# IDENTIFICATION OF NATURAL ANTIFUNGAL CONSTITUENTS FROM *AGARICUS BISPORUS* (J. E. LANGE) IMBACH

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**Abstract.** In agriculture, fungal invasions cause heavy loss and affect food security and food safety. Chemical pesticides are available to control these pathogens but these are associated with environmental and public health concerns. In recent years, there is a growing trend towards nature friendly methods of pathogen control. Use of allelochemicals from mushrooms is a promising field. In the present study, antifungal activity of an edible mushroom, *Agaricus bisporus* was investigated. For this purpose, different organic solvent extracts of *A. bisporus*, were evaluated against plant pathogenic fungal species viz. *Macrophomina phaseolina, Aspergillus flavus, Fusarium oxysporum, Drechslera australiensis* and *Alternaria alternata.* To check the antifungal activity of *A. bisporus,* serial dilution method was used. Six levels of treatments of each *n*-hexane, chloroform and ethyl acetate (0, 5, 10, 15, 20 and 25 mg/ml) were applied against all fungal species. Generally, all organic solvent extracts reduced the fungal biomass significantly with the increase in concentration but, ethyl acetate fraction exhibited better results and reduced the fungal growth in some species up to 50% in *D. australiensis* and 44% each in *A. alternata* and *F. oxysporum.* From Gas Chromatography Mass Spectrometry (GCMS) analysis of ethyl acetate fraction, 10 compounds were identified. Out of these 10 compounds, only one compound [1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester], molecular formula = $C_{16}H_{22}O_4$ and molecular weight = 278, showed 91.31% peak value. It was concluded that this compound being in the highest concentration in the ethyl acetate fraction of *A. bisporus* mushroom was responsible for its antifungal activity, recorded in the present study. The present investigation concluded that *A. bisporus* has bioactive compounds that can be exploited to develop ecofriendly fungicides against a number of plant pathogens.
**Keywords:** *button mushroom, bioactive compounds, allelochemicals, GCMS*

## Introduction

Bioactive compounds from mushrooms have been the most important source of biologically active compounds (Mishra and Tiwari, 2011; Kothari, et al., 2018). Mushrooms are recognized as important source of pharmacologically active compounds (Dimitrijević et al., 2017). These biologically active compounds are used in different forms like oils (Mehrparvar et al., 2016) and remedies and many of these biologically natural active compounds are still unidentified (Matijaševic et al., 2016). Moreover, in agriculture, fungal invasions bring about serious reduction in the quality and yield of crops and incur enormous economic losses. At present the most reliable method to control these fungal pathogens is the use of chemical/synthetic fungicides. But the repeated use of these synthetic compounds causes number of ill effects, e.g. health


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hazards, environmental pollution and antifungal resistance (Revie et al., 2018). So, there is dire need to seek nature friendly alternatives to these synthetic chemicals and mushrooms are good source of these alternatives (Gargano et al., 2017).

In several cases, mushroom bioactive compounds show very high activity than the synthetic antifungals frequently used now-a-days (Heleno et al., 2013; Thatoi et al., 2018). Similarly, *Pleurotus ostreatus* and *Lentinula edodes* extracts exhibit antifungal activity against plant pathogenic fungal species viz. *Fusarium oxysporum.*

*Agaricus bisporus* (Button mushroom) is a Basidiomycete fungus, native to Europe and America. This mushroom is the most cultivated mushroom of the world (Maa et al., 2018). At present it accounts for 35-45% of throughout world production of edible mushrooms (Masoumi et al., 2015). Moreover, studies show that this mushroom has antifungal properties (Hamid and Rezaeian, 2016). In an investigation, methanolic extract of *A. bisporus* showed 21 mm zone of inhibition against *F. oxysporum* (Waithaka et al., 2017). Öztürk et al. (2011) reported that methanolic extract of three *Agaricus* species viz. *Agaricus essettei, Agaricus bitorquis* and *A. bisporus,* exhibited 76.50%, 77.44%, and 78.72% antifungal activity against *Penicillium verucosum.* As there are numerous investigations regarding isolation of bioactive compounds having antifungal activity of *A. bisporus* as well as other mushrooms throughout world but such reports are missing on Pakistani mushroom species. Moreover, past investigations regarding antifungal activity of *A. bisporus* were mostly conducted on human/animal related pathogens and very few on plant pathogens. So, in the present investigation, antifungal activity was determined on some notorious plant pathogenic fungi viz. *Macrophomina phaseolina, Aspergillus flavus, Fusarium oxysporum, Drechslera australiensis* and *Alternaria alternata*. These fungi cause a number of serious plant diseases, e.g., some of the fungal diseases which largely affect crops are, *A. alternata* that causes Alternaria black spot of rose, *Colletotrichum gloeosporioides* infects loquat and *Fusarium solani* causes strawberry fruit rot (Abbas, et al., 2017; Naz, et al., 2017; Mehmood et al., 2017). *A. flavus* produces maize ear rots and mycotoxin contamination (Shu et al., 2015). *Acacia nilotica* is affected by *D. australiensis* (Ahmad et al., 2017). Fusarium head blight of wheat caused by *Fusarium graminearum* (Li et al., 2015), *M. phaseolina* is a phytopathogen causing stalk rot in soybean crop (Ramos et al., 2016). *M. phaseolina* has been reported to infect about 500 plant species and no commercial fungicide has been recommended so far to combat this fungal pathogen (Javaid et al., 2017). These fungal pathogens have been difficult to control by synthetic fungicides. So, the present study was undertaken to evaluate *in vitro* antifungal activity of constituents of *A. bisporus* partitioned through organic solvents like *n*-hexane, chloroform and ethyl acetate. This study would help to identify natural eco-friendly antifungal compounds in *A. bisporus.*

## Materials and methods

Experiments were performed in Medicinal Flora Evaluation, Characterization and Metabolomics Lab., Department of Botany, University of Gujrat, Gujrat, Pakistan during the year 2017.

### *Culturing of fungal isolates and storage*

Isolates of fungal species with accession number viz. *Macrophomina phaseolina* (FCBP-PTF-1156), *Aspergillus flavus* (FCBP-SF-1261), *Fusarium oxysporum*, (FCBP-


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SF-1175), *Drechslera australiensis* (FCBP-AF-482) and *Alternaria alternata* (FCBP-PTF-1285) were purchased from culture bank of University of the Punjab, Lahore, Pakistan. These fungal cultures were subcultured on Potato Dextrose Agar (PDA) medium for mass culturing and stored in a refrigerator (Haier) at 4 °C for further use.

### *Preparations of mushroom extracts*

*Agaricus 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 L methanol (Company; BDH) in a glass jar for 7 days and after that the mixture was filtered by using Whatman no. 1 and evaporated by using rotary evaporator (Heidolph Laborata 4000 efficient) at 45 °C. This methanolic extract was reconstituted with distilled water ($dH_2O$) 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. Organic solvents were evaporated under vacuum in rotary evaporator as above to obtain organic solvent extracts. Resulting extracts were stored at 4 °C for further use (Akbar et al., 2017).

### *Antifungal assays*

Stock solutions of all partitioned extracts viz. *n*-hexane, chloroform and ethyl acetate were synthesized following procedure as described by Ali et al. (2017) and Azhar et al. (2018), with slight modifications like use of lower concentrations of extracts. 150 mg of mushroom extract in each organic solvent extracted was dissolved into 166 µl of Dimethyl sulfoxide (DMSO) and final volume was raised to 500 µl with the addition of distilled autoclaved water. Control mixture comprised of 166 µl DMSO and its final volume raised to 500 µl by the addition of distilled autoclaved water (333 µl). Potato Dextrose Broth (PDB) was prepared by following standard procedures. Overall six concentrations were investigated as follow; 0, 5, 10, 15, 20 and 25 mg/ml of each extract separately. The experiment was done in glass vials for 72 h at 27 °C. The experiment was performed using Completely Randomised Design (CRD) with three replications. At harvest fungal biomass was measured with the help of digital balance (AY120, Shimadzu Corporation, Japan). Fungal biomass was filtered on preweighed filter papers, dried in an electric oven (UNE-600, Memmert, Germany) at 70 °C till constant weight. After drying, these filter papers were again weighed to calculate fungal biomass.

### *Gas chromatography mass spectrometry (GCMS) analysis*

The biochemical constituents of ethyl acetate extract of *A. viridis* were analysed by using GCMS with model number 7890A/5975C of Agilant Technologies in the Department of Chemistry, Forman Christian College University, Lahore, Pakistan. GCMS instrument was equipped with column # HP-5MS (30 m × 250 µm × 0.25 µm). Helium gas with 99.99% purity was used with constant flow rate at 1 mL $min^{-1}$. Material was employed with injection of 2 µL volume. In injector the temperature was 240 °C. Oven temperature was programmed first from 60 °C for 2 min with increase of 5 °C $min^{-1}$ to 80 °C, then 10 °C $min^{-1}$ to 310 °C for 5 min. Total GC running time was 34 min. Relative quantities of all constituents were computed by using same method as described by Jananie et al. (2012).


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### *Statistical analysis*

For statistical analysis, ANOVA was performed followed by Duncan's Multiple Range Test (DMRT) to analyze the treatment means by using computer software, CosStat.

## Results and discussion

### *Antifungal activity of n-hexane, chloroform and ethyl acetate extract against Macrophomina phaseolina*

Data regarding the effect of *n*-hexane extract of *A. bisporus* on the growth of *M. phaseolina* are shown in *Figure 1A. n*-hexane extract of *A. bisporus* concentrations ranging from 5, 10, 15, 20 and 25 mg/ml showed slight significant reduction in fungal biomass.

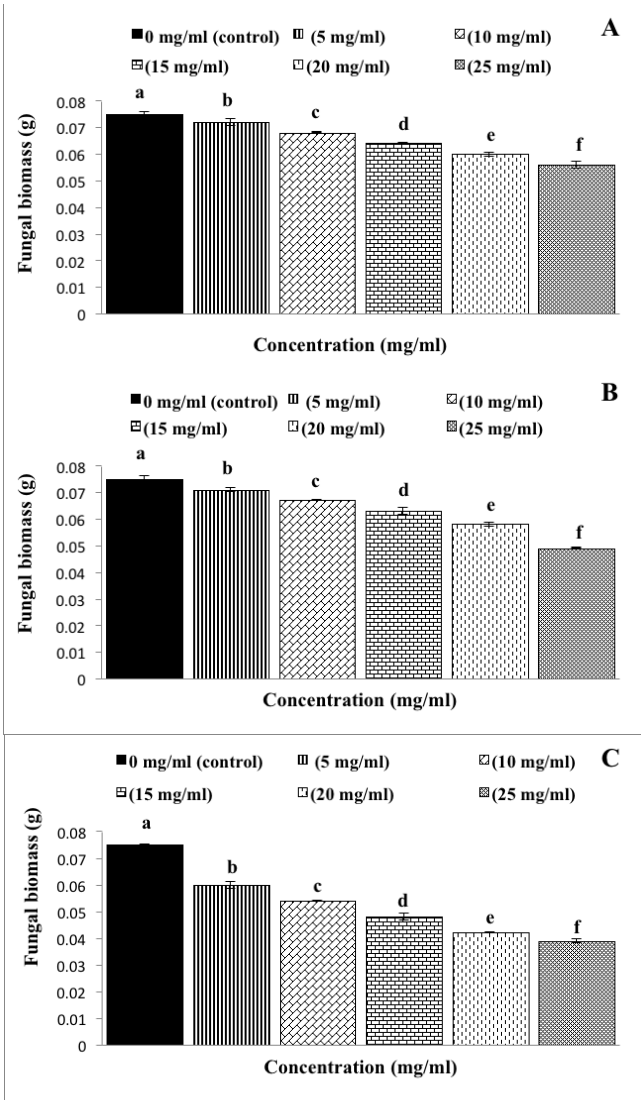


***Figure 1.*** *Effect of different concentrations of (A) n-hexane, (B) Chloroform and (C) ethyl acetate extract of Agaricus bisporus on biomass of Macrophomina phaseolina. Values topped with distinctive letters show a significant variance (P ≤ 0.05), as analyzed by DMRT*


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These extract concentrations caused 6, 9, 14, 20 and 25% reduction in fungal biomass as compared to control (DMSO). Different *n*-hexane extract concentrations ranging from 5 to 25 mg/ml of *A. bisporus* showed slight reduction in fungal biomass from 4 to 25% as compared to control (DMSO). The effect of chloroform extract of *A. bisporus*, on the biomass of plant pathogenic fungi, *M. phaseolina* with same concentrations is shown in *Figure 1B*. According to data, gradual decrease in fungal biomass from 5, 10, 16, 22 and 34% was observed with an increase of extract concentration. The effect of chloroform extract of mushroom *A. bisporus* on the biomass of plant pathogenic fungi, *M. phaseolina* with same concentrations was higher as compared to *n*-hexane extract and there was a gradual decrease in fungal biomass from 5 to 34%. Data about the antifungal activity of ethyl acetate extract of *A. bisporus* on *M. phaseolina* showed pronounced reduction in fungal biomass. In this case, a decline of 10, 19, 28, 37 and 41% was noted as compared to control. Antifungal activity of ethyl acetate extract of *A. bisporus* on *M. phaseolina* showed clear-cut reduction in biomass from 10 to 41% as compared to control. Maximum 41% decrease in biomass was noted against 25 mg/ml concentration. It was seen that ethyl acetate extract showed better response at all levels against the growth of *M. phaseolina* (*Table 1* and *Fig. 1C*). Previously no results were reported against this fungus in literature.

***Table 1.*** *ANOVA of the data for the effect of n-hexane, chloroform and ethyl acetate extract of Agaricus bisporus on biomass of Macrophomina phaseolina*

| Source | df | *n*-hexane | | Chloroform | | Ethyl acetate | |
|---|---|---|---|---|---|---|---|
| | | F | P | F | P | F | P |
| Main effects | | | | | | | |
| *Fusarium oxysporum* | 5 | 181.71 | .00 *** | 801.77 | .00 *** | 495.63 | .00 *** |
| Error | 12 | | | | | | |
| Total | 17 | | | | | | |

### *Antifungal activity of n-hexane, chloroform and ethyl acetate extract against Aspergillus flavus*

Data in *Figure 2A* show that different concentrations of *n*-hexane extract did not show potent antifungal effects on *A. flavus*. Different concentrations of this extract exhibited different levels of inhibition in terms of reduction in fungal biomass. Maximum 18% inhibition was measured at 25 mg/ml concentration as compared to control. While, in other concentrations, the fungal biomass reduced in a very low quantity. Whereas, data about the results of chloroform extract of *A. bisporus* against plant pathogenic fungus, *A. flavus* are shown in *Figure 2B*. The fungal biomass was decreased from 3 to 21% in comparison with control. Very low decrease was seen at 5 mg/ml concentration but this activity further increases with the uplift level of extract concentration. Effect of ethyl acetate extract on *A. flavus* showed significant results as shown in *Figure 2C*. In this case different concentrations of ethyl acetate extract caused 13, 18, 23, 32 and 42% decline in fungal biomass at 5, 10, 15, 20 and 25 mg/ml ethyl acetate extract concentration (*Table 2*). These results are in the agreement with findings of previous workers (Dutta et al., 2013; Kumar and Yadav, 2014) that reported 77.12% antifungal activity against *A. flavus* when extract of *A. bisporus* was investigated in diffusion disk method.


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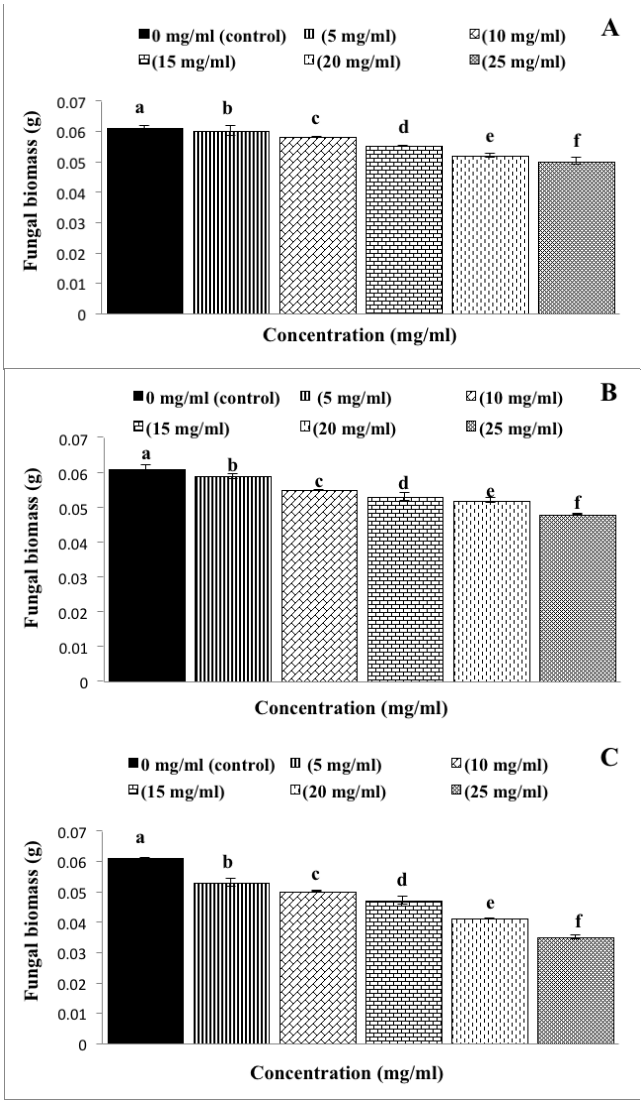


***Figure 2.*** *Effect of different concentrations of (A) n-hexane, (B) Chloroform and (C) ethyl acetate extract of Agaricus bisporus on biomass of Aspergillus flavus. Values topped with distinctive letters show a significant variance (P ≤ 0.05), as analyzed by DMRT*

***Table 2.*** *ANOVA of the data for the effect of n-hexane, chloroform and ethyl acetate extract of Agaricus bisporus on biomass of Aspergillus flavus*

| Source | df | *n*-hexane | | Chloroform | | Ethyl acetate | |
|---|---|---|---|---|---|---|---|
| | | F | P | F | P | F | P |
| Main effects | | | | | | | |
| *Fusarium oxysporum* | 5 | 100.02 | .00 *** | 166.62 | .00 *** | 800.7 | .00 *** |
| Error | 12 | | | | | | |
| Total | 17 | | | | | | |

In past investigation, methanolic extract of mushroom, *Meripilus giganteus* also exhibited antifungal activity against some plant pathogenic fungal species viz. *Aspergillus versicolor*, *Aspergillus niger*, *Aspergillus ochraceus*, *Penicillium*


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*funiculosum, Aspergillus fumigatus, Trichoderma viride* and *Penicillium ochrochloron*, keeping bifonazole and Ketoconazole as control. The extract showed only 10% inhibition in case of *A. ochraceus* as compared to bifonazole while, only 1% minimum inhibitory concentration (MIC) as compared to ketoconazole. Mushroom *M. giganteus* showed very poor antifungal activity against all other fungal pathogens (Stojkovi et al., 2017).

### *Antifungal activity of n-hexane, chloroform and ethyl acetate extract against Fusarium oxysporum*

*n*-hexane extract of test mushroom did not reduce the fungal biomass to great extent. *n*-hexane extract of *A. bisporus* showed only 3, 5, 9, 13 and 17% reduction in fungal biomass in contrast to control (*Fig. 3A*). The chloroform extract of *A. bisporus* depicted significant reduction on biomass *F. oxysporum.* Different concentrations of test extract incurred 3, 7, 11, 18 and 24% decline in fungal biomass as compared to control (*Fig. 3B*).

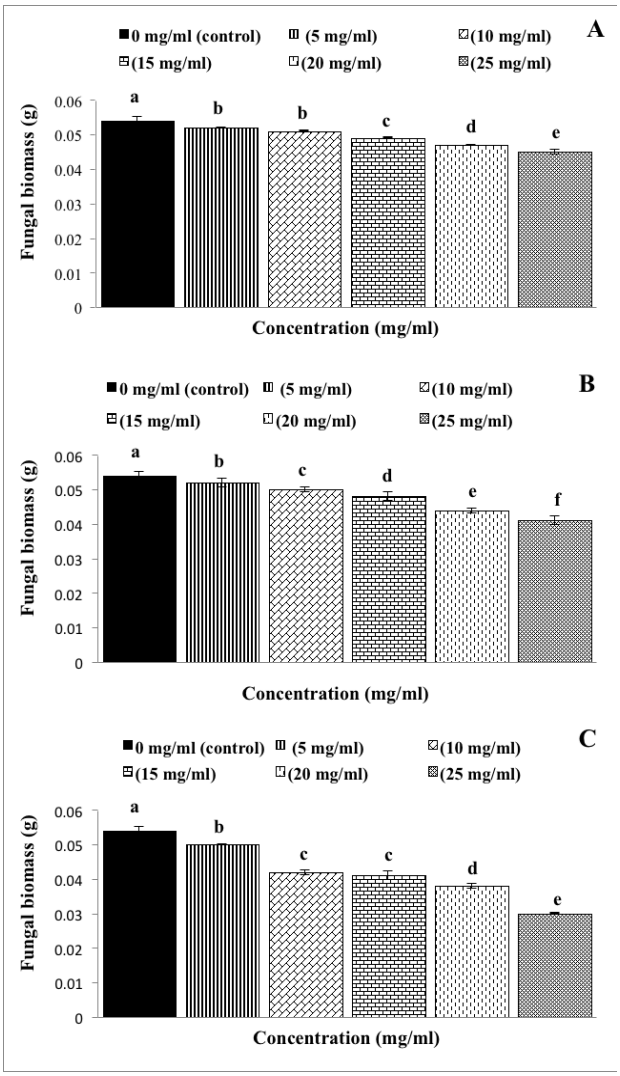


***Figure 3.*** *Effect of different concentrations of (A) n-hexane, (B) Chloroform and (C) ethyl acetate extract of Agaricus bisporus on biomass of Fusarium oxysporum. Values topped with distinctive letters show a significant variance (P ≤ 0.05), as analyzed by DMRT*


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On the other hand ethyl acetate extract of *A. bisporus* revealed relatively higher significant reduction in fungal biomass as compared to control. Different employed concentrations caused 7, 22, 24, 29 and 44% decrease in test fungal biomass when compared with control (*Table 3* and *Fig. 3C*). In this experiment, ethyl acetate showed 44%, chloroform 24% and *n*-hexane 17% reduction in biomass at 25 mg/ml concentration and similar results were reported by Jin and Huang (2011) and Waithaka et al. (2017) in *A. bisporus* that showed 37% antifungal activity against *F. oxysporum*. This enhanced activity of extracts used in the present study might be due the presence of different chemical constituents in the mushroom sample used herein. As the environment plays a major role in the abundance of different metabolites in a particular species, it may be envisaged that the pronounced bioactivity observed in *A. bisporus* grown in Pakistan may have different metabolite profile.

***Table 3.*** *ANOVA of the data for the effect of n-hexane, chloroform and ethyl acetate extract of Agaricus bisporus on biomass of Fusarium oxysporum*

| Source | df | *n*-hexane | | Chloroform | | Ethyl acetate | |
|---|---|---|---|---|---|---|---|
| | | F | P | F | P | F | P |
| Main effects | | | | | | | |
| *Fusarium oxysporum* | 5 | 67.09 | .00 *** | 152.4 | .00 *** | 143.61 | .00 *** |
| Error | 12 | | | | | | |
| Total | 17 | | | | | | |

## *Antifungal activity of n-hexane, chloroform and ethyl acetate extract against Drechslera australiensis*

*n*-hexane extract of *A. bisporus* caused a decline of 2, 6, 13, 19 and 25% at different concentrations viz. 5, 10, 15, 20 and 25 mg/ml *n*-hexane extract concentrations, respectively (*Fig. 4A*). Whereas, extract in chloroform showed 4, 8, 13, 21 and 29% biomass reduction against fungus, *D. australiensis* at these corresponding extract concentrations (*Fig. 4B*). On the other hand, better antifungal activity was recorded in case of ethyl acetate extract at diverse concentrations ranging from 5 to 25 mg/ml. There was significant reduction of 6, 12, 21, 33 and 50% at 5, 10, 15, 20 and 25 mg/ml ethyl acetate extract concentrations, respectively (*Fig. 4C*). Though all treatments in *n*-hexane, chloroform and ethyl acetate showed antifungal effect but ethyl acetate showed better potency as compared to other two extracts at all levels of treatments. Before this, in literature no bioactivity was reported against *D. australiensis* in terms of fungal metabolites (*Table 4* and *Fig. 4A, B, C*).

***Table 4.*** *ANOVA of the data for the effect of n-hexane, chloroform and ethyl acetate extract of Agaricus bisporus on biomass of Drechslera australiensis*

| Source | df | n-hexane | | Chloroform | | Ethyl acetate | |
|---|---|---|---|---|---|---|---|
| | | F | P | F | P | F | P |
| Main effects | | | | | | | |
| *Fusarium oxysporum* | 5 | 46.64 | .00 *** | 67.58 | .00 *** | 157.98 | .00 *** |
| Error | 12 | | | | | | |
| Total | 17 | | | | | | |


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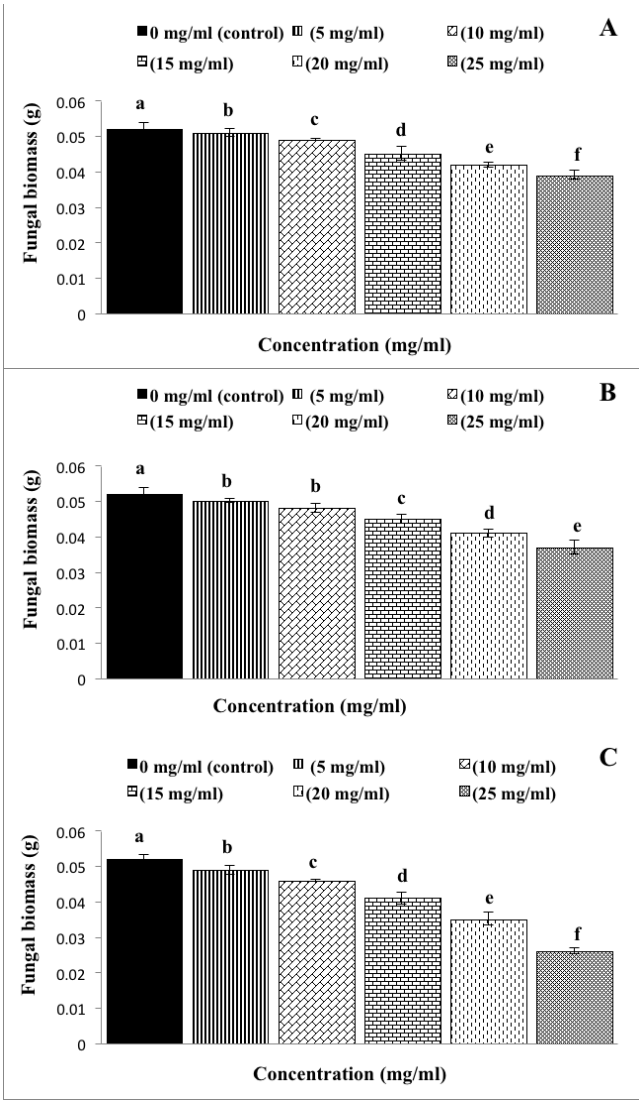


***Figure 4.*** *Effect of different concentrations of (A) n-hexane, (B) Chloroform and (C) ethyl acetate extract of Agaricus bisporus on biomass of Drechslera australiensis. Values topped with distinctive letters show a significant variance (P ≤ 0.05), as analyzed by DMRT*

## *Antifungal activity of n-hexane, chloroform and ethyl acetate extract against Alternaria alternata*

*n*-hexane extract of test mushroom did not show significant results in response to fungal biomass at all concentrations of test extract as shown in *Figure 5A.* There was non-significant reduction in fungal biomass at 5 and 10 mg/ml extract concentration. While at higher concentrations of 15, 20 and 25 mg/ml, there was 9, 14 and 20% significant reduction in biomass of *A. alternata* as compared to control (*Fig. 5A*). In case of chloroform extract of *A. bisporus* at different concentrations of 5, 10, 15, 20 and 25 mg/ml, there was a general trend of significant reduction of fungal growth at all employed concentrations of test extract except at the lowest employed concentration of extract at 5 mg/ml, that yielded non significant effect in terms of fungal growth inhibition. At higher concentrations of 10, 15, 20 and 25 mg/ml, there was a significant inhibition of 11, 18, 24 and 33% (*Fig. 5B*).


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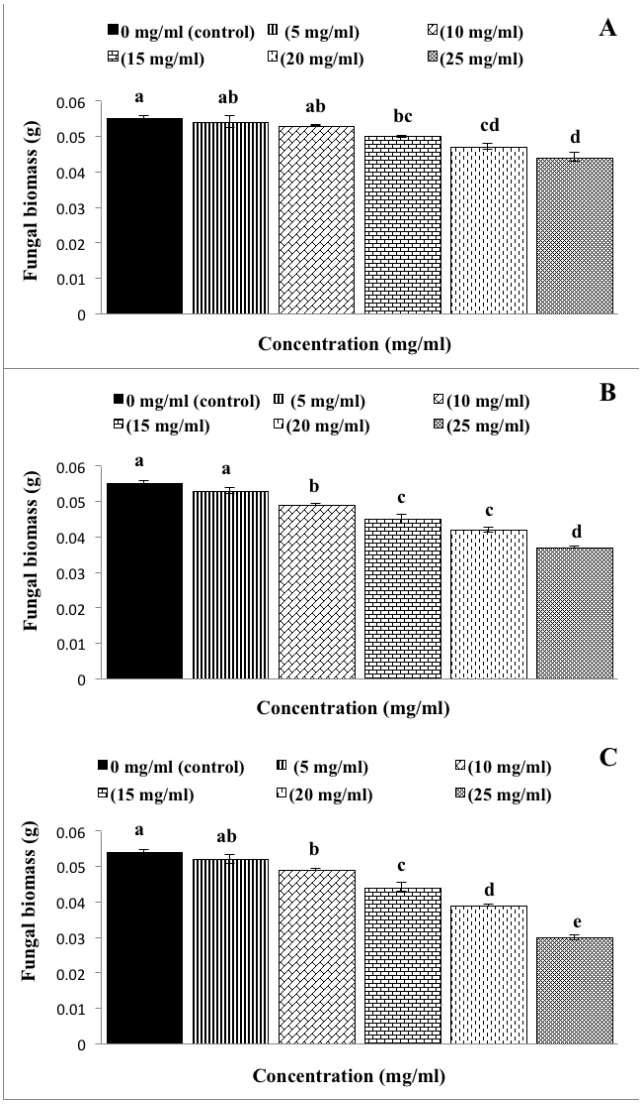


***Figure 5.*** *Effect of different concentrations of (A) n-hexane, (B) Chloroform and (C) ethyl acetate extract of Agaricus bisporus on biomass of Alternaria alternata. Values topped with distinctive letters show a significant variance (P ≤ 0.05), as analyzed by DMRT*

Data regarding antifungal activity of ethyl acetate extract against concentrations from 5 mg to 25 mg/ml are shown in *Figure 5C*. A clear reduction in *Alternaria* biomass was seen. Ethyl acetate showed better response as compared to *n*-hexane and chloroform extract at 25 mg/ml concentration and reduced the fungal biomass up to 44% as compared to control. Bioactivity of ethyl acetate extract against concentrations ranging from 5 mg to 25 mg/ml was more pronounced as compared to *n*-hexane and chloroform extracts. A clear reduction in *Alternaria* biomass was seen in both cases. Ethyl acetate showed better response as compared to *n*-hexane and chloroform extract at 25 mg/ml concentration and reduced the fungal biomass up to 44% as compared to control (*Table 5*). The bioactivity recorded in the present investigation was promising as results are better and consistent with the findings of Senka et al. (2011) on *A. Alternata* where 41.12% inhibitory activity was reported. Other species of Agaricus, e.g. *Agaricus brunnescens* are also known to possess antifungal activity. Chloroform, acetone and


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methanol extracts of *A. brunnescens* exhibited antifungal activity against *Candida albicans* at MIC 39, 19, 39 mg/mL, respectively (Doğan et al., 2013).

***Table 5.*** *ANOVA of the data for the effect of n-hexane, chloroform and ethyl acetate extract of Agaricus bisporus on biomass of Alternaria alternata*

| Source | df | *n*-hexane | | Chloroform | | Ethyl acetate | |
|---|---|---|---|---|---|---|---|
| | | F | P | F | P | F | P |
| Main effects | | | | | | | |
| *Fusarium oxysporum* | 5 | 11.52 | .00 *** | 39.48 | .00 *** | 89.22 | .00 *** |
| Error | 12 | | | | | | |
| Total | 17 | | | | | | |

### *Gas chromatography mass spectrometry analysis (GCMS)*

GCMS analysis demonstrated total 10 compounds were present in the ethyl acetate fraction of *A. bisporus* mushroom that is shown in *Figures 6* and *7*. The Retention time (RT), concentration of component (%) Molecular weight and their molecular formulas are presented in *Table 6*.

***Table 6.*** *Compounds identified in gas chromatography mass spectrometry analysis*

| Sr. # | R. T. (min) | Name of compound | Molecular formula | Molecular weight | Peak area % |
|---|---|---|---|---|---|
| 1 | 16.239 | Benzene, (1-butylheptyl)- | $C_{17}H_{28}$ | 232 | 0.832 |
| 2 | 17.338 | Benzene, (1-butyloctyl)- | $C_{18}H_{30}$ | 246 | 0.850 |
| 3 | 17.481 | Benzene, (1-propylnonyl)- | $C_{18}H_{30}$ | 246 | 0.554 |
| 4 | 18.323 | Benzene, (1-pentyloctyl)- | $C_{19}H_{32}$ | 260 | 0.689 |
| 5 | 18.397 | Benzene, (1-butylnonyl)- | $C_{19}H_{32}$ | 260 | 0.595 |
| 6 | 19.348 | Pentadecanoic acid, 14-methyl-, methyl ester | $C_{17}H_{34}O_2$ | 270 | 1.0211 |
| 7 | 19.932 | 1-Hexadecanol, 2-methyl- | $C_{17}H_{36}O$ | 256 | 0.522 |
| 8 | 20.957 | 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester | $C_{19}H_{34}O_2$ | 294 | 2.864 |
| **9** | **24.799** | **1,2- Benzenedicarboxylic acid, mono (2-ethylhexyl) ester** | **$C_{16}H_{22}O_4$** | **278** | **91.310** |
| 10 | 27.874 | Anthiaergostan-5,7,9,16,22-penten | $C_{28}H_{40}$ | 376 | 0.761 |

R. T. = retention time; min = minutes
Note: Compound highlighted in boldface was present in the highest concentration

Following compounds were identified in the ethyl acetate fraction of *A. bisporus*. viz. Benzene, (1-butylheptyl)- (0.832%), Benzene, (1-butyloctyl)- (0.850%), Benzene, (1-propylnonyl)- (0.554%), Benzene, (1-pentyloctyl)- (0.689%), Benzene, (1-butylnonyl)- (0.595%), Pentadecanoic acid, 14-methyl-, methyl ester (1.0211%), 1-Hexadecanol, 2-methyl- (0.522%), 9, 12-Octadecadienoic acid (Z, Z)-, methyl ester (2.864%), 1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester (91.310%), Anthiaergostan-


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5,7,9,16,22-penten (0.761%). Out of these 10 compounds, only one compound (1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester) with molecular weight (278) showed 91.31% peak value. Out of these 10 compounds, only one compound (1,2-Benzenedicarboxylic acid, mono (2-ethylhexyl) ester) with molecular weight (278) showed 91.31% peak value (*Figs. 6* and *7*). Alshammaa (2017) reported similar compound during their research work. It was concluded that this compound being in highest concentration in the ethyl acetate fraction of *A. bisporus* mushroom is responsible for antifungal activity, recorded in the present study. The present study concludes that *A. bisporus* collected from Pakistan has better antifungal potency that can be exploited as nature friendly alternatives to commercial/synthetic compounds.

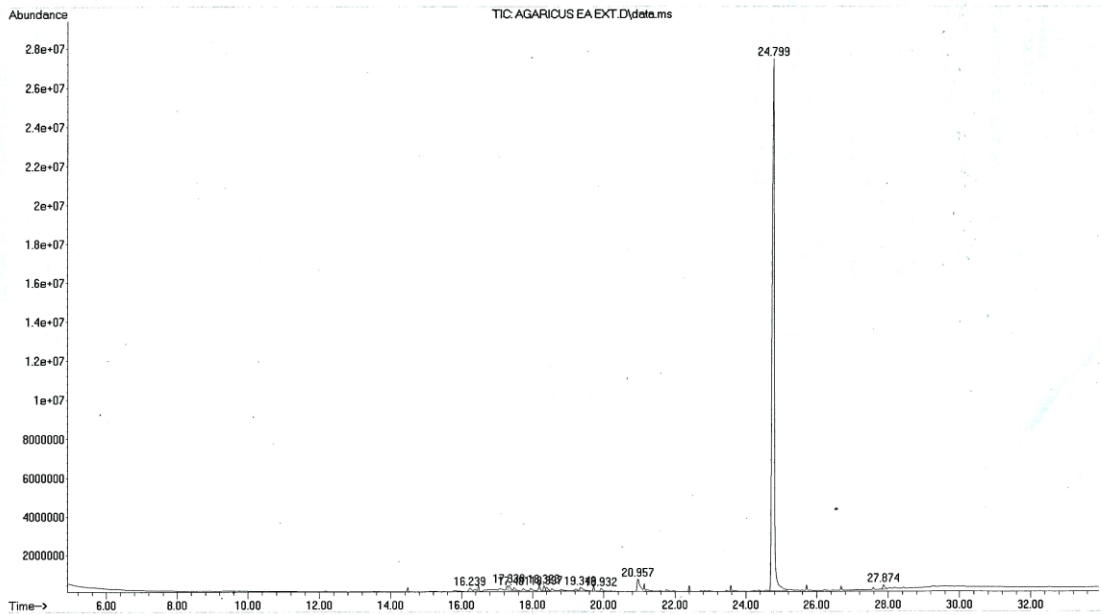


***Figure 6****. GCMS analysis of ethyl acetate fraction of Agaricus bisporus*

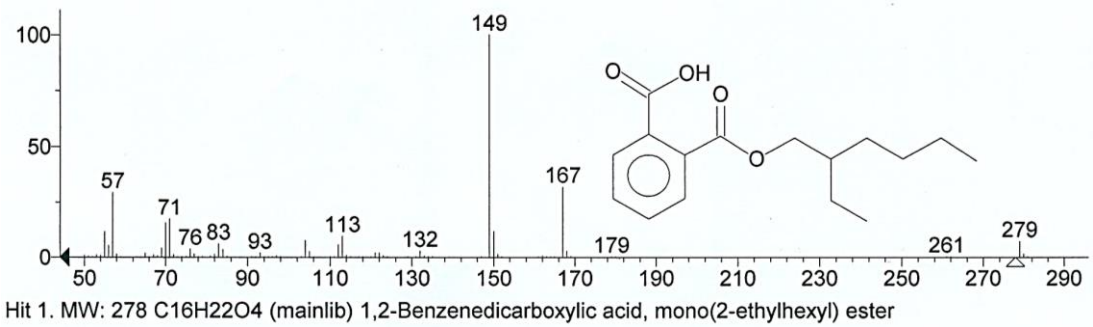


***Figure 7.*** *GCMS spectrum and structure of 1,2- Benzenedicarboxylic acid, mono (2-ethylhexyl) ester*

## Conclusions

The present study concluded better antifungal activity of ethyl acetate extract of *A. bisporus* collected from Pakistan. GCMS analysis of this fraction revealed the presence of a compound named as 1,2- Benzenedicarboxylic acid, mono (2-ethylhexyl) ester


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having the highest concentration. It may be concluded that this compound was responsible for antifungal activity against a number of phyto-pathogenic fungi. The most susceptible fungal species was *D. australiensis* where ethyl acetate extract caused 50% reduction in growth. The structure of this compound can be used as structural lead for the synthesis of bioactive antifungal compounds by pesticide/biotechnological companies.

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