Ann. For. Res. 65(1): 2854-2867, 2022
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
https://www.e-afr.org/

DETERMINATION OF THE INHIBITORY ACTIVITY OF GONDERMA EXTRACT AGAINST SOME TYPES OF BACTERIA THAT ARE RESISTANT TO MULTIPLE ANTIBIOTICS.

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Abstract:

200 median urine samples were collected from patients and visitors to Kirkuk Hospital for the period between 11/18/2020 to 2/17/2021. The samples were taken from different age groups ranging from 20-50 years of both sexes. The results of bacterial culture showed that 138 samples and 69% of the samples gave bacteria growth on the media MacConkey agar, Mannitol salt agar, Blood agar, while 62 and 31% of the samples did not give bacteria growth. Using the Vitek 2 Compact System, 7 different types of Gram-negative bacteria and 9 different types of Gram-positive bacteria were diagnosed, which included 25 Escherichia isolates. coli by 18.8%, followed by Staphylococcus aureus and its isolates numbered 20 by 14.4%, and Staphylococcus bacteria. Epidermidis and saprophyticus Staphylococcus, the number of their isolates was 19, or 13.7%, and 15, or 10.8%, respectively. As for Klebsiella pneumoniae, the number of its isolates was 11, or 8%, while the number of isolates of Proteus bacteria was. vulgaris, Pseudomonas aeruginosa and Serratia.marcescens 5 isolates with a percentage of 3.7%, respectively. Mirabilis. Proteus and. Faecalis Enterococcus and Streptococcus.pneumoniae The number of its isolates was 4 and 2.9%, respectively, for Acinetobacter. baumannii, Staphylococcus.lentus and Streptococcus.pyogenes, the number of their isolates was 3 by 2.1%, while Staphylococcus.warneri bacteria were two isolates by 1.4%. The sensitivity of bacterial isolates were tested using (13) antibiotic that included quinolones, aminoglycosides, beta- lactams and tetracyclines antibiotics. Imipenem and levofloxacin are more effective, While the effectiveness of the rest of the antibiotic range from medium to ineffective The fungus Ganoderma lucidum (in terms of science/ Salah al-Din governorate north-central Iraq) was collected between August 2021-September 2021. The inhibitory activity of the secondary metabolite extract of Ganoderma lucidum was tested against twenty different types of bacteria under study, which were characterized by their multiple resistance to antibiotics.

The highest inhibitory activity was against Staphylococcus. epidermidis With a diameter of inhibition of 30 mm for the aqueous extract of the old fungus by boiling for 72 hours at a concentration of 2% and at a concentration of 3%, the highest inhibitory activity against Staphylococcus. As for the inhibitory activity of the aqueous extract of the old mushroom by adding for 24 hours at a concentration of 2%, the highest inhibitory activity was against Enterococcus faecium1 bacteria with a diameter of 30 mm, and at a concentration of 3%, the highest inhibitory activity was against Serratia bacteria with a diameter of 27 mm. As for the extract of young mushrooms by steeping in boiling water for both periods 72 and 24 hours at a

concentration of 3%, the highest activity was recorded against Escherichia.coil bacteria with an inhibition diameter of 36 mm. As for the secondary alcoholic extract of Ganoderma lucidum at a concentration of 100% for 24 hours, it had the highest inhibitory activity against Staphylococcus bacteria. lentus, 28 mm in diameter.

Keywords: ; Catalase; *S. aureus* ;G.lucidum; *E. coli*

Introduction:

Urinary tract infections are defined as an inflammatory response to the urinary system as a result of the invasion and settlement process that occurs from pathogenic microorganisms. Urinary Tract infection (UTI) is one of the most common bacterial infections in humans, if urinary tract infections include many pathological conditions, including cystitis ((Urinary tract infection (urethritis) and pyelonephritis (Forsyth et al., 2018) Urinary tract infection is one of the most common infections in the community (acquired community) and in hospitals (nosocomial infection), and it affects different ages annually. The path of bacterial reproduction in the urinary tract (Foxman, 2020).

The use of antibiotics in clinical treatments is one of the most successful developments in modern medicine (Munita and Arias 2016,). Its use has reduced child mortality and increased life expectancy. However, the number of infections caused by multidrug-resistant bacteria has begun to increase globally and has become The specter of irreversible injuries is a reality (Blair et al, 2015).

As bacteria use complex and creative mechanisms to evade the attack of antibiotics, which led to the development of antibiotic resistance very rapidly during the past few decades to become now one of the greatest public health threats in the twenty-first century, which requires the search for new materials with anti-bacterial activity and from new sources Other than the well-known traditional sources represented by fungi, and large fungi are those that produce fruiting bodies that can be seen with the naked eye, and large fungi were classified into two main divisions: Basidiomycota and cystic fungi) De silva et al 2013. Fong and Fisher, 2014). In the last four decades, interest in this group of fungi has increased for several reasons, the most important of which is that it is widespread in the world and that it is a new source of effective compounds (alkaloids, polysaccharides, terpenoids, phenols, glycosides, tannins, flavonoids) (Deka et al, 2017; Culliao et al. ,2020) It has a promising effect in the fields of medicine and pharmacology, especially in the treatment of chronic diseases (such as cancer and diabetes) and its anti-bacterial role.)Deka et al, 2017; Badalyan,2020 Rapior and).

MATERIAL AND METHODS

Samples Culture

He samples were planted by taking the filling of the sterile bacteria vector for the urine sample from urine samples - and before centrifugation process - and with a cotton swab for other infections, on mannitol salt agar, blood agar and MacConkey agar to differentiate between positive and negative isolates, and the dishes were incubated. Under air conditions at a temperature of 37

°C for a period of 24-48 hours, the samples in which growth appeared were transferred to the graduate laboratory in the Department of Life Sciences - College of Education for Pure Sciences, to be purified and then diagnosed.

Diagnosis using the Vitek 2 compact system

This device was used to diagnose most types of bacteria in a confirmatory diagnosis through: The Vitek 2 compact system consists of two main components, the instrument and the computer, and the machine consists of five basic components.

Antibiotic sensitivity test using disc diffusion method Sensitivity

Testing was conducted for all isolates under study by the Disc diffusion method using the Kirby-Bauer method) described by the Laboratory and Clinical Research Institute (CLSl, 2015). The bacterial suspension was prepared by transferring (2-3) colonies to the liquid medium and incubated for 18 hours at a temperature of 35°C, then its turbidity was compared with 0.5 McFarland 0.5 standard, which equals 1810 x 1.5 cells/ml, then the bacterial suspension was spread using a squeegee. Cotton swab was placed on the surfaces of the dishes in the center of Muller Hinton and left to dry for 15 minutes. Then the tablets of the antibiotics shown in Table (3-4) were distributed on the dishes using sterile forceps and incubated for 24 hours at a temperature of 37 ° C. Then the results were read by measuring the diameter of the inhibition area around each tablet and compared with global measurements described by the Laboratory and Clinical Research Institute (CLSI 2015, 2015).

Mushroom collection and identification:

Mushrooms were collected in terms of science / Salah al-Din governorate, north-central Iraq, between August 2021-September 2021, according to the morphological characteristics (shape, color, dimensions of the mushroom cap and its upper and lower surface characteristics) and microscopic (such as the shape and dimensions of the basidia and basidial spores, the number of holes / mm and the interaction With potassium hydroxide (KOH) and the environment (the time of its appearance, the nature of its growth and the plants accompanying it). In light of these characteristics, the fungus was diagnosed and confirmed according to Al-Khazraji and his group (2017), after which the mushrooms were dried in an electric oven, then ground by an electric grinder, and then kept in powder form in plastic containers away from light in the freezer until use

Preparation of mushroom extracts

Preparation of the aqueous extract of mushrooms

The method adopted in the study of Hu and his group (2009) was followed with some modifications, weighing 10 gm of mushroom powder and placing it in a clean glass flask, to which 200 ml of distilled water was added, then heated for two hours at 80 °C, then placed in a vibrating incubator for 24 hours at a temperature of 037 °C. The mixture was filtered by means of filter paper in glass tubes and the filtrate was dried in a lypholyzer for 24 hours to obtain the dry powder of the extract, which was placed in a sealed package and kept in the freezer until use. The process

was repeated several times for the purpose of obtaining a sufficient amount of the extract. Then dissolve it in 20% dimethyl sulfoxide (DMSO) solution, then put it in Plastic Tubes and keep it in the refrigerator until use.

Preparation of the alcoholic extract of mushrooms:

The method used in the study (Huet al 2009) was followed with some modifications, with a weight of 10 g of powder per 200 ml of ethyl alcohol at a concentration of 70% in a 1 liter glass beaker and its nozzle was closed with a rubber stopper to prevent evaporation, then it was placed in a vibrating incubator for 24 An hour at a temperature of 37 0 C, then the mixture was filtered using filter paper in glass tubes, then lyophilized the filtrate in a lyophilizer for 24 hours to obtain the dry powder of the alcoholic extract. The process was repeated several times for the purpose of obtaining a sufficient amount of the extract. Close in the freezer until use. Then the precipitate was dissolved in 20% dimethyl sulfoxide (DMSO) solution, then placed in plastic tubes and kept in the refrigerator until use.

Etermination of the inhibitory activity of G.lucidum extract:

The culture medium was prepared mullar agar on plates, and after solidification of the culture medium, all plates were cultured with bacteria and grown for 24 hours, then the fungal extract (100 mg/ml) was mixed with DMSO dissolved medium at 45°C. Then the medium was punctured by a cork drill with a diameter of 10 mm, the fungal extract was placed in the hole and the dishes were incubated at a temperature of 37 °C. According to the diameter of the inhibition after 48 hours of incubation.

RESULTS AND DISCUSSION

Sample collection ((200) median urine samples were collected from patients and patients visiting Kirkuk Hospital for the period between 11/18/2020 to 17/2/2021. The samples were taken from different age groups ranging from 20-50 years and of both sexes. They were used to develop these samples in the middle of MacConkey agar and middle Blood agar and Mannitol salt agar. The information for each patient was collected in a form that includes a number of information, including the patient's name, gender, age, date of infection, the presence of previous infections with one of these infections or more, and taking antibiotics for each patient separately as shown in Appendix No. (1). The results showed, as in Table (4-1), that the number of samples that gave positive bacteria growth on the media used is 138 samples, or (69%) of the total samples, in the neighborhood of 62 samples did not give bacteria growth, with a percentage of 31% of the total samples. The reason for the absence of bacterial growth may be attributed to the fact that the pathogen may be a virus, a fungus, or a type of anaerobic bacteria, which cannot be isolated by the usual culture methods of the aerobic bacteria used in this study. It needs culture media and special conditions, or because patients have taken doses of antibiotics, as was proven in the information collection form for each patient (Balat and Hill., 2003).

Total number and percentage of samples collected from UTI patients

UTI	Number	percentage %
Growth	138	69
No Growth	62	31
Total	200	100

The result of our study agreed with the results obtained by Al Jomard (2015) in his study of wound infections, respiratory system and urinary tract in the city of Kirkuk, which found that the number of positive samples of growth reached 112 isolates with a percentage of (62.22%) and the number of samples that did not show growth reached 68 isolates with a percentage of (37.77%). It was also similar to the results of Legha and his group (2018) and Khanam and his group (2018), which were 54.66% and 55.4, respectively, while they differed from the results of the study of Patel and his group (2019), which was 45.69% in their study, and these percentages were much lower in Other studies, as it was found by 9.19% (2017 Alam et al.) and 36% (Bitew et al. 2017,) that the discrepancy in the rate of urinary tract infections in the different studies may be due to the difference in the length of the study period and the size and type of the community.

gender	Number	Percentage
Male	130	65 %
Female	70	35 %

The total number of samples was distributed between 130, females 65%, and males 70, 35%, as shown in Table (4-2), and the result of our study coincided with the results of Al-Mayahi and Almohana (2015). They found that injuries in females constituted 65%, and they agreed with Hellen (2019) study, where the rate of infection in females was 69.8%, while in males it was 30.2%.

The high rate of injuries in females may be due to differences in physiological nature. Adult women have a urinary tract infection 30 times more than men, as nearly 50% of women are exposed to a urinary tract infection at least once during their life, and one in three women gets it for the first time before the age of 24 years, and urinary tract infections increase in women Sexually active young women are also seen in the elderly and patients who need a urinary catheter (2016, Tan and Chlebicki).

Identification of bacterial isolates

The results in table (4-2) showed the morphological and biochemical tests that performed on isolates under study. These results were consistent with those of the approved diagnostic systems (MacFaddin, 2000; Forbes *et al*., 2007; Mahon *et al*., 2014).

Isolates which were identified as *E. coli* and *K. pneumoniae* was identified by their growth on MacConkey agar, showed recognizable odor, rosy colony result from fermenting lactose sugar in the medium. The microscopic examination demonstrated gram negative rod-shaped, non-spore forming bacteria. While Isolates which were identified as *P.mirabilis* appeared as small pale colonies, little convex and circular with smooth edges on MacConkey's agar plates and were lactose non fermenter, so they turned the media to yellow.

In addition, *P. mirabilis* culture has a special smell (fish odor). On blood agar *P. mirabilis* isolates showed swarming motility. *P. aeruginosa* was identified by its growth on the cetrimide medium. *P. aeruginosa* exhibit blue green colour due to the secretion of pyocins. Pale zone in blood agar around the colonies appeared, this observation indicate the ability of the bacteria to lyses the blood completely. On MacConkey agar, the colonies appeared as pale colour because it was not ferment lactose. The biochemical testing was positive oxidase, and catalase. *C. freundii* had the ability to ferment lactose. These are collectively termed as coliform bacteria. *C. freundii* are rods, usually non-capsulated, motile, all are lactose fermenters, non-haemolysin producer. All Gram negative bacilli were motile except *K. pneumoniae* non motile. The *Staph. aureus* identified by their growth on blood agar producing β-hemolysin and they were differentiated from other *staph.*by their ability of fermenting mannitol; after incubation the color of the medium turned from pink to yellow due to the change of pH, which indicate the fermentation of mannitol and the growth of the *Staph. aureus* appeared as smooth convex colonies on the medium. The coagulase test confirm the identification of *Staph. aureus*, and, aids in differentiating them from other *Staph*. Since only *Staph. aureus* produce coagulase enzyme. The response of *Staph. epidermidis* and *Staph*. *lentus* to gram stain, motility, catalase and oxidase tests are similar to that of other *Staphylococus spp*. It produce α-hemolysis on blood agar, not ferment mannitol, and didn't produce coagulase. Also, *Staph. lentus* produce urease, and ferment manose. Hence it can be differentiate from other coagulase negative *staph*.

Table (4-2):- Biochemical and morphological properties results of isolated bacteria.

Bacteria species / **tests**	***E. coli***	***P. mirabilis***	***K. pneumonia***	***C. freundii***	***P. aeruginosa***	***S. aureus***	***S. epidermidis***	***S. lentus***
Gram stain	G^- ve	G^- ve	G^- ve	G^- ve	G^- ve	G^+ ve	G^+ ve	G^+ ve
catalase	+	+	+	+	+	+	+	+

oxidase		-	-	-	-	+	-	-	-
pigment production		-	-	-	-	+	-	-	-
Urease production		-	+	-	V	-	-	-	+
Hemolysis		-	-	-	-	+	+	-	-
mannitol fermentation		-	-	-	-	-	+	-	-
Motility		+	+	-	+	+	-	-	-
IMVIC	IND	+	-	-	-	-	ND	ND	ND
	MR	+	-	-	+	-	ND	ND	ND
	VP	-	+	+	-	-	ND	ND	ND
	C	-	+	+	+	+	ND	ND	ND
lactose fermentation		+	-	+	+	-	-	-	-
coagulase production		ND	ND	ND	-	-	+	-	-

G^- ve: Gram negatine bacteria, G^+ ve: Gram positive bacteria, IND: indole, MR:methyl red, VP: Voges proskaur, C: Citrate utilization, ND: not done

Sensitivity of bacterial isolates to antibiotics

The sensitivity of bacterial isolates to antibiotics has been identified according to the Clinical and Laboratory Standards Institute (CLSI) (Wikler, 2007(. The results in table (4-5) showed that all isolates are resistant to Tobramycin, Metronidazole, Cloxacillin, Clarithromycin, Ceftazidime, Carbencillin and Azithromycin while they are sensitive to Imipenem, Levofloxacin and Augmentin while their resistance to Norfloxacin, Tigecycline and Amikacin are variable.

In recent years, it has been emphasized that there is a remarkable increase in the incidence of infection by antibiotic resistance bacteria in different parts of the world. For example Mayer, (2005) indicated that *P. aeruginosa* infections are difficult to treat as this organism displays a high level of intrinsic antibiotic resistance. Additionally, he found that treatment with fluoroquinolone antibiotics led to dramatic increases in the MIC which may be associated with treatment failure. Antibiotic resistance in Enterobacteriaceae and some of gram-positive cocci undergo a remarkable change in characters with the widespread occurrence of resistance transfer factors (RTF). RTF may transfer to drug-sensitive strains by conjugation in much the same way and with much the same type of kinetics as F transfer in *E. coli*. (Small *et al*., 1993).

Table (4-5) The susceptibility of bacterial isolates to antibiotics

antibiotics / bacteria	TN	TGC	NOR	MET	LEV	IMP	CX	CLA	CAZ	PY	ATH	AMC	AK
E.co+li	R	R	R	R	S	S	R	R	R	R	R	R	R
K.pneumoniae	R	R	R	R	S	S	R	R	R	R	R	R	S
P.mirabilis	R	S	S	R	S	S	R	R	R	R	R	S	S
C. freundii	R	R	R	R	S	S	R	R	R	R	R	S	S
Pseudomonas aeruginosa	R	S	S	R	S	S	R	R	R	R	R	S	S
S.aureus	R	R	S	R	S	S	R	R	R	R	R	S	S
S. epidermidis	R	S	S	R	S	S	R	R	R	R	R	S	S
S. lentus	R	S	S	R	S	S	R	R	R	R	R	S	S

Ak: Amikacin, AMC: Augmentin, ATH: Azithromycin, PY: Carbencillin, CAZ: Ceftazidime, CLA: Clarithromycin, CX: Cloxacillin, IMI: Imipenem, LEV: Levofloxacin, MET: Metronidazole, NOR: Norfloxacin, TGC: Tigecycline, TN: Tobramycin

Inhibitory activity of fungal extracts against multiple antibiotic-resistant bacterial species:

The emergence of antibiotic resistance and its spread in disease-causing bacteria calls for the search for new substances with antibacterial activity and from new sources other than the traditional known sources. Different from the bacteria under study that were characterized by their multi-resistance to antibiotics, the effectiveness of the secondary metabolite extract of the fungus Ganoderma lucidum differed greatly depending on the concentration of the extract (2 and 3%) and the method of preparation (boiled and soaked with boiling water) in addition to the time required to complete the extraction process (72 and 24), as well as Shown in Tables (4-9), (4-10) and (4-11), the inhibitory activity of the secondary metabolic extract of Ganoderma lucidum increased with the increase in the concentration used, as well as on the type of bacteria used in the test.

The aqueous extract of old mushrooms by boiling for 42 hours at a concentration of 2% showed the highest inhibitory activity against Pseudomonas aeruginosa with an inhibition diameter of 22 mm, while the highest inhibitory activity was by boiling for 24 hours at a concentration of 3% against Pseudomonas aeruginosa with a diameter of 23 mm. Soaking with boiled water at a concentration of 2% for old mushrooms recorded the highest inhibition diameter for a period of 24 against Staph. The old fungus was boiled for 72 hours at a concentration of 2% with the highest inhibitory activity against staph.hea bacteria with a diameter of 23 mm, while the highest inhibitory activity was by boiling for 72 hours at a concentration of 3% against staph.hea bacteria with a

diameter of 24 mm, and the extraction method was using boiling water. And at a concentration of 2% for the old fungus, the highest inhibition diameter was recorded for the infusion period of 72 against E.colil bacteria, with a diameter of 26 mm, while the highest inhibitory activity was at the time of 72 infusion against E.coli and Ente bacteria. rococcus faecium with an inhibition diameter of 30 mm at a concentration of 3%.

Fig. 1 Inhibitory activity of *Ganoderma lucidum* extract by soaking method in *Staph.aureus*
.

He inhibitory activity of the young mushroom extract by steeping in boiling water for both periods 72 and 24 hours at a concentration of 2% against each of Staph.aureus1 and strip was with a diameter of 28 mm and Pseudomonas aeruginosa 1 with a diameter of 34 mm, while the inhibitory activity was shown at a concentration of 3% against Staph.aureus1 with a diameter of 30 mm. 24 hours, while the diameter of the inhibition was 36 mm for 72 hours. While the alcoholic secondary metabolite extract of mushroom at 100% concentration for 24 hours showed the highest inhibitory activity against Staph bacteria. lentus with a diameter of inhibition of 28 mm, while the diameter of inhibition was recorded less with a concentration of 50% inhibition towards Staph. epidermidis and it reached 24 mm.

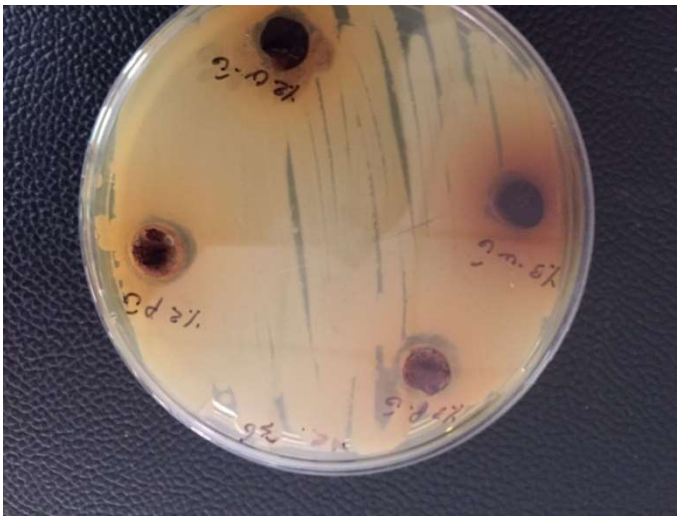

Fig. 2 Inhibitory activity of the young mushroom extract Ganoderma lucidum by soaking E.coli.

The above results showed that the highest inhibitory activity against multi-types of bacteria resistant to antibiotics, whether at a concentration of 2 or 3%, was for the young secondary metabolite extract. Effectiveness of G.lucidum against human pathogenic bacteria and fungi if (Shihabul e.,tal2018) (aqueous extract of G. lucidum has inhibitory activity for negative and positive bacteria and with inhibitory diameters of 6, 10 and 11 Ps. aeruginosa Staph. aureus E. coli, respectively, which is less than that of We reached it in this study as alcohol and aqueous solvents were used with different concentrations and different lining diameters were obtained against the used bacteria. Produces more active extracts . (Soniamol joseph2008) While the alcoholic secondary metabolite extract of the old lucidum.G fungus at 100% concentration for 72 hours showed the highest inhibitory activity against both Proteus mirabilis 2 and Enterococcus faecium1 with an inhibition diameter of 28 mm, while the lowest inhibition diameter was recorded against sar.mar bacteria and reached 12 mm as shown in the figure (4-22). The inhibitory activity of the extract of the fungus G. lucidum is due to its important compounds, and this was confirmed by a number of studies that focused on revealing these compounds, as they indicated the presence of carbohydrates, glycosides, triterpenoids, phenolic compounds and tannins, which increase its effectiveness as an anti-bacterial. (Kumar and his group, 2019) Of the most important triterpenes that have antimicrobial and antiviral properties are ganoderic acids, ganodereni acids, ganoderols and lucideric acids (Bishop and his group, 2015), as well as containing polysaccharides and its antioxidants,) Ferreira 2016)., as indicated by Hexaiang (2006) to the presence of Ganodermin protein in the fungus G. lucidum, which he isolated and confirmed its antagonistic role against plant pathogenic fungi B. cinerea, F. oxysporum and P. piricola. In addition, other proteins have been isolated that have biological activity and have antibacterial effect against pathogenic fungi and yeasts such as lectin protein ribonuclease protein (Wang and co., 2006).

Table 1: Antimicrobial activity of aqueous concentration 2% extract of *Ganoderma lucidum.*

Concentration, method of extraction and time Types of bacteria	S . M . 24C 200%	S . M . 72C 2%	S. ١24C 2%	S. ١72C 2%	F . 24C 2%	F . 72C 2%
Staph.epidermidis	22	30	11	15	24	30
Staph. saprophyticus	18	20	18	22	28	25
Staph. lentus	21	23	18	10	23	20
Proteus mirabilis 1	16	18	22	16	18	20
Ecoil- 1	18	22	20	12	16	25
Ecoil-2	19	20	20	20	12	15
Ecoil-3	11	18	20	8	13	20
Pseudomonas aeruginosa 1	23	23	15	25	16	29
Klebsiella pneumonia	14	15	22	20	12	25
Staph.aureus1	23	24	18	12	14	33
Staph.aureus2	8	5	21	25	23	25
Ser.mar 2	22	23	10	22	23	25
Ser. mar 1	18	22	9	15	18	22
Acinets	21	24	9	15	22	24
Enterococcus faecium 1	20	21	30	19	22	22
Enterococcus faecium 2	10	23	12	15	15	20
Staph. Haemolyticus	22	23	20	14	14	18
Streptococcus pyogenes	21	22	22	16	14	23
Streptococcus.pnemoniae	18	18	21	18	14	20
Staph.warneri	21	20	18	21	13	23

Table 2: Antimicrobial activity of aqueous concentration 3% extract of *Ganoderma lucidum*

Concentration, method of extraction and time Types of bacteria	**S . M . 72 C 3%**	**S . M . 24 C 3%**	**S . 27 C 3%**	**S . 24 C 3%**	**F . 72 C 3%**	**F . 24 C 3%**
Staph.epidermidis	36	25	26	25	14	12
Staph. saprophyticus	20	18	19	22	22	28
Staph. lentus	20	20	19	22	16	18
Proteus mirabilis1	`17	19	18	19	18	22
Ecoil- 1	19	20	24	22	25	36
Ecoil-2	18	19	18	19	13	10
Ecoil-3	11	13	11	10	10	18
Pseudomonas aeruginosa	25	16	19	21	12	18
Klebsiella pneumonia	14	15	20	16	10	10

Staph.aureus1	34	30	16	13	15	30
Staph.aureus2	10	12	20	20	25	30
Ser.mar 2	12	18	11	22	25	23
Ser. mar 1	12	21	23	27	20	15
Acinets	11	8	15	10	10	22
Enterococcus faecium1	24	19	25	24	16	18
Enterococcus faecium2	11	9	11	18	17	20
Staph. Haemolyticus	13	14	12	14	16	17
Streptococcus pyogenes	17	20	15	21	22	23
Streptococcus.pnemoniae	16	18	14	16	20	21
Staph.warneri	13	14	12	11	13	16

Table 3: Antimicrobial activity of aqueous extract of *Ganoderma lucidum*

Types of bacteria	**Inhibitory activity of the alcoholic extract of the old mushroom at a concentration of 50%/4 hours**	**Inhibitory activity of the alcoholic extract of old mushrooms at a concentration of 100%/4 hours**
Staph.epidermidis	24	24
Staph. saprophyticus	21	23
Staph. lentus	23	28
Proteus mirabilis1	20	21
Ecoil- 1	21	22
Ecoil-	19	20
Ecoil-3	18	20
Pseudomonas aeruginosa 1	19	20
Klebsiella pneumonia	19	20
Staph.aureus1	22	23
Staph.aureus2	20	21
Ser.mar 2	21	22
Ser. mar 1	19	20
Acinets	19	20
Enterococcus faecium1	21	22
Enterococcus faecium2	20	21
Staph. Haemolyticus	19	20
Streptococcus pyogenes	22	23
Streptococcus.pnemoniae	20	21
Staph.warneri	19	20

Conclusion:

The results from the present study supported the usage of Ganoderma lucidum fruit body as an ideal bio-pharmaceutics and suggested that the methanol and aqueous extract exert strong antimicrobial activity. All the extracts in this study exhibited potent antioxidant activity.

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