Original Research Article

Nutritional valorization of Côte d'Ivoire Savannah tea plant leaves

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This work was conducted to quantify biochemical compound in tilled and dried *Lippia multiflora* Mold leaves. The study evaluated the geographical variation of this plant in term of its proteins, cellulose, vitamin, minerals, mixed phenolic compounds on leaf materials collected from Abidjan, Toumodi and Bondoukou. The results showed a variability in the physicochemical composition from *L. multiflora* leaves. Correlation analysis revealed significant differences in protein, cellulose, vitamin minerals and mixed phenolic compound. Our results indicated the importance of geographic origin of cultivation areas and leaves' ripeness. Proteins content is more than 9% of DM (Dry Matter). C vitamin and carotenoids content ranged from 17.34-27.71 mg/100g DM and 113.94- 136.97 µg /100 mg of DM, respectively. The leaves had high values for potassium (13.32 - 26. 72 mg /100 mg of DM). Calcium and iron ranged from 7. 87 - 29.25 mg/100g of DM, 0.09 - 0.18 mg/100g of DM. The investigated phenolic compounds showed substantial polyphenols, flavonoids and alkaloids with the highest compound in younger leaves and lowest in older leaves. Nutrient concentrations of *L. multiflora* leaves in Abidjan were greater than the other two locations.

Keywords: Biochemical compound, savannah tea, *Lippia multiflora*, agro-ecological locations

INTRODUCTION

Lippia multiflora Mold is a multifunction tree species that belongs to the the Verbenaceae family and is mainly spontaneously distributed in in Sudanese savannah of western and central Africa. It is vulgarly called savannah tea plant or Gambia tea. *L. multiflora* a native is an aromatic woody shrub plant also distributed in Côte d'Ivoire. It grows with increasing interest because of its biomedical virtues: Tea-like infusions are traditionally used: feeding (Terblanché and Kornelius, 1996), medicinal (Benoit-Vical et al., 1996), pesticides (Porspi, 1992), pharmacologic (Noamesi et al., 1985a and b; Mwangui et al., 1991) antibacterial (Samba et al., 2021) and cosmetic (Oladimedji et al., 2000). Its leaves are used as numerous traditional

medicines based on plants for different results moving from therapeutic febrifuge tea to non- therapeutic endings as tea beverages favoring relaxation sedation, condiments (Oladimedi et al., 2000), and as mouth disinfectant (Menut et al., 1993).

Despite its multifunction values and the deriving great interest, *L. Multiflora* is still a savage harvesting plant. It is also to be noted that though its main oils have been subjects of many studies (Kanko et al., 1999), research on its biochemical content as far as vitamins, compound polyphenolic, proteins and minerals are concerned, are short (Yao-Kouamé and Kane, 2008). The objective of this study is to determine macro and micromolecules in young

Figure 1: Location of the studied research area

- Research area
 - District (research area)
 - Other district
- (Administrative names and boundaries Main district)

and mature leaves of *Lippia multiflora* and the influence of some agro-ecological zones of Côte d'Ivoire.

MATERIALS AND METHODS

Plant material

The plant material was cultivated *Lippia multiflora* leaves as affected by three agro-ecological zones (Abidjan, Toumodi and Bondoukou).

Location of the studied sites

This work was carried out in three agro-ecological cultivated *Lippia multiflora* sites (Figure 1). *L. multiflora* is cultivated in the eastern rainforest (Abidjan) having for coordinates N: 5° 20' 28" / W: 4° 1' 41", the tree savannah in the west (Toumodi) having for coordinates N: 6° 33' 0" / W: 5° 1' 0" and the herbal savannah in the North-east (Bondoukou) having for coordinates N: 8° 1' 60" / W: 2° 48' 0".

Leaves sampling

500g of leaves were randomly harvested according to two picking during the plant's growth (fine and coarse picking) as described by Mariage (2003). After flowering 6 months (first cycle) after seeding, the plant is ripened. At flowering stage of *L. multiflora* (six months after seeding)

corresponding to the physiological maturity, 5 fresh and mature leaves were sampled around (morning) from randomly selected 16 plants dispersed within (30 m²).

The leaves were air-dried, grounded using a mixer and the resulting powders were stored in sealed plastic boxes for physicochemical analysis.

Physicochemical analysis

The separation of phenolic compounds five components (vitamins (C and carotene β), proteins, minerals, cellulose and phytochemical mixtures (flavonoids, polyphenols, alkaloids and tannins) were performed. Carotene β compounds was performed according to Tee and Lim (1996) and is based on determination of carotene beta associated with ethanol, hydroxide potassium and hexane. C vitamin is determined by Poncragz et al. (1971). The C vitamin was extracted by metaphosphoric-acetic acid from 2,6-dichlorophenol indophenol. Proteins compounds were and determined by sulfuric acid performed according to AOAC standard methods (AOAC, 2000). 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. The total phenol content was determined by Folin-Ciocalteu reagent and total flavonoids by AlCl₃ (Wong et al., 2006). Polyphenol content was determined colorimetrically based on the Folin-Ciocalteu (F-C) (Folin reagent). The flavonoids compounds extracted from *L. multiflora* leaves were calculated according to Sockmen et al. (2005). The tannins were

Figure 2: C vitamin contents of leaves from cultivated *Lippia multiflora* as affected by three agro-ecological zones and plucking methods.

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

Figure 3: Beta-carotene of leaves from cultivated *Lippia multiflora* as affected by three agro-ecological zones and plucking methods.

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

Figure 4: Protein of leaves from cultivated *Lippia multiflora* as affected by three agro-ecological zones and plucking methods

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

extracted from methanol (70 %) (Baindrige et al., 1996). The total alkaloids are quantified according to Harbone (1973) and Obadoni and Ochuko (2001).

Statistical analysis

Results were statistically tested for analysis of variance (ANOVA) followed by Duncan's test using SPSS 16.0 software. Statistical significance for all data was set according to values for P < 0.05.

RESULTS

The results of proximate analysis are depicted in figures

and table below. Data showed significant variation (P < 0.05) such as C vitamin in Abidjan *L. multiflora* leaves with the highest compound (Figure 2). C vitamin content ranged from 20.03 - 27.71 mg/ 100g of DM in younger leaves and 17.34 - 20.34 mg/ 100g of DM in older leaves.

The highest levels of carotene beta (Figure 3) and protein (Figure 4) were founded in leaves from Abidjan (123,51 to 136,97 µg/ 100 g of DM and 12,5 to 13%, respectively), whereas lowest levels of these compounds were observed in Toumodi (113.94 to 124.52 µg/ 100 g of DM and 9,87 to 12%) and Bondoulou (117,46 to 123,51 µg/ 100 g of DM and 9,55 to 10,87%). Data showed significant variation (P < 0.05) among Carotene beta content according to the picking method and cultivation zones. Younger leaves levels of carotene beta ranged from 123.51 - 136.97 µg/ 100 g of DM

Table 1. Mineral content of leaves from cultivated *Lippia multiflora* as affected by three agro-ecological zones in mg/ 100g of DM

Site	leaves	Na	Cl	S	P	Ba	Si
ABJ	YL	0.15 ± 0.02b	1.67 ± 0.67b	1.9 ± 0.81c	4.38 ± 0.21bc	0	7.41 ± 0.69c
	AL	0.26 ± 0.04c	3.90 ± 0.36c	1.96 ± 0.84c	9.62 ± 0.78d	0.61 ± 0.35b	9.53 ± 0.92c
TMDI	YL	0.09 ± 0.005a	1.71 ± 0.31b	0.98 ± 0.42b	4.38 ± 0.72bc	0	8.43 ± 0.06c
	AL	0.15 ± 0.005b	1.88 ± 0.88b	1.92 ± 0.83c	5.4 ± 0.98c	0	9.19 ± 0.92c
BDKOU	YL	0	0	0.47 ± 0.02a	1.84 ± 0.03a	0	1.29 ± 0.15a
	AL	0	0.03 ± 0.00a	0.51 ± 0.02a	6.74 ± 0.04c	0	3.51 ± 0.02b

Table 1 (cont.)

Site	leaves	Iron	Mn	Ca	K	Mg	Al
ABJ	YL	0.11 ± 0.02d	0	25.69 ± 0.28e	23.17 ± 0.64c	07.17 ± 0.78c	0.2 ± 0.07a
	AL	0.18 ± 0.03d	0.19 ± 0.02c	29.25 ± 0.54f	26.72 ± 0.48d	11.85 ± 0.93d	3.37 ± 0.07c
TMDI	YL	0.09 ± 0.02c	0	15.70 ± 0.61c	16.69 ± 0.55b	4.4 ± 0.09b	0.18 ± 0.06a
	AL	0.11 ± 0.02d	0	17.75 ± 0.97d	23.72 ± 0.52c	07.85 ± 0.88c	1.38 ± 0.05b
BDKOU	YL	0.02 ± 0.00a	0.001 ± 0.01a	2.68 ± 0.05a	13.94 ± 0.17a	2.1 ± 0.11a	0
	AL	0.05 ± 0.001b	0.04 ± 0.00b	7.79 ± 0.04b	15.95 ± 0.13b	4.75 ± 0.09b	0

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

Figure 5: Cellulose of leaves from cultivated *Lippia multiflora* as affected by three agro-ecological zones and plucking methods

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

Figure 6 : Total poliphenols of leaves from cultivated *Lippia multiflora* as affected by three agro-ecological zones and plucking methods

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

for and older leaves content varied from 113.94 to 124.52 µg/ 100 g of DM (Figure 3). Concerning proteins, the highest percentage (13 % dm) was observed in fresh leaves from Abidjan and 12.5% in mature ones (Figure 4).

Magnesium, potassium, phosphorus, calcium and silicon had the highest concentration in samples from Abidjan, whereas lowest levels of these compounds were observed in Toumodi and Bondoukou. The estimation of the mineral nutrient of the leaves of *L. multiflora* that have been tested

are illustrated in Table 1. Minerals content was founded in the leaves and concentrations were higher than 1mg/100g of DM. Iron (0.02- 0.18 mg/ 100 g of DM) and sodium (0.09- 0.26 mg/ 100 g of DM) were shown lowest content.

The older leaves were significantly high levels of cellulose (19.7- 24.63 % of DM) than the younger leaves (17.99- 18.76 % of DM) (Figure 5).

Phenolic profiles of *L. multiflora* from different locations were shown in figure 6. The highest ranges ranged from

Figure 7 : Total flavonoids of leaves from cultivated *Lippia multiflora* as affected by three agro-ecological zones and plucking methods

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

Figure 8 : Total alkaloids of leaves from cultivated *Lippia multiflora* as affected by three agro-ecological zones and plucking methods

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

Figure 9 : Total tanins of leaves from cultivated *Lippia multiflora* as affected by three agro-ecological zones and plucking methods

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

39.31 mg.L⁻¹ EAG, in mature leaves, to 69.30 mg.L⁻¹ EAG in younger leaves were shown in Abidjan (Figure 6).

The flavonoids are found in *Lippia multiflora* leaves (Figure 7), mainly with young leaves. The young leaves represent values of 14.57 mg.L⁻¹ EQ ; 11.67 and 10.82 mg.L⁻¹ EQ on the three farming sites, Abidjan, Toumodi and Bondoukou.

The results of alkaloids content show high quantities

among the young leaves of 47.84 %; 22.07 % and 32.82 %. There is an important gap in alkaloids' content on harvesting sites (Figure 8).

The tanins' results bring out a substantial difference on the farming sites. The young leaves have relative high proportions (15.46- 27.88 % of DM) than the mature ones (10.36- 23.96 % of DM). There are higher values in Toumodi's samples than the remaining sites (Figure 9).

DISCUSSION

The physicochemical results' analyses reveal significant chemical characteristics variation in relation to the development step of the leaves and farming areas. As a matter of fact, vitamins, proteins, polyphenols, flavonoids, alkaloids and tannins quantities are higher in fresh leaves than in mature ones. The minerals and cellulose quantities are higher in mature leaves than in fresh ones. Variations in vitamin content are due to environmental factors such as temperature. Indeed, Lefsrud et al. (2005) have shown that temperature had effect on plants vitamin variations. Temperature being one of environmental factors influencing growth of plants, it affects physical and chemical processes and controls biological reactions occurring in plants. In addition, it impacts physiological cycles like diffusion rates, liquid transformations, substances solubility, equilibrium and stability of varied systems like mixtures and enzymes (Hasan et al., 1999), explaining the noticed changes of C vitamin quantity and β -carotene between young and adults' leaves. The proteins values obtained are similar to that reported by Yao-Kouamé and Kane (2008) on *Lippia multiflora*. Young leaves having more content than mature ones. This can be explained by proteins mobilization in the growing areas of young leaves. Hence Young leaves' quantity is higher than mature ones.

The cellulose rate relatively low with young leaves and very high with adult leaves corroborates the privilege given to young leaves for tea making. In fact, as cellulose does not spread in infusions, it is relevant that its high rate in adult leaves influences the rate of extracts of *Lippia multiflora* leaves and give a light colour during infusion of adult leaves, whereas that of young leaves is darker and very astringent (Ekissi et al., 2011).

Noticed phytochemical variations in leaves are due to high requirements of carbohydrates produced by plants. According to Khang et al. (2005) and Phengvilaysouk and Wanapat (2008), carbohydrates produced by plants push back the substratum of the synthesis of secondary metabolite. This accounts for the important quantity of phenolic mixtures in young leaves in relation to adult leaves. Peters and Constabel (2002) have revealed that the concentration in phytochemical (polyphenols, flavonoids, alkaloids and tannins) considerably varies among the vegetal species.

As for farming areas, a variability is also observed at the level of the physico-chemical composition of the leaves. Interactions between geographical different farming sites, displayed significant differences ($p < 0.05$) in the proteins, cellulose, vitamins, minerals and mixed phenolic. These noticed differences could be ascribed to environmental factors. For Chweya and Mnzava (1997), the nutritional component of plants can vary according to the soils fertility and the environment. As a matter of fact, soils acidification has a lot of effects on plants' growth. It is a concrete case with soils, the three studied sites, which have a water pH between 5.75 and 6.8. Moderate acid in the studied soils is suitable for a good development of the vegetal species

(Koné et al., 2010). It could explain the biochemical composition variation of *L. multiflora* leaves.

There is C vitamin in our samples. It is vital for the body functioning (Okwu, 2004) and beneficial, given the nutritious aspect of *Lippia multiflora*. The variability in composition is due to sunny weather of the farming area or the salty water irrigated in it. This has been witnessed by Leonardi et al. (2000 a, b) who show that the high salinity of irrigated water can improve the ascorbic acid content, dried matter and titratable acid. The quantities of carotene spread out a variation between the farming areas. Few data are available in the literature about the effect of seasonal fluctuations and soils composition nor on carotenoid content in *Lippia multiflora*. Thus, the Abidjan site has a ferralsol feature of forestry soils with a clayey aspect and curdled structure (M'Lan, 1979). The forest environment is associated with an Atteaan humid climate (two dry seasons and two huge rainy seasons). That of Toumodi, is made of a sandy and shale like soil with average humidity. The pre-forestry environment goes along with a Baouleian climate (two rainy seasons and two dry seasons). The Bondoukou site is characterized by a ferruginous soil. The Savannah environment has a dry sub-soudanean climate (The dry season is longer than the rainy one).

The protein values obtained are similar to those reported by Yao-Kouamé and Kane (2008) on *Lippia multiflora*, but differ from that found in the leaves collected in the department of Korhogo. The highest levels of proteins (26.73% DM) were founded in LsFP from Korhogo (Kane et al., 2016).

Proteins are key organic mixtures with heavy molecular weight which dwell in all living tissues (Osei, 2003). The interactions between geographically different farming sites have portrayed significant differences depending on proteins content. These differences can be environmental. According to Chweya and Mnzava (1997), the nutritional composition of plants can vary on account of the fertility of the soils, the environment and the cropping techniques used. In fact, the studied soils' nitrogen has effect on the protein component of the *Lippia multiflora* leaves. This was proved by Mudau et al., (2007a, 2007b) who demonstrated that there was a significant link between the nitrogen of *Athrixia phylicoid* (asteraceae) and organic matter composition. Ibrahim et al. (2011) think that the poorer the soil is in nitrogen, the greater the foliar nitrogen tissue of *Labisia pumila* plants significantly increases.

There are meaningful interactions in the analyzed samples of cellulose. The recorded differences can be due to gaps about the soils' fertility and environment, as seen in the composition of the soils studied. The geographical locations and conditions in which plants were cultivated, influence their development and their properties (Zaouli, 2005). It has been proved in the mineral composition of the analyzed samples of three farming sites which raised substantial amounts of magnesium, potassium, phosphore, calcium, silicium, less iron, sulfur sodium and a few traces of manganese and barium. The content of macro-minerals such as potassium, calcium, magnesium, and found

phosphorus are relatively close to the values of certain vegetables, Amaranthus, spider plant etc. (Fasuyi and Noyerem, 2005)

The results of the phytochemical mixtures obtained have shown that alkaloids, phenols, flavonoids, and tannins are within *Lippia multiflora* leaves. The data from the phytochemical exam sustain that environmental factors, have a tiny effect on the phytochemical content of plant in the farming areas of research. As a matter of fact, the environmental factors are said to be responsible of changes and secondary metabolite determination in a plant (Waterman et al., 1989). A single plant cultivated in different environments, can have a different phytochemical content. That confirms our phytochemical one we got in *Lippia multiflora* tilled in the areas of Abidjan, Toumodi and Bondoukou. Many authors have also made reports on the way the different climatic conditions could affect the different climatic conditions of plants (Howard et al., 2002; Vallejo et al., 2003). Previous studies have also showed that some environmental factors could be responsible for secondary metabolites production variation with plants. The interaction of the phytochemical composition with environment as mentioned by these researchers corresponds to our data. Out of environmental factors, many others likely to have impact on the phytochemical content of the plants, include the use of pesticides, genetic factors, diseases and scavengers, fertilization, harvesting period and so on (Zhao et al., 2006). Indeed, seasonal fluctuations being different from an area to another, that is, forest environment of Abidjan together with a humid climate (two dry seasons and two abundant rainy seasons), the pre-forestry environment of toumodi along with a Baouleian climate (two rainy seasons and two dry seasons), and the savannah environment of Bondoukou with a sub soudanese dry climate (longer dry season than rainy season) explain the effect of *Lippia multiflora* in the phytochemical content.

Conclusion

The biochemical parameters analysis of *Lippia multiflora* leaves farmed in different ecological locations had displayed a quantitative variability in relation to the farming areas and the development step of the leaves (younger and mature leaves). At development time of the leaves, the quantities of proteins, polyphenols, flavonoids, alkaloids and tannins are higher with young leaves than mature ones. The amounts of minerals and cellulose are higher with adult leaves than young ones. As far as farming areas are concerned, a variability is also noticed at the physico- chemical levels composition of leaves. Interactions between geographically different farming sites, have revealed deep gaps ($p < 0.05$) within proteins' content, cellulose, vitamin, ashes, minerals, phenolic mixtures etc. Our results confirmed the hypothesis that geographical location significantly affects the biochemical compound of *L. multiflora* leaves.

Conflict of interests

The authors declare that they have no conflicting interests

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