



Assessment of *Ganoderma lucidum*'s Antimicrobial Efficacy Against a Few Human Pathogenic Microorganisms

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تقييم فعالية جانوديرما لوسيدوم المضادة للميكروبات ضد بعض الكائنات
الحية الدقيقة المسببة للأمراض في الانسان

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ABSTRACT

Background:

Growing antibiotic resistance is one of the major worldwide issues endangering human health. As a result, many approaches to lowering pathogens are being studied. *G. lucidum*'s impact on some pathogenic species is the focus of the current investigation.

Materials and Methods:

In our study the effect of *G. lucidum* powder fungi compared with antibiotics. we prepared *G. lucidum* in three different concentrations (80, 100, and 200 mg/ml). The sensitivity of pathogenic fungi and positive and negative bacteria species to Gram stain was tested, and the results were compared to the antibiotic sensitivity of the same species used in the study.

Result:

The study showed that *S. aureus* bacteria were sensitive to all the used types of antibiotics, whereas *E. coli* were resistant to most of them; the remaining species had varied sensitivities.

While *S. aureus* showed greater sensitivity to an increasing concentration of the utilized powder, *E. Coli*, *Bacillus*, and *Klebsiella* bacteria all demonstrated resistance to the effects of the powder. The *P. aeruginosa* bacteria and the fungal species employed in the investigation exhibited no discernible sensitivity to the powder utilized.

Conclusion:

In contrast to the bacteria and fungi being studied, *S. aureus* had a higher sensitivity to increasing powder concentration.

Keywords: *Ganoderma lucidum*; sensitive test; Gram positive; Gram negative.



INTRODUCTION

A fungus is a basidiomycetes genus belonging to the polyporales Ganodermataceae [1]. In Asia, Ganoderma products are widely used as nutritional supplements, particularly in China, and Ganoderma species [2]-[6] are very rich in China. A fungus is one of the most widely used medicinal mushrooms in traditional medicine as a preventative and functional food supplement medicinal item [7,8]. *Ganoderma.lucidum* is rich in the nutraceutical portion with possible medicinal benefits for mushrooms [9]. The problem of antibiotic resistance has arisen over the last three decades. Pathogens that are bacterial or fungal have developed mechanisms of protection against antimicrobial medicines and additional research was now necessary to discover novel and more potent antibacterial substances to supplement or replace antibiotic treatment. Natural compounds are currently at the forefront of the focus of several biotechnology firms searching for new antimicrobial drugs [10,11].

Mushroom is a rich source biologically active substances having a wide range of chemical compounds. In this way, the search for novel, highly effective antibacterial drugs may benefit from utilizing isolated mushroom components [12]. Flavonoids and phenolic compounds are the most prevalent bioactive components found in mushrooms [13]. Among these, the flavonoid demonstrates the most diverse range of pharmacological and physiological effects, including anti-inflammatory qualities, of all secondary metabolite categories [14], antibacterial [15], antifungal [16], antioxidant, and anticarcinogenic [17]. *Ganoderma lucidum*'s biological and pharmacological properties are there anti-tumor functions, including effects on cell cycle arrest, cell proliferation inhibition, and apoptosis activation in human colonic carcinoma and cell lines of breast cancer [18]. Ganoderic acid, triterpenes, and polysaccharides are the principal compounds with important pharmacological activity. Over the past three decades, this mushroom has yielded more than 150 triterpenes and it is believed that over fifty carcinostatic polysaccharides are distinct compounds. Consequently, it is probable that products of *Ganoderma lucidum* that include unique triterpenes and polysaccharides, or combinations of these two classes, will contribute to unique pharmacological action [19-21].

The widespread acceptance of *Ganoderma lucidum* and its potential medical benefits have greatly fueled the search for pharmacological compounds in these edible Ganoderma mushrooms. Since the lucidum extract is taken orally by people as a possible preventive measure, it appears to be very safe, showing no toxicity and having positive effects [22].

MATERIALS AND METHOD

Ganoderma was acquired via DXN. The following concentrated doses of three concentrations of 80, 100, and 200 mg/ml were made. In this investigation, five distinct bacterial cultures—of *Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella* are examples of gram-negative bacteria. *Staphylococcus aureus* and *Bacillus* are examples of gram-positive bacteria, and two fungal species (*Candida albicans* and *Candida tropicalis*)—are employed.

After being subcultured into nutrient agar petri dishes, bacterial and fungal cultures were maintained for a whole day at 37°C and 28°C, respectively. (sabouraud dextrose agar). Five milliliters of sterile saline were used to inoculate three to five bacterial colonies obtained from agar plates. The bacterial cultures in the saline test tubes were then compared to 0.5 MacFarland's standard to ensure that the bacteria were uniform. 9.5 grams of Muller Hinton agar and were dissolved in 250 milliliters of distilled water to create 250 milliliters of Muller Hinton agar.



The microorganisms were subcultured into nutrient agar petri plates. Additionally, it was cultured for 24 hours at 37°C for bacteria and 28°C for fungi. (sabouraud dextrose agar). Test tubes containing 5 milliliters of sterile saline were filled with three to five bacterial colonies obtained from the agar plates. The bacterial cultures in the saline test tubes were then compared to 0.5 MacFarland's standard to ensure that the bacteria were uniform.

Ganoderma disc preparation is utilizing sterile Whatman filter paper for antibacterial activities. Before the discs were impregnated in Ganoderma (sterile in a water bath for 15 minutes at 63°C), they were placed on the plate's surface using sterile forceps and gently pressed to make sure that the extract was in contact with the agar surface. Following a 24-hour incubation period at 37°C, the zones of inhibition on the plates were measured and recorded in millimeters.

The fungus is grown using the same technique; the only difference is that the incubation is carried out at 28°C for 24 hours as opposed to 37°C.

Sensitivity test for antibiotics

The Kirby-Bauer method is used. Three to five bacterial colonies were taken from the agar plates and seeded into test tubes holding five milliliters of sterile saline. The bacterial cultures in the saline test tubes are then compared to 0.5 MacFarland's standard to standardize the bacteria. 3.8 grams of Muller Hinton agar that were dissolved in 100 milliliters of distilled water to create 100 milliliters of Muller Hinton agar. Agar was autoclaved at 121°C for 15 minutes to sterilize it.

Muller Hinton agar was aseptically added to the sterile Petri dishes following sterilization. After the agar had solidified for a short while, each bacterial culture was aseptically swabbed onto the Muller-Hinton agar surface and allowed to dry with the use of sterile swabs. Using sterile forceps, the disc containing the antibiotics (Trimethoprim, Azithromycin, Bacitracin, Vancomycin, Ciprofloxacin, and Gentamicin) was placed on the plate's surface and gently pressed to make contact with the agar. For twenty-four hours, the plates underwent 37°C incubation. The millimeter-scale A zone of inhibition measurement was made and observed.

RESULTS AND DISCUSSION

S. aureus was found to be sensitive to every antibiotic used in the study, ranging in sensitivity from 16 mm for Bacitracin to 31 mm for Gentamicin, as Table 1 illustrates. In contrast, *Klebsiella* exhibited resistance to both Vancomycin and Bacitracin, high sensitivity to Ciprofloxacin, and variable sensitivity to the remaining antibiotics. All of the antibiotics used to treat *E. Coli* bacteria demonstrated high resistance, except gentamycin, which proved to be successful.

Bacillus Spp exhibited resistance to Trimethoprim alone while remaining susceptible to other antibiotic classes. Its sensitivity ranged from 9 mm for Bacitracin to 31 mm for Azithromycin. *P. aeruginosa* exhibited resistance to Trimethoprim and Vancomycin, but a medium sensitivity towards Bacitracin and Gentamycin, and a high sensitivity towards Ciprofloxacin and Azithromycin. To compare these antibiotics with Ganoderma extract in this study, sensitivity tests were conducted.



Table (1); sensitivity of bacteria species used in the research against different types of antibiotics.

Antibiotic	<i>S. aureus</i>	<i>Klebsiella</i>	<i>E. coli</i>	<i>Bacillus</i>	<i>P. aeruginosa</i>
Trimethoprim	S	S	R	R	R
Azithromycin	S	S	R	S	S
Bacitracin	S	R	R	I	I
Vancomycin	S	R	R	S	R
Ciprofloxacin	S	S	I	S	S
Gentamicin	S	S	S	S	I

The effects of varying doses of Ganoderma powder on the identical microorganisms employed in the study are shown in Table 2. The *S. aureus* bacteria showed a significant degree of sensitivity, reaching 24 mm at a concentration of 200 mg/ml; this sensitivity was higher than that of Bacitracin but lower than that of Azithromycin and Gentamicin, and it was comparable to that of the antibiotics Ciprofloxacin, Trimethoprim, and Vancomycin, and this demonstrates the efficacy of this powder against *S. aureus* compared to some types of antibiotic taking into consideration the safety of using Ganoderma powder as a natural antibiotic and nutrient compared to the use of the other antibiotics which may have side effects on the body [23] such as diarrhoea, nausea, headache, dizziness, and nephrotoxicity [24]. A study performed proved the occurrence of side effects of the antibiotics Azithromycin and Ciprofloxacin [25],[26]. Another study proved that Ganoderma powder is free of any side effects when used within certain concentrations [27].

Bacillus bacteria showed resistance to Ganoderma powder of concentrations (80 and 100 mg/ml) and medium sensitivity (11 mm) to the concentration of (200mg/ml) compared to the types of antibiotics used in the research where the sensitivity of the bacteria varied between (9 mm-31mm), and despite the medium sensitivity of this bacteria to Ganoderma powder, its use as an antibiotic against infections caused by *Bacillus* gives an effective and safe treatment in case the body responds to it, compared to the side effects that may occur when using other antibiotics, not to mention the other benefits offered by this powder to the body such as acting as an antioxidant and also as a healthy organic nutrient agent [28] and this may make its use preferable over the other antibiotics despite that *Bacillus* is more sensitive to them.

Klebsiella bacteria exhibited resistance to Ganoderma powder of concentrations (100 and 200 mg/ml) while its sensitivity was medium (10 mm) to the concentration of (80 mg/ml) compared to the antibiotics used in the research where it showed evident resistance towards Vancomycin and Trimethoprim, but high sensitivity to Azithromycin and Ciprofloxacin, and a similar sensitivity to Bacitracin. Ganoderma powder's use is preferred instead of antibiotics where this fungus is used traditionally as both food and medicine in several Asian nations [29]. Reports mentioned the use of fungus as useful in treating food to protect from blood pressure and high blood cholesterol diseases, and these features are mainly due to the chemical composition of Ganoderma [30].

E. coli bacteria exhibited medium sensitivity (8mm) to the concentration of (100mg/ml) of Ganoderma compared to Ciprofloxacin, and high sensitivity compared to the antibiotics to which it showed evident resistance; Trimethoprim, Azithromycin, Bacitracin and Vancomycin, and a less effect than Gentamycin. Ekiz et al. studied researchers looked into the bioactivity of aqueous extracts from Ganoderma fruiting bodies and discovered that the extract had an inhibitory effect



on types of bacilli [31]. It was found that parts of *G. applanatum* [32] and *G. pfeifferi* that has high activity against *E. coli* [33].

P. aeruginosa exhibited high sensitivity (17mm) to the Ganoderma powder of (100mg/ml) compared to Bacitracin, and a lower sensitivity (14mm) than that of Ciprofloxacin and Azithromycin at the concentrations of (80 and 200mg/ml), whereas *P. aeruginosa* exhibited evident resistance to Trimethoprim and Vancomycin the thing that indicates the efficacy of Ganoderma powder compared to some types of antibiotics, and one of the interesting merits of Ganoderma fungus is its anti- effect against microbes which is attributed to its extracts that contain bacteriolytic, lysozyme, acid protease enzyme [34]. This fungus draws interest from around the world as a significant natural substance, considering the vast diversity of its biologic effects and uses such as anti- tumours, heart and vascular diseases, respiratory system and anti-depression (active against pain) [35,36].

Candida sp. exhibited resistance to Ganoderma powder of concentrations (80 and 200mg/ml), whereas its sensitivity was medium (15-16 mm) to the concentration of (100 mg/ml) compared to the antifungal fluconazole [37].

A study performed by Gangwar et al. showed the activity of samples of Ganoderma particularly against positive and negative Gram bacteria, and it is also a group of antibiotics, and it also exhibited good efficacy against *Candida albicans* [38].

Table (2), the effect of Ganoderma powder on different species of microbes.

Ganoderma	<i>S. aureus</i>	<i>Klebsiella</i>	<i>E. coli</i>	<i>Bacillus</i>	<i>P. aeruginosa</i>	<i>Candida albicans</i>	<i>Candida tropicalis</i>
80 mg/ml	I	I	R	R	S	R	R
100mg/ml	S	R	I	R	S	S	S
200mg/ml	S	R	R	I	S	R	R

CONCLUSION

The study demonstrated that *P. aeruginosa*, *Bacillus*, and the employed species of fungi demonstrated medium sensitivity to the same concentration of the powder, while *E. coli* and *Klebsiella* were resistant to its effects. The concentration of Ganoderma powder, at 200 mg/ml, was found to have a greater inhibitory effect on *S. aureus* bacterial growth than other concentrations. This leads us to the conclusion that *S. aureus* infections can be treated with Ganoderma powder at a dosage of 200 mg/ml rather than using harmful antibiotics.

Because the powder has no negative effects on the body, it can also be used to treat other infections brought on by other pathogenic species of bacteria that were utilized in the research and showed a medium sensitivity to it.

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Conflict of interests.

Non conflict of interest

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الخلاصة

المقدمة:

إن تزايد مقاومة المضادات الحيوية يعد من القضايا الرئيسية التي تهدد صحة الإنسان على مستوى العالم. لذلك يتم البحث عن طرق بديلة للحد من مسببات الأمراض، وتهدف الدراسة الحالية إلى دراسة تأثير *G. lucidum* على بعض الأنواع المسببة للأمراض.

طرق العمل:

بالمقارنة مع المضادات الحيوية. *Ganoderma lucidum* لقد بحثنا في تأثير مسحوق فطر بواقع (٢٠٠، ١٠٠، ٨٠) ملغم/مل واختبار حساسية أنواع *G. lucidum* تم تحضير (٣) تراكيز مختلفة من مسحوق فطر من البكتريا الموجبة والسالبة لصبغة الكرام وأنواع من الفطريات الممرضة لهذا المسحوق ومقارنتها مع حساسية نفس الاجناس المستخدمة في الدراسة مع أنواع من المضادات الحيوية.

الاستنتاج:

كانت حساسة لجميع أنواع المضادات الحيوية المستخدمة في حين كانت بكتريا *S. aureus* أظهرت الدراسة ان بكتريا مقاومة لاغلبهم فيما تفاوتت الحساسية للاجناس الأخرى في البحث. *E. coli*. مقاومة لتأثير مسحوق الفطر في حين ازدادت حساسية بكتريا (*E. coli*, *Bacillus*, *Klebsiella*) وظهرت كل من (مع زيادة تركيز المسحوق المستخدم . *S. aureus*) وأنواع الفطريات المستخدمة حساسية واضحة ضد المسحوق المستخدم بالبحث . *P. aeruginosa* ولم تظهر بكتريا , اختبار الحساسية,

الكلمات المفتاحية: البكتريا الموجبة لصبغة كرام, البكتريا السالبة لصبغة كرام, *Ganoderma lucidum*.