Research Article

Isoquinoline Alkaloids from the Stem Barks of *Isolona cooperi* (Annonaceae) and Their Antiplasmodial Activity

Akpa Salemon Agnes [1], Aboua Timothée Okpekon [1, *], Seri Chardin Seri [1], Yao Hermann Koffi [1], Affoué Yvette Kouadio [1], Tapé Ulrich Aldelph Kouamé [1,2], François Nicaise Bony [3]

[1] *Laboratoire de Constitution et Réaction de la Matière (LCRM), UFR Sciences des Structures de la Matière et de Technologie, Université Félix Houphouët-Boigny, 22 BP 582 Abidjan 22, Côte d'Ivoire*

[2] *Laboratoire de Chimie Bioorganique et de Substances Naturelles (LCBOSN), UFR Sciences Fondamentales Appliquées, Université Nangui Abrogoua, 02 BP 0801 Abidjan 02, Côte d'Ivoire.*

[3] *Département de Chimie Analytique, Minérale et Générale, Technologie Alimentaire, UFR Sciences Pharmaceutiques et Biologiques, Univ. FHB, BP 34 Abidjan, Côte d'Ivoire.*

***Corresponding author's E-mail:** okpekon@yahoo.fr

Received: 06-06-2025; **Revised:** 28-08-2025; **Accepted:** 08-09-2025; **Published online:** 20-09-2025.

ABSTRACT

Isolona cooperi Hutch. & Dalziel (Annonaceae) is a shrub growing under the dense canopy of the West African rain forest where it is used in traditional medicine as antiparasitic against malaria, African trypanosomiasis, helminthes and scabies. Thus, the MeOH crude extract of its stem barks was investigated in order to determine the alkaloids composition as soon us their antiplasmodial activity. This led to the isolation of five isoquinoline alkaloids from which one is pseudo novel, iso-N-methyl stepholidine (1). The known compounds are N-methyl stepholidine (2), N-methyl tetrahydrocolumbamine (3), magnocurarine (4) and desmethyl magnocurarine (5). Their structures were established by their spectral data analysis including MS, IR, UV, 1D and 2D-NMR spectroscopy. All the isolated compounds, reported for the first time from the stem barks of Isolona cooperi, could be considered to have chemotaxonomic value. Furthermore, these compounds showed antiplasmodial activity with CI50 values ranging from 7.05 ± 0.34 to 69.63 ± 1.97 µM. The new compound, iso-N-methyl stepholidine (1), is one of the most active compounds with activity higher than that of quinine and lumefantrine, two molecules still used in antimalarial chemotherapy. The presence of these compounds could justify the use in folk medicine leafy stems of the plant in magistral preparations against malaria in Côte d'Ivoire.

Keywords: *Isolona cooperi*; Annonaceae; Tetrahydroprotoberberine alkaloids; Benzylisoquinoline alkaloids; Antiplasmodial activity.

INTRODUCTION

Isolona cooperi Hutch. & Dalziel (Annonaceae) is a shrub, up to 2 m tall, growing under the dense canopy of the West African rain forest from Ghana to Liberia [1, 2]. It is used in traditional medicine as tonic, antidote and antiparasitic. In the southern Côte d'Ivoire, this plant is used against malaria, African trypanosomiasis, helminthes and scabies[3, 4, 5].

Phytochemical investigations carried out to date on *Isolona cooperi* showed that the species consisted of alkaloids, monoterpenes, sesquiterpenes and lactones [6]. In our ongoing analyses of this species for its bioactive constituents [7], we hereby reported resulted of our studies for alkaloid constituents of this plant.

MATERIALS AND METHODS

Plant Material

Stem of *Isolona cooperi* were harvested in Yapo-Abbé forest (5°44'25"N 4°3'33"W), in Mai 2019, at Agboville Department, south-eastern of Côte d'Ivoire. The plant samples were identified by Professor Ipou Ipou Joseph of Centre National de Floristique (CNF), Félix Houphouët Boigny University, Cocody-Abidjan, where a sample of herbarium (N° AAS-IC-2019) was kept.

Phytochemical Study

1. General Experimental Procedures

Optical rotations were measured at 25°C on a Polar 32 polarimeter. UV spectra were recorded on a Philips PU 8720 spectrometer. IR spectra were recorded with a Brüker Vector 22. The NMR spectra were recorded on a Brüker AM-400 (400 MHz) NMR spectrometers, using CD_3OD as solvent. EIMS was recorded on a Varian MAT-312 mass spectrometer. ESI-HRMS data were gained using a Micromass Q-TOF high-resolution mass spectrometer. Mass spectra were recorded in the positive-ion mode in the range *m/z* 100-2000, with a mass resolution of 20000 and an acceleration voltage of 0.7 kV. TLC were carried out using silica gel $60F_{254}$ pre-coated aluminium plates (0.2 mm, Merck). Spots were visualized through Dragendorff's reagent and chromogenic agent (Vanillin-H_2SO_4) subsequent heating.

2. Chromatographic and Mass-Spectrometric Analysis

Samples were analyzed using an Agilent 6530 Accurate-Mass Q-TOF coupled with a 1260 Agilent Infinity LC system equipped with a Sunfire® C_{18} column (150 × 2.1 mm ; i.d. 3.5 µm, Waters, Milford, MA, USA) with a flow rate of 0.25 mL/min. Full scan mass spectra were acquired in the positive-ion mode in a mass range of *m/z* 100 to 1200 Da, with the capillary temperature at 320 °C, source voltage at 3.5 kV, and a sheath gas flow rate at 10 L/min. Capillary,

fragmentor, and skimmer voltages were set at 3500 V, 175 V, and 65 V respectively. Four scan events were used: positive MS, mass range encompassing *m/z* 100–1200, and three data-dependent MS/MS scans of the five most intense ions from the first scan event. Three collision energies (*viz.* 30, 50, and 70 eV) were used for MS/MS data generation. Purine ($C_5H_4N_4$, *m/z* 121.050873 and HP-0921 (hexakis (*1H*, *1H*, *3H*-tetrafluoropropoxy)- phosphazene $C_{18}H_{18}F_{24}N_3O_6P_3$, *m/z* 922.009798) were used as internal lock masses. Full scans were acquired at a resolution of 10,000 (*m/z* 922) and 4000 (*m/z* 121). A permanent MS/MS exclusion list criterion was established to prevent oversampling of the internal calibrant.

3. Extraction and Isolation

The powder of dried stem barks (964 g) was extracted by lixiviation in a Soxhlet apparatus using first dichloromethane (3 L) and then methanol (3 L) for six hours, respectively. The alcoolic layer filtered and evaporated to dryness led to a viscous brown MeOH extract (9.4 g). The MeOH extract (4.0 g) was separated by a chromatography over silica gel using CH_2Cl_2/MeOH/HCOOH (95:5:0 to 80:20:5) as eluant, to give ten fractions (F_1-F_{10}) established according to their TLC profile and a positive reaction with Dragendorff's reagent. Fraction F_3 (284.9 mg), successively chromatographied on silica gel (CH_2Cl_2/MeOH/HCOOH, 95:5:3) and Sephadex LH-20 (CH_2Cl_2/MeOH, 2:1) columns, led to compound **3** (37.5 mg). Fraction F_5 (575.0 mg) was purified by chromatography over silica gel, eluted with CH_2Cl_2/MeOH/HCOOH (95:5:3), to afford seven sub-fractions (F_{51}-F_{57}). Sub-fraction F_{57} (40.0 mg) was further successively purified on silica gel and Sephadex LH-20 columns using respectively CH_2Cl_2/MeOH/HCOOH (90:10:2) and (CH_2Cl_2/MeOH, 2:1) as eluant, to yield compound **1** (3.0 mg). Fraction F_6 (630.0 mg) was chromatographied over silica gel, eluted with CH_2Cl_2/MeOH/HCOOH (90:10:2), to afford eleven sub-fractions (F_{61}-C_{611}). Sub-fraction F_{66} (58.5 mg) was chromatographied over silica gel column, eluted with CH_2Cl_2/MeOH/HCOOH (90:10:3) to yield five sub-fractions (F_{661}-F_{665}). Sub-fraction F_{662} (24.6 mg) was purified by chromatography over silica gel using the later eluant and on Sephadex LH-20 (CH_2Cl_2/MeOH, 2:1) column, to yield the inseparable compounds **2** and **4** (2.4 mg). Fraction F_8 (439.0 mg) was purified successively by chromatography over silica gel, eluted with CH_2Cl_2/MeOH/HCOOH (90:10:2; 90:10:2.5 and 90:10:4, respectively), and on Sephadex LH-20 (CH_2Cl_2/MeOH, 2:1) to afford compound **4** (1.5 mg). Fraction F_9 (176.6 mg) was chromatographied over silica gel (CH_2Cl_2/MeOH/HCOOH, 80:20:4) to yield six sub-fractions (F_{91}-F_{96}). Sub-fraction F_{94} (38.8 mg) was first purified by chromatography over silica gel, eluted with AcOEt/MeOH/HCOOH (70:30:5), then on a Sephadex LH-20 column using CH_2Cl_2/MeOH (2:1) as eluant, to afford compound **5** (11.4 mg).

*Iso-N-methyl stepholidine (**1**)*

Yellow oil°; UV (MeOH) λ_{max} (logε): 215 (4.79), 286 (4.35); IR ν_{max} (cm^{-1}): 3325, 2927, 1609, 1510, 1439, 1360, 1284, 1114, 1082, 876, 753; ^1H and ^{13}C NMR (CD_3OD) data, see Table 1; HR- ESI-MS-Q-TOF *m/z*: 342.1704 $[M]^+$ (Calcd. for $C_{20}H_{24}NO_4$, 342.1705); Spray reagents for TLC: Dragendorff's and Vanillin-H_2SO_4 reagents.

Antiplasmodial Assay

Antiplasmodial activity assays were performed *ex vivo* according to the protocol of Trager and Jensen adapted by Koffi *et al.* [8]. This method is based on the culture of *Plasmodium falciparum* isolates in the presence of a series of concentrations of the antimalarial drug studied for the duration of the parasite's life cycle or part of this cycle. The isolates used in the present study were obtained from blood samples from individuals with a proven uncomplicated *Plasmodium falciparum* malaria following a positive RDT validated by positive thick and thin smear tests confirming *P. falciparum* monoinfection at the schizont and gametocyte stages. These parasites present in the parasitized red blood cells were used to carry out the chemosensitivity tests with a parasite density between 0.1 and 0.3%. Stock solutions of the drugs to be tested were prepared in DMSO. The substances were serially diluted two times with 100 µL of RPMI 1640 culture medium in 96-well plates. Then, the parasitized red blood cells were exposed to the different drugs and then incubated for 72 hours at 37°C under a candle bell. The Rieckmann microtest technique adopted by the WHO [9] was used and the fluorescence was measured after exposure to SYBR green in the dark for one hour at room temperature. The antimalarial activity or inhibition of erythrocyte schizogony was determined by measuring the fluorescence of SYBR green intercalated in the parasite DNA, using a spectrofluorometer. Then, IC_{50} values (concentration of the extract inhibiting 50% of parasite growth) were determined by the online software ICEstimator antimalarial version 1.2 [8, 10]. Lumefantrine and quinine were used as positive controls.

RESULTS AND DISCUSSION

Identification of Compounds

The MeOH extract of powder of dried leaves and stem bark of *Isolona cooperi* was subjected on chromatography columns to obtain five isoquinoline alkaloids (**1-5**) from which compound **1** was previously undescribed. The known compounds were identified to N-methyl stepholidine (**2**) [11, 12], N-methyl tetrahydrocolumbamine (**3**) [13], magnocurarine (**4**) [14, 15] and desmethyl magnocurarine (**5**) [16]. Their structures (**Figure 1**) were elucidated on the basis of spectroscopic evidence.

Compound **1** was obtained as a yellowish oil and showed a positive reaction with Dragendorff's reagent. Its IR spectrum showed phenolic hydroxyl (3325 cm^{-1}) and aromatic (1510, 1609 cm^{-1}) absorption bands. It showed UV maxima at 215 and 286 nm. The molecular formula determined as $C_{20}H_{24}NO_4$ was the same of that of compound **2** (HR-ESI-MS-Q-TOF at *m/z* 342.1704 $[M]^+$; calcd. 342.1705). Its ^1H NMR and ^{13}C NMR spectra (**Table 1**) also showed characteristic signals very similar to that of compound **2** which were subsequently confirmed by 2D

NMR (COSY and HMBC) experiments (**Figure 2**). However, there were slight differences observed in the resonance of C-6 (δ_C 63.1 in **1** versus 53.5 in **2**) and N-methyl (δ_C 39.2 in **1** versus 50.8 in **2**). These observed differences in chemical shifts, indicating one shielded while other deshielded, could arise from the *cis* or *trans* conformation adopted by the fusion of the B/C rings specifically including the nitrogen in position 7. Since in N-methyl stepholidine (**2**) we have a B/C-*cis* fusion corresponding to the α-form then the compound **1** could be the β-form with a B/C-*trans* fusion [17]. This was confirmed by the NOE experiment which showed no cross peak between the N-methyl protons and H-13a (**Figure 2**). From this relative arrangement, the absolute configurations 7*R*, 13a*S* of compound **1** were determined. We named this compound iso-N-methyl stepholidine.

Table 1: ^{1}H, ^{13}C and 2D NMR Spectroscopic Data for **1** in CD_3OD

N° Atome	^{13}C (δ, ppm)	^{1}H (δ, ppm), m (J, Hz)	COSY	HMBC
1	113.3	6.88, s (1H)		C-2, C-3, C-4a, C-5, C-13a
1a	123.3	-		
2	147.8	-		
3	149.7	-		
4	112.6	6.87, s (1Hs)		C-1a, C-2, C-5
4a	123.1	-		
5	24.4	3.38, m (1H, Hα) 3.13, m (1H, Hβ)	H-6	
6	63.1	3.99, m (1H, Hα) 3.87, m (1H, Hβ)	H-5	
8	62.9	4.84, d (16.2) (1H, Hα) 4.66, d (16.2) (1H, Hβ)		C13a
8a	121.4	-		
9	145.6	-		
10	150.2	-		
11	118.8	6.93, d (8.3) (1H)	H-12	C-9, C-12a
12	125.8	7.01, d (8.3) (1H)	H-11	C-10, C-8a
12a	122.4	-		
13	29.6	3.85, m (1H, Hα) 3.02, m (1H, Hβ)	H-13a	
13a	67.3	4.95, dd (15.0, 4.0) (1H)	H-13	
3-OCH_3	56.5	3.93, s (3H)		C-3
9-OCH_3	60.9	3.89, s (3H)		C-9
NCH_3	39.2	2.93, s (3H)		C-8, C-13a

Table 2: Antiplasmodial activity of extract and isolated compounds on *P. falciparum* isolates

Substances	*IC_{50}*	
	µg/mL	**µM**
MeOH extract	5.02 ± 0.20	-
Iso-N-methyl stepholidine (1)	2.57 ± 0.19	7.51 ± 0.56
N-methyl stepholidine (2)	NT	-
N-methyl tetrahydrocolumbamine (3)	2.51 ± 0,12	7.05 ± 0.34
Magnocurarine (4)	NT	-
Desmethyl magnocurarine (5)	20,89 ± 0.59	69,63 ± 1.97
Lumefantrine	4.93 ± 0.12	9.33 ± 0.23
Quinine	3.88 ± 0.08	11.95 ± 0.25

NT: Note tested

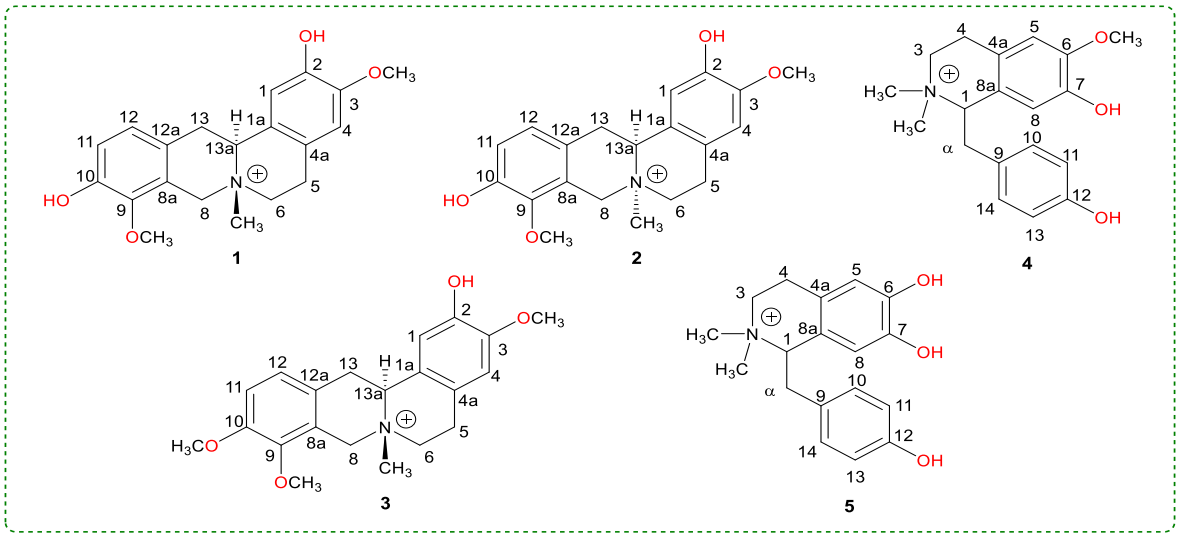

Figure 1: Structures of isolated compounds (**1-5**)

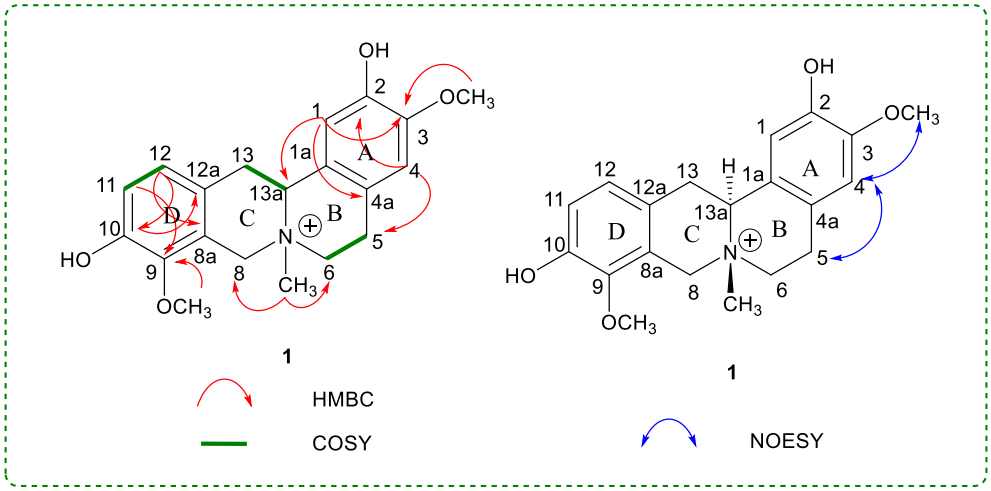

Figure 2: Important Key COSY, HMBC and NOESY Correlations for **1**

From a chemotaxomomic viewpoint, similar benzylisoquinoline alkaloids, such as **1-5** were obtained in limited plant families [18, 19]. N-methyl stepholidine (**2**) was isolated from the tubers of *Stephania venosa* (Menispermaceae) [12] while N-methyl tetrahydrocolumbamine (**3**) was obtained from the stems of *Tinospora hainanensis* (Menispermaceae) [13]. Magnocurarine (**4**) was previously isolated from the bark of *Magnolia obovata* (Magnoliaceae) [20], the stems of *Cryptocarya konishii*, *Litsea cubeba* (Lauraceae) [15], the bark of *Evodia cf. trichotoma* (Rutaceae) [14] and from the bark of *Cissampelos pareira* (Menispermaceae) [21]. As for desmethyl magnocurarine (**5**), it has only recently been isolated from the leaves of *Ocotea paranapiacabensis* (Lauraceae) along with benzylisoquinoline alkaloid **4** and two other known analogues [16]. They are to the best of our knowledge described here for the first time in *Isolonna cooperi* (Annonaceae). Therefore, they could be used to establish a relationship between these species.

Antiplasmodial Activity of Compounds

The *ex vivo* antiplasmodial assay performed on these compounds showed that iso-N-methyl stepholidine (**1**) and N-methyl tetrahydrocolumbamine (**3**) were the most active (**Table 2**). Structurally, these benzylisoquinoline alkaloids differ only on position 10, where **1** has a hydroxy group and **3** has a methoxy group. The presence of a methyl group in this position weakly increased activity from 7.51 ± 0.56 (**1**) to 7.05 ± 0.34 µM (**3**). Moreover, these activity values are higher than that of lumefantrine and quinine (**Table 2**) which are currently used in antimalarial chemotherapy. However, although the MeOH extract is more active against *P. falciparum*, this difference in activity between extract and the isolated compounds could be explained by the possible presence of other antiplasmodial molecules not yet elucidated, or by the complementary or synergistic activity of these molecules which would be little active separately.

CONCLUSION

A pseudo new benzylisoquinoline along with four other known ones were isolated from the stem barks of *Isolona cooperi*. These compounds that were isolated for the first time from the plant could be have a chemotaxonomic importance that would support phylogenetic relationships between Annonaceae and certain plant families such us Lauraceae, Magnoliaceae and Menispermaceae. Certain of these molecules have revealed very promising antiplasmodial activities, comparable to molecules

currently used in antimalarial chemotherapy. Further, these molecules will be evaluated in combination to establish possible synergies that could justify the promising activity of the crude MeOH extract. The study of their toxicity is also to be considered.

Acknowledgment: The authors wish to thank Prof. Pierre Champy and Mrs. Blandine Séon-Méniel in the Pharmacognosy Laboratory of the UFR of Pharmacy of Paris-Saclay University (France), for the recording of NMR spectra and HR-ESI-MS-Q-TOF of the isolated compounds. They are grateful to the CNF (Centre National de Floristique) of the Félix Houphouët-Boigny University, Abidjan-Cocody, Côte d'Ivoire, for the plant authentication.

Source of Support: The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest: The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

REFERENCES

1. Aubreville A. La flore forestière de la Côte d'Ivoire. 1. Paris : Centre Technique Forestier Tropical ; 1959.
2. Hutchinson J., Dalziel J.M. Flora of West Tropical Africa. 1. London: Kew Publishing; 1954.
3. Ivrine .F.R. Woody Plants of Ghana. Oxford: Oxford University Press; 1961.
4. Aké A.L., Adjanohoun E.J. Contribution au recensement des plantes médicinales Côte d'Ivoire. I. Abidjan : Centre National Floristique ; 1979.
5. Okpekon A.T. Chemical and biological study of Ivorian plants used in traditional medicine as antiparasitics: *Aframomum sceptrum* K. Schum. (Zingiberaceae), *Bridelia ferruginea* Benth. (Euphorbiaceae), *Isolona cooperi* Hutch. & Dalz. and *Uvaria afzelii* Sc. Elliot (Annonaceae) [PhD thesis]. Paris, France: Paris-11 University; 2006.
6. Boti J.B., Koukoua G., N'Guessan Y.T., Muselli A., Bernardini A.F., Casanova J. Composition of the leaf, stem bark and root bark oils of Isolona cooperi investigated by GC (retention index), GC-MS and ^{13}C-NMR spectroscopy. *Phytochem. Anal*., 2005;16: 357–363.
7. Okpekon T.A., Kabran F.A., Say V.M., Evanno L., Maciuk A., Loiseau P, Champy P. Figadère B. Apoprunellelactone (APL), an antiprotozoal lactone from the stem barks of *Isolona cooperi* Hutch. & Dalziel (Annonaceae). *Nat. Prod. Res.*, 2021;35(23): 5112–5119. https://doi.org/10.1080/14786419.2020.1781116
8. Koffi J.A., Silué K.D., Tano D.K., Dable T.M., Yavo W. Evaluation of antiplasmodial activity of extracts from endemic medicinal plants used to treat malaria in Côte d'Ivoire. *BioImpacts*, 2020;10:151–157.
9. Rieckmann K.H., Sax L.J., Campbell G.H., Mrema J.E. Drug sensitivity of *Plasmodium falciparum*. An *in vitro* microtechnique. *Lancet*, 1978;1:22–23.
10. Offoumou M.R., Kipre G.R., Kigbafori D.S., Camara D., Djaman A.J., Zirihi G.N. *In vitro/ ex vivo* antiplasmodial activity and phytochemical screening of crude extracts *Entandrophragma angolense* (Welw.) C. DC., *Griffonia simplicifolia* (Vahl ex DC.) Baill. et *Uapaca guineensis* Müll. Arg., three plants of Ivorian Pharmacopeia in the treatment of malaria. *Int. J. Curr. Microbiol. Appl. Sci.*, 2018;*7*:2088–2095.
11. Huang Z., Zhu J., Zhou Y. Intermolecular O-methylation during EIMS of quaternary methoiodides of isoquinoline alkaloids. *Org. Mass Spectrom*., 1984;19:605-609.
12. Ingkaninan K., Changwijit K., Suwanborirux K. Acetylcholinesterase inhibitors from *Stephania venosa* tuber. *J. Pharm. Pharmacol.*, 2006;58:695–700.
13. Guo Y., Kojima K., Lin L., Fu X., Zhao C., Hatano K., Chen Y., Oguihara Y. A New N-methyltetrahydroprotoberberine alkaloid from *Tinospora hainanensis*. *Chem. Pharm. Bull.,* 1999; 47(2):287–289.
14. Arbain D., Ahmad A., Nelli Z., Sargent M.V. (R)-Magnocurarine from *Evodia cf. trichotoma. Planta Med*., 1993;59:290.
15. Lee S.S., Lin Y.J., Chen C.K., Liu K.C.S., Chen C.H. Quaternary alkaloids from *Litsea cubeba* and *Cryptocarya konishii*. *J. Nat. Prod.*, 1993;56(11):1971–1976.
16. Freitas L.D., Vallée M., Dametto A.C., Pennacchi P.C., Andricopulo A.D., Maria-Engler S.S., Bolzan V.S. Advanced glycation end product inhibition by alkaloids from *Ocotea paranapiacabensis* for the prevention of skin aging. *J. Nat. Prod.,* 2020;83(3):649–656.
17. Yoshikawa K., Morishima I. Conformational analysis of quaternary alkaloids by carbon-13 NMR spectroscopy. *Chem. Lett*., 1975;9:961–964.
18. Zhao W., Liu M., Shen C., Liu K., Liu H., Ou C., Dai W., Liu X., Liu J. Biosynthesis of plant-specific alkaloids tetrahydroprotoberberines in engineered *Escherichia coli*. *Green Chem.*, 2021;23:5944–5955. https://doi.org/10.1039/D1GC01670A
19. Zhao W., Liu J., Chen Y. Advances in the biosynthesis of naturally occurring benzylisoquinoline alkaloids. *Front. Plant Sci.*, 2025;16:1548471. https://doi.org/10.3389/fpls.2025.1548471
20. Ogiu K., Morita M. Curare-like action of magnocurarine, isolated from *Magnolia obovate*. *Jap. J. Pharmacol.*, 1953;2:89–96.
21. Bala M., Kumar S., Pratap K., Verma P.K., Padwad Y., Singh B. Bioactive isoquinoline alkaloids from *Cissampelos pareira*. *Nat. Prod. Res.*, 2019;33(5):622–627. https://doi.org/10.1080/14786419.2017.1402319