

# Natural Product Research

Formerly Natural Product Letters

ISSN: (Print) (Online) Journal homepage: <https://www.tandfonline.com/loi/gnpl20>


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To cite this article: Cengiz Sarikurkcü, Mehmet Halil Solak, Petr Tarkowski & Sanja Ćavar Zeljković (2022): Minerals, phenolics, and biological activity of wild edible mushroom, *Morchella steppicola* Zerova, Natural Product Research, DOI: [10.1080/14786419.2022.2050910](https://doi.org/10.1080/14786419.2022.2050910)

To link to this article: <https://doi.org/10.1080/14786419.2022.2050910>

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SHORT COMMUNICATION



## Minerals, phenolics, and biological activity of wild edible mushroom, *Morchella steppicola* Zerova

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### ABSTRACT

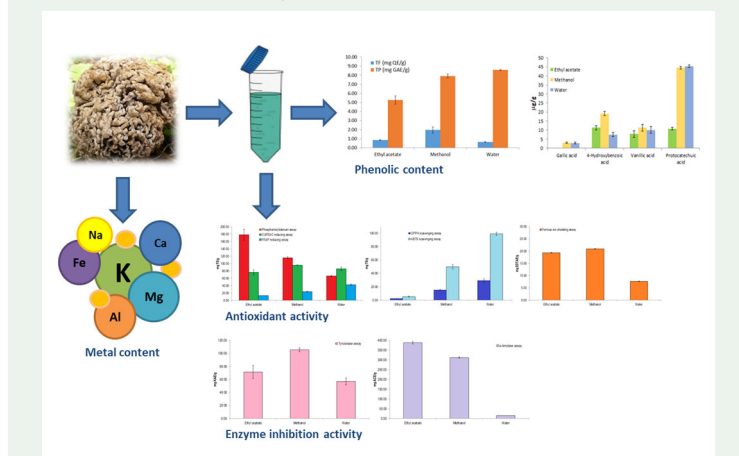
Tasteful morels (*Morchella* sp.) are one of the most popular mushrooms, both economically and scientifically. Due to their beneficial constituents, they are classified as functional foods. This study focuses on the chemical composition and biological activities of a wild edible mushroom, *Morchella steppicola* Zerova. The metal composition of this species reveals high levels of biogenic elements. However, according to the levels of iron and cobalt, and calculated health risk indices, this mushroom can be recommended for occasional consumption. Also, *M. steppicola* is found to be rich in gallic acid, protocatechuic acid, 4-hydroxybenzoic acid, and vanillic acid. Statistical analysis showed that mainly 4-hydroxybenzoic and vanillic acids are responsible for both antioxidant and inhibition of enzymes  $\alpha$ -amylase and tyrosinase. Presented work acknowledges the fact that this mushroom has significant potential to be used for the treatment of several human disorders, similarly to other members of this genus.

### ARTICLE HISTORY


Received 27 November 2021  
Accepted 3 March 2022

### KEYWORDS

*Morchella steppicola* Zerova; metal content; health risk indices; phenolic compounds; antioxidant activity; enzyme inhibition activity



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 Supplemental data for this article can be accessed online at <https://doi.org/10.1080/14786419.2022.2050910>.

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## 1. Introduction

Mushrooms possess high nutraceutical and pharmaceutical potential, and the majority of them have been used as food or medication (Rai et al. 2005; Valverde et al. 2015; Ma et al. 2018). They are rich sources of valuable compounds like proteins, carbohydrates, fats, vitamins, and minerals.

*Morchella* genus attracts great attention due to its taste, but also as a rich source of different biologically active compounds (Gursoy et al. 2009; Heleno et al. 2013; Türkekul et al. 2017; Acar et al. 2021; Taşkın et al. 2021). The steppe morel, *Morchella steppicola* Zerova, is an ecologically distinctive species that is widely distributed in central Eurasia, especially in temperate grasslands and East European steppes on calcareous and clay soils (Yatsiuk et al. 2016). Unlike most true morels, this species can be readily identified morphologically by the densely packed labyrinth of irregular ridges on a cerebriform pileus.

However, the chemical composition of this mushroom species is still unknown. Therefore, this study aims to provide data on chemical composition regarding elements and phenolic compounds of *M. steppicola* found in Turkey. In addition, antioxidant activity and inhibition of the extracts of *M. steppicola* for tyrosinase and  $\alpha$ -amylase were also assayed. The chemical composition and bioactivities of the isolated extracts were correlated, and it was confirmed that the carriers of the beneficial biological activities of *M. steppicola* are mainly simple phenolic acids. In addition, daily intake of metal and health risk index values is also calculated.

## 2. Results and discussion

### 2.1. Mineral content and health risk assessment

Thirty-two elements were determined in *M. steppicola* (Supplementary Table S1), including essential macro (K, Fe, Mg, Ca, and Na) and microelements (Mn, Cu, Co, Zn, etc.), but also some heavy metals, such as Pb, Cr, Ag, etc. Potassium was found to be the most abundant. This metal was also found in high concentrations in other *Morchella* species (Haro et al. 2020). Besides, this mushroom was found to be very rich in Mg, Fe, and Ca. The uptake and presence of heavy metals in mushrooms depend on the environment, and also the mushroom genetics (Sarikurkcu et al. 2015).

The Health Risk Index (HRI) and Daily Intake of Metal (DIM) values given here are theoretical data. The daily amounts of selected metals to be taken into the body as a result of the consumption of the mushroom species examined in this study are summarized in Supplementary Table S2. According to these results, daily consumption of *M. steppicola* could make some health issues considering the HRI values for Fe and Co (USEPA 2002). Overall, according to metal composition, this mushroom can be recommended for occasional consumption. It could provide a beneficial effect due to the high levels of essential biogenic elements, especially magnesium and calcium, which are necessary for the bones but also associated with lowering the risk of diabetes (Haro et al. 2020).

## 2.2. Phenolic composition of *M. steppicola* extracts

To get a detailed picture of the phenolic composition of *M. steppicola*, three solvents of different polarities were used for the preparation of the extracts. The yields of the ethyl acetate, methanol, and water extracts of dry *M. steppicola* were 3.12%, 13.35%, and 20.75% (w/w), respectively. The results of the total phenolic content of these extracts are presented in [Supplementary Table S3](#), and they ranged from  $5.26 \pm 0.44$  to  $8.57 \pm 0.06$  mg GAE/g. The presented results show slightly lower levels of total phenolics determined in the methanolic extracts of other *Morchella* species from Turkey (Gursoy et al. 2009; Taşkın et al. 2021). Furthermore, among 31 phenolic compounds analyzed chromatographically ([Supplementary Table S4](#)), only four were detected in *M. steppicola* extracts ([Supplementary Figure 1](#), [Supplementary Table S5](#)), with protocatechuic acid as the major phenolic found.

Lower amounts of protocatechuic acid were found in the extracts of *M. pulchella* from Turkey (Acar et al. 2021). This mushroom also contains caffeic acid, which was not detected in *M. steppicola*. In addition, few simple phenolic acids were found in *M. esculenta* both from Serbia and Portugal (Heleno et al. 2013), but their levels were again lower than those found in *M. steppicola*. To the best of our knowledge, *M. esculenta* from the Kashmir area is found to be the richest mushroom in phenolic compounds among all morrels studied so far (Wagay et al. 2019). The also authors found flavonoids in this species. However, the presence of flavonoids in mushrooms is questionable since they do not possess the enzymes required for flavonoid biosynthesis (Gil-Ramírez et al. 2016).

## 2.3. Biological activities of *M. steppicola* extracts

Antioxidant activity of *M. steppicola* extracts was evaluated via six assays that are based on different reaction mechanisms, i.e. cation reduction assays (phosphomolybdenum, CUPRAC, and FRAP), radical scavenging assays (DPPH and ABTS), as well as on ion chelating assay ([Supplementary Table S3](#)).

*Morchella steppicola* aqueous extract possesses the highest ability to reduce both Fe and Cu ions, but ethyl acetate extract shows the best results in a reduction of Mo cations. In addition, again water extracts show the best potential to scavenge stable radicals, but scavenging ABTS radical seems easier than scavenging DPPH radical. Taşkın et al. (2021) investigated antioxidant activities of six different *Morchella* species and their results agree with those presented here. Comparison of the presented results with others found in the literature regarding other *Morchella* mushrooms (Gursoy et al. 2009; Jeong-Ah et al. 2011; Heleno et al. 2013; Türkekul et al. 2017; Acar et al. 2021) is not very convenient because those authors were using different units for the antioxidant activities. But, in all mentioned studies, authors found very high correlations of phenolic content and composition with the antioxidant activities of the extracts. Moreover, Jeong-Ah et al. (2011) isolated antioxidant compounds from *M. esculenta*, but those actives do not have phenolic character, i.e. they are fungal sterols. Also, the authors assayed these compounds for inhibition of NF- $\kappa$ B activation, which is closely connected with the primary oxidative stress-response pathway, and they found that fungal sterols from this mushroom inhibit this activation in  $\mu$ M levels.

In the presented study, both CUPRAC and FRAP assays showed quite a high correlation with the total phenolic content found in the examined extracts (Supplementary Table S6), while, according to the phosphomolybdenum assay, phenolic compounds found in these extracts are not the main carriers of the total antioxidant activity. In addition, it seems that vanillic acid is the main compound responsible for the reduction of transition Cu metals. In addition, very high correlations were found for total phenolic content and both radical scavenging assays, DPPH and ABTS (Supplementary Table S6). These findings are in the agreement with the literature (Heleno et al. 2013; Taşkın et al. 2021). Also, the content of both gallic and protocatechuic acids correlate with the radical scavenging activities of examined extracts.

In addition, *M. steppicola* extracts are tested for inhibitory activity of tyrosinase and  $\alpha$ -amylase. Both enzymes are associated with human diseases that are very frequent in modern time, i.e.  $\alpha$ -amylase is associated with diabetes mellitus, and tyrosinase is associated with different dermatological disorders.

Regarding inhibitory activities for tyrosinase, an enzyme that catalyzes the production of melanin, all three extracts showed different activities (Supplementary Table S3). The activity of the extracts is ordered water > ethyl acetate > methanol. Moreover, the Pearson coefficients in the correlation of the 4-hydroxybenzoic acid contents in these extracts and enzymatic inhibitions were very high (Supplementary Table S6), indicating this acid might be responsible for these activities, especially in the case of tyrosinase inhibition. It should be emphasized that, to the best of our knowledge, this is the very first report of tyrosinase inhibitory activity of any of *Morchella* species.

On contrary, ethyl acetate and methanolic extracts showed very high and similar inhibitory activity against  $\alpha$ -amylase, a hydrolytic enzyme that cleaves  $\alpha$ -bonds of polysaccharides, while the aqueous extract had very poor activity. Similarly, Acar et al. (2021) also found the weak  $\alpha$ -amylase inhibitory activity of the water extract of *M. pulchella*.

### 3. Conclusions

This study presents the very first results on the chemistry and biological activities of *M. steppicola* mushroom. This species contains high concentrations of biogenic metals that are closely related to different metabolic processes. However, according to the levels of iron and cobalt, and calculated health risk indices, this mushroom can be recommended for occasional consumption. Since the levels of these metals could be closely related to the soil composition, controlled cultivation is needed. Furthermore, *M. steppicola* is found to be rich in simple phenolic acids, which seem to be responsible for both antioxidant and enzyme inhibition activities. This work acknowledges the fact that this *Morchella* species have some potential to be used for medicinal purposes, similarly to other members of this genus.

### Disclosure statement

The authors declare that they have no conflict of interest.

## Funding

This work was funded by project No. RO0418 (Sustainable systems and technologies, improving crop production for a higher quality of production of food, feed, and raw materials, under conditions of changing climate) funded by Ministry of Agriculture, Czechia.

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