Journal of Coastal Life Medicine

journal homepage: www.jclmm.com

Original article doi: 10.12980/jclm.4.2016J6-158

Phytochemicals and heavy metals analysis of methanolic extract of edible mushrooms collected from Karak District, Khyber Pakhtunkhwa, Pakistan

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ARTICLE INFO

Article history:
Received 23 Aug 2016
Received in revised form 6 Sep 2016
Accepted 13 Sep 2016
Available online 14 Sep 2016

Keywords:
Phytochemicals
Alkaloids
Saponins
Glycosides
Heavy metals

ABSTRACT

Objective: To qualitatively evaluate the phytochemicals and quantitatively determine the heavy metals of three species of edible mushrooms collected from the Karak area of Khyber Pukhtoonkhwa, Pakistan.

Methods: The plant sample was subjected to methanolic extraction. The extraction was then concentrated by using rotary evaporator. The methanolic extract was screened for the qualitative study of various phytochemicals and quantitative measurement of heavy metals.

Results: A maximum of phytochemicals were confirmed by carring out different tests. Among the different phytochemicals, alkaloids, flavonoids, proteins and carbohydrates were found to be present in the extracts, while saponins and glycosides were not detected. Similarly quantitative study of heavy metals was also conducted on the same extracts of the edible mushrooms. The results suggested that iron was present in maximum concentration than all other metals and nickel was found to be present in little amount when compared with other metals. All the metals were found present.

Conclusions: The concentrations of heavy metals were investigated in the samples which were different in all samples. The presence of different phytochemicals in the mushroom is the key for its active biological profile.

1. Introduction

Phytochemicals are naturally occurring and biologically active compounds found in plants which provide health insurance to human being and also protect plants from various diseases. Phytochemicals also gives color, flavor, and aroma to the plants. Mushroom is rich in vitamins and proteins but low in calories[1,2].

Medicinal plants containing phytochemicals are used for the purpose of medicine and provide a complete house of medicine for the treatment of all diseases of mankind[3-5]. The value of traditional medical system and medicine industry has become greatly popular over the past few decades. About 70–80 percent the world populations are dependent on plant medicine[6,7]. Mushrooms are characterized as macroscopic fungi which are recently introduced into the plant kingdom having spores and cell wall. Mushroom has been treated as a special kind of food, flavors, sauces and medicine for thousands of years[8-10]. Edible mushroom means that this fungus has no dominant poisonous effect on human health and has aroma and pleasing taste. Mushroom is a type of macro fungi, due to their large structure seen with naked eye. They can appear either in hypogenous or epigeous form[11].

It is cleared from the literature review that mushrooms are not only used for foodstuff but also used as medicine because they contain phytochemicals like alkaloids, saponins, tannins, triterpenes, sterols and flavonoids[12,13]. Medicinal mushrooms are used for the purpose of medicine. According to the literature survey, there are 1.5 million species of mushrooms of which

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The journal implements double-blind peer review practiced by specially invited international editorial board members.

70 000 species are described. About 10 000 species are considered the macro fungi of which 5 000 species are used for food purpose and 1 800 species have medicinal properties[14].

The aim of this study was to quantitatively and qualitatively determinate the heavy metals and phytochemicals in the three editable mushrooms (morchella, boletus, gasteromycetes).

2. Materials and methods

2.1. Sample collection

The mushroom materials (morchella, boletus, gasteromycetes) were collected from the rural local area of Karak and identified by taxonomist in the Department of Botany, Kohat University of Science and Technology, Kohat. After collection the materials were placed in the Department of Chemistry, Kohat University of Science and Technology, for further study.

2.2. Sample processing

The samples were dried and soaked in ethanol for methanolic extraction. The extraction was concentrated by using rotary evaporator at 50 ˚C.

2.3. Qualitative analysis of mushroom samples

Specific amount of each mushroom was taken in separate china dish and heated in furnace up to 550–600 ˚C for 4–5 h to remove all the moisture contents from the samples. The powder content of plants was then placed in dessicator for cooling purpose. HNO_3 stock solution (3 mL) was added dropwise into each china dish having mushroom samples. The samples were then filtered into separate flasks by using Whatman filter paper 42 and diluted up to 25 mL with distilled water. For heavy metals analysis all these samples were placed for atomic absorption spectrophotometer.

Different phytochemical tests of the methanolic extract of edible mushrooms were carried out to study the presence of phytochemicals as described by Krishnaiah *et al.*[15] and Edeoga *et al*.[16].

3. Results

The aim of this study was to study the heavy metals and medicinally active phytochemicals in the three edible mushrooms from Karak District, Khyber Pukhtoonkhwa, Pakistan. The results of heavy metal analysis and phytochemicals are given in Tables 1 and 2.

Six heavy metals were analyzed in this experiment. All the six metals were found to be present in the edible mushroom. Morchella is one of the species of edible mushrooms, containing higher value of iron (Fe), followed by chromium (Cr), cadmium (Cd), zinc (Zn), nickel (Ni) and lead (Pb) in the range of 45.30 mg/L, 10.98 mg/L, 5.09 mg/L, 4.62 mg/L, 3.56 mg/L and 3.25 mg/L, respectively. Lead was present in low quantity in morchella plant. Boletus is an edible mushroom and it was subjected to heavy metal analysis. In this case, higher concentration of iron (Fe) was noted, which is followed by cadmium (Cd), chromium (Cr), lead (Pb), zinc (Zn) and nickel (Ni) in the range of 34.14 mg/L, 9.29 mg/L, 8.28 mg/L, 7.39 mg/L, 5.01 mg/L and 1.39 mg/L, respectively. Lower concentration of nickel was found in boletus mushroom. Similarly, gasteromycetes were the third species of edible mushrooms present in the local area of Karak, Pakistan. It was also investigated for metal analysis and it was found to contain higher quantity of iron (Fe), *i.e.* 43.23 mg/L, followed by zinc (10.26 mg/L), chromium (9.31 mg/L), nickel (5.49 mg/L), cadmium (3.98 mg/L), and lead (1.90 mg/L), which is present in low quantity.

The results clearly indicated that alkaloids are present in all (morchella, boletus and gasteromycetes) the species of mushrooms. Saponins were found totally absent in all the three species. Proteins were also checked in the mushroom species; as a result, all the species contained proteins. Glycosides were also tested but they were not found in any of the three species.

Table 2
Phytochemical analysis of edible mushrooms.

Phytochemical analysis	Qualitative tests	Inference
Alkaloids	Mayer's test	+
	Wagner's test	+
	Hager's test	+
Saponins	Foam test	-
Proteins	Biuret's test	+
	Millen's test	+
Glycosides	Legal's test	-
	Liebermann's test	-
Flavanoids	Lead acetate	+
Carbohydrates	Molish's test	+
	Benedict's test	+
	Fehling's test	+

4. Discussion

The presence of alkaloids in these three species of mushroom was confirmed by carried out Mayer's test, Wagner's test and

Table 1
The heavy metal analysis in edible mushrooms. mg/kg.

Samples	Nickel	Chromium	Lead	Zinc	Cadmium	Iron
Morchella	3.560 ± 0.002	10.980 ± 0.132	3.250 ± 0.002	4.620 ± 0.001	5.090 ± 0.210	45.300 ± 0.124
Boletus	1.390 ± 0.210	8.280 ± 0.058	7.390 ± 0.011	5.012 ± 0.003	9.290 ± 0.231	34.140 ± 0.212
Gasteromycetes	5.490 ± 0.129	9.310 ± 0.214	1.900 ± 0.023	10.260 ± 0.011	3.980 ± 0.219	43.230 ± 0.124

Hager's test. The absence of saponin was confirmed by using foam test. The presence of proteins was confirmed by using Biuret's test and Millen's test. Similarly, flavonoids and carbohydrates were also confirmed by using lead acetate test for flavonoids and Molish's, Benedict's and Fehling's tests for carbohydrates. Glycosides were also tested but they were not found in any of the three spcies. This was confirmed by carried out Legal's and Liebermann's tests. The above results indicate that the plants are rich in alkaloids, proteins, carbohydrates and flavonoids. But saponins and glycosides were found absent in the edible mushrooms.

Element analysis was also investigated in the mushroom species to study the concentrations of different heavy metals by using atomic absorption spectrometry. The overall results show that gasteromycetes have higher average values of all heavy metals, followed by morchella, and boletus. But iron was found in higher concentration in all the species of mushroom. It means that these mushrooms grow in areas rich in higher amount of iron. Therefore, they absorbed the iron with maximum concentration than other heavy metals.

The aim of the present study was to identify the concentrations of heavy metals and to screen the phytochemicals in the three edible mushrooms. The concentrations of heavy metals were investigated in the samples which were different in all samples. The presence of different phytochemicals in the mushrooms is the key for their active biological profile.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

All the group members express special thanks to Mr. Hameed-Ur-Rehman, Department of Chemistry for help in publication.

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