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RESEARCH ARTICLE

VARIABILITY IN PHYSICOCHEMICAL COMPOSITION OF CULTIVATED BROADLEAF MORPHOTYPE OF *LIPPIA MULTIFLORA* MOLDENKE AS AFFECTED BY PICKING METHODS AND AGRO-ECOLOGICAL ZONES OF CÔTE D'IVOIRE

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ABSTRACT

This investigation was undertaken to assess the influence of three cultivation zones and two picking methods on the biochemical composition of broadleaf morphotype of *Lippia Multiflora* (*blmLM*), in order to determine the area and method likely to give leaves for a tea of a good quality. Results showed significant variability in leaves composition according to cultivation area and plucking method. Leaf samples of fine picking (*LsFP*) from Beoumi were the most rich in protein (26.73%), tannins (14.26 mg/g), flavanone (13.33 mg/g), while the highest amount of total phenolic compounds (TPC) content (2061 mg GAE/100g), ash (10.25%), fat (8.46 %) and caffeine (7.06 mg/g) were found in leaf samples of coarse picking (*LsCP*) from the same area. Ca (9072.00-14981.17 mg/kg), K (2843.00-13565.67 mg/kg) and Mg (3122.64-4952 mg/Kg), were the most abundant minerals of *blmLM* leaves. This study revealed that Beoumi was the suitable cultivation area which may provide leaves with best nutritional qualities. Bondoukou and Korhogo showed similar general leaves biochemical characteristics. On the other hand fine plucking method was most profitable than coarse plucking on biochemical point of view.

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INTRODUCTION

Tea is an important economic crop in many Asian and African countries, due to its taste, aroma and health effects (Khokhar and Magnusdottir, 2002). The high consumption of tea is attributed to its richness in important substances having cool, bitter flavor, antioxidant properties and health benefits (Dimitrios, 2006). These benefits are linked to chemical compounds including alkaloids (theobromine, caffeine and theophylline), polyphenols (catechin, flavonoids), amino acids, polysaccharides, lipids and inorganic elements (Xion et al., 2012). Although *Camellia sinensis* is the plants mainly

recognized as true tea, some aromatic plants are used as tea, due to their pleasantly aroma and therapeutic properties, such as *Lippia multiflora* Moldenke which belongs to the wide family of verbenaceae, comprising 41 genera of perennial herbaceous plants with about 200 species (Owolabi et al., 2009). *Lippia multiflora* Moldenke commonly called "savannah tea" or "Gambia tea" (Adjanohoun et al., 1980) is a herbaceous, perennial and very fragrant plant which exists in the wild form in several agro-ecological regions of West Africa, mainly in Guinea savannah and coastal areas of the pre-forest savannah (Jim et al., 2001). In Côte d'Ivoire, it is naturally founded in central localities such as Bouaflé, Tiébissou, Bouaké, Toumodi and in northern grassy savannah localities namely Bondoukou,

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Ferkessedougou and Korhogo (Hien et al., 2012), where people use it for its essential oil and as beverage. Its leaves are used for the treatment of several illnesses (Jigam et al., 2009) due to its pharmacological, medicinal, pesticides, insecticides, food and cosmetics properties (Oladimeji et al., 2000) and because it generates new income (N'guessan and Yao-Kouamé, 2010). Many authors reported the biochemical variability of essential oil of *Lippia multiflora* leaves according to the growth areas (Kanko et al., 1999) and soils physico-chemical characteristics (Diomandé et al., 2015), but few data existing concerning the biochemical composition of leaves and no data for their variability depending on growing area. Literature reported that the phenolic and mineral components of *Camellia sinensis* vary significantly according to the geographical zone, clone, harvesting time and stage of plucking (Adnan et al., 2013). Although widely sold in markets and in supermarkets, there is very little information on the biochemical quality of *Lippia multiflora* leaves according to their origin, whereas it was revealed that the biochemical composition of tea leaves and infusion may vary from one region to another. Thus this work aimed to investigate the influence of some agro-ecological zones of Côte d'Ivoire on the chemicals composition of leaves from *blmLM*.

MATERIAL AND METHODS

Sites of the study

This study was conducted in three areas of Côte d'Ivoire which are: Bondoukou (East), Béoumi (Central) and Korhogo (North). The environmental characteristics of each site are reported in table 1.

Table 1 Characteristics of the study sites

	Bondoukou	Korhogo	Béoumi
Latitude	8°06'43"N	9°23'23"N	7°42'28"N
Longitude	2°42'23"O	5°48'49"O	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 vegetation	Tree savannah	shrubby savannah	Tree Savannah
Soil type	Cambisols	Cambisols	ferralitique revamped

Plant material and experimental device of plots

The *blmLM* was identified by the Centre National de Floristique (CNF) of Félix Houphouët-Boigny University (Abidjan, Côte d'Ivoire) and the plants were grown in the three agro-ecological zones described above (Table 1). The experimental design was a randomized complete block with three repetitions for each morphotype. Each plot of 400 m² at each test site was subdivided into 6 subplots each measuring 54 m² (9 m x 6 m), and containing 45 feet of *blmLM*. The plots were maintained by regular weeding.

Sampling

Leaves were harvested in September 2013 according to two picking (fine and coarse picking) methods as described by

Mariage Frères (2003). The fine picking consisted in removing the bud (pekoe) and the two leaves below, and the coarse picking consisted to pick from buds to fourth leaves below. Leaf samples were dried in the open air out of the sun for a week to prevent moisture and preserve flavor and organoleptic quality of the derivative tea (Yao-Kouamé and Kané, 2008). The dried leaves were ground using a mixer and the resulting powders were stored in sealed plastic boxes for physicochemical analysis.

Physicochemical analysis

Moisture, ash, crude protein, fat and crude fiber

Moisture, ash, crude protein (microKjeldahl N x 6.25), fat and crude fiber were determined by AOAC (2000) standard methods.

Determination of phenolics and Caffeine contents

The TPC contents were assessed by the spectrophotometric method as described by Singleton and Rossi (1965) using the Folin-Ciocalteu. Phenolics were extracted from pulverized samples of *blmLM* leaves. Exactly 5 g of samples were weighed out and extracted using 20 mL of 80 % (by volume) aqueous ethanol. The mixture was extracted for 20 min in inert atmosphere (N₂), filtered through Whatman No 4 filter paper (Whatman International Ltd, Kent, UK) using a Büchner funnel. Extraction of the residue was repeated using the same conditions. The filtrates were combined and adjusted to 50 mL in a volumetric flask with 80 % aqueous ethanol. The obtained extract was used for determination of total phenols (TPC).

Extract (0.5 ml) was added with 2.5 ml of Folin-Ciocalteu reagent followed by addition of 2 ml sodium carbonate (Na₂CO₃) (75 g/l). The sample was incubated for 5 min at 50°C. The absorbance was then measured at 760 nm using Shimadzu UV-1650 PC Spectrophotometer (Kyoto, Japan). The results were expressed as mg gallic acid equivalents per gram of dm (mg GAE/g) that was derived from a calibration curve.

The separation of phenolic compounds was performed according to method of Donovan et al. (1998). The samples and standard solutions were filtered through Whatman paper 0.45 µm, and then through millipore membrane 0.45 µm (CARL ROTH, Karlsruhe, Allemagne). The equipment used is a HPLC system (Shimadzu, France) provided with a binary pump (LC-20A) coupled with a UV-VIS detector (SPD-20A). The used column (Thermo, Runcom, Angleterre) for this analysis was Hypersil ODS type C18, 250 mm x 4.6 mm, 5µm (Thermo, Runcom, Angleterre). The separation was carried out in elution gradient. The used mobile phase consisted of 50 mM NH₄H₂PO₄ at pH 2.60 (solvent A), acetonitrile solution/NaH₂PO₄ (80:20, V/V) (solvent B) and 200 mM o-phosphoric acid at pH 1.50 (Solvent C). The flow rate was 0.5 mL / min. The solvent gradient elution program 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 20-25 min; 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 compound was analyzed by matching the retention time and its spectral characteristics against those of standards.

Mineral contents

Ca, Mg, Na, K, Mn, P, Fe, Zn and Cu were determined by Atomic Absorption Spectrophotometer (AAS), according to AOAC (2000) standard method.

Data analysis

Measurements were performed in triplicate. Data were expressed as means ± standard deviation. The data were subjected to two-way analysis of Variance (ANOVA) models. Means were separated according to Duncan's multiple range analysis (P 0.05), with the help of the software STATISTICA 7 (Statsoft Inc, Tulsa-USA Headquarters) and XLSTAT-Pro 7.5.2 (Addinsoft Sarl, Paris-France).

RESULTS

Proximate analyses

The results of proximate analysis are depicted in table 2. Data showed significant variation (P<0.05) in different component such as moisture (14.53-16.75 %), ash (6.93-10.25 % dm), protein (14.98-26.73 % dm), fat (6.21-8.46 % dm) and crude fiber (9.70-11.61 % dm) in leaf samples according to the picking methods and agro-ecological zones. *LsFP* presented the highest percentage of moisture (16.75 % dm) and protein (26.73 %), while the highest amount of ash (10.25% dm), fat (8.46 % dm) and crude fiber (11.61 % dm) were of observed in *LsCP*.

Table 2 Biochemical composition of leaves from cultivated *blmLM* as affected by three agro-ecological zones and plucking methods

Zone	Sample types	moisture (%)	ash (%)	Protein (%)	Fat (%)	Crude fiber (%)
BK	<i>LsFP</i>	14,81±0,25 ^a	6,93±0,24 ^b	18,02±0,79 ^b	6,62±0,39 ^b	9,70±0,11 ^a
	<i>LsCP</i>	14,53±0,02 ^a	9,55±0,31 ^d	14,98±0,77 ^c	7,47±0,25 ^d	10,15±0,09 ^a
KG	<i>LsFP</i>	16,75±0,06 ^d	8,10±0,06 ^c	20,48±0,57 ^a	8,30±0,06 ^a	10,70±0,11 ^a
	<i>LsCP</i>	16,20±0,11 ^c	9,14±0,54 ^a	19,07±0,54 ^{bc}	8,26±0,04 ^a	11,61±0,15 ^a
BM	<i>LsFP</i>	15,90±0,47 ^{bc}	9,04±0,24 ^a	26,73±1,56 ^d	6,21±0,12 ^c	10,84±0,44 ^a
	<i>LsCP</i>	15,59±10,25 ^b	10,25±0,04 ^e	20,49±0,23 ^a	8,46±0,04 ^a	11,53±0,51 ^a

Value with different superscript letter in columns are statistically significant (P<0.05)

BK=Bondoukou, KG= Korhogo, BM= Béoumi, *LsFP*= leaf samples of fine picking, *LsCP*= Leaf samples of coarse picking, dm=dry matter

The highest levels of moisture (16.75 %) and protein (26.73% dm) were founded in *LsFP* from Korhogo and Béoumi respectively, while the lowest amounts were observed in *LsCP* from Bondoukou. *LsCP* from Béoumi presented the highest amount of ash (10.25 % dm) and fat (08.46 % dm), whereas lowest levels of these compounds were observed in *LsFP* from Bondoukou and Béoumi respectively. Concerning crude fiber, the highest percentage (11.53 % dm) was observed in *LsCP* from Korhogo and the lowest level (09.70 % dm) was founded in *LsFP* from Bondoukou.

Caffeine and phenolics

Caffeine and phenolic compounds contents are depicted in table 3. Results showed significant variability (p<0.05) according to the picking methods and agro-ecological zones. *LsCP* presented high amount of TPC and caffeine than those of *LsFP* and inversely for the phenolic compounds.

The highest level of caffeine (7.06 mg/g dm) was observed with *LsCP* from Beoumi. TPC levels ranged from 1126 to 2061 mg GAE/100g dm and also varied significantly according picking method and cultivation zone. The highest TPC value (2061 mg GAE/100g dm) was founded in *LsCP* from Beoumi, while *LsFP* from Bondoukou contained the lowest content (1126 mgGAE/100g dm). Phenolics levels ranged from 0.47 to 37.97 mg/g dm for catechin, 7.16 to 14.26 mg/g dm for tannins, 01.95 to 13.33 mg/g dm for flavanone and 0.39 to 0.95 mg/g dm for quercetin. *LsFP* from Beoumi showed the highest amount of tannins (14.26 mg/g dm), flavanone (13.33 mg/g dm) and quercetin (0.95 mg/g dm), while the highest amount of catechin (37.97 mg/g) were found in *LsFP* from Korhogo.

Mineral composition of savannah tea leaves

Results regarding mineral content are depicted in tables 4. The results indicated significant variations (P<0.05) among mineral levels according to the picking method and cultivation zones. P (2843.33-13565.00 mg/kg dm), Na (1162.00-1347.00 mg/kg dm), Ca (9072.00-14981.17 mg/kg dm), and Mg (3122.64-4932.67 mg/kg dm) were in high concentration, while Ca (14.83-31.67 mg/kg dm), Mn (34.67-166.23 mg/kg dm) and Zn (22.17-51.33 mg/kg dm) were low. The levels of P and Fe were ranged from 200 to 250 mg/kg and 100 to 200 mg/kg respectively.

Ca and Mg had the highest concentration in samples with levels of 9072.00 mg/kg dm for *LsCP* from Beoumi and 14981.17 mg/Kg dm for *LsCP* from Korhogo, while *LsFP* from Bondoukou showed the highest concentration of Na (1347.00 mg/kg), Fe (200.17 mg/kg) and Zn (51.33 mg/kg). Sample of *LsCP* from Beoumi had the highest concentration of K (13565.00 mg/kg).

Table 3 Caffeine and phenolics contents of leaves from cultivated *blmLM* as affected by three agro-ecological zones and plucking methods

Zones	Sample types	Caffeine (mg/g)	TPC (mgGAE/100 g dm)	Catechin (mg.g ⁻¹)	Tannin (mg.g ⁻¹)	Flavanone (mg.g ⁻¹)	Quercetin (mg.g ⁻¹)
BK	<i>LsFP</i>	1.85±0.03 ^b	1126±0.70 ^a	06.21±0.01 ^b	12.15±0.31 ^d	05.75±0.05 ^c	0.73±0.01 ^b
	<i>LsCP</i>	2.93±0.04 ^c	1560±0.10 ^c	05.47±0.02 ^a	09.16±0.09 ^b	04.59±0.06 ^{cd}	0.39±0.06 ^a
KG	<i>LsFP</i>	1.45±0.02 ^a	1438±0.75 ^b	37.97±0.01 ^f	07.16±0.06 ^a	01.95±0.11 ^a	0.74±0.01 ^b
	<i>LsCP</i>	4.12±0.02 ^d	1642±1.04 ^c	13.99±0.02 ^d	10.27±0.05 ^c	02.88±0.08 ^b	0.92±0.01 ^c
BM	<i>LsFP</i>	4.05±0.01 ^d	1842±0.57 ^d	12.94±0.02 ^c	14.26±0.09 ^c	13.33±0.08 ^e	0.95±0.01 ^c
	<i>LsCP</i>	7.06±0.03 ^e	2061±1.89 ^e	19.93±0.01 ^e	10.09±0.09 ^c	05.40±0.05 ^c	0.63±0.02 ^b

Value with different superscript letter in columns are statistically significant (P<0.05)

BK=Bondoukou, KG=Korhogo, BM= Béoumi, *LsFP*= Leaf samples of fine picking, *LsCP*= Leaf samples of coarse picking, TPC: Total Phenolic Compounds, dm: dry matter

Table 4 Mineral contents of leaves from cultivated *blmLM* as affected by three agro-ecological zones and plucking methods (mg/kg dm)

Zones	Sample types	Na	K	Ca	P	Mg	Cu	Mn	Fe	Zn
BK	<i>LsFP</i>	1347.00±0.40 ^f	2983.33±0.70 ^b	9793.73±0.05 ^c	248.60±0.23 ^b	4474.33±0.15 ^c	25.33±0.00 ^c	82.83±0.06 ^c	200.17±0.02 ^f	51.33±0.00 ^a
	<i>LsCP</i>	1192.00±0.50 ^b	2843.33±0.70 ^a	13183.67±0.50 ^e	253.32±0.06 ^d	3944.33±0.15 ^b	14.83±0.00 ^a	34.67±0.01 ^a	34.17±0.03 ^a	22.17±0.00 ^c
KG	<i>LsFP</i>	1162.00±0.40 ^a	6510.00±0.80 ^d	14981.17±0.50 ^f	244.71±40.47 ^c	4606.00±0.20 ^e	31.67±0.00 ^e	89.63±0.06 ^d	176.33±0.03 ^d	43.17±0.00 ^c
	<i>LsCP</i>	1238.47±0.91 ^c	4130.00±0.40 ^c	11916.17±0.10 ^d	248.33±0.03 ^b	4932.67±0.05 ^f	31.17±0.00 ^d	34.83±0.04 ^a	130.33±0.15 ^b	38.33±0.00 ^d
BM	<i>LsFP</i>	1205.33±0.40 ^c	2845.33±8.50 ^a	9074.00±0.57 ^a	246.67±0.40 ^a	4552.60±0.50 ^d	31.69±0.02 ^f	166.23±0.38 ^e	181.50±0.10 ^e	51.13±0.00 ^a
	<i>LsCP</i>	1225.60±0.46 ^d	13565.00±2.9 ^e	9072.00±0.20 ^a	246.67±0.05 ^a	3122.64±0.05 ^a	20.67±0.00 ^b	57.17±0.20 ^b	175.33±0.02 ^c	19.17±0.00 ^b

Value with different superscript letter in columns are statistically significant (P<0.05)

BK=Bondoukou, KG=Korhogo, BM= Béoumi, *LsFP*= Leaf samples of fine picking, *LsCP*= Leaf sample of coarse picking, dm=dry matter

DISCUSSION

Leaves of *blmLM* had higher values of moisture (14.53 -16.75 %), protein (14.98 -26.73% dm), ash (06.93-10.25 % dm), fat (06.21-08.46 % dm) and crude fiber (09.70-11.61% dm) than those reported by Yao-Kouamé and Kané (2008) and Ekissi et al. (2011) in the leaves of savannah tea. Moisture and protein levels were high in fine picking samples, while the amounts of ash, fat and crude fiber were found to be high in the coarse picked ones. This result is in agreement with the finding of Owuor and Kwach (2012) and may be due to the high proportion of young leaves and bud which contain more water and protein than mature leaves, which are wealthy in ash, fat (Ekissi et al., 2011) and crude fiber (Yao-Kouamé and kané, 2008).

The high levels of moisture observed in leaf samples after drying could be due to the fact that samples were dried in open air out of the sun, to preserve their organoleptic quality, seeing that high temperatures cause loss of volatiles compound responsible of the perfume of the leaves (Yao-Kouamé and Kané, 2008). However, it should be noticed that high moisture content tends to promote microbial activities and fungal growth (Mohamed and Sulaiman, 2009) and does not ensure good conservation of the tea leaves.

As far as concern crude fiber, its content may vary in plant material depending on the development stage of the plant (Yao-Kouamé and Kané, 2008). It has also been reported that its level determines the quality of teas (Owuor and Kwach, 2012). Thus, high crude fiber content in *blmLM* leaves obtained by coarse picking method would indicate their lower quality than fine picked samples, which could be recommended for making tea.

Ash content is an indication of mineral content (Nigam and Niambar, 2014). Thus, higher ash content in *LsCP* corroborates their richness in minerals as founded by results. In general, mineral contents of *blmLM* leaves were in agreement with those of Olivier et al. (2012) who found Ca (5252 mg/kg), Mg (1956 mg/kg), P (2534 mg/kg) and K (13896 mg/kg) as major mineral in the leaves of green tea of *Camellia sinensis*. Data also showed that Cu, Zn, Mn and Fe levels were lower in our samples, as well as in the leaf samples of different herbal teas and their infusions analyzed by Olivier et al. (2012). The variation of mineral concentrations in *blmLM* according to the picking method is in agreement with that of Carr et al. (2003) who showed that mineral contents of *Camellia sinensis* leaf were correlated with the age of the leaf, with old leaves accumulating about 10 times more than young leaves. It should also be underlined that mineral contents in *blmLM* varied significantly according to agro-ecological zone as reported by Annan et al. (2010) for *Lippia multiflora* from three geographic zones of Ghana. This kind of variation in moisture, protein, ash and fat levels, and in the other hand mineral profiles in *blmLM* leaves according to the growing areas may be due to some factors like the differences in the soil characteristics and climatic condition (Fraga, 2005, Kottur et al., 2010).

TPC values founded in *blmLM* were higher than TPC content (20.20 mg GAE/100g) in leaves extract of *Lippia multiflora* reported by Bangou et al. (2011). Polyphenols in vegetable and fruit are responsible for many biological activities (Arct and Pytkowska, 2008). Indeed polyphenols provided antioxidant properties to *Lippia multiflora* and *Camellia sinensis* leaves (Mello and Quadros, 2014; Dabire et al., 2015). Therefore, high amount of TPC founded in the leaves of *blmLM* would indicate their functional value and mostly those from Beoumi. Caffeine content (1.45-7.06 mg/g) in *blmLM* is substantially in line with mean of 3.80% of caffeine in green tea (Adnan et al., 2013), except *LsCP* from Beoumi which showed high amount

of caffeine. Moreover, result concerning tannins content corroborates the finding of Bangou *et al.* (2011), but superior to those of *Lippia chevalieri* (7.62 mg/g) reported by these authors. The amounts of quercetin founded in *blmLM* were similar to the result of Kirakosyan *et al.* (2004), who obtained 0.92 mg/g of quercetin in the leaves of *Crataegus laevigata* (Hawthorn). Our study however showed that in the same growing area, caffeine levels were higher in *LsCP* than in *LsFP*, while phenolic compounds which are one of quality parameter of tea were founded to be high in *LsFP*.

This trend may be also attributed to the ratio between proportion of young leaves and mature leaves of the samples which can influence caffeine and phenolics content. There are different reports on major compound changes in green tea depending on the leaf age. Chen *et al.* (2003) reported that young tea leaves were richer in caffeine, epigallocatechin gallate (EGCg) and epicatechin gallate (ECg) than were mature leaves, but old leaves had higher levels of epigallocatechin (EGC) and epicatechin (EC). In addition, the influence of picking standard on caffeine content in tea has been demonstrated by Owuor and Chavanchi (1986). The presence of caffeine and phenolic compounds such tannins; catechin, quercetin and flavanone in leaves of *blmLM* may be the basis of the nutritional, medicinal and pharmacological properties of this plant as reported by Jigam *et al.* (2009).

Caffeine provide central nervous system stimulating, mental alertness and improving reasoning power (Lieberman *et al.*, 1987) while catechin and tannins contribute to the bitterness and astringency of tea infusion (Wang *et al.*, 2000) and its antioxidant, radical scavenging activities, protection against cancers and cardiovascular diseases (Santos-Buelga and Scabert, 2000). As for the picking method, samples harvested according fine picking method which showed high levels of phenolic compounds may be the best for making prime quality of tea since phenolic compounds content are recognized as an important parameter in tea quality (Yilmaz *et al.*, 2004). In addition, earlier studies have reported that two leaves and a bud plucking standard is the best compromise between yield and quality (Owuor and Kwach, 2012). However, the gaps between caffeine and phenolic compound contents of *blmLM* from the three zones can be attributable to soil properties and environmental factor (climate, temperature and rainfall), since these factors varied from one area to another as mentioned in table 1. Many studies had demonstrated that the origin as well as other factors such as soil and climate, influence biochemical composition of tea leaves (Yao *et al.*, 1992; Kwach *et al.*, 2013) and particularly savannah tea leaves (Kanko *et al.*, 1999; Ekissi *et al.*, 2014).

CONCLUSION

This study revealed the richness of *blmLM* leaves in organic compounds and minerals which are very important for human health. The composition of leaves varied according to the growing zone and the picking method. The leaves of *blmLM* are a good source of protein and mineral such as Ca, Mg, Fe, K, Zn, Na and P which contribute to their dietary values. Moreover, their richness in phenolics (tannins, catechin,

quercetin and flavanone) and caffeine provide them nutritional, medicinal and pharmacological properties.

Samples from Beoumi give the high levels for most of the beneficial compounds such as caffeine, tannins, flavanone and essential minerals. This area is therefore the most suitable for the cultivation of *blmLM*, followed by Korhogo area.

Moreover, the fine plucking is the best harvesting method because of its high content of young leaves which are rich in essential biochemical component.

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