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Physiochemical screening and comparison of mineral composition of leaves from broadleaf and longleaf morphotypes of *Lippia multiflora* Moldenke (Verbenaceae) cultivated of Côte d'Ivoire

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Abstract

The phytochemical screening and mineral analyze of longleaf morphotype (llmLM) and broadleaf morphotype (blmLM) of *Lippia multiflora* cultivated in 3 localities of the Côte d'Ivoire such Bondoukou (Bdk), Béoumi (Bmi) and Korhogo (Krg) were carried out. The aims of this study were to provide information on the therapeutic and nutritional values of this herbal tea according the plant origin. Results showed presence of bioactive compounds like alkaloids, tannins and flavonoids in the extracts. All of the extracts were rich in condensed tannins. Concerning flavonoids, flavone was high grade in extracts from Bondoukou and Béoumi while the highest levels of flavanols were found in extracts from Korhogo. Essentials minerals like calcium, magnesium, sodium, potassium, manganese, phosphorus, zinc, iron, and Cooper was presents in leaves and extracts. In addition, the levels of both of this mineral in extract were below the maximum level permissible suggested by World health organization (1989). We noted also the variability of both components according the cultivation areas. The presence of phytochemicals components and mineral elements in the aqueous extracts of *Lippia multiflora* contribute to their therapeutics application in medicinal practices and their nutritional importance. However, the variability among the phytochemical profiles of samples from different areas express their different medicinal, pharmacologic and nutritional properties.

Key worlds: phytochemical screening, minerals, cultivated, *Lippia multiflora*

1. Introduction

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Herbal medicines are important in the culture and tradition in African societies [1]. Although many of them have been studied, the biochemical composition of most is less known. Among these we have *Lippia multiflora* which belong the verbenaceae's family, comprising about 200 reported species [2]. In Africa Lippia multiflora is found in Ghana, Côte d'Ivoire and Senegal as a lasting fragrant bush, of which the imbuement is utilized as a home grown tea generally known as "Shrub Tea" or "Tea of Gambia" [3]. *L. multiflora* can grow on various soil types and in different agro-ecological zones. In Côte d'Ivoire, it's found in the central and northern parts of

The aqueous extracts of are leaves are traditionally consumed as tea which used to treat some illnesses such fever, coughs, influenza [5], malaria and blood pressure [6]. It's likewise utilized as sanitizer, antipyretic and diuretic [7] and filled in as a sudorific, purgative, febrifuge, and for the colic treatment [8]. This plant is known for its pesticidal properties [9] and the decoction or infusion of its leaves has a muscle relaxant effect and analgesic, sedative, hypertensive and diuretic properties [3]. Studies on the essential oils have demonstrated their antimicrobial [8], pediculocide and scabicide [10], antioxidant [11], antibacterial and antifungal, antipyretic and anti-inflammatory properties [3]. However, most of the phytochemical studies of *L. multiflora* have focused on the volatile constituents of essential oils, resulting in limited information on non-volatile secondary metabolites and minerals. Still, many studies have reported the presence of tannin, flavonoids, alkaloids, glycosides, caffeine and saponins in the leaves [8-12]. Some of these compounds have been isolated and characterized, but their contents remain unknown, while studies have shown that they can be used as a marker to elucidate the quality of teas [13]. In addition, [14] reported that the level of catechins in tea plants depends on the variety of the plant, as well as environmental conditions which can also affect flavonoid synthesis. Also, the substance piece of tea differs relying upon environment, season, farming practices, assortment, age and position of the leaf [15]. Hence, an assessment of the biochemical arrangement of the leaves of Lippia multiflora as per the developing regions could give data on their quality and the executives procedures that could work on its development. This study aimed to determine the quality and quantities of polyphenolics compound and minerals in the leaves and infusion of cultivated *Lippia multiflora* and study the variability of this composition according the cultivation places.

2. Material and strategies

2.1. Description of the review destinations

The study has investigated in 3 localities of Côte d'Ivoire. East Bondoukou, central Béoumi and the north Korhogo. The natural qualities of each site are accounted for in the table 1.

Table 1. Characteristics of the study sites

characteristics	Localities of cultivation		
	Bondoukou	Korhogo	Béoumi
Latitude **Longitude**	8°06'43''N 2°42'23''O	9°23'23''N 5°48 ''49O	7°42'28''N 5°35'50''O
Annual rainfall (mm)	1100 à 1700	1000 à 1200	1200
Annual average temperature (°C)	22 à 27	26	25,4
Climate	Baouléen	Soudanais	Baouléen

Relative Humidity (%)	80	61,5	75
Natural Végétation	Tree savana	shrubby savannah	Tree savana
Soil type	Cambisols	Cambisols	ferralitique revamped

2.2. Plant material

Leaves from two morphotypes of *Lippia multiflora* were collected on plants grown in three different areas cited above. Sampling was performed in September; the period which the leaves are traditionally harvested before flowering by users. The morphotypes (figure 1) were identified by [16].

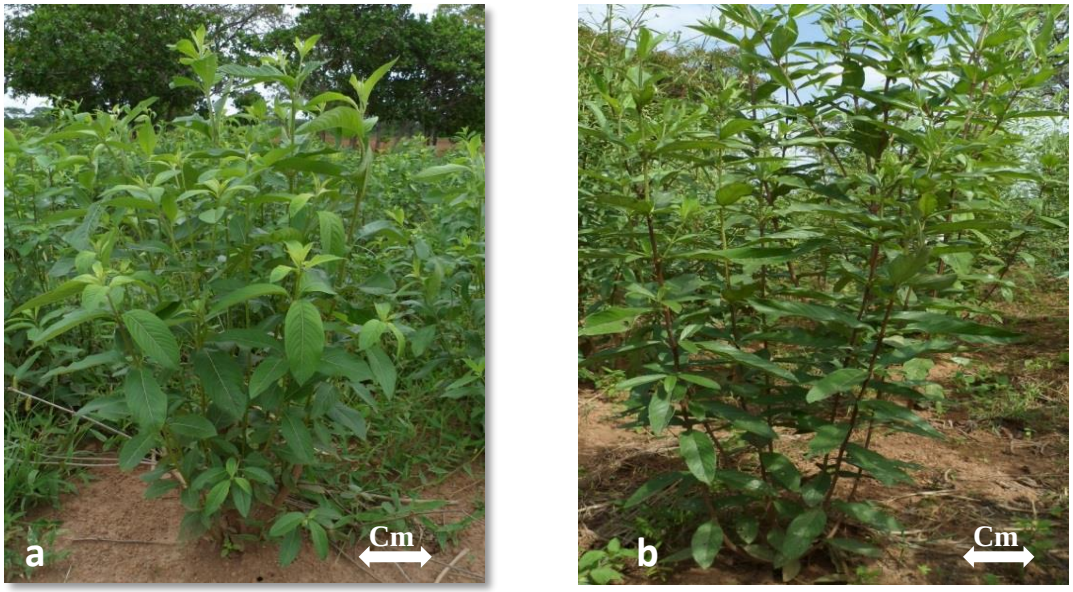

Fig 1. Morphotypes of *Lippia multiflora*: (a): broadleaf (b): Long leaves

2.3. Collection method and aqueous extract preparation

2.3.1. Harvested method

The leaves were collected involving a fine picking technique as portrayed by [17] which comprises of eliminating the bud that is pekoe and 2 leaves underneath. The leaf tests were dried out of the sun for seven days, as suggested by [18]. The dried leaves were pummeled utilizing a Blender FAR BL514X Cl blender and subsequent powders got put away in fixed boxes of plastic for biochemical investigations.

2.3.2. Preparation of infusion

Infusions were prepared according to the method of [19]. For each sample, 5 g of powder were weighed with an accurate balance Mettler Type A 100, in a 500 mL beaker and added with 250 mL of boiled water and the whole was infused for exactly 5 minutes. The suspension has been stirred periodically with a glass rod in order to promote maximum extraction of soluble

compounds of the sheets. At the end of the allotted time, the mixture got filtered by using the filter paper and then the filtrate kept in the fridge for biochemical analyses.

2.4. Phytochemical screening

For research of chemical compounds groups showing pharmacological interest, leaves powder extracts were tested for alkaloids, flavonoids, tannins and total phenolic compounds.

2.4.1. Test for the alkaloids

The portrayal of alkaloids was performed by technique for [20], utilizing the Dragendorff's reagent. In a container, dry dissipated 6 mL of the plant extricate. The buildup is taken up in 6 mL of ethanol at 60 ° and the alcoholic arrangement subsequently got is appropriated in two test tubes. In the main cylinder, two drops Dragendorff's reagent, was then added. The presence of an accelerate or orange tone demonstrates the alkaloids presence.

2.4.2. Test for polyphenols

The absolute polyphenols were featured by the response with ferric chloride, [20] technique. A 2 mL part of the plant remove was added with 2 drops of alcoholic arrangement of 2 % of the ferric chloride. The presence of a pretty much green or dull blackish-blue tone shows the polyphenolic compounds presence.

2.4.2.1. Test for tannins

Tannin got portrayed by technique for [21]. Around 5 mL of watery concentrates were vapored. 15 mL of reagent Stiany was then added to the dry buildup. The blend was then kept under the water shower at 80°C for minute. Then it got cooled under the running water. The perception of huge accelerates chips described catechin tannins. The arrangement containing the chips is separated and the gathered filtrate was then soaked with sodium acetic acid derivation. 3 drop of ferric chloride was then added to the blend. The appearances of dark blue-dark tone demonstrate the gallic tannins presence.

2.4.2.2. Test for flavonoids

Flavonoids can be characterized by Ferric chloride test according method of [22]**.** About 0.1g of *Lippia multiflora* leaf powder was get dissolved in 1ml of ethanol and 1 ml of 10% ferric chloride was the added. A solution of brown color with precipitate of dirty green color indicated presence of flavonoids.

2.4.2.3. Total phenolic determination

Complete phenolic compound were controlled by the Folin-Ciocalteau technique [23]. To 100µl of the examples, 500µl of Folin-Ciocalteau reagent and 5 ml of bidestilated water were added. Later 30 sec to 8 min, sodium carbonate solution of 1.5 ml (20% w/v) was added. The concentrates were represent 30 min at 40° C. The absorbance of the response combination was then estimated at 765 nm with an UV-Vis spectrometer (model-synstronics 2202). Gallic corrosive was utilized as standard and the outcomes were communicated as gallic corrosive counterparts (GAE) in milligrams per 100 gram of dry matter of plant material.

2.5. Quantitative determination of chemical constituency by High Performance Liquid Chromatography

The phenolic compound (catechin, tannins, flavanone, quercetin) and alkaloids include caffeine, were measured using High Performance Liquid Chromatography (HPLC). HPLC investigation was performed with Beckman HPLC with a Model 127 pump, 32 KARAT Software working framework and a Model 166 UV indicator. The phenolic builds were recognized at 280 nm with a stream pace of 1 ml/min. The segment was worked at 25°C temperature. Partitions were completed in a double pumping framework by differing the extent of 2.5% (v/v) acetic acid in water (versatile stage A) and 70% methanol in water (portable stage B). The dissolvable slope elution program was as per the following: 10% to 26% B (v/v) in 10 min, to 70% B at 20 min lastly to 90% B at 25 to 31 min. The infusion volume for all examples was 100 µL. The compounds of phenolic were examined by matching the maintenance time and their otherworldly attributes against those of norms.

2.6. Mineral composition of leaves and infusion

Mineral analysis: Minerals such as calcium, sodium, zinc, potassium, phosphorus, iron, and cooper were determined in leaves and infusion by Atomic Absorption spectrophotometer, according to standard methods of [24]. Measurements got performed in triplicate.

2.7. Data analysis

Data got expressed as the means ± of SD. The information were exposed to examination of difference a0nd means were isolated by Duncan various range of tests at $P < 0.05$ significant levels.

3. Results and discussion

3.1. Phytochemical screening of extracts of *L. multiflora* leaves

The phytochemical screening results are presented in table 2. We noted the presence of tannins, flavonoids and alkaloids in all of the extracts. Several authors had previously identified these components in the leaves extracts of *Lippia multiflora* [8-12]. All the extracts were characterized by the presence of condensed tannins. As for flavonoids, aqueous extracts from Bdk and Bmi were rich in flavone while those from Kgo contained flavanols.

Tannins in aqueous extracts contribute to its organoleptic quality because there are responsible of astringency and bitterness [25] of infusions. The presence of tannins in aqueous extracts of *Lippia multiflora* could explain uses of this plant in the treatment of skin infection [12]. Tannin is one of the phytochemicals in plants which had been reported to inhibit the growth of various bacteria and virus [26]. Condensed tannins have beneficial effect on vascular health and can suppress production of the peptide responsible for hardening arteries [27]. [2] reported that the majority of flavonoids identified in genus Lippia are flavones, frequently 6-hydroxylated flavones and methoxyflavone, which is in agreement with our result. Presence of flavone and flavanols in extracts contribute to their medicinal value because flavonoids are used in medicine as antimicrobial, anti-inflammatory and antioxidant agent [28]. Alkaloids has a pharmacological effect and are used in medicine as antioxidant [29].

Table 2: phenolic composition of the aqueous extract of *Lippia multiflora*

	Tannins		Flavonoids		Alkaloids
samples	presence	kinds	Presence	kinds	Presence
Bdk blmLM	+++	Condensed	+++	Flavone	+++
Bdk llmLM	+++	Condensed	+++	Flavone	+++
Bmi blmLM	+++	Condensed	+++	Flavone	+++
Bmi llmLM	+++	Condensed	+++	Flavone	+++
Kgo blmLM	+++	Condensed	+++	Flavanols	+++
Kgo llmLM	+++	Condensed	+++	Flavanols	+++

+ Positive – negative, Bdk: Bondoukou; Bmi: Beoumi; Kgo: Korhogo: blmLM: broadleaf morphotype of *Lippia multiflora*; llmLM: longleaf morphotype of *Lippia multiflora*

3.2. Total phenolics determination

The results concerning total phenolics (TP) are presented in figure 2. There is a significant variation ($p<0.05$) among samples according areas and varieties. The TP content varie from 112.5 to197.4 mg/100g DW. The amounts in this study are higher than those obtained with *Lippia chevalieri* by [30] who found 17.88 mEq/100mg extract. Results exhibit variation of TP content among areas and varieties. Highest levels were obtained in the longer leaves from Korhogo and the lowest levels were found in larger leaves from Bondoukou. This difference may be due to soils types, climatic conditions, plant variety which are factors that can strongly influence teas polyphenols [19]. Thus in shaded tea flushes the concentration of total phenolic are much lower.

Generally, *Lippia multiflora* is characterized by its highest amount of phenolic compounds which contributes to its various beneficial health effect such as antioxidant properties and anti-fatigue effect as reported by [31]. Indeed, phenolic compounds are herbal substances which possess free radical scavenge capacity, because of their hydroxyl groups [32]. Thus, extract from Korhogo must be more antioxidant than those from Béoumi and Bondoukou regarding his high levels of phenolic compounds.

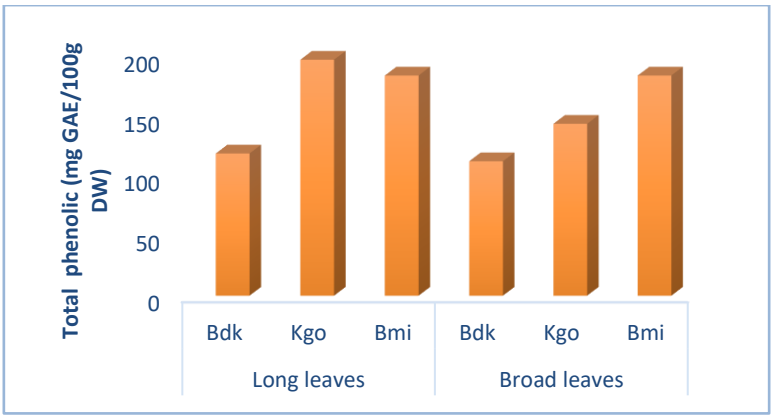

Fig. 2. Total phenolic levels in aqueous extracts two morphotypes of *Lippia multiflora* from different areas.

3.3. Quantitative determination by High Performance Liquid Chromatography (HPLC).

Data regarding, alkaloids (caffeine), tannins and flavonoids (catechin, flavanone, quercetin) content of *Lippia multiflora* extracts is depicted in table 3.

Caffeine content showed significant variation ($p<0.05$) among areas and varieties. Its levels were found in range of 14.59 to 40.05 mg.100g-1. The highest amount was obtained in broad leaves of Béoumi and the lowest quantity was obtained with leaves from Korhogo. The amounts obtained in our study are high than the results (2.34-4.33 mg.100g-1) obtained by [19] in green tea from *Camellia sinensis* leaves. This difference may be due to the previously non transformation of our samples, difference of analytical methods or plants species. However, difference in caffeine levels of extracts of *L. multiflora* from different areas in our study may be due to factors such as, plants varieties, soils chemistry and climate condition. Indeed, [33] reported that caffeine content in greens teas is affected by plant clone, stage of plucking, geographical locations. Whereas [34] noticed that caffeine levels can also varied signicantly with clones and location but not with season, while [14] reported in contrary highest level of caffeine during the dry season. Thus, the high caffeine amount in Beoumi leaves may be due to low soil water content in this region characterized by it low rainfall. The wealth of caffeine in extracts of *Lippia multiflora* contribute to their organoleptic qualities and their pharmacological and medicinal properties. Caffeine is the most important alkaloid in tea that keeps people awake [35] and contributes to the efficiency in treatment of some diseases, to other taste characteristic and regarded as an important parameter for commercial tea evaluation [36]. Presence of caffeine in *Lippia multiflora* extracts were previously reported by [37].

Catechins content depict also significant variations ($P<0.05$) among different locations (Table 3). Catechin content in *L. multiflora* ranged between 8.89-59.51 $mg.100g^{-1}$ with maximum amount (59.51 $mg.100g^{-1}$) observed in sample of Korhogo, while minimal amount was observed in leaves from Bondoukou containing 8.89 $mg.100g^{-1}$ and 6.21 $mg.100g^{-1}$ in long and broad leaves respectively. Korhogo is characterized by it low rainfall and high temperatures; this could explain the high catechin content in this locality unlike localities Bondoukou and Béoumi which are characterized by a humid climate and relatively elevated rainfall. Previously studies reported effects of light and humidity on levels of catechin in tea. [38] founded that the content and the composition of green tea catechins vary with climate and growth condition. Catechin contributes to the antioxidant and antimicrobial properties of infusion, because the correlation between their scavenging and antibacterial capacities and their ability to modulate membrane physical properties were founded by [39].

The levels of tannins varies significantly from 6.02 to 14.33 $mg.100g^{-1}$according plant origin. There amounts vary. The highest amounts were observed in the extracts from Korhogo, followed by those of Bondoukou. Levels obtained in this study are in agree with that obtained in *Lippia multiflora* but superior than those in *Lippia chevalieri* reported by [30]. Content of tannin are affected by various factors such as plants species; plant age, the growth site characteristics and season [40].

Quercetin and flavanone are both characterized by their low content in extracts of *Lippia multiflora*. Their concentration varied according variety and the highest level (13.33 $mg.100\ g^{-1}$) was obtained in broad leaves from Béoumi. Despite their lowest levels, it has been reported that many flavonoids, including quercetin, display anti-inflammatory and antimicrobial activities [41].

Table 3: photochemical analysis of *Lippia multiflora* leaves aqueous extract

Sample	Caffeine (mg.100g^{-1})	Catechins (mg.100 g^{-1})	Tannins (mg. 100 g^{-1})	Flavanone (mg. 100 g^{-1})	Quercetin (mg. 100 g^{-1})
***llmLM*Bdk**	20.14±0.05^{f}	08.89±0.05^{d}	11.95±0.09^{d}	06.11±0.15^{h}	0.23±0.01^{a}
***llmLM*Kgo**	14.59±0.03^{a}	59.51±0.10^{l}	14.33±0.28^{e}	04.02±0.55^{b}	1.06±0.01^{c}
***llmLM*Bmi**	19.07±0.01^{e}	19.07±0.02^{h}	06.02±0.50^{f}	04.25±0.15^{b}	0.25±0.33^{a}
***blmLM*Bdk**	18.57±0.03^{d}	06.21±0.01^{b}	12.15±0.31^{d}	05.75±0.05^{g}	0.73±0.01^{b}
***blmLM*Kgo**	14.59±0.02^{a}	37.97±0.01^{k}	07.16±0.06^{a}	01.95±0.11^{d}	0.74±0.01^{b}
***blmLM*Bmi**	40.05±0.01^{i}	12.94±0.02^{e}	14.26±0.09^{e}	13.33±0.08^{i}	0.95±0.01^{c}

Value with different superscript letters in columns are statistically significant ($p<0.05$)
Bdk: Bondoukou; Bmi: Béoumi; Kgo: Korhogo: *blmLM*: broadleaf morphotype of *Lippia multiflora*; *llmLM*: longleaf morphotype of *Lippia multiflora*

3.4. Mineral composition of *Lippia multiflora* leaves

The mineral contents of the leaves are given in Tables 4. A total of nine (9) elements were determined. Six of them in particular; Ca (8231.17-14981.17 mg.kg-1), K (2740.00-7949.87 mg.kg-1), Mg (3414.33-4731.00 mg.kg-1) and Na (1046.76-1206.33 mg.kg-1) and P (244.71 - 250.00 mg.Kg-1) had the highest concentration. Similar macroelement levels have been determined by [42] in popular consumed tea. Calcium was most abundant (8231.66–14981.17 mg.kg-1) in both samples while for Mg, K, and Na the order of magnitude varied among growing areas. [43] founded the highest amount of calcium (2100 mg, 100 g-1) in *Moringa oleifera* leaves. Iron (Fe) has average contents ranging from 62.50 to 200 mg. kg-1 when three trace elements Mn, Zn and Cu were present at the lowest concentrations. There are significant differences in mean content of most determined mineral in *Lippia multiflora* leaves according areas and varieties. These results are in agreement with findings of [44] who show variability among concentration of Fe, Zn, Mn and Cu of *Lippia multiflora* from 3 locations of Ghana. The variation of mineral levels may be due to factors such as soil chemistry and climate [46]. According to [46], the concentrations of element in soil are reflected in elemental contents of tea leaves.

This study showed that leaves of *Lippia multiflora* are a rich source of Ca, K, Mg and Na which can improve human health. Calcium can be valuable for the oxygen transport and cell movement [43], sodium and potassium are useful in the transmission of the impulse of nerve and balance of electrolyte, while magnesium is include in the digestive ingestion, different substance response and practically working of 90 proteins in the body [43].

A few helpful impacts have been credited to normal utilization of tea and incorporate; serum cholesterol decrease, diminished danger of malignant growth and cardiovascular infections [47]. These medical advantages have been ascribed to the solid cancer prevention agent action of catechins that secure the body against free radical initiated oxidative pressure [48].

Table: 4. Concentration (mg/Kg) of mineral in dry leaves of two varieties *Lippia multiflora* from different geographical areas.

Sample	Mineral elements								
	K	Na	Mg	Ca	P	Fe	Mn	Zn	cu
llmLMBdk	4396.67±0.200^{b}	1083.67±0.4^{b}	4247.67±5.00^{f}	10823.66±0.027^{i}	248,33±5,00^{abc}	142.00±0.03^{g}	38.67±0.80^{d}	41.33±0.05^{j}	20.67±0.0^{a}
llmLMKrg	7949.87±0.416^{i}	1190.33±0.40^{e}	4731.00±0.150^{j}	8796.17±0.500^{e}	248.33±5.00^{abc}	62.50±0.03^{b}	54,33±0.02^{e}	30.17±0.05^{f}	32.17±0.0^{k}
llmLMBmi	2740.00±0.200^{d}	1046.76±0.4^{a}	3414.33±0.150^{c}	8231.17±0.500^{d}	250.00±5.000^{abc}	187.02±0.01^{k}	21.67±0.02^{b}	39.00±0.03^{h}	16.00±0.0^{d}
blmLMBdk	2983.33±0.700^{e}	1347.00±0.40^{l}	4474.33±0.150^{g}	9793.73±0..058^{h}	248.60±0.232^{abc}	200.17±0.02^{l}	82.83±0.06^{i}	51.33±0.01^{a}	2533±0.0^{f}
blmLMKrg	6510.00±0.800^{h}	1162.00±0.40^{d}	4606.00±0.200^{i}	14981.17±0.500^{l}	244.71±40.474^{a}	176.33±0.03^{i}	89.63±0.08^{j}	43.17±0.02^{j}	31.67±0.0^{i}
blmLMBmi	2845.33±8.505^{a}	1205.33±0.40^{g}	4552.60±0.503^{h}	9074.00±0.577^{f}	246.67±0.400^{ab}	181.50±0.10^{j}	166.23±0.38^{k}	51.13±0.01^{a}	31.69±0.2^{i}

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

llmLMBdk: longleaf morphotype of Bondoukou; *llmLMBmi*: longleaf morphotype of Béoumi; *llmLMKgo*: longleaf morphotype of Korhogo; *blmLM*:Bdk: broadleaf morphotype of Bondoukou; *blmLM*:Bmi: broadleaf morphotype of Béoumi; *blmLM*:Kgo: broadleaf morphotype of Korhogo

4. Conclusion

Lippia multiflora is a rich source of phenolic compounds and mineral elements. Given this richness, tea made from the leaves of this plant may help improve health and treat certain nutritional ailments. However, significant differences were observed between morphotypes and culture areas. Thus, each sample from each location had its own phenolic and mineral profile, thus confirming the effect of cultivation areas on the phenolic and mineral composition of *Lippia multiflora*. This can be used as a marker indicating the origin of the leaves of their characteristics for the user of Lippia products. Results demonstrated also the ability of the Lippia multiflora plant to accumulate large amounts of essential minerals and highlight that the consumption of its leaf infusion may be a potential source of the essential nutrients.

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References

1. C.W. Fennell, K.L. Lindsey, L.J. McGaw, S.G. Sparg, G.I. Stafford, E.E. Elgorashi, O.M. Grace, J. van Staden. Assessing African medicinal plants for efficacy and safety: pharmacological screening and toxicology. J. Ethnopharmacol. 94 (2004) 205–217
2. M.E. Pascual, K. Slowing, E. Carretero, D. Sánchez Mata, A. Villar. *Lippia*: traditional uses, chemistry and pharmacology: a review. *J. Ethnopharmacol* 76 (2001) 201-214.
3. A. A. Abena, M. Diatewaa, G. Gakossoa, M. Gbeassorc, T.H. Hondi-Assaha, J.M. Ouamba. Analgesic, antipyretic and anti-inflammatory effects of essential oil of *Lippia multiflora*. Fitoterapia (2003) 74: 231–236.
4. K.E. Adou, A.S.P. N'guetta, A. Kouassi, C. Kanco, A. Yao-Kouamé, D.P. Sokouri, M.Y. Coulibaly. Caractérisation agro-morphologique et identification de quelques populations de *Lippia multiflora*, une verbénacée sauvage. J. Appl. Biosci. 37(2011) 2441-2452.
5. F.R. Irvine. Woody Plants of Ghana. Oxford Univ. Press Ltd., London. 1961, 863 pp.
6. B.K.Noamesi, G. Adebayo, S.D.A. Bamgbose. Muscle relaxant properties of aqueous extract of *Lippia multiflora*. Planta Med. 3 (1985) 253-255.
7. C. Kanko. Contribution à l'étude phytochimique de *Lippia multiflora* (Verbenaceae). Thèse de Doctorat 3ème cycle. Université de Cocody-Abidjan, (1995).
8. O. Kunle, J. Okogun, E. Egamana, E. Emojevwe, M. Shok. Antimicrobial activity of various extracts and carvacrol from *Lippia multiflora* extract. Phytomed. 10 (2003) 59-61.
9. T. Silou, J.M. Ouamba. Evolution of the content and the composition of the essential oils of *Lippia multiflora* according to the period of harvest. Rev. Méd. Pharm. Afr. 6 (2) (1993) 103-108.
10. F.A. Oladimeji, O.O. Orafidiya, T.A. Ogunniyi, T.A. Adewunmi. Pediculocidal and scabicidal properties of *Lippia multiflora* essential oil. J. Ethnopharmacol. 72 (2000) 305-311.
11. H. Agnaniet, T. Makani, A. Akagah, C. Menut, J.M. Bessière. Volatile constituents and antioxidant activity of essential oils from *Lippia multiflora* Mold. Growing in Gabon. Flavour Fragr. J. 20 (2005) 34-38.
12. D. Toyosi, M. Olu, EA Alamu. Preliminary antibacterial and phytochemical screening of three medicinal plants used in the folkloric treatment of skin infections against three bacteria. J Ethnobiol. Ethnopharmacol. 2 (1) (2013) 1-4.

13. G.N. Magoma, F. Wachira, M. Obanda. The use of catechins as biochemical marker in diversity studies of teas (camellia sinensis). Genet. Resour. Crop Evol. 47 (2) (2000) 107-114.

14. L. Cherotich, S.M. Kamunya, A. Alakonya, S.W. Msomba, M.A. Uwimana, J.K. Wanyoko, P.O. Owuor . Variation in catechin composition of popularly cultivated tea clones in east Africa (Kenya). Am. J. Plant. Sci. 4 (2013) 628-640.

15. S.A. Aherne, N.M. O'Brien. Dietary flavonols: chemistry, food content, and metabolism. Nutrition. 18 (1) (2002) 75– 81.

16. K.A. Alui, A. Yao-Kouamé, K.C. Ballo, K.P. Kouadio, K.A. N'guessan, K.Y. Nangah Comportement de deux morphotypes de *Lippia multiflora* (Verbenaceae) sur ferralsols de la région de Yamoussoukro, Côte d'Ivoire. J. Appl. Biosci. 8 (2011) 2592 – 2601.

17. P.O. Owuor, B.O. Kwach B.O. Quality and yields of black tea *Camellia sinensis* L.O. Kuntze in response to harvesting in Kenya: a review. Asian j. Biol. Life Sci. 1 (1) (2012) 1-7.

18. A. Yao-Kouamé, F. Kané. Biochemical characteristics of *Lippia multiflora* (Verbenaceae) leaves with respect to fertilizer applied to the soil. J. Plant Sci. 3 (4) (2008) 287-291.

19. M. Adnan, A. Ahmad, A. Ahmed, N. Khalid. Chemical composition and sensory evaluation of tea (camellia sinensis) commercialized in Pakistan. Pak. J. Bot. 45 (3) (2013) 901-907.

20. A.A. Obouayeba, M. Diarrassouba, E.F. Soumahin, T.H. Kouakou. Phytochemical analysis, purification and identification of hibiscus anthocyanins. J. Pharm. Chem. Biol. 3 (2) (2015) 156-168.

21. A.P. Bidie, B.B. N'Guessan, A.F. Yapo, J.D. N'Guessan, A.J. Djaman. Activité antioxydante de dix plantes medicinales de la pharmacopé ivoirienne. Sci. Nat. 8 (1) (2011) 1-11.

22. A. Sofowora. Medicinal plants and traditional medicine in Africa 3rd Ed. Spectrum Books Ltd; Nigeria. (1993) 150-153.

23. V.L. Singleton, R. Orthofer, R. Lamuela- Raventós. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteau reagent. Meth Enzymol. 299 (1999) 152-178.

24. Association of Analytical Chemists. Official Methods of Analysis. 17th ed., Arlington, Virginia. USA. (2000).

25. I. Spranger, B. Sun, A.M. Mateus, V. De Freitas, J. Ricardo-Da-Silva. Chemical characterization and antioxidant activities of oligomeric and polymeric procyanidin fractions from grape seeds. Food Chem. 108 (2008) 519–532.

26. T. Okuda, T. Yoshida, T. Hataio. Chemistry and biological activities of tannins in medicinal plants. Med Plants Res. 5 (1999) 29-165.

27. P.K. Ashok, K. Upadhyaya. Tannins are Astringent. J. Pharmacogn. Phytochem. 1 (3) (2012) 45-50.

28. A.D. Agrawal. Pharmacological Activities of Flavonoids: A review. Int. J. Pharm. Sci. Nanotech. 4 (2) (2011) 1394-1398.

29. J.U. Rehman, N. Akhtar, Y.M. Khan, K. Ahmad, M. Ahmad, S. Sultana, H.M. Asif. Phytochemical screening and hepatoprotective effect of *Alhagi maurorum* Boiss (Leguminosae) Against Paracetamol-Induced Hepatotoxicity in Rabbits. Trop. J. Pharm. Res. 14 (6) (2015) 1029-1034.

30. M.J. Bangou, M. Kiendrebeogo, M. Compaoré, A.Y. Coulibaly, N.T.R. Méda, N. Almaraz-Abarca, B. Zeba, J. Millogo-Rasolodimby, O.G. Nacoulma. Enzyme inhibition effect and polyphenolic content of medicinal plant extracts from Burkina Faso. J. Biol. Sci., 11 (1) (2011) 31-38.
31. F. Liudong, Z. Feng Z, S. Daoxing, QXiufang, F. Xiaolong, L. Haipeng. Evaluation of antioxidant properties and anti- fatigue effect of green tea polyphenols. Sci. Res. Essays. 6 (13) (2011) 2624-2629.
32. E. Miser-Salihoglu, G. Akaydin, E. Caliskan-Can, S. Yardim-Akaydin. Evaluation of antioxydant activity of various herbal lolk medecines. J. Nutr Food Sci. 3 (2013) 5-9.
33. S. Klegou. Le théier, *Camellia sinensis* : Données pharmacologiques récentes. Thèse de Pharmacie. Université Paris XI, Chatenay-Malabry, (2005).
34. B.O. Kwach, P.O. Owuor, D.M. Kamau, J.K. Wanyoko, S.M. Kamunya. Influence of localion of production, season and genotype on caffeine and flavan-3_ols in young green tea (*Camellia sinensis*) leaves in Kenya. J. Agri. Sci. Techn. 3 (2013) 557- 574.
35. Y. Zhang, L.V. Hao, Y. Sun, G. Wang, H. Tan. Study on Simultaneous Determination of potassium, sodium, phosphorous, sulfur, iron, manganese, copper, zinc, calcium, magnesium in Tea Infusion by Inductively Coupled Plasma Atomic Emission Spectrometry. Chem. Rapid Communications. 2, (2) (2014) 41-47.
36. S. Khokhar, and S.G.M. Magnusdottir. Total phenol, catechin and caffeine contents of teas commonly consumed in the United Kingdom. J. Agric. Food Chem. 50 (3) (2002) 565 570.
37. B.K. Noamesi. Power tea (*Lippia multiflora*) a potent hypertensive therapy. West African Journal of Pharmacology Drug Research, 4(1) (1977) 33-36.
38. D.S Wheeler, W.J. Wheeler. The medicinal chemistry of tea. Drug Develop. Res. 61 (2004) 45-65.
39. N. Caturlia, A. Evavera-Samper, M.J. Reyes, E. Mateo, V. Micol. The relationship between the antioxidant and the antibacterial properties of galloylated catechins and the structure of phospholipid model membranes. Free Radic. Biol. Med. 34 (6) (2003). 648-662.
40. H. Mehanso, L.G. Butler, D.M. Carlson. Dietary tannins and salivary proline rich proteins, interaction, induction and defense mechanism. Annu. Rev. Nutr. 7 (1987) 423-440.
41. J.R. Nijveld, E. Van-Nood, D.E.C. Van-Hoorn, P.G. Boelens, K. Van-Norren, P.A.M. Van-Leeuwen. Flavonoids: a review of probable mechanisms of action and potential applications 1-3. Am. J. Clin. Nutr. 74 (2001) 418-425.
42. A. Donkor, C. Kuranchiea, P. Osei-Fosua, S. Nyarkoa, L. Doamekpora.. Assessment of essential minerals and toxic trace metals in popularly consumed tea products in Ghana, a preliminary study. Res. J. Chem. Environ. Sci. 3 (1) (2015) 49-55.
43. M. Ilyas, M.U. Arshad, F. Saeed, M. Iqbal. Antioxidant potential and nutritional comparison of Moringa leaf and seed powder and their tea infusion. J. Plant Sci. 25 (1) (2015) 226-233.
44. K. Annan, A.I. Kojo, A. Cindy, A. Samuel, B.M. Tunkumgnen. Profil of heavy metals in some medicinal plants from Ghana commonly used as components of herbal formulation. Pharmacogn. Res. 2 (1) (2010) 41-44.
45. W.Y. Han, F.J. Zhao, Y.Z. Shi, L.F. Ma, J.Y. Ruan. Scale and causes of lead contamination in Chinese tea. Environ. Pollut. 139 (1) (2006) 125-132.
46. N. Jalbani, T.G. Kazi, M.K. Jamali, B.M. Arain, H.L. Afridi, A. Baloch. Evaluation of aluminum contents in different bakery foods by electrothermal atomic absorption spectrometer. J. Food Comp. Anal. 20 (2007) 226-231.

47. F.L. Chung, J. Schwartz, C.R. Herzog, Y.M. Yang. Tea and cancer prevention: Studies in animals and humans. J. Nutr. 133 (10) (2003) 3268-3274.
48. F. Pourmorad, S.J. Hosseinimehr, N. Shahabimajd. Antioxidant activity, phenol and flavonoid contents of some selected Iranian medicinal plants. Afr. J. Biot. 5 (11) (2006) 1142-1145.