Impact of Ginger Enrishment on Biochemical Characteristics of Tisane from *Aloysia Citrodora* Leaves, Cultivated at a small scale in the Area of Man (West Region of Côte d'Ivoire)

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Abstract— *This study confirm one of the official missions attributed to the University of Man, those to enhance natural's resources of the region of Man and its properties. The assessment of the biochemical characterization of a tisane from Aloysia citrodora's leaves enriched at ginger has been conducted.*

To reach this goal, tisane has been prepared with Aloysia Citrodora. An aqueous extract of Zingiber rhizomes has been also produced. Ginger extract incorporated in tisane of Aloysia citrodora at 2.5 % level. The results have shown that many studied parameters increased highly. The dry matter increased from 88.92±3.92% to 90.07±2.91%. Incorporated ginger in tisane of Aloysia citrodora improved total phenolic content, antioxidant capacity, insoluble solids. Total amount of phenolic compounds was 11.68±4.05mg GAE/g and it's increased to 15.90±0.42 mg GAE /g. Antioxidant activity of this enriched tisane was also 3.96±1.58 µM Trolox Eq / Kg for the ABTS method. The analysis concerning mineral content of obtained tisane has noted a high content particularly those of Calcium. Its content were three time (535 130.4 ppm) important in enriched ginger tisane at 2.5% than Aloysia Citrodora's tisane (192888.9 ppm).

Keywords— Aloysiacitriodora, tisane, Zingiber officinale, Man, Côte d'Ivoire.

I. INTRODUCTION

Nowadays, several aspects of research data point out that specific dietary compound act like preventive agent against some diseases like cardiovascular disorders, some types of cancer, inflammatory circumstances, and obesity. Functional foods, which have specific physiological benefits, contain bioactive ingredients recognize as prebiotics, probiotics, flavonoids, phenolic compounds, phytosterols, bioactive peptides (Arvanitoyannis, 2005).

Aloysia citrodora belongs to the family Verbenaceae and has several other synonyms for the scientific name including *A. triphylla* (L'Hér.) Britton, *A. citridora* Paláu, *A. citriodora* Paláu, *Lippia citriodora* Kunth, *L. citrodora* Kunth, and *L. triphylla* (L'Hér.) (Somanchi *et al*. 2017).

The plant is a perennial shrub with up to 3 m height, striate and scabrous branches, and lanceolate 7-10 cm margined leaves with short petioles. The tiny flowers have white or light blue color which appears on a hairy calyx with four tips in the panicle-like spikes. The petals form 4-5 mm funnel at the base which covers 2 long and 2 short stamens (Vitali *et al*. 2009).

This plant has a wide geographical distribution from South America to North Africa and South of Europe. It should be mentioned that due to the pleasant lemony fragrance and its application in food industries and cosmetics, as well as its use as a home remedy for several health problems, the plant is currently available in other parts of the world, as well (Carnat *et al*. 1999). One of these species is Erica arborea.

Aloysia Citrodora leaves contains several phytochemicals, which show high antioxidant activity, and may be the cause of several health benefits such as a hypotensor, diuretic. Several parts of this herb are preferred in various regions as a urinary antiseptic, and against constipation (Pascual *et al.* 2001).

Also, the processed leaves are used in tea manufacturing and as an ingredient of alcoholic or non-alcoholic herbal drinks. The plant "cedrón" is also included in the Código Alimentario Argentino (CAA) as a corrective and coadjutant, in the section referred to vegetal condiments (Código Alimentario Argentino) (Ghédira, 2017). Tisane is not only a source of water (Goetz, 2004). According to all kind properties of *Aloysia Citrodora* and the goal to preserve the health of the people of « tonpki »'s region, the polytechnic University of Man has planned to describe different tisanes from local plant or plant from another area.

The present investigation is undertaken to evaluate biochemical properties, antioxidant activity and sensory evaluation of *Zingiber officinale* enriched tisane from *Aloysia citrodora* leaves, cultivated at a small scale in the west region (Man) of Côte d'Ivoire. To reach the objectives, we must:

- Prepare the leaves of *Aloysia citrodora.*
- Produce the tisane from *Aloysia citrodora'*s leaves.
- Extract the juice of Zingiber officinale.
- Assess biochemical, microbiological load and of the resulting tisane.
- Evaluate sensory evaluation.

II. MATERIALS AND METHODS

2.1 Materials

Dried leaves of *Aloysia Citrodora* and fresh ginger rhizomes were the materials of this study. The fresh leaves were harvested from those which were cultivated on 600 m^2 space in the village called Kouitongouiné in the department of Gbangbégouiné Yati, the region of Man (Côte d'Ivoire). The camp is 4 km from Man's University. Fresh ginger rhizomes were obtained from local « Ermankono » market (Côte d'Ivoire).

2.2 Methods

2.2.1 Preparation of *Aloysia citrodora's* leaves

Fresh leaves were harvested and quickly send to the laboratory. The leaves were manually cleaned to remove stones and other unwanted debris, washed under tap water to remove dust, dirt and adhered material. They were also washing thoroughly in sterile de-ionized water and 1% salted water during 1 to 5 minutes to remove germs. They are rinsed again after the previous step (De Saint Sauveur, 2010). The sample was placed in a nettle for 15 minutes. The fleshed leaves were dried at room temperature without ligth (Bidima, 2016).

2.2.2 Production of Herbal Tea Beverage

The herbal tea beverage was prepared according to Felgines *et al.* (2014). 40 g of dried leaves of *A. citrodora* were soaked in 1L boiling water for 30 min. the infusions leaves were liquated and keep at -20°C before different analysis.

2.2.3 Production of aqueous extract of ginger

Clean fresh ginger rhizomes were chopped to small pieces using a knife by cutting cross with a thickness. Furthermore, the rhizomes were put into 50 mL of de-ionized water and the overall were grounded by using laboratory scale blender (Kenwood blender) until to get a fine extract. 100 mL of water was added to the sample of ginger, furthermore, filtration using filter paper was conducted to separate filtrate / extract from the solid waste. Ginger extracts were stored in sterile dark amber bottles at 5°C (Yeo *et al.* 2014).

2.3 Biochemical characterization

- pH was determined with a pH meter (Testo 230 type 4, France) (Guerra and Mujica 2010).
- Dry matters were determined following the methods of Official AOAC (AOAC, 1990).

- Ash was determined following the methods of Official BIPEA (BIPEA, 1976).
- Insoluble Solids quantified by Weende's method (Multon, 1991).
- Fat acid was determined by extraction with soxhlet (AOAC, 1975).
- Total sugar and Reducing sugar, content of was measured following the methods described by the official AOAC (AOAC, 1995).
- The total phenol content of the solution was determined spectrophotometrically using Folin-Ciocalteu's method described by Singleton et al. Rossi (1965) modified by Wood *et al.* (2002).
- Antioxidant Activity was evaluated as ABTS according the method described by par Choong *et al.* (2013).
- Micronutrients such as : Phosphorus (P), Potassium (K), Zinc (Zn), Calcium (Ca), Iron (Fe) and Iod (I) by fluorescence X, following the method described by Sawadogo *et al.* (2014).

2.4 Microbiological analysis

Microbial analysis was carried out on the tisane control and tisane of Ginger (*Zingiber officinale)* and *Aloysia Citrodora*. Different dilutions have been conducted according to NF ISO 7218 (AFNOR. 1996).

2.4.1 Total viable count

Microbial count was carried out using plate count agar (PCA) for total viable (TVC), incubated at 30 °C for 72h. The method used was described by AFNOR rules NF V 08-051 (AFNOR, 1999).

2.4.2 Fungal count

Potato dextrose agar (PDA) was those used for fungal count and incubated at 37°C overnight and enumerated while PDA was incubated for 48 h according to NF-V08-022 rules.

2.4.3 Enumeration of indicator bacteria, contamination

2.4.3.1 Staphylococcus aureus.

Mannitol salt agar (MSA) for staphylococci count was used by modified AFNOR V-08-014 method.

2.4.3.2 Thermotolerant Coliform and Escherichia coli

The count of thermotolerant coliforms was done by AFNOR (1974), by AFNOR V-08-013 and sulfite-reducing anaerobes by NF EN 15213.

2.5 Sensory Attributes

Characterizations evaluated for sensory attributes were color, texture, hardness, chewiness, sweetness, saltiness, pleasantness, spicy and overall acceptance by unstructured scaling method or quantitative descriptive analysis (QDA) (Stone *et al*. 1974). The panel consisted of 17 people comprising both male and female, often participated in sensory evaluation. The descriptors of sensory attributes explained orally to the panelists. The scorecard consisted of a scale from 0 to 10. Panelists asked to record each evaluation, by marking it (0 to 10) according to their intensity or perception of the magnitude of each attribute.

2.6 Statistical Analysis

All the experiments were carried out in triplicates (n = 3) and the results expressed as mean ± standard deviation and percentage (SD) using Microsoft Excel software. Post hoc comparisons made by least significant difference (LSD). IBM SPSS Statistical software version 20.0 used to analyze the results. The comparisons of mean were made by the analysis of variances (ANOVA) at 0.05 significance level.

III. RESULTS

3.1 Biochemical properties of Ginger (*Zingiber officinale*), control tisane of *Aloysia citrodora* leaves (T0) and enriched tisane of *Aloysia citrodora* at 2.5% *Zingiber officinale* (T2.5)

The results of physical properties like pH, color, dry matter, turbidity, filterability, insoluble solids, water activity and ash content of Z. officinale enriched jaggery are represented in Table 1.

It was noted an improvement of the studied parameters of tisane enriched by ginger. This improvement concerned dry matter content which was 88.92±3.92% with control tisane (T0) increased to reach 90.07±2.91% with tisane enriched at 2.5% ginger.

Results have also shown increased phenolic content, crude fiber and antioxidant capacity in enriched tisane compared to it respective control. Its where respectively 11.68±4.05mg gallic Acid Eq /g, 5.12±2.7% et 3.96±1.58 µM Trolox Eq / Kg versus 15.90±0.42 mg Gallic Acid Eq /g, 3.07±0.40% et 6.06±0.51 µM Eq Trolox/ Kg.

TABLE 1:
BIOCHEMICAL PROPERTIES OF GINGER (*ZINGIBER OFFICINALE*), CONTROL TISANE OF ALOYSIA CITRODORA LEAVES (T0) AND ENRICHED TISANE OF *ALOYSIA CITRODORA* AT 2.5% *OFFICINALE* (T2.5) *ZINGIBER*

Parameters	Solution of *Zingiber officinale*	Control Tisane of *Aloysia citrodora* (T0)	Enriched tisane of *Aloysia citrodora* at 2,5% *Zingiber officinale* (T2.5)
pH	5.94±0.07a	6.9±2.17b	6.04±0.17c
Dry matter (%)	19.07±6.22a	88.92±3.92b	90.07±2.91c
Total phenol content (mg GAE* /g)	5.91±0.32a	11.68±4.05b	15.90±0.42c
Total sugar (mg equivalent glucose/g)	1.20±0.12a	47.78±3.06b	48.50±4.22c
Reducing sugar (mg equivalent glucose/g)	0.08±0.00a	0.10±0.03a	0.11±0.00a
Fat (%)	10.63±2.58a	13.13±3.72b	12.67±3.68c
Ash (%)	3.57±0.05a	1.64±1.29c	3.07±0.40b
Insoluble solids (%)	2.02±0,02a	5.12±2.70b	6.07±0.02c
Antioxidant Activity (µM Eq Trolox/ Kg)	5.01±0,01a	3.96±1.58b	6.06±0.51c

**** GAE: Gallic acid equivalent***

3.2 Minerals assessment

Significant results were noticed for the mineral analysis of ginger enriched tisane as depicted in table 2.

The controlled sample exhibited lower values for all the minerals as presented in table 2. The level of Ca particularly increased three times in enriched ginger tisane compared to the control tisane (192888.9 ppm vs 535 130.4 ppm). The observation was the same concerning Zn, the content was the twice of those of the control tisane (991.2 ppm vs 1920.6 ppm). The level of phosphorus was also increased 9 times in the enriched ginger tisane.

TABLE 2
MINERALS COMPOSITION OF CONTROL TISANE OF *ALOYSIA CITRODORA* LEAVES (T0) AND ENRICHED TISANE OF *ALOYSIA CITRODORA* AT 2.5% *ZINGIBER OFFICINALE* (T2.5)

Parameters	Composition (ppm)	
	T0	**T2.5**
P	10.7	98,58
K	15977.0	247730.7
Zn	991.2	1920.6
Ca	192888.9	535130.4
Fe	50062.5	6641.7
I	9.18	9,8

3.3 Microbial quality of different tisane samples

Results of the microbial load of tisane of Aloysia citrodora with or without ginger were presented in Table 3. A kind of reduction in microbial load was also found for each sample for yeast, fungus and coliform (Table 3). Coliform load was not found in all samples. Fecal coliform is very harmful to human health and in this study fecal confirmation test was done and fecal coliform was not observed. The same results were found for staphyloccocus aureus, Sulfite-reducing Anaerobes, Salmonella spp, Yeasts and moulds. So, it can postulate that properly boiled tea is safe to drink for human health.

TABLE 3
MICROBIALS LOAD OF CONTROL TISANE OF *ALOYSIA CITRODORA* LEAVES (T0) AND ENRICHED TISANE OF *ALOYSIA CITRODORA* AT 2.5% *ZINGIBER OFFICINALE* (T2.5)

Microbial load (CFU)	Samples		
	T0	T2.5	E U criteria 2005
Total viable count 30°C	< 10cfu/ml	< 10cfu/ml	
Total Coliforms /g	No growth	No growth	10 ufc/ml
Thermotolerant coliforms	No growth	No growth	10 ufc/ml
Staphylococcus aureus /g	No growth	No growth	No growth
Sulfite-reducing Anaerobes	No growth	No growth	No growth
Salmonella spp	No growth	No growth	No growth
Yeasts and moulds	No growth	No growth	No growth

CFU* = Colony forming unit

3.4 Sensory Attributes of control tisane of *Aloysia citrodora* leaves (T0) and enriched tisane of *Aloysia citrodora* at 2.5% *Zingiber officinale* (T2.5)

Sensory attributes such as color, texture, appearane, taste, Aroma and overall acceptance of control tisane and tisane of *Aloysia citrodora* at 2.5% *Zingiber officinale* (T2.5) were evaluated by a quantitative descriptive analysis method (Figure 1).

Concerning the color, the score obtained was respectively 6.8 and 6.7 for the T2.5 and T0. There was no difference in taste among T2.5 and T0 (5 vs 5.1). But it has been noted another trend about texture and appearance. Texture's score of T0 was 3.6 but 4.4 for T2.5. Those of appearance has increased from 3.4 (T0) to reach 4.5 (T2.5: tisane with incorporate ginger), it was an improvement of appearance.

The result has showed that aroma has changed positively. The score was 4 for control tisane and 6 for tisane T2.5

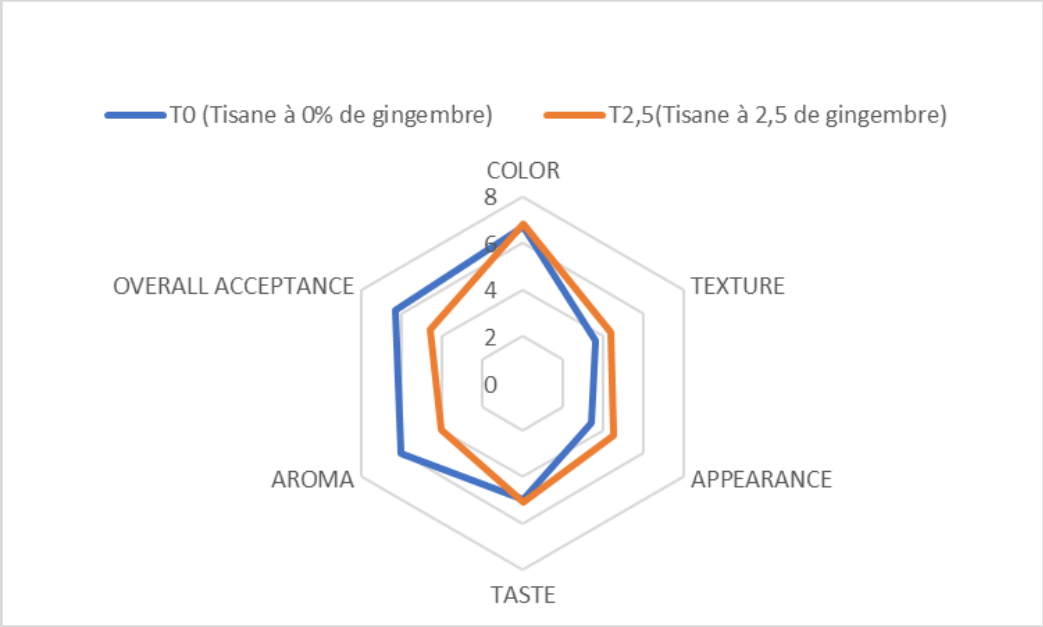

FIGURE 1: Star diagram of sensory attributes of control tisane and enriched tisane at 2.5 % Ginger (*Z. officinale*) Enriched

IV. DISCUSSION

Since a long time, Aloysia citrodora is a medicinal plant which is used by native people for several indications such as asthma, flu, diarrhea, flatulence, insomnia, and rheumatism (Abuhamdah *et al.* Mohammed, 2013). Lemon verbena is also used in the treatment of nervous condition, tachycardia, migraine headache (Pascual *et al.* 2001). According to Yousef zadeh *et al.* Meshkatalsadat, (2013) and Abdi and co-workers (2020), it's also used to treat hyperglycemia and certain cancers.

The high antioxidant activity of *Aloysia citriodoara* tisane would be due to the trapping activity of the superoxide radical and a moderate activity vis à vis to the hydroxyl radical and hypochlorous acid (Valentao *et al.*, 2002).

Add aqueous extract of *Zingiber officinale* at the tisane from *Aloysia citriodora* improved the phenol content of the obtained tisane. Lenoir (2011) showed that Polyphenols enhance total oxidant-scavenging. Indeed, during the inflammation, hydroxyl radical increased in tissues and plasma. The study done on mice, have indicated that, increasing of hydroxyl radical was reduced among them by using luteonin (Ashok kumar *et al.*, 2008). Phenolic compounds have been widely studies not only for its antioxydants properties but also for its ability to improve Cellular Antioxidant Defense. Furthermore, the study conducted among human has shown that tisane at 2.5% of ginger did not presented an anxiety and sedative effect mechanistically (Marty 2017).

V. CONCLUSION

This study confirms one of the official missions attributed to the University of Man. One of the goals is to generate an innovative beverage by local products from the region or not. The study consist to formulate and analyse the herbal infusions prepared from formulations of indigenous herbs (*Aloysia Citrodora*) and spices (*Zingiber officinale*) and to analyse for its biochemical, nutrient content and evaluate sensoials properties.

At the end of this study, the results have showed that the formulate beverage which have high amounts of bioavailable bioactive compounds and mineral contents. The elemental content of tea beverage was found to be high for Calcium and Zinc. Microbial load were found in acceptable limit. The sensory qualities were better with incorporate aqueous extract of ginger.

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