University of Basra
College of Pharmacy

Antibacterial and anti-inflammation activity of Hericium *erinaceus mushroom* extract

A Graduation Project Research
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Abstract

Infectious disease is one of the major threats to humans and it is the second leading cause of death worldwide. Edible mushrooms have many nutritional and medicinal values to human health. The medicinal properties of edible mushroom extract in inhibiting pathogenic microorganisms had advantages over the use of chemically synthetic antimicrobial compounds due to less unwanted side effects and can combat microbial resistance. This study aimed to show the effects of methanol extraction (80%) of mushroom Hericium *erinaceus* fruiting bodies, which was prepared and subsequently affects its activity as an antimicrobial against two tested microorganisms, and anti-inflammatory potential. Finding showed methanol extract has positive tests of flavonoids, alkaloids, tannins and appeared more antibacterial activity against *Staphylococcus aureus* (16) mm compared with *Escherichia coli* (12) mm. The *H. erinaceus* extract has non-significant anti-inflammatory IC_{50} reach (493.7) µg/ml compared with (479.7) µg/ml of positive control (Aspirin). This study was concludes that mushroom extracts contain an important compounds may be using as antibacterial and anti-inflammatory to treatment of infection diseases.

Keywords: Lion's Mane Mushroom, alkaloid, tannin, flavonoid, Staphylococcus aureus, Escherichia coli, Aspirin

1-Introduction:

Infectious diseases are considered a major threat to human health, and it is the second leading cause of death worldwide. Many of these deaths occur because patients do not have access to life-saving antimicrobial compounds when and where these are needed[1].

Moreover, infections due to resistant bacteria are now too common and some pathogens have even become resistant to multiple types or classes of antibiotics, such as methicillin-resistant Staphylococcus aureus (MRSA) and vancomycin resistant Staphylococcus aureus (VRSA), thus resulting in losing effective antibiotics for treatment of serious infection and can lead to death. So the continuous need for natural bioactive compounds that combat the microbial resistance to antibiotics was considered an admitted challenge, so many studies had been created to present some bioactive compounds especially from plants or from other sources, like microorganisms[2].

Many mushrooms have high-quality protein content with essential amino acids and are considered a good source of vitamins, such as thiamine, riboflavin, and ascorbic acid[3]. In the last few years, several antimicrobial and antioxidant compounds were discovered in the fungi kingdom[4]. Moreover, mushrooms have a wide range of secondary metabolites of high therapeutic value, such as antioxidant, antiviral, antithrombotic, anti-inflammatory, and antitumor activities[5].

Mushrooms are macro fungi with distinctive basidiomata or ascomata, which can be either hypogeous or epigeous, large enough to be seen with the naked eye and to be picked up by hand. Mushrooms have been traditionally appreciated due to their excellent sensory characteristics, including the pleasant flavor and texture. Biologically active compounds isolated from mushrooms include polypeptides, polysaccharides, glycopeptides, ribonucleases, proteases, and lectins as well as low molecular weight compounds, such as lactones, terpenoids, and alkaloids[6]. In general, fungi could represent sources of several valuable compounds[7].

In particular, mushrooms could be considered a good source of antioxidants that worked as protector agents against oxidative damage such as ascorbic acid and gallic acid[8] .In addition, many edible and wild mushrooms, such as Pleurotus ostreatus and Laetiporus sulphureus, showed high antimicrobial effects when extracted with polar sol vents against both pathogenic bacteria and fungi[9].

Figure 1: Fruit body of Hericium erinaceus

Hericium erinaceus, also known as Lion's Mane Mushroom or Hedgehog Mushroom, figure (1) is an edible mushroom with historical usage in traditional Chinese medicine. This mushroom belongs to the class of Agaricomycetes under the phylumbasidiomycota. It also has antitumor activities against HepG-2, MCF-7, El-4, and EC-109. Additionally, *H. erinaceus* has other therapeutic uses and biological activities, such as antioxidant, antimicrobial and anti-inflammatory potential[10].

The study was aimed to evaluate antibacterial and anti-inflammatory effects of fungus *Hericium erinaceus* extract.

2-Material and method

1.2- Extraction

Grind the dry fruiting bodies of the mushrooms, figure (2) with a grinder . Fifty grams of air-dried fruiting bodies were extracted three successive times with 80% methanol, then filtered, and the combined filtrates were concentrated and then used for the following tests (Suleiman *et al*., 2022)*.

Figure 2: Dry fruit body of H. erinaceus

2.2- Qualitative chemical assessment

Chemical analyses were carried out on the *H. erinaceus* extract to identify their primary components[11]. The following procedures were followed during the tests:

1- Dragendorff"s test for alkaloids

By adding 1 mL of Dragendorff"s reagent to 2 mL of extract, an orange red precipitate was formed, indicating the presence of alkaloids.

2-Shinoda's test for flavonoids

One piece of magnesium chips was then added to the filtrate followed by few drops of conc. HCl. A pink, orange, or red to purple colouration indicates the presence of flavonoids.

3- Ferric chloride test for tannins

1 ml of the filtrate was diluted with distilled water and added 2 drops of ferric chloride. A blue-black, green, or blue-green precipitate indicates the presence of tannins.

2.3- Antibacterial assay

Agar well diffusion method[12] was applied to determine the antibacterial activity of fungal extract against two pathogenic bacteria the first belonged to Gram-positive bacteria (*Staphylococcus aureus*) and the second belonged to Gram-negative bacteria (*Escherichia coli*). Working solution extract (20 mg/ml) were provided by dissolving 20 mg of fungal extract in 1 ml of DMSO.

The assay was started by activation two isolates of bacteria on nutrient broth media for three hours in incubator 37 c, by soup bacterial isolates were spread on Muller Hinton Agar, then left for 5 minutes to absorption after that create agar wells (6 mm in diameter). 100 µl of fungal extract were loaded into wells. After allowing the agents to diffuse into the agar media, the plates were underwent a 24- hour incubation at 37c.

2.4- Anti- inflammation activity

Anti-inflammatory bioassay in vitro of mushroom *H. erinaceus* extract was done according to Dharmadeva et al., (2018)[13].Serial dilution from (250 µg/ml, 500 µg/ml, and 1000µg/ml) was performed for mushroom extract and for reference drug (Aspirin). All samples contained 5.0 ml of total volume. Reaction mixtures were prepared using 2.8 ml of phosphate buffered saline (pH 6.4) and 0.2 ml of Bovine Serum albumin. Then 2 ml of extract from each different concentration were mixed gently with reaction mixtures. A similar procedure was used for reference drugs (Aspirin). In addition, distilled water was used as negative control. Reaction mixtures were incubated in a water bath at 37°C for 20 min, and later, it was heated at 70°C at which the reaction mixture was maintained for 5 min.

Then, the reaction mixture was allowed to cool down at room temperature for 15 min. Absorbance of reaction mixture after denaturation was measured for each concentration at 680 nm. The percentage of inhibition of protein was determined on a percentage basis with respect to control using the following formula:

Percentage inhibition (%) =

(Absorbance of control - Absorbance of test /Absorbance of control) X100

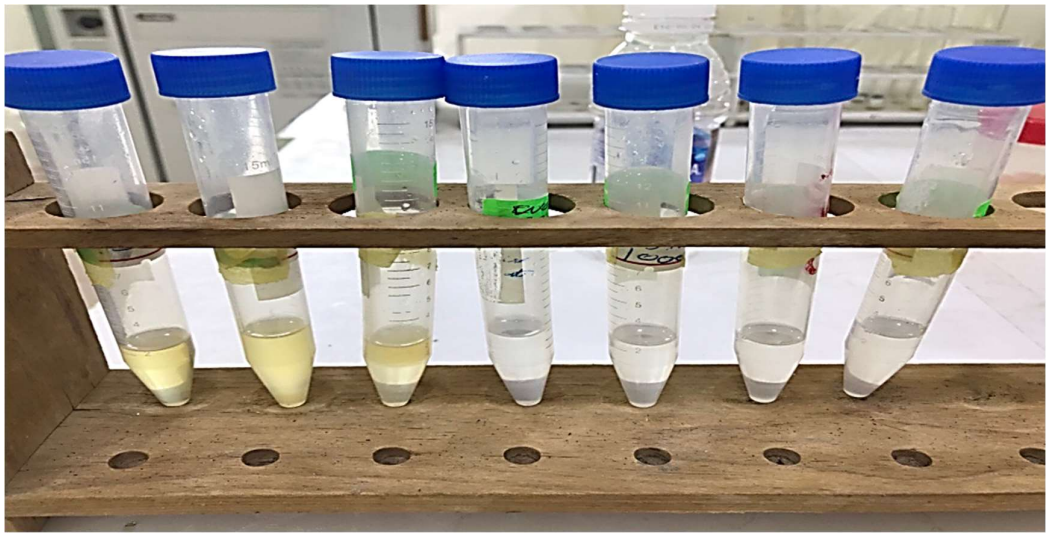

Figure 3: Anti-inflammation test

3-Result and Discussion

Qualitative chemical tests of the *H. erinaceus* extract showed positive results for all tests, figure (4).

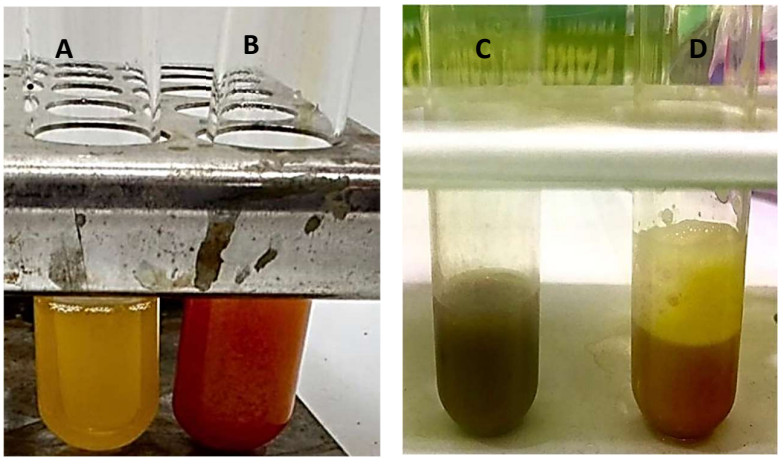

Figure 4: Figure 4: Phytochemical test for H. erinaceus A: blank, B: Alkaloid, C: Tannin, D: Flavonoids

The presence of alkaloid, tannin and flavonoid compounds have inhibitory effects on mutagenesis and carcinogenesis in humans when up to 1.0 g are ingested daily from a diet rich in fruits and vegetables (Mujić *et al*., 2011)**.

Antibacterial activity

The extract of *H. erinaceus* showed inhibitory activity against two type of pathogenic bacteria with inhibition zone reached about 16 , 12 mm of the diameter for *S. aruues* and *E. coli* respectively. Figure (5)

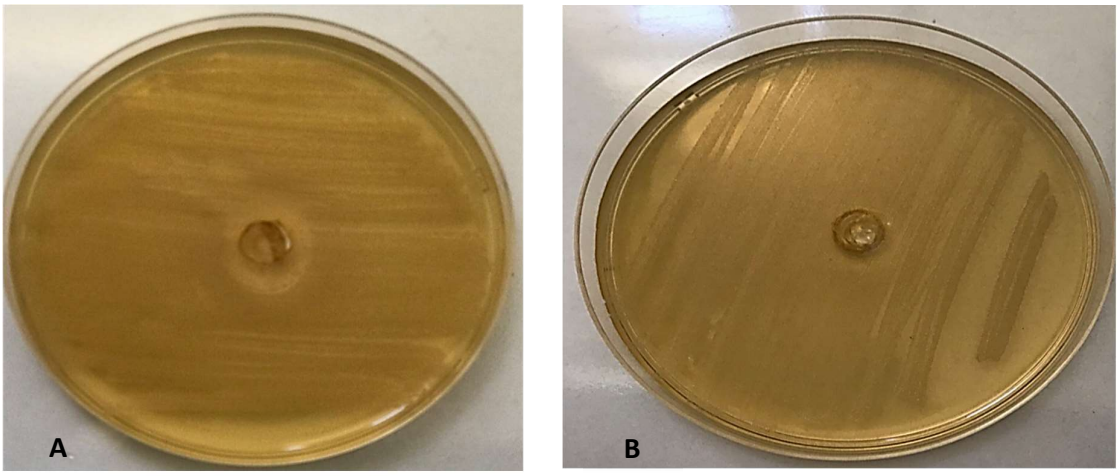

Figure 5: Antibacterial activity of H. erinaceus extract: A: S. aruues, B: E. coli

This result is agree with results reported by Sridhar *et al*.(2011)[14] showed the methanol and aqueous extract of mushroom fruit bodies showed high antimicrobial activity against *Salmonella typhi* and *S. aureus.* The changeable antimicrobial activity of mushroom extracts may be indicating the presence of different broad-spectrum antimicrobial compounds in the mushroom. Similar reports by other researchers return the variable of antimicrobial activity of mushroom extracts may arise from the genetic structure of mushroom species, physical, biochemical constituents, chemical differences of mushroom extracts, solvents, and test microorganisms that used when its antimicrobial properties compared to the other mushroom species (Suleiman *et al*., 2022)*.

Anti-inflammatory activity

Figure (6) shows anti-inflammatory activity of three concentrations of *H. erinaceus* extract, in which the percentage of protein inhibition ranged 96.15, 93.75, 90 % compared with 69.23, 60, 20 % as an inhibition percentage of Aspirin as the positive control for three contraction. Hence, the *H. erinaceus* extract displayed good protein protection.

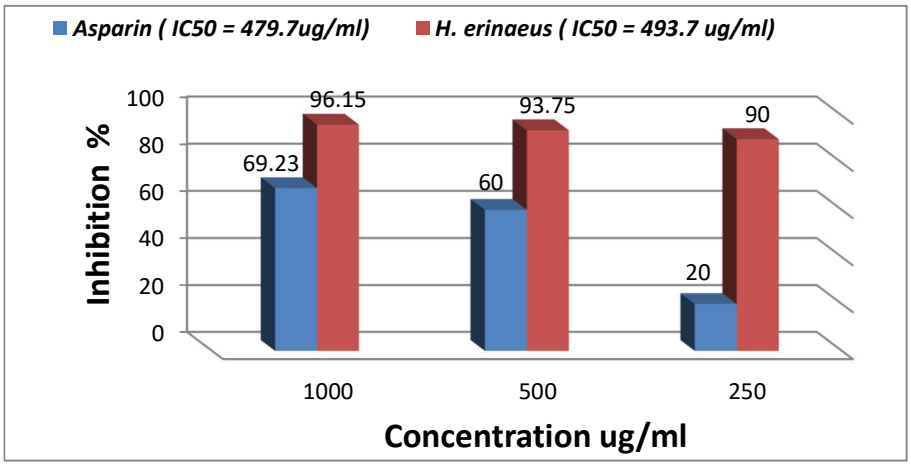

Figure 6: Anti- inflammatory activity percentage of fungus H. erinaceus extracts under three different concentrations compared with Aspirin

Protein denaturation is the most common cause of prolonged inflammation. Therefore, inhibition of such denaturation can have a clinically favorable effect on inflammation. Anti-inflammatory activity of n-hexane, chloroform, ethyl acetate, and methanol extracts of mycelia in submerged culture of *H. erinaceus*, indicated that theses extracts from medicinal mushrooms exhibited anti-inflammatory activity that might be attributable to the inhibition of nitrous oxide (NO) generation and can therefore be considered a useful therapeutic and preventive approach to various inflammation-related diseases[15].

The *H. erinaceus* extract demonstrated the ability of this extract to be used for medical treatment of inflammation, and bacterial infection as they both considered flavonoid compounds and have antibacterial and anti-inflammatory effect. More studies will be needed to isolate and identify the pure active compounds as well as determination of the mode of action of these antimicrobial compounds, we believe that it is worthwhile to exploit the potential of these antimicrobial compounds in treating the infectious bacterial and fungal diseases.

4. Conclusion

Our present study concules that Prosopis farcta possess anti-inflammatory properties which could be due to presence of active constituents present in the plant extract such as luteolin, rutin and hesperidin flavonoids .

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