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# Polyphenols content and microbial load of stem barks of *Mitragyna ledermannii* (K. Krause) Ridsdale (Rubiaceae), during storage on markets of the District of Abidjan (Côte d'Ivoire)

**Kouamé Kouassi Bernadin[1,2], Coulibaly Kalpy Julien[3], Kabran Guy Roger Mida[4], Mamyrbekova-Békro Janat[4], Koné Mamidou Witabouna[1,2*]**

[1]*UFR Sciences de la Nature, Université Nangui Abrogoua, BP 801 Abidjan 02, Côte d'Ivoire*
[2]*Unité de Botanique et Médecine traditionnelle, Institut Pasteur de Côte d'Ivoire, BP 490 Abidjan 01, Côte d'Ivoire*
[3]*Unité de Chimie et Microbiologie de l'Environnement, Institut Pasteur de Côte d'Ivoire, BP 490 Abidjan 01, Côte d'Ivoire*
[4]*UFR Sciences Fondamentales et Appliquées, Laboratoire de Chimie Bio-organique et Substance Naturelles, Université Nangui Abrogoua, BP 801 Abidjan 02, Côte d'Ivoire*

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***Corresponding Author***

Name: Koné Mamidou Witabouna
E-mail: konemamidou.sn@univ-na.ci

**ABSTRACT**

Contamination of plant-based drugs by microorganisms is a common problem in many countries. However, reports on the influence of microflora on chemical quality of medicinal plants are scarce. The present study was conducted to access the evolution of polyphenols content of the stem bark of *Mitragyna ledermannii* (K. Krause) Ridsdale, a medicinal plant stored and sold on the markets of the Abidjan District. The polyphenols content was assessed using spectrophotocolorimetry on 32 samples that had microbial load ≥105 CFU/g. Polyphenols content ranged from 1366.67 µg EAG/g Dry Matter to 17266.67 µg EAG/g Dry Matter. The correlation established between the polyphenol content and microbial loads revealed three trends: increase of polyphenols content and decrease of the load of microflora or inversely, increase of polyphenols content and increase of the load of microflora and finally decrease of polyphenols content and load of microflora. These results show that in overall the microbial contamination may alter polyphenol content of raw plant material sold on markets. However, other factors as temperature can be incriminated.

## Introduction

The therapeutic efficiency of medicinal plants depends on quality of their metabolites. These chemical compounds can be damaged by various factors linked to the conditions of storage. According to WHO (OMS, 2002; 2005), the quality and safety of raw plant materials and finished products depend on intrinsic (genetics) or extrinsic factors such as environment, method of harvest, culture and processing after harvest, transport and storage. For example, the accidental contamination by microorganisms or chemicals, weather conditions, duration of day, have a great influence on chemical and biological qualities of medicinal plants (OMS, 2002; 2005).

Chemical modifications related to the storage can occured in plant organs (Fennell et al., 2004). Stafford et al. (2004) observed a reduction or loss of some compounds such as chlorophylls in *Vernonia colorata* (Willd.) Drake, *Leonotis leonurus* (L.) R. Br. and *Helichrysum cymosum* (L.) Less, plants used in traditional medicine. Also Mohamed and Nezam Deldin (1985) show that during the storage, the relative quality of acid phenols and soluble tannins decreases, flavones disappear and give oxidated compounds of brown color. Several factors can be responsible for these chemical modifications. According to Eloff (1999), a high microbial load can damage the active constituents of plants. Recent microbiological analysis of stem bark of *Mitragyna ledermannii* (K. Krause) Ridsdale (Rubiaceae) revealed high microbial loads largely beyond the WHO (≥$10^5$ CFU/g) limits (Kouamé et al., 2018). This microbial flora include Total Aerobic Mesophilic Flora, *Staphylococcus aureus*, *Escherichia coli*, thermotolerant colifomes, total coliforms, yeasts and molds, *Enterococcus* spp and *Pseudomonas* spp. *M ledermannii* is one of the most marketed medicinal plants on the markets of the District of Abidjan.

The stem bark and leaves of *M. ledermannii* are used against common diseases such as dysentery, fever, malaria and gonorrhea (Fofana, 2004). The phytochemical screening of various organs of this plant showed the presence of several phytocompounds such as alkaloids, tannins, flavonoids, sterols and terpenes, saponosides, reducing compounds, mucilages, anthracene derivatives, cardiotonic heterosides, coumarins (Bidie et al., 2011; Mouellet, 2004; Obouayeda et al., 2014), polyphenols, anthocyanins, proteins, carbohydrates, glycoproteins and polysaccharides (Fofana, 2004). Polyphenols are compounds exhibiting many properties including antioxidant (Gurbuz et al., 2009), anti-mutagenic (Witchtl et al., 2003), anti-bacterial (Sannomiya et al., 2005), anti-carcinogenic (Vafeiadou et al., 2009) and anti-inflammatory (Sutradhar et al., 2008). Within the framework of the study undertaken by our team on *Mitragyna ledermannii*, the present study concentrates on the evolution of polyphenols in connection with the microbial loads of its stem barks.

## Materials and methods

### Sampling

The samples were collected from January to April 2016 on markets stalls of three settings (Abobo, Adjamé and Yopougon) of the Abidjan District. A total of 47 traders were visited : 20 from Abobo, 17 from Adjamé and 10 in Yopougon. Briefly, four samples were collected as followed:
Batch 1: at arrival of plants on the wholesale markets,
Batch 2: one month later in the saleswomen's shop
Batch 3: two months later (same stock),
Batch 4: three months (same stock).
For this present study, only the most contaminated stem barks of *M. ledermannii* (≥$10^5$ CFU/g) were selected.

### Study of microbial load

From each sample, 25 g of powder were mixed with 225 mL of peptone water. The mixture was homogenized and kept at room temperature for 10 min. Then three serial dilutions ($10^{-1}$, $10^{-2}$ $10^{-3}$) were made from this initial suspension. Samples were tested for count of aerobic mesophilic bacteria (Plate count Agar) (NF V08-051, 1999), coliforms (Violet Red Bile Lactose Agar) (NF V08-050, 2009), *Escherichia coli* (Trypone Bile X Glucuronide) (NF V08-060, 2009), *Enterococci* (Bile Esculine Azide Agar) (NF EN ISO 6888-1, 1999), *Staphylococcus aureus* (Baird Parker) (NF ISO 21528-2, 2004), *Pseudomonas* (Cetrimide, King A, King B) (NF EN ISO 13720, 2010), yeast and mould (Chloramphenical Sabouraud Agar) (NF V08-059, 2002). The samples were analyzed using standard laboratory methods and procedures (Kouamé et al., 2018). The number of colony-forming units per gram (CFU/g) were counted and the average of all microbial loads was conducted.

### Preparation of plant extracts

The crude extracts were prepared from 15 g of powders in ethanol-water (50: 50) for 24 h under mechanical stirring. The mixture was filtered and the filtrate was dried in an oven at 40 °C.

### Determination of total polyphenols

Total polyphenols content were determined by the Folin-Ciocalteu method (Singleton et al., 1999; Heilerová et al., 2003). A volume of 1.5 mL of $Na_2CO_3$ (17 %, m/v) and 0.5 mL of Folin-Ciocalteu reagent (0.5 N) were added to 1 mL of plant extract (0.1g/mL). The mixture was incubated at 37 °C for 30 min then the absorbance was measured at 765 nm (blanc = methanol). Gallic acid was used as standard at a range of concentrations (0 to 1000 µg/mL). The content in total polyphenols (Q) was calculated using the following formula:

**Q = (V x C x d) /m ( µg EAG/g Dry Matter )**

**V**: final volume of the extract (mL); **C** : concentration of the extract (µg/mL); **d** :factor of dilution; **m** : mass of dry matter (g)

### Statistical Analysis

The results were analyzed using a variance analysis (one factor) performed with STATISTICA 7.1 software. The comparison of the means was carried out with the test of the Least significant difference (LSD) for classification of microbial loads and total polyphenols content. The differences were significant ($P$ < 0.05) and highly significant ($P$ < 0.001).

## Results

### Microbial load of samples studied

The average loads of microflora in the 32 samples are reported in Figure 1. For batches 1, 2, 3 and 4 (Adjame), the highest microbial load was obtained for batch 3 (P3). The values were respectively 2.9 x$10^7$, 3.7 x$10^7$ and 3.8 x$10^7$ CFU/g. For batches 3, 9 and 12 (Adjame), the highest microbial load was obtained for batch 2 (P2). The average loads of microorganisms were 3.2 x$10^6$ CFU/g for stock 3 and 2.7 x$10^7$ CFU/g for stock 9 and 12. For batches 15 (Adjame) and 5 (Yopougon), the highest microbial load was obtained for batch 1 (P1). The average loads of microorganisms were 3.0 x$10^7$ CFU/g for stock 15 and 1.6 x$10^7$ CFU/g for stock 5.

### Total phenols content of the plant extracts studied

The extract of the stem barks collected in the stock 1 (Williamsville/Adjame), showed a high total phenols content (17266.67 µg EAG/g DM). From the second to the third sampling, a progressive decrease of polyphenols was observed, with respective contents of 9433.33 µg EAG/g DM and 8033.33 µg EAG/g DM. On the contrary, for the fourth sampling, a light increase of the content was noticed. However, this value remains lower than that of sample 1. The analysis of variance showed a high significant difference ($P$ < 0.001) between the contents in total phenols of the various samples, with the highest content (17266.67 µg EAG/g DM) obtained for sample 1 (Table 1).

For batch 2 (Williamsville), a very low content in total phenols was recorded for sample 1 (1366.67 µg EAG/g DM) followed by a strong increase of polyphenols for sample 2 (15500 µg EAG/g DM). Then a decrease was observed from sample 3 (9300 µg EAG/g DM) to sample 4 (6233.34 µg EAG/g DM). There was a high significant difference ($P$ < 0.001) between the contents of the four samples (Table 1).

For batch 3 (Williamsville), the contents in total phenols obtained ranged from 9633.33 to 13833.33 µg EAG/g DM. The content decreased from 9633.33 µg EAG/g DM for sample 1 to 3233.33 µg EAG/g DM (sample 2) and 2466.67 µg EAG/g DM for sample 3. However, a strong increase of the content was observed in sample 4 (13833.33 µg EAG/g DM). The analysis showed a high significant difference between the contents of the four

samples, with the highest for sample 4 (Table 1). For batch 4 (Williamsville), the total phenols contents of sample 1 (5966.67 µg EAG/g DM) and sample 2 (5766.67 µg EAG/g DM) were approximately the same. An increase of this content was observed for sample 3 (8166.67 µg EAG/g DM) followed by a reduction in sample 4 (4200 µg EAG/g DM). The analysis of variance showed no significant difference between the contents of the first two samples. However, this difference ($P < 0.001$) was highly significant for sample 3 and 4, with the highest content (8166.7 µg EAG/g DM) for sample 3 (Table 1). The study of the stem barks collected in williamsville (stock 9), revealed a high content in total phenols for sample 1 (7833.34 µg EAG/g DM). This content decreased for sample 2 (5433.34 µg EAG/g DM) followed by a light increase for sample 3 (5800 µg EAG/g DM), then dropped down for sample 4 (5366.67 µg EAG/g DM). There was a high significant difference ($P < 0.001$) between the contents of the first three samples. No significant difference was observed between the contents of the samples 2 and 4 (Table 1).

The analysis of the stem barks of batch 12 ("quartier rouge"/Adjame), showed a progressive increase of the polyphenols content from sample 1 (2166.67µg EAG/g DM) to sample 3 (8033.34 µg EAG/g DM). For sample 4, the content decreased to 3800 µg EAG/g DM. The statistical analysis revealed a high significant difference ($P < 0.001$) between the four samples, with the greatest content for sample 3 (Table 1). For batch 15 ("quartier rouge" /Adjame), the contents were 4933.34 µg EAG/g DM, 5033.34 µg EAG/g DM and 4700 µg EAG/g DM respectively for samples 1, 2 and 3. There was no significant difference between these contents. An increase of the content (9233,34 µg EAG/g DM) was observed for sample 4 and the difference was highly significant ($P < 0.001$) with the other samples (Table 1). The stem barks collected in Wassakara/ Yopougon (batch 5) showed gradual increases in contents from sample 1 (9566.67 µg EAG/g DM) to sample 3 (11100.00 µg EAG/g DM). A reduction in the content was noticed in sample 4 (4500 µg EAG/g DM). The contents were significantly different ($P < 0.001$) (Table 1).

**Table 1.** Polyphenols content of the extracts of the stem barks of *Mitragyna ledermannii*

| Setting/stock | Batches | Means±SD (µg EAG/g DM) | Statistical parameters |
|---|---|---|---|
| Adjame stock 1 | 1 | 17266.67±58$^{d}$ | $P < 0.001$ |
| | 2 | 9433.33±115$^{b}$ | |
| | 3 | 8033.33±115$^{a}$ | |
| | 4 | 11766.67±115$^{c}$ | |
| Adjame stock 2 | 1 | 1366.67±115$^{a}$ | $P < 0.001$ |
| | 2 | 15500.00±200$^{d}$ | |
| | 3 | 9300.00±200$^{c}$ | |
| | 4 | 6233.33±115$^{b}$ | |
| Adjame stock 3 | 1 | 9633.33±115$^{c}$ | $P < 0.001$ |
| | 2 | 3233.33±115$^{b}$ | |
| | 3 | 2466.67±58$^{a}$ | |
| | 4 | 13833.33±115$^{d}$ | |
| Adjame stock 4 | 1 | 5966.67±115$^{b}$ | $P < 0.001$ |
| | 2 | 5766.67±115$^{b}$ | |
| | 3 | 8166.67±115$^{c}$ | |
| | 4 | 4200.00±100$^{d}$ | |
| Adjame stock 9 | 1 | 783.33±115$^{c}$ | $P < 0.001$ |
| | 2 | 5433.33±115$^{a}$ | |
| | 3 | 5800.00±100$^{b}$ | |
| | 4 | 5366.67±115$^{a}$ | |
| Adjame stock 12 | 1 | 2166.67±115$^{a}$ | $P < 0.001$ |
| | 2 | 2633.33±115$^{b}$ | |
| | 3 | 8033.33±115$^{d}$ | |
| | 4 | 3800.00±100$^{c}$ | |
| Adjamé stock 15 | 1 | 4933.33±58$^{a}$ | $P < 0.001$ |
| | 2 | 5033.33±115$^{a}$ | |
| | 3 | 4700.00±115$^{a}$ | |
| | 4 | 9233.33±115$^{b}$ | |
| Yopougon stock 5 | 1 | 9566.67±115$^{b}$ | $P < 0.001$ |
| | 2 | 10233.33±115$^{c}$ | |
| | 3 | 11100.00±115$^{d}$ | |
| | 4 | 4500.00±200$^{a}$ | |

The values bearing the same letter as exponent are not statistically different. SD = Standard Deviation

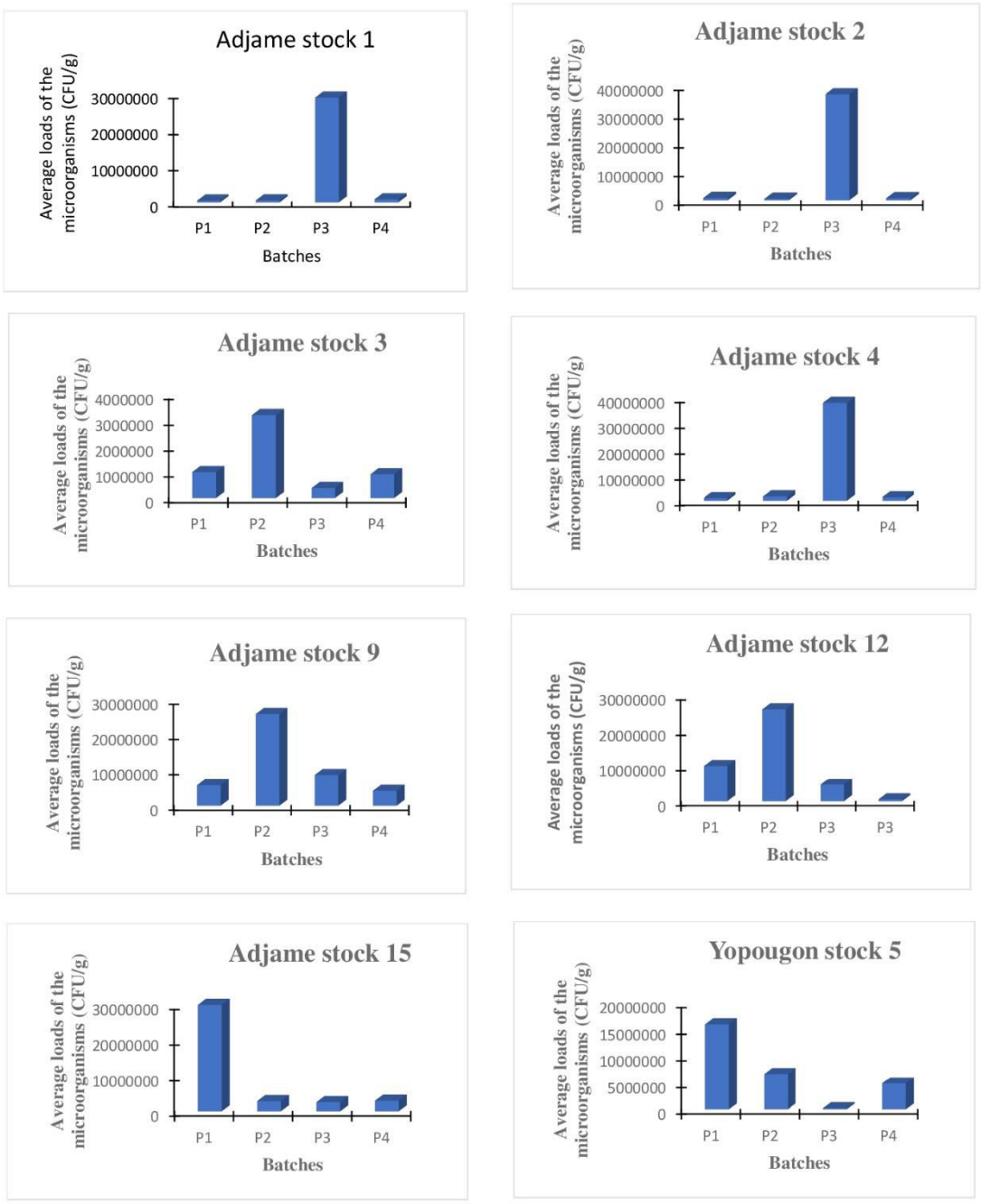


**Fig. 1**. Microbial loads of the stem barks of *Mitragyna ledermannii* collected in the District of Abidjan.

**CFU:** Colony Forming Unit; **P1**: Batch 1; **P2**: Batch 2; **P3**: Batch 3: **P4**: Batch 4

**Evolution of the polyphenols content and microbial loads**

The evolution of the content in total phenol and microbial load were analyzed. The results showed that some interaction may be established between these two parameters.

**Increase of polyphenols content and decrease of the load of microflora or inversely**

The results revealed that for some samples microbial load was low while total phenols content was high. This case was observed with samples of stocks 1 and 2. For batch 1 (Williamsville), samples collected at arrival had the lowest microbial load ($4.9x10^5$ CFU/g) while its content in total phenols was very high (17266.67 µg EAG/g DM). For sample 2, the microbial load increased ($5.5x10^5$ CFU/g) and the polyphenols content decreased (9433.34 µg EAG/g DM). A same trend was observed for sample 3. For sample 4, a high decrease of microbial load (from $2.9x10^7$ CFU/g to $8.1x10^5$ CFU/g) was associated with an increase of total phenols content (11766.67 µg EAG/g DM) (Figures 2 and 3).

**Increase of polyphenols content and increase of the load of microflora**

In this case, the results showed that the two parameters increased. This trend was observed with samples of batches 4 and 5. For batch 4 (Williamsville/Adjame), samples collected at arrival on market had high microbial load ($1.0x10^6$ CFU/g) and polyphenols content was 5966.67 µg EAG/g DM. Between samples 2 and 3, an increase of microbial load from $1.7x10^6$ to $3.8x10^7$ CFU/g was associated with, an increase of total phenols content from 5766.67 to 8166.67 µg EAG/g DM (Figure 4). For batch 5 (Wassakara/Yopougon), microbial loads of sample 1 were $1.6x10^7$ CFU/g and polyphenols content were 9566.67 µg EAG/g DM. For sample 2, microbial loads ($6.7x10^7$ CFU/g) and polyphenols content (10233.33 µg EAG/g DM) increased (Figure 5).

**Decrease of polyphenols content and load of microflora**

In this case, load of microflora and polyphenols content decreased. This situation was observed with samples of batches 4 and 9. For batch 9 (Williamsville), microbial loads of samples 1 was $5.8x10^6$ CFU/g and polyphenols content was 7833.34 µg EAG/g DM. For samples 3 and 4, microbial loads decreased from $8.6x10^6$ to $4.2x10^6$ CFU/g and the polyphenols content has also decreased from 5800 to 5366.67 µg EAG/g DM (Figure 6). A same trend was observed for stock 4 between samples 3 and 4 (Figure 4).

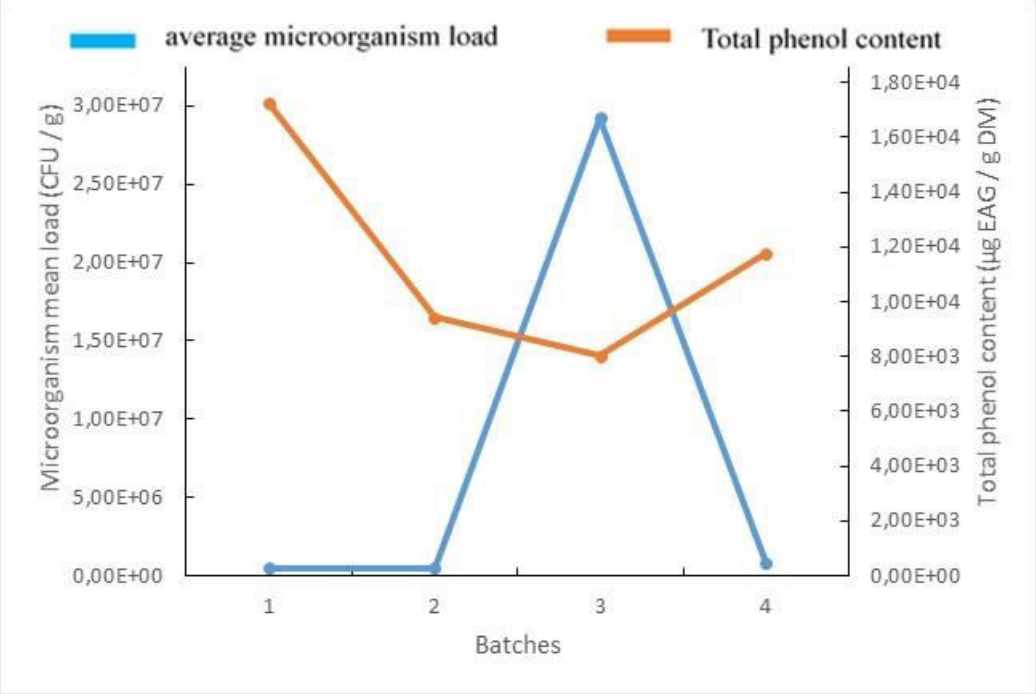


**Fig. 2.** Evolution of the microbial load and polyphenols content of the extracts of the stem barks of *Mitragyna ledermannii* from stock 1

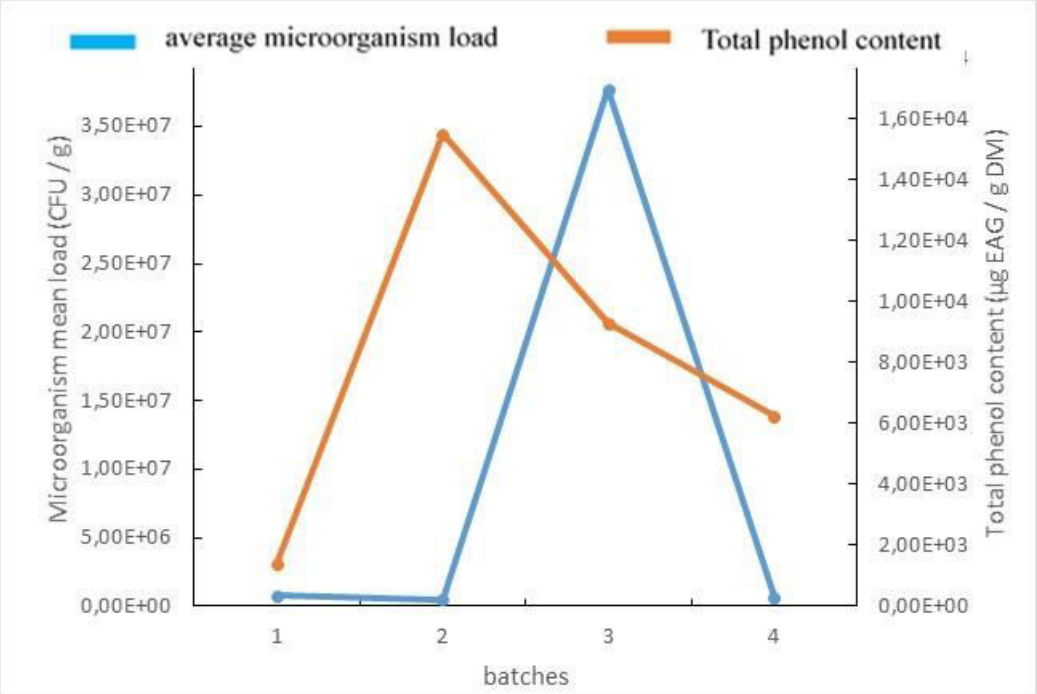


**Fig. 3.** Evolution of the microbial load and polyphenols content of the extracts of the stem barks of *Mitragyna ledermannii* from stock 2

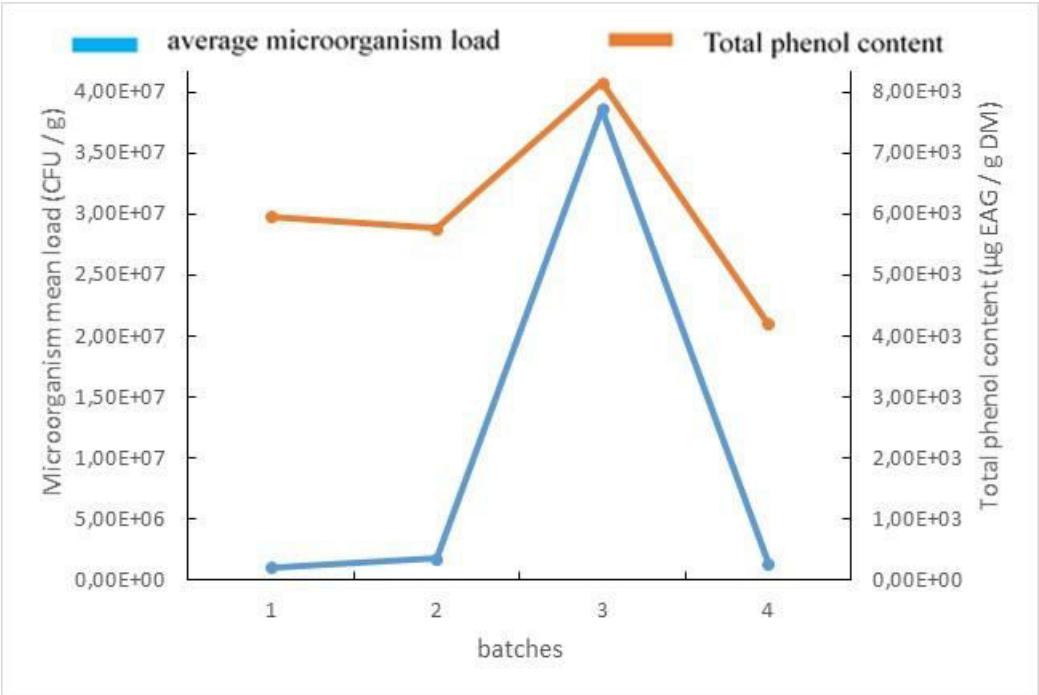


**Fig. 4.** Evolution of the microbial load and polyphenols content of the extracts of the stem barks of *Mitragyna ledermannii* from stock 4

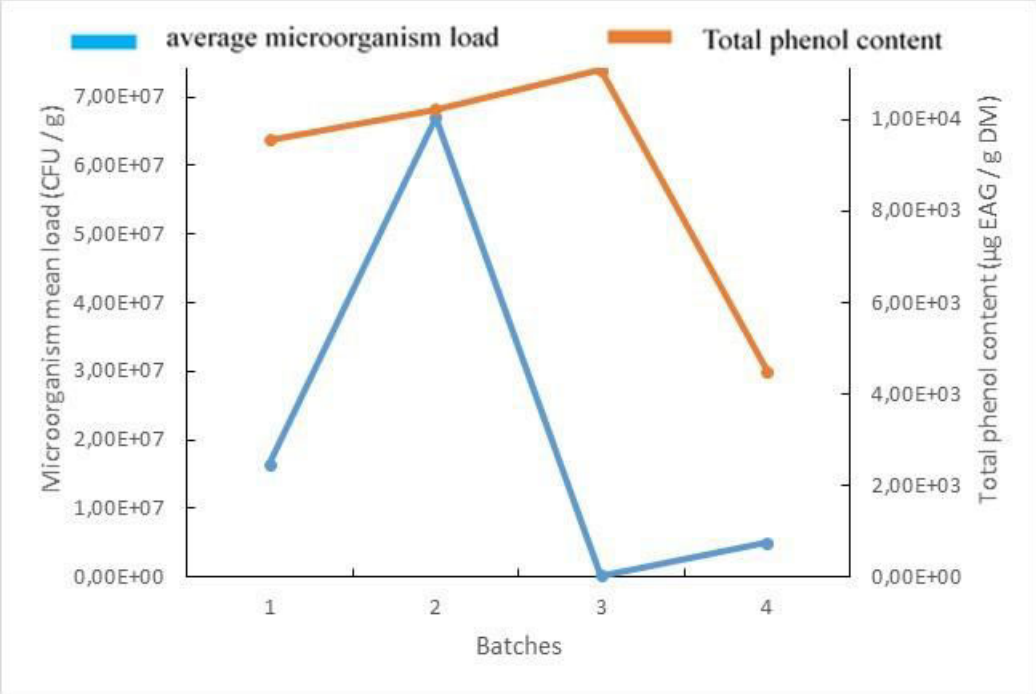


**Fig. 5.** Evolution of the microbial load and polyphenols content of the extracts of the stem barks of *Mitragyna ledermannii* from stock 5

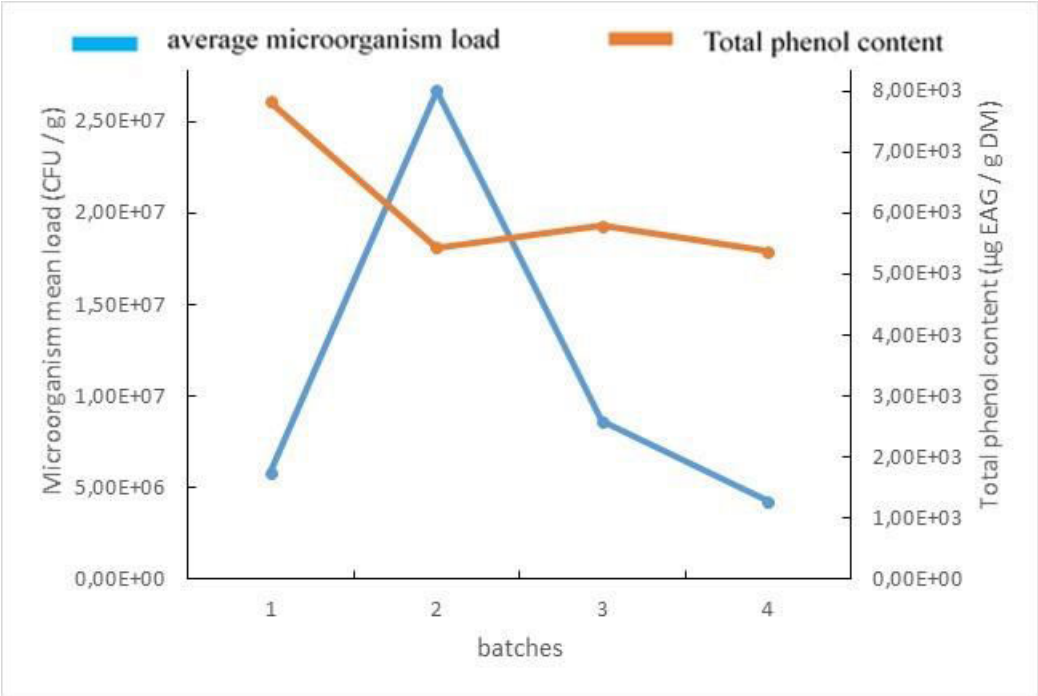


**Fig. 6.** Evolution of the microbial load and polyphenols content of the extracts of the stem barks of *Mitragyna ledermannii* from stock 9

## Discussion

The present study was conducted to access the evolution of polyphenols content of the stem bark of *Mitragyna ledermannii* a medicinal plant stored and sold on the markets of the Abidjan District. The polyphenols content of samples varied from 1366.67 µg EAG/g DM to 17266.67 µg EAG/g DM. By superimposing evolution of microbial load to polyphenols content, three trends were raised: increase of polyphenols content and decrease of the load of microflora or inversely, increase of polyphenols content and increase of the load of microflora and finally decrease of polyphenols content and decrease of the load of microflora.

An increase of polyphenols content and decrease of the load of microflora and inversely was observed for batch 5 (Yopougon) and batches 1, 2, 3, 4, 9, 12 and 15 (Adjame). These results show that the microbial flora could have a negative impact on some metabolites, in particular phenolic compounds in the stem barks of *Mitragyna ledermannii*. Similar evolution was also reported in several plants for other compounds. For *Glycyrrhiza glabra* L., Manisha et al. (2014) reported that the quantity of glycyrrhizin, a saponin decrease when microbial loads increase. Dutta and Roy (1992) showed that microbial load reduced percentage of strychnine and brucine, two alkaloids present in *Strychnos nux-vomica* L. The study of Lutomski et Kedzia (1980) revealed that when the load of *Aspergillus* and *Penicillium* is beyond $10^4$ CFU/mg, these moistures are growing in the plant tissues. Such a situation may be responsible for degradation of the active constituents. Microbes degrade sugars and starch components of plants for their growing. Prasad et al. (1985; 1988) reported degradation of sugars and starch by three moistures in *Terminalia belerica Roxb*. and *Terminalia chebula* Reiz. A total of 32 samples used for this study showed that moisture loads ($4.4x10^4$ to $2.4x10^7$ CFU/g) were superior to $10^4$ CFU/mg.

For samples 1 and 2 in batches 5 (Yopougon), 12 (Adjamé), and samples 2 and 3 in batch 4 (Adjame), results showed an increase of polyphenols content despite an increase of the load of microflora. This increase could be understood by activity of certain microorganisms. Several microbial such as *Penicillium, Mucor, Saccharomyces, Lactobacillus, Streptococcus, Pediococcus* and *Lactococcus* may increase degradation of carbohydrates, proteins and lipids (Ul-Haq, 2002; Guiraud and Rosec, 2004; Juszczyk et al., 2005; Kermiche, 2013). This degradation is slowly achieved according storage conditions (Multon, 1982) due to amylolytic, lipolytic and proteolytic enzymes (Popoola, Adeoti and Idakwo, 2003; Savijoki, Ingmer and Varmanen, 2006; Shahidan et al., 2011; Tiwari and Upadhyay, 2013). The products of degradation of these primary metabolites are involved in polyphenols biosynthesis (Akroum, 2011). The stem barks of *Mitragyna ledermannii* are rich in carbohydrates and proteins (Fofana, 2014). An increase of microbial load may accelerate the mechanism of degradation that will lead to an increase in the content of polyphenols. Wang and Zheng (2001) and Najem (2003) also noticed an increase of certain phenolic compounds such as flavonoids during storage.

From batches 2, 4 and 9 (Adjame), the microbial loads decreased and the polyphenols content decreased. This trend revealed that microorganisms are not the only factors that could influence the quantities of the secondary metabolites in medicinal plants stored and sold on markets. According to Aganga and Mosase (2001) and Pedneault et al. (2001), phenolic compounds can be modified by extrinsic factors such as the geographical, climatic and genetic factors.

The degree of maturation of plants, duration of drying and storage can cause modifications of their chemical composition. The losses are more important when the duration of the drying is long (Benjilali and Zrira, 2005) and when the temperature is high. The variations of polyphenol contents of the stem barks of *Mitragyna ledermannii* may be explained by the bad conditions of storage on markets.

## Conclusion

This study revealed that the microbial load of medicinal plants sold on markets may influence the content in phenolic compounds. However, other factors such as (temperature, humidity) may also play a role in chemical modifications.

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**Conflict of interest statement**

The authors declare that they have no conflict of interests.

**Contribution detail**

KONE Mamidou Witabouna conceived the study. KONE Mamidou Witabouna, COULIBALY Kalpy Julien, KABRAN Guy Roger Mida, MAMYRBEKOVA- Békro Janat designed the experiments. KOUAMÉ Kouassi Bernadin collected samples on markets, performed the experiments. All authors analysed the data, discussed the results and drafted the manuscript, the main contributor being KOUAMÉ Kouassi Bernadin. All the authors read and approved the final manuscript.

**Abbreviations**

WHO: World Health Organization
CFU: Colony Forming Unit
EAG/DM: Equivalent Acid Gallic/Dry Matter

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