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ANALYSIS OF MINERAL COMPOSITION BY X-RAY FLUORESCENCE AND IN VITRO ANTI-AMYLASE ACTIVITY OF EXTRACTS OF *AFRAMOMUM MELEGUETA* K. SCHUM, *CURCUMA LONGA* L., AND PIPER GUINEENSE SCHUMACH. & THONN. MEDICINAL PLANTS

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Abstract

The objective of this study was to highlight the mineral composition of *Aframomum melegueta, Curcuma longa*, and *Piper guineense* and to evaluate the anti-amylase activity of plant extracts from these plant species. The mineral composition was determined by X-ray fluorescence (XRF). This method allows for rapid and accurate identification of the elements present. The extracts of these species were also tested in vitro for their ability to inhibit amylase, a key enzyme in starch digestion and blood sugar regulation. Several essential minerals were found in the species analysed: potassium, calcium and iron, with varying concentrations depending on the plant. The XRF spectra reveal the elemental composition of three extracts from the species studied. These elements are potassium, iron, zinc and rubidium. These are present in all

the samples analysed, confirming a similar mineral base. The most pronounced anti-amylase activity was observed with *Curcuma longa* extract, followed by *Piper guineense* and *Aframomum melegueta* extracts. The various properties observed justify the traditional use of these plants in diabetes management and reinforce their potential as nutraceutical ingredients. The plants studied were found to inhibit the activity of the enzyme α-amylase, which is essential for the intestinal absorption of glucose. This action, therefore, helps to regulate glucose absorption, improve insulin sensitivity, and maintain optimal functioning of pancreatic β cells. The use of these plants may be a wise choice among the strategies for combating diabetes, namely phytotherapy.
Keywords: *mineral composition analysis, anti-amylase activity, Aframomum melegueta K. Schum., Curcuma longa L., Piper guineensis Schumach. & Thonn, medicinal plants*

1. Introduction

Since ancient times, human societies have exploited the properties of plants for various purposes, particularly in the areas of health and nutrition. Plant species used to prevent or treat different ailments are commonly referred to as medicinal plants. Currently, phytotherapy, defined as the medicinal use of plants, is experiencing a resurgence of interest, due to both growing evidence of its effectiveness and increasing public acceptance (Ngbolua *et al.*, 2011; Mbemba, 2020; Gbolo, 2023). Historically, plants and their extracts have been used to treat a wide range of diseases and disorders. In recent years, several factors have contributed to their resurgence, including their relatively low cost compared to synthetic drugs and a certain disillusionment with modern medicine (Chanda *et al.*, 2015; Ngbolua *et al.*, 2011; Boukeria *et al.*, 2019). Plants are capable of producing a wide variety of bioactive compounds, including mainly terpenoids, alkaloids, and phenolic compounds. These constitute one of the most studied groups due to their low toxicity and their multiple beneficial effects, whether therapeutic, pharmaceutical, cosmetic, or nutritional (Boukeria *et al.*, 2019).

The purpose of this study was to analyse and evaluate the anti-amylase activity of *Aframomum melegueta* K. Schum and *Curcuma longa* L. (both of Zingiberaceae), and *Piper guineense* (of Piperaceae). *A. melegueta* is a herbaceous and perennial species of approximately 1.5 m in height, with simple, alternate, lanceolate leaves about 40 cm long and 12–15 cm wide. Fleshy fruits, small, aromatic, and spicy, are used in cooking and in traditional medicine (Oludare Osuntokun, 2020). *C. longa* L. is a perennial, rhizomatous, herbaceous, and flowering plant native to India and Southeast Asia. It bears large, sheathing, elliptical, and lanceolate leaves up to 50 cm long and 7–25 cm wide. Flowers are yellow, arranged in spikes, white to green, sometimes tinged reddish-purple, and described as sterile (Grugeau, 1995). *P. guineense* is a climbing vine of about 20 m long, clinging to trees, with lanceolate and alternate leaves, up to 40 cm long and 12–15 cm wide, native to West Africa. Its Flowers are greenish-yellow and arranged in spikes. Fruits are small, red-brown drupes, black when dried, and often used as a spicy and aromatic spice in cooking.

Aframomum melegueta K. Schum., *Curcuma longa* L., and *Piper guineense* Schumach. & Thonn. are commonly used in traditional medicine. They have therefore been the subject of much scientific research. Yu Shen et *al.* (2025) report that *A. melegueta* is used to treat several types of bodily pain, such as diarrhea, sore throat, catarrh, congestion, rheumatism, and infectious diseases, including urinary tract infections Yu Shen et *al.* (2025). Different parts of this plant contain specific phytochemicals, such as flavonoids, phenolic compounds, alkaloids, tannins, terpenoids, saponins, and cardiotonic glycosides (Ibarue et al., 2021). These compounds act as anti-inflammatory, antimicrobial, anti-allergic, anticoagulant, anti-cancer, anti-diabetic, and hepatoprotective agents. Fuloria et al. (2022) assert that *C. longa* is a powerful medicinal plant due to its pharmacological properties and chemical components, such as starch, essential elements, proteins, vitamins, volatile oils, curcuminoids, and curcumin, which have beneficial effects on humans. This is why it is often used to treat various ailments, such as inflammation, digestive disorders, skin diseases, and

pain caused by blood stagnation. Fuloria et al. (2022) confirm its usefulness in relation to heart health, liver protection, and wound healing. *C. longa* also has antioxidant, hepatoprotective, antimicrobial, antibacterial, antifungal, and anti-allergic properties. Mbemba (2020) also asserts that it contains a group of phenolic compounds known as curcuminoids, the main one being curcumin. These compounds act by inhibiting the activity of the enzymes α-glucosidase and α-amylase, which are essential for the intestinal absorption of glucose. This activity helps regulate glucose absorption, improve insulin sensitivity, and maintain optimal pancreatic β-cell function. *C. longa* also possesses a broad spectrum of biological activities, including antioxidant, anti-inflammatory, antitumor, and anti-sickle cell properties. This plant is often associated with black pepper (*Piper nigrum*), which significantly increases the bioavailability of curcumin, thereby enhancing its therapeutic effects (Mbemba, 2020; Mbadiko *et al*., 2024).

On the other hand, Mbadiko et al. (2023) report that several species of the genus *Piper* have a wide range of biological properties, including anti-inflammatory, antioxidant, antibacterial, antifungal, antiplasmodial, analgesic, immunomodulatory, antitumor, amoebicidal, and antiviral effects. As far as *P. guineense* is concerned, Chinwendu *et al*. (2016) identified the following phytochemicals in the leaves: alkaloids, saponins, flavonoids, tannins, phenolic compounds, steroids, glycosides, and essential oils. They also mentioned the antioxidant (flavonoids and phenolic compounds), antibacterial, anti-inflammatory, and anticonvulsant properties, and those relating to reproductive health. For their part, Sikhuemene and Ongbomwan (2020) highlighted the following phytochemical elements (alkaloids, cyanogenic glycosides, saponins, tannins, flavonoids, anthraquinones, and phenols). They also mentioned the following mineral elements (Ca, Mg, Na, K, Fe, Zn, P). All these findings confirm its use as a medicinal plant.

Several previous studies have shown that combining extracts from different medicinal plants can improve the efficacy of pharmacological action by inducing synergy, acting simultaneously on multiple targets, reducing the doses of each component, and reducing side effects (Carabajal et al. 2019; Sharma et al. 2020; Deciga-Campos 2021; Gufe et al. 2023). Similar studies have also been conducted on *A. melegueta, C. longa*, and *P. guineense* in the treatment of various diseases. Shoba et al. (1998), for example, report that the combination of *Curcuma longa* and *Piper spp*. enhances anti-inflammatory effects by improving the absorption of curcumin. It is therefore plausible that the combination of *C. longa* and *P. guineense*, on the one hand, and of *C. longa* and *A. melegueta*, used as a substitute for black pepper, on the other hand, may enhance their medicinal properties. Furthermore, De Ruijter (2008) mentions the combination of *Strychnos congolana* Gilg roots and Aframomum melegueta K.Schum. seeds in the Democratic Republic of Congo for treating dysmenorrhea. On the other hand, Schmelzer (2008) reports the combination of *Hilleria latifolia* (Lam.) H. Walter leaves and *Piper guineense* Schumach. & Thonn. leaves for treating body swellings and leprosy. Ogbunugafor *et al*. (2017) and Mbadiko *et al*. (2024) report that *C. longa* exhibits numerous biological activities, including anti-inflammatory, antioxidant, and hypoglycemic properties. Mayele et al. (2025) demonstrated, through their study of the qualitative and quantitative composition of certain metabolites and the in vitro evaluation of their antioxidant activities, that the combination of *Aframomum melegueta*, *Curcuma longa*, and *Piper guineense* contained all the metabolites studied and that the concentrations of polyphenols, flavonoids, and tannins were higher than those of the individual plants. According to them, combining extracts of these plant species could make the treatment of diabetes and other diseases more effective.

The above demonstrates that the combined use of plant substances or herbal preparations has the potential advantage of increasing the benefit/risk ratio, either by enhancing or improving the therapeutic effects of their active ingredients. This approach also helps simplify the treatment protocol, thereby promoting patient adherence (HMPC, 2018). In this study, we will analyse the mineral composition of the extracts of *A. melegueta*, *C. longa*, and *P. guineense* and

evaluate their anti-amylase activity. These extracts will be analysed separately and in combination to assess the efficacy of these medicinal plants.

2. Materials and methods

2.1 Material

The seeds of the species *Aframomum melegueta*, and the rhizomes of the species *Curcuma longa* were collected in the Bateke Plateau, in the Commune of Maluku, located near Kinshasa, the capital city of the DR Congo, and the fruits of *Piper guineense* analysed in this study come from the village of Koko in the province of Kikwit in DR Congo. These three species were formally identified in the Herbarium of the University of Kinshasa.

2.2 Methods

2.2.1 Analysis of mineral composition by X-ray fluorescence

The mineral composition of plant samples of A. melegueta, C. longa and P. guineensis was analysed by X-ray fluorescence at the molecular biology laboratory of the General Commission for Atomic Energy (CGEA), located at the Kinshasa Regional Centre for Nuclear Studies (CGEA/CREN-K). The X-ray fluorescence method was used. The basic principle of an X-ray fluorescence (XRF) spectroscopy system involves a primary radiation source, either a radioisotope or an X-ray tube, and a detector that records the secondary X-rays emitted by the sample. When an atom is irradiated by a photon in the X-ray region, an inner-shell electron can be ejected, creating a vacancy. During the subsequent de-excitation process, an electron from a higher energy level fills this vacancy. The energy released during this transition is emitted as X-ray photons, which are characteristic of the element involved. The resulting X-ray fluorescence spectrum appears as a series of lines. Analysis of their positions reveals the mineral elements present in the sample (qualitative analysis). Conversely, the relative or absolute intensity of these lines indicates their concentration (semi-quantitative or quantitative analysis).

2.2.2 Evaluation of anti-amylase activity

The in vitro evaluation of anti-amylase activity was performed according to the method reported by Wickramaratne *et al.* (2016). Digestive enzymes are now recognised as major therapeutic targets in the treatment of obesity and certain associated metabolic disorders, such as type 2 diabetes (Prieto-Rodríguez *et al.*, 2022). The hydrolysis of starch by amylases results in the release of reducing sugars, mainly glucose. Free aldehyde groups convert oxidised 3,5-dinitrosalicylic acid (yellow in colour) into 3-amino-5-nitrosalicylic acid and nitrosalicylic acid, which are easily recognisable by their red-orange colour and maximum absorption at 540 nm.

The intensity of this colouring is directly proportional to the concentration of reducing sugar produced in the reaction medium. However, when an extract with amylase-inhibiting potential is present, it prevents starch hydrolysis, thereby limiting the release of glucose and the reduction of 3,5-dinitrosalicylic acid. The inhibitory power of the extract is then assessed by comparing the measured absorbance of the colored complex (3-amino-5-nitrosalicylic acid) in the presence and absence of the extract.

2.2.3 Operating procedure

a. Extraction of plant amylases

The amylases were extracted from 10 g of germinated corn flour macerated in 100 mL of distilled water. The resulting suspension was homogenized by magnetic stirring for 15 minutes, then filtered through Whatman No. 1 paper. The filtrate was then centrifuged at 4,000 rpm for 20 minutes at 4 °C. The resulting supernatant containing the enzymes was collected and stored in a refrigerator at 4 °C for immediate use or at –20 °C for later use.

b. Preparation of different extract concentrations

To prepare the plant extracts for testing, 20 mg of each extract was dissolved in 10 mL of distilled water or 80% ethanol to obtain a stock solution of 2 mg/mL, from which successive dilutions were made.

c. Preparation of 3,5-dinitrosalicylic acid (DNSA) colororimetric solution

Colorimetric solution of 3,5-dinitrosalicylic acid (DNSA) was prepared by dissolving 12 g of sodium potassium tartrate tetrahydrate in 8 mL of 2 M NaO H. Then, 20 mL of a 96 mM solution of 3,5-dinitrosalicylic acid was added to this solution.

d. Screening for anti-amylase activity

To evaluate antidiabetic activity, 200 µL of enzyme extract was mixed with 200 µL of plant

extract and incubated at 37 °C for 10 minutes. Next, 200 µL of a 1% pure starch solution was added and incubated for 3 minutes at 37 °C. The reaction was then stopped by adding 200 µL of DNSA. This mixture was then boiled in a water bath at 90 °C for 10 minutes. After cooling to room temperature, the solution was diluted by adding 5 µL of distilled water. Absorbance was measured at 540 nm using a UV-visible spectrophotometer. An enzyme blank, representing 100% activity, was prepared by replacing the plant extract with 200 µL of the solvent used. A second blank, specific to each concentration level of the extracts, was prepared in the absence of the enzyme.

e. Amylase activity inhibition calculation

The percentage inhibition of amylase activity was calculated using the following formula:

% A*mylase*inhibition = Abs control–Abs extract Abs control *x* 100

Where Abs = Absorbance

2.2.4 Statistical analysis

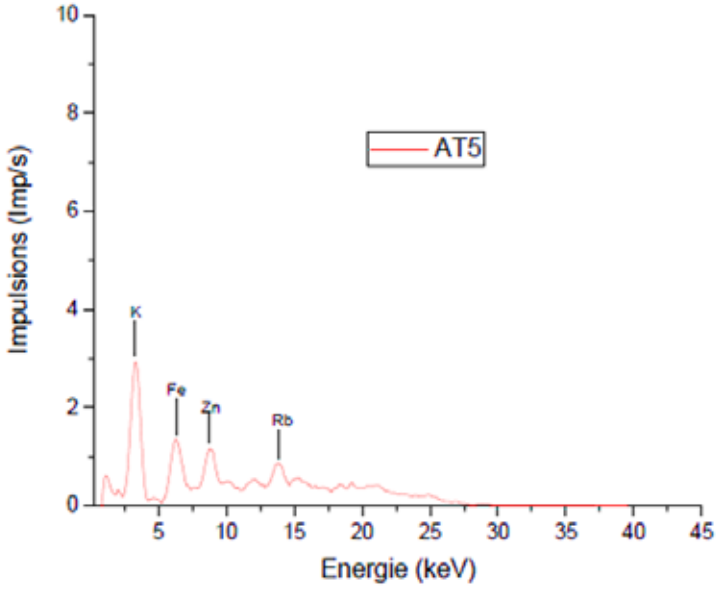
GraphPad Prism 6.0 and Statistix 8.0 software were used for all statistical analyses, including the determination of IC_{50} values. Results were expressed as mean ± standard deviation. One-way analysis of variance (ANOVA) was used to compare the means of the different samples, followed by Tukey's test for multiple comparisons. The threshold significance was set at α =0.05.

3. Results and discussion

3.1 Plant mineral composition

Figures 1, 2, and **3** below illustrate the mineral composition of *Aframomum melegueta*, *Curcuma longa,* and *Piper guineense* species. They reveal the presence of potassium, iron, and zinc in the *A. melegueta* sample, potassium, iron, and zinc in that of *C. longa*, and potassium, iron, and selenium in that of *P. guineense*. Previous studies also highlighted the presence of magnesium, calcium, manganese, chromium, zinc, copper, iron, phosphorus, and sodium in the various plants analysed (Khan *et al.*, 2024; Jansen *et al.*, 2005). Potassium, an essential mineral, plays a crucial role in maintaining resting membrane potential and regulating osmolarity within cells. It is also well established that potassium has an impact on the functions of endothelial cells and vascular smooth muscle cells. Numerous studies have also shown that increasing dietary potassium intake can lower blood pressure to more physiologically favourable levels (Haddy 2006; Chan 2024). The hypotensive effects of this element have been documented in several intervention trials. These effects are reported in various meta-analyses (Biff and Deborah 2020). Furthermore, epidemiological data accumulated over the last decade show a link between low dietary potassium intake – or low serum concentrations – and an increased risk of insulin resistance and the development of type 2 diabetes.

Figure 1. Quantification of mineral elements by X-ray fluorescence of *Aframomum melegueta* extract



Furthermore, zinc deficiency and changes in the homeostasis of this essential trace element are linked to several chronic diseases, including diabetes and its complications, notably diabetic retinopathy. Indeed, zinc contributes to the structural stability of copper-zinc superoxide dismutase (Cu-Zn SOD). This is an important antioxidant defence enzyme. It protects thiol (–SH) groups from oxidation by competing with iron, inhibits the activity of NADPH oxidase, a major source of free radicals, and reduces the production of reactive oxygen species (ROS) by interfering with Fenton reactions, in particular by competing with iron and copper (Mbemba *et al.*, 2023; Mayele et *al.*, 2025). Prasad and Bao (2019) attest that zinc also plays a major direct and indirect antioxidant role, which helps to maintain cellular redox balance.

Figure 2. *Quantification of mineral elements in Curcuma longa* L.

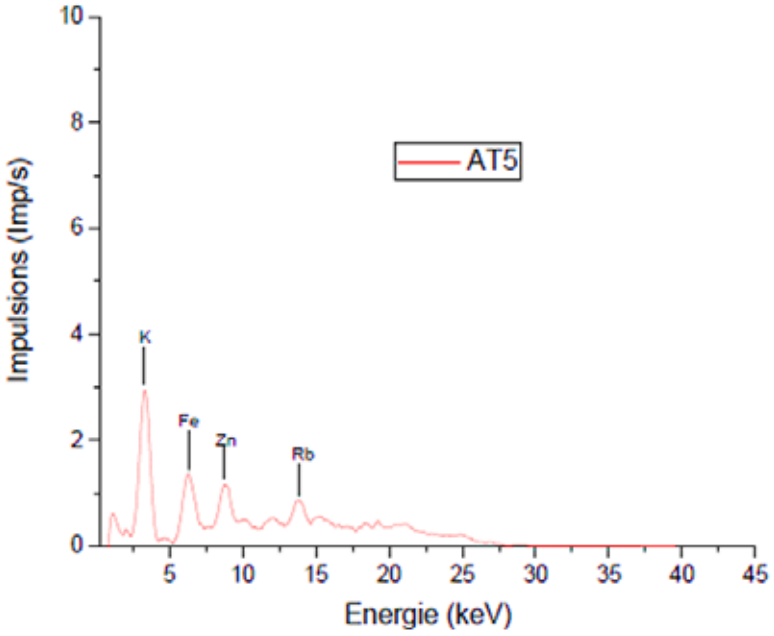
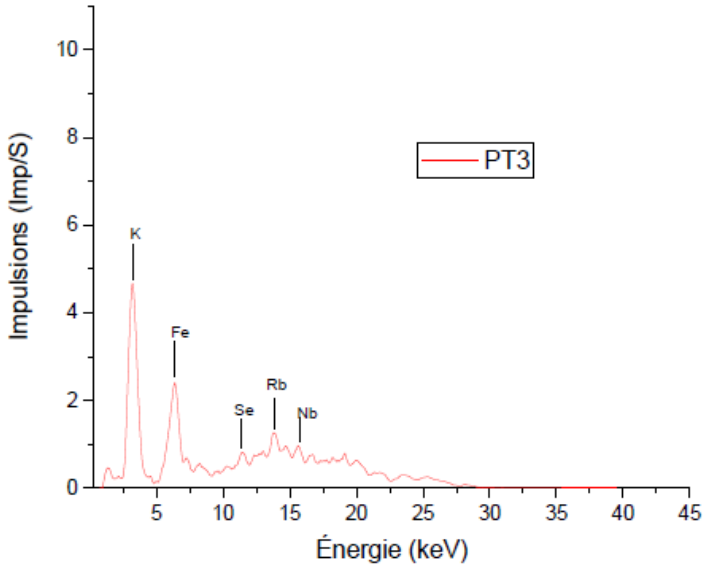


Figure 3. Quantification of mineral elements by X-ray fluorescence of *Piper guineensis* extract



Selenium, for its part, plays a fundamental role in defending cells against damage caused by free radicals. Ceci est dû à la présence de glutathion peroxydase sélénium-

dépendante dans le site actif et à l'activité antioxydante des selenoproteins (Burk, 2002). Le sélénium contribue également à l'amélioration de la fonction des cellules effectrices cytotoxiques, essentielles à la maturation des lymphocytes T et à la production d'anticorps T-dépendants (Zhang et al., 2020).

However, selenium intake in excess of nutritional requirements could inhibit the replication and mutation of SARS-CoV-2 into more virulent forms, while mitigating oxidative stress, organ damage, and the cytokine storm associated with infection (Zhang *et al., 2020*). This protective effect is particularly crucial in older individuals, in whom selenium deficiency is linked to an increase in pro-inflammatory cytokines.

Iron is an essential element in many biological processes, including the synthesis of haemoglobin, cytochromes, and key enzymes such as catalase, which plays a role in antioxidant defence (Mbemba, 2023). A recent study conducted by Oleiwi *et al.* (2024) highlighted a significant positive correlation between iron concentrations and catalase activity in subjects with diabetes, suggesting a close link between iron status and the regulation of oxidative stress in this pathological context.

Furthermore, organic germanium is known for its various pharmacological activities and is often used in medicine for anti-tumour, antiviral, antibacterial, antioxidant, immune regulation, and hypoglycaemic purposes. Wang et al. (2020) report that germanium can inhibit inflammation by suppressing the activation of the NF-kB and MAPK pathways, and reducing the expression of TNF-α, IL-1β and IL-6. Traces of germanium keep hydrogen peroxide at a low level, inhibiting oxidative stress and thus preventing its activity. It has been observed that germanium is part of the active centers of certain enzymes and participates in oxidation, mainly with hydrogen peroxide, without producing harmful oxygenated species (Menchikov et al., 2023). In fact, previous results suggest that Ge-132 has the potential to act as an antioxidant supplement by protecting cells. (Menchikov and Popov, 2023).

The spectrum shows that the CT4 sample contains beneficial elements (K, Fe, Zn), but also heavy metals of concern (Pb), which implies: potential nutritional or therapeutic value, but also a toxicological risk to be monitored, particularly when used for medical purposes (Purwadi *et al.*, 2022). The PT3 spectrum reveals a profile rich in bioactive elements (K, Fe, Se), supporting potential use in phytotherapy or supplementation. However, the presence of non-essential elements (Rb, Nb) calls for further analysis to assess toxicological safety before any medicinal or dietary use. Niobium (Nb) is rarely present in biological matrices. However, its detection could be linked to environmental contamination or a particular geological origin. It has no known role in human metabolism (Tchounwou *et al.,* 2019).

The *Curcuma longa* and *Piper guineense* spectra show interesting nutritional potential, particularly due to the presence of essential elements. However, the presence of non-essential or even toxic trace metals requires toxicity assessment before any therapeutic or food use. The spectra obtained by XRF show the elemental composition of three extracts (*Aframomum melegueta, Curcuma longa, and Piper guineense*). Potassium (K), Iron (Fe), Zinc (Zn) and Rubidium (Rb) are present in all samples, thus confirming a similar mineral base. These elements are important for health: K: electrolyte balance and muscle contraction; Fe: oxygen transport; Zn: immunity and antioxidant enzyme; Rb: is a non-essential trace element, considered as an indicator of bioaccumulation, often associated with the bioavailability of minerals and trace elements (Palmar *et al.*, 2023). For their part, Tchounwou *et al.* (2019) also report the presence of specific elements in *A. melegueta*, *C. longa,* and *P. guineense*. However, *A. melegueta* does not contain any toxic heavy metals, making it safer for therapeutic use. *C. longa,* on the contrary, has a peak of Pb (lead), a toxic element, which may pose safety problems for prolonged therapeutic use. On the contrary, *Piper guineense* contains Se (selenium) and Nb (niobium), which are absent in other species. Selenium is known as an essential trace element in low doses and an antioxidant, while Niobium is a non-essential element, as it is rarely found and must be monitored.

3.2 Anti-amylase activity

Table 1 below reveals that, among the aqueous extracts, the *P. guineense* extract

tested individually shows significant inhibition of amylase activity, with an IC_{50} of 49.0 ± 0.20 mg/ml. This activity is followed by that observed for the combination of *C. longa* and *A. melegueta* (**CA**) samples, then that of the combination of *C. longa*, *A. melegueta*, and *P. guineense* (**CAP**), with respective inhibition percentages of 46.2 ± 0.60 mg/mL and 46.1 ± 0.20 mg/mL. The inhibitory effects of these samples are statistically significant compared to those of the other plant extracts analysed individually or in combination.

Concerning the hydro-ethanolic extracts, analysis of Table 1 below indicates that the extract of *P. guineense*, tested individually, exhibits significant inhibitory activity, with an IC_{50} of 47.7 ± 0.80 mg/ml. This activity is followed by that of the *A. melegueta* extract analysed separately, with an IC_{50} of 41.0 ± 0.20 mg/mL, then that of the combination of *C. longa, A. melegueta*, and *P. guineense* (CAP), which revealed an IC_{50} of 40.0 ± 0.80 mg/mL. The other extracts, used alone or in combination, showed less marked inhibitory activity compared to the samples mentioned above.

Table 1. *Anti-amylase activity of extracts from A. melegueta, C. longa, and P. guineensis, analysed separately or in combination*

	Concentrations (mg/mL)					
Samples	**AM**	**CL**	**PG**	**CA**	**CP**	**CAP**
Aqueous extracts	14.2 ± 0.4^{e}	23.1 ± 1.21^{d}	49.0 ± 0.20^{a}	46.2 ± 0.60^{b}	44.7 ± 1.0^{c}	46.1 ± 0.20^{b}
Organic extracts	41.0 ± 0.2^{b}	39.0 ± 0.20^{c}	47.7 ± 0.80^{a}	15.0 ± 0.60^{f}	24.8 ± 0.40^{d}	40.0 ± 0.80^{bc}
Acarbose (Control)			22.29 ± 1.24^{de}			
p-value			0.0000			

Caption: AM: A. *melegueta;* **CL***: C. longa;* **PG***: P. guineense; CA: Combination of C. longa and A. melegueta;* **CP***: Combination of C. longa and P. guineense;* **CAP***: Combination of C. longa, A. melegueta, and P. guineense. For each line, the superscript letters indicate the degree of anti-amylase activity of the aqueous or ethanolic extracts of the different samples analysed. These letters correspond to activity levels ranked in descending order according to the following sequence: a > b > c > d > e > f*

The fact that the CA and CAP combinations also exhibited notable inhibitory activity suggests the existence of a synergistic effect between the phytochemicals of the plants studied. Such observation opens up an interesting avenue for the use of these combinations in the formulation of nutraceuticals for anti-diabetic purposes. Indeed, these combinations could inhibit the action of amylases involved in the digestion of glucose polymers, thereby delaying the intestinal absorption of this monosaccharide. This mechanism contributes to the regulation of postprandial hyperglycaemia and is strongly recommended in the management of diabetes (Kifle *et al.*, 2021).

Scientific literature remains limited about the study of the anti-amylase potential of the combination of extracts of *C. longa* and *A. melegueta* or *C. longa* and *P. guineense*, or even the combination of these three plant species, compared to the action of each of them evaluated separately. With respect to *C. longa*, Lekshmi *et al.* (2012) showed that its aqueous and organic extracts exhibit anti As for C. longa*, Lekshmi et al. (2012) showed that its aqueous and organic extracts exhibit remarkable anti-amylase activity. Furthermore, the inhibition of glycosidase, another key enzyme in carbohydrate digestion, has been attributed to its essential oils, as well as to curcuminoids, primarily curcumin and bisdemethoxycurcumin (Wildowati et al., 2018; Ramkumar et al., 2021).-amylase activity. Lekshmi et al. (2012) showed that aqueous and organic extracts of C. longa exhibit remarkable anti-amylase activity. Furthermore, Wildowati et al. (2018) and Ramkumar et al. (2021) indicate that the inhibition of glycosidase, another key enzyme in carbohydrate

digestion, is due to its essential oils, as well as to curcuminoids, primarily curcumin and bisdemethoxycurcumin. Meanwhile, Omosa *et al.* (2017) attributed the antidiabetic activity of *C. longa* to the presence of curcuminoids and sesquiterpenoids.

In addition, the literature reports that curcumin, the main representative of curcuminoids and the major active constituent of *C. longa*, has a hypoglycemic effect by reducing hepatic glucose production and stimulating glucose absorption through the activation of several genes, particularly those coding for the glucose transporters GLUT4, GLUT2, and GLUT3 (Ghorbani *et al.,* 2014). It may also induce the activation of the nuclear receptor PPAR-γ, decrease plasma glucose levels, and stimulate enzymes involved in glycolysis, such as hepatic glucokinase. In addition, curcumin promotes the accumulation of hepatic glycogen and downregulates neo-glucogenesis enzymes, including phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (Vafaeipour *et al.*, 2022). Curcumin improves insulin expression and secretion by activating phosphatidylinositol-3-kinase (PI3K), protein kinase B (Akt), and the signalling pathway involving the GLUT2 glucose transporter. This effect is associated with increased activity of GLUT2 and glucokinase (GCK), two key elements in the regulation of glucose uptake and intracellular metabolism (Zhang and Kitts, 2021). According to Ramkumar et al. (2021), bisdemethoxycurcumin, another representative of the curcuminoids, is capable of inhibiting human pancreatic α-amylase in vitro, as it regulates blood glucose and is targeted in the treatment of diabetes.

Mohammed et al. (2017) indicate that the leaves and seeds of *A. melegueta* contain antihyperglycemic activity. This helps improve pancreatic β-cell dysfunction and reduces other complications associated with diabetes. Karlsson *et al.* (2013) report that extracts from certain species of the genus *Aframomum* (*A. Aulacocarpus*, *A. citratum, A. deniellii*) not only limit weight gain, but also lower total and LDL cholesterol levels, while increasing HDL cholesterol, which has a well-established protective effect against obesity (*Karlsson et al., 2013*). According to these authors, obesity is now recognised as one of the main risk factors for type 2 diabetes.

Platel and Srinivasan (2000) report that extracts of *Piper* species (black pepper) stimulated food digestion by stimulating the secretion of digestive enzymes (pancreatic amylase, trypsin, and chymotrypsin). This activity significantly reduces food transit time in the gastrointestinal tract and increases both saliva production and gastric secretions. Furthermore, Liu et al. (2020) reported that administering piperine, an alkaloid isolated from species of the genus Piper, to obese mice resulted in a significant decrease in fasting blood glucose, total serum cholesterol, and triglycerides, while also improving glucose intolerance and insulin resistance. Furthermore, the effect of piperine on improving the bioavailability of curcumin, as well as enhancing its therapeutic properties, has been widely documented in the literature (Partial *et al., 2015*; Mbadiko *et al.*, 2024).

Results from this study indicate that the anti-amylase activity observed for the extracts of A. melegueta, C. longa, and Piper guineense species, whether evaluated individually or in combination, is closely linked to the presence of phytochemicals capable of inhibiting amylases. These compounds include certain alkaloids such as piperine, polyphenols including curcuminoids, particularly curcumin and its derivatives, as well as terpenoids and saponins. These classes of compounds have been reported in the literature as having antidiabetic effects, particularly through their ability to inhibit pancreatic and salivary α-amylase, thereby contributing to the regulation of blood glucose levels in diabetic patients (Wildowati *et al.*, 2018; Teng *et al.*, 2018; Liu *et al.*, 2020; Ramkumar *et al.*, 2021; Aurelio *et al.*, 2022).

4. Conclusion and recommendation

This study focused on the fundamental analysis and evaluation of the anti-amylase activity of Curcuma, *Aframomum melegueta*, and *P. guineensis*. Based on fundamental analysis, the mineral composition revealed that the hydro-ethanolic extracts of C. longa and A. melegueta (CA) present an interesting profile: potassium (K) is normally present in medicinal plant extracts; iron (Fe) is known as an enzyme, which suggests a potential

therapeutic interest, especially as an antioxidant. Zinc (Zn) is a cofactor for enzymes and plays a crucial role in the immune response. The spectrum therefore shows that sample CT4 contains not only beneficial elements (K, Fe, Zn), but also heavy metals of concern (Pb), which not only have potential nutritional or therapeutic value, but also possess a toxicological risk that needs to be monitored, particularly when used for medical purposes. The PT3 spectrum reveals a profile rich in bioactive elements (K, Fe, Se), supporting potential use in phytotherapy or supplementation. However, the presence of non-essential elements (Rb, Nb) calls for further analysis to assess toxicological safety before any medicinal or food use.

X-ray fluorescence (XRF) spectra show the elemental composition of three extracts (*Aframomum melegueta, Curcuma longa, and Piper guineense*). The resulting XRF spectra of *C. longa* and *Piper guineense* show interesting nutritional potential, which is particularly due to the presence of essential elements. However, the presence of traces of non-essential elements, or even toxic metals, necessitates a toxicity assessment before any therapeutic or food use. An integrated approach combining X-ray fluorescence, biological, and toxicological analyses is recommended.

Piper guineense contains selenium (Se) and niobium (Nb), which are absent in *A. melegueta* and *C. longa*. Selenium is an essential trace element in small doses, acting as an antioxidant. Niobium, on the other hand, is a non-essential element whose presence is rare and must be monitored. However, *Aframomum melegueta* does not contain toxic heavy metals, making its therapeutic use safer. The trace elements present in the studied plants also play a role in various biochemical processes, particularly in antioxidant mechanisms.

Regarding the anti-amylase activity of the aqueous extracts, the *P. guineense* extract, tested alone, showed significant inhibition of α-amylase, followed by the combination of *C. longa* and *A. melegueta* (CA), and then the combination of the three samples (CAP). For hydroethanolic extracts, the *P. guineense* extract tested individually also showed the strongest inhibitory activity, followed by the *A. melegueta* extract, and then by the combination CAP of the three species.

This study presents interesting perspectives for the development of medicinal plants with a favourable mineral salt profile and pronounced anti-amylase activity. This will enable improved formulation of nutraceuticals for the prevention of chronic non-communicable diseases associated with oxidative stress. An integrated approach combining XRF, biological, and toxicological analyses is therefore recommended.

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Declaration of available data

The sharing of available data does not apply to this article, as no new data was created as part of this article. However, we are willing to answer any questions regarding this article.

Author contributions

Project conception and supervision - **MT.** Project supervision – **NNKJP**. Writing-original project, and execution - **MMB.** Writing-original project execution - **MMB.** Writing–revision - **MMB, MMD.** Editing- English translation - **MMD.** Laboratory product funding - **MMB, MA, KMJ.** All authors have read and accepted the final version of the manuscript.

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Conflict of interest

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