International Journal of Chemistry Studies
www.chemistryjournal.in
ISSN: 2581-348X
Received: 07-04-2026, Accepted: 08-05-2026, Published: 23-05-2026
Volume 10, Issue 2, 2026, Page No. 55-60

Phytochemical study using HPLC-ESI-QTOF and FT-IR, antioxidant activity of aqueous and hydroethanolic extracts of three traditional medicines PN, MChim, MChim2

Akoubet-Ouayogode Aminata[1], N'guessan Ocho Esther[2], Kablan Ahmont Landry Claude[2], Kablan Richmond Jean - François[3], Adiko N'dri Marceline[1]

[1] Department of Analytical, Faculty of Pharmaceutical and Biological Sciences, Inorganic and General Chemistry, Food Technology, Félix Houphouët-Boigny University, Abidjan, Ivory Coast

[2] Department of Mathematics-Physics-Chemistry, UPR of Organic Chemistry, UFR of Biological Sciences, Peleforo GON Coulibaly University, Korhogo, Ivory Coast

Corresponding Author: Kablan Ahmont Landry Claude

Abstract

Diabetes is a major health issue in Ivory Coast. Traditional remedies, often made from plants, are used to treat this condition. However, the chemical composition of these remedies and their ability to fight free radicals remain poorly understood. This study aims to evaluate antioxidant activity by DPPH and ABTS tests, to identify the families of compounds present in the extracts by infrared and to carry out a chemical study by HPLC-ESI-QTOF-MS/ MS for the traditional medicine Mchim. All three extracts exhibit significant free radical scavenging activity, with an inhibitory concentration at 50% (IC_{50}) of 2.01 ± 0.017 mg/ mL for the PN extract, 0.12 ± 0.02 mg/mL for the Mchim extract, and 0.097 ± 0.005 mg/ mL for Mchim2. At a concentration of 1.59 µM Trolox eq, the Mchim extract inhibited 50.89% of the Abts radical. The amount of polyphenols is higher in the Mchim extract (7.67 ± 0.28 mg GAE/g) compared to the PN extract (0.67 ± 0.28 mg GAE/g). HPLC-ESI-QTOF-MS/MS analysis revealed the presence of N1-acetylspermin in Mchim. Research shows that these traditional medicines possess antioxidant activities. which could explain their use in the treatment of diabetes.

Keywords: Traditional medicine, antioxidant activity, FT-IR, HPLC-ESI-QTOF-MS/ MS, diabetes

Introduction

Diabetes is a chronic condition that occurs when the pancreas does not produce enough insulin or when the body fails to effectively use the insulin produced (WHO, 2015). Currently, this disease is classified as a pandemic by the World Health Organization (WHO). It is among the most common non-communicable diseases worldwide, with approximately 463 million people affected in 2019 (FID, 2019). Expenditure related to diabetes in Africa is considerable, reaching approximately 23% of the total health budget (FID, 2019) [12]. In Côte d' Ivoire, diabetes is a major public health issue due to its high prevalence (6.2%), which equates to 700,000 individuals affected in the population (AIP, 2019).

Diabetes treatments are expensive for these low - resource populations. These high costs, along with the distance to healthcare facilities for some people who struggle to access modern medications, are pushing patients to turn to traditional medicines. In this context, the WHO encourages intensified research into avenues that also include traditional treatments based on medicinal plants.

In the city of Korhogo, located in northern Ivory Coast, herbal treatments such as PN, Mchim and Mchim2 are available to people with diabetes. The manufacturers of these remedies claim they may help regulate blood sugar levels. However, little scientific data exists to confirm their chemical composition and antioxidant activity. It is within this framework that this research was conducted with the aim of identifying the bonds of families of phytochemical compounds by infrared and finally to evaluate the antioxidant activity as well as the structure of a molecule present in the extract from Mchim by mass spectrometry.

Materials and methods

1. Plant material

PN

The plant material is composed of:

The PN powder was purchased from a manufactured goods herbalist located in the district of Korhogo in the North of Ivory Coast in December 2023. According to the herbalist, this powder is mainly made up of the fruits of *Ficus vallis-choudae.*

The phytomedicine Mchim is a liquid remedy made from plant extracts. It is primarily composed of *Chrysanthellum. indicum,* of dark green colour coded Mchim. It was bought in Korhogo.

The phytomedicine Mchim2 is a liquid remedy based on extracts of *Cnestis leaves ferruginea, Anogeissus leiocarpus* And of *Aloe vera.* It was purchased from a traditional medicine practitioner in January 2024 in the city of Korhogo

2. Methods

Mchim and Mcim2 powder

The PN powder was weighed and carefully placed in a container. It was subsequently used for various chemical tests. Mchim and Mchim2 in liquid form were incubated for 24 hours at a temperature of 50 °C. The resulting extracts were used to perform our various analyses.

3. Antioxidant activities

DPPH Method

To measure the antioxidant potential of the extracts, the method proposed by N' guessan *et al.*, (2025 b) was adopted. DPPH is dissolved in pure ethanol to create a 0.3 mg/ mL solution. 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 are prepared in pure ethanol. In sterile, dry tubes, 2.5 mL of plant extract is mixed with 1 mL of the DPPH solution. After shaking, the tubes are placed in a dark place for thirty minutes. Next, the absorbance of the mixture is measured at 517 nm, using as a blank a solution composed of 2.5 mL of pure ethanol and 1 mL of DPPH. Vitamin C (ascorbic acid) serves as a positive control. The following formula is used to determine the percentages of DPPH:

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

I: Percentage of inhibition; A _b : Absorbance of the blank; A _e : Absorbance of the sample

The concentrations to capture 50% (IC_{50}) of DPPH. are determined on the graphs illustrating the percentage of DPPH inhibition as a function of extract or vitamin C concentrations.

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

This method is based on the ability of substances to reduce the ABTS+ radical cation (2,2-azinobis-3-ethylbenzothiazoline-6-sulfonic acid). The experiment was carried out according to the protocol established by Choong *et al.*, 2007 [6]. The ABTS+ radical cation was generated by a reaction between 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 ratio of 1: 1 (v/v). Subsequently, the mixture was placed in the dark at room temperature for 12 to 16 hours. This ABTS+ solution was then diluted with methanol to obtain a solution with an absorbance of 0.7±0.02 at 734 nm. Indeed, a 3.9 mL sample of this diluted ABTS+ solution was combined with 100 µL of the compound to be evaluated. After mixing, the mixture was incubated in the dark for 6 minutes (T=30±2°C). Subsequently, the residual absorbance of the ABTS+° radical was measured at 734 nm using a UV-visible spectrophotometer, and this value is expected to be between 20% and 80% of the absorbance of the blank. The measurements were carried out in triplicate, and the results were expressed in µmol of Trolox equivalent per liter of extract (µmol TE/L). From the following concentrations : 0-3. 75-5-6. 25-10-11. 25-12. 5-13. 75-15 of Trolox, a calibration curve was created. The calculation of the inhibition rate (%I) of ABTS+ is established by the following formula:

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

A_0 = absorbance ABTS dilute,

Extracted ABS = absorbance of diluted ABTS + sample

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

Concentration or antioxidant activity (µM eqTrolox) = (%I x fd)/ (4.99 x 10)

Fd : dilution factor

Extract concentration before dilution = 10 mg/mL

Infrared (IR) Spectroscopy

The concept of infrared spectroscopy is based on the absorption of light by most molecules in the infrared region of the electromagnetic spectrum, which results in molecular vibrations. This method is used to identify the nature of chemical bonds as well as the different functions present within a molecule. Infrared spectra were obtained using a Perkin Elmer Spectrum 2 instrument. 2 mg samples were prepared by dissolving them in methanol, then the solvent was removed to create a homogeneous film on the cell. Information was collected over a frequency range of 400 cm^{-1} to 4000 cm^{-1} using Spectrum 2 software. (Johnny *et al.,* 2021).

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

The recently developed HPLC-ESI-Q-TOF-MS system has become an essential tool for examining complex systems of chemical substances (Han *et al.,* 2012 [14]). This device combines different analytical methods:

- HPLC provides a fast and efficient approach for the analysis of samples and the separation of their constituent elements.
- ESI spectrometry: is a soft ionization method capable of generating both protonated and deprotonated ions. It generally gives the maximum of the ionic peak. This is a particularly useful technique for determining the mass of heavy molecules.
- The Q-TOF-MS technique offers high-resolution mass spectrometry and allows confirmation of the elemental composition of parent ions as well as that of ionic fragments (Quirantes *et al.*, 2013).

Results and discussion

1. Results

Infrared Spectroscopy

Mchim 's IR spectrum

In the infrared spectrum of the Mchim extract, visible absorption bands can be noted at 3307.3 cm^{-1} (OH); 2929.2 cm^{-1} (CH); 1597.9 cm^{-1} (NH); 1374 cm^{-1} (OH); 1019.2 cm^{-1} (CO) and 706.5 cm^{-1} (CH aromatic) (Figure 1). The evaluation of the measurements reveals the presence of sterols (OH), polyphenols (OH), alkaloids (NH) as well as tannins (OH; CO).

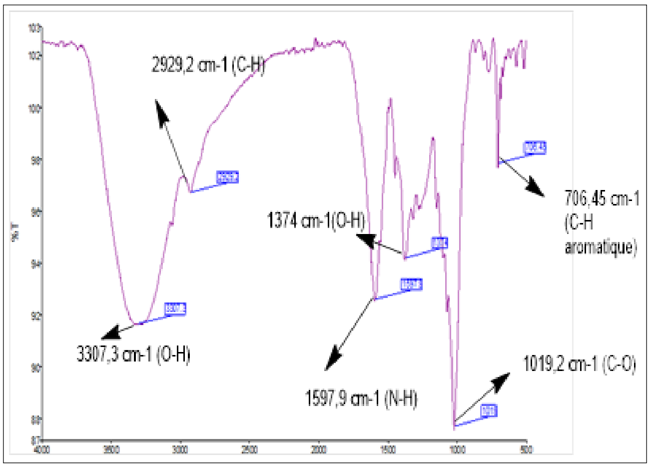

Fig 1: Mchim IR spectrum

1. Antioxidant activity

Antiradical activity by the DPPH method

When a DPPH solution is combined with a substance capable of releasing a hydrogen atom, this interaction produces the reduced form which has a still observable yellowish color of the picryl group. This technique was implemented to measure the antiradical potential of the extracts. The results concerning the antiradical capacity of the PN and Mchim and Mchim2 extracts, analyzed using the DPPH test, are presented as a percentage of inhibition (I%) of the DPPH radical by the extracts, and these data are illustrated graphically (I% as a function of concentration).

Ascorbic acid (Vitamin C) serves as a reference antioxidant. The concentration capable of neutralizing 50% of the DPPH radical (IC_{50}) is 0.04 ± 0.00 mg/mL for ascorbic acid (vitamin C). We therefore note that the extracts PN (2.01 ± 0.017), (Mchim (0.12 ± 0.02 mg/ mL) and Mchim2 (0.097 ± 0.005 mg/ mL) exhibit significant antiradical activities compared to the reference molecule, vitamin C (IC_{50} = 0.04 ± 0.00 mg/ mL) (Table 1).

Table 1: Antiradical activity of extracts tested by DPPH

Extract	PN	Mchim	Mchim2	Vitamin C
IC $_{50}$ in mg/ mL	2.01±0.017	0.12±0.02	0.097±0.005	0.04±0.00

Evaluation of antioxidant capacity using the ABTS method.

The technique Abts relies on the ability of an antioxidant to stabilize the blue-green cationic radical Abts+ by transforming it into colorless Abts +, by capturing a proton thanks to the antioxidant. We applied this method to evaluate the analyzed extracts. The antioxidant capacity of the extracts examined shows variations. In light of these observations, it appears that the extract Mchim consistently possesses the strongest antioxidant capacity. Compared with previous methods, it is evident that the Mchim2 extract displays a relatively low antioxidant capacity (Figure 2).

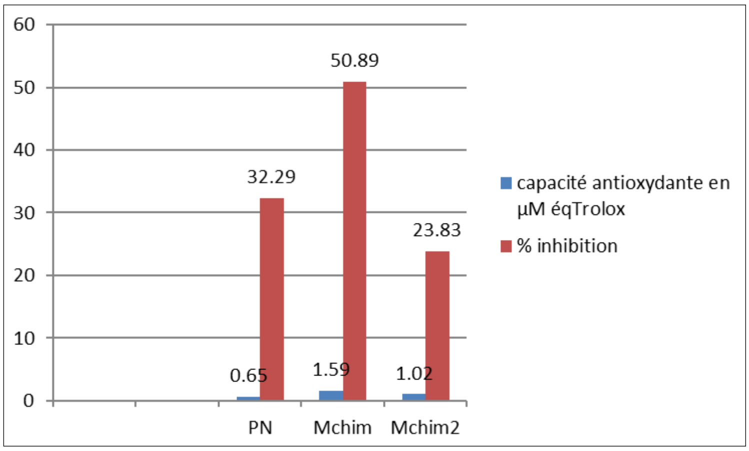

Fig 2: Percentage of inhibition and antioxidant capacity of extracts by the Abts test

Mass spectrometry (HPLC-ESI-QTOF-MS/MS) of the Mchim extract

The examined Mchim extract possesses good antioxidant properties. In order to identify and understand the major compounds present in the extract, a dereplicative approach based on the coupling of High-Performance Liquid Chromatography (HPLC) with Quadrupole Time-of-Flight (Q/TOF) tandem mass spectrometry was implemented.

Mass spectrometry analysis revealed the presence of N1-acetylspermine in the Mchim extract (Figure 3).

The MS chromatographic profile, which shows the molecular ion at *m/z* : 245 and the fragments resulting from the collision-induced dissociation of this ion, is obtained from the fragmentation spectrum of the compound. Examination of this spectrum reveals the presence of main ion fragments at *m/z* : 185 [M+H-60], *m /z* : 172 [M+H-73], *m/z* : 128 [M+H-117], *m/z* : 112 [M+H-133] and *m/z* : 99 [M+H-146].

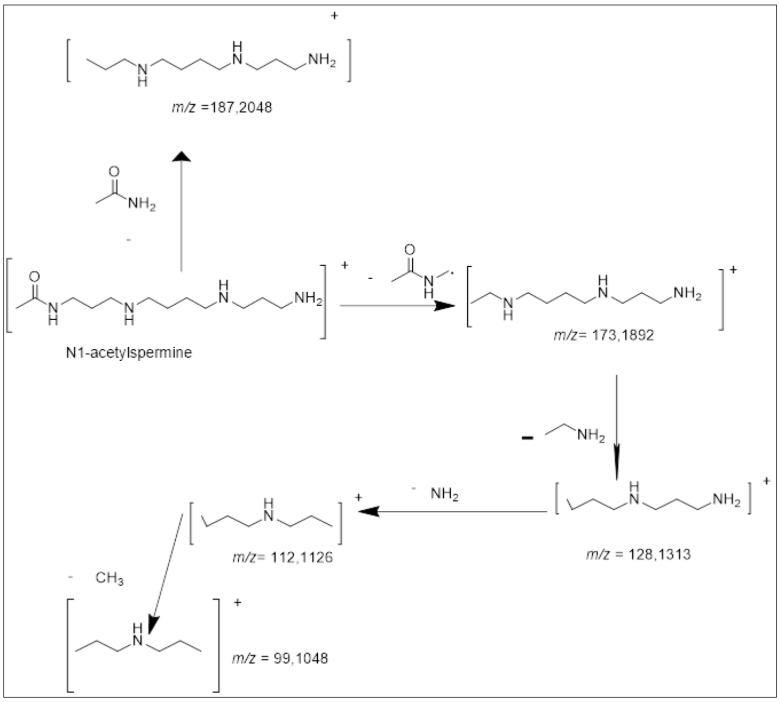

Fig 3: Mode of N1-acetylspermin fragmentation

Discussion

Chrysanthellum indicum is a medicinal plant that contains a large number of flavonoids and saponosides (Cissé *et al.*, 2019). This composition allows it to have positive effects on the blood system. It helps to alleviate the feeling of heavy legs and supports the liver after overindulging in food (Honoré-Thorez, 1985) [16]. In Burkina Faso, this plant is recognized for its antioxidant benefits, and recent research has revealed that it is used to treat kidney conditions caused by oxidative stress (Guenne *et al*., 2012) [36]. Other research has shown that its extracts are used to combat cystic stones, venous insufficiency and circulatory disorders in the lower limbs (Mevy *et al.*, 2012) [26].

Extraction methanolic from the root of *Cnestis ferruginea* possesses analgesic and anti-inflammatory properties, probably through peripheral and central mechanisms that involve the inhibition of the release and/or action of vasoactive substances such as histamine, serotonin, kinins and prostaglandins. This plant contains glycosidic anthraquinones, sterols, phenolic compounds, tannins, alkaloids, saponins and flavonoids, and is considered non - toxic (Ishola *et al.,* 2011) [17].

Anogeissus leiocarpus It contains alkaloids, glycosides, phenols, steroids, tannins, ellagic acids, anthraquinones, saponins, and flavonoids (Mann *et al*., 2008) [24]. This plant possesses antioxidant, antiangiogenic, and antitumor properties and is used against colon cancer (Hassana *et al.*, 2018) [15]. Its leaves are used in Nigeria and Guinea as an antimalarial (Adjanohoun *et al.*, 1991, Bhat *et al.*, 1990) [1, 4]. *A. leiocarpus also possesses* antiprotozoal and antileishmanial properties (Attioua *et al.*, 2011) [3].

Aloe Vera is a plant antibacterial, antiviral, anticancer, immunoregulatory hepatoprotective (Gao *et al.,* 2019) [13]

Leaves of *Ficus vallis-choudae* has antidiabetic properties (Kolefer *et al.*, 2021) [21] and the bark of its trunk possesses antiplasmodial properties (Chouna *et al.*, 2022) [7].

Polyphenols also play a role in assimilation and the fight against free radicals, single oxygen and the degradation of peroxides (Conte *et al*., 2003) [10].

These compounds have beneficial effects on brain degeneration as well as anti-inflammatory properties (Vergé *et al.*, 1999) [40]. As for flavonoids, they have the ability to influence the action of certain enzymes and to change the functioning of various cellular systems, which suggests that they may have many biological properties, including significant antioxidant, vasculoprotective, antihepatotoxic, antiallergic, anti-inflammatory, anti-ulcer, antitumor (Ghedira, 2005) and antimicrobial (Cushnie *et al.*, 2005) [11] properties.

Antioxidants can be beneficial to the body. Recent research has revealed that there may be variations in the assessment of a product 's antioxidant activity depending on the method used (Schlesier *et al.,* 2002; Nsimba *et al.*, 2008) [29, 37]. To evaluate the antioxidant activity of our extracts, we therefore use two approaches : DPPH and ABTS. The two techniques used to measure the antioxidant activities of our extracts allowed us to better appreciate Our results. Polyphenolic compounds possess biological activities (anti-inflammatory, anti- carcinogenic, and anti-atherosclerotic). These activities may be linked to antioxidant activity (Chung *et al.*, 1998) [8]. A daily diet rich in fruits and vegetables, containing a minimum of 1g of polyphenolic compounds, could have inhibitory effects on mutagenesis and carcinogenesis in the body (Tanaka *et al.,* 1998).

Mass spectrometry analysis allowed us to understand the structure of the N1-acetylspermine molecule in the Mchim extract.

N1-acetylspermine represents a variant of the polyamines. These compounds, such as spermidine and spermine, could influence diabetes, both in terms of its formation and progression, and could also play a role in its prevention. Abnormal concentrations of polyamines in the beta cells of the pancreas, which are responsible for insulin production, could disrupt the release of this hormone and be a contributing factor to diabetes. Research also shows that variations in polyamine levels could affect the immune response related to type 1 diabetes. This substance could be involved in the treatment of various conditions such as cancer, kidney disease, strokes, and neurological disorders (Ramos-Molina *et al.*, 2018; Casero). *et al.*, 2018) [5, 34]. Consequently, it could stimulate long-term insulin release (Sjoholm, 1993) [38]. However, several studies conducted on obese animal models have recently revealed abnormal levels of polyamines in the liver (Kwak *et al.*, 2015; Yun *et al.*, 2013) [23, 43], adipose tissue (Jamdar *et al.*, 1996 [18]), urine (Pelantová *et al.*, 2016) [32] and pancreatic islets (Sjoholm *et al.*, 2001) [39]. Consequently, N1-acetylspermin (exogenous spermin) has proven effective in reducing body weight and fasting blood glucose, and has also been shown to improve glucose tolerance in obese mice (Sadasivan). *et al.,* 2014) [35].

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

Studies indicate that the extracts have better free radical scavenging activity compared to vitamin C, considered the reference molecule, which has an IC50 of 0.04 ± 0.00 mg/mL. The study by HPLC-ESI-QTOF-MS/MS of the extract from MChim enabled the identification of N1-acetylspermine. The results obtained suggest that the frequent use of these drugs in the traditional treatment of diabetes could be attributed to their antioxidant activity.

References

1. Adjanohoun F, Ahyi MRA, Aké AL. Contribution to Ethnobotanical and Floristic Studies in Western Nigeria. CSTR-OUA, 1991, 220.
2. AIP. Diabetes will be the 7th leading cause of death worldwide by 2030. www.aip.ci., 2019.
3. Attioua B, Lagnikab L, Yeoc D, Antheaumed C, Kaisere M, Wenigerf B, *et al*. *In vitro* antiplasmodial and antileishmanial activities of flavonoids from *Anogeissus leiocarpus* (*Combretaceae*). Int J Pharmaceut Rev Res,2011:11:1-6.
4. Bhat RB, Eterjere EO, Olapido VT. Ethnobotanical studies from Central Nigeria. Journal of Economic Botany,1990:44:382-390.
5. Casero JRA, Murray Stewart T, Pegg AE. Polyamine metabolism and cancer: treatments, challenges and opportunities. Nature Reviews Cancer,2018:18(11):681-695.

6. Choong C, Van-Den TT, Roger F. Antioxidant Activities, Phenolic and Beta-Carotene Contents of Sweet Potato Genotypes with Varying FleshColours. Food Chemistry,2007:103:829-838.
7. Chouna HSD, Dize D, Kagho DUK, Bankeu JJK, Fongang YSF, Tali MBT, *et al*. Constituents of ripe figs of *Ficus vallis-choudae* Delile (*Moraceae*) with antiplasmodial activity. Research in parasitology.,2022:121(7):2121-2127.
8. Chung KT, Lu Z, Chou MW. Mechanism of inhibition of tannic acid and related compounds on the growth of intestinal bacteria. Food and Chemical Toxicology,1998:36(12):1053-1060.
9. Cisse M, Somboro A, Samake D, Traore N, Sidibe L. Ethnobotanical and phytochemical studies of *Chrysanthellum* plant powder *americanum* (l.) vatke (*asteraceae*). Malian Journal of Science and Technology,2019:(22):11-17.
10. Conte A, Pellegrini S, Tagliazucchi D. Synergistic protection of PC12 cells against β-amyloid toxicity by resveratrol and catechin. Brain research bulletin,2003:62(1):29-38.
11. Cushnie TP, Lamb AJ. Antimicrobial activity of flavonoids. Int J Antimicrob Agents,2005:26(5):343-56.
12. FID. FID Diabetes Atlas, 9th ed. FID. Brussels, 2019, 167.
13. Gao Y, Kuok KI, Jin Y, Wang R. Biomedical applications of *Aloe vera*. Critical reviews in food science and nutrition.,2019:59(sup1):S244-S256.
14. 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.
15. Hassana LEA, Al-Suadea FS, Fadul SM, Majida AMSA. Evaluation of antioxidant, antiangiogenic and antitumor properties of *Anogeissus leiocarpus* against colon cancer. Angiotherapy,2018:1(2):56-66.
16. Honoré-Thorez D. Description, identification and therapeutic uses of *Chrysanthellum Americanum*, *Chrysanthellum indie* Ul1J De. Subsp *Afroamerieanum*, BL. TURNER. Journal de Pharmacie de Belgique,1985:40(5):323-331.847980, 8 pages, February 2011.
17. Ishola IO, Akindele AJ, Adeyemi OO. Analgesic and anti-inflammatory activities of *Cnestis ferruginea* Vahl ex DC (*Connaraceae*) methanolic root extract. Journal of ethnopharmacology,2011:135(1):55-62.
18. Jamdar SC, Cao WF, Samaniego E. Relationship between adipose polyamine concentrations and triacylglycerol synthetic enzymes in lean and obese Zucker rats. Enzyme and Protein,1996:49(4):222-230.
19. Beaugrand J, Goudenhooft 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.
20. (https://www.sciencedirect.com/science/article/pii/S0926669021010219)
21. Kolefer K, Miaffo D, Ponka R. Evaluation of the antidiabetic properties of *Ficus vallis-choudae* leaf extract Delile in a model of type 2 diabetes induced by a high-fat diet and streptozotocin. The Scientific World Journal,2021:(1):1502230.
22. 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* (*Caesalpiniaceae*). International Journal of Biological and Chemical Sciences,2021:15:97-105.
23. Kwak HC, Kim YM, Oh SJ, Kim SK. Sulfur amino acid metabolism in Zucker fatty diabetic rats. Adipogenesis. Journal. Biological. Chemical.,2015:290:27384–27392.
24. Mann A, Yahaya Y, Banso A, Ajayi GO. Phytochemical and antibacterial screening of *Anogeissus leiocarpus* against some microorganisms associated with infectious wounds. African Journal of Microbiology Research,2008:2(1):60-62.
25. Marinova D, Ribarova F, Atanassova M. Total phenolics and total flavonoids in Bulgarian fruits and vegetables. Journal of the University of Chemical Technology and Metallurgy,2005:40(3):255-260.
26. Mevy JP, Bessiere JM, Dherbomez M. Composition, antimicrobial and antioxidant activities of *Chrysanthellum* essential oil *americanum* (Linn.) Vatke. Journal of Essential Oil Bearing Plants,2012:15(3):489-496.
27. N'guessan OE, Kablan ALC, Akoubet OA, Kablan RJF, Boni AP, Adiko NDM, *et al*. Phytochemical screening, evaluation of the antiradical activity and acute toxicity of a traditional antidiabetic drug based on *Anacardium occidentale* L., *Alternanthera repens* (L.) link., *Ageratum conyzoides* L. sold in Korhogo. Pharmacopoeia and traditional African medicine,2025:24(1):54-66.
28. N'guessan OE, Kablan ALC, Akoubet OA, Kablan RJF, Sima OC, Adiko NDM, *et al*. Chemical study of a traditional medicine (KAB. MC) for antidiabetic purposes sold in Korhogo (Côte d'Ivoire). Pharmacopoeia and traditional African medicine,2025:24(1):85-95.
29. Nsimba RY, Kikuzaki H, Konishi Y. Antioxidant activity of various extracts and fractions of *chenopodium quinoa* and *Amaranthus* SSp. Seads, Food chem.,2008:106:760-766.
30. World Health Organization (WHO). WHO Strategy for Medicine, 2015.
31. traditional for 2014-2023, 45.
32. Pelantová H, Bugáňová M, Holubová M, Šedivá B, Zemenová J, Sýkora D, *et al*. Urinary metabolomic profiling in mice with diet-induced obesity and type 2 diabetes mellitus after treatment with metformin, vildagliptin and their combination. Molecular and cellular endocrinology,2016:431:88-100.
33. Quirantes-Piné R, Lozano-Sánchez J, Herrero M, Ibáñez E, Segura-Carretero A, Fernánde Gutiérrez A. HPLC-ESI-QTOF-MS as a powerful analytical tool for characterizing phenolic compounds in olive leaf extracts. Phytochemical. Analysis,2013:24:213-223.
34. Ramos-Molina B, Lambertos A, Peñafiel R. Antizyme inhibitors in polyamine metabolism and beyond: pathophysiological implications. Medical Sciences,2018:6(4):89.

35. Sadasivan SK, Vasamsetti B, Singh J, Siddaraju N, Khan KM, Oommen AM, *et al*. Modulation of de novo purine biosynthesis results in AMPK activation and improves glucose handling and insulin sensitivity. Journal of Diabetes & Metabolism Disorders,2014:13:1-9.
36. Guenne S, Hilou A, Ouattara N, Nacoulma OG. Antibacterial activity and phytochemical composition of extracts from three medicinal species of *Asteraceae* from Burkina Faso. Asian Journal of Pharmaceutical Clinical Research,2012:5(2):0974-2441.
37. Schlesier K, Harwat M, Bohm V, Bitch R. Assessment of antioxidant activity by using different *in vitro* methods. Free radical Research,2002:36:177-187.
38. Sjoholm A. Role of polyamines in the regulation of proliferation and hormone production by insulin-secreting cells. American Journal of Physiology-Cell Physiology,1993:264(3):501-518.
39. Sjoholm A, Arkhammar P, Berggren PO, Andersson A. Polyamines in pancreatic islets of hyperglycemic obese mice (ob / ob) of different ages. American Journal of Physiology - Cell Physiology.,2001:280:317–323. 24.
40. Vergé S, Soulet S, Lacan F, Mas T, Arnaudinaud V, Nay B, *et al*. Polyphenols in wine : chemistry for life. Bulletin- Societe De Pharmacie De Bordeaux,1999:138(1/4):75-90.
41. Tanaka M, Kuei CW, Nagashima Y, Taguchi T. Application of antioxidant products from the Maillard reaction of histidine and glucose to sardine products. Nippon Suisan Akaishi,1988:54(8):1409-1414.
42. Wood JE, Senthilmohana ST, Peskinb AV. Antioxidant activity of procyanidin-containing plant extracts at different pHs. Food Chemistry,2002:77(2):155–161.
43. Yun KU, Ryu CS, Lee JY, Non JR, Lee CH, Lee HS, *et al*. Hepatic metabolism of sulfur amino acids in db / db mice. Food Chem. Toxicol.,2013:53:180–186.