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Original Research Article

Anti-inflammatory and Analgesic Investigations of Methanol Extract of *Ganoderma lucidum*

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ABSRTACT

Ganoderma lucidum is a popular woody and spongy mushroom (fungi) widely distributed throughout the world. It is commonly used in the production of nutriceuticals, functional foods and also serves as a therapeutic herb used in the treatment of several diseases. This study was aimed at evaluating the phytochemicals, proximate composition, antioxidant, anti-inflammatory and analgesic activities as well as acute toxicity of the crude methanol extract of *G. lucidum*. The phytochemicals, proximate composition and antioxidant potential were determined using already established methods. The formalin-induced inflammation and acetic acid-induced writhing techniques were applied to evaluate the anti-inflammatory and analgesic activities respectively. Phytochemicals detected were saponins, flavonoids and terpenoids. The moisture content, acid insoluble ash, water soluble ash, total ash, alcohol extractive value and water extractive value were 12.53 ± 0.18%, 1.45 ± 0.21%, 2.68 ± 0.51%, 3.31 ± 0.22%, 1.41 ± 0.00% and 1.07 ± 0.01% respectively. The IC_{50} values for the DPPH radical scavenging capacity of the extract and ascorbic acid (standard) were 31.56 ± 1.30 and 18.84 ± 2.06 µg/mL respectively. The crude extract at the dose of 50 mg/kg body weight showed the highest % inhibition of edema after 4 hours and there was a significant decrease ($p < 0.05$) in the number of writhes in a dose dependent manner. In the oral administration of the crude extract to Swiss mice, 100% mortality was recorded at 5000 mg/kg. The study confirms that *G. lucidum* is a potential source of phytomedicine with substantial pharmacological and antioxidant properties but however, could be toxic at higher doses.

Keywords: *Ganoderma lucidum*, Anti-inflammatory, Analgesic, Antioxidant, Phytochemicals.

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Introduction

Nature has provided sufficient supply of medicine to cure diseases by imbibing them in vast warehouses called medicinal plants. These "warehouses" (medicinal plants) contain chemicals which though may not be nutritional, but exhibit some characteristics that make them disease fighting agents.[1,2] Medicinal plants are a rich spring of bioactive phytochemicals and bio- nutrients which are used for the curing of several human diseases. It has been shown in the last two to three decades that these phytochemicals play important roles in preventing diseases like cancer and diabetes.[1] It is estimated that approximately one quarter of prescribed drugs are plant derived drugs.[3] Phytochemicals (from Greek *phyto*, meaning "plant") are compounds which are produced by plants through primary or secondary metabolism. These compounds shield plants from diseases and damages and also contribute to the aroma, flavour and colour of plants. The medicinal attributes of different plants e.g. antioxidant, anti-inflammatory and analgesic activities are as a result of the active phytochemicals and useful chemical composition in different parts of the plant.[4]

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Inflammation can be viewed as the composite genetic reaction of vascular tissues to destructive stimuli like irritants or pathogens, through a protecting effort by the organism to get rid of the damaging stimuli and also commence the restorative development for the tissue.[5] Inflammation has become the focus of international scientific exploration due to its impact on both animal and human illnesses.[5]

Also, analgesics are medications that relieve pain. Meanwhile, pain is an unfriendly emotive and sensual experience which is triggered through actual or prospective tissue impairment.[6] The emotive manifestation of pain differs from one individual to the other and from time to time in the same individual. In therapeutic application, pain is classified into nociceptive and neuropathic.[6] Although pain is advantageous to our immune system, yet, it brings severe grief and distress to the sufferers, reducing life expectancy and as a result, needs to be treated. In order to subdue pain, non-steroidal anti-inflammatory drugs (NSAIDs) are prescribed all over the world.[7,8] However, prolong application of these NSAIDs can only offer asymptomatic relief but one of their grave consequences is toxicity to the gastrointestinal linings, kidney and liver.[9] Based on this, herbal medications sourced from plants are being used in corresponding and substitute medicines (CSM) for the cure and management of inflammations, pains and other related diseases.[10] It is important to note that several synthetic analgesics, anti-pyretic and anti-malarial drugs like morphine, aspirin, atrophine, artemisinin, and chloroquine were all obtained from plants.[11] *Ganoderma lucidum* is the binomial name given to the wild mushroom which is known as "Lingzhi" in Chinese, "Reishi" by the Japanese, "Hangul" or "Yeongji" in Korea. It is as well referred to "Glossy ganoderma" or "shiny polyporus" in English, and usually called "Leman kwado" or "Burtuntuna" in Hausa.[12] It is a basidiomycete white rot fungus[13] that belongs to the family *Polyporaceae* (or *Ganodermataceae*) of *Aphyllophorales*. In the rainy season, it is rarely found growing at the base of stumps of deciduous

Plate 1: *Ganoderma lucidum*

trees.[14] This mushroom belongs to the kingdom "fungi" from the phylum - *Basidiomycota* and in the class – *Agaricomycetes* and order - polyporales, family - *Ganodomataceae* and genus - Ganoderma.[15] This mushroom is a polypore which is soft when fresh, flat and corky with an obvious red-vanished kidney shaped cap, and white to dull brown pores are found underneath depending on the age.[16] Its therapeutic importance is well documented in the Chinese literature dated back closely two thousand years to the Shen Nong Materia Medica (102–200AD). In the Chinese custom, it is considered as a sign of immortality, happiness, good health and even good fortune.[17]
The mushroom has a universal distribution and found growing in both tropical and temperate geographical regions including Europe, North and South America, Africa and Asia. Its fundamental medicinal value is based on its acknowledged health benefits along with no side effects.[18] Among its several curative doles, the mushroom has been reported to possess anti-tumor activity, immunomodulatory and immunotherapeutic application. It is also documented to have activity against diabetes and hypertension along with microbial activity on several micro-organisms such as *Bacillus cereus*, *Aspergilus niger*, *Escherichia coli* and *Candida albicans*. This mushroom was also reported to possess antiviral activity with an exact action on HSV-1 and HSV-2, vesicular stomatitis, influenza virus and HIV type 1 or a "fix it all" therapy for diseases.[19,20,21,22] Since these reports were mostly based on aqueous extracts, there is the need to analyze the methanol extract of this wild mushroom in order to further assess its bioactivities. [23] Hence, the aim of this research work was to evaluate the phytochemicals, proximate, acute toxicity, antioxidant, anti-inflammatory and analgesic activities of the extract from *Ganoderma lucidum* fruiting body.

Materials and Methods

Reagents, materials and instruments
All the reagents used were of analytical grade and solutions were prepared from distilled water. These include: 99.8% methanol (JHD, England), dilute HCl, distilled water, ascorbic acid, DPPH, indomethacin, aspirin, acetic acid, ethanol, precision weighing balance, mechanical grinder (British gally hamp), rotary evaporator, vacuum pump, UV-visible spectrophotometer (JENWAY 6320D), water bath, desiccator, muffle furnace, mechanical shaker, laboratory oven, syringe, gavage needles, glass wares and among others.

Collection of plant materials and preparation
The fresh fruiting bodies of *G. lucidum* were collected in May, 2018 from the Nigeria Institute for Oil-Palm Research (NIFOR), Benin City, Nigeria. The fungi samples were identified and authenticated by Dr. Akinnibosun of the Department of Plant Biology and Biotechnology, Faculty of Life Sciences, University of Benin, Nigeria and a voucher specimen number (UBH_G 430) was deposited. The fruiting bodies were rinsed with deionized water, air dried and pulverized by means of a mechanical grinder at the Department of Pharmacognosy, Faculty of Pharmacy, University of Benin. The powdered fruiting bodies were stored in an air-tight container and kept for analysis.

Extraction of plant material
The pulverized plant materials (500 g) were macerated in 2.5 L of methanol with continuous stirring and shaking for 72 hours. The extract was concentrated to dryness using rotary evaporator at reduced pressure and temperature of 40^{o}C. The weight of the extract was taken and then stored in the fridge-freezer for analysis.

Phytochemical Screening
The secondary metabolites contained in the concentrated crude extract were qualitatively screened. The phytochemicals were assessed using established methods.[24,25,26] The presence of glycosides, saponins, flavonoids, tannins, steroids, terpenoids, and alkaloids were investigated.

Proximate analysis
Quantitative parameters such as moisture content, water extractive value, alcohol (ethanol) extractive value, total ash, acid insoluble ash and water soluble ash were analyzed using standard methods.[27]

Antioxidant activity
The scavenging potential of the crude methanol extract of *G. lucidum* on DPPH free radical was estimated by applying the technique designed by Jain *et al.*[28] The capacity to scavenge DPPH radical was estimated using the equation below:

$$\text{DPPH radical scavenging ability (in percent)} = \frac{A0-A1}{A0} \times 100$$

Where: A_0 = DPPH radical absorbance in methanol
A_1 = DPPH radical absorbance + sample extract/standard in methanol.

The IC_{50} value, otherwise known as the 50% inhibitory concentration was determined by an exponential equation to match data into the concentration-response.

Experimental animals
Forty-five (45) healthy Swiss mice (male and female) weighing between 19 and 25 g were utilized for the anti-inflammatory, analgesic and acute toxicity studies. These animals were acquired from the animal breeding unit in the Department of Biochemistry, University of Benin. The mice were kept in the Animal House section of the Department of Animal and Environmental Biology, University of Benin and were acclimatized for two weeks. They were sustained under the typical environmental settings: 12 hours-day/night cycle and were fed with typical mash grower feeds and water *ad libitum*. They were handled in accordance with the standard protocols guiding handling of experimental animal(s) throughout the experimental period.[29]

Evaluation of acute toxicity
The acute toxicity determination was conducted by applying the new approach to practical Acute Toxicity Testing according to Igbe *et al.*,[30] as revised by Ogbeide *et al.*[31] The mice were distributed into five distinct groups of three mice each which was labeled 1-5. Groups 2-5 were the test groups, while group 1 was the control. Groups 2-5 were administered 5, 50, 500, 5000 mg/kg of the methanol crude extract suspended in gum acacia respectively via oro-gastric syringe, while group 1 (control) was administered 10% gum acacia solution via oral route. The animals were observed for common symptoms of toxicity and mortality within a period of 24 hours, and the animals that survived after 24 hours were further observed for any signs of delayed toxicity for another period of 14 days. The median lethal dose (LD_{50}) value was estimated by the application of the equation below:

$$LD_{50} = \sqrt{D_0 \times D_{100}}$$

Where: LD_0 = Maximum dose without death;
LD_{100} = minimum dose with death

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Anti-inflammatory study (Formalin-induced rat hind paw edema)
The effect of methanol extract of *G. lucidum* on formalin-induced inflammation in rat paw was investigated by following the method described by Winter *et al*,[32] as revised by Ogbeide *et al.*[33] Rats were randomly distributed into five distinct groups, each consisting of three animals. Group 1 serving as a negative control was given only distilled water. Groups 2-4 received 50, 100 and 200 mg/kg of the extract respectively and Group 5, which served as a positive control received the standard drug, aspirin (100 mg/kg). The animals were fasted 12 hours prior to the experiment. All drugs were administered orally. Thirty (30) minutes after oral administration of the test materials, 0.1 mL 1% formalin suspension was injected subcutaneously in the left hind paw of each animal, resulting to edema formation (localized inflammation) *in situ*. The volume of paw edema was measured hourly for four hours using a vernier calliper after administration of formalin. The average percentage increase in paw volume with time was calculated and compared against the control group. Percentage inhibition was calculated using the formula:

$$\% \text{ inhibition} = \frac{D0-Dt}{D0} \times 100.$$

Where, D_0 = average value of the inflammation or hind paw edema of the control group of mice at a particular time;
D_t = average inflammation of the treated group (extracts or reference Aspirin) of mice at a given time.

Evaluation of analgesic activity (Acetic acid-induced writhing in mice)
The peripheral analgesic activity of the methanol extract of the fruiting bodies of *G. lucidum* was determined by the acetic acid-induced writhing inhibition method as described by Akuodor *et al.,*[34] with little adjustments. The prescreened Swiss mice employed for the experiment were distributed into five distinct groups. The extracts were administered orally at 50, 100 and 200 mg/kg body weight. Inhibition of writhing in mice by the plant extract was compared with the inhibition of writhing by a standard analgesic (indomethacin) which was orally given at a dose of 20 mg/kg. Acetic acid (0.7%) at a dose of 0.1 mL/10 g was administered intraperitoneally (i.p.) to generate sensation of pain. The number of writhes was calculated for 10 minutes immediately after the acetic acid injection. Decrease in the writhing when compared with that of the control group was taken as a reflection for analgesia. Analgesia index was evaluated as percentage writhing inhibition and estimated from the equation given below:

$$\% \text{ Writhing inhibition} = \frac{C-D}{C} \times 100$$

Where, C = average writhing number for the control group of mice;
D = average writhing number for the drug and extract treated mice.

Statistical Analysis
The results are presented as mean ± standard error of mean (SEM). The significant difference was evaluated by applying "Newman-Keuls Multiple Comparison Test ANOVA". Values with $p \leq 0.05$ were taken to be significant.

Results and Discussion

Phytochemical Screening
Phytochemicals have continued to be a major source of drugs for pharmaceuticals due to their several medicinal potentials. The qualitative phytochemical investigation of the crude methanolic extract of *G. lucidum* revealed the presence of saponins, flavonoids and terpenoids (Table 1). The presence of these three secondary metabolites in the extract confirms its uniqueness as essential medicinal plant. Saponins, flavonoids and terpenoids have been known to exhibit several biological activities such as haemolyses in erythrocytes, anti-inflammatory, antibacterial, antifungal, antimutagenic, antioxidant and antiviral effect according to Aghedo *et al*.,[35] and several researchers.

Proximate analysis
The results of the proximate analysis are presented in Table 2. The value obtained for moisture content was found to be 12.53 ± 0.18% w/w. The lower the moisture content of a drug, the better its capacity to prevent the growth of yeast, bacteria and fungi during storage.[36] It has been recommended and is generally accepted that the moisture content of crude drugs should not exceed 14%.[36] The moisture content helps us to evaluate the vulnerability of a crude plant sample to microbial and hydrolytic degradation.[37] This implies that the phytochemicals present in the crude drug sample might not be susceptible or vulnerable to microbial and hydrolytic degradation. The mean total ash obtained from the analysis was 3.31 ± 0.22% w/w. The total ash is an amount of the residue left after ashing. This residue is made up of non-volatile inorganic constituents. The mean acid insoluble ash obtained was 1.45 ± 0.21% w/w. Acid insoluble ash is the degree of the sandy matter in the crude drug sample.[38]. Water soluble ash obtained was 2.68 ± 0.51% w/w which gives a strong quantitative indication of purity and quality of the material.[37] The extractive values give an information on which of the solvent is a better extractor. Hence, from the result obtained, alcohol is a better solvent extractor for the sample than water.

Table 1: Phytochemical constituents of crude extract of *G. lucidum*

Phytochemicals	Results
Glycosides	–
Saponins	+
Flavonoids	+
Tannins	–
Steroids	–
Terpenoids	+
Alkaloids	–

(+) = Present and (-) =Absent

Table 2: Results of proximate analysis of crude extract of *G.lucidum*

Parameters	Values %w/w ± S.D
Moisture content	12.53 ± 0.18
Total ash	3.31±0.22
Acid insoluble ash	1.45 ± 0.21
Water soluble ash	2.68 ± 0.51
Alcohol extractive value	1.41 ± 0.00
Water extractive value	1.07 ± 0.01

Table 3: DPPH-scavenging potential of crude extract of *G.lucidum*

Concentrations (µg/mL)	Ascorbic acid (% Inhibition)	Crude extract (% Inhibition)
50	40.31 ± 0.07	30.54 ± 1.20
100	50.10 ± 1.09	38.70 ± 0.00
150	60.80 ± 1.59	40.80 ± 1.07
200	80.51 ± 1.00	42.55 ± 0.05
250	90.04 ± 2.00	45.60 ± 0.50

Values are mean ± standard error of the mean of triplicate analysis

Antioxidant estimation

DPPH free radical scavenging activity of *G. lucidum* extract showed an appreciable and dose-dependent increase in scavenging effect for the standard (ascorbic acid) and crude extract (Table 3). At the highest concentration (250 µg/mL), the percentage inhibition of the crude extract obtained was 45.60 ± 0.50% while the percentage inhibition of ascorbic acid (standard) obtained was 90.04 ± 2.00%. The IC_{50} value is the concentration which will inhibit 50 percent of the original DPPH radical. It should be noted that the lesser the concentration of inhibition at IC_{50} (50%) value, the greater the strength in scavenging free radicals.[39] From Table 4, the extract showed appreciable antioxidant activity with an IC_{50} value of 31.65 ± 1.30 µg/mL. However, this was less active than the reference, ascorbic acid with an IC_{50} value of 18.84 ± 2.06 µg/mL. This antioxidant activity displayed by the extract could be attributed to the phytochemicals earlier detected.

Anti-inflammatory and analgesic studies

Formalin-induced inflammation is one of the suitable experimental techniques used for determining the anti-inflammatory properties of compounds or natural products.[40] It shows some level of reproducibility. Inflammation induced by formalin resulted in the formation of the rat paw edema, which is, swelling of the paw. The result in Table 5 shows the level of significance across the control and treated groups in the formalin induced edema in mice. The methanolic extract of *G. lucidum* at doses of 50, 100 and 200 mg/kg body weight and the standard drug (100 mg/kg Aspirin) was found to significantly ($p < 0.05$) decrease the licking time compared with that of the control within a specified time interval.

The result of this study shows dosage-dependent activity of the extract in inhibiting inflammation with the highest % inhibition of 30.36 at 50 mg/kg (least dosage) and lowest % inhibition of 26.00 at 200 mg/kg (highest dosage) after 4 hours (Table 6). The % of inhibition with reference to the drug, aspirin, was 33.59, hence the plant showed anti-inflammatory activity in comparison with the standard drug. Therefore, it can be inferred that this extract might have provided defense to counter the activities of chemo-irritants and inflammatory agents and also the activation of chemoreceptors.[37,41]

Acetic acid-induced writhing test is a widely applied model of instinctual pain and it is a highly sensitive test for analgesics. It mainly comprises histamine mediators, histaminic peritoneal receptors, cholinergic and acetylcholine. It finds application in assessing the peripherally acting analgesics.[10,40] Similarly, from the acetic acid-induced writhing experiment, results obtained reveal that the extracts considerably lowered the writhing response, as intraperitoneal inoculation of the acid formed abdominal writhing through the activation of chemo-sensitive nociceptors.[41] However, the extract acted as a defense to the animals, thereby, displaying analgesic effect. The maximum analgesic effect of the pod extract was manifested at a dose of 200 mg/kg with a reduction of the amount of writhes by 72.61%. Whereas 100 mg/kg dose reduced the amount of writhes by 58.13% and 50 mg/kg reduced the amount of writhes by 54.21% showing a dose-dependent pattern (Table 7). This dose-dependent pattern of inhibiting acetic acid-induced writhing exhibited by the extract is a reflection of the outlying effect. Hence, it is evocative of the dose related mode of general herbal formulations in the management of pain and diseases relating to pain.[42] Acetic acid writhing inhibition indicates that the extract could have crucial repercussion and depressant influence on the nervous system; this is because depressants of the central nervous system are well-known to prevent or decrease the amount of writhes in the acetic acid pain experiments.[43,44] The presence of alkaloids, and flavonoids in *G. lucidum* could be attributed for its anti-nociceptive property because these phytoconstituents have been shown to possess anti-inflammatory as well as analgesic activities.[45,46]

Acute toxicity study

From the results of the acute toxicity study (Table 8), it was observed that 5 and 50 mg/kg body weight produced 0% mortality. Similarly, 500 mg/kg body weight produced 33% mortality which implies that only one animal died at this dose level. However, it was observed that 5000 mg/kg body weight produced 100% mortality. Since Hodge and Sterner[47] and Lorke[48] reported toxicity standard stating that any compound having an oral intake LD_{50} between 500 and 5000 mg/kg should be considered as virtually "slightly toxic". Hence, the *G. lucidum* extract could be said to be slightly toxic.

Table 4: IC_{50} values of *G. lucidum* extract and standard (ascorbic acid)

Sample	IC_{50} value (µg/mL)
Ascorbic acid	18.84 ± 2.06
Crude extract	31.56 ± 1.30

Table 5: Effect of *G. lucidum* extract on formalin induced inflammation in rat paw

Groups	Doses (mg/kg)	1hr	2hrs	3hrs	4hrs
Control	DW	5.53 ± 0.15	5.77 ± 0.15	5.50 ± 0.29	5.27 ± 0.15
Aspirin	100	4.00 ± 0.23	3.90 ± 0.06	3.90 ± 0.06	3.50 ± 0.17
G.l extract	50	4.50 ± 0.29	4.50 ± 0.00	4.00 ± 0.06	3.67 ± 0.09
G.l extract	100	4.50 ± 0.29	4.50 ± 0.29	4.27 ± 0.15	3.80 ± 0.00
G.l extract	200	4.00 ± 0.00	4.20 ± 0.15	4.20 ± 0.12	3.90 ± 0.06

G.l = *Ganoderma lucidum*, Values are mean ± SD. (n= 3 mice); P-value > 0.05

Table 6: %Inhibition of *G. lucidum* extract on formalin Induced paw edema on rat

Groups	Doses (mg/kg)	% inhibition 1hr	% inhibition 2hrs	% inhibition 3hrs	% inhibition 4hrs
Control	DW	0	0	0	0
Aspirin	100	27.67	32.41	29.09	33.59
G.l extract	50	18.63	22.01	27.27	30.36
G.l extract	100	18.63	22.01	22.36	27.89
G.l extract	200	27.67	27.21	23.64	26.00

Table 7: Effect of *G. lucidum* extract on acetic acid induced peripheral pain in mice

Group	Dose (mg/kg)	Number of writhing per 10 minutes	Inhibition of pain (%)
Control (DW)	0.5 mL	38.33 ± 4.04	-
Indomethacin	20	8.05 ± 1.10**	79.00
G.l extract	50	17.55 ± 1.50**	54.21
G.l extract	100	16.05 ± 0.00**	58.13
G.l extract	200	10.50 ± 2.00**	72.61

Table 8: Acute toxicity result of methanol extract of *G. lucidum* on mice.

Group	Doses (mg/kg)	Number of lethality	Percentage mortality
Control	DW	0/3	0
G.l extract	5	0/3	0
G.l extract	50	0/3	0
G.l extract	500	1/3	33
G.l extract	5000	3/3	100

Conclusion

The results illustrated that the methanol extract of *G. lucidum* fruiting body contains certain phytochemicals which could be responsible for its antioxidant activity, dose-dependent anti-inflammatory and analgesic activities. However, it could be slightly toxic at a higher dose, so therefore, great caution must be taken when being administered for medicinal purposes. It is recommended that bioactive compounds present in the extract be isolated, purified and characterized.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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