

Nutritional and toxicological assessment of *Piliostigma thonningii* leaves and Tubaani food samples: a preliminary investigation

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Abstract Tubaani is a local delicacy prepared with *Piliostigma thonningii* leaves. The leaves may contain trace/heavy metals and important phytonutrients that could impact consumers' health. Concerns over the nutritional and toxicological implications of *Piliostigma thonningii* leaves are critical. Tubaani food and *Piliostigma thonningii* leaf samples were investigated using Neutron Activation Analysis (NAA) and Spectrophotometry technique. The health risk of Tubaani was also assessed by calculating the target hazard quotient (THQ) and hazard index (HI) of potentially toxic elements. Fifteen trace elements were detected at non-toxicological concentrations in the samples analyzed. No

significant difference ($p > 0.05$) was observed between the samples' mean concentrations. The phenolic content in leaf extracts was higher as compared to the flavonoids. However, the flavonoids in the leaves had an effect on the food samples, unlike the phenols. The THQ and HI of the elements were below 1.0. There is no reason to be concerned about the current dietary intake of the potentially toxic elements in the routine consumption of Tubaani as portrayed in data obtained in this investigation by NAA, THQ, and HI.

Keywords *Piliostigma thonningii* · Flavonoids · Polyphenols · Neutron activation analysis · Target health quotient · Hazard index

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Introduction

Tubaani is a local delicacy made from steamed cowpea (black-eyed beans) flour mostly consumed by indigenes of some parts of West Africa. In northern Ghana, Tubaani is considered one of the staple foods because of its regular consumption by many people. It is usually served with powdered pepper and salt immersed in vegetable oil. It is a high-energy food laden with nutrients such as vitamins, proteins, iron, and soluble fiber (Abizari et al. 2012; Bosland and Votava 2012). It is prepared by wrapping a slurry of cowpea (black-eyed beans) flour with suitable leaves and boiling over steam to cook. The most common leaves used are those obtained from *P. thonningii* a leguminous plant. The leaves add colour and improve the flavour characteristics of the food (Tubaani) but are also known to be endowed with important minerals and nutrients that could be beneficial for human health. Nevertheless, they may also contain vital nutrients such as dietary antioxidants (Romani et al. 2004) and trace elements capable of ameliorating cellular oxidant

stress thereby reducing the risk of chronic diseases such as diabetes, cancer, cardiovascular diseases, neurodegenerative diseases, thyroid diseases, and allergic diseases (Zujko and Witkowska 2023; Pizzino et al. 2017; Attar 2020).

Traditionally, the raw or cooked tender young leaves of *P. thonningii* are either chewed to relieve thirst or eaten as cooked vegetables. In some jurisdictions, the leaves are cooked in water which is then used to cook millet (Fern 2023). It has been used in preparing various herbal remedies to manage conditions such as fever, coughs, wounds, and various ulcerations. The leaves have been found to contain certain compounds with anti-inflammatory and antibacterial properties (Ighodaro and Omole 2012). Chemical analysis reveals that the plant contains phytochemicals such as tannins, steroids, volatile oils, minerals, phenols and flavonoids, and carbohydrates, reducing sugars and proteins. *P. thonningii* leaves are also known to be rich in flavonoids some of which are effective at reducing cholesterol levels (Madara et al. 2010; John et al. 2020).

Aside from nutritional benefits, *P. thonningii* may also contain toxic elements consumption of which may result in a variety of health conditions such as gastrointestinal and kidney dysfunction, cancers, nervous system disorders, immune system dysfunction, miscarriages, birth defects, and genetic mutations among others (Itanna 2002). Health risks arising from heavy metal contamination along the food chain is currently of global concern. Toxic elements (e.g., mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl), and lead (Pb)) are metallic elements with relatively high

density and poisonous to cells at very low concentrations (Tchounwou et al. 2012).

Therefore, to provide risk–benefit in food safety and nutrition associated with the consumption of Tubaani daily but also demonstrate if antioxidants in the *P. thonningii* leaves survive cooking, the cooked and uncooked leaves, raw beans flour, and Tubaani were subjected to multi-elemental analysis via NAA while nutrients were assayed spectrophotometrically.

Materials and methods

Samples preparation

Uncooked and cooked leaves of *P. thonningii*, black-eyed bean flour, and Tubaani, were obtained from Navrongo in the Kassena Nankana Municipal Assembly at the site of production (10° 50' 53.7" N and 1° 00' 57.6" W) and food market (10° 52' 42.1" N and 1° 5' 4.2" W) from the same vendor. Leaves and food samples were air dried at ambient temperature for 7 days. Three of the samples i.e., the cooked leaves, Tubaani and uncooked leaves Fig. 1a–c were pulverized using a laboratory blender. The beans powder was obtained already pulverized. (Fig. 1d). For the purpose of NAA, three sets of each of the four powdered samples were wrapped in thin sheets of polyethylene and heat-sealed into pellets weighing 150 mg (Fig. 1e).

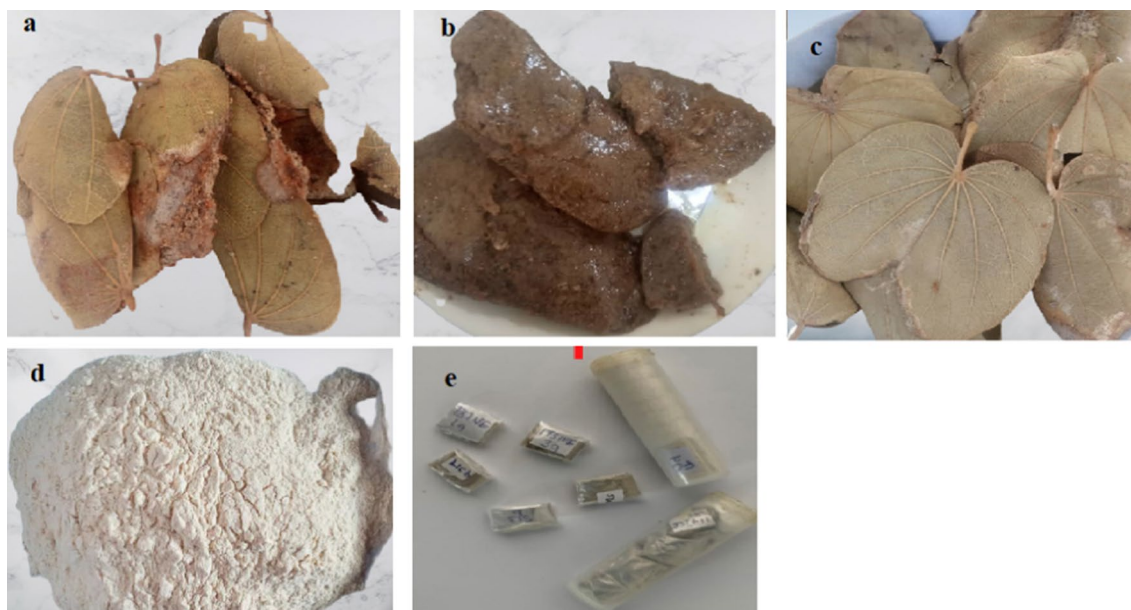


Fig. 1 Images of samples used for the analysis. a = cooked leaves, b = Tubaani meal, c = raw uncooked leaves, d = black-eyed bean flour, e = Pulverized samples pelleted for NAA

Water content analysis

Water content in four of the food samples were directly determined by measuring the weight loss after removing the moisture. Each chunk of Tubaani after peeling off the leaves was weighed on an electronic balance. The chunks were then cut into slices and dried to a constant weight at ambient temperature (30–31 °C) in an analytical laboratory and thereafter ground, and homogenized using an automated agate mortar and pestle. Moisture content was then determined by Eq. 1.

$$\text{moisture content} = \frac{\text{weight of wet sample} - \text{weight of dry sample}}{\text{weight of wet sample}} * 100\% \quad (1)$$

Neutron activation analysis

Samples for irradiation weighing 150 mg each were packed in clean polyethylene bags and heat-sealed into pellets. Pellets filled with sample material, together with a pellet filled with a certified reference material, and an empty capsule (the “blank”) were sealed together in polyethylene foil. Comparators are added to measure the neutron flux during irradiation. The whole package was packed in an irradiation container, and irradiated by shooting it to a position close to the reactor’s core Ghana research miniature neutron source reactor 1 (GHARR-1 MNSR) at the Ghana Atomic Energy Commission using a pneumatic irradiation tube system. The pellets were irradiated using a thermal power of 17 kW, at neutron flux of 5.0×10^{11} n/cm² s². The irradiations conducted was to analyze for short-lived, medium-lived and long-lived species. The samples were allowed to cool after irradiation and mounted directly on high purity germanium (HPGe) detector-based gamma-ray spectrometry system for counting. The detector has a relative efficiency of 45% at 1.33 MeV and a resolution of 2.0 keV full width at halve maximum (FWHM). The k_0 -standardization method and the k_0 - International Atomic Energy Agency (IAEA) software was used to calculate concentrations, as described elsewhere (Osei et al. 2021).

Preparation of extracts for phenolic and flavonoid content determination

0.05 g of each powdered sample was weighed into a falcon tube and 10 mL of distilled water was added. The mixture in the falcon tube was agitated for 15 h on a shaker to extract and the supernatant was decanted into fresh tubes. 5 mL of fresh distilled water was then added to the resulting residue and vigorously shaken for 5 min to re-extract the marc and the resulting extract was pooled with the previous

supernatant. The process was repeated until a clear solution was obtained. The pooled fractions were clarified by passing through Whatman No 1 filter paper.

Spectrophotometry technique

Determination of total phenolic content

Total phenolic content was determined quantitatively using the Folin Ciocalteu method, with Gallic acid as standard as described by Ainsworth and Gillespie (Ainsworth and Gillespie 2007). Serial dilutions of gallic acid solutions were prepared from an initial 1 mg/mL stock solution for the calibration curve described by the calibration equation $y = 0.9756x - 0.0483$ with an $R^2 = 0.9883$. The reaction mixture for each crude extract contained 50 µL of extract, 250 µL of 10% Folin–Ciocalteu’s reagent, 3 mL of deionized water, and 750 µL of 20% Na₂CO₃ solution which was made to react in the dark at room temperature for 30 min. The absorbance of each of the reaction mixtures was captured at 760 nm. Four chemical blank samples were also prepared and treated as extracts and their absorbance was recorded. The procedure was repeated thrice and the average value of absorbance was calculated. Finally, the total phenolic content expressed as milligram gallic acid equivalent (mg GAE) per gram of the dried samples was computed by fitting absorbance values of unknown samples into the calibration equation.

Determination of flavonoid content

The total flavonoid content of the extracts was determined using aluminium chloride calorimetric method (Afiy et al. 2012). A quercetin standard curve was prepared using different concentrations of quercetin. Initially, 0.01 g of quercetin was dissolved in 50 mL of EtOH to produce a stock solution of 0.2 mg/mL from which serial dilutions were prepared. 0.5 mL of each of the sample extracts was then mixed with 1.5 mL of 98% ethanol, 0.1 mL of 10% aluminum chloride, 0.1 mL of potassium acetate (1 M), and 3.0 mL of distilled water. The resulting homogeneous mixture was incubated for 30 min after vigorous shaking. The absorbance of the homogeneous mixture was read at 415 nm on a UV–VIS spectrometer. The total flavonoid content was expressed in terms of the quercetin equivalent (µg QE/g) of the sample. The procedure was repeated three times and the average value of absorbance was determined.

Quality control

The samples were analyzed in triplicate. Validation of the analytical technique was done using NIST (National Institute of Standards and Technology, MD, USA) 1573a – Tomato

Leaves, NIST 1547–Peach Leaves and IAEA (International Atomic Energy Agency, Vienna, Austria) 336–Lichen to relate the observed peak areas, received neutron dose, and sample mass to the sample concentrations. Recovery for reference materials used was within Y%

Health risk assessment: estimated daily intake, target hazard quotient, hazard index

Estimated daily intake

The estimated daily intake (EDI) of Tubaani was based on potential toxic elements. Two major factors are used in estimating the daily intake; the concentration of the toxic elements (C_{toxic}) and reference body weight (R_{bw}). In this study, the concentration of toxic elements (Cobalt, Vanadium, Chromium, Aluminium and Scandium) in the dry food samples were used to calculate the EDI. In addition, 70 kg was used as the reference body weight. The EDI was calculated by Eq. 2.

$$EDI = \frac{C_{toxic} * DAC}{R_{bw}} \quad (2)$$

where DAC is the daily average consumption (g/day).

Target hazard quotient

Individual health risks posed by potential toxic elements in Tubaani were assessed using the target hazard quotient (THQ). The THQ was calculated by Eq. 3 as the ratio of the potential exposure to toxic substances and the threshold at which no unfavorable effects are anticipated. Non-carcinogenic health effects are not anticipated if the THQ is less than 1. There is a chance that negative health effects could occur if the THQ is greater than 1. However, a THQ greater than 1 does not indicate a statistical likelihood that unfavorable non-carcinogenic health effects may materialize (Antoine et al. 2017).

$$THQ = \frac{FETE * PoE * DAC * C_{toxic}}{ORDTE * R_{bw} * AET} \quad (3)$$

where FETE is the frequency of exposure to trace elements (365 days/year), PoE is period of exposure (70 years), ORDTE is oral reference dose of trace elements ($\mu\text{g}/\text{kg } R_{bw}/\text{day}$) (World Health Organization 1996), and AET is the average exposure time to no unfavorable effects of trace elements (365 days \times 70 years).

Hazard index

Hazard index (HI) is the effect of total target hazard quotient (THQ) of all elements on a target organ or organ system.

Similar to the THQ, cumulative exposures below a HI of 1.0 based on target organ specific hazard quotients are probably not going to have a negative impact on non-cancer health over the course of exposure (Antoine et al. 2017).

Statistical analysis

One-way analysis of variance (ANOVA) test in Statistical Package for the Social Sciences (SPSS) software (version 20, IBM Corp., USA) was used to statistically analyze the experimental data and estimate the significance of differences between samples. A p value of <0.05 was considered statistically significant.

Results

Water content

The water content in the four food samples was determined directly from their weight. The mean wet weight, dry weight, and water content were 64.35 ± 4.83 (g), 25.93 ± 2.52 (g), and 58.28 ± 1.37 (%) respectively.

Neutron activation analysis

A total of 15 elements were identified in all four samples (i.e., beans flour, Tubaani, uncooked and cooked leaves of *P. thonningii*) through their characteristics γ -ray lines except cobalt and titanium which were non detected in the cooked and raw leaves respectively. The irradiation scheme was based on elements of interest and their half-life. These are; $1 \text{ min} < t_{1/2} < 2.5 \text{ h}$ for short lived-, $12 \text{ h} < t_{1/2} < 3 \text{ days}$ for medium lived- and $t_{1/2} < 2.5 \text{ days}$ for long lived elements. For this reason, aluminum (Al), chlorine (Cl), titanium (Ti), vanadium (V), manganese (Mn), and magnesium (Mg) were evaluated with short-term irradiation; potassium (K), scandium (Sc), chromium (Cr), iron (Fe), cobalt (Co), zinc (Zn), bromine (Br), and sodium (Na) for long term irradiation and calcium (Ca) for medium-term irradiation. The k_0 method was successfully implemented and validated at the GHARR-1 MNSR reactor, as shown by Osei et al. (2021). This guarantees that the results are independent and are not influenced by the chemical state of the elements (International Atomic Energy Agency 1990).

The highest elemental concentration (i.e., calcium = $16,590 \mu\text{g}/\text{g}$) was found in sample C whereas, the lowest concentration (i.e., titanium = below our detection limit) was detected in sample B. Scandium, cobalt, manganese, vanadium, bromine, chromium, and zinc were determined to be less than $100 \mu\text{g}/\text{g}$ in all the four samples. However, titanium and cobalt were not detected in samples C and A respectively (Table 1). There was no statistical

Table 1 Elemental composition in (µg/g) of the samples analyzed

Elements	Concentration in (µg/g) dry weight			
	A	B	C	D
Na	178.20±4.5	6655.00±2.5	147.30±6.4	8091.00±2.4
Mg	1958.00±3.7	2153.00±4.8	2131.00±5.4	2139.00±5.4
Al	973.90±2.5	704.00±2.6	648.80±2.5	650.20±2.7
Cl	231.60±3.3	471.10±3.2	270.10±3.6	498.60±3.8
K	7243.00±8.6	15,350.00±9.2	13,860.00±5.3	13,160±4.1
Ca	16,590.00±2.7	14,900.00±2.8	6895.00±3.3	8175.00±3.2
Ti	ND	657.00±35.5	16.81±44.3	0.00±0.0
V	0.65±20.9	0.40±14.0	0.21±33.5	0.48±23.0
Mn	20.27±4.3	22.57±3.2	22.17±3.4	21.50±3.7
Br	0.97±13.0	1.56±15.2	1.57±5.9	1.58±21.0
Co	0.16±32.0	ND	0.47±15.0	0.16±16.9
Cr	2.12±26.0	1.16±28.0	0.86±32.0	1.28±26.0
Fe	356.00±4.6	437.00±13.0	256.00±16.1	220.00±6.7
Sc	0.08±18.0	0.09±12.8	0.06±13.0	0.026±7.9
Zn	45.30±4.4	45.80±13.0	70.30±13.0	67.40±4.0

A = uncooked dried leaves, B = cooked leaves covering the food, C = beans flour, D = Tubaani, “ND” = not detected

difference ($p > 0.05$) in the mean concentrations of the elements between the samples. However, Post Hoc Turkey HSD test revealed that, in all four samples investigated, there was a significant difference ($p < 0.05$) in concentration between the elements Ca, K and Al, Br, Cl, Co, Cr, Fe, Mg, Mn, Na, Sc, Ti, V, Zn.

Spectrophotometry analysis

Total phenolic content

The total phenol content of the four sample extracts was estimated using the Folin Ciocalteu method in terms of the Gallic acid equivalent (GAE) in mg/g. The total phenolic content was calculated using the standard curve equation given by $y = 0.9758x - 0.0483$, where $R^2 = 0.9883$. The total phenolic content determined was in the range of 21.94 and 40.50 GAE mg/g. The largest amount of phenolics was identified in cooked leaves covering the food extract and the least amount was found in the food. Table 2 shows the total phenolic content in the various samples.

Table 2 Total phenolic and flavonoid contents of raw black-eyed beans flour, Tubaani, and cooked and uncooked leaves of *P. thoninigi*

Sample extract	Polyphenol concentration (mg/mL)	Gallic acid equivalence (mg GAE/g)	Flavonoid concentration (µg/mL)	Quercetin equivalence (mg QE/g)
A	0.133±0.004	39.887±1.081	4.24±0.23	12.72±0.69
B	0.135±0.003	40.503±0.940	5.98±0.26	17.94±0.77
C	0.075±0.001	22.554±0.308	10.94±0.11	32.83±0.33
D	0.073±0.003	21.939±0.081	11.24±0.28	33.72±0.84

A = Uncooked leaves, B = Cooked leaves covering the food, C = Beans flour, D = Tubaani

Total flavonoid content

With some modification of the aluminum chloride calorimetric technique (Affify et al. 2012), the total flavonoid content expressed as quercetin equivalents in mg QEg⁻¹ dry extracts were determined using a standard quercetin curve, $y = 0.009x - 0.0255$ and $R^2 = 0.9982$ for all four sample extracts as described above (see methodology). The values of the total flavonoid content of the four samples ranged from 1272.22 to 3372.22 mg QE/g. The highest concentration of total flavonoid was detected in Tubaani, while the lowest concentration was observed in raw dried leaves. Table 2 shows the total flavonoid content in the various samples.

Health risk assessment: estimated daily intake, target hazard quotient, hazard index

If we assume that the average weight of an adult man is 70 kg and, in a day, this 70 kg man consumes 6 chunks of Tubaani (i.e., DAC). This translates to 155.58 g (i.e.,

25.93 × 6) dry weight of Tubaani. Table 3 shows the results of the health risk assessment.

Discussion

Neutron activation analysis

In this study, the elements determined from the four samples through NAA technique can be categorized as trace elements (Zn, Sc, V, Cr, Co, Fe, Mn, Br), macro elements (Mg, Ca, Na, K, Cl), and other elements (Ti, Al). Among these elements' chromium, cobalt, manganese, iron, calcium, magnesium, sodium, potassium, vanadium, and chlorine are considered essential trace elements (Prashanth et al. 2015). The elements potassium, chloride, calcium, magnesium, iron, and zinc are nutritionally essential minerals. The average daily requirement of these nutritional essential elements for an adult human should be above 100 mg/day. The concentration of these elements in the current study was far lower than those reported in World Health Organization (1996) and Prashanth et al. (2015). Similarly, manganese levels in the four samples were also far below the daily intake (2.5–3.0 mg) of many people, as well as those reported in Canada, the United States, and New Zealand, where daily manganese intake among individuals eating self-selected diets was 3.1, 2.9, and 2.7 mg, respectively. Insufficient levels of essential minerals can lead to loss of consciousness, neurological disorders, and anemia, among others, and may prove fatal unless intervened properly (World Health Organization 1996; Abbott et al. 2003).

Table 3 Results of concentrations, estimated daily intake, target hazard quotient of elements in Tubaani

Element	Concentration (µg/g)	ORDTE (µg/kg/day)	EDI (µg/kg/day)	THQ
Na	8091	3,510,000	17,982.825	0.005
Mg	2139	300,000	4754.080	0.016
Al	650.2	4500	1445.116	0.321
Cl	498.6	5,670,000	1108.174	0.000
K	13,160	2,670,000	29,249.040	0.011
Ca	8175	760,000	18,169.521	0.024
Ti	0	Not accessed	–	–
V	0.48	16	1.067	0.067
Mn	21.5	3100	47.785	0.015
Br	1.58	4000	3.512	0.001
Co	0.16	15	0.356	0.024
Cr	1.28	50	2.845	0.057
Fe	220	13,000	488.966	0.038
Sc	0.03	0.38	0.058	0.152
Zn	67.40	10,000	149.801	0.015

In situations whereby the concentration of elements measured in the food sample was higher than those in the leaves (e.g., chloride and magnesium), it is possible that they could be an uptake of these elements by the food from the leaves. On the contrary, for elements whose concentrations were higher in the leaves as compared to the food (e.g., potassium, calcium, and iron), it is possible that these elements might have leached into the stock during the steaming process.

Despite the fact that vanadium, cobalt, chromium, and aluminium are essential elements, above a certain level, they are considered potentially toxic elements (Haidu et al. 2017). Scandium is non-essential for human health and has been found in a few toxicity investigations to be mildly hazardous (Cleave and Crans 2019). For the potentially toxic elements identified in this study, Aluminium, chromium, and cobalt had a lower concentration in the food signifying that there was no migration of these elements from the leaves into the food. There appears to be a three-fold decrease in the cobalt from the beans to the Tubaani suggesting that the cooking process leaches Cobalt from the beans. Also, no migration of cobalt from the leaves was observed as the concentration was the same in both uncooked leaves and the food. This may account for why cobalt was not detected in the cooked leaves as shown in Table 1. For Vanadium, it was realized that its concentration increased from 0.129 in the uncooked beans to 0.488 in the Tubaani, while it decreased from 0.654 in the uncooked leaves to 0.409 in the cooked leaves, suggesting that there was a flow of vanadium from the leaves into the Tubaani. Exposure to potentially toxic elements in excess concentrations could lead to the development of intoxication syndromes (Skalnaya and Skalny 2018). For example, excessive levels of cobalt metal may enhance thyroid and bone marrow activity, resulting in erythrocyte overproduction, lung fibrosis, and asthma (Domingo 1989; Czarnek et al. 2015). Also, chromium has been linked to digestive problems such as non-cancerous illnesses of the lungs and upper respiratory tract, as well as teratogenic and dermatological consequences (Baruthio 1992).

Titanium on the other hand was not detected in the dry raw leaf nor measured in the food. However, it was found in trace quantities in the dry cooked leaf and the flour. During rock weathering, small quantities of titanium end up in the water. As a result, the high concentration value in the dry cooked leaves may be due to the uptake of traces of titanium in the water (used in processing the beans flour into a slurry) and the beans flour itself. This may account for why traces of titanium were not detected in the food (Tubaani). In summary, the findings indicate that the concentration of all the elements determined in the four samples through the NAA technique was lower than the range of daily dietary intake reported in the literature and IAEA data (World Health Organization 1996).

Polyphenol and flavonoids content in samples

All the extracts were positive for polyphenols and flavanoids confirming earlier findings of Dluya et al. (2015). The maximum and minimum phenolic levels were obtained in cooked leaves and the food respectively. Similarly, the maximum level of flavonoids was obtained in food and the minimum was in raw dry leaves (Table 2). Quantitative analysis of total phenolic revealed that the cooked leaves covering the food and raw dry leaves extracts contain 40.503 and 39.887 mg g⁻¹ respectively of total polyphenols expressed as gallic acid equivalent (GAE, mg g⁻¹). Similarly, the total flavonoid of the dry cooked leaves covering the food and raw dry leaves were 1794.440 and 1272.220 mg g⁻¹ of total flavonoids expressed as quercetin equivalent (QE, mg g⁻¹). Although the concentration of the phenols was higher as compared to the flavonoid, their values were far lower than those reported in Ogbiko et al. (2020). This may be attributed to differences in the variety, environmental condition, stage of growth of the plant, extraction method, and age of plants. Plants that have high phenolic and flavonoid content have a lot of antioxidant activity (Alagbe and Oluwafemi 2019).

The flavonoid content in the *P. thonningii* leaf extracts was found to be lower than that of the food and flour. A difference of 522.22 mg QE/g between the raw dry leaves and cooked dry leaves, and 88 mg QE/g between food and flour in flavonoid content was observed. There was an increase in the flavonoid content of the flour when cooked, suggesting the possible absorption of flavonoids by the food from the leaves. This accounts for the increase in the flavonoid content of cooked leaves (Table 2). Similarly, the phenolic content in the leaf extracts was higher than that of the food and flour. However, both cooked and raw dry extracts were approximately equal. This was the same in the case of the flour (22.554 mg GAE/g) and food extracts (21.939 mg GAE/g). This indicates that the phenolic content in the *P. thonningii* leaves has no nutritional contribution to the food (Tubaani) due to the reduction of phenols in the food, unlike the flavonoids.

The majority of phytochemicals are physiologically active and may have a favorable effect on the human body. Complex polysaccharides, flavonoids, terpenoids, proteins, numerous organic acids, alcohols and their corresponding complexes, oils, tannins, saponins, glycosides, glucosides, and other phytochemicals have a wide spectrum of favorable effects on the human body (Hossain et al. 2019). An in vivo research of *P. thonningii* leaves' antioxidant function revealed that they can protect the liver from carbon tetrachloride-induced oxidative damage by scavenging free radicals and inhibiting lipid peroxidation (Boualam et al. 2021). Because of their phenolic hydroxyl groups, phenolic substances are known to scavenge radicals such as singlet

oxygen, superoxide, and hydroxyl. They have antioxidant capabilities due to their redox characteristics. Flavonoids, on the other hand, are a class of plant polyphenolic chemicals found in photosynthetic cells that have a wide range of biological features, including a role in uncontrolled lipid peroxidation. This is due to their unique interaction capacity with protein phosphorylation, iron chelation, and free radical scavenging activities (Ogbiko et al. 2020).

Health risk assessment

The estimated daily intake of Tubaani by adults was calculated using the element's average concentration. The total food intake of all the elements was less than 1. By comparison, the estimated daily intake was lower than the recommended oral reference dose of trace elements (World Health Organization 1996). No individual target hazard quotient for the food's potentially dangerous elements examined was determined to be higher than 1. In the order of increasing target hazard quotient, the individual potentially toxic elements were; Al (0.321), Sc (0.152), V (0.067), Cr (0.057), and Co (0.024). The findings of this study are in agreement with previous studies (Antoine et al. 2017; Filippini et al. 2020) that found the target hazard quotient of these potentially toxic elements to be lower than 1. Similar to the target hazard quotient, the hazard index that takes into account the combined effects of consuming these toxic substances were also found to be less than 1. This demonstrates that there is no extra risk of non-carcinogenic health consequences associated with the consumption of Tubaani. However, there is no guarantee that intakes at or below the references are risk-free or that intakes above them present excessive risks (Han et al. 1998).

Conclusion

The purpose of the study was to assess the nutritional value and health risks *Piliostigma thonningii* leaves used for preparing Tubaani. Through the NAA technique, the concentrations of all elements in all the samples were lower than the recommended daily dietary intake. Also, individual THQ and HI values of potentially toxic elements were less than 1. Spectrophotometrically, the flavonoid content in the flour extract was found to be lower than in the food (Tubaani). On the other hand, spectrophotometrically, the total flavonoid concentration was found to be lower in all four samples than that of the phenols. Therefore, the high phenolic concentration in the leaf extracts could be exploited for the creation of novel medicinal compounds as well as antioxidant and antibacterial medications. Our results suggest that the dietary intake of the potentially toxic elements identified in this study is not a cause for worry, however, the plant

samples were sourced from only a section of Ghana (Navrongo), thus broader investigations with samples from different geographical location are warranted to buttress our current findings.

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Author contributions DGA, ENM, RAN, DAW and AMG conceived the presented idea and design. ENM, EKA, ASKA, DAW, KTA, FZ, and EKD gathered the literature. ENM and DGA drafted the manuscript. RAN, DAW, EKD, ASKA and AMG contributed to the write-up and verified the analytical methods. All authors contributed to the interpretation and final approval of the manuscript to be published.

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Code availability Not applicable.

Declarations

Conflict of interest The authors declare that they have no competing interests" in this section.

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Consent to participate Not applicable.

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