EVALUATION OF POTENT SILVER NANOPARTICLES PRODUCTION FROM Agaricus bisporus AGAINST Helicobacter pylori

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Agaricus bisporus have long been used in ancient times to treat diseases like cancer, diabetes, antimicrobial and inflammatory throughout the world. The objectives of present study were to evaluate the anti-Helicobacter pylori profile of AgNPs produced by A. Bisporus (AB). Materials and Methods: H. pylori bacteria were isolated from patients with duodenal ulcer by using (Endoscope) and identified using biochemical tests. Silver nanoparticles (AB-AgNPs) were prepared using aqueous extract of A. Bisporus as bioreductant. The characterization of AB-AgNPs was also determined by UV-Visible. Well diffusion assay was used to determine the anti-Helicobacter effect of A. Bisporus extracts and AB-AgNPs at concentrations 100, 75, 50 and 25 mg mL⁻¹. Result: AB-AgNps were recorded to be highest in zone of inhibition as compared to ethanolic (EOH) and aqueous (EQ) extracts in all concentration. Conclusion: This is the first report that highlights the anti-H. pylori activity of AB-AgNPs. The overall result showed that AB-AgNPs could be controlled the H. pylori and are presumed to be promising therapeutic agent in ulcerative diseases.

Keywords: Anti-Helicobacter pylori, AgNO3, UV-Visible spectroscopy.

INTRODUCTION

H. pylori, one of the primary risk factors of gastric ulcer and gastric cancer, as with many other organisms, H. pylori resistance is on the constantly increase around the world (Mustafa et al., 2015). Therefore, there is a need to discover new antibacterial agents proposing fight the side-effects caused by antibiotic treatment, especially for H. pylori (Albaayit et al., 2019; Li et al., 2017). Nowadays, nanotechnology has become a standout area for researchers in the research for drugs with potent antibacterial properties because of the unique physical and chemical properties of the nanomaterial (Albaayit et al., 2020; Hussain et al., 2018). Recently, researchers are focusing on the effectiveness of mushroom in the search of natural materials (Ma et al., 2018; Al-Ani and Albaayit, 2018). A. bisporus (Family: Agaricaceae) is one of the species widely distributed in North America that has been used in traditional medicine in China, and Mexico for the treatment of cancer and diabetic (Hyde et al., 2019). This mushroom also contributed to improve immunity and tumor-retardation and contain compounds comprising a wide range of antimicrobial such as butyl ester, behenic alcohol, 1-amino cyclopentane hydroxamic acid and methylphenidate (Gautam et al., 2016). A. bisporus was recently used in the synthesis of silver nanoparticles (AgNPs) to improve the effectiveness of mushroom (Evans & Markose, 2014). The AgNPs are among the most common sources of disinfectants. Recently, research to determine the potential of AgNPs for the significantly biocides bacterial infections had flourished with the discovery of many drug candidates (Greenwell and Rahman, 2015). The potent biocides of these superior disinfectants are attributed to several mechanisms including induce the production of reactive oxygen species (ROS) and alter the mechanisms of signal transduction (Ahmad et al., 2015).

AB-AgNPs was reported to exhibit various in vitro antibacterial activities including anti- salmonella typhi, anti-Staphylococcus aureus (Dhanasekaran et al., 2013), anti-Escherichia coli (Sriramulu et al., 2017), anti-Pseudomonas aeruginosa and anti-Proteus mirabilis (Román et al., 2020), and anti-antitumor effect (Owaid et al., 2015). Thus, the AB-AgNPs were prepared and the effect of the formulation determined on H. pylori, which might explore value drug for treatment of H. pylori-related disease.

MATERIALS AND METHODS

Collection and isolation of H. pylori: Fifty clinical samples (histological biopsies) had been collected from patients with duodenal ulcer. The samples were kept in the 2ml normal saline using ice box to transport to the laboratory. The collection was conducted from Baghdad Teaching Hospital during the period of 1/12/2018-1/5/2019. Two selective media were applied for the preliminary isolation of bacteria (Al-Ammar et al., 2011). A single isolated colony of each isolate was subjected for confirmative routine diagnosis of H. pylori via applying; (1) Gram stain sensitivity test to Caphalothin and Nalidixic acid and (2) Biochemical tests based on the standards of microbiological techniques (Carroll, 2012; Holt et al., 1994).
Preparation of A. bisporus: Oven dried A. bisporus was powdered, and soaked in (95%) ethanol, and boiling distilled water (D.W) under agitation at (60 ± 2) °C for (30) minutes. The extraction at a 1:10 dried mushroom weight to volume ratio. The filtrate was collected through Whatman (No.1) filter paper, subjected to freeze dryer, and stored at 4± 2°C °C until use (Albaayit et al., 2020; Albaayit et al., 2019).

Biosynthesis and Characterization of silver nanoparticles (AB-AgNPs): AB-AgNPs were made according to the method described by (Gurunathan et al., 2013). A stock solution of Silver nitrate (1x10⁻³) AgNO₃ was prepared in sterile deionised water. Approximately 100 mg/ml of aqueous extract solution was prepared in sterile distilled water and filtered through (0.2) µm syringe filter. Based on the result of a preliminary trial, (5) ml of (100) mg/ml of aqueous extract of A. bisporus were added to (5) ml of (1x 10⁻³) M aqueous AgNO₃ solution and kept at room temperature for 7 days. The yellow colour of mixture solution turned to dark yellow indicating the formation of silver nanoparticles. The Characterization for the AB-AgNPs was determined using colorimetric indices in UV-Visible spectroscopy.

Screening of crude extract and AB-AgNPs for anti H. pylori activity: Agar well diffusion method was used as described previously (Al-Azzawi et al., 2012). Briefly, 100 µl of bacterial suspension was dispensed in each nutrient agar plate. Four agar wells of 6 mm diameter were prepared using cork borer in each Petri plate, after loading with 25, 50, 75 and 100 µg/mL of the EQ, EOH and AB-AgNPs and the plate incubated for 4h at 37°C. The inhibition zone (mm) of bacteria was measured by using millimeters.

DISCUSSION AND RESULT

A total of fifty samples two H. pylori were isolated from histological biopsies of a patient with duodenal ulcer using morphological and biochemical tests Fig. 1. The antibacterial assay of A. bisporus against H.pylori showed that EOH effective than EQ in all concentration (25, 50, 75 and 100 mg/ml). The inhibition zone at concentration 100 mg /ml was 12.33 ± 1.45mm but at concentration 25 mg /ml was 5.33± 0.33 mm, the inhibition zone of EQ at concentration 100 mg /ml was 6.67 ± 0.67mm but at concentration 25 mg /ml was 4.33 ± 0.33 mm. This result could be due to the solubility of substances that is responsible for the antibacterial action is more with polar solvent compared to water (Albaayit et al., 2014). Our outcome is in agreement with findings reported by Gonelimali et al., who stated that ethanolic extract has a significant antibacterial effect compared to aqueous, and hence EQ was selected for AB-AgNps synthesis in this study. Nowadays, fungal synthesised-nanoparticles have posed a great platform to the scientists because of their stability and non-toxicity compared to other organism synthesised nanoparticles (Chandrappa et al., 2016). In this study, the silver nanoparticles were synthesized using the A. bisporous

confirm the stability of metal nanoparticles. Ag nanoparticles exhibit strong absorption of electromagnetic waves in the visible range due to surface plasmon resonance (SPR) phenomenon which causes collective oscillation of the conduction electrons (Narasimha et al., 2011). Due to the excitation of AgNPs in UV-visible spectrum, it exhibits a yellowish-brown color in aqueous solution Fig. 2.

![Colloid of mushroom and AgNO₃](image1)

**Figure 2.** a) Colloid of mushroom and AgNO₃ b) UV-Vis absorption spectra of silver nanoparticles after bio-reduction by aqueous extract of A. bisporus.

Anti-bacterial potency of the AB-AgNPs was tested against *H. pylori*. The silver nanoparticles have shown highest zone of inhibition compared with the other extracts Fig. 3. One of the most possible mechanisms of antibacterial is that Ag⁺ released from AgNPs, can interfere with cell division by strongly binding to thiol groups of protein found on the cellular surface thus causing bacterial cell death (Abdussalam-Mohammed, 2020). In addition, AgNPs can cause oxidative damage via generation reactive oxygen species (ROS), resulting in attack enzymes and proteins leading to irreversible damage to DNA replication (Albaayit et. al., 2018; Albaayit et. al., 2016; Abdal Dayem et. al., 2017).

![Inhibition zone vs Concentration](image2)

**Figure 3.** Antibacterial evaluation following Well diffusion assay on *H. pylori*. Abbreviations: EQ, aqueous; EOH, ethanol; AB-AgNPs, A. bisporus nanoparticles.

**Conclusion:** AB-AgNPs have a great possible to be developed into a highly active therapy in the treatment of *H.pylori*-related conditions. For this, more investigations have to be done to understand the mechanistic effects on bacterial cells.

**REFERENCES**


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Inhibition Helicobacter pylori using Silver Nanoparticles


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