



Evaluation of the phytoconstituents of *Auricularia auricula-judae* mushroom and antimicrobial activity of its protein extract

Angus Nnamdi Oli^a, Peter Anyigor Edeh^a, Reham M. Al-Mosawi^b, Nancy Amara Mbachu^c, Hussein O.M. Al-Dahmoshi^d, Noor S.K. Al-Khafaji^d, Uchechukwu Onyeukwu Ekuma^e, Ugochukwu Moses Okezie^a, Morteza Saki^{f,g,*}

^a Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Agulu, Nnamdi Azikiwe University, Awka, Nigeria

^b Department of Microbiology, Dentistry College of Basic Science, Basrah University, Basrah, Iraq

^c Department of Human Biochemistry, Faculty of Basic Medical Sciences, Nnamdi Azikiwe University Nnewi, Anambra State, Nigeria

^d Biology Department, College of Science, University of Babylon, Babylon Province-Hilla City, Iraq

^e Department of Microbiology, Eastern Palm University, Ogboko, Imo State, Nigeria

^f Department of Microbiology, Faculty of Medicine, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

^g Student Research Committee, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran

ARTICLE INFO

Keywords:

Auricularia auricular-judae

Chemical constituents

Minimum inhibitory concentrations

Mushroom

Nigeria

Protein extract

ABSTRACT

Introduction: The growing resistance to antibiotics and the complexity of defeating multi-drug resistant bacteria have led to an increase in the search for novel and effective antimicrobials from various plants. This study aimed to determine the bioactive contents of *Auricularia auricular-judae* mushroom and evaluate the antimicrobial potential of its protein extract against some selected human bacterial and fungal pathogens which could serve as a lead to the discovery of new antimicrobial agents.

Methods: The constituents of the *A. auricular-judae* were evaluated by standard phytochemical analysis methods. The agar well diffusion, micro-broth dilution, and time-kill kinetic assays were used to determine the antimicrobial activity of the extracts against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, yeast (*Candida albicans*), and dermatophytic pathogens.

Results: The preliminary phytochemical analysis of the extracts revealed the presence of carbohydrate (43.15 %; 38.30 %) proteins (23.75 %; 23.75 %), flavonoids (1.20 %; 0.80 %), alkaloids (0.60 %; 1.00 %), saponin (6.00 %; 2.40 %), tannin (1.65 %; 1.57 %), cyanide (0.24 %; 0.40 %), ash (12.40 %; 10.40 %), moisture (6.00 %; 6.00 %), lipids (6.00 %; 6.00 %), and fiber (8.70 %; 6.45 %) for the Tris buffer and warm aqueous extracts, respectively. The Tris and warm aqueous protein extracts showed antimicrobial effects toward all the human bacterial pathogens and two fungal isolates.

Conclusions: This study revealed the potential ability of *A. auricular-judae* for use as a herbal antimicrobial in the treatment of human bacterial and fungal pathogens.

1. Introduction

Irrespective of the discovery and wonders of antimicrobial drugs, microbial infection is still a leading cause of morbidity and mortality worldwide. The menace has been aggravated by the lack of new antimicrobials and the persistent emergence and re-emergence of resistant microbial strains in the last few decades. Despite the impact and benefits of antimicrobial chemotherapy in modern medicine, microbial resistance is now a global threat [1]. Today, uncritical use of antibiotics has been the leading cause in the development of drug-resistant bacteria. In addition, the consequence of acquired mutations assisting

bacterial survival has also contributed to the non-susceptibility of microorganisms to commonly used antimicrobials [1]. This has increased the prevalence and incidence of opportunistic infections significantly beyond hospitalized patients, to the general population. The complexity in treating multi-drug resistant infections has led to an increase in the search for novel and effective antimicrobials, especially structures originating from natural products [2]. Research into natural products has demonstrated significant progress in the discovery of new compounds with antimicrobial activity because nature has generously handed us a source of compounds from medicinal plants, marine and terrestrial organisms, including fungi and bacteria which all has the potential to

* Corresponding author at: Ahvaz Jundishapur University of Medical Sciences, Golestan Bld., P.O. Box: 159, Ahvaz, Khuzestan, 61357-15794, Iran.

E-mail address: mortezasaki1981@gmail.com (M. Saki).

treat diseases, including infectious diseases [2]. Today, the need for novel antimicrobials has become greater than ever in the face of increasing resistance to the older ones, which has led to the exploitation of mushroom as an alternative source of protein both as food and antimicrobials [3–5]. Mushrooms are non-photosynthetic macrofungi with a fleshy distinctive fruiting body which can be either epigenous or hypogenous and visible to the naked eye [6,7]. The biological value of mushrooms, as a food product containing a unique complex of biologically active substances, has been proved in recent decades [8,9]. Edible fungi grown on plant residues contain proteins, all essential amino acids, unsaturated fatty acids, vitamins, macro and micro elements, polysaccharides, and melanin [10]. Mushrooms are superior nutritional supplements with excellent medicinal values and have been evaluated for their nutritional status and medicinal values based on their chemical composition [3,8,11]. Outside the nutritional and medicinal properties of edible mushrooms, they've also been reported to have antimicrobial activities [12,13]. Like other bioactive compounds found in mushrooms, the proteins developed by these fungi have shown several biological activities including immunomodulatory effects, antiviral, antifungal, and antibacterial properties [3,6,8,12,14]. To date, one of the edible fungi that has biological properties is *Auricularia auricular-judae*. The *A. auricular-judae*, a species that is well known as wood ear, tree ear, or black fungus originated from China where it grows mainly in decayed trees in the mountainside and accounts for more than 98 % of the overall production of this mushroom [15,16]. The fruiting bodies of members of the genus *Auricularia* are waxy and cartilaginous, and the color ranges from purplish-brown to black, especially when they are dried. The fruiting bodies are not only shaped like a human ear but also feel like ears, due to their rubbery, gelatinous state when fresh [10,14,17].

According to the previous studies [13,18] that have shown antimicrobial effects of protein extract of some fungal species, this study hypothesized that the protein content of *A. auricular-judae* is a potential source of an alternative treatment for microbial pathogens, but its therapeutic potential against microorganisms has not been evaluated till now. Hence this study aimed to evaluate the potential activities of the protein content of this mushroom against some Gram-positive and Gram-negative bacteria, *Dermatophytes* and *Candida albicans* fungus.

2. Materials and methods

2.1. Mushroom material source

This *in vitro* study was performed from January 2020 to March 2020. The *A. auricular-judae* mushroom sample material was purchased from Abakaliki Ebonyi State, Nigeria, and authenticated by a qualified taxonomist in the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Agulu, Nnamdi Azikiwe University, Awka, Nigeria. A voucher specimen was deposited in the herbarium of the department (HER-608). The mushroom was shade-dried for 2 weeks, pulverized and stored till ready for use.

2.2. Preparation of microbial isolates

A total of 10 microbial isolates comprising of Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus subtilis*), Gram-negative bacteria (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*), yeast (*Candida albicans*) and dermatophytic pathogens (*Trichophyton schoenleinii*, *Trichophyton mentagrophytes*, *Microsporum gypseum*, and *Microsporum ferugineum*), previously collected from clinical samples in the postgraduate laboratory of the Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, were used in this study. Each of the collected isolates was re-identified and confirmed using the standard protocols as described in the literature [19–25].

2.3. Preparation of mushroom protein extracts for antimicrobial activity

2.3.1. Tris buffer extraction

To extract with Tris buffer, the protocol provided by Tehrani et al. was used [13]. The fruiting bodies of the mushroom (500 g) were washed with distilled water, then the samples were shade dried, sliced into small fragments and homogenized in 1000 ml of cold Tris buffer, 50 mM, pH 7.3 and stirred for 3–4 h. The homogenate was passed through a sieve cloth and centrifuged at 4500 rpm, for 30 min. The supernatants were saturated by adding cold acetone (80 % v/v) and left overnight in the fridge (4 °C). The collection of protein yield was done by centrifugation at 4000 × g for 20 min. This extract stored in the freezer at –20 °C until it needs.

2.3.2. Warm aqueous extraction

A 500 g of air-dried *A. auricular-judae* powder was poured into a beaker containing boiled warm water (50 °C) so that all the powder was immersed in water. The beaker was placed on a hot plate at 70 °C and allowed to stand for 4 h with intermittent stirring. After 4 h, the sample was filtered using sieve cloth. The homogenate was passed through a sieve cloth and centrifuged at 4500 rpm, for 30 min. The supernatants were saturated by adding cold acetone (80 % v/v) and left overnight in the fridge (4 °C) [7]. The protein precipitate was collected by centrifugation at 4000 × g for 20 min and stored in the freezer at –20 °C until it was needed.

2.4. Preliminary phytochemical screening

The Tris buffer and warm aqueous extracts were subjected to qualitative and quantitative preliminary phytochemical screening to determine the bioactive compounds present in the mushroom using the following described methods [7,26,27].

2.4.1. Alkaloids

Qualitative analysis was performed by adding of few drops of Mayer's reagent to solution comprised of a 0.5 g of extracts in 5 ml HCL (1%). The pale precipitate indicated the presence of alkaloids. For the quantitative evaluation of alkaloid, 200 ml of 10 % acetic acid in ethanol was mixed with 2.5 g of extract, and stored at 27 °C for 4 h. After heating on a water bath, NH₄OH was added until precipitation was finalized. After the settlement of the yield for 3 h and washing with 20 ml of 0.1 M NH₄OH, the precipitate was recovered by decanting using Gem filter paper (12.5 cm) [7]. The filtrate was oven-dried until a constant weight was achieved. The alkaloid content was calculated as:

$$\% \text{ Alkaloid} = (\text{Initial Wt of Gem filter paper} - \text{Final Wt}) 100 / \text{Wt of Crude extract used}$$

2.4.2. Flavonoids

The qualitative assay was done by adding a 0.1 g of the extract to 2 ml of distilled water. The addition of a few drops of sodium hydroxide generated an intense yellow color which became colorless on the addition of a few drops of dilute acid; suggesting the presence of flavonoids. For quantitative determination, 2.50 g of the extract was mixed with 50 ml of 80 % aqueous methanol and stored at 25–27 °C overnight. The sediment was recovered by decanting and re-extraction carried out using 50 ml ethanol. The whole solution of ethanol extract was filtered with the Whatman paper number 42 (125 mm). The filtrate was evaporated to dryness until a constant weight was gained [7]. The flavonoids content was calculated as:

$$\% \text{ Flavonoid} = (\text{Initial Wt of Crucible} - \text{Final Wt of Crucible}) 100 / \text{Wt of Crude extract used}$$

2.4.3. Saponins, tannins, carbohydrates, proteins, and other constituents

The qualitative and quantitative protocols were performed exactly according to the references mentioned [7,26,27]. The value of each ingredient was calculated based on quantitative estimation per 100 g of the dry weight of the sample.

2.5. Culture media and reference antibiotics

The malt extract agar (MEA), Mueller-Hinton agar (MHA), malt extract broth (MEB), and Mueller-Hinton broth (MHB) were purchased from Merck, Darmstadt, Germany. The ciprofloxacin (98 % HPLC) and fluconazole (98 % HPLC) were procured from Sigma-Aldrich, Michigan, USA.

2.6. Preparation of inoculums

All bacterial test isolates were cultured on MHB and incubated at 37 °C for 18–24 hours, while fungal isolates were grown in MEB at 30 °C for 48–72 hours. Then the turbidity of the suspensions was adjusted to 1.0×10^6 CFU/mL for agar well diffusion assay and micro-broth dilution [28].

2.7. Antimicrobial assay of the crude mushroom protein extract on test isolates

2.7.1. Determination of the diameter of the inhibition zone (DIZ)

The DIZ was determined by agar well diffusion. The MHA and MEA plate surfaces were inoculated by spreading a 100 µl volume of the microbial inoculum (1.0×10^6 CFU/mL) over the agar surface. Then, an 8 mm hole punched aseptically with a sterile cork borer, and a volume of 30 µl of the extract solutions was poured into the wells. Ciprofloxacin (5 µg/mL) and fluconazole (12 µg/mL) were used as a control for comparison of the results. The agar plates were incubated at 37 °C or 30 °C for 24 or 72 h depending on the test microorganism. The clear zone around the wells was measured and recorded in mm post-incubation and considered as the DIZ. This assay was done in triplicate and the results were shown as the mean value with the parallel standard deviation (SD) (28).

2.7.2. Minimum inhibitory concentrations (MIC) by micro-broth dilution

The MIC of the protein extracts was determined using micro-broth dilution in sterile 96-well microplates. A 100 µl of protein extract with concentrations of 5 µg/mL, 2.5 µg/mL, 1.25 µg/mL, and 0.62 µg/mL, were poured in the wells 1, 2, 3, and 4 of each column, respectively. Then, 100 µl of 1.0×10^6 CFU/mL of bacterial or fungal suspension inoculums was added to each well of columns 1–4. For the sample growth control, column 5 was filled by 200 µl of bacterial or fungal suspension. For the medium and protein sample sterility controls, column 6 was filled with 200 µl MHB or MEB, and column 7 was prepared with 200 µl of protein extract, respectively. Finally, column 8 was filled by 100 µl MHB containing ciprofloxacin (5 µg/mL) plus 100 µl bacterial suspensions, as a positive antibiotic control marker. For fungi, column 8 was filled by 100 µl MEB containing fluconazole (12 µg/mL) plus 100 µl of fungal suspensions, as a positive antibiotic marker. Each row of the microplate was allocated to one pathogen. The microplates containing bacteria were covered and incubated at 37 °C for 24 h while those with fungi were incubated at 30 °C for 72 h. The MIC was determined as the lowest concentration which showed no visible growth. The procedure was repeated in triplicates to confirm the antimicrobial effects.

2.7.3. Time-kill kinetics assay

The time-kill kinetics assay was performed according to the previously described method with slight modification [28]. Various values of the protein extracts were adjusted equal to MIC, $2 \times$ MIC, and $4 \times$ MIC. An inoculum of 1.0×10^6 CFU/mL was added and incubated at 37

Table 1

Phytochemical analysis of the *Auricularia auricula-judae* extracts.

Parameters	Extracts			
	Quantitative estimation (µg/100 g weight of dry sample)		Qualitative estimation (100 g weight of dry sample)	
	Tris buffer	Warm water	Tris buffer	Warm water
Moisture %	6.00	6.00	+	+
Crude protein %	23.75	23.75	++	++
Crude ash %	12.40	10.40	+	+
Crude fiber %	8.70	6.45	+	+
Crude lipid %	6.00	6.60	+	+
Carbohydrate %	43.15	38.30	++	++
Saponins %	6.00	2.40	+	+
Alkaloid %	0.60	1.00	+	+
Tannins %	1.65	1.57	+	+
Cyanide %	0.24	0.40	+	+
Flavonoid %	1.20	0.80	+	+

+ = trace, ++ = moderate.

°C. A value of 1.0 mL of the sample were taken at time intervals of 0, 1, 2, 3, 4, 5, 6, 8 and cultured aseptically into 20 mL nutrient agar and incubated at 37 °C for 24 h. Finally, the CFU of the pathogens was calculated and a graph of the log CFU/mL against time was made.

3. Results

3.1. Phytochemical analysis

In this study, the preliminary phytochemical analysis of the extracts revealed the presence of carbohydrate (43.15 %; 38.30 %), proteins (23.75 %; 23.75 %), flavonoids (1.20 %; 0.80 %), alkaloids (0.60 %; 1.00 %), saponin (6.00 %; 2.40 %), tannin (1.65 %; 1.57 %), cyanide (0.24 %; 0.40 %), ash (12.40 %; 10.40 %), moisture (6.00 %; 6.00 %), lipids (6.00 %; 6.00 %), and fiber (8.70 %; 6.45 %) for the Tris buffer and warm aqueous extracts, respectively (Table 1). The amount of extracted compounds using Tris buffer did not differ significantly (p -value > 0.05) from that obtained from warm water.

3.2. Antimicrobial activity of extracts

3.2.1. Agar well diffusion

The results of agar well diffusion are presented in Table 2. Both protein extracts showed antimicrobial effects against studied pathogens except for *T. mentagrophytes*, *M. gypseum*, and *M. ferugineum*. The Tris buffer protein extract showed the strongest effect against *E. coli* with DIZ of 6.00 ± 0.00 mm, followed by *P. aeruginosa* (4.00 ± 0.00 mm), and *S. aureus* (3.66 ± 0.53 mm). The warm aqueous extract exhibited the most effectiveness against *E. coli* and *S. aureus* with the DIZ of 3.66 ± 0.53 mm. Although ciprofloxacin and fluconazole antibiotics developed a larger inhibition zone diameter against studied pathogens than protein extracts, this difference was not significant (p -value > 0.05).

3.2.2. The MIC of the protein extracts

The MIC of protein extracts was equal to 5 µg/mL for all pathogens except for *E. coli* (2.5 µg/mL) (Table 3). In addition, the inhibitory effects of both Tris buffer and warm aqueous extracts were similar. Also, the two mushroom extracts had no activity against *T. mentagrophytes*, *M. gypseum*, and *M. ferugineum*. Among the test pathogens, the *E. coli* was the most sensitive microorganism to Tris and warm aqueous extracts.

3.2.3. Time-kill kinetics assay

The killing rate kinetics of the Tris buffer compared with that of water protein extract (at $2 \times$ MIC) against test isolates are shown in

Table 2

Diameter of the inhibition zone (mm) of Tris and warm aqueous protein extract of *Auricularia auricula-judae* against studied pathogens.

Studied pathogen	Tris buffer protein extract	Warm aqueous protein extract	Ciprofloxacin (5 µg/mL)	Fluconazole (12 µg/mL)
<i>Staphylococcus aureus</i>	3.66 ± 0.53	3.66 ± 0.53	8.66 ± 0.65	–
<i>Escherichia coli</i>	6.00 ± 0.00	3.66 ± 0.53	8.33 ± 0.65	–
<i>Pseudomonas aeruginosa</i>	4.00 ± 0.00	2.66 ± 0.65	8.33 ± 0.65	–
<i>Klebsiella pneumoniae</i>	2.33 ± 0.65	2.33 ± 0.65	7.66 ± 0.65	–
<i>Bacillus subtilis</i>	3.00 ± 0.00	3.00 ± 0.00	6.33 ± 0.65	–
<i>Candida albicans</i>	3.33 ± 0.65	1.66 ± 0.65	–	7.00 ± 0.00
<i>Trichophyton schoenleinii</i>	2.33 ± 0.65	2.00 ± 0.00	–	6.66 ± 0.65
<i>Trichophyton mentagrophytes</i>	0.00 ± 0.00	0.00 ± 0.00	–	5.66 ± 0.65
<i>Microsporium gypseum</i>	0.00 ± 0.00	0.00 ± 0.00	–	7.33 ± 0.65
<i>Microsporium ferugineum</i>	0.00 ± 0.00	0.00 ± 0.00	–	6.33 ± 0.65

Table 3

Minimum inhibitory concentrations of Tris and warm aqueous protein extract of *Auricularia auricula-judae* against studied pathogens.

Isolates	Tris buffer of protein extract	Warm aqueous of protein extract
<i>Staphylococcus aureus</i>	5 µg/mL	5 µg/mL
<i>Escherichia coli</i>	2.5 µg/mL	2.5 µg/mL
<i>Pseudomonas aeruginosa</i>	5 µg/mL	5 µg/mL
<i>Klebsiella pneumoniae</i>	5 µg/mL	5 µg/mL
<i>Bacillus subtilis</i>	5 µg/mL	5 µg/mL
<i>Candida albicans</i>	5 µg/mL	5 µg/mL
<i>Trichophyton schoenleinii</i>	5 µg/mL	5 µg/mL
<i>Trichophyton mentagrophytes</i>	–	–
<i>Microsporium gypseum</i>	–	–
<i>Microsporium ferugineum</i>	–	–

Figs. 1 and 2 (the data of protein extracts equal to 1 × MIC, and 4 × MIC are not shown). The killing rate kinetic of both extracts was time- and concentration-dependent (data are not shown). Both extract were bacteriostatic at 1 ×, 2 ×, and 4 × MIC levels. Linear regression

analysis of the killing kinetics on *S. aureus* showed that the slopes of two extracts were not significantly different (p -value = 0.071). However, the elevations or intercepts were very significantly different (p -value = 0.007) meaning that a known microbial load of the organisms was going to be cleared at significantly different times. For the *E. coli*, slopes were quite significantly different (p -value = 0.005). The Tris buffer protein extract killed the organism significantly faster than the water extract. For the *P. aeruginosa*, the slopes and the elevations (or intercepts) were not significantly different (p -value = 0.329 and 0.677 respectively). The rates of killing were similar. Also, a known microbial load of the organisms was going to be cleared at equal times. The anti-pseudomonads activities of the extracts were similar. For the *K. pneumoniae*, the slopes were extremely significantly different (p -value = 0.004). The Tris-buffer protein extracts extremely killed the organism much faster than the water protein extract. For the *B. subtilis*, the killing rates of the two extracts did not differ significantly (p -value = 0.158). Also, the elevations or intercepts were not significantly different (p -value = 0.350). For *C. albicans*, the differences between the slopes were significantly different (p -value = 0.028). The Tris-buffer extract performed better. For *T. schoenleinii*, the slopes and intercepts did not differ

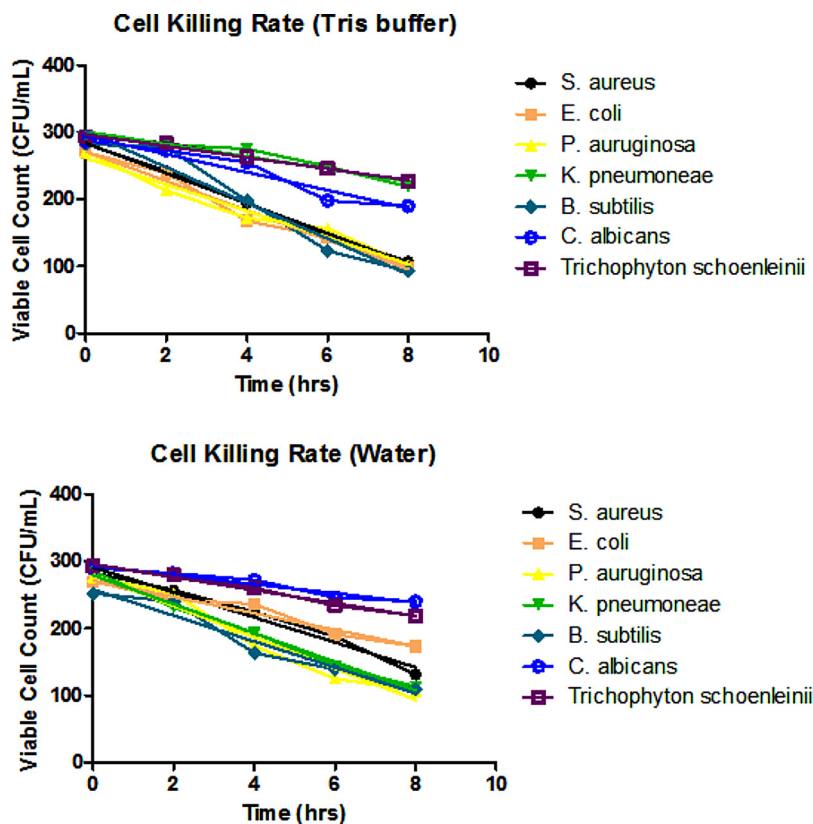


Fig. 1. Cells killing rates of the Tris buffer and warm water protein extract (at 2xMIC) against test isolates.

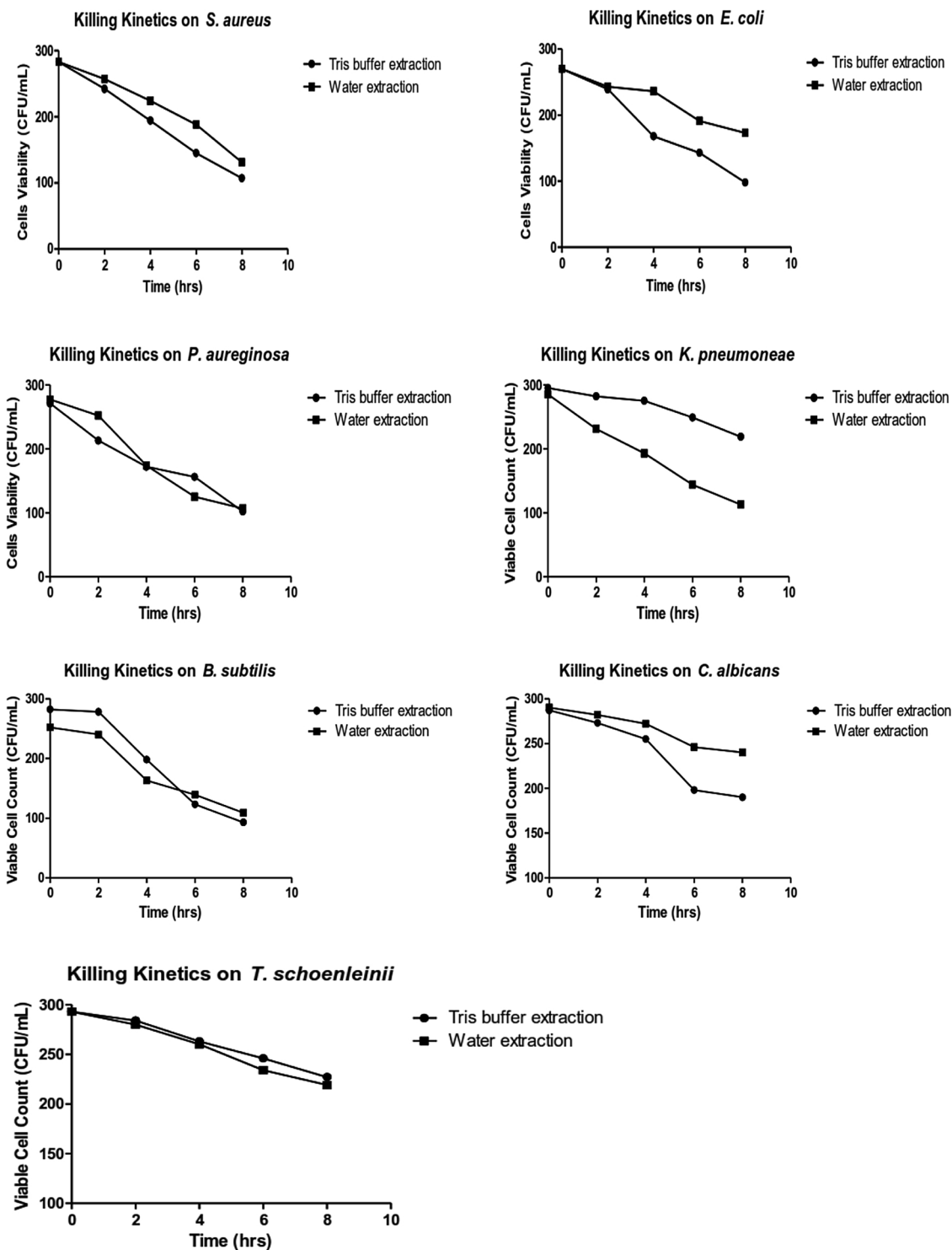


Fig. 2. Killing rate kinetics of the Tris buffer compared with that of water protein extract (at 2xMIC) against test isolates.

significantly (p -value > 0.05). Both extracts have similar killing kinetics.

4. Discussion

The development of resistance to commonly used antibiotics amongst human pathogens is partly due to the misuse of these antimicrobial agents, which has led humans to seek new and alternative therapies. In this study, the phytochemical properties and anti-microbial effects of Tris buffer and warm water protein extracts of dry *A.*

auricula-judea mushroom were evaluated. The phytochemical analysis of crude extracts revealed the presence of carbohydrate (43.15 %; 38.30 %), proteins (23.75 %; 23.75 %), flavonoids (1.20 %; 0.80 %), alkaloids (0.60 %; 1.00 %), saponin (6.00 %; 2.40 %), tannin (1.65 %; 1.57 %), cyanide (0.24 %; 0.40 %), ash (12.40 %; 10.40 %), moisture (6.00 %; 6.00 %), lipids (6.00 %; 6.00 %), and fiber (8.70 %; 6.45 %), which was in line with the works of Essien et al. [29], Khan et al. [30], and Deka et al. [31]. Although the amount of protein extracted by the two solvents did not differ in this study, previous reports have shown that the type of solvent used in the extraction, temperature, pH, duration of

extraction, and the presence of interfering materials can affect the extraction process [32,33]. The results of this study revealed variable antimicrobial effects of the protein extracts on the selected organisms confirming the works of Tehrani et al. [13], and Kavitha et al. [34] which recorded that Tris buffer and ultrasonic-assisted protein extracts of *Agaricus* mushroom were active against *S. aureus*, *B. subtilis*, *P. aeruginosa*, *K. pneumoniae*, and *E. coli*. However, in the work of Zheng et al. [35], the mushroom protein extracts were not active against *Pseudomonas batatae*, *Erwinia herbicola*, *E. coli*, and *S. aureus*. In another study by Afzal et al. [36], the antimicrobial ability of protein extracts from six medicinal plants (*Feniculum vulgare*, *Peganum harmala*, *Azadirachata indica*, *Ocimum basilicum*, *Cichorium intybus*, and *Ricinus communis*) was reported against *S. aureus* 6736152. Irrespective of the fact that the Tris buffer and warm water protein extracts had no antifungal effects on the *T. mentagrophytes*, *M. gypseum*, and *M. ferugineum*, they showed slight activity against *C. albicans*, and *T. schoenleinii*. These weak antifungal effects were in line with the study by Appiah et al. [28], who reported that methanol extracts of *Trametes elegans*, *Trametes gibbosa*, *Volvariella volvacea*, and *Schizophyllum commune* mushrooms did not affect *Aspergillus niger*, *Aspergillus flavus*, and *Aspergillus tamaritii*. It appears that the antibacterial properties of mushrooms are stronger than their antifungal properties. The Tris buffer and warm water protein extracts of *A. auricula-judea* indicated activity against all the bacteria isolates, *C. albicans*, and *T. schoenleinii* with a MIC of 5 µg/mL except for *E. coli*, whose MIC was 2.5 µg/mL. There was no significant difference between the effects of the extracts on Gram-negative and Gram-positive bacteria, while previous studies reported that mushroom extracts have more antimicrobial activity against Gram-positive bacteria in comparison to Gram-negative bacteria [37]. It seems that some fungi have genes that can secrete peptides and proteins with antibiotic-like properties that can eradicate the both Gram-positive and Gram-negative bacteria [18]. In the current research, the diameter of inhibition zone of studied protein extracts against the tested pathogens was lower than the ciprofloxacin and fluconazole antibiotics, while in a study by Al Akeel et al. [38], the results have revealed a very robust effect of *A. ascolonicum* medicinal plant against *E. coli* and *Proteus vulgaris* with an inhibition zone of 17 mm, which was more than the control antibiotic chloramphenicol (25 µg) with a zone of inhibition equal to 8 mm.

So far, few studies have examined the time-kill kinetics of mushrooms against microorganisms. In this study, the time-kill kinetic assay revealed that Tris buffer and warm water protein extracts of *A. auricula-judea* had bacteriostatic properties that were contrary to findings of Tinrat [39] who reported bactericidal activity of some mushrooms in his study. In line with the finding of the present research, Appiah et al. [28], showed that methanol extracts of *T. elegans*, *S. commune*, *V. volvacea*, and *T. gibbosa* mushrooms had bacteriostatic action against *E. coli* ATCC 25922, *S. aureus* ATCC 25923, and *C. albicans* ATCC 25923. Besides, this study showed that both Tris buffer and warm water protein extracts had similar killing kinetics which approved that irrespective of the extraction solvent, the killing rate is dependent on the duration of exposure and concentration.

5. Limitations

In this study, due to the traffic restrictions because of the Coronavirus disease COVID-19-related pandemic, we were unable to perform work on more samples and the study was completed in less than the allotted time. Due to the lack of enough financial sources the protein fractions extracted from mushroom were not analyzed and determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and chromatography. Besides, the mechanisms of action of *A. auricula-judea* against the test pathogens were not investigated. Further work should address these issues.

6. Conclusions

This study documented that Tris and warm aqueous protein extracts of *A. auricula-judea* had antimicrobial effects against all the human bacterial pathogens and two fungal isolates; and therefore can be concluded that *A. auricula-judea* protein may have a potential antimicrobial fraction that can be used as an antimicrobial protein/peptides (AMP) in the treatment of human bacterial and fungal infections. The results showed that although the extracts are active against these organisms, they require enough time to exhibit their inhibition properties on the organisms. Before this study, *A. auricula-judea*, an edible tender mushroom that has quite a significant amount of protein has only been studied on a whole extract basis. The activities of the mushrooms bioactive substances have been published by several authors but nothing has been done on the proteins of this therapeutic and nutritional mushroom to evaluate its antimicrobial properties against some important human pathogens. With the results presented in this research, the alternate hypothesis is accepted that *A. auricular-judea* proteins have antimicrobial effects on human pathogens. Determining the active fractions of these proteins and their antimicrobial effect in the *in vivo* studies or in combination with other chemicals or nanoparticles can be the subjects of future research.

Financial support

None.

Data availability

Supplementary material related to this article can be obtained from the corresponding author on request.

CRediT authorship contribution statement

Angus Nnamdi Oli: Conceptualization, Data curation, Investigation, Validation, Visualization, Formal analysis, Software, Supervision, Writing - review & editing. **Peter Anyigor Edeh:** Conceptualization, Data curation, Investigation, Validation, Visualization, Formal analysis, Software, Supervision, Writing - review & editing. **Reham M. Al-Mosawi:** Methodology, Project administration, Software, Supervision, Writing - review & editing. **Nancy Amara Mbachu:** Conceptualization, Data curation, Investigation, Validation, Visualization, Formal analysis, Methodology, Project administration, Supervision, Writing - review & editing. **Hussein O.M. Al-Dahmishi:** Methodology, Project administration, Writing - original draft, Software, Supervision, Writing - review & editing. **Noor S.K. Al-Khafaji:** Methodology, Project administration, Writing - original draft, Software, Supervision, Writing - review & editing. **Uchekukwu Onyeukwu Ekuma:** Methodology, Project administration, Software, Supervision, Writing - review & editing. **Ugochukwu Moses Okezie:** Methodology, Project administration, Software, Supervision, Writing - review & editing. **Morteza Saki:** Writing - original draft, Software, Supervision.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Acknowledgments

None.

References

- [1] B. Li, T.J. Webster, Bacteria antibiotic resistance: new challenges and opportunities for implant-associated orthopedic infections, *J. Orthop. Res.* 36 (1) (2018) 22–32.
- [2] M.A. Hayashi, F.C. Bizerra, P.I.D.S. Junior, Antimicrobial compounds from natural

- sources, *Front. Microbiol.* 4 (2013) 195.
- [3] M.E. Valverde, T. Hernández-Pérez, O. Paredes-López, Edible mushrooms: improving human health and promoting quality life, *Int. J. Microbiol.* 2015 (2015) 376387.
- [4] C. Paul, T. Roy, N. Das, Potentiality of oyster mushroom (*Pleurotus* spp.) in medicine—a review, *Ann. Food. Process. Preserv.* 2 (2) (2017) 1014.
- [5] H.S. Shen, S. Shao, J.C. Chen, T. Zhou, Antimicrobials from mushrooms for assuring food safety, *Compr. Rev. Food Sci. Food Saf.* 16 (2) (2017) 316–329.
- [6] S. Parihar, et al., Phytochemical screening, total phenolic content, antibacterial and antioxidant activity of wild edible mushroom *Pleurotus ostreatus*, *Int. Res. J. Pharm.* 6 (1) (2015) 65–69.
- [7] A.N. Oli, et al., *Pleurotus ostreatus* extract enhances the phagocytic actions of neutrophils against *Streptococcus pneumoniae*, *Ann. Med. Health Sci. Res.* 9 (2) (2019) 499–508.
- [8] J.J. Zhang, Y. Li, T. Zhou, D.P. Xu, P. Zhang, S. Li, H.B. Li, Bioactivities and health benefits of mushrooms mainly from China, *Molecules* 21 (7) (2016) 938.
- [9] S. Khatua, S. Ghosh, K. Acharya, Chemical composition and biological activities of methanol extract from *Macrocybe lobayensis*, *Int. J. Appl. Pharm. Sci.* 7 (10) (2017) 144–151.
- [10] A.R. Bandara, S. Rapior, P.E. Mortimer, P. Kakumyan, K.D. Hyde, J. Xu, A review of the polysaccharide, protein and selected nutrient content of *Auricularia*, and their potential pharmacological value, *Mycosphere* 10 (1) (2019) 579–607.
- [11] C.S. Eze, J.E. Amadi, A.N. Emeka, Survey and proximate analysis of edible mushrooms in Enugu State, Nigeria, *Ann. Exp. Biol.* 2 (3) (2014) 52–57.
- [12] H.T. Feleke, A. Doshi, Antimicrobial activity and bioactive compounds of Indian wild Mushrooms, *Indian J. Nat. Prod. Resour.* 8 (3) (2018) 254–262.
- [13] M.H. Tehrani, E. Fakhrohoseini, M.K. Nejad, H. Mehregan, M. Hakemi-Vala, Search for proteins in the liquid extract of edible mushroom, *Agaricus bisporus*, and studying their antibacterial effects, *Iran. J. Pharm. Res.* 11 (1) (2012) 145–150.
- [14] P.G. Miles, S.T. Chang, *Mushrooms: Cultivation, Nutritional Value, Medicinal Effect, and Environmental Impact*, CRC press, 2004.
- [15] Y. Zhao, et al., Comparative transcriptome analysis reveals the relationship of three major domesticated varieties of *Auricularia auricula-judae*, *Sci. Rep.* 9 (1) (2019) 78.
- [16] F.J. Yao, et al., Development of a molecular marker for fruiting body pattern in *Auricularia auricula-judae*, *Mycobiology* 46 (1) (2018) 72–78.
- [17] H. Bao, S. You, L. Cao, R. Zhou, Q. Wang, S.W. Cui, Chemical and rheological properties of polysaccharides from fruit body of *Auricularia auricular-judae*, *Food Hydrocoll.* 57 (2016) 30–37.
- [18] A. Kombrink, et al., Induction of antibacterial proteins and peptides in the coprophilous mushroom *Coprinopsis cinerea* in response to bacteria, *ISME J.* 13 (3) (2019) 588–602.
- [19] P. Afrough, M.R. Pourmand, A.A. Sarajian, M. Saki, S. Saremy, Molecular investigation of *Staphylococcus aureus*, *coa* and *spa* genes in Ahvaz hospitals' staff nose compared with patients clinical samples, *Jundishapur J. Microbiol.* 6 (4) (2013) e5377.
- [20] S. Yazdaneshtad, et al., Preliminary survey of extended-spectrum β -lactamases (ESBLs) in nosocomial uropathogen *Klebsiella pneumoniae* in north-central Iran, *Heliyon* 5 (9) (2019) e02349.
- [21] A. Farajzadeh Sheikh, et al., Prevalence and antimicrobial resistance of *Shigella* species isolated from diarrheal patients in Ahvaz, southwest Iran, *Infect. Drug Resist.* 12 (2019) 249–253.
- [22] C. Ejikegwu, et al., Characterization of metallo- β -lactamases-encoding genes *bla_{IMP-1}* and *bla_{VIM-1}* amongst *Klebsiella pneumoniae* from abattoir samples of Ebonyi state, southeastern Nigeria, *Gene Rep.* 16 (2019) 100428.
- [23] M. Moosavian, et al., Prevalence of enterotoxin-encoding genes among diverse *Shigella* strains isolated from patients with diarrhea, southwest Iran, *Acta Microbiol. Immunol. Hung.* 66 (1) (2019) 91–101.
- [24] L.V. de Sousa, et al., Isolation and identification of *Candida* species in patients with orogastric cancer: susceptibility to antifungal drugs, attributes of virulence in vitro and immune response phenotype, *BMC Infect. Dis.* 16 (1) (2016) 86.
- [25] M.F. Shalaby, A.N. El-Din, M.A. El-Hamd, Isolation, identification, and *in vitro* antifungal susceptibility testing of dermatophytes from clinical samples at Sohag University Hospital in Egypt, *Electron. Physician* 8 (6) (2016) 2557–2567.
- [26] G.E. Trease, W.C. Evans, *Textbook of Pharmacognosy*, Bailliere Tindall, London, UK, 2002.
- [27] J.B. Harborne, *Phytochemical Methods-A Guide to Modern Techniques of Plant Analysis*, Chapman & Hall, London, UK, 1998.
- [28] T. Appiah, Y.D. Boakye, C. Agyare, Antimicrobial activities and time-kill kinetics of extracts of selected Ghanaian mushrooms, *Evid. Based Complement. Alternat. Med.* 2017 (2017) 4534350.
- [29] E.E. Essien, I.I. Udoh, N.S. Peter, Proximate composition and anti-nutritive factors in some wild edible medicinal macrofungi, *J. Nat. Prod. Plant. Resour.* 3 (6) (2013) 29–33.
- [30] A.A. Khan, M.M. Jahangir, G.S. Fozia, K. Ziaf, Nutritional and chemical profiles of *Auricularia auricular* mushrooms: a review, *Int. J. Agric. Environ. Res.* 2 (2016) 185–191.
- [31] A.C. Deka, I. Sarma, S. Dey, T.C. Sarma, Antimicrobial properties and phytochemical screening of some wild macrofungi of Rani-Garbhanga reserve forest area of Assam, India, *Adv. Appl. Sci. Res.* 8 (3) (2017) 17–22.
- [32] A. Altemimi, N. Lakhssassi, A. Baharlouei, D.G. Watson, D.A. Lightfoot, Phytochemicals: extraction, isolation, and identification of bioactive compounds from plant extracts, *Plants (Basel)* 6 (4) (2017) 42.
- [33] É.R. Oliveira, G.R. Carvalho, M.Á. Cirillo, F. Queiroz, Effect of ecofriendly bio-based solvents on oil extraction from green coffee bean and its industrial press cake, *Braz. J. Chem. Eng.* 36 (4) (2019) 1739–1753.
- [34] R. Kavitha, N. Damodharan, D. Uwadokakelechi, B.B. Olabiyi, Ultrasonic-assisted extraction and antibacterial activities of protein recovered from white button mushroom (*Agaricus bisporus*), *Int. J. Pharm. Tech. Res.* 8 (5) (2015) 991–995.
- [35] S.Y. Zheng, Q.H. Liu, G.Q. Zhang, H.X. Wang, T.B. Ng, Purification and characterization of an antibacterial protein from dried fruiting bodies of the wild mushroom *Clitocybe sinopica*, *Acta Biochim. Pol.* 57 (1) (2010) 43–48.
- [36] M. Afzal, A. Bakhsh, M.R. Ahmad, I. Manzoor, S. Liaquat, Comparison of the antimicrobial activity of seed protein extracts from six medicinal plants against *Staphylococcus aureus* 6736152, *Emir. J. Food Agric.* 23 (1) (2011) 103–109.
- [37] D. Matijašević, et al., The antibacterial activity of *Coriolus versicolor* methanol extract and its effect on ultrastructural changes of *Staphylococcus aureus* and *Salmonella Enteritidis*, *Front. Microbiol.* 7 (2016) 1226.
- [38] R. Al Akeel, Y. Al-Sheikh, A. Mateen, R. Syed, K. Janardhan, V.C. Gupta, Evaluation of antibacterial activity of crude protein extracts from seeds of six different medicinal plants against standard bacterial strains, *Saudi J. Biol. Sci.* 21 (2) (2014) 147–151.
- [39] S. Tinrat, Antimicrobial activities and synergistic effects of the combination of some edible mushroom extracts with antibiotics against pathogenic strains, *Int. J. Pharm. Sci. Rev. Res.* 35 (2) (2015) 253–262.