GSC Biological and Pharmaceutical Sciences

eISSN: 2581-3250 CODEN (USA): GBPSC2

Cross Ref DOI: 10.30574/gscbps

Journal homepage: https://gsconlinepress.com/journals/gscbps/

(Research Article)

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Levels of minerals and trace elements in leaves of cultivated "Long leaf morphotypes" of *Lippia multiflora* and their transfer to infusions

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GSC Biological and Pharmaceutical Sciences, 2025, 33(01), 031-038

Publication history: Received on 22 August 2025; revised on 30 September 2025; accepted on 03 October 2025

Article DOI: https://doi.org/10.30574/gscbps.2025.33.1.0378

Abstract

Lippia multiflora Moldenke leaves are used for the preparation of tea infusions in traditional medicine. In this study, the mineral composition of the leaves of the long-leaf morphotype of *Lippia multiflora*, collected from different cities of Ivory Coast, and their infusions were determined by Atomic Absorption Spectroscopy (AAS). The results showed that calcium (8231.17±0.5-10823.66±0.02 $mg.kg^{-1}$), potassium (2740.00±0.2-7949.87±0.41 $mg.kg^{-1}$), magnesium (3414.33±0.15-4731.00±0.15 $mg.kg^{-1}$) and sodium (1046.76±0.45-1190.33±0.4 $mg.kg^{-1}$) were the most predominant macroelements in the leaves, compared to the microelements Fe (62.50±0.0-142.00±0.0 mg. kg^{-1}), Mn (21.67±0.0-54.33±0.02 $mg.kg^{-1}$), Zn (30.17±0.00-41.33±0.00 $mg.kg^{-1}$) and Cu (16±0.00-32.17±0.0 $mg.kg^{-1}$). In the infusions, the order of magnitude of the minerals was: Na > K > Ca > Mg > P > Zn > Mn > Cu > Fe. However, the mineral contents of the infusions were very low compared to their respective daily doses recommended by the World Health Organization; thus demonstrating a safe consumption of this herbal tea.

Keywords: Mineral contents; Infusions; Longleaf morphotype; *Lippia multiflora*

1. Introduction

Tea is the most consumed beverage after water, due to its refreshing and mildly stimulating effects [1]. It is prepared from the leaves of a shrub, *Camellia sinensis* [2]. About 75 % of the approximately 2.5 million tons of dried tea harvested worldwide each year is processed into tea [3]. One kilogram of tea is consumed on average per person per year, and an average consumer drinks 2 to 3 cups of tea per day [4]. However, besides the Camellia sinensis tea plant, several other medicinal plants are used for the preparation of refreshing drinks. Among them we have the plant *Lippia multiflora*, commonly known as savannah tea or Gambia tea. *Lippia multiflora* Moldenke belongs to the Verbenaceae family, which includes about 200 known species of small trees, shrubs, and grasses. It is generally found in tropical regions of West Africa, Central American countries, and South American countries [5]. It is an aromatic and perennial plant with more or less woody stems reaching 2.7 to 4.0 m in height [6]. Its aerial and underground parts are commonly used by traditional healers in Côte d'Ivoire and other African countries for the treatment of health problems such as hypertension, enteritis, lymphatic cleansing, chest pain, diabetes, colds, gastrointestinal disorders, stomach aches, jaundice, cough, fever, nausea, bronchial inflammation, conjunctivitis, malaria and venereal diseases [7,8].

Many studies have focused on the phytochemical [9], botanical [10] and medicinal [11] aspects of the plant. From a biochemical point of view, authors have reported that the leaves of *Lippia multiflora* are very rich in essential minerals

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(potassium, calcium, magnesium, iron, manganese, zinc and phosphorus) and bioactive compounds [12], but data on the composition of their infusions are scarce. Indeed, their decoctions and infusions constitute the main forms of use of *Lippia multiflora* leaves. However, studies have shown that some minerals are essential for the functioning of the human body, but their deficiencies and sometimes their excesses can lead to serious consequences for humans [13], and others are harmful, even in small quantities [14]. In these conditions and given the significant consumption of herbal teas made from *Lippia multiflora* leaves, it is important to determine their mineral composition, in order to assess its possible effects on the health of the consumer. It is in this context that we conducted this study with the aim of analyzing and comparing the mineral composition of leaves from three regions, as well as that of their infusions, in order to determine whether they could contribute to the daily mineral nutritional needs of consumers.

2. Material and methods

2.1. Material

The leaves were harvested in September 2018 from the broad-leaved variety of *Lippia multiflora* (Figure 1), grown in the center, north and northeast localities of Côte d'Ivoire, namlely Béoumi (7°42’28’’N ; 5°35’50’’O), characterised by a Baouléan climate and desaturated ferralitic soil; Korhogo (9°23’23’’N ; 5°48’49’’O), characterised by a Sudanese climate and Cambisols soil and Bondoukou (8°06’43’’N ; 2°42’23’’O), charactersed by a Baouléen climatie, cambisol.

Figure 1 Longleaf morphotype of *Lippia multiflora*

3. Methods

3.1. Leaves harvesting method

The leaves were harvested using the fine picking method described by [15], which consists of removing the bud (pekoe) and the two leaves below it. The samples collected were dried in the laboratory at room temperature (37 °C) until their weight stabilised. The dried leaves were pulverised using a Blender FAR BL514X Cl mixer and the resulting powders were stored in airtight plastic boxes for biochemical analysis.

3.2. Preparation of infusions

The extracts were prepared according to the method described by [16]. For each sample, 20 g of powder was weighed using a Mettler Type A 100 precision balance in a 500 ml beaker, then 1000 mL of boiling water was added and the mixture was infused for exactly 5 minutes. The suspension was stirred periodically with a glass rod to promote maximum extraction of soluble compounds from the leaves. At the end of the allotted time, the solutions were filtered using filter paper and the filtrate was stored in the refrigerator for analysis.

3.3. Mineral Analysis of leaves and infusions

Mineral analysis were performed in leaves powder and extracts according the methods of [17]. The analysis involved determination of the content of 9 elements (Na, K, Ca, Mg, Zn, Cu, Fe, P and Mn in mineralised tea samples (n = 3) and extracts (n = 3). The contents of the elements were determined in the tea leaves (2 g of herbal material) after incineration in a muffle furnace at 450 °C. The ashes obtained were solubilised in crucibles using spectrally pure hydrochloric acid at 6 mol l^{-1} (POCH, Poland). Na and K were analysed using a Flame Atomic Emission Spectroscope (FAES) with a flame photometer at wavelengths of λ = 589.0 and λ = 766.5 nm, respectively. The concentrations of Ca, Mg, Zn, Cu, Fe, P and Mn were determined using Atomic Absorption Spectroscopy (AAS) with a SOLAAR 939/959 spectrophotometer. Calcium was determined at λ = 422.7 nm, magnesium at λ = 285.2 nm, zinc at λ = 213.9 nm, copper at λ = 324.8 nm, iron at λ = 248.3 nm and manganese at λ = 279.5 nm. The Na and K determinations were made by adding caesium chloride to the standards and samples as an ionisation buffer at a concentration of 0.2 % w/v. Ca and Mg were analysed by adding 0.4 % w/v lanthanum oxide as a correction buffer to bind the analysed element to the matrix. The percentage extraction of elements from the leaves during brewing was also determined.

3.4. Data analysis

Data were expressed as means ± standard deviation. The data were subjected to analysis of variance (ANOVA) and means were separated by Duncan multiple range tests at $P < 0.05$ significant levels.

4. Results

The ranges of total element contents in the dried leaf samples and the element concentrations in the infusions analyzed in this study are summarized in Tables 1 and 2, respectively. The mean element concentrations in the leaves and in the tea infusion differed significantly (ANOVA, $P = 0.05$). The amount of a specific mineral therefore varies from one locality to another, revealing a unique mineral profile of the samples from each locality. Analysis of the data in Table 1 reveals that leaves from Korhogo are richer in most minerals, including Mg (4731.00±0.15), K (7949.87±0.41), Na (1190.33±0.4), Cu (32.17±0.0) and Mn (54.33±0.02) ; while those from Béoumi yielded the highest levels of P (250.00±5.00) and Fe (187.02±0.01); in contrast, leaves collected at Béoumi contain more Ca (10823.66±0.02) and Zn (41.33±0.00) than other localities. It is also evident that the dried leaves and the corresponding infusions exhibit different mineral profiles.

In the dried savannah tea leaves (Table 1), the most abundant macronutrient was calcium, followed bypotassium, magnesium, sodium, and phosphorus. Among the trace elements tested, Fe was the most abundant, followed by Zn, Mn, and Cu. However, Na, a non-essential alkali metal, was more abundant than Zn.

The concentrations of elements in the infusions Table 2, were determined in order to assess the actual exposure to these elements when consuming these drinks. Infusions showed significant differences from the leaves in terms of the order of magnitude of the concentrations of the elements in Table 2, which were as follows: Na > K > Ca > Mg > P > Zn > Mn > Cu > Fe.

Table 1 Mineral composition of dry leaves of *Lippia multiflora*

	Localities		
Minerals $mg.kg^{-1}$	**Bondoukou**	**Beoumi**	**Korhogo**
Calcium (Ca)	$10823.66±0.02^{f}$	$8231.17±0.5^{c}$	$8796.17±0.5^{d}$
Potassium (K)	$4396.67±0.2^{e}$	$2740.00±0.2^{b}$	$7949.87±0.41^{f}$
Magnesium (Mg)	$4247.67±5.0^{d}$	$3414.33±0.15^{b}$	$4731.00±0.15^{e}$
Sodium (Na)	$1083.67±0.4^{b}$	$1046.76±0.45^{a}$	$1190.33±0.4^{d}$
Phosphorus (P)	$248.33±5.0^{a}$	$250.00±5.00^{a}$	$248.33±5.0^{a}$
Copper (Cu)	$20.67±0.00^{d}$	$16±0.00^{b}$	$32.17±0.0^{f}$
Iron (Fe)	$142.00±0.0^{e}$	$187.02±0.01^{f}$	$62.50±0.0^{a}$
Manganese (Mn)	$38.67±0.8^{c}$	$21.67±0.0^{a}$	$54.33±0.02^{d}$
Zinc (Zn)	$41.33±0.00^{e}$	$39.00±0.0^{d}$	$30.17±0.00^{c}$

Value with different superscript letters in columns are statistically significant ($p<0.05$)

Table 2 Mineral composition of infusion

	Localities		
Minerals $mg.kg^{-1}$	**Bondoukou**	**Beoumi**	**Korhogo**
Calcium (Ca)	44,96±0,76b	54,04±0,60c	50,10±3,06c
Potassium (K)	81,99±0,03^{c}	103,46±3,91^{d}	97,78±1,46^{c}
Magnesium (Mg)	27,41±3,26^{bd}	18,80±6,51^{ab}	34,33±4,07^{cd}
Sodium (Na)	143,01±8,30^{bd}	116,29±1,57a	156,03±4,84d
Phosphorus (P)	3,09±0,18^{b}	3,77±0,13^{b}	3,46±0,04^{b}
Copper (Cu)	0,002±0,00^{a}	0,072±0,09^{a}	0,090±0,11^{a}
Iron (Fe)	0,003±0,00^{a}	0,001±0,00^{a}	0,001±0,00^{a}
Manganese (Mn)	0,13±0,01^{a}	0,17±0,00^{a}	0,27±0,05^{b}
Zinc (Zn)	0,13±0,17^{a}	0,75±0,15^{a}	0,28±0,23^{a}

Value with different superscript letters in columns are statistically significant ($p<0.05$)

The extraction rates of minerals in the infusions are presented in **Table 3**. These results show a very significant variability of solubility of metals in the infusion. The highest average extraction rates were observed for sodium with an average extraction rate ranging from 11.11 to 13.20 %, followed by K (1.23-3.77 %) and P (1.24-1.51 %). Minerals such as Mg (0.55-0.73 %), Mn (0.33-0.78 %), Cu (0.009-0.45 %), and Fe (0.0005-0.002 %) gave extraction yields below 1%. For all the minerals, Fe with an extraction rate ranging from 0.0005 to 0.002% was the least soluble mineral. Taking into account their solubilities in infusions, the minerals of *Lippia multiflora* leaves can be classified in the following order: Na > K > P > Zn > Ca > Mg > Mn > Cu > Fe

Table 3 Extraction rates of minerals from leaves to infusions

	Transfer rates (%)		
Minerals	**Bondoukou**	**Beoumi**	**Korhogo**
Calcium (Ca)	0.42	0.66	0.57
Potassium (K)	1.96	3.77	1.23
Magnesium (Mg)	0.64	0.55	0.73
Sodium (Na)	13.20	11.11	13.10
Phosphorus (P)	1.24	1.51	1.39
Copper (Cu)	0,009	0.45	0,28
Iron (Fe)	0,002	0,0005	0.0016
Manganese (Mn)	0,33	0.78	0.50
Zinc (Zn)	0,31	1.92	0.93

In order to assess he mineral intake of savana tea, the amount of minerals available in a cup were calculated from the data in Table 2, using a conversion factor of 1 mg/kg = 0.0025 mg/cup, assuming that 1 g of tea leaves is used for 100 ml of boiling water at home and that a cup contains 250 ml of liquid. Table 4 presents the contents of mineral elements in a 250 mL cup of tea. The results show that a cup of *Lippia multiflora* infusion contains very low amounts of Na (29.07-39.01 mg/250 mL), K (20.50-25.86 mg/250 mL), Ca (11.24-13.51 mg/250 mL), P (0.77-0.94 mg/250 mL), Mg (4.70-8.58 mg/250 mL), Cu (0.00-0.02 mg/250 mL), Mn (0.03-0.07 mg/250 mL), Fe (0.00-0.01 mg/250 mL) and Zn (0.00-0.23 mg/250 mL). Sodium, averaging 34.61 mg per 250 mL, is the most abundant mineral in a cup of *Lippia multiflora* infusion, followed by potassium (23.60 mg/250 mL), calcium (12.42 mg/250 mL), and magnesium (6.51 mg/250 mL). Iron, zinc, copper, and manganese are almost nonexistent in the cup of tea. Comparing the content of each mineral with its recommended daily intake (RDI) reveals a very significant difference.

Table 4 Minerals content of a Cup of tea and Recommended Daily Intake (RDI)

	Localities				
Minerals mg/250 mL	**Bondoukou**	**Beoumi**	**Korhogo**	**Average**	**RDI (mg/J)**
Ca	11.24	13.51	12.52	12.42	1000
K	20.50	25.86	24.45	23.60	4700
Mg	6.25	4.70	8.58	6.51	400–420
Na	35.75	29.07	39.01	34.61	1500
P	0.77	0.94	0.87	0.86	700
Cu	0.00	0.02	0.02	0.01	900
Fe	0.00	0.00	0.00	0.00	8-18
Mn	0.03	0.04	0.07	0.05	1.8-2.3
Zn	0.03	0.19	0.07	0.09	8-11

RDI : Recommanded Daily Intake

5. Discussion

The contents of Ca (8231.17±0.5–10823.66±0.02 mg/kg), K (2740.00±0.2–7949.87±0.4 mg/kg), Mg (3414.33±0.15–4731.00±0.15 mg/kg), Na (1046.76±0.45–1190.33±0.4 mg/kg), P (248.33±5.0–250.00±5.00 mg/Kg), Cu (16±0.00–32.17±0.0 mg/Kg), Fe (62.50±0.0–187.02±0.01 mg/kg), Mn (21.67±0.0–54.33±0.02 mg/kg) and Zn (30.17±0.00–41.33±0.00 mg/kg) founded in this study are significantly low compared to those found in *Lippia multiflora* leaves by [18]. Furthermore, our values found for potassium (K) and manganese (Mn) are substantially identical to the concentrations of K (152.52±47.36-10029.62±48.1 mg/kg) and Mn (31.15±3.68 and 60.61 ± 0.73 mg/kg) found by [19], in young leaves of *Lippia multiflora*. However, our values are higher than the contents of Ca (20911.54 to 49979.44 mg/kg), Mg (5087.42 to 6027.12 mg/kg) and Fe (130.21 to 272.24 mg/kg) reported by these authors. On the other hand, our contents of Na (401.22 to 503.61 mg/kg) and Cu (8.11 to 13.46 mg/kg) are higher than those of [19]. The differences between our values and those of [18], could be related to the methods of leaf collection and the age of the leaves harvested. Indeed, according to Kané et al [20] and Ekissi et al [19] the contents and minerals of the leaves vary according to the harvesting method and the stage of maturity of the leaves. The variation in the concentrations of each mineral according to the origin of the leaves is in agreement with the results of [19] and [12]. Indeed, these authors have shown that the mineral content of the leaves of wild plants and cultivated plants of *L. multiflora* harvested in different localities. This variation in the concentrations of mineral elements in the leaves of *Lippia multiflora* could be linked to the composition of the soil as reported by [21]. High concentrations of macronutrients in *Lippia multiflora* leaves make them an important source of dietary supplements. Indeed, the K/Na ratio was 4.95, 2.62 and 6.68 in leaves from Bondoukou, Beoumi and Korhogo respectively. This indicates a major nutritional advantage, since the higher intake of K compared to Na has been linked to a lower incidence of hypertension [22].

Furthermore, the main use of the plant's leaves in rural and urban areas is the preparation as herbal teas (infusion and decoction).

Analysis of the infusions showed that the mineral elements founded in the leaves are also present in the infusions but at lowers concentrations. The decrease in the concentration of elements in infusion compared to the dry leaf could be explained by their fixation to the tannic acid and tannins in the leaves which exude them during the infusion or decoction. The precipitation of these chelates leads to a notable decrease in the concentration of metals in the infusion extract [3]. Indeed Street et a [23] reported that in teas with lower tannin content, there is a better leaching of the elements in the infusion.

In the infusion, the most abundant macroelement is sodium, followed by potassium, calcium, magnesium and phosphorus. Regarding trace minerals, Zn is the most predominant mineral in the infusion. Our results are consistent with those of [24], who found Na and Zn as major macroelement and microelement respectively in the infusion of differents types of tea. However, our results differ from those of Olivier et al [25] and Malick et al [26] who found K as the major macroelement in green tea infusions. The difference between our results and those of these authors could be related to the method of preparation of the infusions [27] but also to the nature of the leaves used and their biochemical compositions. Indeed, according to Rusinek [28] and Pytlakowska et al [29], the rate of extraction of minerals in the infusion is modified by several factors, including the infusion time, the type of plant used, the composition of the tea in tannins and oxalic acid.

Although containing low minerals amounts, consuming *Lippia multiflora* leaf tea can help meet nutritional needs for several essential elements. Indeed, some macronutrients, such as potassium, calcium, sodium, magnesium, iron, and manganese, among others, play important roles in the human body [25], and their deficiency or imbalance can lead to physiological disorders. For example, calcium and magnesium are the main mineral components of bones and are necessary for normal growth and physiological function [30].

Sodium and potassium are essential for many regulatory systems in the body. The minimum daily intakes of Na and K are 2.4 g and 3.5 g, respectively [31].

Phosphate is essential for various cellular processes, such as ATP production, intracellular signal transmission, bone mineralization, cell membrane formation, and DNA and RNA synthesis [32,33]. High dietary phosphate intake can precipitate diseases such as type 2 diabetes [34]. The Recommended Dietary Allowance (RDA) for phosphorus is approximately 700 mg/day [35].

Although poorly present in infusions compared to macroelements, trace elements have both a curative and preventive role in the fight against diseases [36], and their deficiencies can lead to serious consequences. For example : Iron is an integral component of hemoglobin, found in red blood cells, which transports oxygen throughout the body for energy metabolism [13]. Manganese is a key component of the antioxidant enzyme superoxide dismutase (SOD) ; it plays a role in blood clotting and hemostasis; it acts as a cofactor for various enzymes [13]. Zinc is necessary for the production of proteins and genetic material and plays a role in taste perception, wound healing, normal fetal development, sperm production, normal growth and sexual maturation, and immune system health [13]. Copper is important for many enzymes involved in iron metabolism [13]. Copper deficiency can cause anemia and neutropenia, affecting oxygen transport and immune function, and zinc deficiency may contribute to complications during pregnancy and childbirth [37]. Sodium is the most extractable ion in the leaf of *Lippia multiflora*, with an extraction rate of 13 %, which explains its high content in the infusion. Our results are similar to those of Soares et al [37] who report the high extractability of sodium compared to other ions present in the leaves of *Verbena minutiflora*.

Considering the concentration of different minerals in infusions, it is clear that they do not really contribute to significantly covering human mineral needs. The low concentrations of potentially harmful minerals in infusions compared to their recommended daily intake can be considered an advantage. For example, sodium, although predominant in infusions, its content is very low compared to its required daily intake. This higher sodium content compared to potassium, could therefore not be considered as constituting a health risk [38].

6. Conclusion

The leaves of the "long leaf" morphotype of *Lippia multiflora* are rich in essentials elements for humans. The majors macronutrients were Ca, K, Mg, and Na, compared to the trace elements Fe, Mn, Zn, and Cu. The order of magnitude of these minerals is as follows:

Ca > K > Mg > Na > P > Fe > Zn > Mn > Cu. The infusions contain the same types of essential minerals, but in smaller quantities, with an order of magnitude : Na > K > Ca > Mg > P > Zn > Mn > Cu > Fe. Thus, consumption of leaf tea can help compensate for deficiencies in certain nutrients. Furthermore, excessive consumption of this herbal tea does not pose any risk of poisoning, as the concentration of all essential elements is well below the recommended daily intake. However, the locality of Korhogo, which provided the leaves richest in most minerals, seems to be the most suitable area for cultivation if we want to benefit from the nutrients of this plant.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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