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Isolation, Purification and NMR-Based Structural Elucidation of Sterol Derivatives from *Ganoderma resinaceum* (Fr.) Karst

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Abstract: This study presents a detailed phytochemical investigation of the medicinal mushroom *Ganoderma resinaceum* (Fr.) Karst., collected from Mazandaran Province, Iran. Sequential maceration using *n*-hexane, ethyl acetate (EtOAc), and methanol (MeOH) yielded crude extracts of differing polarity. Chromatographic purification of the EtOAc fraction produced four major sterol derivatives: ergosterol, ergosterol peroxide, 9,11-dehydroergosterol peroxide, and ergosta-7,22-dien-3β-ol. Their structures were confirmed using comprehensive spectroscopic techniques, including ^{1}H NMR, ^{13}C NMR, and DEPT. To the best of our knowledge, this is the first report of these sterol derivatives from Iranian *G. resinaceum*. The findings highlight the chemical diversity of the species and underscore the relevance of medium-polarity fractions as reservoirs of pharmacologically significant sterols.

Keywords: *Ganoderma resinaceum*; NMR; mushroom; phytochemical analysis; ergosterol derivatives; chromatography

1. Introduction

Mushrooms are recognised as nutritional resources and as important agents in both traditional and contemporary medicine. Their earliest documented therapeutic applications appear in ancient Eastern medical systems, and their relevance has broadened through modern Western scientific research. Approximately 16,000 mushroom species have been described globally, and more than 2000 are considered safe for human consumption [1]. Mushrooms remain an underexplored reservoir of physiologically active metabolites with considerable therapeutic potential. They produce structurally diverse compounds exhibiting antibacterial, immunomodulatory and anti-inflammatory activities [2]. Recent investigations have also identified hepatoprotective, hypoglycaemic and neuroprotective effects, reinforcing their suitability as promising candidates for drug discovery and development [3].

Medicinal mushrooms represent an important source of structurally diverse natural products. Phytochemical investigations have revealed that mushrooms produce a wide variety of secondary metabolites, including polysaccharides, terpenoids, sterols, phenolic compounds, fatty acids, alkaloids and peptides [4]. Among these, polysaccharides and triterpenoids have historically received the greatest research attention due to their immunomodulatory and anticancer properties [5]. However, other classes of metabolites such as sterols and sterol derivatives are increasingly recognised as biologically active constituents contributing to the pharmacological effects of medicinal mushrooms [4].

Species belonging to the genus *Ganoderma* are particularly rich in bioactive metabolites and have been widely investigated for their chemical diversity and therapeutic potential. Numerous phytochemical studies have reported that *Ganoderma* species contain lanostane-type triterpenoids, ergostane-type sterols, meroterpenoids, polysaccharides and phenolic compounds that collectively contribute to their biological activities [6–9]. Sterols represent one of the major lipid components of fungal cell membranes, with ergosterol being the predominant sterol found in higher fungi [10]. In recent years, considerable attention has been directed toward ergosterol and its oxidised derivatives due to their diverse pharmacological activities.

Several studies have demonstrated that fungal sterols possess anti-inflammatory, antioxidant, antimicrobial and anticancer properties [4]. In particular, ergosterol peroxide and related sterol derivatives have been reported to inhibit inflammatory mediators, suppress tumour cell proliferation and modulate key signalling pathways such as NF-κB and MAPK [11]. These findings suggest that sterol compounds may play an important role in the therapeutic properties attributed to medicinal mushrooms.

Ganoderma lucidum and related species within the *Ganoderma* genus are widely recognised for strong therapeutic efficacy and have been the subject of extensive pharmacological and chemical investigations. The genus name is derived from the Greek words *ganos* and *derma*, referring to the characteristic glossy surface of the fruiting body. Petter Adolf Karsten formally defined the genus in 1881 [12].

Although species of the genus *Ganoderma* have been extensively investigated for their lanostane-type triterpenoids, comparatively fewer studies have focused on their sterol composition. Sterols represent an important class of fungal metabolites that may contribute to the pharmacological properties attributed to medicinal mushrooms. However, the sterol profile of *Ganoderma resinaceum* remains insufficiently characterised, particularly for specimens originating from different geographical regions.

Ganoderma resinaceum occurs naturally in the forested regions of northern Iran and remains comparatively underexplored from a phytochemical perspective. While *G. lucidum* has been extensively studied for its bioactive constituents, limited information is available regarding the secondary metabolites of *G. resinaceum*. Since environmental conditions and geographical variation may influence fungal metabolite biosynthesis, investigation of locally collected specimens may reveal additional sterol derivatives and related metabolites. Therefore, the present study aimed to isolate and structurally characterise sterol constituents from *Ganoderma resinaceum* through chromatographic purification and spectroscopic analysis.

2. Materials and Methods

2.1. Materials

All solvents and reagents were analytical grade unless stated otherwise, ensuring reproducible extraction and chromatographic performance. The extraction solvents included *n*-hexane, ethyl acetate and methanol (Arian Sina, Iran), selected to achieve polarity-based separation of non-polar, semi-polar and polar constituents. Anisaldehyde reagent, glacial acetic acid and concentrated sulfuric acid were obtained from Merck (Darmstadt, Germany) and used for TLC visualisation due to their high sensitivity toward sterol-type structures.

Column chromatography employed silica gel (70–230 mesh; Merck, Darmstadt, Germany) and Sephadex LH-20 (Fluka, St. Gallen, Switzerland) as stationary phases, enabling both adsorption-based and size-exclusion-based separations. Precoated silica gel 60 F_{254} plates (Merck) were used for TLC profiling. TLC spots were inspected under UV light at 254 and 366 nm using a UV-visible spectrophotometer (CAMAG, Muttenz, Switzerland), providing rapid assessment of compound purity and mobility. Laboratory glassware included beakers (25, 50 and 100 mL), volumetric flasks (100 mL), graduated pipettes (1, 2, 5 and 10 mL), funnels and TLC developing chambers, all cleaned and dried thoroughly to avoid contamination.

Instrumentation included a Heidolph rotary evaporator (Heidolph Instruments, Schwabach, Germany) for solvent removal under reduced pressure, an AND-GF300 analytical balance (±0.001 g; A&D Company, Tokyo, Japan) for precise mass measurements, a Rihan Teb oven (Rihan Teb, Tehran, Iran) for controlled drying, an IKA-Werke electric grinder (IKA-Werke, Staufen, Germany) for sample pulverisation and an Electromantle heating mantle (Electrothermal Engineering Ltd., Essex, UK) for controlled heating. Freeze-drying was performed using a laboratory lyophiliser to preserve thermolabile constituents. NMR spectra (^{1}H, 400 MHz; ^{13}C, 125 MHz) were recorded on a Bruker (Bruker Corporation, Billerica, MA, USA) spectrometer using standard deuterated solvents.

2.2. Sample Collection

Fruiting bodies of *Ganoderma resinaceum* were collected in November 2018 from forested regions of Chalmardi village, Hezarjarib district, Neka County, Mazandaran Province, Iran, at an elevation of approximately 400 m above sea level (36°33′42″ N 53°23′31″ E). The specimens were located on fallen trunks of hornbeam

(*Carpinus betulus*) and beech (*Fagus orientalis*), consistent with the species' preference for hardwood substrates. Each specimen was examined for morphological integrity prior to collection to ensure accurate taxonomic identification and chemical reliability.

The herbarium sample was deposited under the code IRAN, MZ.298F. Taxonomic identification was performed and confirmed by MSc Saeed Ali Mousazadeh (Pasand Forest and Rangeland Research Station, Agricultural and Natural Resources Research and Education Centre of Mazandaran, AREEO, Behshahr, Iran), ensuring correct species assignment before chemical analysis.

2.3. Preparation of Extracts

2.3.1. Drying and Grinding

Fresh fruiting bodies were manually cleaned to remove soil, bark fragments and other surface debris. The material was air-dried in shade at ambient temperature to prevent degradation of thermolabile metabolites. Once fully dried, the fruiting bodies were ground to a fine, homogeneous powder using an IKA-Werke electric grinder. The powdered material was stored in a refrigerator to maintain chemical stability until extraction.

2.3.2. Extraction and Crude Extract Preparation

Sequential extraction using solvents of different polarity is widely applied in natural product research to efficiently separate metabolites according to their polarity. Non-polar solvents such as *n*-hexane and ethyl acetate are particularly suitable for extracting lipophilic compounds including sterols and fatty acids, whereas solvents with intermediate polarity can recover moderately polar metabolites [13].

A total of 1.1 kg of dried powdered mushroom material underwent sequential maceration with *n*-hexane, ethyl acetate and methanol to ensure comprehensive extraction across a polarity gradient. Each maceration step was repeated three times, using 2 L of solvent, each lasting at least 72 h at room temperature, with periodic agitation to enhance solvent penetration. After each cycle, the extracts were filtered, and the combined filtrates for each solvent were separately concentrated under reduced pressure using a rotary evaporator under reduced pressure at 40 °C, and the evaporation process typically required 30-45 min depending on the solvent volume. The concentrated extracts were lyophilised to obtain dry crude extracts.

The dried mushroom powder (1100 g) was extracted with *n*-hexane to obtain a crude extract (10.5 g), corresponding to a yield of 0.9 % (*w*/*w*). Subsequent fractionation afforded the ethyl acetate fraction (4.2 g, 0.4 %) and methanol fraction (5.4 g, 0.5 %) based on the initial dry material weight. The overall extraction yield was 1.83% relative to the initial dry weight, consistent with the expected distribution of sterol-type compounds in the non-polar and semi-polar fractions.

2.4. Fractionation and Chromatographic Purification

Column chromatography on silica gel together with thin-layer chromatography (TLC) was used for the separation and purification of sterol derivatives according to the procedure previously reported by our group [14].

The ethyl acetate extract (4.2 g), which displayed the richest TLC profile, was selected for purification. Initial fractionation was performed using silica gel column chromatography (70–230 mesh). The extract was dry loaded by adsorbing it onto coarse silica gel, removing the solvent and applying the dried mixture onto the column. A silica gel column (20 × 5 cm) was eluted with gradient mixtures of *n*-hexane and ethyl acetate at ratios of 19:1, 8.5:1.5, 8:2, 7:3, 6.5:3.5, 6:4, 1:1 and 0:1. This was followed by ethyl acetate-methanol (1:1) and a final wash with 100% methanol. Fractions with similar TLC profiles were combined into eleven pooled fractions (E1–E11). TLC plates were examined under UV_{254} and UV_{365} and visualised with anisaldehyde-sulfuric acid. Fractions E2, E4 and E5 were selected for further purification due to their distinct sterol-type TLC features.

2.4.1. Purification of Compound E2a

Fraction E2 (20 mg) was purified on a silica gel column (40 × 2 cm). TLC monitoring produced fifteen subfractions; vials 5-7 yielded a pure compound designated E2a. The compound yielded 12 mg of white crystalline material. Optimal separation was obtained using *n*-hexane-ethyl acetate (8.5:1.5). TLC showed an R_f of 0.50 in *n*-hexane:ethyl acetate (7:3), strong UV_{254} absorption and a yellow anisaldehyde-derived spot.

2.4.2. Purification of Compound E4c

Fraction E4 (62 mg) was purified using a silica gel column (40 × 2 cm) under gradient elution. Nineteen vials were collected; vials 6-10 contained a homogeneous compound designated E4c (22 mg). Optimal separation used *n*-hexane-ethyl acetate (17.75:2.25). TLC showed an R_f of 0.45 in *n*-hexane:ethyl acetate (6:4) with pink anisaldehyde staining and strong UV_{254} absorption.

2.4.3. Purification of Compound E4d

Further purification of fraction E4 (60 mg) yielded compound E4d. Column chromatography (40 × 2 cm) generated nineteen vials; vials 11–16 were combined to give 17 mg of pure material. Separation used mixtures of *n*-hexane, dichloromethane and ethyl acetate (20:0:0, 17:2:1, 16:2:2 and 15:2:3). TLC displayed an R_f of 0.82 with strong UV_{254} and UV_{365} absorption.

2.4.4. Purification of Compound E5b

Fraction E5 (43 mg) was purified using a silica gel column (40 × 2 cm), yielding eleven vials. Vials 8–11 contained a homogeneous compound designated E5b (11 mg). Optimal purification used *n*-hexane:ethyl acetate (7:3). TLC showed an R_f of approximately 0.50 in *n*-hexane:ethyl acetate (6:4) with clear $UV_{254/365}$ visibility and a black anisaldehyde-derived spot.

2.5. Spectroscopic and Analytical Characterisation

Structural characterisation of compounds E2a, E4c, E4d and E5b employed NMR spectroscopy. ^{1}H NMR (400 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker spectrometer using deuterated solvents selected according to compound solubility. Chemical shifts (δ, ppm) and coupling constants (*J*, Hz) were reported using standard conventions. Purity assessment throughout purification was monitored using TLC under UV light and anisaldehyde staining.

3. Results

The present study was primarily designed to isolate and characterize sterol constituents from *Ganoderma resinaceum*. Comprehensive metabolite profiling of the crude extract using advanced chromatographic techniques such as HPLC-MS or GC-MS was beyond the scope of the current investigation and may be considered in future studies.

A total of four steroidal compounds were isolated from the ethyl acetate extract of *G. resinaceum*. Sequential chromatographic purification yielded structurally related ergostane-type metabolites. The complete NMR spectra of the isolated compounds are provided in the Supplementary Materials (Figures S1–S4).

The structures of the isolated sterol derivatives were established based on detailed analysis of ^{1}H NMR, ^{13}C NMR and DEPT spectroscopic data and confirmed through comparison with previously published spectral data for related sterol compounds [14,15]. The complete ^{13}C NMR spectroscopic data for all isolated compounds are summarised in Table 1.

Table 1. ^{13}C NMR data (δ_C, ppm) for ergostane-type sterols isolated from *G. resinaceum* ($CDCl_3$, 100 MHz).

Carbon No.	Ergosterol (E2a)	Ergosterol Peroxide (E4c)	9,11-Dehydro-EP (E4d)	Ergosta-7,22-dien-3b-ol (E5b)
C-1	38.3	34.6	33.51	37.1
C-2	32	30.1	31.9	31.5
C-3	70.4	66.4	66.3	71.0
C-4	40.8	36.9	37.9	38.0
C-5	139.8	82.1	82.7	40.2
C-6	119.6	135.4	135.5	29.6
C-7	116.3	130.7	130.9	117.4
C-8	141.4	79.4	38.3	139.5
C-9	46.2	51.6	142.5	49.4
C-10	37.1	36.9	39.0	34.2
C-11	21.1	20.7	119.2	21.5
C-12	39.1	39.3	41.1	39.4
C-13	42.8	44.5	43.6	43.3
C-14	54.5	51.0	48.1	55.9
C-15	23.0	23.4	29.1	22.9
C-16	28.3	28.6	20.7	28.2
C-17	55.7	56.1	55.8	55.1
C-18	12.0	12.8	12.9	12.1

Table 1. *Cont.*

Carbon No.	Ergosterol (E2a)	Ergosterol Peroxide (E4c)	9,11-Dehydro-EP (E4d)	Ergosta-7,22-dien-3b-ol (E5b)
C-19	16.3	18.2	25.6	13.1
C-20	40.4	39.7	39.9	40.5
C-21	21.1	19.6	20.5	19.6
C-22	135.6	19.66	135.1	131.9
C-23	132	132.5	132.4	135.7
C-24	43.3	43.1	44.7	42.8
C-25	33.1	33.4	33.5	33.1
C-26	21.5	20.1	19.65	19.9
C-27	19.6	19.9	19.65	21.1
C-28	17.6	17.9	18.7	17.63

3.1. Compound E2a: Ergosterol

Compound E2a was obtained as a white crystalline powder (12 mg). TLC analysis showed a single spot at R_f 0.50 in *n*-hexane:EtOAc (7:3), appearing dark under UV_{254} and developing a yellow stain after anisaldehyde-sulfuric acid treatment. The ^{1}H NMR spectrum displayed characteristic olefinic protons at δ_H 5.60 (H-7), 5.40 (H-5), 5.25 (H-23) and 5.17 (H-22), consistent with a $\Delta^{5,7,22}$-triene system. The proton attached to the hydroxyl-bearing carbon (H-3) appeared at δ_H 3.60 as a broad resonance typical of sterols. Six methyl singlets and doublets occurred between δ_H 1.00–1.64, matching the expected ergostane framework (Table 2, Figure S1).

The ^{13}C NMR spectrum showed 28 carbon signals, including olefinic carbons corresponding to C-5/C-6, C-7/C-8 and C-22/C-23, as well as the oxygenated C-3 at δ_C 70.48. DEPT-90 and DEPT-135 experiments confirmed the presence of six methyl groups, nine methine carbons and the expected methylene and quaternary carbons for an ergostane nucleus (Figure S1). The phase discrimination in DEPT-135 allowed clear verification of the sterol substitution pattern and provided unambiguous confirmation of the carbon skeleton. Spectral data agreed fully with literature values for ergosterol [14,15], confirming E2a.

Table 2. ^{1}H NMR shifts (ppm) in $CDCl_3$ for identified compounds of *G. resinaceum*.

Position	Ergosterol	Ergosterol Peroxide	9,11-Dehydroergosterol Peroxide)	Ergosta-7,22-dien-3*β*-ol
H-3	3.60 (1H, br m)	3.99 (1H, br m)	4.00 (1H, br m)	3.62 (1H, br m)
H-5	5.40 (1H, m)	-	-	-
H-6	-	6.26 (1H, d, *J* = 8.4 Hz)	6.35 (1H, d, *J* = 8.4 Hz)	-
H-7	5.60 (1H, m)	6.05 (1H, d, *J* = 8.8 Hz)	6.53 (1H, d, *J* = 8.4 Hz)	5.20 (1H, m)
H-11	-	-	5.4 (1H, m)	-
H-22	5.17 (1H, m)	5.22 (1H, dd)	5.23 (1H, dd)	5.20 (1H, m)
H-23	5.25 (1H, m)	5.14 (1H, dd)	5.23 (1H, dd)	5.20 (1H, m)
CH_3-18	0.67 (3H, s)	0.83 (3H, s)	0.82 (3H, s)	0.57 (3H, s)
CH_3-19	0.83 (3H, s)	0.89 (3H, s)	0.93 (3H, s)	0.81 (3H, s)
CH_3-21	1.03 (3H, d, *J* = 6.5 Hz)	1.00 (3H, d, *J* = 6.5 Hz)	1.02 (3H, d, *J* =6.4 Hz)	1.03 (3H, d, *J* = 6.8 Hz)
CH_3-26	0.87 (3H, d, *J* = 7 Hz)	0.81 (3H, d, *J* = 6.8 Hz)	0.85 (3H, d, *J* = 6.8 Hz)	0.84 (3H, d, *J* = 6 Hz)
CH_3-27	0.85 (3H, d, *J* = 7 Hz)	0.85 (3H, d, *J* = 6.8 Hz)	0.86 (3H, d, *J* = 6.8 Hz)	0.82 (3H, d, *J* = 6.8 Hz)
CH_3-28	0.92 (3H, d, *J* = 6.5 Hz)	0.91 (3H, d, *J* = 6.4 Hz)	0.94 (3H, d)	0.94 (3H, d, *J* = 6.8 Hz)

3.2. Compound E4c: Ergosterol Peroxide

Compound E4c was isolated as white crystals (22 mg) with R_f 0.45 in *n*-hexane:EtOAc (6:4). After anisaldehyde treatment, the compound formed a pink spot, and strong absorption was observed under UV_{254}. The ^{1}H NMR spectrum displayed diagnostic olefinic signals at δ_H 6.26 (H-6), 6.05 (H-7), 5.22 (H-22) and 5.14 (H-23), each appearing as doublets or doublet-of-doublets with coupling constants consistent with adjacent unsaturated carbons. The hydroxyl-bearing H-3 appeared at δ_H 3.99. Six methyl groups resonated between δ_H 0.81–1.00, as expected for ergosterol derivatives (Table 1, Figure S2).

The ^{13}C NMR spectrum (Figure S2) contained 28 distinct carbons, with downfield signals at δ_C 82.18 (C-5) and δ_C 79.40 (C-8) characteristic of the 5,8-epidioxysterol peroxide bridge. Olefinic carbons between δ_C 130–135 supported the Δ^6 and Δ^{22} double bonds. DEPT spectra confirmed the expected distribution of CH, CH_2 and CH_3 groups. Oxygen-adjacent methine carbons were well differentiated in DEPT-90, and DEPT-135 provided strong negative-phase CH_2 signals, reinforcing the peroxide-linked ring structure. Data were fully consistent with reported spectra of ergosterol-5,8-peroxide [14,15], confirming E4c.

3.3. Compound E4d: 9,11-Dehydroergosterol Peroxide

Compound E4d was isolated as a white crystalline powder (17 mg) with R_f 0.82 in *n*-hexane:EtOAc (7.5:2.5). The TLC spot developed as a dark band after anisaldehyde spraying and heating. Strong UV_{254} absorption and

faint UV_{365} fluorescence were observed. The ^1H NMR spectrum displayed olefinic doublets at δ_H 6.35 (H-6) and δ_H 6.53 (H-7), and unsaturated protons at δ_H 5.20 corresponding to H-22 and H-23, each appearing as dd consistent with a Δ^{22} double bond. The hydroxyl-bearing H-3 resonated at δ_H 4.00. A deshielded multiplet at δ_H 5.45 was assigned to H-11, supporting the presence of a $\Delta^{9(11)}$ double bond. Methyl resonances occurred between δ_H 0.76–0.98 (Table 1, Figure S3).

The ^{13}C NMR spectrum (Figure S3) confirmed the peroxide bridge through downfield shifts at δ_C 82.73 (C-5) and δ_C 78.36 (C-8). Additional olefinic carbons at δ_C 148.56 (C-9) and δ_C 119.20 (C-11) supported the $\Delta^{9(11)}$-system. DEPT analyses confirmed six methyl groups, multiple sterol-core methines and the expected substitution pattern. DEPT-135 distinguished peroxide-bearing quaternary carbons lacking CH signals in DEPT-90. Combined data established E4d as 9,11-dehydroergosterol peroxide, consistent with previously reported sterols [14,15].

3.4. Compound E5b: Ergosta-7,22-dien-3β-ol

Compound E5b was isolated as white crystals (11 mg) with R_f 0.50 in *n*-hexane:EtOAc (6:4). After anisaldehyde treatment and heating, the TLC plate showed a dark spot. In the ^1H NMR spectrum, olefinic protons between δ_H 5.20–5.24 corresponded to H-7, H-22 and H-23. The hydroxyl-bearing H-3 appeared as a broad signal at δ_H 3.62. Six methyl groups resonated between δ_H 0.57–1.03, characteristic of sterols bearing Δ^7 and Δ^{22} unsaturation (Table 1, Figure S4).

The ^{13}C NMR spectrum (Figure S1) showed 28 carbons, including olefinic carbons at δ_C 139.59/117.49 (C-8/C-7) and δ_C 135.70/131.89 (C-22/C-23), confirming double bonds at C-7 and C-22. The oxygenated C-3 appeared at δ_C 71.08. Remaining carbons corresponded to typical sterol methylene and methine signals. DEPT-90 and DEPT-135 spectra supported the expected carbon distribution, with clear CH_3 signals, negative-phase CH_2 peaks and well-assigned quaternary ring-junction carbons. Comparison with reference spectra [14,15] verified E5b as ergosta-7,22-dien-3β-ol (Figure 1).

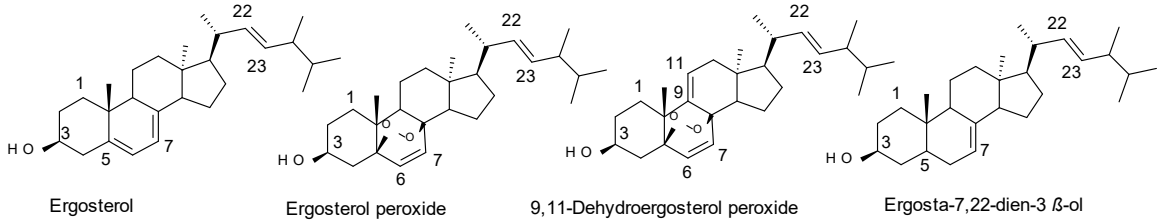

Figure 1. Chemical structures of ergosta derivatives.

4. Discussion

Mushrooms are recognised globally for their nutritional value and wide range of therapeutic effects. More than 130 biological activities have been reported, including anti-diabetic, antioxidant, antibacterial, anticancer, prebiotic, immunomodulatory, anti-inflammatory and cardiovascular effects [16]. Medicinal mushrooms, particularly species belonging to *Ganoderma*, possess marked potential for producing structurally diverse bioactive metabolites with distinct pharmacological profiles. The biochemical properties of region-specific indigenous strains may also contribute to the discovery of novel variants with enhanced biological activity [17]. *G. resinaceum* is an edible and medicinal mushroom that has attracted increasing attention due to its abundant secondary metabolite composition.

Existing phytochemical studies of *Ganoderma* have primarily focused on lanostane-type triterpenoids, which are widely recognised as major contributors to the biological properties of this genus [18]. However, sterols and associated ergostane derivatives represent an additional chemical class of importance. Although structurally simpler than triterpenoids, they remain comparatively under-investigated despite growing evidence supporting their contribution to the pharmacological characteristics of *Ganoderma* species.

In this study, chromatographic purification of the EtOAc fraction of *G. resinaceum* yielded four ergostane-type steroidal compounds: ergosterol (E2a), ergosterol peroxide (E4c), 9,11-dehydroergosterol peroxide (E4d) and ergosta-7,22-dien-3β-ol (E5b). Their enrichment in the EtOAc extract demonstrates the suitability of medium-polar solvents for isolating sterol constituents from fungal matrices. Comprehensive spectroscopic analysis using ^1H NMR, ^{13}C NMR and DEPT experiments enabled confident assignment of proton and carbon environments and allowed clear confirmation of substitution patterns characteristic of ergostane derivatives.

Ergosterol and its oxidised derivatives are among the most frequently documented fungal sterols and are consistently reported in *Ganoderma* species and other higher basidiomycetes [19]. The identification of ergosterol

(E2a) in *G. resinaceum* agrees with previous phytochemical studies in which it appears as a major structural component of fungal membranes [20]. Ergosterol peroxide (E4c) and 9,11-dehydroergosterol peroxide (E4d) are oxidised sterols that have often been associated with stronger biological activity than their non-oxidised counterparts [11]. The peroxide bridge at C-5 and C-8, confirmed in the present study by characteristic downfield ^{13}C NMR shifts, is a distinguishing structural feature contributing to their pharmacological properties [19]. The presence of ergosta-7,22-dien-3β-ol (E5b) further supports the chemical diversity of sterols in *G. resinaceum* and aligns with previous reports from related *Ganoderma* species [21].

Ergosterol and its derivatives represent an important class of fungal sterols that have attracted increasing attention due to their diverse biological activities. Several studies have reported that ergosterol derivatives exhibit anti-inflammatory, antioxidant, antimicrobial, and anticancer properties. In particular, ergosterol peroxide has been shown to inhibit the proliferation of several cancer cell lines and to modulate key signalling pathways involved in inflammation and oxidative stress. These findings suggest that sterol constituents present in medicinal mushrooms may contribute to their pharmacological properties and therapeutic potential [10,14].

Several chromatographic investigations of *Ganoderma* have shown that lipid-rich fractions contain a particularly high diversity of ergostane-type sterols exhibiting a range of biological activities. For example, Chen et al. isolated fourteen ergosterol derivatives from the lipid fraction of *G. lucidum*, including one novel compound, through bioassay-guided fractionation combined with silica gel, ODS chromatography and preparative HPLC. Structural elucidation was achieved through ^{1}H NMR, ^{13}C NMR, MS and HMBC correlations [22]. Their findings demonstrated the effectiveness of multistep chromatographic workflows in separating closely related sterol analogues from complex fungal matrices.

Baby et al. (2015) conducted an extensive chromatographic study of sterols and triterpenoids from multiple *Ganoderma* species using petroleum ether and EtOAc fractions followed by silica gel and Sephadex LH-20 column chromatography. Ergosterol and ergosterol peroxide were consistently reported among the major sterols across species. Structural identification relied on ^{1}H NMR, ^{13}C NMR, and DEPT analyses supported by literature comparison. The authors emphasised that, although sometimes overshadowed by triterpenoids, sterols are chemically stable and biologically meaningful metabolites that substantially contribute to the pharmacological profile of *Ganoderma* mushrooms [6]. Their repeated detection across studies reinforces their role as characteristic secondary metabolites of this genus.

The ergostane-type sterols isolated here, particularly ergosterol and ergosterol peroxide, continue to receive increasing research interest due to their anti-inflammatory and antioxidant activity. Ergosterol peroxide has shown protective effects in LPS- and UVA-induced inflammatory models in human keratinocytes, where it reduced intracellular ROS levels, suppressed IL-1β and IL-8 secretion and modulated key signalling mediators involved in inflammatory cascades [23]. Sterol fractions from *G. lucidum* (GLS) have also inhibited LPS-induced inflammation in RAW264.7 macrophages by decreasing nitric oxide production and downregulating pro-inflammatory cytokines such as TNF-α, IL-1β and IL-6 through suppression of the MAPK and NF-κB pathways [24]. In addition, specific phytosterols such as ergosterol have been shown to suppress NO production, COX-2 and iNOS expression and ERK phosphorylation in macrophage models, demonstrating structure-dependent modulation of intracellular signalling pathways [25]. Overall, these findings indicate that ergostane-type steroids significantly contribute to the bioactivities often attributed to *Ganoderma* species. The present study, by confirming their occurrence in *G. resinaceum*, reinforces their pharmacological relevance and supports future investigations into their functional and therapeutic potential.

Although the present study successfully isolated and characterized sterol derivatives from *G. resinaceum*, several limitations should be acknowledged. The investigation focused primarily on the isolation and structural identification of sterol constituents, and comprehensive metabolite profiling of the crude extract using advanced analytical techniques such as LC-MS or GC-MS was not performed. In addition, biological activity evaluation of the isolated compounds was beyond the scope of the present work. Therefore, further studies involving detailed metabolomic profiling and biological activity assessment of the isolated sterols would provide deeper insight into the pharmacological potential of this species.

5. Conclusions

The present study reports the isolation and structural characterization of sterol derivatives from *G. resinaceum*. The results contribute to the phytochemical knowledge of this comparatively underexplored species. The findings also highlight the presence of sterol constituents that may contribute to the biological properties attributed to medicinal mushrooms. Further investigations focusing on comprehensive metabolite profiling and biological evaluation of these compounds are warranted to better understand their pharmacological potential.

Supplementary Materials

The additional data and information can be downloaded at: https://media.sciltp.com/articles/others/26041413 58041155/NPA-26030079-supplementary.zip.

Author Contributions

E.H.: conceptualization, validation, and writing—original draft preparation, review and final editing; M.A.: writing—original draft preparation, review and final editing; Z.D.: investigation, collection of resources and data analysis; A.B.: software; writing—original draft preparation; L.N.: validation, and writing—original draft preparation, review and final editing. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest.

Use of AI and AI-Assisted Technologies

During the preparation of this manuscript, the authors used Grammarly to improve grammar, spelling, and overall language clarity. The authors carefully reviewed and edited the output and take full responsibility for the content of the publication.

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