

Exploring Bagworm (Psychidae) as a Source of Bioactive Natural Products

Pamela Oluwafunke AKINDEKO¹, Isiaka MOHAMMED², Adeola Anuoluwapo FADAIRO³,
Onyebuchi John Kennedy NWOKOJI⁴, Ibrahim Adebayo OLADOSU⁵

^{1,2,3,4,5}University of Ibadan, Ibadan, Nigeria

*Corresponding Author

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ABSTRACT

Interest in natural products research has expanded beyond plants to include insects as potential reservoirs of bioactive secondary metabolites. While previous studies on bagworms have primarily focused on their ecology and biology, their chemical constituents remain largely unexplored. In this study, the bagworm and its protective case were investigated for their secondary metabolites. Extraction was carried out using cold methanol, followed by qualitative screening for secondary metabolites. Selective acid-base extraction and liquid-liquid partitioning were employed for isolation, while preparative thin-layer chromatography (PTLC) was used for purification. Spectroscopic characterization was carried out using Nuclear Magnetic Resonance (NMR) and Fourier Transform Infrared (FTIR) spectroscopy. **Qualitative screening for secondary metabolites** revealed the presence of alkaloids, flavonoids, tannins, triterpenoids, and steroids. The FTIR spectrum revealed absorption bands at 3373 cm⁻¹ (O–H stretch), 2925 cm⁻¹ (C–H stretch), and 1715 cm⁻¹ (C=O stretch), indicative of hydroxyl and carbonyl functional groups. The ¹H NMR spectrum showed signals between δ 0.8–2.0 ppm (aliphatic protons of a terpene skeleton) and δ 3.0–5.5 ppm (protons of a sugar moiety, including an anomeric proton at δ ~5.2 ppm). The ¹³C NMR spectrum displayed peaks at δ ~20–40 ppm (terpene carbons), δ 60–80 ppm (oxygenated carbons of sugar), and δ ~100 ppm (anomeric carbon), confirming the presence of a glycosylated terpene derivative (C₁₂H₁₈O₆) as the major isolated metabolite. Conclusively, this study reveals the bagworm as a potential source of novel bioactive secondary metabolites, underscoring the importance of insects in natural product discovery.

Keywords: Secondary Metabolite, PTLC, NMR, FTIR, Glycosylated terpene, Bagworm.

INTRODUCTION

Natural products remain important sources of structurally diverse molecules for drug discovery and biomedical research. Their chemical diversity arises from the wide range of ecological and physiological functions that natural products perform, including defense, communication, adaptation, and interactions with other organisms. Historically, plants and microorganisms have dominated natural-product research; however, increasing attention has been directed towards animals and, particularly, insects as sources of chemically diverse natural products with potential biological and pharmacological relevance (Dossey, 2010; Vilcinskas, 2013).

Insects represent an exceptionally diverse group of organisms and possess sophisticated chemical systems for survival. They produce, modify, store, or sequester a wide range of compounds involved in defense against predators and pathogens, reproduction, communication, and interactions with their environment. These chemical systems include small-molecule metabolites as well as peptides and other bioactive substances, providing opportunities for the discovery of structurally novel compounds. Although insect-derived natural products have historically received less attention than plant- and microorganism-derived compounds, advances

in natural-product isolation, spectroscopy, metabolomics, and other analytical approaches have increased the feasibility of investigating their chemical diversity (Dossey, 2010; Seabrooks & Hu, 2017).

The chemical potential of insects is particularly relevant to drug discovery because their ecological adaptations can give rise to unusual molecular structures and biological activities. Insect-derived compounds have been investigated for antimicrobial, anticancer, anti-inflammatory, immunomodulatory, and other biological properties. This diversity is associated not only with compounds produced directly by insects but also with substances obtained from their diet, accumulated through sequestration, or contributed by insect-associated microorganisms. Consequently, insects constitute a broad and comparatively underexplored reservoir of natural products that can complement conventional sources of bioactive compounds (Dossey, 2010).

Within insects, the order Lepidoptera, comprising butterflies and moths, provides an especially interesting group for natural-product investigations. Lepidopteran larvae interact closely with their host plants and are exposed to predators, parasitoids, pathogens, and other environmental pressures. These interactions are associated with diverse chemical defence strategies, including the sequestration and utilisation of plant secondary metabolites. Reported compounds associated with Lepidoptera include terpenoids, iridoid glycosides, cardenolides, and other plant-derived defensive substances, demonstrating the capacity of these insects to accumulate and utilise chemically diverse metabolites. (Nishida, 2002; Opitz & Muller, 2009)

Evidence from individual lepidopteran species further demonstrates this chemical potential. Franke et al. (1986), for example, isolated the iridoid glycosides aucubin, catalpol, and 6-O- β -glucopyranosylaucubin from larvae and pupae of the butterfly *Euphydryas cynthia*. The occurrence of these glycosides in the insect was associated with chemical defence, with preliminary feeding experiments indicating some degree of unpalatability. Such findings demonstrate that lepidopteran insects can contain chemically defined secondary metabolites with ecological significance and provide a basis for investigating other, less-studied members of the order.

Bagworms (Family: Psychidae) are lepidopteran insects distinguished by the construction of self-enclosing protective cases during larval development. The family contains approximately 1,000 species, and larvae remain within these cases throughout development. Many species are polyphagous and interact with a broad range of host plants, placing them within ecological environments rich in diverse plant secondary metabolites (Rhainds et al., 2009).

Despite the biological and ecological interest surrounding Psychidae, their chemical constituents have received considerably less attention than their life history, behaviour, and agricultural importance. This represents an important gap in natural-product research because the distinctive lifestyle of bagworms, their close association with plants, and their protective cases provide several possible avenues for chemical investigation. In particular, the chemical composition of bagworm larvae and their protective cases remains insufficiently characterised compared with better-studied insect groups.

The investigation of bagworms may therefore contribute to the broader search for insect-derived natural products by identifying secondary metabolites that have not been extensively documented in this group. Characterisation of such compounds is important not only for expanding knowledge of the chemical diversity of Psychidae but also for establishing a foundation for subsequent biological evaluation. In this context, chromatographic separation combined with spectroscopic techniques such as Fourier-transform infrared spectroscopy (FTIR) and nuclear magnetic resonance (NMR) provides an appropriate approach for the isolation and structural characterisation of metabolites from complex insect extracts.

Therefore, this study investigated the secondary metabolites present in bagworm larvae and their protective cases through methanolic extraction, qualitative phytochemical screening, selective isolation, chromatographic purification, and spectroscopic characterisation. The study specifically sought to contribute to the limited chemical information available on bagworms and to evaluate their potential as a source of structurally diverse natural products.

MATERIAL AND METHOD

Sample Collection and Extraction

Twenty-five bagworm larvae, intact within their protective cases, were procured from Ibode Market, Ibadan, Nigeria. The samples were placed in a 1-litre reagent bottle and macerated in 500 mL of methanol for seven days at room temperature. The resulting mixture was filtered through fluted filter paper to separate the liquid from the insect and plant debris. The liquid filtrate was then concentrated using a rotary evaporator set to 50°C for exactly three hours (5:00 a.m. to 8:00 a.m.) under monitored vacuum pressure to prevent the degradation of thermolabile compounds.

Qualitative Screening

The concentrated methanolic extract was subjected to standard qualitative screening tests to identify the presence of major secondary metabolite classes. Alkaloids were detected using Dragendorff's reagent. Flavonoids were screened by the addition of 10% sodium hydroxide (NaOH), and anthocyanins were tested using dilute hydrochloric acid. The presence of tannins was evaluated using ferric chloride and potassium hydroxide solutions. Saponins were assessed via the frothing test, while triterpenoids and steroids were identified using the Liebermann-Burchard test (involving chloroform, glacial acetic acid, and concentrated sulfuric acid). Finally, phenols were tested using ferric chloride, reducing sugars with Fehling's solutions A and B, and glycosides via the Keller-Killiani test.

Selective Acid-Base Extraction

A modified acid-base extraction was employed to selectively isolate the secondary metabolites. First, 20 mL of a 0.1 M lead (II) acetate solution was added to the methanolic extract to precipitate out interfering substances like tannins. The mixture was acidified with 10 mL of glacial acetic acid, transferred to a separatory funnel and partitioned with 50 mL of chloroform. The organic (chloroform) layer was separated and set aside. To recover the desired metabolites, the aqueous phase was neutralized by the incremental addition of sodium hydrogen carbonate (NaHCO₃). The neutralized solution was then partitioned with diethyl ether. The resulting upper organic layer, containing the neutral target compounds, was collected and concentrated for purification.

Preparative Thin-Layer Chromatography (PTLC)

Fractions showing similar TLC profiles, as well as the organic extract recovered from the diethyl ether partition, required further purification. This was achieved using Preparative Thin-Layer Chromatography (PTLC). The samples were applied to PTLC plates and developed in a solvent system consisting of hexane and ethyl acetate in a 70:30 ratio. The major target band was visually identified, carefully scraped from the silica plate, eluted with a suitable solvent, and concentrated to yield the isolated compound as a colourless amorphous solid.

Spectroscopic Characterization

The purified compound was characterized using Fourier Transform Infrared (FTIR) spectroscopy and Nuclear Magnetic Resonance (NMR) spectroscopy. The FTIR spectrum was recorded to identify the presence of specific functional groups. For NMR analysis, the isolated solid was dissolved in deuterated chloroform (CDCl₃). Both Proton (¹H) and Carbon-13 (¹³C) NMR spectra were acquired to determine the structural framework, chemical shifts, and proton-carbon connectivity of the compound.

¹H NMR (CDCl₃): δ (ppm) 4.95, 4.94 (2H, d, geminal, C-1), 1.79 (3H, s, C-5), 5.49 (1H, dd, C-3), 6.55 (1H, dd, C-4), 5.29 (1H, d, anomeric, C-1'), 3.64 (br, s, OH, C-2'), 1.30 (3H, s, C-3'), 4.01, 3.97 (2H, m, overlapping, C-4', C-5'), 4.52 (1H, m, C-6'), 9.68 (1H, s, aldehydic C-7').

^{13}C NMR (CDCl_3): δ (ppm) 199.23 (C=O, C-7'), 142.19 (CH, vinylic, O-substituted, C-4), 139.57 (sp^2 quaternary, C-2), 118.01 (CH, vinylic, C-3), 111.62 (CH_2 , vinylic, C-1), 101.85 (CH, anomeric, C-1'), 79.62 (CH-O, C-6'), 78.20 (CH-O, C-4'), 73.82 (CH-O, C-2'), 71.16 (CH-O, C-5'), 19.95 (CH_3 , C-3'), 19.06 (CH_3 , C-5).

RESULTS AND DISCUSSION

Qualitative Screening Composition

The qualitative screening of the methanolic extract of the bagworm insect and its protective casing contained alkaloids, flavonoids, tannins, triterpenoids, and steroids, whilst glycosides, phenols, saponin, and anthocyanin were not. (See Table 1)

Table 1: Qualitative Analysis Result

Qualitative Analysis Result

Alkaloids	+
Flavonoids	+
Tannins	+
Saponins	–
Triterpenoids	+
Steroids	+
Phenols	–
Glycosides	–
Anthocyanins	–

(+ = Present, – = Absent)

FTIR Analysis

The purified compound (Figure 1) showed a sharp absorption at 3373 cm^{-1} which is assigned to O–H stretching vibration, consistent with the hydroxyl groups present in the compound. Peaks at 2956 cm^{-1} and 2918 cm^{-1} are due to aliphatic C–H stretching, confirming the presence of saturated hydrocarbon chains. The absorption at 1512 cm^{-1} is attributed to C=C stretching of the alkenyl bond (C16=C17). Bands at 1467 cm^{-1} and 1376 cm^{-1} correspond to CH_2 and CH_3 bending vibrations, while the absorptions at 1240 cm^{-1} and 1214 cm^{-1} , together with 1081 cm^{-1} , represent C–O stretching from alcohol and ether functionalities. Additional features at 833 cm^{-1} and 755 cm^{-1} are associated with =C–H out-of-plane bending of the alkenyl group. The spectrum therefore confirms the presence of hydroxyl groups, aliphatic chains, ether linkages, and an alkenyl double bond in the proposed structure.

Table 2: Table of Fourier transform infrared analysis on isolated compound

Absorbance (cm^{-1})	Assignment	Structural feature
3373	O-H stretching	Hydroxyl groups
2956, 2917	C-H stretching(aliphatic)	Aliphatic chains
1511	C=C stretching	Alkene bond

1467, 1376	CH ₂ /CH ₃ bending	Aliphatic groups
1240, 1214	C-O stretching	Alcohols/ethers
1081	C-O stretching	Alcohols/ethers
833, 755	=C-H out-of-plane bending	Alkene group

NMR Analysis

In the ¹H NMR (Table 3), the spectrum shows a downfield aldehydic signal at δ 9.68 (1H, s, C-7'), directly supporting the presence of a -CHO group in the molecule. In the vinylic region, δ 6.55 (1H, dd, C-4) and δ 5.49 (1H, dd, C-3) confirm a double bond system, indicating unsaturation in the aglycone portion. The two nonequivalent protons at δ 4.95 and 4.94 (2H, d, C-1) are geminal hydrogens on the same carbon, further supporting the presence of a terminal -CH₂ group adjacent to a double bond. The anomeric proton appears at δ 5.29 (1H, d, C-1'), a typical feature of glycosidic linkage. Other sugar protons resonate in the range δ 4.52–3.97 (C-4', C-5', C-6'). Due to their highly similar oxygen-rich electronic environments, the methine protons for C-4' and C-5' overlap to form a combined 2H multiplet at δ 4.01 and 3.97, respectively. A broad singlet at δ 3.64 (C-2') represents an exchangeable OH group. Two methyl singlets at δ 1.79 (3H, s, C-5) and δ 1.30 (3H, s, C-3') correspond to aliphatic CH₃ substituents. Altogether, the proton spectrum confirms the structural elements: an aldehyde, a vinylic system, a terminal CH₂ group, two methyl substituents, and a sugar moiety.

In the ¹³C NMR spectrum, the most deshielded resonance at δ 199.23 corresponds to an aldehyde carbonyl carbon (C-7'), which is in agreement with the aldehydic proton at δ 9.68. One quaternary sp² carbon is observed at δ 139.57 (C-2), confirming the unsaturated and substituted backbone. The olefinic carbon at δ 118.01 (C-3) supports the presence of a vinylic CH group, while the signal at δ 111.62 (C-1) represents a vinylic CH₂ group, consistent with the geminal protons at δ 4.94 and 4.95. C-4 resonates at δ 142.19 and is assigned as an O-substituted vinylic CH; its downfield position reflects oxygen substitution on the double bond. The anomeric carbon at δ 101.85 (C-1') is characteristic of a sugar unit. Additional oxygenated carbons at δ 79.62, 78.20, 73.82, and 71.16 confirm the presence of a glycosidic moiety. Crucially, the ¹³C data clearly resolves the overlapping 4' and 5' sugar protons from the ¹H spectrum into two distinct oxygenated carbons at δ 78.20 (C-4') and 71.16 (C-5'). Finally, two aliphatic carbons at 19.95 (C-3') and δ 19.06 (C-5) support the methyl groups detected in the ¹H spectrum.

Table 3: ¹H (400 MHz) and ¹³C (100 MHz) NMR Spectroscopic Data for the isolated compound in CDCl₃

Position	¹³ C δ (ppm)	Carbon Type	¹ H δ (ppm)	Multiplicity	Integration	Assignment / Feature
1	111.62	CH ₂	4.94, 4.95	d	2H	Vinylic / Allylic
2	139.57	qC	-	-	-	Quaternary sp ²
3	118.01	CH	5.49	dd	1H	Vinylic
4	142.19	CH-O	6.55	dd	1H	O-substituted vinylic
5	19.06	CH ₃	1.79	s	3H	Methyl group
1'	101.85	CH	5.29	d	1H	Anomeric
2'	73.82	CH-O	-	-	-	Oxygenated methine
OH (on 2')	-	-	3.64	s (br)	1H	Hydroxyl proton
3'	19.95	CH ₃	1.3	s	3H	Methyl group
4'	78.2	CH-O	4.01	m	1H*	Oxygenated methine
5'	71.16	CH-O	3.97	m	1H*	Oxygenated methine
6'	79.62	CH-O	4.52	m	1H	Oxygenated methine
7'	199.23	C=O	9.68	s	1H	Aldehydic

Note: The protons at positions 4' and 5' appear as overlapping multiplets integrating to 2H in total.

The isolated compound shared glycosylation features with the iridoid glycosides reported from *Euphydryas cynthia*, particularly the characteristic anomeric proton and carbon signals, but differed in its aglycone framework, which contained an aldehyde functionality (Franke et al., 1986).

Proposed Structure

The isolated compound was recovered as a colourless amorphous solid.

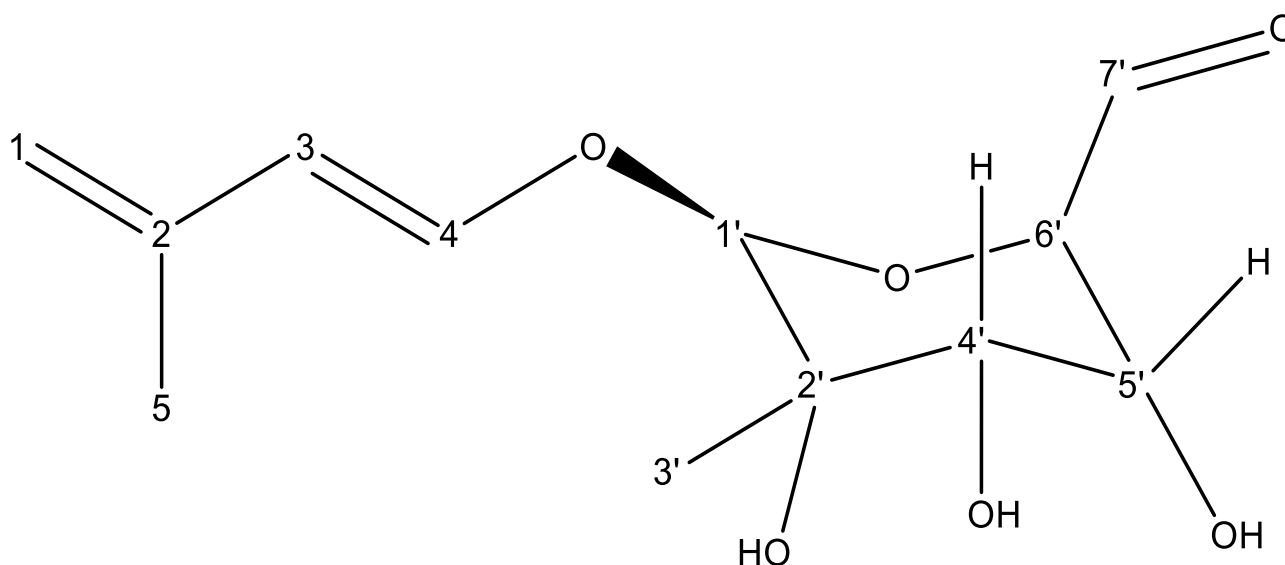


Fig 1: Proposed isolated compound

DISCUSSION

The isolation of a glycosylated terpenoid derivative from the bagworm extract highlights the chemical complexity inherent in the Psychidae family. Insects, particularly within the order Lepidoptera, frequently utilize diverse chemical defense strategies, which include the sequestration, modification, and utilization of plant secondary metabolites. The isolated compound shares distinct glycosylation features, specifically the characteristic anomeric proton and carbon signals with iridoid glycosides such as aucubin and catalpol, which have been previously isolated from the larvae and pupae of the butterfly *Euphydryas cynthia*. However, the bagworm isolate differs structurally in its aglycone framework; while iridoid glycosides typically possess a cyclopentane-pyran fused ring system, the isolated compound features an unsaturated terpenoid chain bearing an aldehyde functionality. Because bagworms are highly polyphagous and interact with a broad spectrum of host plants, the presence of this functionalized glycoside suggests that the larvae may sequester structurally diverse plant-derived metabolites from their ecological environment, potentially utilizing them for defense against predators or environmental adaptation.

Despite providing foundational chemical data on this underexplored insect group, the current study has several limitations. First, the structural elucidation relies primarily on 1D NMR (^1H and ^{13}C) and FTIR spectroscopy. While these techniques strongly support the proposed glycosylated terpenoid framework, they are insufficient to establish the complete absolute stereochemistry and carbon-carbon connectivity of the molecule. Second, the specific botanical identity of the host plants utilized by the collected larvae was not documented. Consequently, it remains undetermined whether the isolated compound is synthesized *de novo* by the bagworm, sequestered directly from its diet, or metabolically altered following ingestion. Finally, while the qualitative screening confirmed the presence of bioactive chemical classes, no specific *in vitro* biological assays were conducted to evaluate the pharmacological potential of the isolated compound.

CONCLUSION AND RECOMMENDATIONS

This study provides the first definitive chemical profiling of bagworm (Psychidae) larvae and their protective cases in this region, contributing crucial data to the limited chemical literature available on this insect family. By successfully isolating and characterizing a glycosylated terpenoid compound featuring an unsaturated framework and an aldehyde functionality, this research demonstrates that bagworms act as reservoirs for structurally complex secondary metabolites. This underscores the broader importance of investigating insects as viable, underexplored resources for natural product discovery that can complement conventional plant and microbial sources. To advance these foundational findings, future research must employ advanced spectroscopic techniques—specifically two-dimensional (2D) NMR (such as COSY, HSQC, and HMBC) and High-Resolution Mass Spectrometry (HRMS)—to unambiguously confirm the complete molecular connectivity and stereochemistry of the isolated compound. Furthermore, comprehensive bioactivity studies, including antimicrobial, antioxidant, and cytotoxicity assays, are essential to evaluate the therapeutic potential of this metabolite. Finally, comparative ecological studies examining the chemical constituents of different bagworm species across various host plants, developmental stages, and collection locations will clarify the origins and ecological roles of these diverse chemical arsenals.

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