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Examination of the chemical profile of methanolic extract of *Agaricus bisporus* wild edible mushroom, Zarnagh region (East Azerbaijan province, Iran)

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ABSTRACT

Purpose: The main purpose of this study is to investigate the chemical, physicochemical, and antibacterial properties of *Agaricus bisporus* collected from the Zarnagh region (province of East Azerbaijan, northwestern Iran), to enhance health quality, and economic exploitation in pharmaceutics, food, and agriculture industries. The methanol extracts of *Agaricus bisporus* mushroom (maceration extraction method), was screened for chemicals properties. **Research Method:** All chemicals were purchased from Merck (Darmstadt, Germany). Fruiting bodies of wild edible mushrooms (*A. bisporus*) were collected from the Z. region. The methanolic extracts were evaluated by Gas Chromatography-Mass Spectrometry and High-performance liquid chromatography coupled to the ultraviolet detector analysis. The antibacterial activities were carried out by using three bacteria. **Findings:** The results of chemical and nutritional factors include crude protein (46.62 ± 0.19 g/100g), crude fat (10.59 ± 0.13 g/100g), fiber (17.76 ± 0.32 g/100g), carbohydrate (1.56 ± 0.27 g/100g) and total energy (288.3 ± 2.61 Kcal). Potassium, iron, calcium, phosphorus, sodium, and copper in considerable quantities were found in the wild edible mushroom from the Z. region. The eight compounds were identified in the crude methanolic extract by GC-MS analysis. Rutin, myricetin, quercetin, and kaempferol identified by HPLC-UV analysis. The highest inhibitory activity was noticed against *E. coli* (PTCC 1399) with 22±0.2 mm (diameter of inhibition zone) for methanol extract.**Limitations:** There was no significant limitation to the report. **Originality/Value:** Briefly, there is no much information about the chemical profile of wild mushrooms of Iran, and this work is the first study on the chemical and antibacterial properties of *Agaricus bisporus* mushrooms.

INTRODUCTION

Mushrooms are heterotrophs, and they are generally found to grow in all types of soils, forests, open fields, mountains and hills, deserts, deadwood logs, tree stumps, or with same decomposed organic residues. The beneficial properties of mushrooms are widely used for medicinal and therapeutic purposes because most chemical compounds naturally found in the mushroom acted as clinically proven drug models to prevent infectious diseases (Rimantas, 2015). At least 2,000 species (from 38000 mushroom varieties) showing various degrees of edibility, and they have been recognized to have antibacterial properties. Today, the increase of resistance by pathogens to many common antibiotics is motivating further efforts to seek new antimicrobial drugs to combat infectious diseases. Most of the discovered antibacterial compounds are derived from plants and microorganisms (Sjollema et al., 2018). According to reports (Liktor-Busa et al., 2016; Stojkoví et al., 2014; Gogavekar et al., 2014), antibacterial properties of mushroom extracts are commonly determined using bioassays, including agar well diffusion tests, disk diffusion method, and microdilution assays that define the inhibitive activities against targeted microorganisms. One of the main problems in identifying novel sources of antimicrobial mushrooms is the difficulty of extrication them from their complex matrix and intracellular location, avoiding the use of large amounts of solvent and long extraction time. Conventional extraction techniques are prone to longer extraction time, the need for costly and high purity solvent, evaporation of the considerable value of solvent, and low extraction metabolite. In the meantime, novel methods have been grounded in reducing extraction time and solvent consumption, increasing extraction yield and enhancing extracts quality. Water, ethanol, methanol, and several other organic solvents, including chloroform, acetone, dichloromethane, hexane, and ethyl acetate, are the dominant solvents utilized in extracting mushroom chemical properties. Temperature is one of the significant factors, although a higher temperature may also result in the degradation of temperature-sensitive phenolic compounds. The nature of plant matrices, the analyte of interest, and pertinent analytical purpose can affect extraction kinetics, choice of the right solvent, and efficiencies of modern extraction techniques, however, no universal extraction is ideal, and each extraction procedure is unique to each plant (Khoddami et al., 2013). Other studies, indicated that Gas chromatography-mass spectrometry analysis of flavonoids and non-flavonoids standards was more efficient than that of other methods for quantification of phenolics, providing a brief analysis with better resolution and baseline separation of all means with minimum co-elution but, the high yield liquid chromatography (HPLC) quantification assay is significantly more common than the GC method, as it can lower temperatures during the analysis, thereby reducing the risk of double bonding of methyl esters without needing preliminary derivatization, It makes to facilitate the analysis time (Alara et al., 2021). Phenolic compounds are defense factors in plants and mushrooms that have been studied in various studies on mushrooms. It can be seen that a wide range of these compounds have been identified by high-performance liquid chromatography (Dulay et al., 2017; Cvetanović et al., 2019). In these studies, it has been proven that the consumption of these compounds acts as antioxidants for humans and helps prevent cardiovascular disease, reduce inflammation and ulcers, prevent cancer and diabetes, and also reduce the rate of mutation in human cells (Dzah et al., 2020; Gründemann et al., 2020).

The aims of this study are to investigate the metabolites that make up the *Agaricus bisporus* including phenolic compounds, proteins, carbohydrates, and a group of mineral elements. In addition, the antibacterial properties of the fungus evaluated against three types of gram-positive and gram-negative bacteria.

MATERIALS AND METHODS

Mushroom and chemicals

All chemicals used in this study were analytical grade and were purchased from Merck (Darmstadt, Germany). Four flavonoid compound standards, including myricetin, quercetin, kaempferol, and rutin, were purchased from Sigma Aldrich (MO, USA). Fruiting bodies of wild edible mushrooms (*A. bisporus*) were collected from the Z. region (East Azerbaijan in northwestern Iran) during April and May of 2018 (Table 1). Figure 1 shows the geographical location of the Z. region.

Preparation of macro fungi extracts

The fresh edible wild mushrooms have cut into near 0.5 cm^2 pieces and repeatedly washed for 15 min, with distilled water to clear any organic impurities available on them. The cleaned mushrooms are then crushed into small pieces. The chopped parts of wild edible mushrooms are dried for seven days in a dark and dry place (Ren et al., 2014). After, it was powdered with an electronic mill, which shown in Figure 2. A fair dried mushroom powder sample (15 g) was extracted by stirring with 150 mL of solvent (methanol, ethanol, acetone, water, and ethyl acetate) at 25 °c at 150 rpm for 72 h and filtered through Whatman No. 4 paper. The combined solvent extracts were evaporated at 40 °c to dryness and redissolved in a known concentration (Borokini et al., 2016). Other solvents such as (ethanol, ethyl acetate, acetone, and aqueous) were also extracted to evaluate the antibacterial properties of the fungus by the above method (Borokini et al., 2016).

Table 1. Details of wild edible collected mushroom and GPS status

Class	Basidiomycetes
Subclass	*Hymenomycetes*
Order	*Agaricales*
Family	*Agaricaceae*
Genus	*Agaricus*
Scientific name	*Agaricus bisporus*
English name	Button mushroom
Local name	Çimen evelek mantari
Date of collected	April and May of 2018
Region of collected	Zarnagh region (province of East Azerbaijan)
Country of origin	Iran

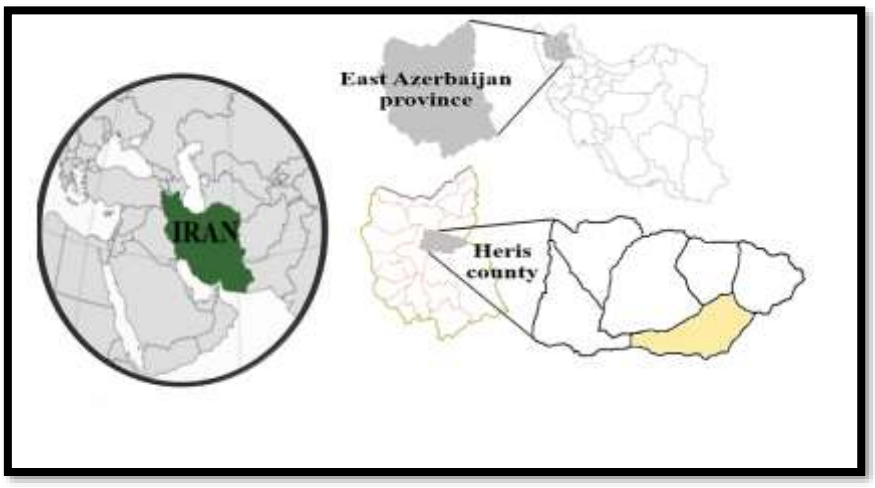

Fig. 1. Geographical location of the Z. region (East Azerbaijan province, Heris county, Iran).

Fig. 2. Steps for making the powder from *A. Bisporus* (left to right).

Proximate composition and mineral analysis

Fiber, fat, moisture, protein, and ash amounts of the fruiting bodies of *A. bisporus* mushrooms were determined by the methods of AOAC International (Gogavekar et al., 2014). Ash amount was analyzed by weighing the samples after and before the ignition at 570 °C for eight hours. Protein content was determined by the Kjeldahl method, its amount was gained by multiplying the corresponding total nitrogen content by a factor of 6.25. Carbohydrate amount was defined by difference, whereas energy was calculated using Atwater's calorie conversion factors: four kcal/g for crude protein, nine kcal/g for fat, and four kcal/g for available carbohydrate. Potassium, iron, calcium, phosphorus, sodium, and copper were determined according to the method of AOAC International. Analysis of minerals was performed using the atomic absorption spectrophotometer of the Shimadzu (AA-6300) model (Gogavekar et al., 2014).

Quantification of chemical compounds by Gas chromatography-mass spectrometry (GC-MS)

Gas chromatography-mass spectrometry analysis was carried out to study the significant bio-compounds present in the methanolic extract of *A. bisporus*. Summarily for GC-MS analysis of samples, 1µl of the methanolic extract of *A.bisporus* was injected into Agilent 7890B gas chromatograph connected with a mass detector (Model 5977A, Agilent Technologies, USA). The gas chromatograph was equipped with an HP-5MS capillary column (phenylmethyl siloxane, 30 m × 0.25 mm ID 0.25um, Agilent technologies). The injector temperature was 270 ℃. The oven temperature was tuned from 60 ℃ to 200 ℃ at the rate of 5 ℃/min. Helium was selected as the carrier gas. Also, the mass spectrometer was set in EI mode at 70 eV. The spectrum of unknown compounds was compared with the known compounds stored in the National Institute Standard to ascertain the name and structure of the compounds (Khoddami et al., 2013).

Qualitative analysis of phenolic compounds by high-performance liquid chromatography with ultraviolet detection (HPLC-UV)

The HPLC system (Agilent, 1260 Infinity II model, USA) was equipped with a reverse-phase chromatographic analysis, Shimpak C18 column (4.6 mm x 250 mm) packed with 5µm diameter particles. The mobile phase contained methanol: acetonitrile: acetic acid: phosphoric acid: water in a ratio, 20:10:1:1:20 (v:v:v:v:v), at a flow rate of 1.0 ml/min and the injection volume was 20 µl. The UV detector wavelength was set at 352 nm. The analytical column used was kept at 25℃. The presence of 4 compounds was investigated in *A. bisporus*. Four flavonoid compound standards, including myricetin, quercetin, kaempferol, and rutin, were purchased from Sigma Aldrich (MO, USA). The stock solutions of standards were prepared with analytical accuracy dissolving 1 mg of standard substance (a mix of quercetin, myricetin, kaempferol, and rutin) in 10 mL of methanol. The peaks and retention time of stock solution of standard chromatogram by HPLC-UV was compared with the methanolic extract

chromatogram prepared from *A. bisporus* for identification and qualitative analysis of phenolic compounds (quercetin, myricetin, kaempferol, and rutin) in this wild edible mushroom (Khoddami et al., 2013; Thanasekaran et al., 2009).

Antibacterial activity determination

The antibacterial activity of aqueous, methanolic, ethanolic, ethyl acetate, and acetone extracts of the *A. bisporus* mushroom against three bacteria were challenged in this study by using the agar well diffusion method (Table 2).

In this study, the agar plate surface is inoculated by spreading a volume of the microbial inoculum over the entire agar surface, Then, a hole with a diameter of 6 to 8 mm is punched aseptically with a sterile corn borer or a tip, and a volume (20–100 µL) of the antimicrobial agent or extract solution at desired concentration is introduced into the well. So, agar plates are incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested. Briefly, the inoculated plates were incubated for 48-72 hr, at 37 °C, a diameter of the inhibition zone was measured and expressed in millimeters (Thanasekaran et al., 2009; Balouiri et al., 2016; Dulay et al., 2017).

RESULTS AND DISCUSSION

Chemical profile

The chemical composition results of *A. bisporus* wild edible mushrooms analysis was presented in Table 3. The content of macronutrients (dried weight basis) is 46.62 ± 0.19 g/100 g for protein, 1.56 ± 0.27 g/100 g for carbohydrate, 10.59 ± 0.12 g/100 g for lipid, 17.76 ± 0.32 g/100 g for fiber, and total energy was 288.3 ± 2.61 Kcal/100 g. According to Table 3, the moisture amount of the *A. bisporus* mushroom was 13.42 ± 0.28 g/100 g. the ash amount of mushroom was 10.53 ± 0.07 g/100 g on a dry weight basis. Essential elements in wild mushrooms play a vital role in the proper development of human health. Six minerals (Cu, Fe, Ca, K, P, and Na) were determined in *A. bisporus* wild edible mushroom (collected from Z. region).

According to the results in Table 3, the most abundant elements are potassium and phosphorus; respectively, copper was the lowest element presented in this mushroom (36.50 ± 0.75 mg/100 g on a dry weight basis). In a study that was done (Wang et al., 2014), showed that the amount of fatty acids among locally wild edible mushrooms is higher than the local species that are cultivated, the amount for local wild edible mushrooms is between 4.3 to 12.7 percent of the dry weight of the mushrooms (g /100 g). This amount was recorded for cultivated fungi between 0.7 to 4.5 percent of the dry weight of the mushrooms (g /100 g). The average amount of protein in mushrooms is reported to be between 12 to 30 percent of the dry weight (g /100 g) of the fungus. In addition, the carbohydrate content of different mushrooms (g /100 g) was reported to be between 13 to 65 percent of the dry weight of mushrooms on average (Wang et al., 2014). Therefore, this mushroom is a good source of protein, carbohydrate, fat, and fiber for human consumption at nutritional and medicinal levels.

Table 2. Characteristics of the bacteria used

Bacteria	Standard number	Garam
Escherichia coli	PTCC 1399	Negative
Pseudomonas aeruginosa	PTCC1430	Negative
Staphylococcus aureus	ATCC25925	Positive

GC-MS analysis

GC-MS analysis for the *A. bisporus* methanolic extract was shown in (Fig. 3). In this study, eight compounds are identified in the crude methanolic extract.

A variety of compounds has been detected in *A. bisporus* including 1-Propanol, 2-(2-hydroxypropoxy), Ethylene brassylate, Methyl pimar-7-en-18-oate, Methyl isodextropimarate, Methyl 8-isopimaren-18-oate, Methyl 8-pimaren-18-oate, Formic acid, 2-bromomethyl-4,4-dimethyl-3-(3-oxobut-1-enyl) cyclohexyl-2-enyl ester, and Methyl pimar-8(14)-en-18-oate (Table 4). According to Table 4, among the identified compounds, only Ethylene brassylate (Butron et al., 2019), has a lot of use in the health and cosmetics industry and has been found in some plants that have an acceptable percentage in this mushroom. Other compounds are also found in some articles (Costa et al., 2013), on mushrooms in small quantities.

Table 3. Macronutrients profile of the *A. bisporus* wild edible mushroom collected from the Z. region basis)

Components	Amount[a]
Moisture (g/100 g)	13.42 ± 0.28
Ash (g/100 g)	10.53 ± 0.07
Protein (g/100 g)	46.62 ± 0.19
Carbohydrate (g/100 g)	1.56 ± 0.27
Fat (g/100 g)	10.59 ± 0.13
Fiber (g/100 g)	17.76 ± 0.32
K (mg/100 g)	22615.25 ± 2.18
P (mg/100 g)	1194.38 ± 2.12
Ca (mg/100 g)	107.12 ± 3.83
Na (mg/100 g)	357.0 ± 2.43
Fe (mg/100 g)	62.5 ± 1.76
Cu (mg/100 g)	36.50 ± 0.75
Energy (Kcal/100 g)	288.3 ± 2.61

[a] Values represent means of triplicate readings ± S.D. Values with the same superscript along the row are not significantly different ($p \geq 0.05$).

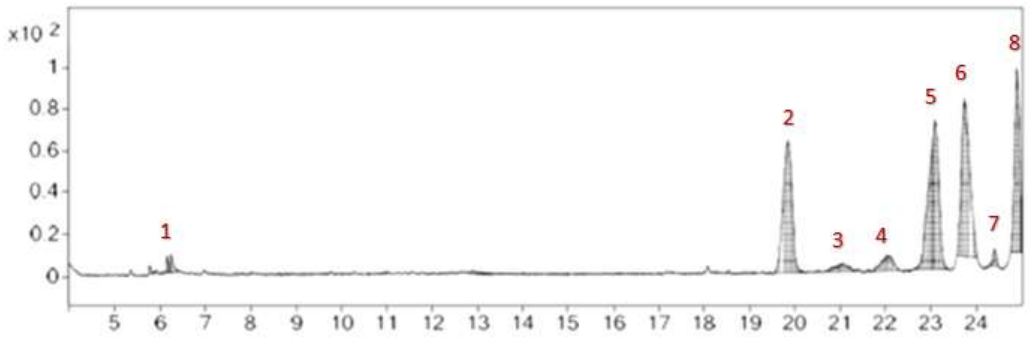

Fig. 3. GC-MS analysis of *A. bisporus* methanolic extract.

Phenolic compound qualitative analysis wild edible mushroom by HPLC-UV

Analysis of phenolic compounds in the methanolic extract of *A. bisporus* mushroom was carried out using HPLC-UV. Four flavonoid compounds (myricetin, quercetin, kaempferol, and rutin) were analyzed. Figure 4 shows the chemical structure of the flavonoid compounds identified by HPLC. All of them were detected in the methanolic extract of *A. bisporus* wild edible mushroom at a wavelength of 352 nm.

Peaks monitored by an HPLC system equipped with an ultraviolet (UV) detector were identified by comparison of retention time and UV spectra with a standard mixture of four flavonoid compounds (Fig. 5 and Table 5). As shown in Table 5, qualitative analysis was performed to identify the type of flavonoid compounds. Since the retention time for each flavonoid compound is constant in a specific laboratory system and conditions, it can be used to determine the type of flavonoid in the analyte. For this purpose, the analyte chromatogram is compared with the chromatogram obtained from the standard of that analyte. If the retention time is the same, the type of material can be determined with certainty. By examining and comparing the standard solution chromatograms of flavonoid compounds and methanolic extract of *A. bisporus*, four flavonoid compounds including quercetin, myricetin, kaempferol, and rutin were identified in the methanolic extract of the mushroom by HPLC-UV (Fig. 5).

Quercetin, myricetin, kaempferol, and rutin are some of the most potent antioxidants among polyphenols. They have been proven to be beneficial in the treatment of specific ailments (such as the treatment of various cancers, cardiovascular disease, and viral diseases). Therefore, the reverse-phase high-performance liquid chromatography profile of *A. bisporus* methanolic extract revealed rutin, myricetin, quercetin, and kaempferol (Fig. 4). Other studies (Yahia et al., 2017; Alara et al., 2021), have shown on flavonoid compounds in different mushrooms, which rutin is the base of all polyphenols and plays an important role in the treatment of diseases. It has also been proved that routin reduces pain and pressure on the eye (Barros et al., 2009). Thus, in the studied mushroom, the rutin has the highest comparative percentage of components, while kaempferol has the lowest relative percentage in the fungus.

Table 4. Bio-compounds found in *A. bisporus* by gas chromatography and mass spectrophotometer

Name	Retention time (min)	Area Sum %
1-Propanol, 2-(2 hydroxypropoxy)	6.241	1.06
Ethylene brassylate	19.852	22.34
Methyl pimar-7-en-18-oate	21.054	1.89
Methyl isodextropimarate	22.031	3.35
Methyl 8-isopimaren-18-oate	23.084	28.86
Methyl 8-pimaren-18-oate	23.742	24.47
Formic acid, 2-bromomethyl-4,4-dimethyl-3-(3-oxobut-1-enyl) cyclohexyl-2-enyl ester	24.401	1.19
Methyl pimar-8(14)-en-18-oate	24.889	16.84

Table 5. Comparison of HPLC-UV chromatograms, standard mixture of four phenolic compounds (A), and methanol extract of *A. bisporus* (B), at wavelength 352 nm

Name	Retention time (min) (A)	Retention time (min) (B)	Area Sum %
Rutin	4.387 min	4.477 min	76.85
Myricetin	5.896 min	5.29 min	6.81
Quercetin	7.406 min	7.297 min	13.23
Kaempferol	10.176 min	10.171 min	3.09

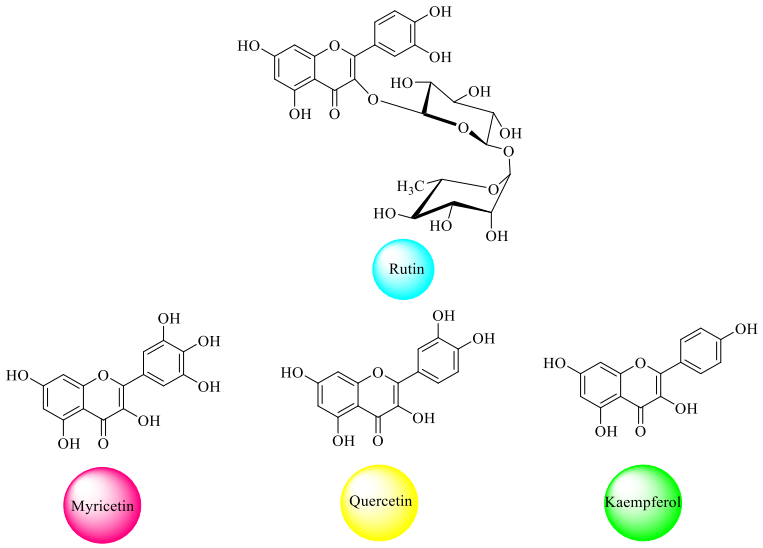

Fig. 4. Chemical structures of myricetin, quercetin, kaempferol, and rutin which determined in the structure of the *A. bisporus* with using the method HPLC-UV.

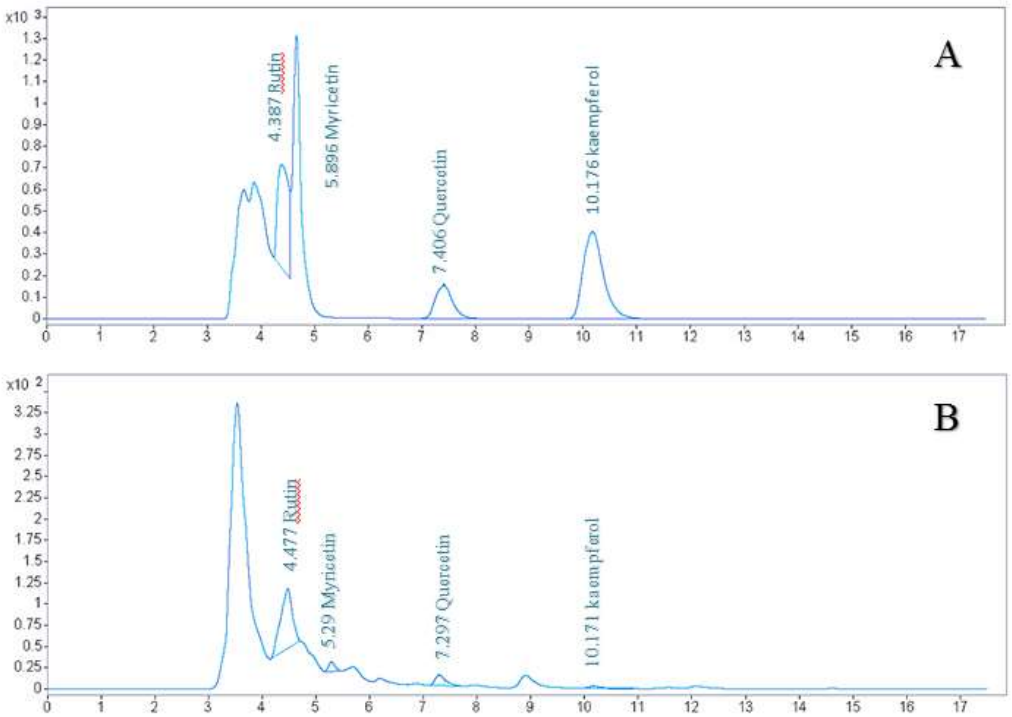

Fig. 5. Comparison of HPLC-UV chromatograms at 352 nm. Standard mixture of four phenolic compounds (A), and methanol extract of *A. bisporus* (B).

Table 6. Antibacterial activity of solvent (methanol, ethanol, acetone, water, and ethyl acetate) extracts of *A. bisporus* wild edible mushroom (collected from Z. region)

Bacteria	Garam	Methanol extract	Ethanol extract	Ethyl acetate extract	Aqueous extract	Acetone Extract
Escherichia coli	Negative	22 ± 0.2 mm	16 ± 0.1 mm	17 ± 0.1 mm	15 ± 0.2 mm	21 ± 0.1 mm
Pseudomonas aeruginosa	Negative	16.6 ± 0.1 mm	15 ± 0.1 mm	16 ± 0.1 mm	15 ± 0.2 mm	16 ± 0.2 mm
Staphylococcus aureus	Positive	20 ± 0.1 mm	17 ± 0.1 mm	18 ± 0.1 mm	15 ± 0.2 mm	20 ± 0.1 mm

(±) Values represent means of triplicate readings ± S.D. Values with the same superscript along the row are not significantly different ($p \geq 0.05$).

Antibacterial activity

The antibacterial effect of solvents extract (methanol, ethanol, acetone, water, and ethyl acetate) of *A. bisporus* was tested against *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Escherichia coli* bacteria. As summarized in Table 6, the range of the inhibition zones of *A. bisporus* solvent extracts, which were obtained against all tested bacteria, was reported between 15±0.1 to 22±0.2 mm. The highest inhibitory activity was noticed against *E. coli* (PTCC 1399) with 22±0.2 mm (diameter of inhibition zone) for methanol extract, and the weakest inhibitory activity was determined against *P. aeruginosa* (PTCC 1430) with 15±0.1 mm (diameter of inhibition zone) for ethanol extract. The results show that the studies mushroom compared to other mushrooms in other articles (Yaltirak et al., 2009; Ren et al., 2014) has shown significant antibacterial activity.

CONCLUSION

Based on our results, *A. bisporus* wild edible mushroom collected from the Z. region (province of East Azerbaijan in northwestern Iran) is found to be a rich source of protein. It has considerable values in carbohydrates, fiber, fat, and important major minerals like potassium, phosphorus, and calcium with some minor minerals. *A. bisporus* mushrooms contain various phenolic compounds that make them of great importance as functional foods and for medicinal and therapeutic purposes. Briefly, GC-MS analysis and HPLC-UV analysis also confirm the presence of various phenolic bioactive metabolites in the methanolic extract of *A. bisporus*. Phenolic compounds in it are very important in terms of nutrition and health, which shows inhibitory, affects against a group of diseases, among which rutin shows a special place. These phenolic compounds also have strong antioxidant effects. This topic shows the nutritional importance of this mushroom.

It was proven antibacterial effects by using the agar well diffusion method in this wild edible mushroom. Therefore, consumption of fruiting bodies of *A. bisporus* would seem to be of great health benefit to combat various deficiency diseases, malnutrition, and viral diseases. Our findings may be of use to assess structural insights of bioactive compounds towards the development of pharmaceutical industries, food, and biochemicals compound.

Conflict of interest

The authors declare that there are not conflicts of interest.

Acknowledgments

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