Phytochemical Study and Evaluation of the Nutritional Potential of *Glyphaea brevis* leaves from the flora of Togo

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ABSTRACT

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| Glyphaea brevis is a food plant used in traditional medicine to treat hypertension and a number of infectious diseases. It has been the subject of very few studies and there is little data on its nutritional value. The aim of the present study is therefore to exploit the leaves of Glyphaea brevis from Togolese flora. Aqueous and hydroethanolic extracts were obtained by maceration of leaf powder. Phytochemical tests and polyphenol and flavonoid assays were carried out using colorimetric methods. Antioxidant activity was assessed in vitro using the DPPH radical scavenging test and the FRAP test. The nutritional value of Glyphaea brevis leaves was assessed using standard AOAC methods, and toxicity was assessed using an acute toxicity test in wistar rats. Phytochemical screening revealed the presence of flavonoids, saponosides, tannins, gallic tannins, terpenoids and mucilages in the aqueous extract. In addition to these compounds, anthocyanins and terpenes were found in the hydroethanol extract. The determination of polyphenols and flavonoids in the considered extracts showed that the hydroethanolic extract of the leaves contained the highest levels of these phenolic compounds: 200.893 ± 4.805 mgEAG/gE for polyphenols and 74.034 ± 2.815 mgEQ/gE for flavonoids respectively. The IC50 for antiradical compounds (DPPH●) were 1271.136 ± 3.204 μg/mL for the hydroethanolic extract and 1535 ± 2.310 μg/mL for the aqueous extract and the antioxidant activity with the FRAP test was 914.333 ± 2.205 μmol/L and 440.966 ± 1.105 μmol/L respectively. The levels of biochemical compounds were 64.99% for digestible carbohydrates, 12.14% for fibre, 19.03% for protein and 3.84% for fats. The energy value was 393.85 Kcal/100g DM. Spectrophotometer assays revealed the presence of minerals such as Na, Ca, Mg, K and Fe at significant levels, with a Na/K ˂ 1 ratio. The acute toxicity test did not reveal any signs of toxicity. All the results obtained in this study therefore justify the therapeutic and dietary use of Glyphaea brevis leaves.  |

***Keywords****: Glyphaea brevis, phytochemical characteristics, antioxidant activity, nutritional value.*

1. INTRODUCTION

Human beings use plants for a wide variety of purposes. Plants are used in many areas, including health and food. In addition, the nutritional importance of leafy vegetables has been highlighted by the advent of chronic diseases such as cancer, diabetes, obesity and cardiovascular disease (Vodouhe et al. 2012). It has also been observed that 25-60% of cancers can be prevented by diet, with better integration of fibre (Afolayan 2008). In this respect, the consumption of leafy vegetables, which are an important source of dietary fibre, vitamins and minerals, is becoming a necessity (De Fremicourt 1996). Improving the nutritional status of people living in urban, rural and peri-urban areas therefore depends on incorporating leafy vegetables into their daily diet. These plants are consumed by different peoples for their therapeutic and dietary value. Daily consumption of fresh green vegetables contributes to a balanced diet and good health. Vegetables are therefore an important source of food and medicine, thanks to their nutrients and antioxidant compound, which are not found in staple foods alone (Organization 2011). They are therefore an endogenous source of food and nutritional security that is essential for sustainable development. The need for vegetables is growing in cities, and they are constantly being grown to meet ever-increasing consumer demand. Then, vegetables are of considerable economic and social interest, given their relatively accessible cost and the ease with which they can be processed (Dansi et al. 2008). In Togo, and more specifically in the maritime region, tropical and exotic plants are grown to meet the need for fresh vegetables. This is the case for the leaves of Adansonia digitata and Moringa oleifera (Abdel-Ghaffar et al. 2011) which not only have therapeutic potential but also interesting nutritional characteristics (Ferreira et al. 2008) alongside the usual leafy vegetables produced for the same purpose. However, due to problems associated with declining soil fertility and high pest pressure, traditional vegetable cropping systems without chemical inputs cannot easily meet the challenge of food security (Afolayan & Jimoh 2008). It is therefore becoming necessary to further diversify vegetable sources by considering other under-used species of nutritional interest. Among these under-used species is Glyphaea brevis, a species of leafy vegetable found in the Togolese flora, known for its diverse properties and used in traditional medicine to treat various illnesses such as malaria, gastric disorders, intestinal parasites, infections, dyspepsia and diarrhoea. This species is also used as food by the Togolese population. However, very little data is available on this interesting species. This justifies its low value, even though it could contribute to the availability of leafy vegetables and hence to food security. In this context, there is a need for data on G. brevis. The present study therefore set out to determine the phytochemical characteristics and nutritional potential of this species in order to contribute to its development in Togo.

Provide a factual background, clearly defined problem, proposed solution, a brief literature survey and the scope and justification of the work done.

2. material and methods

**2.1. Hardware**

**2.1.1. Plant material**

The material consisted of the leaves of *Glyphaea brevis* (Fig.1), collected in southern Togo in the plateau region at Danyi-Kpévé (Danyi prefecture). The identity of the plant was botanically verified at the Department of Botany, University of Lomé, where a voucher specimen (TOGO15930) was deposited in the Herbarium of Togolese Flora. The harvested leaves were spread out and dried at room temperature (25-28°C) at the Laboratory of Organic Chemistry and Natural Substances (Lab COSNat) at the University of Lomé for 7 days. After drying, they were ground to a powder using an electric grinder.



**Figure 1. Leaves of *Glyphaea brevis***

**2.1.2. Animal material**

The animal material consisted of Wistar rats supplied by the animal house of the Faculty of Science at the University of Lomé (Togo). All procedures involving animals were carried out in accordance with the guidelines of the national ethics committee (No. SBM/UL/15/NS0009)

**2.2. Preparation of extracts**

Extraction was carried out by maceration of 100 g of powder in 1 L of distilled water for the aqueous extract and in 1 L of hydroethanol solvent (ethanol - distilled water, 4:1, v/v) for the hydroethanol extract. Each maceration was renewed every 24 hours for a total duration of 72 hours. The mixtures were stirred intermittently. After filtration through cotton and then Whatman paper, the resulting filtrates were concentrated using a rotary evaporator at 40 °C for the hydroethanolic extract and at 45 °C for the aqueous extract, in order to obtain the dry extracts. The extracts obtained were then stored at 4°C. The extraction yield was calculated using the following formula**:**

**R= (Mex / Mp) x 100**

**R** is the extraction yield in %; **Mex** is the mass of dry extract; **Mp** is the mass of powder used.

**2.3. Phytochemical screening**

For the phytochemical screening, alkaloids, flavonoids, anthocyanins, anthraquinones, saponosides, tannins, terpenes, terpenoids and gallic tannins were sought using various methods (Wuivi et al. 2023) for determining the main chemical groups based on colouring and/or precipitation tests.

**2.3.1 Test for alkaloids**

5 g of the plant powder was mixed with 25 ml of 5% hydrochloric acid. The mixture was macerated for 24 h. Then, 1 ml of filtrate was collected and 5 drops of Mayer's reagent was added. The formation of a pale yellow or squinty precipitate demonstrates the presence of alkaloids.

**2.3.2 Test for flavonoids**

5 ml of extract was treated with 5 ml of hydrochloric alcohol (SHINODA reagent) and a pinch of magnesium powder. The formation of orange colour indicates the presence of flavones, red colour indicates flavonols and violet colour indicates flavonones.

**2.3.3 Test for saponosides**

Saponosides are detected by the foam index, which is determined by the degree of dilution of an aqueous decoction of the drug which, under the given conditions, produces a persistent foam. A few volumes of plant extract were adjusted to 100 ml, is distributed into 10 test tubes in arithmetical series of 1/10 concentration. After 30 longitudinal shakes for 15 seconds, the tubes are left to stand for 15 minutes. The height of the foam is measured. If it is greater than 1 cm in one of the tubes, the dilution in these tubes is the foam index sought.

**2.3.4 Test for tannins**

1 ml of extract was treated with a few drops of 1% FeCl3. A dark blue, green or black coloration indicates the presence of tannins.

**2.3.5 Test for gallic tannins**

1 ml of extract was saturated with sodium acetate and treated with a few drops of 1% FeCl3. A blue or black blue reveals the presence of gallic tannins.

**2.3.­­6 Test for anthocyanins**

1 ml of extract was treated with a few drops of 5% HCl. The mixture was then alkalinized by adding a few drops of diluted ammonia. A deepening red color, turning violet-blue or greenish, indicates the presence of anthocyanins.

**2.3.7 Test for triterpenes**

10 ml of EtOH at 70 °C to 1 g of powder and shake for 30 minutes. To this mixture, was added 10 ml of distilled water, then 2 ml of 10% lead acetate, equal volume V/V. After a 15-minute rest, 2 ml of 10% aqueous disodium phosphate solution was added to the filtrate. After 15 minutes' rest, the filtrate was collected in a separating funnel and extracted three times with 5 ml chloroform. The chloroform solutions were dried over anhydrous sodium sulfate and then evaporated. The first portion was solubilized with a few drops of acetic acid. To the resulting mixture was added 3 ml of a mixture of acetic anhydride-sulfuric acid. A purple, blue or green color indicates the presence of triperpenoids.

**2.3.8 Test for mucilages**

1 ml of 10% diluted extract was introduced into a tube and 5 ml of absolute alcohol was added. The appearance of a flaky precipitate indicates the presence of mucilages after around ten minutes.**2.4. Determination of phenol contents**

The determination of total phenol contents, based on quantification of the total concentration of hydroxyl groups present in the extract, was carried out spectrophotometrically, using the colorimetric method with the Folin-Ciocalteu reagent (Evenamede et al. 2019).

The protocol used is based on that described by Ali-Rachedi et al. (2018). A calibration curve was performed using gallic acid at different concentrations. The total phenol contents of *Glyphaea brevis* extracts were determined from the linear regression line (Y= 0.011173X2 + 0.022972; $R^{2}$ = 0.9891) of the gallic acid calibration curve.

**2.4.1.** [**Flavonoid assay**](https://popups.uliege.be/0037-9565/index.php?id=7398#tocfrom3n3)

Flavonoids were quantified using a method based on the formation of a highly stable complex between aluminium chloride and the oxygen atoms present on carbons 4 and 5 of the flavonoids. The protocol used is based on that described by Ali-Rachedi et al. ( 2018). A calibration curve was performed using quercetin at different concentrations. The flavonoid contents of *Glyphaea brevis* extracts were then determined from the linear regression line (Y= 0.0008X+ 0.0021; $R^{2}$ = 0.9967) of the quercetin calibration curve.

**2.5. Assessment of antioxidant activity**

**2.5.1. DPPH radical reduction test**

This activity was assessed by free radical scavenging. The DPPH radical , which is a free radical that is stable at room temperature and soluble in ethanol, was used to determine the antiradical potential of the extracts using the method described byBrukum Florance et al. (2023).

The percentage inhibition (PI) of DPPH free radicals was calculated according to the formula:

 **PI (%) = ((A0 - A1)/A0) × 100**

* A0: absorbance of DPPH
* A1: absorbance after addition of the test products at a given concentration.

The concentration of the sample required to neutralise 50% of the free radicals (IC50) was determined graphically by linear regression.

The anti-free radical activity of the different extracts was assessed by their inhibitory activity on an ethanolic solution of DPPH●, measured at 517 nm. The standard used was quercetin. The reducing power of the 2,2'-diphenyl-1-picryhydraqyl radical (DPPH) was determined from the linear regression line (Y = $-$0.0053973X2 + 0.79431; $R^{2}$ = 0.99726) of the quercetin calibration curve.

**2.5.2. Ferric ion reduction test (FRAP)**

The FRAP test involves measuring the ability of extracts to reduce ferric iron Fe3+ to ferrous iron Fe2+. Fe3+  is involved in the formation of the hydroxyl radical via the Fenton reaction (Karagözler et al. 2008). It is used to determine the antioxidant potential of extracts.

In this test, the electron donor capacity of the antioxidant is measured by the change in absorbance at 593 nm when a blue ferrous tripyridyltriazine complex (Fe2+ TPTZ) is formed from a colourless oxidised Fe3+ form.

The iron reducing power of the considered extracts was determined from the linear regression line with equation Y = 0.0005280X2 - 0.0005; R2 = 0.9925 from the Fe2+ calibration curve.

**2.6. Determination of biochemical compounds contents**

**2.6.1. Determining water content**

The method involved drying 1 g of sample in the oven at a temperature of 105 ± 2°C until the weight of the sample became constant.

The water content was determined according to the formula:

Te (%) = $\frac{m1-m2}{m1} x 100$

With Te = water content; m1 = mass of fresh sample and m2 = mass of dry sample.

**2.6.2. Determining dietary fibre content**

Dietary fibre content was determined using the cellulose insoluble method in accordance with French standard NF V 03-040.

**2.6.3. Determination of crude ash contentes**

The samples were incinerated progressively in a muffle furnace at a temperature of $550°C$. The organic substances were transformed into $H\_{2}O$ and$ CO\_{2}$and the residue obtained was raw ash, made up of mineral elements.

**2.6.4. Fat contents**

Fats were determined using the AOAC (2005) method**.** The principle consists of repeatedly bringing hexane into contact with the samples in the presence of heat (in a heating flask).

**2.6.5. Protein assay**

Total nitrogen content was determined using the Kjeldahl method (AOAC 2005).

**2.7. Determination of total carbohydrate content**

The total carbohydrate content of the sample was deduced from the difference between the dry matter content and the sum of the protein, lipid, fibre and crude ash contents using the following formula:

$$GT\left(\%\right)=MS\left(\%\right)-\left[P\left(\%\right)+MG\left(\%\right)+F(\%)+CB\left(\%\right)\right]$$

**2.8. Determination of minerals**

The content of minerals ($Fe, K, Na, Mg, Ca et Zn$) were determined by flame atomic absorption spectrophotometry at wavelengths of 372.0 nm, 766.6 nm, 589 nm, 403 nm, 422.7 nm and 885 nm respectively.

**2.9. Assessment of the acute toxicity of Glyphaea brevis leaves**

Toxicity was assessed using the acute toxicity test at a dose of 5000 mg/Kg body weight with Wistar rats (Teichler and Bürger 2008). The extracts in question were administered orally to the rats in a single dose. The rats treated in this way were monitored for 14 days to note any signs of observable toxicity.

**2.10. Data processing**

The results were analysed using Graphpad prism 8 software, and graphs and histograms were produced using the same software. The CIs50 were calculated from the linear or logarithmic equations of the curves plotted.

3. results and discussion

The aim of this study was to carry out a phytochemical study and assess the nutritional potential of *Glyphaea brevis* leaves.

**3.1. Physical characterisation of the extract**

The hydroethanol and aqueous extracts obtained after evaporation were brown in colour with a pasty consistency. The aqueous and hydroethanol extracts obtained had yields of 4.46$\%$ and 10.25$\%$ respectively.

**3.2. Phytochemical screening of different extracts**

Phytochemical screening of the aqueous and hydroethanol extracts showed the presence of flavonoids, saponosides, tannins, gallic tannins, terpenoids and mucilages, but did not reveal the presence of alkaloids (Table 1). These results are similar to those reported by Dickson et al. (2011) who found alkaloids, flavonoids, tannins, gallic tannins, terpenoids and mucilages in the hydroethanol extract of the leaves. Oloruntobi and Johnson (2015) reported the presence of alkaloids, saponins, tannins, flavonoids and terpenoids in the ethanolic extract of *Glyphaea brevis* leaves. The absence of alkaloids observed in both extracts may be due to the solvents used (Hosni et al. 2020). The presence of these secondary metabolites suggests the biological activities of *Glyphaea brevis* leaves, hence the need for a quantitative study.

**Table 1. Results of phytochemical screening of extracts from *Glyphaea brevis***

|  |  |  |
| --- | --- | --- |
| Compounds sought | Results with aqueous extract | Results with hydroethanol extract |
| Alkaloids | $$-$$ | $$-$$ |
| Flavonoids | $$+$$ | $$+$$ |
| Saponosides | $$+$$ | $$-$$ |
| Tannins | $$+$$ | $$+$$ |
| Gall tannins | $$+$$ | $$+$$ |
| Anthocyanins | $$-$$ | $$+$$ |
| Terpenes | $$-$$ | $$+$$ |
| Terpenoids | $$+$$ | $$+$$ |
| Mucilage | $$+$$ | $$+$$ |

 + = Present ; $-$ = absent

**3.3. Quantitative tests**

**3.3.1. Total phenol content**

Total phenol content was expressed for each extract in mgEqAG/g dry extract. The quantity of total phenols in the extracts in the present study is reported in milligrams of gallic acid equivalents per gram of dry matter (mgEqGAg dry extract). The results obtained with the hydroethanolic and aqueous extracts of *Glyphaea brevis* leaves show respective contents of 200.893 $\pm $ 4.805 mgEqAG/g and 99.33$0\pm $ 4.640 mgEqAG/g dry extract. These values are shown in Fig.2a. The results of the total phenol assay (Fig.2a) showed that the hydroethanol extract of *Glyphaea brevis* leaves had a higher phenolic compound content than the aqueous extract. The presence of these phenolic compounds in the two extracts at different levels could be explained by several factors that could influence their levels. Recent studies have shown that the polarity of the solvent, the duration of storage and the extraction method used have a strong influence on the phenolic compound content. These high levels of phenolic compounds bode well for the antioxidant properties of *Glyphaea* **brevis** leaves ( Mahita et al. 2014).

**3.3.2. Total flavonoid content**

The total flavonoid contents obtained were 74.034$ \pm $2.815 mgEQ/g dry extract for the hydroethanol extract and 28.547$ \pm $ 1.141 mgEQ/g dry extract for the aqueous extract, as shown in Fig. 2b. Analysis of the extracts showed that the total flavonoid content of the hydroethanolic extract of *Glyphaea brevis* leaves was higher (74.034 ± 2.815 mgEqQ/g ES) than that of the aqueous extract (28.547 $\pm $1.141 mgEQ/g ES). This difference may also be linked to the different solvents used for the extractions. In fact, the concentration of flavonoids in the extracts depends on the polarity of the solvents used in extract preparation (Djeridane et al. 2010). Flavonoids, among many others, are known for their antioxidant, anti-inflammatory, diuretic and artery-protecting properties ( Ksouri et al. 2007). The presence of flavonoids in the leaves is thought to play a positive role in the treatment of cardiovascular and neurodegenerative diseases, and suggests that the leaves have anti-tumour activity (Badiaga 2011).

 

**Figure 2. Total phenol content of the extracts (a), Flavonoid con****tent of the extracts (b)**

**3.4. Assessment of antioxidant activity**

**3.4.1. DPPH radical reduction test**

The anti-free radical activity of the different *G. brevis* extracts and that of the standard are shown in Fig.3a. The antioxidant activity of *Glyphaea brevis* extracts was expressed in mgEqQ/g ES. The CI values (50% inhibitory concentration) obtained were used to compare the DPPH radical scavenging capacity of the extracts tested versus quercetin. The quercetin with the lowest IC50 value, 43.130 ± 1.055 µg/mL, had the highest free radical scavenging activity compared with the hydroethanol and aqueous extracts tested. The hydroethanol extract of the leaves (IC50: 1271.136 ± 3.204 µg/mL) has a higher anti-free radical activity than the aqueous extract. This difference in potency confirms the difference between the phenolic compound contents of these two extracts (Hosni et al. 2020). The results of the present study are consistent with those reported by Dickson et al. (2011).This antioxidant power of *Glyphaea brevis* leaf extracts is probably due to the presence of hydroxyl groups in the polyphenols and flavonoids, which can act as electron donors. It may also be due to the presence of other antioxidant classes such as anthocyanins and certain minerals known for their antioxidant activity (Adida et al. 2016). Previous studies have also reported that phenolic compounds, generally flavonoids, phenolic acids and tannins, possess remarkable antioxidant properties due to the existence of conjugated double bonds within their intrinsic structures, enabling them to stabilise free radicals by acquiring several resonance-stabilised mesomeric forms.

**3.4.2. FRAP test**

The reducing power of the extracts was expressed in equivalents Fe2+ shown in Fig.3b. The results show that the capacity to reduce iron is proportional to the increase in sample concentration. However, the hydroethanol and aqueous extracts of *Glyphaea brevis* leaves showed different antioxidant activities, with values of 914.333$ \pm $2.205 μmol/L and 440.966$ \pm $ 1.105 μmol/L, indicating that the antioxidant activity of the hydroethanol extract was higher than that of the aqueous extract. This antioxidant power is due to the presence of secondary metabolites.

 

**Figure 3. Anti-free radical compound content (a)****, Ferrous Fe 2+ ion content (b)**

**Data were expressed as mean ± MSE (n = 3)**

**3.5. Nutritional potential of *Glyphaea brevis* leaves**

Assessment of the nutritional potential of *G. brevis* leaves shows that they have nutritional value due to the presence of primary and secondary metabolites. The average mineral content, expressed in terms of total ash, was 9.92 mg/100 g of dry matter in *Glyphaea brevis* leaves. These levels are higher than those reported by Ndong et al. (2007) for *Moringa oleifera* leaves (2.42 ± 0.30%). This indicates that *Glyphaea brevis* leaves are rich in minerals. In addition, the average Na, K, Mg, Ca and Fe contents obtained in this study were 71.58, 525.75, 17.92, 5080 and 0.15 mg/100 g respectively, apart from trace zinc, compared with 70.87 ± 0.48, 254.44 ± 7.74, 97.27 ± 3.25, 423.19 ± 25.90 and 21.72 ± 0.61 mg/100 g dry matter respectively reported for young *M. oleifera* leaves (Ndong et al. 2007) and 7.43, 54.20, 231.22, 44.15**,** 13.58 and 3.80 mg/100 g respectively for Na, K, Mg, Ca, Fe and Zn in the leaves of *Amaranthus hybridus* (Akubugwo et al. 2007). These levels are therefore comparable to those found in certain commonly consumed vegetables such as the leaves *of Amarantharus hybridus*, *Moringa Oleifera* and *Adansonia digitata*(Akubugwo et al. 2007). Minerals are essential elements required for the proper functioning and maintenance of the body. The presence of these minerals in appreciable quantities in *G. brevis* leaves is therefore a nutritional asset. Consequently, their use in food could have beneficial effects on osteoporosis, the prevention of ageing and the strengthening of the immune system (Melila et al. 2021). In addition, the presence of calcium, potassium, iron, sodium and magnesium could prevent certain deficiency diseases. In addition, the average Na/K ratio was less than 1, making it beneficial for the prevention of arterial hypertension (Houndji et al. 2018).

Considering 100 g of dry matter from *G. brevis* leaves, this study shows that the contribution to the recommended daily allowance (RDA) is low for zinc ($< $0.01%), iron (0.2-0.6%), magnesium (4.27%) and sodium (4.77%). However, it is relatively high for potassium (11.18%) and very high for calcium (564.44%).

The water content of *G. brevis* leaves (70.68%) was relatively close to those reported for *Amarantharus hybridus* leaves (83.48%) and M. oleifera leaves (73%).$\%),$ *M. oleifera* (73$\%) $and *A. digitata* (74$\%)$ and much higher than that observed in cereals (10-20%) (Melila et al. 2021). The average total carbohydrate content of *G.brevis* leaves was 77.13 g/100 g dry matter. This is higher than that of *Amarantharus hybridus* leaves (52.18$ \%$) (Akubugwo et al. 2007) *A. digitata* leaves (16.1$ \%$) (Atchibri et al. 2012) and young *M. oleifera leaves* (14.1$ \%)$ (Ndong et al. 2007). This carbohydrate content would justify the contribution of *Glyphaea brevis* leaves to the Recommended Daily Allowance (RDA) for this nutrient. This shows that these leaves have a good nutritional value in relation to carbohydrates. The fat content obtained was 3.84%. This is lower than that of *Amarantharus hybridus* (4.65$ \%) $(Akubugwo et al. 2007) and higher than that of *Adansonia digitata* (0.42%) (Atchibri et al. 2012) and *Moringa oleifera* leaves (0.60%) (Ndong et al. 2007). *G. brevis* leaves would then contribute between 3.95$\%$ to 8.72$\%$ to the lipid RDA for a 70 kg adult. This is in line with the fat content of fruit and vegetables and has a positive effect on the fight against chronic diseases. The average protein content is 19.03%. It is higher than that of *Amarantharus hybridus,* close to that of *A. digitata* leaves, but lower than that obtained with *M. oleifera* leaves (57.79 ± 0.24) (Akubugwo et al. 2007). *G. brevis* leaves contributed 33.98$\% $to the protein RDA. This result confirms that these leaves are a significant source of plant protein in the fight against protein-energy malnutrition. The energy value of *G. brevis* leaves is higher than that observed in *A. digitata* (Atchibri et al. 2012)and *Moringa oleifera* leaves (Ndong et al. 2007). This result shows that their use in human food could contribute to the fight against malnutrition. Given the overall organic matter and mineral content, it can be said that the leaves of *Glyphaea brevis* have significant nutritional value, similar to that of commonly consumed vegetables such as *M. oleifera* and *Amarantharus hybridus*. These leaves make an effective contribution to combating mineral deficiencies and protein and energy malnutrition.

**Table 2. Mineral content of *Glyphaea brevis* leaves (DM = dry matter) (a),** **Macronutrient content and energy value of *G. brevis* leaves analysed (DM = dry matter; FM = fresh matter) (b)****, Results of tests for anti-nutritional substances in the leaves of *G. brevis* (c)**

(**a**)

|  |  |
| --- | --- |
| Minerals considered | Contents and values of the ratios considered |
| Sodium (Na) | $71.58$ mg/100 g DM |
| Potassium (K) | $525.75$ mg/100 g DM |
| Calcium (Ca) | $5080$ mg/100 g DM |
| Magnesium (Mg) | $$17.92 mg/100 g DM$$ |
| Iron (Fe) | $$0.15 mg/100 g DM$$ |
| Zinc (Zn) | $<0.01$ mg/100 g DM |
| Na/K ratio  | $$0,13$$ |
| Ca/Mg ratio | $$283.48$$ |

(**b**)

|  |  |
| --- | --- |
| Parameters determined | Leaves of *Glyphaea brevis* |
| Protein content ($g/100 g DM$) | $$19.03$$ |
| Fat ($g/100 g DM$) | $$3.84$$ |
| Total carbohydrates ($g/100 g DM$) | $$67.21$$ |
| Digestible carbohydrates ($g/100 g DM$) | $$64.99$$ |
| Water content ($g/100 g MF$) | $$7.68$$ |
| Total ash ($g/100 g DM$) | $$9.92$$ |
| Total fibre ($g/100 g DM$) | $$12.14$$ |
| Metabolizable energy ($Kcal/100 g DM$) | $$379.52$$ |

(**c**)

|  |  |
| --- | --- |
| Elements considered |  Test results  |
| Cyanides | $$-$$ |
| Nitrites | $$-$$ |

 $ - $= Absent, DM = dry matter; FM = fresh

 **Table 2c** shows the absence of cyanides and nitrites in the leaves analysed

**Table 3. Contribution to RDI in organic and mineral substances from 100 g of *Glyphaea brevis* leaf dry matter (Recommended nutritional intake for a body weight of 70 kg (Frenot & Vierling 2002)AFSSA (Oliveira et al*.* 2021)) (a), Comparison of the mineral content of *Glyphaea brevis* leaves with that of *Amarantharus hybridus*, *Moringa oleifera* and *Adansonia* digitata leaves (b), Comparison of macronutrient content and energy value of *G. brevis* leaves with those of *Amaranthus hybridus* (Akubugwo et al. 2007*)*, *Moringa oleifera* (Ndong et al. 2007) and *Adansonia digitata* (Atchibri et al. 2012) (c)**

**(a)**

|  |  |  |  |
| --- | --- | --- | --- |
| Items analysed | RDA for adults (Male/Female) | Content in 100 g DM of *G. brevis* leaves | Contribution to RDA of 100 g DM of *G. brevis* leaves (%) |
| Fats (g) | 44-97 | 34.84 | 3.95 - 8.72 |
| Protein (g) | 56 | 19.03 | 33.98 |
| Energy (Kcal) | 2500/2000 | 379.52 | 15.18-18.97 |
| Na (mg) | 4700 | 525.75 | 11.18 |
| K (mg) | 4700 | 525.75 | 11.18 |
| Ca (mg) | 900 | 5080 | 564.44 |
| Mg (mg) | 420 | 17.92 | 4.27 |
| Zn (mg) | 750 | $<$ 0.01 | $<$ 0.01 |
| Fe (mg) | 27.40/58.80 | 0.15 | 0.2-0.6 |

**(b)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Items analysed | *G. brevis* | *A. hybridus* | *M. oleifera* | *A. digitata* |
| Na | $$71.58$$ | $$7.43$$ | $$70.87\pm 0.48$$ | $$156.65\pm 6.74$$ |
| K | $$525.75$$ | $$54.20$$ | $$254.44\pm 7.74$$ | $$1584\pm 65.46$$ |
| Ca | $$5080$$ | $$44.15$$ | $$423.19\pm 25,90$$ | $$231.21\pm 29.40$$ |
| Mg | $$17.92$$ | $$231.22$$ | $$97.27\pm 3.25$$ | $$77.69\pm 28.99$$ |
| Fe | $$0.15$$ | $$13.58$$ | $$21.71\pm 0.61$$ | $$6.31 \pm 1.75$$ |
| Zn  | $$< 0.01$$ | $$3.80$$ | $$1.13\pm 0.14$$ | $$1.13\pm 0.14$$ |

**(c)**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Elements considered | *G. brevis* | *A. hybridus* | *M. oleifera* | *A. digitata* |
| Total carbohydrates (g/100 g DM) |  |  |  |  |
| Protein (g/100 g DM) | 19.03 | 17.92 | 57.79 | 28.80 |
| Fat (g/100 g DM) | 3.84 | 4.65 | 0.60 | 0.42 |
| Ash content (g/100 g DM) | 9.92 | 13.8 | 2.42 | 5.61 |
| Water content (g/100 g MF) | 70.68 | 83.48 | 73 | 74 |
| Energy (Kcal/100 g DM) | 379.52 | 268.92 | 390.11 | 305.86 |

MS = dry matter, FM = fresh matter, DM = dry matter

**3.6. Acute toxicity of aqueous and hydroethanol extracts of *Glyphaea brevis* leaves**

Administration of the extracts at a single dose of 5000 mg/kg body weight showed no signs of toxicity in the rats during the 14 days of observation. In fact, during this period, no signs of morbidity were observed in relation to the coat, trembling, breathing, appearance of faeces, mobility, feeding habits or mass of the rats, and no deaths were recorded. If these leaves are to be used as a vegetable, their content in anti-nutritional substances needs to be determined. The results obtained show that toxic substances such as nitrites and cyanides are absent in the leaves analysed, justifying their possible use in human food. Furthermore, the results of the acute toxicity test after 14 days of observation revealed no signs of toxicity. There were no behavioural changes and no deaths in rats. These results show that the LD50 of the extracts is greater than 5000 mg/kg. It can therefore be suggested that *G. brevis* leaves are non-toxic (Kennedy et al. 1986).

4. Conclusion

Phytochemical screening of G. brevis leaves revealed the presence of several phytochemical groups with the exception of alkaloids, which were found to be absent in the two extracts considered in the present study. Assessment of the antioxidant activity of Glyphaea brevis leaves also showed that they have significant antioxidant capacity, in conjunction with their content of phytochemicals such as phenolic compounds and flavonoids. The phytochemical and biochemical compositions of the studied leaves showed that they have a high nutritional value, making a significant contribution to the recommended daily intake. In addition, tests to identify toxic substances in the extracts were negative. The acute toxicity test on wistar rats concluded that the extracts had an LD50 greater than 5000 mg/kg. The use of Glyphaea brevis leaves as a leafy vegetable in the diet could therefore make an effective contribution to promoting health and combating malnutrition. Taken together, these results justify the food and therapeutic uses of Glyphaea brevis leaves and show that this species can be taken into account in the search for endogenous sources of food and nutritional security in Togo.

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Competing interests

The authors declare no conflicts of interest.

Authors’ Contributions

Yaovi Samuel APETI and Kodo Selom EVENAMEDE contributed to the phytochemical study and to the evaluation of the nutritional potential of the leaves of *Glyphaea brevis* from the Togolese flora as well as to the drafting of the original manuscript under the supervision of the other authors. All the authors participated in the conceptualisation, methodology, critical comments and contributed to the drafting of the manuscript.

Ethical approval

All procedures involving animals were carried out in accordance with the guidelines of the national ethics committee (No. SBM/UL/15/NS0009)

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