



Biochemical Composition and Therapeutic Values of Leaves of *Lippia Multiflora* Moldenke Grown in Côte D'Ivoire



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Abstract

Phytochemical analysis of leaves from two varieties of *Lippia multiflora* cultivated in two localities of Côte d'Ivoire, such as Béoumi and Korhogo, were carried out to provide information on the therapeutic value of this herbal tea. Results showed the 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 Béoumi, while the highest levels of flavanols were found in extracts from Korhogo. Essential minerals like calcium (Ca), sodium (Na), magnesium (Mg), potassium (K), phosphorus (P), manganese (Mn), zinc (Zn), copper (Cu), and iron (Fe) were present in leaves and extracts. In addition, the levels of both of these minerals in the extract were below the maximum level permissible suggested by the World Health Organization (1989). We also noted the variability of both components according to the cultivation areas. The presence of phytochemical components and mineral elements in the aqueous extracts of *Lippia multiflora* contributes to their therapeutic application in medicinal practices and their nutritional importance. However, the variability among the phytochemical profiles of samples from different areas expresses their different medicinal, pharmacologic, and nutritional properties.

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1 Introduction

Traditional medicine has been the dominant healthcare system in Africa. For people living in rural areas, traditional medicine is easily available, accessible, and affordable (Edet et al., 2019). Although a very small number of medicinal plants are cultivated and their biochemical compositions elucidated, the majority remain in the wild, and knowledge of their compositions in bioactive compounds is insufficient. Among these plants is *Lippia multiflora*, which belongs to the Verbenaceae family, comprising some 200 species of herbs and shrubs (Pascual et al., 2001). In Africa, *Lippia multiflora* is found in Ghana, Senegal, and Côte d'Ivoire, where it is commonly known as “Bush Tea” or “Tea of Gambia” (Abena et al., 2003). The *Lippia multiflora* plant adapts well to different soil types and agroecological zones. In Côte d'Ivoire, the plant is found in the central, north-eastern and north-western regions, where infusions and decoctions of the leaves are traditionally consumed as a tea to treat illnesses such as fever, coughs and influenza (Irvine, 1961), malaria (Valentin et al., 1995) and anxiety disorders (Ngaibi et al., 2021). They are also used as a disinfectant, antipyretic, and diuretic (Kanko et al., 2004), and as a sudorific, febrifuge, laxative, and colic treatment (Kunle et al., 2003). *Lippia multiflora* leaves are also known for their pesticidal properties (Wangrawa et al., 2024), their muscle relaxant effect and their analgesic, sedative, hypertensive, and diuretic properties (Abena et al., 2003). Studies on essential oils have demonstrated their antimicrobial (Kunle et al., 2003), pediculicidal and scabidicidal (Oladimeji et al., 2000), antioxidant (Agnaniet et al., 2005), antibacterial and antifungal, antipyretic and anti-inflammatory properties (Abena et al., 2003). 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 (Kunle et al., 2003; Toyosi et al., 2013). 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 (Magoma et al., 2000). In addition, Cherotich et al. (2013), 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. In addition, the chemical composition of tea varies depending on climate, season, agricultural practices, variety, age, and position of the leaf (Aherne & O'Brien, 2002).

Thus, an evaluation of the biochemical composition of the leaves of *Lippia multiflora* according to the growing areas could provide information on their quality and management strategies that could improve their cultivation. This study aimed to determine the quality and quantities of polyphenolic compounds and minerals in the leaves and infusion of cultivated *Lippia multiflora* and study the variability of this composition according to the cultivation areas.

2 Materials and Methods

Description of the study sites

The study was carried out in two localities (Béoumi and Korhogo), whose environmental characteristics are presented in Table 1.

Table 1
Characteristics of the study sites

Environnemental parameters	Cultivation localities	
	Korhogo	Béoumi
Latitude	9°23'23"N	7°42'28"N

Environnemental parameters	Cultivation localities	
	Korhogo	Béoumi
Longitude	5°48'490	5°35'50"O
Annual rainfall (mm)	1000-1200	1200
Annual average temperature (°C)	26	25.4
Climate	Soudanais	Baouléen
Relative Humidity (%)	61.5	75
Natural Vegetation	Shrubby savannah	Tree savana
Soil type	Cambisols	Ferralitique revamped

Plant material

Leaves were collected from two morphotypes of *Lippia multiflora* distinguished by leaf shape (Figure 1), as identified by [Alphonse et al. \(2011\)](#). Leaves were harvested in September, before flowering.



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

Collection method and aqueous extract preparation

Harvested method

Leaves were harvested using the fine-picking method as described by [Owuor & Kwach \(2012\)](#), which involves sampling the bud and the two leaves below. Samples were dried in the shade for a week, pulverized using a Blender FAR BL514X CI and the resulting powders stored in sealed plastic boxes for biochemical analysis.

Aqueous extract preparation

Leaf extracts were prepared according to the method of [\(Adnan et al., 2013\)](#). For each sample, 5 g of powder was weighed into a 500 mL beaker using a Mettler Type A 100 precision balance, then 250 mL of boiled water was added. The suspension was infused for exactly 5 minutes and stirred periodically with a glass rod to promote maximum extraction of soluble compounds. At the end of the allotted time, the extract was filtered through filter paper and the filtrate kept refrigerated for biochemical analysis.

Phytochemical screening

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

Test for alkaloids

The characterization of alkaloids was performed by method of [Obouayeba \(2015\)](#), using the Dragendorff's reagent. In a capsule, dry evaporated 6 mL of the plant extract. The residue is taken up in 6 mL of ethanol at 60 ° and the alcoholic solution thus obtained is distributed in two test tubes. In the first tube, two drops Dragendorff's reagent (Potassium Bismuth Iodide) was added. The appearance of a precipitate or orange color indicates the presence of alkaloids.

Test for polyphenols

The total polyphenols were highlighted by the reaction with ferric chloride. A volume of 2 mL of the leaves extract was added two drops of alcoholic solution of 2 % ferric chloride. The appearance of a more or less dark blackish-blue or green color indicates the presence of polyphenolic compounds.

Test for tannins

Tannins were characterized by the method of [Bidie et al. \(2011\)](#). Exactly 5 mL of aqueous extracts were vaporized and 15 mL of Stiany reagent (10 mL 40% formalin added to 5 mL concentrated HCl) added to the dry residue. The mixture was kept in a water bath at 80°C for one minute, then cooled under running water. The observation of large precipitated flakes characterizes catechin tannins. The solution containing the flakes is filtered and the filtrate collected is then saturated with sodium acetate. Three (3) drops of 2 % ferric chloride are added to the mixture. The appearance of a deep blue-black color indicates the presence of gallic tannins.

Test for flavonoids

The ferric chloride test characterized flavonoids according to the method of [Sofowora \(1993\)](#). 0.1 g of leaf powder was dissolved in 1 mL of ethanol, and then 1 mL of 10% ferric chloride was added. A brown solution with a dirty green precipitate indicates the presence of flavonoids.

Total phenolic determination

Total phenolic compounds were determined by the Folin-Ciocalteau method of [Singleton et al. \(1999\)](#). To 100 µL of the samples, 5 mL of bidistilled water and 500 µL of Folin-Ciocalteau reagent were added. After 30 seconds to 8 minutes, 1.5 mL of sodium carbonate (20 % w/v) was added. The extracts were incubated for 30 min at 40 °C. The absorbance of the reaction mixture was then measured at 765 nm with a UV-Vis spectrometer (model-synstronics 2202). Gallic acid was used as a standard, and the results were expressed as gallic acid equivalents (GAE) in milligrams per 100 grams of dry matter of plant material.

Quantitative determination of chemical constituency by High Performance Liquid Chromatography

The phenolic compounds (catechin, tannins, flavanone, quercetin) and caffeine content were measured using HPLC. This analysis was performed with a Beckman HPLC with a Model 127 pump, a Model 166 UV detector, and 32 KARAT Software operating system. The phenolic compounds were detected at 280 nm with a flow rate of 1 ml/min. The column was operated at a temperature of 25 °C. Separations were carried out in a dual pumping system by varying the proportion of 2.5 % (v/v) acetic acid in water (mobile phase A) and 70 % methanol in water (mobile phase B). The solvent gradient elution program was as follows: 10 % to 26 % B (v/v) in 10 min, to 70 % B at 20 min, and finally to 90 % B at 25 to 31 min. The injection volume for all samples was

100 μ L. The phenolic compounds were analyzed by matching the retention time and their spectral characteristics against those of standards.

Mineral composition of leaves

Minerals such as calcium, magnesium, sodium, potassium, manganese, phosphorus, iron, zinc, and copper were determined in leaves by Atomic Absorption spectrophotometer, according to standard methods of [AOAC \(2000\)](#).

Data analysis

Data were expressed as means $\pm$ 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.

3 Results and Discussions

Phytochemical screening of extracts

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 leaf extracts of *Lippia multiflora* ([Toyosi et al., 2013](#); [Germame & Mekuria, 2021](#)). All the extracts were characterized by the presence of condensed tannins. As for flavonoids, aqueous extracts from Bmi were rich in flavone while those from Korhogo contained flavanols. Tannins are responsible for the astringency and bitterness of aqueous extracts ([Ma et al., 2014](#)). Their presence in *Lippia multiflora* leaf extracts may explain the use of this plant in the treatment of skin infections ([Toyosi et al., 2013](#)). Tannins are phytochemical compounds that inhibit the growth of various bacteria and viruses ([Okuda et al., 2011](#)). Condensed tannins have a beneficial effect on vascular health and can suppress the production of the peptide responsible for hardening of the arteries ([Ashok & Upadhyaya, 2012](#)). Flavonoids identified in the *Lippia* genus are predominantly flavones, often 6-hydroxylated flavones and methoxyflavones ([Pascual et al., 2001](#)), which is in agreement with our results. The presence of flavones and flavanols in extracts contributes to their medicinal value, as flavonoids are used medicinally as antimicrobial, anti-inflammatory, and antioxidant agents ([Agrawal, 2011](#)). Alkaloids have a pharmacological effect and are used medicinally as antioxidants ([Rehman et al., 2015](#)).

Table 2
Phenolic composition of the aqueous extract of *Lippia multiflora*

Samples	Tannins		Flavonoids		Alkaloid
	Presence	Kinds	Presence	Kinds	Presence
BmibLm	+	Condensed	+	Flavone	+
BmilLm	+	Condensed	+	Flavone	+
KrgbLm	+	Condensed	+	Flavanols	+
KrglLm	+	Condensed	+	Flavanols	+

BmibLm: broad leaf morphotype of Beoumi; **BmilLm**: long leaf morphotype of Beoumi;
KrgbLm: broad leaf morphotype of Korhogo; **KrglLm**: long leaf morphotype of Korhogo;
 Key + Means presence - Means not observed

Quantitative determination by High Performance Liquid Chromatography (HPLC)

Data regarding caffeine, tannins, catechin, flavanone, and quercetin content in leaves aqueous extract of *Lippia multiflora* is depicted in Table 3. Caffeine content showed significant variation ($p < 0.05$) among areas and varieties. Its levels were found in a range of 14.59 to 40.05 mg.100g⁻¹. The highest amount was obtained in the

broad leaves of Béoumi. The amounts obtained in our study are higher than the results (2.34-4.33 mg.100g⁻¹) obtained by [Adnan et al. \(2013\)](#) in green tea from *Camellia sinensis* leaves. This difference may be due to the previously non-transformation of our samples, or a difference in plant species. However, differences in caffeine levels of extracts of leaves from different areas may be due to factors such as plant varieties, soil chemistry, and climate conditions. Indeed, [Mutuku et al. \(2016\)](#) reported that caffeine and catechins contents in green teas are affected by plant clone, stage of plucking, and geographical locations. Whereas [Cherotich et al. \(2013\)](#), reported 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 its low rainfall. The wealth of caffeine in extracts of *Lippia multiflora* contributes to their organoleptic qualities and their pharmacological and medicinal properties. Caffeine exhibits neuroprotective properties, potentially serving as a preventive measure against the onset of neurodegenerative conditions such as Alzheimer's and Parkinson's disease ([Reddy et al., 2024](#)). This compound is the most important alkaloid in tea that keeps people awake ([Rajput et al., 2022](#)).

Catechins content also depicts 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 a maximum amount (59.51 mg.100g⁻¹) observed in a sample of Korhogo. This locality is characterized by its low rainfall and high temperatures; this could explain the high catechin content in this locality, unlike Béoumi which is characterized by a humid climate and relatively elevated rainfall. Previously, studies reported effects of light and humidity on levels of catechin in tea. [Wheeler & Wheeler \(2004\)](#), found that the content and composition of green tea catechins vary with climate and growth conditions. Catechin in tea extract contributes to its antioxidant, antibacterial, antifungal, antidiabetic, anti-inflammatory, antiproliferative, and antitumor properties ([Cosarca et al., 2019](#)). It possesses anti-hypertensive, antiobesity, anticarcinogenic, cardioprotective properties ([Swarnalatha et al., 2021](#)).

The levels of tannins vary significantly from 6.02 to 14.33 mg.100g⁻¹ according plant origin. The highest amounts were observed in the extracts from Korhogo. Levels obtained in this study are in agreement with those obtained in *Lippia multiflora* but are superior to those in *Lippia chevalieri* reported by [Bangou et al. \(2011\)](#). Content of tannins is affected by various factors such as plant species, plant age, the growth site characteristics, and season ([Mehanso et al., 1987](#)).

Tannins are used in the treatment of skin inflammation, injuries and may prevent the inception of many chronic diseases. On the other hand, internally tannin compounds may produce effects on the gastrointestinal tract including antioxidant, free radical scavenging, antimicrobial, antiviral, anti-mutagenic, anti-carcinogen, anthelmintic, hepatoprotective effects, an inhibitor of the harmful pro-oxidative enzyme, and anti-nutrient effects ([Hossain et al., 2021](#)).

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 ([Lesjak et al., 2018](#)).

Table 3
Photochemical analysis of *Lippia multiflora* leaves aqueous extract

Samples	Caffeine (mg.100g ⁻¹)	Catechins (mg.100 g ⁻¹)	Tannins (mg. 100 g ⁻¹)	Flavanone (mg. 100 g ⁻¹)	Quercetin (mg. 100 g ⁻¹)
KrglLm	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
BmilLm	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
KrgbLm	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
BmibLm	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 ($p<0.05$)
BmibLm: broad leaf morphotype of Beoumi; *BmilLm*: long leaf morphotype of Beoumi;
KrgbLm: broad leaf morphotype of Korhogo; *KrglLm*: long leaf morphotype of Korhogo

Mineral composition of Lippia multiflora leaves

The mineral contents of the leaves are given in Table 4. A total of nine 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 [Donkora et al. \(2015\)](#), in popularly 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. [Ilyas et al. \(2015\)](#), found 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 the mean content of the most determined mineral in *Lippia multiflora* leaves according to areas and varieties. These results are in agreement with the findings of [Annan et al. \(2010\)](#), who show variability among the concentrations of Fe, Zn, Mn, and Cu of *Lippia multiflora* from three locations in Ghana.

The richness of *Lippia multiflora* leaves in macro and trace elements augurs their nutritional and therapeutic values. Calcium can be useful for the transport of oxygen and cellular activity ([Ilyas et al., 2015](#)), sodium and potassium are helpful in the transmission of nerve impulses and electrolyte balance, while magnesium is involved in the intestinal absorption, various chemical reaction and almost functioning of 90 enzymes in the body ([Ilyas et al., 2015](#)). Trace elements are necessary for the normal development and functioning of humans. Zinc is an essential trace element that functions as a cofactor for certain enzymes involved in metabolism and cell growth. it is found in nearly 300 specific enzymes ([Plum et al., 2010](#); [Osredkar et al., 2011](#)). As a component of many enzymes, Zn is involved in the metabolism of proteins, carbohydrates, lipids, and energy ([Al-Fartusie & Mohssan, 2017](#)). Copper is essential to all living organisms as a trace dietary mineral because it is a key constituent of the respiratory enzyme complex. Copper is a constituent of the blood pigment hemocyanin, which is replaced by the iron-complexed haemoglobin ([Olafisoye et al., 2017](#)).

Iron is an essential component needed to develop antioxidants to fight reactive oxygen species (ROS). It's also needed to generate the microbiocidal hypochlorite acids, where iron deficiency has been observed to reduce average concentrations of blood T cells in blood bloodstream ([Shah et al., 2019](#)). Manganese is a mineral that is used by the body as an enzyme cofactor in glucose metabolism ([Zeece, 2020](#)). It is also one of the important heavy metals essential for normal growth and metabolism ([Lahhob et al., 2023](#)).

Tabla 4
Concentration (mg/Kg) of mineral in leaves of two varieties *L. multiflora* from different geographical areas.

Minéraux (mg.kg ⁻¹)	Zones de culture			
	Béoumi		Korhogo	
	<i>MfLa</i>	<i>MfLg</i>	<i>MfLag</i>	<i>MfLg</i>
K	2740,00±0,2 ^d	2740,00±0,2 ^d	6510,00±0,8 ^h	7949,87±0,4 ⁱ
Na	1205,33±0,4 ^g	1046,76±0,4 ^a	1162,00±0,4 ^d	1190,33±0,4 ^e
Mg	4552,60±0,5 ^h	3414,33±0,1 ^c	4606,00±0,2 ⁱ	4731,00±0,5 ^j
Ca	9074,00±0,6 ^f	8231,17±0,5 ^d	14981,17±0,5 ^l	8796,17±0,5 ^e
P	246,67±0,4 ^{ab}	250,00±5,0 ^{abc}	244,71±4,5 ^a	248,33±5,0 ^{abc}
Fe	181,50±0,1 ^j	187,02±0,0 ^k	176,33±0,0 ⁱ	62,50±0,0 ^b
Mn	166,23±0,4 ^k	21,67±0,0 ^b	89,63±0,1 ^j	54,33±0,0 ^e
Zn	51,13±0,0 ^a	39,00±0,0 ^h	43,17±0,0 ⁱ	30,17±0,1 ^f
Cu	31,69±0,2 ⁱ	16,00±0,0 ^d	31,67±0,0 ⁱ	32,17±0,0 ^k

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

llmLMBmi: longleaf morphotype of Béoumi; *llmLMKgo*: longleaf morphotype of Korhogo;

blmLM:Bmi: broadleaf morphotype of Béoumi; *blmLM*:Kgo: broadleaf morphotype of Korhogo

4 Conclusion

Lippia multiflora leaves are rich in bioactive compounds and minerals essential for human health. Because of this richness, consuming infusions and decoctions of the plant's leaves can help treat many pathologies and mineral deficiencies. However, the study showed that each sample had its biochemical profile, depending on the area of cultivation and plant variety. This specificity of leaf composition could explain their different uses in different regions of Côte d'Ivoire, and could be used as a geographical indicator for users of teas made from *Lippia multiflora* leaves, like those made from *Camellia sinensis*.

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