

ANTIBACTERIAL AND ANTIOXIDANT ACTIVITIES OF *AGARICUS BISPORUS* (J.E. LANGE) IMBACH FROM PAKISTAN

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Keywords: Bioactive constituents, Mushroom, Bactericide, DPPH, GCMS

Abstract

In the present study, antibacterial and antioxidant (DPPH free radical scavenging activity) activity of an edible mushroom, *Agaricus bisporus* collected from Pakistan was investigated. In antibacterial assays, n-hexane showed 4.83, 3.33, 16 and 3.33 mm inhibition zone diameter (IZD), chloroform showed 6.33, 16.17, 17 and 1.67 mm (IZD) while, ethyl acetate exhibited 30, 20, 19 and 28.67 mm (IZD) against four test plant pathogenic bacteria viz., *Pseudomonas syringae* Van Hall, *Ralstonia solanacearum* Yabuuchi *et al.* *Xanthomonas axonopodis* Dowson and *Erwinia carotovora* (Jones) Waldee, at 100 mg/ml concentration. Optical density (OD) was recorded at 517 nm. n-hexane extract showed 60.73, 47.6 and 33.05%, chloroform showed 89.97, 82.91 and 77.68% whereas, ethyl acetate exhibited 89.83, 65.82 and 23.59% free radical effects as compared to control. Gas chromatography mass spectrometry (GCMS) analysis showed the highest concentration of mono(2-ethylhexyl) phthalate.

Introduction

Natural compounds from mushrooms have been the most important source of biologically active compounds (Mishra and Tiwari *et al.* 2011, Dimitrijević *et al.* 2017). These compounds are used in different forms and many of these are still unidentified (Matijašević *et al.* 2016). In agriculture, bacterial invasions bring about serious reduction in the quality and yield of crops and incur enormous economic losses (Navarro *et al.* 2018). At present the most reliable method to control these bacterial pathogens is the use of chemical/synthetic bactericides (Waithaka *et al.* 2017). But the repeated use of these synthetic compounds causes number of ill effects e.g., health hazards, environmental pollution and antibacterial resistance (Ali and Nejad 2016, Marie *et al.* 2016, Erjavec *et al.* 2016). So, there is urgent need to seek nature friendly alternatives to these synthetic chemicals and mushrooms are good source of these alternatives (Gargano *et al.* 2017). As an example, antibacterial activity of *A. bisporus* was checked by using acetone, ethanol and water extract against *Escherichia coli* and *Staphylococcus aureus*. Acetone, ethanol and water extracts caused 33.9, 61.9 and 78.6% inhibition in case of *E. coli*, while there was 35.4, 56.6 and 65.4% reduction in case of *S. aureus* (Ndungutse *et al.* 2015). Methanol extracts of wild edible mushrooms, namely *Agaricus arvensis*, *Agaricus campestris*, *Armillaria mellea*, *Fomes fomentarius*, *Coprinus micaceus*, *Coriolus versicolor* and *Lactarius deliciosus* possess antibacterial and antioxidant properties (Dundar *et al.* 2016). Extracts of three mushrooms, *Terfezia boudieri*, *Agaricus brunnescens* and *Lactarius vellereus* were tested for their antibacterial potency against bacteria. The chloroform extract of *T. boudieri* exhibited the strongest minimum inhibitory concentration (MIC 2.4 µg/ml) value against the test microorganism, *Streptococcus pyogenes*. Maximum antimicrobial effects were observed with the acetone extracts of *T. boudieri* and *L. vellereus* (MIC 4.8 µg/ml) against *Bacillus subtilis* (Doğan *et al.* 2013).

Antioxidants are the compounds that protect the cell from destruction caused by free radicals through redox reaction carried out by peroxide or oxygen (Hossain *et al.* 2015). Free radicals destroy the proteins, DNA and sugars present in the plants and antioxidants are the chemicals that

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delay or stop the formation of these radicals by scavenging effect (Hussein *et al.* 2015). Various bioactive substances extracted from edible mushrooms have been used against chronic diseases such as cancer, to reduce the oxidative stress and for enhancing the immune function (Jayasuriya *et al.* 2015, Gargano *et al.* 2017). Mushrooms have phenolic compounds and vitamins which are known to be excellent antioxidants (Muruke 2014). Important iron chelators that act as free radical's scavengers are found in some mushrooms (Pal *et al.* 2010).

Agaricus bisporus (Button mushroom) is a Basidiomycete fungus, native to Europe and America. Many studies show that this mushroom has antibacterial (Dániel *et al.* 2016) and antioxidant activity (Xu *et al.* 2016). As there are numerous investigations regarding isolation of bioactive compounds having antibacterial and antioxidant activity of *A. bisporus* throughout the world but such reports are missing on Pakistani mushroom species. Moreover, previous studies regarding antibacterial and antioxidant activity of *A. bisporus* were mostly conducted on human/animal related pathogens and very few on plant pathogens. So, in the present investigation, antibacterial activity was determined on some notorious plant pathogenic bacteria *viz.* *Pseudomonas syringae*, *Ralstonia solanacearum*, *Xanthomonas axonopodis* and *Erwinia carotovora*. These bacteria cause a number of serious plant diseases e.g., canker, necrotic spots, bacterial wilt, rot diseases and lesions in number of crop plants e.g., tomato, pepper, egg plant and citrus. These bacteria have been difficult to control by synthetic pesticides (Doolotkeldieva *et al.* 2016). So, the present study was undertaken to evaluate bioactivities of constituents of *A. bisporus* partitioned through organic solvents like *n*-hexane, chloroform and ethyl acetate. This study would help to recognize natural eco-friendly bioactive constituents in *A. bisporus*.

Materials and Methods

Isolates of bacterial species with accession number *viz.* *Pseudomonas syringae* (FCBP-PB-0405), *Ralstonia solanacearum* (FCBP-PB-0407), *Xanthomonas axonopodis* (FCBP-PB-0001) and *Erwinia carotovora* (FCBP-PB-0421) were purchased from culture bank of University of the Punjab, Lahore, Pakistan. These bacterial cultures were subcultured on Lysogeny broth (LB) medium for mass culturing and stored in a refrigerator at 4°C for further use.

A. bisporus mushroom, (white, cultivated), was purchased from Margalla Mushroom Industries, Islamabad, Pakistan. The mushroom was sun dried for 7 days. The dried mushroom was ground to powder in pestle and mortar. This dried mushroom powder (220 g) was first soaked into 1.5 litre methanol (BDH) in a glass jar for 7 days and after that the mixture was filtered by using Whatman no.1 filter paper and evaporated by using rotary evaporator (Model: Heidolph Laborata 4000 efficient) at 45°C. This methanolic extract was further reconstituted with distilled water dH₂O and then partitioned with different organic solvents *viz.* *n*-hexane, chloroform and ethyl acetate, in order of their increasing polarity in separating funnels to obtain the *n*-hexane, chloroform and ethyl acetate extracts. Resulting extracts were stored at 4°C for further use.

For the determination of antibacterial activity of different solvent extracts, bioassays were performed by using disk diffusion method as described by (Shameem *et al.* 2017) with some modifications. All bacterial species were grown on Lysogeny broth (LB) medium and then 6 mm disk of filter paper was cut out with the help of paper punch. After that, this filter paper was soaked into different concentrations of various solvent extracts of *A. bisporus* and placed onto the streaked test bacterial cultures and incubated at 30°C. All treatments were replicated thrice. Antibacterial activity was measured with the help of scale after 72 hrs in terms of zone of inhibition (IZD).

For the determination of antioxidant activity of test mushroom, *A. bisporus*, concentrated extracts in *n*-hexane, chloroform and ethyl acetate were used as described by Rashid *et al.* (2017),

with little modifications. All partitioned extracts @ 10, 20 and 30 µg/ml were dissolved in 100% methanol and raised the final volume up to 4.0 ml. After that 1 ml of 0.2 mM 2,2-diphenyl-1-picrylhydrazyl (DPPH) solution was added into these solutions and shaken the solution vigorously. The solutions were kept at dark for 15 min and after that absorbance was measured at 517 nm by using spectrophotometer (Model: UV3000 Spectrophotometer). DPPH radical scavenging activity was calculated by using the formula;

$$\text{Inhibition (\%)} = [(Control\ absorbance) - (Sample\ absorbance)/(control\ absorbance)] \times 100$$

The biochemical constituents of organic solvent extracts viz. *n*-hexane, chloroform and ethyl acetate of *A. bisporus* showing higher potency were analyzed by using GCMS.

For statistical analysis, ANOVA was performed followed by DMRT to delineate the treatment means.

Results and Discussion

Agaricus bisporus mushroom samples collected from Pakistan were investigated for its potency regarding antibacterial activity against four notorious plant pathogens as well as its antioxidant 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging potential. For this purpose, mushroom extracts were prepared in methanol and then partitioned with other organic solvents viz. *n*-hexane, chloroform and ethyl acetate. The potency of these organic solvent extracts was investigated using different concentrations, 50 and 100 mg/ml. DPPH was recorded in terms of optical density (OD) of 10, 20 and 30 µg/ml extracts solutions as compared with control. The most active fraction was analysed by GCMS analysis.

Data about the antibacterial effect of *n*-hexane, chloroform and ethyl acetate extracts are shown in Fig. 1 (A, B, C and D). Different concentrations of *n*-hexane, chloroform and ethyl acetate extracts ranging from 50 to 100 mg/ml showed antibacterial activity and exhibited significant effect over control. Antibacterial activity was evaluated in terms of measuring zone of inhibition caused by known quantity as well as concentration of particular organic solvent extract e.g., *n*-hexane, chloroform and ethyl acetate organic solvent extracts. *n*-hexane extract caused 2.17, 1, 10.67 and 1 mm inhibition zone at 50 mg/ml concentration while, at 100 mg/ml concentration it exhibited 4.83, 3.33, 16 and 3.33 mm inhibition zone diameter (IZD) against four test plant pathogenic bacteria viz. *Pseudomonas syringae*, *Ralstonia solanacearum*, *Xanthomonas axonopodis* and *Erwinia carotovora*, respectively. At 50 mg/ml concentration, chloroform extract showed 2.33, 11.67, 14 and 1.17 while at 100 mg/ml it showed 6.33, 16.17, 17 and 1.67 mm (IZD). Ethyl acetate extract exhibited 15, 7.83, 15.17 and 16.17 at 50 mg/ml and at 100 mg/ml it revealed pronounced inhibitory effects causing 30, 20, 19 and 28.67 mm (IZD) against four test plant pathogenic bacteria, *P. syringae*, *R. solanacearum*, *X. axonopodis* and *E. carotovora*, respectively. The present results showed resemblances with the reports of earlier workers (Kang *et al.* 2015, Soltanian *et al.* 2016, Waithaka *et al.* 2017) on *A. bisporus* during their research and exhibited 4 mm IZD against *R. solanacearum*. Ashok *et al.* (2013) also reported antibacterial activity that showed conformity with the present results, where petroleum ether, chloroform and methanolic extract of mushroom *Hygro cybeparvula* showed 4 mm inhibition zone against *P. syringae*. Recently, Dimitrijević *et al.* (2017) investigated the antibacterial activity of six mushroom species from Serbia. Out of these six mushrooms only four mushrooms, namely *Boletus fechtneri*, *Boletus rhodoxanthus*, *Boletus appendiculatus* and *Xerocomus badius* exhibited antibacterial activity against *Pseudomonas aeruginosa*, a plant and human pathogen. In this case, disc diffusion bioassays were conducted by methanolic extracts of mushrooms but these were able to induce 14, 12, 14 and 11 mm inhibition zone against *E. coli*. The inhibition zone observed

during the present studies are more pronounced as compared to earlier research work performed by other workers throughout the world. Moreover, *A. bisporus* extracts partitioned by different organic solvents used in the present study and tested against a number of plant pathogenic bacteria have not been reported earlier. As different solvent extracts used in the present investigation exhibited very pronounced inhibitory effects against a number of plant pathogenic bacteria as compared to previous studies, this difference in more promising results can be attributed to more quantity of antibacterial compounds present in mushroom sample grown and collected from Pakistan as compared to Agaricus mushroom samples used in previous studies in other parts of the world. This argument is further strengthened by the fact that habitat where mushrooms and plants are being grown greatly affects the metabolism of plants/mushrooms thereby developing different metabolites both in terms of quantity and quality (Tsongai *et al.* 2016). This change in metabolite profile leads to differential bioactivities that can be harnessed on industrial scale for which mushrooms samples having pronounced bioactivities like used in this study are better options in future.

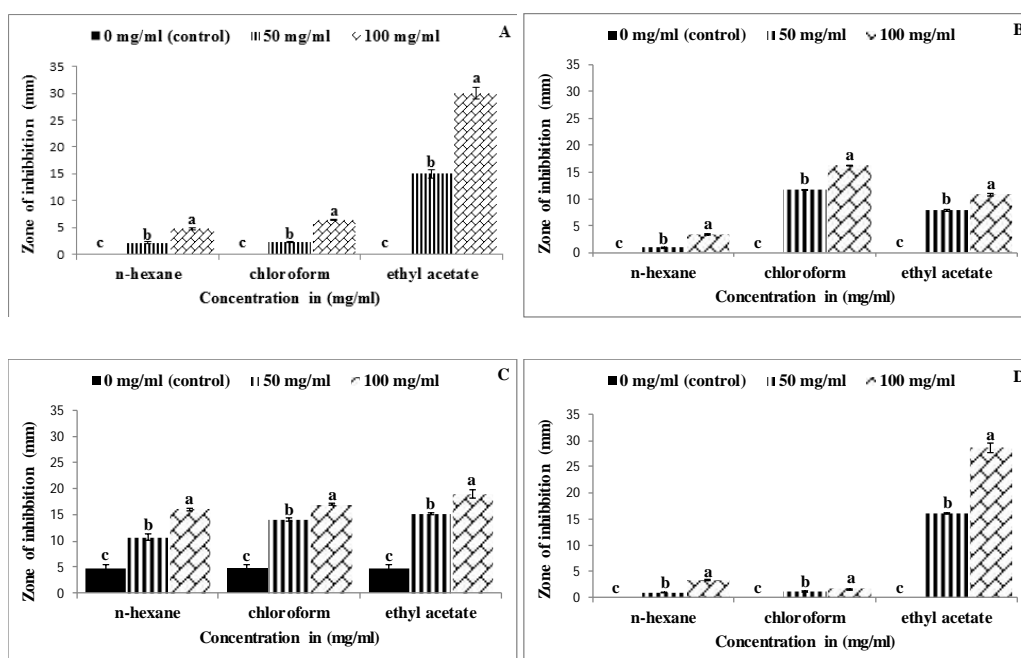


Fig. 1. Effect of different concentrations of *n*-hexane, chloroform and ethyl acetate extract of *Agaricus bisporus* on growth of (A) *Ralstonia solanacearum*, (B) *Pseudomonas syringae*, (C) *Erwinia carotovora* and (D) *Xanthomonas axonopodis*.

Values with different letters at their top show a significant difference ($P \leq 0.05$), as determined by DMRT. Vertical bars show standard errors of means of three replicates.

To record the antioxidant activity of *n*-hexane, chloroform and ethyl acetate extracts of *A. bisporus*, optical density (OD) was taken at 517 nm. *n*-hexane extract showed 60.73, 47.6 and 33.05%, chloroform depicted 89.97, 82.91 and 77.68% whereas ethyl acetate exhibited 89.83, 65.82 and 23.59% free radical effects at 10, 20 and 30 $\mu\text{g/ml}$ as compared to control (Fig. 2A and B). In case of antioxidant bioassays, similar results were reported in methanolic and aqueous extract of dry fruiting bodies of *A. bisporus* collected from Kodaikanal, India that resulted 88.2,

87.5 and 67.57% free radical scavenging activity against control DPPH (Razia *et al.* 2016). In another investigation, *A. bisporus* (brown, cultivated) water extract showed 88.64% DPPH radical-scavenging activity (Buruleanu *et al.* 2017). The presence of antioxidant activity in a number of mushrooms belonging to different species having variable activity have also been reported (Magdziak *et al.* 2017).

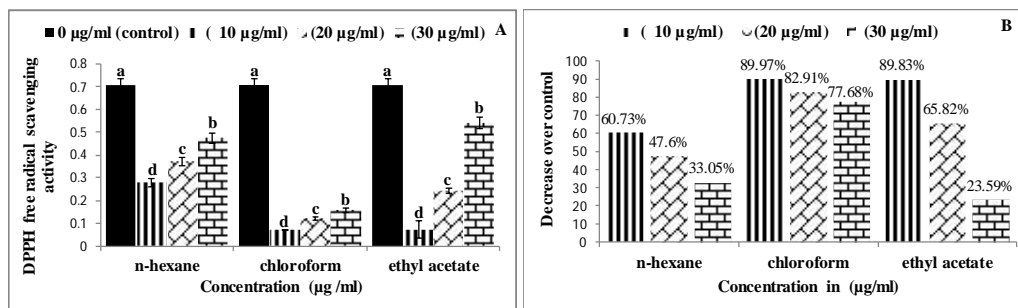


Fig. 2. (A) Antioxidant (DPPH free radical scavenging) activity of n-hexane, chloroform and ethyl acetate extract of *Agaricus bisporus*, (B) Percentage reduction in DPPH free radical scavenging activity due to different concentrations of n-hexane extract over control.

Values with different letters at their top show a significant difference ($P \leq 0.05$), as determined by DMRT. Vertical bars show standard errors of means of three replicates.

In the present study, GCMS analysis of ethyl acetate fraction of *A. bisporus* showed the presence of one compound, mono(2-ethylhexyl) phthalate which showed the highest concentration. Alshammaa (2017) reported similar compound during their research work. Phthalates are naturally occurring bioactive constituents. These have diverse properties from being used as curative drugs, antimicrobial (Ramasany *et al.* 2010) and anticancer (Rahdary and Sobati 2012), e.g., Diisobutyl phthalate, Dibutyl phthalate and 1,2-benzene-dicarboxylic acid, mono(2-ethylhexyl) phthalate. It was concluded that this compound being in highest concentration in the ethyl acetate fraction of *A. bisporus* mushroom is responsible for different bioactivities like antibacterial and antioxidant activity, recorded in the present study. The present study concludes that *A. bisporus* collected from Pakistan has better antibacterial and antioxidant potency that can be exploited as nature friendly alternatives to commercial/synthetic compounds.

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((Manuscript received on 5 February, 2018; revised on 26 July, 2018))