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Trends in Assessment of *Ganoderma lucidum* Methanol Extract Against MRSA Infection *In Vitro* and *In Vivo* with Nutrition Support

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ABSTRACT

Objectives: *Ganoderma lucidum*, a traditional Chinese mushroom, has been widely used for the management of antibacterial infection and nutrition. This study was conducted to investigate the protective effect of *Ganoderma lucidum* methanol extract against MRSA infection *in vitro* and *in vivo* in case of made special diet for albino rates. **Methods:** *Ganoderma lucidum* was collected then form extracted using methanol and characterized using GC-MS. *In vitro* assay to evaluate anti-MRSA activity was done using Agar well diffusion method with MIC determined. *In vivo*, total of 32 male and female albino rats of 6-8 weeks with a body weight of (115 – 120) g were divided into four groups with 8 rats in each: negative control, positive control injected IP with 200 micro of MRSA (8×10^8 CFU/mouse) and 2 groups have injected IP with 200 micro of MRSA (8×10^8 CFU/mouse) with *G. lucidum* methanol extract dose of (125 and 250 mcg respectively) for 3 weeks, blood samples and lungs were collected for examination. **Results:** Treatment of albino rats with *Ganoderma lucidum* methanol extract reduced the histological changes due to lung inflammation induced by MRSA infection and significantly reduced increasing in the lactate dehydrogenase (LDH) levels. Also, it shows a good antibacterial effect against MRSA colonies growth which supports the antibacterial therapeutic effect of *G. lucidum*. **Conclusion:** Present study concluded that *G. lucidum* methanol extract has potent antibacterial effect against MRSA infected albino rats, and it's effective as protective against lung inflammation induced by MRSA infection.

Keywords: *G. lucidum*; Nutrition; Diet; MRSA; Antibacterial activity.

INTRODUCTION

Mushrooms contain compounds as source of nutrition and play role as bioactive compounds¹. *Ganoderma lucidum* (*G. lucidum*) is a kind of mushroom contains several triterpenoid substances as nutrients with an evaluation of their therapeutic effects that exert a certain degree of antibacterial activity against rang of Gram-positive as well as Gram-negative bacteria like

Bacillus cereus, *Enterobacter aerogenes*, *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*². These compounds include triterpenoids, alkaloids, nucleosides, fatty acids and amino acids that play an important role in traditional application and pharmacological activities. Currently, *G. lucidum* is used as a powder, tea and nutritional complement. Methicillin-resistant *Staphylococcus aureus* (MRSA) has become one of the most important

current threats to public health due to its increased virulence and resistance to an increasingly broad spectrum of antibiotics³. MRSA is being reported as main cause of lung infection and inflammation as well as respiratory failure associated with respiratory infections and pneumonia caused by MRSA⁴. In recent years, a great deal of progress has been made in the management of bacterial infection by using natural or chemical substances that may have antibacterial activity^{5,6,7}. So, the current study aimed to investigate the possible antimicrobial activity of *G. lucidum* methanol extract against multidrug resistant MRSA *in vitro* and *in vivo* with study the impact of nutrition *in vivo* study.

MATERIAL AND METHODS

Chemicals, microorganism strain and experimental animals

All chemicals and Muller Hinton Agar media and Muller Hinton Broth media that used in this study were purchased from Sigma-Aldrich, Egypt. Methicillin-Resistant *Staphylococcus aureus* (MRSA) ATCC® BAA-2313™ were collected from Culture Collection Unite, the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University, Egypt. Thirty Two albino rats (16 male and 16 female) of Sprague dawley, aged 6-8 weeks with a body weight of 115-120g were obtained from EGY VAC (The Egyptian Company for production of vaccines, sera and drugs (Vacsera)

Collection and Identification of mushroom

Mushroom samples were collected from USA. Mushroom was identified by fruiting body, gills, stem, smell, texture and habitat.

Formation of mushroom methanol extraction

The fresh fruiting bodies of *G. lucidum* were washed with sterile water, cut into small piece using a stainless-steel blade and lyophilized with a Lyphilyzer Labconco Free Zone 2.5 Liter Benchtop Freeze Dry System (Kansas City, MO, USA). Samples free of moisture with crunchy appearance were separately crushed into fine powders using a blender. 50 g of crushed samples were mixed with 200 ml of methanol and were kept in a shaker at 120 rpm and 30 °C for 48 hrs. The extract was filtered using Whatman No. 4 filter paper. The residue was extracted with two additional 200ml of methanol, as described earlier. Extract were evaporated using a rotary evaporator to remove the solvents and obtained the soluble components of the samples in a paste form⁸.

Characterization of mushroom methanol extract by (GC-MS) analysis

The chemical composition of (*Ganoderma lucidum*) methanol extract samples were performed using Trace GC1310-ISQ mass spectrometer (Thermo Scientific, Austin, TX, USA) with a direct capillary column TG-5MS (30 m x 0.25 mm x 0.25 μm film thickness). *Ganoderma lucidum* methanol extract samples were taken to the Regional Center for Mycology and Biotechnology (RCMB), Al-Azhar University for GC-MS characterization.

Antimicrobial (Anti-MRSA) Susceptibility Testing

30 ml of solidified Muller Hinton Agar media (MHA) containing the suspension of MRSA cultures were poured in sterilized petri dishes with (150 x 20 mm Sterile - Thomas Scientific, NJ, USA). Holes were made by 1 cm cork-borer. The methanol extract were diffused in 5% dimethyl sulfoxide (DMSO) (Sigma-Aldrich) to the concentration of 10 mg/mL then 100 μl of sample was added into well in petri dish. Also, Vancomycin was used as reference antibiotics as positive control and DMSO as negative control. Three replicates were done. The plates were incubated for 2 hrs at 4 ± 2 °C followed by 24 hrs at 37 ± 2 °C⁹.

Determination of Minimum Inhibitory Concentration (MIC)

The MIC values were obtained by assaying visible growth (turbidity) with a micro plate reader (Bio-TekuQuantmicroplate spectrophotometer, VR USA). In brief, a stock of 0.5 mg/ml of extract dissolved in 5% DMSO was diluted serially (1:2) with Muller Hinton Broth media contains 5% DMSO to prepare six dilutions (250, 125, 62.5, 31.2, 15.6 and 7.8 μg/mL). 10 μL of prepared MRSA suspensions (0.5 McFarland) was added. The plates were incubated for 18 hrs at 28 ± 2 °C¹⁰.

Determination of antimicrobial (Anti- MRSA) activity using *In vivo* assay with nutrition support

Animal experimental design

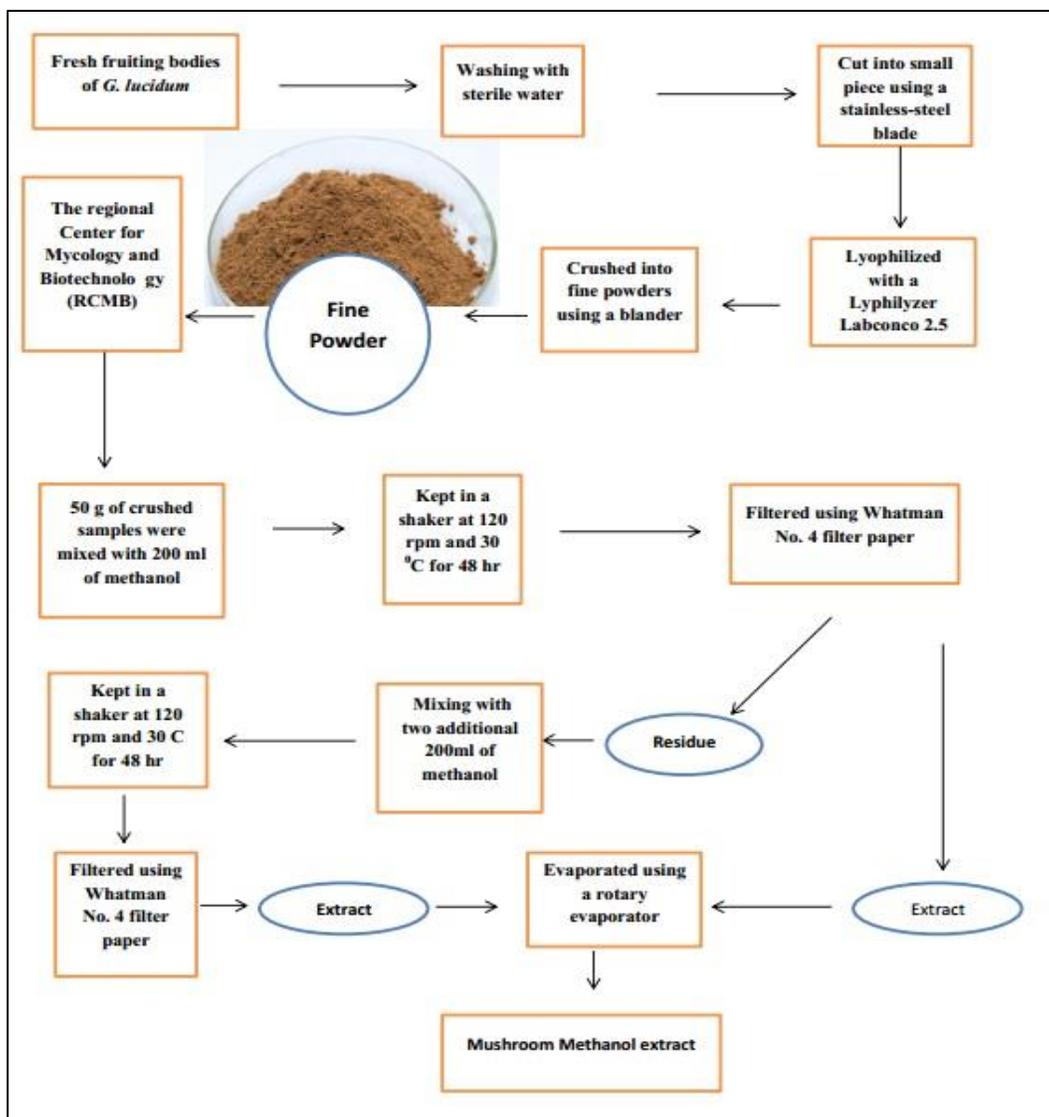
Wistar albino rats were randomly selected and divided into four groups with eight animals in each group.

Group 1: Animals received standard diets and Intra Peritoneal injected (IP) with 200 micron saline (**healthy group**).

Group 2: Animals received standard diets and injected IP with 200 micron of MRSA (8*10⁸ CFU/mouse) (**infected group**).

Group 3: Animals received standard diets containing 125 mcg of mushroom extract and injected IP with 200 micro of MRSA (8*10⁸ CFU/mouse).

Group 4: Animals received standard diets containing 250 mcg of mushroom extract and injected IP with 200 micron of MRSA (8*10⁸ CFU/mouse).



Flow chart: Mushroom (*Ganoderma lucidum*) collection and methanol extraction

All groups received standard diets; both group 3 and group 4 were received standard diets containing 125 & 250 mcg respectively mushroom extract for five days before injected IP with MRSA as a prophylaxis. These were maintained at the animal house of faculty of science, Al-Azhar University, where they put in standard cages with standard food and water at optimum conditions included temperature, light and ventilation. After three weeks of experimental period, rats were anaesthetized using diethyl ether.

Nutrition: Diets used

Standard diet was prepared according to Reeves *et al.*, (1993)¹¹ with some modifications. Diet was purchased from Egyptian local market. Protein was

supplied as Casein (80% protein) which was added to diet in a proportion of 14%. Starch was supplied as corn starch 65.5, while fat (source of vitamin E) was supplied as corn oil 1.0. Salt mixture 4.0, cellulose 5.0 and choline chloride 0.2 g, methionine 0.3 g (g/100g diet) are shown in the following **Table 1**.

Collection of blood Samples, biochemical, hematological analysis

Rat was dissected and 5 ml of blood samples were collected from retinal vein. 2ml were collected in EDTA tube to measure haematological analysis (Complete blood count), 3ml were collected in plain tubes and were allowed to clot then centrifuged at 4000 rpm for 15 minutes to obtain serum for estimation of

biochemical analysis: Lactate dehydrogenase, Liver enzymes (AST and ALT) and Kidney functions (Creatinine and Urea).

Histopathological examination

The lung tissues were fixed in 10% buffered formalin for 2 hrs and embedded in paraffin. Approximately 4 μ m thick sections were prepared from tissue paraffin block and stained with Haematoxylin and Eosin (H & E). Pathological changes were checked by light microscope BX 53 (Olympus) at 600_x magnification. The severity of changes was quantitated based on the degree of inflammation, all slides were examined to assess the degree of inflammation as mild, moderate or severe ¹².

Statistical Analysis

All results were recorded in the form of means of three replicates. By using SPSS v17, Data were inserted to analysis by the statistical. The difference mean comparison between the groups was analysed by (ANOVA) at $p < 0.05$.

RESULTS AND DISCUSSION

Identification of mushroom

Fruiting bodies of mushroom were collected from wild growth on trees stems at Richmond, Virginia, Maryland; USA. Identification was performed by comparing their characteristics with authentic specimens present in Virginia State University herbarium according to (Sharma, 2012) ¹³.

Characterization of (*Ganoderma lucidum*) methanol extract by (GC-MS) analysis

The protective efficacy of *Ganoderma lucidum* methanol extracts may be due to the presence of active components such as (alkaloids and flavonoids) which have the ability to reduce lung inflammation and improve the general health. 5-fluorouracil (5-FU) a pyrimidine derivative shows interesting biological activity against tumors and cancer. It used in the treatment of a variety of carcinomas including colon, breast, and other cancers like those of head and neck cancers ¹⁴. 5-FU has broad-spectrum antimicrobial activity and inhibits virulence factor and biofilm production against several bacterial pathogens. Its antibacterial activity was reported against *S. aureus* and *S. epidermidis* at (MIC $< 0.8 \mu\text{g/mL}$). Also, It inhibits the growth of *S. epidermidis* at concentration well below its MIC ¹⁵. Chlorogenic acids (CQAs), an esters of caffeic acid and quinic acid, are biologically important phenolic compounds present in many plant species ¹⁶. Numerous studies have reported that CQAs have favorable effects on human health, exhibiting several biological properties such as antioxidants and anti-inflammatory properties ¹⁷, anticancer ¹⁸, neuro-

protective effects ¹⁹ protect from Alzheimer's disease, hepato-protective effect ²⁰ and antibacterial ²¹. Also, CQAs is known from their pro health properties, including that they may be helpful in fighting obesity, modify glucose – 6 -phosphatase involved in glucose metabolism and reduction of the risk of cardiovascular disease ²². Linoleic acid (LA) is an essential polyunsaturated fatty acid (PUFA) required for normal growth and development and has been reported to inhibit the synthesis of prostaglandin resulting in reduced inflammation and prevention of certain chronic diseases however presents evidence that excess LA in the food supply might adversely affect the brain ²³. Linolenic acids have antibacterial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Escherichia coli* ²⁴. Glycolic (GA) acid is a water - soluble alpha hydroxy acid (AHA) used for skincare applications. There is some evidence that glycolic acid have anti-inflammatory, keratolytic and antioxidant effects and might reduce acne-related inflammation. GA exhibits antibacterial activity against *C. acnes* at suitable pH-condition ²⁵.

Antimicrobial (Anti-MRSA) Susceptibility Testing and MIC determination

Results of *G. lucidum* methanol extract in **Figure 3** showed a potent inhibitory activity against Methicillin-resistant *Staphylococcus aureus* (MRSA). *G. lucidum* exhibited inhibitory activity 29.0 ± 1.9 mm at MIC 125 ($\mu\text{g/ml}$) as low dose and inhibition zone 30.0 ± 0.7 mm at 250 mcg (MIC) as high dose without significant difference between two doses compared with results of vancomycin which inhibition zone recorded 21.5 ± 0.99 mm and DMSO without any inhibition recorded. Acute lung inflammation is a potentially life-threatening complication of infections due to methicillin-resistant *Staphylococcus aureus* MRSA, a worldwide emerging pathogen, which causes necrotizing pneumonia and acute respiratory distress syndrome (ARDS), MRSA virulence factors encompass immunotoxins termed super antigens that contribute to lung inflammation. Due to MRSA will continue to develop resistance to antibiotics, we require new therapeutic strategies to be found ⁴. *G. lucidum* mushroom is herb plants have been proven to be effective anti-bacterial and anti-inflammatory compounds with no toxicity ²⁶, The reasons of various therapeutic properties of *G. lucidum* are may be attributed to its polysaccharides and triterpenes main components ²⁷. In the present study, antibacterial activity of 125 and 250 mcg of methanol extract of *G. lucidum* showed a moderate efficacy on MRSA colonies. Our results agreed with that reported by ²⁸ who studied the antioxidant and antimicrobial activities of *G. lucidum* mushroom and found that MeOH extracts were effective on tested microorganisms *S. aureus* and MRSA

Table 1. Diet used and contents according to RDA for nutrition of albino

Ingredients	Corn starch	Protein	Salt mixture	Vitamin mixture	Cellulose	Choline chloride	Methionine
RDA/g	65.5	14.0	4.0	1.0	5.0	0.2	0.3

Table 2. Characterization of (*Ganoderma lucidum*) methanol extract by (GC-MS) analysis

Compound	CF	MW	RT	Area (%)
2,2,2-TRIFLUORO-N-(TRIMETHYLSILYL)ACETAMIDE	C ₅ H ₁₀ F ₃ NOSi	185	5.12	13.7
5-Fluorouracil	C ₄ H ₃ FN ₂ O ₂	114	6.77	40.3
PYRROLO	C ₉ H ₁₅ NO ₂	169	8.84	0.31
trans-Epoxy succinyl-L-leucylamido butane (4-guanidino)	C ₁₅ H ₂₇ N ₅ O ₅	357	9.01	0.98
1-Methoxy-2-propyl acetate	C ₆ H ₁₂ O ₃	132	9.22	0.99
5-PYRIMIDINE CARBOXALDEHYDE	C ₆ H ₆ N ₂ O ₃	154	9.35	0.5
N-(1-METHYLPROPYL)-2,2,2-TRIFLUOROACETAMIDE	C ₇ H ₁₂ F ₃ NO	183	9.73	0.49
D-(-)-Lactic acid, 2TMS derivative PROPANOIC ACID,	C ₉ H ₂₂ O ₃ Si ₂	234	9.98	0.47
Glycolic acid	C ₈ H ₂₀ O ₃ Si ₂	220	10.94	0.42
1,3-Dioxane	C ₉ H ₂₀ O ₃ Si	204	11.08	0.26
Pentasiloxane, dodecamethyl	C ₁₂ H ₃₆ O ₄ Si ₅	384	11.77	0.10
Linolenic acid	C ₂₇ H ₅₂ O ₄ Si ₂	496	12.06	4.18
Benzenesulfonamide, TMS derivative	C ₉ H ₁₅ NO ₂ SSi	229	12.70	0.34
SILANOL, TRIMETHYL, PHOSPHATE	C ₉ H ₂₇ O ₄ PSi ₃	314	14.92	5.27
D-Mannitol, 1,2:3,4:5,6-tris-O-(1-methylethylidene)	C ₁₅ H ₂₆ O ₆	302	22.64	0.32
5,8,11-Eicosatrienoic acid, tert-butyl dimethylsilyl ester	C ₂₆ H ₄₂ O ₂ Si	414	23.22	0.19
MANNURONIC ACID, 2,3,5,6-TETRAKIS-O-(TRIMETHYLSILYL)-, LACTONE	C ₁₈ H ₄₂ O ₆ Si ₄	466	23.93	6.07
Sebacic acid, 2TMS derivative	C ₁₆ H ₃₄ O ₄ Si ₂	346	24.75	0.91
D-(-)-Ribofuranose, tetrakis (trimethylsilyl) ether (isomer1)	C ₁₇ H ₄₂ O ₅ Si ₄	438	25.25	5.16
D-Pinitol, pentakis(trimethylsilyl) ether	C ₂₂ H ₅₄ O ₆ Si ₅	554	27.82	0.37
Chlorogenic acid (6TMS)	C ₃₄ H ₆₆ O ₉ Si ₆	786	28.50	10.49
Palmitic Acid, TMS derivative	C ₁₉ H ₄₀ O ₂ Si	328	31.92	5.18
Octadecenoic acid, (E)-, TMS derivative	C ₂₁ H ₄₂ O ₂ S	354	35.06	5.71

as well as *E. coli* at 50-200µg/mL concentrations. Furthermore, Prasad and Wesely, (2008) ²⁹ investigated the antibacterial activity of *G. lucidum* methanol extract from Iran and showed that methanol extract of *G. lucidum* possess efficient antibacterial effects toward MRSA. Also, Iftekhar *et al.*, (2011) ³⁰ tested the antimicrobial activity of 50 mg/ml of different extracts of *G. lucidum* against 30 strains of MRSA and MSSA

and reported that methanol extracts of *G. lucidum* displayed Weak to moderate activities against MRSA and MSSA. The results of this study provides an evidence that there is no difference between low dose(125mcg) and high dose (250 mcg) of *Ganoderma* sp. extracts, therefore the low dose(125mg) is recommended as prevention of MRSA infections and low side effects.



Figure 1. Identification of mushroom

Table 3. Lactate dehydrogenase parameters in the studied groups

Parameters	Control group (n= 8) (4M/4F) (G1)	MRSA group (n= 8) (4M/4F) (G2)	MRSA groups (n= 24)	
			MRSA+150mg mushroom extracts group (n= 8) (4M/4F) (G3)	MRSA+200mg mushroom extracts group (n= 8) (4M/4F) (G4)
LDH (U/L)				
Rang	750 - 1951	3092 - 3720	2010 - 3001	1950 - 2990
Mean ±S.D	1165.5± 352	3465.1± 229	2371.4± 300	2389.1± 320
P value		< 0.0001**** (G2 vs G3, G2 vs G4) P<0.001		

*Significant (P<0.05); NS= Non-significant; LDH, Lactate dehydrogenase

Table 4. Liver functions parameters in the studied groups

Parameters	Control group (n= 8) (4M/4F) (G1)	MRSA groups (n= 24)		
		MRSA group (n= 8) (4M/4F) (G2)	MRSA+125mcg mushroom extracts group (n= 8) 4M/4F (G3)	MRSA+250mcg mushroom extracts group (n= 8) (4M/4F) (G4)
ALT (U/L)				
Range	32 - 59	44 - 78	27 - 40	30 - 48
Mean ±S.D	46± 4.22	57.8± 4.53	33.25± 1.75	35.5± 2.03
AST (U/L)				
Range	129 - 160	185 - 222	132 - 170	130 - 190
Mean ±S.D	138.16±4.75	199.75± 4.74	149.87± 4.93	154.12± 6.28
P value		< 0.0001**** (G2 vs G3, G2 vs G4) P<0.001		

*Significant (P<0.05); NS= Non-significant

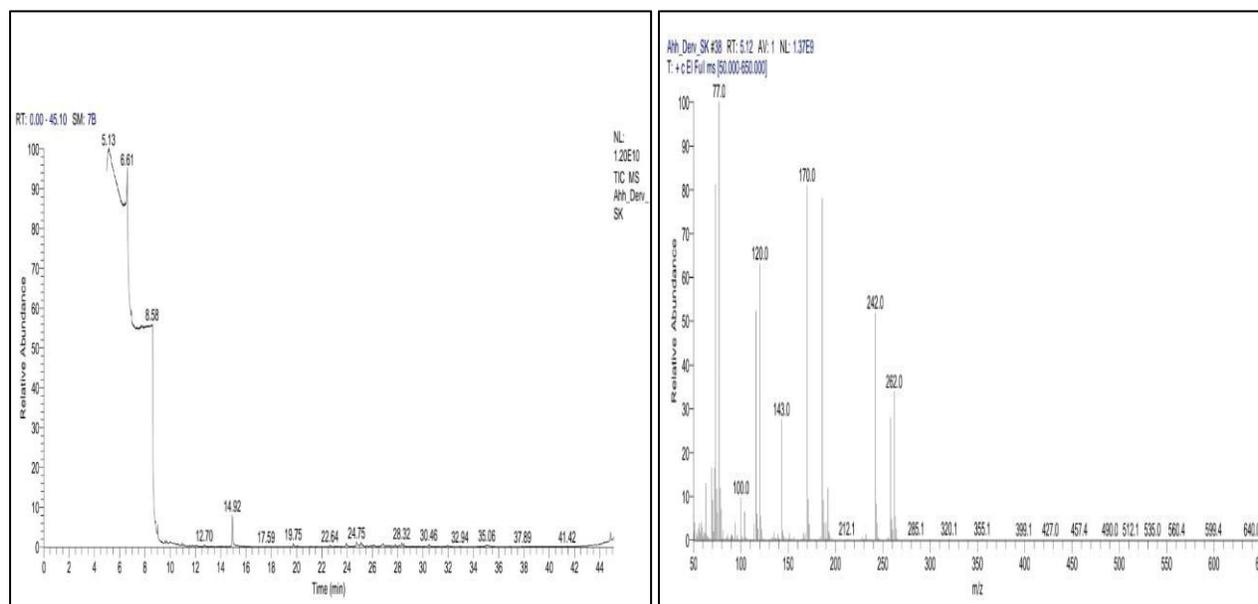


Figure 2. Characterization of (*Ganoderma lucidum*) methanol extract by (GC-MS) analysis.

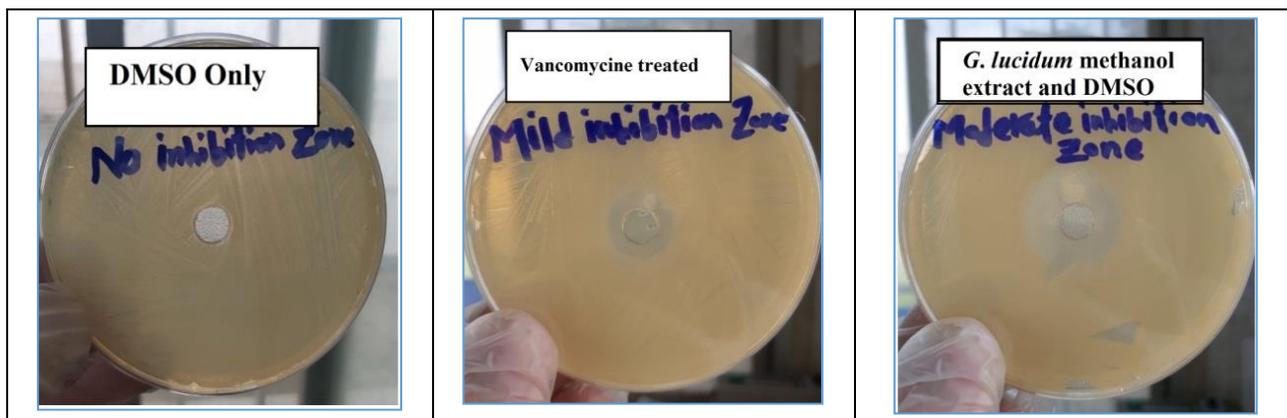


Figure 3. Antimicrobial (Anti-MRSA) Susceptibility

Determination of antimicrobial (Anti- MRSA) activity using *In vivo* assay with nutrition support

Biochemical parameters

Lactate dehydrogenase (LDH) level

The variation of lactate dehydrogenase (LDH) level between all the studied groups in (Table 2) showed that G2 members were the highest LDH with a mean value of 3465.1 ± 229 U/L, while G1 members were the lowest LDH with a mean value of 1165.5 ± 352 U/L. In comparison G3 and G4 showed almost the same LDH level with a mean value 2371.4 ± 300 and 2389.1 ± 320 U/L, respectively. The *G. lucidum* treated groups (Group

3 and 4) treated with 125 mcg and 250 mcg respectively showed statistically significant decline in LDH levels compared to the positive control group (Group 2) ($P < 0.0001$). From our results, the *G. lucidum* treated groups showed statistically significant decline in LDH levels compared to those of positive control group. This decline in LDH levels suggesting a potent protective effect of *G. lucidum* against lung inflammation. 125 mcg *G. lucidum* extract is the dose-dependent manner which significantly reduced serum LDH levels compared with that of positive control group. These results are in agreement with Rahman and Hossain, (2013)³¹ who has studied the protective effect of *G. lucidum* on acute hepatotoxicity

using paracetamol for the induction of liver damage and demonstrate that the *G. lucidum* has significant hepatoprotective activity against paracetamol - induced acute hepatotoxicity and reported that *G. lucidum* significantly decreased the plasma levels of LDH in rats nearly to those of the normal control. This coincides also with Xu *et al.*, (2017)³² who has studied cardioprotective effects of *G. lucidum* polysaccharides (GLPS) on DOX-induced cardiotoxicity and associated serum LDH release which is also an indicator of cell injury and found that DOX injection significantly elevated serum levels of LDH ; however GLPS pre-treatment significantly reversed the levels of these cardiac enzymes and obviously prevented LDH leakage as well as significantly decreased the serum levels of LDH.

Liver enzymes

Liver function (ALT and AST) were carried out in the studied groups. The variation of ALT level between all the studied groups in (Table 3) showed that G1 members were the highest ALT with a mean value of 46 ± 4.22 U/L, while G3 members were the lowest ALT with a mean value of 33.25 ± 1.75 U/L. In addition, G2 and G4 showed ALT level with a mean value 57.8 ± 4.53 and 35.5 ± 2.03 U/L, respectively. *G. lucidum* treated groups (Group 3 and 4) treated with 150 mcg and 200 mg/kg respectively showed statistically significant decline in ALT activities compared to the positive control group (Group 2) $P < 0.001$). The variation of AST level between all the studied groups in (Table 4) showed that G2 members were the highest AST with a mean value of 199.75 ± 4.74 U/L, while G1 members were the lowest AST with a mean value of 138.16 ± 4.75 U/L. In addition, G3 and G4 showed almost the same AST level with a mean value 149.87 ± 4.93 and 154.12 ± 6.28 U/L, respectively. *G. lucidum* extract -treated groups (Group 3 and 4) treated with 125 mcg and 250 mcg respectively showed statistically significant decline AST activities compared to the positive control group (Group 2) ($P < 0.001$).

Hematological parameters

Complete Blood Count (CBC)

The complete blood count (white blood cells, red blood cells, hemoglobin, hematocrit, platelet count) were carried out in the studied groups. The variation of white blood cells (WBC) count between all the studied groups in (Table 4) showed that G2 members were the highest WBC with a mean value of 19.1 ± 2.2 ($10^3/\text{cmm}$), while G4 members were the lowest WBC with a mean value of 13.05 ± 1.53 ($10^3/\text{cmm}$). In addition, G1 and G3 showed WBC count with a mean value 15.1 ± 1.49 and 14.5 ± 1.1 ($10^3/\text{cmm}$), respectively. WBC showed no significant change between studied groups as compared to in controls ($P > 0.05$). The variation of red blood cells

(RBC) count between all the studied groups in (Table 5) showed that G2 members were the highest RBC with a mean value of 8.17 ± 0.23 ($10^6/\text{cmm}$), while G4 members were the lowest RBC with a mean value of 7.43 ± 0.09 ($10^6/\text{cmm}$). In addition, G1 and G3 showed RBC count with a mean value 7.69 ± 0.31 and 7.84 ± 0.23 ($10^6/\text{cmm}$), respectively. RBC showed no significant change between studied groups as compared to in controls ($P > 0.05$). The variation of hemoglobin (HG) level between all the studied groups in (Table 4) showed that G2 members were the highest HG with a mean value of 15.18 ± 0.31 (g/dL), while G3 members were the lowest HG with a mean value of 13.83 ± 0.43 (g/dL). In addition, G1 and G4 showed HG level with a mean value 14.38 ± 0.35 and 14.16 ± 0.22 (g/dL), respectively. HG showed no significant change between studied groups as compared to in controls ($P > 0.05$). The variation of Hematocrit (HCT) level between all the studied groups in (Table 3) showed that G2 members were the highest HCT with a mean value of 45.63 ± 1.27 %, while G4 members were the lowest HCT with a mean value of 41.2 ± 0.9 %. In addition, G1 and G3 showed HCT level with a mean value 43.45 ± 1.21 and 41.75 ± 1.51 %, respectively. HCT showed no significant change between studied groups as compared to in controls ($P > 0.05$). The variation of Platelet (PLT) count between all the studied groups in (Table 5) showed that G1 members were the highest PLT with a mean value of 839.3 ± 46.28 ($10^3/\text{cmm}$), while G3 members were the lowest PLT with a mean value of 683.5 ± 35.07 ($10^3/\text{cmm}$). In addition, G2 and G4 showed PLT count with a mean value 764 ± 59.97 and 748 ± 60.67 ($10^3/\text{cmm}$), respectively. PLT showed no significant change between studied groups as compared to in controls ($P > 0.05$).

Histopathology examination

Biopsies were fixed into formalin 10 % for 2 hours followed by paraffin inclusion for histological analysis paraffin sections were cut at 4 microns thick and subjected to routine hematoxylin and eosin staining. All slides are examined to assess the degree of inflammation as mild, moderate or severe. Group (1) Female and group (1) Male their pathological tissues were showing mild inflammation as seen in Figure 4A. Microscopy foci of inflammatory condensation centred by a bronchiole with vascular engorgement, and the accumulation of alveolar fluid rich in infective organisms. Group (2) Female, their pathological tissues were severely inflamed, their as seen in Figure 4B. Microscopy foci of inflammatory condensation with marked infiltration of superlative exudate rich in red blood cells, neutrophils, and fibrin into the alveolar fluid associated with fibrin purulent exudates neutrophils in the lumen, and foci of ulceration of the epithelium and parietal inflammation. Group (2) Male was showing moderate inflammation as seen in Figure 4C. Microscopy foci of inflammatory

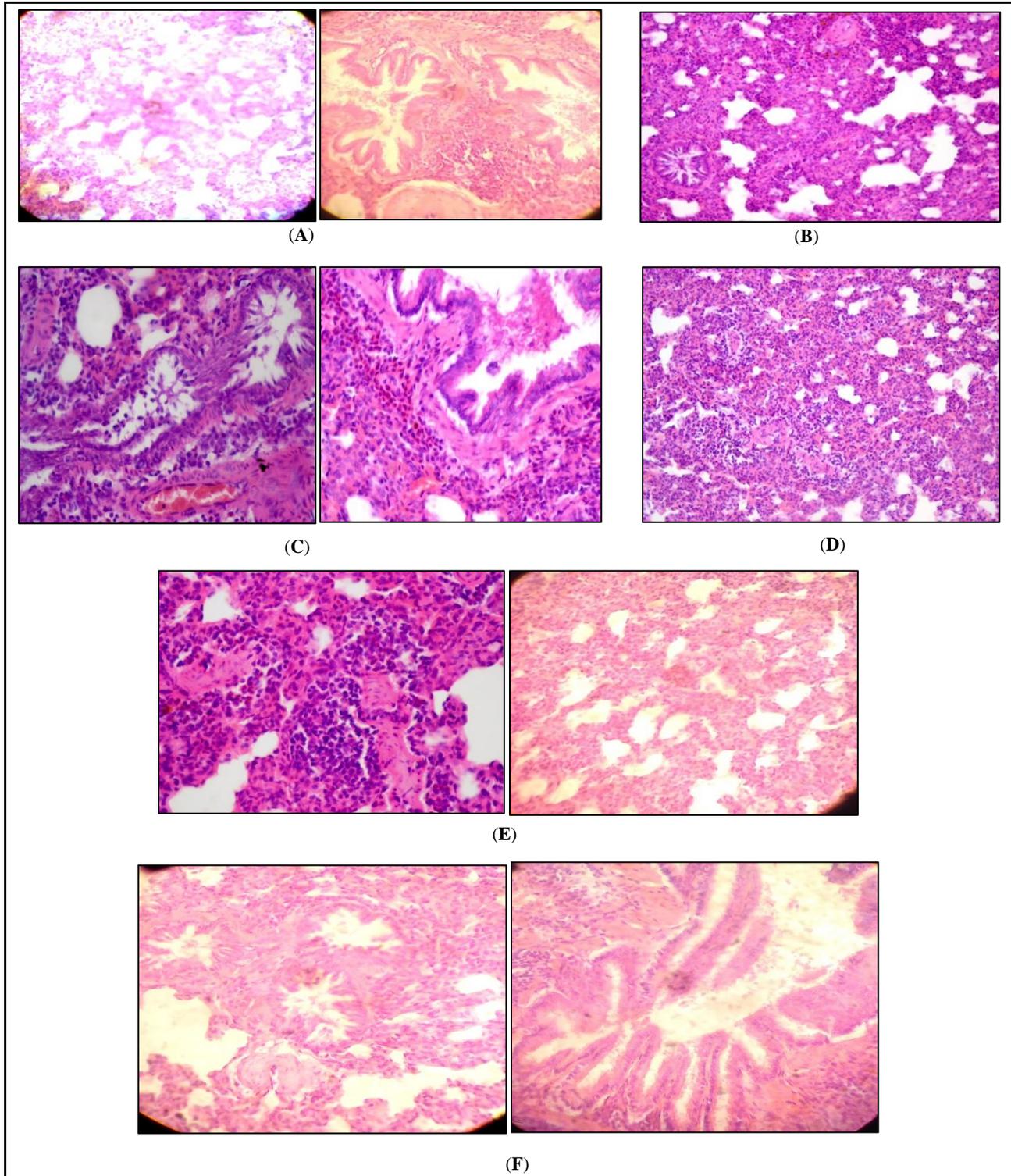


Figure 4. (A) Histopathology examination: Mild inflammatory cellular infiltrate. (B) Histopathology examination: Severe inflammatory cellular infiltrate. (C) Histopathology examination: A: Bronchial lumen shows inflammatory cells, B: Neutrophilic rich inflammatory cellular infiltrate. (D) Histopathology examination: moderate inflammatory cellular infiltrate. (E) Histopathology examination: Mixed inflammatory cells mainly lymphocytes, neutrophils, plasma cells and histiocytic (Female left & Male right). (F) Histopathology examination: mixed inflammatory cells mainly lymphocytes, neutrophils, plasma cells and histiocytic (Female left & Male right).

Table 5. Hematological parameters in the studied groups

Parameters	MRSA groups (n= 24)			
	Control group (n= 8) (4M/4F) (G1)	MRSA group (n= 8) (4M/4F) (G2)	MRSA+125mcg mushroom extracts group (n= 8) (4M/4F) (G3)	MRSA+250mcg mushroom extracts group (n= 8) (4M/4F) (G4)
WBC (10³/cmm)				
Range	10.1- 19.5	15.5 - 28.4	10.2 - 17.5	6.1- 16.2
Mean ±S.D	15.1± 1.49	19.1± 2.2	14.5± 1.1	13.05± 1.53
P value			0.089 ^{NS}	
RBC (10⁶/cmm)				
Range	6.9-8.48	7.34-9.14	7.01- 8.4	6.98- 7.63
Mean ± S.D	7.69±0.31	8.17± 0.23	7.84± 0.23	7.43± 0.09
P value			0.187 ^{NS}	
HG (g/dL)				
Range	13.1-15.8	14.3- 16.6	12.7- 15.4	13.6- 15
Mean ± S.D	14.38± 0.35	15.18± 0.31	13.83± 0.43	14.16± 0.22
P value			0.066 ^{NS}	
HCT (%)				
Range	37.6-45.4	41.6- 50.3	37.9- 47	37.7- 44
Mean ± S.D	43.45± 1.21	45.63± 1.27	41.75± 1.51	41.2± 0.9
P value			0.083 ^{NS}	
PLT (10³/cmm)				
Range	693-992	627- 1024	594- 822	555- 928
Mean ± S.D	839.3±46.28	764± 59.97	683.5± 35.07	748± 60.67
P value	0.235 ^{NS}			

*Significant (P<0.05); NS= Non significant ; WBC, white blood cells; RBC, red blood cells; HG, hemoglobin; HCT, hematocrit; PLT, platelet count.

condensation are centred by a bronchiole with vascular engorgement and accumulation of alveolar fluid rich in infective organisms. Group (3) Female, Group (3) Male, Group (4) Female and Group (4) Male were mild inflamed, their pathological tissues were showing mild inflammation as seen in Figures 4D, 4E and Figure 4F, Microscopy foci of inflammatory condensation centered by a bronchiole with vascular engorgement, and the accumulation of alveolar fluid rich in infective organisms, mixed inflammatory cells mainly lymphocytes, neutrophils, plasma cells and histolytic.

CONCLUSION

The present study suggests that *G. lucidum* methanol extract has antibacterial effect against MRSA *in vitro* and also when infected albino rats as *in vivo*. Additionally, it is effective in protecting against lung inflammation induced by MRSA infection. Also, *G. lucidum* methanol extract contain bioactive compounds serve as nutrition support with potency anti – MRSA. The future prospective for this work is using *G. lucidum* methanol extract in drug delivery.

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Conflict of interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

REFERENCES

1. Heleno, S. A.; Barros, L., Martins, A.; Morales, P.; Fernández-Ruiz, V.; Glamoclija, J.; Ferreira, I. C. F. R. Nutritional value, bioactive compounds, antimicrobial activity and bioaccessibility studies with wild edible mushrooms. *LWT - Food Science and Technology* **2015**, *63* (2), 799–806. <https://doi.org/10.1016/j.lwt.2015.04.028>
2. Cör D.; Knez Ž.; and Knez Hrnčič M. Antitumour, antimicrobial, antioxidant and antiacetylcholinesterase effect of *Ganoderma Lucidum* terpenoids and polysaccharides: A review. *Molecules* **2018**, *23* (3), 649.
3. Peng K.-T.; Huang T.-Y.; Chiang Y.-C., Hsu Y.-Y.; Chuang F.-Y.; Lee C.-W.; and Chang P.-J. Comparison of Methicillin-Resistant

- Staphylococcus aureus Isolates from Cellulitis and from Osteomyelitis in a Taiwan Hospital, 2016-2018. *J. Clin. Med.* **2019**, 8(6), 816.
- Dahal M.; Schwan W. R. Management of methicillin-resistant Staphylococcus aureus mediated ventilator-associated pneumonia. *Curr. Trends Microbiol.* **2018**, 12: 95.
 - Lu L.; Hu W.; Tian Z., Yuan D.; Yi G., Zhou Y., Cheng Q.; Zhu J.; Li, M. Developing natural products as potential anti-biofilm agents. *Chin. Med.* **2019**, 14 (1), 11.
 - Salam A. M., Quave C. L. Opportunities for plant natural products in infection control. *Curr. Opin. Microbiol.* **2018**, 45, 189–194.
 - Rossiter S. E., Fletcher M. H., and Wuest W. M. Natural products as platforms to overcome antibiotic resistance. *Chem. Rev.* **2017**, 117 (19), 12415–12474.
 - Lloyd A.L., Barnes C.W., Held B.W., Schink M.J., Smith M.E. Elucidating lucidum: Distinguishing the diverse laccate Ganoderma species of the United States. *PLOS ONE* **2018** 13 (7): e0199738. <https://doi.org/10.1371/journal.pone.0199738>.
 - Ramesh C. H.; Pattar M. G. Antimicrobial properties, antioxidant activity and bioactive compounds from six wild edible mushrooms of western ghats of Karnataka, India. *Pharmacognosy Res.* **2010**, 2 (2), 107.
 - Souza L. K. H., Fernandes O. de F. L., Kobayashi C. C. B. A., Passos X. S., Costa C. R., Lemos J. A., Souza-Júnior A. H., Silva M. do R. R. Antifungal susceptibilities of clinical and environmental isolates of Cryptococcus neoformans in goiânia city, Goiás, Brasil. *Rev. Inst. Med. Trop. Sao Paulo* **2005**, 47, 253–256.
 - Reeves P. G.; Niesen F. H.; Fahey Jr G. C. AIN-93 purified diets for laboratory rodents: final report of the American Institute of Nutrition ad hoc writing committee on the reformulation of the AIN-76A rodent diet. *J. Nutr.* **1993**, 123 (11), 1939.
 - Akpera, M. T., Oguntayo, B. O., Jombo, G. T. A. Impact of Ganoderma applanatum extraction on haematological profiles of laboratory rats: A preliminary study in Nigeria. *Asian Pacific Journal of Tropical Disease* **2012**, 2 (5), 390–394. [https://doi.org/10.1016/S2222-1808\(12\)60085-1](https://doi.org/10.1016/S2222-1808(12)60085-1).
 - Sharma, J.R.. Aphylllophorales of Himalaya, Botanical Survey of India, Salt Lake, Kolkata. **2012** DOI.org/10.11646/phytotaxa.246.2.5.
 - Zhang N., Yin Y., Xu S.-J., Chen W.-S. 5-Fluorouracil: mechanisms of resistance and reversal strategies. *Molecules* **2008**, 13(8), 1551–1569.
 15. Rangel-Vega A., Bernstein L. R., Mandujano Tinoco E.-A., García-Contreras S.-J., and García-Contreras R. Drug repurposing as an alternative for the treatment of recalcitrant bacterial infections. *Front. Microbiol.* **2015**, 6, 282.
 16. Olthof M. R., Hollman P. C. H., and Katan M. B. Chlorogenic acid and caffeic acid are absorbed in humans. *J. Nutr.* **2001**, 131 (1), 66–71.
 17. Girsang E., Lister I. N. E., Ginting C. N., Nasution S. L., Suhartina S., Munshy U. Z., Rizal R., and Widowati W. Antioxidant and anti-inflammatory activity of chlorogenic acid on lead-induced fibroblast cells. *J. Phys. Conf. Ser.* **2019**, 1374 012006.
 18. Santana-Gálvez J., Vilella Castrejón J., Serna-Saldívar S. O., and Jacobo-Velázquez D. A. Anticancer potential of dihydrocaffeic acid: a chlorogenic acid metabolite. *CyTA-Journal Food* **2020**, 18 (1), 245–248.
 19. Fernandes M. Y. D.; Dobrachinski F.; Silva H. B.; Lopes J. P., Gonçalves F. Q., Soares F. A. A., Porciúncula L. O., Andrade G. M., Cunha R. A.; Tomé A. R. : Neuromodulation and neuroprotective effects of chlorogenic acids in excitatory synapses of mouse hippocampal slices. *Sci. Rep.* **2021**, 11 (1), 1–11.
 20. Chen Z.; Yang Y.; Mi S.; Fan Q., Sun X.; Deng B., Wu G., Li Y.; Zhou Q.; ; Ruan Z. Hepatoprotective effect of chlorogenic acid against chronic liver injury in inflammatory rats. *J. Funct. Foods* **2019**, 62, 103540.
 21. Sun Z., Zhang X., Wu H., Wang H., Bian H., Zhu Y., Xu W., Liu F., Wang D., and Fu L. Antibacterial activity and action mode of chlorogenic acid against Salmonella Enteritidis, a foodborne pathogen in chilled fresh chicken. *World J. Microbiol. Biotechnol.* **2020**, 36 (2), 1–10.
 22. Fazel Nabavi S., Tejada S., N Setzer W., Gortzi O., Sureda A., Braidy N., Daglia M., Manayi A., and Mohammad Nabavi S.: Chlorogenic acid and mental diseases: from chemistry to medicine. *Curr. Neuropharmacol.* **2017**, 15 (4), 471–479.
 23. Taha A. Y. Linoleic acid—good or bad for the brain? *NPJ Sci. Food* **2020**, 4 (1), 1–6.
 24. Agoramoorthy G., Chandrasekaran M., Venkatesalu V., and Hsu M. J. Antibacterial and antifungal activities of fatty acid methyl esters of the blind-your-eye mangrove from India. *Brazilian J. Microbiol.* **2007**, 38, 739–742.
 25. Valle-González E. R., Jackman J. A., Yoon B. K., Mokrzecka N., and Cho N.-J. pH-dependent antibacterial activity of glycolic acid: implications for Anti-Acne Formulations. *Sci Rep.* **2020**, 10, 7491
 26. Sarnthima R.; Khammaung S., and Sa-ard P. Culture broth of Ganoderma lucidum exhibited antioxidant, antibacterial and α -amylase inhibitory activities. *J. Food Sci. Technol.* **2017**, 54 (11), 3724–3730.

27. Sohretoglu D.; Huang S. Ganoderma lucidum polysaccharides as an anti-cancer agent. *Anti-Cancer Agents Med. Chem.* **2018**, 18 (5), 667–674.
28. Celal B. Antioxidant and antimicrobial capacities of Ganoderma lucidum. *Med. Crave* **2019**; 7(1), 5–7.
29. Prasad Y.; Wesely W. E. G. Antibacterial activity of the bio-multidrug (Ganoderma lucidum) on Multidrug resistant *Staphylococcus aureus* (MRSA). *J. Adv. Biotechnol.* **2008**; 10, 16.
30. Iftekhar A. F.; Hassan M.; Choudhry Z. K.; Khan M. D. I.; Saleh A. A. In vitro evaluation of anti-staphylococcal activity of Ganoderma lucidum, Ganoderma praelongum and Ganoderma resinaceum from Pune, India. *Bangladesh J Pharmacol.* **2011**, 6, 14–17.
31. Rahman M. M., and Hossain S. Preventive effect of Ganoderma lucidum on paracetamol-induced acute hepatotoxicity in rats. *J. Sci. Res.* **2013**, 5 (3), 573–578.
32. Xu F.; Li X.; Xiao X.; Liu L.; Zhang L.; Lin P.; Zhang S.; Li Q. Effects of Ganoderma lucidum polysaccharides against doxorubicin-induced cardiotoxicity. *Biomed. Pharmacother.* **2017**, 95, 504–512.