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

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Abstract

Background: The research objective is to learn about inhibitory effect of the alcoholic extract for Ganoderma lucidum against five kinds of isolated bacteria from children's' tonsils which included Bacillus cereus, Streptococcus pyogenes, Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa. Research Method: Alcoholic extract showed Inhibitory effect towards the growth of studied bacterial isolates and it was different by bacteria type and concentration by using the turbidity test method. Results: It reached on the concentration 200 mg/mL was the lowest value of turbidity recorded in B. cereus. Which gave a turbidity score (0.233) O.D. The inhibitory effect was in the turbidity way and at 630 nanometer wavelength and by using the alcoholic solvent (ethanol) in concentration 200 mg/ml best than the other concentrations. Effective anti-bacterial chemical compounds have also been detected in the alcoholic extract for G.lucidum using the GC-MS technique ,Special in chemical compounds separation and indicates the presence of the compound (E)-9- octadecenoic acid ethyl ester nearly 48.92%, It is an unsaturated fatty acid anti - bacterial inflammation. Through molecular diagnosis, a new strain of studied bacteria obtained and registered B. cereus in the World Genetic Data Bank (NCBI) by Bacillus cereus As-A-M gene for 16S ribosomal RNA, partial sequence. Conclusion: Alcoholic extract also showed inhibitory activity of bacteria especially at concentration 200 mg/ml.

Keywords: Ganoderma lucidum, Alcoholic extract, antibacterial activity, GC- MS.

1. Introduction

Mushrooms are a rich source of natural antibiotics for example the Ganoderma lucidum and considered a rich source in pharmacologically effective compounds, It is one of the types for mushrooms commonly used in traditional Chinese medicine and in Asian countries, It is an important source of effective antibacterial chemical compounds and the medically active part, polysaccharide contains of anti-bacterial activities and inflammation and anti-oxidant(1,2). The G. lucidum belongs to the Basidiomycetes (3).

Studies indicate that G.lucidum plays a helpful role in the management of bacterial and viral infections, different pathogenic bacteria were selected from which antibacterial activity was determined by using crude extracts of macrofungi, which showed the strongest antibacterial activity(4). There are several studies on G. lucidum that showed that the active compounds of the fruiting bodies of the fungus have an inhibitory ability for different types of Gram-positive bacteria and Gram-negative bacteria (5).

It was observed that mushroom extracts are more active against Gram-positive bacteria such as Bacillus cereus, Staphylococcus aureus than Gram-negative bacteria, As the crude mushroom extracts showed different degrees of inhibition of the microorganisms, thus G. lucidum is an alternative to antibiotics and antifungals (6).

2. Materials and Methods

Ethical consent

The study protocol was assessed and approved by the Ethics Committee of our institution, the research protocol did not interfere with any medical recommendations or prescriptions. Informed consent was taken from the patient with keeping the patients` records confidential in all stages of the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Source of the mushrooms

Mushroom isolation Ganoderma lucidum was obtained from local markets of Mosul/Iraq and mushroom origin (India).

Preparation of alcoholic extract for mushrooms

50 g of G. lucidum powder taken and put in a beaker capacity 1000 ml and then added to it petroleum ether400 ml to remove defatted fat, and put on a

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Stirrer for a 72 hour after that filter the mix with filtration paper whatmann No.1, The precipitate was taken and put again in beaker capacity 1000ml and it added 400 ml of ethyl alcohol at concentration 95% and put in shaker machine for 72 hours(7). Filtered the mix with filtration paper whatmann No.1, The extract is concentrated in Rotary vacuum evaporator (RVE) machine to get the extract crude,The extract was dried in an oven at 40 °C, It is then kept in the refrigerator at 4 ℃ until used(8) .

Preparation of concentrations for mushroom extract and sterilize.

Been thawed 1 g of alcoholic extract powder in 5 ml of dimethyl Sulfoxid (DMSO),Thus a concentration of 200 mg/ml was obtained as a standard concentration from which the rest of the concentrations were prepared, Alcoholic extract was sterilized by pasteurization way at 62 ℃ for 10-15 minutes(9).

Source of bacterial isolates

Bacterial isolates were obtained (Staphylococcus aureus, Streptococcus pyogenes, Bacillus cereus, Pseudomonas aeruginosa, Escherichia coli) from children with tonsillitis and the diagnosis was confirmed by using the biochemical tests and PCR.

Test the Inhibitory effect of the alcoholic extract for *G. lucidum* by using the turbidity test method.

turbidity method have been used to determine the extent which alcohol extract influences G. lucidum on isolated bacterial and this will be done through the visible light spectrophotometer at a wavelength 630 nanometers, Pipes containing 9.8 ml have been prepared of sterile nutrient broth and the environment was then inoculated with 0. 1 ml from the bacterioplankton and with concentration 108 cell/ ml comparison in McFarland standard and at a rate of three repeats per concentration, then it was added 0.1 ml of alcohol extract for G.lucidum and with a concentrations 12.5, 25, 50, 100, 200 mg/ml and then the tubes were incubated at 37°C and for 24 hour and the turbidity was then measured by the optical spectrometer, In comparison with the control sample of 9. 8 ml the nutrient broth and 0.1 ml microbial plankton with a concentrate 108cell/ ml and 0.1 ml of solvent DMSO, The effect of the extract on the growth of the isolated bacteria was determined (10).

Gas chromatography_Mass Spectrometry

A sample has Sent of raw alcohol extract for G. lucidum to Samarra University Faculty of Applied Sciences Laboratory to diagnose the alcoholic extract ingredients using chromatography gas related to mass spectrometer, The separation process was performed through injection the alcoholic extract for G. lucidum70% of ethanol in the injector so that it turns into steam and the survey was conducted for one hour (11).

Micrograms of the sample are placed in the separation column of the device At 40 temperature and for 1 minute and then up to 150, That's 5 per minute, And then up to 280, That's 5 per minute, Then the samples were injected by automatic injector and upon obtaining the mass spectrum of each compound, the separated curves of each compound were diagnosed based on the spectrum database in a (NSTAO8) library(12).

3. Statistical Analysis

Results were analyzed statistically using the statistical analysis system (SAS) program, The drift rate of the inhibition zone was calculated by using Duncan's multiple test at a probability level of 0.01 where the different transactions were morally marked with different satire letters (13).

4. Results

Fig (1) shows that there are significant differences between different concentrations of alcoholic extract G.lucidum against pathogenic bacterial isolates., It found that the highest value of turbidity was in the transaction of control that exceeded all other transactions, The shapes indicated that the lowest value of the turbidity was recorded at concentration 200 mg/ml, And treat it B. cereus bacteria which gave a turbidity score (0.233) O.D compared to the control that reached (1.762) O.D, and then the S.aureus bacteria the turbidity was estimated in it (0.412) O.D, And compared to the control sample which reached 1.861 O.D. The charts show that inhibitory effect by the turbidity way and by using alcohol (ethanol) solvent was in a concentration 200 mg/ml better than the other concentrators. while in the E. coli and S. pyogenes and P. aeraginosa the turbidity grade has estimated 0.535- 0.481- 0.589 O. D on respectively in comparison with the control sample which reached 1.928, 1.958, 1.906 O.D on respectively at concentration 200 mg/ml.

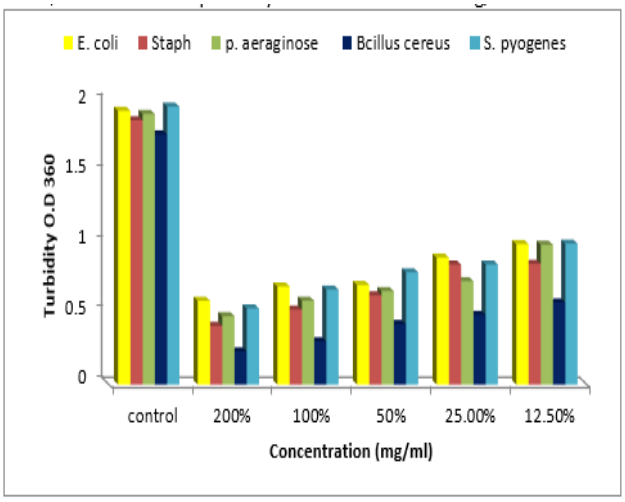

Fig. (1): turbidity test to know the bacteria sensitivity for alcohol(ethanol) extract G. lucidum.

Fig (2) shows results of (GC-MS) Gas chromatography-Mass Spectrometey technique that used to separate effective compounds in the alcohol extract for G. lucidum and depending on the survival

of fatty acids and phenolic compounds in the separation column for compounds which depends on the length of the hydrocarbon chain in the acid based on molecular and polar weight in the separation column for the compounds, It resulted in 70 chemical compounds that play a role in the inhibitory effect of bacteria in G. lucidum , We selected five chemical compounds in G. lucidum, And these five compounds show the largest area under the curve in terms of concentration and as follows: (E)-9-Octadecenoic acid ethyl ester compound and It's a synonymous name for palmitic acid accounted for 48.92% unsaturated fatty acid and anti-bacterial inflammation, and Hexadecanoic acid, ethyl ester compound (which called palmitic acid ethyl ester) accounted for 7.28%, subsequent by 2- Benzothiazol amine accounted for 7.23%, While appeared the Ethanol, 2, 2-diethoxy accounted for 5.41%, The G. lucidum containment on these ingredients explains its ability to inhibit bacterial isolates.

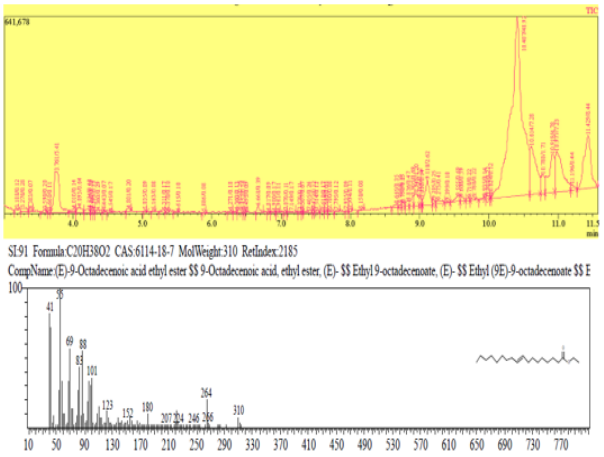

Fig (2): Active compounds separated from alcoholic extract of G. lucidum using GC-MS technique.

New strain obtained in Bacillus cereus bacteria and this strain was registered in the World Gene Bank (NCBI) by Bacillus cereus As-A-M gene for 16S partial sequence, ribosomal RNA as in (fig.3 and 4).

9/26/22, 11:23 PM Bacillus cereus As-A-M gene for 16S ribosomal RNA, partial sequence - Nucleotide - NCBI

An official website of the United States government

Here's how you know

Nucleotide

GenBank

Bacillus cereus As-A-M gene for 16S ribosomal RNA, partial sequence

GenBank: LC729073.1

FASTA Graphics

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REFERENCE   1
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  TITLE     Effective inhibitors of Edible Mushroom Extracts against pathogenic
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  JOURNAL   Unpublished
REFERENCE   2  (bases 1 to 894)
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  TITLE     Direct Submission
  JOURNAL   Submitted (14-SEP-2022) Contact:Aseel Khazal Aldawoode University
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      841 gggagtacgg ccgcaaggct gaaactcaaa ggaattgacg gggcccgcac aagc
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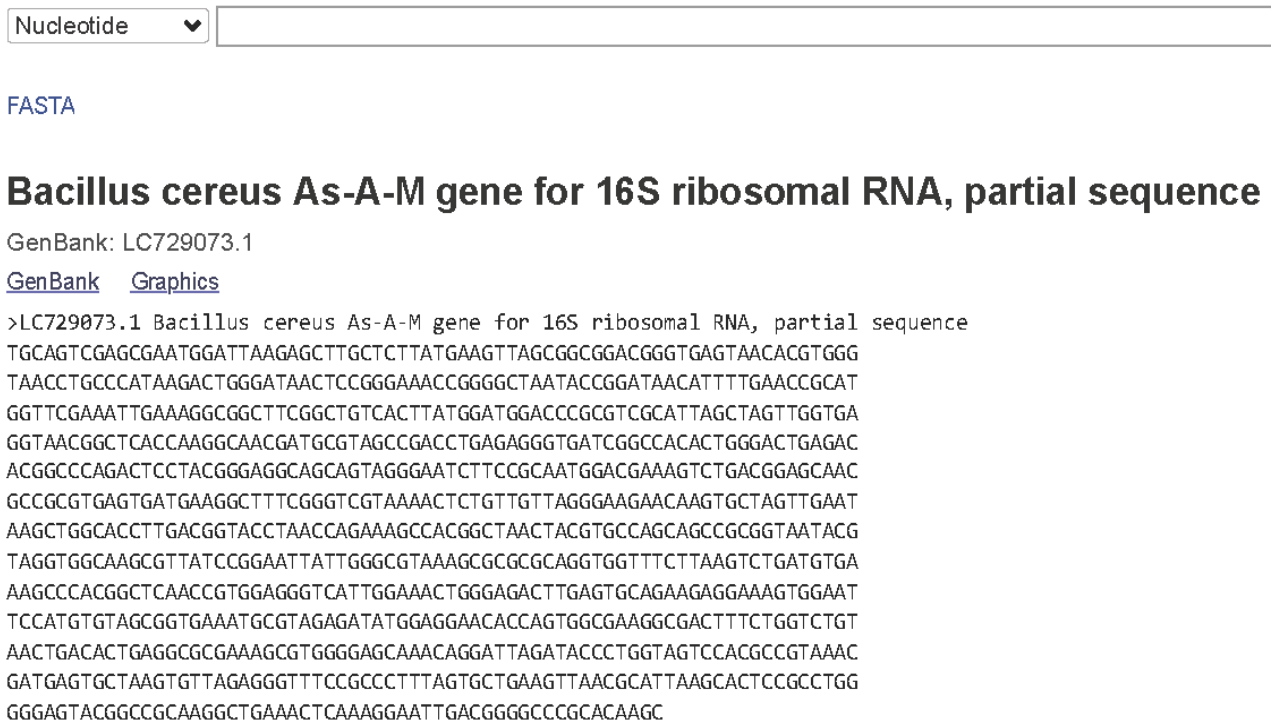
https://www.ncbi.nlm.nih.gov/nuccore/lc729073

Fig. (3): PCR molecular diagnosis of a new strain of B. cereus


Nucleotide

FASTA

Bacillus cereus As-A-M gene for 16S ribosomal RNA, partial sequence

GenBank: LC729073.1

GenBank Graphics

>LC729073.1 Bacillus cereus As-A-M gene for 16S ribosomal RNA, partial sequence
TGCAGTCGAGCGAATGGATTAAGAGCTTGCTCTTATGAAGTTAGCGGCGGACGGGTGAGTAACACGTGGG
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GGGAGTACGGCCGCAAGGCTGAAACTCAAAGGAATTGACGGGGCCCGCACAAGC

https://www.ncbi.nlm.nih.gov/nuccore/LC729073.1?report=fasta

Fig. (4): Genetic sequence of the new strain of B. cereus

5. Discussion

The researcher found Ćilerdžić et al that both hot water and alcoholic extracts for G. lucidum was effective However, the effectiveness of hot water extract is less efficient (14). Our results were close to those obtained by the researcher (Radhika, 2021) he indicated that the ethanol extract G.lucidum has a good antibacterial effect tested in his study, such as S.pyogenes, P.aeruginosa and E.coli, He considered G.lucidum extracts to be an effective antibacterial agent(15).

Studies have shown that 2-Benzothiazolamine compound it has a toxic effect on bacterial cells through its effect on the formation of bacterial biofilms (16).

6. Conclusions

Study results showed alcoholic extract effect of G. lucidum different types of bacteria causing tonsillitis, Alcoholic extract also showed inhibitory activity of bacteria especially at concentration 200 mg/ml. It was also discovered effective antibacterial chemical compounds in G. lucidum mushroom extract by GC-Mass technique, which showed the presence of the (E)-9-Octadecenoic acid ethyl ester compound and ethyl ester compound, Hexadecanoic acid also 2-hydroxy Cyclopentadecanone compound and 2-Benzothiazol amine and 2-Diethoxy, 2-Ethanol.

Conflict of interest: The declaration of no conflict of interest by the authors is stated clearly.

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