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PHYTOCHEMICAL PROFILE AND ANTIOXIDANT POTENTIAL OF FOUR CULTIVARS OF CORCHORUS OLITORIUS L: TRADITIONAL AFRICAN VEGETABLES UNDERUSED IN CÔTE D'IVOIRE

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Abstract

Corchorus olitorius L, a traditional leafy vegetable of the Malvaceae family, is well appreciated in the central region of Côte d'Ivoire, where there are 5 cultivars whose phytochemical profile and antioxidant potential remain poorly understood. To better characterise them, phytochemical analysis and evaluation of antioxidant activity by DPPH and ABTS were carried out. The analyses revealed that the leaves of these cultivars are good sources of nutrients, with high levels of protein (19 - 25%), ash (8 - 12%), fibre (3 - 9%), total carbohydrates (40 - 48%), energy (252 - 288 Kcal/100g), iron (29 - 192 mg/100g), calcium (2167 - 2574 mg/100g) and potassium (6799 - 10692 mg/100g). The leaves are also rich in bioactive compounds such as polyphenols (11 - 81 mg GA eq/g extract), flavonoids (268 - 474 mg Q eq/g extract) and condensed tannins (8 - 75 mg TA eq/g extract). High antioxidant activity was observed (IC_{50} DPPH = 61 - 173 µg/mL and IC_{50} ABTS = 12 - 29 µg/mL). The best phytochemical profile and antioxidant potential were obtained in the CONB cultivar, which is also the most widely consumed by the population.

Keywords: phytochemical analysis, antioxidant activity, plant minerals, Corchorus olitorius, Ivory Coast

Introduction

Tropical developing countries are largely characterised by high frequencies of protein-energy malnutrition (PEM) and micronutrient deficiency (Koffi *et al*., 2019).

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Consumption of the leafy vegetables could address global food and nutrition security concerns, according to FAO recommendations (FAO, 2002; Yao *et al.*, 2015), because they are good sources of vitamins, minerals, fibre and phytochemicals (Tanimonure and Naziri, 2021) that would play a role antioxidant against the process of aging and the genesis of certain diseases (cancers, cardiovascular diseases, etc.) (Choudhary *et al*., 2013).

Efforts are being made at national level to improve scientific knowledge of leafy vegetables so that they can be integrated into the food systems and consumption habits of the Ivorian population in order to fill the scientific knowledge gap on their nutritional potential (Ehile *et al*., 2019). In Côte d'Ivoire, leafy vegetables are traditionally cooked and eaten as a sauce (Acho *et al*., 2014). The most widely marketed are spinach (*Basella alba*), taro (*Colocasia esculenta*), aubergine (*Solanum melongena*), African spinach (*Talinum triangulare*) and Jew's mallow (*Corchorus olitorius*). Although available in Ivory Coast, *Corchorus olitorius*, better known under the name "kplala" or "Kroala", seems to be the least valued. In Nigeria, Egypt, Tunisia, China, Italy and the Philippines, previous studies have shown that kplala leaves are excellent sources of nutrients such as proteins, lipids, carbohydrates, minerals, vitamins (A, B1, B2, B3, B5, B9 and C), α-linolenic acid and essential amino acids (phenylalanine, leucine, threonine, valine, lysine, methionine and tyrosine) (Mahmoud *et al.*, 2016; Athanase *et al.*, 2018; Soliman & Mohamed, 2020; Harouna Dieté *et al.*, 2023).

Kplala leaves are also rich in bioactive substances including flavonoids, alkaloids, polyphenols, anthraquinones, sterols, terpenes, glycosides and saponosides (Giro, 2017; Biswas et al., 2020). Regular consumption of kplala leaves has also been shown to protect the liver and pancreas (Saliu *et al.*, 2019), help control blood pressure and reduce cholesterol (Anyasor *et al*., 2020), the risk of obesity and heart disease (Lee *et al.*, 2020) and cancer (Tosoc *et al*., 2021). Unfortunately, the phytochemical profile and antioxidant potential of five kplala cultivars identified in the central regions of Côte d'Ivoire have not been studied in depth (Figure 1) (Harouna Dieté *et al*., 2023). Given the potential importance of these cultivars for the health of the population of central Côte d'Ivoire, it is important to determine their phytochemical profile and antioxidant potential. To this end, a study was carried out on samples taken from various departments in central Côte d'Ivoire.

Materials and methods

Experimental site and analysis laboratories

The various analyses of leaf samples of kplala cultivars taken from different departments in central Côte d'Ivoire were carried out at the Laboratory for Industrial Processes, Synthesis, the Environment and New Energies (LAPISEN) of the Institut National Polytechnique Félix HOUPHOUËT-BOIGNY (INP-HB) in Yamoussoukro (Côte d'Ivoire). Phytochemical screening and *in vitro* antioxidant

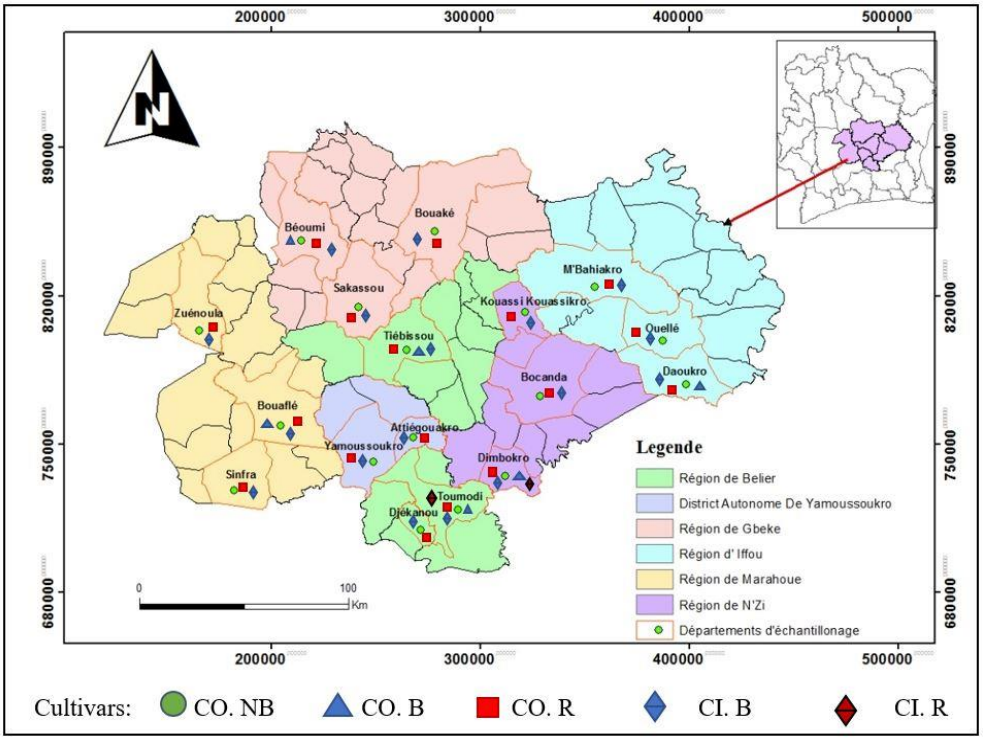
potential were studied at the Laboratory of Organic Chemistry and Natural Products at the "Dunarea de Jos" University of Galati.

Chemicals and reagents

All reagents and organic solvents were purchased from Sigma-Aldrich (Milan, Italy) and Fisher Scientific SAS (Strasbourg, France).

Plant material

The plant material consists of 44 leaf samples of kplala cultivars collected in different departments of the central regions during an ethnobotanical survey carried out from June 17 to July 18, 2021. The 44 samples taken were classified by cultivar (Figure 1). A random draw was then made from each class to obtain a sample of each cultivar, which was washed to remove debris and other dirt. After drying for three weeks at room temperature in the laboratory, the samples were crushed, sieved and stored in aluminium food packaging. These kplala cultivars were collected at the flowering stage, and were described in our previous work (Harouna Dieté *et al*., 2023).



Legend: **CO. NB**: *Corchorus olitorius* variety *olitorius* green non-bright; **CO. R**: *Corchorus olitorius* variety *olitorius* red; **CO. B**: *Corchorus olitorius* variety bright green *olitorius*; **CI. B**: *Corchorus olitorius* variety *incisifolius* shiny green and **CI. R**: *Corchorus olitorius* variety *incisifolius* red.

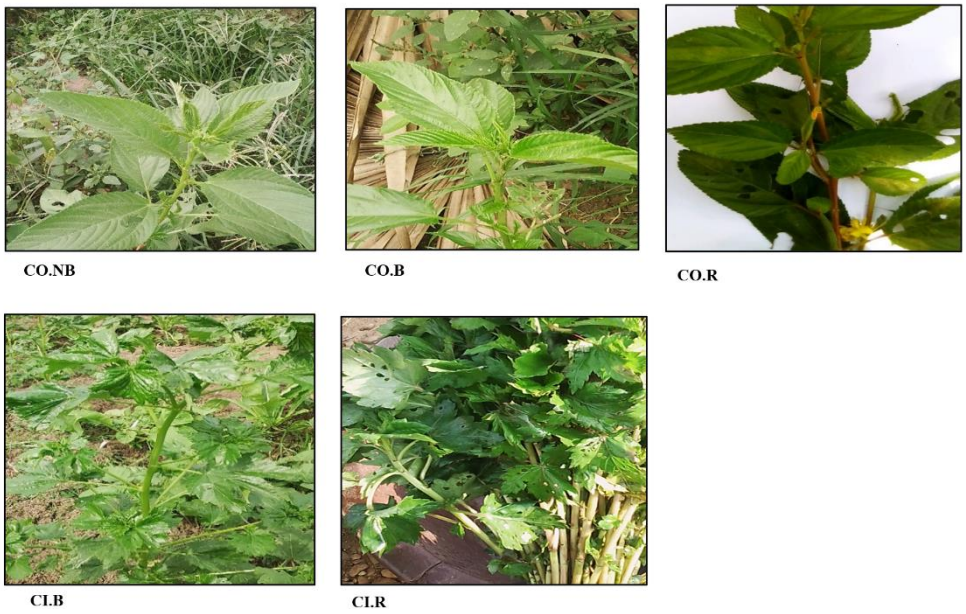


Figure 1. Map of the various departments sampled in central Côte d'Ivoire.

Dosage of macronutrients (%)

The water (H%), dry matter (DM%), protein, crude fibre and crude ash content were determined using the methods described by AOAC (1990). Total carbohydrates and energy values were determined using the calculation method recommended by the FAO (2002). This method takes into account moisture, fat, protein and ash content on the one hand, and energy coefficients for leafy vegetables on the other.

Carbohydrate: 100 - (% moisture + % protein + % fat + % ash + % fibre) (1)

The total energy value in Kcal per 100g of dry matter is given by :

$$E\ (Kcal) = (4 \times P) + (4 \times G) + (9 \times L) \quad (2)$$

where: E - the total energy value per 100 g of sample; P - total protein content of the sample; G - total carbohydrate content; L - total fat content.

Determination of anti-nutritional compounds

Determination of oxalate

The method used for the determination of oxalate in leafy vegetable samples is that described by Day and Underwood (1986). To do this, one (1) g of leafy vegetable powder was homogenised in 15 mL of H_2SO_4 (3M) under magnetic stirring for one hour. The resulting mixture was filtered through Whatman filter paper. The filtrate was titrated under heat with a solution of $KMnO_4$ (0.05 M) until the colour changed to a persistent pink. The oxalate content of the sample is given in mg/100 g by the following expression :

$$\text{Oxalates (mg/1 00 g)} = \frac{1.1 \text{ x Veq x } 100}{\text{ms x DM}} \quad (3)$$

with : Veq - volume of $KMnO_4$ (mL) added at equivalence; ms - mass (g) of sample; DM- dry matter.

Determination of phytate

Briefly, 4 g of each sample was soaked in 100 mL of 2% HCl for 5 hours and then filtered. 25 mL of the filtrate was measured into a conical flask and 5 mL of a 0.3% ammonium thiocyanate solution (NH_4SCN) was added as an indicator and 53.5 mL of distilled water was also added to achieve a pH of 3.5. The mixture was titrated with ferric chloride ($FeCl_3$) until a brownish-yellow colour was obtained, which persisted for 5 minutes. Phytate content (mg/100 g) was calculated as follows: (Reddy *et al.*, 1982).

$$\text{Phytate content} = \frac{T \times 0.195 \times 3.55 \text{ x } 100}{94.5} \quad (4)$$

Where: T = titrated; and 0.195; 3.55 and 94.5 are constants.

Determination of mineral salt content

Mineral salts Fe, Ca, K, Cu and Zn were determined in triplicate in all samples by X-ray fluorescence spectrometry (XRF) using a Niton XL3 XRF spectrometry instrument (Thermo Fisher Scientific, Germany) according to the method described by Clay (2022). This data was recovered using a powerful reader called Niton XL3t coupled with NDF software and the results are expressed in (mg/Kg). Mineral salts Mg, Na and P were analysed by ICP RQ ICP-MS inductively coupled plasma mass spectrometry (Thermo Fisher Scientific, Germany) using the method described by AOAC (1990) after digestion and mineralisation of the samples.

Preparation of the extract

Briefly, a 50 g powder fraction of the plant material was weighed using a precision balance and then dissolved in 500 mL of 70% aqueous ethanol solution (Ethanol, 70% (w/v), Water, 28 - 32% (v/v), Merck KGaA, Darmstadt, Germany). In a 500 mL erlenmeyer flask, the mixture was sonicated in an ultrasonic bath to two hours, then left to macerate for 24 hours. The extract obtained was filtered twice through cotton wool. The filtrate was evaporated to obtain residues on which phytochemical analyses and antioxidant activities were carried out (Kouamé *et al*., 2021). Before each analysis, the obtained residue was rehydrated in the above stated hydroalcoholic solution to a 1 mg/mL concentration.

Determination of extraction yield

The yield (%) of each crude extract was determined by dividing the mass of the crude extract by the mass of the sample powder and multiplying by 100.

$$R\ (\%) = (me/ms) \text{ x } 100 \quad (5)$$

R - yield in %; me - mass of crude extract in g; ms - mass of sample powder in g.

Phytochemical screening of secondary metabolites

Qualitative analysis of alkaloids, flavonoids, polyphenols, quinones, saponins, sterols and triterpenes, tannins and cardiac glycosides was carried out after hydroethanolic extraction using the methods (Table 1) described by Edeoga *et al* (2005), De *et al* (2010) and Bassène (2012).

Table 1. Methods of phytochemical screening of secondary metabolites

Secondary metabolites	Methods
Sterols and triterpenes	Libermann-Buchard Test
Alcaloides	Dragendorff Reagent Test
	Wagner Reagent Test
	Mayer Reagent Test
Polyphenols	Phosphotungustic test
Flavonoids	Alkaline
	Shibata Test
Tannins	$FeCl_3$ Test
Quinones	Borntraëger Test
Saponins	Foaming power
Cardiac glucosides	Keller-Killani Test

Detection of sterols and triterpenes

Libermann-Buchard test: 5 mg of the dry extract was treated with 5 drops of acetic anhydride and boiled in a water bath for 5 min. After cooling, 0.5 mL of sulphuric acid was added to the sides of the test tube. A brown ring was observed at the junction of two layers. The green colour of the upper layer indicates the presence of steroids, while a red colour shows the presence of triterpenoids

Detection of polyphenols

Polyphenols were detected using the phosphotungustic acid method. To 1 mL of the alcoholic solution, 1 mL of a phosphotungustic acid solution and 9 mL of a 25% aqueous sodium carbonate solution were added. A blue colouration of the mixture indicates the presence of polyohenols.

Detection of flavonoids

Alkaline flavonoid test: For 0.5 mL of the test solution, a few drops of concentrated sodium hydroxide solution were added. The formation of an intense yellow colour, which became colourless after the addition of a few drops of dilute acid, indicates the presence of flavonoids.

Cyanidin test: Flavonoids were detected in the residues by the cyanidin reaction. Two to three magnesium chips were added to an aliquot of the extract dissolved in 5 mL hydrochloric alcohol (2:1, v/v), followed by the release of heat and the appearance of a pink-orange or purplish colour. The addition of 3 drops of isoamyl alcohol intensifies this coloration, confirming the presence of flavonoids.

Detection of tannins

Ferric chloride test: For 0.5 mL of the extract solution, 1 mL of 1% (v/v) ferric chloride ($FeCl_3$) was added. The positive test for tannins gave a blue-green colour.

Detection of alkaloids

Dragendorff and Wagner reagent test: 1 g of the extract was taken up in 6 mL of 60% ethanol, then divided into 2 test tubes. 2 drops of Dragendorff reagent are added to the first tube, and the appearance of an orange-red precipitate indicates a positive test. 2 drops of Wagner reagent are added to the second tube. The appearance of a reddish-brown precipitate indicates a positive test.

Mayer test. For 0.5 mL of extract, 1 mL of Mayer (potassium mercuric iodide solution) was added. A cream-coloured precipitate indicates the presence of alkaloids.

Quinone test

Quinones were detected in extracts using the Borntraëger reagent. A few drops of 10% NaOH were added to 2 mL of a solution of crude extract (0.1 g in 10 mL of distilled water). The appearance of a yellow, red or violet hue in the aqueous phase indicates a positive reaction.

Detection of cardiac glycosides

The Keller-Killani method was used: 0.4 mL glacial acetic acid was added to 0.5 mL of the sample extract. Next, a quantity of ferric chloride was added. Finally, 0.5 mL of concentrated sulphuric acid was carefully added next to the test tube containing the mixture. The blue colour of the acetic acid layer indicates the presence of cardiac glycosides.

Saponoside test

Foaming power: Saponins were detected in the extract through the foam test. 5mL of the alcoholic extract was evaporated to dryness and taken up in 5 mL of water. The solution was transferred to a test tube and shaken for 2 minutes. The appearance of a column of foam at least 1cm high and lasting at least 15 minutes indicates the presence of saponosides.

Determination of total polyphenol content

Polyphenol content was assessed using the method described by Cudalbeanu et al (2018). Briefly, 10 µL of hydroalcoholic extract (1 mg/mL) and 25 µL of Folin-Ciocalteu reagent were mixed and the resulting mixture was incubated at room temperature for 5 minutes. After incubation, 25 µL of 20% Na2CO3 solution and 140 µL of distilled water were added and the whole mixture was incubated at room temperature for 30 minutes. The absorbance was then read at 760 nm using a microplate reader, the Tecan Pro 200. The results were expressed as mg gallic acid equivalents per gram of extract (mg GAE/g extract) using a gallic acid calibration curve. All experiments were performed in triplicate.

Determination of total flavonoid content

The flavonoid content was determined using the method described by Marquardt et *al*. (2020) with a few modifications. Briefly, 130 µL of hydroalcoholic extract (1 mg/mL) and 10 µL of 5% $NaNO_2$ were mixed and incubated for 5 min. Next, 10 µL of 10% $AlCl_3$ solution was added and the mixture was incubated at room temperature

for 6 minutes. Absorbance was read at 510 nm using a microplate reader, the Tecan Pro 200. The results were expressed as mg quercetin equivalents per gram extract (mg QE/g extract) using a quercetin calibration curve. All experiments were performed in triplicate.

Determination of condensed tannin content

Condensed tannin content was determined in triplicate using the method described by Zongo *et al* (2023). Briefly, a quantity of 500 µL (1 mg/mL) of diluted hydroalcoholic extract (1/100) and 1 mL of freshly prepared vanillin (1 g in 100 mL of 70% sulphuric acid) were mixed and the whole mixture was homogenised and incubated in a water bath at 30°C for 15 min in the dark. Absorbance was recorded at 500 nm against a blank using a spectrophotometer. The results were expressed as mg tannic acid equivalents per gram of extract (mg TAE/ g extract) using a tannic acid calibration curve.

DPPH (2,2-diphenyl-1-picrylhydrazyl) test

The antioxidant effects of the extracts tested were assessed by DPPH radical scavenging in a 96-well plate, as detailed in Zongo *et al.* (2023). The absorbance of the samples was measured at 517 nm (Tecan Pro 200 multi-well plate reader) after 20, 35 and 50 min incubation at room temperature. The control sample used was the solvent mixed with the DPPH solution. Trolox was used as a positive control. The percentage inhibition of DPPH was calculated using the following formula:

$$\%\ Inhibition = \frac{(Absorbance\ of\ Control - Absorbance\ of\ sample)}{(Absorbance\ of\ Control)} \times 100 \quad (6)$$

ABTS (2,2-Azino-bis (3-ethylbenzothiazoline-6-sulfonic acid)) test

The ABTS test was performed using the microplate reader test, as detailed in Zongo *et al*. (2023). The absorbance was read at 760 nm at different time intervals. The percentage of ABTS free radical scavenging activity was calculated using the following formula:

$$\%\ Inhibition = \frac{(Absorbance\ of\ Control - Absorbance\ of\ sample)}{(Absorbance\ of\ Control)} \times 100 \quad (7)$$

Statistical analysis

Results were expressed as mean ± standard deviation for triplicate analyses. A one-way ANOVA was applied to the data to analyse the statistical differences between the results. A one-way ANOVA using Fisher's test ($p < 0.05$) was used to determine the differences between the means values of each parameter analyzed. Next, a principal component analysis (PCA) followed by a hierarchical ascending classification (HAC) with standard parameters (Euclidean distance, Ward's method, automatic truncation) was used to classify cultivars according to their profile on the same dendrogram in order to form homogeneous groups. The input data for the HAC were the coordinates of the individuals weighted by the percentages of inertia on the axes, not centred reduced. Finally, the phytochemical profile and antioxidant potential of *Corchorus olitorius* cultivars (kplala) and those of *Basela alba* (Spinach), *Colocasia esculentus* (Taro) leaves, *Hibiscus sabdariffa* (Guinea sorrel)

and *Moringa oleifera* (Moringa) identified in the literature (Sharma *et al.*, 2012; Acho *et al.*, 2014; Kaur *et al.*, 2014; Anel *et al.*, 2016; Zihad *et al.*, 2019; Peñalver *et al.*, 2022; Sheik *et al*., 2023) were subjected to principal component analysis (PCA) followed by hierarchical ascending classification (HAC). These statistical analyses were carried out using R Studio software version 2021.09.0 Build 351.3. Histograms were plotted using the software Graph Pad Prism 8.0.2 (263) 2019.

Results and discussion

Nutritional constituents of the leaves of five C. olitorius cultivars

The nutritional and anti-nutritional parameters of the leaf of kplala cultivars generally varied significantly ($p < 0.05$) from one cultivar to another. Analysis of these parameters shows that the five kplala cultivars are excellent sources of protein and total carbohydrates (Table 2). The leaves of kplala cultivars have higher protein contents (19.34 - 25.66% of DM) than those of spinach (9.86% of DM), aubergine (12.34% of DM) and Guinea sorrel (9.44% of DM), but similar to those of Moringa leaves (25.3% of DM) (Sharma *et al*., 2012; Acho *et al*., 2014; Peñalver *et al*., 2022). They could play an important role in providing cheap and available protein for rural communities (Acho *et al*., 2014; Zihad *et al*., 2019). The high protein, carbohydrate and energy contents of kplala cultivar leaves could be an alternative in the fight against the high frequencies of protein-energy malnutrition (PEM) prevalent in our African populations (Koffi *et al*., 2019). They would also help to address global food and nutritional security concerns, as recommended by the FAO (FAO, 2002; Yao *et al.*, 2015). The leaves of kplala cultivars have fibre contents (3.99 - 9.90% ofDM) comparable to those of amaranth leaves (2.1 - 4.9% of DM) (Jan *et al*., 2023). Fibres play an important role in the absorption of cholesterol, and sugars from the diet and, above all, in the management of diabetes and obesity (Zhao *et al*., 2018; Adeyeye *et al*., 2018). The low levels of lipids in the kplala samples studied (0.46 - 1.90% of DM) could be advantageous for people suffering from obesity and other related diseases. In fact, a diet providing 1 to 2% of its calorific energy in the form of lipids is sufficient in humans, as excessive consumption has been implicated in certain cardiovascular disorders, such as atherosclerosis, cancer and ageing. Health disorders such as appendicitis, haemorrhoids, gallstones, heart disease and constipation are corrected or treated by abundant consumption of vegetables due to their low fat content (Acho *et al*., 2014; Adeyeye *et al*., 2018).The average ash content obtained in the leaves of kplala cultivars is in agreement with the results presented by Sha'a *et al*. (2019) in kplala leaves from Nigeria (11.18±0.00%).

In terms of mineral content, kplala cultivars are also rich in iron and potassium, proving their importance for human nutrition (Table 2). Iron levels in the studied samples (29.20 - 192.75 mg/100g of DM) were similar to those found in spinach (63.33 mg/100g of DM) and taro (70.00 mg/100g of DM) (Acho *et al*., 2014) and celosia (27 - 44 mg/100g of DM) (Atchibri *et al*., 2012). Iron (Fe) is a micronutrient that is essential for haemoglobin synthesis, central nervous system development and protection against infection. Iron deficiency is even more common worldwide than

iodine deficiency. It is the cause of iron deficiency anaemia, a serious public health problem that can affect up to half of all women and children. The populations most at risk are pregnant women, infants and the elderly, mainly due to a lack of iron-rich foods (Baudin, 2021a). In addition, the potassium content of the leaves of the kplala cultivars (6799.51 - 10692.26 mg/100g of DM) was higher than the levels obtained by Acho *et al*. (2014) in the leaves of kplala harvested at maturity in eastern Côte d'Ivoire (1669.06 mg/100g of DM). This difference would be due not only to the type of kplala variety or the harvesting stage of the plant, but also to the nature of the soil. It was significantly higher than in green leafy vegetables such as spinach (4014.11 mg/100g of DM), taro (2892.64 mg/100g of DM), and aubergine (3380 mg/100g of DM), but similar to that of tropical purslane (9348.42 mg/100g of DM) (Acho *et al*., 2014). Potassium and sodium are important intracellular and extracellular cations respectively, being the minerals that contribute to water balance and acid-base regulation, nerve and muscle contraction (Ferry, 2012). For example, it has been reported that a Na/K ratio of less than one can reduce diseases associated with high blood pressure (FND, 2005). In kplala leaves, Na/K ratio (0.01 - 0.016) respects this rule (Table 2) and was significantly better than those of leafy vegetables consumed in Côte d'Ivoire such as spinach, taro and aubergine (Acho *et al.* 2014). The high calcium levels in the leaves of kplala cultivars (2167.36 - 2574.36 mg/100g of DM) (Table 2) was significantly higher than the results reported by Acho *et al*. (2014) and Dappah *et al*. (2018) respectively 1159.07 mg/100g of DM and 30.55 mg/100g of DM in kplala leaves harvested at maturity in eastern and central Côte d'Ivoire. However, the calcium content of kplala cultivars is similar to that found by Atchibri *et al*. (2012), ranging from 1546 to 2489 mg/100g of DM in kplala leaves harvested at the market garden site in Abidjan (a city on the southern Atlantic coast of Côte d'Ivoire). This similarity could be justified by the fact that the Abidjan market is generally supplied with kplala by growers in central Côte d'Ivoire, the area where the kplala cultivars were harvested (Harouna Diété *et al*. 2023). Calcium is a mineral that is involved in numerous metabolic and signalling processes, such as the functioning of cell membranes, the transmission of nerve impulses, neuromuscular excitability and blood coagulation (Ferry, 2012). In addition, calcium and phosphate are two minerals that form a powerful duo for bone health, they are associated with the growth and maintenance of bones, teeth and muscles, they are found in the form of hydroxyapatite, $[Ca_{10}(PO_4)_6 (OH)_2]$ in the mineral phase of bone (Ferry, 2012). It has been reported that low calcium intakes (< 500 mg/d) aggravate calcium leakage, which is common in the elderly population. It is secondary to the bone demineralisation responsible for osteoporosis (Ferry, 2012). However, a Ca/P ratio greater than 1 may be advantageous for the consumption of the leaves studied because a diet is considered good if the Ca/P ratio is > 1 and bad if it is < 0.5 (Acho *et al*., 2014; Adeyeye and Aye, 2005; FND, 2005). In fact, in the five kplala cultivars, the Ca/P ratio is well above 1 (6.42 - 14.28) (Table 2). The Ca/P ratio of the kplala cultivars was in line with the recommended dietary allowances (Source: FAO, 2004; Konare *et al*., 2022), which proves that consumption of these cultivars is highly beneficial for human well-being (Acho *et al*., 2014; Adeyeye and Aye, 2005; FND,

2005).To sum up, these mineral salts are involved in many important biological functions, most of them being cofactors of enzymes supplied by the diet (of which leafy vegetables are important sources), helping to meet the body's needs (Baudin, 2021).

The anti-nutritional factors most frequently found in leafy vegetables are oxalate and phytates (Acho *et al.*, 2014). Due to their negative charges, oxalate and phytic acid have a strong affinity to bind to metal ions such as calcium, zinc and iron. This interferes with the absorption of these minerals by the small intestine and negatively affects various metabolic processes (Petroski & Minich, 2020; Siener *et al*., 2020). The oxalate content of the leaves of kplala cultivars varied from 45.80 ± 0.14 to 16.65 ± 0.18 mg/100g of DM and that of phytate (0.93 ± 0.03 - 1.86 ± 0.03 mg/100g DM with a significant difference ($p < 0.05$) (Table 2). On the other hand, phytate levels in the leaves of kplala cultivars are significantly lower than those found by Acho *et al*. (2014) and Dappah *et al*. (2018) 38.75 and 50 mg/100g of DM respectively in the kplala leaves harvested in Abidjan and Gagnoa (Côte d'Ivoire). The same is true of the content in aubergine (41.67 mg/100g of DM) (Acho *et al*., 2014). However, to impair calcium bioavailability, the molar mass ratio of these anti-nutrients to calcium must be less than 2.5 (Dappah *et al*., 2018). Thus, the oxalate/Ca and phytate/Ca molar ratios of the five kplala cultivars studied were well below the critical threshold. Similarly, phytate levels could not impede the iron bioavailability of five kplala cultivars, given that the phytate/Fe ratio was below the critical level of 1 (Table 2). On the other hand, Acho *et al*.(2014) reported a high oxalate content in kplala leaves (780.00 mg/100g of DM) taken at maturity in Dabou, which is a coastal area or Atlantic coast located in the east of Côte d'Ivoire whose soil is thought to be loaded with NaCl. NaCl is known to be involved in increasing oxalte biosynthesis in plants (Briens, 1978). In addition to the effect of the soil, the oxalate content in the Dabou leaves could also be attributed to the development stage of the kplala leaves, because kplala leaves are often harvested and consumed during the flowering period (as in the case of the kplala samples in this study) (Harouna Dieté *et al*., 2023). Moreover, in Côte d'Ivoire, the cooking method for kplala leaves would reduce these anti-nutritional factors, as they are sensitive to long boiling (Agbo *et al*., 2019; Harouna Dieté *et al*., 2023).

Definitively, the differences observed in the nutritional and anti-nutritional composition of the leaves of kplala cultivars compared with the data found in the literature of kplala harvested in other areas could be explained by the variability of cultivation practices, climate, soil type, growth stage, use of natural or chemical fertilisers and the analysis period (Soliman and Mohamed, 2020).

Table 2. Nutrient and anti-nutrient content of the leaves of five cultivars of *C. olitorius* found in central Côte d'Ivoire

	CI. B	CI. R	CO. B	CO. NB	CO. R	RDA*
Macronutrients (%DM)						
Dry matter	35.70±0.42^{a}	84.70±0.99^{a}	85.20±0.28^{a}	85.31±0.31^{a}	82.18±0.00^{b}	-
Protein	25.66±0.41^{a}	21.74±0.09^{b}	19.57±0.44^{c}	20.30±0.25^{c}	19.34±0.37^{c}	48-50g
Fat	1.29±0.04^{a}	0.63±0.01^{b}	0.46±0.02^{b}	1.90±0.11^{c}	1.17±0.03^{a}	25-32g
Ash	11.15±0.02^{a}	11.41±0.10^{ab}	11.77±0.18^{b}	8.48±0.04^{c}	12.33±0.00^{d}	-
Fibre	3.32±0.28^{a}	3.99±0.00^{b}	9.90±0.07^{c}	6.59±0.14^{d}	8.31±0.03^{a}	19-29g
Carbo-hydrates	40.89 ±0.08^{a}	47.17 ±0.03^{b}	43.37 ±0.36^{c}	48.54 ±0.08^{d}	41.32 ±0.04^{a}	189 - 212 g
Energy, Kcal/100g	276.77 ±0.15^{a}	280.69 ±0.08^{b}	256.27 ±0.06^{c}	288.18 ±0.02^{d}	252.66 ±0.14^{e}	2120 - 2600
Micronutrients (mg/100g DM)						(mg/day)
Ca	2459.40 ±0.32^{a}	2303.52 ±0.47^{b}	2574.36 ±0.12^{c}	2167.36 ±0.35^{d}	2241.64 ±0.29^{e}	800 - 1000
K	9067.52 ±0.57^{a}	9946.47 ±0.06^{b}	10692.26 ±0.05^{c}	8979.54 ±0.22^{d}	6799.51 ±0.24^{e}	1000 - 1200
Mg	0^{a}	23.61^{b}	15.74^{c}	94.45^{d}	55.09^{e}	200 - 230
P	227.68^{a}	288.4^{b}	273.22^{c}	151.79^{d}	349.11^{e}	800 - 1000
Fe	192.75 ±0.25^{a}	29.20±0.15^{b}	33.53±0.09^{c}	60.47±0.41^{d}	56.37±0.35^{e}	20 - 58
Na	128.76^{a}	101.78^{b}	114.09^{c}	110.11^{d}	110.2^{e}	3000 - 4000
Cu	2.14±0.03^{a}	0.00±0.00^{b}	0.00±0.00^{b}	1.83±0.08^{c}	1.72±0.08^{c}	2-5
Zn	5.61±0.06^{a}	2.96±0.02^{b}	3.71±0.04^{c}	3.35±0.02^{d}	3.07±0.01^{b}	15
Na/K	0.014	0.01	0.011	0.012	0.016	< 1
Ca/P	10.8	7.99	9.42	14.28	6.42	>1
Anti-nutrients (mg/100g DM)						
Oxalate	16.65±0.18^{a}	37.48±0.04^{b}	28.43±0.03^{c}	25.30±0.01^{d}	45.80±0.14^{e}	
Phytate	1.21±0.36^{ab}	1.86±0.03^{a}	1.40±0.11^{ab}	0.93±0.03^{b}	1.16±0,01^{b}	
Oxalate/Ca	0.01	0.02	0.01	0.01	0.02	
Phytate/Ca	0.0005	0.0008	0.0005	0.0004	0.0005	
Phytate/Fe	0.01	0.06	0.04	0.02	0.02	

Means sharing no letters are significantly different (P < 0.05). RDA*: Recommended Dietary Allowance (Source: FAO, 2004) (Konare *et al.*, 2022) and (FND, 2005). DM: Dry Matter

Extraction yield

The yield of hydroalcoholic extracts from the leaves of five kplala cultivars varied from 14.4 ± 0.20 to 15.2 ± 0.26% of DM ($p < 0.05$). This variation in yield is thought to be due to the amount of dry matter in the plant material used. All the extracts had a pasty, viscous appearance with a blackish-green colour (Table 3).

Table 3. Extraction yield of *C. olitorius* cultivars

Cultivars of *C. olitorius*	Yield (% of DM)
CO.NB	14.4 ± 0.20^{b}
CO.R	15.2 ± 0.26^{a}
CO.B	14.8 ± 0.44^{ab}
CI.B	14.4 ± 0.35^{b}
CI.R	14.8 ± 0.66^{ab}

Means sharing no letters are significantly different ($P < 0.05$).

Phytochemical analysis of secondary metabolites in the leaves of five Kpala cultivars

Secondary metabolites are molecules synthesised by plants to play a role in adaptation (Hatcher *et al.*, 2020) and are modified by humans to produce medicines, cosmetics, plant protection products or biodegradable plastics (Wang *et al*., 2019; Kallscheuer *et al*., 2019). Phytochemical screening of the leaves of the five cultivars of kplala showed that they contain sterols and triterpenes, alkaloids, polyphenols, flavonoids, tannins, quinones, saponosides and cardiac glycosides (Table 4).

Table 4. Phytochemical screening of secondary metabolites in hydro-ethanolic extracts from leaves of *C. olitorius* cultivars

Secondary metabolites	CI. B	CI. R	CO. B	CO. NB	CO. R
Sterols and triterpenes	+	+	+	+	+
	+	+	+	+	+
Alcaloides	+	+	+	+	+
	+	+	+	+	+
Polyphenols	+	+	+	+	+
Flavonoids	+	+	+	+	+
	+	+	+	+	+
Tannins	+	+	+	+	+
Quinones	+	+	+	+	+
Saponins	+	+	+	+	+
Cardiac glucosides	+	+	+	+	+

Legend: +: presence.

Apart from sterols and triterpenes, Anyasor *et al*. (2020) had identified in Nigeria, in an ethanolic extract of kplala leaves, all the same secondary metabolites detected in the leaves of the five cultivars. Among the secondary metabolites quantified, flavonoids remained the most important of the five kplala cultivars. The highest

levels of polyphenols, flavonoids and condensed tannins were obtained in the cultivar CO. NB with significant difference ($p < 0.05$) (Figure 2).

The average polyphenol content of kplala leaf cultivars (Figure 2) was 7 times higher (2.44 mg AGE/g extract) than that found by Acho *et al*. (2014) in kplala leaves harvested in Dabou (Côte d'Ivoire). On the other hand, it was slightly higher than that reported by Gomaa *et al*. (2019) 22.41 mg AGE/g extract in kplala leaves purchased on the local market in Egypt and similar to that presented by Obeng *et al*. (2020) 9.82 mg AGE/g DW in kplala leaves harvested in Ghana.

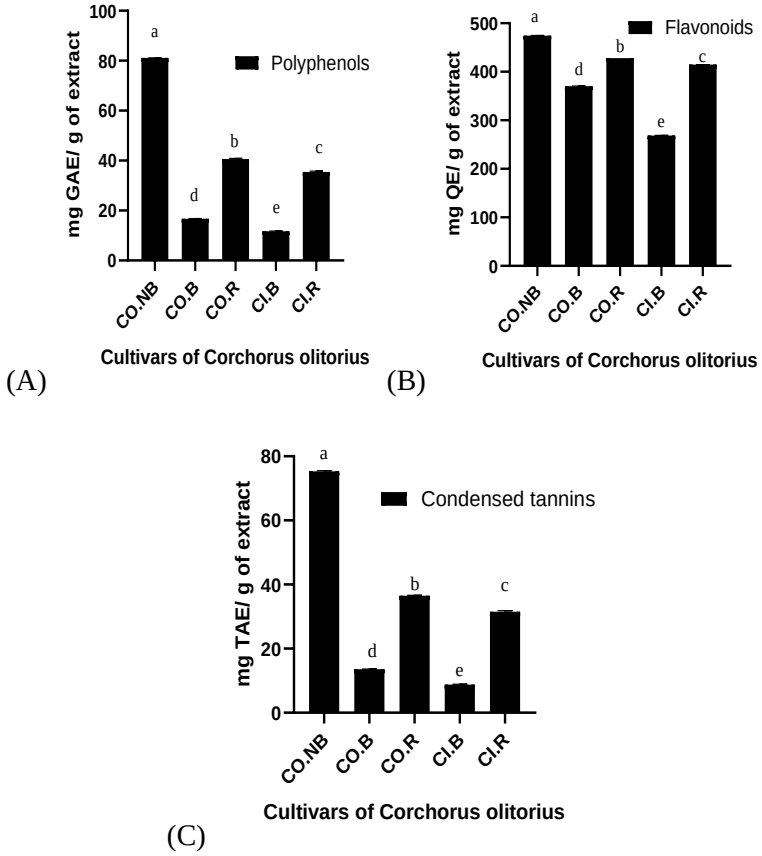


Figure 2. The content of polyphenols (A), flavonoids (B) and condensed tannins (C) in the hydroethnolic extracts of the leaves of five *Corchorus olitorius* cultivars.

The flavonoid content of kplala leaves was significantly higher than that reported by Acho *et al*. (2014) 1.69 mg/g in kplala leaves harvested in Dabou (Côte d'Ivoire), and also higher than that reported by Mokhtar & Morsy (2014) 117.88 mg QE/g kplala leaf extract from Egypt. On the other hand, the flavonoid content of the leaves

of the kplala cultivars was lower than the content found by Obeng *et al*. (2020) 1033.45 mg QE/g kplala leaf extract from Ghana. Biswas et al. (2020) reported that the tannin content of leaves of 11 kplala cultivars from Mali, India and China varied from 13.08 to 26.95 mg TAE/g DW. Furthermore, Biswas *et al.* (2023) found levels varying from 16.51-26.89 mg TAE/g DW in kplala leaves (from China) analysed at different stages of development (seedling, vegetative growth, flowering and post-flowering). These authors showed that kplala leaves contained more polyphenols, flavonoids and tannins during the flowering period than during the sowing, vegetative growth, post-flowering or ripening stages. Moreover, this variability in the content of these bioactive compounds is linked to climate variability, soil type, the cultivation technique used, extraction methods, the type of solvent used and the analysis methodology (Biswas *et al*., 2020). These biologically active compounds are considered to be reducing agents and hydrogen donors, giving them a powerful antioxidant capacity to attenuate the effects of lipid and protein oxidation, water oxidation, etc. (Biswas *et al.*, 2023).

Antioxidant activity of the hydroethanolic extract of the leaves of C. olitorius cultivars determined by the DPPH and ABTS test.

The DPPH and ABTS radical test is based on the assumption that an electron or proton donor is an antioxidant, and the antioxidant capacity is proportional to the disappearance of the free radical in the studied sample (Molyneux, 2004; Biswas *et al*., 2020; Flieger e*t al*., 2021). Evaluation of the antioxidant activity by inhibition of the DPPH radical and the ABTS radical showed after comparison with the reference (Trolox) that the leaves of *C. olitorius* cultivars had an antioxidant capacity capable of inhibiting the free radicals with generally a significant difference ($p < 0.05$). With the exception of the cultivar CI. B, the other four cultivars CO.B, CO.NB, CO.R and CI.R had very low IC_{50} values (the lowest concentration capable of inhibiting DPPH and ABTS radicals) (Table 5), which shows that these *C. olitorius* cultivars leaves could be considered as food supplements with the capacity to prevent oxidative stress or prevent oxidation. Thus, the antioxidant activity of these *C. olitorius* cultivars would be due to the individual or synergistic action of the phytochemical compounds analysed. In short, in the absence of effective therapies for the treatment of diseases caused by oxidative stress such as diabetes, atherosclerosis, coronary heart disease, cancer, inflammation, cardiovascular diseases, neurodegenerative diseases, etc., antioxidants or sources of natural antioxidants such as these leafy vegetables may prove to be an effective alternative (Flieger *et al.*, 2021).

Choudhary *et al* (2013) showed that there is a positive correlation between oxidative stress, antioxidants and the development of diseases such as diabetes. It would be interesting to evaluate the anti-diabetic activity of the leaves of these *C. olitorius* cultivars in order to help reduce the prevalence of type 2 diabetes, which rose from 4.2 to 8.4% between 2015 and 2020 in Côte d'Ivoire (Christiane, 2018; Bakary, 2020).

However, it should be added that these *in vitro* evaluation methods are highly dependent on pH, solvent, the sample collection conditions and the extract

preparation methods, which are non-exhaustive elements that could considerably influence the results obtained (Pinelo *et al*., 2004; Flieger *et al*., 2021). Additionally, it has been reported that poor absorption of exogenous antioxidants to the body is due to restrictions in penetration of cell membranes and their degradation in the stomach and intestines (Molyneux, 2004; Flieger *et al.*, 2021). On the other hand, it is now generally accepted that the high *in vitro* chemical reactivity of plant antioxidants does not constitute proof of their effectiveness *in vivo* (Flieger *et al*., 2021). For this reason, it would be interesting to evaluate the *in vivo* antioxidant activity of the leaves of these five kplala cultivars in terms of future work in order to confirm their real potential antioxidant capacity.

Table 5. Antioxidant activity of the hydroethanolic extract of the leaves of five cultivars of *C. olitorius* determined by the DPPH and ABTS test.

	DPPH (IC_{50} µg/mL)	**ABTS (IC_{50} µg/mL)**
CI.B	173.09±3.78^{a}	17.72±0.24^{a}
CI.R	77.75±0.29^{b}	29.60±0.49^{b}
CO.B	60.69±0.05^{c}	18.72±0.30^{a}
CO.NB	61.55±0.33^{c}	16.31±0.04^{c}
CO.R	63.37±0.22^{c}	12.31±0.32^{d}
Standard (Trolox)	10.57 ± 0.0019	32.56 ± 0.0016

Means sharing no letters are significantly different ($P < 0.05$).

Comparative analysis of the constituents and antioxidant potential of different kplala cultivars

Principal component analysis (PCA) performed on the variables (or cultivars) explained 69.28% of the total variance of the parameters studied. The hierarchical ascending classification carried out on the coordinates of the individuals shows three classes that can be distinguished by affinity (C1, C2, C3). The first class (C1) corresponds to the cultivar CO. R cultivar, characterised by high levels of anti-nutritional factors (oxalate and phytate) and phosphorus (P), with low levels of dry matter, energy, protein, fibre, mineral salts (Zn, Fe, Na, Cu and K) and antioxidant activity (ABTS). The second division comprises classes C2 and C3. Class C2 is made up of the cultivars CO. NB and CI. B, which have similar properties, particularly in terms of nutrients, secondary metabolites and antioxidant activity. In-depth analysis shows that the cultivar CO. NB is rich in bioactive compounds such as polyphenols, flavonoids, tannins and rich in antioxidant activity (ABTS and DPPH) with the lowest IC_{50} values. It is also rich in total carbohydrates (responsible for the plant's gooey character, much appreciated by the local population) and lipids. The cultivar CI. B has the best nutritional profile, being rich in dry matter, protein, fibre and minerals (Zn, Fe, Na, Cu and K), but low in bioactive compounds (polyphenols, flavonoids, tannins, DPPH antioxidant activity). Finally, the third class (C3) is made up of the cultivars CI. R and CO. B, which are characterised by high calcium, phosphorus and crude ash values (Figure 3). Of the five cultivars, the CO. NB, which

is the most widely consumed by people in central Côte d'Ivoire (Harouna Dieté *et al*., 2023), has the best profile in terms of bioactive constituents and antioxidant activity, with high levels of nutrients (Figure 3).

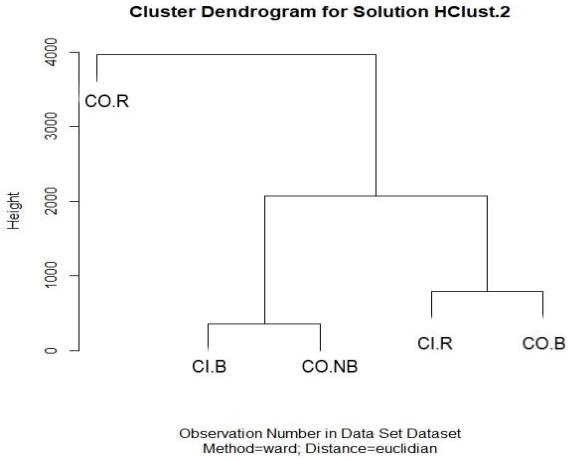


Figure 3. Dendrogram of *C. oltorius* cultivars classified according to their phytochemical profile and antioxidant activity

Furthermore, the phytochemical profile and antioxidant potential of the kplala cultivars are closer to those of aubergine leaves, spinach and taro, which are the most widely consumed and marketed by the Ivorian population (Acho *et al*., 2014; Sheik *et al*., 2023) (Figure 4).

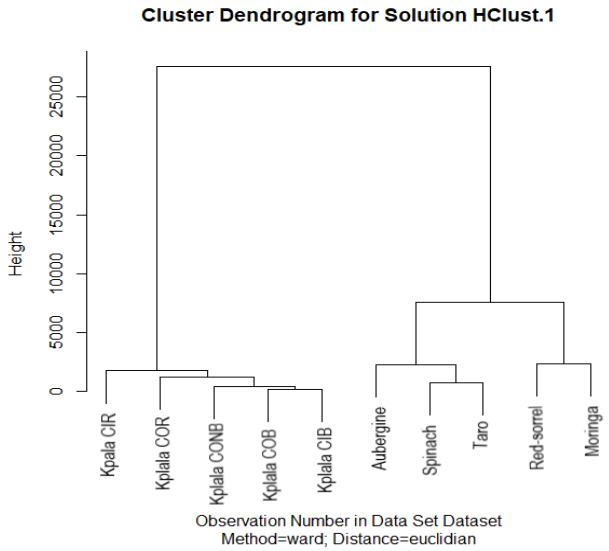


Figure 4. Classification of different leaves consumed in Côte d'Ivoire with kplala

Conclusions

This study, which analysed the phytochemical composition and antioxidant potential of the leaves of five kplala cultivars from central Côte d'Ivoire, showed that they are good sources of protein, energy, iron, calcium and potassium, and that they are low in phytate and oxalate. In terms of secondary metabolites, the leaves of these kplala cultivars also contain sterols and triterpenes, alkaloids, polyphenols, flavonoids, tannins, quinones, saponosides and cardiac glycosides. Quantitative analysis showed that flavonoids were the main compounds among the secondary metals quantified. The results of the antioxidant activity assessment show that these cultivars are sources of natural antioxidants that could contribute to the fight against diseases linked to oxidative stress. However, it is important to assess the antioxidant activity of these kplala cultivars *in vivo*, particularly the one with the best phytochemical profile and antioxidant potential, in order to prove their real effectiveness in the living organism.

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