Effect of aqueous extract edible mushroom *Ganoderma lucidum* on pathogenic bacteria isolated from children's' tonsils

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Abstract

The aim of the research is to find natural alternatives to antibiotics that have the ability to inhibit pathogenic bacteria by identifying the inhibition of a hot water extract of Ganoderma lucidum against bacteria isolated from children's' tonsils, which included Staphylococcus aureus, Streptococcus pyogenes, Bacillus cereus, pseudomonas aeruginosa and Escherichia coli. The hot aqueous extract showed a significant inhibitory effect against the growth of isolated bacteria at a concentration of 200 mg/ml, where the highest inhibition diameter was 21.33 ± 0.577 mm in S. aureus, while the lowest inhibition diameter was 13.67 ± 0.57 mm in P. aeraginosa at the same concentration. All types of bacteria showed resistance to a concentration of 12.5 mg / ml. The results show the difference in the inhibitory effectiveness with different concentrations. Effective antibacterial chemical compounds were identified in the aqueous extract of G. lucidum by Gas Chromatography-mass spectrometry (GC-MS), which showed the compound 7-Hexadecenoic acid, methyl ester, (z)- (30.52%), which is an unsaturated fatty acid, It has antibacterial activity.

Keywords: Ganoderma lucidum, aqueous extract, antibacterial activity, GC. MS.

1. Introduction

Macrofungi (mushrooms) are one of the main sources of many medicinal products, Studies have shown that fungal extracts have medical importance for living organisms, including humans and animals, as they showed several pharmacological activities such as anti-bacterial and anti-inflammatory activity (AL- Obaidy et al.,2019). Many species of macrofungi are used in traditional medicine, but Ganoderma lucidum is considered the most important (Frljak et al., 2021). It is a type of mushrooms polypore that grows on live and dead wood and is considered one of the causes of white wood rot, G. Lucidum produces fruiting bodies with a diameter of 30 to 250 mm (Siwulski et al., 2015). It has been used for decades in China to treat various types of diseases, as an antibacterial agent, and as an antioxidant (Taofiq et al., 2017). Studies have shown that G.lucidum has antibacterial activity, and other studies have shown that mushroom extracts (containing different types of triterpenes, polysaccharides, alkaloids and steroids are known for their antibacterial effect) it was also active against Gram-negative bacteria such as E.coli (L et al.,2013).

One of the most important bacterial genera causing tonsillitis is S.pyogenes, which causes recurrent tonsillitis and is responsible for about 10-20% of cases, followed by S.aureus , one of the most common causes of respiratory infections, including tonsillitis (Hurst et al., 2018; Cheung, et al., 2021). Bacillus cereus E.coli and p. aeruginosa is an opportunistic human pathogen (Alhazmi, 2015; Glasset et al.,2018; Santos et al.,2020).

2. Materials and Methods

mushroom source

The fungus G.lucidum was obtained from the local markets of Mosul/Iraq, the origin of the fungus (India).

Preparation of the aqueous extract of *Ganoderma lucidum*

50 gm of G.lucidum powder was taken and placed in a 1000 ml beaker and petroleum ether was added to it in a volume of 400 ml to remove defatted fats, Then the baker was tightly wrapped with aluminum foil and placed on the magnetic stirrer for 72 hours, after which the mixture was filtered with Whatmann No.1 filter paper (Le Grand et al., 1988). The precipitate was taken and soaked in a beaker by adding 400 ml of distilled water and placed in a rocking water bath at a temperature of 60 °C for 6 hours to obtain the hot aqueous extract. Then the mixture was filtered with Whatmann No.1 filter paper. Then the aqueous extract is concentrated by a rotary vacuum evaporator (RVE) at a temperature of 50 °C. The aqueous extract was dried in an oven at a temperature of 40 ° C to obtain a powder of the crude extract, then kept

doi.org/10.31838/hiv23.02.30 Received: 16.10.22, Revised: 24.11.22, Accepted: 11.12.22

in the refrigerator at 4 ° C until use (Harborne, 1984).

Preparation and sterilization of mushroom aqueous extract concentrations

Concentrations of G.lucidum extract by dissolving 1 gm of the aqueous extract powder of G.lucidum in 5 ml of sterile distilled water, thus obtaining a concentration of 200 mg / ml as a standard concentration from which the rest of the concentrations were prepared , then it is sterilized with Millipor (Filters Membrane 0.22 µg) and used in to effect on bacterial isolates (Al- Numan,1998; Miladinovic and Milabinovic, 2000).

Source of bacterial isolates

100 pathological samples (swab) were collected from children with tonsillitis. Bacterial isolates (Staphylococcus aureus, Streptococcus pyogenes, Bacillus cereus, pseudomonas aeruginosa and Escherichia coli) were diagnosed and their diagnosis was confirmed using the VITEK2 test.

Measurement of the inhibitory effect by well agar diffusion method

The inhibition activity of the aqueous extract of G. lucidum was tested against the growth of isolated bacteria. Mueller-Hinton agar medium was inoculated with the bacteria to be tested. The suspension was diluted using Normalsilane solution and by comparison with the standard Macferland control tube, which is equivalent to 108 cells / ml, After that, 0.1 ml of the bacteria suspension was spread on a medium Mueller-Hinton agar plate using a sterile cotton swab, and after waiting 10 minutes for the dish to dry, holes are made in the dish containing the Mueller-Hinton agar using a 6 mm Stainiess steel bore, then put in each hole 0.1 ml of each concentration of aqueous extract. Then the dishes were incubated at 37 °C for 24 hours, and the inhibition diameters were measured around each hole, then the results were recorded (Valgas et al ., 2007 ; Annamalia et al.,2009; Balouir et al., 2016).

Gas chromatography

The separation process was carried out by injecting an aqueous extract of 70% ethanol into the injector so that it turns into steam, and scanning was carried out for one hour (Hübschmann, 2015), The gaseous sample is transported by the carrier gas at a constant flow rate towards the separation column, as the components of the sample separate during their passage through the column due to the difference in the degree of absorption in the components of the cell, In the second step micrograms of the sample are placed in the separation column of the device at a temperature of 40 °C for one minute, then the temperature is raised to 150 °C, at a rate of 5 °C per minute, and then raised to 280 °C, at a rate of 5 °C per minute (Al-Fekaiki et al., 2017).

3. Results and Discussion

The results showed table (1) the difference in the inhibitory effect of the hot aqueous extract of G. Lucidum mushroom against the activity of pathogenic bacteria isolated from tonsillitis. The table showed that the highest inhibitory activity was in S. aureus at a concentration of 200 mg/ml and with an average diameter of inhibition of 21.33±0.5 mm, compared to the control group (distilled water), which did not show any effect (0.00 mm) as in (Fig. 1), The zones of inhibition converged against E.coli, B.cereus and S.pyogens, at the same concentration where the average diameter of inhibition was 15.67±0.57 - 14.67±0.57 -14.6±0.57 respectively.While it was the lowest value in P. aeraginosa with an average diameter of inhibition 13.67±0.57mm at a concentration of 200 mg/ml as in (Fig. 2). The inhibitory effect was directly proportional to the concentration, and all bacterial isolates showed resistance to a concentration of 12.5 mg/ml. By detecting the active compounds using the GC-MS technology, the presence of Heptadecanoic acid, 15-methyl-methyl ester, was found to be present with an area under the curve estimated at (2.71%). It is a fatty acid that has the ability to inhibit bacteria. Studies have shown that fatty acids have antibacterial activity, as they prevent the formation of membranes in Gram-positive and Gram-negative bacteria, Unsaturated fatty acids have higher activity than saturated ones (Yuyama et al.,2020). The results of the current study are similar to those of the researcher (Migahed et al., 2018) that the aqueous extract of G.lucidum showed an inhibitory effect against species including E.coli, S.aureus, S.typhi, Streptococcus sp. and Erwinia carotovora , and showed that G.lucidum has anti-bacterial activities and some types of pathogenic bacteria.

Table 1. Inhibitory effect of hot aqueous extract of G.lucidum on the growth of isolated bacteria

%12.5 (mg/ml)	%25 (mg/ml)	%50 (mg/ml)	%100 (mg/ml)	%200 (mg/ml)	Control	Concentration Bacteria
0.00±0.00 n	7.33±0.57 m	10.33±0.57 j	12.67±0.57 ef	15.67±0.57 b	0.00±0.00 n	E. coli
0.00±0.00	10.33±0.57 j	13.33±0.57 de	15.67±0.57 b	21.33±0.57 a	0.00±0.00 n	S. aureus
0.00±0.00 n	0.00±0.00 n	10.67±0.57 ij	12.33±0.57 fg	13.67±0.57 d	0.00±0.00 n	P.aeragina
0.00±0.00	0.00±0.00n	9.33±0.57 k	11.67±0.57 gh	14.67±0.57 c	0.00±0.00 n	B.cereus
0.00±0.00 n	8.33 l±0.57l	10.33±0.57 j	11.33±0.57 hi	14.6±0.577 c	0.00±0.00 n	S. pyogenes

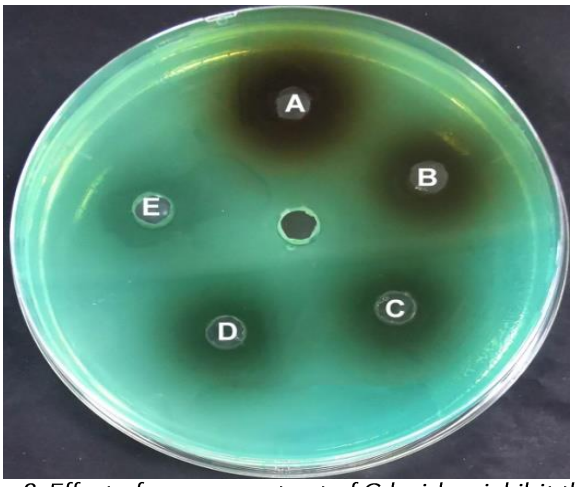


Fig 2. Effect of aqueous extract of G.lucidum inhibit the growth of bacteria P. aeraginosa Concentrations: A-200%, B. 100%, C. 50%, D. 25%, E. 12.5%

Fig1. Effect of aqueous extract of G. lucidum inhibit the growth of bacteria S. aureus Concentrations: A-200%, B. 100%, C. 50%, D. 25%, E. 12.5%

4. GC-MS technology

The results of the Gas Chromatography-Mass Spectrometey (GC-MS) technique used to separate the active compounds in the aqueous extract of G.lucidum, depending on the survival of fatty acids and phenolic compounds in the separating column, showed, It resulted in (70) chemical compounds that have a role in the inhibitory activity of bacteria in G.lucidum. We chose five chemical compounds in G.lucidum based on the highest concentration. These five compounds show the largest area under the curve in terms of concentration, which are as follows: Compound 7-Hexadecenoic acid, methyl ester, (z)- with the highest area under the curve, which was estimated at (30.52%) as in (Fig. 4). It is an unsaturated fatty acid that has antibacterial activity as it works to disrupt the bacterial cell wall and membrane, and compound 2-Benzothiazo amine with an area under the curve, which was estimated at (22.95%) as in (Fig. 4), is a heterocyclic compound that has antibacterial activity. Whereas, the compound Ricinoleic acid had the highest area under the curve (5.47%), and it is an unsaturated fatty acid. The compound, Hexadecanoic acid, ethyl ester, is a fatty acid with an area under the curve estimated at (5.40%). It is a fatty acid ester, an antibacterial agent, Heptadecanoic acid, 15-methyl-methyl ester, with an area under the curve estimated at (2.71%), is a fatty acid that has the ability to inhibit bacteria as in (Fig. 3). The study conducted by the researcher (Garuba et al., 2020) showed that G.lucidum is considered one of the nutrients that contain biologically active compounds useful in the pharmaceutical industry.

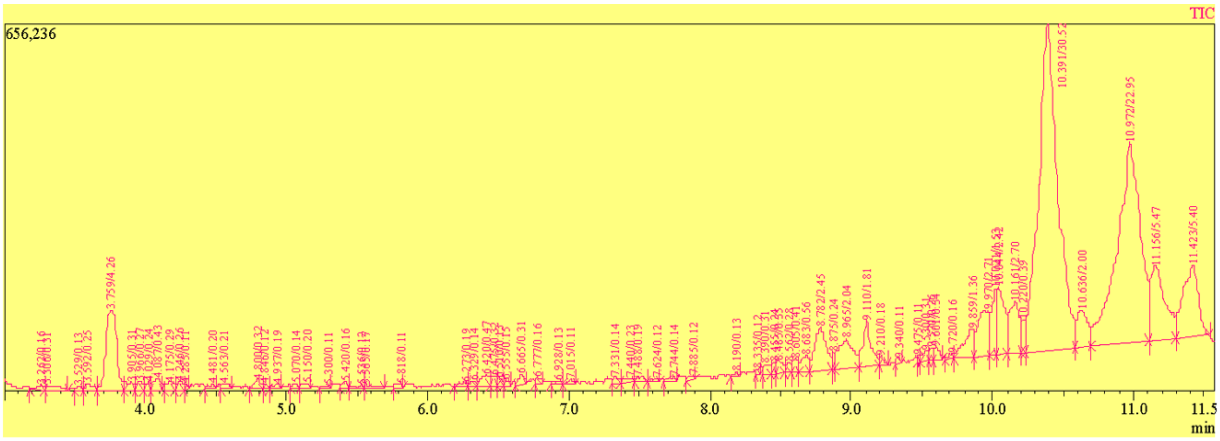
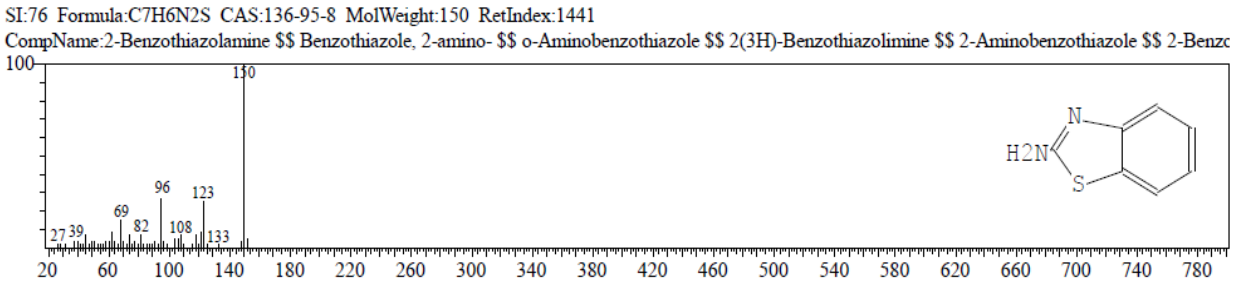


Fig 3. Analysis curve of the active compounds separated from the aqueous extract of G. lucidum using GC-MS technique.



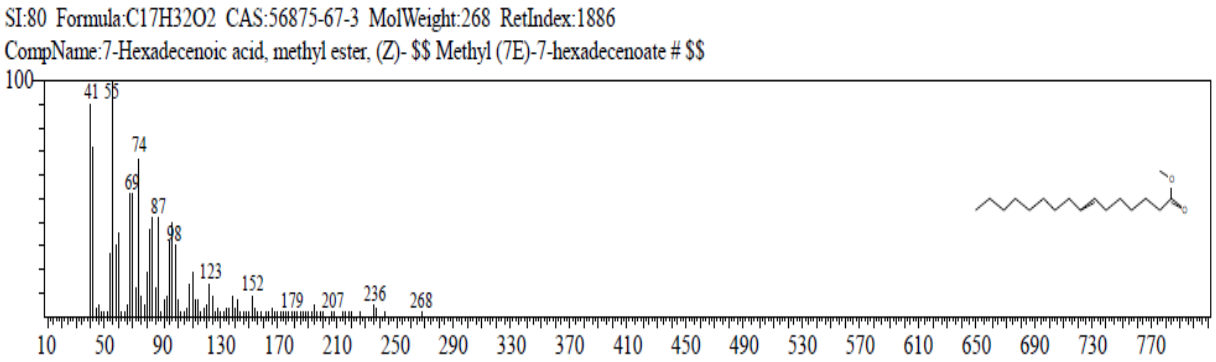


Fig4. The curve shows the structural formula of the chemical compounds separated from the aqueous extract of G. lucidum

5. Conclusions

The results of the research showed the effect of the aqueous extract of G. lucidum on pathogenic bacteria isolated from children's' tonsils, as the concentration of 200 mg / ml showed the highest inhibition rate for all isolated bacterial species. The active chemical compounds were detected by GC-MS technology, which showed the presence of the compound 7-Hexadecenoic acid, methyl ester, (z)- as well as the compound 2-Benzothiazo amine, Ricinoleic acid, Hexadecanoic acid, ethyl ester, and the compound Heptadecanoic acid,15-methyl-methyl ester, which had a role in inhibiting pathogenic bacteria that cause tonsillitis.

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