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Evaluation of a tea bag formulation of *Tapinanthus bangwensis* (Engl. and K. Krause) Danser leaves, meant for the management of diabetes

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ABSTRACT

The incorporation of traditional medicine into the mainstream health care delivery system particularly in some developing countries such as Ghana has resulted in reliable access to herbal medicines and their safe use. This has also led to a surge in the search for herbal alternatives, reformulation, and development of herbal medications in treating and managing diseases such as diabetes. Currently, the surging price of orthodox antidiabetics and their reported adverse effects from long term use has contributed to many Ghanaians switching to the use of phytomedicines as alternatives. The aim of this study is to formulate tea from the leaves of *Tapinanthus bangwensis,* family *Loranthaceae* to serve as an additional remedy for the management of diabetes.

For standardization, quality control, and authentication of the formulation, FTIR, and HPLC analysis were conducted. The safety profile of the tea *via* determination of median lethal dose (LD_{50}), microbial load, essential elements and toxic metal contents were also assessed. In addition, the pH, uniformity of mass, total water extractive and optimization of the extraction method were also investigated.

The FTIR showed the presence of alcohols, esters, phenols and aromatic compounds. The HPLC fingerprint also showed 23 peaks with the highest peak having a retention time of 15.4 min. The extract also recorded a pH of 7.43 ± 0.02 at 26.1 °C and total water extractive of 39.37 ± 0.05 $\%^{w}/_{w}$. The tea passed the uniformity of mass test with an average net mass of 3.0 ± 0.02 g. Optimum extraction of the tea was realised with 250 mL of freshly boiled water. The formulated tea was found to be safe since the determined LD_{50} was 50 times more than the daily dose. The microbial load, elemental and toxic metal contents were also within acceptable limits.

This study has shown the possibility of introducing this tea at a safe dose as an additional herbal remedy for the management of diabetes.

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Introduction

Herbal teas are widely consumed globally not only to treat various health conditions but also as food [1,2]. Herbal teas which are usually supplied in sachets or bulk form [3] are mostly prepared immediately before use, and comprises one (monoherbal) or more herbs (polyherbal) purposely for oral aqueous preparations *via* infusion, decoction or maceration. They vary in colour, clarity and aroma depending on the composition of constituents present in them [4]. Herbal teas can be obtained from the leaves, seeds, bark, flowers or any parts of the plants depending on how the compounds dissolve in aqueous solution [5]. The increasing awareness in the consumption of herbal teas has led to the expansion of the herbal tea industry which has eventually helped to provide more remedies to diseases in addition to creating more employment opportunities [6,7].

Diabetes is among the world's prevalent diseases with an estimated 537 million persons between the ages of 19 to 80 years old living with this condition [8]. This figure is expected to rise to 643 million by 2030 and 783 million by 2045 if the current trend persists [8]. In addition, diabetes has been reported to be the cause of 6.7 million deaths and has cost the world more than 966 billion dollars in health expenditure only in 2021 [8]. The increasing cost of orthodox antidiabetics and their failure to address the fundamental issue of dying beta cells in diabetics has resulted in people, especially, those in the low and middle income earning countries like Ghana to seek herbal remedies [9]. In Ghana, the integration of phytomedicine into the mainstream healthcare delivery system has also contributed to the increase in the use of alternative medicines in the management of diabetes and other ailments [10].

Tapinanthus bangwensis, family *Loranthaceae*, is a sycophantic plant typically found in Africa. It grows on different plants such as pear, neem, cassava, kolanut, calabash, citrus, cocoa, guava, mango and rubber [11,12]. Folkloric uses include the treatment of; arthritis, diabetes, cancer, menopausal symptoms, malaria, respiratory, inflammation, hypertension and other diseases [13,14]. Studies on the aqueous and methanolic extracts of the leaves of this plant has demonstrated antidiabetic property *via* its insulin-like action [9,12]. The aim of this study was to formulate and evaluate tea bags from *Tapinanthus bangwensis* leaves as an additional herbal remedy in the management of diabetes.

Materials and methods

Plant collection, identification and authentication

Tapinanthus bangwensis leaves parasitic on *Persea americana* were collected from Mampong Akuapem (5° 55′ 25.6′ N, 0° 7′ 58.5 W) in July 2021. These identified leaves were authenticated by a botanist from the Centre for Plant Medicine Research (CPMR), Department of Plant Development. A specimen with voucher number CPMR 503 has been deposited at CPMR's herbarium for future reference.

Processing of Tapinanthus bangwensis leaves for tea bagging

The harvested *Tapinanthus bangwensis* leaves were washed in distilled water to remove unwanted debris. The leaves were then dried in an oven at 60 °C for two hours, milled and sifted into a fine powder suitable for tea bagging using an electric grinder (Amcon mechanical blender DT-710, China) [15,16]. They were then bagged using a tea bagging machine (GATBPM manufactured by GMP machineries Prahaladnagar, Ahmedabad-Indian) as shown in Fig. 1.

Phytochemical analyses of formulated Tapinanthus bangwensis tea

The preliminary phytochemical screening was evaluated using established methods as detailed by Houngbeme et al. [17]. Shinoda's test for flavonoids, ferric chloride solution test for phenolics, Mayer's test was used to screen for alkaloids, froth test for saponins, Liebermann-Burchard's test for phytosterol and triterpenes, Borntrager's test for free anthraquinones and Fehling's test for free reducing sugars.

Instrumental characterization of Tapinanthus bangwensis tea

Fingerprinting using FTIR of Tapinanthus bangwensis tea

This was carried out by placing the lyophilised aqueous extract on the diamond crystal of the PerkinElmer Fourier transform infrared spectrophotometer (FTIR) (spectrum 2, SR. No. 94133, UK). Pressure was applied using the force gauge to ensure maximum

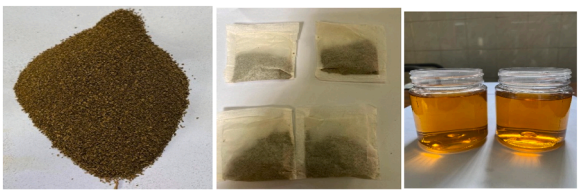

Fig. 1. Milled *Tapinanthus bangwensis* leaves (i). Developed *Tapinanthus bangwensis* tea bag (ii). Prepared *Tapinanthus bangwensis* infusion from tea bag (iii).

contact with extract. A spectrum was obtained between 4000 and 400 /cm [18].

Fingerprinting using HPLC

HLPC fingerprint analysis was performed using a Perkin Elmer (USA) HPLC-UV system with a Flexar solvent manager, supplied with a Phenomenex C18 column (250 × 4.6 mm, 5 μm). The solvent system used was 0.05 % aqueous trifluoroacetic acid (TFA) labelled as solution T and methanol (solution M) as the mobile phase with a flow rate of 1 mL/min and a volume of injection of 20 μL. The temperature of the column was maintained at 26 °C. The linear gradient started from 10 % of methanol for 5 min; 10 %–90 % of methanol in 25 min and then decreased to 10 % of methanol, held for 5 min. The chromatogram was monitored at a wavelength of 320 nm.

Determination of elemental contents in the Tapinanthus bangwensis tea

Digestion of extract

One gram of lyophilised aqueous extract was weighed into a digestion tube. A volume of 2 mL of distilled water was mixed well with it. Ten millilitres of Nitric-Perchloric acid (1:1), followed by concentrated sulphuric acid of volume 5 mL were added. The hot plate at 200 °C constant temperature was used to heat the mixture till it became a clear solution. The tube was removed and allowed to cool to room temperature. To make up to the 50 mL mark, distilled water was added, shaken well and transferred into prewashed PET bottle for the elemental analysis.

Measurement of elemental content using the flame atomic absorption spectroscopy (FAAS)

The Flame Atomic Absorption Spectroscopy (FAAS) (analytikjena model novAA400P) was used for the measurements using a single-beam optical mode. Hollow cathode lamp (HCL) for each element was used as the source of light for this analysis. Acetylene (N26 quality, Air liquid, Ghana) and compressed air were respectively employed as fuel gas and oxidant for the flame.

The pneumatic nebulizer was used to aspirate small volumes of the analytes into the flame. The ions were reduced to elements and vaporised by the flame. The elements present absorb the light generated by HCL at specific wavelength in the ultraviolet or visible spectrum; thus, Cu at 324.8 nm, Pb at 283.3 nm, Cd at 228.8 nm, Zn at 213.9 nm, Mn at 279.5 nm, K at 766.5 nm, Fe at 248.3 nm, Ni at 232 nm, Mg at 285.2 nm, Ag at 328.1 nm, Na at 589 nm, Al at 309.3 nm, Cr at 357.9 nm and Ca at 422.8. The absorbed light was then transmitted through a monochromator where it was detected with a detector [19].

Determination of pH of aqueous extract of Tapinanthus bangwensis tea bag

The pH of a 1 % w/v prepared infusion from the tea bags was determined at 26.1 °C after the calibration of the pH meter (Oaklon pH/mv/conductivity meter (PC 700)) with standard buffer solutions pH 4 (Reagacon, Ireland) followed by buffer with pH 10 and 7 (Riedel-de Haen, Germany). The procedure was done in triplicate [20].

Determination of moisture content of Tapinanthus bangwensis tea bag

A quantity of 3 g of *Tapinanthus bangwensis* tea was weighed into a crucible. The total weight of the tea in the crucible was determined (W_1). It was then dried at 105 °C in an oven until a constant weight was obtained. It was then removed, cooled in a desiccator and weighed (W_2). The loss in weight on drying is determined by;

$$\text{Moisture content} = \frac{\text{W1} - \text{W2}}{W}\ x\ 100$$

where, W = weight of sample

Evaluation of total water extractive of Tapinanthus bangwensis tea bag

A quantity of 1 g of the content in the tea bag was weighed into a stoppered 250 mL flask. One hundred millilitres of distilled water was added to the mixture and heated for 1 h on a water bath. It was then filtered with Johnson test paper (Qualitative filter paper, grade 304; 125 mm). Ten millilitres of the filtrate was evaporated to dryness to obtain a constant weight at 105 °C for 1 h. The weight of the extract was obtained by;

$$Weightofresidue = (weightofdish + residue) - (weightofemptydish)$$

The total water extractive value was calculated as; $(\%w/w) = \frac{weight\ of\ residue}{initial\ weight\ in\ 10\ mL}\ x\ 100$ [21].

Optimization of extraction method of aqueous extract of Tapinanthus bangwensis tea bag

A tea bag of net weight 3 g was infused in 150 mL of freshly boiled water for 10 min, filtered and measured. The total solid residue as well as the total extract in the filtrate were evaluation. This extraction process was repeated for 200 mL, 250 mL and 300 mL of freshly boiled water. This analysis was done in triplicate for each extraction volume [21].

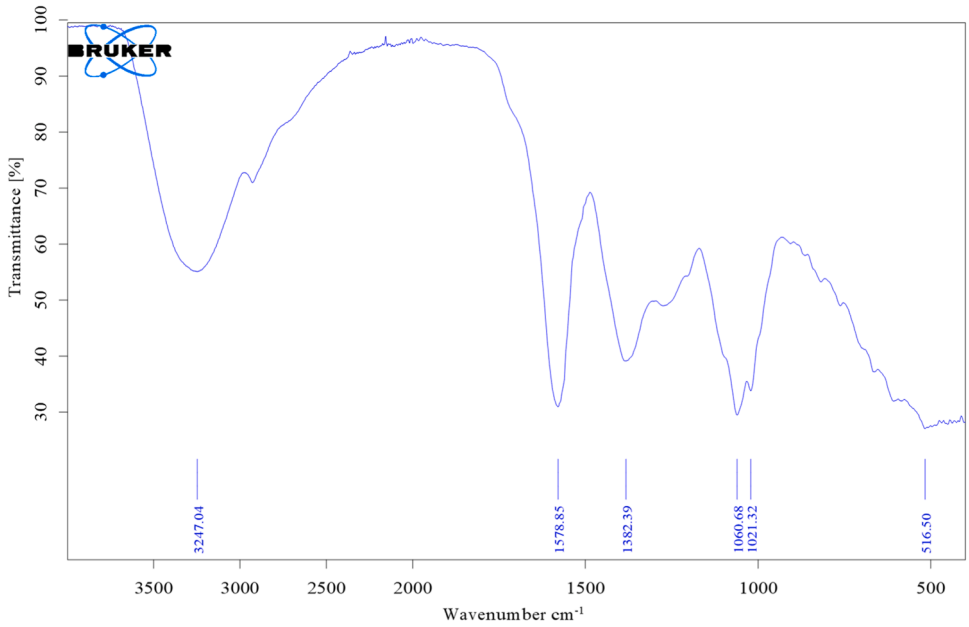

Fig. 2. FTIR spectrum of *Tapinanthus bangwensis* tea.

Uniformity of mass of formulated of Tapinanthus bangwensis tea bags

Twenty (20) randomly selected tea bags were weighed. A single filled tea bag was weighed and recorded. It was then opened and completely emptied, ensuring that no fragments were lost. The quantity of the content in the tea bag was calculated by subtraction of the mass of the empty bag from the filled bag. This procedure was repeated on nineteen more tea bags. The average mass of the twenty tea bags was then determined. The uniformity of mass was deduced from this readings [3].

Microbial load test on aqueous extract of Tapinanthus bangwensis tea bag

The microbial load of the tea bags was evaluated using the [22]. The test is aimed at quantifying the level of fungi and aerobic bacteria that may be present in 1 g or 1 mL of a sample, as well as detecting the presence of some specific pathogenic bacteria as stated in the BP [22]. Viability was evaluated *via* the pour plate method using the malt extract agar for fungi and plate count agar for bacterial enumeration. Incubation was done at 32.5 ± 2.5 °C for 24–72 h and 22.5 ± 2.5 °C for 3–7 days for bacterial and fungal enumeration respectively. The colony-forming units per gram (CFU/g) was computed after the incubation period. Specific pathogenic bacteria; *Salmonella spp, Staphylococcus spp.* and *Escherichia coli* were isolated employing their respective selective media as described by the BP [22].

Determination of median lethal dose (LD_{50}) of aqueous extract of Tapinanthus bangwensis tea bag in ICR mice

Eighteen (18) healthy male mice were randomly selected for this study. They were allowed to acclimatise themselves to a temperature of 22 ± 3 °C, 55 ± 5 % relative humidity and a lighting sequence of 12 h darkness and 12 h light for 7 days prior to the study. The mice were grouped into three ($n = 6$); the first group was given distilled water, the second and third groups were given 2.5 g/kg and 5 g/kg lyophilized extract respectively. The individual animals were observed for the first 30 min, every 2 h within the first 24 h and then daily for 14 days. Observation for physical signs of toxicity such as death, changes in fur, skin, mucous membrane, heart rate, respiration, movements, salivation, diarrhoea, pattern of behavioural changes, convulsion, tremor and others were observed [23,24]

Statistical analyses

The data was analysed using GraphPad Prism for windows version 5 (Graph Pad software Inc., San Diego, CA, USA) and Excel. Results were measured in triplicate and were expressed as mean ± standard deviation.

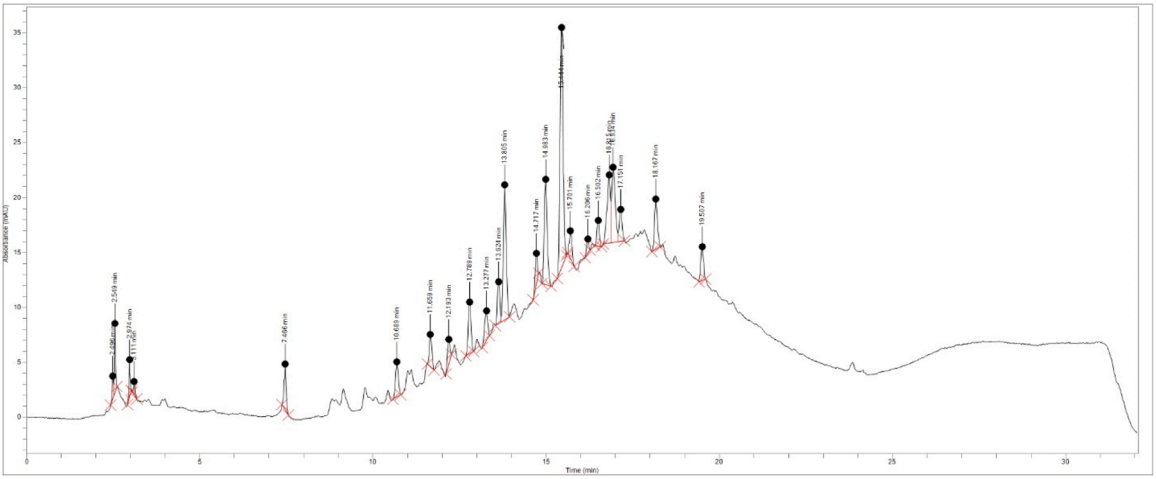

Fig. 3. HPLC chromatogram of Tapinanthus tea detected at 320 nm.

Table 1
Essential elemental content of *Tapinanthus bangwensis* tea.

Minerals	Limit of detection (mg/L)	Content (mg/L)	concentration per daily dose (mg/L)	RDA (mg/day)
Ag	0.1628	BDL	BDL	–
Na	0.1214	0.3498 ± 0.0092	0.7276	1500 [34]
K	0.1943	0.8603 ± 0.1568	1.7894	4700 [34]
Mn	0.0774	0.6905 ± 0.0337	1.4362	11 [35]
Cr	0.8620	BDL	BDL	0.02 [36]
Fe	0.1214	BDL	BDL	18^{f} /8^{m} [34]
Ca	0.2463	0.5066 ± 0.0645	1.0537	1000 [34]
Zn	0.0904	1.150 ± 0.0209	2.3920	10–20 [37]
Mg	0.2914	1.764 ± 0.0279	3.6691	320^{f} / 420^{m} [34]
Cu	0.2861	BDL	BDL	12 [37]

[f] female.
[m] males; RDA –recommended Daily Allowance; BDL – Below Detectable Limit.

Result and discussion

Phytochemical screening of aqueous extract of Tapinanthus bangwensis tea

From the test carried out, the tea demonstrated the presence of flavonoids, reducing sugars, alkaloids, phenolic compounds, phytosterols and saponins. This result has also been reported in previous research findings [12,25–29]. These phytoconstituents may be responsible for its antihyperglycaemic activity [24,28].

Instrumental characterization of Tapinanthus bangwensis tea

Fingerprinting of this extract will help in the identification, authentication, standardization and quality control of the formulation.

FTIR functional group analysis and fingerprinting of extract

The FTIR spectroscopy analysis was carried out to obtain a fingerprint for quality control of the *Tapinanthus bangwensis* tea. Fig. 2 shows a unique spectrum which can be used as reference fingerprint to help authenticate the formulation. The spectra also revealed the presence of a broad band at 3247.04 cm^{-1} (O—H stretching) indicating the possible presence of alcohols, phenols or flavonoids. A strong peak observed at 1578.85 cm^{-1} shows the presence of a C═C bond stretch of aromatic compounds. A peak at 1382.39 cm^{-1} suggesting C—H bending of a methyl group and broad bands at 1060.68 cm^{-1} and 1021.32 cm^{-1} expressing C—O stretch of ethers.

HPLC fingerprinting of extract of Tapinanthus bangwensis leaves

The HPLC fingerprint of the tea at wavelength 320 nm will aid in quality control and proper identification of the constituent of this formulation. The HPLC fingerprint chromatogram showed twenty-three (23) peaks (Fig. 3), with the largest peak having a retention time of 15.4 min. This fingerprint chromatogram represents the phytochemical constituents present in the tea. Though the wavelength of maximum absorption of the extract was determined at 268 nm in distilled water, the detection wavelength selected for the HPLC fingerprinting was 320 nm. This was due to the fact that, the latter produced a chromatogram with a better resolved peaks. Such an

Table 2
Toxic metal content of *Tapinanthus bangwensis* tea.

Toxic metals	Limit of detection (mg/L)	Content (mg/L)	RDA (mg/day)
Pb	0.6762	BDL	BDL
Ni	0.3183	BDL	BDL
Cd	0.0944	BDL	BDL
Al	0.3225	BDL	BDL

RDA –recommended Daily Allowance; BDL – Below Detectable Limit.

Table 3
Physicochemical properties of *Tapinanthus bangwensis* tea.

Physical characteristics	
pH (°C)	7.43 ± 0.02
Moisture content (%)	5.14 ± 0.14
Total water extractive (%$^{w}/_{w}$)	39.37±0.05
Colour	Melon yellow
Odour	Aromatic
Texture	rough

occurrence may be expected since this sample is an extract. Plant extracts could have compounds with extinction coefficients which vary to different extents between the chosen wavelengths.

Elemental content of the Tapinanthus bangwensis tea

Macro and micronutrients are nutraceuticals which are useful in reversing oxidative stress associated with chronic diseases such as diabetes, with subsequent delay in the progression of the disease [30,31]. They have also been reported to help in glucose homeostasis [32] as well as help to reduce the occurrence of common infections in diabetics [33]. The content of Ag, Ni, Pb, Cr, Fe, Cu, Cd and Al in *Tapinanthus bangwensis* tea were below the detection limit of the FAAS (Tables 1 and 2). This means that, these elements are either absent or their content is too small to be detected by the FAAS. However, *Tapinanthus bangwensis* tea demonstrated the presence of Na, K, Mn, Ca, Zn and Mg with Mg being the predominant mineral. Even though their respective content is below the recommended daily dose (RDA), the consumption of this tea by diabetics offers them an added medical advantage.

This research has proven that a daily intake of tea from *Tapinanthus bangwensis* leaves collected from Mampong-Akuapem will pose no potential lead, nickel, cadmium and aluminium toxicity to consumers since their concentrations were all below detection limits.

Physicochemical properties of aqueous extract of Tapinanthus bangwensis tea bag

The macroscopic assessment showed that, *Tapinanthus bangwensis* tea bag had a rough texture and its content was brown. The prepared tea was melon yellow in colour, and had aromatic smell. The pH of the prepared 1 %$^{w}/_{v}$ infusion is basic as shown in Table 3 with a pH value of 7.43 ± 0.02. This obtained pH value is within the acceptable range of 4.0–7.5 for grasses, trees, annuals, shrubs, fruits, flowers and vegetables [38,39]. Also, they fall within the acceptable pH range for food, thus 2–9 (https://cooking.stackexchange.com/questions/6418/safe-pH, Accessed on 18/05/21). This means that, *Tapinanthus bangwensis* tea will pose no danger with respect to pH upon consumption. The total water extractive is about compounds such as alkaloids, salt, amino acids, sugar, acid, phenolics compounds, minerals and others which are solubilised and can be extracted by water. The high extractive value of *Tapinanthus bangwensis* tea, 39.37 ± 0.05 %$^{w}/_{w}$ as shown in Table 3 may be the reason for its pharmacological activities due to the high solubility of the biochemical compounds in water. For any commercial tea, moisture content determination is a vital quality parameter that ought to be investigated. This is because it affects not only the quality of the tea but also its shelf life. Thus, tea with higher moisture content has shorter shelf lives due to an increased risk of microbial contamination and decomposition. *Tapinanthus bangwensis* tea bag also reported a moisture content of 5.14 ± 0.14 % which falls within the acceptable limit of less than 20 % [40]. This means that, the developed *Tapinanthus bangwensis* tea bag will have a relatively long shelf life, a low risk of microbial contamination and a low probability of the finely stiffed powder of *Tapinanthus bangwensis* leaves clumping together to form hard aggregates. The results reported in Table 3 support the development of the *Tapinanthus bangwensis* leaves as tea bags.

Uniformity of mass of Tapinanthus bangwensis tea bag

The formulated *Tapinanthus bangwensis* tea bag recorded an average net weight of 3 ± 0.02 g. It also passed the uniformity of mass test since no tea bag deviated by 10 % or more and none also deviated by more than 20 % from the mean weight. The passing of this test could be due to even filling of the tea bag by the tea-bagging machine. Uniformity of the mass will ensure constant dosing of medicament. This is because, the right weight of a tea bag will offer a high probability of producing tea bags with the right therapeutic dose, which will ensure the achievement of the desired therapeutic outcome upon administration.

Table 4
Results of optimization of extraction from *Tapinanthus bangwensis* tea bags.

Weight of tea bags used for extraction (g)	Volume of water used in extraction (mL)	Volume of filtrate obtained (mL) $n = 3$	Total extract in filtrate (g) $n = 3$	Total solid residue (% w/v) $n = 3$
3	300	270 ± 4	1.08 ± 0.03	0.40 ± 0.01
3	250	225 ± 2	1.13 ± 0.02	0 0.50 ± 0.01
3	200	175 ± 3	1.05 ± 0.02	0.60 ± 0.01
3	150	130 ± 2	0.98 ± 0.01	0.75 ± 0.01

Table 5
Microbial load analysis of *Tapinanthus bangwensis tea* bags.

Test conducted	Results (cfu/g)	Maximum acceptable count [22]
TAMC	1.2×10^3	$\leq 5.0 \times 10^7$
TYMC	6.0×10^2	$\leq 5.0 \times 10^5$
E. coli	–	Absence
Salmonella spp.	Absent	Absence

TYMC: Total Yeast and moud Counts, TAMC: Total Aerobic Microbial Counts, - absent.

Optimization of extraction of Tapinanthus bangwensis tea bags

The extent of water extraction of tea depends on factors such as constituents of the tea, infusion temperature, water and tea ratio and particle size of the tea. In order to achieve maximum extraction of the tea bag, this test was carried out. It can be observed in Table 4 that, optimum extraction was realized by infusing a tea bag in 250 mL of freshly boiled water for 10 min. This information will give patients and prescribers informed decisions on how to prepare this tea to get the most phytochemical constituents in solution in order to achieve an optimal therapeutic outcome.

Microbial load analysis of Tapinanthus bangwensis tea bags

Microbial contamination cannot be overlooked when it comes to herbal teas. Processing of raw plant materials and packaging of herbal teas must be properly done with the right pre-treatments to reduce their microbial load to acceptable levels in order not to pose a health risk to consumers. Adopting proper drying methods for raw plant material before processing could lead to low microbial contamination. Kumadoh and her team have discovered that oven-dried and microwave-dried methods for drying raw plant materials could reduce microbial contamination if adopted by the food industry [21]. The result as demonstrated in Table 5 shows that, the levels of yeast and mold as well as aerobic bacterial counts in the tea were within the acceptable specifications, with the advantage of stability enhancement [41].

Toxicity of Tapinanthus bangwensis tea in ICR mice

Evaluation of the safety of natural product formulations is essential in ensuring the attainment of a desirable therapeutic outcome [42]. Form the acute toxicity studies, *Tapinanthus bangwensis* extract showed no death within 48 h suggesting that, the estimated LD_{50} which is greater than 5000 mg/kg is greater or equal to level 5 on the Hodge and Sterner Scale and is also 50 times more than the recommended dose (one tea bag twice daily equivalent to 99.99 mg/kg). Also, there were no other signs of weakness, as supported by normal lachrymatory, respiratory and locomotor activities and the absence of pilo-erection after 14 days monitoring. Thus, at recommended dose, the formulated *Tapinanthus bangwensis* tea bag may not be toxic and is within the acceptable margin of safety (Hodge and Sterner Scale).

Conclusion

Herbal tea from the leaves of *Tapinanthus bangwensis* has successfully been formulated. The reported LD_{50} indicates its high safety profile. Also, the reported toxic and essential elemental contents in the extract of the *Tapinanthus bangwensis* leaves harvested at Mampong-Akuapem are within the permissible limits. The preparation also passed all quality tests subjected. The addition of *Tapinanthus bangwensis* tea as a nutraceutical for the management of diabetes will help in its safe and effective management among the Ghanaian population.

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CRediT authorship contribution statement

Doris Kumadoh: Conceptualization, Formal analysis, Data curation, Writing – original draft. **Michael O. Kyene:** Formal analysis, Writing – review & editing. **Mary-Ann Archer:** Methodology, Formal analysis, Writing – review & editing, Data curation. **Genevieve N. Yeboah:** Formal analysis, Writing – review & editing. **Emmanuel Adase:** Methodology, Formal analysis, Writing – review & editing, Data curation. **Maxwell Mamfe Sakyiamah:** Formal analysis, Writing – review & editing. **Susana Oteng-Mintah:** Formal analysis, Writing – review & editing. **Ofosua Adi-Dako:** Supervision, Formal analysis, Writing – review & editing. **Christina Osei-Asare:** Supervision, Formal analysis, Writing – review & editing. **Esther Eshun Oppong:** Supervision, Formal analysis, Writing – review & editing.

Declaration of Competing Interest

Authors declare no conflict of interest.

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