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Isolation and spectroscopic characterization of Catechin from the leaves and stems of *Landolphia owariensis* P. Beauv. (Apocynaceae) from Côte d'Ivoire

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***Abstract*:**

The investigation of medicinal plants constitutes a valuable approach for the discovery of bioactive compounds with pharmacological relevance. In this work, the leaves and stems of *Landolphia owariensis* (Apocynaceae) were investigated for their secondary metabolite composition with the aim of isolating and characterizing bioactive constituents. Crude methanolic and ethyl acetate extracts were obtained using appropriate extraction procedures, followed by chromatographic fractionation, which led to the isolation of a major compound with yields of 0.043 % (AcDFM) and 0.033 % (AcDTM). The structure of the isolated compound was elucidated using complementary spectroscopic techniques, including nuclear magnetic resonance spectroscopy (^{1}H, ^{13}C NMR, DEPT, HSQC, HMBC), infrared (IR) spectroscopy, high-resolution mass spectrometry, GC–MS analysis, and optical rotation measurements. The compound was identified as (+)-catechin, a flavan-3-ol widely recognized for its antioxidant and bioactive properties. The identification of (+)-catechin in *L. owariensis* represents a notable phytochemical finding, as it expands the known chemical profile of the species and contributes to the chemotaxonomic understanding of the genus *Landolphia*. Although a direct causal relationship cannot be established within the scope of this study, the presence of this compound may partly support the traditional medicinal uses of the species. The results highlight the phytochemical potential of *L. owariensis* and provide a scientific basis for further studies aimed at exploring its pharmacological properties and possible applications in drug discovery and natural product development.

***Keywords* :** *Landolphia owariensis* ; Apocynaceae ; (+)-Catechin ; Phytochemistry ; Spectroscopic characterization.

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1. Introduction

Medicinal plants constitute an invaluable source of bioactive compounds that have been used for centuries in traditional pharmacopoeias. Among these compounds, specialized metabolites (formerly referred to as secondary metabolites), such as flavonoids, occupy a prominent place owing to their numerous biological properties, including antioxidant, anti-inflammatory, cardioprotective, and anticancer activities [1–3]. These compounds are distributed across several botanical families, notably the Apocynaceae, which are recognized for their chemical richness and the diversity of their traditional uses.

Landolphia owariensis P. Beauv. is a tropical African liana belonging to the Apocynaceae family. Commonly known as "Gbéi" or "Pôpôni" in the Dioula language (Côte d'Ivoire), this plant is traditionally used to relieve menopausal symptoms in the localities of Grand-Lahou and Katiola and is also employed in traditional pediatric care in Ferkessédougou [4–7]. In other regions of Africa, *L. owariensis* is used for its antimicrobial and anti-inflammatory properties, as well as in the management of various conditions such as malaria, infections, digestive disorders, and certain metabolic disturbances [8–10].

Previous phytochemical studies carried out on this species have revealed the presence of several groups of specialized metabolites, including alkaloids, flavonoids, tannins, and iridoids in the leaves and stems [11–14]. These studies also highlighted high levels of total phenolic compounds as well as significant antioxidant activity of the methanolic extracts and their ethyl acetate fractions [7]. Despite these investigations, studies focusing on the isolation and structural characterization of individual chemical constituents from this plant remain limited.

Among phytochemicals of interest, catechin, a flavan-3-ol widely distributed throughout the plant kingdom, is considered an important compound in natural products chemistry because of its antioxidant properties and its involvement in several biological activities. Catechins are involved in the scavenging of free radicals through their phenolic groups and play an important role in the prevention of oxidative stress and associated diseases [8,9,15]. They also constitute the basic structural units of proanthocyanidins and are present in numerous medicinal and dietary plants [16,17]. Their occurrence in plant extracts is frequently associated with significant biological activities, thereby justifying the interest in their isolation and characterization.

The isolation of such compounds from complex plant matrices requires the use of appropriate fractionation techniques followed by advanced analytical methods for structural identification. In this regard, spectroscopic techniques such as nuclear magnetic resonance (NMR) and mass spectrometry play an essential role in molecular structure elucidation [18,19]. In this context, the present study aimed to isolate catechin from extracts of the leaves and stems of *L. owariensis* P. Beauv. and to perform its structural characterization using appropriate spectroscopic techniques. This study contributes to a better understanding of the chemical composition of *L. owariensis*, expands the phytochemical knowledge of the genus *Landolphia*, and provides a scientific foundation for future pharmacological and chemotaxonomic studies.

2. Materials and methods

2.1. Plant material

The leaves and stems of *Landolphia owariensis* were collected from a forest located near the National Center for Agronomic Research (CNRA, 5°19′48.807″ N, 4°7′51.767″ W) and the Swiss Center for Scientific Research in Adiopodoumé (CSRS, 5°19′49.841″ N, 4°7′40.703″ W), in southern Côte d'Ivoire. The species was identified and authenticated at the Botany Laboratory of the CSRS in Côte d'Ivoire (herbarium no. 7, SIG Ivoire database reference: 2315542). Plant samples were carefully cleaned, then dried in an air-conditioned room at 20 °C (one week for leaves and two weeks for stems) before being ground into powder.

2.2. Preparation of extracts

- **Crude methanolic extracts**
  A total of 154.5 g of powdered leaf material and 107 g of powdered stem material were separately subjected to sequential extraction. Delipidation was first carried out by two successive 24 h macerations in petroleum ether (1600 mL for leaves; 1000 mL for stems), followed by two successive 24 h macerations in dichloromethane under identical volume conditions. After drying of the marc, methanolic extraction was performed by three successive decoctions of 30 min each (1600 mL for leaves; 1000 mL for stems). Solvent removal using a rotary evaporator at 40 °C (Büchi, Rotavapor R300) yielded two crude methanolic extracts: leaf decoction (DFM; 25 g) and stem decoction (DTM; 15 g).

- **Ethyl acetate fractions**
  One gram of each crude methanolic extract (DFM, DTM) was dissolved in 150 mL of distilled water and subjected to liquid–liquid partitioning. The aqueous phase was exhaustively extracted by three successive treatments with 150 mL of ethyl acetate. This operation was repeated several times until obtaining 3.3815 g of AcDFM and 1.799 g of AcDTM.

2.3. Fractionation and isolation

2.3.1. Fractionation and isolation of the compound from AcDFM

The AcDFM extract (3.3815 g) was fractionated on a silica gel column (Kieselgel 60 M, 169.075 g) using a dichloromethane/methanol (DCM/MeOH) solvent gradient with varying proportions (100:0; 97:3; 95:5; 90:10; 85:15; 80:20). Several fractions were obtained: F1–F166, F167–F289, and F290–F350. Fractions F167–F289 (522 mg) were further fractionated on a silica gel column with DCM/MeOH (95:5), yielding subfractions

F'1–F'45, F'46–F'80, and F'81–F'125. Purification of fractions F'46–F'80 (86 mg) led to the isolation of catechin (14.5 mg), which exhibited an intense red coloration under visible light after spraying with vanillin–sulfuric acid reagent.

2.3.2. *Fractionation and isolation of the compound from AcDTM*

The AcDTM extract (1.799 g) was fractionated on a silica gel column (Kieselgel 60 M, 90 g) using a DCM/MeOH solvent gradient (100:0; 97:3; 95:5; 90:10; 85:15; 80:20). Fractions obtained were F1–F77, F78–F190, and F191–F226. Fractions F78–F190 (232.7 mg) were further fractionated with DCM/MeOH (95:5), yielding subfractions F'1–F'132, F'133–F'143, and F'144–F'150. Purification of fractions F'133–F'143 (23 mg) resulted in the isolation of catechin (6 mg), also showing an intense red coloration under visible light after spraying with vanillin–sulfuric acid reagent.

2.4. Elucidation of the isolated compound

NMR spectra (^{1}H, ^{13}C, DEPT, COSY, HSQC, HMBC) were recorded in CD_3OD using a Bruker spectrometer operating at 400 MHz. High-resolution mass spectrometry (HRMS) data were obtained with a Bruker maXis mass spectrometer in positive electrospray ionization mode (ESI+). Infrared (IR) spectra were recorded using a Fourier-transform infrared spectrometer (Perkin Elmer Spectrum UATR Two). Optical rotation was determined with an Anton Paar MPC 100 automatic polarimeter.

3. Results and discussion

3.1. Extraction yields

The extraction yields obtained (Table 1) indicate that the methanolic extracts exhibited the highest values, with 16.18 % for leaves (DFM) and 14.22 % for stems (DTM). This observation can be attributed to the high polarity of methanol, which facilitates the extraction of a broad spectrum of specialized metabolites, particularly phenolic compounds, flavonoids, and tannins.

In contrast, the ethyl acetate extracts displayed lower yields (13.45 % for AcDFM and 11.77 % for AcDTM), reflecting the semipolar nature of this solvent, which promotes a more selective extraction of specific bioactive compounds.

The yields of the isolated compound from the ethyl acetate extracts were very low (0.043 % for AcDFM and 0.033 % for AcDTM). Such values are typical of isolation processes targeting individual molecules, which are generally present in small quantities within complex plant matrices. Furthermore, leaf extracts consistently showed higher yields compared to stem extracts, suggesting a greater abundance of extractable metabolites in the leaves of *Landolphia owariensis*.

Table 1
Extraction and isolation yields.

Extract type	Extract	Yield (%)
Methanolic extracts	DFM	16.18
	DTM	14.22
Ethyl acetate extracts	AcDFM	13.45
	AcDTM	11.77
Compound isolated from ethyl acetate extracts	AcDFM	0.043
	AcDTM	0.033

3.2. Structure elucidation of isolated catechin

The chromatograms (Figure 1) showed the presence of a catechin molecular fingerprint in subfractions F'46–F'80 (for AcDFM) and F'133–F'143 (for AcDTM) with an R_f of 0.17. Its detection using vanillin–sulfuric acid reagent generated an intense red coloration, characteristic of flavan-3-ols such as catechin. The isolated phytochemical compound appeared as a dark powder after purification.

High-resolution mass spectrometry analysis (Figure 2) in positive electrospray ionization mode (ESI+) of the isolated compound was performed. The spectrum shows a major peak at $m/z = 291.0863$, corresponding to a pseudomolecular ion $[M+H]^+$. The measured exact mass is in excellent agreement with the molecular formula $C_{15}H_{14}O_6$ (error below 1 ppm), confirming the accuracy of the assignment. The neutral molecular mass of the compound is thus estimated to be $M = 290.0786$ g mol^{-1}.

Gas chromatography–mass spectrometry (GC–MS) analysis of the purified compound after derivatization with BSTFA allowed its identification as catechin in the form of a trimethylsilylated derivative. The chromatogram (Figure 3) reveals a main peak eluting at a retention time of 16.48 min, characteristic of a highly silylated polyphenol, indicating good volatility after derivatization.

The mass spectrum recorded (Figure 4) in electron impact mode (EI, 70 eV) shows a base peak at m/z 73, attributed to the $Si(CH_3)_3^+$ ion, a typical signature of trimethylsilylated compounds. The high intensity of this signal confirms the efficiency of the silylation reaction and the presence of multiple TMS groups on the molecule.

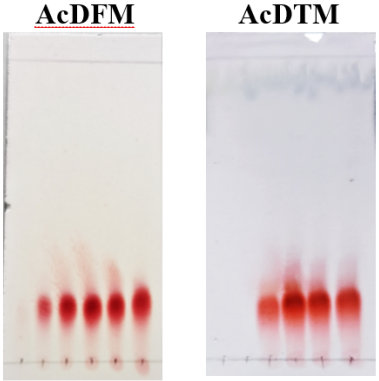


Fig. 1. Chromatograms of catechin isolated from subfractions F'46–F'80 (AcDFM) and F'133–F'143 (AcDTM).

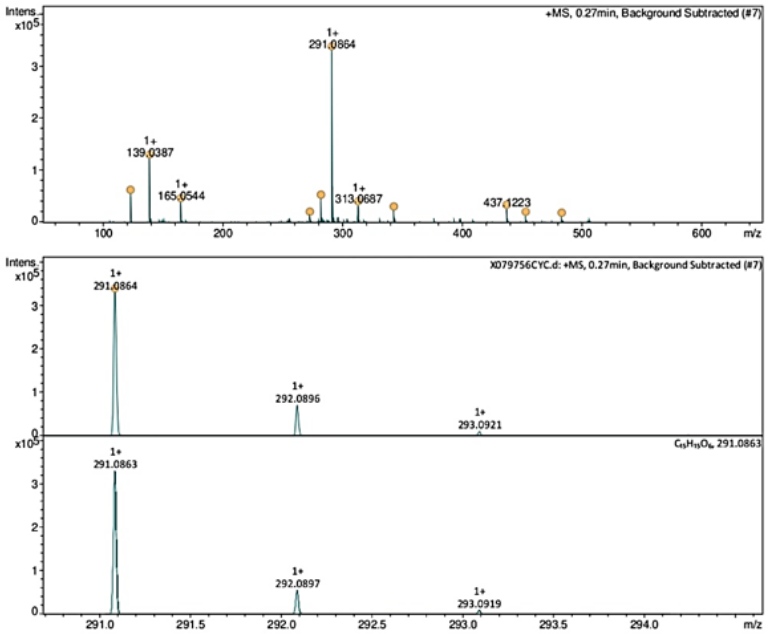


Fig. 2. HRMS profile of the isolated compound.

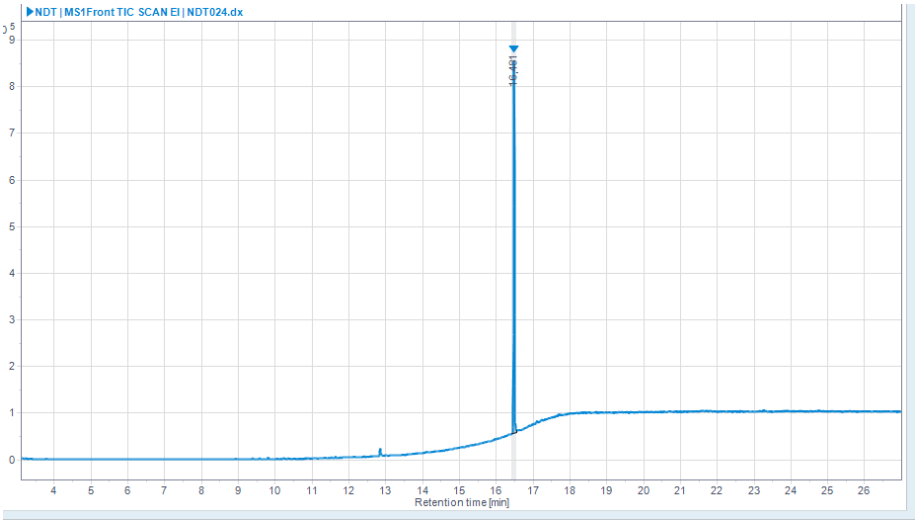


Fig. 3. GC–MS chromatogram of catechin.

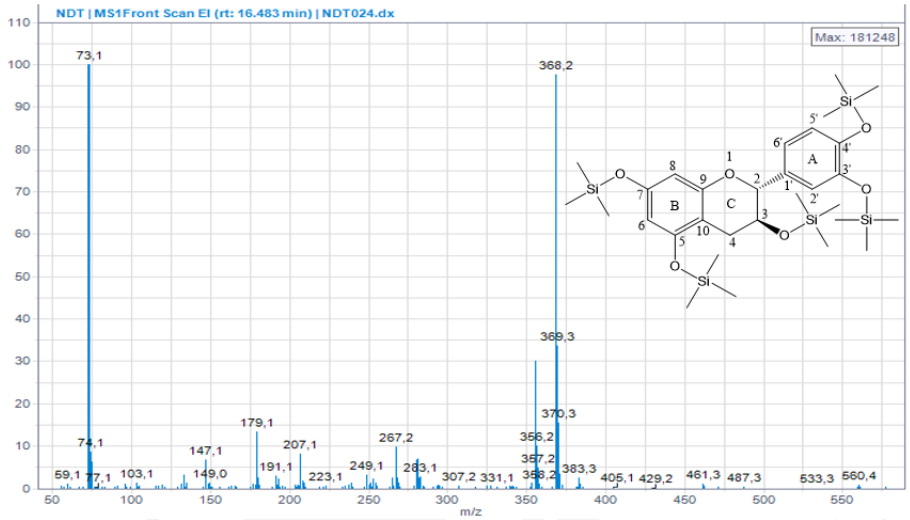


Fig. 4. Mass spectrum of catechin in positive mode.

Several diagnostic fragments are observed at m/z 147.1, 179.1, 191.1, 207.1, 249.1, 267.2, and 283.1, corresponding to aromatic fragments resulting from the cleavage of the catechin flavanol skeleton. These ions are characteristic of TMS-derivatized flavonoids and arise mainly from cleavages of the A, B, and C rings of the flavan-3-ol structure. The presence of intense peaks in the high mass region, notably at m/z 368.2–370.3, is attributed to pseudomolecular fragments resulting from the successive loss of TMS groups. These ions are typically observed for pentakis-trimethylsilylated (5TMS) polyphenols analyzed in EI mode, where the theoretical molecular ion of catechin-5TMS (m/z = 650.28) is generally weak or absent due to the extensive fragmentation induced by electron ionization. All these spectral data, combining the presence of characteristic TMS ions, aromatic fragments of the flavanol skeleton, and heavy fragments related to the loss of TMS groups, are consistent with reference spectra reported in the literature [20]. These results were confirmed by complementary spectroscopic analyses, notably NMR (^{1}H, ^{13}C, DEPT, COSY, HSQC, HMBC).

The ^{1}H NMR spectrum (400 MHz, CD_3OD, δ, ppm) of catechin showed various signals (Figure 5): signals at 6.84 (d, J = 2.0 Hz, 1H), 6.76 (d, J = 8.1 Hz, 1H), and 6.72 (dd, J = 8.2 Hz, 1H) attributable to the aromatic protons of ring B corresponding to H-2′, H-5′, and H-6′, respectively. Signals at 5.93 (s, J = 2.3 Hz, 1H) and 5.85 (s, J = 2.3 Hz, 1H) attributable to the aromatic protons of ring A corresponding to H-8 and H-6, respectively. The signal at 4.56 (d, J = 7.5 Hz, 1H) attributable to the oxygenated proton H-2 of ring C. Signals at 3.97 (m, J = 7.9, 5.4 Hz, 1H), 2.85 (dd, J = 16.2, 5.5 Hz, 1H), and 2.50 (dd, J = 16.1, 8.2 Hz, 1H) attributable to the aliphatic protons of ring C corresponding to H-3, H-4a, and H-4b, respectively. These results are consistent with the data for catechin isolated from *Albizia lebbeck* [16].

The ^{13}C NMR spectrum (101 MHz, CD_3OD, δ, ppm) (Figure 6) allowed the enumeration of 15 carbons with the following signals: 157.83 ppm (C-9); 157.57 ppm (C-7); 156.90 ppm (C-5); 146.24 ppm (C-4′); 146.21 ppm (C-3′); 132.20 ppm (C-1′); 120.03 ppm (C-6′); 116.06 ppm (C-5′); 115.23 ppm (C-2′); 100.79 ppm (C-10); 96.26 ppm (C-8); 95.48 ppm (C-6); 82.84 ppm (C-2); 68.80 ppm (C-3); and 28.51 ppm (C-4).

Comparison of the signals observed in the ^{13}C NMR spectrum (Figure 6), the DEPT-135 spectrum (Figure 7), and literature data [21] allowed the identification of quaternary carbons, CH, and CH_2 groups, with an absence of CH_3 groups consistent with the catechin structure. Specifically, one CH_2 (28.51 ppm; C-4), seven CH groups (120.03 ppm, C-6′; 116.06 ppm, C-5′; 115.23 ppm, C-2′; 96.26 ppm, C-8; 95.48 ppm, C-6; 82.84 ppm, C-2; 68.80 ppm, C-3), and seven quaternary carbons (157.83 ppm, C-9; 157.57 ppm, C-7; 156.90 ppm, C-5; 146.24 ppm, C-4′; 146.21 ppm, C-3′; 132.20 ppm, C-1′; 100.79 ppm, C-10) were identified.

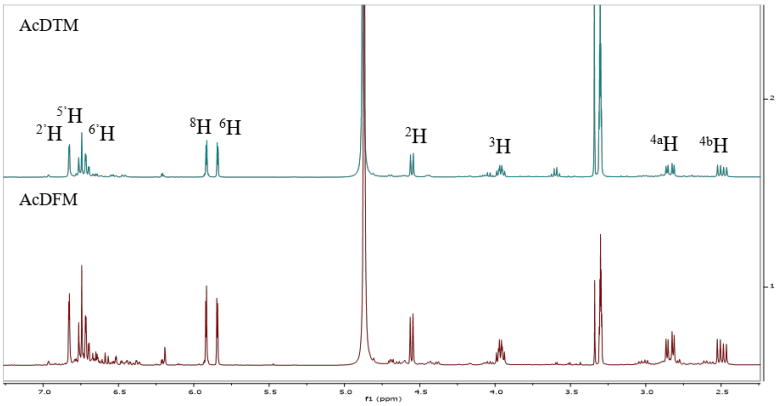


Fig. 5. ^{1}H NMR spectrum (400 MHz, CD_3OD, δ, ppm) of catechin.

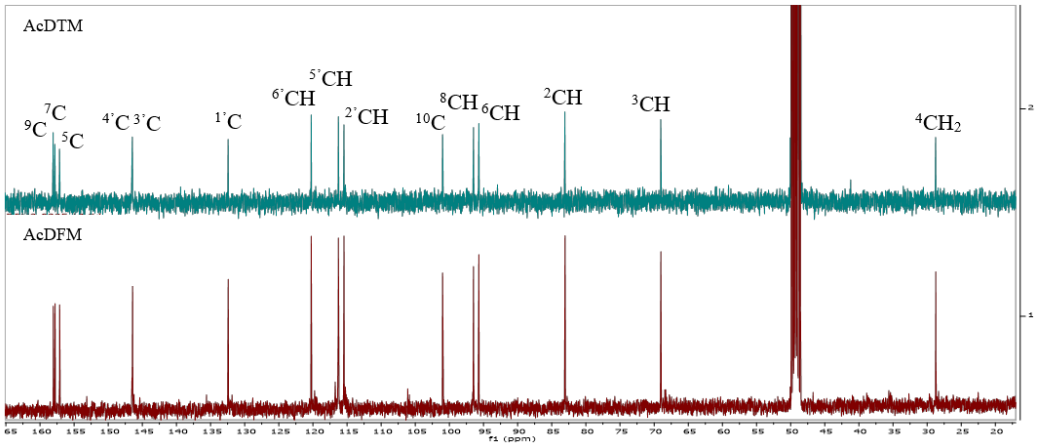


Fig. 6. ^{13}C NMR spectrum (101 MHz, CD_3OD, δ, ppm) of catechin.

Using HSQC (Figures 8(A) and 8(B)), several observations were made. A geminal coupling ($J = 16$ Hz) between two H atoms, each resonating as a doublet of doublets at $\delta_H = 2.85$ ppm and $\delta_H = 2.50$ ppm, is carried by carbon C-4 ($\delta_C = 28.94$ ppm), corresponding to the CH_2 of ring C. The proton resonating as a multiplet at $\delta_H = 3.97$ ppm is carried by the chiral carbon C-3 ($\delta_C = 68.80$ ppm) at position 3 of ring C, corresponding to a CH. A proton resonating as a doublet at $\delta_H = 4.56$ ppm is carried by the chiral carbon C-2 ($\delta_C = 82.84$ ppm) at position 2 of ring C, corresponding to a CH. Furthermore, the protons resonating as singlets at $\delta_H = 5.93$ ppm (1H) and $\delta_H = 5.85$ ppm (1H) are carried respectively by carbons at position 8 ($\delta_C = 96.26$ ppm) and position 6 ($\delta_C = 95.48$ ppm) of ring A, corresponding to the CH of the ring. Finally, the protons resonating as a doublet at $\delta_H = 6.84$ ppm (1H), a doublet at $\delta_H = 6.76$ ppm (1H), and a doublet of doublets at $\delta_H = 6.72$ ppm (1H) are carried respectively by carbons at position 2′ ($\delta_C = 115.23$ ppm), position 5′ ($\delta_C = 116.06$ ppm), and position 6′ ($\delta_C = 120.03$ ppm) of ring B, corresponding to the CH of this ring.

The IR spectrum of catechin (Figure 9) provides information on the presence or absence of certain functional groups. The spectrum showed a moderately broad absorption band between 3500 and 3000 cm^{-1}, consistent with the presence of hydroxyl (OH) groups. An absorption band recorded between 1610 and 1520 cm^{-1} reflects the existence of C=C double bonds of an aromatic ring. Finally, the absorption bands observed at 1279.66 cm^{-1} and 1242 cm^{-1} correspond to the C–O–C group between C-9 and C-2 of ring C.

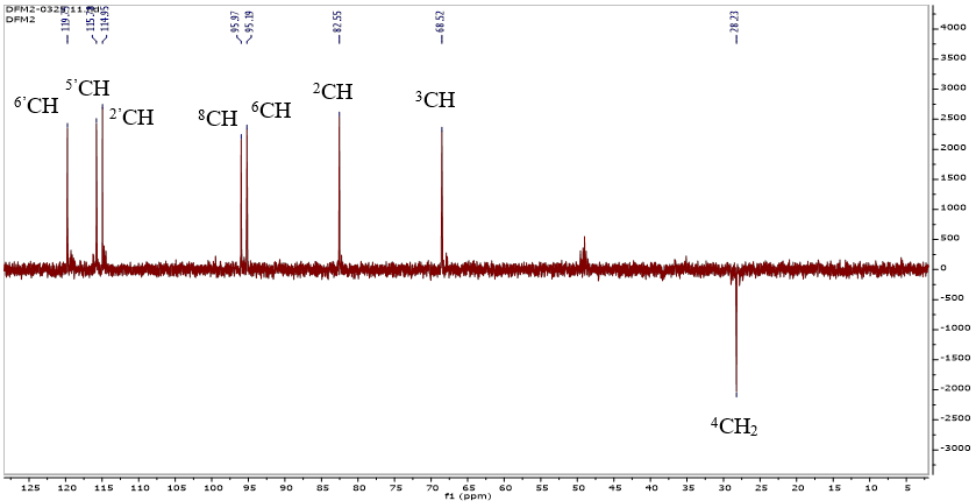
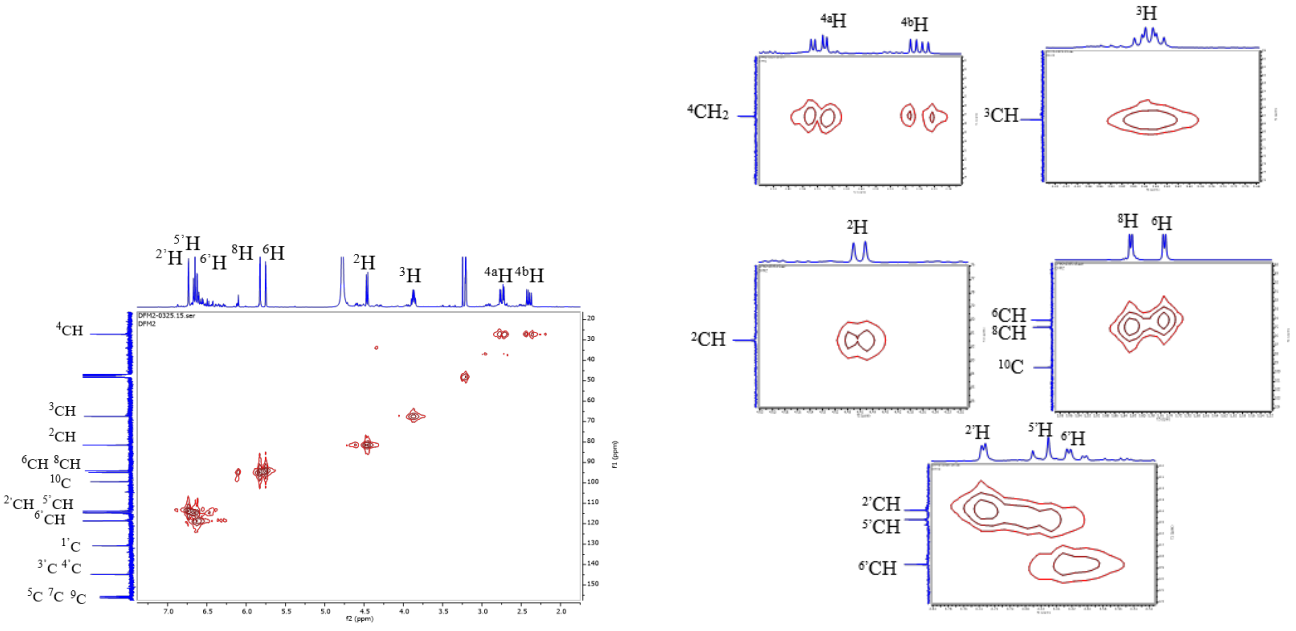


Fig. 7. DEPT-135 spectrum of isolated catechin: CH and CH_3 positive, CH_2 negative.



(A) **(B)**

Fig. 8. HSQC spectra (^1H–^{13}C correlations; ^1J): (A) and (B).

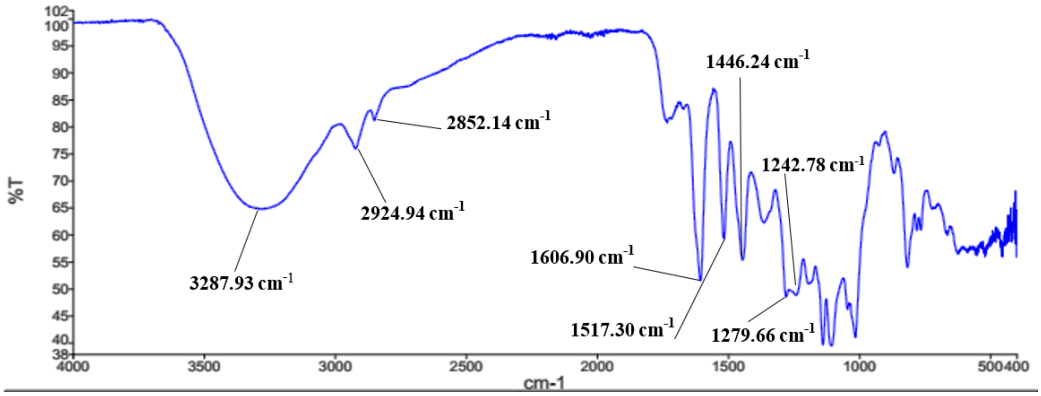


Fig. 9. IR spectrum of catechin.

The chemical structure of the molecule was confirmed by comparison with the structural data of catechin isolated from *Albizia lebbeck* (Fabaceae) reported by Desai *et al.* [16]. To resolve the stereochemical ambiguity, the specific optical rotation of the isolated compound ($[\alpha]_D^{25}$) was determined:

$$[\alpha]_D^{25} = +15.15° \quad (c = 0.013;\ Me_2CO;\ AcDFM)$$

$$[\alpha]_D^{25} = +14.89° \quad (c = 0.055;\ Me_2CO;\ AcDTM)$$

Optical rotation measurement allows interpretation of the deviation of the polarization plane of incident light passing through an optically active substance; a positive value indicates a dextrorotatory (+) character, while a negative value indicates a levorotatory (−) character. Comparing our results with the work of El-Razek [22], who reports the following values for catechin epimers in acetone:

(+)-catechin: $[\alpha]_D^{25} = +14°$ $(c = 0.4;\ Me_2CO)$

(−)-catechin: $[\alpha]_D^{25} = -57°$ $(c = 0.5;\ Me_2CO)$

(+)-epicatechin: $[\alpha]_D^{25} = +69°$ $(c = 0.5;\ Me_2CO)$

(−)-epicatechin: $[\alpha]_D^{25} = -55°$ $(c = 0.5;\ Me_2CO)$

The values obtained for our compound are reasonably close to those of (+)-catechin. In light of these results, we conclude that the isolated compound is (+)-catechin (Figure 10).

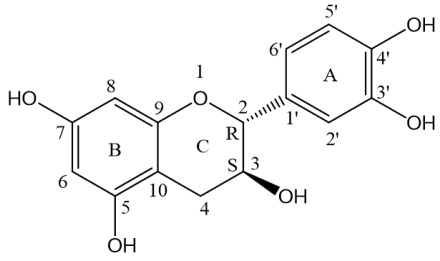


Fig. 10. Molecular structure of catechin: (2*R*,3*S*)-2-(3,4-dihydroxyphenyl)chromane-3,5,7-triol.

Phytochemical investigations conducted on the genus *Landolphia* have revealed a remarkable diversity of secondary metabolites, including flavonoids, phenolic compounds, fatty acids, terpenoids, lignans, coumarins, alkaloids, saponins, and steroids [6]. The most extensively studied species are *Landolphia owariensis*, *L. dulcis*, *L. kirkii*, *L. heudelotii*, and *L. membranacea* [6]. In *L. dulcis*, phytochemical studies have demonstrated the presence of flavonoids, tannins, phenolic compounds, alkaloids, saponins, and steroids in the leaves and stem bark [23], while essential oil analyses revealed the occurrence of terpenoid compounds and fatty acid derivatives, including phytol, eucalyptol, *n*-hexadecanoic acid, and caryophyllene oxide [24]. In *L. kirkii*, phenolic compounds, lignans, and various fatty acids have been reported, whereas the available phytochemical information on *L. senegalensis* and *L. violacea* remains relatively scarce [6].

Among these species, *L. owariensis* appears to be one of the best documented from ethnobotanical, pharmacological, and phytochemical perspectives. Previous studies have led to the identification of several bioactive metabolites, including chlorogenic acid, protocatechuic acid, β-sitosterol, myricitrin, quercitrin, α-amyrin, and daucosterol [4, 6]. However, despite the richness of the phytochemical profile already reported for this species and the well-documented occurrence of phenolic compounds within the genus *Landolphia*, no previous study had reported the isolation of catechin [6].

Therefore, the detection of catechin in *L. owariensis* represents, to the best of our knowledge, the first identification of this flavan-3-ol in this species and likely within the genus *Landolphia*. This finding significantly enriches the phytochemical profile of the plant and provides new insights into the chemotaxonomy of the genus. It also suggests that flavan-3-ols may constitute a class of secondary metabolites that has hitherto been underestimated in *Landolphia* species [6].

The significance of this discovery extends beyond its chemotaxonomic implications. Indeed, *L. owariensis* is widely used in African traditional medicine for the treatment of gastrointestinal disorders, infections, and inflammatory conditions [6, 25]. Catechin is well recognized for its antioxidant, anti-inflammatory, antimicrobial, cardioprotective, and neuroprotective properties, which have been extensively documented in recent pharmacological studies [26, 27]. Its occurrence in *L. owariensis* may therefore contribute, at least in part, to the therapeutic activities traditionally attributed to this species. Although no direct causal relationship can be established within the scope of the present study, the identification of this compound provides a plausible chemical basis for some of the ethnomedicinal uses of

the plant.

Furthermore, catechin has attracted increasing interest in the pharmaceutical, nutraceutical, and cosmetic industries owing to its potent antioxidant activity and its ability to mitigate oxidative stress-induced damage [26,27]. As a result, it is being investigated as a natural ingredient in the development of dietary supplements, functional products, and formulations intended for the prevention or attenuation of various disorders associated with oxidative stress and chronic inflammation [27].

Beyond these biological applications, catechin also holds considerable potential in the field of green nanotechnology. Catechin-type polyphenols have been employed as reducing and stabilizing agents in the eco-friendly synthesis of metallic nanoparticles, particularly silver nanoparticles, owing to their phenolic hydroxyl groups, which are capable of reducing metal ions and stabilizing the resulting nanostructures [28]. Catechin derivatives, such as epigallocatechin gallate (EGCG), have also been investigated for incorporation into nanostructured drug-delivery systems and therapeutic biomaterials [29]. Consequently, the isolation of catechin from *L. owariensis* opens promising avenues for the future valorization of this species, not only in the pharmaceutical sector but also in nutraceuticals, nanotechnology, and the development of functional materials.

4. Conclusion

In this study, the leaves and stems of *Landolphia owariensis* (Apocynaceae) were investigated for their secondary metabolite composition. Sequential extraction using methanol and ethyl acetate, followed by chromatographic fractionation, led to the isolation of a major compound with yields of 0.043 % (AcDFM) and 0.033 % (AcDTM). Comprehensive spectroscopic analyses, including 1D and 2D NMR (^{1}H, ^{13}C, DEPT, HSQC, HMBC), IR spectroscopy, high-resolution mass spectrometry, GC–MS, and optical rotation measurements, allowed the unambiguous identification of the compound as (+)-catechin, a flavan-3-ol.

The identification of (+)-catechin in *L. owariensis* is noteworthy from both a phytochemical and chemotaxonomic perspective, as it represents the first report of this compound in this species and potentially within the genus *Landolphia*. This finding expands the known chemical diversity of the genus and suggests that flavan-3-ols may have been previously underreported in this taxonomic group.

From a pharmacological standpoint, the occurrence of (+)-catechin provides a plausible chemical basis that may partially support some of the traditional medicinal uses of *L. owariensis*, particularly those related to inflammatory and infectious conditions, given the well-documented antioxidant and bioactive properties of catechin in the literature.

Overall, this work contributes to a better understanding of the phytochemical profile of *L. owariensis* and highlights its potential as a source of bioactive phenolic compounds. Nevertheless, further investigations, including the isolation of additional secondary metabolites and *in vitro*/*in vivo* biological evaluations of the isolated catechin, are required to fully elucidate the pharmacological potential and possible applications of this species in drug discovery and related fields.

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