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Antioxidant and Chromatographic Profile of *Ganoderma lucidum*

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Abstract

Ganoderma lucidum is a medicinal fungus which possesses a wide range of biological activities due to its nutritional, antioxidant and therapeutic values. This study examine the antioxidant and phytochemical properties of *G. lucidum* aqueous extracts in Sample A (boiled for 1 hour) and Sample B (boiled for 3 hours). The antioxidant activity and DPPH radical-scavenging assay were determined using standard procedures, while further phytochemical composition of the extracts was determined through chromatographic analysis. Sample A demonstrated higher DPPH radical scavenging activity (67.85%) while Sample B, displayed an increased trend with time compared with ascorbate. Total Antioxidant Capacity (TAC) analysis, revealed Sample A with higher TAC (56.79 mg/ml) than Sample B (51.71 mg/ml). Phytochemical composition of *G. lucidum* aqueous extracts exhibited higher percentage of alkaloids at Sample A (3.00 ± 0.12%) and TAC (56.79 ± 0.04 µg/ml), while Sample B displayed higher percentages of saponins (8.00 ± 0.12%), flavonoids (51.50 ± 0.77 µg/ml), proanthocyanidins (38.23 ± 0.18 µg/ml), phenols (16.18 ± 0.07 µg/ml), and FRAP (64.00 ± 2.00 mg/ml). Chromatographic profiles illustrated that both samples displayed 32 peaks, with varying constituents. The highest peak in Sample A was recorded at PEAK 22 (31.317), while in Sample B was observed at PEAK 37 (35.927). These peaks represented different identified constituents, including Pyrrolidine, 1-methyl-3, 2'-spiro-benzo-1, 3-dioxolane-Benzene, 1,3,5-tris(2,2-dimethylpropyl)-2-iodo-4-nitro-Propanamide, and 2,2-dimethyl-N-(3-methylphenyl)-. These findings provide insights into the

potential health benefits associated with *G. lucidum* and guide further exploration of its bioactive compounds.

Keyword: Antioxidant, DPPH radical-scavenging activity, Total Antioxidant Capacity, Extract

INTRODUCTION

It was estimated that there are 1.5 million different types of mushrooms in the world (Dharmaraj et al., 2015). They are produced and eaten worldwide because of their excellent nutritional value and medicinal advantages (Ferdousi et al., 2020). They are called "Macrofungi" because, in contrast to other fungus, they have a big fruiting body that is visible to the naked eye. Because they can carry toxins, some can be ingested while others cannot. Some species are useful as meals because of their nutritional content, while others have been extensively employed in traditional medicine and as a source for the creation of medications and nourishing therapeutic compounds (Alves et al., 2012). A variety of pharmacological characteristics exist in mushrooms such as antimicrobial activity, which is reflected in the diversity and abundance of bioactive chemicals that have been extracted and found in them (Matijaevic et al., 2016).

The medicinal resource of the genus Ganoderma contains a number of known bioactivities; exhibited antibacterial, antifungal, antiandrogenic, anti-HIV, antiplatelet aggregation, and other activities, which features numerous triterpene metabolites such as lucidenic and ganoderic acids, (Chan and Chong, 2022).. The bioactive -1-3-glucan polysaccharides separated from *Ganoderma lucidum*'s fruiting body displayed a wide spectrum of bioactivities, including antitumor and anticancer properties (Sharma et al., 2019). Mushrooms, like many plants, protect living things from oxidative stress, therefore they may be regarded as antioxidants that are crucial for the chemoprevention of illnesses brought on by reactive oxygen species (Kamra and Bhatt, 2012; Kaur et al., 2015). *G. lucidum* has reportedly been used for these treating numerous health challenges (Paterson, 2006). However, there has been relatively little pharmacological research done on the discovered substances. Thus, the aim of this study is to examine the antioxidant activity and phytochemical content of aqueous extracts of *G. lucidum* prepared Sample A (boiled for 1 hour) and Sample B (boiled for 3 hours) for effective extraction and identification of bioactive components.

MATERIALS AND METHODS

Sample Collection

Freshly grown *Ganoderma lucidum* was collected from Mycofarms and Allied Synergy Limited, Benin City, Edo State and identified and at the African Centre for Mushroom Research and Technology Innovations (ACMRTI) by Prof. J. A Okhuoya of the Mycology Unit in the Department of Plant Biology and Biotechnology, University of Benin with voucher number LS23511.

Preparation of *Ganoderma lucidum* Extract

The mushroom fruiting bodies were cleaned and washed with distilled water to get rid of any leftover compost. Following constant weight air drying, they were crushed into a coarse powder in a laboratory scale mill and mixer (Bristy et al., 2022). Small parts of the sporocarps were broken up, dried at 40 °C for 48 hours, and then pulverized. The plant material was dried at each stage to get rid of the moisture and get rid of the fungus. For future usage, the air-dried powder was kept in an airtight container (Liua et al., 2009). 200 grams of materials that were pulverized and dried were taken using 2000 milliliters of distilled water by boiling the mixture for one hour (Sample A) and three hours (Sample B). A rotary evaporator was used to concentrate the filtrate at a temperature below 75°C (Millipore, Hong Kong). Extracts were kept at 4°C. For this work, the necessary concentrations were made using dried fractions (Uddin et al., 2018).

Antioxidant Analysis of *Ganoderma lucidum* Extract

Total Antioxidant Capacity

The amount of total antioxidant activity was calculated using a phosphomolybdenum assay. A green phosphate/molybdenum (V) complex is formed at an acidic pH as a result of the extract's reduction of molybdenum (IV) to molybdenum (V), which is the basis for the method. Each of the following solutions was combined in one (1) ml: 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate. 3 ml of the Molybdate reagent solution were mixed with 1 mg/ml of the extracts. 90 min of incubation at 95 °C were spent on these tubes. The reaction mixture's absorbance was measured at 695 nm after these tubes had been incubated and had been brought to room temperature for 20 to 30 minutes. For comparison, ascorbic acid was used.

Estimation of Diphenyl-2-Picryl-Hydrazyl (DPPH)

The ability of the extracts to scavenge free radicals against the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was assessed using a method of Brand-Williams et al. (1995) with slight modified. The assay is based on the antioxidant compounds' capacity to lower DPPH by donating hydrogen, which causes a change in color from deep violet to golden yellow. Spectrophotometry was used to measure the transition from deep violet to light yellow at 517nm. In a nutshell, 2 mL of various doses of the extracts (0.2 - 1.0 mg/mL) were combined with 0.5 mL of a 0.3 mM DPPH solution in methanol. The reaction tubes were shaken and let to sit at room temperature in the dark for 15 minutes while the absorbance was measured at 517 nm. Every test was run in triplicate. Ascorbic acid was used as a standard control at concentrations comparable to those used in the preparation of the test samples. The test samples were a blank made up of 0.5 mL of 0.3 mM DPPH and 2 mL of methanol. The radical scavenging activity was determined using the formula: DPPH radical scavenging activity (%) = [(A0A1)/ (A0)] 100, where A0 was the absorbance of the DPPH radical + methanol and A1 was the absorbance of the DPPH radical + sample extract or standard. The amount of extract that must be present in order for it to be effective in scavenging 50% of DPPH free radicals is known as the 50% inhibitory concentration value (IC50).

Phytochemical Analysis of *Ganoderma lucidum*

Estimation of Total Phenolic Content (TPC)

The Folin and Ciocalteau's method was used to determine the total phenolic content. Gallic acid concentrations (10–120 g/mL) were made in methanol. Then, 2.5 mL of a ten-fold diluted Folin-Ciocalteau reagent and 2 mL of 7% sodium carbonate were combined with 0.5 mL of the sample (1 mg/mL). The mixture's absorbance was measured at 760 nm after it had been allowed to stand at room temperature for 30 minutes. Gallic acid was used as the reference control in all determinations, which were carried out in triplicates. Gallic acid was used as the positive control throughout all determinations, which were carried out in triplicates. Gallic Acid Equivalent was used to express the overall phenolic content (GAE).

Estimation of Total flavonoids Content

The scavenging of free radicals, chelation of metal ions like iron and copper, and inhibition of enzymes that produce free radicals are only a few of the methods by which flavonoids'

antioxidative activities are brought about (Benavente-Garcia, 1997). Flavonoids have the potential to neutralize nearly every known ROS, depending on their structural makeup. Utilizing the aluminum chloride method, one can calculate the total flavonoid content (Chang et al., 2002). After incubating the reaction mixture (3.0 ml) containing 1.0 ml of extract, 0.5 ml of aluminium chloride (1.2% in ethanol), and 0.5 ml of potassium acetate (120 mM) at room temperature for 30 minutes, the absorbance at 415 nm is measured. Catechin (Kim et al., 2003) or quercetin (Ordonez et al., 2006) in methanol can be used as positive controls. In terms of standard equivalent, the flavonoid content is stated (mgg-1 of extracted compound).

Estimation of Proanthocyanidin Content

The Sun et al. (1998) technique was used to determine the presence of proanthocyanidin . It is based on the butanol-HCl reagent's oxidative depolymerization of condensed tannins. 1 mL of 4% vanillin in methanol solution and 0.75 ml of strong hydrochloric acid were added to 0.5 ml of each extract that contained 1.0 mg/ml. After 15 minutes of untouched mixing, the absorbance was measured at 500 nm. Ascorbic acid served as the benchmark.

Estimation of Saponin Content

The method used by Ifemeje et al. (2014) to determine the saponin content was used. A water bath at 50°C was used to soak 1 g of the sample in 20% acetic acid in ethanol for 24 hours. A water bath was used to concentrate the extract to one-fourth of its original volume after it had been filtered. Then, until the precipitate was fully formed, concentrated NH_4OH was added drop by drop to the extract. The entire solution was given time to settle, and the precipitate was then filtered, collected, and weighed. The following formula was used:

Where, W1 = Weight of empty filter paper

W2 = Weight of filter paper + Saponin

Estimation of Alkaloids Content

The method used by Ifemeje et al. (2014) to determine alkaloid content was used. 40 ml of 10% acetic acid in ethanol and one (1 g) of the plant sample were each added to a 250 ml beaker. At 25°C, or room temperature, the mixture was covered and let to stand for 4 hours. Following filtration using filter paper No. 42, the filtrate was concentrated on a water bath until it was one-fourth the size of its initial volume. Until the precipitation was

finished, concentrated NH_4OH was applied drop by drop. After allowing the mixture to settle, the precipitate was collected on filter paper that had been weighed, and was washed out with weak NH_4OH. After drying, the precipitate was weighed. The following formula was used:

Where, W1 = Weight of empty filter paper

W2 = Weight of filter paper + Alkaloid

Chromatography Analysis of the mushroom Extract

Fragmentation analysis was done after the chemical makeup of *Ganoderma lucidum* extracts was examined using the GC/MS technique. The HP5MS capillary column (bonded and cross-linked 5% phenyl-methylpolysiloxane, 30 mm x 0.25 mm, coating thickness 0.25 m) and Thermo Scientific DSQ II Single Quadrupole GC/MS were employed in the electron impact (EI) ionisation mode (70 eV) (Restek, Bellefonte, PA). Temperatures were set at 220 C for the injector and detector. Oven temperature was maintained at 50°C for 30 minutes before being increased to 240°C at a rate of 3°C/min. At a flow rate of 1 ml/min, helium (99.99%) served as the carrier gas. The molecular weight of the breakdown products was measured using a scan range of 60-200 g/mol. Manual injection of diluted samples (1/100 in chloroform, v/v) in quantities of 1.0 L was carried out in accordance with the comparison of their mass spectra (Avci et al., 2014).

Statistical Analysis

Using SPSS, descriptive and inferential statistics were applied to the collected data (v20 incorporation, Chicago, Illinois, USA).

RESULTS

As shown in figure 1, Vitamin C demonstrated higher percentage DPPH radical-scavenging activity compared to aqueous extracts of *G. lucidum* in this study, with the highest activity recorded at 100 hours (89.79%). Comparing the samples, it was found that Sample A had higher percentage (%) DPPH radical scavenging activity than Sample B, with the highest value being recorded at 100 hours (67.85%). The graph also demonstrated how rising time reflected rising scavenging activity in sample B which could potentially rise above Sample A with respect to increasing time and duration. Total Antioxidant Capacity of aqueous *G. lucidum* extracts is reported in figure 2. According to the study's data, Sample A had a higher

total antioxidant capacity (56.79 mg/ml) than Sample B, which had the lowest capacity (51.71 mg/ml). Sample A was made with a shorter boiling time.

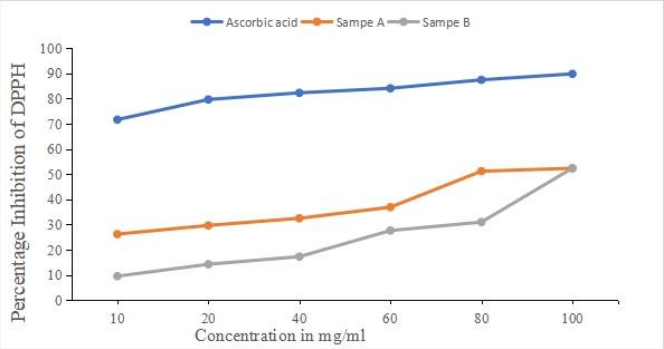

Figure 1: Percentage DPPH radical-scavenging activity of aqueous *G. lucidum* extracts

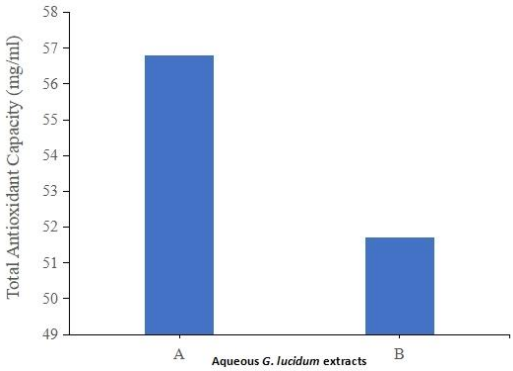

Figure 2: Total Antioxidant Capacity of aqueous *Ganoderma lucidum* extracts

Phytochemical Composition of *Ganoderma lucidum* Extract

Table 1 provides information on the phytochemical composition of aqueous extracts from *G. lucidum*. The table compares the composition of two different samples, labeled as Sample A and Sample B. As shown in table 1, higher percentage alkaloids (3.00 ± 0.12 %) and TAC (56.79 ± 0.04 µg/ml) were only recorded in sample A while percentage Saponins (8.00 ± 0.12 %), Flavonoids (51.50 ± 0.77 µg/ml), Proanthocyanidin (38.23 ± 0.18 µg/ml), Phenol (16.18 ± 0.07 µg/ml) and FRAP (64.00 ± 2.00 mg/ml) were recorded in sample B. As shown in the table, Sample B prepared at a higher boiling time generally exhibits higher values for saponins, flavonoids, proanthocyanidins, phenols and FRAP compared to Sample A which recorded higher values only in alkaloids and TAC, suggesting a potentially greater antioxidant and bioactive profile in sample B.

Table 1: Phytochemical composition of aqueous *G. lucidum* extracts

Phytochemical Components	Composition of aqueous *G. lucidum* extracts	
	Sample A	Sample B
Alkaloids (%)	3.00 ± 0.12	2.47 ± 0.15
Saponins (%)	4.27 ± 0.18	8.00 ± 0.12
Flavonoids (µg/ml)	32.82 ± 0.20	51.50 ± 0.77
Proanthocyanidin (µg/ml)	19.13 ± 0.04	38.23 ± 0.18
Phenol (µg/ml)	6.08 ± 0.02	16.18 ± 0.07
FRAP (mg/ml)	22.67 ± 0.88	64.00 ± 2.00
TAC (µg/ml)	56.79 ± 0.04	51.58 ± 0.07

Values were expressed in mean ± SEM across the parameters

Chromatographic profile of the phytochemical constituents of aqueous *G. lucidum* Extracts

Figure 3 and 4 shows the chromatographic profile of the phytochemical constituents of aqueous *G. lucidum* extracts Sample A (boiled for 1 hour) and B (boiled for 3 hours) respectively. Figure 3 showed that there were 32 peaks with the highest peak recorded at PEAK 22 (31.317). Constituents identified were Pyrrolidine; 1-methyl-3, 2'-spiro-benzo-1, 3-dioxolane-Benzene; 1,3,5-tris(2,2-dimethylpropyl)-2-iodo-4-nitro-Propanamide and 2,2-dimethyl-N-(3-methylphenyl)- while the lowest observable peak was recorded at PEAK 1 with constituents identified as hexadecane. Figure 4 showed that there were also 32 peaks with the highest peak recorded at PEAK 37 (35.927). Constituents identified were Spiro[2.3]hexan-4-one, 5,5-diethyl 9-Octadecenoic acid (Z)-, 2-hydroxy-1-(hydroxymethyl)ethyl ester Oleic acid and 3-hydroxypropyl ester while the lowest observable peak was recorded at PEAK 1 (8.773) with constituents identified as Undecanoic acid, 10-methyl-, methy l ester Dodecanoic acid, methyl ester Dodecanoic acid and methyl ester.

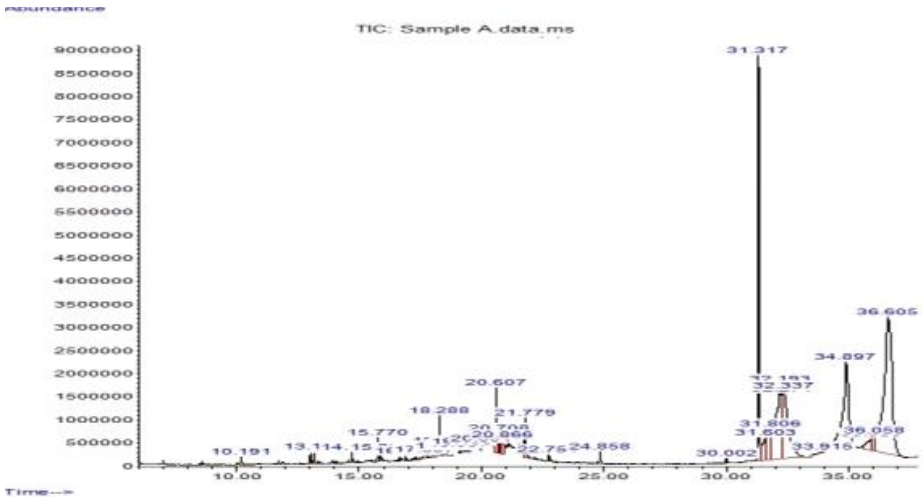

Figure 3: HPLC chromatogram of aqueous *Ganoderma lucidum* extract (Sample A)

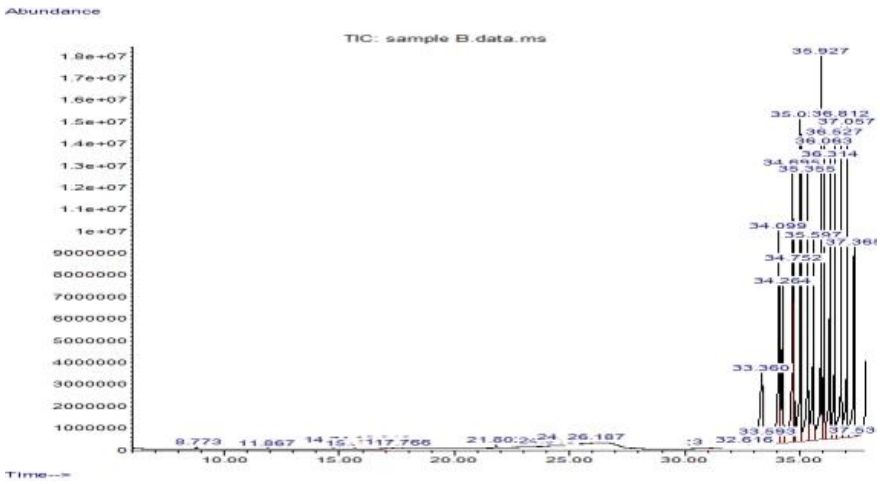

Figure 4: HPLC chromatogram of aqueous *Ganoderma lucidum* extract (Sample B)

DISCUSSION

The nutraceutical and therapeutic investigations of mushrooms provides the impression that they are the next generation food, not only in providing quality daily dietary required protein but also in fighting against diseases with high mortality rate such as emerging bacterial, fungal and viral diseases, tumours, cancers and nervous disorders (Sankaranarayanan et al., 2020). Many researches have confirmed the extraction of antioxidant, phytochemical, and antibacterial properties from a variation of plants, including mushrooms. Aqueous extraction at two different starting times was identified in the current investigation. According to the findings of the current investigation, aqueous *G.*

lucidum extract that was cooked for three hours demonstrated larger percentages of DPPH radical scavenging activity and antioxidant capacity. In addition, it was shown that these values were inferior to ascorbate. Jonathan and Awotona (2010) investigated the antibacterial properties of an aqueous and an ethanol extract of *G. lucidum* in a different investigation. According to the study, these Mushroom (Ganoderma) species' crude and pure extracts both showed varying degrees of inhibition against the test organisms. The implications of these findings in the current study shown and demonstrated that an aqueous *G. lucidum* extract that has been cooked for three hours has significant potential to treat a variety of illnesses and disorders, including tumours, inflammations, and malignancies, Sankaranarayanan et al. (2020) obtained similar results. The findings of this investigation, which include DPPH radical scavenging activity and antioxidant capacity are in line with those of Kang et al. (2019) and Uddin *et al.* (2021). The outcomes also agreed with a previous study by Mahdi-Pour et al. (2012) that showed *Ganoderma* species were employed in the creation of functional meals.

Various *Ganoderma spp*. extracts have been shown to have varying levels of scavenging efficacy capability. The extraction solvent and the environmental circumstances under which the mushroom was gathered may have contributed to these variances in antioxidant activity. In conclusion, Ogidi and Oyetayo (2016) have shown that the ability of aqueous extracts of *Ganoderma spp* to scavenge H_2O_2 suggests that they may be used in the creation of herbal medications and as nutraceutical agents. In this investigation, the phytochemical components phenol, proanthocyanin, alkaloid, saponin, and flavonoid were reported. Phenol, Proanthocyanin, saponin, and flavonoid concentrations were shown to rise with longer boiling times, but alkaloid composition increased with less heat treatment. According to Kothiyal and Singh (2022), the mushroom fruiting body includes a variety of bioactive compounds, including alkaloids, flavonoids, polyphenols, and polysaccharides. The majority of the antioxidant activities noted by various writers may be attributed to these substances.

In this work, the aqueous *G. lucidum* extractions were found to contain phenol. According to Rahimah et al. (2019), phenol is the primary substance believed to be connected to the antioxidant benefits of mushrooms. Singh et al. (2015) revealed that phenol has been shown to protect the liver, lungs, and kidneys, indicating that it may be the main factor eliciting the antioxidant activity of *Ganoderma spp* as seen in this study. In the current investigation, longer boiling times of the *Ganoderma spp* extract resulted in larger

concentrations of flavonoids and proanthocyanin. Flavonoids and proanthocyanin are significant because they are effective in regulating and preventing tissue damage brought on by the presence of triggered, radical, or singlet oxygen species, claim Sankaranarayanan et al. (2020).

Low concentrations of alkaloid (3.2 mg/ml) and saponin (8.2 mg/ml) were found in the current investigation. According to Ihayere and Okhuoya (2022), the alkaloids concentration in *Ganoderma spp* was dissimilar from what Wood et al. (2021) had claimed. According to the study, variations in culture and fruiting circumstances, substrate, the mushroom's developmental stage, and the maturity of the fresh mushrooms sample all have an impact on the bioactive compounds in mushrooms like *Ganoderma spp*. The findings of this study concurred with a study by Sitati et al. (2021), which found that the aqueous extract of wild edible termitophilous mushrooms had a greater percentage yield and included alkaloids. Although the extraction solvent was specified, Ogidi et al. (2021) observed that the mushroom samples had the greatest alkaloids content (0.440.01%), adding that alkaloids are most potent therapeutically significant bioactive components. According to the study, alkaloids are used as medicines because of their antispasmodic, analgesic, and antimicrobial properties in pure form or as synthetic drug derivatives. In conclusion, alkaloids have been described as a class of fundamental organic compounds with microbial and plant origins that interfere negatively with the functioning of microorganisms' DNA by possessing at least one nitrogen atom in a ring structure. Several structurally similar chemicals with a steroid or triterpenoid aglycone make up the broad class of substances known as saponins (Wandati et al., 2013). In a previous work, Ogidi and Oyetayo (2016) found 16.60 mg/g of saponin in ethyl acetate fraction of *G. lucidum*. The ethanol extract reported by Ogidi and Oyetayo (2016) produced more saponin than the aqueous extract when compared to the values found in the current investigation. This could be due to the saponins' steroid component's solubility in ethanol. These results led Lee et al. (2012) to conclude that saponins possess a wide range of pharmacological qualities that have a variety of positive effects, such as anti-inflammatory and anti-diabetic capabilities.

The results of this analysis revealed that both samples contained 32 peaks, with PEAK 22 (31.317) in sample A, which had been boiling for one hour, having the greatest peak. The lowest apparent peak was observed at PEAK 1, with ingredients classified as hexadecane. Other contents were pyrrolidine, 1-methyl-3, 2'-spiro-benzo-1, 3-dioxolane-benzene, 1, 3,

5-tris (2, 2-dimethylpropyl)-2-iodo-4-nitro-propanamide, and 2, 2-dimethyl-N-(3-methylphenyl). Additionally, sample B, which was cooked for 3 hours, revealed that PEAK 37 was the highest peak (35.927). The discovered components were Spiro [2 .3] Hexan-4-one, 5, 5-diethyl-9-Octadecenoic acid (Z), 2-hydroxy-1-(hydroxymethyl) ethyl ester Oleic acid, and 3-hydroxypropyl ester. PEAK 1 (8.773) had the lowest detectable peak, which was composed of Undecanoic acid, 10-methyl-, methy-, and methyl ester Dodecanoic acid, as well as methyl ester.

CONCLUSION

The search for new antimicrobials is on-going since the routinely prescribed medications have unpleasant side effects and because their indiscriminate and improper usage has increased the number of antimicrobial-resistant strains. Currently, research is being done on edible mushrooms in the hunt for novel antibiotics. In the current investigation, two Ganoderma aqueous extracts significantly the presence of phytochemical components such phenols, proanthocyanin, flavonoids, saponins, and alkaloids. Before using these extracts to produce any new drugs, more study is required to evaluate their toxicological impact on animal models and to identify the dose.

Recommendation

Further findings as regards the active phytochemical to synthesized novel drug development. The plant should therefore be considered for compound isolation and elucidation.

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Competing Interests

Authors have declared that no competing interests exist.

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