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Research Article

Ganoderma Lucidum - A Potential Medicinal Mushroom Against MDR Isolates from The Secondary Infections of Covid-19

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Abstract: The severe acute respiratory syndrome produced by COVID-19 is a highly infectious and pathogenic viral infection. Many COVID-19 patients have secondary bacterial infections, which enhance disease and increase death, particularly when requiring invasive mechanical ventilation. One of the most important medicinal mushrooms, *Ganoderma lucidum*, has been used for food, feed, and medication since the dawn of humanity. The present investigation aims to discover the potential of the medicinal mushroom *Ganoderma lucidum* inhibited multidrug-resistant isolates from secondary infection of Covid-19 patients. Isolation and identification of urine samples from secondary infection of post-Covid-19 patients and evaluate the antibiotic sensitivity assay, as identification of bioactive compounds, anti-inflammatory and antioxidant activity from *Ganoderma lucidum*. Totally 6 clinical urine samples were collected from the age group 45 to 60; 3 were male, and 3 were female. In total, nine bacteria and 10 fungi were isolated and identified. As antibiotic sensitivity assays of ceftriaxone, fluoroquinolones, azithromycin and amphotericin, nystatin and fluconazole were performed by the disc diffusion method against bacteria and fungi, the zone of inhibition was maximal in *Klebsiella pneumoniae* and *Fusarium oxysporum*. The aqueous and ethanolic extracts of *Ganoderma lucidum* were analyzed for the bioactive compounds, viz., steroids, flavonoids, alkaloids and phenolic compounds. The effect of the anti-inflammatory activity of the aqueous extract was excellent. The activity of the DPPH assay was maximum in aqueous and ethanolic extracts of all concentrations (100 to 500 ml). Antibiotic resistance could probably rise due to the frequent prescription of broad-spectrum empiric antimicrobials to COVID-19 patients. Hence, *Ganoderma lucidum* can be exploited to prevent secondary infection in COVID-19 patients.

Keywords: Post Covid-19, *Ganoderma lucidum*, Antibiotic Sensitivity Assay, Bioactive Compounds, Antioxidant and Anti-Inflammatory.

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1. INTRODUCTION

Medical practitioners are advised by the World Health Organization (WHO) clinical management guidelines for the Covid-19 disease to collect specimens from the upper samples from the respiratory system and blood taken for bacterial cultures, and only in extreme situations should empirical antibiotic therapy be started. WHO Guidance provision[1]. The most frequently identified causative pathogens among Covid-19 patients are bacteria, including *Klebsiella pneumoniae, Acinetobacter* spp., *Pseudomonas* spp., *Escherichia coli, and Staphylococcus* spp.[2]. Treatment of Covid-19 patients and responsible antibiotic usage are both essential for lowering rates of antibiotic-resistant bacteria; thus it is critical to recognize the percentage of Covid-19 patients with acute respiratory bacterial coinfection[3]. Despite the clinical importance of secondary bacterial infections, it is still unknown how much they influence4 the severity and mortality of Covid-19. Several studies have addressed this issue, but variations in testing methodologies, site-specific nosocomial infections, definitions of early vs late conditions, and different treatment methods [4,5] complicate the analysis of this data. Antibiotics are medicines used to fight bacterial infections. There are different types of antibiotics. Each type is only effective against certain bacteria. An antibiotic sensitivity test can help determine which antibiotic will be most effective in treating your infection. The test can also help find a treatment for antibiotic-resistant disorders. Antibiotic resistance happens when standard antibiotics become less effective or ineffective against certain bacteria. Antibiotic resistance can turn once easily treatable diseases into serious, even life-threatening illnesses [6]. In oriental medicine, the woody Basidiomycota mushroom *Ganoderma lucidum*, a part of the polypores family of Ganodermaceae, is widely used to promote longevity and overall wellness [7]. It is commonly bought for its therapeutic and spiritual benefits and is widely formed on a commercial scale [8]. The presence of more than 200 bioactive elements, classified into water-soluble, organic-soluble, and volatile-soluble chemicals, provides *G. lucidum*its pharmacological activity [9]. Antioxidants supply an electron to hold a free radical in control. A free radical that has been neutralized cannot harm our cells [10]. Due to their antioxidative and anti-inflammatory characteristics, preparations made from *G. lucidum* mycelium, fruiting bodies, and spores have recently been marketed as nutriceuticals or dietary supplements [4]. Mushrooms have been functional nutrition for ages and are a naturally occurring source of fibre, proteins, vitamins, and minerals. Many mushroom species have natural medicinal potential and function as beneficial nutrients[11]. The international fungus *Ganoderma lucidum*is a polypore rack mushroom that turns from orange-white to bright red as it develops until maturity[12]. In addition*, G. lucidum* has a long history of usage in traditional Asian medicine, stretching back hundreds of years[13]. The Chinese herbal supplement *Ganoderma lucidum* has been studied extensively during the past two decades [14]. Some reports demonstrate *G. lucidum* additive properties in addition to its anti-inflammatory benefits [15]. This study aims to evaluate the Isolation and identification of secondary infection of urine samples in covid-19 patients and the effect of antibiotics sensitivity assay, identification of bioactive compounds, antioxidants, and anti-inflammatory from *Ganoderma lucidum*.

2. MATERIALS AND METHODS

2.1 Collection of Urine Sample

The six clinical urine samples from people aged 45 to 60 were investigated; 3 male and 3 female samples were collected from the Government Medical College, Thanjavur. The sample collection survey number is 64347, and the certificate of the ethical committee is TGMC-EC-2021-0196. Samples were screened by CLSI standards (Clinical and Laboratory Standards Institute). The gold standard for urine analysis is urine culture with pathogen identification of pathogens. Mid-stream urine was used for the study. Inoculation for urine culture was done on a MacConkey agar, a Hi-Chrome UTI agar and CLED (cysteine lactose electrolyte deficient) agar. Primary were subjected to identification via microscopy, growth characteristics and biochemical tests.[16-17]

2.2 Antibiotic Sensitivity Assay

Muller Hinton Agar Plates: Prepared; Inoculated with Procured Gram Positive and Gram Negative Bacteria The antibiotic disc was impregnated with Muller Hinton agar on the plate surface. Plates were incubated for 24 hours at 37 °C, forming a clearing zone around the disc[18]. In the agar disc diffusion method, antibiotic sensitivity of ceftriaxone, fluoroquinolones, azithromycin and amphotericin, nystatin, and fluconazole testing against bacterial and fungal pathogens is part of Standard Method [19].

2.3 Collection of Ganoderma lucidum

Ganoderma lucidum was collected as wild from the ordinary soil of Thanjavur (Dt), Tamil Nadu and authenticated in the Department of Plant Pathology, Tamil Nadu Agriculture University, Coimbatore. The selected strains were multiplied on potato dextrose agar (PDA) Petri plates, and slant culture was also maintained for further analysis.

2.4 Preparation for Mushroom Extract

Ten grams of mushroom powder were mixed with 50 ml of aqueous and ethanol separately in a beaker and placed in a shaker for 24 hours. The aqueous solutions were filtered through Whatman No. 1 filter paper and then placed in the rotary evaporator vacuum for 15 minutes at 37°C. Then the residue was dissolved in 10 ml of dimethyl sulfoxide (DMSO) and stored at 40°C for further analysis [20].

2.5 Bioactive Compounds of Ganoderma lucidum

2.5.1 Qualitative Bioactive Analysis

Freshly prepared aqueous and ethanol extracts were tested for bioactive compounds using standard methods (Harborne (1973)[22].

2.5.2 Quantitative Bioactive Analysis

Preliminary bioactive substances like alkaloids [21], aminoacids[21], coumarins[21], flavonoids [22], phenols [21], quinones[21], saponins[23], steroids[21], tannins [24] and terpenoids[21] were analysed by using standard methods.

2.6 In Vitro Anti - Inflammatory Activity[25]

*T*he reaction mixture (5 mL) consisted of 0.2 mL of egg albumin (from fresh hen's egg), 2.8 mL of phosphate-buffered saline (PBS, pH 6.4) and 2 mL of varying concentrations of extracts (100, 200, 300, 400 and 500µg/ mL respectively). A

similar volume of double-distilled water served as a control. Then the mixtures were incubated at (37± 2°C) in an incubator for 15 min and then heated at 70°C for 5 min. After cooling, their absorbance was measured at 660 nm by using the blank. Diclofenac sodium at the final concentrations (100-500µg/ mL) was used as a reference drug and treated similarly to determine absorbance. The percentage inhibition of protein denaturation was calculated by using the following formula:

$$\% \text{ inhibition} = 100 \times (Vt / Vc - 1)$$

Where Vt = absorbance of the test sample, Vc = absorbance of control. The extracts concentration for 50% inhibition (IC50) was determined by plotting percentage inhibition with respect to control against treatment concentration.

2.7 Antioxidant Activity

2.7.1 Hydrogen Peroxide (H_2O_2) Radical Scavenging Activity Assay [26]

Solution of 0.2 M potassium dihydrogen phosphate and 0.2 M sodium hydroxide solutions were prepared per the Indian Pharmacopoeia 1996 standards. 50 ml potassium dihydrogen phosphate solution was placed in a 200 ml volumetric flask, and 39.1 ml of 0.2Msodium hydroxide solution was added. Finally, the volume was made up to 200ml with distilled water to prepare phosphate buffer (pH-7.4). 50 ml phosphate buffer solution was added to an equal amount of hydrogen peroxide and generated free radicals. The solution was kept at room temperature for 5min to complete the reaction. Extracts (1 ml) in distilled water were added to 0.6 ml hydrogen peroxide solution. The absorbance was measured at 230 nm in a spectrophotometer against a blank solution containing phosphate buffer solution without hydrogen peroxide. The percentage of scavenging of H_2O_2 of the extract was calculated. Ascorbic acid (0.1 mg/ml) was used as a standard, and the same concentrations were prepared as the test solutions. The ability to scavenge the H_2O_2 radical was calculated using the following equation:

$$H_2O_2 \text{ scavenging activity } (\%) = (A0 - A1) / A0 \times 100$$

Where A0 is the absorbance of the control and A1 is the absorbance in the presence of an extract sample. A standard of ascorbic acid was run using the same concentrations as that of extract. The antioxidant activity of the sample was expressed as a concentration (mg/ml) of the sample that inhibited the formation of H_2O_2radicals by 50%.

2.7.2 DPPH Assay [27]

The antioxidant activity of the *Ganoderm alucidum* based on the scavenging activity of the stable 2, 2- diphenyl-2-picrylhydrazyl (DPPH) free radical was determined according to the method described with slight modification. The extraction solvents, such as extract, were prepared in 100, 200, 300, 400 and 500µg/mL. Five ml of each solution was prepared, and the concentration was mixed with 0.5 mL of 1 ml DPPH solution. The experiment was done in triplicate. The test tubes were incubated for 30 min at room temperature, and the absorbance was measured at 517nm. The lower absorbance of the reaction mixture indicated higher free radical scavenging activity. Vitamin C (0.1 mg/ml) was used as a standard, and the same concentrations were prepared as the test solutions. The difference in absorbance between the test and the control (DPPH in methanol) was calculated and expressed as % scavenging of DPPH radical.

$$\% \text{ Scavenged [DPPH]} = [(AC - AS)/AC] \times 100$$

2.7.3 Thiobarbituric Acid (TBA) Assay [28]

Preparation of TBA Reagent. The standard solution of 4.0 mm TBA was prepared in glacial acetic acid. For this purpose, 57.66 mg of TBA was dissolved in 100 mL of glacial acetic acid. Samples of *Ganoderma lucidum* extract with 100% glacial acetic acid (AA) and 50% glacial acetic acid with water (AW). The extract of leaf sample (1 mL) was mixed with 1 mL TBA reagent, and the above procedure was repeated five times (nn = 5). Ferric Thiocyanate (0.1 mg/ml) was used as a standard, and the same concentrations were prepared as the test solutions. The TBARS was calculated using the formula as $\mu\mu$M/g of the sample:

$$\text{TBARS } (\mu\mu\text{M/g}) = (\text{Ac} \times VV) / WW, \quad (1)$$

Where Ac is the amount determined from the calibration curve and WW is the weight of the sample taken, and VV is the volume in mL or dilution factor of the total leaf extract prepared.

3. STATISTICAL ANALYSIS

3.1 Quantitative Analysis of Bioactive Compounds

Experiments were carried out in triplicate and the results are expressed as mean values with standard deviation.

3.2 Antibiotic Sensitivity Assay

Pearson's correlation coefficients were analyzed and used to assess the antibiotic sensitivity assay against infected bacteria and fungi in post-Covid-19 patients. Statistical Package for Social Sciences (SPSS) software computed and analysed the data.

4. RESULTS

Table 1: Incidence of microbial infection from urine samples

No of patients	Age group	Strain code	Male	Female	percentage of disease (%)
1.	45	TPGS101	0	1	56
2.	60	TPGS102	1	0	22
3.	58	TPGS103	1	0	52
4.	48	TPGS104	0	1	57
5.	52	TPGS105	1	0	59
6.	60	TPGS106	0	1	31

During the study period, urine samples were collected from post-COVID-19 patients in the Government Medical College, Thanjavur. Six urine samples were collected and processed to isolate bacteria and fungi. The strain code is denoted as TPGS 101 to TPGS 106 (Table 1). All the samples were collected from people aged 45 to 60; among them, 3 samples were from men and 3 from women. The maximum percentage of infection was 59% for males in TPGS-105 and 57% for females in TPGS-104. Similarly, the minimum for males in TPGS102 (22%) and females in TPGS106 (31%), The overall percentage of infections was below 60. Identification and biochemical test of bacteria from secondary infected of Post Covid-19 Patients are shown in Plate-1 and Table 2.

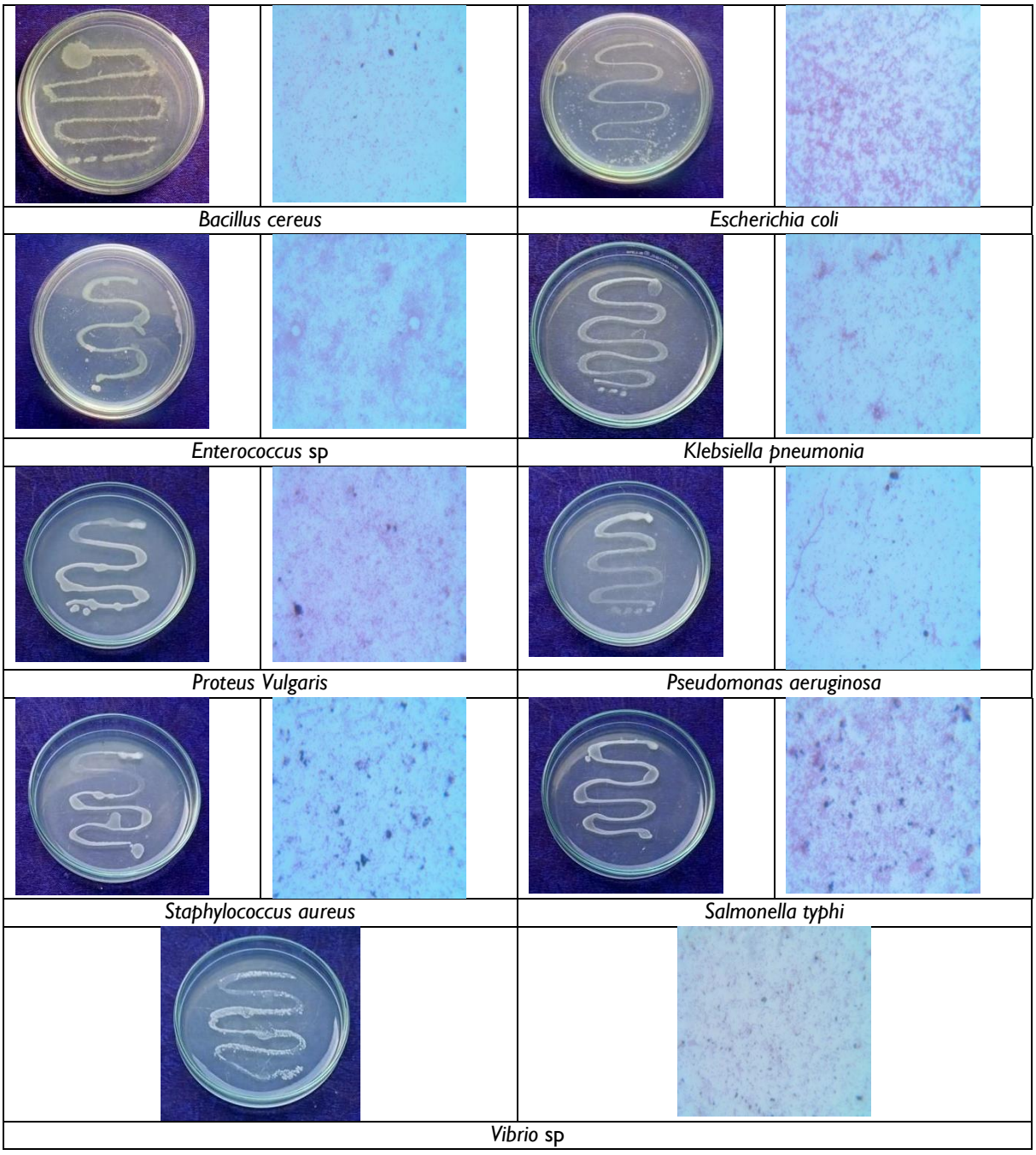


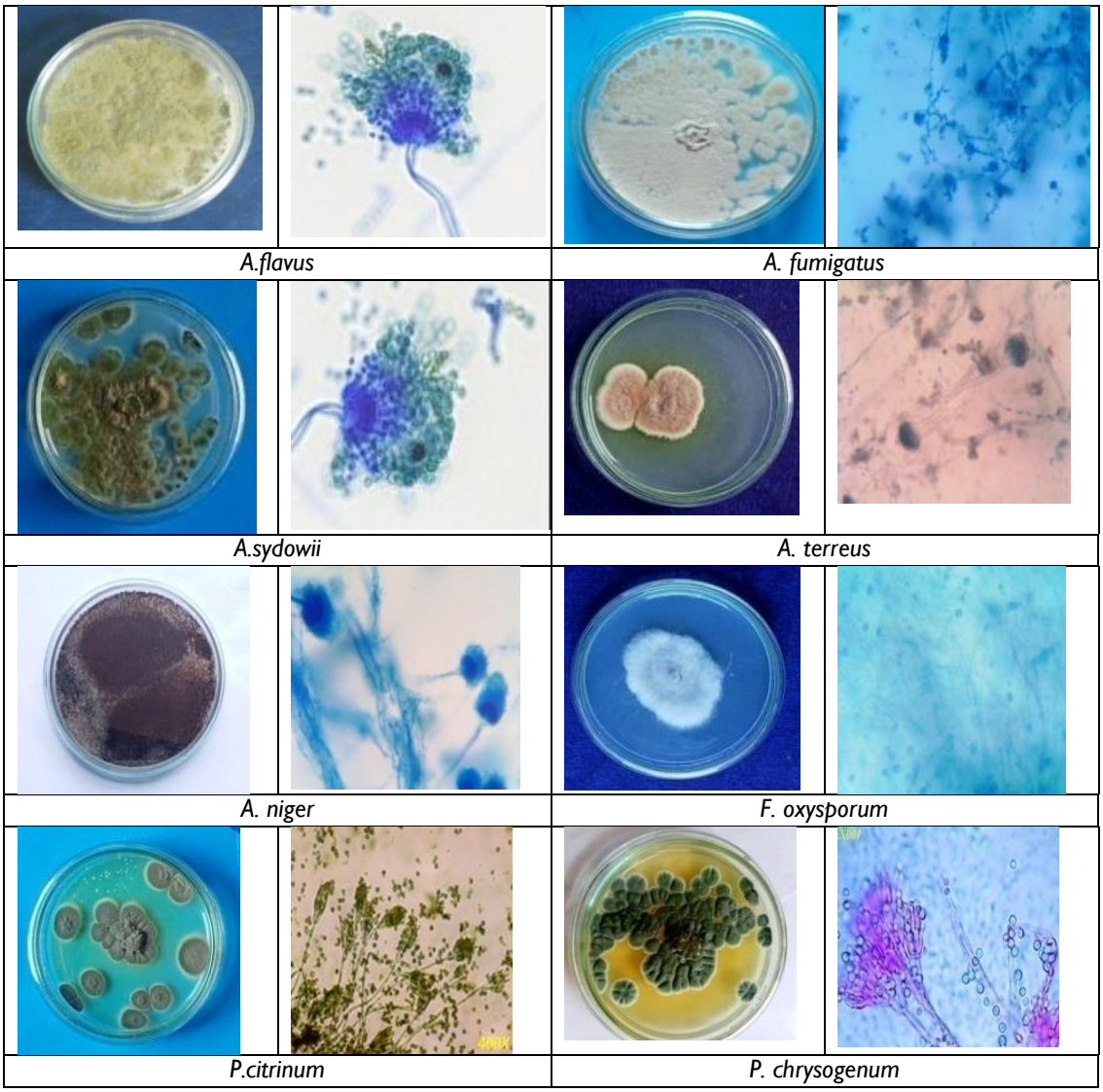
Plate 1: Identification of bacteria from post-Covid-19 patients

Table 2: Biochemical features of bacteria isolated from secondary infection of Post Covid-19 Patients

Strain	Gram stain	Motility	I	MR	VP	C	Cat	L	G	Oxidase	Identification of Bacteria
TPGS1	+	-	-	-	+	-	+	-	+	+	*Bacillus cereus*(IBRI131)
TPGS2	-	+	+	-	+	-	-	-	-	+	*Escherchia coli*(IBRI102)
TPGS3	+	+	-	+	-	-	-	-	+	+	*Enterococcus* sp (IBRI135)
TPGS4	+	-	-	+	-	-	-	-	+	-	*Klebsiellapneumoniae*(IBRI105)
TPGS5	+	-	+	+	+	-	-	-	-	+	*Pseudomonas aeruginosa*(IBRI108)
TPGS6	-	+	+	+	-	-	+	-	-	+	*Proteus vulgaris*(IBRI198)
TPGS7	-	+	-	-	+	-	-	-	+	+	*Staphylococcus aureus*(IBRI112)
TPGS8	+	-	-	+	-	+	-	+	+	+	*Salmonella typhi*(IBRI197)
TPGS9	+	-	-	+	-	-	-	-	+	+	*Vibrio* sp (IBRI199)

(I-Indole, MR-Methyl Red, VP-VogesProskauer, C-Citrate, Cat-Catalase, L-Lactose, G-Galactose)
(TP- T. Pushpa&GS-G.Senthilkumar)

When the samples were analyzed using the Clinical and Laboratory Standards Institute (CLSI) method, the strains of TPGS1, TPGS2, TPGS3, TPGS4, TPGS5, TPGS6, TPGS7, TPGS8, and TPGS9 identified in organisms are *Bacillus cereus, Escherichia coli, Enterococcus* sp., *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus, Salmonella typhi*, and *Vibrio* sp. Identified the organisms were confirmed by their growth on nutrient agar medium, microscopic examinations, and biochemical tests approved by Bergey's manual determinative bacteriology. Pneumoniae mainly causes COVID-19. *Klebsiella pneumoniae* was negative, and other positive organisms were identified. (Table - 2 and plate - 1). The identification of fungi in the post-Covid-19 urine sample are shown in Plate-2, Table-3 and fig-1.



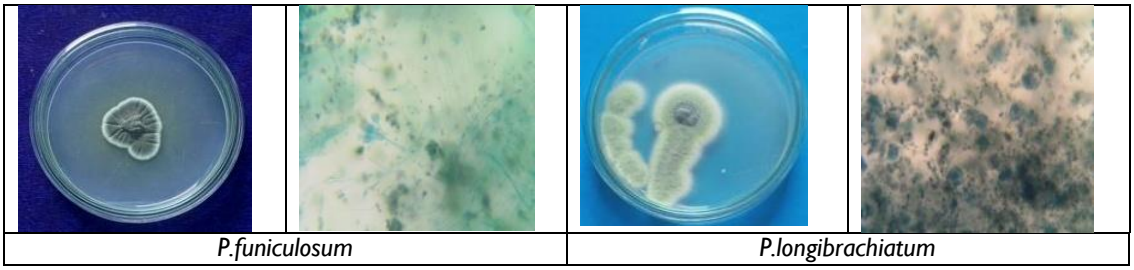


Plate 2: Identification of fungi in secondary infected of Post Covid-19 patients

Table 3: Identified list of fungi from secondary infection of Post Covid-19 patients

Name of the Fungi	Urine samples					
	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Sample 6
Aspergillus flavus	2	6	1	2	4	5
A. fumigatus	1	2	3	3	1	1
A. niger	6	1	1	1	4	5
A. terreus	2	4	1	3	6	1
A. sydowii	1	3	6	3	1	2
Fusariumoxysporum	4	4	3	6	7	5
Penicilliumcitrinum	2	5	1	5	1	4
P. funiculosum	3	3	2	1	3	6
P. chrysogenum	1	2	5	3	4	1
P. longibrachiatum	5	3	7	4	6	5
Total number of colonies	27	33	30	31	37	35

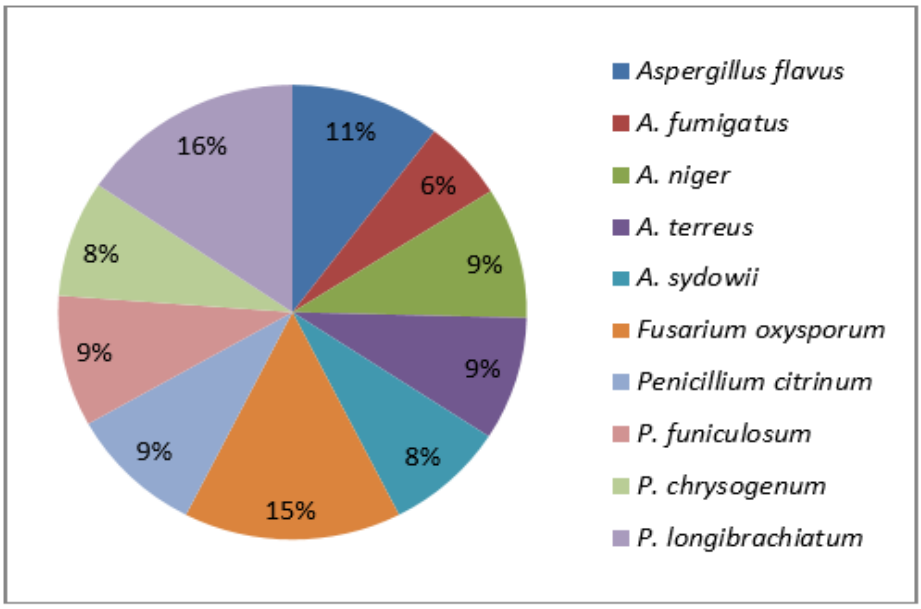


Fig 1: Distribution of fungal pathogens isolated from post-Covid-19 patients

Ten different fungal species were identified from the urine samples like *Aspergillus flavus, A. fumigatus, A. niger*, *A.terreus*, *A. sydowii, Fusarium oxysporum, Penicillium citrinum, P.funiculosum, P.chrysogenum* and *P.longibrachiatum* were isolated from the urine samples of post Covid-19 cases(Table-3, Plate-2and fig-1).All the fungi were identified via microscopic examination as well as growth characteristics on PDA(Potato dextrose agar) medium. It was observed that the maximum percentage of fungal isolates was *P.longibrachiatum* (16%) and *Fusarium oxysporum* (15%). The minimum percentage of fungi isolates was *A. fumigates* (6%). The maximum number of colonies(37) was presented in sample 5 and the minimum number of colonies (27) was shown in sample 1.

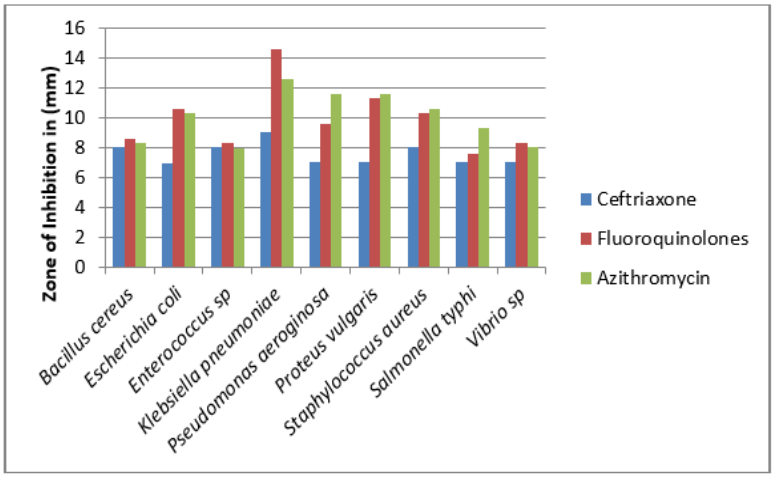


Fig 2: Determination of antibiotic sensitivity assay against infected bacteria of Post-Covid -19 patients

Table 4: Correlation analysis of antibiotic sensitivity assay against infected bacteria of post -Covid-19 patients

Correlation	*Bacillus cereus*	*Escherichia coli*	*Enterococcus* sp	*Klebsiella pneumonia*	*Pseudomonas aeruginosa*	*Proteus Vulgaris*	*Staphylococcus aureus*	*Salmonella typhi*	*Vibrio* sp
Bacillus cereus	1								
Escherchia coli	0.8923	1							
Enterococcus sp	0.8612	0.5391	1						
Klebsiella pneumonia	0.9838*	0.9586*	0.7565	1					
Pseudomonas aeruginosa	0.5405	0.8621	0.0380	0.6823	1				
Proteus vulgaris	0.8237	0.9909*	0.4212	0.9118*	0.9223*	1			
Staphylococcus aureus	0.7952	0.9833*	0.3768	0.8908	0.9399*	0.9988*	1		
Salmonella typhi	0.2185	0.6354	-0.3076	0.3894	0.9390*	0.7333	0.7653	1	
Vibrio sp	0.9420*	0.9920*	0.6409	0.9868*	0.7914	0.9661*	0.9525*	0.5331	1

- Correlation significance at 0.05 levels, respectively.

Urinary isolates were assessed to check antibacterial potentials. Ceftriaxone, fluoroquinolones and azithromycin were tested for antibiotic sensitivity using the well diffusion method. Both the antibiotics showed productive efficiency against gram-positive and gram-negative uropathogens: *Bacillus cereus*, *Escherichia coli*, *Enterococcus sp.*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa, Proteus vulgaris*, *Staphylococcus aureus*, *Salmonella typhi* and *Vibrio* sp. The efficiency of antibiotics was varied from 9.050 mm to 14.61 mm and 12.61 mm by ceftriaxone, fluoroquinolones and azithromycin, which produced the best activity against *Klebsiella pneumoniae (Fig. 2)*. Among the bacteria, fluoroquinolones and azithromycin antibiotics showed the highest resistance. Mainly, fluoroquinolones are indicated for treating *K.. pneumoniae* bacterial infections.

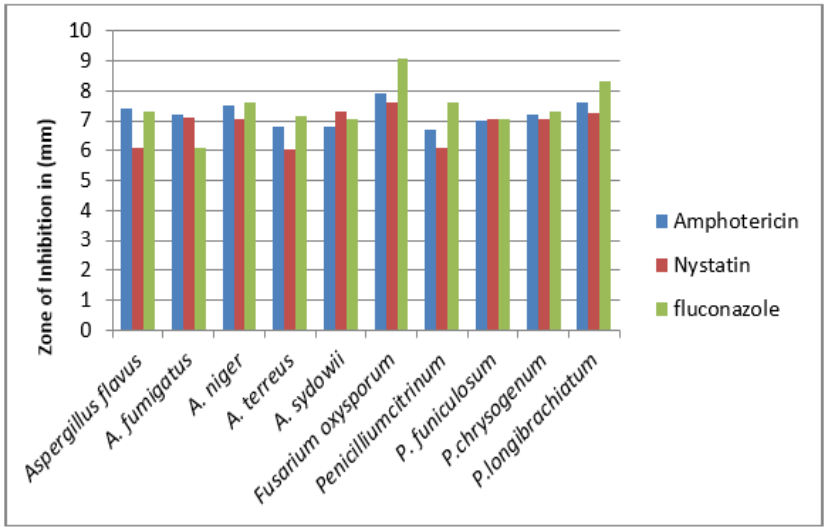


Fig 3: Determination of antibiotic sensitivity assay against infected fungi of Post-Covid -19 Patients

Table 5: Correlation analysis of antibiotic sensitivity assay against infected fungi of post Covid-19 patients

Correlation	*Aspergillus flavus*	*A. fumigatus*	*A. niger*	*A. terreus*	*A. sydowii*	*Fusarium oxysporum*	*Penicillium citrinum*	*P. funiculosum*	*P.chrysogenum*	*P.longibrachiatum*
Aspergillus flavus	1									
A. fumigatus	-0.4409	1								
A. niger	0.4922	-0.9983*	1							
A. terreus	0.5036	-0.9974*	0.9999*	1						
A. sydowii	0.1850	-0.9636	0.9465	0.9422	1					
Fusarium oxysporum	0.1985	-0.9671	0.9508	0.9467	0.9999*	1				
Penicillium citrinum	0.4845	-0.9987*	0.9999*	0.9997*	0.9493	0.9535	1			
P. funiculosum	0.9999*	-0.4490	0.5	0.5113	0.1938	0.2072	0.4923	1		
P.chrysogenum	-0.5077	-0.5493	0.5	0.4885	0.7526	0.7435	0.5075	-0.5	1	
P.longibrachiatum	-0.5294	-0.5279	0.4778	0.4663	0.7357	0.7263	0.4856	-0.5217	0.9996*	1

**** - Correlation significance at 0.05 levels, respectively.***

Antibiotic sensitivity assay fungi of amphotericin and nystatin along with standard fluconazole were done against 10 different fungi *Aspergillus flavus*, *A. fumigatus, A. niger, A.terreus, A. sydowii, Fusarium oxysporum, Penicillium citrinum, P.funiculosum, P. chrysogenum*, and *P. longibrachiatum*, isolated from urine samples of post- Also provide an efficient zone of inhibition, which ranges from 6.90mm, 7.30mm the best inhibition against *Fusarium oxysporum*, in amphotericin, nystatin, and fluconazole. Similarly, almost maximum inhibition zones are presented at *P.longibrachiatum* 7.60mm, 7.26 mm and 8.30mm (Fig 3). On the other hand, the fluconazole and amphotericin antibiotics showed the highest resistance.

Table 6: Qualitative analysis of bioactive compounds of *Ganoderma lucidum*

Bioactive compounds	Different solvents	
	Aqueous	Ethanol
Alkaloids	+	+
Amino acids	+	+
Coumarins	-	-
Flavonoids	+	+
Glycoside	-	-
Phenols	+	+
Phlobatannins	-	-
Quinones	-	-
Saponin	-	-
Steroids	+	+
Tannin	-	-
Terpenoids	+	+

(+)Present (-)Absent

Table 7: Quantitative analysis of bioactive compounds of *Ganoderma lucidum*

Bioactive compounds	Quantity (mg/g)	
	Aqueous	Ethanol
Alkaloids	6.14±0.08	5.70±0.09
Amino acids	3.31±0.03	4.63±0.06
Flavonoids	4.10±0.07	2.59±0.08
Phenols	1.80±0.18	1.41±0.09
Steroids	7.00±0.40	7.02±0.28
Terpenoids	6.49±0.05	6.29±0.02

Standard deviation ± error

Analysis of bioactive compounds in *Ganoderma lucidum* Two types of solvents are used. The solvents are aqueous and ethanol. Qualitative bioactive compounds include alkaloids, amino acids, Coumarins, flavonoids, glycosides, phenols, phlobatannins, quinones, saponins, steroids, tannins and terpenoids are tested in both the extracts (Table 6). Alkaloids, amino acids, flavonoids, phenols, steroids, and terpenoids were commonly present in both solvents. Quantitative analysis

of bioactive compounds indicated higher availability of steroids (7.00±0.40)mg/g, terpenoids (6.49±0.05)mg/g and alkaloids (6.14±0.08)mg/g in aqueous solution, respectively. On the other hand, ethanol showed higher quantities of steroids (7.02±0.28)mg/g and terpenoids (6.29±0.02)mg/g (Table 7). Aqueous solvents are highly effective in *Ganoderma lucidum*.

Table 8: Efficacy of anti-inflammatory activity of *Ganoderma lucidum*

The concentration of Extract (µg/ml)	Diclofenac Sodium (Standard %)	% of activity	
		Aqueous	Ethanol
100	10.2±0.36	12.3±0.50	13.9±0.02
200	10.6±0.69	15.1±0.96	11.4±0.93
300	11.3±0.32	18.9±0.09	10.2±0.13
400	11.8±0.12	14.0±0.32	14.4±0.05
500	12.0±0.36	16.7±0.48	13.4±0.82

Values are expressed in mean ±S.D

The *Ganoderma lucidum* mushroom extracts of aqueous and ethanolic media exhibited a dose-dependent inhibition of protein (albumin) denaturation and were standard at 100, 200, 300, 400 and 500 µg/ml concentrations. Both extracts and diclofenac sodium exhibited concentration-dependent inhibition of protein denaturation. The increased absorbance in both the extracts and the standard drug indicated protein stabilizing activity (denaturation was inhibited) with increased dose. The standard diclofenac sodium inhibition of 10.2±0.36, 10.6±0.69, 11.3±0.32, 11.8±0.12 and 12.0±0.36%respectively.The 300 µg/ml(18.90.09 %) concentration of aqueous solvent is extremely inhibiting. The 400µg/ml (14.4±0.05 %) concentration of ethanol solvent is extremely inhibited, respectively (Table 8).

Table 9: Effect of antioxidant activity of *Ganoderma lucidum* aqueous extract by various methods

The concentration of extract (µg/ml)	Standard (ascorbic acid)(%) activity	Hydrogen peroxide scavenging (H_2O_2) assay (%) activity	Standard (Vitamin C) (%) activity	DPPH assay (%) activity	Standard (ferric thiocyanate) (%) activity	TBA (%) activity
100	11.3±0.04	13.7±0.06	10.4±0.10	17.7±0.19	12.2±0.13	14.6±0.13
200	11.5±0.03	11.5±0.77	11.2±0.16	16.4±0.25	13.3±0.15	11.3±0.35
300	12.6±0.02	10.7±0.08	12.3±1.14	16.3±0.09	14.3±0.17	13.2±0.18
400	13.3±0.05	9.05±0.06	13.3±0.10	18.6±0.80	15.4±0.13	16.6±0.08
500	14.5±0.03	10.6±0.62	14.2±0.12	16.5±0.02	16.3±0.14	15.2±0.14

Values are expressed in mean ± S.D

Different concentrations of antioxidant activity, such as 100 to 500µg/ml were analyzed. The three standards used in the activity of H_2O_2 corresponding to a standard ascorbic acid, DPPH related to vitamin C and thiobarbituric acid corresponding to ferric thiocyanate were recorded with the percentage of activity. Hydrogen peroxide assay was presented at the aqueous extract of concentration 400 µg/ml for minimum, but DPPH and TBA assay was maximum in the same concentration. All methods are standard in same for gradually increased in this activity. H_2O_2 assay was highly inhibited in 100 µg/ml concentration (13.7±0.06%). DPPH and TBA assay maximums at 400µg/ml were (18.6±0.80%) and (16.6±0.08%) respectively (Table 9).

Table 10: Effect of antioxidant activity of *Ganoderma lucidum* with ethanol extract by various methods

The concentration of extract (µg/ml)	Standard (ascorbic acid) (%)	Hydrogen peroxide scavenging (H_2O_2) assay (%) activity	Standard (Vitamin C) (%)	DPPH assay (%) activity	Standard (ferric thiocyanate) (%)	TBA (%) activity
100	11.3±0.04	10.5±0.84	10.4±0.10	16.5±0.64	12.2±0.13	15.6±0.15
200	11.5±0.03	13.4±0.22	11.2±0.16	15.4±0.18	13.3±0.15	13.1±0.68
300	12.6±0.02	14.8±0.28	12.3±1.14	17.9±0.36	14.3±0.17	14.6±0.44
400	13.3±0.05	13.3±0.19	13.3±0.10	20.1±0.68	15.4±0.13	15.1±0.69
500	14.5±0.03	12.7±0.80	14.2±0.12	19.9±0.33	16.3±0.14	12.7±0.43

Values are expressed in mean ± S.D

The antioxidant activity of *Ganoderma lucidum*is maximum in ethanol extract compared to the other solvent. The maximum percentage of DPPH assay was observed in ethanol solvent at concentrations of 100 µg/ml (16.5±0.64%), 200µg/ml (15.4±0.18%), 300µg/ml (17.9±0.36%), 400 µg/ml (20.1±0.68%)and 500µg/ml (19.9±0.33%).The antioxidant activity of Hydrogen peroxide scavenging (H_2O_2) assay was(14.8±0.28%) at 300 µg/ml concentration and thiobarbituric acid(TBA)
the assay was (15.6±0.1%) at 100µg/ml recorded respectively.

5. DISCUSSION

Scientists from all over the world indicated the prevalence of UTI among female than male, whereas in this study more samples were collected from male post-Covid-19 cases. Inappropriate usage of antibiotics and other immunosuppressive drugs for the treatment of Covid-19 could reduce immunological features of the Covid-19 cases; hence, in this study, more bacteria and fungi organisms were isolated from the post-Covid-19 urine samples. The most common bacterial infections in female outpatients are urinary tract infections[29]. The inappropriate use of antibiotics and, consequently, increased incidence of antimicrobial resistance leads to many complications and prolongs the problem of UTI[30]. This results in an extremely negative impact on the patients' quality of life and represents a significant financial implication. Urinary tract infections are a major public health problem worldwide, with an estimate 150 million cases per year. With an incidence of 12% in females and 3% in males, urinary tract infections are still the most common in females and in all age groups. In primary health care, 50% to 80% of women with typical symptoms have UTI[31,32]. According to our study of the total number of samples, the percentage referring to females is significantly lower compared to male samples. This data is more variable than many current reports as the samples were collected from post-Covid-19 Cases, and the incidence of Covid admission was higher among males; hence variable report was noted in this study. According to various studies all around the world, *E.coli* was the most common isolate, followed by *Enterococcus faecalis, Staphylococcus saprophyticus, K. pneumonia* and *Proteus mirabilis.* In this study also indicated that *Bacillus cereus, E.coli, Enterococcus* sp., *Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, Staphylococcus aureus, Salmonella typhi* and *Vibrio* sp.*,* Presence of *E.coli* in UTI is a constant for a long time and with its trend of increasing resistance to different groups of antibiotics, the treatment becomes more challenging. The increasing presence of resistant strains, combined with various virulent factors, makes *E. coli* the most dominant cause of UTI. After *E. coli*, according to our study, the most common isolates in the female population are *Proteus* sp.*, Enterococcus faecalis, Enterobacter, Pseudomonas* sp. and *Klebsiella* sp., which represent a range of bacteria which, with small differences, makes most common isolates worldwide. Urinary isolates were assessed to check antibacterial potentials. ceftriaxone, fluoroquinolones and azithromycin were tested for analyzing antibiotic sensitivity assay well diffusion method. ceftriaxone, fluoroquinolones and azithromycin produced the best activity against *Klebsiella pneumonia.* Anibiotic sensitivity assay fungi of amphotericin and nystatin along with standard fluconazole were done against 10 different fungi.The best inhibition against *Fusarium oxysporum* in amphotericin, nystatin and fluconazole. It was estimated that about 1.5 million fungal species[33] around the world are the good source of natural bioactive compounds[34]. This study evidenced the antibacterial efficiency of ethanol and aqueous. These extracts produced effective antibacterial efficiency than the commercial drug chloramphenicol. Antimicrobial drugs have long been used for prophylactic and therapeutic purposes [35]. Unfortunately, the recent increase in the occurrences of drug-resistant bacterial strains is creating serious treatment problems. Consequently, the antimicrobial activity of various anti-tumour polysaccharides from medicinal mushrooms is being re-evaluated in terms of their clinical efficacy. Such compounds would be expected to function by mobilizing the body 's humoral immunity to ward off viral, bacterial, fungal and protozoal infections resistant to current antibiotics. Polysaccharide Keratin has been shown to induce potent antimicrobial activity against *Escherichia coli*, *Listeria monocytogenes* and *Candid*[36,37]succeeded in the Isolation and identification of Pleuromutilin, a diterpene that is especially useful for the treatment of mycoplasma infections in animals and served for the development of the first commercial antibiotic of basidiomycete origin. With the development of new fermentation and purification technologies, basidiomycetes are again receiving attention as potential sources of new classes of antibiotics[38,39,40]. The antimicrobial activity of aqueous and ethanol extracts from *Ganoderma lucidum* against nine UTI MDR bacterial strains, all the extracts provide positive assays against gram positive and gram negative UTI isolates. Gram-positive bacteria are more sensitive than gram-negative bacteria to fungal extracts. This study also incurred insights from the reports of[41,42,43]. They also stated antimicrobial potentials of mushroom fungi against different microbial species. Our research also has shown that all the isolates from the Post Covid-19 cases of urine showed MDR property, hence all the organisms are subjected for antibiotic resistance efficacy study using medicinal mushroom *Ganoderma lucidum*. In general, denaturation of protein leads to the production of autoantigens, damage of tissues, which could lead to inflammatory mediators and inflammation. Inhibition of protein denaturation prevents the formation of inflammatory mediators, thereby reducing inflammation. They[44] reported that extracts exhibit anti-inflammatory activity by reducing histamine and serotonin production, which is responsible for inflammatory activity. They also stated that extracts also block cyclooxygenase enzyme there by prevents inflammation. Flavonoids, tannins and penolic compounds are responsible for this kind of activity. Overall, flavonoids may prevent protein denaturation. Our results were also in line with[45-46]stated that anti-inflammatory power of the extract could be due to glycosides or steroids. Monoterpenoids[17], sesquiterpenes[47], diterpenes[48], terpenoids[49]; flavonoids[50], glycosides[51], steroids[50], cyclohexylethanoids[49], Anthocyanins[52] could be responsible for anti-inflammatory activity. In our study also been alkaloids, amino acids, flavonoids, phenols, steroids and terpenoids were commonly presented in both of the solvents. The anti-inflammatory activity was 300 g/ml concentration of aqueous solvent extremely inhibitate respectively. Mushrooms are usually used as —Biological Response Modifiers – which will modify the host 's biological response by a stimulation of the immune system which may result in various therapeutic effects. Mushrooms have anticancer, liver protective, analgesic, sedative and anti-radiation properties and have therapeutic effects in gastric and duodenal ulcer. Mushrooms are low-calorie, high protein diet with almost no sugars and starch and thus suitable for diabetic people and also for people with obesity, hypertension and heart diseases. Oxidation is vital to living organisms for the production of energy to provide fuel in biological process. Oxidative damage caused by free radicals may be related to aging and disease. A free radical is any species which contains one or more unpaired electrons and is capable of independent existence. Free radicals that are produced during the natural metabolism of aerobic cells are mostly in the form of reactive oxygen species (ROS). The most reactive oxygen species (ROS) include superoxide anion (O), hydroxyl radical (OH-), hydrogen peroxide radical (ROO-). The nitrogen derived free radicals are nitric oxide anion (NO-) and peroxynitrite anion (ONOO-)[53]. Most of the free radicals once produced are neutralized by cellular antioxidant defenses (enzymes and non-enzymatic molecules). Maintenance of equilibrium between

free radicals production and antioxidant defense is an essential condition for adequate organism functioning[54,55]. In fact, the non-controlled production of free radicals has been related to more than one hundred diseases including several kinds of cancer, diabetes etc, Tissues contain several compounds called antioxidants that inhibit free radicals. The reason is antioxidants are important to an organism's physical well-being comes from the fact that oxygen is a potentially toxic element since it can be transformed by metabolic activity into more reactive forms such as the superoxide anion, hydrogen peroxide, singlet oxygen and the hydroxyl radical. Almost all organisms are well protected from free radical damage by enzymes such as superoxide dismutase and catalase, or compounds such as ascorbic acid, tocopherols, glutathione and flavonoids. Xanthine oxidase is one of the main enzymatic sources of reactive oxygen species (ROS) in vivo[56]. Xanthine oxidase in normal tissue is a dehydrogenase enzyme that transfers electrons to nicotinamide adenine dinucleotide (NAD+) as it oxidizes xanthine or hypoxanthine to uric acid.

6. CONCLUSION

It can be concluded that the biological activities are the most important characters of Medical Microbiology. Medicinal mushrooms are rich sources of potential antibiotic agents. Preliminary myco chemical screening revealed the presence of some specific bioactive compounds in *G.lucidum* which are highly remarkable potential sustainable activities in the field of medicinal industry. According to the antioxidant property that promotes free radicals of molecules to create a chain reaction of cellular disruption, when cells grow and reproduce at an abnormal rate of breaking down the natural defence and repair process. This is because, which ingestion is extremely beneficial for mechanism in antioxidant productive capabilities topical use is solely focused on booting our health and appearance. One of the most important effects of the *G. lucidum* mushroom is that it can boost the immune system and ant-inflammatory activities. This type of research in biological properties of *G. lucidum* with respective cancer patients has shown that some of the bioactive molecules found in the mushroom can increase the activity of a type of white blood cells in the form of natural killer cells. However, it is clear that *G. lucidum* bioactive compounds which form the potential candidature for biological activates including cancer disease and Immune system. More research is needed to determine the extent of the benefits in the healthy and ill.

7. AUTHORS CONTRIBUTION STATEMENT

Dr. G. Senthilkumar designed and finalized the manuscript of study, Dr. A. Panneerselvam and Dr. V. Ambikapathy provided valuable suggestions for this work, Mrs. T. Pushpa collected samples and analyzed the work and prepared the draft manuscript, Mr. P. Prakash helped for analysis and alignment of manuscript, Ms. A. Kanmani discussed the methodology. All authors read and approved the final version of the manuscript.

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9. CONFLICT OF INTEREST

Conflict of interest declared none.

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