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ORIGINAL ARTICLE

Investigation of *Morchella esculenta* and *Morchella conica* for their antibacterial potential against methicillin-susceptible *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* and *Streptococcus pyogenes*

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Abstract

Antimicrobial resistance is an alarming problem, especially due to emergence of methicillin-resistance *Staphylococcus aureus* (MRSA). World Health Organization (WHO) has already listed MRSA as a top priority pathogen for the development of novel antibacterial agents. Presently, different therapeutic approaches against bacterial infections are in practice which includes targeting bacterial virulence factors, bacteriophage therapy, and manipulation of the microbiome. Natural products have been efficiently used for centuries to combat bacterial infections. *Morchella* is a natural fungal product which has been reported to possess broad-spectrum biological activities against bacterial infections. Hence, this study was aimed to evaluate the antibacterial efficacy of two macro-fungi against *S. aureus,* MRSA, *and Streptococcus pyogenes* (*S. pyogenes*). The antibacterial potential of both fungal extracts (*Morchella esculenta* and *Morchella conica*) was evaluated using disk diffusion and standard broth microdilution methods. The chemical compounds of both fungi were investigated using ultra-performance liquid chromatography mass spectroscopy (UPLC–MS) analysis. All fungal extracts inhibited growth of tested bacteria with inhibitory zone ranging from 10.66 ± 0.3 to 21.00 ± 1.5 mm. The minimum inhibitory concentration (MIC) of tested bacterial growth ranged from 03.33 to 16.0 mg/ml. It was noteworthy that *Morchella* extracts prevented *S. aureus* growth in a bactericidal manner with minimal bactericidal concentration (MBC) of 8–16 mg/ml. The extracts were also more effective against MRSA than currently available antibiotics. In conclusion, the growth inhibition of tested bacteria by fungal extracts revealed their potential as antibacterial agents and their compounds may be used as drug candidates.

Keywords Antibacterial agents · Antimicrobial resistance · Minimum inhibitory concentration · Fungal extract · MRSA · UPLC–MS

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Introduction

Antimicrobial resistance has become an emerging problem for human health and it has now reached a crises stage worldwide. The emergence of bacterial resistance against conventional antibiotics is also slowing the development of novel therapeutic options by the pharmaceutical industries (Hauser et al. 2016). Thus, two main problems are associated with the increased rate of infectious diseases caused by resistant bacteria. First, increase in resistance to new antibiotics. Second, the gap between new drug development and increasing antimicrobial resistance is growing (Ferri et al. 2017). As conventional antibiotics become increasingly unreliable, therefore, alternative therapeutic options are getting more attention. Most of the currently

available antibiotics are either naturally derived or modified drugs (Gil-Gil et al. 2019; Xu et al. 2019). Thus, screening of fungal extracts for the presence of bioactive compounds and antibacterial potential is important in the current era of antimicrobial resistance.

Gram-positive cocci (*Staphylococci* and *Streptococci*) are responsible for both community- and hospital-associated infections (Pfaller et al. 2018). *Staphylococcus aureus* (*S. aureus*) is the major Gram-positive bacteria in clinical setup and the causative agent of a broad range of illnesses running from skin infection to life-threatening pneumonia and bacteremia (Kanayama et al. 2016; Qin et al. 2020). *Streptococcus pyogenes* (*S. pyogenes)* causes a wide range of diseases, e.g., pharyngitis, meningitis, septicemia, pneumonia, erysipelas, *Streptococcal* toxic shock syndrome (STSS), and necrotizing fasciitis. Glomerulonephritis and acute rheumatic fever are the major post-infection sequelae of *S. pyogenes* (Imöhl et al. 2017). These bacteria are notorious for the acquisition of numerous antimicrobial resistance which complicates the treatment options. Methicillin-resistance *Staphylococcus aureus* (MRSA) infection is also continuously emerging especially in healthcare settings (Lee et al. 2018a). The remarkable emergence of MRSA is a special concern and still considered as a superbug in the era of beta-lactam antibiotics (Zhang et al. 2019; Qin et al. 2020). In fact, the world is facing a MRSA crisis which is rapidly spreading among humans causing multiple complications such as gangrene, painful abscesses, cytokine storms, and multiple organs failure (Lowy 1998; Yagnik et al. 2021). WHO gives high priority to MRSA for research, discovery and development of novel antibacterial agents (Organization 2017; Tacconelli et al. 2018). The growing emergence of such resistant organism highlights the clinical demands for further research on development of effective antibacterial agents (Houri et al. 2017).

First- and second-generation cephalosporins, glycopeptides, and penicillin are the most commonly used antibiotics against Gram-positive cocci infections (Kollef 2005; Falagas et al. 2008). The increased incidence of Gram-positive cocci infections has been subjected to a sharp increase in resistance to cephalosporins, penicillin, and glycopeptides (Doern et al. 1999; Smith et al. 1999; Falagas et al. 2008). In addition, the infections caused by resistant Gram-positive cocci can lead to the excess duration of hospital stay, high morbidity, and increased cost of care (Vincent et al. 1995; Niederman 2001). Since last 40 years, the only class of daptomycin and linezolid has been added to the current list of antibiotics. Poor hygiene, lack of infection control systems, and misuse of antibiotics by humans and animal farms have contributed to the antibacterial resistance (Tickell et al. 2020; Yagnik et al. 2021). Most of the currently available antibiotics are discovered from fungi and bacteria (Durand et al. 2019).

Fungi are considered a potential source of therapeutic bioactive compounds, nutrients, and medicinal foodstuff. Due to the presence of bioactive compounds, they are used as antibacterial, antifungal, and antiviral agents since hundreds of years. In the last decade, there was tremendous progress in human medicines, but infectious diseases are still a threat to public health in developing countries (Canli et al. 2019; Atila et al. 2021). About 300,000 natural products had been identified, of which 19,869 natural products are from the fungi kingdom (Chassagne et al. 2019; Mussagy et al. 2019). Specifically, in the Basidiomycota phylum, 4280 natural products have been identified (Vargas-Sinisterra and Ramírez-Castrillón 2021). Since 1981 to 2014, approximately half of the new drugs are derived from natural products, of which 73% of all anticancer compounds and 65% of all antibacterial compounds are derived from fungi (Newman and Cragg 2016; Chassagne et al. 2019). The estimated number of mushrooms species on the earth is 140000 but only 22,000 are described in literature data (Lindequist et al. 2005). There are a large number of fungi and mushroom species not yet investigated for their antibiotic potentials (Hassan et al. 2019). Mushrooms are a great source of bioactive compounds containing phenolics, terpenes, steroids, β-glucans, and nitrogen compounds (De Silva et al. 2013; Zhu et al. 2015). These compounds protect mushrooms against various microorganisms in the soil to survive in their environment (Rai et al. 2015).

Fungal organisms and mushrooms are widely known for their therapeutic values and food products (Volcão et al. 2021). The scientific community investigated wide number of mushroom species to develop novel therapies (Lindequist et al. 2005). Mushrooms possess antioxidant, antibacterial, antifungal, antiparasitic, antiviral, antitumor, antidiabetic, immunomodulating, cardiovascular-protective, radical scavenging, and hepatoprotective effects (Niego et al. 2021). Mushrooms belonging to the genus *Morchella* are the choicest edible species. Among them, *Morchella esculenta* (*M. esculenta*) commonly called morel is important for their therapeutic valve (Shameem et al. 2017). It is one of the precious edible fungi, well known for its nutritional values and delicate taste. Investigations have shown that *M. esculenta* demonstrates a wide range of biological activities, including antibacterial, anti-inflammatory, antitumor, antioxidant, and hepatoprotective activities (Shameem et al. 2017; Li et al. 2019). *Morchella conica* (*M. conica)* is another fungal species of the same genus in the *Morchellaceae* family, commonly known as black morels (Yang et al. 2019; Wu et al. 2020). It is reported to possess antioxidant, immunomodulatory, and antitumor properties (Xu et al. 2018). Few of its polysaccharides inhibit nitric oxide production in macrophages (Huang et al. 2012).

Fungi produce a variety of biomolecules, including peptides, alkaloids, polyketides, and terpenes with potential

medicinal applications (Liu and Liu 2018). Purified carotenoids from yeast work as potential antibacterial biomolecules against several genera of bacteria including *S. aureus* (Vargas-Sinisterra and Ramírez-Castrillón 2021). Demand for scale-up of various naturally derived biomolecules from fungi has been increased due to rapid antibacterial resistance. There is limited information on the antibacterial potential of *M. conica* and *M. esculenta,* especially against MRSA and *S. pyogenes*. The raised bacterial resistance and health benefits of fungi led us to assess the antibacterial efficacy of *M. conica* and *M. esculenta* against methicillin-susceptible *S. aureus,* MRSA and *S. pyogenes*.

Materials and methods

Fungal material

The two fungal species used in this study, *Morchella esculenta* and *Morchella conica* were collected from Swat, Khyber Pakhtunkhwa Pakistan. Both species were identified based on their morphological characteristics as previously described (Hamayun et al. 2006; Sher et al. 2015).

Preparation of fungal extract

The fungi samples were air-dried and protected from sunlight exposure. The dried powder samples were suspended in methanol (MeOH) and ethanol (EtOH) (extracts/solvent ratio 1:10 weight/volume) and shaken at 120 RPM for 72 h in a shaking incubator at 25 °C temperature. After incubation, both extracts were filtered via a Whatman No 1 filter paper and the solvent was evaporated using a rotary evaporator under reduced pressure at 37 °C. The MeOH extracts were further fractioned in n-hexane. The resulting compounds from each solvent were dissolved in DMSO to prepare a stock solution (25 mg/ml).

Bacterial strains

S. aureus American-type culture collection (ATCC-29213) strain, MRSA (clinical strain), and *S. pyogenes* (clinical isolates) were obtained from the Department of Microbiology, University of Health Sciences Lahore, Pakistan. The stored bacteria from stock solution were initially streaked on blood agar.

Determination of the antibacterial activity of *Morchella* extracts

Antibacterial activity assays (disk diffusion method)

The antibacterial potential of *M. esculenta* and *M. conica* extracts were performed using disk diffusion method according to clinical and laboratory standards institute 2021 (CLSI 2021). The *S. aureus* and MRSA were cultured on nutrient agar, while *S. pyogenes* were cultured on sheep blood agar for 24 h. After incubation, the bacterial suspension was adjusted to 0.5 McFarland turbidity standards equivalent to a concentration of 1.5×10^8 CFU/ml. Bacterial suspensions were inoculated on Mueller–Hinton agar (MHA) plates except *S. pyogenes* which were inoculated on blood agar plates. Sterile plain disks (6 mm) impregnated in *M. esculenta* and *M. conica* extracts were placed on the inoculated plates. Ciprofloxacin and tetracycline were used as positive controls for *S. aureus* and *S. pyogenes*, respectively. Plates were incubated at 37 °C for 18–24 h. The inhibition zones were measured in millimeters (mm). Each test was performed in triplicate.

Minimum inhibitory concentration (MICs)

Standard broth microdilution methods were performed to assess the minimal inhibitory concentrations (MICs) of the tested compounds in accordance with the guidelines of CLSI, 2021 (CLSI 2006). In brief, 100 µl of Mueller–Hinton broth (MHB) was added to each well of the 96-well microdilution tray except sterility control. Then, 100 µl of all mushroom extract was added to each well in a series of twofold dilutions yielding different concentrations which ranged from 16 to 0.0625 mg/ml, except those wells which were acting as drug free and negative controls. 10 µl of bacterial suspension (approximately $2–8 \times 10^5$ CFU/ml) was poured into each well of microtiter plates except the wells of negative and sterility controls. Ciprofloxacin and oxacillin were used as a standard antibiotic for tested bacteria at the concentration of 128 µg/ml serially diluted to 0.5 µg/ml in accordance with the recommendations of CLSI 2021. The microtiter plates were incubated for 18–24 h at 37 °C. MIC was defined as the lowest concentration of antimicrobial agent which inhibited the visible growth of the bacteria in the wells. For determining MICs, the plates were visually examined for turbidity in comparison with the control well.

Spectrophotometric broth microdilution method

In addition to visual interpretation, spectrophotometric broth microdilution method was also used for the analyses of bacterial growth inhibition. After loading the microtiter plate, the optical density (OD) was taken through a microplate ELISA reader (Thermo Scientific) at 620 nm. The OD of each well was taken before incubation ($t0$) and after 24 h incubation ($t24$). Any negative value was assigned zero value, while value greater than 100 was taken as an accurate 100 value (Green et al. 2020). Following formula was used for the analyses of bacterial growth and growth inhibition (Sulej et al. 2019):

$$\text{Bacterial growth (\%)} = \frac{(\text{OD test } t24 - t0)}{(\text{OD control } t24 - t0)} \times 100$$

$$\text{Growth inhibition (\%)} = 100 - \frac{(\text{OD test } t24 - t0)}{(\text{OD control } t24 - t0)} \times 100$$

Minimum bactericidal concentration (MBC)

To determine the minimal bactericidal concentration (MBC), following microdilution test, a loopful of inoculum from each well of no visible bacterial growth was plated on Mueller–Hinton agar. The plates were incubated for 18–24 h at 37 °C. The MBC was determined as the lowest concentration of antimicrobial agents that can kill > 99.9% bacteria.

Preparation of samples for UPLC–MS analyses

The fungal extract (01 mg) from the stock solution was dissolved in 500 µl methanol. The methanolic solution was five times diluted in 50% acetonitrile and filtered through 2 µm pore size disposable syringe filters (Sartorius Minisart® SRP-15 syringe filters). The filtrate was centrifuged at 14,000 g for 10 min to remove any remaining particles. The working samples were transferred to special LC–MS glass vials before operating on UPLC–MS chromatography system.

Ultra-performance liquid chromatography mass spectroscopy (UPLC–MS) analyses

LC–MS analyses of *M. esculenta* and *M. conica* were performed on UPLC–MS system (Waters Corporation, USA) equipped with a sample manager, solvent manager, and column heater. The temperature of the sample manager was maintained at room temperature and the column temperature was set at 60 ◦C. The sample column was washed with acetonitrile after each run to remove any remaining compound. The mass spectrum measurement was performed in positive as well as negative mode (ESI−, ESI+) as the sample was run for 10 min.

Statistical analysis

For all bacterial isolates' disk diffusion, MICs and MBCs results were expressed using means, standard deviations, and standard error of the mean (SEM). One-way analysis of variance (ANOVA) was performed to determine statistically significant differences among the means of tested agents. All statistical analyses were performed using Statistical Package for the Social Sciences (SPSS) version 20.0. The two-tailed P value ≤ 0.05 was considered to be statistically significant for all tests.

Results

Determination of antibacterial effectiveness of *Morchella* extracts via disk diffusion method

Antibacterial activity of *M. esculenta* extracts

The *M. esculenta* and *M. conica* extracts (ethanolic, methanolic, and n-hexane) were analyzed for their antibacterial potential against *S. aureus*, MRSA, and *S. pyogenes* using disc diffusion assay and depicted in Table 1. All the three types of extracts *M. esculenta* were active against all tested bacteria with inhibitory zone ranging from 10 to 21 mm. The activity of *M. esculenta* extracts was excellent against *S. aureus* with zones of inhibition ranging from 17.33 ± 0.3 mm to 21.00 ± 1.5 mm. The organism was also sensitive to ciprofloxacin with a clear zone of 24.33 ± 0.3 mm. The zone of inhibition of *M. esculenta* extracts for *S. pyogenes* ranged from 18.66 ± 0.3 mm to 20.33 ± 0.8 mm which was in parallel to tetracycline (19.33 ± 0.3 mm). Antibacterial activity of *M. esculenta* extracts against MRSA was faint as lower inhibitory zone of n-hexane fraction was 10.66 ± 0.3 mm. The maximum zone of inhibition of MeOH extract of *M. esculenta* against MRSA was 14.66 ± 0.3 mm, while MRSA was resistant to ciprofloxacin.

Antibacterial activity of *M. conica* extracts

MeOH extracts of *M. conica* spp. produced a clear zone of 19 mm against *S. aureus* and *S. pyogenes* as shown in Table 1. Likewise, the EtOH extracts showed an inhibitory zone of 17.00 ± 0.5 mm and 16.66 ± 0.3 mm against *S. aureus* and *S. pyogenes*, respectively. MRSA was also susceptible to *M. conica* extracts with an inhibition zone of 10–13 mm. Collectively, all morel extracts presented promising antibacterial potential against *S. aureus* and *S. pyogenes* as compared to MRSA.

Minimal inhibitory concentration and minimal bactericidal concentration of fungal extracts

The inhibitory and bactericidal potential of the fungal extracts against *S. aureus* and *MRSA* was determined by MIC and MBC methods. The extracts of *M. conica* confer inhibitory potential against *S. aureus* and *MRSA*. All three types of *M. conica* extracts inhibited *S. aureus* with MIC varied from ≥ 3.33 ± 0.6 to ≥ 16.0 ± 0 mg/ml (Table 2).

The MIC of *M. esculenta* against *S. aureus* both for MeOH and EtOH extracts was the same i.e., ≥ 6.66 ± 1.3 mg/ml, while for n-hexane fraction, it was ≥ 13.33 ± 2.6 mg/ml as shown in Table 2. The MIC and MBC values of MeOH

Table 1 Antibacterial efficacy of two macro-fungi against *S. aureus*, MRSA and *S. pyogenes* using disk diffusion method

Tested bacteria	Inhibition zone (mm)				
	MeOH extract	EtOH extract	n-Hexane fraction	Ciprofloxacin 10 µg	Tetracycline (15 µg)
Morchella esculenta					
S. aureus (ATCC isolate)	17.33 ± 0.3	17.66 ± 0.8	21.00 ± 1.5	24.33 ± 0.3	–
MRSA (clinical isolate)	14.66 ± 0.3	11.66 ± 0.3	10.66 ± 0.3	R	–
S. pyogenes (clinical isolate)	19.66 ± 0.6	18.66 ± 0.3	20.33 ± 0.8	–	19.33 ± 0.3
Morchella conica					
S. aureus (ATCC isolate)	19.00 ± 1.1	17.00 ± 0.5	16.66 ± 1.2	24.33 ± 0.3	–
MRSA (clinical isolate)	10.33 ± 0.3	10.33 ± 0.3	13.66 ± 0.3	R	–
S. pyogenes (clinical isolate)	19.33 ± 0.8	16.66 ± 0.3	19.66 ± 0.6	–	19.33 ± 0.3

Data are presented as mean ± standard error of mean. Values expressed as mean within row and column are statistically significantly different in ANOVA (p values ≤ 0.05)

MeOH methanol, *EtOH* ethanol, *mm* millimeter, *R* resistant

Table 2 Minimal inhibitory concentration and minimum bactericidal concentration of two fungi against *S. aureus* and MRSA using broth microdilution

Fungal extracts	*S. aureus*		MRSA	
	MIC	MBC	MIC	MBC
Morchella conica				
MeOH (mg/ml)	≥ 3.33 ± 0.6	≥ 8	≥ 6.66 ± 1.3	≥ 16
EtOH (mg/ml)	≥ 16.0 ± 0	≥ 16	≥ 13.33 ± 2.6	n.d
N-Hexane (mg/ml)	≥ 13.33 ± 2.6	≥ 16	≥ 13.33 ± 2.6	≥ 16
Ciprofloxacin (µg/ml)	≥ 1.00 ± 0	≥ 2	≥ 128 ± 0^{R}	n.d
Oxacillin (µg/ml)	–	–	≥ 64 ± .0^{R}	n.d
Morchella esculenta				
MeOH (mg/ml)	≥ 6.66 ± 1.3	≥ 16	≥ 8.0 ± 0	≥ 16
EtOH (mg/ml)	≥ 6.66 ± 1.3	≥ 16	≥ 16.0 ± 0	n.d
N-Hexane (mg/ml)	≥ 13.33 ± 2.6	≥ 16	≥ 16.0 ± 0	n.d
Ciprofloxacin (µg/ml)	≥ 1.0 ± 0	≥ 2	≥ 128 ± 0^{R}	n.d
Oxacillin (µg/ml)	–	–	≥ 64 ± .0^{R}	n.d

Data are presented as mean ± standard error of mean. Values expressed as mean within row and column are statistically significantly different in ANOVA (p values ≤ 0.05)

MeOH methanol, *EtOH* ethanol, ≥ greater than or equal to, *n.d* not detected, *MIC* minimal inhibitory concentration, *MBC* minimal bactericidal concentration, *R* resistant

^{R}Considered as resistant according to CLSI 2021 MIC breakpoints

extracts were twice higher for MRSA as compared to MIC and MBC values for *S. aureus*. Overall, the MIC values of both fungal extracts were considerably higher for MRSA than *S. aureus*.

S. aureus was sensitive to ciprofloxacin (MIC ≥ 1.00 ± 0 µg/ml); however, MRSA was resistant to ciprofloxacin (MIC ≥ 128 ± 0 µg/ml). MRSA also demonstrated a resistant pattern towards oxacillin (MIC ≥ 64 ± 0 µg/ml) (Table 2). It is noteworthy that all the fungal extracts inhibited *S. aureus* growth in a bactericidal manner with MBC value of 8–16 mg/ml) as shown in Table 2. Thus, all *Morchella* extracts showed statistically significant antibacterial potential against *S. aureus* ($p \leq 0.05$).

Spectrophotometric broth microdilution method for inhibitory effects

The anti-bacterial potential of both fungal extracts was further analyzed by growth curve as shown in Figs 1, 2, 3 and 4. The bacterial growth was observed at different concentrations using spectrophotometric analysis. The growth of *S. aureus* cultures treated with MeOH *M. conica* extracts was gradually increased at concentrations ≤ 02 mg/ml, while for EtOH and N-hexane extracts of *M. conica*, the tested bacterial growth start increasing at concentrations ≤ 16 mg/ml. Similarly, the bacterial growth was increasing after treating with *M. esculenta* extract at concentrations ≤ 04 mg/ml. The MRSA growth slightly raised at the concentration of ≤ 08 mg/ml. Ciprofloxacin completely restricted the *S. aureus* growth up to the concentration of 02 µg/ml. There was 100% MRSA growth in the presence of oxacillin (32 µg/ml) and ciprofloxacin (04 µg/ml) as shown in Fig. 5.

The susceptibility pattern of *S. aureus and* MRSA treated with various concentrations of fungal extracts and antibiotics are presented in Tables 3 and 4. All three types of *M. esculenta* extracts suppressed more than 90% growth of *S. aureus* and MRSA at a concentration of 04 mg/ml. A significant bacterial growth inhibitory potential of *M. conica* was noted for its MeOH extract which 100% inhibited bacterial growth at the concentration of 04 mg/ml. While the growth of *S. aureus* was 99% limited by ciprofloxacin at the concentration of 0.250 µg/

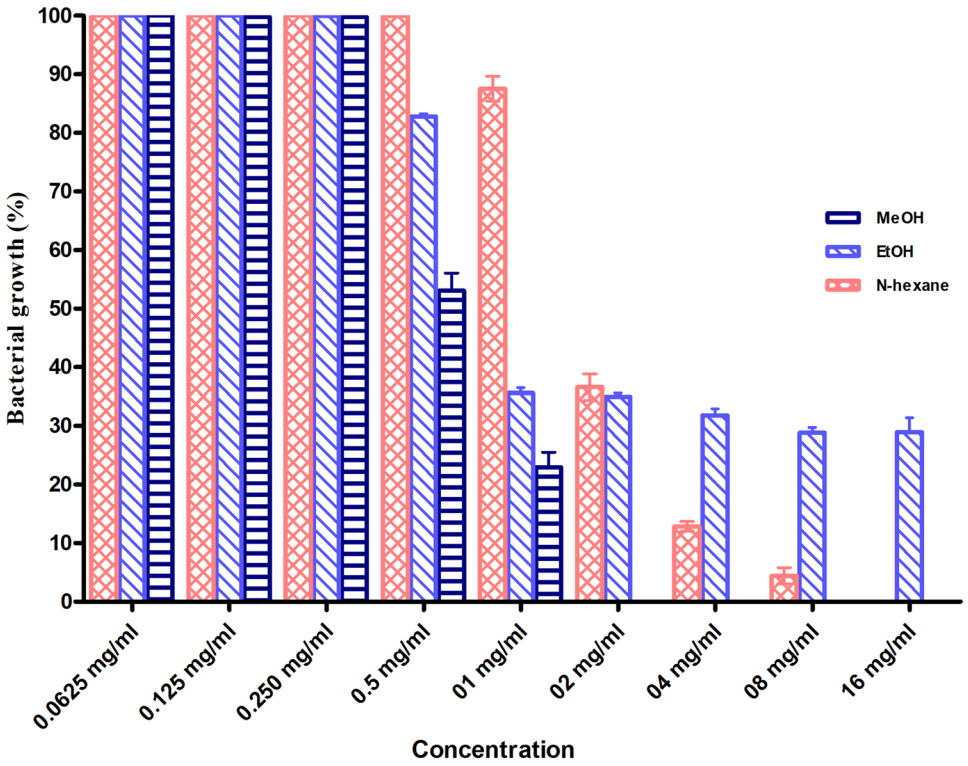

Fig. 1 Effects of different *M. conica* extracts (methanolic, ethanolic, and n-hexane) at various concentrations on *S. aureus* isolates (color figure online)

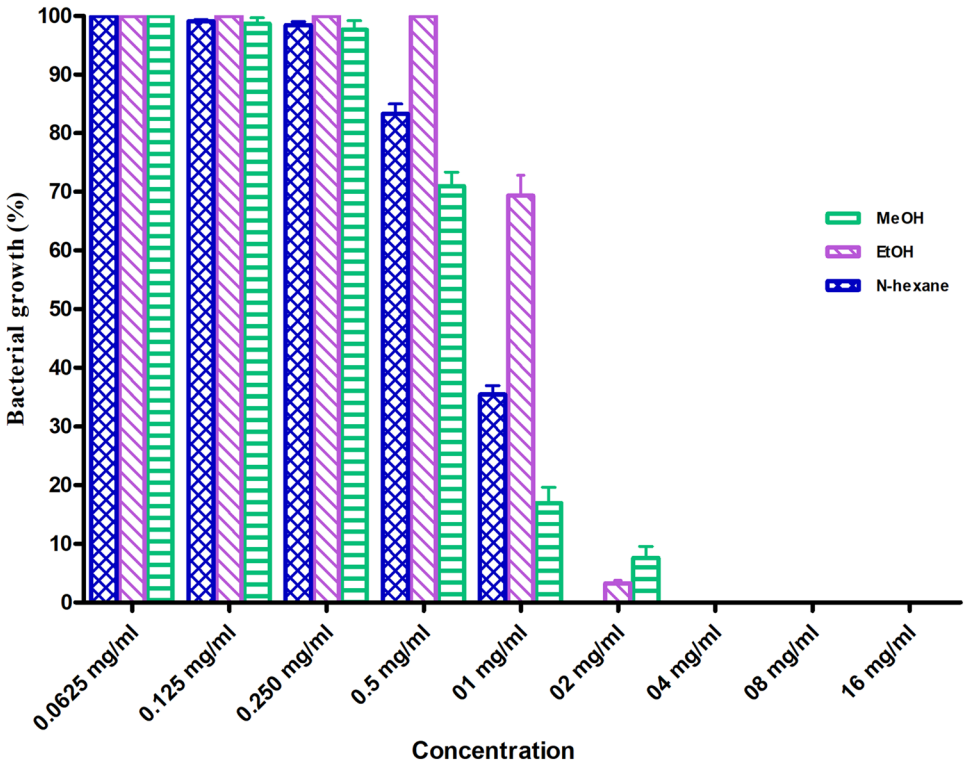

Fig. 2 Effects of different *M. esculenta* extracts (methanolic, ethanolic, and n-hexane) at various concentrations on *S. aureus* isolates (color figure online)

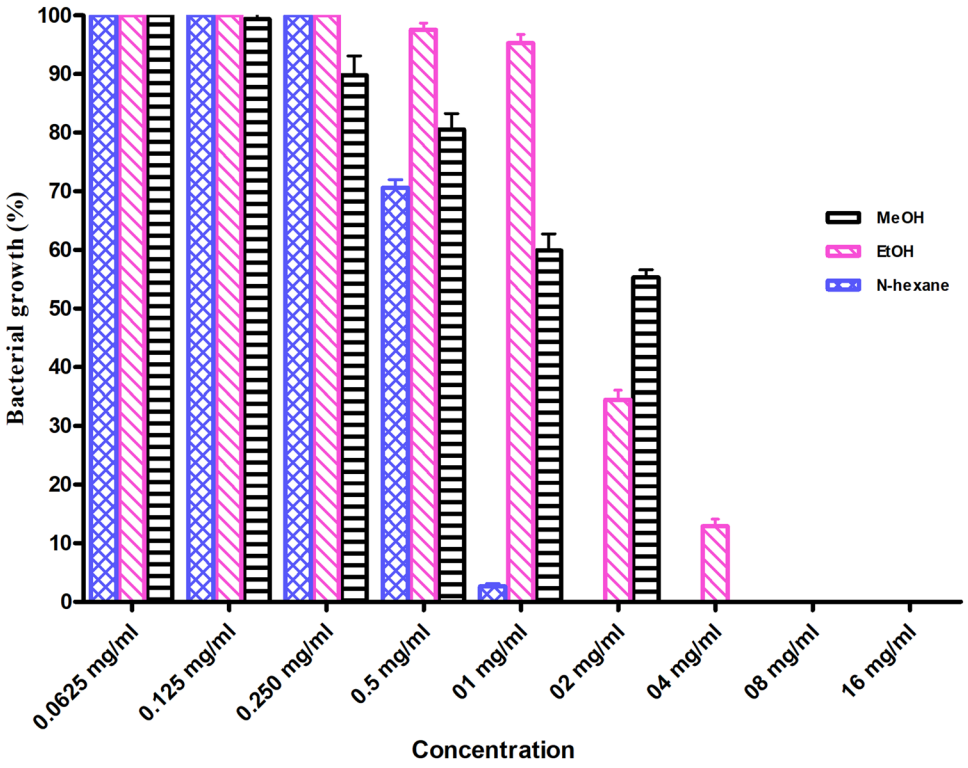

Fig. 3 Growth of MRSA clinical isolate treated with various concentrations of *M. conica* (methanolic, ethanolic, and n-hexane extracts) (color figure online)

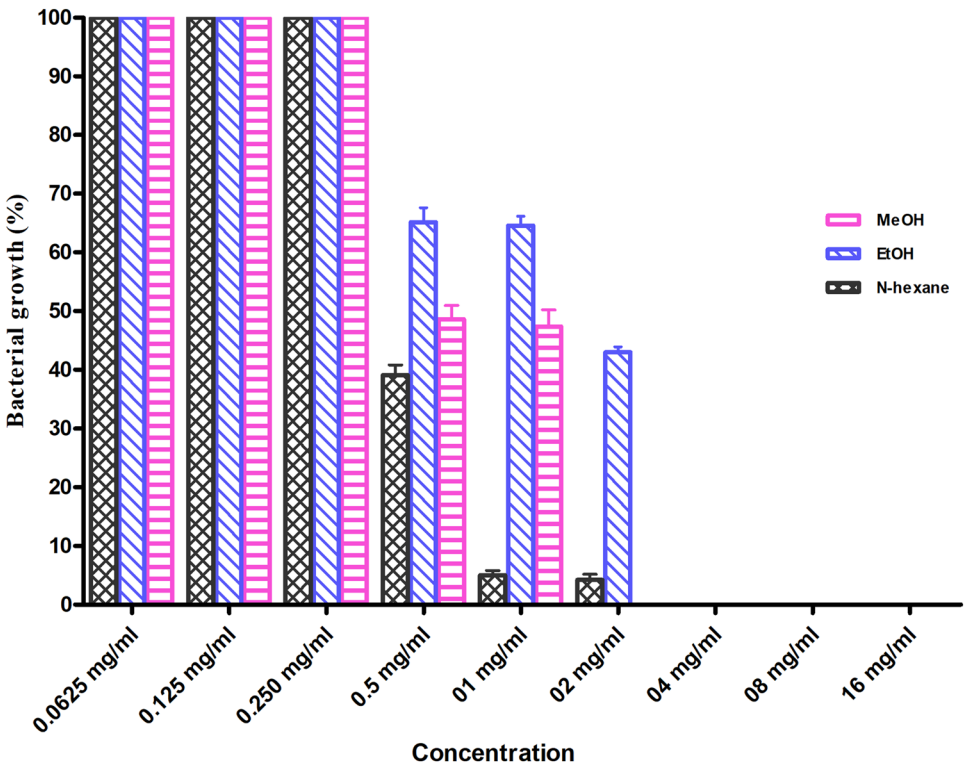

Fig. 4 Growth of MRSA clinical isolate treated with various concentrations of *M. esculenta* (methanolic, ethanolic, and n-hexane extracts) (color figure online)

ml, however, none of its concentrations suppressed MRSA

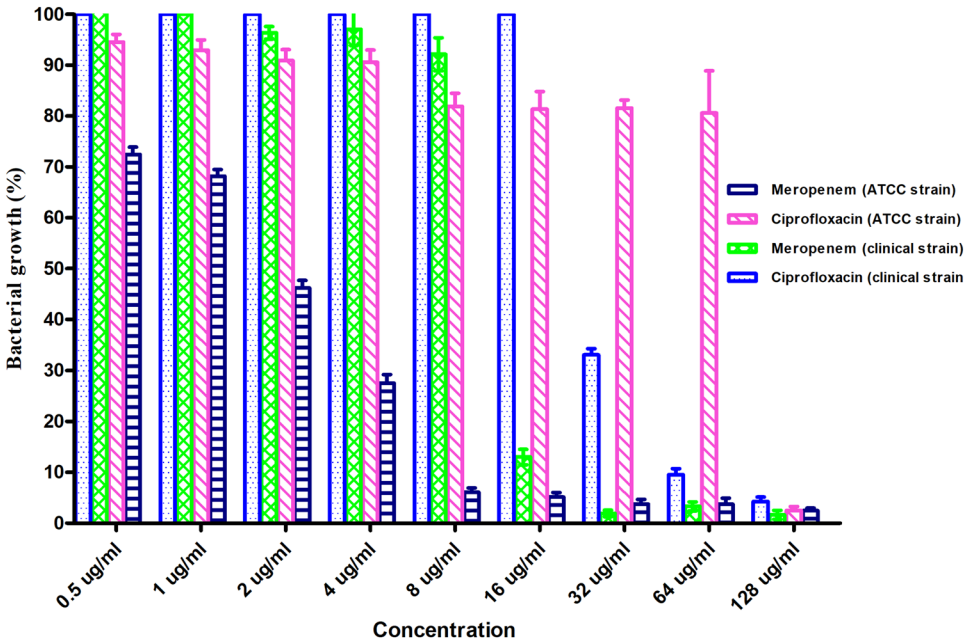

Fig. 5 Growth of *S. aureus* and MRSA in the presence of ciprofloxacin and oxacillin antibiotics (color figure online)

Table 3 *S. aureus* growth inhibition measurement (%) using optical density (OD) after exposure to fungal extract and antibiotics at different concentration

Concentration	*M. esculenta*			*M. conica*			Antibiotic Ciprofloxacin[a]
	EtOH	MeOH	N-Hexane	EtOH	MeOH	N-Hexane	
16 mg/ml	100	100	100	70	100	100	100
08 mg/ml	100	100	100	72	100	94.05	100
04 mg/ml	100	100	93.07	52	100	86.48	100
02 mg/ml	96.84	91.44	43.29	40	100	62.70	100
01 mg/ml	27.36	82.23	63.23	22	79.08	10.81	100
0.5 mg/ml	N.I	26.31	15.68	18	47.71	N.I	98.79
0.250 mg/ml	N.I	N.I	1.96	N.I	N.I	N.I	99.19
0.125 mg/ml	N.I	N.I	0.49	N.I	N.I	N.I	88.30
0.0625 mg/ml	N.I	N.I	N.I	N.I	N.I	N.I	50

N.I No inhibition

[a]Different concentration was used for antibiotic (ciprofloxacin) than fungal extracts ranging from 128 µg/ml to 0.5 µg/ml

Table 4 Measurement of growth inhibition (%) of MRSA after treatment with mushroom extracts and antibiotics at different concentration by spectrophotometer

Concentration	*M. esculenta*			*M. conica*			Control antibiotics	
	EtOH	MeOH	N-Hexane	EtOH	MeOH	N-Hexane	Oxacillin[a]	Ciprofloxacin[a]
16 mg/ml	100	100	100	100	100	100	100	87.91
08 mg/ml	100	100	100	100	100	100	100	67.58
04 mg/ml	100	100	100	86.12	100	100	N.I	48.90
02 mg/ml	56.97	100	100	64.73	45.45	100	N.I	32.96
01 mg/ml	34.30	51.16	68.26	N.I	59.74	97.61	N.I	17.03
0.5 mg/ml	59.88	50.58	59.60	N.I	18.18	28.57	N.I	3.84
0.250 mg/ml	N.I	N.I	N.I	N.I	35.71	N.I	N.I	4.94
0.125 mg/ml	N.I	N.I	N.I	N.I	N.I	N.I	N.I	2.74
0.0625 mg/ml	N.I	N.I	N.I	N.I	N.I	N.I	N.I	4.94

N.I No inhibition

[a]Different concentration was used for antibiotic (ciprofloxacin, oxacillin) then fungal extracts ranging from 128 µg/ml to 0.5 µg/ml

Table 5 Compounds identified in the *M. conica* and *M. esculenta* using UPLC–MS analysis

S. no	RT (min)	Name of compound	ESI mode	Base peak (m/z)	Molecular formula	Molecular weight (g/mol)
M. conica						
01	0.33	3-Quinolinecarboxylic acid, 7-amino-1-ethyl-6-fluoro-1,4-dihydro-4-oxo	–	233.08	$C_{12}H_{11}FN_2O_3$	234.08
02	7.79	Oleic acid	–	281.42	$C_{18}H_{34}O_2$	282.42
03	7.45	Linoleic acid	–	279.42	$C_{18}H_{32}O_2$	280.42
04	7.68	Palmitic acid	–	255.33	$C_{16}H_{32}O_2$	256.33
05	7.92	2-Hydroxy-3-(4-methoxy ethyl phenoxy)-propanoic acid	–	245.79	$C_{12}H_{16}O_5$	246.79
06	8.30	Ecgonine-methyl ester	–	222.14	$C_{10}H_{17}NO_3$	223.14
07	6.78	Rhoifolin	+	579.22	$C_{27}H_{30}O_{14}$	578.22
08	0.33	Aspartame	–	299.16	$C_{14}H_{18}N_2O_5$	300.16
09	0.33	Zopiclone N-oxide	–	387.19	$C_{17}H_{17}C_lN_6O_4$	388.19
M. esculenta						
01	7.68	Palmitic acid	–	255.33	$C_{16}H_{32}O_2$	256.33
02	7.79	Oleic acid	–	281.27	$C_{18}H_{34}O_2$	282.25
03	8.30	Naringenin-7-O-glucoside	–	417.12	$C_{21}H_{22}O_{10}$	418.12
04	8.30	Acenocoumarol	–	352.89	$C_{19}H_{15}NO_6$	353.89
05	6.59	N-Succinyl-L-diamino pimelic acid	–	291.91	$C_{11}H_{18}N_2O_7$	292.91
06	7.92	Palmitic acid	–	255.92	$C_{16}H_{32}O_2$	256.92
07	7.41	Linoleic acid	–	279.42	$C_{18}H_{32}O_2$	280.42
08	7.41	Oleamide	–	280.38	$C_{18}H_3N_O$	281.38

RT Retention time, *ESI* electrospray ionization

growth by 90%.

Compounds identification in extracts of *M. conica* and *M. esculenta* through UPLC–MS

The potential compounds identified in different extracts *M. conica* and *M. esculenta* are shown in Table 5. Nine compounds with different retention times were identified in *M. conica,* while 08 compounds were identified in *M. esculenta* using UPLC–MS. The known compounds identified in *M. conica* were: 3-Quinolinecarboxylic acid, 7-amino-1-ethyl-6-fluoro-1,4-dihydro-4-oxo, oleic acid, linoleic acid, palmitic acid, 2-Hydroxy-3-(4-methoxy ethyl phenoxy)-propanoic acid, ecgonine-methyl ester, rhoifolin, aspartame, and zopiclone N-oxide. The major compounds identified in the *M. esculenta* were: palmitic acid, oleic acid, naringenin-7-O glucoside, acenocoumarol, N-Succinyl-L-diamino pimelic acid, linoleic acid, and oleamide. Molecular weight, base peak (m/z), retention time, and molecular formula are given in Table 5.

Discussion

Decades of evolution have helped bacteria to survive in the magical era of antibiotic actions (Yagnik et al. 2021). MRSA is a high priority pathogen of WHO due to its surprisingly high resistance to currently available antibiotics. Vancomycin is the last choice antibiotic to treat MRSA infection; however, its severe side effects and emergence of bacterial resistance have limited its usage (Xu, 2020). Diseases caused by resistant organisms are a global health issue which has created the situation of dire need for the development of novel antimicrobial agents. Some approaches on antimicrobial natural products might overcome the bacterial resistance and may accelerate the discovery process of new antibiotics (Alves et al. 2014). The current study was focused on two macro-fungi (*M. conica* and *M. esculenta*) for the presence of anti-bacterial compounds and their potential effects against *S. aureus,* MRSA, and *S. pyogenes*. The results showed positive bacteriostatic and bactericidal activities of these fungal extracts against tested bacteria.

Biomolecules derived from fungal products are considered a diverse group of natural products. Secondary metabolites produced by fungi play an important role against microbial virulence by competing with them. Some of the natural products exhibit a broad spectrum of biocidal activity against human pathogenic bacteria (Jakubczyk and Dussart 2020). The discovery of penicillin by Sir Alexander Fleming in 1928 was an example of the importance of fungi in drug discovery. Screening for bioactive natural products led to the discovery of multiple antibiotics from *Streptomyces* species (Ligon, 2004; Zhu et al. 2014). Low molecular weight and

also high molecular weight compounds found in the fungus are considered to be the most responsible agents for antibacterial activity (Heleno et al. 2013). Fruiting bodies and mycelia of mushrooms accumulate various types of bioactive metabolites with, anti-inflammatory, immunomodulatory, and antimicrobial properties (Alves et al. 2012). The results of the current study for the first time showed different compounds in *M. esculenta* and *M. conica* including: oleic acid, linoleic acid, palmitic acid, and propanoic acid. Presence of bioactive compounds including polyphenolic compounds, protein and polysaccharides in *M. esculenta* are responsible for strong biological activities (Wu et al. 2021).

Both *M. esculenta* and *M. conica* extracts associated *S. aureus* and *S. Pyogenes* growth inhibition was comparable to the inhibition presented by standard antibiotics, ciprofloxacin, and tetracycline commonly used to treat *S. aureus* and *S. Pyogenes* infections respectively (Aburawi et al. 2019; Ahmad et al. 2019). Among tested solvents, methanolic extract was excellent for its promising bacterial growth inhibition. We found various concentrations of active compounds in the crude extracts which were responsible for their antibacterial activity, but the anti-bacterial potentials of these compounds were also dependent on extract preparation. For example, some fungal extracts have promising antibacterial activity when methanol was used as the extraction solvent instead of water. This property is well documented by an author from the USA, who reported the significant antibacterial activity of 25 mushrooms against bacterial pathogens when methanol was used as an extracted solvent (Hassan et al. 2019). Fungal extracts used in the current study possessed low inhibitory effects against MRSA as compared to *S. aureus* and *S. pyogenes,* but its activity was better than ciprofloxacin. The lower inhibitory effects of fungal extracts against MRSA could possibly be attributed to the presence of virulence factors, plasmid, and acquisition of resistant genes in the bacteria (McClure et al. 2018; Kumar et al. 2020). However, antibacterial activity of both mushroom extracts (*M. conica* and *M. esculenta*) against MRSA could be a notable result of the study, whereas previous studies were limited to *S. aureus* (Shameem et al. 2017; Canli et al. 2019).

The current study evaluated mushrooms efficacy using standard broth microdilution method. Several authors have declared that the broth microdilution method is more suitable for the determining potential of plant extracts. This method is ideal when given compounds are administered orally because both the compound and microorganisms have a direct contact with each other (Abbes et al. 2012; Etame et al. 2019). In the present study, the MIC values of *M. esculenta* and *M. conica* extracts were higher for MRSA than the MSSA. Importantly, the growth of MRSA was not prevented by oxacillin. These results showed that the tested MRSA are multidrug-resistant strain (Kim et al. 2005). Varying degrees of bacterial inhibition might be linked to the presence of resistance factors such as transposons, plasmid as well as the genetic makeup of the organisms, and environmental factors (Bala et al. 2011; Gebreyohannes et al. 2019). The clinical isolates are more resistant than the standard strains due to the production of enzymes, resistant genes and efflux pumping (Gebreyohannes et al. 2019).

M. esculenta extracts demonstrated ≥ 99% inhibition of *S. aureus* growth with concentration of 6.66 mg/ml. The previous study has shown that *M. esculenta*-mediated gold nanoparticles (AuNPs) effectively inhibited *S. aureus* growth (Acay 2021). The MIC was 0.4421 mg/ml and this low MIC value may possibly be explained by the positive charge on AuNPs necessary for bacterial cell content leak, formation of holes in cell walls and cell death (Rai A et al. 2010; Acay 2021). It is known that fruiting bodies of *M. esculenta* are important source of polysaccharides, sterols, fatty acids, amino acids, and several secondary metabolites which are responsible for many biological effects (Lee et al. 2018b). The results of methanolic extract of *M. conica* were of particular interest as it showed excellent growth inhibitory effects against *S. aureus* at a very low concentration. This is in parallel to a study from Serbia where the *S. aureus* was more sensitive to the *M. conica* extracts (Vieira et al. 2016). Collectively, all mushroom extracts (*M. esculenta* and *M. conica*) suppressed the *S. aureus* growth in a bactericidal manner. Shameem et al. reported no bactericidal activity of the *M. esculenta* using ethyl acetate as a solvent against *S. aureus* (Shameem et al. 2017). However, the present results are in line with a study from Serbia and Portugal where methanolic extracts of mushrooms were used (Heleno et al. 2013; Vieira et al. 2016).

Spectrophotometric reading demonstrated excellent results for the presence or absence of bacterial growth after being treated with tested compounds. A similar result was demonstrated in a previous study on *S. aureus* and other bacteria (Erdoğan Eliuz 2021). Spectrophotometric broth microdilution provides precise and accurate results for the interpretation of MIC endpoints (Arthington-Skaggs et al. 2002).

Importantly, this is the first study to report the therapeutic effects of *Morchella* extracts against MRSA. Previously, *Morchella* extracts have been studied for their hypoglycemic, immunomodulatory, antifungal effects, and anti-bacterial effects other than MRSA (Su et al. 2013; Shameem et al. 2017; Canli et al. 2019; Begum et al. 2021). It is well established that fungal extracts are a rich source of antibacterial agents and bioactive compounds with high therapeutic outputs (Gebreyohannes et al. 2019). Since centuries, the consumption of *Morchella* mushrooms as a functional food is an additional benefit (Nitha and Janardhanan 2008). The use of standard broth microdilution method, spectrophotometric analyses, UPLC–MS of mushroom extracts in the current

study is proposing its potential use in the pharmaceutical industry for the discovery of novel antimicrobial agents. Although, this small-scale study demonstrated the potential effects of *Morchella* extracts against tested bacteria, however, further research is required to elucidate their inhibitory mechanisms, toxicity and effectiveness.

Conclusion

Following conclusions can be drawn from this study: first, both fungal species (*M. conica* and *M. esculenta*) are edible mushrooms that can be considered a potential source of antibacterial agents, specifically against *S. aureus, S. pyogenes,* and MRSA. Second, the better growth inhibition of MRSA by the mushroom extracts as compared to available antibiotics is a promising result. These results highlight the use of these mushrooms in further studies. Further studies about the chemical composition, in vivo studies, and clinical trials on mushrooms must be conducted to expand the current results.

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Author contributions FUH and MI designed study and developed the original idea and wrote the manuscript; FUH performed the experiments and wrote the manuscript. MI, SS, UA, and AG contributed with the experiment's setup. All the authors read and approved the final draft.

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Data availability All the authors of the study had full access to all data and take responsibility to submit for publication.

Declarations

Conflict of interest All the authors declare that they have no competing interests.

Ethical approval The research project was approved by the University of Health Sciences Lahore, Pakistan Ethical review committee (No: UHS/Reg-21/ERC/1481).

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