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ANTIMICROBIAL ACTIVITY OF SOME MALAYSIAN MUSHROOMS ON *ESCHERICHIA COLI* AND *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Background and Objective: Nowadays, the world is facing significant challenges in modern healthcare services because many antimicrobial agents have lost their effectiveness in treating infectious diseases primarily due to the development of microbial resistance. Mushroom species release various bioactive compounds and yet largely untapped resource of useful natural compounds. This study aimed to assess the primary screening for antimicrobial activity of selected Malaysian mushroom's against *Escherichia coli* and *Staphylococcus aureus.*

Methodology: The mycelia biomass extracts of the 8 collected Basidiomycetes mushrooms. Antibacterial activity was measured using Disc diffusion and Well diffusion methods against standard reference strains *E. coli* MTCC 740 and *S. aureus* MTCC 501. **Results:** The potential inhibition of *Agaricus cupreobrunneus*, which gave a reading of 4.6 cm for disc diffusion and 3.4 cm for well diffusion of inhibition against *E. coli* MTCC 740 and against *S. aureus* MTCC 501 that, gave a reading of 4 cm for disc diffusion and 3.1 cm for well diffusion approaches. The second highest bioactivity mushroom against *E. coli* MTCC 740 the highest inhibition was shown by extract of brown *Agaricus bisporus* which gave 3.5 cm and give 3.2 cm *S. aureus* MTCC 501 by disc diffusion. For well diffusion the zone of inhibition by same mushroom for *E. coli* MTCC 740 which gave reading 3.0 cm and gave 2.7 cm against *S. aureus* MTCC 501. **Conclusions:** The species studied showed potential inhibition of *A.cupreobrunneus* more than other mushrooms by disc diffusion method especially against *E. coli* more than *S. aureus*. Further studies are required to ascertain their toxicity against mammalian cells and potential side effects.

Keywords: Antibacterial, Mushroom, *Agaricus cupreobrunneus, Agraicus bisporus, E. coli, S. aureus*

INTRODUCTION

Medicinal mushrooms have a long history of use in folk medicine. It is suitable to use as a nutritional supplement in our daily life. They have prophylactic impact, therapeutic impact as well as benefits in the food industry, as alternative food for human and animal consumption and as biocatalyst. Most tropical mushrooms or basidiomycetes have not been studied for their bioactive compound composition. Majority of studies were done on fruit bodies of known and well-researched basidiomycetes such as *Ganoderma spp*. and *Pleurotus spp*. Our tropical forest holds a vast range of tropical basidiomycetes, which have not been tapped for its unique and unlimited potential for various uses such as in food, pharmaceutical, nutraceutical, biochemical and therapeutic industries (Alves, *et al*., 2012). According to Kakon (Kakon, *et al*., 2012), Smith, (Smith, *et al*., 2002), Ganeshpurkar (Ganeshpurkar, *et al*., 2010), and Ferreira (Ferreira, *et al*., 2010), approximately 700 species of higher Basidiomycetes had found to possess significant pharmacological activities. Mushrooms have reported to have great potential as a nutritionally functional food and a source of physiologically valuable and non-toxic medicines (Zaidman, *et al*., 2005; Raina, 2013). Current problem of microbial drug resistance and increased concern on opportunistic infections makes the alternative drugs especially those originating from plants and Basidiomycetes to be prospective. Since few studies have reported on the antimicrobial activity of different wild mushroom extracts in Malaysia, this study objectives to do isolation and screening of antibacterial-producing mushrooms

by primary screening of bioactive extracted compounds from different species of mushrooms as antibacterial agents against standard reference strains bacteria and concentrate to identify the genus and species of this mushrooms. We focused oneight mushrooms species that originated from four families, namely *Cantharellus subalbidus*, white *Hypsizygus tessellatus*, brown *Hypsizygus tessellatus*, *Agaricus bisporus* and *Agaricus cupreobrunneus.*

MATERIALS AND METHODS:

Mushroom samples preparation: Mushrooms (*Cantharellus subalbidus*, white *Hypsizygus tessellatus*, brown *Hypsizygus tessellatus*, *Agaricus bisporus* and *Agaricuscu preobrunneus*) purchased from a local food store in Kelana Jaya, China town and wild from Genting highland, Malaysia start from May 2017 to August 2017 at the Laboratory of Biotechnology School, University of Lincoln, Malaysia. The mushrooms were incubited in an oven at 40 ºC for 48 hrs or until constant weight was observed. Each dried mush-room sample then macerated for 24 hrs in water at room temperature. After removing the water, maceration repeated with fresh water. The aqueous extracts were combined and filtered, concentrated and concentrate under reduced pressure. The aqueous extract was stored at -20 ºC until tested as well diffusion (Wong, *et al*., 2013). For disc diffusion the mushroom material wascatted into smalller pieces' size 1 cm then stored at -20 ºC until lab work (Almahdi, 2006, Poh-Hwa, *et al*., 2011).

Kirby-Bauer antimicrobial susceptibility test: The antibacterial activities of the selected mushrooms were tested using Kirby-Bauer disc diffusion method (DDM) and well diffusion methods (WDM), against both Gram-positive (*Staphylococcus aureus* MTCC 501) and Gram-negative (*Escherichia coli* MTCC 740) bacterial strains(Wong, *et al*., 2013).

Briefly, bacterial culture inoculum was adjusted to 0.5 McFarland standard and swapped onto the nutrient agar. Sterile forceps were used to apply filter paper disks containing mushroom aqueous extracts onto the agar surface. Tetracycline antibiotic disks (Oxoid Ltd.) were used as positive control. Nutrient agar plates were incubated for 18-24 h at 37 ºC, and diameter of the inhibition zone was measured in cm (Alves, *et al*., 2012).

Conformation of strong antibacterial activity of Mushrooms: The antibacterial mushroom identities were confirmed morphologically and biochemical properties by referring to literature (Hall, *et al*., 2010).

RESULTS

Antibacterial Activity by disc diffusion Method against Pathogenic Bacteria: Out of 8 mushroom with antimicrobial activity 2 (25 %) wild mushrooms from location site no 1 (Kelana Garden Ke) show no activity against *E.coli* MTCC 740 and S. aureus MTCC 501, as show in Table 1, Figure 1.

Table 1: Antimicrobial activity by disc diffusion and well diffusion methods against targets pathogenic bacteria with mushroom types.

No.	Type	Zone of inhibition by Mushroom (cm)	
		E. coli MTCC 740	*S. aureus* MTCC 501
1	Wild	**1.1**	**1.2**
2	Cultivated	**1.6**	**1.2**
3	Cultivated	**1.1**	**1.5**
4	Cultivated	**2.5**	**1.7**
5	Wild	**1.4**	**1.5**
6	Cultivated	**1.2**	**1.4**
7	Cultivated	**3.5**	**3.2**
8	Cultivated	**4.6**	**4.0**

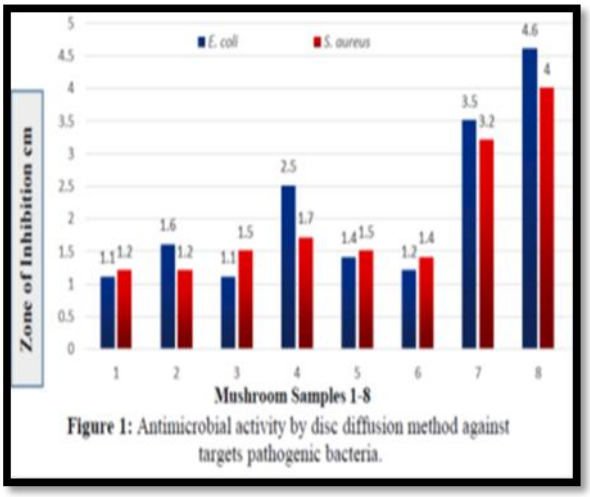


Figure 1: Antimicrobial activity by disc diffusion method against targets pathogenic bacteria.

Cultivated mushroom no 2 show slight activity against *E.coli* MTCC 740 (1.6 cm) and less activity against *S. aureus* MTCC 501 (1.2 cm). Cultivated mushroom no 3 show low activity against *E.coli* MTCC 740 (1.1 cm) and little activity against *S.aureus* MTCC 501 (1.5 cm). Cultivated mushroom no 4 show moderate activity against *E. coli* MTCC 740 (2.5 cm) and less activity against *S. aureus* MTCC 501 (1.7 cm). Cultivated mushroom no 4 show moderate activity against *E. coli* MTCC 740 (2 cm) and less activity against *S. aureus* MTCC 501 (1.5 cm). Other wild mushroom no 5 collected from location site (Genting Highlands Gh) show antibacterial activity against *E. coli* MTCC 740 (1.4 cm) and slightly higher activity against *S. aureus* MTCC 501 (1.4 cm). Culti-

vated mushroom no 6 show low activity against *E. coli* MTCC 740 (1.1 cm) and slightly higher activity against *S. aureus* MTCC 501 (1.2 cm).
Cultivated mushroom no 7 strong activity against *E. coli* MTCC 740 with zone of inhibition (3.0 cm) but less than DDM and less activity against *S. aureus* MTCC 501 (2.7 cm). The highest and strong activity of cultivated mushroom no 8 with zone of inhibition against *E. coli* MTCC 740 (3.4 cm) and higher zone of inhibition against *S. aureus* MTCC 501 (3.1 cm) as shown in Plate 1.

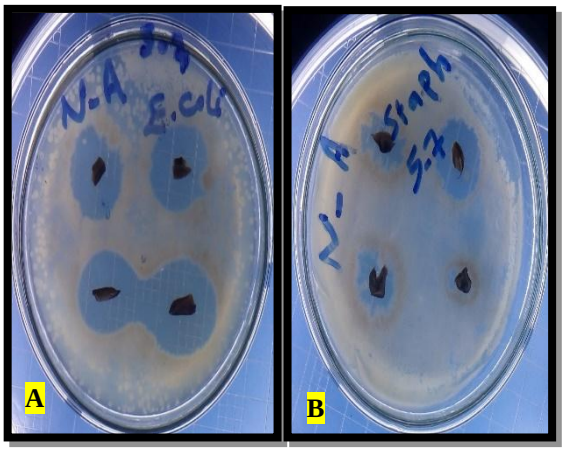


Plate 1: Antibacterial activity by disc diffusion method of mushroom 8 against *E. coli* MTCC 740 (A) and *S. aureus* MTCC 501 (B).

Antibacterial Activity by Well Diffusion Method Against Pathogenic Bacteria: Out of 8 mushrooms with antibacterial activity 2 (25 %) wild mushrooms from location site no 1 (Kelana Garden Ke) show no activity against standard reference strains *E. coli* MTCC 740 and *S. aureus* MTCC 501, as show in Table 1 and Fig. 2.

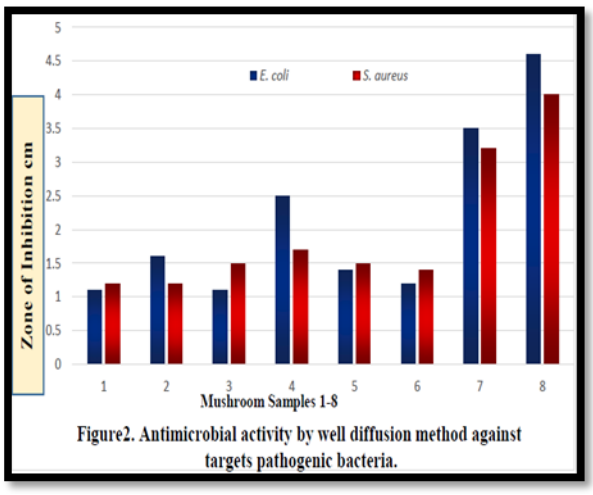


Figure2. Antimicrobial activity by well diffusion method against targets pathogenic bacteria.

Cultivated mushroom no 2 show slightly activity against *E. coli* MTCC 740 (1.5 cm) less than DDM and less activity against *S. aureus* MTCC 501 (1.3 cm). Cultivated mushroom no 3 show low activity against *E. coli* MTCC 740 (1.1 cm) and little activity against *S. aureus* MTCC 501 (1.5 cm) similar to DDM. Cultivated mushroom no 4 show moderate activity against *E. coli* MTCC 740 (2 cm) and less activity against *S. aureus* MTCC 501 (1.5 cm). Wild mushroom no 5 collected from location site (Genting Highlands Gh) show antibacterial activity against *E. coli* MTCC 740 (1.4 cm) and slightly higher activity against *S. aureus* MTCC 501 (1.4 cm).
The highest strong activity of cultivated mushroom no 8 with zone of inhibition against *E. coli* MTCC 740 (3.4 cm) and higher zone of inhibition against *S. aureus* MTCC 501 (3.1 cm) as shown in Plate 2.

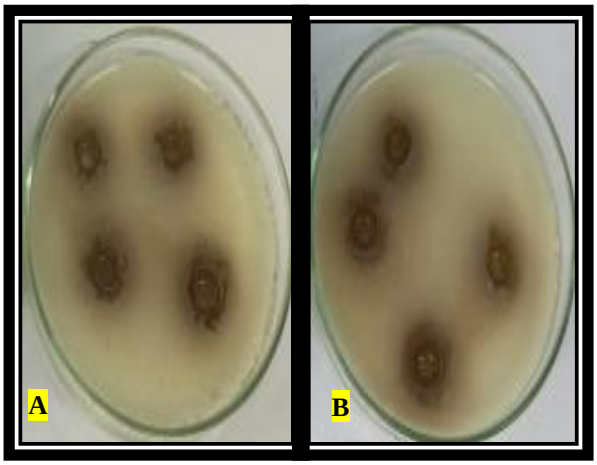
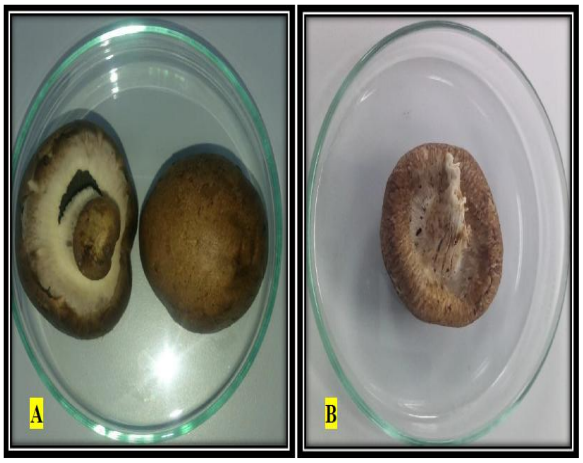


Plate 2: Antibacterial activity of mushroom 8 by well diffusion method against *E. coli* MTCC 740 (A) and *S. aureus* MTCC 501 (B).

Conformation of strong antibacterial activity of Mushrooms: The mushroom identities were confirmed morphologically by referring to literature. Cultivated mushroom no. 7 was identified as brown *Agaricus bisporus* and classified. The conidia chains were from down. The cap and spore were brown. Diffusible and melanin pigments were produced on potato dextrose agar. Strong antimicrobial activity against standard reference strains *E. coli* MTCC 740, as Gram-positive bacteria and *S. aureus* MTCC 501 was observed as shown in Plate 3. Cultivated mushroom no. 8 was identified as brown *Agaricus cupreobrunneus* and classified. The conidia chains were from down. The cap brown and spore is white. Diffusible and melanin pigments were producedon potato dextrose agar. Strongly antimicrobial activity against *E. coli* MTCC 740, as Gram-negative bacteria and *S. aureus* MTCC 501 was observed as shown in Plate 3.



DISCUSSIONS

In this study, an assay of the inhibition of standard reference strains Gram-positive (*Staphylococcus aureus* MTCC 501), Gram-negative *(Escherichia coli* MTCC 740) were studied. Basidiomycetes produced a series of biologically active compounds when grown in pure culture (Clericuzio, *et al*., 2008). They provide a rich and varied source of compounds that have antibacterial properties (Rahi and Malik, 2016). In the measurement done against Gram-positive bacteria, for all basidiomycetes inhibition temperature and pH was noticed at 37ºC and pH 7 for bacteria. The highest inhibition was shown by the extract of *Agaricus-cupreobrunneus,* which gave a reading of 4.6 cm for DDM and 3.4 cm for WDM of inhibition against *E. coli* MTCC 740. *Agaricus cupreobrunneus* mushroom have shown activity against *S. aureus* MTCC 501 which gave a reading of 4 cm for DDM and 3.1 cm for WDM of inhibition similar results reported by Buckingham (Buckingham, 2020) and Waithaka (Waithak, *et al*., 2017). For second highest bioactivity mushroom as for *E. coli* MTCC 740 the highest inhibition was shown by extract of brown *Agaricus bisporus* which gave 3.5 cm and give 3.2 cm *S. aureus* MTCC 501 by DDM. For WDM the zone of inhibition by same mushroom for *E. coli* MTCC 740 which gave reading 3.0 cm and gave 2.7 cm against *S. aureus* MTCC 501 which reported by (Gardner, 2015; Shittu, *et al*., 2015; Soboleva, *et al*., 2006). All 8 species of Basidiomycetes show low inhibition of growth for WDM which indicated as lower concentration similar to previous reports (Al-Mahdi, 2006; Al-Mahdi, *et al*., 2019). Although the results were obtained, the concentration used to inhibit was higher by DDM (Al-Mahdi, 2019). Strains of Agaricales showed high antibacterial properties and high yields of water-soluble polysaccharides in their mycelium extract which is believed to contribute to the antibacterial effect (Soboleva, *et al*., 2006). The mushroom mycelium contains steroids, lactones, alkaloids, polysaccharides and triterpenes. Pharmacologically, a number of the water-soluble polysaccharides have demonstrated antibacterial and immunostimulant activities (Middleton, *et al*., 2005). The value of crude extract did not differ in all types of solid substrate were added. Potato and yeast were found to be the best complex Carbone and Nitrogen source for the growth of mycelia (Gbolagade, *et al*., 2006).

Conclusion and recommendations: The antimicrobial activity was also promising since the species studied were also low in their toxicity levels. Although the high concentrations were used and the human pathogenic bacteria's activity against fungi was more. Therefore, further investigation on using them as natural antimicrobial products is important. The modification of the extraction method can contribute to the availability of various other total soluble extracts, which can contribute to their bioactive form. We recommend more studies are required for determine the interaction of antibacterial and antifungal antibiotics which compare it with control antibiotics.

LIMITATIONS: Due to the retrospective nature of our study, there was limitation of describing the various types of mushrooms, morphological and biochemical characteristics of the mushrooms and pathogenic bacteria usage.

Conflict of interest- None

Significance Statement: This study discovers the strong potential inhibition of *A.cupreobrunneus* mushroom against Gram-negative bacteria that can be beneficial for use. *A. cupreobrunneus* mushroom in traditional medicine are potentially effective as antimicrobial agents. This study will help the researcher to uncover the critical areas of brown mushroom as antibacterial, antifungal and anticancer potential that many researchers were not able to explore. Thus, a new theory on antimicrobial and anticancer bioactivity from the secondary metabolism of this species of mushroom may be arrived at.

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