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Phytochemical profiling, antioxidant activity, cytotoxicity and GC-MS characterization of *Ganoderma applanatum*

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Abstract. *Invento JB, Benida GMV, Tejano JCM, Enojales JJC, Pagente MJO, Dologan KCR, Plimaco SMY. 2026. Phytochemical profiling, antioxidant activity, cytotoxicity and GC-MS characterization of* Ganoderma applanatum*. Nusantara Bioscience 18 (1): n180104. https://doi.org/10.13057/nusbiosci/n180104.* This study investigated the medicinal potential of *Ganoderma applanatum* ethanolic extracts by focusing on antioxidant capacity, cytotoxicity, and bioactive compound profiling. Samples were collected, authenticated, and subjected to phytochemical screening, revealing the presence of flavonoids, steroids, saponins, tannins, and alkaloids. The free-radical scavenging ability of the extracts was determined using a modified DPPH assay, where dilutions (10-1,000 µg/mL) were mixed with 0.1 mM ethanolic DPPH, vortexed, incubated in the dark for 1 h at room temperature, and absorbance read at 517 nm in triplicates to calculate % inhibition and IC50. The extract demonstrated a 97% inhibition at 500 µg/mL, indicating strong free radical neutralization. GC-MS analysis identified 26 bioactive compounds, including fatty acids, hydrocarbons, phenolics, terpenoids, and steroids, many of which are associated with antioxidant and cytotoxic effects. Cytotoxicity was evaluated via the Brine Shrimp Lethality Test (BSLT) by the MSU-IIT Chemistry Department, exposing 10 *Artemia salina* nauplii per replicate to triplicate dilutions (1-10,000 µg/mL) for 24 h, and calculating % mortality. LC50 values were determined to be 37 µg/mL, signifying potent toxicity. These results collectively highlight *G. applanatum's* rich phytochemical composition and significant bioactivities, supporting its traditional medicinal use. The evidence-based findings suggest that the ethanolic extract possesses promising antioxidant and cytotoxic properties, warranting further investigation for therapeutic applications.

Keywords: Bioactive compounds, Brine Shrimp Lethality Test, ethanolic extract, *Ganoderma applanatum,* GC-MS analysis

INTRODUCTION

Mushrooms, as fruiting bodies of filamentous fungi, represent a vast and ecologically important group within the kingdom Fungi, primarily classified into Ascomycetes and Basidiomycetes based on reproductive and ecological traits (Zeid et al. 2011). They have historically been important as both food and traditional medicine across diverse cultures worldwide (Diansambu et al. 2015). Despite estimates of 2.2 to 3.8 million fungal species globally, only about 10% have been thoroughly studied, revealing a significant knowledge gap in fungal biodiversity and bioactivity (Hawksworth et al. 2017). Contemporary research increasingly acknowledges their broad medicinal potential, including antioxidant, anti-diabetic, hypocholesterolemic, anti-tumor, immunomodulatory, and antimicrobial activities (Barros et al. 2007). However, conventional treatments often face limitations such as high costs, lack of specificity, and adverse side effects due to damage to healthy cells (Ochwang'i et al. 2004). Consequently, mushrooms remain a valuable source for discovering novel therapeutic agents, prompting extensive phytochemical investigations linked to their ethnomycological uses (Lindequist et al. 2010). However, many species remain underexplored pharmacognostically, particularly in specific locales such as the Philippines.

Phytochemicals, the bioactive compounds abundant in mushrooms, represent a prolific reservoir of potential natural therapeutics. The growing concerns over side effects and microbial resistance associated with synthetic drugs have intensified the search for alternative natural antibiotics and antioxidants (Wang et al. 2022; Aljubiri et al. 2021). Notably, many mushroom fruiting bodies exhibit significant antioxidant properties by scavenging harmful free radicals, underscoring their relevance in combating oxidative stress.

Ganoderma applanatum (Pers.) Pat., a basidiomycete widely distributed and known for its thick, corky fruiting bodies, is traditionally used medicinally but lacks comprehensive phytochemical and bioactivity profiling in the Philippine context (Cör et al. 2018; Suansia and John 2021). While Ganoderma species have demonstrated anticancer, anti-inflammatory, and antioxidant activities globally (Barbieri et al. 2017), detailed studies on *G. applanatum's* bioactive compounds and their functional properties remain limited.

In the Philippines, a diverse array of mushroom species, including *G. applanatum*, is traditionally utilized in ethnomedicine for treating ailments like wounds, infections, and inflammation, reflecting rich indigenous knowledge systems. Ganoderma is a dark reddish-brown basidiomycete that belongs to the Ganodermaceae family, Aphyllophorales order, and Hymenomycetes class, that has a distinctive spore-bearing fruiting bodies and stalks. Numerous species of Ganoderma are found worldwide, and they usually grow as facultative or saprophyte parasites on the living and decomposing wood of deciduous trees (Cör et al. 2018). Higher fungi are defined as those whose carpophores are visible to the unaided eye, such as Ganoderma species. They have important ecological and economic implications (Hapuarachchi et al. 2018). Ganoderma species are not considered edible mushrooms since their fruiting bodies are always thick, corky, and gritty. These species are renowned worldwide for being an incredibly therapeutic mushrooms, even if they cannot be eaten fresh (Priyanka et al. 2024). The therapeutic characteristics of several species within the genus are the subject of extensive investigation. The pharmaceutical sector has recently shown interest in Ganoderma species' many conventional medicinal uses (Loyd et al. 2018). A number of research have shown that the genus Ganoderma has anticancer, anti-inflammatory, immune-regulating, anti-oxidation, antiviral, anti-hyperglycemic, and anti-hyperlipidemic qualities (Wang et al. 2020).

Despite this general knowledge, there remains a specific gap regarding the phytochemical composition and bioactivity of *G. applanatum* from the Philippine region. To address this, the present study aims to (i) conduct qualitative phytochemical screening to identify key secondary metabolites; (ii) quantify Total Phenolic and Flavonoid Contents (TPC and TFC); (iii) characterize bioactive compounds through GC-MS analysis; (iv) evaluate antioxidant capacity using the DPPH radical scavenging assay; and (v) assess cytotoxicity via the Brine Shrimp Lethality Test (BSLT). These targeted methods will provide a foundational pharmacognostic profile of *G. applanatum,* supporting its traditional medicinal claims and guiding future biomedical research.

MATERIALS AND METHODS

Study area

Tangub City, located in the tropical province of Misamis Occidental, Philippines, at the coordinates 8.07 N and 123.75 E, has a diverse topography, according to the city government's 2012 report. Approximately 40% of the land is flat along the Panguil Bay coastline, while 6% is rolling and hilly terrain that gradually becomes more mountainous towards the Mt. Malindang National Forest Reservation area. Barangay Matugnaw serves as the sampling location because of the abundance of *G. applanatum* in the area.

Collection and extract preparation

Fruiting bodies of *G. applanatum* were sampled from a single accession site in Barangay Matugnaw, Tangub City, Misamis Occidental, Philippines (coordinates 8.07°N, 123.75°E; elevation ~50 m), harvesting 200 g of fresh material from multiple fruiting bodies on decaying hardwood hosts. No additional samples from other locations were used in this study, limiting genetic diversity representation. Photographs and documentation were submitted to Dr. Jennifer M. Niem, PhD (Curator, Mycological Herbarium, University of the Philippines Los Baños), confirming taxonomic identity as *G. applanatum*. Samples were air-dried at room temperature (25-30°C) for 2-3 weeks until constant weight, chopped into small fragments (~1 cm), and pulverized to fine powder (<1 mm) using an electric grinder. Extraction involved macerating 200 g powder in 500 mL absolute ethanol (95-99.9%) with constant agitation (orbital shaker, 150 rpm) for 7 days at room temperature. The mixture was filtered through Whatman No. 1 filter paper, and the filtrate concentrated via rotary evaporation to semisolid residue. The ethanolic extract was stored in airtight amber glass containers at 4°C for subsequent phytochemical and bioactivity assays.

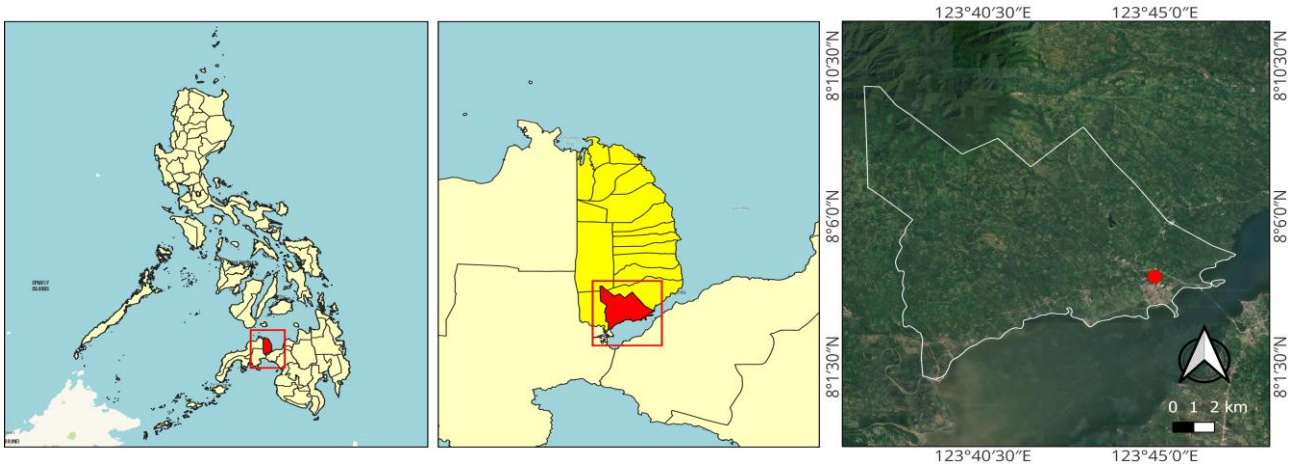

Figure 1. Geographical location of the sampling site. The map of the Philippines is in the upper left portion, the map Barangay Matugnaw, Tangub City, Misamis Occidental is inset in the right portion

Phytochemical screening analysis

The qualitative evaluation of *G. applanatum* ethanolic extract for phytoconstituents, including alkaloids, saponins, flavonoids, tannins, cyanogenic glycosides, steroids, and anthraquinones, was conducted following the standardized procedures of the Department of Chemistry at Mindanao State University-Iligan Institute of Technology (MSU-IIT), Philippines. The samples were sent to the MSU-IIT Chemistry laboratory for analysis, where the presence of these compounds was assessed and recorded using a 3-point scale (+ for turbid, ++ for moderate, and +++ for heavy), as outlined in the Handbook of Philippine Medicinal Plants (Peña et al. 2019).

Determination of total flavonoids and total phenolics

The total flavonoid content of the ethanolic mushroom extract was determined using a modified aluminum chloride colorimetric assay based on Kumari et al. (2011). Briefly, 10 mg of the extract was dissolved in methanol, and 10 µL of this solution was mixed with 100 µL of 1% aluminum nitrate and 100 µL of 1 M aqueous sodium acetate in a test tube. The mixture was incubated at room temperature for 40 minutes to allow the reaction to proceed. Absorbance was then measured at 545 nm using a spectrophotometer. Quercetin served as the standard, and flavonoid content was expressed as milligrams of quercetin equivalents per gram of dry weight (mg QE g^{-1} dw), providing an accurate quantification of total flavonoids in the extract.

Total phenolic content was assessed using the Folin-Ciocalteu assay following the procedure described by Gan et al. (2013). In this method, 1 mL of the ethanolic extract was combined with 1 mL of Folin-Ciocalteu's reagent diluted 1:9 with distilled water. After a 5-minute reaction period, 1 mL of 10% sodium carbonate solution was added, and the volume was adjusted to 10 mL with distilled water. The mixture was incubated in the dark for 90 minutes to develop color fully, and absorbance was recorded at 663 nm. A gallic acid calibration curve was used to quantify phenolic content, expressed as milligrams of gallic acid equivalents per gram of dry weight (mg GAE g^{-1} dw), ensuring a reliable estimation of total phenolics in the sample.

Gas Chromatography-Mass Spectrometry (GC-MS) analysis

The GC-MS analysis of the sample was conducted to identify the compounds present in the ethanolic extracts of *G. applanatum*. The compounds were identified by directly comparing the mass spectrum of the analyte at a particular retention time to that of a reference standard found in the National Institute of Standards and Technology (NIST) library where at least 80% similarity index was considered significant. The mushroom ethanolic extracts was qualitatively analyzed at the Chemistry Analytical and Research Laboratory of the Ateneo de Davao University in Davao City.

Network analysis

The network analysis in this study was carried out using the Gephi software (0.10.1 202301172018 released under the dual license CDDL and GNU General Public Version 3, (©2008-2023). Data is imported as edges table, separator as comma, and graph type as directed. For statistical description, network overview, node overview, edge overview, and community detection were run using Gephi software. Gephi analyzed every dataset separately. This is because each dataset has a distinct community and network graph (Aziz 2022). In this study, the bioactive compounds and biological functions are nodes, and the interconnection between are the edges. The layout of the resulting graph was changed using the Fruchterman Reingold. The Gephi software ran network centrality and modularity. The resulting network was analyzed for *G. applanatum* bioactive compounds identified and its biological functions through the visualized outcomes of the network analysis.

Free-radical scavenging activity (DPPH)

The antioxidant activity of *G. applanatum* extracts was evaluated by their capacity to scavenge the stable free radical 1,1-diphenyl-2-picrylhydrazyl (DPPH). This assay was adapted with modifications from Jacinto et al. (2011). Various concentrations of the extract (1,000, 500, 300, 200, 100, 50, 30, 20, and 10 µg/mL) were each mixed with 3 mL of 0.1 mM ethanolic DPPH solution. The mixtures were vortexed vigorously for 10 seconds and then incubated in the dark at room temperature for one hour. Absorbance was measured at 517 nm, with ethanol-DPPH solution serving as the control. Each sample was tested in triplicate. The degree of radical scavenging was determined by the reduction in DPPH absorbance, calculated as the percentage inhibition using the formula: % Inhibition = [(Acontrol - Asample) / Acontrol] × 100. A control refers to the absorbance values of the control, while A sample indicates the absorbance values of the sample. The antioxidant potency was expressed as the IC50 value, representing the extract concentration required to inhibit 50% of the DPPH radicals.

Cytotoxicity Test using Brine Shrimp Lethality Test

MSU-IIT Chemistry Department assessed the lethality effects on brine shrimp (*Artemia salina* (Linnaeus, 1758)) to evaluate the potential bioactivity of the ethanolic extracts. Various concentrations of the extract (1, 10, 100, 500, 1000, and 10000 µg/mL) were prepared in triplicate, and each replicate were exposed to ten (10) brine shrimp nauplii. After 24 hours of exposure, the number of deceased nauplii were recorded, and the percentage of mortality were calculated. To determine the cytotoxicity level of the extract, the LC50 were computed based on the rating of (Alhadi et al. 2015).

RESULTS AND DISCUSSION

Numerous secondary metabolites were found in the current study's *G. applanatum* ethanolic extracts after phytochemical profiling with ethanol as the solvent. Based on the phytochemical components of *G. applanatum's* secondary metabolites, high concentrations of antioxidant chemicals, including flavonoids and saponins, were found in the extracts. Furthermore, there were trace levels of

alkaloids, steroids, and tannins but none of anthraquinone and cyanogenic glycosides (Table 1). Other researchers (Poojah et al. 2014; Wood et al. 2021; Ihayere and Okhuoya 2022) have also reported the existence of these chemicals in members of the Ganodermataceae family, with a few minor deviations. The current findings are consistent with their findings. These phytochemicals are thought to be responsible of the therapeutic and medicinal qualities of mushrooms.

The ethanolic extract of *G. applanatum* was examined for its major components, specifically phenolics and flavonoids (Table 2). The total phenolic content was calculated at 41.34 ± 0.26 µg/g, which is comparable to the standard reference of gallic acid. As well, the total flavonoid content was measured at 4.87 ± 0.11 mg/g, expressed as quercetin equivalents. These results highlight the substantial presence of bioactive compounds in *G. applanatum,* which may aid to its potential health benefits and therapeutic applications. The quantification of these phytochemicals is fundamental, as both phenolics and flavonoids are recognized for their antioxidant properties and may hold a function in the mushroom's anticancer effects. Further research is necessary to discover the exact mechanisms beyond which these compounds exert their biological activities.

Flavonoids were one of the phytochemical substances that Mohammadifar et al. (2020) found to be relatively abundant in the ethanolic extracts of *Ganoderma lucidum* (Curtis) P.Karst. and *G. applanatum*. This finding is consistent with a prior examination that contrasted *G. lucidum* with *G. applanatum*. Flavonoids have been connected to a wide range of pharmacological and therapeutic actions, such as antiviral, antibacterial, anticancer, and antioxidant properties (Ullah et al. 2020). The biochemical and antioxidant characteristics of flavonoids have been connected to a number of diseases, including atherosclerosis, cancer, and Alzheimer's Disease (AD) (Lee et al. 2009). Furthermore, the capacity of these flavonoids to scavenge and block potentially harmful free radicals has drawn the most significant attention to their antioxidant activity. This prevents oxidative stress and lowers the incidence of heart attacks and cardiovascular disease (Sharma et al. 2021). Future research into the pharmacological characteristics of flavonoids might lay the groundwork for the development of powerful medications.

One of the secondary metabolites found in *G. applanatum* extract is saponin, and it has a wide variety of antioxidant properties. According to Lee et al. (2012), mushroom glycosides, such as saponins, have pharmacological properties that include antiviral, anti-inflammatory, and anticarcinogenic activities. Saponins have anticancer characteristics that include inhibiting tumor development, metastasis, and angiogenesis, as well as reversing multidrug resistance (MDR). These include bile acid binding, immune modulatory effects, apoptosis induction, cell differentiation promotion, and suppression of carcinogen-induced cell proliferation (Xu 2016). In addition to their variety of biological activities, saponins have been shown to have anti-diabetic qualities (El Barky et al. 2017). Because of this, saponins are now regarded as promising compounds that may be used to create novel anti-diabetes drugs.

Steroids are present in *G. applanatum* extracts, which is noteworthy as they have been shown to alleviate inflammation, cure a number of autoimmune diseases, and produce anesthesia. Because of their wide range of effects on several physiological systems, steroids are being employed in current anesthetic therapy (Shaikh et al. 2012). This diversity of effects may account for the remarkable efficacy of steroids in treating chronic inflammatory disorders, such as rheumatoid arthritis and asthma (Barnes 2006).

Alkaloids were also detected as a phytochemical compound (Kaur and Arora 2015). Alkaloids are necessary for maintaining human health as well as an organism's natural defenses. Numerous pharmacological actions are exhibited by alkaloids, such as anticancer, hypnotic, psychotropic, antibacterial, antimitotic, antimalarial, anti-inflammatory, and antiviral properties (Hossen et al. 2022). Well-known alkaloids, including morphine, narceine, strychnine, quinine, ephedrine, and nicotine, are used in therapeutic settings. Two analgesic alkaloids are morphine and narceine; narceine is used to alleviate congestion, whereas morphine may be used to ease pain (Tadeusz 2007).

Tannins can increase glucose absorption and decrease adipogenesis by boosting the pathological oxidative state of a diabetic condition, making them suitable therapeutic agents for the management of Non-Insulin-Dependent Diabetes Mellitus (NIDDM) (Kumari and Jain 2012). Because of their astringent qualities, tannins are useful in medicine for treating frostbite, minor burns, varicose ulcers, hemorrhoids, and gum irritation. They also encourage the formation of new tissues and the quick healing of injuries and inflammatory mucosa (Soliman and Barreda 2022).

Table 1. Phytoconstituents profile of the ethanolic extracts of *Ganoderma applanatum*

Phytoconstituents	Interference
Flavonoids	+++
Steroids	++
Saponins	+++
Tannins	+
Alkaloids	++
Cyanogenic glycosides	-
Anthraquinone	-

Note: "+" indicates present: "+" turbid, "++" moderate, "+++" heavy; "-'' indicates absent

Table 2. The total flavonoids content and total phenolic content of the ethanolic of *Ganoderma applanatum*

Sample	Total flavonoids content (mg Quercetin per gram of extract)	Total phenolic content (µg Gallic acid per gram extract)
Ganoderma applanatum	4.87 ± 0.11	41.34 ± 0.26

A phytochemical study of many wild mushroom extracts obtained from different solvents produced the following findings. Phytochemical screening of *G. applanatum* in related research by Manasseh et al. (2012) showed the presence of steroids, flavonoids, heart glycosides, and saponins, but no measurable amounts of anthraquinone, alkaloids, or tannins in the aqueous extracts of the mushroom. Using methanol as the extraction solvent, Islam et al. (2015) investigated the secondary metabolite components of *G. lucidum* mushrooms. The fruiting body extracts included eleven different phytochemicals, with polyphenols, flavonoids, tannins, coumarins, vitamin C, and anthocyanins present in more significant amounts. *Ganoderma lucidum* samples were subjected to phytochemical examination, which identified the presence of triterpenoids, glycosides, carbohydrates, and phenolic compounds. Methanol and methanol ethyl acetate separate the most active ingredients with the least amount of polarity, as Modi et al. (2014) showed.

Depending on the extraction solvent, the secondary metabolites of mushrooms with bioactive characteristics might have different characteristics. Different solvents can extract different phytochemical constituents based on how polar or soluble they are in the solvent (Nagaraj et al. 2013). These findings were corroborated by a study conducted by Thapa et al. (2022), in which the phytochemical constituents of various extracts in various solvents yielded varying results. The affinity between the polarity of the solvent and the compounds utilized has a significant effect on the extraction procedure, which explains why the ethanolic extracts of *G. lucidum* have a low overall yield. The nature of the compounds to be isolated determines the appropriate solvent.

Numerous phytochemicals, including flavonoids, saponins, alkaloids, steroids, and tannins, are present in the ethanolic extracts of *G. applanatum* and may be separated and examined for a range of biological activities. These secondary metabolites are physiologically active and play significant roles in the bioactivity of medicinal mushrooms because of their medicinal properties. These phytochemicals affect the human body in a unique and particular way. Researchers are becoming increasingly interested in isolating and determining the structures of phytochemicals because they can be used to create new pharmaceuticals that are less harmful to humans and have fewer side effects, in addition to being used to treat various diseases directly. The antioxidant, antimicrobial, anti-inflammatory, and anticancer properties of *G. applanatum* in relation to their reported ethnomycological and therapeutic applications are supported by the results of the phytochemical screening of the extract in the present study.

Gas Chromatography-Mass Spectrometry analysis

Gas Chromatography-Mass Spectrometry (GC-MS) was chosen over Liquid Chromatography-Mass Spectrometry (LC-MS) for evaluating the ethanolic extract attributable to its suitability for volatile, non-polar, and thermally stable compounds usually found in fungal extracts. Unlike LC-MS, which focuses in separating polar or larger biomolecules (e.g., proteins or hydrophilic metabolites) in aqueous matrices, GC-MS provides exceptional resolution for small-molecule volatiles over vaporization and capillary column separation, allowing precise identification of bioactive components like terpenes and fatty acids. This selection supports with the extract's composition, as demonstrated by the 26 identified compounds, enhancing sensitivity and efficacy for qualitative profiling without the need for extensive sample preparation typical in LC-MS workflows. Table 3 showed the bioactive compounds that were identified qualitatively through GC-MS analysis; it includes its molecular weight, Similarity Index, Retention time, molecular formula, and the reported biological activities from the ethanolic extracts of *G. applanatum.*

The 9-Octadecane, Phenol, 3,5-bis(1,1-dimethylethyl), Butric acid, 4-tridecyl ester, Heneicosane, 7,9-di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione, Ethyl Oleate, Octadenoic Acid, and Ethyl Ester were the most common compounds that possessed antimicrobial properties. Generally, other identified biological activities found in the mushroom extract were anesthetic agent, antifungal, antibacterial, antioxidant, anti-cancer, insecticidal role, anti-inflammatory, analgesic effects, hemolytic, hypocholesterolemic, flavor, nematicide, anti-androgenic, antifouling, anti-neuroinflammatory, antiasthma, and diuretic properties, and an anti-HIV compound used to prevent the HIV virus. However, the identified compounds with no reported bioactivity included 1s,4R,7R11R-1,3,4,7-Tetramethyltricyclo, 1H-Pyrrolo[2,3-f] quinolin-9-ol,1,2,3,5,7-pentamethyl, Hexadecanoic acid, ethyl ester, 1-Norvaline, N-(2-methoxyethoxycarbonyl)-hexyl ester, Linoleic acid ethyl ester, 2H-1-benzopyran,33,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-(2.alpha,4a.alpha, 8a.alpha.) and 9,19-Cyclolanostan-3-ol, 24-methylene-,(3-beta) as shown in the table below.

Ganoderma applanatum's therapeutic effects are derived from a variety of bioactive components such as fatty acids, hydrocarbons, phenolic compounds, terpenoids, triterpenoids, polysaccharides, and steroids, found in their fruiting body, spores, and mycelium. These substances have been linked to the mushroom's pharmacological qualities (Cör et al. 2018). In addition, considering the proved ethnomycological and therapeutic applications of *G. applanatum,* the findings from the GC-MS analysis of the extract in this study supported the mushroom's antioxidant, antibacterial, anti-inflammatory, and anticancer properties.

One of the substances found in this study, ergosterol, is the most common sterol found in the cell membranes of fungi, including edible mushrooms. Like cholesterol in mammal cell membranes, it maintains the integrity of fungal cell membranes. The composition and operation of cell plasma membranes depend on steroids. Ergosterol has been shown in earlier studies to have antioxidant properties that protect yeast from the free radicals produced by tert-butyl hydroperoxide (Dupont et al. 2021). Furthermore, from the lipid-enriched fraction of *G. lucidum*, fourteen ergosterol derivatives were identified and purified. These substances showed anti-angiogenesis and anti-tumor effects on HepG2, MDA-MB231, and HUVEC cells. None of the chemicals had any effect on normal cells (Chen et al. 2017).

Table 3. Bioactive compounds qualitatively isolated from the ethanolic extracts of *G. applanatum* through GC-MS analysis

Compound name	Mol. formula	SI[a]	Mol. Wt.[b]	Retention time (min)	Biological properties with reference
Cyclopropane, nonyl-	$C_{12}H_{24}$	95	168	5.660	Antiproliferative (Al-Mansoub et al. 2021, Mostofa et al. 2021); Antioxidant (Al-Mansoub et al. 2021); Cytotoxic (Mostofa et al. 2021), Anethetic agent (Mary and Giri 2017); Antibacterial (Okwu and Ighodaro 2009);
Hexadecane	$C_{16}H_{34}$	93	226	6.190	Antibacterial (Hsouna et al. 2011; Yogeswari et al. 2012; Arora et al. 2017; Nyalo et al. 2023); Antioxidant (Hsouna et al. 2011; Aziz et al. 2022; Chen et al. 2022); Enzyme inhibition (Aziz et al. 2022); Antimicrobial (Khan et al. 2016; Teoh et al. 2021); Anticancer (Khan et al. 2016; Arora et al. 2017; Kim et al. 2020; Teoh et al. 2021); Antifungal (Hsouna et al. 2011; Arora et al. 2017)
9-Octadecene, €-	$C_{18}H_{36}$	95	252	6.995	Antioxidant (Matthew et al. 2021; Raslan et al. 2022); (Lee et al. 2007; Mishra and Shree 2007); Cytotoxic (Raslan et al. 2022); Antibacterial (Rouis-Soussi et al. 2014; Karanja et al. 2021); Anticancer and Antimicrobial; (Lee et al. 2007; Mishra and Shree 2007)
1s,4R,7R,11R-1,3,4,7-Tetramethyltricyclo[5.3.1.0(4,11)]undec-2-en-8-one	$C_{15}H_{22}0$	77	218	7.795	Antioxidant (Chen et al. 2022); Antimicrobial (Eltayeib and Ismaeel 2014)
Phenol, 3,5-bis (1,1-dimethylethyl)-	$C_{14}H_{22}O$	91	206	8.050	Antibacterial (Lalthanpuii and Lalchhandama 2019; Addai et al. 2022; Nyalo et al. 2023); Antimicrobial (Dhanya et al. 2016; Das et al. 2018; Teoh et al. 2021; Trivedi and Thumar 2021; Vega-Portalatino et al. 2023); Antioxidant (Kim et al. 2020; Addai et al. 2022; Chen et al. 2022; Raslan et al. 2022); Cytotoxic (Al-Shwyeh Hussah Abdullah et al. 2011; Mostofa et al. 2021; Raslan et al. 2022); Anti-diabetic (George et al. 2018); Anticancer (Mostofa et al. 2021; Teoh et al. 2021); Antiparasitic (Lalthanpuii and Lalchhandama 2019)
Butyric acid, 4-tridecyl ester	$C_{17}H_{34}O_2$	87	270	8.880	Antimicrobial and Antifungal (Suerbaev et al. 2014)
Heneicosane	$C_{21}H_{44}$	91	296	10.185	Antibacterial (Nyalo et al. 2023; Aljubiri et al. 2021); (Vanitha et al. 2020); Antioxidant (Aziz et al. 2022); Enzyme inhibition (Aziz et al. 2022); Antimicrobial (Kawuri and Daramaysa 2019; Abu ElKhair et al. 2020; Ghavam et al. 2021; Trivedi and Thumar 2021); Antifungal (Teoh et al. 2021); Anticancer (Teoh et al. 2021); Cytotoxicity (Aljubiri et al. 2021); Anti-inflammatory and Analgesic (Okechukwu 2020); Anti-diabetic (George et al. 2018); Insecticidal role (Seenivasagan et al. 2009; Bhutia et al. 2010)
1H-Pyrrolo[2,3-f]quinolin-9-ol, 1,2,3,5,7pentamethyl-	$C_{16}H_{18}N_2O$	66	254	11.010	No known activity
Docosanoic acid, ethyl ester	$C_{24}H_{48}O_2$	88	368	11.405	Antioxidant and Antimicrobial (Almalki et al. 2022); Hemolytic, Hypocholesterolemic, Flavor, Nematicide, Anti-androgenic (Mohan et al. 2012)
Bicyclo[4.1.0]hepta-2,4-diene, 2,3,4,5-tetraethyl-7,7-diphenyl-	$C_{27}H_{32}$	56	356	12.340	Antioxidant and Insecticidal effect (Al-Harbi et al. 2021)
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	$C_{16}H_{22}O_4$	95	278	12.415	Antimicrobial (Kandagalla and Krishnappa 2019); Cytotoxic activity (Kandagalla and Krishnappa 2019); Antifouling (Kandagalla and Krishnappa 2019); Anticancer activity activity (Shobi and Viswanathan 2018); Antibacterial (Sivakumar et al. 2014; Arora et al. 2017; Shobi and Viswanathan 2018); (Sivakumar et al. 2014)
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	$C_{17}H_{24}O_3$	89	276	13.115	Antioxidant (Arora et al. 2017; Kim et al. 2020; Aziz et al. 2022); Enzyme inhibition (Aziz et al. 2022); Anticancer (Teoh et al. 2021); Antimicrobial (Arora et al. 2017; Teoh et al. 2021); Antibacterial (Arora et al. 2017)
Dibutyl phthalate	$C_{16}H_{22}O_4$	96	278	13.825	Antimicrobial (Jemimma et al. 2017; Beulah et al. 2018; Das et al. 2018; Trivedi and Thumar 2021; Almalki et al. 2022); Antioxidant (Arora et al. 2017; Almalki et al. 2022); Antibacterial (Arora et al. 2017; Kandagalla and Krishnappa 2019); Cytotoxicity (Kandagalla and

					Krishnappa 2019); Antifouling (Beulah et al. 2018); Antifungal (Arora et al. 2017)
Hexadecanoic acid, ethyl ester	$C_{18}H_{36}O_2$	94	284	14.340	Antibacterial (Okwu and Ighodaro 2009; Arora et al. 2017; Lalthanpuii and Lalchhandama 2019; Diep et al. 2021; Addai et al. 2022; Nyalo et al. 2023); Antioxidant (Rouis-Soussi et al. 2014; Khan et al. 2016; Arora et al. 2017; Kim et al. 2020; No and Kampus 2020; Al-Mansoub et al. 2021; Addai et al. 2022; Almalki et al. 2022); Antimicrobial (Jemimma et al. 2017; Prayitno et al. 2021); Antiproliferative (Al-Mansoub et al. 2012); Hypocholesterolemic (Chukwu et al. 2020; Prayitno et al. 2021); Antifungal (Rouis-Soussi et al. 2014; Khan et al. 2016; Arora et al. 2017); Antiparasitic (Lalthanpuii and Lalchhandama 2019); Anti-diabetic (George et al. 2018)
1-Norvaline, N-(2-methoxyethoxycarbonyl1)-, hexyl ester	$C_{15}H_{29}NO_5$	79	303	16.475	No known activity
Linoleic acid ethyl ester	$C_{20}H_{36}O_2$	93	308	16.870	Antibacterial (Hasan et al. 2014; Nyalo et al. 2023); Antimicrobial (Khan et al. 2016; Prayitno et al. 2021); Hypocholesterolemic (Prayitno et al. 2021); Antioxidant (Al-Mansoub et al. 2012; Kim et al. 2020); Antiproliferative (Al-Mansoub et al. 2012)
Ethyl Oleate	$C_{20}H_{38}O_2$	93	310	16.970	Antibacterial (Diep et al. 2021); Antioxidant (Kim et al. 2020); Antimicrobial (Akin-Osanaiye et al. 2011; Jemimma et al. 2017)
Octadecanoic acid, ethyl ester	$C_{20}H_{40}O_2$	92	312	17.360	Antimicrobial (Teoh et al. 2021; Almalki et al. 2022); (Abubakar and Majinda 2016); Anticancer (Teoh et al. 2021); Antioxidant (Almalki et al. 2022; Kim et al. 2020; Arora et al. 2017) Antiparasitic (Lalthanpuii and Lalchhandama 2019); Antibacterial (Okwu and Ighodaro 2009; Arora et al. 2017; Lalthanpuii and Lalchhandama 2019); Antifungal (Arora et al. 2017); Anti-diabetic (George et al. 2018)
Tributyl acetylcitrate	$C_{20}H_{34}O_8$	92	402	18.120	Antibacterial (Nyalo et al. 2023); Antimicrobial (Abu ElKhair et al. 2020); Antifungal (Peña et al. 2019; Hadi and Hussein 2016)
Ergosterol	$C_{28}H_{44}O$	91	396	19.120	Antibacterial (Kandagalla and Krishnappa 2019); Cytotoxicity (Kandagalla and Krishnappa 2019); Anti-neuro inflammatory (Kushairi et al. 2020); Anticancer (Hapuarachchi et al. 2017; Ikarashi et al. 2020)
2H-1-Benzopyran, 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-(2.alpha.,4a.alpha.,8a.alpha.)-	$C_{13}H_{22}O$	61	194	19.430	Antimicrobial (Balla et al. 2017; Sharma et al. 2021)
9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-	$C_{31}H_{52}O$	88	440	19.965	Antifungal (Khan et al. 2016; Sobhy et al. 2023); Antibacterial (Hasan et al. 2014; Aljubiri et al. 2021); Cytotoxicity (Aljubiri et al. 2021); Antioxidant (Khan et al. 2016; No and Kampus 2020); Anti-HIV compound used to prevent the HIV virus (Arora et al. 2017) Antimicrobial (Khan et al. 2016); Antilisterial activity (Penduka et al. 2014)
Ergosta-7,22-dien-3-ol, (3.beta.,5.alpha.,22E)-	$C_{28}H_{46}O$	91	398	20.290	Antibacterial (Zhu et al. 2017; Kandagalla and Krishnappa 2019). Cytotoxicity (Hapuarachchi et al. 2017; Kandagalla and Krishnappa 2019; Settu and Arunachalam 2020); Anti-hypercholesterolemia (Chukwu et al. 2020); Anti-fungal (Zhu et al. 2017).
9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)-	$C_{31}H_{52}O$	91	440	21.210	Antifungal (Sobhy et al. 2023); Antibacterial (Hasan et al. 2014; Aljubiri et al. 2021); Cytotoxicity (Aljubiri et al. 2021); Antioxidant (Khan et al. 2016; No and Kampus 2020); Antimicrobial (Khan et al. 2016); Antilisterial activity (Penduka et al. 2014)
Ethyl iso-allocholate	$C_{26}H_{44}O_5$	79	436	21.650	Antioxidant (Addai et al. 2022; Almalki et al. 2022); Antimicrobial (Halliwell 1995; Huang et al. 2005; Addai et al. 2022; Almalki et al. 2022); Anti-inflammatory, anticancer, antimicrobial, antiasthma and diuretic properties (Halliwell 1995; Huang et al. 2005)
7,22-Ergostadienone	$C_{28}H_{44}O$	77	396	21.650	No known activity

The potential as anticancer agents has been demonstrated by the specific activity of the seven most common bioactive components of *G. lucidum* extract against several human cancer cell types (solid and blood malignancies), Inflammatory Breast Tumors (IBC), and triple-negative breast cancers (TNBC). Three of the seven substances that showed significant in vitro anticancer effects were ergosterol, 5,6-dehydroergosterol, and ergosterol peroxide (Martínez-Montemayor et al. 2019).

Fatty acids not only provide a significant energy source but also have a important biological effect on the specialized functions that cells and tissues play throughout life. The fatty acids found in the ethanolic extract of *G. applanatum* included butyric acid, 4-tridecyl ester, docosanoic acid, hexadecanoic acid, ethyl ester, linoleic acid, ethyl ester, ethyl oleate, and octadecanoic acid. These findings confirmed prior research indicating extensive fatty acid contents in a number of mushroom species, mainly linoleic acid (Günc Ergonül et al. 2013). Extracts from *G. lucidum* spores incorporated a considerable number of fatty acids, such as oleic and palmitic acids, concurrent to Salvatore et al. (2020). Furthermore, Taskin et al. (2013) found that *G. lucidum* samples included hexadecanoic acid, benzenedicarboxylic acid, and octadecanoic acid, with the most prevalent compound being hexadecanoic acid. Various researches on the properties of these fatty acid-rich oils in the remedy of inflammatory, autoimmune, and cancer-related conditions have proved the theory that they employ a varied range of biological properties.

The current analysis has also identified 3,5-bis (1,1-dimethylethyl) phenol as a prospective inhibitor of the polo-like kinases-1 enzyme, with anticancer properties based on Ki and G values (Rizvi et al. 2014). The phenol was prior acquired from a methanolic extract of *Ageratum houstonianum* Mill. leaves. Utilizing three definite solvent fractions prepared in n-hexane (PSHE), diethyl ether (PSDE), and *Persicaria strigosa* (R.Br.) Nakai ethyl acetate (PSEE), Swargiary et al. (2023) also examined the phytochemical constituents, antiproliferative, and apoptosis-inducing properties of *P. strigosa* methanolic extract (PSME). Subsequent to the study, all solvent fractions exhibited dose-dependent mortality of Dalton's lymphoma cells. While PSEE displayed the maximum cytotoxicity, PSHE demonstrated the minimum activity. From the ethyl acetate extract, 12 compounds were extracted for the analysis. Of these, 3,5-bis (1,1-dimethylethyl) phenol proved the highest binding affinity with every protein and drug-like potential in accordance with the Lipinski rule, indicating the compound's potential to treat cancer.

Tributyl acetyl citrate was found in the ethanolic extracts of *G. applanatum* in the present study. The root extract of *Lobularia maritima* (L.) Desv., a perennial plant species significantly exploited in conventional medicine and recognized as sweet alyssum, was observed to inhibit the excessive concentration of this bioactive component. The study found that the ethyl acetate fraction from the roots was the most potent extract, with an EC50 value of 0.08 mg/mL, extensively unique from the EC50 values of the flower, leaf, and stem fractions. The roots of *L. maritima* displayed remarkable antiradical activity against DPPH free radicals in comparison to the BHA standard, implying its intriguing potential as an antioxidant extract in nutritional, medicinal, and biocosmetic formulations (Kouidhi et al. 2021).

The present study has also found heneicosane compound that presented important antibacterial activity against *Streptococcus pneumoniae* and *Aspergillus fumigatus*, showing its promise as a probable antimicrobial or therapeutic agent. Research classified itself by separating and recognizing a bioactive molecule from a natural basis (Vanitha et al. 2020). In a rat model, heneicosane, along with other n-alkanes, showed analgesic influences, theoretically via inflammation suppression (Okechukwu 2020). These compounds promise for producing innovative analgesic or anti-inflammatory medications. The stem extract of *Dendrobium crepidatum* Lindl. & Paxton includes triacontane, a main source of its detected cytotoxic action. The study assessed the cytotoxic impacts on HeLa and U251 cancer cell lines. Results implied that the chloroform extract was most efficient in lowering HeLa cell production, while the hexane extract displayed the effective inhibitory result on U251 cell growth.

The evaluation uncovered a compound known as 2H-1-Benzopyran, 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-(2.alpha.,4a.alpha.,8a.alpha.), which is documented for its effective antimicrobial properties (Sharma et al. 2021; Balla et al. 2017). This compound belongs to the class of benzopyrans, which are distinguished for their varied healing treatments. Benzopyran derivatives are found in several natural products, involving flavonoids and tocopherols. Corresponding to Tiwari and Singh (2023), the benzopyran compound 2H-1-Benzopyran, 3,4,4a,5,6,8a-hexahydro-2,5,5,8a-tetramethyl-(2.alpha.,4a.alpha.,8a.alpha.) is valuable for ailments linked to oxidative stress, such as cardiovascular and cerebral vascular diseases. Reports by Sebille et al. (2005) emphasized the capability of this compound not only in healing oxidative stress-related illnesses but also in pharmaceutical formulations for antiviral and antitumor therapies. The growing attention in novel drugs, predominantly benzopyran compounds, is essential to existing and forthcoming innovations and beneficial approaches, as deliberated in the review “New Insights into the Origin and Therapeutic Implications of Benzopyran and Their Derivatives” (2023).

As well, the compound 23,9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.), for which the Table 1 is represented, has been reported to obtain the following supplementary activities involving antimicrobial activity (Khan et al. 2016); anti-fungal activity (Khan et al. 2016; Sobhy et al. 2023); antioxidant activity (Khan et al. 2016; No and Kampus 2020); anticoccal activity (Hasan et al. 2014; Aljubiri et al. 2021); cytotoxic activity (Aljubiri et al. 2021) and anti listerial activity (Penduka et al. 2014). The work of Ralf and Maria (2003) also highlighted variety of triterpene compounds and their potential exploitation as they show the structural features of some steroid derivatives, including 3-methylene steroid derivatives.

Furthermore, Yulin et al. (2017) noted that the so far described novel drugs of the lanostane type triterpene series have a distinct healing effect on the inhibition of osteoclast cells which possibly will offer the opportunity for new treatment alongside bone illnesses. The study of these compounds sets them in the focus of their attention in biological as well as chemical production.

Moreover, ergosta-7,22-dien-3-ol was found in the assays as a sterol class compound, which is remarkable due to its potential medical applications and bioactive qualities, specifically its anti-inflammatory effects. Isolated from marine species like Marthasterias glacialis, this compound regulates key inflammatory mediators like COX-2 and NF-κB, reducing inflammation pathways (Pereira et al. 2014). Also, Lindequist (2017) showed that ergosta-7,22-dien-3-ol was structurally linked to other ergosterol derivatives found in medicinal mushrooms, which have been confirmed to have a variety of health benefits, with cytotoxic and antimicrobial properties. Present research on the synthesis and biological assessment of ergosterol derivatives, such as ergosta-5,7,22-trien-3-ol, also contributes credence to this idea (Hu et al. 2014; Zhang et al. 2016).

The research revealed that the compound 9,19-Cyclolanostan-3-ol, 24-methylene-, (3.beta.)- is a type of triterpenoid known as lanostane and established for its pointed biological activities, particularly its anti-inflammatory effects. Studies have revealed that lanostane triterpenoids, comprising this specific compound, can influence key inflammatory pathways, making them possible candidates for therapeutic application in situations considered by chronic inflammation, such as rheumatoid arthritis and neurodegenerative diseases (Yang et al. 2020). The structural alterations of lanostanes, particularly the presence of specific functional groups, are helpful in influencing their pharmacological properties, thus enhancing their beneficial efficiency (Dembitsky 2024). Additionally, Sidjui et al. (2017) determined that compounds derived from lanostanes have showed cytotoxic properties against a range of cancer cell lines, implying their possible role in cancer therapy. According to Wal et al. (2011), the varied biological activities of 9,19-Cyclolanostan-3-ol, 24-methylene-, and (3.beta.)- pointed out their importance in medicinal chemistry and drug advancement.

Also, the current study detected Ethyl iso-allocholate, a bioactive compound known for its significant antimicrobial and antifungal properties and found in various plants. Studies have revealed its capability to prevent dihydropteroate synthase, which is a vital target in fighting antibiotic resistance, mostly in *Escherichia coli*, as it shows a vital part in bacterial folate synthesis, hence contributing a new approach to address antibiotic resistance (Malathi et al. 2017). In laboratory experiments, it has been exhibited to successfully hinder several fungi, such as *Curvularia lunata* and *Microsporum canis,* underlining its widespread antifungal activity. Ethyl iso-allocholate has proven to be efficient in the study of (Abubacker and Palaniyappan 2013) against both plant and human pathogenic fungi, indicating to its probable use in herbal antifungal formulations.

The findings of this report suggest that there may be a biochemical substance for the traditional medicinal uses of *G. applanatum*, incorporating the therapy of diseases and its use as an antioxidant and anticancer agent. For further investigation, the above-mentioned bioactive compounds that have not described biological activities ought to be considered and discovered; henceforth, they can boost our knowledge concerning their biological uses and can influence a possible detection of their therapeutic practice in the field of medicine. Moreover, the result of this study should be a basis for further examination concerning its biological make up and usage.

The figure above represents the interconnectedness of the scientific properties of the 27 distinct bioactive compounds identified in the ethanolic extracts of *G. applanatum*. Gephi analysis showed that a total of 53 nodes corresponding to biological features or individual bioactive molecules, and 128 edges displaying the relationship among each bioactive molecule and the associated biological feature were identified (Figure 2). The mean degree, m, that represents the average connectivity of the network is estimated as 2.24, using the statistical properties of the network. Degree is the total number of interactions a node has. This value indicates that, on average, each node within the network is linked to two other nodes. As per Figure 2 the prominent therapeutic role in the network are antimicrobial properties, which are followed by antibacterial and antioxidant. In this network, core node has a prominent centrality, indicating its rich information across multiple bioactive compounds. Figure 3 showed the mass peak of the bioactive compounds against its retention time

DPPH radical scavenging method

This study employs the DPPH radical scavenging method, which is a valuable assay for predicting antioxidant activity by inhibiting lipid oxidation. DPPH radical scavenging determines the amount of free radicals that have been scavenged. Table 4 displays the outcome of *G. applanatum* ethanolic extracts' inhibition of the DPPH radical's scavenging activity. In the current investigation, the scavenging abilities of the extracts were concentration-dependent; their activity increased as the concentration increased.

Table 4. DPPH radical scavenging activity of the ethanolic extracts of *Ganoderma applanatum*

Ganoderma applanatum	
Concentration (µg/mL)	**%Inhibition**
10	12.43
20	22.09
30	23.87
50	35.11
100	53.35
200	83.63
300	94.77
500	95.07
1000	94.67
IC50	97 µg/mL

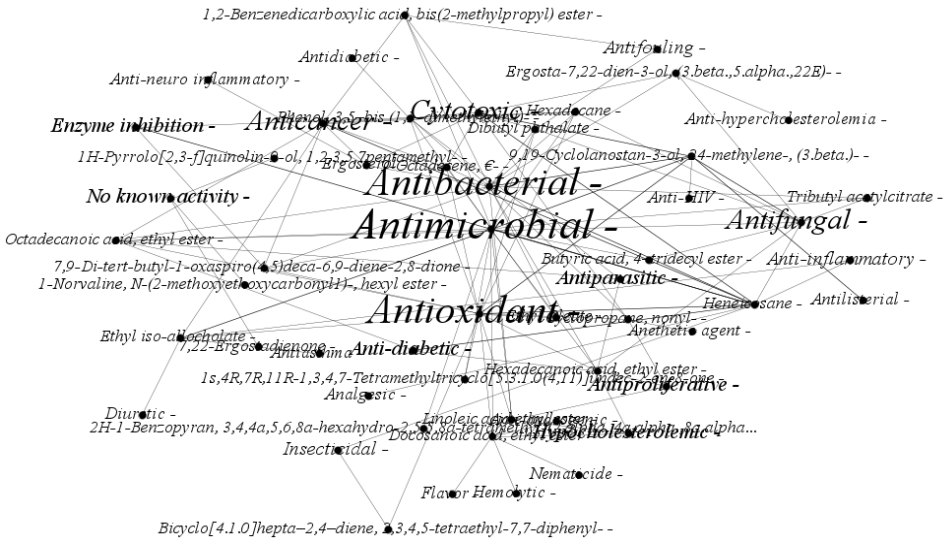

Figure 2. Network analysis of the relationship of the ethnopharmacological properties of *G. applanatum* and the bioactive compounds detected

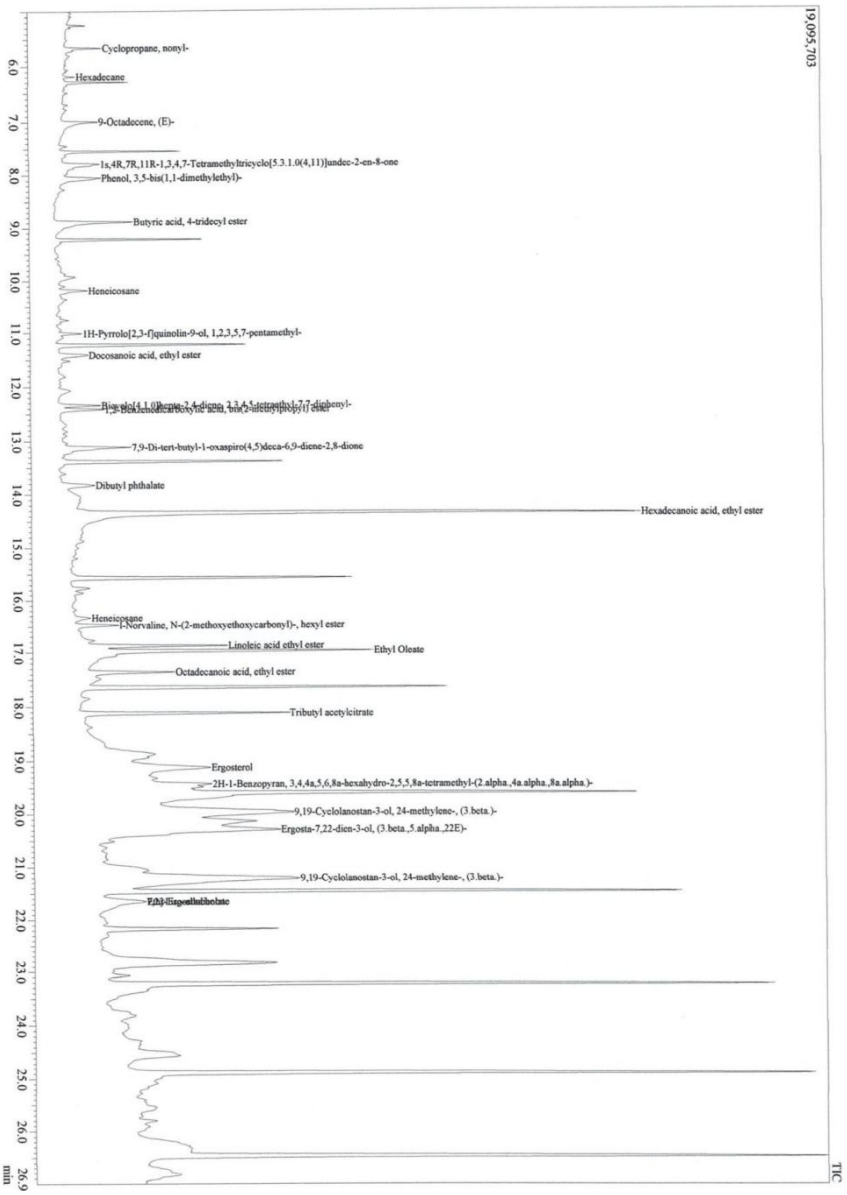

Figure 3. The mass peak of the bioactive compounds against its retention time

The phytochemical screening revealed that *G. applanatum* has secondary metabolites, including flavonoids, steroids, saponins, tannins, and alkaloids, according to the findings of this study. It is believed that these compounds are responsible for the antioxidant activity of mushrooms. Several bioactive components, each with specific biological effects, are responsible for the antioxidant properties of the mushroom extract used (Wasser 2010). Various ganoderma extracts have reportedly demonstrated scavenging properties. Four ganoderma species collected from diverse Indian forests exhibited DPPH free radical scavenging capacities ranging from 91.64 to 95.51 percent. *Ganoderma tsugae* Murrill (95.51%) and *Ganoderma* sp. (94.43%) exhibited the highest levels of radical scavenging activity at IC50 values of 12 and 10 mg/mL, respectively (Rajoriya et al. 2015). According to Bristy et al. (2022), *G. lucidum* extracts had the greatest scavenging potential, with 93% scavenging activity at a concentration of 500 g/mL and the lowest IC50 value of 40 g/mL among the mushroom extracts studied. Variations in scavenging activity may be attributable to geographical origin, environmental growing conditions, and extraction solvent type.

Nagaraj et al. (2014) discovered that extracting *G. applanatum* with various solvents such as methanol, chloroform, and petroleum ether resulted in varying levels of free radical scavenging efficiency. The methanol extract had the highest amount of free radical scavenging activity and the lowest IC50 values when compared to the other solvent extracts, suggesting stronger free radical scavenging activity. This study found that the geographic location of the species affects *G. applanatum*'s capacity to scavenge free radicals even when employing ethanol extracts. Popovych et al. (2019) confirmed that optimal moisture and temperature conditions increased the production of mushroom fruiting bodies. There are numerous options for antioxidant analysis, one of which is the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) test, which uses the IC50 value to determine the concentration of extracts required to inhibit 50% of radicals.

At a concentration of 500 g/mL, the antioxidant activity of the ethanolic extract inhibited DPPH by as much as 95.07%. According to Peña et al. (2019), compounds with 80% inhibition are regarded as potent antioxidants, moderate antioxidants with 50-80% inhibition, and weak antioxidants with less than 50% inhibition. The half-maximal inhibitory concentration measures the ability of a substance to interfere with biological function. A sample with an IC50 value of less than 50 ppm is considered to be a powerful antioxidant, 50-100 ppm as a strong antioxidant, 101-150 ppm as a medium antioxidant, and greater than 150 ppm as a feeble antioxidant (Abdulaziz et al. 2019). Olugbami et al. (2014) found that the lower the IC50 value, the greater the antioxidant activity of the extracts, and the more effective a substance is at scavenging DPPH. The fact that the extract achieved an IC50 value of 97 ppm demonstrates conclusively that it possesses powerful antioxidant properties. The results of this study imply that there may be a biochemical foundation for the traditional medicinal uses of *G. applanatum*, including the treatment of infections and its use as an antioxidant and anticancer agent.

Cytotoxity using Brine Shrimp Lethality Test

The Brine Shrimp Lethality Test (BSLT) is an essential method utilized for assessing the lethal properties of diverse chemical substances. The test measures the mortality rates of brine shrimp larvae during a specific interval in order to assess the potential toxicity of substances. The lethality test findings for brine shrimp offers significant information about the intriguing properties of the substances under consideration; higher fatality rates imply higher levels of toxicity. Table 5 showed that within 24 hours, the mortality rates of brine shrimp larvae exposed to *G. applanatum* ethanolic extracts presented a concentration-dependent pattern. The results emphasized the extracts' potential toxic properties by showing that higher extract concentrations had a more evident effect on brine shrimp larvae mortality.

The Brine Shrimp Lethality Test is utilized to evaluate the toxicity of ethanolic extracts, showing significant toxic properties. It often has a strong association with cytotoxic and anti-tumor properties (Baravalia 2012; Hasan et al. 2023; Syamsurizal et al. 2024). BSLT has also been recommended for screening pharmacological activities in various extracts and is used to verify the LC50 value of the active compound (Carballo et al. 2002; Astuti et al. 2005). In addition, the study of Krishnaraju et al. (2005) has shown the efficiency of the brine shrimp lethality test as a method in detecting fungal toxins and the evaluation of mushroom extract toxicity.

This test was used to evaluate the toxicity of the ethanolic extract of *G. applanatum*. The significant toxicity of the ethanolic extract as evidenced by the increase in mortality of brine shrimp larvae over a 24-hour period with the increasing concentration was highlighted. The outcome revealed a pronounced increase in lethality, with 30, 70, and 83% of deaths at 10, 100, and 1000 µg/mL, respectively. LC50 is an important marker in toxicology which means the concentration of a substance that kills 50% of a test population, usually aquatic organisms. LC50 values are needed for monitoring purposes and are used for evaluating and permitting the classification of chemicals into toxicity categories according to their lethal concentrations (Pillai 2023). *Ganoderma applantum* extract was at a concentration of 37 (µg/mL) (ppm).

Table 5. Brine Shrimp Lethality Test of the ethanolic extract of *Ganoderma applanatum*

Concentration (µg/mL)	Mortality rate (%) 24 hours exposure	LC50 (µg/mL)
10	30	
100	70	37
1000	83	

This classification considers their LC50 values, which range from 0 to 100 g/mL (extremely toxic); from 100 to 500 g/mL (medium toxic); from 500 to 1000 g/mL (low toxic). Yurasbe et al. (2023) define non-toxic substances as having LC50 values > 1000 g/mL. *Ganoderma applanatum* extract was determined to be highly toxic to this organism (LC50 = 37 ppm, for 24h), showing a high toxicity for this organism.

In conclusion, through an in-depth analysis of ethanolic extracts, significant insights into the cytotoxic characteristics and identified bioactive compounds of *Ganoderma applanatum* have been revealed. A total of 26 bioactive compounds were identified by GC-MS analysis performed in this research, which comprises fatty acids, hydrocarbons, phenolic compounds, terpenoids, triterpenoids, and steroids. These compounds exhibitsmajor biological activitiess, such as antimicrobial, antioxidant, and anticancer activities, which makes them useful for medicinal purposes. Moreover, the Brine Shrimp Lethality Test showed that the extract proved to be highly toxic with an LC50 value of 37 ppm. Furthermore, the results revealed that, the total phenolics was 41.32 ± 0.26 µg/mg expressed as gallic acid equivalents and the total flavonoid content was 4.87 ± 0.11 mg/g expressed as quercetin equivalents, this extract exhibits significant antioxidant activity, making it a promising natural remedy for oxidative stress-related diseases. Additionally, these findings emphasize the rich composition of bioactive components in *G. applanatum* and their potential contribution to the health benefits and therapeutic properties associated with this species.

REFERENCES

Abdulaziz AA, Dapar MLG, Manting MME, Torres MAJ, Aranas AT, Mindo RAR, Cabrido RK, Demayo CG. 2019. Qualitative evaluation of the antimicrobial, antioxidant, and medically important phytochemical constituents of the ethanolic extracts of the leaves of *Gliricidia sepium* (Jacq.) Walp. Pharmacophore 10 (4): 72-83.

Abu ElKhair RM, Omran GA, Yasser M, Seif ElDin AA. 2020. Qualitative GC-MS analysis and antimicrobial activity of volatiles from *Carthamus lanatus* (L.) growing in Egypt. Rec Pharm Biomed Sci 4 (1): 6-12. https://doi.org/10.21608/rpbs.2019.15957.1036.

Abubacker MN, Palaniyappan K. 2013. In vitro antifungal potentials of bioactive compounds heptadecane, 9-hexyl and ethyl iso-allocholate isolated from *Lepidagathis cristata* willd. (Acanthaceae) leaf. British Biomedical Bulletin 3: 336-343.

Abubakar MN, Majinda RRT. 2016 GC-MS analysis and preliminary microbial activity of *Albizia adianthifolia* (Schumach) and *Pterocarpus angolenis* (DC). Medicines 3 (1): 3. https://doi.org/10.3390/medicines3010003.

Addai ZR, Abood MS, Hlail SH. 2022. GC-MS profiling, antioxidants and antimicrobial activity of prickly pear (*Opuntiaficus-indica*) pulp extract. Pharmacognosy J 14 (2): 262-267. https://doi.org/10.5530/pj.2022.14.32.

Akin-Osanaiye CB, Gabriel AF, Alebiosu RA. 2011. Characterization and antimicrobial screening of ethyl oleate isolated from *Phyllanthus amarus* (Schum and Thonn). Ann Biol Res 2 (2): 298-305.

Alhadi A, Khalid H, Alhassan, M, Ali A, Babiker S, Albdeen E, Kabashi A. 2015. Antioxidant and cytotoxic activity of *Cordia africanian* Sudan. Adv Med Plant Res 3 (2): 29-32.

Al-Harbi NA, Al Attar NM, Hikal DM, Mohamed SE, Abdel Latef AA, Ibrahim AA, Abdein MA. 2021. Evaluation of insecticidal effects of plants essential oils extracted from basil, black seeds and lavender against *Sitophilus oryzae*. Plants 10 (5): 829. https://doi.org/10.3390/plants10050829.

Aljubiri SM, Mahgoub SA, Almansour AI, Shaaban M, Shaker KH. 2021. Isolation of diverse bioactive compounds from *Euphorbia balsamifera*: Cytotoxicity and antibacterial activity studies. Saudi J Biol Sci 28 (1): 417-426. https://doi.org/10.1016/j.sjbs.2020.10.025.

Almalki G, Rabah S, Al-Faifi Z, Alharbi A, Sharma M. 2022. Phytochemistry screening, antioxidant and antimicrobial activities of *Euphorbia inarticulata* Schweinf plant extract. Pharmacophore 13 (1): 91-9. https://doi.org/10.51847/S7eqwFEBAX.

Al-Mansoub MA, Asif M, Revadigar V, Hammad MA, Chear NJ, Hamdan MR, Majid AM, Asmawi MZ, Murugaiyah V. 2021. Chemical composition, antiproliferative and antioxidant attributes of ethanolic extract of resinous sediment from *Etlingera elatior* (Jack.) inflorescence. Braz J Pharm Sci 57: e18954. https://doi.org/10.1590/s2175-97902020000418954.

Al-Shwyeh Hussah Abdullah AS, Mohamed Elwathig SM, Parveen Jamal PJ. 2011. Antibacterial activity of Malaysian mango kernel. Afr J Biotechnol 10 (81): 18739-18748. https://doi.org/10.5897/AJB11.2746.

Aniszewski T. 2007. Alkaloids-Secrets of Life. Alkaloid Chemistry, Biological Significance, Applications and Ecological Role. Elsevier B.V., Amsterdam.

Arora S, Kumar G. 2017. Gas Chromatography-Mass Spectrometry (GC-MS) determination of bioactive constituents from the methanolic and ethyl acetate extract of *Cenchrus setigerus* Vahl (Poaceae). Pharma Innov J 6 (11): 635-640.

Astuti D, Latif F, Wagner K, Gentle D, Cooper WN, Catchpoole D, Grundy R, Ferguson-Smith AC, Maher ER. 2005. Epigenetic alteration at the DLK1-GTL2 imprinted domain in human neoplasia: Analysis of neuroblastoma, phaeochromocytoma and Wilms' tumour. Br J Cancer 92 (8): 1574-1580. https://doi.org/10.1038/sj.bjc.6602478.

Aziz M, Ahmad S, Khurshid U, Pervaiz I, Lodhi AH, Jan N, Khurshid S, Arshad MA, Ibrahim MM, Mersal GA, Alenazi FS. 2022. Comprehensive biological potential, phytochemical profiling using GC-MS and LC-ESI-MS, and in-silico assessment of *Strobilanthes glutinosus* Nees: An important medicinal plant. Molecules 27 (20): 6885. https://doi.org/10.3390/molecules27206885.

Balla OY, Ali MM, Garbi MI, Kabbashi AS. 2017. Chemical composition and antimicrobial activity of essential oil of *Mentha viridis*. Biochem Mol Biol 2 (5): 60-66. https://doi.org/10.11648/j.bmb.20170205.12.

Baravalia Y, Vaghasiya Y, Chanda S. 2012. Brine shrimp cytotoxicity, anti-inflammatory and analgesic properties of *Woodfordia fruticosa* Kurz flowers. Iran J Pharm Res 11 (3): 851.

Barnes PJ. 2005. How corticosteroids control inflammation: Quintiles prize lecture 2005. Br J Pharmacol 148 (3): 245-254. https://doi.org/10.1038/sj.bjp.0706736.

Barros L, Ferreira M-J, Queiro´s B, Ferreira ICFR, Baptista P. 2007. Total phenols, ascorbic acid, b-carotene and lycopene in Portuguese wild edible mushrooms and their antioxidant activities. Food Chem 100: 413-419. https://doi.org/10.1016/j.foodchem.2006.07.038.

Beulah GG, Soris PT, Mohan VR. 2018. GC-MS determination of bioactive compounds of *Dendrophthoe falcata* (LF) Ettingsh: An epiphytic plant. Intl J Health Sci Res 8: 261-269.

Bhutia YD, Jain N, Ahmed F, Sharma M, Singh R, Kumar S, Mendki MJ, Kumar P, Vijayaraghavan R. 2010. Acute and sub-acute toxicity of an insect pheromone, N-heneicosane and combination with insect growth regulator, diflubenzuron, for establishing No Observed Adverse Effect Level (NOAEL). Indian J Exp Biol 48: 744-751.

Bristy AT, Islam T, Ahmed R, Hossain J, Reza HM, Jain P. 2022. Evaluation of total phenolic content, HPLC analysis, and antioxidant potential of three local varieties of mushroom: A comparative study. Int J Food Sci 2022: 1-11. https://doi.org/10.1155/2022/3834936.

Carballo JL, Hernández-Inda ZL, Pérez P, García-Grávalos MD. 2002. A comparison between two brine shrimp assays to detect in vitro cytotoxicity in marine natural products. BMC Biotechnol 2 (1): 17. https://doi.org/10.1186/1472-6750-2-17.

Chen G, Pan F, Gao Y, Li H, Qin X, Jiang Y, Qi J, Xie J, Jia S. 2022. Analysis of components and properties of extractives from *Alnus cremastogyne* pods from different provenances. Molecules 27 (22): 7802. https://doi.org/10.3390/molecules27227802.

Chen X, Zhang L, Cheung PCK. 2017. *Ganoderma lucidum* polysaccharides protect fibroblasts against ultraviolet B-induced photoaging. Mol Med Rep 15 (4): 2431-2440.

Chukwu E, Osuocha KU, Iwueke AV. 2020. Phytochemical profiling, body weight effect and anti-hypercholesterolemia potentials of

Cnidoscolus aconitifolius leaf extracts in the male albino rat. J Pharm Phytotherapy 12 (2): 19-27. https://doi.org/10.5897/JPP2016.0436.

Cör D, Knez Ž, Knez Hrnčič M. 2018. Antitumour, antimicrobial, Antioxidant and antiacetylcholinesterase effect of *Ganoderma lucidum* terpenoids and polysaccharides: A review. Molecules 23 (3): 649. https://doi.org/10.3390/molecules23030649.

Das R, Romi W, Das R, Sharma HK, Thakur D. 2018. Antimicrobial potentiality of actinobacteria isolated from two microbiologically unexplored forest ecosystems of Northeast India. BMC Microbiol 18: 1-16. https://doi.org/10.1186/s12866-018-1215-7.

Dembitsky VM. 2024. Bioactive diepoxy metabolites and highly oxygenated triterpenoids from marine and plant-derived fungi. Microorganisms 15 (1): 66-90. https://doi.org/10.3390/microbiolres15010005.

Dhanya KI, Swati VI, Vanka KS, Osborne WJ. 2016. Antimicrobial activity of *Ulva reticulata* and its endophytes. J Ocean Univ China 15: 363-369. https://doi.org/10.1007/s11802-016-2803-7.

Diansambu MI, Dibaluka MS, Lumande KJ, Degreef J. 2015. Culture de trois espèces fongiques sauvages comestibles du groupement de Kisantu (R D Congo) sur des substrats ligno-cellulosiques compostés. Frique Science: Revue Internationale des Sciences et Technologie 11 (3): 241-261. (French)

Diep TT, Yoo MJ, Pook C, Sadooghy-Saraby S, Gite A, Rush E. 2021. Volatile components and preliminary antibacterial activity of tamarillo (*Solanum betaceum* Cav.). Foods 10 (9): 2212. https://doi.org/10.3390/foods10092212.

Dupont S, Fleurat-Lessard P, Cruz RG, Lafarge C, Grangeteau C, Yahou F, Gerbeau-Pissot P, Abrahão Júnior O, Gervais P, Simon-Plas F, Cayot P. 2021. Antioxidant properties of ergosterol and its role in yeast resistance to oxidation. Antioxidants 10 (7): 1024. https://doi.org/10.3390/antiox10071024.

El Barky AR, Hussein SA, Alm-Eldeen A, Hafez ya, Mohamed T. 2017. Saponins and their potential role in diabetes mellitus. Diabetes Manag 7 (1): 148-158.

Eltayeib AA, Ismaeel HU. 2014. Extraction of *Cyperus rotundus* rhizomes oil, identification of chemical constituents and evaluation of antimicrobial activity of the oil in North Kordofan state. Intl J Adv Res Chem Sci 1 (9): 18-29.

Gan CH, Nurul AB, Asmah R. 2013. Antioxidant analysis of different types of edible mushrooms (*Agaricus bisporous* and *Agaricus brasiliensis*). Intl Food Res J 20 (3): 1095-1102.

George LO, Radha HR, Somasekariah BV. 2018. In vitro anti-diabetic activity and GC-MS analysis of bioactive compounds present in the methanol extract of *Kalanchoe pinnata*. Indian J Chem 57 (B): 1213-1221.

Ghavam M, Afzali A, Manconi M, Bacchetta G, Manca ML. 2021. Variability in chemical composition and antimicrobial activity of essential oil of *Rosa damascena* Herrm. from mountainous regions of Iran. Chem Biol Technol Agric 8 (1): 1-16. https://doi.org/10.1186/s40538-021-00219-6.

Hadi I, Hussein HM. 2016. Antimicrobial activity and spectral chemical analysis of methanolic leaves extract of *Adiantum capillus-veneris* using GC-MS and FTIR spectroscopy. Intl J Pharm Phytochem Res 8: 369-385.

Halliwell B. 1995 Antioxidant characterization: Methodology and mechanism. Biochem Pharmacol 49 (10): 1341-1348. https://doi.org/10.1016/0006-2952(95)00088-H.

Hapuarachchi KK, Cheng CR, Wen TC, Jeewon R, Kakumyan P. 2017. Mycosphere essays 20: Therapeutic potential of *Ganoderma* species: Insights into its use as traditional medicine. Mycosphere 8 (10): 1653-1694. https://doi.org/10.5943/mycosphere/8/10/5.

Hasan A, Artika I, Kuswandi TG. 2014. Analysis of active components of *Trigona* spp. propolis from Pandeglang Indonesia. Glob J Biol Agric Health Sci 3: 215-219.

Hasan MM, Islam M, Sikdar B, Acharjee UK, Hasan MF. 2023. Screening of antibacterial, cytotoxic and pesticidal activities of *Abroma augusta* (L.) seeds extract. S Asian J Biol Res 5 (1): 15-25.

Hawksworth DL, Lücking R. 2017. Fungal diversity revisited: 2.2 to 3.8 million species. Microbiol Spectrum 5 (4): 10-1128. https://doi.org/10.1128/9781555819583.ch4.

Hossen SMM, Yusuf ATM, Emon NU, Alam N, Sami SA, Polash SH, Nur MA, Mitra S, Uddin MH, Emran TB. 2022. Biochemical and pharmacological aspects of *Ganoderma lucidum*: Exponent from the in vivo and computational investigations. Biochem Biophys 32: 101371. https://doi.org/10.1016/j.bbrep.2022.101371.

Hsouna AB, Trigie M, Mansour RB, Jarraya RM, Damak M, Jaoua S. 2011. Chemical composition, cytotoxicity effect and antimicrobial activity of *Ceratonia silisqua* essential oil with preservative effects against listeria inoculated in minced beef meat. Intl J Food Microbiol 148 (1): 66-72. https://doi.org/10.1016/j.ijfoodmicro.2011.04.028.

Hu H, Zhang Z, Lei Z, Zhang Y, Zhang Y. 2014. Comparative study of antioxidant activity and antiproliferative effect of hot water and ethanol extracts from the mushroom *Inonotus obliquus*. J Biosci Bioeng 107 (1): 42-48. https://doi.org/10.1016/j.jbiosc.2008.09.004.

Huang D, Ou B, Prior RL. 2005. The chemistry behind antioxidant capacity assays. J Agric Food Chem 53 (6): 1841-1856. https://doi.org/10.1021/jf030723c.

Ihayere CA, Okhuoya JA. 2022. Phytochemical analysis of cultivated medicinal mushroom-*Ganoderma* sp. NJB 35 (1): 11-18.

Ikarashi N, Hoshino M, Ono T, Toda T, Yazawa Y, Sugiyama K. 2020. A mechanism by which ergosterol inhibits the promotion of bladder carcinogenesis in rats. Biomedicines 8 (7): 180. https://doi.org/10.3390/biomedicines8070180.

Islam MJ, Hossain MS, Barua A, Tanim MA, Hasan MS, Hossain MR, Emon NU, Hossen SM. 2021. *Ganoderma applanatum* mushroom provides new insights into the management of diabetes mellitus, hyperlipidemia, and hepatic degeneration: A comprehensive analysis. Food Sci Nutr 9: 4364-4374. https://doi.org/10.1002/fsn3.2407.

Jemimma HL, Arumugasamy K, Kumar RN, Kaffoor HA. 2017. GC-MS analysis of root and aerial parts ethanolic extract of *Phyllanthus vasukii* Parthipan et al. Sp. Nov.(Phyllanthaceae). Intl J Ayurvedic Herbal Med 7 (4): 2672-2684.

Kandagalla S, Krishnappa M. 2019. Exploring the ethnomycological potential of *Lentinus squarrosulus* Mont. through GC- MS and chemoinformatics tools. Mycology 11: 78-89. https://doi.org/10.1080/21501203.2019.1707724.

Karanja LN, K'Owino IO, Wangila PT, Ramkat RC. 2021. Phytochemical composition and antibacterial activity of fruit extract of *Solanum incanum* L. against *Ralstonia solanacearum*. Asian J Appl Chem Res 9 (4): 1-16. https://doi.org/10.9734/ajacr/2021/v9i430218.

Kaur R, Arora S. 2015. Alkaloids-important therapeutic secondary metabolites of plant origin. J Crit Rev 2 (3): 1-8. https://doi.org/10.31838/jcr.02.01.01.

Kawuri R, Darmayasa IBG. 2019. Bioactive compound of *Streptomyces capoamus* as biocontrol of bacterial wilt disease on banana plant. In IOP Conf Ser Earth Environ Sci 374 (1): 012054. https://doi.org/10.1088/1755-1315/347/1/012054.

Khan K, Firdous S, Ahmad A, Fayyaz N, Nadir M, Rasheed M, Faizi S. 2016. GC-MS profile of antimicrobial and antioxidant fractions from *Cordia rothii* roots. Pharm Biol 54 (11): 2597-2605. https://doi.org/10.3109/13880209.2016.1172320.

Kim BR, Kim HM, Jin CH, Kang SY, Kim JB, Jeon YG, Park KY, Lee IS, Han AR. 2020. Composition and antioxidant activities of volatile organic compounds in radiation-bred Coreopsis cultivars. Plants 9 (6): 717. https://doi.org/10.3390/plants9060717.

Kouidhi S, Zidi O, Abdelwahed S, Souissi Y, Trabelsi N, Redissi A, Hamdi M, Trabelsi E, Amara Y, Bhiri T, Khrouf R. 2021. Investigation of the chemical composition and antioxidant and antimicrobial activities of *Lobularia maritima*: Potent therapeutic applications. J Chem 2021 (1): 1981680. https://doi.org/10.1155/2021/1981680.

Krishnaraju AV, Rao TV, Sundararaju D, Vanisree M, Tsay HS, Subbaraju GV. 2005. Assessment of bioactivity of Indian medicinal plants using brine shrimp (*Artemia salina*) lethality assay. Intl J Appl Sci Eng 3 (2): 125-134.

Kumari D, Reddy MS, Upadhyay RC. 2011. Antioxidant activity of three species of wild mushroom genus *Cantharellus* collected from North-Western Himalaya, India. Intl J Agric Biol 13: 415-418.

Kumari M, Jain A. 2012. Tannins: An antinutrient with positive effect to manage diabetes. Res J Recent Sci 1 (12): 70-73.

Kushairi N, Tarmizi NAKA, Phan CW, Macreadie I, Sabaratnam V, Naidu M, David P. 2020. Modulation of neuroinflammatory pathways by medicinal mushrooms, with particular relevance to alzheimer's disease. Trends Food Sci Technol 104: 153-162. https://doi.org/10.1016/j.tifs.2020.07.029.

Lalthanpuii PB, Lalchhandama K. 2019. Chemical profiling, antibacterial and antiparasitic studies of *Imperata cylindrica*. J Appl Pharm Sci 9 (12): 117-121. https://doi.org/10.7324/JAPS.2019.91216.

Lee J, Lim S, Kang S-M, Min S, Son K, Lee HS, Park EM, Ngo HTT, Tran HTL, Lim Y-S, Hwang SB. 2012. Saponin inhibits hepatitis C

virus propagation by up-regulating the suppressor of cytokine signaling 2. PLoS One 7 (6): e39366.
Lee Y, Yuk D, Lee J et al. 2009. Epigallocatechin-3-gallate prevents lipopolysaccharide-Induced elevation of β-amyloid generation and memory deficiency. Brain Res 1250: 164-174.
Lee YS, Kang MH, Cho YS, Jeong CS. 2007. Effect of constituents of amomun Xanthioides on gastritis in rats and on growth of gastric cancer cells. Arch Pharm Res 30 (4): 436-43. https://doi.org/10.1007/BF02980217.
Lindequist U, Rausch R, Füssel A, Hanssen HP. 2010. Higher fungi in traditional and modern medicine. Medizinische Monatsschrift fur Pharmazeuten 33 (2): 40-48.
Lindequist U, Venturella G, Gargano ML. 2017. Medicinal mushrooms: Valuable biological resources of high exploitation potential. Phytochem Rev 16 (3): 459-480.
Loyd AL, Richter BS, Jusino MA, Truong C, Smith ME, Blanchette RA, Smith JA. 2018. Identifying the "Mushroom of Immortality": Assessing the *Ganoderma* species composition in commercial reishi products. Front Microbiol 16 (9):1557. https://doi.org/10.3389/fmicb.2018.01557.
Malathi K, Anbarasu A, Ramaiah S. 2017. Ethyl iso-allocholate from a medicinal rice Karungkavuni inhibits dihydropteroate synthase in *Escherichia coli*: A molecular docking and dynamics study. Indian J Pharm Sci 78 (6): 780-8. https://doi.org/10.4172/pharmaceutical-sciences.1000184.
Manasseh AT, Godwin JTA, Emanghe EU, Borisde OO. 2012. Phytochemical properties of *Ganoderma applanatum* as potential agents in the application of nanotechnology in modern day medical practice. Asian Pac J Trop Biomed 2 (2): 580-583. https://doi.org/10.1016/S2221-1691(12)60277-9.
Martínez-Montemayor MM, Ling T, Suárez-Arroyo IJ, Ortiz-Soto G, Santiago-Negrón CL, Lacourt-Ventura MY, Valentín-Acevedo A, Lang WH, Rivas F. 2019. Identification of biologically active *Ganoderma lucidum* compounds and synthesis of improved derivatives that confer anti-cancer activities in vitro. Front Pharmacol 10: 115. https://doi.org/10.3389/fphar.2019.00115.
Mary PA, Giri RS. 2017. GC-MS Analysis of bioactive compounds of *Acryranthes aspera*. World J Pharm Res 7 (1): 1015-1056.
Matthew O, James A, Akogwu I, Fabunmi T, Idoko O, Ebun B, Mohammed Z, Dorathy O. 2021. Evaluation of in vitro antioxidant, phytochemical and GC-MS analysis of aqueous extract of *Solanum dasyphyllum* fruits. J Med Biol Sci Res 7 (3): 10-14. https://doi.org/10.36630/jmbsr_21008.
Mishra PM, Shree A. 2007. Antibacterial activity and GC-MS analysis of the extract of leaves of *Finlaysonia obovata* (a mangrove plant). Asian J Plant Sci 6: 168-172. https://doi.org/10.3923/ajps.2007.168.172.
Modi HA, Shah P, Shukla MD, Lahiri SK. 2014. Determination of total phenolic content and antioxidant activity of *Ganoderma lucidum* collected from Dang district of Gujarat, India. Nat Prod Indian J 10 (3): 75- 83.
Mohammadifar S, Gharaghoz SF, Asef MR, Vaziri A. 2019. Antioxidant Activity and some biochemical properties of *Ganoderma applanatum* (Pers.) Pat. from Iran. Advanced Research in Microbial Metabolites & Technology 2 (2): 61-69. https://doi.org/10.22104/armmt.2021.4454.1047.
Mohan VR, Jegadeswari P, Nishanthini A, Muthukumarasamy S. 2012. GC-MS analysis of bioactive components of *Aristolochia krysagathra* (Aristolochiaceae). J Curr Chem Pharm Sci 2 (4): 226-232.
Mostofa MG, Reza AA, Khan Z, Tsukahara T, Alam AK, Sadik MG. 2021. The apoptosis-inducing antiproliferative activity and quantitative phytochemical profiling of polyphenol-rich part of *Leea aequata* L. leaves. Preprint Version 1 at Research Square. https://doi.org/10.21203/rs.3.rs-830741/v1.
Nagaraj K, Mallikarjun N, Raja N, Venugopal TM. 2014. Antioxdative activities of wild macro fungi *Ganoderma applanatum* (PERS.) PAT. Asian J Pharm and Cli Res 7 (2): 166-171.
No JB, Kampus USU. 2020. Bioactivity and phytochemical constituents of extract etanol from stem *Musa paradisiaca* Linn. Proceedings of the 1st International Conference on Chemical Science and Technology Innovation (ICOCSTI 2019): 89-95.
Nyalo PO, Omwenga GI, Ngugi MP. 2023. Antibacterial properties and GC-MS analysis of ethyl acetate extracts of *Xerophyta spekei* (Baker) and *Grewia tembensis* (Fresen). Heliyon 9 (3): e14461. https://doi.org/10.1016/j.heliyon.2023.e14461.
Ochwang'I DO, Kimwele CN, Oduma JA, Gathumbi PK, Mbaria JM, Kiama SG. 2004. Medicinal plants used in treatment and management of cancer in Kakamega County, Kenya. J Ethnopharmacol 151 (3): 1040-1055. https://doi.org/10.1016/j.jep.2013.11.051.
Okechukwu PN. 2020. Evaluation of anti-inflammatory, analgesic, antipyretic effect of eicosane, pentadecane, octacosane, and heneicosane. Asian J Pharm Clin Res 13 (4): 2020. https://doi.org/10.22159/ajpcr.2020.v13i4.36196.
Okwu DE, Ighodaro BU. 2009. GC-MS evaluation of the bioactive compounds and antibacterial activity of the oil fraction from the stem barks of *Dacryodes edulis* G. Don Lam. Intl J Drug Dev Res 1 (1): 117-125.
Olugbami JO, Gbadegesin MA, Odunola OA. 2014. In vitro evaluation of the antioxidant potential, phenolic and flavonoid contents of the stem bark ethanol extract of *Anogeissus leiocarpus*. Afr J Med Med Sci 43 (1):101-109.
Peña JF, Dapar ML, Aranas AT, Mindo RA, Cabrido CK. 2019. Assessment of antimicrobial, antioxidant and cytotoxic properties of the ethanolic extract from *Dracontomelon dao* (Blanco) Merr. and Rolfe. Pharmacophore 10 (2-2019): 18-29.
Penduka D, Buwa L, Mayekiso B, Basson AK, Okoh AI. 2014. Identification of the anti listerial constituents in partially purified column chromatography fractions of *Garcinia kola* seeds and their interactions with standard antibiotics. Evidence-Based Complement Altern Med 2014. https://doi.org/10.1155/2014/850347.
Pereira DM, Correia-da-Silva G, Valentão P, Teixeira N, Andrade PB. 2014. Anti-inflammatory effect of unsaturated fatty acids and ergosta-7, 22-dien-3-ol from *Marthasterias glacialis*: Prevention of CHOP-mediated ER-stress and NF-κB activation. PLoS One 9 (2): e88341. https://doi.org/10.1371/journal.pone.0088341.
Pillai KS. 2023. Classifying chemicals into toxicity categories based on LC50 values-Pros and cons. J Environ Biol 44 (5): I-III. https://doi.org/10.22438/jeb/44/5/Editorial.
Prayitno TA, Widyorini R, Lukmandaru G. 2021. Chemical variation of five natural extracts by non-polar solvent. Maderas. Ciencia y Tecnología 23: 0-0. https://doi.org/10.4067/S0718-221X2021000100401.
Rajoriya A, Tripathy SS, Gupta N. 2015. In vitro antioxidant activity of selected *Ganoderma* species found in Odisha, India. Tropical Plant Research 2(2): 72–77.
Ralf P, Bagchus WM. 2003. 3-Methylene Steroid Derivatives for the Treatment of Autoimmune Diseases. https://patents.google.com.iPopovych V, Les M, Shuplat T, Bosak P, Fitak M, Popovych N. 2019. The effects of temperature and moisture stress content on theextensive cultivation of the oyster mushroom. Bull Iraq nat Hist Mus 15 (4): 473-489.
Raslan A, Abdel-Motaal F, Abou-Ellail M, Mohamed AEH. 2022. Antioxidant and cytotoxic activity of ethyl acetate extract from *Thermomyces lanuginosus* and *Aspergillus nidulans* isolated from rhizospheric region of peanut (*Arachis hypogaea*). Preprint Version 1 at Research Square. https://doi.org/10.21203/rs.3.rs-2315764/v1.
Rizvi SM, Shakil S, Zeeshan M, Khan MS, Shaikh S, Biswas D, Ahmad A, Kamal MA. 2014. An enzoinformatics study targeting polo-like kinases-1 enzyme: Comparative assessment of anticancer potential of compounds isolated from leaves of *Ageratum houstonianum*. Pharmacognosy Mag 10 (Suppl 1): S14. https://doi.org/10.4103/0973-1296.127333.
Rouis-Soussi LS, El Ayeb-Zakhama A, Mahjoub A, Flamini G, Jannet HB, Harzallah-Skhiri F. 2014. Chemical composition and antibacterial activity of essential oils from the Tunisian *Allium nigrum* L. EXCLI J 13: 526.
Salvatore MM, De Gregorio V, Gallo M, Corsaro MM, Casillo A, Vecchione R, Andolfi A, Naviglio D, Netti PA. 2020. Evaluation of two extraction methods for the analysis of hydrophilic low molecular weight compounds from *Ganoderma lucidum* spores and antiproliferative activity on human cell lines. Appl Sci 10 (11): 4033. https://doi.org/10.3390/app10114033.
Sebille S, Pirotte B, Boverie S, de Tullio P, Lebrun P, Antoine MH. 2009. U.S. Patent No. 7,507,761. Patent and Trademark Office, Washington DC, US..
Seenivasagan T, Sharma KR, Sekhar K, Ganesan K, Prakash S, Vijayaraghavan R. 2009. Electroantennogram, flight orientation, and oviposition responses of *Aedes aegypti* to the oviposition pheromone n-heneicosane. Parasitol Res 104: 827-833. https://doi.org/10.1007/s00436-008-1263-2.

Settu S, Arunachalam S. 2020. Fungal endophytes: A blooming reservoir for future products. Intl J Pharm Sci Rev Res 65 (1): 169-178.

Shah P, Modi HA, Shukla MD, Lahiri SK. 2014. Preliminary phytochemical analysis and antibacterial activity of *Ganoderma lucidum* collected from Dang District of Gujarat, India. Int J Curr Microbiol App Sci 3 (3): 246–255.

Shaikh S, Verma H, Yadav N, Jauhari M, Bullangowda J. 2012. Applications of steroid in clinical practice: A review. Int Sch Res Notices 2012: 1-11. https://doi.org/10.5402/2012/985495.

Sharma PK, Fuloria S, Alam S, Sri MV, Singh A, Sharma VK, Kumar N, Subramaniyan V, Fuloria NK. 2021. Chemical composition and antimicrobial activity of oleoresin of *Capsicum annuum* fruits. Mindanao J Sci Technol 19 (1). https://doi.org/10.61310/mndjstecbe.1030.21.

Shobi TM, Viswanathan MBG. 2018. Antibacterial activity of di-butyl phthalate isolated from *Begonia malabarica*. J Appl Biotechnol Bioeng 5 (2): 97-100. https://doi.org/10.15406/jabb.2018.05.00123.

Sidjui SI, Tane P, Ngameni B, Dimo T, Kamtchouing P. 2017. Four lanostane-type triterpenes from the fruiting bodies of mushroom *Laetiporus sulphureus* var. *miniatus*. Phytochem Lett 7: 1-4.

Sivakumar SR. 2014. GC-MS analysis and antibacterial potential of white crystalline solid from red algae *Portieria hornemannii* against the plant pathogenic bacteria *Xanthomnas axonopodis* pv. Citri (Hasse) Vauterin et al. and *Xanthomonas campestris* pv. Malvacearum (smith 1901) dye 1978b. Intl J Adv Res 2 (3): 174-183.

Sobhy S, Al-Askar AA, Bakhiet EK, Elsharkawy MM, Arishi AA, Behiry SI, Abdelkhalek A. 2023. Phytochemical characterization and antifungal efficacy of camphor (*Cinnamomum camphora* L.) extract against phytopathogenic fungi. Separations 10 (3): 189. https://doi.org/10.3390/separations10030189.

Soliman AM, Barreda DR. Acute Inflammation in Tissue Healing. Intl J Mol Sci 24 (1): 641. https://doi.org/10.3390/ijms24010641.

Suansia A, John P. 2021. Mycelial biomass production of medicinal mushroom *Ganoderma* P. Karst. Int J Agric Sci 17 (1): 15-18. https://doi.org/10.15740/HAS/IJAS/17.1/15-18.

Suerbaev KA, Zhaksylykova GZ, Appazov NO. 2014. Biological active esters of the isovaleric acid. Eurasian Chemico-Technol J 16 (4): 299-302. https://doi.org/10.18321/ectj4.

Swargiary A, Roy MK, Boro H, Verma AK, Daimari M, Das JK. 2023. Phytochemical analysis, antiproliferative and apoptosis-inducing properties of *Persicaria strigosa* Nakai. J Appl Pharm Sci 13 (5): 162-170. https://doi.org/10.7324/JAPS.2023.106106.

Syamsurizal S, Utami DT. 2024. The combination of fraction A3 of *Cyrtostachys renda* Blume with doxorubicin improved cytotoxicity against T47D cell line through cell cycle arrest and apoptotic induction. J Appl Pharm Sci 14 (9): 270-278. https://doi.org/10.7324/JAPS.2024.173607.

Taskin H, Kafkas E, Çakiroglu Ö, Büyükalaca S. 2013. Determination of volatile aroma compounds of *Ganoderma lucidum* By Gas Chromatography Mass Spectrometry (HS-GC/MS). Afr J Tradit Complement Altern Med 10 (2): 353-355. https://doi.org/10.4314/ajtcam.v10i2.22.

Teoh L, Gnanasegaran N, Adnan AFM, Taha RM. 2021. The comparative antimicrobial and anticancer of chemical extract from in vitro and in vivo *Peperomia pellucida* plantlet. J Appl Biol Biotechnol 9 (2): 115-123.Thapa R, Maharjan R, Tamang P, Gautam P, Adhikari R, Maharjan S. 2022. Antimicrobial assessment and phytochemical screening of medicinal plants and *Ganoderma lucidum*. International Journal of Applied Sciences and Biotechnology 10: 228-236. https://doi.org/10.3126/ijasbt.v10i4.49508.

Tiwari AK, Singh MV. 2023. Insights into the origin and therapeutic implications of benzopyran and its derivatives. Chem Select 8 (20): e202300220. https://doi.org/10.1002/slct.202300220.

Trivedi N, Thumar J. 2021. Chemical profiling of antimicrobial metabolites from halophilic actinomycete *Nocardiopsis*_sp. Al-H10-1 (KF384482) isolated from Alang, Gulf of Khambhat, India. BioRxiv 2021-06. https://doi.org/10.20546/ijcmas.2021.1006.080.

Ullah A, Munir S, Badshah SL, Khan N, Ghani L, Poulson BG, Emwas AH, Jaremko M. 2020. Important flavonoids and their role as a therapeutic agent. Molecules 25(22): 1-39. https://doi.org/10.3390/molecules25225243.

Uma Gowrie S, Chathurdevi G, Rani K. 2014. Evaluation of bioactive potential of basidiocarp extracts of *Ganoderma lucidum*. Int J Pharm Res Allied Sci 3 (1): 36-46.

Vanitha A, Sangeetha VS, Kanmani M, Priya RS. 2020. Chemical constituents from ethanol extract of abrus precatorius by using gcms techniques. Scholar: National School of Leadership 9 (1.4).

Vega-Portalatino EJ, Rosales-Cuentas MM, Valdiviezo-Marcelo J, Arana-Torres NM, Espinoza-Espinoza LA, Moreno-Quispe LA, Cornelio-Santiago HP. 2023. Antimicrobial and production of hydrolytic enzymes potentials of bacteria and fungi associated with macroalgae and their applications: A review. Front Mar Sci 10: 1174569. https://doi.org/10.3389/fmars.2023.1174569.

Wal JM, Wal JM, Wal JM. 2011. Medicinal mushrooms in prevention and control of diabetes mellitus. Crit Rev Food Sci Nutr 51 (7): 624-634.

Wang L, Li J, Zhang J, Li Z, Liu H, Wang Y. 2020. Traditional uses, chemical components and pharmacological activities of the genus *Ganoderma* P. Karst.: A review. RSC Adv 10: 42084-42097. https://doi.org/10.1039/D0RA07219B.

Wasser SP. Weis AL. 1999. Medicinal properties of substances occurring in higher basidiomycetes mushrooms: Current perspectives (review). Intl J Med Mushrooms 1: 31-62. https://doi.org/10.1615/IntJMedMushrooms.v1.i1.30.

Wood TT, Rowaiye AB, Okwu CO, Popoola OA, Unaeze CH, AkienAlli IJ, Iheka SC, Braide W. 2021. Phytochemical screening and antibacterial effects of wild *Ganoderma* species on selected foodborne bacteria. Int J Adv Res Biol Sci 8 (1): 128–137.

Xu XH, Li T, Fong CM, Chen X, Chen XJ, Wang YT, Huang MQ, Lu JJ. 2016. Saponins from Chinese medicines as anticancer agents. Molecules 21 (10): 1326. https://doi.org/10.3390/molecules21101326.

Yang Y. Tasneem S, Daniyal M, Zhang L, Jia Y, Jian Y, Li B, Wang W. 2020. Lanostane tetracyclic triterpenoids as important sources for anti-inflammatory drug discovery. World J Tradit Chin Med 6 (3): 229-238. https://doi.org/10.4103/wjtcm.wjtcm_17_20.

Yaoyao W. 2016. CN105524134-Novel Lanostane Type Triterpene Compound, Method for Preparing Same and Medical Application of Novel Lanostane Type Triterpene Compound. https://patentscope.wipo.int.

Yogeswari S, Ramalakshmi S, Neelavathy R, Muthumary JY. 2012. Identification and comparative studies of different volatile fractions from *Monochaetia kansensis* by GCMS. Global J Pharmacol 6 (2): 65-71.

Yurasbe NQ, Din NA, Palaniveloo K, Manikam S, Nagappan T. 2023. Phytochemical diversity and biological activities of *Curcuma* species from the East Coast of Peninsular Malaysia. Biodiversitas 24 (8): 4243-4252. https://doi.org/10.13057/biodiv/d240805.

Zeid DC, Savoie JM, Pardo-Gime A. 2011. Soybean the main nitrogen source in cultivation substrates of edible and medicinal mushrooms soybean and nutrition. Soybean Nutr 22: 433-452.

Zhang Y, Zhang Y, Zhang X, Zhang Y. 2016. The antioxidant properties of mushroom polysaccharides can be enhanced by fermentation. Front Pharmacol 7: 476.

Zhu F, Li J, Xie W, Wang C, Liu Y. 2017. Identification and antibacterial activity of two steroids secreted by the fungus beetle *Xylographus bostrichoides* (Dufour, 1843). Bangladesh J Bot 46: 1171-1176.