ISSN 1062-3590, Biology Bulletin, 2022, Vol. 49, Suppl. 1, pp. S101–S108. © Pleiades Publishing, Inc., 2022.
ISSN 1062-3590, Biology Bulletin, 2022. © Pleiades Publishing, Inc., 2022.

BOTANY

Chemical Characterization, Antibacterial, Antifungal, Antioxidant and Oxidant Activities of Wild Mushrooms *Rhizopogon luteolus* and *Rhizopogon roseolus*

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Received November 1, 2021; revised May 19, 2022; accepted May 19, 2022

Abstract—Mushrooms are natural materials with nutritious, poisonous, hallucinogenic and medicinal properties. Wild mushrooms are known to have many medicinal properties. In this study, antioxidant, oxidant and antimicrobial potentials, phenolic and element contents of wild mushrooms *Rhizopogon roseolus* (Corda) Th. Fr. and *R. luteolus* Fr. were determined. Antioxidant (TAS) and oxidant status (TOS) were determined using Rel Assay Diagnostics kits. Antimicrobial activities were measured against bacterial and fungal strains using the agar dilution method. Element contents (Cr, Cu, Mn, Fe, Ni, Cd, Pb and Zn) were measured using atomic absorption spectrophotometer. Phenolic contents were screened using LC-MS/MS device. As a result of the study, TAS values of *R. luteolus* and *R. roseolus* were determined as 2.327 ± 0.132 and 3.260 ± 0.119, TOS values was 27.057 ± 1.128 and 19.850 ± 0.433, and OSI values was 1.163 ± 0.116 and 0.611 ± 0.032, respectively. It was determined that the extracts of mushrooms were effective against standard bacterial and fungal strains at 25–400 μg/mL concentrations. Fe, Cu, Pb, Ni, Mn, Co and Cr levels were found to be higher in *R. roseolus*. Zn and Cd levels were higher in *R. luteolus*. As a result of the analysis, acetohydroxamic acid, fumaric acid, salicylic acid and luteolin were determined in both mushroom species. Phloridzindyhrate was determined only in *R. roseolus*. Ellagic acid and Curcumin were also found in *R. luteolus*. As a result, it was determined that *R. luteolus* and *R. roseolus* could be a natural source for the determined compounds. It has also been found that mushrooms have antioxidant potentials.

Keywords: antimicrobial, antioxidant, phenolic contents, medicinal mushrooms, *Rhizopogon luteolus*, *Rhizopogon roseolus*

DOI: 10.1134/S1062359022130180

INTRODUCTION

Mushrooms are cosmopolitan living organisms that spread in all ecosystems. Mushrooms, which are generally seen after the rains, attract attention with their nutritive properties (Sevindik, 2020; Wasser, 2021). They are indispensable dietary products in world cuisine due to their high protein content and unique flavors. In addition to their nutritional properties, mushrooms also attract attention with their medicinal properties (Cerletti et al., 2021). It has been previously reported by different researchers that mushrooms have many biological activities such as antioxidant, antimicrobial, anticancer, antialergic, antiproliferative, antiaging, hepatoprotective, immunomodulatory and DNA protective effects (Chang et al., 2007; Weng et al., 2011; Islek et al., 2021; Sarıdoğan et al., 2021; Sevindik, 2021; Sevindik and Bal, 2021; Song et al., 2021; Venturella et al., 2021). These effects of mushrooms are seen thanks to the bioactive compounds they contain (Chakraborty et al., 2021). In this context, it is very important to determine the biological activities of mushrooms and the important compounds in it. In this study, *Rhizopogon luteolus* Fr. and *R. roseolus* (Corda) Th. Fr. mushrooms were used as material.

Rhizopogon (Rhizopogonaceae) is a genus of ectomycorrhizal Basidiomycetes. *Rhizopogon* species are often referred to as "false truffles." It is generally ectomycorrhizal with coniferous trees (Binder and Hibbett, 2006). It plays an important role in the ecology of coniferous forests, especially pine and fir. *Rhizopogon* species survive in old growth stands by colonizing the roots of trees during seedling formation. Its spores can remain in the soil for a long time and are revived when suitable conditions occur (Twieg et al., 2007). *Rhizopogon* species play an important role in the recovery of degraded forests in the ecosystem (Bruns et al., 2009). In addition to all these features in the ecosystem, it

also has nutritive properties. Many species of *Rhizopogon* are considered edible. But it is not widely credited for the discovery of gastronomically better mushroom species. In addition, *R. roseolus* species has been commercially developed in Japan and New Zealand and significant results have been obtained (Yun and Hall, 2004). In this context, antioxidant, oxidant, antimicrobial potentials and element and phenolic contents of *R. luteolus* and *R. roseolus* species were determined in our study.

MATERIALS AND METHODS

R. luteolus and *R. roseolus* specimens were collected from Oğuzeli/Gaziantep (Turkey). Mushrooms were dried in an oven. After drying, 30 g were taken from the samples and extracted with ethanol (EtOH) for approximately 6 hours at 50°C in a soxhlet apparatus (Gerhardt EV 14). The resulting mushroom extracts were concentrated with a rotary evaporator (Heidolph Laborota 4000 Rotary Evaporator).

Antioxidant and Oxidant Tests

Antioxidant and oxidant potentials of mushroom samples were determined using Rel Assay TAS and TOS kits. The manufacturer's procedures were followed when performing the tests. Trolox in TAS kits and hydrogen peroxide in TOS kits were used as calibrators (Erel, 2004, 2005). Oxidative stress index (OSI) TOS values were determined by proportioning TAS values (Sevindik, 2019).

Antimicrobial Tests

Antimicrobial activities of ethanol extracts of mushroom samples against bacterial and fungal strains were measured by the agar dilution method recommended by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Antibacterial activities of mushroom extracts were determined against *Staphylococcus aureus* ATCC 29213, *S. aureus* MRSA ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Acinetobacter baumannii* ATCC 19606, which were pre-cultured on Muller Hinton Broth medium. Antifungal activities were determined against *Candida albicans* ATCC 10231, *C. krusei* ATCC 34135 and *C. glabrata* ATCC 90030 which were pre-cultured on RPMI 1640 Broth medium. Standard solutions from mushroom samples were adjusted with distilled water at 800, 400, 200, 100, 50, 25 and 12.5 μg/mL concentrations. Fluconazole and Amphotericin B were used as reference drugs for fungi. Amikacin, Ampicillin and Ciprofloxacin were used as reference drugs for bacteria. The lowest extract concentration that inhibits the growth of bacteria and fungi was determined as MIC (Minimal inhibitory concentration) (Bauer et al., 1966; Hindler et al., 1992; Matuschek et al., 2014).

LC-MS/MS Analysis

The presence of 26 phenolic compounds was investigated by LC-MS/MS device in *R. luteolus* and *R. roseolus*. In this context, analyzes were performed using Shimadzu LC-MS 8040 model mass spectrometer equipped with ESI source operation in triple, quadrupole and both positive and negative ionization modes. Three applications were made for each compound analysis in the experiment. The first quantitative results were performed for the second and third analyzes for confirmation. Optimum Electrospray Ionization (ESI) parameters; 350°C interface temperature, 250°C DL temperature, 400°C heatsink temperature, 3 L/min. Nebulizer gas flow and 15 L/min. was determined as the drying gas flow (Köksal et al., 2017).

Element Analysis

Element contents of mushroom samples were determined using atomic absorption spectrophotometer device (Agilent 240FS AA). In order to determine the Cr, Cu, Mn, Fe, Ni, Cd, Pb and Zn contents accumulated in the mushrooms, the samples were dried at 80°C until constant weighing. Then, 0.5 g was taken from the samples and mineralized in a mixture of 9 mL HNO_3 + 1 mL H_2O_2 in a microwave solubilization device (Milestone Ethos Easy). Then the measurement process was performed (Baba et al., 2020).

RESULTS AND DISCUSSION

Total Antioxidant and Oxidant Status

Mushrooms have been used in the treatment of many diseases in different communities. It draws attention especially with its antioxidant properties (Sevindik, 2018; Maity et al., 2021). Oxidizing compounds are compounds produced as a result of environmental factors and metabolic activities in living organisms. The increase in the levels of oxidant compounds causes oxidative stress (Jayakumar et al., 2008). Cancer, Alzheimer's, Parkinson's, and cardiological disorders are diseases caused by oxidative stress (Giridharan et al., 2011). The antioxidant defense system plays an active role in suppressing and reducing the effects of oxidative stress. In cases where the antioxidant defense system is insufficient, the use of supplemental antioxidants is inevitable (Wu et al., 2020). In this context, the determination of the antioxidant potential of mushrooms is very important in the determination of supplemental antioxidants. In this study, antioxidant and oxidant status of *R. luteolus* and *R. roseolus* were determined. The obtained results are shown in Table 1.

In previous studies, it has been reported that methanol, ethanol, water extracts of *R. roseolus* have antioxidant activity using β-carotene/linoleic acid method, Reducing power, Chelating effects on ferrous ions methods (Gursoy et al., 2010; Kalyoncu et al., 2010). In our study, antioxidant and oxidant potentials of *R. luteolus* and *R. roseolus* were determined for the first time using Rel Assay kits. As a result of the studies, it was determined that the antioxidant potential of *R. roseolus* was higher than that of *R. luteolus*. TAS values of *Infundibulicybe geotropa* (TAS: 1.854), *Lepista nuda* (TAS: 3.102), *Leucoagaricus leucothites* (TAS: 8.291), *Helvella leucopus* (TAS: 2.181), *Suillus granulatus* (TAS: 3.143), *Ramaria stricta* (TAS: 4.223) and *Tricholoma virgatum* (TAS: 3.754) have been reported in TAS studies on different wild mushrooms species (Sevindik et al., 2018, 2020; Bal et al., 2019; Sevindik and Akata, 2019; Krupodorova and Sevindik, 2020; Mushtaq et al., 2020; Selamoglu et al., 2020). TAS value is an indicator of all endogenous antioxidants produced in the organism. The high TAS value indicates that the antioxidant potential of the organism is high (Korkmaz et al., 2018). In this context, it is seen that the TAS value of *R. luteolus* is higher than *I. geotropa* and *H. leucopus*, and lower than *L. nuda, L. leucothites, S. granulatus, R. stricta* and *T. virgatum*. In addition, it was determined that the TAS value of *R. roseolus* was higher than *I. geotropa, L. nuda, S. granulatus* and *H. leucopus*, and lower than *L. leucothites, R. stricta* and *T. virgatum*. In this context, it was determined that *R. luteolus* and *R. roseolus* had antioxidant potentials.

When TOS values are examined, it is seen that the TOS value of *R. luteolus* is higher. In previous TOS studies, TOS values of *I. geotropa* (TOS: 30.385), *L. nuda* (TOS: 36.920), *L. leucothites* (TOS: 10.797), *H. leucopus* (TOS: 14.389), *S. granulatus* (TOS: 18.933), *R. stricta* (TOS: 8.201) and *T. virgatum* (TOS: 8.362) have been reported in wild mushrooms (Sevindik et al., 2018, 2020; Bal et al., 2019; Sevindik and Akata, 2019; Krupodorova and Sevindik, 2020; Mushtaq et al., 2020; Selamoglu et al., 2020). Compared to these studies, the TOS values of *R. luteolus* and *R. roseolus* were lower than *I. geotropa* and *L. nuda*, and higher than *L. leucothites, H. leucopus, S. granulatus, R. stricta* and *T. virgatum*. TOS value is an indicator of the whole of the oxidant compounds produced by the living thing as a result of environmental factors and metabolic activities (Korkmaz et al., 2018). It is seen that TOS values are high in both mushroom species used in our study. It is thought that this is due to the fact that they are underground mushrooms and that underground mushrooms are more affected by environmental factors.

The OSI value shows how much the oxidant compounds produced in the living body are suppressed by the antioxidant defense system. The increase in the OSI value is an indication that the organism is insufficient in suppressing oxidant compounds (Korkmaz et al., 2018). In our study, it was determined that the OSI value of *R. luteolus* was higher. In this context, it is seen that *R. luteolus* is more inadequate than *R. roseolus* in suppressing oxidant compounds. In previous OSI studies, OSI values of *I. geotropa* (OSI: 1.639), *L. nuda* (OSI: 1.190), *L. leucothites* (OSI: 0.130), *H. leucopus* (OSI: 0.661), *S. granulatus* (OSI: 0.603), *R. stricta* (OSI: 0.194) and *T. virgatum* (OSI: 0.223) have been reported in wild mushrooms (Sevindik et al., 2018, 2020; Bal et al., 2019; Sevindik and Akata, 2019; Krupodorova and Sevindik, 2020; Mushtaq et al., 2020; Selamoglu et al., 2020). Compared to these studies, the OSI value of *R. luteolus* was lower than *I. geotropa* and *L. nuda*, and higher than *L. leucothites*, *H. leucopus, S. granulatus, R. stricta* and *T. virgatum*. In addition, it was observed that *R. roseolus* was lower than *I. geotropa, L. nuda* and *H. leucopus*, and higher than *L. leucothites, S. granulatus, R. stricta* and *T. virgatum*. As a result, it was observed that both mushrooms species were at normal levels in suppressing oxidant compounds.

Table 1. TAS, TOS and OSI values

	TAS	TOS	OSI
R. luteolus	2.327 ± 0.132	27.057 ± 1.128	1.163 ± 0.116
R. roseolus	3.260 ± 0.119	19.850 ± 0.433	0.611 ± 0.032

Values are presented as mean ± S.D.; $n = 6$ (experiments were made as 5 parallel).

Antimicrobial Activity

In recent years, there has been an increase in diseases caused by microorganisms. Antimicrobial drugs are used against these diseases. Especially as a result of unconscious drug use, microorganisms become resistant to drugs. For this reason, the discovery of new antimicrobial drugs is inevitable. Researchers are turning to natural products for new antimicrobial drugs (Sami et al., 2020; Takó e al., 2020; Kumla et al., 2021). In this study, antimicrobial potentials of *R. luteolus* and *R. roseolus* against bacterial and fungal strains were determined. The obtained results are shown in Table 2.

In our study, antimicrobial activities of ethanol extracts of *R. luteolus* and *R. roseolus* on standard bacterial (*Staphylococcus aureus, S. aureus* MRSA, *Enterococcus faecalis, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii*) and fungal strains (*Candida albicans, C. glabrata, C. krusei*) were investigated. According to the results obtained, it was observed that *R. luteolus* was more effective against bacteria and fungus strains than *R. roseolus*. In previous studies, it has been reported that ethanol, methanol, ethylacetate, diethyl ether and n-hexane extracts of *Rhizopogon roseolus* are effective against *Bacillus cereus, B. subtilis, Escherichia coli, Saccharomyces cerevisiae, Micrococcus luteus, Enterobacter aerogenes* and

Table 2. MIC values of *R. luteolus* and *R. roseolus*

	A	B	C	D	E	F	G	H	J
R. luteolus	100	100	200	50	50	25	50	50	50
R. roseolus	100	100	400	50	50	25	100	100	100

(A) *S. aureus*, (B) *S. aureus* MRSA, (C) *E. faecalis*, (D) *E. coli*, (E) *P. aeruginosa*, (F) *A. baumannii*, (G) *C. glabrata*, (H) *C. albicans*, (J) *C. krusei*. 25, 50, 100, 200 and 400 extract concentrations.

Table 3. Phenolic compounds of *R. luteolus* and *R. roseolus*

	R. luteolus	*R. roseolus*
Acetohydroxamic acid	3.53	1.58
Catechin hyrate	n.d.	n.d.
Vanilic acid	n.d.	n.d.
Syringic acid	n.d.	n.d.
Thymoquinone	n.d.	n.d.
Resveratrol	n.d.	n.d.
Fumaric acid	28.25	42.91
Gallic acid	n.d.	n.d.
Caffeic acid	n.d.	n.d.
Hydoxycinamic acid	n.d.	n.d.
Hydroxyben	n.d.	n.d.
Protocatechuic acid	n.d.	n.d.
Salicylic acid	1.07	1.08
Oleuropein	n.d.	n.d.
Phloridzindyhrate	n.d.	2.07
2-Hyroxy 1,4Naphthaquinone	n.d.	n.d.
Myricetin	n.d.	n.d.
Ellagic acid	3.27	n.d.
Quercetin	n.d.	n.d.
Butein	n.d.	n.d.
Naringenin	n.d.	n.d.
Silymarin	n.d.	n.d.
Luteolin	0.20	0.19
Kemferol	n.d.	n.d.
Alizarin	n.d.	n.d.
Curcumin	0.11	n.d.

n.d.: Not detected.

Candida albicans (Solak et al., 2006). It has been reported that water, methanol, chloroform, acetone and n-hexane extracts of *R. luteolus* have antimicrobial activities against *Escherichia coli, Bacillus subtilis, Bacillus licheniformis, Staphylococcus aureus, Agrobacterium tumefaciens* (Sadi et al., 2015). In our study, it was determined that *R. luteolus* and *R. roseolus* were effective against bacterial strains at 25–400 μg/mL concentrations. It was also effective against fungal strains at 50 and 100 μg/mL concentrations. Both mushroom extracts showed the highest activity against *A. baumannii* at 25 μg/mL concentration. The lowest activity of the extracts was exhibited against *E. faecalis* at 200 and 400 μg/mL concentrations. In this context, it has been determined that *R. luteolus* and *R. roseolus* have important antimicrobial potentials. In this context, it is thought that it can be used as a natural antimicrobial agent.

Phenolic Contents

Mushrooms are natural materials responsible for many biological activities. These biological activities take place thanks to the bioactive compounds they produce in their bodies. These bioactive compounds are medically important compounds that do not have nutritional properties (Rašeta et al., 2020). In our study, phenolic compounds of *R. luteolus* and *R. roseolus* were screened. The obtained results are shown in Table 3.

As a result of the analysis, it was determined that *R. luteolus* contains acetohydroxamic acid, fumaric acid, salicylic acid, ellagic acid, luteolin and curcumin. It has been determined that *R. roseolus* contains acetohydroxamic acid, fumaric acid, salicylic acid, phloridzindyhrate and luteolin. In previous studies, it was reported that *R. luteolus* contains fumaric acid (Tel-Çayan et al., 2016). In our study, fumaric acid was determined as the major compound in both *R. luteolus* and *R. roseolus* mushrooms in parallel with this study. Acetohydroxamic acid has previously been reported to have antioxidant activity (Liu et al., 2017). It has been reported that fumaric Acid has antibacterial, antitumor, anti-poisoning, antiproliferative, antipsoriatic activities (Kuroda and Akao, 1981; Sebok et al., 1994; He et al., 2011). It has been reported that salicylic acid has antifungal, antioxidant and cytotoxic effects (Amborabé et al., 2002; Shim et al., 2003; Egorova et al., 2015). It has been reported that ellagic acid has antioxidant, antifungal, DNA protective, anti-inflammatory, hepatoprotective and antiproliferative activities (Xu et al., 2003; Losso et al., 2004; Han et al., 2006; Girish et al., 2009; Marín et al., 2013; Li et al., 2015). It has been reported that luteolin has antitumor, antioxidant, antibacterial, anti-inflammatory and antiviral effects (Wang and Xie, 2010; Lee et al., 2012; Roy et al., 2015; Fan et al., 2016; Aziz et al., 2018). Curcumin has been reported to have antiviral, antimicrobial, antidepressant, anti-inflammatory, immunomodulatory, anti-metastatic, and antioxidant activities (Menon et al., 1998; Antony et al., 1999; Barclay et al., 2000; Rao et al., 2013; Zorofchian Moghadamtousi et al., 2014; Kulkarni et al., 2018). In this context, it is thought that the *R. luteolus* and *R. roseolus* mushrooms used in our study may be a source in terms of the compounds determined in their structures. In addition, it is thought that the determined activities are caused by the phenolic compounds in their structure.

Table 4. Element contents of *R. luteolus* and *R. roseolus*

Elements	*R. luteolus*, mg kg^{-1}	*R. roseolus*, mg kg^{-1}	Literature ranges, mg kg^{-1}
Fe	284.82 ± 7.84	459.66 ± 8.78	14.60–835.00
Cu	19.46 ± 0.58	40.97 ± 2.47	60.33–95.00
Zn	20.16 ± 3.05	12.96 ± 0.70	29.80–158.00
Pb	4.36 ± 0.97	18.22 ± 1.38	2.86–16.54
Ni	1.42 ± 0.21	6.01 ± 0.68	0.67–5.14
Mn	28.29 ± 2.59	61.06 ± 2.65	18.10–103.00
Co	7.30 ± 1.60	11.08 ± 1.21	0.01–8.27
Cd	5.41 ± 0.76	3.45 ± 0.74	2.71–7.50
Cr	10.3 ± 2.23	16.82 ± 0.80	9.63–42.70

Values are presented as mean ± S.D. (experiments were made as 3 parallel).

Element Contents

Mushrooms accumulate elements at different levels in their bodies depending on the region they are in and the substrate they use (Mleczek et al., 2021). In this context, mushrooms can be used as pollution indicators. In addition, they can contain levels of elements that can be harmful when consumed (Falandysz et al., 2021). For this reason, it is very important to determine the levels of the elements that mushrooms contain. In our study, element levels in the bodies of *R. luteolus* and *R. roseolus* mushrooms were determined. The obtained results are shown in Table 4.

In previous studies, the lowest and highest values were reported for the elements determined in the structures of wild mushrooms and are shown in Table 4 (Svoboda and Chrastný, 2008; Zhu et al., 2011; Sevindik et al., 2017; Eraslan et al., 2021). In general, it was determined that *R. roseolus* accumulated elements at higher levels than *R. luteolus*. In our study, it was determined that the Fe, Mn, Cd and Cr levels determined in *R. luteolus* and *R. roseolus* were within the range of the literature. It was observed that the Cu and Zn contents were lower than the literature ranges. It was determined that Pb, Ni and Co contents were higher than the literature ranges in *R. luteolus* and higher than the literature ranges in *R. roseolus*. In this context, it was observed that the element levels of *R. luteolus* and *R. roseolus* used in our study were generally at normal levels.

CONCLUSIONS

In this study, antioxidant, antimicrobial, oxidant potentials, phenolic and element contents of ethanol extracts of *R. luteolus* and *R. roseolus* mushrooms were determined. As a result of the study, it was determined that mushrooms have antioxidant potentials. In addition, it was determined that they have significant antimicrobial activity. In addition, it has been determined that they can be natural sources in terms of compounds detected at different levels in their structures. Element levels were generally found to be within the range of the literature. As a result, it has been determined that *R. luteolus* and *R. roseolus* mushrooms are important natural materials.

COMPLIANCE WITH ETHICAL STANDARDS

The authors declare that they have no conflicts of interest. This article does not contain any studies involving animals or human participants performed by any of the authors.

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