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Antimicrobial activity of crude fractions and morel compounds from wild edible mushrooms of North western Himalaya

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ABSTRACT

The antimicrobial properties of morel compounds from wild edible mushrooms (*Morchella esculenta* and *Verpa bohemica*) from Kashmir valley was investigated against different clinical pathogens. The butanol crude fraction of most popular or true morel *M. esculenta* showed highest 19 mm IZD against *E.coli* while as same fraction of *Verpa bohemica* exhibited 15 mm IZD against same strain. The ethyl acetate and butanol crude fractions of both morels also exhibited good antifungal activity with highest IZD shown against *A. fumigates*. The three morel compounds showed quite impressive anti bacterial and fungal activities. The Cpd 3 showed highest inhibitory activity almost equivalent to the synthetic antibiotics used as control. The MIC/MBC values revealed the efficiency of isolated compounds against the pathogenic strains. In the current study significant inhibitory activity of morel compounds have been obtained paying the way for their local use from ancient times.

1. Introduction

Infectious diseases remain one of the major threats to human health throughout the world [17]. Although numerous antibiotics have been used against pathogens, antimicrobial resistance is an increasing public health problem. In recent years, Basidiomycetes and other higher fungi including some recognized medicinal mushrooms have been re-investigated as sources of novel antibiotics mainly as a result of increasing difficulty and the cost of isolating novel bioactive compounds from the Actinomycetes and Streptomycetes [13]. Mushrooms need antibacterial and antifungal compounds to survive in their natural environments. Therefore, antimicrobial compounds could be isolated from many mushroom species and could be of great benefit for humans [27]. Mushrooms are a rich source of natural antibiotics [3].The glucans found in the cell wall are well known for their immunomodulatory properties, and the secondary metabolites have been found to be active against bacteria and viruses [15,25]. In general, humans and fungi share common microbial antagonists such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, so humans can benefit from the natural defence strategies of fungi to produce antimicrobials [9,21].

Mushrooms have been considered as ingredient of gourmet cuisine across the globe, especially for their unique flavour and have been valued by humankind as a culinary wonder, besides this they are well recognized for their functional food attributes. All together with a long history as food source, mushrooms are important for their healing capacities and properties in traditional medicine. In order to add further scientific knowledge to this aspect, an important study was carried out on two wild edible mushrooms *Morchella esculenta* and *Verpa bohemica* for evaluating their medicinal value (antimicrobial activity). Mushrooms of the genus *Morchella* are considered among the choicest edible mushrooms in the world. *M. esculenta*, (commonly known as common morel, morel, yellow morel, true morel, morel mushroom, and sponge morel) is a species of fungus in the Morchellaceae family of the Ascomycota. From 2000 years *Morchella* species have been used in traditional Chinese medicine as well as in Japan and Malaysia for the treatment of various diseases [10].*Verpa bohemica* is a species of fungus in the same family of Morchellaceae. Commonly known as the early morel (or early false morel) or the wrinkled thimble-cap, it

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is one of several species known informally as a “false morel”. Due to their unique flavour and taste local people cook the fruiting body of these mushrooms mixed with rice and vegetable and consider it as nutritious as fish or meat.

2. Material and methods

2.1. Collection and identification of mushroom species

The mushrooms *V. bohemica* and *M. esculenta* were collected manually in bulk from Aparhwat range (Gulmarg area 3748 m a.s.l), Kashmir Himalaya, J&K. *V. bohemica* was collected in the last week of April while *M. esculenta* was collected in the month of May to first week of June 2014. All the mushrooms were collected at fully mature stage with complete fruit body formation.

2.2. Preparation and phytochemical analysis of mushroom extracts

All the mushroom species were shade dried at the temperature of 30 °C in a well conditioned rooms fitted with dehumidifiers so as to protect them from the disease due to their higher water content. The dried powder (100 g) of each mushroom species was subjected to rigorous extraction via soxhlet apparatus. Thereafter, the material was pre-extracted with petroleum ether to remove oily substances. The resulting petroleum ether extract contained large amounts of secondary metabolites. The pre-extracted extracts were then refluxed with ethyl acetate and butanol. Extracts were concentrated using rotary evaporator, which were later completely dried, weighed and kept for further usage in sterilized sealed vials at 4 °C. With the aim of optimization in the complete extraction of samples, effects of different extraction solvents were premeditated via ultrasound-assisted extraction as a fast and proficient extraction tool Nowacka et al., 2014 [23,24]. The polar fraction of the samples was also preliminarily analyzed by TLC and specific reagents, according to the methodology previously described [28]. The particular fractions were chromatographed with a silica gel (Merck) column using an suitable solvent system, sequentially to pay for the pure compounds Da Silva et al., 2001, Isaias et al., 2004, [19,21]. The purity of all the isolated compounds was examined by TLC using Merck silica pre-coated aluminum plates of 200 μm thickness with several solvent systems of different polarities. Spots were visualized by sulfuric acid, sulfuric anisaldehyde and $FeCl_3$ reagents. The identification of isolated compounds was performed by analyses of melting points, IR spectra, ^{1}H and ^{13}C NMR spectra as well as the comparison of physical data.

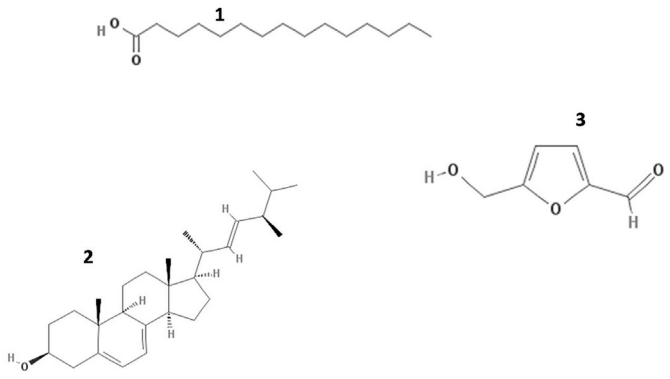

Fig. 1. Molecular structure of isolated morel compounds from *M.esculenta and V.bohemica.* 1. Pentadecanoic acid 2. Ergosterol (Ergosta-5,7,22-trien-3?-ol) 3.5–Hydroxymethylfurfural.

2.3. Antimicrobial susceptibility testing

2.3.1. Test microorganisms

Escherichia coli CD0002, *Pseudomonas aeruginosa CD0012, Staphylococcus aureus CD0003 Bacillus subtilis CD0005, Proteaus vulgaris CD0009* and all fungal strains viz, *Aspergillus niger CD0014*, *Candida albicans MRD3212*, *Candida kruesie MRD3623, Candida. paraloposis MRD1546* and *Aspergillus fumigates CD0083* were provided by Bacteriological and Mycological section (Deptt. of Microbiology, SKIMS, Soura, Srinagar) and Microbiology Laboratory, CORD University of Kashmir. All the strains were maintained and grown on their respective media.

2.3.2. Disc diffusion method [4]

In this method sterile discs with antimicrobial agents are poured on microbial cultures and inhibition zone diameters around the discs are measured as the potency of crude mushroom extracts/isolated compounds.

2.3.3. Micro-broth dilution method

2.3.3.1. Minimum inhibitory concentration (MIC). The method followed by Sette et al., 2006 with some modifications were used for MIC and MBC determination using Muller-Hinton Broth NCCLS 1990b on a tissue culture test plate (96 wells) for bacterial cultures. The stock solutions of all extracts/compounds were diluted and transferred into the first well, and serial dilutions were performed in order to have concentrations in the range of 10 mg/l to 0.05 mg/ml. Erythromycin was used as the reference antibacterial agent while as 0.5% DMSO was used as vehicular control. The inoculums (100 μl) were added to all wells and the plates were incubated at 34 °C for 24 h. Antibacterial activity was detected by checking the visibility of growth. MIC was defined as the lowest concentration of substances that inhibited visible growth, as indicated by the visual inspection. The bacterial suspensions were adjusted with sterile saline to a concentration of 1.0×10^5 CFU/ml. The inocula were prepared daily and stored at 4 °C until use. Dilutions of the inocula were cultured on solid medium to verify the absence of contamination and to check the validity of the inoculum.

Table 1
Antibacterial activity (IZD mm) of crude fractions and morel compounds from *M. esculenta and V. bohemica* against pathogenic bacterial strains.

Bacterial Strains	Crude extracts of *V.bomeica*		Crude extracts of *M.esculenta*		Morel compounds			Erythromycin
	Ethyl acetate fraction	Butanol fraction	Ethyl acetate fraction	Butanol fraction	Cpd 1	Cpd 2	Cpd 3	
Staphylococcus aureus	$^{b}12 \pm 1.0$	$^{a}9 \pm 1.52$	$^{b}14 \pm 1.2$	$^{a}8 \pm 1.25$	$^{c}17 \pm 2$	$^{d}25 \pm 1.2$	$^{de}28 \pm 2.9$	$^{b}15 \pm 3.2$
Bacillus subtilis	$^{b}15 \pm 0.577$	$^{b}12 \pm 0.5$	$^{a}8 \pm 1.6$	$^{b}15 \pm 1.3$	$^{c}22 \pm 1.9$	$^{c}23 \pm 1.6$	$^{d}26 \pm 3.2$	$^{d}29 \pm 2.7$
Pseudomonas aeruginosa	$^{a}9 \pm 1.52$	$^{a}9 \pm 1.15$	$^{a}8 \pm 1.52$	$^{b}11 \pm 0.9$	$^{c}17 \pm 2.6$	$^{c}18 \pm 2.7$	$^{c}20 \pm 3.1$	$^{c}20 \pm 2.5$
Escherichia coli	$^{a}11 \pm 1.15$	$^{b}15 \pm 1.7$	NA	$^{c}19 \pm 1.5$	$^{cd}21 \pm 2$	$^{cd}21 \pm 1.5$	$^{e}27 \pm 2.6$	$^{e}30 \pm 3.2$
Proteus vulgaris	$^{b}13 \pm 0.577$	$^{a}11 \pm 1.0$	NA	$^{a}12 \pm 0.6$	$^{d}20 \pm 3.6$	$^{bc}16 \pm 1.6$	$^{d}22 \pm 3.1$	$^{e}29 \pm 3.4$

Values are represented as mean ± SD(=3), the Data is analyzed by one way ANOVA, the values with different superscripts along the rows are statically significant at $P < 0.05$. Cpd1 = Pentadecanoic acid; Cpd2 = Ergosterol (Ergosta-5,7,22-trien-3β-ol; Cpd3 = 5-Hydroxymethylfurfural.