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Biochemical composition and sensory characteristics of infusions of leaves from two morphotypes of *Lippia multiflora* (verbenaceae) grown in Côte d'Ivoire

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ABSTRACT

Objectives: this study aims to compare composition and sensorial characteristics of infusions of leaves from broad leave morphotype (blmLM) and long leave morphotype (llmLM) of *Lippia multiflora* (Savannah tea) cultivated in three localities, in order to identify the morphotype and/or locality, which offer the better biochemical composition and organoleptic qualities of tea.

Methodology and results: Leaves harvested were dried and pulverized. Infusions were prepared using standard methods and sensorialy analysed by Mossion methods. Elements were determined according to AOAC methods. Phenolics compounds and caffeine was analysed by a standard method. Infusions of blmLM are rich in tannins (12.15-14.26 mg/L), quercetin (0.33-0.95 mg/L) and flavanone (05.75-13.33 mg/L), while those of llmLM are rich in caffeine (19.07-20.14 mg/L) and catechin (08.89-59.56 mg/L). Calcium (50.96-51.54 mg/L) and magnesium (35.10-37.08 mg/L) contents are highest in infusions of BlmLM, while llmLM infusions are richer in K (97.78-103.46 mg/L) and Na (143.01-156.03 mg/L). Globally, infusions are brown to greenish yellow, astringent, slightly bitter with a pleasant smell. Infusions of Béoumi and Korhogo have a mint scent; those of Béoumi have a scent of lemongrass.

Conclusion and Application of results: The qualities of infusions depend on the morphotype and the growing area. Their richness in phenolic compounds, caffeine and essential minerals gives them nutritional, medicinal and pharmacological properties and can be a replacement for ordinary teas sold on the market. This study provides information on the choice of the plant morphotype and the cultivation area according to the needs of the consumer. Consumption of infusions of leaves from Korhogo and Béoumi that are richer in tannins, catechin, quercetin and essential minerals (calcium, magnesium and iron) will be beneficial for human nutrition, bones strengthening and prevention of cardiovascular diseases which represent a public health problem. Taking *Lippia multiflora* into account in the agricultural and eating habits of farmers will constitute for them a source of income and also a source of permanent raw material for the agro-food processing industries.

Keywords: *Lippia multiflora*, leaves infusion, biochemical composition, sensory characteristics

INTRODUCTION

Tea is an infusion of *Camellia sinensis* leaves, which is the most consumed beverage in the world after water because of its medicinal and nutritional properties (Senthilkumar *et al.,* 2015). However, apart from *Camellia sinensis*, several other plants such as *Cymbopogon citratus* and *Lippia multiflora* are used as tea because of their pleasant odour. *Lippia multiflora* belonged to the verbenaceae that is a large family with about 70 to 80 genera and over 3000 species. It is a perennial and aromatic shrub mainly distributed in tropical Africa, South America and Central America (Kunle & Egharevba, 2012). In Côte d'Ivoire, it grows in the center, north and north-east (Hien *et al*., 2012). Thus, because of this adaptability to different ecosystems and different soils types, *Lippia multiflora* presents morphological variability called "morphotypes ". Alui *et al.* (2011) through their studies on the agronomic characteristics of different morphotypes of the plant, revealed two main morphotypes: plants with oblong, rigid, long, dark green leaves and plants with elliptical, shiny leaves, short, light green in colour. From a chemical point of view, the essential oils of *Lippia multiflora* have been largely characterized. Several authors indicated that their chemical composition is influenced by plant origin and genetic factors (Mann, 2014). However, literature on other biochemical components of leaves, such as alkaloids, polyphenols (flavonoids, tannins and catechins) and minerals, is very rare. This study aims to compare caffeine, phenolic and mineral composition of the leaves of two morphotypes: long leaves morphotypes (*llm*LM) and broad leaves morphotype (*blm*LM) of *Lippia multiflora* cultivated in Côte d'Ivoire as well as their infusions. The objective is to identify the morphotypes and localities, which offer leaves of better organoleptic qualities for the preparation of tea.

MATERIAL AND METHODS

Characteristic of the different study sites: The study was carried out in three localities of Côte d'Ivoire, which are Béoumi (Center), Bondoukou (East) and Korhogo (North), chosen on the basis of their differences from pedoclimatic point of view (table 1).

Table 1. Natural characteristics of the study sites

Characteristics	Localities of culture		
	Béoumi	Bondoukou	Korhogo
Latitude **longitude**	7°42'28''N 5°35'50''O	8°06'43''N 2°42'23''O	9°23'23''N 5°48 ''49O
Annual rainfall (mm)	1200	1100 à 1700	1000 à 1200
Annual average temperature (°C)	25,4	22 à 27	26
Climate	Baouléen	Baouléen	Soudanais
Relative humidity (%)	75	80	61,5
Natural vegetation	Tree savannah	Tree savannah	Shrubby savannah
Soil types	Ferralitique revamped	Cambisols	Cambisols

Plan material and experimental device of plots: The plants were grown in the three agro-ecological zones described above (Table 1). The experimental design was a complete random block with two replicates for each morphotype. The 400m^2 plot at each test site was subdivided into 6 sub-plots measuring 54 m^2 (9 mx 6 m) containing 45 feet of plant, maintained by regular weeding.

Sampling: Leaves were harvested according to the fine picking method as described by Owuor & Kwach (2012). Leaf sample were dried in the open air out of the sun for a week and pulverized using a Blender FAR BL514X Cl mixer to obtain a fine dry powder that was stored in sealed plastic boxes.

Method of preparing the infusion: Leaves samples (5 g) were infused with 250 mL freshly boiled deionized water for five minute. The resulting solution was filtered through filter paper (Whattman No. 42) and then poured into 250 mL tea tasting porcelain bowl for quality assessment (Adnan *et al*, 2013).

Mineral composition analysis: Minerals in infusions such as calcium, magnesium, sodium, potassium, phosphorus, copper, manganese, iron and zinc were determined by Atomic Absorption spectrophotometer, according to standard methods of AOAC (2000).

Analysis of caffeine and phenolic compounds by HPLC: Phenolic compounds and caffeine was performed according to method of Donovan *et al.* (1998). The sample and standard solutions were filtered through Whattman paper 0.45 µm, and then through millipore membrane 0.45 µm (CARL ROTH, Karlsruhe, Allemagne). The equipment used is a HPLC (Shimadzu, France) system provided with a binary pump (LC-20A) coupled with a UV-VIS detector (SPD-20A). The used column (Thero, Runcom, England) for this analysis was Hypersil ODS type C18, 250 mm x 4.6 mm, 5µm (Thero, Runcom, England). The separation was carried out in elution gradient. The used mobile phase consisted of 50 mM NH4H2PO4 at pH 2.6 (solvent A), acetonitrile solution/ NaH2PO4 (80:20, V/V) (solvent B) and 200 mM o-phosphoric acid at pH 1.5 (Solvent C). The flow rate was 0.5 mL / min. Elution profile was as follows: 100% A for 0-5 min, 92% A/8% B for 5-8 min; 14% B/86% C for 8-20 min; 16.5% B/83.5% C for 20-25min; 21.5% B/78.5% C for 25-35 min, 50% B/50% C for 35-70 min and 100% A for 70-75 min. Each peak detected in the sample solutions was identified by comparing the retention time with chromatogram of reference substance.

Sensory evaluation of infusions: Sensory evaluation were realised at the Laboratory of Biocatalysis and Bioprocesses of Nangui Abrogoua University. Quantitative Descriptive Analysis was conducted by 12 trained judges to determine aroma, appearance, flavour and overall acceptability of infusions. Those parameters was analysed by the method of Mossion (2007), by assigning a score of between 0 and 20 for each descriptor, according to the sensitivities of panellists. Zero corresponds to little and 20 correspond to a lot. Products as references were also identified for each characteristic (Table 2). Panellists received three-digit randomized coded samples in a randomized and monadic order.

Table 2: References products offered for the different descriptors

Odour	**References**
Mint	Spearmint leaves
Lemongrass	Lemongrass leaves
Sweet	Vanilla powder
Fresh grass	Fresh cut grass
citrus	Lemon fruit
Appearance	**References**
Browning	Coffee solution
Yellow colour	Colour disc
Flavour	**References**
Bitterness	Caffeine solution
Acid	Lemon juice (citric acid)
Astringency	Green plantain banana

Statistical analyses of data: All analyses were performed in triplicate. The results were expressed as the mean ± standard deviation. Analysis of variance (ANOVA) was performed to test the cultivation area on biochemical composition. The means were compared using Duncan's test (P≤0.05), using STATISTICA 7.1 software.

RESULTS

Mineral composition: The infusions contain all the analysed minerals (table 3). Sodium (16.29 to 156.03 mg/L) is the most abundant mineral in infusions, followed by potassium (80.50-103.46 mg/L), calcium (39.76-54.04 mg/L) and magnesium (15.91-37.08 mg/L). Phosphorus and the weakest macro-element present in the infusions with a content varying from 3.09 to 3.77 mg/L. For the macro-elements (Ca, K, Mg, Na), a significant difference is observed (P ≤ 0.05) between the two morphotypes for each locality. Contents of copper, iron, zinc and manganese are less than 0.05 mg/L in infusions.

Table 3: Mineral composition of infusions of leaves from llmLM and blmLM of *Lippia multiflora* from three agro-ecological zones (mg/L)

Localities	Bondoukou		Korhogo		Beoumi	
Parameter	llmLM	blmLM	llmLM	blmLM	llmLM	blmLM
Ca	44.96±0.76^{b}	50.96±3.11^{c}	50.10±3.06^{c}	51.84±3.00^{c}	54.04±0.60^{c}	39.76±2.66^{a}
K	81.99±0.03^{c}	87.31±3.12^{cd}	97.78±1.46^{c}	91.50±6.89^{c}	103.46±3.91^{d}	80.50±1.75^{c}
Mg	27.41±3.26^{bd}	37.08±6.46^{c}	34.33±4.07^{cd}	35.10±4.43^{cd}	18.80±6.51^{ab}	15.91±4.91^{a}
Na	143.01±8.30^{bd}	127.81±1.87^{b}	156.03±4.84^{d}	124.84±7.08^{b}	116.29±1.57^{a}	136.46±1.57^{c}
P	3.09±0.18^{b}	3.41±0.22^{b}	3.46±0.04^{b}	3.29±0.12^{b}	3.77±0.13^{b}	3.29±0.08^{b}
Cu	0.002±0.00^{a}	0.014±0.01^{a}	0.090±0.11^{a}	0.017±0.00^{a}	0.072±0.09^{a}	0.022±0.00^{a}
Fe	0.003±0.00^{a}	0.0003±0.00^{a}	0.001±0/00^{a}	0.010±0.00^{ab}	0.001±0.00^{a}	0.002±0.00^{a}
Mn	0.13±0.01^{a}	0.19±0.01^{a}	0.27±0.05^{b}	0.21±0.01^{b}	0.17±0.00^{a}	0.15±0.00^{a}
Zn	0.13±0.17^{a}	0.09±0.01^{a}	0.28±0.23^{a}	0.32±0.27^{a}	0.75±0.15^{a}	0.03±0.00^{a}

Each value is an average of three replicate. Values are mean ± standard deviation. Means not sharing a similar letter in a line are significantly different p = 0.05 as assessed by the test of Duncan. llmLM: longleaf morphotype of *Lippia multiflora*; blmLM: broadleaf morphotype og *Lippia multiflora*

Contents of caffeine and phenolics compound in infusions: Caffeine and phenolic compounds contents are depicted in table 4. Results showed significant variability ($p<0.05$) according to the morphotypes and agro-ecological zones. Caffeine content varies from 14.59 to 40.05 mg/L, the highest level (40.05 mg/L) was observed with blmLM from Beoumi. llmLM from Korhogo showed the highest amount of catechin (59.51 mg/L), tannins (14.33 mg/L), and quercetin (1.06 mg/L), while the highest amount of flavanone (13.33 mg/L) were found in blmLM from Korhogo.

Table 4: Caffeine and phenolics contents of infusions from llmLM and blmLM of *Lippia multiflora* from three agro-ecological zones (mg/L)

Parameter	**Bondoukou**		**Korhogo**		**Beoumi**	
	llmLM	blmLM	llmLM	blmLM	llmLM	blmLM
Caffeine	20.14±0.05^{e}	18.57±0.03^{b}	14.59±0.03^{a}	14.59±0.02^{a}	19.07±0.01^{d}	40.05±0.01^{d}
Catechin	08.89±0.05^{b}	06.21±0.01^{b}	59.51±0.10^{f}	37.97±0.01^{f}	19.07±0.02^{d}	12.94±0.02^{c}
Tannins	11.95±0.09^{e}	12.15±0.31^{d}	14.33±0.28^{f}	07.16±0.06^{a}	06.02±0.50^{a}	14.26±0.09^{e}
Flavanone	06.11±0.15^{e}	05.75±0.05^{c}	04.02±0.55b^{c}	01.95±0.11^{a}	04.25±0.15^{cd}	13.33±0.08^{e}
Quercetin	0.23±0.01^{a}	0.73±0.01^{b}	1.06±0.01^{c}	0.74±0.01^{b}	0.25±0.33^{a}	0.95±0.01^{c}

Each value is an average of three replicate. Values are mean ± standard deviation. Means not sharing a similar letter in a line are significantly different p = 0.05 as assessed by the test of Duncan. llmLM: longleaf morphotype of *Lippia multiflora*; blmLM: broadleaf morphotype og *Lippia multiflora*

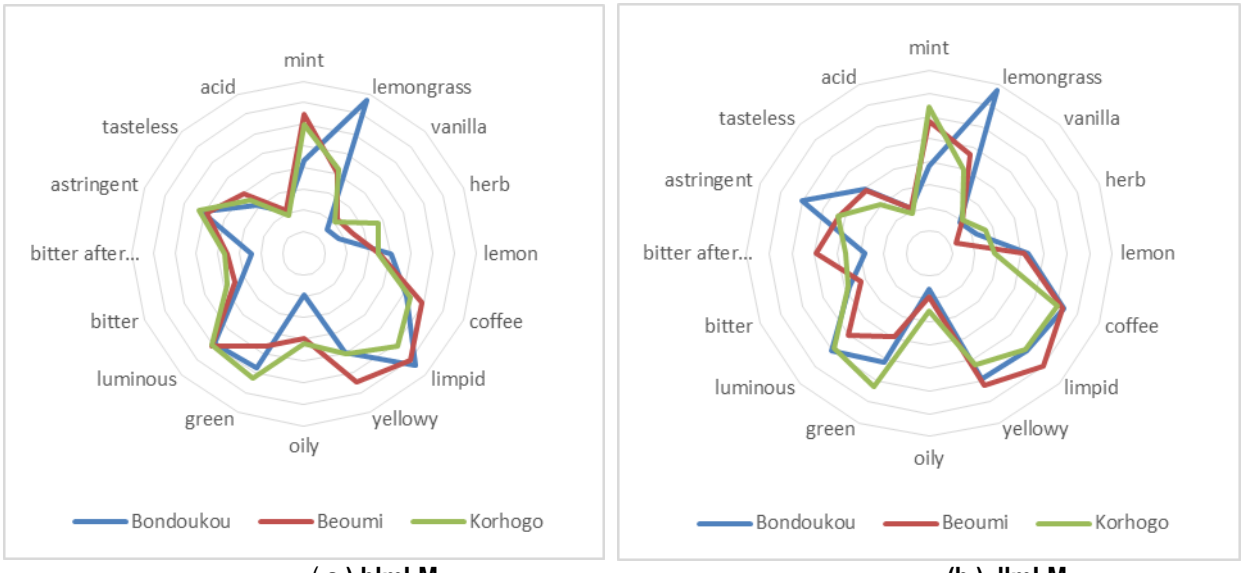
Sensory evaluation and sensorial profiles of infusions: After the sensory analysis, the parameters most perceived by the panellists with a score greater than 8 were considered important and taken into account for the representation of the sensory profile of the infusions of each morphotype are recorded in table 5.

Flavour: Bitterness is weakly perceived in all infusions with marks from 6.25 to 9.83. There is no significant difference ($P\leq0.05$) between the culture zones and the morphotypes. Astringency is a sensation that was also detected in all infusions with a significant difference ($P\leq0.05$) between culture areas and morphotypes. Regarding the morphotypes, the average marks vary

from 9.75 to 10.50 for blmLM and from 8.50 to 12.00 for llmLM. In general, in each of the localities, the infusions of the blmLM are more astringent than those of the llmLM.

Appearance: The most pronounced appearance characters of infusions are: coffee, limpid, yellow, green and bright (Table 5). In all localities, the brown (coffee) colour is more pronounced in infusions of llmLM compared to those of blmLM. As for the "yellowish" colour, it is seen in all infusions at significantly varying intensity (P≤0.05), between the cultivation areas. Indeed, Beoumi infusions are more yellowish compared to those of Bondoukou and Korhogo. However, in the same locality, there is no significant difference between the morphotypes. The green colouring is also seen in all infusions with varying intensities. For this character, the scores vary significantly (P≤0.05), depending on the growing area. Thus, the infusions of Korhogo are more greenish compared to those of Bondoukou and Beoumi. For each, there is no difference between the morphotypes.

Aroma: Concerning the olfactory descriptors, the aromas of "mint" and "lemongrass" were the most dominant scents in all the infusions to varying degrees. For each character, there is a significant difference between the cultivation areas. The highest scores for the "mint" aroma (11.50-12.91) were obtained with the infusions of Béoumi and Korhogo, while the infusions of Bondoukou had the highest scores for the "lemongrass" aroma (13.41-15.50). These results show that Béoumi and Korhogo leaves have a "mint" aroma, while those of Bondoukou have a "lemongrass" aroma. In each of the localities, the averages are roughly identical regardless of the morphotype, showing that the aroma depends very little on the variety of the plant. Sensorial profile of infusion of each the morphotype represented by figure 1 (a & b) was established taking into account the main descriptors wich obtained the highest scores by the panellists. The profiles show that whatever the morphotype, the infusions of Beoumi and Korhogo have an aroma of mint, while those of Bondoukou have an aroma of lemongrass. In addition, the Bondoukou infusion is much more astringent compared to those of the other two localities. In addition, while Bondoukou and Korhogo infusions have a more greenish colour, Beoumi's is more yellowish regardless of the morphotype.



(a) blmLM **(b) llmLM**

Figure 1. Sensory profiles of infusions of the leaves from the broad leaf (blmLM) and long leaf (llmLM) morphotypes of *Lippia multiflora*, cultivated in three localities in Côte d'Ivoire

Table 5. Notes on the flavour, appearance and aroma descriptors of the infusions of the two morphotypes according to the cultivation area

		Bondoukou		Beoumi		Korhogo	
		llmLM	blmLM	llmLM	blmLM	llmLM	blmLM
Flavour	Bitter	6.25±1.43^{a}	7,58±0,86^{ab}	7.00±0.93^{a}	6.50±1.20^{a}	7.66±1.20^{ab}	7.66±1.20^{ab}
	Astringency	10.25±1.19^{ab}	12.00±0.76^{e}	9.75±1.28^{c}	8.50±0.93^{ab}	10.50±1.46^{d}	8.58±1.46^{b}
Appearance	Brown (coffee)	10.33±0.91^{a}	12.75±0.63^{c}	11.83±0.93^{abc}	12.58±0.67^{bc}	10.66±1.87^{ab}	12.08±1.97^{abc}
	Limpid	14.75± 0.81^{bc}	12.08±0.91^{abc}	14.00±1.07^{bc}	14.00±0.78^{bc}	12.25±0.93^{a}	11.91±1.09^{a}
	Yellowy	10.00±0.90^{abcd}	11.91±0.97^{bcd}	12.91±1.05^{d}	12.50±1.26^{bc}	10.16±0.74^{abc}	10.50±1.15^{abcd}
	Green	11.58±1.10^{abc}	10.25±0.94^{bcd}	9.33±1.09^{cd}	7.91±1.15^{d}	12.50±1.11^{a}	12.66±0.89^{ab}
Aroma	Mint	8.58±0.99^{bcd}	7.66±0.83^{b}	12.91±1.50^{bc}	11.50±1.40^{bc}	11,91±0,92^{acd}	12,58±1,11^{a}
	lemongrass	15.41±1.17^{b}	15.50±0.97^{b}	8.08±1.19^{b}	9.41±0.83b	8.50±1.37^{ab}	7.91±1.25^{a}

Each value is an average of three replicate. Values are mean ± standard deviation. Means not sharing a similar letter in a line are significantly different p = 0.05 as assessed by the test of Duncan. blmLM: broadleaf morphotype of *Lippia multiflora;* llmLM: longleaf morphotype of *Lippia multiflora*

DISCUSSION

Mineral composition: The main form of food use of *L. multiflora* leaves is infusion and decoction (Kunle & Egharevba, 2012). Mineral elements in the aqueous extracts are considered an asset for the health of the consumer, their presence reflects the nutritional value of the infusion of *Lippia multiflora* and its benefit for the health. The calcium, magnesium and sodium contents obtained in this study are higher than those determined by Mayouf *et al.* (2015) in infusion of *C. sinensis*.

Contents of caffeine and phenolics compound in infusions: Caffeine contents found in this study are lower than those found by Adnan *et al.* (2013) in the infusion of *C. sinensis*, which vary from 38 to 40.6 mg/L. The tannin contents (6.02 of 14.33 mg/L) are lower than those found by Chakrabarty *et al.* (2017) in infusion of *C. sinensis* leaves (22 mg/L). Difference between morphotypes at the same site may be explained by the specific metabolic behavior of each morphotype with respect to environmental factors, in accordance with the findings made by Anuraga *et al.* (1993) in the genus Lotus. The catechin contents (6.21 to 59.51 mg/L) infusions of *L. multiflora* are much higher than those of the leaves of *C sinensis* (3.31 mg/L) reported by Oliveira (2012). Quercetin and flavanones of has been previously revealed in the leaves of *L. multiflora* and they are with tannins and catechins responsible of the medicinal and nutritional properties of the infusions (Toyosi *et al.,* 2013).

Sensory evaluation and sensorial profiles of infusions: Tea Aroma, appearance and taste are the main parameters of it quality (Zheng *et al.,* 2016). The variability of colour of infusion according localities corroborate result of Liang *et al.* (2007) who showed the variability of the colour of tea liqueurs from different origins. Infusions colour can be influenced by cultivar of plant, harvest season, climate and environmental conditions (Gao *et al.,* 2016), that would explain the significant differences between the culture zones and the morphotypes. The variability of the aroma depending on the growing area has been observed by He *et al.* (2009) in infusions of *C. sinensis* leaves. Results differ from those of Ekissi *et al.* (2014) who working on spontaneous *L. multiflora* showed that infusions of Bondoukou leaves smelled of *Ocimum gratissimum*. This difference would be because the leaves were not picked during the same periods. The sensory profiles of the infusions of the leaves from the three localities show a significant difference ($P \leq 0.05$) between the infusions, certainly because of the biochemical composition of the leaves, which varies according to the cultivation area. Indeed, any factor affecting the composition of tea leaves also influences that of infusions (Zheng *et al*., 2016).

CONCLUSION AND APPLICATION OF RESULTS

The qualities of infusions depend on the morphotype and the growing area. Their richness in phenolic compounds, caffeine and essential minerals gives them nutritional, medicinal and pharmacological properties and can be a replacement for ordinary teas sold on the market. This study provides information on the choice of the plant morphotype and the cultivation area according to the needs of the consumer. Consumption of infusion of leaves from Korhogo and Béoumi that are richer in tannins, catechin, quercetin and essential minerals (calcium, magnesium and iron) will be beneficial for human nutrition, bones strengthening and prevention of cardiovascular diseases which represent a public health problem. Taking *Lippia multiflora* into account in the agricultural and eating habits of farmers will constitute for them a source of income and also a source of permanent raw material for the agro-food processing industries

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