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# Phytochemical and mineral characterization of the leafy stems of *Mezoneuron benthamianum* Baill: A traditional chewing stick plant from western Côte d'Ivoire

Monyn épouse Kouamé Ebalah Delphine [1,*], Kouamé N'Guessan Brigitte [1], Kouassi Kouamé Seraphin [2] and Koné Mamidou Witabouna [3]

[1] *Agronomic, Forestry, and Environmental Engineering, University of Man, PO Box 20 Man, Côte d'Ivoire.*
[2] *Fundamental and Applied Sciences, Nangui Abrogoua University, 02 BP 801 Abidjan 02, Côte d'Ivoire.*
[3] *Swiss Center for Scientific Research in Côte d'Ivoire, 01 BP 1303 Abidjan 01, Côte d'Ivoire.*

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## Abstract

Traditional chewing sticks are widely used in West Africa for oral hygiene and constitute a source of bioactive phytochemicals. *Mezoneuron benthamianum* Baill. is traditionally used in western Côte d'Ivoire for oral care, but its chemical profile remains poorly documented. This study aimed to characterize the phytochemical constituents, mineral composition, and chromatographic profile of its leafy stems. Aqueous and hydroethanolic extracts were analyzed through qualitative colorimetric reactions, thin-layer chromatography (TLC), and scanning electron microscopy coupled with energy-dispersive X-ray spectroscopy (SEM–EDS). The hydroethanolic extract showed a higher yield (17.6%) than the aqueous extract (13.7%). Qualitative colorimetric reactions revealed polyphenols, flavonoids, tannins, coumarins, sterols/polyterpenes, reducing sugars, and proteins, while TLC confirmed several secondary metabolite classes. SEM–EDS showed a mineral profile dominated by calcium (26974.9 mg/kg), potassium (8201.6 mg/kg), phosphorus (968.4 mg/kg), and magnesium (934.1 mg/kg). These findings provide preliminary scientific evidence supporting the traditional use of *Mezoneuron benthamianum* as a chewing stick for oral hygiene.

**Keywords:** *Mezoneuron benthamianum*; Chewing Stick; Phytochemical Profile; Mineral Composition; Thin-Layer Chromatography

## 1. Introduction

Medicinal plants remain an important source of bioactive compounds and continue to contribute significantly to traditional healthcare systems, particularly in developing countries where access to modern therapeutic products may be limited [1]. Among these resources, plants used for oral hygiene, including chewing sticks, have received increasing scientific interest because of their potential to contain secondary metabolites with antimicrobial, antioxidant and protective properties against oral diseases [2,3]. Chewing sticks derived from different plant species have been traditionally used in several African communities for tooth cleaning and maintenance of oral health. Their biological properties are generally associated with the presence of phytochemicals such as polyphenols, flavonoids, tannins and other phenolic compounds, which may contribute to their beneficial effects in the oral environment [4]. In addition, the mineral composition of plant materials represents an important parameter for their biological characterization, as elements such as calcium, magnesium, phosphorus, iron and zinc are involved in various physiological functions related to tissue integrity and general health [5]. Among the plant species traditionally used as toothbrush sticks in western Côte d'Ivoire, *Mezoneuron benthamianum* Baill. (Fabaceae) is widely employed by local populations for oral hygiene purposes. This species belongs to the Fabaceae family, which includes numerous plants known for their richness in secondary metabolites and their diverse biological activities [6]. However, information regarding its detailed

* Corresponding author: Monyn épouse Kouamé Ebalah Delphine

phytochemical profile, mineral composition and chromatographic characteristics remains limited. A better understanding of its chemical composition is necessary to provide scientific support for its traditional use and contribute to the valorization of indigenous plant resources. Therefore, this study aimed to investigate the phytochemical profile, mineral composition and chromatographic characteristics of *M. benthamianum* Baill. leafy stems used as a traditional toothbrush plant in western Côte d'Ivoire.

## 2. Materials and Methods

### 2.1. Plant material, study area and sample preparation

The plant material consisted of leafy stems of *Mezoneuron benthamianum* Baill., a species traditionally used as a chewing stick for oral hygiene by local populations in western Côte d'Ivoire. The samples were collected in Man, Tonkpi Region, western, Côte d'Ivoire (Figure 1). This region is characterized by a tropical humid climate and rich floristic diversity, including several medicinal plant species traditionally used in primary healthcare.

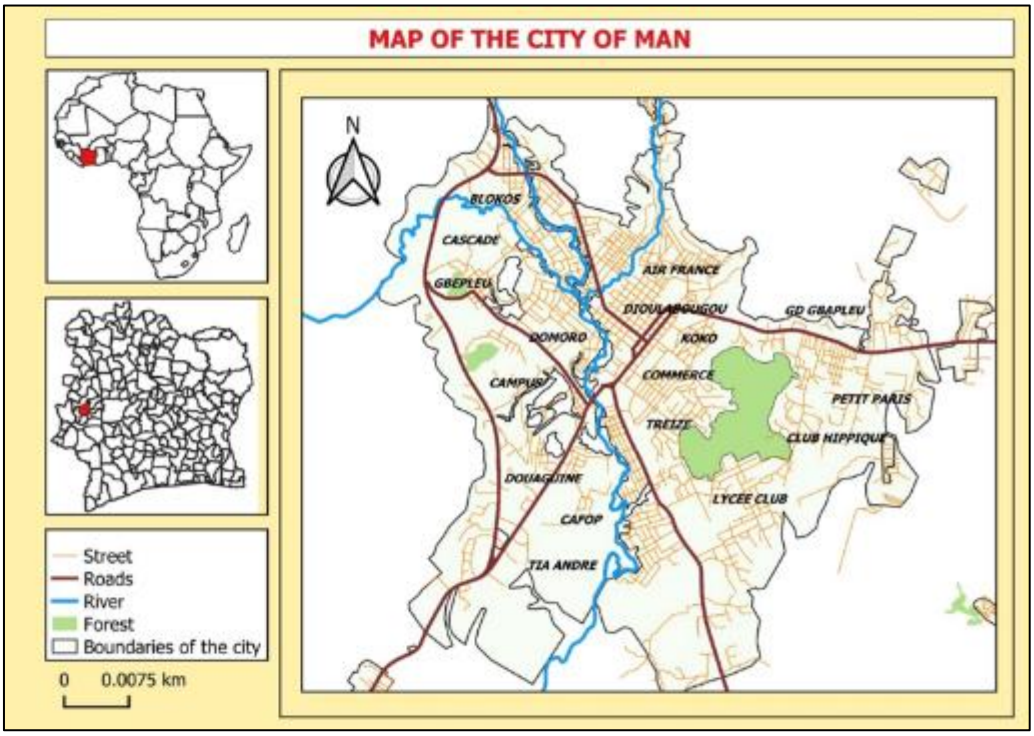


**Figure 1** Location map of the sampling site of Mezoneuron benthamianum Baill. in Man, western Côte d'Ivoire

The collected plant samples were authenticated at the National Floristic Center (CNF) of Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire. After botanical identification, a voucher specimen was deposited in the CNF herbarium under the accession number UCJ009456 for future reference. The leafy stems of *M. benthamianum* were washed with distilled water, air-dried at room temperature under shade, and then ground into a fine powder using an electric grinder. The obtained powder was stored in airtight containers until extraction and chemical analyses. A photograph of the studied species is presented in Figure 2.

(Source : Authors)

**Figure 2** Morphological appearance of *Mezoneuron benthamianum* Baill. collected in western Côte d'Ivoire

## 2.2. Extraction and preparation of plant extracts

### *2.2.1. Aqueous extraction*

Aqueous extraction was performed according to conventional procedures used for medicinal plant studies [7]. Briefly, one hundred grams (100 g) of *Mezoneuron benthamianum* powdered leafy stems were macerated in 1 L of distilled water for 24 h under continuous magnetic stirring. The obtained macerate was filtered successively through sterile cotton and filter paper to remove plant debris and obtain a clear filtrate. The filtrate was then concentrated and dried in an oven at 50 °C until a constant weight was obtained. The dried aqueous extract was weighed, transferred into airtight containers, and stored at 4 °C until further chemical analyses.

### *2.2.2. Hydro-ethanolic extraction*

Hydro-ethanolic extraction was carried out according to previously described procedures for medicinal plant extracts [7]. Briefly, one hundred grams (100 g) of powdered leafy stems were macerated in 1 L of 70% ethanol (v/v) for 24 h under magnetic stirring. The macerate was filtered using the same procedure described for the aqueous extract. The obtained filtrate was concentrated under reduced pressure using a rotary evaporator and dried at 40 °C. The resulting hydro-ethanolic extract was stored in airtight containers at 4 °C until chemical analyses.

## 2.3. Determination of extraction yield

The extraction yield was calculated according to the following formula :

$$\text{Extraction yield (\%)} = \frac{\text{Mass of dry extract (g)}}{\text{Mass of plant powder used (g)}} \times 100$$

where:

Mass of dried extract represents the weight of the dried extract obtained;

Mass of plant powder used represents the initial amount of plant powder subjected to extraction.

### 2.4. Mineral composition analysis

The mineral composition of *Mezoneuron benthamianum* leafy stems was determined after mineralization of the plant material. The powdered samples were incinerated at 550 °C for 24 h in a Naberthern MORE THAN HEAT furnace at the Centre National de Recherche Agronomique (CNRA), Bingerville, Côte d'Ivoire. The obtained ashes were analyzed at the Centre d'Analyses et de Recherche (CAR) of PETROCI, Abidjan, Côte d'Ivoire. The elemental composition was determined using a variable-pressure scanning electron microscope coupled with an energy-dispersive X-ray spectroscopy system (SEM-EDS) (MEB FEG Supra 40 VP Zeiss) equipped with an Oxford Instruments X-ray detector and an Inca Dry Cool microanalysis platform. The analyses were performed using variable accelerating voltages ranging from 0.1 to 30 keV. Different magnifications (12× to 1,000,000×) were used according to sample characteristics, with a resolution of 2 nm. The mineral concentrations were expressed directly as elemental concentrations, in percentage (%) and milligrams per kilogram of dry matter (mg/kg DM). Mineral analysis was performed in triplicate, and results were expressed as mean values.

### 2.5. Phytochemical screening

*2.5.1. Qualitative colorimetric reactions*

The phytochemical screening of *Mezoneuron benthamianum* leafy stem extracts was performed to identify the main classes of secondary metabolites using qualitative colorimetric reactions according to previously described methods [8,9]. The analyses focused on the detection of polyphenols, flavonoids, tannins, coumarins, sterols/polyterpenes, alkaloids, saponins, anthocyanins, reducing sugars and proteins. Specific chemical reagents were used to reveal each group of compounds. The presence or absence of metabolites was determined based on the development of characteristic colors or precipitates after reaction with the corresponding reagents [8]. The results were recorded as positive (+) or negative (−) according to the observed reactions.

*2.5.2. Thin-layer chromatography (TLC) analysis*

Thin-layer chromatography (TLC) was performed to characterize the chemical profiles of the *Mezoneuron benthamianum* extracts according to standard procedures used for the analysis of plant secondary metabolites [10], with slight modifications. The extracts were dissolved in methanol at a concentration of 10 mg/mL. Twenty microliters (20 µL) of each extract solution, corresponding to 200 µg of extract, were applied onto silica gel 60 F254 aluminium plates (Merck) using capillary tubes. After application, the plates were developed in a saturated chromatographic chamber containing the mobile phase chloroform/ethyl acetate/acetic acid/water (2.5:7:0.2:1: v/v/v/v), with two drops of ammonia solution added to the mixture. After migration, the plates were removed, dried, and visualized under visible light and ultraviolet radiation at 254 and 365 nm.

Specific spraying reagents were used for the detection of different groups of secondary metabolites, including ferric chloride ($FeCl_3$, 2%) for phenolic compounds and tannins, ethanolic aluminium chloride ($AlCl_3$ 5%) for flavonoids, a methanolic solution of potassium hydroxide (KOH, 5%) for anthraquinone derivatives and ammonia solution (25%) for coumarins [7,8]. The retention factor (Rf) values of the detected spots were calculated using the following equation:

$$\text{Rf} = \frac{\text{Distance travelled by the compound}}{\text{Distance travelled by the solvent front}}$$

### 2.6. Statistical analysis

Data obtained from the extraction yields and mineral composition analyses were recorded and processed using Microsoft Excel software. The results were expressed as mean values of the measurements performed. Descriptive statistical analysis was used to summarize the obtained data and present the mineral concentrations and extraction yields of *Mezoneuron benthamianum* extracts.

## 3. Results and discussion

### 3.1. Extraction yields of *Mezoneuron benthamianum* extracts

The extraction yields obtained from the leafy stems of *Mezoneuron benthamianum* varied according to the solvent used. The aqueous extract showed a yield of 13.7%, whereas the hydro-ethanolic extract exhibited a higher yield of 17.6% (Table 1).

**Table 1** Extraction yields of aqueous and hydro-ethanolic extracts of *Mezoneuron benthamianum*

| Extract of Mezoneuron benthamianum | Powder mass (g) | Extract mass (g) | Yield (%) |
|---|---|---|---|
| Hydro-ethanolic | 100 | 17.6 | 17.6 |
| Aqueous | 100 | 13.7 | 13.7 |

The higher yield obtained with the hydro-ethanolic extract may be attributed to the intermediate polarity of the solvent mixture, which facilitates the extraction of a wider range of plant constituents compared with water alone [11]. Similar differences in extraction yields according to solvent polarity have been reported in medicinal plant studies [7].

### 3.2. Mineral composition of *Mezoneuron benthamianum*

The mineral composition of *Mezoneuron benthamianum* leafy stems was determined by elemental analysis. The results revealed the presence of several macroelements and trace elements, indicating a diversified mineral profile (Table 2).

**Table 2** Mineral composition of leafy stems of *Mezoneuron benthamianum* Baill. (mg/kg dry matter)

| Symbol | Element | Concentration (%) | Concentration (mg/kg DM) | Absolute error (%) |
|---|---|---|---|---|
| Na | Sodium | 0.0141 | 141 | 0.0015 |
| Mg | Magnesium | 0.09341 | 934.1 | 0.0016 |
| Al | Aluminium | 0.07331 | 733.1 | 0.0008 |
| Si | Silicon | 0.12788 | 1278.8 | 0.0009 |
| P | Phosphorus | 0.09684 | 968.4 | 0.0005 |
| S | Sulfur | 0.14707 | 1470.7 | 0.0005 |
| Cl | Chlorine | 0.04311 | 431.1 | 0.00007 |
| K | Potassium | 0.82016 | 8201.6 | 0.0021 |
| Ca | Calcium | 2.69749 | 26974.9 | 0.004 |
| Mn | Manganese | 0.00799 | 79.9 | 0.00018 |
| Fe | Iron | 0.0353 | 353 | 0.00025 |
| Cu | Copper | 0.00062 | 6.2 | 0.00004 |
| Zn | Zinc | 0.00086 | 8.6 | 0.00003 |
| Se | Selenium | <0.00005 | <0.5 | 0 |

DM: dry matter

Among the macroelements, calcium was the most abundant element with a concentration of 26974.9 mg/kg dry matter, followed by potassium (8201.6 mg/kg), sulfur (1470.7 mg/kg), silicon (1278.8 mg/kg), phosphorus (968.4 mg/kg) and magnesium (934.1 mg/kg). Sodium was detected at a lower concentration (141 mg/kg) (Table 2). The distribution of the main macroelements is illustrated in Figure 3.

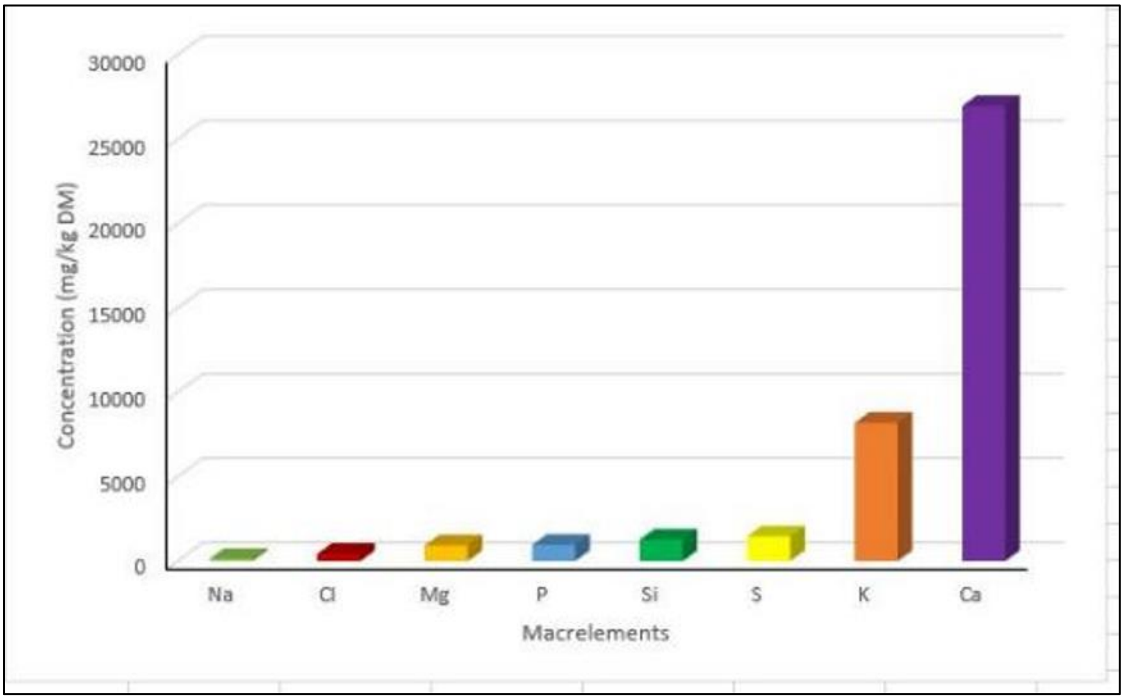


**Figure 3** Distribution of macroelements detected in *Mezoneuron benthamianum* leafy stems

Regarding trace elements, aluminium showed the highest concentration (733.1 mg/kg), followed by iron (353 mg/kg), manganese (79.9 mg/kg), zinc (8.6 mg/kg) and copper (6.2 mg/kg) (Table 2). The distribution of these elements is presented in Figure 4.

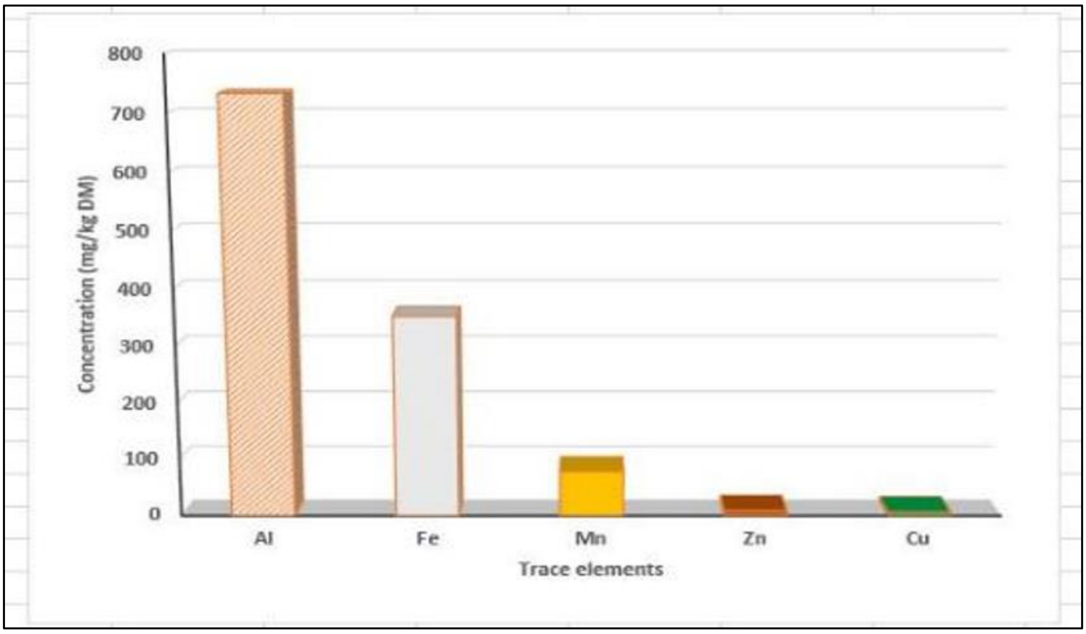


**Figure 4** Distribution of trace elements detected in *Mezoneuron benthamianum* leafy stems

The high calcium content observed in *M. benthamianum* leafy stems is of particular interest because calcium is an essential mineral involved in tooth mineralization and the maintenance of dental structure [12]. The concomitant presence of phosphorus and magnesium, which are involved in calcium metabolism, may contribute to the mineral value of this plant material although the contribution of these minerals to oral effects requires further biological investigations. Potassium, detected at a high level, is also an important mineral involved in cellular homeostasis and physiological functions [13]. The presence of silicon, chlorine and sodium further contributes to the diversified mineral profile of the leafy stems, although their specific contribution to oral health cannot be established from mineral composition alone. The presence of trace elements such as iron, manganese, zinc and copper highlights the mineral richness of *M. benthamianum*. These elements are essential cofactors for several enzymes and participate in important biological processes, including antioxidant defense and metabolic regulation [14, 15]. Zinc may be of particular interest in the oral context because of its involvement in the oral environment. However, the mineral composition alone does not demonstrate a direct anticariogenic or therapeutic effect. Similar mineral diversity has been reported in medicinal plants used traditionally in healthcare, suggesting that plant-derived materials may represent valuable sources of mineral nutrients [16]. Although aluminium was detected at a relatively high concentration (733.1 mg/kg), this element is not considered essential for human nutrition, and its biological significance requires cautious interpretation [17]. The mineral profile obtained in this study provides additional information on *M. benthamianum* and may partly contribute to understanding its traditional use as a chewing stick for oral hygiene. However, the biological properties of this species are likely associated with the combined contribution of mineral elements and secondary metabolites identified through phytochemical investigations. Further biological studies are required to clarify the contribution of these constituents to its oral-health-related effects.

### 3.3. Phytochemical screening

*3.3.1. Qualitative colorimetric reactions*

Qualitative phytochemical screening revealed the presence of polyphenols, flavonoids, tannins, coumarins, sterols/polyterpenes, reducing sugars and proteins in both extracts, whereas alkaloids, saponins and anthocyanins were absent (Table 3).

**Table 3** Qualitative phytochemical screening of aqueous and hydroethanolic extracts of *Mezoneuron benthamianum*

| **Phytochemical groups** | **Aqueous extract** | **Hydroethanolic extract** |
|---|---|---|
| Polyphenols | + | + |
| Flavonoids | + | + |
| Tannins | + | + |
| Coumarins | + | + |
| Sterols/Polyterpenes | + | + |
| Reducing sugars | + | + |
| Proteins | + | + |
| Alkaloids | – | – |
| Saponins | – | – |
| Anthocyanins | – | – |

(+): Present; (–): Absent.

The predominance of phenolic compounds agrees with recent reports indicating that chewing-stick plants are important sources of bioactive metabolites involved in oral health protection. Polyphenols, flavonoids and tannins are known for their antioxidant and antimicrobial activities, while coumarins and terpenoids also contribute to plant defense and biological activity [18–20]. These findings support the traditional use of *M. benthamianum* as a chewing stick and are consistent with previous studies reporting the presence of bioactive phenolic compounds responsible for its anticaries activity [21].

*3.3.2. Thin-layer chromatographic characterization of secondary metabolites in Mezoneuron benthamianum*

Thin-layer chromatography (TLC) was used as a complementary method to characterize the main groups of secondary metabolites detected during phytochemical screening. The TLC analysis of aqueous (MBaq) and hydroethanolic (MBeth) extracts of *Mezoneuron benthamianum* revealed the presence of tannins, flavonoids, coumarins and anthrone/anthraquinone derivatives (Table 4)

**Table 4** Thin-layer chromatographic profile of aqueous and hydroethanolic extracts of *Mezoneuron benthamianum*

| Reagents / Extract | Tannins ($FeCl_3$ 2%) | Anthrones/Anthraquinones (KOH 5%) | Flavonoids ($AlCl_3$ 5%) | Coumarins ($NH_3$ 25%) |
|---|---|---|---|---|
| MBeth | Rf: 0.90; 0.20; 0.10 (black spots) | Rf: 0.90; 0.70; 0.10 (yellow-orange spots) | Rf: 0.90; 0.60; 0.50 (yellow-orange fluorescence) | Rf: 0.90; 0.60; 0.10; 0.40; 0.20 (green/blue fluorescence) |
| MBaq | Rf: 0.80; 0.20; 0.10 (black spots) | Rf: 0.70 (yellow-orange spots) | Rf: 0.90; 0.80 (yellow-orange fluorescence) | Rf: 0.20 (green/blue fluorescence) |

MBeth: hydroethanolic extract of *Mezoneuron benthamianum* ; MBaq: aqueous extract of *Mezoneuron benthamianum*

Tannins were detected after spraying with 2% ferric chloride ($FeCl_3$), showing dark spots in both extracts. Flavonoids revealed yellow-orange spots after aluminum chloride ($AlCl_3$ 5%) treatment, while coumarins produced green and blue fluorescent spots after ammonia ($NH_3$ 25%) revelation. Anthrone/anthraquinone derivatives were detected after treatment with 5% potassium hydroxide (KOH), showing yellow-orange spots.

The hydroethanolic extract exhibited a more diversified chromatographic profile compared with the aqueous extract, with a greater number of spots and Rf values detected for the different metabolite groups. This suggests that the hydroethanolic solvent allowed the extraction of a broader range of compounds detectable under the TLC conditions used. These observations agree with previous reports showing that hydroethanol facilitates the extraction of phenolic compounds, particularly flavonoids and tannins, due to its intermediate polarity [6,7]. Representative TLC profiles illustrating tannin and anthrone/anthraquinone detection are presented in Figure 5.

These results provide additional qualitative evidence of the phytochemical richness of *M. benthamianum* and support its traditional use as a chewing stick for oral hygiene.

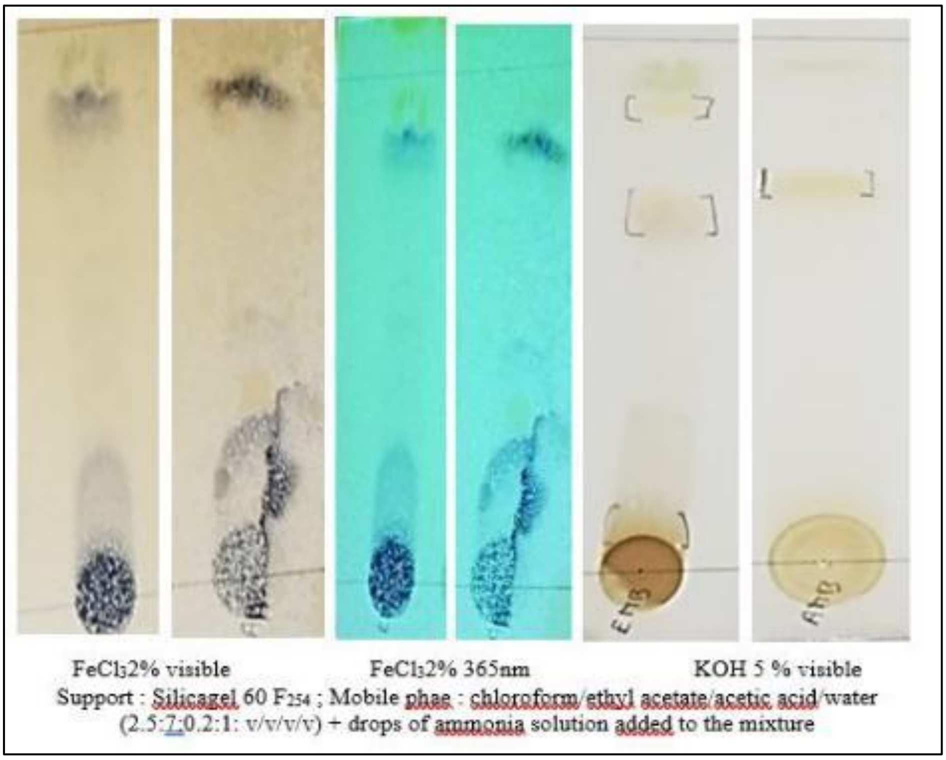


**Figure 5** Representative thin-layer chromatographic profiles of *Mezoneuron benthamianum* extracts

### 3.4. Overall phytochemical and mineral significance of *Mezoneuron benthamianum*

The combined phytochemical and mineral characterization of *Mezoneuron benthamianum* provides valuable information on the chemical profile of this plant traditionally used as a chewing stick in western Côte d'Ivoire. The presence of several secondary metabolites, including polyphenols, flavonoids, tannins and coumarins, together with the detection of various mineral elements, highlights the phytochemical and mineral richness of this species.

Among the detected minerals, calcium was the most abundant element, followed by potassium, sulfur, phosphorus and magnesium. These minerals are important for plant metabolism and may contribute to the nutritional and biological properties of plant-derived preparations. Calcium and phosphorus are particularly associated with mineralized tissues, while magnesium and potassium participate in numerous physiological processes [22]. The detected trace elements, including aluminium, iron, manganese, zinc and copper, also contribute to the overall mineral profile of the leafy stems. The occurrence of tannins and flavonoids is of particular interest because these phenolic compounds are widely recognized for their antioxidant properties and their ability to interact with biological targets. In plants traditionally used for oral hygiene, these metabolites may contribute to protective effects through astringent properties and modulation of oxidative processes in the oral environment [23].

The combination of phenolic compounds, coumarins and mineral constituents may partly explain the traditional importance of *M. benthamianum* as a chewing stick. However, further investigations, including quantitative analysis of individual bioactive compounds and biological assays, are necessary to establish direct relationships between its chemical composition and potential oral health benefits.

## 4. Conclusion

This study provided a comprehensive phytochemical and mineral characterization of *Mezoneuron benthamianum* Baill., a plant traditionally used as a chewing stick for oral hygiene in western Côte d'Ivoire. The results revealed the presence of several secondary metabolites, including polyphenols, flavonoids, tannins, coumarins and other bioactive compound groups, as well as a diverse mineral profile characterized by high levels of calcium and potassium. Thin-layer chromatographic analysis further supported the presence of specific secondary metabolite classes in both aqueous and hydroethanolic extracts. These findings highlight the chemical richness of *M. benthamianum* and provide preliminary chemical evidence consistent with its traditional use. Further studies involving quantitative determination of individual compounds, evaluation of biological activities and safety assessment are required to better establish its potential value in oral health applications.

## Compliance with ethical standards

### *Acknowledgments*

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### *Disclosure of conflict of interest*

The authors declare that they have no conflict of interest.

### *Statement of ethical approval*

This study did not involve human participants, animals or clinical samples. Therefore, ethical approval and informed consent were not applicable.

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