International Journal of Secondary Metabolite
2021, Vol. 8, No. 3, 214–226
https://doi.org/10.21448/ijsm.953923
Published at https://dergipark.org.tr/en/pub/ijsm
Research Article

Chemical compositions and antioxidant activities of four different mushroom species collected from Turkey

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Abstract: Many researchers agree that edible mushrooms have beneficial effects on human health. The aim of this study is to investigate the chemical composition and antioxidant activities of methanol extracts obtained from *Coprinus comatus*, *Hydnum repandum*, *Agaricus impudicus* ve *Sarcodon imbricatus* collected from Yuvacık-İzmit, Kastamonu, and Uzungöl-Trabzon. Spectrophotometric analysis showed that the total phenolic (64.69 mg GAEs/g) and flavonoid (1.73 mg QEs/g) content of *S. imbricatus* was higher than that of the others. In line with its superiority in phytochemical composition analyzes, *S. imbricatus* extract showed the highest activity in β-carotene bleaching, DPPH free radical scavenging and reducing power assays (836.0, 89.0 and 267.0 mg TEs/g extract, respectively). Metal chelating test resulted in the superiority of *A. impudicus* (1282.0 mg TEs/g). Relative antioxidant capacity index (RACI) value of *S. imbricatus* was quite high compared to other extracts (0.90). Apart from the metal chelating assay, there was a high correlation between the antioxidant activities of the extracts and their RACI values. Pearson correlation analysis showed that the correlation between the phenolic/flavonoid contents of the extracts and their antioxidant activities was over 0.9. The antioxidant activity of *A. impudicus* was brought to the literature for the first time with this study. When the data obtained from the current study were evaluated as a whole, it was concluded that *S. imbricatus* could be a rich source for new and alternative antioxidant compounds.

ARTICLE HISTORY
Received: June 17, 2021
Accepted: July 27, 2021

KEYWORDS
Agaricus impudicus, *Coprinus comatus*, *Hydnum repandum*, *Sarcodon imbricatus*, *Antioxidant activity.*

1. INTRODUCTION

In recent years, searches for the addition of new supplements to existing food supplements have started to intensify among both researchers and end consumers. In this quest, mushrooms have gained great importance due to their nutritional properties and powerful bioactive chemicals (Mujić *et al*., 2011; Bowe, 2013; Falandysz & Borovička, 2013; Zhang *et al*., 2016; Jayachandran *et al*., 2017). For hundreds of years, mushrooms have been used by humans both to meet their nutritional needs and to treat various diseases (Gaglarirmak, 2011; Gustafson, 2016; Chatterjee *et al*., 2017). Edible mushrooms are considered among the indispensable elements of diets due to their rich nutritional contents, as well as being delicious enough to be consumed by many people (Csóka *et al*., 2017; Luo *et al*., 2017; Sharma & Gautam, 2017;

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e-ISSN: 2148-6905 / **© IJSM** 2021

Politowicz *et al*., 2018). In recent studies, it has been reported that especially phenolic compounds contribute significantly to the biological activities of mushrooms (Sevindik *et al*., 2020).

During metabolic reactions in organisms, the amount of free radicals increases. Organism uses endogenous defense mechanisms against this increase. However, insufficient endogenous antioxidant enzyme systems cause free radicals to damage biological molecules. It has been reported that free radicals underlie many metabolic, chronic or neurological disorders in organisms (Khansari *et al*., 2009; Salminen *et al*., 2012; Cencioni *et al*., 2013; Nowak, 2013; Polidori & Scholtes, 2016; Tan *et al*., 2018). When the results obtained from both epidemiological and clinical studies are evaluated as a whole, one of the most effective ways to deal with this problem is to support the organism with external antioxidants. Many researchers agree that there is a decrease in the incidence of diseases caused by free radicals in those who are fed diets rich in antioxidants (Cherubini *et al*., 2005; Fletcher, 2010; Sevindik *et al*., 2020).

In many studies, it has been documented that edible mushrooms have many useful effects on human health. It is known that mushrooms are foods rich in antioxidant compounds and thus both help to scavenge free radicals and increase the activities of endogenous antioxidant enzymes (Liu *et al*., 2004; Asatiani *et al*., 2007a, b; Oyetayo, 2009). Researchers suggest that some edible mushrooms contribute to the reduction of DNA damage caused by oxidative stress on various cell lines (Shi *et al*., 2002; Miyaji *et al*., 2004). In addition, some mushroom compounds are known to inspire the development of various bioactive molecules in the pharmacology industry (Orangi *et al*., 2016). In addition to their antioxidant activities, mushrooms also accumulate various minerals depending on the mineral substance concentration in the soil, and therefore are among the important sources for meeting the mineral needs of organisms (Kosanić *et al*., 2016).

The aim of this study is to investigate the chemical composition and *in vitro* antioxidant activities of the methanol extracts of *Coprinus comatus* (O.F. Müll.) Pers., *Hydnum repandum* L., *Agaricus impudicus* (Rea) Pilát and *Sarcodon imbricatus* (L.) P. Karst, collected from Yuvacık-İzmit, Kastamonu, and Uzungöl-Trabzon locations. As a result of the literature search, no study was found on the chemical composition and antioxidant activity of *A. impudicus*. In addition, the data obtained in the β-carotene bleaching test of *H. repandum* and metal chelating tests of *S. imbricatus* were also brought to the literature for the first time with the present study. Therefore, it is thought that the presented data would fill an important gap in the literature.

2. MATERIAL and METHODS

2.1. Mushrooms and The Extraction Process

The fruit bodies of the edible mushrooms analyzed in the present study were collected in July 2019 from the localities detailed in Table 1. Dried fruit bodies (5 g) out of direct sunlight and in an environment with good air flow were extracted with 100 ml of methanol at 25 °C at 150 rpm by stirring for 24 hours. The obtained extract was purified from its residues by passing it through filter paper. The same plant material was extracted with methanol two more times using the above-mentioned method, and the extracts were combined with the first methanol extract. The methanol in the combined extracts was removed under low vacuum with the aid of a rotary evaporator. Extract yields are given in Table 2. Extracts were stored in the dark at +4 °C until tests were performed.

2.2. Determination of Total Phenolics of Extracts

The total amount of phenolic compounds of the extracts was determined by using Folin-Ciocalteu Reagent (FCR) (Sarikurkcu *et al*., 2008). Sample solutions containing 1 mg of extract

were made up to 46 ml with distilled water. 1 ml of FCR was added to this mixture and 3 ml of 2% Na_2CO_3 solution was added 3 minutes later. The mixture was shaken at room temperature for 2 hours. Then the absorbance of the samples at 760 nm was read. The total amounts of phenolic compounds in the extracts were determined using the following equation (1) obtained from the standard gallic acid plot, and the results were given in gallic acid equivalents (mg GAEs/g extract):

$$A = 34.16 \text{ (mg gallic acid)} - 0.04 \ (R^2: 0.999) \quad (1)$$

Table 1. Mushrooms analyzed in the current study.

Mushrooms	Family	Habitat	Substrate	Herbarium no	Locality
C. comatus	Agaricaceae	Roadside	Soil	A&HA. 051	Yuvacık-İzmit
H repandum	Hydnaceae	Beech forest	Soil	A&HA. 090	Yuvacık-İzmit
A impudicus	Agaricaceae	Near fir forest	Soil	Akata 7234	Kastamonu
S. imbricatus	Bankeraceae	Spruce forest	Soil	A & Y. 965	Uzungöl-Trabzon

2.3. Determination of Total Flavonoids Extracts

The total amount of flavonoid compounds in the extracts were determined using the $AlCl_3$ method (Dewanto *et al*., 2002; Sarikurkcu *et al*., 2008). 1 ml of $AlCl_3$ solution prepared in 2% methanol was mixed with the samples containing the same volume of extract. The absorbance was read against the blank after 10 minutes of incubation at 415 nm at room temperature. The blank was prepared to contain 1 ml of extract solution and 1 ml of methanol or water. The total amounts of flavonoid compounds in the extracts were determined using the following equation (2) obtained from the standard quercetin plot, and the results were given in quercetin equivalents (mg QEs/g extract):

$$A = 29.54 \text{ (mg quercetin)} - 0.027 \ (R^2: 0.996) \quad (2)$$

2.4. Determination of Total Antioxidant Activity by β-carotene Bleaching Test

The antioxidant activity was determined by the β-carotene-linoleic acid test system based on the measurement of the inhibition of conjugated diene hydroperoxides and volatile organic compounds resulting from linoleic acid oxidation (Dapkevicius *et al*., 1998; Dewanto *et al*., 2002; Sarikurkcu *et al*., 2008). The β-carotene solution was prepared by dissolving 1 mg of β-carotene in 2 ml of chloroform. 50 µg of linoleic acid and 400 mg of Tween 20 were added to this solution. After chloroform was removed by rotary evaporator, it was mixed with 200 ml of oxygenated distilled water. 2.5 ml of this emulsion was added to 0.5 ml of the extracts. For control, 0.5 ml of methanol was added to the test tube instead of the extract. As soon as the emulsion was added to the test tubes, the initial absorbance was measured at 490 nm using a spectrophotometer. Tubes were incubated at 50 ºC. Incubation was continued until the color of the β-carotene disappeared (120 minutes). The β-carotene bleaching ratio (R) was calculated according to equation (3) and the results were given as trolox equivalent (mg TEs/g extract):

$$R = \text{In } (a/b)/t \quad (3)$$

where, ln: natural logarithm, a = initial absorbance and b = absorbance after 120 min incubation.

Antioxidant activity (AA) was calculated according to equation (4):

$$AA = [(R_{control}-R_{sample})/R_{control}] \times 100 \quad (4)$$

2.5. Determination of Free Radical Scavenging Activities of Extracts

Free radical scavenging activities of the extracts were determined using 1.1-diphenyl-2-picrylhydrazil (DPPH) free radical (Cuendet *et al*., 1997; Dapkevicius *et al*., 1998; Dewanto *et al*., 2002; Sarikurkcu *et al*., 2008). 1 ml of 0.4 mM methanol solution of DPPH was mixed with 1 ml of the extracts and their absorbance was measured at 517 nm after 30 minutes of incubation at room temperature. The absorbance values of the samples were evaluated against the blank (1 ml methanol). Results were given as trolox equivalent (mg TEs/g extract).

2.6. Determination of Reducing Power Potentials of Extracts

The reducing power potential tests of the extracts were carried out according to the method of Oyaizu (1986). 2.5 ml of the extracts were added to the test tubes. 2.5 ml of 0.2 M phosphate buffer (pH: 6.6), and 2.5 ml of 1% potassium ferricyanide were added to each tube and the mixture was incubated at 50 ºC for 20 minutes. Then, 2.5 ml of 10% trichloroacetic acid was added to the reaction mixture and 2.5 ml of sample was taken from the solution. After adding 2.5 ml of distilled water and 0.5 ml of 0.1% $FeCl_3$ on the sample, absorbance values were determined at 700 nm. As a control, methanol was used instead of the sample. Results were given in trolox equivalents (mg TEs/g extract).

2.7. Determination of Chelating Capacity of Extracts

The chelating capacity of the extracts on Fe^{2+} were determined according to the method specified by Dinis *et al.* (1994). 2 mM 0.05 ml $FeCl_2$ solution was added to the test tubes containing 2 ml of the extract solution. The reaction was initiated by the addition of 0.2 ml of 5 mM ferrozine. After mixing, the mixture was incubated for 10 minutes at room temperature and then absorbance was measured at 562 nm. Results were given in trolox equivalents (mg TEs/g extract).

2.8. Statistical Analyzes

All tests were performed in triplicate and results are given as the mean and standard deviation of triplicate experiments. The level of significance between the results was determined by Tukey's test by choosing 95% confidence interval (α=0.05) with the help of ANOVA analysis of variance using SPSS v22 program. Correlation analyzes between tests were performed using the Pearson correlation test. Another statistical analysis, called the relative antioxidant capacity index (RACI), was applied to rank the extracts in terms of their antioxidant activity potentials, taking into account the cumulative effects of phenolics and flavonoids. This test was carried out to ensure that the results obtained from different antioxidant activity tests were comparable with each other (Sun & Tanumihardjo, 2007).

3. RESULTS / FINDINGS

Information on the family, habitat, substrate, herbarium number and localities of the mushroom species evaluated in the current study are given in Table 1. While *C. comatus* was collected from the roadside in Yuvacik-İzmit, other mushroom species were obtained from areas with high vegetation density such as beech, fir or spruce forests. All of the mushroom samples evaluated in the current study are grown in the soil.

Extraction yields obtained as a result of the extraction process are given in Table 2. According to the data in the table, a minimum extraction yield of 26.23% was obtained as a result of the extraction of mushroom species with methanol (*H. repandum*). Although the extract yields of *C. comatus*, *A. impudicus* and *S. imbricatus* were over 30%, it was determined that *C. comatus* had the highest yield with 36.84%.

3.1. Chemical Compositions of Mushroom Extracts

Total phenolic and flavonoid contents of methanol extracts obtained from *C. comatus*, *H. repandum*, *A. impudicus* and *S. imbricatus* are given in Table 2. It was determined that the phenolic contents of the samples were higher than the flavonoid contents. According to the data in the table, the methanol extract richest in phenolic compounds belongs to *S. imbricatus* [64.69 mg GAEs/g extract]. It was followed by *C. comatus*, *A. impudicus* and *H. repandum*, respectively (total phenolic contents were 21.6, 9.82 and 5.29 mg GAEs/g extract, respectively).

Table 2. Extract yields, total flavonoid and phenolic contents of methanol extracts of mushrooms.[1]

Mushrooms	Extract yield (%)	Total phenolics (mg GAEs/g extract)	Total flavonoids (mg QEs/g extract)
C. comatus	36.84	21.6 ± 0.52^{b}	0.15 ± 0.02^{b}
H. repandum	26.23	5.29 ± 0.04^{c}	0.21 ± 0.02^{b}
A. impudicus	33.16	9.82 ± 0.68^{c}	0.004 ± 0.001^{c}
S. imbricatus	35.11	64.69 ± 3.25^{a}	1.73 ± 0.05^{a}

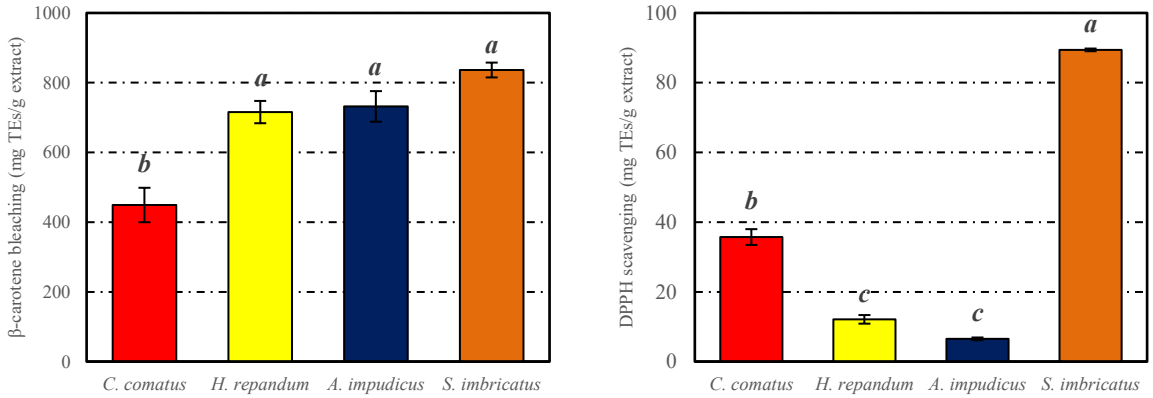
[1]Different superscripts in the same column indicate that the data is statistically significantly different from each other.

As understood from the total flavonoid content data in Table 2, *S. imbricatus* was the richest mushroom extract with 1.73 mg QEs/g. However, it was determined that the total flavonoid content of other mushroom extracts remained below 1.0 mg QEs/g. It was determined that *A. impudicus* was the poorest extract in terms of this compound class, with a flavonoid value of 0.004 mg QEs/g.

3.2. Antioxidant Activities of Mushroom Extracts

Mushroom extracts were subjected to four different test systems called β-carotene bleaching, DPPH free radical scavenging, reducing power and chelating effect in order to evaluate their antioxidant activities in a multi-dimensional manner. The results obtained from the β-carotene bleaching and DPPH free radical scavenging tests are given in Figure 1, while the data on the reducing powers and chelating effects of the extracts are presented in Figure 2.

Figure 1. Total antioxidant and radical scavenging activities of mushrooms.



According to the data in Figure 1, the extract that showed the highest activity in protecting linoleic acid against oxidation stress in the β-carotene bleaching test belonged to *S. imbricatus*. The activity value of this extract obtained from the test system in question was 836.0 mg TEs/g. *S. imbricatus* was followed by *A. impudicus* (732.0 mg TEs/g) and *H. repandum* (716.0 mg TEs/g) extracts, whose activity values were quite close to each other, respectively. *C. comatus*

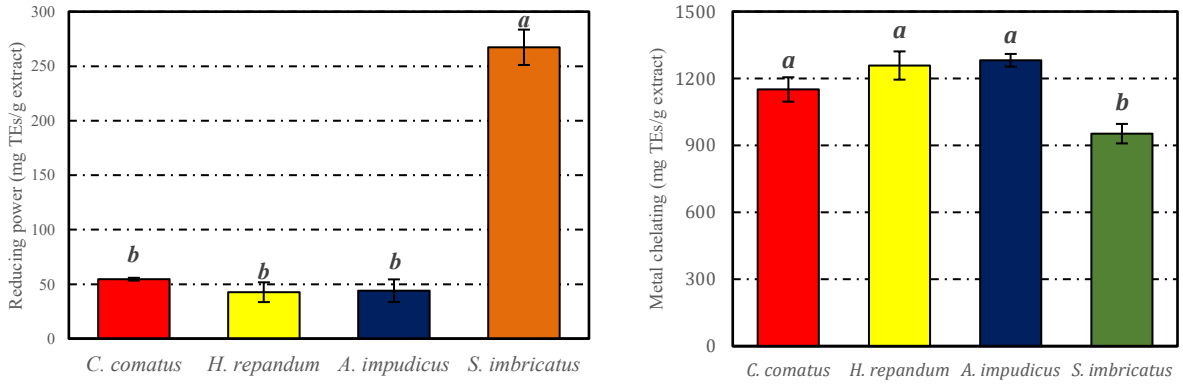
exhibited the lowest activity in this test system (449.0 mg TEs/g). Statistical analyzes showed that there was no significant difference between the activities of *H. repandum* and *A. impudicus*. However, the total antioxidant activities of the other extracts differed statistically significantly.

Data on DPPH radical scavenging activities of mushroom extracts are also presented in Figure 1. As can be seen from the figure in question, the extract exhibiting the most effective scavenging activity on the DPPH free radical was *S. imbricatus* (89.0 mg TEs/g), as in the β-carotene bleaching test. This value is almost three times higher than the free radical scavenging activity of *C. comatus* (36.0 mg TEs/g), the closest follower of the aforementioned mushroom species. In this test system, *H. repandum* and *A. impudicus* showed weak DPPH scavenging activity (12.0 and 7.0 mg TEs/g, respectively). The data obtained from the Tukey's test revealed that the DPPH radical scavenging activities of *H. repandum* and *A. impudicus* were not significantly different from each other, but other mushroom extracts showed statistically different activities.

According to Figure 2, which includes data on the reducing powers of the extracts, *S. imbricatus* again showed the highest activity (267.0 mg TEs/g), as in the β-carotene bleaching and DPPH free radical scavenging tests. However, the reducing powers of *C. comatus*, *H. repandum* and *A. impudicus* extracts were found to be too low and close to each other compared to *S. imbricatus* (55.0, 43.0 and 44.0 mg TEs/g, respectively). While the reducing power of *S. imbricatus* was statistically significantly different from the other three mushroom species, no statistical difference was found between the reducing powers of *C. comatus*, *H. repandum* and *A. impudicus*.

The final test system in which the antioxidant activities of the mushroom extracts evaluated in the present study was metal chelating assay. According to the data in Figure 2, the mushroom extracts showed a different potential than the activity profiles they exhibited in the first three test systems. In this test system, it was determined that the extract with the highest chelating capacity of metal ions belonged to *A. impudicus* (1282.0 mg TEs/g). However, the chelating capacity of *H. repandum* was found to be too close to that of *A. impudicus* (1258.0 mg TEs/g). The chelating activities of these two mushroom species were followed by *C. comatus* (1151.0 mg TEs/g) and *S. imbricatus* (953.0 mg TEs/g), respectively. Statistically, no difference was found between the metal chelating activities of *H. repandum* and *A. impudicus*. Moreover, it was determined that the results obtained from these two species and the metal chelating data of *C. comatus* were statistically partially similar. The metal chelating capacity of *C. comatus* was also found to be partially similar to that of *S. imbricatus*.

Figure 2. Reducing power and chelating activities of mushrooms.



3.3. Antioxidant Activity/RACI Correlations of Mushroom Extracts

As a result of the RACI calculations carried out in order to make the data obtained from different antioxidant activity tests and expressed in different units mathematically comparable with each other (Figure 3), when the data obtained from the four test systems are evaluated as a whole, it is seen that the antioxidant activity potential of *S. imbricatus* was found to be significantly higher than the other species (RACI value: 0.90). This mushroom species was followed by *H. repandum* and *A. impudicus* with the same RACI value (RACI value: - 0.24). *C. comatus* was in the last place in terms of antioxidant activity (RACI value: -0.42).

Figure 3. RACI values of methanol extracts of mushrooms.

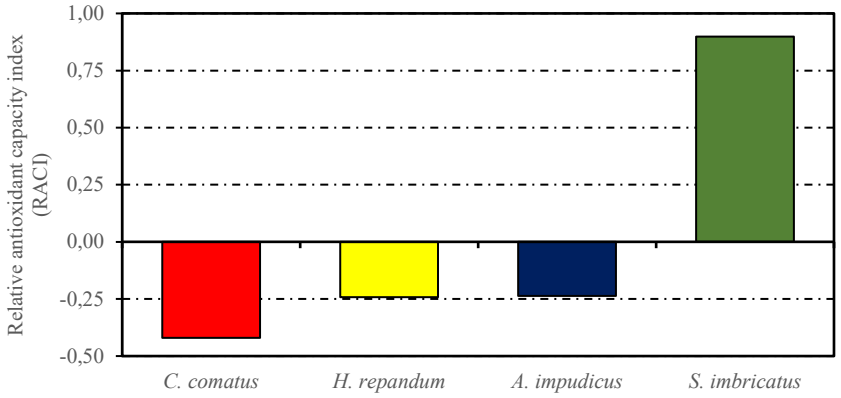
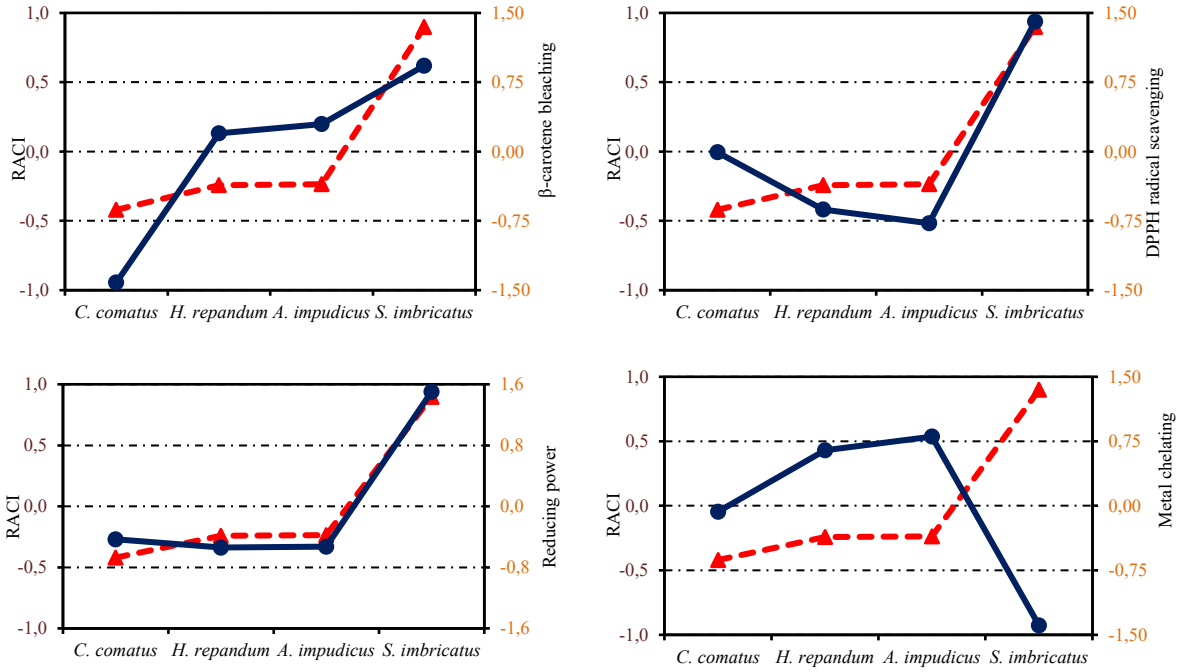


Figure 4 shows the correlation between the RACI values and the data obtained from the antioxidant activity tests of each of the extracts. It was determined that there was a high correlation between the data obtained from β-carotene bleaching, DPPH free radical scavenging, and reducing power tests and the RACI values of the extracts. However, it was found that there was an inverse correlation between the activity data of the extracts and the RACI values in the metal chelating test. This situation is thought to be a rational basis for the fact that the activity profile of the extracts in the metal chelating test is different from the activity profiles exhibited in other tests.

Figure 4. Correlation between RACI values and antioxidant activities of mushroom extracts.



3.4. Pearson Correlations Between Parameters

The data obtained as a result of the Pearson correlation analysis, which was carried out in order to statistically determine to what extent phenolics and flavonoids contribute to the antioxidant activities of the extracts and to reveal the correlation between the applied test systems, are given in Table 3. According to the data in the table, it was determined that there was a high correlation between the total phenolic and flavonoid compound amounts of the extracts and their DPPH free radical scavenging capacity and reducing power (correlation coefficients above 0.9). It was found that the correlation between the groups of compounds in question and the activities of the extracts in the β-carotene bleaching test is lower than the ones given above (correlation coefficients 0.401 and 0.573, respectively). It was also found that the correlation between the RACI values of the extracts and their reducing power was significant (correlation coefficient: 0.976).

Table 3. Pearson correlations between parameters.

Tests	β-carotene bleaching	DPPH radical scavenging	Reducing power	Metal chelating
DPPH radical scavenging	0.320	-	-	-
Reducing power	0.577	0.952	-	-
Metal chelating	-0.165	-0.920	-0.844	-
RACI	0.698	0.886	0.976	-0.807
Toal phenolics	0.401	0.986	0.976	-0.906
Total flavonoids	0.573	0.952	0.990	-0.868

4. DISCUSSION and CONCLUSION

4.1. Chemical Compositions of Mushroom Extracts

As a result of the literature search, no study was found investigating the chemical composition of *A. impudicus*. Therefore, the data presented for this mushroom species in the present study is the first record for the literature. On the other hand, there are some studies in the literature to elucidate the total phenolic/flavonoid contents and chemical compositions of *C. comatus*, *H. repandum* and *S. imbricatus*. In a study by Seklic *et al*. (2016), total phenolic and flavonoid contents of *C. comatus* were reported as 11.96 mg GAEs/g and 10.0 mg RUEs/g, respectively, while in another study by Sadi *et al*. (2015), these values were 76.32 and 3.67 µg/g, respectively. According to Kalaw & Albinto (2014), the total phenolic compound content of this species is 17.82 mg GAEs/g. There are also data on the quantitative chemical composition of *C. comatus* in the literature. Studies have reported that this mushroom species contains quinic (Karaman *et al*., 2019), vanillic, gallic, gentisic and cinnamic acids (Tesanovic *et al*., 2017). There is also a report in the literature regarding the total phenolic and flavonoid content of *H. repandum*. In this study by Ozen *et al*. (2011), the total phenolic and flavonoid contents of the mentioned species were reported as 3.67 mg pyrocatechol/g and 0.102 mg QEs/g, respectively. In addition, a study by Vasdekis *et al*. (2018) reported that *H. repandum* contains piceatannol. It is also reported in the literature that the total phenolic and flavonoid content of *S. imbricatus* is 1.50-13.20 and 1.46-5.45 mg/g, respectively (Barros *et al*., 2007a,b; Shomali *et al*., 2019) and contains gallic acid (0.75 mg/g) and myrcetin (2.89 mg/g) (Shomali *et al*., 2019). It is seen that the total phenolic/flavonoid compound data obtained from the present study differ from the literature data. It is a fact accepted by many researchers that the chemical compositions of both mushroom and plant samples are affected by environmental conditions such as climate, soil structure, pH, altitude, humidity, locality, collection time, etc. (Boira *et al*., 1998; Yatin *et al*., 2000). Therefore, it is thought that this difference arises due to environmental conditions.

4.2. Chemical Compositions of Mushroom Extracts

There is no data on the antioxidant activity of *A. impudicus* in the literature. In addition, the data obtained in the β-carotene bleaching of *H. repandum* and metal chelating tests of *S. imbricatus* were also brought to the literature for the first time with the present study.

There are some findings in the literature regarding the antioxidant activities of *C. comatus* obtained through the test methods used in the current study (Asatiani *et al*., 2007a,b; Liv *et al*., 2010; Akata *et al*., 2012; Kalaw & Albinto, 2014; Sadi *et al*., 2015; Seklic *et al*., 2016; Tesanovic *et al*., 2017; Cao *et al*., 2019). According to the data in these studies, the mushroom species in question showed 72.06% activity at a concentration of 2.0 mg/ml in β-carotene assay (Asatiani *et al*., 2007a, b). In DPPH free radical scavenging test, the mushroom species in question exhibited moderate or high activity according to some researchers (Li *et al*., 2010; Akata *et al*., 2012; Kalaw & Albinto, 2014; Sadi *et al*., 2015; Tesanovic *et al*., 2017; Seklic *et al*., 2016). According to Cao *et al.* (2019), polysaccharides contribute significantly to the DPPH free radical scavenging activity of *C. comatus*. In a study conducted by Akata *et al.* (2019), it was reported that the activity values of *C. comatus* in reducing power and metal chelating tests were 12.40-28.83 mg TEs/g and 7.76 mg EDTAEs/g, respectively. Another supporting finding that the species has metal chelating ability was reported by Sadi *et al.* (2015) (0.842 mg/ml).

The only available source in the literature regarding DPPH, reducing power, and metal chelating capacity of *H. repandum* is the study by Ozen *et al.* (2011). According to the results of this study, the related mushroom species exhibited 53.10%, 0.307 absorbance and 80.36% activity values in DPPH, reducing power and metal chelating tests at 50 µg/ml concentration, respectively.

According to a study carried out by Barros *et al.* (2017b), the activity values of *S. imbricatus*, another mushroom species analyzed in the present study, in β-carotene bleaching, DPPPH radical scavenging and reducing power tests, were 3.97, 1.44 and 2.79 mg/ml, respectively. In a study conducted by Barros *et al.* (2017c), it was reported that cooking and processing processes reduced the effectiveness of *S. imbricatus* in the β-carotene bleaching test. In other studies on the DPPH radical scavenging activity of *S. imbricatus*, it has been reported that methanol extract exhibited remarkable efficacy (Marcotullio *et al*., 2008; Luo *et al*., 2017; Shomali *et al*., 2019).

When the literature data given above are evaluated as a whole, it is understood that the data obtained from the present study generally share the same common denominator with those reported by other researchers, although some differences have been detected with some of the literature data. In particular, *S. imbricatus* is thought to be a potential source for new and alternative antioxidant compounds in the food industry. However, it was concluded that more detailed techniques such as fractionation accompanied by biological activity should be applied to detect the chemical compounds responsible for the activity.

Declaration of Conflicting Interests and Ethics

The author declares no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the author.

Authorship Contribution Statement

Arzuhan Sihoglu Tepe: Investigation, Methodology, Resources, Visualization, Software, Formal Analysis, Validation, and Writing -original draft.

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