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ANTIMICROBIAL POTENTIAL OF DIFFERENT SOLVENT EXTRACTS OF *MORCHELLA ESCULENTA* (L.) PERS.

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Keywords: Extracts, Disc diffusion, Antimicrobial activity, Extraction solvent

Abstract

The antimicrobial activity of different extracts (methanolic, ethyl acetate, hot and cold water extracts) of *Morchella esculenta* against bacterial strains including *Agrobacterium tumefaciens*, *Bacillus atrophaeus, Bacillus subtilis, Citrobactor freundii, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Staphyllococcus aureus, Staphyllococcus epidermidis, Pseudomonas aeruginosa* and *Xanthomonas oryzae* and some fungal strains such as *Trychophyton rubrum, Rhizopus stolonifer,Trichoderma citrinoviride, Aspergillus fumigatus* and *Alternaria alternata* was evaluated. Disc diffusion assay was used for antibacterial activity and well diffusion method was applied for measuring the antifungal activities of different extracts. Hot water extract was found more effective against bacterial strains and produced maximum zone of inhibition of 90% against *Bacillus atrophaeus* at 3 mg/disc, while cold water extract showed strong activity against the majority of selected fungal strains; *Trichophyton rubrum, Trichoderma citrinoviride* and *Alternaria alternata*. Ethyl acetate extract of *M. esculenta* revealed least antimicrobial activities against the tested microbes.

Introduction

Scarcity of new antimicrobial and increasing antibiotic resistance are major risks to global health care (Walsh and Toleman 2012). Development of antimicrobial resistance in both humans and animals is due to number of factors which include excessive and inappropriate use of antibiotics, high application of these agents as growth enhancers in animal feed (Lowy 2003, Andersson and Hughes 2011). In this situation, the development of novel and alternative classes of drugs with least side effect to treat microbial infection is urgently needed (Srivastava *et al.* 2013).

M. esculenta is also one of the medicinal mushroom having high medicinal value, and commercially available in Pansara markets. In some research reports, *M.esculenta* has shown antioxidant, antiallergenic, antitumor, anti-inflammatory, antiseptic, neuroprotective and antimicrobial properties (Baati *et al.* 2011, Mahmood *et al.* 2011, Halliwell 2012, Heleno *et al.* 2013). So far, data regarding the effect of extraction solvents on antimicrobial potential of *M.esculenta* have not been reported. Therefore, in the present study antibacterial potential of different solvent extracts of *M.esculenta* was investigated.

Material and Methods

M. esculenta was collected from district Swat and taxonomically identified by Assistant Prof. Dr. Zahid Khan, Department of Botany, University of Swat, Khyber-Pakhtunkhwa (KPK) Pakistan.

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Analytical grade solvents were used in the extraction (Merck & Co., Inc., Kenilworth, NJ, USA). Nutrient agar and nutrient broth were purchased from Musaji Adam & Sons. Ciprofloxacin and Terbinafine were provided by Meditech Pharmaceuticals Peshawar, Pakistan. Whatman filter paper was used for filtration and Rotary evaporator (Rotavapor R-R 210/R215; BUCHIL Labortechnik AG) for drying solutions.

Three hundred gram of dried crushed mushrooms (*M. esculenta*) was mixed with one liter of analytical grade methanol in extraction flask. The flask was shaken twice a day for four consecutive days. At the end of the shaking period, the solution was filtered through Whatman filter paper. The filtered solution contained methanol soluble compounds. Methanol was separated from solution through rotary evaporator at 35°C, leaving paste which was then dried in China dish. The dried extract known as crude methanol extract was weighted and stored in clean and sterilized vials. The solid residues in the extraction flask was treated with other solvents (n hexane, ethyl acetate, Hot and cold water) using the same methodology of extraction.

Nutrient agar and nutrient broth were prepared according to standard procedure for antimicrobial bioassay (Khan *et al.* 2017). For fungal bioassay, potato dextrose medium was prepared from 300 g of potatoes extract, 20 g of dextrose, 20 g of agar and 1 litre of water. Other accessories and media containing bottle were autoclaved at 15 psi for 15 min at 121°C. Media were poured into Petri plates under sterile condition.

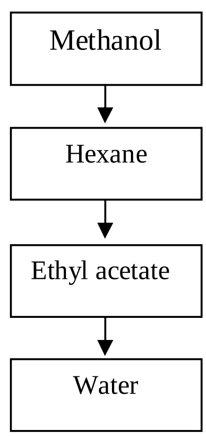

Fig. 1. Solvents used for extraction on the ascending order of polarity.

Well diffusion method was used for antifungal bioassay while disc diffusion method was performed for antibacterial bioassay. Bacterial species used in the present study were *Agrobacterium tumefaciens, Bacillus atrophaeus, Bacillus subtilis, Citrobactor freundii, Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Staphyllococcus aureus, Staphyllococcus epidermidis, Pseudomonas aeruginosa* and *Xanthomonas oryzae.* Among the fungal species were *Trichophyton rubrum, Trichoderma citrinoviride* and *Alternaria alternata.* Bacterial strains were cultured and standardized on nutrient agar medium and nutrient agar broth respectively, while potato dextrose agar and broth were used for culturing and standardization of fungal strains (Khan *et al*. 2016).

Results and Discussion

Selection of the suitable solvents for the optimum anti-microbial activity and antioxidant activity of the extract is one of the challenging tasks due to the difference in solubility of bioactive compounds in various solvent (Khan *et al*. 2018) . Thus preliminary screening is an essential to select the best solvent that finalizes the optimum bio activity of extract (Khan *et al*. 2017). The present study was conducted to find out the effect of different extraction solvents on antibacterial and antifungal activities of *M. esculenta* against selected bacterial and fungal strains. Four different solvent extracts were prepared and tested for antibacterial and antifungal activity. During the study *M. esculenta* was found to be more effective against bacteria as compared to fungi. The results showed that hot water extract was more effective against *B. atrophaeus, C. freundii, P. aeruginosa, S. aureus* and *E. coli, Xanthomonas oryzae, S. aureus, E. coli* as compared to other tested samples (Figs 1B, 2A, 2B) while the same extract was found to be ineffective against *A. tumefaciens* and *S. typhi* (Figs 1A, 3). Effective inhibitory activity against *E. coli* and *S. aureus* was found in ethanol extract (Badsha *et al*. 2012, Kalyoncu *et al*. 2010). The effectiveness of hot water might be due to the activation of strong bioactive compounds at high temperature or it might be possible that high temperature degrade the bio active compounds and the residuals of the degraded compounds might have strong antimicrobial efficacy as compared to the parental compounds. Cold water extract was effective against *S. typi, S. epidermidis* and *K. penumoniae* (Figs 3A, 5B, 6A). Similar findings were also reported in ethanol extract by Badsha *et al*. (2012). However, the cold water extract did not produce ZI against *C. freunjii* and *A .tumefaciens.*

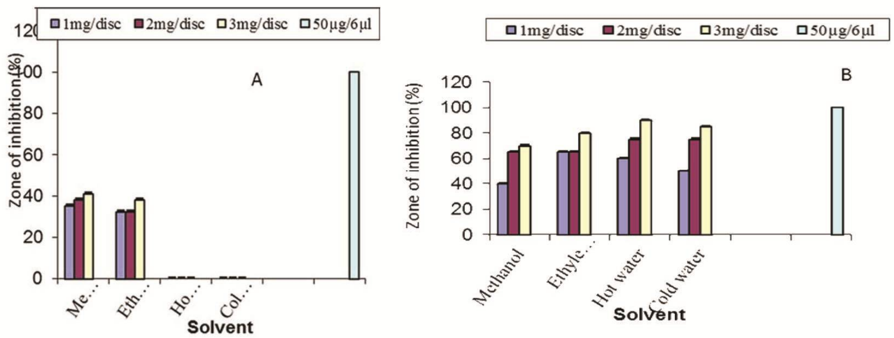

Fig. 1. Antibacterial efficacy of different solvent extracts of *M. esculenta* extract against *Agrobacterium* (A) and *B. autropus* (B) (Bar represents mean ± Sd).

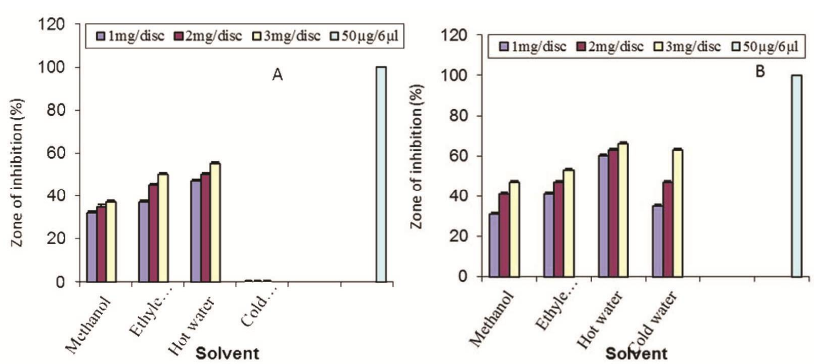

Fig. 2. Antibacterial activities of different solvent extracts of *M. esculenta* extract against *Citrobacter freundii* (A) and *P. aerginosa* (B), (Bar represents mean ± Sd).

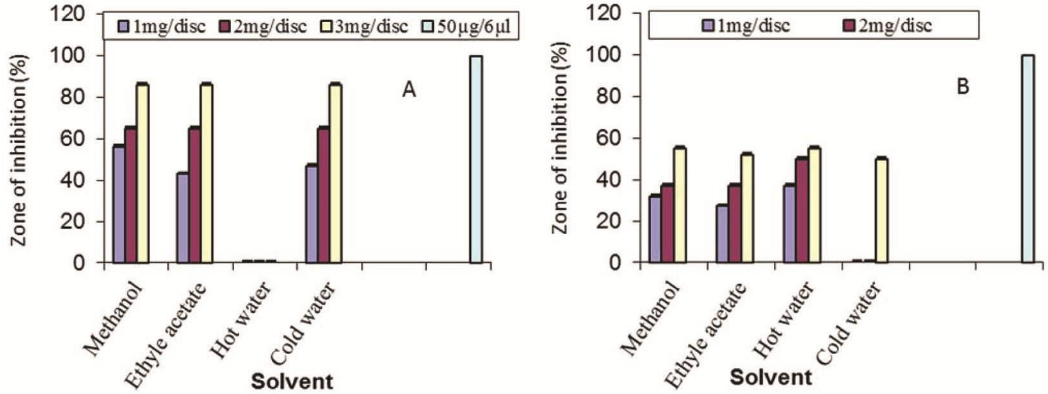

Fig. 3. Antibacterial activities of different solvent extracts of *M. esculenta* extract against *S. typhi* (A) and *X. oryzae* (B), (Bar represents mean ± Sd).

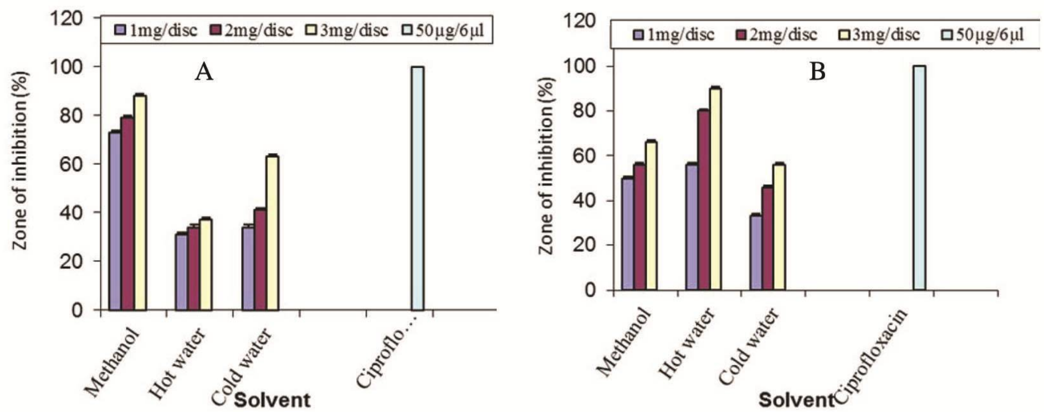

Fig. 4. Antibacterial activities of different solvent extracts of *M. esculenta* extract against *B. subtilis* (A) and *S. aureus* (B), Bar represents mean ± Sd).

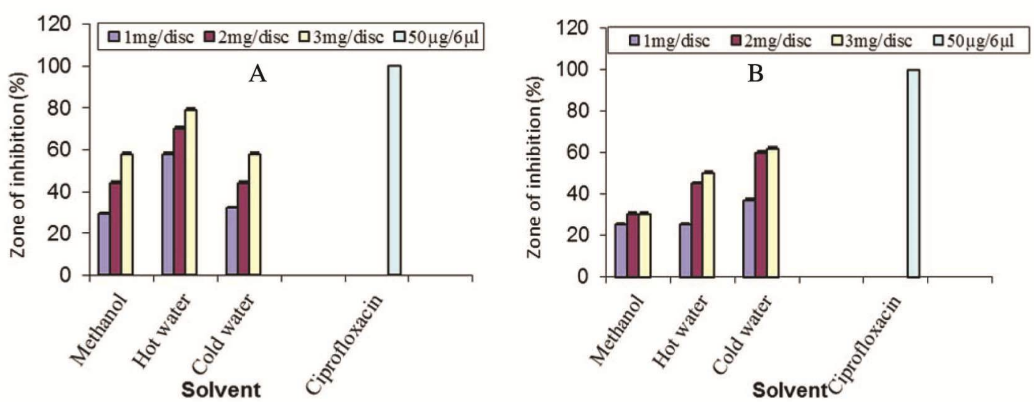

Fig. 5. Antibacterial activities of different solvent extracts of *M. esculenta* extract against *E. coli.* (A) and *S. epidermidis* (B), (Bar represents mean ± Sd).

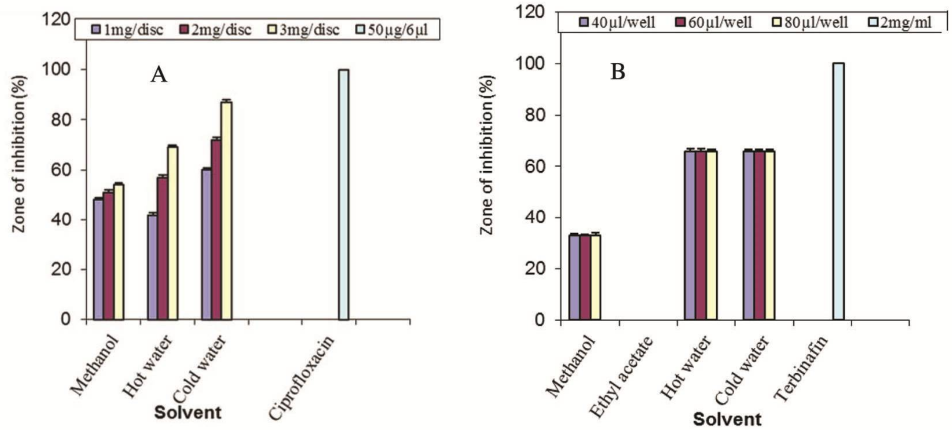

Fig. 6. Antibacterial activities of different solvent extracts of *M. esculenta* extract against *K. pneumonia* (A) and *T. rubrum* (B). (Bar represents ± Sd).

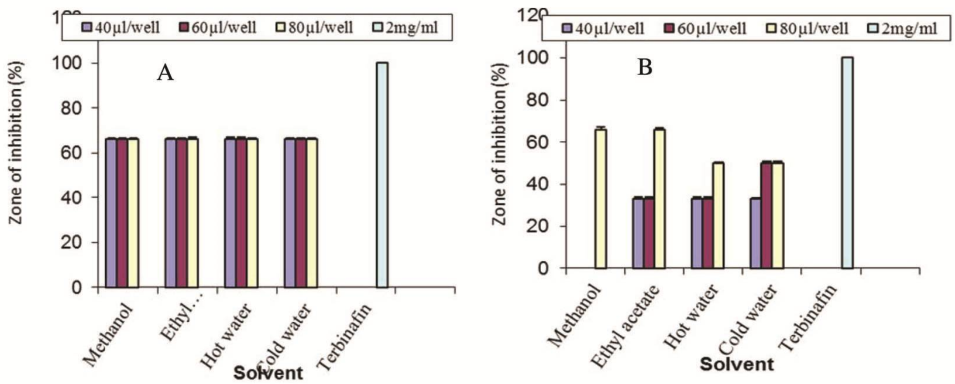

Fig. 7. Antifungal efficacy of the different solvents extracts of *M. esculenta* against *R. stolonifer* (A) and against *T. citrinoviride* (Bar represents ± Sd).

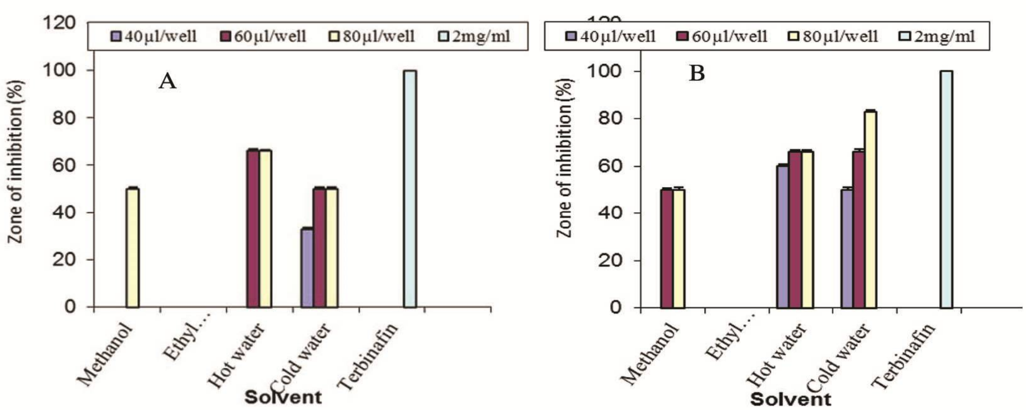

Fig. 8. Antifungal efficacy of the different solvents extracts of *M. esculenta* against *A. fumigatus* (A) and *A. alternata* (Bar represents ± Sd).

Antifungal efficacy of different extracts (mthanolic, ethyl acetate, hot and cold water extracts) of *M. esculenta* against fungal strains namely *T. citrinoviride*, *A. fumigatus* and *A. alternata* are presented in Figs 7B, 8A, 8B. Maximum zone of inhibition (66% ZI) was exhibited by methanol and ethyl acetate extract at 80 µl/well of the tested concentration (1mg). Hot water and ethyl acetate extracts of 1mg of *M. esculenta* revealed equal ZI (33%) at both 40 and 60 µl/well. The same ZI (33%) was measured by cold water extract of 1mg at 40 µl/well. At 80 µl/well, 1mg of both hot and cold water extract produced equal ZI of 50% against *T. citrinoviride,* the same ZI was measured for the cold water extract of at 60 µl/well.

Furthermore, ethyl acetate extract of *M. esculenta* did not inhibit the growth of *A. fumigatus* and *A. alternata* at any concentration (Figs 8A, 8B). Hot water extracts of 1 mg revealed equal ZI of 66% at 60 and 80 µl/well concentration and the same extract did not inhibit the growth of *A.fumigatus* at 40 µl/well. Methanol extract of 1 mg produced 50% ZI at 80 µl/well against *A. fumigatus*. However, the same extract did not affect the growth of the same fungus at 40 and 60 µl/well. Cold water extract (1 mg) produced the same zone of inhibition (50% ZI) at both 60 and 80 µl/well. The same extract produced 33% ZI at 40 µl/well. Cold water extract was more affective (83% ZI at 80 µl/well) against *A. alternata* as compared to other tested samples. Hot water extract produced 66% ZI while 50% ZI was noted for methanolic extract at both 60 and 80 µl/well. Both hot and cold water extracts revealed equal ZI of 66% at 60 µl/well, the same extracts produced 60 and 50% ZI, respectively at 40 µl/well. However Badsha *et al.* (2012) reported that methanolic, ethanolic and chloroform extracts were ineffective against *A. fumigatus* and *A. niger.* Furthermore, all the four extracts of the mushroom showed similar activity against *R. stolonifer* during this study. Results also revealed that water extract and methanol extracts were comparatively more affective against fungal species as compared to other extracts. However, cold water extracts is generally more effective than cold extracts for controlling fungal pathogens. Similar findings were also reported by Touba *et al.* (2011)

Hot water is comparatively best solvent for antibacterial activity and cold water and methanol extract is better for antifungal efficacy. Furthermore *M. esculenta* is more effective against bacteria as compared to fungi.

Acknowledgments

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References

Andersson DI and Hughes D 2011. Persistence of antibiotic resistance in bacterial populations. FEMS Microbiol Rev. **35**: 901-11.

Baati T, Horcajada P, Gref R, Couvreur P and Serre C 2011. Quantification of fumaric acid in liver, spleen and urine by high-performance liquid chromatography coupled to photodiode-array detection. J. Pharm. Biomed. Anal. **56**: 758-762.

Badshah H, Qureshi, RA, Khan J, Ullah F, Fahad S, Ullah F and Khan N 2012. Pharmacological screening of *Morchellae sculenta* (L.) Pers., Calvatiagigantea (Batsch ex Pers.) Lloyd and *Astraeus hygrometricus* Pers., mushroom collected from South Waziristan (FATA.). J. Med. Plants Res. 6: 1853-1859.

Halliwell B 2012. Free radicals & antioxidants: updating a personal view. Nutr. Rev. **70**: 257- 265.

Heleno SA, Stojkovic D, Barros L, Glamoclija J, Sokovic M, Martins A, Queiroz MJRP and Ferreira ICFR 2013. A comparative study of chemical composition, antioxidant and antimicrobial properties of *Morchella esculenta* (L.) Pers. from Portugal and Serbia. Food Res. Int. **51**: 236-243.

Kalyoncu F, Oskay M, Saglam H, Erdogan TF and Tamer AU 2010. Antimicrobial and antioxidant activities of mycelia of 10 wild mushroom species. J. Med. Food. **13**: 415-419.

Khan W, Bakht J and Khan BM 2017. *In vitro* antifungal, anti-oxidant and HPLC analysis of the extracts of *Physalis phyladephica*. Bangladesh J. Pharmacol. **12**: 313-318.

Khan W, Bakht J and Shafi M 2016. Antimicrobial potentials of different solvent extracted samples from *Physalis ixocarpa.* Pak. J. Pharm. Sci. **29**: 467-475.

Khan W, Bakht, J, Nair MG, Uddin MN and Shafi M. 2018. Extraction and isolation of important bioactive compounds from the fruit of *Physalis ixocarpa*." Pak. J. Pharm. Sci. **31**: 2463-2469.

Lowy F 2003. Antimicrobial resistance: the example of *Staphylococcus aureus*. J. Clin. Invest. **111**: 1265-73.

Mahmood A, Malik RN, Shinwari ZK and Mahmood A 2011. Ethnobotanical survey of plants from Neelum, Azad Jammu & Kashmir, Pakistan. Pak. J. Bot. **43**: 105-110.

Srivastava J, Chandra H, Nautiyal AR and Kalra SJS 2013. Antimicrobial resistance (AMR) and plant-derived antimicrobials (PDAms) as an alternative drug line to control infections. Biotech. **4**: 451-60.

Touba EP1, Zakaria M and Tahereh E 2011.Anti-fungal activity of cold and hot water extracts of spices against fungal pathogens of roselle (*Hibiscus sabdariffa*) *in vitro*. Microb. Pathog. **52**:125-9.

Walsh T and Toleman M. 2012. The emergence of pan-resistant Gram-negative pathogens merits a rapid global political response. J. Antimicrob Chemother. **67**: 1-3.

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