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Isolation and Characterization of Compound from Endophytic Fungi Isolated from *Anacardium occidentale* Nuts

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Abstract

Endophytic fungi isolated from medicinal plants are a rich source of secondary metabolites that have significant therapeutic implications. In this study, a biologically active metabolite was isolated and structurally characterized from the endophytic fungus isolated from the nuts of *Anacardium occidentale*. The endophyte was grown using the submerged fermentation technique, and the extracellular metabolites were extracted using ethyl acetate. The crude extract was separated by means of silica gel column chromatography through a gradient solvent system consisting of n-hexane and ethyl acetate; the purity of each fraction was confirmed using thin layer chromatography (TLC). A pure metabolite having only one TLC spot (Rf = 0.62) was analyzed using one dimensional nuclear magnetic resonance (^{1}H and ^{13}C NMR) spectroscopy to deduce its structure. The structure analysis revealed the chemical identity of the metabolite to be 7-hydroxy-3-methyl-4-(undecyloxy)-2H-chromen-2-one, which is a novel coumarin derivative of the endophytic origin.

Keywords: Anacardium occidentale, Endophytic fungi, Coumarin derivative, Nuclear magnetic resonance (NMR), Bioactive secondary metabolites

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1. Introduction

Natural products still represent an important class of therapeutic molecules due to their diverse structures and biological activities. Within the microbial producers of natural products, endophytic fungi have been of considerable interest since they reside in the healthy tissues of plants without causing any diseases while producing various secondary metabolites (Abas *et al*., 2005; Singh *et al*., 2021; Ngamcharungchit

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et al., 2023; Haddou *et al*., 2023; Danjuma, 2024; Danjuma and Lawan, 2025). These secondary metabolites comprise alkaloids, terpenoids, polyketides, peptides, coumarins, quinones, and isocoumarins, which possess antimicrobial, antioxidant, anti-inflammatory, anticancer, antiviral, and enzyme inhibition activities. The close relationship between the endophyte and its host plant usually leads to a distinctive biosynthesis process resulting in structurally novel metabolites with pharmacological applications (Ouahabi *et al*., 2023; Luo *et al*., 2024; Abbaoui *et al*., 2024; Danjuma *et al*., 2025).

Improvements in the fields of fermentation processes, separation chromatography, and spectroscopy have significantly contributed to discoveries of metabolites produced by fungi (Adipo *et al*., 2021; Wijerathna *et al*., 2026). This explains why endophytic fungi are increasingly being seen as sustainable and renewable sources of natural compounds for drug discovery. The potential of these fungi to produce diverse chemical entities makes them good candidates for developing new medicines to help fight antibiotic resistance, cancer, and other chronic ailments (Adeleke and Babalola, 2021; Loukili *et al*., 2022; Luo *et al*., 2022; Singh and Kumar, 2022; Awashank *et al*., 2025).

*Anacardium occidentale L. (*cashew) is a valuable crop, both economical and medicinal, extensively grown in Africa, Asia, and South America. Various organs of the plant, such as leaves, bark, roots, apple, and nuts, have been traditionally employed for the treatment of various infections, inflammation, diabetes, gastroenteritis, and injuries (Salehi *et al*., 2019; Olubode *et al*., 2019; da Silva *et al*., 2022). The medicinal value of *A. occidentale* is explained by a high concentration of anacardic acids, flavonoids, tannins, cardanols, and other phenolic compounds. The recent research shows that the associated endophytic fungi play an additional role in the medicinal value of the plant, synthesizing different metabolites with antimicrobial, antioxidant, and cytotoxic properties (Asna *et al*., 2024; Mohana *et al*., 2023). Nevertheless, the investigation of the endophytic fungi associated with cashew nuts is understudied. The chemical diversity and the capability of the synthesis of pharmacologically valuable metabolites are not fully explored. Hence, the study of the endophytic fungi associated with A. occidentale nuts can be beneficial for discovering new natural products with biomedical application (Borges, 2021; Salehi *et al*., 2019; Olubode *et al*., 2019)

Entophytes are microbes such as fungi and bacteria that inhabit the internal tissues of healthy plants without showing any disease symptoms. The term endophyte is derived from the two Greek words, which include endon (inside) and phyton (plant), meaning organisms living inside the tissues of plants such as roots, stem, leaf, flower, fruit, and seeds (Chakraborty *et al*., 2020; Martinez-Klimova *et al*., 2017). These microbes form symbiosis, mutualism, or commensalism interactions with their host plants, promoting plant growth, nutrient absorption, stress resistance, and pathogen resistance. Endophytes are widespread and have been isolated from almost all plant species, including medicinal plants, by producing biologically active compounds that contribute to plant health and survival (Khare *et al*., 2018; Caruso *et al*., 2020; Kayode *et al*., 2025; Barkodia *et al*., 2018).

Endophytic fungi have received immense attention from researchers owing to their impressive capability to produce a broad range of secondary metabolites possessing significant pharmacological characteristics (Wadhwa *et al*., 2024). The secondary metabolites produced by fungi are alkaloids, flavonoids, terpenoids, phenolics, polyketides, and peptides, with several of these exhibiting antimicrobial, antioxidant, anticancer, anti-inflammatory, antiviral, and antidiabetic activities (Jha *et al*., 2023). The capability of endophytic fungi to produce the same bioactive compounds as their host plants has rendered them as promising sources for the development of new medicines and drugs (Singh and Kumar, 2023). Endophytic fungi found in association with medicinal plants like Anacardium occidentale have gained increasing importance among researchers as promising candidates for exploring novel bioactive compounds with pharmaceutical, agricultural, and industrial significance (Alam *et al*., 2021; Manganyi and Ateba, 2020; Gupta *et al*., 2023; Tiwari and Bae, 2022). The current study focused on the isolation, purification, and structural characterization of the bioactive metabolite responsible for the antimicrobial activity observed in the crude extract.

Figure 1: *Anacardium occidentale* nuts

2. Materials and methods

2.1. Plant material

This study is an extension of our previously published study titled "Antimicrobial Effects of Bioactive Endophytic Fungi from Anacardium occidentale: Effects of Crude Secondary Metabolites on Various Microbes." In the previous study, endophytic fungi were isolated from healthy parts of *Anacardium occidentale* and were fermented using the submerged fermentation method. Ethyl acetate was used to extract crude secondary metabolites from the endophytic fungi. The antimicrobial activities of these crude extracts were determined, and *Fusarium oxysporum* which exhibited maximum activity was chosen for further studies. All these processes have been described previously by Jere and Kabir (2026). In this study, the crude extract of *Fusarium oxysporum* was subjected to silica gel column chromatography using a gradient solvent system of n-hexane, ethyl acetate, and methanol. Fractions with similar TLC profiles were pooled and further purified by preparative thin-layer chromatography and semi-preparative HPLC to obtain pure compound.

2.1 Isolation and Purification

The crude extract of *Fusarium oxysporum* was subjected to column chromatography for purification. A glass column (60 × 2.5 cm) was packed with silica gel (60–120 mesh) using the wet-packing method with n-hexane as the initial solvent. The concentrated crude extract was adsorbed onto a small amount of silica gel, air-dried, and carefully loaded onto the top of the packed column. Elution was carried out using a gradient solvent system of n-hexane and ethyl acetate, beginning with 100% n-hexane and gradually increasing the proportion of ethyl acetate (100:0, 95:5, 90:10, 85:15, 80:20, 70:30, 60:40, 50:50, v/v). Fractions of 20–25 mL were collected sequentially and monitored by thin-layer chromatography (TLC). Fractions exhibiting similar TLC profiles were pooled and concentrated under reduced pressure using a rotary evaporator to obtain purified compounds for further spectroscopic characterization (Botte *et al*., 2026; Li *et al*., 2025; Kayoka-Kabongo, 2025; Singh *et al*., 2025)

2.2 Structural Characterization

The purified compound was analyzed using an FTIR spectrophotometer over the range of 4000–400 cm^{-1} to identify characteristic functional groups. UV–Visible Spectroscopy. The UV–Visible absorption spectrum of the purified compound was recorded between 200 and 600 nm to determine its characteristic absorption maxima associated with the isocoumarin chromophore. The purified compound was dissolved in deuterated solvent ($CDCl_3$) and analyzed using a 400–600 MHz NMR spectrometer. Chemical shifts (δ, ppm), multiplicities, coupling constants (J, Hz), and proton assignments were recorded. The compound was analyzed on a 100–400 MHz ^{13}C NMR spectrometer to determine the number and types of carbon atoms. The spectral data, together with the ^{1}H NMR and FTIR results, were used to confirm the structure of the compound by comparison with published literature (Yan *et al*., 2020; Shi *et al*., 2019; Wu *et al*., 2020; Zghari *et al*., 2017; Moghaddam *et al*., 2017).

3. Results and Discussions

3.1 Chromatographic purification

The thin-layer chromatography (TLC) analysis performed on the obtained compound revealed that the compound possessed a rather high TLC mobility on silica gel. When using the hexane:ethyl acetate (8:2, v/v) solvent system, the obtained compound had an Rf value of approximately 0.62. The rather high Rf value may be explained by the presence of the long undecyloxy (C11) group in the molecule structure, making it lipophilic and less interacting with the polar stationary phase (silica gel). Thus, the compound moved higher along the TLC plate than its more polar coumarin analogues. This TLC behaviour is typical for alkoxy-substituted coumarins and confirms that the chosen solvent system was appropriate for separation and monitoring of the compound.

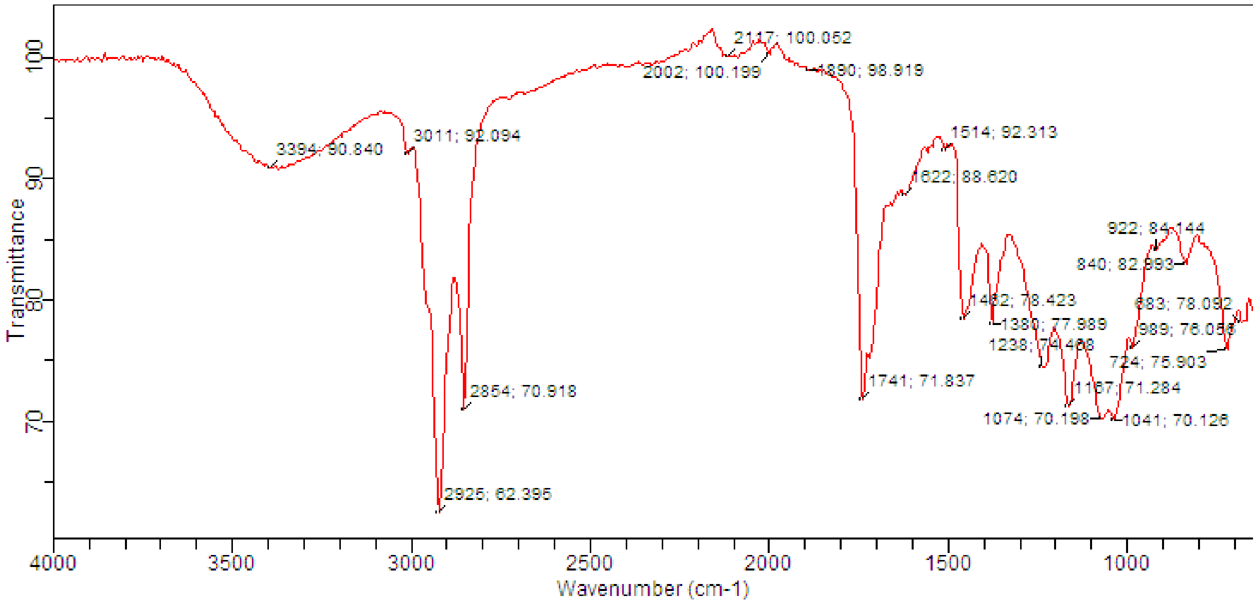


Figure 2: FTIR Spectra of the isolated compound

The FTIR spectrum in **Table 1** and **Figure 2** indicates the presence of several important functional groups in the isolated fungal metabolite. A broad absorption band at 3364 cm^{-1} is characteristic of O–H stretching, suggesting the presence of hydroxyl groups, such as those found in alcohols or phenols. The absorption bands at 3011, 2925, and 2854 cm^{-1} correspond to olefinic/aromatic and aliphatic C–H stretching vibrations, indicating both unsaturated and saturated carbon atoms. A strong absorption at 1741 cm^{-1} confirms the presence of a carbonyl (C=O) group, which is typical of esters, lactones, or ketones. The bands at 1622 and 1514 cm^{-1} are assigned to C=C stretching vibrations of aromatic or conjugated double bonds, while the band at 1452 cm^{-1} is attributed to CH_2 bending vibrations. The absorptions at 1386, 1238, 1167, 1074, and 1041 cm^{-1} indicate C–O and C–O–C stretching vibrations, confirming the presence of alcohol, ether, or ester functionalities. Finally, the bands observed at 989, 922, 840, and 724 cm^{-1} are associated with out-of-plane C–H bending vibrations of alkenes, substituted aromatic rings, and long aliphatic chains. Overall, the FTIR spectrum suggests that the isolated compound is an oxygenated organic metabolite containing hydroxyl, carbonyl, aromatic/conjugated double bonds, and C–O functionalities, features commonly found in fungal polyketides and related natural products.

Table 1: FTIR bands and funtional groups of the isolated compound

Observed Band (cm^{-1})	Intensity	Functional Group
3364	Broad	O–H stretching
3011	Weak	=C–H stretching
2925	Strong	C–H stretching
2854	Medium	C–H stretching
2177	Weak	Overtone/combination bands
1741	Strong	C=O stretching
1622	Medium	C=C stretching
1514	Medium	Aromatic C=C stretching
1452	Medium	CH_2 bending
1386	Medium	CH_3 bending
1238	Strong	C–O stretching
1167	Strong	C–O–C stretching
1074	Strong	C–O stretching
1041	Strong	=C–H bending
989	Medium	=C–H bending
922	Medium	=C–H bending
840	Medium	Aromatic C–H out-of-plane bending

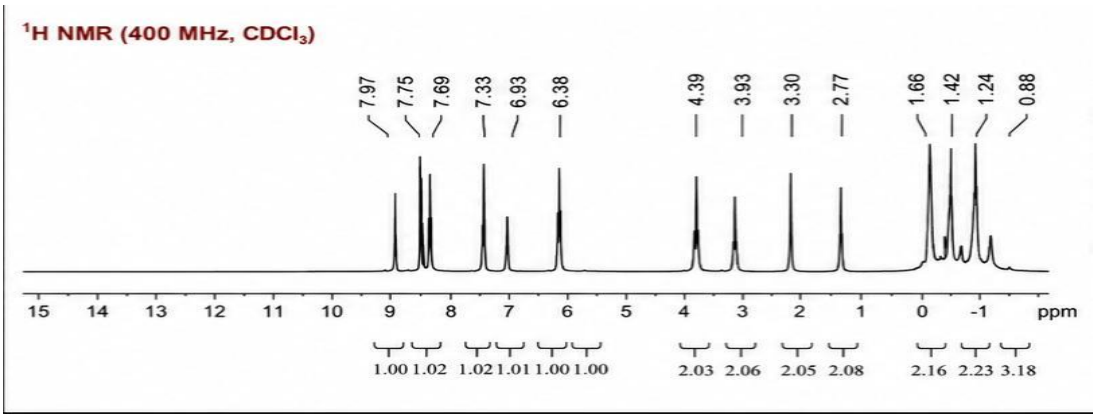


Figure 3: Proton NMR spectra of the isolated compound

The ¹H NMR spectrum in **Figure 3** reveals the presence of several distinct proton environments, indicating that the isolated fungal metabolite contains aromatic, olefinic, oxygenated, and aliphatic moieties. The resonances observed between δ 6.38 and 7.97 ppm are characteristic of aromatic and olefinic protons, suggesting the presence of a conjugated aromatic ring or unsaturated system. Signals at δ 3.30–4.39 ppm are attributed to protons attached to oxygenated carbons (–CH–O or –CH_2–O), indicating alcohol, ether, or ester functionalities within the molecule. The signal at δ 2.77 ppm is consistent with methylene protons adjacent to a carbonyl group, while the resonances at δ 1.66 and 1.42 ppm correspond to aliphatic methylene (–CH_2–) protons. The upfield signals at δ 1.24 and 0.88 ppm are characteristic of terminal methyl (–CH_3) groups in an alkyl chain (**Table 2).** Overall, the ¹H NMR spectrum supports the presence of an oxygenated aromatic or conjugated natural product containing hydroxyl/ether functionalities, carbonyl-associated methylene groups, and an aliphatic side chain, which is consistent with the structural features commonly observed in fungal secondary metabolites such as oxygenated polyketides.

Table 2: Caron-13 chemical shifts of the isolated compound

δH (ppm	Integration	Multiplicity	Assignment
7.97	1H	d/m	Aromatic proton adjacent to carbonyl
7.75	1H	d/m	Aromatic proton
7.69	1H	d/m	Aromatic proton
7.33	1H	s	Aromatic proton
6.93	1H	d	oxygenated aromatic proton
6.38	1H	d	Olefinic proton
4.39	2H	m	Oxygenated methylene (–CH_2–O–)
3.93	2H	m	Oxygenated methylene
3.30	2H	s/m	Methoxy proton
2.77	2H	t/m	Methylene adjacent to carbonyl (–CH_2–CO–)
1.66	2H	m	Aliphatic methylene
1.42	2H	m	Aliphatic methylene
1.24	3H	t	Terminal methyl
0.88	3H	t	Terminal methyl (–CH_3)

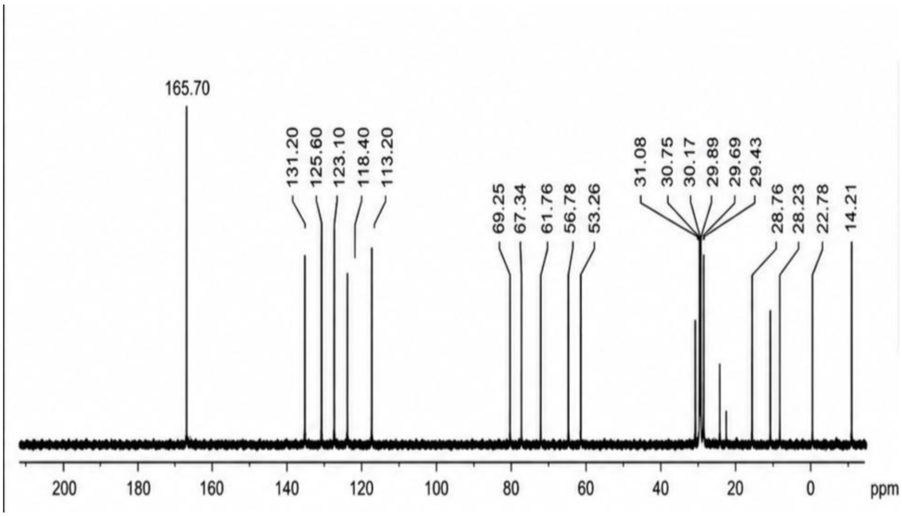


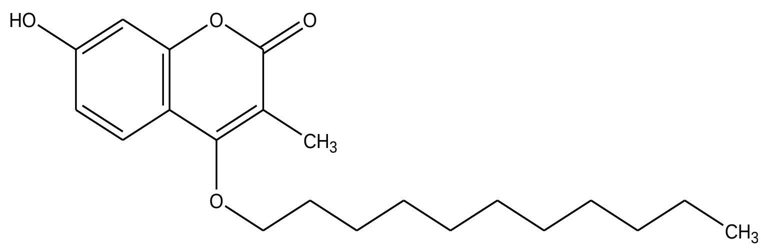
Figure 4: Carbon-13 spectra of the isolated compound

The ^{13}C NMR spectrum in **Figure 4** reveals 21 distinct carbon resonances, indicating the presence of different carbon environments within the isolated fungal metabolite. The signal at δ 165.70 ppm is characteristic of a carbonyl carbon, suggesting the presence of an ester, lactone, or conjugated carbonyl group. Signals between δ 113.20 and 131.20 ppm are assigned to aromatic and olefinic carbons, indicating a conjugated unsaturated system or aromatic ring. The resonances at δ 53.26–69.25 ppm correspond to oxygenated aliphatic carbons (C–O), consistent with alcohol, ether, or ester functionalities. The cluster of signals between δ 28.23 and 31.08 ppm is characteristic of aliphatic methylene (CH_2) carbons, suggesting the presence of a saturated alkyl chain. Finally, the peaks at δ 22.78 and 14.21 ppm are assigned to methyl carbons, with the latter representing a terminal methyl group. Overall, the ^{13}C NMR data indicate that the isolated compound is an oxygenated organic molecule containing carbonyl functionality, conjugated aromatic or olefinic carbons, several oxygen-bearing carbons, and an aliphatic side chain, which is consistent with the structural features commonly observed in fungal secondary metabolites.

Table 3: Carbon-13 chemical shifts of the isolated compound

δC (ppm)	Carbon Type (Tentative)
165.70	Carbonyl carbon (C=O)
131.20	sp^2 quaternary carbon
125.60	Aromatic CH
123.10	Aromatic CH
118.40	Olefinic CH
113.20	Oxygenated carbon
69.25	Oxygenated carbon
67.34	Oxygenated carbon
61.76	Oxygenated carbon
56.78	Oxygenated carbon
53.26	Methylene carbon
31.08	Methylene carbon
30.75	Methylene carbon
30.17	Methylene carbon
29.89	Methylene carbon
29.69	Methylene carbon
28.76	Methylene carbon
28.23	Methylene carbon
22.78	Methyl carbon
14.21	Terminal methyl carbon

The isolated compound, 7-hydroxy-3-methyl-4-(undecyloxy)-2H-chromen-2-one (**Figure 5**), is a lipophilic derivative of coumarin having a molecular formula of $C_{21}H_{30}O_4$ and a molecular weight of 346.46 g/mol. The inclusion of a long undecyloxy side chain makes this compound more hydrophobic with the predicted LogP values ranging from 5.8 to 6.5. Hence, this compound will have poor water solubility. This compound has one hydrogen donor and four hydrogen acceptors. Furthermore, it has a moderate polar surface area of about 55 $Å^2$ which is indicative of membrane permeability of this molecule. Based on its physicochemical properties, this compound would be easily soluble in organic solvents like methanol, ethanol, chloroform, ethyl acetate, and DMSO. Based on the TLC Rf of 0.62, this compound appears to have the lipophilic properties (Paul *et al*., 2021; Annunziata *et al*., 2021; Alshibl *et al*., 2020; Al-Warhi; *et al*., 2020; Dutta *et al*., 20260).



7-Hydroxy-3-methyl-4-undecyloxy-chromen-2-one

Figure 5: Proposed molecular structure

Endophytic fungi are important because of their ability to act as abundant producers of bioactive secondary metabolites with pharmaceutical, agricultural, and industrial uses. The organisms live in healthy plant tissue without causing any harm to the host and biosynthesize various structurally diverse metabolites, such as alkaloids, terpenoids, polyketides, phenolics, flavonoids, coumarins, peptides, and quinones, which have high biological activities as antimicrobials, antioxidants, anti-inflammatories, anticancer agents, antivirals, antidiabetics, and immunomodulators. Besides providing an increased protection for their host plants from pathogens and environmental stressors, biosynthesis of such metabolites is an important resource for discovering novel lead molecules. Moreover, endophytic fungi have the capability to biosynthesize metabolites that are produced by their host plants and serve as an environmentally friendly alternative to obtaining natural products from medicinal plants. The fast growth of endophytic fungi and their ability to produce valuable compounds under artificial fermentation conditions have resulted in a potential for producing therapeutically active molecules and developing pharmaceuticals, agrochemicals, and industrial bioproducts (Venugopalan & Srivastava, 2018; Kusari *et al*., 2019; Nicoletti & Fiorentino, 2021; Rai *et al*., 2021).

Conclusion

This research successfully isolates and elucidates the structure of a natural product based on coumarin from an endophytic fungus in relation with the nuts of Anacardium occidentale L. Bioactivity-guided purification through silica gel column chromatography, coupled with thin-layer chromatography, produced a chromatographically pure natural product, while the structure of the purified metabolite was elucidated based on one-dimensional (^1H and ^13C) nuclear magnetic resonance spectroscopy as 7-hydroxy-3-methyl-4-(undecyloxy)-2H-chromen-2-one. This identification clearly reveals the amazing capability of endophytic fungi to produce natural products with structurally unique pharmacological importance. These results provide further emphasis on the significance of A. Occidentale-associated endophytes as sustainable sources of novel chemical scaffolds in natural product-based drug discovery programs.

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