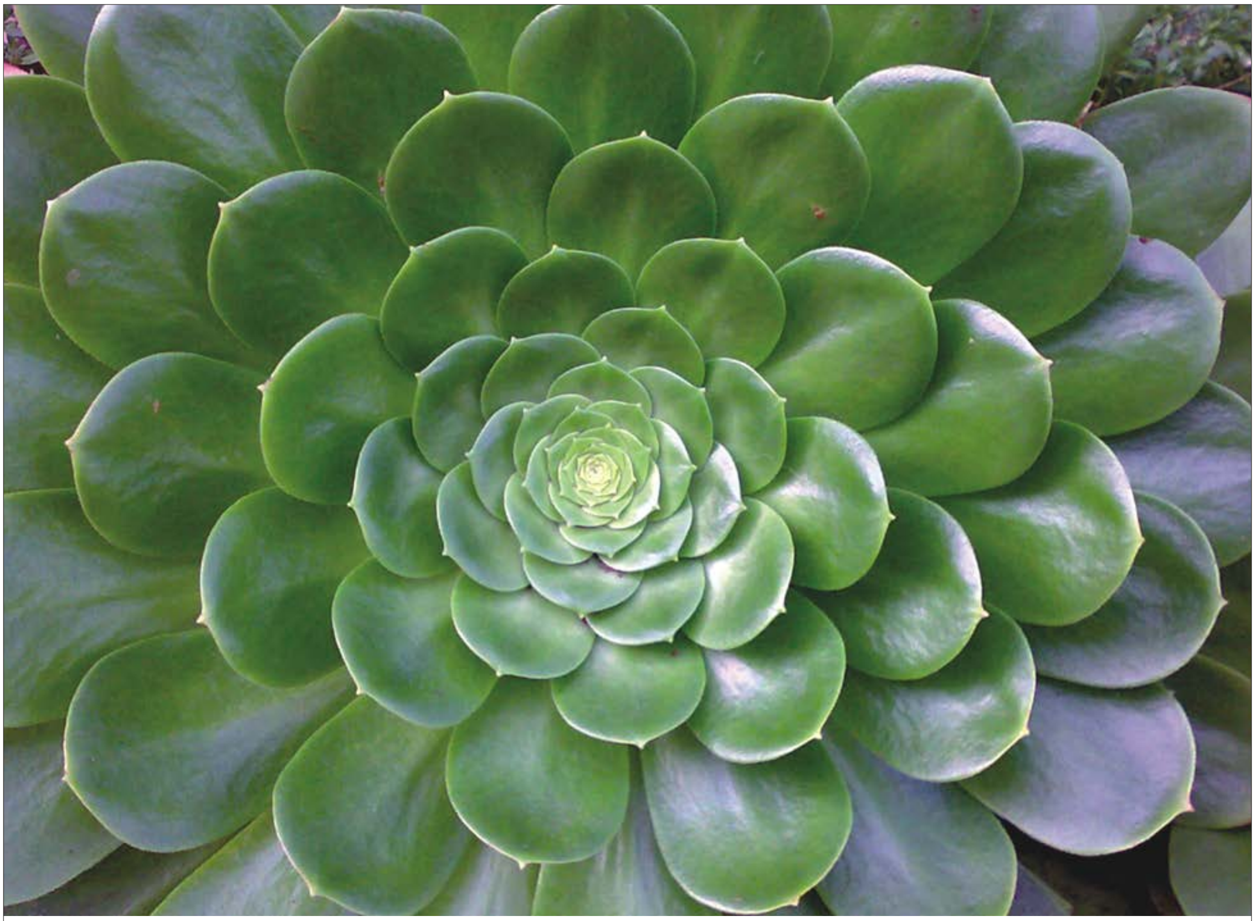

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Biochemical Characteristics of *Lippia multifora* (Verbenaceae) Leaves with Respect to Fertilizer Applied to the Soil

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Abstract: This study is part of the general domestication of *Lippia multiflora*, a plant which may contribute to the diversification of African agriculture and traditional pharmacopeia. The study focuses on determining certain characteristics of leaves at different vegetative stages. Thus, analysis were performed on *Lippia multiflora* (Verbenaceae) leaves taken from plants a month and a half old (young leaves) and one year old (old leaves), cultivated on field plots with different amounts of fertilizer (urea). The results show that the amount of urea applied to the soil did not affect the analyzed elements; the measured values of which are 16.5% of humidity level for young leaves and 12% for old leaves. The result shows also that ash rate is 10% for young leaves and 14% for old leaves; when proteins represent 12.25% for young leaves and 8.75% for old leaves. We measured out the carbohydrate and obtain that there rate is 0.40% for young leaves and 0.60% for old leaves. As for reducing sugar it represent 0.013% for young leaves and 0.008% for old leaves.

Key words: *Lippia multiflora*, biochemical components, leaves, fertilizer

INTRODUCTION

Lippia multiflora Moldenke is a shrubby plant which grows in the tropical regions of Africa. It is commonly called Savanna tea or Gambia tea. *Lippia multiflora* is traditionally used to cure inflammation of bronchial tubes, fever, rhinopharyngitis, conjunctivitis, icteris (Coffi *et al.*, 2003) and high blood pressure (Etou *et al.*, 2005). This plant would also possess pesticide properties.

The aqueous extract of *Lippia multiflora*'s leaves have also analgesic, antipyretic and anti-inflammatory activities (Abena *et al.*, 2003). Aside those properties, *Lippia multiflora* are also an aromatic plant that increases consumption.

Despite the diversity in its possible usages, *Lippia multiflora* is not yet domesticated leading to conservation and brush fire problems which prevent its development and its sustainability.

Except for studies dealing with essential oils extraction and their analysis, no detailed study has been conducted on the plant biochemical parameters such as temperature and drying time, humidity level, ash and proteins contents, carbohydrate and total sugar contents, caffeine and tannins contents. The objective of this study is to provide data on the biochemical characteristics of *Lippia multiflora* leaves at different plant growth stages with respect to different soil fertilizer (urea) levels.

MATERIALS AND METHODS

Experimental Site

The study was conducted from March to September 2006 at the experimental farm of the Abobo-Adjamé University in the Northern part of Abidjan in The Ivory Coast, under equatorial climate with four seasons: two rainy seasons (May-July) and (October-November) and two dry seasons (December-April) and (August-September).

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Fig. 1: Soil profile of the experimental site

(a) (b)

Fig. 2: (a) young plant (b) old plant

At the beginning, the land was covered with shrubby vegetation composed of *Panicum maximum* and *Eupatorium odoratum*. The soil at the site is ferrallitic, characteristic of forest land. The soil has a clayey texture and cluster structure with a low content (24 to 37%) of coarse sand. The major soil chemical characteristics are the following: barely acid pH, low nitrogen content, average phosphorous content, low cationic exchange capacity and relatively low base saturation rate (Fig. 1).

Lab Material

Regular lab material and various reagents were used: 95-96% sulfuric acid, 2% boric acid, 0.1 N sodium hydroxide, 6 N sodium hydroxide, 30% zinc acetate, 12 N hydrochloric acid, copper II sulfate, caustic alkali, double tartrate of sodium and potassium, iron III sulfate, 0.1 N potassium permanganate and 10% lead acetate.

Biologic Material

The dosages were made on leaves taken at two growth stages of plants cultivated on experimental plots at the University of Abobo-Adjamé (Fig. 2a, b). The plots have received various doses of fertilizer (urea). The sampling was made on plants of one and a half month old and on plants of one year old.

Sampling Methods

The study plots were divided in 3 Fischer plots with respectively 500, 1000 and 1500 kg ha^{-1} of urea fertilizer. The leaves samples were distributed according to plant growth stage and applied fertilizer level. Every sample was divided into two parts: one part was dried inside the laboratory at an average temperature of 25°C during 7 days and the other part was dried in the sun at an average temperature of 39°C during 4 days. The samples were weighed at the start and the end of the drying periods. The various samples were again weighed then ground and preserved separately at ambient temperature in closed plastic containers.

The humidity of fresh plant material was determined by weighing samples before and after a drying period. While in the dry product it was determined by weighing samples before and after a heating period of 2 h at 130°C temperature.

The ash content was measured by cremating 5 g of every sample in an oven at 500°C for 5 h. Leaf proteins contents were measured using the KJELDALL Method, by the mineralization of 1 g of every sample and titration of the total nitrogen after distillation.

Carbohydrates and total sugars were measured by Bertrand's method (Audigie *et al*., 1977).

RESULTS AND DISCUSSION

Humidity level of leaves dried in the shade was higher than that of leaves dried in the sun (Table 1-4). Ashes contents of leaves dried in the sun were quite similar to those of leaves dried in the shade. Proteins, cellulose, carbohydrate and total sugars contents did not vary no matter the drying method and the soil fertility level. Proteins content was higher in young than in old leaves (Table 2, 3). Cellulose content was relatively low (about 6%) in young leaves and very high (about 9%) in old leaves (Table 1-4). Carbohydrate contents were very low in *Lippia multiflora* leaves (0.40% for young leaves and 0.60% for old leaves. Reducing sugars were almost nonexistent for both young (0.007 to 0.013%) and old leaves (0.008 to 0.009%).

Table 5 indicates that fertilizer (urea) application led to a neutral pH of the soil. Nitrogen and phosphorus have increased cationic exchange capacity while the exchangeable cations (Ca^{++} ; Mg^{++}) contents have decreased, except for potassium (K^{+}) with higher content at 500 kg ha^{-1} of urea and remained constant at 1000 and 1500 kg ha^{-1} of urea. The change in soil chemical status due to fertilizer application did not affect the chemical composition of the plant leaves.

This study shows that tea infusion composition depends on several parameters such as temperature (Khokhar and Magnusdottir, 2002). The study of leaves of *Lippia multiflora* also shows

Table 1: Physicochemical parameters of young leaves dried in the sun as affected by soil fertility level

	Urea (kg ha^{-1})			
Contents (%)	0	500	1000	1500
Humidity	16.400±0.8400	16.800±0.000	17.200±0.000	17.220±0.890
Ashes	8.800±0.8300	10.000±1.000	10.000±0.890	10.000±0.890
Proteins	12.420±0.6100	12.920±0.480	12.600±0.380	12.950±0.730
Celluloses	6.000±0.4400	7.500±0.000	7.000±0.570	5.000±0.570
Glucides	0.420±0.0100	0.410±0.016	0.410±0.008	0.400±0.051
Total sugars	0.013±0.0019	0.013±0.002	0.015±0.000	0.014±0.000

Table 2: Physicochemical parameters of young leaves dried in the shade as affected by soil fertility level

	Urea (kg ha^{-1})			
Contents (%)	0	500	1000	1500
Humidity	18.800±1.670	19.000±0.000	19.000±1.0900	19.200±1.090
Ashes	8.400±1.000	8.400±1.090	6.000±1.0000	6.800±1.000
Proteins	12.250±0.410	12.950±0.390	12.250±0.3800	12.420±0.730
Cellulose	6.500±0.650	6.000±0.410	7.000±0.5000	6.500±0.220
Glucides	0.400±0.010	0.410±0.005	0.490±0.0000	0.510±0.030
Total sugars	0.007±0.008	0.007±0.005	0.012±0.0034	0.013±0.004

Table 3: Physicochemical parameters of old leaves dried in the sun as affected by soil fertility level

	Urea (kg ha^{-1})			
Contents (%)	0	500	1000	1500
Humidity	13.000±1.41	12.200±1.6700	12.000±1.410	12.400±1.09
Ashes	1.400±0.54	15.200±1.0000	15.200±1.000	14.800±2.04
Proteins	8.570±0.48	8.050±0.7300	8.400±0.390	8.400±0.39
Cellulose	8.500±0.35	8.500±0.2200	90.500±0.220	8.500±0.00
Glucides	0.680±0.01	0.660±0.0160	0.690±0.008	0.650±0.051
Total sugars	0.008±0.00	0.008±0.0054	0.008±0.000	0.008±0.00

Table 4: Physicochemical parameters of old leaves dried in the shade as affected by soil fertility level

	Urea (kg ha^{-1})			
Contents (%)	0	500	1000	1500
Humidity	17.800±1.78	16.800±1.0900	17.200±1.6700	16.400±1.090
Ashes	12.000±0.20	12.000±0.5300	12.800±0.1400	12.800±1.190
Proteins	8.050±0.48	8.570±0.7300	8.570±0.3900	8.750±0.470
Cellulose	9.000±0.00	9.000±0.0000	8.500±0.0000	80.000±0.000
Glucides	0.680±0.01	0.650±0.0160	0.650±0.0000	0.680±0.048
Total sugars	0.008±0.00	0.009±0.0054	0.008±0.0054	0.008±0.000

Table 5: Chemical characteristics of the soil before and after fertilizer application

Soil samples with kg ha^{-1} urea	pH	Nitrogen	Phosphorus	CEC	Ca^{2+}	Adsorbent complex (me/100 g) Mg^{2+}	K^+
Control	6.80	0.51	29.66	15.83	1.29	0.27	0.15
500	6.90	0.08	46.83	6.18	0.59	0.21	0.21
1000	7.10	0.09	55.16	5.74	0.75	0.12	0.12
1500	7.06	0.07	31.66	6.12	0.54	0.10	0.14

an irregular distribution and different chemical forms of several mineral elements (Carr *et al*., 2003). Carmen *et al*. (2003) showed that the composition of catechins, gallic acid, caffeine and trace elements in commercial teas varied depending on species, season and horticultural conditions and particularly, with degree of fermentation during the manufacturing process.

The results reveal that humidity level of leaves dried in the shade was higher than that of young leaves dried in the sun which was higher than that of old leaves. The difference is due to the amount of energy necessary for water extraction from the leaves. As a matter of facts, drying the leaves in the sun allows the evaporation of a larger quantity of water from the leaf; therefore, the remaining linked water which is eliminated at 130°C temperature in a dryer. On the other hand, drying the leaves in the shade allows the evaporation of a small part of the constitution water in the leaf. Leaf conservation strongly depends upon the interaction between its water content and micro-organisms activity. Therefore, leaves dried in the sun will withstand longer conservation periods as compared to those dried in the shade. However, drying in the sun leads to less aromatic dry matter. As a matter of facts, aromatic components in the leaves are very volatile and are easily lost under the action of high temperatures. It is necessary to use other drying method which better conserves aromatic components while significantly eliminates leaf water. For the meantime it is preferable to make the drying to the shade if one wants to optimize the aroma of the leaves, because the flavoured leaves are better quality for the preparation of tea.

Similar ashes contents in both type of drying methods, suggest that there is no mineral lost during drying in high temperature conditions since ashes constitute the mineral part of organic materials (Audigie *et al.*, 1977).

High proteins content in young leaves, may be the basis of their strong aromatization, suggests a high protein synthesis level, (Guignard *et al.*, 1985). Young leaves have a better taste than old leaves and therefore should be recommended for tea preparation. Statistical analysis indicate that proteins contents were not affected neither by the amount of fertilizer applied to the soil, nor the leaf drying methods.

Relatively low cellulose content in young leaves, farther more, justifies the use of young leaves for tea preparation. Cellulose does not dilute in infusions and so, its high content in old leaves affects the extract for tea, giving light colour to infusions made from old leaves, while those from young leaves are dark and very astringent.

Very low carbohydrate content of *Lippia multiflora* leaves compared to *Camellia sinensis* tea which contains up to 25% could be explained by the nature of the leaves, the experimental methods used for carbohydrate dosage, or field experimental conditions (Audigie *et al.*, 1977). The very low content of reducing sugars in the leaves could be explained by their quick transfer and storage in the fruits, grains, tuber roots through sap water.

CONCLUSION

The study shows that *Lippia multiflora* leaves are characterized by high water, ashes and cellulose contents and by low proteins, carbohydrate and reducing sugars contents. Biochemical components of *Lippia multiflora* are mainly located in young leaves good for tea preparation. Leaves should, therefore, be harvested on young plants 3 to 4 months after planting. The application of urea fertilizer on the soil did not significantly affect the contents of biochemical components of the leaves.

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