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Kinkeliba and Mbor Mbor: Safe, Nutritional Herbal Teas of the Gambia, and Rich in Phytoconstituents

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Abstract

Combretum micranthum (Kinkeliba) and *Lippia chevalieri* Moldenke (Mbor mbor) are herbal plants commonly referred to as the "Teas of The Gambia." The mature and young leaves of both plant species were collected from various villages across The Gambia, at locations distanced from human activities. Approximately 300 g of air-dried and pulverized samples were extracted with 500 cm^3 of ethanol over a period of 48 h and subsequently concentrated using a rotary evaporator. Both qualitative and quantitative phytochemical analyses of the leaf extracts, along with the determination of heavy metals Pb, Cd, and Cu, were conducted according to standard protocols. The findings revealed that both plants possess similar phytoconstituents, although their quantities varied depending on the sample locations. No significant variation within the 95% confidence interval for *L. chevalieri* was observed between the young and mature leaf samples, except concerning phlobatannins and glycosides. Mbor mbor exhibited higher levels of saponins and alkaloids compared to Kinkeliba, which contained elevated quantities of flavonoids, tannins, and phenols. No evidence of toxicity from heavy metals was detected, as their concentrations were significantly below the permissible limits established by FAO/WHO standards.

The health risk assessment of the herbal teas indicated that both the hazard quotient and hazard index were below 1, suggesting safety within acceptable thresholds. The consumption of these herbal teas is recommended due to their availability, cost-effectiveness, safety, and associated nutritional and health benefits.

Keywords: *Hazard index, herbal teas, Gambia, phytochemicals*

Introduction

Combretum micranthum and *Lippia chevalieri* Moldenke are herbal plants widely distributed and used in West Africa (Pascual *et al*., 2001). Both plants, commonly known as the "teas of The Gambia" (Mevy *et al*., 2007), are called "Kinkeliba" and "Mbor Mbor" respectively by most tribes in The Gambia (Eloff *et al.,* 2008; Etonihu, 2022). Teas from these plants are simply made by infusing or steeping their leaves in boiling water. Ubiquitous in tropical and sub-tropical African countries like Senegal, Gabon, Gambia, Mali, Nigeria, Niger, and Ghana, where they are widely used in traditional medicine (Odoh *et al*., 2020), the plants hold significant cultural and nutritional values in the region. Beyond their culinary use, both plants are valued for their potential health benefits. *C. micranthum* (Kinkeliba), for example, was selected as one of the 50 most important African medicinal plants by the Association for African Medicinal Plant Standard (AAMPS) (Welch, 2010). In addition to their use as herbal teas, *L. chevalieri* has been reported (Pascual *et al*., 2001) to exhibit antimalarial activities.

No deleterious effects have been reported about *C. micranthum,* suggesting its safety as an herbal tea and for traditional medicinal uses (Amadi *et al*., 2020; Kola *et al*., 2002; Akeem *et al*., 2012). Toxicity resulting from heavy metals could depend on numerous factors such as the chemical species of the heavy metal, dose, contact manner, as well as the sex, age, genetics, and nutritional state of the consumer (Mehrandish *et al*., 2019). When metallic ions meet nuclear proteins and DNA, they can cause damage to the latter and structural alterations that can precipitate apoptosis and cancer (Beyersmann & Hartwig, 2008). Contamination or pollution via heavy metals is the most harmful because they are non-biodegradable, persistent in the ecosystem, and are subject to bioaccumulation in the food chain (WHO, 2007). The harmful consequences of heavy metals, even at low concentrations, can endanger people's health when they consume contaminated herbal remedies orally, whether as medicines or food (as teas) (Dghaim *et al*., 2015). Consequently, a dire need for the WHO and other regulatory bodies to ensure compliance with the permissible limits for heavy metals in consumable medicinal plants (Ghani *et al*., 2012).

Table 1 shows that fifteen amino acids have been identified in the leaves and seeds of *C. micranthum*. Among these are eight essential amino acids, precursors for valuable protein (Bougma *et al*., 2021; Zeitoun *et al*., 2020). Minerals play an important role in the medicinal properties of plants. Calcium, magnesium, potassium, sodium, iron, and zinc are present in the seeds (Bougma *et al*., 2021), and potassium nitrate in the leaves (Tine *et al*., 2024). Among the carbohydrates present in the leaves of *C. micranthum* are glucose, galactose, rhamnose, arabinose, xylose, galacturonic acid, *myo-inositol,* and *Sorbitol* (Yapo *et al*., 2014).

The presence of thirteen flavonoids has been reported (Welch *et al*., 2017) in the leaf extracts of Kinkeliba. From the leaves and roots of *C. micranthum*, thirty-four flavonoids were found to belong to the structural groupings of bioflavonoids, anthocyanins, flavans, flavanols, flavones, flavanonols, and flavanol polymers (Tine *et al*., 2024).

Table 1: Amino acid composition of *C. micranthum*

S/N	**Amino Acid (g/100 g Protein)**
1.	Threonine
2.	Valine
3.	Methionine + Cysteine
4.	Isoleucine
5.	Leucine
6.	Phenylalanine + Tyrosine
7.	Lysine
8.	Histidine
9.	Aspartic acid and asparagine
10.	Glutamic acid and glutamine
11.	Serine
12.	Glycine
13.	Arginine
14.	Alanine
15.	Proline

Source: Bougma *et al.* (2021); essential amino acid (EAA)

Extracts of both plants are rich in phenolic compounds. Jean *et al.* (2012) reported the presence of 20 phenolic compounds in the aerial parts (stems and leaves) of *L. chevalieri* plant and with phenolic acids as the most abundant constituent. 11 compounds were of cinnamic acid derivatives, 4 flavones, 2 Rosmarinus acid derivatives, 2 luteolin derivatives, and 1 Saponaria derivative. A study by Bangou (2012) identified the presence of rutin, flavonoid aglycones, tannins, saponosides, sterols and triterpenes and the absence of anthraquinones, alkaloids, and coumarins in the methanolic extracts of *L. chevalieri*. Sixteen phenolic acids were identified in the leaves of *C. micranthum*, 6 of which are known to be *hydroxybenzoic acids*, 1 *phenylacetic acid*, and 9 *hydroxycinnamic acids* (Tine *et al.*, 2024; Jean *et al.*, 2012; Nergard *et al.*, 2015). Phenolic acids are known for their antioxidant properties and are an important class of bioactive substances in medicinal plants.

Welch (2010) reported the presence of fourteen alkaloids in the leaves of *C. micranthum,* including new types of piperidine flavans alkaloids called kinkeloids. These kinkeloids are eight in total namely *kinkeloid A1, kinkeloid A2, kinkeloid B1, kinkeloid B2, kinkeloid C1, kinkeloid C2, kinkeloid D1,* and *kinkeloid D2.* Other alkaloids include *Choline, Betanidine, Stachydrine, 4-Hydroxystachydrine, Betaine,* and *Desacetylcolchicine* (Tine *et al.*, 2024).

Both *C. micranthum* and *L. chevalieri* are amongst the medicinal plants popular in The Gambia and widely consumed as tea. However, their scientific information on nutrition, phytochemistry, and heavy metal compositions remains very sparse.

Materials and Methods

Identification and Collection of Samples

The plants *Combretum micranthum* (Kinkeliba) and *Lippia chevalieri* (Mbor mbor) were identified by both the traditional practitioners and a Plant Taxonomist at the Ministry of Agriculture in The Gambia. Their morphological traits, including Colour, size, texture, and position on the branches, were used to identify and collect the young and mature leaves. The mature leaves of *C. micranthum* (kinkeliba) were collected from the shrub from three villages in The Gambia (Faraba Banta in the Kombo East District, Jarra Pakalinding in the Lower River Region and Fass in the North Bank Region). Similarly, both mature and young leaves of *L. chevalieri* were collected from the plant from Mayamba and Sanyang in The Gambia, and at sites isolated from human settlements and activities. While Mayamba is in Lower Nuimi District in the North Bank Region (NBR), Sanyang is located along the Atlantic coast in Kombo South District in the West Coast Region (WCR).

Preparation of Samples

The collected leaves of *C. micranthum* (Kinkeliba) and *L. chevalieri* (Mbor mbor) were rinsed with water to remove dirt on the surface, air-dried separately at room temperature for two weeks and pulverized into fine powders using a clean mechanical blender.

Extraction of the Plant Samples

Approximately 300 g each of the pulverized leaf samples of *C. micranthum* (Kinkeliba) and *L. chevalieri* (Mbor mbor) were packed into a thimble and placed inside a Soxhlet extractor and extracted with 500 cm^3 ethanol for 48 h. The resulting extracts were concentrated using a rotary evaporator (Büchi Rotavapor, R-205; Quick fit, England), evaporated to dryness under vacuum, and carefully stored.

Phytochemical Analysis

Both qualitative and quantitative phytochemical analyses of leaf extracts of *C. micranthum* (kinkeliba) and *L. chevalieri* (Mbor mbor) were done using standard methods (Sofowora *et al.*, 2013; Sathya *et al.*, 2013).

Determination of Heavy Metals

The heavy metals Pb, Cd, and Cu were determined according to the method of the Association of Official Analytical Chemists (AOAC, 1990) using the atomic absorption spectrophotometer (model 230ATS). The analysis of each sample was repeated three times.

Health Risk Assessment

The risk associated with the consumption of heavy metal-contaminated herbal products was assessed based on the estimated daily intake (EDI), the hazard quotient (HQ), and the hazard index (HI). EDI was calculated using the following equation:

$$EDI = \frac{MC \times IR}{BW} \quad (1)$$

Where, MC = metal content in mg/L (ppm), BW = body weight. IR = the ingestion rate, and this was assumed to be 0.25L/day in adults for Mbor-mbor tea. The mean weight of 64.4 kg in adults ages 25- 64 was used as body weight as described by a method (Cham *et al.*, 2018).
Hazard quotient (HQ) is used to assess the non-carcinogenic risk of a metal. HQ is determined using the following equation:

$$HQ = \frac{EDI}{RfD} \quad (2)$$

Where, EDI = estimated daily intake, and RfD = reference dose. The following RfD were used: Cu (0.040 mg/kg/day), Cd (0.001mg/kg/day), and Pb (0.004mg/kg/day) (USEPA, 2011).
Hazard index (HI) is the sum of individual hazard quotients.

$$HI = HQ_{Cu} + HQ_{Cd} + HQ_{Pb} \quad (3)$$

Method of Data Analysis

Statistical analysis of the data was performed using SPSS (Statistical Package for the Social Sciences) version 27.0. Descriptive statistics were calculated for each group, including means, standard deviations, and standard errors. Levene's test was conducted to assess the homogeneity of variances across groups for both phytochemical measurements (mg/g) and heavy metal concentrations (ppm). A one-way ANOVA was performed to compare the means of the different groups for both phytochemical measurements and heavy metal concentrations. This analysis aimed to determine if there were statistically significant differences among the group means. The Post Hoc Tests using Tukey's HSD (Honestly Significant Difference) test were applied to identify specific group differences. This test allows for pairwise comparisons between group means. In addition to the ANOVA, robust tests such as Welch's and Brown-Forsythe tests were conducted to evaluate the equality of means. Means plots were generated to visualize and represent the differences in means across groups, aiding in the interpretation of the results.

Results and Discussion

Qualitative Phytochemical Analysis

Tables 2 and 3 show similar phytoconstituents between *C. micranthum* (Kinkeliba) and *L. chevalieri* (Mbor mbor), but with variations among the samples from the different regions in The Gambia. Bioactive compounds such as saponins, alkaloids, flavonoids, tannins, phlobatinins, phenols, glycosides, carbohydrates, and balsams were present in *C. micranthum* leaves (Table 2). This corroborates the findings of Abdullahi *et al.* (2014). The results of the leaf extracts showed that while glycosides and phlobatannins were absent in the samples from the North Bank Region (KNBR) and West Coast Region (KWCR), they were present in the Lower River region (KLRR).

Table 2: Qualitative Phytochemical Composition of Ethanolic Leaf Extracts of *Combretum micranthum* (Kinkeliba)

		Samples		
S/N	**Parameter**	**KNBR**	**KLRR**	**KWCR**
1.	Saponins	+	+	+
2.	Alkaloids	+	+	+
3.	Flavonoids	+	+	+
4.	Tannins	+	+	+
5.	Phlobatanins	–	+	–
6.	Phenols	+	+	+
7.	Glycosides	–	+	–
8.	Carbohydrates	–	+	+
9.	Balsams	+	+	+

KNBR = Kinkeliba from North Bank Region; KLRR = Kinkeliba from Lower River Region; KWCR = Kinkeliba from West Coast Region; + means present; – means absent

Table 3 showed the presence of phlobatannins in the mature leaf samples from Sanyang (MMMS) but absent in both the young leaf samples from Sanyang (YMMS) and the mature leaf samples from Mayamba (MMMM), but absent in both the young leaf samples from Sanyang (YMMS) and the mature leaf samples from Mayamba (MMMM).

Table 3: Qualitative Phytochemical Composition of Ethanolic Leaf Extracts of *L. chevalieri* (Mbor mbor)

		Samples		
S/N	**Parameter**	**MMMS**	**YMMS**	**MMMM**
1.	Saponins	+	+	+
2.	Alkaloids	+	+	+
3.	Flavonoids	+	+	+
4.	Tannins	+	+	+
5.	Phenols	+	+	+
6.	Glycosides	–	+	+
7.	Carbohydrates	+	+	+
8.	Balsams	+	+	+
9.	Phlobatanins	+	–	–

MMMS = Mature mborr mborr leaves from Sanyang; YMMS = Young mborr mborr leaves from Sanyang; MMMM = Mature mborr mborr leaves from Mayamba; + means present; – means absent

Statistical tests at a 95% confidence level using their mean variations showed that there was no significant difference in *L. chevalieri* (Mbor mbor) between the young leaf samples (YMMS) and the mature leaf samples (MMMS) from Sanyang, except in their phlobatannins and glycosides. The presence of bioactive compounds, carbohydrates, and glycosides in both plants is responsible for their known medicinal and culinary values and explains the roles of the plants in both dietary and medicinal applications. The presence of phlobatannins in mature leaves from Sanyang, but their absence in young leaves from Sanyang and mature leaves from Mayamba suggests maturity and location-specific variation. Yoda *et al.* (2020) reported the presence of phenolic compounds, flavonoids, phenolic acids, and the absence of terpenes and saponins in the leaf extracts of *L. chevalieri*. On the other hand, the methanolic extracts of *L. chevalieri* leaves from Cameroon contain saponins, terpenoids, flavonoids, tannins, and polyphenols, and the absence of alkaloids (Ignareki *et al.*, 2023).

Quantitative Phytochemical Analysis

Figures 1 and 2 show the results of quantitative phytochemistry of the ethanolic leaf extracts of *C. micranthum* (Kinkeliba) and *L. chevalieri* (Mbor mbor), respectively.

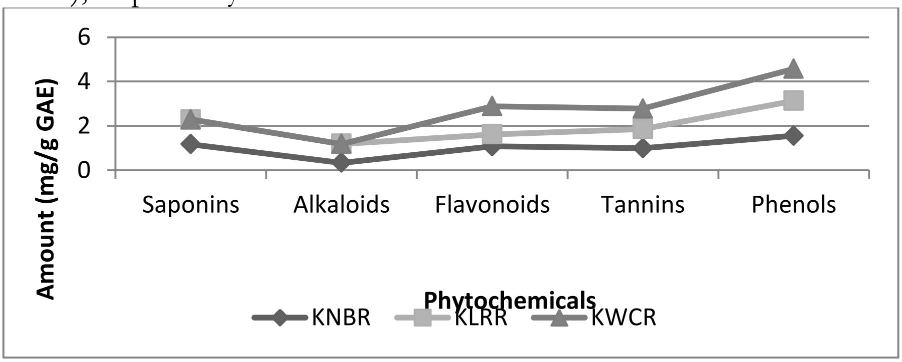

Figure 1: Quantitative Phytochemical Composition of Ethanolic Leaf Extracts of *Combretum micranthum* (Kinkeliba) in mg/g GAE

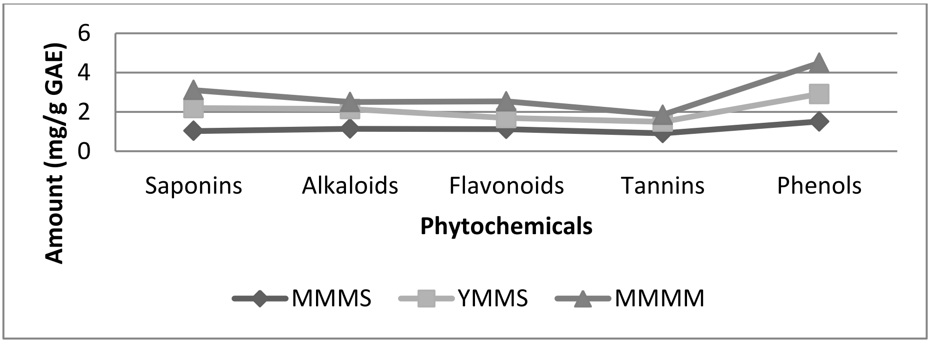

Figure 2: Quantitative Phytochemical Composition of Ethanolic Leaf Extracts of *L. chevalieri* (Mbor mbor) in mg/g GAE

The quantitative phytochemicals in the Gambia samples compared favourably to the findings in the literature. The total phenolic content, total flavonoids, and tannin content as 17.88, 3.19, and 7.62 mg gallic acid equivalent/100 mg, respectively, had been reported (Bangou *et al.*, 2011). As the use of acetone extracts, decoction, and ethanol-water extracts to assess phenol and flavonoid contents of stems and leaves of *L. chevalieri* from Burkina Faso is known (Jean *et al.*, 2017). The phenol and flavonoid content showed the following in the extracts: 7.86 mg EAG/g dry extracts and 0.90 mg EQ/g dry extracts for phenol and flavonoid contents in acetone; 16.10 mg EAG/g dry extracts and 0.77 EQ/g dry extracts for the phenol and flavonoid contents in decoction; 19.48 mg EAG/g dry extracts and 1.62 EQ/g dry extracts in ethanol-water. Similarly, Wangrawa *et al.* (2023) collected samples from Ouagadougou in Kenya in a study to ascertain larvicidal and adulticidal properties of methanol and acetone extracts of *L. chevalieri.* They reported that in the methanolic extracts, the total terpenoids content was 18.09 mg ursolic acid equivalent/mg of extracts; total polyphenols were $1.69. 10^{-2}$ μg gallic acid equivalent/ 100 mg of extracts, while total flavonoids were $1.56.10^{-1}$ mg quercetin equivalent/ 100 mg of extracts.

Figure 3 shows that, by quantitative phytochemistry, Mbor mbor contains more saponins (3.111 mg/g) and alkaloids (2.496 mg/g) than Kinkeliba (3.056 mg/g and 1.898 mg/g, respectively). Conversely, Kinkeliba had higher contents of flavonoids (2.890 mg/g), tannins (2.776 mg/g), and phenols (4.568 mg/g) than Mbor mbor 2.528, 1.850, and 4.486 mg/g, respectively.

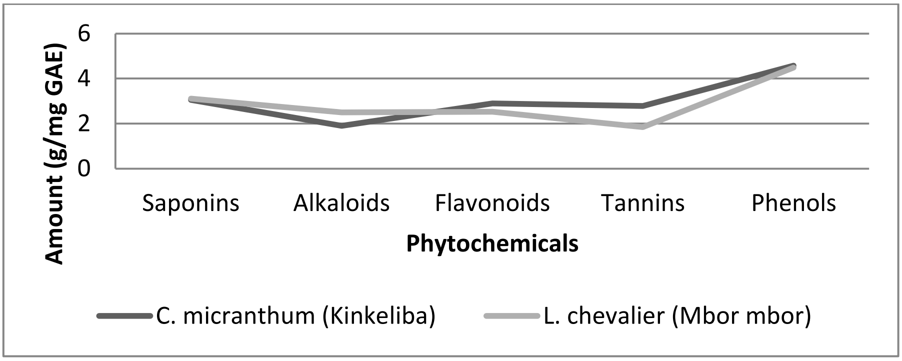

Figure 3: Comparative Quantitative Phytochemical Compositions of Ethanolic Leaf Extracts of *C. micranthum* (Kinkeliba) and *L. chevalieri* (Mbor mbor) in mg/g GAE

All the leaf samples contained saponins, with the highest content observed in the young leaves from Sanyang (1.161 mg/g). This may indicate that young leaves accumulate more of this compound due to their potential role in plant defense, as corroborated by Ignareki *et al.* (2023), who reported similar observations in *L. chevalieri* leaves from Cameroon, but in contrast with the results from Yoda *et al.* (2020).

Alkaloids were found in all the samples, with the highest content in the mature leaves from Sanyang (1.142 mg/g). The difference observed with the mature leaves from Mayamba (0.352 mg/g) suggests location variation due to differences in environmental factors. Although Ignareki *et al.* (2023) and Wangrawa *et al.* (2023) did not detect the presence of alkaloids in the samples from Cameroon and Burkina Faso, respectively, this difference in phytoconstituent can be attributed to environmental factors and/or climate change. Flavonoids and phenols were present in all the samples from the various regions, with the highest content in mature leaves from Sanyang (1.130 mg/g) and Mayamba (1.578 mg/g), respectively. Flavonoids are well-known for their strong antioxidant capabilities and are important for shielding plants from environmental stress. This phytoconstituent also provides cardiovascular protection and reduces inflammation. This shows that both plant leaves are rich sources of natural antioxidants. Despite the observed phytochemical variations in both plant leaves, the calculated p-value of 0.823 (greater than 0.005) showed that there is no statistically significant difference between the means of Mbor mbor and Kinkeliba at the 95% confidence level.

Heavy Metal Content

The levels of heavy metals in ethanolic leaf extracts of *L. chevalieri* (Mbor mbor) are shown in Table 4. The amounts for cadmium ranged from 0.003 ppm for the MMMM sample to 0.028 ppm for the MMMS sample. The young leaves from Sanyang (YMMS) had higher levels of copper (0.277) than the mature leaves 0.254 ppm from Sanyang (MMMS) and 0.179 ppm from Mayamba (MMMM). Lead levels ranged from 0.169 ppm for the MMMM sample to 0.302 ppm for the MMMS sample.

Table 4**:** Levels of Heavy Metals in the Ethanolic Leaf Extracts of *L. chevalieri* (Mbor mbor) in ppm

		Samples			WHO
S/N (ppm)	**Parameter**	**MMMS**	**YMMS**	**MMMM**	**Limits**
1.	Cadmium	0.028 ± 0.001	0.016 ± 0.001	0.003 ± 0.001	**0.300**
2.	Copper	0.254 ± 0.001	0.277 ± 0.002	0.179 ± 0.001	**20.000**
3.	Lead	0.302 ± 0.002	0.242 ± 0.001	0.169 ± 0.001	**10.000**

MMMS = Mature mborr mborr leaves from Sanyang; YMMS = Young mborr mborr leaves from Sanyang; MMMM = Mature mborr mborr leaves from Mayamba; ± means standard deviation

From the results, the contents of cadmium, copper, and lead in all the samples were well below their World Health Organization (WHO, 2007) [12] toxicity limits. This shows that the leaves of *L. chevalieri* are potentially safe for therapeutic and dietary applications as they are unlikely to pose any threat due to heavy metal toxicity.

Health Risk Assessment

The risk associated with the consumption of heavy metal-contaminated herbal products was assessed based on the estimated daily intake (EDI), the hazard quotient (HQ), and the hazard index (HI).

Estimated Daily Intake

The estimated daily intake (EDI) for the heavy metals (cadmium, copper, and lead) was calculated based on the metal content in the leaf extracts and the assumed ingestion rate for adults consuming the Mbor mbor tea. The values were compared to the tolerable daily intake (TDI) (Table 5). The Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommended a provisional maximum tolerable daily intake (PMTDI) of 0.5 mg/kg/day for copper (FAO/WHO, 2023). The EDI of copper in all samples was significantly below the tolerable daily intake (TDI). The provisional tolerable monthly intake (PTMI) of cadmium is 0.025 mg/kg bw (FAO/WHO, 2023). The EDI values of cadmium in all samples were below the calculated tolerable daily intake (TDI). JECFA withdrew the previously established provisional tolerable weekly intake (PTWI) of 0.025 mg/kg bw for lead, as evidence suggests the value could no longer be considered health protective. The tolerable daily intake value of 0.00003 mg/kg/day for lead (Pb) (Reis *et al.*, 2014) was used in the current study. The calculated EDI for lead in all samples exceeded the tolerable daily intake.

Table 5: Estimated Daily Intake for Adults (mg/kg body weight per day)

Sample	**Cadmium**	**Copper**	**Lead**
YMMS	0.00010	0.00110	0.00090
MMMS	0.00010	0.00100	0.00120
MMMM	0.00000	0.00070	0.00070
TDI [34]	0.02500	0.50000	NA
TDI [35]	0.00083	0.50000	0.00003

*TDI = Tolerable Daily Intake; NA = Not Available

Hazard Quotient and Hazard Index

The hazard quotient (HQ) was determined for each heavy metal to assess the non-carcinogenic risk associated with their consumption, while the hazard index (HI) provided an overall assessment of the potential health risk associated with the consumption of the plant (Table 6). An HQ value less than 1 is safe and no negative health impacts are expected; an HQ value equal to or greater than 1 (≥ 1) is considered unsafe and could have negative health effects.

	HQ			
Sample	Cadmium	Copper	Lead	**HI**
YMMS	0.100	0.028	0.225	0.4
MMMS	0.100	0.025	0.300	0.4
MMMM	0.000	0.018	0.175	0.4

Table 6: Hazard Quotient and Hazard Indices

The HQ values for cadmium (Cd), copper (Cu), and lead (Pb) (Table 6) below 1 indicated that there are no significant health risks on consumption based on the assessed metals. Similarly, the HI value less than 1 indicates that the cumulative exposure to the assessed heavy metals is considered safe. That is, the estimated daily intake of the metals is below the reference dose (RfD). Although this study has not indicated a health danger, further monitoring and assessment are necessary. HI value of 1 indicates that the cumulative exposure to the assessed heavy metals is at the level of the reference dose (RfD); while HI values greater than 1 mean that the cumulative exposure to the assessed heavy metals exceeds the threshold limit that suggesting the possibility of negative health effects and reconsideration of the product's ingestion. The calculated hazard index of all the samples was less than 1 and therefore within the safe threshold.

Conclusions

C. micranthum (Kinkeliba) and *L. chevalieri* (Mbor mbor) are herbal teas commonly consumed in The Gambia. This study shows that both herbal teas are safe from toxic elements, nutritional, and rich in phytoconstituents. Qualitative phytochemistry shows that both *L. chevalieri* (Mbor mbor) and *C. micranthum* (Kinkeliba) have similar phytoconstituents with variations due to differences in sample locations. There was no significant variation in *L. chevalieri* (Mbor mbor) between the young and mature leaf samples, except in the phlobatannins and glycosides. Quantitative phytochemistry (mg/g) shows that *L. chevalieri* (Mbor mbor) contains more saponins and alkaloids than *C. micranthum* (Kinkeliba), but *C. micranthum* (Kinkeliba) has higher contents of flavonoids, tannins, and phenols. The levels of the toxic metals Cd, Pb, and Cu were far below their deleterious limits. The health risk assessment for the herbal teas based on the estimated daily intake (EDI) showed that the hazard quotient (HQ) and hazard index (HI) were below 1 and within the safe threshold. Based on our results, the consumption of these herbal teas is recommended for their ready availability, cost effectiveness, safety, nutritional, and health benefits.

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