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IDENTIFICATION OF β-SITOSTEROL IN A LIPOPHILIC FRACTION OF *KHAYA GRANDIFOLIOLA* C. DC. (MELIACEAE) BY SPECTROSCOPIC ANALYSIS

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ABSTRACT

β-Sitosterol is a widely distributed plant sterol of interest in the phytochemical characterization of natural resources. This study aimed to isolate and spectroscopically characterize a sterol compound from the dichloromethane extract of the stem bark of *Khaya grandifoliola* C. DC. (Meliaceae), collected in April 2025 in Ahougnassou-Allahou, Tiébissou Department, Côte d'Ivoire. After drying and pulverization, the plant material was subjected to successive maceration with n-hexane, dichloromethane, and ethyl acetate. The dichloromethane extract was then fractionated by silica gel column chromatography, with fractions monitored by thin-layer chromatography (TLC). The isolated compound was characterized by combining proton nuclear magnetic resonance (^{1}H NMR), carbon-13 nuclear magnetic resonance (^{13}C NMR), and DEPT-135 spectroscopic data. The ^{1}H NMR spectrum showed an olefinic proton signal at approximately δ_H 5.35 ppm and a multiplet at δ_H 3.5-3.6 ppm, consistent with a proton

associated with a carbon-carbon double bond and a proton attached to an oxygen-bearing carbon, respectively. The ^{13}C NMR spectrum displayed two olefinic carbon signals at δ_C 140.8 and 121.7 ppm, together with an oxygenated carbon signal at δ_C 71.8 ppm. These signals, considered alongside the characteristic aliphatic profile, are consistent with the reported NMR data for β-sitosterol. Accordingly, the spectroscopic findings support the tentative identification of the isolated compound as β-sitosterol. Nevertheless, in the absence of two-dimensional NMR data, mass spectrometric analysis, and an assessment of the isolate's purity, this structural assignment remains provisional and requires further confirmation.

KEYWORDS: *Khaya grandifoliola*; β-sitosterol; dichloromethane extract; silica gel column chromatography; NMR spectroscopy; DEPT-135.

INTRODUCTION

Natural products serve as a primary engine of structural diversity in organic chemistry, critical for scaffolding the discovery of novel chemotypes with biologically, nutritionally, cosmeceutically, or pharmaceutically relevant profiles.[1] Structural characterization requires a multi-tiered analytical strategy encompassing targeted metabolomic extraction, orthogonal chromatographic resolution, and rigorous structure elucidation mediated by a complementary suite of spectroscopic techniques.[2,3] In this context, interrogating the lipophilic profiles of tropical plant species represents a vital avenue for unlocking the latent value of biodiversity hotspots and mapping undocumented chemical spaces.[4] Among plant-derived lipophilic metabolites, phytosterols occupy a distinctive position. Characterized by a rigid tetracyclic steroid nucleus, these compounds exhibit structural divergence through diverse substituent modifications, which directly dictate their downstream physicochemical behaviors.[5,6] As a prominent member of the phytosterol family, β-Sitosterol (stigmast-5-en-3β-ol) occurs widely across diverse plant tissues, serving as a benchmark component in sterol profiling.[7] Its structure comprises a secondary alcohol group at C-3, a double bond between C-5 and C-6, and a branched hydrocarbon side chain. Its molecular formula is $C_{29}H_{50}O$, and its molar mass is approximately 414.71 $g \cdot mol^{-1}$.[8] The identification of β-sitosterol primarily relies on the convergence of several spectroscopic observations. In proton nuclear magnetic resonance (^1H NMR) spectroscopy, the olefinic proton H-6 typically resonates at approximately δ_H 5.3-5.4 ppm, whereas the methine proton H-3, attached to the carbon bearing the hydroxyl group, generally appears at around δ_H 3.5 ppm. In carbon-13 nuclear magnetic resonance (^{13}C NMR)

spectroscopy, characteristic signals include the quaternary carbon C-5 at approximately δ_C 140.8 ppm, the olefinic carbon C-6 at approximately δ_C 121.7 ppm, and the oxygenated carbon C-3 at approximately δ_C 71.8 ppm. These diagnostic parameters necessitate careful interpretation with respect to the extraction solvent matrix, specific experimental conditions, and the potential co-elution of closely related sterol congeners.[9] To refine the structural assignment, DEPT-135 analysis is deployed to stratify protonated carbon centers according to their respective hydrogenation states. This modality yields positive phase signals for CH and CH_3 moieties, selectively inverts CH_2 resonances into the negative phase, and suppresses quaternary carbon signals entirely.[10,11] Comparison of the DEPT-135 spectrum with the conventional ^{13}C NMR spectrum therefore helps assess the consistency of carbon-environment assignments with the proposed molecular structure. However, the presence of characteristic signals from the steroid nucleus alone is insufficient to distinguish β-sitosterol from other structurally related phytosterols. Such discrimination necessitates a rigorous comparative analysis of spectroscopic datasets specifically ^{1}H and ^{13}C NMR chemical shifts while systematically accounting for the subtle structural variations localized within the steroidal side chains.[12]

The genus *Khaya*, which belongs to the family Meliaceae, comprises tropical woody species whose organs have been investigated phytochemically, particularly for limonoids and other specialized metabolites. *Khaya grandifoliola* thus emerges as a compelling candidate for the bioprospecting of novel natural products sourced from West African botanical reservoirs.[13,14]

Nevertheless, studies of this species must distinguish between constituents actually characterized in a particular plant organ and those reported in other organs or related species. The chemical compositions of seeds, leaves, and bark cannot be considered interchangeable in the absence of organ-specific experimental evidence. From this perspective, the stem bark of *K. grandifoliola* represents a relevant material for investigating its lipophilic fraction and characterizing its constituents. The use of solvents of increasing polarity enables metabolites to be distributed according to their solubility properties, while silica gel column chromatography facilitates the separation of constituents from complex extracts. Subsequently, nuclear magnetic resonance spectroscopy serves as the definitive cornerstone for structural elucidation, contingent upon the meticulous interpretation of spectral data and rigorous cross-examination against established literature benchmarks.[15]

The present study follows this approach and aims to characterize a compound isolated from a lipophilic fraction obtained from the stem bark of *K. grandifoliola*, collected in the Bélier Region of Côte d'Ivoire. Specifically, the study has three objectives: (i) to describe the extraction and chromatographic fractionation procedures used to obtain the compound under investigation; (ii) to interpret its main spectroscopic features based on ^{1}H NMR.[13] C NMR, and DEPT-135 spectra; and (iii) to compare the observed diagnostic signals with reference data for β-sitosterol in order to assess the strength of the proposed structural assignment. The significance of this work lies in providing spectroscopic documentation of a lipophilic constituent of *K. grandifoliola* stem bark. It contributes to the chemical investigation of this plant resource and establishes an experimental basis for further studies aimed at confirming the structure, assessing the purity, and exploring the potential applications of the tentatively identified compound.

MATERIAL AND METHODS

Plant Material

The plant material consisted of the stem bark of *Khaya grandifoliola* C. DC., a species belonging to the family Meliaceae. The samples were collected in April 2025 in Ahougnassou-Allahou, Tiébissou Department, Belier Region, Côte d'Ivoire (7.1681° N, 5.0592° W).

Preparation of the Plant Material

Following collection, the plant material was dried in a laboratory oven at 18 °C for three weeks and subsequently ground into a fine, homogeneous powder. A representative portion of the powdered material was then used for extraction and phytochemical analysis.

Maceration and Sequential Solvent Extraction

The extraction procedure was based on general phytochemical extraction methods described by Harborne.[15] and a protocol attributed to Nyigo et al.[16] Briefly, 10 g of powdered *K. grandifoliola* stem bark was macerated in 100 mL of a hydroethanolic mixture consisting of ethanol and water in a 70:30 (v/v) ratio for 24 h. The resulting mixture was filtered, and the ethanol was removed from the filtrate using a rotary evaporator. An aliquot (15 mL) of the resulting hydroethanolic extract was subsequently subjected to successive liquid-liquid partitioning with solvents of increasing polarity, namely n-hexane, dichloromethane, and ethyl acetate. Each solvent was used in three successive portions of 10 mL (3 × 10 mL).

Accordingly, the extract was successively partitioned with n-hexane (C_6H_{14}), dichloromethane (CH_2Cl_2), and ethyl acetate (EtOAc). The dichloromethane fraction was collected, concentrated, and retained for subsequent chromatographic fractionation.

Chromatographic Fractionation of the Dichloromethane Extract

The dichloromethane extract was fractionated by column chromatography using silica gel 60 (particle size: 35-70 µm; A.C.C.). The chromatographic column measured 40 cm in length and 3 cm in internal diameter and was packed with 50 g of silica gel as the stationary phase.

Elution was carried out sequentially, first with n-hexane and subsequently with a mixture of n-hexane and ethyl acetate (4:1, v/v). The eluate was collected in successive fractions, which were monitored by thin-layer chromatography (TLC). The chromatographic spots were visualized by spraying the TLC plates with a 10% potassium permanganate solution. Based on the TLC profiles, fractions of interest were selected, and the isolated compound was subsequently subjected to nuclear magnetic resonance (NMR) spectroscopy for structural characterization.

Nuclear Magnetic Resonance Spectroscopic Analysis

The compound obtained following chromatographic fractionation was characterized using proton nuclear magnetic resonance (^{1}H NMR), carbon-13 nuclear magnetic resonance (^{13}C NMR), and distortionless enhancement by polarization transfer (DEPT-135) spectroscopy. Spectral interpretation focused on chemical shifts, signal multiplicities, and the classification of carbon environments based on the combined analysis of the.[13] C NMR and DEPT-135 spectra. The resulting spectroscopic data were compared with reference values reported in the literature to assess the proposed structural assignment.

RESULTS

Proton NMR (^{1}H NMR) Spectral Profile

The ^{1}H NMR spectrum of the isolated compound (Figure 1) exhibits several signals characteristic of an unsaturated sterol structure bearing an alcohol functional group. Analysis of the downfield region reveals an olefinic signal centered at approximately δ_H 5.35 ppm, integrating for one proton. This signal is attributable to the H-6 proton associated with the double bond between C-5 and C-6 of the sterol nucleus.

A multiplet observed in the δ_H 3.5-3.6 ppm region, corresponding to one proton, is assigned to H-3. This resonance is characteristic of a methine proton attached to a carbon bearing a hydroxyl group, consistent with the structure of β-sitosterol.

In the upfield region, roughly between δ_H 0.65 and 1.05 ppm, there is a set of signals attributable to the methyl groups of the sterol skeleton and the side chain. These signals are an important feature in the characterization of phytosterols, although their partial overlap makes it difficult to assign individual methyl groups based solely on the spectrum described.

The spectrum also displays an intense aliphatic cluster centered around δ_H 1.25 ppm, reflecting the presence of numerous methylene and methine protons within the saturated rings and the hydrocarbon side chain. A signal near δ_H 0.85-0.90 ppm, appearing as a triplet, is consistent with the terminal C-29 methyl group.

Finally, the residual signal observed near δ_H 7.26 ppm is attributable to non-deuterated chloroform present in the $CDCl_3$ solvent. All of these observations are consistent with a phytosterol possessing a double bond within the sterol nucleus and a secondary alcohol functional group. The signals observed at approximately 5.35 and 3.5-3.6 ppm serve as the primary diagnostic features pointing to β-sitosterol.

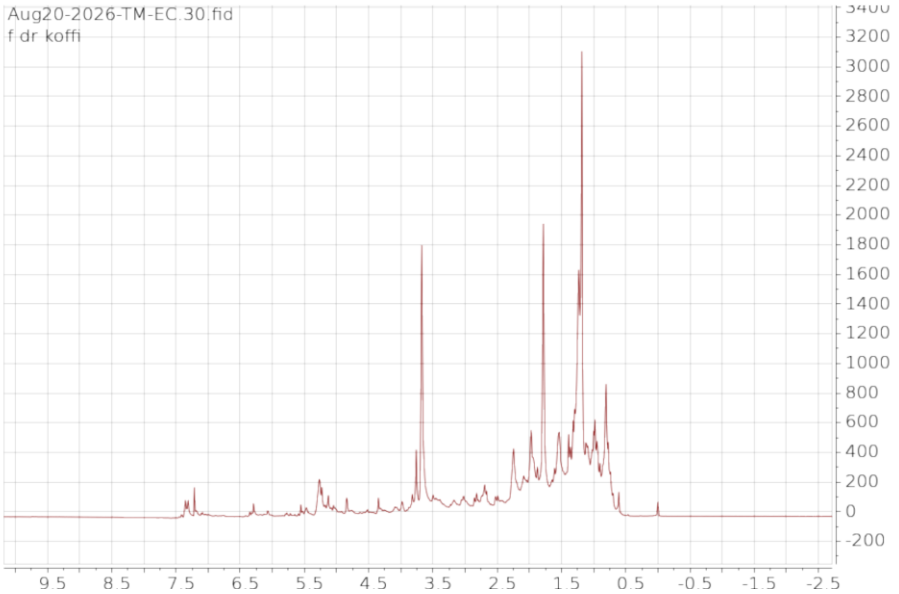


Figure 1. ^{1}H NMR spectrum of the compound isolated from the lipophilic fraction of *Khaya grandifoliola*.

Carbon-13 Nuclear Magnetic Resonance (^{13}C NMR) Characterization

The ^{13}C NMR spectrum of the isolated compound (Figure 2) displays two characteristic signals associated with a carbon–carbon double bond, at δ_C 140.8 and 121.7 ppm. The signal at δ_C 140.8 ppm is assigned to the quaternary carbon C-5, whereas the signal at δ_C 121.7 ppm corresponds to the olefinic methine carbon C-6. Another key diagnostic feature is the signal at δ_C 71.8 ppm. This chemical shift is characteristic of an aliphatic carbon bonded to an oxygen atom and is consistent with C-3, which bears a hydroxyl group in β-sitosterol.

The remainder of the spectrum exhibits a high density of signals in the aliphatic region, predominantly between 19 and 40 ppm. This distribution is consistent with saturated carbon atoms belonging to the rings of the steroid nucleus and its side chain.

The chemical shifts of the two olefinic carbons and the oxygen-bearing carbon represent the most discriminating features of the spectrum. Their agreement with reference values reported for β-sitosterol lends further support to the proposed identification.

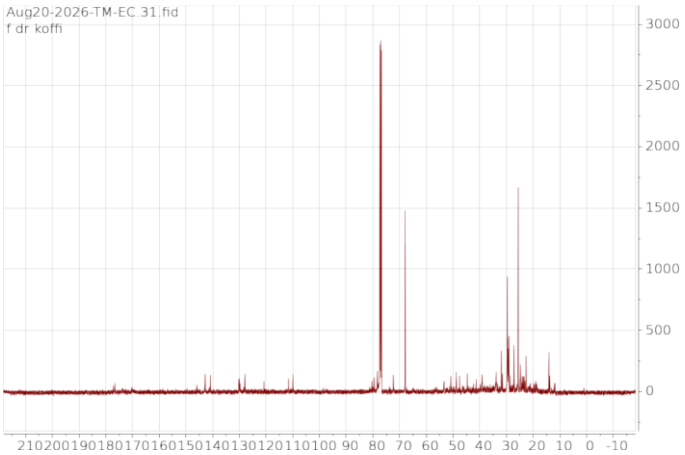


Figure 2. ^{13}C NMR spectrum of the compound isolated from the lipophilic fraction of *Khaya grandifoliola.*

Contribution of the DEPT-135 experiment

The DEPT-135 spectrum (Figure 3) complements the ^{13}C NMR data by enabling the differentiation of methine (CH), methylene (CH_2), and methyl (CH_3) carbons according to the phase of their signals. Under standard experimental conditions, CH and CH_3 groups produce positive-phase signals, whereas CH_2 groups give rise to negative-phase signals. Quaternary carbons are not detected in this experiment.

The signal assigned to C-3, at δ_C 71.8 ppm, appears in the positive phase, indicating that this carbon is a protonated methine carbon rather than a quaternary carbon. This finding is consistent with the presence of a hydroxyl-bearing methine group at position C-3 of the sterol nucleus.

The aliphatic region displays signals attributable to both CH_2 carbons and CH/CH_3 carbons. This distribution is consistent with the polycyclic structure of the sterol nucleus and the branched hydrocarbon side chain of β-sitosterol. Unlike a linear aliphatic chain, the side chain contains branching features that contribute to the molecule's overall spectroscopic profile.

Overall, the DEPT-135 experiment provides additional evidence for the consistency of the proposed carbon classification. However, in the absence of a detailed assignment and count of the positive- and negative-phase signals, the available spectral description does not allow for quantitative verification of all the carbon environments expected for β-sitosterol. Accordingly, the DEPT-135 data support the proposed structural assignment but do not, on their own, establish an unequivocal identification.

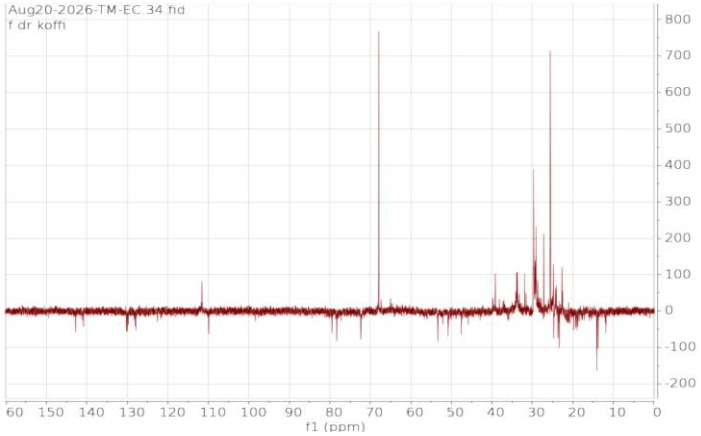


Figure 3. DEPT-135 NMR spectrum of the compound isolated from the lipophilic fraction of *Khaya grandifoliola.*

Comparison of Chemical Shifts with Reference Data

Table 1 delineates the primary spectroscopic parameters acquired for the investigated compound, indexed against established reference data reported for β-sitosterol in $CDCl_3$.[17,18] These reference values are consistent with those published in previous studies investigating the isolation and NMR characterization of this phytosterol.[9]

The signals associated with the densely populated aliphatic region, corresponding to C-1, C-2, C-4, C-7-C-17, and C-20-C-29, overlap to form a complex cluster between 19 and 40 ppm. This signal distribution is consistent with the numerous saturated carbon environments present in the steroid nucleus and its branched side chain.

Table 1: Comparison of the diagnostic chemical shifts observed for the isolated compound with reference values reported for β-sitosterol in $CDCl_3$.

Position	reference δ_C (ppm)	observed δ_C (ppm)	DEPT-135	reference δ_H (ppm)	observed δ_H (ppm)
C-3 (CH-OH)	71.8	~71.8	CH (+)	3.52 (m)	~3.5-3.6
C-5 (=C<)	140.8	~140.8	Cq		
C-6 (=CH-)	121.7	~121.7	CH (+)	5.35 (d, J≈5 Hz)	~5.3-5.4
C-18 (CH_3)	11.9	_	CH_3 (+)	0.68 (s)	0.65-1.05
C-19 (CH_3)	19.4	_	CH_3 (+)	1.01 (s)	0.65-1.05
C-21 (CH_3)	18.8	_	CH_3 (+)	0.92 (d)	0.65-1.05
C-26/C-27 (2×CH_3)	19.0 / 19.8	_	CH_3 (+)	0.80-0.84 (2 d)	0.65-1.05
C-29 (CH_3)	12.0	_	CH_3 (+)	0.84 (t)	0.65-1.05

Cq: quaternary carbon (not detected in the DEPT-135 experiment)

Proposed structural identification

The combined interpretation of the ^1H NMR, ^{13}C NMR, and DEPT-135 spectra reveals a set of structural features consistent with those reported for β-sitosterol. These include an olefinic proton signal, a proton attached to an oxygen-bearing carbon, two olefinic carbon signals attributable to the Δ^5 (C-5/C-6) double bond, and a series of aliphatic carbon signals characteristic of a sterol skeleton. The assignment of the signal at δ_C 71.8 ppm to C-3, together with the signals at δ_C 140.8 and 121.7 ppm attributed to C-5 and C-6, respectively, provides particularly strong support for the proposed structure. Taken together, these spectroscopic findings support the tentative identification of β-sitosterol as the most plausible structural assignment for the isolated compound. However, in the absence of complementary two-dimensional NMR data, mass spectrometric evidence, and an assessment of the isolate's purity, this identification remains provisional and requires further confirmation.

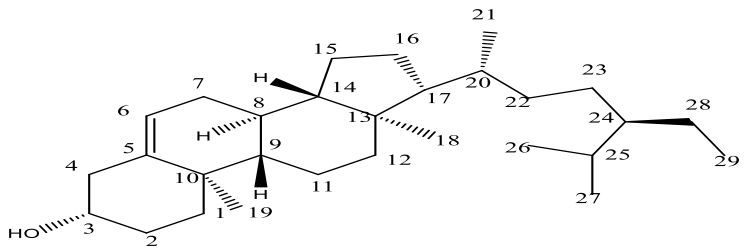


Figure 4. Chemical structure of β-sitosterol, also known as stigmast-5-en-3β-ol.

DISCUSSION

Structural Significance of the Main NMR Signals

The characterization of β-sitosterol relies on the complementary information provided by the ^{1}H and ^{13}C NMR spectra. The olefinic signal observed at approximately δ_H 5.35 ppm indicates the presence of a proton attached to a carbon-carbon double bond in a cyclic environment. In β-sitosterol, this resonance is assigned to the H-6 proton associated with the Δ^5 double bond of the sterol nucleus.

This observation becomes more diagnostically significant when considered alongside the two carbon resonances observed at approximately δ_C 140.8 and 121.7 ppm. The former is assigned to the quaternary carbon C-5, whereas the latter corresponds to the olefinic methine carbon C-6. The presence of these two signals is consistent with the C-5/C-6 double bond in the sterol skeleton and agrees with the spectroscopic characteristics of β-sitosterol reported in the literature. These two signals are structurally diagnostic of the Δ^5 (C-5/C-6) double bond characteristic of the sterol core and align seamlessly with the published spectral attributes of β-sitosterol.[9]

A second key feature is the signal observed at approximately δ_H 3.5-3.6 ppm, which is assigned to the H-3 proton attached to the hydroxyl-bearing carbon. This chemical shift, together with the corresponding oxygenated carbon signal at approximately δ_C 71.8 ppm, is characteristic of a secondary alcohol environment. Furthermore, the positive-phase signal observed at this chemical shift in the DEPT-135 spectrum supports the assignment of C-3 as a protonated methine carbon.

These three structural features C-3, C-5, and C-6 provide the main spectroscopic evidence supporting the proposed structure. Their agreement with reported reference data makes β-sitosterol a plausible identity for the isolated compound. Nevertheless, these signals should not be interpreted in isolation. Reliable structural elucidation requires consistency across the available spectroscopic data and adequate consideration of alternative structural assignments. Accordingly, although the observed NMR features support the proposed identification, further confirmation using complementary techniques, such as two-dimensional NMR and mass spectrometry, would strengthen the structural assignment.

Interpretation of the aliphatic region and the contribution of DEPT-135

The high density of signals observed in the aliphatic region is consistent with the large number of saturated carbon atoms in β-sitosterol, which comprises four fused rings and a branched side chain. The different CH, CH_2, and CH_3 environments give rise to a relatively complex spectrum in which several resonances may overlap or occur in close proximity. Signals associated with the methyl groups provide additional structural information regarding the sterol framework and the branching pattern of the side chain. However, partial overlap in the ^1H NMR spectrum limits the possibility of unambiguous positional assignments without detailed analysis of signal multiplicities, coupling constants, and two-dimensional NMR correlations.

In this context, DEPT-135 spectroscopy provides complementary information by distinguishing methylene carbons from methine and methyl carbons according to their signal phases. Specifically, the positive-phase signal assigned to C-3 supports its classification as a CH group and helps differentiate the various types of saturated carbon environments. The absence of quaternary carbon signals is also consistent with the fundamental principles of the DEPT-135 experiment, which does not detect carbons lacking directly attached hydrogen atoms. Nevertheless, qualitative agreement between the observed spectral features and those expected for β-sitosterol should be distinguished from complete structural elucidation. The available description of the DEPT-135 spectrum does not specify the total number of positive- and negative-phase signals, thereby limiting comprehensive verification of the expected distribution of the 29 carbon atoms in β-sitosterol. Detailed examination of the original spectra, together with a complete assignment table, would strengthen the proposed structural identification.

Specificity of identification and simitations of spectroscopic comparison

The convergence of the observed chemical shifts at δ_C 71.8, 140.8, and 121.7 ppm is consistent with the proposed identification of β-sitosterol. However, several phytosterols share a similar structural framework, including a hydroxyl group at C-3 and a double bond at the Δ^5 position. Campesterol, for example, exhibits similar structural features, whereas stigmasterol contains an additional double bond in its side chain.

Distinguishing between these compounds therefore requires careful examination of the signals arising from the side chain. The presence or absence of additional olefinic resonances, together with the corresponding carbon chemical shifts, can help differentiate these

structures. When signals overlap or are insufficiently resolved, two-dimensional NMR experiments and complementary analytical techniques become particularly valuable.

In the present study, the principal diagnostic signals are consistent with β-sitosterol; however, the reported data do not provide a complete assignment of all carbon and proton signals. Furthermore, no mass spectrometric data are available to support the proposed molecular identity. Consequently, it would be premature to claim that all potential structural alternatives have been unequivocally excluded. The most scientifically appropriate approach is therefore to describe β-sitosterol as the proposed identity of the isolated compound, based on the convergence of the available spectroscopic evidence.

Another limitation concerns the purity of the isolate. The intense aliphatic signal observed at approximately δ_H 1.25 ppm is compatible with several types of saturated proton environments. Although this signal alone does not establish the presence of fatty acids or triglycerides as impurities, it cannot exclude their possible contribution to the spectrum. Further chromatographic analysis, using high-performance liquid chromatography (HPLC) or gas chromatography–mass spectrometry (GC-MS), as appropriate for the sample's properties, would help assess the homogeneity of the isolate and provide additional evidence regarding its composition.

Phytochemical Interest of *Khaya grandifoliola*

The identification of a phytosterol type constituent in a lipophilic fraction of *Khaya grandifoliola* stem bark contributes to our understanding of the chemical composition of this species. It highlights the value of an integrated approach combining selective extraction, chromatographic separation, and spectroscopic characterization to investigate plant materials that warrant further phytochemical exploration.

Although the identification of a compound widely distributed throughout the plant resource may not, in itself, represent a major taxonomic discovery, it remains of analytical and phytochemical interest when documented in a clearly identified plant part using a reproducible experimental procedure and an appropriately qualified level of structural evidence.[19,20] The significance of the present study therefore lies not only in documenting the isolated constituent but also in demonstrating an analytical approach for characterizing the lipophilic fractions of this species.[21]

To fully assess the implications of this finding, it should be considered in the context of previous studies specifically investigating the stem bark of *K. grandifoliola*. Findings concerning the seeds or other plant parts should not be directly extrapolated to the bark, as their chemical compositions may differ. A comprehensive literature search, including possible botanical synonyms and regional scientific publications, would help establish whether this constituent has previously been reported in the stem bark of this species.

Analytical perspectives and scientific valorization

The findings of this study provide a basis for several further experimental investigations. The first priority is to strengthen the structural identification through two-dimensional NMR experiments, particularly heteronuclear single quantum coherence (HSQC) and heteronuclear multiple-bond correlation (HMBC). HSQC would enable the correlation of proton signals with the carbon atoms to which they are directly attached, whereas HMBC would provide information on longer-range proton–carbon correlations. Together, these experiments would refine signal assignments and help verify the consistency of the proposed molecular structure.

A second avenue involves confirmation by mass spectrometry using an analytical method suitable for sterol characterization. Although β-sitosterol has a molar mass of approximately 414.71 $g \cdot mol^{-1}$, agreement with the expected molecular mass alone is insufficient to distinguish it from all possible structural isomers. Combining molecular mass information, characteristic fragmentation patterns, and NMR data would provide stronger evidence for the proposed identification.

A third priority is quantitative assessment. Determining the extraction yield, the mass of the isolated compound, and its purity would help evaluate the efficiency of the isolation procedure and the potential value of this plant resource for further investigation. In addition, assessing variability across individual plants, harvesting periods, and geographical locations would help establish the reproducibility of the compound's occurrence within the species.

Finally, any potential application in the nutritional, pharmaceutical, or cosmetic sectors would require specific evidence concerning the purity, safety, stability, and biological activity of the isolated compound or the preparation under investigation. The identification of a phytosterol alone is insufficient to establish the efficacy or safety of a finished product.

CONCLUSION

This study focused on the spectroscopic characterization of a compound isolated from a lipophilic fraction of the stem bark of *Khaya grandifoliola,* collected in Ahougnassou-Allahou, Tiébissou Department, Côte d'Ivoire. Interpretation of the ^{1}H NMR, ^{13}C NMR, and DEPT-135 data revealed several structural features consistent with β-sitosterol. The main evidence supporting this assignment includes an olefinic proton signal at approximately δ_H 5.35 ppm, an oxygen-bearing methine proton signal at approximately δ_H 3.5-3.6 ppm, two olefinic carbon signals at δ_C 140.8 and 121.7 ppm, and an oxygenated carbon signal at δ_C 71.8 ppm. The aliphatic signal profile and the complementary information provided by DEPT-135 further support this structural hypothesis.

Based on the available spectroscopic evidence, β-sitosterol represents the most plausible proposed identity for the isolated compound. However, the absence of complete signal assignments, two-dimensional NMR data, and mass spectrometric confirmation warrants caution in considering this identification definitive.

Further investigations incorporating additional NMR experiments, assessment of chromatographic purity, and determination of extraction and isolation yields would strengthen these findings and clarify their significance in the phytochemical valorization of Ivorian plant resources. Overall, this study contributes to the chemical characterization of *K. grandifoliola* stem bark and provides a basis for subsequent analytical and phytochemical investigations.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest that could have influenced the conduct of this study or the publication of this article.

REFERENCES

1. Atanasov AG, Zotchev SB, Dirsch VM, & International Natural Product Sciences Taskforce, Supuran CT. Natural products in drug discovery: Advances and opportunities. Nat Rev Drug Discov., 2021; 20(3): 200-216. https://doi.org/10.1038/s41573-020-00114-z.

2. Bitwell C, Sen IS, Chimuka L, Maseka KK. A review of modern and conventional extraction techniques and their applications for extracting phytochemicals from plants. Sci Afr., 2023; 19: e01585. https://doi.org/10.1016/j.sciaf.2023.e01585.
3. Queiroz EF, Guillarme D, Wolfender JL. Advanced high-resolution chromatographic strategies for efficient isolation of natural products from complex biological matrices: From metabolite profiling to pure chemical entities. Phytochem. Rev., 2024; 23: 1415-1442. https://doi.org/10.1007/s11101-024-09928-w.
4. Zhang QW, Lin LG, Ye WC. Techniques for extraction and isolation of natural products: A comprehensive review. Chin Med, 2018; 13(20): 1-26. https://doi.org/10.1186/s13020-018-0177-x.
5. Moreau RA, Whitaker BD, Hicks K. B. Phytosterols, phytostanols, and their conjugates in foods: Structural diversity, quantitative analysis, and health-promoting uses. Prog Lipid Res, 2002; 41(6): 457-500. https://doi.org/10.1016/S0163-7827(02)00006-1.
6. García-Llatas G, Rodríguez-Estrada MT. Current and new insights on phytosterol oxides in plant sterol-enriched food. Chem Phys Lipids, 2011; 164(6): 607-624.
7. Ambavade SD, Misar AV, Ambavade PD. Pharmacological, nutritional, and analytical aspects of β-sitosterol: A review. Adv Tradit Med., 2014; 14: 193-211. https://doi.org/10.1007/s13596-014-0151-9.
8. National Institute of Standards and Technology. (s. d.). β-Sitosterol. Dans NIST Chemistry WebBook (SRD 69). https://webbook.nist.gov/cgi/cbook.cgi?ID=83-46-5.
9. Islam MJ, Hayatullah, Rahman MA, Jahan N, Mostafa MG, Akter B, Uddin MR. Isolation, structural characterization and molecular docking of β-sitosterol from *Trewia polycarpa* root bark: A potential source of bioactive compounds. Discov Chem., 2025; 2(156): 1-17. https://doi.org/10.1007/s44371-025-00236-6.
10. Claridge TDW. High-resolution NMR techniques in organic chemistry (3rd ed.). Elsevier: 2016.
11. Doddrell DM, Pegg DT, Bendall MR. Distortionless enhancement of NMR signals by polarization transfer. J Magn Reson, 1982; 48(2): 323-327. https://doi.org/10.1016/0022-2364(82)90215-3.
12. Evtyugin DD, Evtuguin DV, Casal S, Domingues MR. Advances and Challenges in Plant Sterol Research: Fundamentals, Analysis, Applications and Production. Molecules, 2023; 28(19): 6723: 1-45. https://doi.org/10.3390/molecules28196723.

13. Mukaila YO, Ajao AAN, Moteetee AN. *Khaya grandifoliola* C. DC. (Meliaceae: Sapindales): Ethnobotany, phytochemistry, pharmacological properties, and toxicology J Ethnopharmacol, 2021; 276: 114253. https://doi.org/10.1016/j.jep.2021.114253.
14. Olatunji, T. L., Odebunmi, C. A., & Adetunji, A. E. (2021). Biological activities of limonoids in the genus Khaya (Meliaceae): A review. Future J Pharm Sci., 2021; 7(74): 1-16. https://doi.org/10.1186/s43094-021-00197-4.
15. Harborne JB. Phytochemical Methods: A Guide to Modern Techniques of Plant Analysis, 3e éd. Chapman & Hall, Londres, 1998.
16. Nyigo VA, Peter X, Mabiki F, Malebo HM, Mdegela RH, Fouche G. Isolation and identification of euphol and β-sitosterol from the dichloromethane extracts of *Synadenium glaucescens*. J Phytopharm, 2016; 5(3): 100-104.
17. Chaturvedula VSP, Prakash I. Isolation of Stigmasterol and β-Sitosterol from the dichloromethane extract of Rubus suavissimus. Int Curr Pharm J, 2012; 1(9): 239-242.
18. Goad LJ, Akihisa T. Analysis of Sterols. Blackie Academic & Professional, Londres, 1997 (données RMN de référence des phytostérols usuels).
19. Heinrich M, Jalil B, Abdel-Tawab M, Echeverria J, Kulić Ž, McGaw L J, Pezzuto JM, Potterat O, Wang JB. Best practice in the chemical characterisation of extracts used in pharmacological and toxicological research-The Con Phy MP-Guidelines. Front Pharmacol, 2022; 13(953205): 1-20. https://doi.org/10.3389/fphar.2022.953205.
20. Izzo AA, Hoon-Kim S, Radhakrishnan R, Williamson EM. A practical guide for transparent reporting of research on natural products in the British Journal of Pharmacology: Reproducibility of natural product research. Br J Pharmacol, 2020; 177(10): 2169–2178. https://doi.org/10.1111/bph.15054.
21. Kellogg JJ, Paine MF. Selection and characterization of botanical natural products for research studies: A NaPDI Center recommended approach. Natural Product Reports, 2019; 36(8): 1196-1221. https://doi.org/10.1039/C8NP00065D.