and clinical signs were recorded throughout the study. The animals' weight gain was assessed every three days. The study lasted two weeks (14 days).

## 3. Results

### 3.1. Yield

The results show that the highest yields were obtained by extraction using the binary solvent mixture of distilled water and ethanol. Furthermore, the FMm extract (12.50±0,64%) exhibited a higher yield compared to the other extracts (Table 1).

**Table 1** Yields of the extracts studied

| Extracts | FMe | FMm | Mchim | Mchim2 |
|---|---|---|---|---|
| Extraction efficiency % | 6.75±0.05 | 12.50±0.64 | 10.54±0.01 | 9.60±0.02 |

*3.1.1. Phytochemical Screening*

The results show that all the extracts studied contain polyphenols, flavonoids, tannins, sterols, and terpenes. With the exception of the Mchim extract, these extracts also contain alkaloids, and coumarins and saponins are absent in the majority of the extracts.

**Table 2** Phytochemical composition of the different extracts

| Compounds families | Alkaloids | Polyphenols | Flavonoids | Tannins | Saponins | Sterols | Terpenes | Coumarins |
|---|---|---|---|---|---|---|---|---|
| FMe | + | + | + | + | - | + | + | - |
| FMm | + | + | + | + | - | + | + | + |
| Mchim | - | + | + | + | + | + | + | + |
| Mchim2 | + | + | + | + | + | + | + | - |

*3.1.2. Acute Toxicity*

The following results were obtained from studies conducted over a 24-hour period and a 14-day interval (for delayed effects of the extracts). Neither the control mice nor those treated with various extracts showed any signs of immediate toxicity or death.

Regarding the control mice ($H_2O$), no evidence of immediate toxicity was observed. The mice were in excellent condition, with normal skin and fur, as well as eyes and mucous membranes in perfect condition. Furthermore, no abnormal behavior was noted, such as diarrhea, excessive salivation, fatigue, aggression, or drowsiness. Their heart rates were normal, and they maintained good mobility. No deaths were recorded.

Regarding the mice that received the various extracts, the following observations were made: the mice exhibited a physical condition similar to that of the control group, with no signs of toxicity or adverse effects associated with the FMe, FMm, Mchim, and Mchim2 extracts. In conclusion, no adverse effects were observed in the groups that received the extracts, all of which exhibited a health status similar to that of the control mice throughout the study. The mortality rate was therefore zero percent (0%) during the 14-day experimental period (Table 3).

**Table 3** Observations over 24 hours and 2 weeks in the control and treated groups of mice

| Observations | Sample Lot | | Experimental batch | |
|---|---|---|---|---|
| | 6 hours | 12 hours | 6 hours | 12 hours |
| Skin and fur | Normal | Normal | Normal | Normal |
| Eyes | Normal | Normal | Normal | Normal |
| Mucous membranes | Normal | Normal | Normal | Normal |
| Diarrhea | None | None | None | None |
| Salivation | None | None | None | None |
| Lethargy | None | None | None | None |
| Heart rate | Normal | Normal | Normal | Normal |
| Aggression | None | None | None | None |
| Drowsiness | None | None | None | None |
| Feeding | Yes | Yes | Yes | Yes |
| Mobility | Yes | Yes | Yes | Yes |
| Mortality | None | None | None | None |

The extracts were administered at doses of 5, 50, 300 and 2000 mg/kg body weight. Following administration of the FMe extract, the mean weight of the mice, which was 27.33 g, increased to 29.50 g on the 14th day (Figure 2); following administration of the FMm extract, the mean weight increased from 27.33 g to 29.50 g on the 14th day (Figure 3). Following administration of the Mchim extract at these various doses to the treated groups of mice with an average weight of 27.66 g, the average weight of the mice on the 14th day was 28.33 g (Figure 4). Finally, for the Mchim2 extract, the average weight of the mice, which was 27.33 g, increased to 29.50 g on the 14th day (Figure 5).

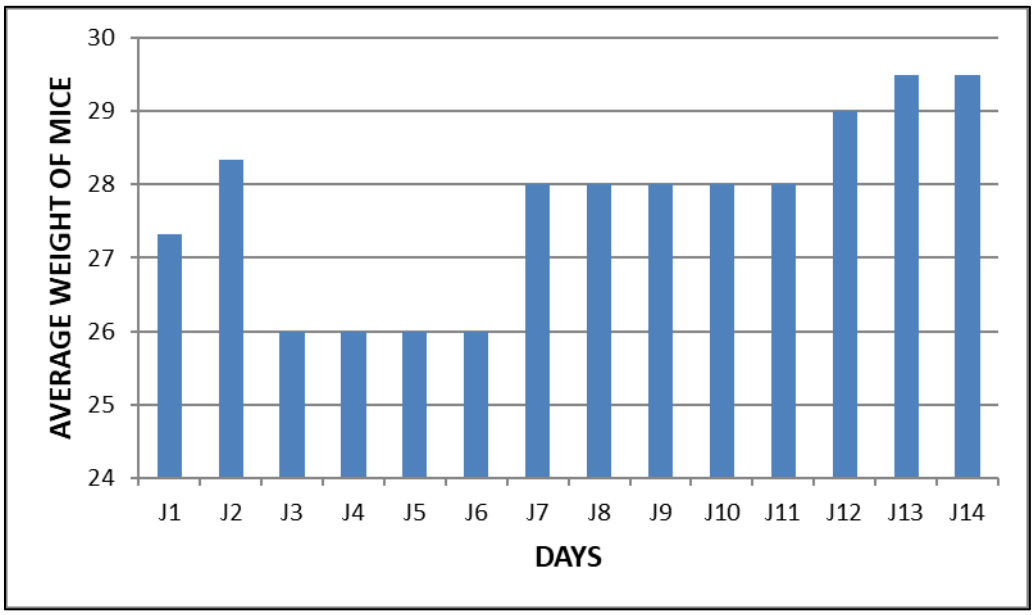


**Figure 2** Changes in the average weight of mice following administration of the Fme extract

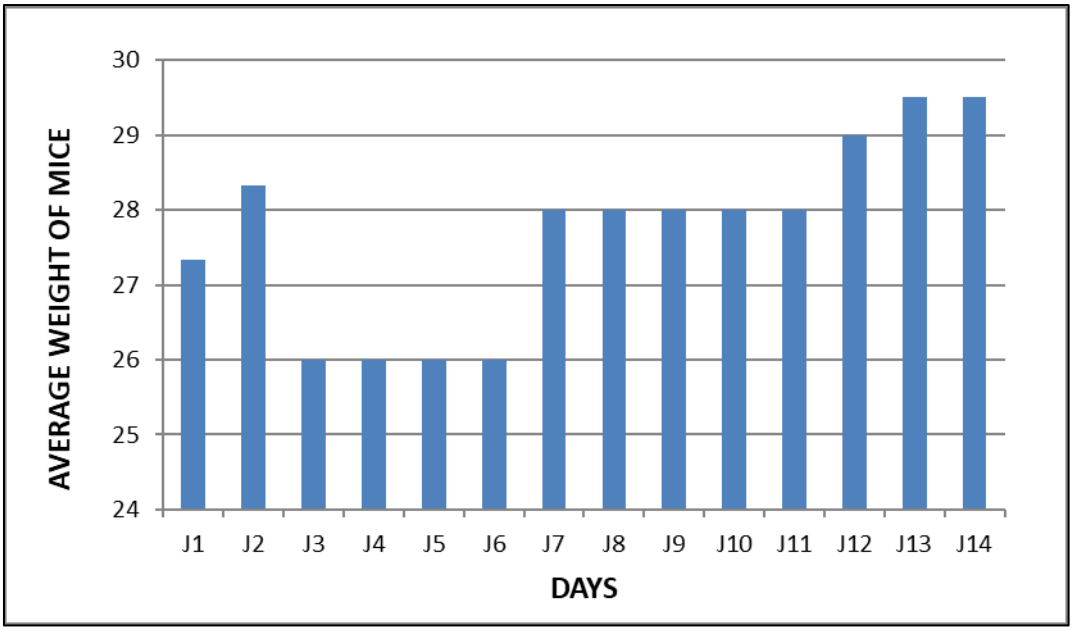


**Figure 3** Changes in the average weight of mice following administration of the FMm extract

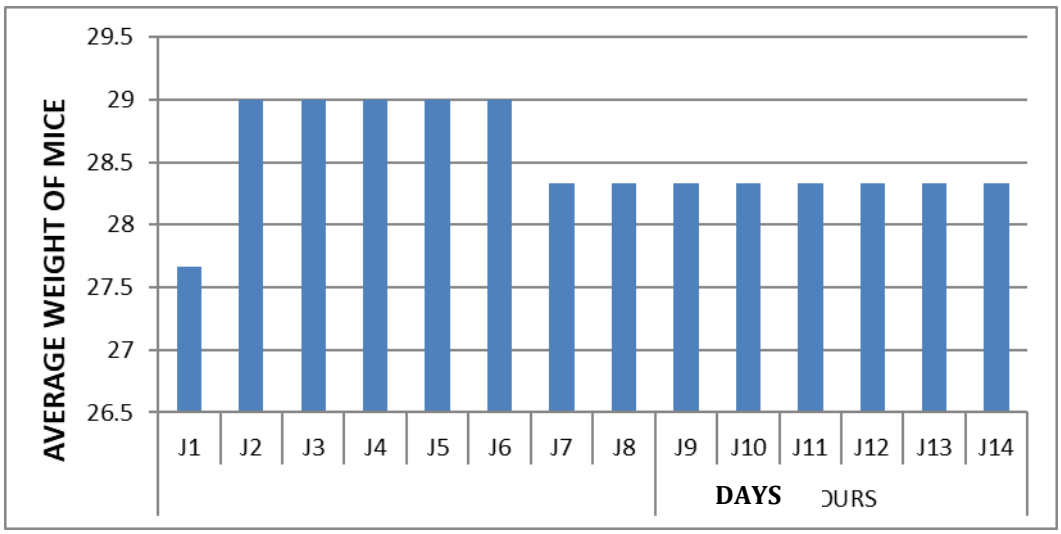


**Figure 4** Changes in the average weight of mice following administration of the Mchim extract

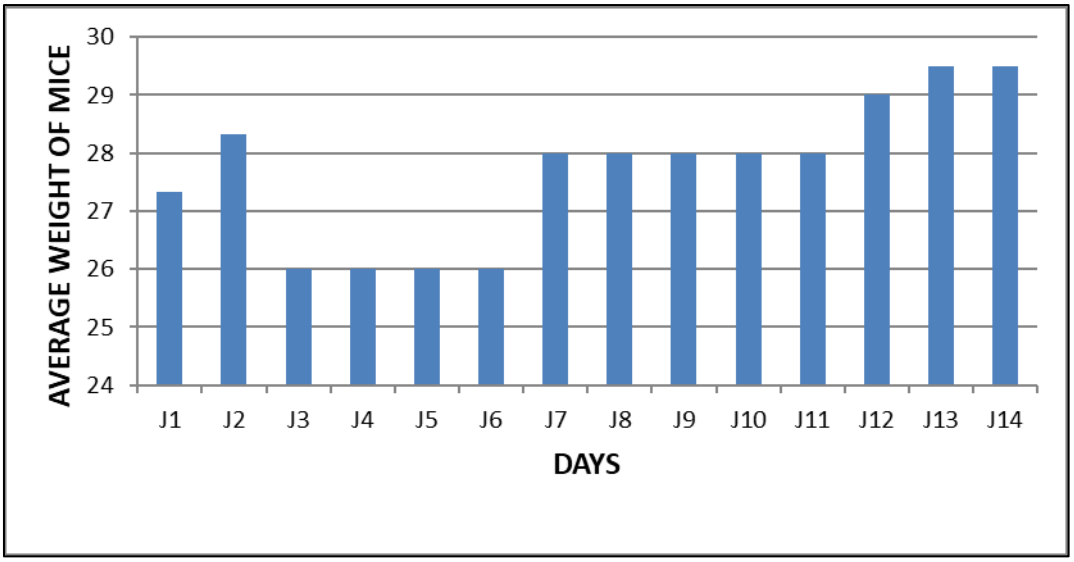


**Figure 5** Changes in the average body weight of mice following administration of the Mchim2 extract

## 4. Discussion

In this study, it was found that extraction using a mixture of water and ethanol was more effective than extraction using water alone. Furthermore, the FMm extract yielded a higher yield compared to the other extracts. This difference in yields could be related to the nature of the solvent used for extraction, as well as to the composition of the sample (N'guessan et *al.*, 2025a).

Phytochemical analysis is very useful for studying certain bioactive compounds found in medicinal plants (Akinsola et *al.*, 2022). Phytochemical analysis revealed the presence of sterols, terpenes, polyphenols, flavonoids, and tannins in all the extracts studied

Flavonoids are found in a variety of fruits, vegetables, medicinal plants, and other types of foods. These are substances that may help prevent and manage type 2 diabetes (Al-Ishaq et *al.*, 2019). Furthermore, they may enhance insulin sensitivity, promote insulin production, and reduce inflammation, all of which are key factors in the prevention and management of diabetes. Tannins are polyphenols found in various plants; they may have a positive impact on blood glucose levels, particularly in rats with type 1 diabetes (Omar et *al.*, 2022). They may lower blood sugar levels while promoting weight gain in insulin-deficient rats. However, their effect may be less pronounced in rats with type 2 diabetes.

As for sterols, they are plant-derived compounds found in various foods. They may help lower cholesterol levels, and certain effects on blood glucose levels have been noted. Terpenes, for their part, may influence blood sugar levels and play a role in regulating carbohydrate metabolism (N'guessan et *al.*, 2025a). As for alkaloids, these are generally nitrogen-containing chemical compounds derived from natural sources that can produce effects on the body, as well as sedative and analgesic effects. They may be beneficial in reducing symptoms of depression and anxiety. Due to their stimulating properties, which can cause agitation linked to cellular and nervous disorders, alkaloids can become dangerous if consumed in excess.

*Chrysanthellum indicum* is a medicinal plant rich in flavonoids and saponins (Cissé et *al.*, 2019). This composition gives it beneficial effects on the circulatory system. It helps reduce heavy legs and supports liver function after overeating (Honoré-Thorez, 1985). In Burkina Faso, this plant is recognized for its antioxidant effects, and recent studies have shown that it is used to treat kidney problems related to oxidative stress (Guenne et *al.*, 2012). Other research has demonstrated that its extracts are used to combat gallstones, venous problems, and circulatory disorders in the lower limbs (Mevy et *al.*, 2012).

Methanol-based extracts of the *Cnestis ferruginea* root possess analgesic and anti-inflammatory effects, likely attributable to peripheral and central mechanisms involving the inhibition of the release or action of vasoactive substances such as histamine, serotonin, kinins, and prostaglandins. This plant contains anthraquinone glycosides,

sterols, phenolic compounds, tannins, alkaloids, saponins, and flavonoids, and is considered non-toxic (Ishola et *al*., 2011).

*Anogeissus leiocarpus* contains alkaloids, glycosides, phenols, steroids, tannins, ellagic acids, anthraquinones, saponins, and flavonoids (Mann et *al.*, 2008). This plant exhibits antioxidant, antiangiogenic, and antitumor properties and is used in the treatment of colorectal cancer (Hassana et *al*., 2018). Its leaves are also used in Nigeria and Guinea as an antimalarial remedy (Adjanohoun et *al*., 1991; Bhat et *al*., 1990). *A. leiocarpus* also possesses antiprotozoal and antileishmanial properties (Attioua et *al*., 2011). *Aloe vera* is a plant known for its antibacterial, antiviral, and anticancer actions, as well as its immunoregulatory and hepatoprotective properties (Gao et *al.*, 2019).

## 5. Conclusion

The present study focused on the phytochemical characterization and acute toxicity evaluation of traditional antidiabetic herbal remedies (FM, Mchim and Mchim2) used in the Korhogo region of Côte d'Ivoire. The phytochemical screening revealed the presence of several bioactive compounds, including polyphenols, flavonoids, tannins, sterols, terpenes, and, in most cases, alkaloids. These secondary metabolites are well known for their potential involvement in glycemic regulation and may justify the traditional use of these remedies in the management of diabetes.

The extraction yields indicated that hydroethanolic solvents were more effective than aqueous extraction alone, highlighting the importance of solvent polarity in the recovery of bioactive compounds. Furthermore, the acute toxicity study conducted according to OECD guideline 423 demonstrated that all tested extracts were non-toxic at a limit dose of 2000 mg/kg body weight, with no mortality or significant behavioral changes observed in treated animals. This suggests a relatively high margin of safety for these herbal preparations under the tested conditions.

However, despite these promising findings, this study presents certain limitations. The work was limited to qualitative phytochemical analysis and acute toxicity assessment, without evaluating the specific antidiabetic activity or identifying the active compounds responsible for the observed effects. Therefore, further investigations are required, including in vivo antidiabetic activity studies, chronic toxicity assessments, and isolation and characterization of active constituents.

In conclusion, the studied traditional remedies appear to be rich in bioactive compounds and relatively safe at high doses, supporting their continued use in traditional medicine. Nevertheless, rigorous pharmacological and toxicological studies are necessary to validate their efficacy and ensure their safe integration into modern therapeutic practices.

## Compliance with ethical standards

### *Acknowledgments*

We would like to thank Peleforo GON COULIBALY University (Ivory Coast) for facilitating the implementation of this project

### *Disclosure of conflict of interest*

The authors declare no conflicts of interest.

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