EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Coden USA: EJBPBO
Volume: 13
Issue: 5
Year: 2026

# FIRST REPORT OF TRIGONELLINE ISOLATION FROM *TAPINANTHUS BANGWENSIS* GROWN ON AVOCADO TREE

**Aka Adjo Francelline[1], Kabran Aka Faustin[1]*, Koné Maïmouna Coura[2], Cebriàn-Torrejòn Gérardo[3]**

[1]Laboratoire de Constitution et réaction de la Matière, UFR Sciences des Structures de la Matière et Technologie, Université Félix HOUPHOUËT-BOIGNY, 22 BP 582 Abidjan 22, Côte d'Ivoire.
[2]Laboratoire de Biologie et Santé, UFR Biosciences, Université Félix HOUPHOUËT-BOIGNY, 01 BPV 34 Abidjan, Côte d'Ivoire.
[3]Laboratoire COVACHIM-M2E EA 3592, Campus de Fouillole, UFR SEN, Département de Chimie, Université des Antilles, B.P. 250, 97157 Pointe-à-Pitre Cedex, Guadeloupe, France.

***Corresponding Author: Kabran Aka Faustin**

Laboratoire de Constitution et réaction de la Matière, UFR Sciences des Structures de la Matière et Technologie, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire.

**DOI:** https://doi.org/10.5281/zenodo.20132504

**How to cite this Article:** Aka Adjo Francelline[1], Kabran Aka Faustin[1]*, Koné Maïmouna Coura[2], Cebriàn-Torrejòn Gérardo[3]. (2026). First Report of Trigonelline Isolation from Tapinanthus Bangwensis Grown on Avocado Tree. European Journal of Biomedical and Pharmaceutical Sciences, 13(5), 324–332.
This work is licensed under Creative Commons Attribution 4.0 International license.

**Article Received on 15/04/2026** **Article Revised on 05/05/2026** **Article Published on 20/05/2026**

## ABSTRACT

The present study focuses on the isolation and structural determination of Trigonelline, a compound with multiple biological properties, from *Tapinanthus bangwensis* leaves. The isolation and purification of this compound from the crude ethanolic extract of the plant's leaves were carried out by combining silica gel column chromatography and Sephadex® LH-20. The elucidation of the structure of Trigonelline, isolated for the first time from the plant, was made possible by the analysis of one- and two-dimensional $^{1}H$ and $^{13}C$ NMR spectra, IR and mass spectrometry. The evaluation of antioxidant activity by the DPPH method of the ethanol, ethyl acetate, and hexane extracts showed that the hexane extract was the most active ($IC_{50}$ = 8.33 µg/mL). Furthermore, phytochemical screening of the different extracts using color and precipitation reactions revealed the presence of leucoanthocyanins, saponins, and sterols and terpenes in all extracts.

**KEYWORDS:** *Tapinanthus bangwensis*, leaves, Trigonelline, NMR, DPPH.

## 1. INTRODUCTION

*Tapinanthus bangwensis* (Loranthaceae) is a well known African mistletoe found growing on a variety of host plants, edible or non-edible (**Orhue et al., 2014**), obtaining part of its food from the latter. Like all mistletoes, it depends on its host for nutrients and water (**Osadebe et Uzochukwu, 2006**). *T. bangwensis* often grows on *Theobroma cacao* (**Ogunbameru et al., 2026**), *Persea americana* (**Sakyiamah et al., 2023**), *Parkia biglobosa*, *Triclisia gilletii*, *Phyllanthus muellerianus* (**Efuntoye et al., 2010**), *Pachyra insignis*, *Cassia occidentalis* (**Njoya et al., 2023**), and citrus plants such as *Psidium guajava* (**Molehin et Adefegha, 2015**), *Citrus aurantifolia* (**Efuntoye et al., 2010**), and *Citrus sinensis* (**Ekhaise et al., 2010**). However, only those that grow on edible plants are used for medicinal purposes (**Orhue et al., 2014**). This plant is used to treat numerous illnesses. Indeed, *T. bangwensis* is used for the treatment and management of ailments such as diabetics, hypertension, blood pressure, liver disorders, syphilis, asthma, epilepsy, cancer of the ovary and breast and acquired immunodeficiency syndrome (**Sakyiamah et al., 2023**; **Ihegboro et al., 2020** ; **Maza et al., 2017**; **Ekhaise et al., 2010**). Various extracts from the leaves showed anti-inflammatory, hypolipidemic, antimicrobial, anti-diabetic, anti-hyperglycaemic, anti-hyperproteinaemic, anticancer, hypotensive, and antioxidant activities (**Sakyiamah et al., 2023**; **Ihegboro et al., 2020**; **Molehin et Adefegha, 2015**; **Adegoke et Oloyede, 2012**). Chemical studies conducted on various organs of *T. bangwensis* have made it possible to highlight different chemical groups and to isolate numerous compounds. Hence, phytochemical analysis carried out on *T. bangwensis* leaves ethanol and aqueous extracts revealed the presence of phenols, alkaloids, tannins, flavonoids, saponins, quinones, carbohydrates, and glycosides (**Alang et al., 2024**). **Efuntoye et al. (2010)** also highlighted the presence of tannins,

alkaloids, saponins, and flavonoids in methanol and chloroform extracts from the same plant organ. In addition, triterpenoids and carbohydrates have been isolated from seeds and stem (**Maza et al., 2017**; **Atewolara-Odule et al., 2020**; **Njoya et al., 2023**) while gallic acid derivatives were isolated from the leaves of the plant (**Patrick-Iwuanyanwu et al., 2014**; **Atewolara-Odule et al., 2020**). As a continuation of our work on the search for new biologically active compounds from medicinal plants, this work was carried out on *Tapinanthus bangwensis* leaves growing on *Persea americana* tree. The present study reports for the first time the isolation and structural determination of Trigonelline from the ethyl acetate fraction of *Tapinanthus bangwensis* leaves.

## 2. MATERIAL AND METHODS

### 2.1. General experimental procedures

Infrared (IR) absorption spectrum was recorded on a Bruker Vector 22 Fourier Transform Spectrometer (Champs-sur-Marne, France). The absorption bands (ν) were expressed in $cm^{-1}$. The NMR spectra were carried out on a Bruker AM-400 spectrometer (400 MHz) using $D_2O$ as solvent. The solvent signals were used as references. HR-ESI/MS spectrum was registered with an Agilent 6530 Q-TOF spectrometer (Les Ulis, France) equipped with an ESI source operating with a positive polarity. The antioxidant activity was assessed using a UV/Vis spectrophotometer (JENWAY Spectrophotometer). Leaves were ground using a Retsch apparatus (Eragny sur Oise, France). Thin-layer chromatographies were carried out on aluminium plates coated with silica gel 60 F254 (Merck) and visualized with UV light (254 and 365 nm) then sprayed with phosphomolybdic acid. The chromatography columns were performed on silica gel (Merck, 40-63 µm) or Sephadex® LH-20 (Pharmacia). Chemicals and solvents were purchased from Sigma-Aldrich.

### 2.2. Plant material

Leaves of *Tapinanthus bangwensis* growing on an avocado tree (*Persea americana*) were harvested in August 2013 in Adiopodoumé, south of Côte d'Ivoire. The plant samples were identified by the botanist TEHE Henry from Centre Suisse de Recherche Scientifique (CSRS, Côte d'Ivoire) where a voucher specimen has been deposited under the reference TB-KABRAN-Adiopodoumé 2013-1.

### 2.3. Extraction and isolation

The dried and powdered leaves of *Tapinanthus bangwensis* (500 g) was extracted by maceration in ethanol 96% (2 L) at room temperature (25°C) three times in a row for 24 hours. After filtration and evaporation of the solvent, 70 g of a greenish residue (TbE) was obtained. This residue was then counter-extracted successively by hexane (100 mLx3) and ethyl acetate (100 mLx3) after dissolution in an ethanol/water (50:50) mixture (200 mL). The organic phases, after filtration and removal of the solvent by rotary evaporator, yielded 12 g of a greenish hexane extract (TbH) and 7 g of a brownish ethyl acetate extract (TbA). 6g of the ethyl acetate extract were fractionated on a silica gel column under atmospheric pressure, using the dichloromethane/ethyl acetate/methanol (10:80:10) system as the mobile phase. After collection and grouping of the eluates according to their chromatographic profiles by TLC (revealed by phosphomolybdic acid and UV 254 nm, and 365 nm), 10 fractions (F1-F10) were obtained. Fraction F10 (12 mg), which had the best chromatographic profile on TLC, was purified by Sephadex® LH-20 gel column chromatography using dichloromethane/ethanol (2:1) mixture as eluent to give 5 mg of compound (**1**).

### 2.4. Identification of compound (1)

*Trigonelline (1)*: white amorphous solid; IR $\nu_{max}$ 3078, 2918, 2360, 2340, 1641, 1610, 1580, 1375, 1215, 1046, 768 $cm^{-1}$; HR-ESI/MS (*m/z*) 138.0551 $[M+H]^+$ (Calculated 138.0555); 160.0377 $[M+Na]^+$; 297.0893 $[2M+Na]^+$ (Molecular formula $C_7H_7NO_2$); $^1H$ and $^{13}C$ NMR data (See **Table 1**).

### 2.5. Phytochemical screening

Phytochemical screening was carried out on TbE, TbA and TbH extracts using color and precipitation reactions in tubes to determine the major secondary metabolites according to the methods described by **Tiwari et al.** (**2015**), **Afolayan** (**2023**), **Yao et al.** (**2026**), and **Chennu et al.** (**2026**) with some slight modifications for certain tests.

#### 2.5.1. Terpenoids and steroids

**Tiwari et al.** (**2015**) method with some modifications was used for this test. A 5 mL aliquot of the solution to be analyzed was evaporated to dryness in a capsule over a sand bath. The resulting residue was dissolved under heat in 1 mL of acetic anhydride and then transferred to a test tube to which 0.5 mL of concentrated sulfuric acid was added. The reaction is positive if a purple or violet ring appears, turning blue and then green.

#### 2.5.2. Alkaloids test

The alkaloids were detected by the method described by **Yao et al.** (**2026**).

#### 2.5.3. Flavonoids

Flavonoids were characterized by the cyanidin reaction according to the method described by **Chennu et al.** (**2026**). Briefly, in a test tube, 5 mL of the extract were mixed with 5 mL of hydrochloric alcohol (an equivolume mixture of ethanol, distilled water, and concentrated hydrochloric acid), 1 mL of n-amyl alcohol, and a few magnesium turnings. The appearance of a pinkish-orange (flavones), pinkish-purple (flavanones), or red (flavonones, flavanonols) color, collected in the supernatant of n-amyl alcohol, indicates the presence of a free flavonoid (genine). The colors are less intense with flavone glycosides.

**2.5.4. Polyphenols**
Polyphenols were determined by the method described by **Yao et al.** (**2026**).

**2.5.5. Saponins**
One gram of plant powder was placed in a 250 mL Erlenmeyer flask, and 100 mL of distilled water was added. The resulting mixture was gently heated, filtered, and cooled. Ten mL of the filtrate was transferred to a test tube and shaken vigorously for 15 seconds. The tube was then placed upright for 15 minutes. If foam persists after this time, the plant material contains saponins.

**2.5.6. Anthocyanins**
To 5 mL of extract, 5 mL of sulfuric acid were added, followed by 5 mL of ammonium hydroxide. The presence of anthocyanins is indicated if the color intensifies upon acidification and then turns blue-violet in a basic medium.

**2.5.7. Leucoanthocyanins**
Leucoanthocyanins were characterized by the cyanidin reaction without the addition of magnesium turnings, after heating for 15 minutes in a water bath. In the presence of leucoanthocyanins, a cherry-red or purplish color develops. Catechols produce a reddish-brown hue.

**2.5.8. Anthraquinones test**
The method described by **Yao et al.** (**2026**) was used to characterize anthraquinones.

**2.5.9. Catechic tannins**
One mL of hydrochloric alcohol solution (an equivolume mixture of ethanol, distilled water, and concentrated hydrochloric acid) was added to 5 mL of infusion. The resulting solution was then boiled for 15 minutes. The formation of a red precipitate soluble in amyl alcohol indicates the presence of catechic tannins.

**2.5.10. Gallic tannins**
5 mL of Stiasny's reagent (a mixture of 10 mL of 40% formaldehyde and 15 mL of concentrated hydrochloric acid) were added to 10 mL of infusion. The resulting solution is boiled for 15 minutes in a water bath. The formation of a precipitate indicates the presence of gallic tannins.

**2.5.11. Phlobatannins**
The method described by **Afolayan** (**2023**) was used for this test.

**2.5.12. Cardiac glycosides**
To 5 mL of extract, 2 mL of glacial acetic acid containing one drop of ferric chloride solution were added. To the resulting solution, 1 mL of concentrated sulfuric acid was added. The formation of a brown ring at the interface indicates the presence of cardenolides. A violet ring appears below the brown ring, while a greenish ring gradually forms within the acetic acid phase.

**2.5.13. Indoles test**
Indoles were characterized by Salkowski's reaction. The method used was that described by **Yao et al.** (**2026**).

**2.5.14. Quinones**
Borntraeger's reagent (ammonia diluted 2:1) allowed for the detection of quinones. Two mL of the extract were evaporated to dryness. The residue was reconstituted with 5 mL of hydrochloric acid (1:5) and then boiled for 30 minutes in a water bath. After cooling under running cold water, the hydrolysate was extracted with 20 mL of chloroform. 0.5 mL of Borntraeger's reagent was added to the collected organic phase. A color change to red or purple indicates the presence of quinones.

**2.6. Antioxidant activity**
The antioxidant activity of the extracts was performed using the DPPH radical scavenging method as described by **Yao et al.** (**2026**). Vitamin C and Quercetin were used as reference compounds.

## 3. RESULTS AND DISCUSSION

### 3.1. Phytochemical analysis

**Table 1** groups together the results of the major chemical groups highlighted in the TbE, TbA and TbH extracts.

**Table 1: Phytochemical screening of *Tapinanthus bangwensis* leaves extracts.**

| | Extracts | | |
|---|---|---|---|
| **Phytochemicals** | **TbE** | **TbH** | **TbA** |
| Polyphenols | ++ | - | ++ |
| Anthraquinones | + | - | - |
| Quinones | - | - | - |
| Alkaloids | + | - | + |
| Terpenoids and steroids | ++ | + | + |
| Flavonoids | ++ | - | ++ |
| Leucoanthocyanins | ++ | + | ++ |
| Saponins | ++ | + | ++ |
| Anthocyanins | - | - | - |
| Catechic tannins | + | - | + |
| Gallic tannins | ++ | - | ++ |
| Phlobatannins | + | - | + |
| Cardiac glycosides | - | - | - |
| Indoles | - | - | - |

(-): Negative; (+): Positive; (++): More positive

The results revealed the presence of leucoanthocyanins, saponins, and terpenes and sterols in all tested extracts. Anthraquinones were detected only in the TbE extract, while quinones, anthocyanins, cardiac glycosides and indoles were absent from all extracts. Our results are partially in agreement with those of **Alang et al.** (**2024**) and **Ekhaise et al.** (**2010**). The first authors showed the presence of quinones and glycosides in the ethanolic and aqueous extracts of the plant's leaves, while the second authors demonstrated the absence of alkaloids and anthraquinones in the methanolic extract of the same plant organ. Similarly, **Efuntoye et al.** (**2010**) showed the absence of anthraquinones in the methanol and

chloroform extracts of the same plant organ, whereas these compounds are present in the TbE extract. These differences could be explained, on the one hand, by the extraction solvents (**Karthiraja et al., 2025**; **Lee et al., 2024**), and on the other hand, by the nature of the host plant on which *T. bangwensis* was harvested (**Edagbo et al., 2024**). Indeed, **Efuntoye et al.** (**2010**) showed that the antibacterial activity of *T. bangwensis* is influenced by the host plant, whereas the biological activity of a plant is linked to its chemical composition. Furthermore, the presence of these compounds in the plant could justify its various biological potentialities (**Alang et al., 2024**; **Efuntoye et al., 2010**) as well as its uses in traditional medicine (**Ogunbameru et al., 2026**).

### 3.2. Structural elucidation of compound (1)

Purification by combination of silica gel column chromatography and Sephadex® LH-20 of the ethyl acetate fraction from the ethanol extract of *Tapinanthus bangwensis* leaves yielded compound (**1**).

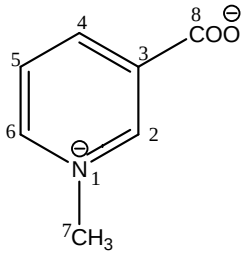



**Figure 1: Structure of Trigonelline isolated from *Tapinanthus bangwensis.***

Compound (**1**) was obtained as a white, amorphous solid (**Fig. 1**). The molecular formula of (**1**) was established as $C_7H_7NO_2$ based on the protonated molecular ion $[M+H]^+$ at *m/z* 138.0551 (calcd for $C_7H_8NO_2$, 138.0555) in HR-ESI/MS (**Fig. 2**). The IR spectrum (**Fig. 3**) showed a large band at 3078 $cm^{-1}$ characteristic of a carboxylic group. Two very close intense bands in the form of a "duck's beak" are also observed at 1610 and 1580 $cm^{-1}$. The band at 1610 $cm^{-1}$ is characteristic of an α, β-unsaturated carbonyl group while the one at 1580 $cm^{-1}$ is attributable to the C=C double bonds of an aromatic ring conjugated with the C=O (**Supudompol et al., 2004**; **Widén et al., 1971**). Furthermore, the bands visible at 2918 and 1641 $cm^{-1}$ are attributable to C-H imine and $C=N^+$ vibrations respectively (**Vildan et al., 2009**).

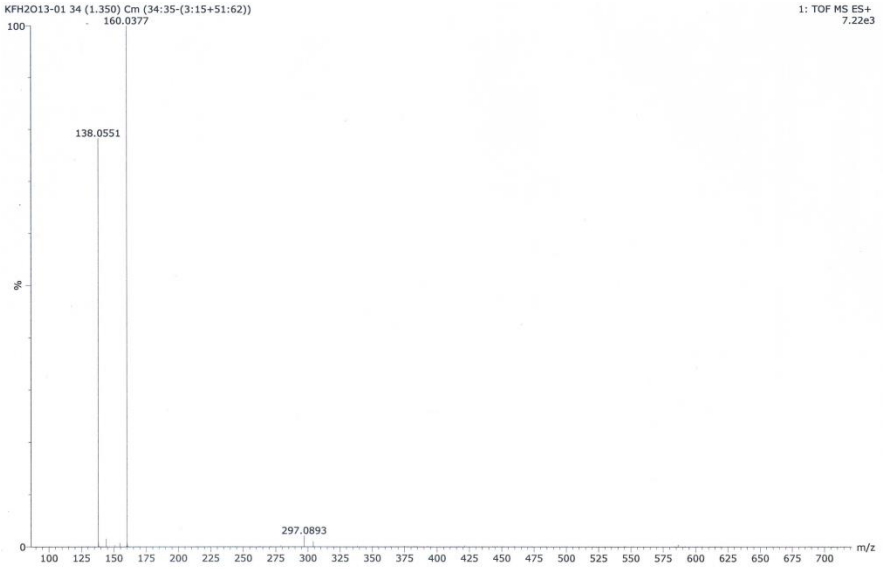



**Figure 2: HR-ESI/MS (+) spectrum of compound. (1)**

The $^1H$ and $^{13}C$ NMR spectra of (**1**) indicated that it is an aromatic compound, specifically a substituted pyridinium derivative (**Pernak et Rogoża, 2000**). Indeed, $^1H$ NMR spectrum (**Fig. 4**) revealed four (4) aromatic protons resonances (**Table 2**) at $\delta_H$ 9.05 (1H; s; H-2), 8.76 (1H; m; H-4), 8.01 (1H; t; $J$ = 6.6 Hz; H-5), and 8.76 (1H; m ; H-6), and a methyl group at $\delta_H$ 4.37 (3H; s; H-7).

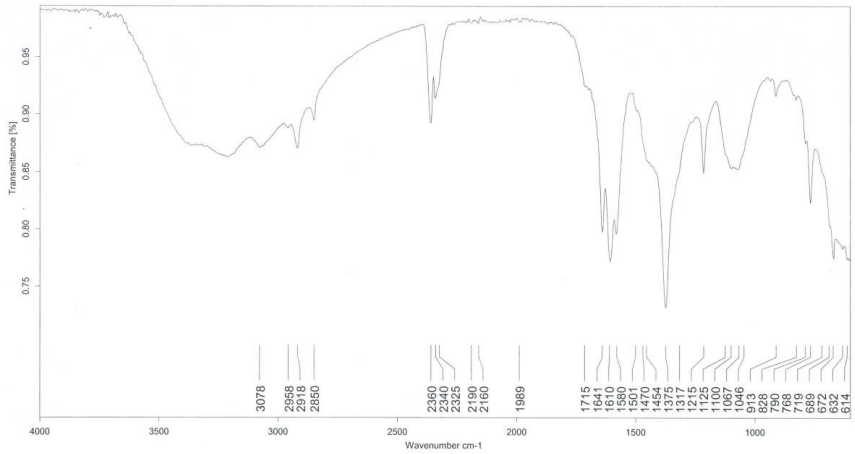



**Figure 3: IR spectrum of compound. (1)**

**Table 2: NMR spectroscopic data (400 MHz, $D_2O$) for compound. (1)**

| Position | $\delta_C$ (ppm) | $\delta_H$, m (*J*, Hz) | HMBC | COSY |
|---|---|---|---|---|
| 1 | - | - | - | - |
| 2 | 144.7 | 9.05, s (1H) | C-3 ; C-4 ; C-6 | - |
| 3 | 136.8 | - | - | - |
| 4 | 146.0 | 8.76, m (1H) | C-5 ; C-6 | H-5 |
| 5 | 127.6 | 8.01, t (1H ; 6.6) | - | H-4 ; H-6 |
| 6 | 145.7 | 8.76, m (1H) | C-4 ; C-5 | H-5 |
| 7 | 48.2 | 4.37, s (3H) | C-2 ; C-6 | - |
| 8 | 177.2 | - | - | - |

The $^{13}C$ NMR spectrum (**Fig. 5**) showed resonances for a carbonyl at $\delta_C$ 177.2 (C-8), five aromatic carbons at $\delta_C$ 144.7 (C-2), 136.8 (C-3), 146.0 (C-4), 127.6 (C-5), and 145.7 (C-6), and a methyl group at $\delta_C$ 48.2 (C-7).

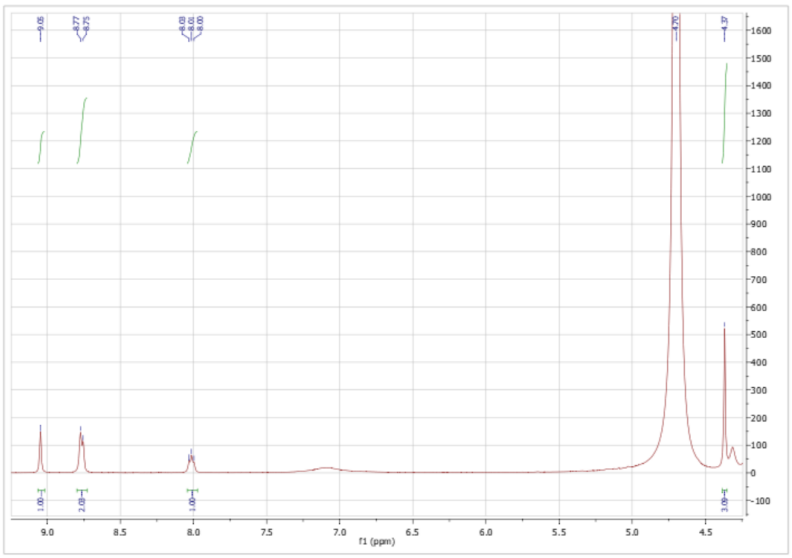



**Figure 4: $^1H$ NMR spectrum in $D_2O$ of compound. (1)**

The different assignments were made using HSQC spectrum. The HMBC spectrum allowed to determine the positions of the different substituents on the pyridinium ring. Indeed, this spectrum showed significant correlations between protons H-7 and carbons C-2 and C-6, indicating that the methyl group is attached to the nitrogen atom. In addition, other important correlations were observed between proton H-2 and carbons C-3, C-4 and C-6 on the one hand, and between proton H-6 and carbons C-4 and C-5 on the other (**Fig. 6**). From the above and in accordance with the mass of compound (**1**), the carboxylate group ($COO^-$) was placed in position 3 on the pyridinium ring, i.e. in beta position with respect to the nitrogen atom (**Fig. 1**). This position is corroborated by the strong deshielding of protons H-2, H-4 and H-6, in ortho and para positions in the pyridinium ring consistent with the mesomeric effect inside the latter.

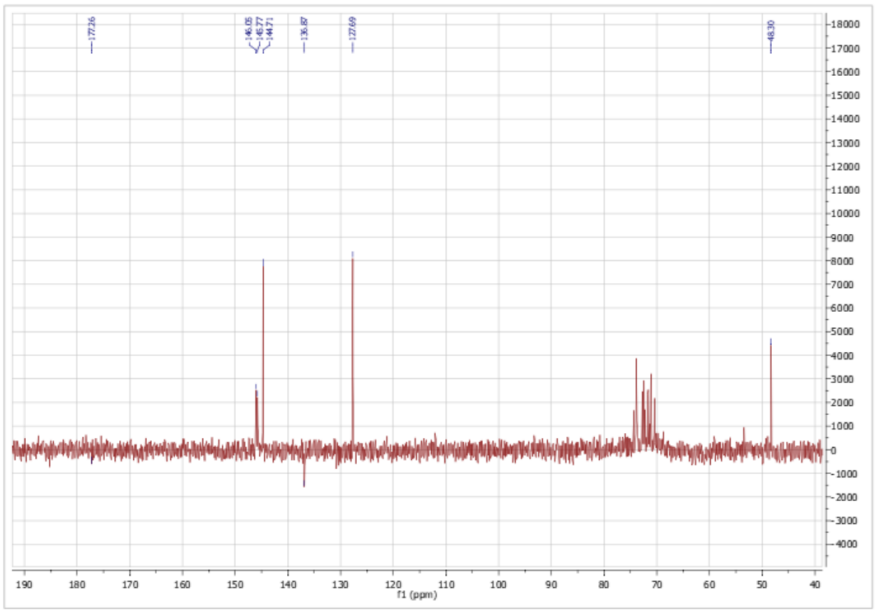


**Figure 5: $^{13}$C NMR (*J*-mod) spectrum in $D_2O$ of compound. (1)**

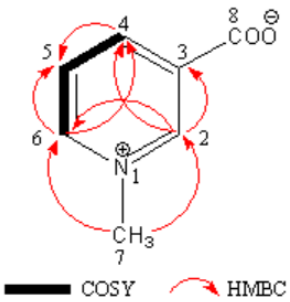



**Figure 6: Important COSY and HMBC correlations for compound. (1)**

The homonuclear correlation spectrum $^1$H-$^1$H COSY made it possible to confirm the positions of the substituents of the pyridinium ring through the correlations between protons H-4, H-5 and H-6 (**Fig. 6**).

Based on spectral data, in agreement with the literature, compound (**1**) was identified as Trigonelline (**Farag et al., 2015**), a methyl betaine of nicotinic acid. This compound is isolated and described for the first time from *Tapinanthus bangwensis* as well as from the genus *Tapinanthus*. It occurs in many plants (**Manas et Raka, 2012**) and is present in all plant organs (**Ashihara et al., 2015**). Indeed, it has already been reported from *Coffea arabica* and *C. canephora* (**Jeszka-Skowron et al., 2020**; **Matsui et al., 2007**), *Trigonella foenum-graecum* (**Sekhar et al., 2024**; **Mounir et al., 2016**), *Raphanus sativus* (**Kuroda et al., 2018**), *Moringa oleifera* (**Manas et Raka, 2012**), from numerous species of the genus *Annona* (**Machado et al., 2013**), Fabaceae family (**Manas et Raka, 2012**), etc. This compound possesses hypoglycaemic, hepatoprotective, cholesterol-lowering, anti-diabetic, antioxidant, anti-cancer, and cardioprotective properties (**Sekhar et al., 2024**; **Costa et al., 2020**; **Kuroda et al., 2018; Farag et al., 2015**). Its estrogenic, neuroprotective, and antibacterial activities have also been described (**Ashihara et al., 2015**). In addition, Trigonelline has nutritional benefits (**Ashihara et al., 2015**; **Viani et Horman, 1974**). The most important commercially available of this molecule is coffee (*Coffea arabica* and *Coffea canephora*) (**Ashihara et al., 2015**).

### 3.3. Antioxidant activity

DPPH method was used to evaluate the antioxidant activity of the extracts (TbE, TbA and TbH) and two references (Vitamin C and Quercetin) according to their mode of action. **Fig. 7** summarizes the results obtained.

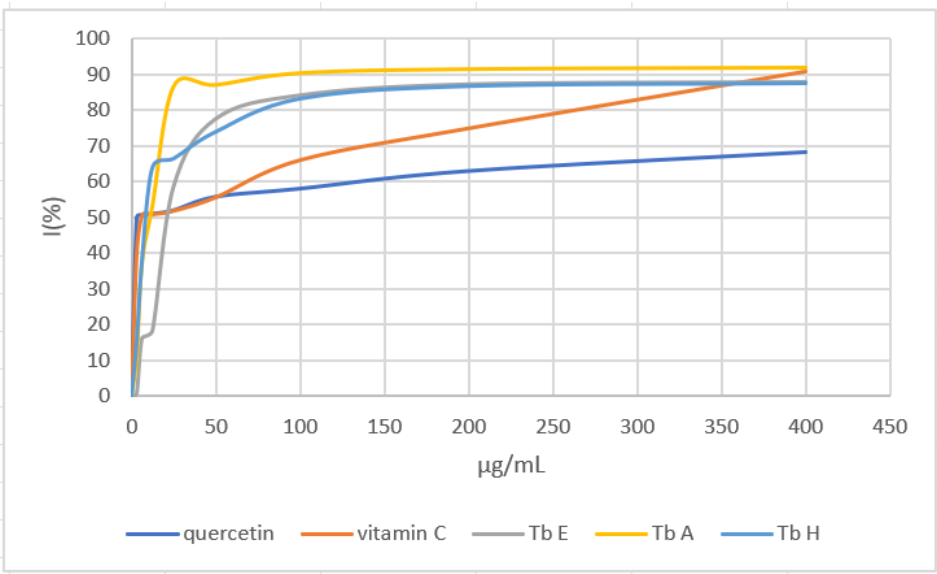



**Figure 7: Antioxidant activity of extracts from *Tapinanthus bangwensis* leaves.**

All tested compounds possess antioxidant activity. The references used, Vitamin C ($IC_{50}$ = 7.08 µg/mL) and Quercetin ($IC_{50}$ = 3.33 µg/mL), are more active than the extracts TbE ($IC_{50}$ = 20.83 µg/mL), TbA ($IC_{50}$ = 10.83 µg/mL), and TbH ($IC_{50}$ = 8.33 µg/mL). Among the extracts tested, TbH is the most active with an activity comparable to that of vitamin C. The antioxidant activity of the hexane extract from leaves has already been demonstrated by **Ihegboro et al.** (**2020**). TbA and TbH were derived from the crude extract TbE and are more active than the latter. This reflects the fact that most of the active compounds in TbE are soluble in ethyl acetate and hexane. Furthermore, the most active extract (TbH) contains a greater number of these compounds, suggesting that they are lipophilic in nature, while TbH is nonpolar. Therefore, TbH and TbA could be a good source of bioactive compounds. In order to isolate and characterize the compounds responsible for the antiradical activity of TbA, which exhibited a relatively simple thin-layer chromatographic profile, this extract was purified. Purification allowed the isolation of Trigonelline, whose antiradical potential has already been demonstrated in the literature (**Sekhar et al., 2024**). The presence of this molecule in TbA could explain its antioxidant activity. Furthermore, the antioxidant activity of the ethyl acetate extract of *T. bangwensis* leaves has already been described by **Bassey et al.** (**2012**). In addition, previous studies showed that this extract possessed hepatoprotective (**Patrick-Iwuanyanwu et al., 2010a**), anti-inflammatory (**Patrick-Iwuanyanwu et al., 2010b**), and cytotoxic (**Bassey et al., 2012**) properties. According to these authors, all these properties are due to the presence of phenolic compounds in this extract (**Patrick-Iwuanyanwu et al., 2014**). The results of our study clearly show that compounds of other chemical natures, such as Trigonelline, which is an alkaloid, could contribute to these activities. In addition.

## 4. CONCLUSION

The aim of this work was the isolation and structural determination of Trigonelline from the crude ethanolic extract of *Tapinanthus bangwensis* leaves. To do this, a phytochemical screening was first carried out to determine the major chemical groups present in the ethanol (TbE), ethyl acetate (TbA), and hexane (TbH) extracts by specific characterization tests in tubes. The results of these tests showed the presence of leucoanthocyanins, saponins, and sterols and terpenes in all extracts, justifying the use of the plant in traditional medicine for the treatment of certain diseases. The purification of the ethyl acetate fraction (TbA) from the crude ethanolic extract (TbE) by combinations of silica gel column chromatography and Sephadex® LH-20 made it possible to isolate and characterize Trigonelline, an alkaloid with numerous biological properties isolated for the first time from the species. The presence of this molecule in TbA constitutes a major contribution to justifying the antioxidant potential of this extract. Indeed, the evaluation of the antioxidant activity of TbE ($IC_{50}$ = 20.83 µg/mL), TbA ($IC_{50}$ = 10.83 µg/mL), and TbH ($IC_{50}$ = 8.33 µg/mL) by the DPPH method showed that TbH and TbA are the most active extracts, with activities comparable to that of vitamin C ($IC_{50}$ = 7.08 µg/mL), used as a reference molecule. Furthermore, this study is a contribution to the chemical knowledge of *T. bangwensis*.

## 5. ACKNOWLEDGEMENTS

The authors are grateful to Blandine Seon-Méniel from UMR BioCIS, CNRS, Paris-Saclay University, 92296 Châtenay-Malabry (France), for NMR analysis. They also thank the botanist TEHE Henry from Centre Suisse de Recherche Scientifique (CSRS, Côte d'Ivoire) for identifying the plant material.

## 6. COMPETING INTERESTS

Authors declared that no competing interests exist.

## REFERENCES


1. Adegoke, M.B., Oloyede, B.O. Antihyperglycaemic and antihyperproteinaemic activity of extracts of *Picralima nitida* seed and *Tapinanthus bangwensis* leaf on alloxan-induced diabetic rabbits. *Int. J. Innov. Appl. Stud*., 2012 ; 3: 1125-1131.
2. Afolayan, M.O. Comparative phytochemical and antioxidant analysis of the leaf extracts of two Nigerian medicinal plants. *J. Sci. Math. Lett*., 2023; 11(1): 30 (38).
3. Alang, M.B., Beka, R.G., Bibila, M.M. Phytochemical screening, antibacterial evaluation and health benefits of *Tapinanthus bangwensis* leaves. *J. Pharmacogn. Phytochem.*, 2024; 13(4): 240-246.
4. Ashihara, H., Ludwig, I.A., Katahira, R., Yokota, T., Fujimura, T., Crozier, A. Trigonelline and related nicotinic acid metabolites : occurrence, biosynthesis, taxonomic considerations, and their roles *in planta* and in human health. *Phytochem. Rev.*, 2015; 14: 765-798.
5. Atewolara-Odule, O.C., Aiyelaagbe, O.O., Olubomehin, O.O., Ogunmoye, A.O., Feyisola, R.T., Sanusi, A.S. Antioxidant activity of some secondary metabolites from *Tapinanthus bangwensis* (Engl., and K. Krause) [Loranthaceae] grown in Nigeria. *Sci. Afr.*, 2020; 8: 1-6.
6. Bassey, P., Sowemimo, A., Lasore, O., Spies, L., Van de Venter, V. Biological activities and nutritional value of *Tapinanthus bangwensis* leaves. *Afr. J. Biotechnol*., 2012; 11(73): 13821-13826.
7. Chennu, M.M.P.R., Tanniru, R., Jyotirmayee, P., Renuka, B., Rupashree, N., Prakash, P. Phytochemical profiling and comparative antioxidant potential of *Enicostemma littorale* leaf extracts using *in vitro* assays. *EJBPS*, 2026; 13(4): 223-229.
8. Costa, M.C., Lima, T.F.O., Arcaro, C.A., Inacio, M.D., Batista-Duharte, A., Carlos, I.Z., Spolidorio L.C., Assis, R.P., Brunetti, I.L., Baviera, A.M. Trigonelline and curcumin alone, but not in combination, counteract oxidative stress and inflammation and increase glycation product detoxification in the liver and kidney of mice with high-fat diet-induced obesity. *J. Nutr. Biochem*., 2020; 76: 108303.
9. Edagbo, D.E., Ajiboye, T.O., Alowonle, A.A., Oyewole, O.B. Infestation and prevalence of the mistletoe, *Tapinanthus bangwensis* on host plants in Moor Plantation, Ibadan, Nigeria. *J. Res. For. Wildl. Environ.*, 2024; 16(1): 105-114.
10. Efuntoye, M.O., Ayodele, A.E., Thomas, B.T., Ajayi, T.O. Does host plant affect the antibacterial activity of *Tapinanthus bangwensis* (Engl. And K. Krause) Danser (Loranthaceae)? *J. Med. Plant Res.*, 2010; 4(13): 1281-1284.
11. Ekhaise, F.O., Ofoezie, V.G., Enobakhare, D.A. Antibacterial properties and preliminary phytochemical analysis of methanolic extract of mistletoe (*Tapinanthus bangwensis*). *BAJOPAS*, 2010; 3(2): 65-68.
12. Farag, M.A., Porzel, A., Wessjohann, L.A. Unraveling the active hypoglycemic agent trigonelline in *Balanites aegyptiaca* date fruit using metabolite fingerprinting by NMR. *J. Pharm. Biomed. Anal.*, 2015; 115: 383-387.
13. Ihegboro, G.O., Ononamadu, C.J., Owolarafe, T.A. Shekwolo, I. Screening for toxicological and anti-diabetic potential of n-hexane extract of *Tapinanthus bangwensis* leaves. *Toxicol. Res. Appl*., 2020; 4: 1-11.
14. Jeszka-Skowron, M., Frankowski, R., Zgoła-Grześkowiak, A. Comparison of methylxantines, trigonelline, nicotinic acid and nicotinamide contents in brews of green and processed Arabica and Robusta coffee beans-Influence of steaming, decaffeination and roasting processes on coffee beans. *LWT*, 2020; 125: 109344.
15. Karthiraja, A.S., Kowsalya, K., Durga Sri, R., Revathi, S., Karthika, A. Extraction of flavonoids from natural sources using modern techniques : A review. *Mediterr. J. Med. Med. Sci*., 2025; 1(3): 43-46.
16. Kuroda, R., Kazumura, K., Ushikata, M., Minami, Y., Kajiya, K. Elucidating the improvement in vascular endothelial function from Sakurajima Daikon and its mechanism of action : A comparative study with *Raphanus sativus*. *J. Agric. Food Chem*., 2018; 66(33): 8714-8721.
17. Lee, J.E., Jayakody, J.T.M., Kim, J.I., Jeong, J.W., Choi, K.M., Kim, T.S., Seo, C., Azimi, I., Hyun, J., Ryu, B. The influence of solvent choice on the extraction of bioactive compounds from Asteraceae : A comparative review. *Foods*, 2024; 13(3151): 1-21.
18. Machado, A.R.T., Lage, G.A., Medeiros, F.S, Filho, J.D.S., Pimenta, L.P.S. Quantitative analysis of trigonelline in some Annona species by proton NMR spectroscopy. *Nat. Prod. Bioprospect.*, 2013; 4: 158-160.
19. Manas, M., Raka, K. Studies on trigonelline from *Moringa oleifera* and its *in vitro* regulation by feeding precursor in cell cultures. *Rev. Bras. Farmacogn. Braz. J. Pharmacogn*., 2012; 22(5): 994-1001.
20. Matsui, A., Yin, Y., Yamanaka, K., Iwasaki, M., Ashihara, H. Metabolic fate of nicotinamide in higher plants. *Physiol. Plant*, 2007; 131: 191-200.
21. Maza, H., Mkounga, P., Fenkam, S.L., Sado, S.K., Ishikawa, H., Nishino, H., Nkengfack, E.A. Triterpenoids from seeds of *Tapinanthus bangwensis*. *Phytochem. Lett.*, 2017; 19: 23-29.
22. Molehin, O.R., Adefegha, S.A. Antioxidants and inhibitory effects of aqueous and ethanolic extract of *Tapinanthus bangwensis* leaves on $Fe^{2+}$-induced lipid peroxidation in pancreas (*in-vitro*). *Int. Food Res. J.*, 2015; 22(1): 257-262.

23. Mounir, O., Khalid, E.B., Saaïd, A. Toxicological properties of fenugreek (*Trigonella foenum graecum*). *Food Chem. Toxicol.*, 2016; 96: 145-154.
24. Njoya, E.M, Maza, H.L.D., Swain, S.S., Chukwuma, C.I., Mkounga, P., Mba, Y.M.N., Mashele, S.S., Makhafola, T.J., McGaw, L.J. Natural compounds isolated from African mistletoes (Loranthaceae) exert anti-inflammatory and acetylcholinesterase inhibitory potentials : *in vitro* and *in silico* studies. *Appl. Sci*., 2023; 13: 2606.
25. Ogunbameru, F.E., Karigidi, K.O., Akintimehin, E.S., Omogunwa, T.S., Makinde, A.P., Adetuyi, F.O. Evaluation of *in vitro* antioxidant and anti-inflammatory potentials of *Tapinanthus bangwensis* leaves. *Mediterr. J. Pharm. Pharm. Sci*., 2026; 6(1): 31-39.
26. Orhue, P.O., Edomwande, E.C., Igbinosa, E., Momoh, A.R.M., Asekomhe, O. Antibacterial activity of extracts of mistletoe (*Tapinanthus dodoneifollus* (DC) Dancer) from cocoa tree (*Theobrama cacao*). *Int. J. Herbs Pharmacol. Res*., 2014; 3(1): 24-29.
27. Osadebe, P.O., Uzochukwu, I.C. Chromatographic and antimotility studies on the extracts of *Loranthus micranthus*. *J. Pharm. Allied Sci*., 2006; 3(1): 263-268.
28. Patrick-Iwuanyanwu, K.C., Onyeike, E.N., Adhikari, A. Isolation, identification and characterization of gallic acid derivatives from leaves of *Tapinanthus bangwensis*. *J. Nat. Prod.*, 2014; 7: 14-19.
29. Patrick-Iwuanyanwu, K.C., Onyeike, E.N., Wegwu, M.O. Anti-inflammatory effect of crude methanolic extract and fractions of African mistletoe (*Tapinanthus bangwensis*) [Engl. & K. Krause] on Wistar albino rats. *Pharm. Lett.*, 2010a; 2(6): 76-83.
30. Patrick-Iwuanyanwu, K.C., Onyeike, E.N., Wegwu, M.O. Hepatoprotective effects of methanolic extract and fraction of African Mistletoes (*Tapinanthus bangwensis* [Engl. & K. Krause] from Nigeria. *EXCLI J*., 2010b; 9: 187-194.
31. Pernak, J., Rogoża, J. Synthesis of 3-substituted pyridinium salts. *ARKIVOC*, 2000; 889-904.
32. Sakyiamah, M.M, Gordon, P.K., Bolah, P., Baffour, P.K., Ehun, E., Quasie, O., Kumadoh, D., Archer, M-A., Mintah, S.O., Appiah, A.A. Assessment of the phytochemical composition and antimicrobial properties of *Tapinanthus bangwensis* leaves hosted by the branches of *Persea americana*. *BMC Complement. Med. Ther.*, 2023; 23(34): 1-10.
33. Sekhar, M.G., Shanmugam, K.R., Chakrapani, I.S. Trigonelline, a fenugreek bioactive protects heart tissue against alcohol intoxication : An *in-vivo* study focusing on antioxidant perspective. *J. Ayurveda Integr. Med.*, 2024; 15 : 100963.
34. Supudompol, B., Likhitwitawuid, K., Houghton, P.J. Phloroglucinol derivates from *Mallotus pallidus*. *Phytochemistry*, 2004; 65: 2589-2594.
35. Tiwari, A.N., Shah, B.K., Gohel, H.R. Determination of presence of various antioxidants in aqueous extract of various plants - A preliminary study. *Int. Res. J. Biological Sci*., 2015; 4(8): 1-5.
36. Viani, R., Horman, I. Thermal behavior of trigonelline. *J. Food Sci*., 1974; 39(6): 1216-1217.
37. Vildan, A., Sülünay, P., Hüseyin, T. Ercin, E. Synthesis and antimicrobial activity of some pyridinium salts. *Molecules*, 2009; 14: 5203-5215.
38. Widén, C.J., Vida, G., Von, E.J., Reichstein, T. Die phloroglucide von Dryopteris villarii (Bell.) woynar und anderer farne der gattung Dryopteris sowie die mögliche. *Helv. Chim. Acta*, 1971; 54(8): 2824-2850.
39. Yao, Y.J.G-C., Kabran, A.F., Dibi, K.J., Ambeu-Loko, N.C.M., Koné, M.C., Cebriàn-Torrejòn, G. Isolation and structural determination of *myo*-inositol from *Coelocaryon preussii* fruit shells: antioxidant activity. *EJBPS*, 2026; 13(4): 230-238.