



# Investigation of the Polyphenol Composition, Biological Activities, and Detoxification Properties of Some Medicinal Mushrooms from Turkey

## Türkiye'deki Bazı Tıbbi Mantarların Polifenol Bileşiminin, Biyolojik Aktivitelerinin ve Detoksifikasyon Özelliklerinin Araştırılması

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### ABSTRACT

**Objectives:** Ethanolic extracts of the mushroom species *Ganoderma adspersum*, *Inonotus hispidus*, *Russula chloroides*, and *Sarcodon imbricatus* were investigated for their polyphenolic contents and biological activities.

**Materials and Methods:** The radical scavenging activity of the extracts was evaluated by 2,2-diphenyl-1-(2,4,6-trinitrophenyl) (DPPH) method and their polyphenolic compounds were determined by high performance liquid chromatography (HPLC) analysis. Furthermore, the activity effects of mushroom extracts on the enzyme glutathione-S-transferase (GST) were also examined. Additionally, the antimicrobial activity of mushroom extracts was evaluated by disc diffusion method.

**Results:** Ethanolic extract of *I. hispidus* demonstrated the highest total phenolic content and total flavonoid contents, with 227.23±4.96 mg gallic acid equivalent/g and 42.14±0.20 quercetin equivalent/g, respectively. The highest DPPH radical scavenging activity was observed for ethanolic extracts of *I. hispidus*, with 10.687±1.643 µg/mL IC<sub>50</sub>. HPLC analysis demonstrated that *R. chloroides* was composed of ferulic acid, gallic acid, and myricetin compounds. The highest GST enzyme activity effect was detected with the ethanol extracts of *I. hispidus* and *S. imbricatus*. None of the mushroom extracts demonstrated significant inhibition of the bacterial strains used.

**Conclusion:** These results indicate that *I. hispidus* may be proposed as a new potential source of natural medicine and its potential may be related to its polyphenolic content, which needs further investigation.

**Key words:** Wild mushrooms, polyphenolic compounds, antioxidant, glutathione-S-transferase, detoxification properties

### ÖZ

**Amaç:** *Ganoderma adspersum*, *Inonotus hispidus*, *Russula chloroides* ve *Sarcodon imbricatus* mantar türlerinin etanolü ekstraktları, polifenolik içerikleri ve biyolojik aktiviteleri açısından araştırılmıştır.

**Gereç ve Yöntemler:** Ekstrelerin radikal süpürücü etkileri 2,2-difenil-1-(2,4,6-trinitrofenil) (DPPH) yöntemi kullanılarak ve polifenolik içerikleri yüksek performanslı sıvı kromatografisi (HPLC) analizleri ile belirlendi. Ayrıca, mantar ekstraktlarının glutatyon-S-transferaz (GST) enzim aktivatör etkisi incelendi. Bunlara ek olarak, mantar ekstraktlarının antimikrobiyal aktivitesi, disk difüzyon yöntemi ile değerlendirildi.

**Bulgular:** *I. hispidus*'un etanol ekstresi sırasıyla 227.23±4.96 mg GAE/g ve 42.14±0.20 QE/g değerleri ile en yüksek toplam fenol ve toplam flavonoid içeriği göstermiştir. DPPH radikalini en yüksek süpürme aktivitesi de *I. hispidus*'un etanol ekstresinde, 10.687±1.643 µg/mL IC<sub>50</sub> değeri ile gözlenmiştir. HPLC analizi, *R. chloroides*'in ferulik asit, gallik asit ve mirisetin bileşiklerini içerdiğini göstermiştir. En yüksek GST enzim aktivatör etki *I. hispidus* ve *S. imbricatus*'un etanol ekstraktlarında belirlenmiştir. Mantar ekstraktlarının hiçbirini kullanılan bakteri suşları üzerinde belirgin bir inhibisyon göstermemiştir.

**Sonuç:** Bu sonuçlar, ileri araştırmalar gerektirip *I. hispidus*'un yeni bir potansiyel doğal ilaç kaynağı olabileceğini ve bu etkinin polifenolik içerik ile ilişkili olabileceğini göstermektedir.

**Anahtar kelimeler:** Yabani mantarlar, polifenolik bileşikler, antioksidan, glutatyon-S-transferaz, detoksifikasyon özellikleri

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## INTRODUCTION

Recently, exploration of natural sources for novel bioactive compounds has gained considerable attention and it has helped to provide therapeutic drugs and principal compounds. Mushrooms, traditionally known as a valuable source of natural bioactive compounds, have been studied widely for their therapeutic capabilities. Medicinal mushrooms have been proved to contain many biologically active compounds, and many effective drugs and agrochemical fungicides are derived due to secondary metabolites extracted and isolated from mushrooms.<sup>1,2</sup> Some of the most recent isolated and identified compounds from mushrooms have shown promising antiviral, antibacterial, antioxidant, antidiabetic, immunomodulatory, antitumor, and hepatoprotective properties. Moreover, they contain a number of valuable nutrients, including protein, enzymes, B vitamins (especially niacin), and vitamin D.<sup>3,4</sup>

*Ganoderma adspersum* is a species of Basidiomycetes. Several species of *Ganoderma* are rich in bioactive compounds such as triterpenoids and polysaccharides. Traditionally, *Ganoderma* species have been widely used in the treatment of hepatopathy, chronic hepatitis, nephritis, hypertension, arthritis, neurasthenia, insomnia, bronchitis, asthma, and gastric ulcers.<sup>5</sup> They were also investigated for a variety of potential therapeutic benefits such as reducing blood pressure, as well as their blood cholesterol, antioxidant, anticancer, antidiabetic, antiviral, and antibacterial properties.<sup>6-9</sup> Wong et al.<sup>10</sup> demonstrated that extract of *Ganoderma lucidum* prepared in hot water had a protective effect on the cardiovascular system. Their study suggested that it reduces superoxide-induced damage to the heart. Additionally, Çayan-Tel et al.<sup>11</sup> isolated applanoxidic acid G, applanoxidic acid E, applanoxidic acid A, and 22-stigmastenol compounds from *G. adspersum* and investigated the antioxidant and anticholinesterase activities of mushroom extracts and isolated pure compounds. They reported that applanoxidic acid E and 22-stigmastenol showed significant antioxidant activities in the inhibition of lipid peroxidation. The same authors also demonstrated that applanoxidic acid G and 22-stigmastenol compounds exhibited moderate inhibiting activity against the enzyme butyrylcholinesterase.

*Sarcodon imbricatus* (Bankeraceae) is an edible mushroom. It is commonly known as the shingled hedgehog or scaly hedgehog. In folk medicine, it is used for lowering cholesterol levels, relaxing muscles, and regulating blood circulation.<sup>12</sup> Many studies have demonstrated that *S. imbricatus* is a good source of sterol compounds. In previous studies, the compounds ergosterol, ergostane, and cholestane have been isolated from *S. imbricatus*.<sup>13,14</sup> In particular, the presence of ergosterol peroxide in the methanol extract of *S. imbricatus* is important because it shows various biological activities, such as antileukemic, anticancer, apoptotic-inducing, and anti-inflammatory.<sup>15-17</sup>

*Inonotus hispidus* (Hymenochaetaceae) is commonly known as shaggy bracket. It is known as a pathogen on plants. However, it has numerous medicinal properties. In previous studies, the antiviral activity of two phenolic compounds, hispolon and hispidin, which were isolated from the fruit bodies

of *I. hispidus*, was investigated. The results obtained showed that hispidin and hispolon exhibit considerable antiviral activity against influenza viruses type A and B.<sup>18</sup>

*Russula chloroides* is a member of the genus *Russula*, which belongs to the family Russulaceae. Even though there is a high number of species, the biological properties of *Russula* have not been investigated in detail.

Glutathione-S-transferases (GSTs) comprise a phase II metabolic isozyme family existing in both eukaryotes and prokaryotes. These isozymes are best known for their ability to catalyze the conjugation of the reduced form of glutathione (GSH) to xenobiotic substrates for the purpose of detoxification. This eukaryotic species has multiple GST isozymes that are found in the cytosol and membrane. They catalyze the process of glutathione conjugation in electrophilic regions using a sulfhydryl group, which increases the solubility of xenobiotic and endogenous compounds. During this process, endogenous compounds like peroxidase lipids are detoxified together with the disintegration of compounds and xenobiotics.<sup>19,20</sup>

The main aims of this work were to investigate the polyphenolic contents and biological activities of ethanol extracts of several wild mushrooms from the local environment (*G. adspersum*, *I. hispidus*, *R. chloroides*, and *S. imbricatus*). Furthermore, the GST enzyme activity of these mushroom extracts was evaluated for the first time.

## MATERIALS AND METHODS

### Chemical materials

All chemicals used in the study were supplied by Sigma Aldrich (USA), except 1-chloro-2,4-dinitrobenzene (CDNB), which was purchased from Gerbu (Germany), and nutrient broth, malt extract, and malt extract agar, which were obtained from Merck (USA).

### Mushroom materials

Mushroom samples of *G. adspersum*, *I. hispidus*, *R. chloroides*, and *S. imbricatus* were collected from Belgrad Forest, İstanbul, and Yomra, Trabzon. They were identified by Dr. Ilgaz Akata and they are kept at the *Fungarium* of Ankara University with the code numbers Akata 6355, Akata 6052, Akata 5895 and Akata & Yuzun 757.

### Extraction of mushrooms

For ethanol extraction, 10 g of dried samples were weighed and ground into a fine powder with liquid nitrogen, and then mixed with 100 mL of ethyl alcohol (96%) at room temperature for 24 h. The extract obtained was filtered using Whatman No. 1 paper. In the next step, the existing ethanol was removed using a rotary evaporator at 40°C and the remaining solution was lyophilized. Finally, the samples were then kept in dark and cold (4°C) conditions to prevent oxidative damage.<sup>21</sup>

### Determination of total phenolic content

The Folin-Ciocalteu method was used to evaluate the content of total phenolic compounds of mushroom extracts.<sup>22</sup> Each 0.1 mL of extract solution was mixed with 2 mL of a 2% (w/v)

sodium carbonate solution using strong vortexing. After 5 min, 0.1 mL of 50% Folin–Ciocalteu reagent (w/v) was added and the resulting mixture was vortexed and then incubated for 1 h at room temperature. Afterwards, the absorbance of each mixture was measured at 750 nm with a UV-VIS spectrophotometer. The results were evaluated using 0.05, 0.1, 0.15, and 0.2 mg/mL gallic acid (GA) as standard curve and recorded as milligrams of GA equivalent per gram of dried sample.

#### Determination of total flavonoid content

The aluminum chloride colorimetric method was utilized to determine the total content of flavonoids in samples.<sup>23</sup> This test was conducted by mixing 0.1 mL of each extract solution with 0.15 mL of 95% ethanol, 0.01 mL of 10% aluminum chloride, 0.01 mL of 1 M sodium acetate, and 0.25 mL of dimethyl sulfoxide. The mixture was then incubated at room temperature for 30 min and the absorbance of the reaction was measured at 415 nm with a UV-VIS spectrophotometer. Finally, the standard curve was produced using different concentrations of quercetin solutions (0.025, 0.05, 0.1, 0.15, and 0.2 mg/mL). The total flavonoid content of the extract was expressed as milligrams of quercetin equivalent per gram of dried sample.

#### High performance liquid chromatography analysis

The high performance liquid chromatography (HPLC) assay was performed to investigate the phenolic compound profiles of ethanol mushroom extract. For this purpose, a lyophilized sample was dissolved in 2 mL of 80% methanol and then filtered using 0.45  $\mu$ m cellulose membrane filters before injection. Aliquots of 20  $\mu$ L were injected into the ultra-performance liquid chromatography system (Shimadzu Nexera X2, Shimadzu Corporation, Kyoto, Japan) equipped with a diode array detector set at 280, 320, and 360 nm. A 250 $\times$ 4.6 mm i.d., 5  $\mu$ m, C18 ODS-3 column (Intersil) was used. The mobile phase was composed of 5% formic acid (A) and methanol (B) at flow rate of 0.9 mL/min. The elution gradient was 5–80% (B) from 0 to 60 min. Calibration curves for each phenolic standard (the best wavelength) were prepared for quantification. For this purpose, myricetin, quercetin hydrate, ferulic, gallic, vanillic, caffeic, chlorogenic, and p-coumaric acids were used as positive controls. The HPLC analysis was carried out by the Central Laboratory of the General Directorate of the Food and Control Institute.

#### Free radical scavenging activity by DPPH assay

The radical scavenging activities of mushroom extracts were measured according to the methods described by Sharma and Bhat<sup>24</sup> with some modifications. The antioxidant activities of the extracts were determined on the basis of the radical scavenging effect of the DPPH-free radical. According to the procedure, 0.2 mL of mushroom extract at different concentrations was mixed with 0.5 mL of DPPH ethanol solution (0.12 mM) and 0.5 mL of ethanol (96%). Then samples were incubated for 30 min at room temperature and in darkness. Next absorbance was read at 517 nm by UV-VIS spectrophotometer with GA employed as reference. The DPPH radical scavenging activity of each sample was expressed as the half maximal inhibitory concentration

(IC<sub>50</sub>) value and calculated from the dose-response inhibition curve.

#### Isolation of cytosol from bovine liver

The bovine liver used in this study was provided by a slaughterhouse in Kazan, Ankara, Turkey. The liver samples were homogenized in 10 mM potassium phosphate buffer (pH 7.0), containing 0.15 M KCl, 1 mM EDTA, and 1 mM DTT, using a glass Teflon homogenizer and then centrifuged at 10,000 $\times$ g for 20 min. The supernatant was filtered through cheesecloth and the filtrate was centrifuged at 30,000 $\times$ g for 60 min. The collected supernatants were filtered again and the resultant filtrate was referred to as cytosol.<sup>25</sup> The prepared homogenates were kept at -80°C for future analysis. Total protein content was determined by the Lowry method.<sup>26</sup>

#### Testing activity of GST

GST activity was determined against the substrate CDNB by monitoring thioether formation at 340 nm.<sup>27</sup> In line with the protocol, an assay mixture composed of mushroom extract solutions (concentration in the range of 10–0.625 mg/mL), 200 mM potassium phosphate buffer (pH 6.5) with 20 mM CDNB and 50 mM GSH, and bovine liver cytosolic fractions was prepared and used as the enzyme source to measure GST activity. GSH-CDNB conjugate formation was followed in 1 mL total volume assay by UV-VIS spectrophotometer at 340 nm for 2 min. Initial rates of enzymatic reactions were determined as nanomoles of the conjugation product of GSH and reported as nmol/min/mL.

#### Antimicrobial assay

The antibacterial activities of mushroom extracts were determined against the gram (+) bacterial strain *Staphylococcus aureus* ATCC 25923 and the gram (-) bacterial strains *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 using the disc diffusion method.<sup>28</sup> The bacterial strains were incubated at 37°C in Nutrient Broth culture for 24 h. Inoculants were prepared by transferring colonies of each organism into 0.9% sterile saline solution until the visible turbidity was equal to 0.5 McFarland standard containing approximately 10<sup>8</sup> cfu/mL bacteria. Nutrient Agar was used as culture for antibacterial activities. Afterwards, 0.02 mL of each extract was applied to 6-mm-diameter sterile paper discs and to eliminate any residual solvent the discs were left to dry overnight at room temperature. The surface of the plates was inoculated by using prepared inoculant containing saline suspension of microorganisms. The discs were placed in the center of the agar surface of each petri plate. Zones of inhibition were measured in mm after incubating the petri plates at 30°C for 24 h. For this study, streptomycin (10 mg) and tetracycline (30 mg) were used as positive controls for all samples.

## RESULTS

The ethanol extracts of *G. adpersum*, *I. hispidus*, *R. chloroides*, and *S. imbricatus* were used to investigate their polyphenolic contents and antioxidant and antimicrobial activities. Moreover, for the first time, their effects on GST activity were evaluated. With this research, each extract was prepared by dissolving

10 g of dry samples in 100 mL of ethanol (96%) solvent. The extraction yields of *G. adspersum*, *I. hispidus*, *R. chloroides*, and *S. imbricatus* were in the range of 3.71% to 13%. In addition, total phenolic contents, total flavonoid contents, and radical scavenging activity of the extract were determined using spectrophotometric assays. The percentage of the yields, total phenolic contents, total flavonoid contents, and results of DPPH scavenging activity are shown in Table 1. The total phenolic contents of extracts were 227.23±4.96 to 3.125±0.12 mg GAE/g of the dry samples. The total flavonoid contents varied from 42.14±0.20 to 1.99±0.27 mg QE/g of the dry samples. Phenolic and flavonoid contents of the ethanol extract of *I. hispidus* were 227.23±4.96 mg GAE/g and 42.14±0.20 QE/g, respectively, and these are higher values compared to the other mushroom species. Free radical scavenging activity results are presented as percentage of DPPH radical scavenging activity of different extracts (mg/mL) according to concentration inhibition curves and IC<sub>50</sub> values. The results showed that the highest amount of free radical scavenging activity was in the extracts of *G. adspersum* and *I. hispidus*, with 48.002±0.861 and 10.687±1.643 µg/mL IC<sub>50</sub>, respectively (Figure 1, Table 1). The IC<sub>50</sub> value for GA solution, which was used as a reference, was 4.000±0.002 µg/mL.

**Table 1. The percent (%) yield, total phenolics content, total flavonoid content, and DPPH results of mushroom extracts**

Mushroom	Yield (%)	TPC (mg GAE/g)	TF (mg QE/g)	DPPH IC <sub>50</sub> µg/mL
<i>Inonotus hispidus</i>	3.71	227.23±4.96	42.14±0.20	10.687±1.643
<i>Ganoderma adspersum</i>	6.44	109.20±8.83	13.6±0.22	48.002±0.861
<i>Sarcodon imbricatus</i>	11.88	13.20±0.1	5.45±0.11	950.878±11.418
<i>Russula chloroides</i>	13.00	3.125±0.12	1.99±0.27	2637.709±55.857
Gallic acid	-	-	-	4.000±0.002

DPPH: 2,2-diphenyl-1-(2,4,6-trinitrophenyl), TPC: Total phenolic contain, TF: Total flavonoid

**Table 2. HPLC analysis of the phenolic profiles of mushrooms extracts**

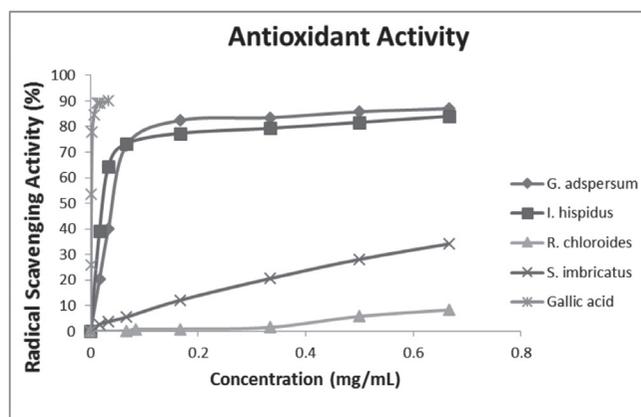
Mushroom	<i>Inonotus hispidus</i>	<i>Ganoderma adspersum</i>	<i>Sarcodon imbricatus</i>	<i>Russula chloroides</i>
Content of selected polyphenol compound mg/g				
Caffeic acid	-	-	-	-
Chlorogenic acid	-	-	-	-
<i>p</i> -coumaric acid	-	-	-	-
Ferulic acid	-	-	-	4.6020±0.23
Gallic acid	-	-	0.7510±0.04	0.3027±0.02
Myricetin	0.6010±0.03	0.6370±0.03	2.8910±0.15	1.7460±0.09
Quercetin hydrate	-	1.3040±0.07	-	-
Vanillic acid	-	-	-	-

HPLC: High performance liquid chromatography

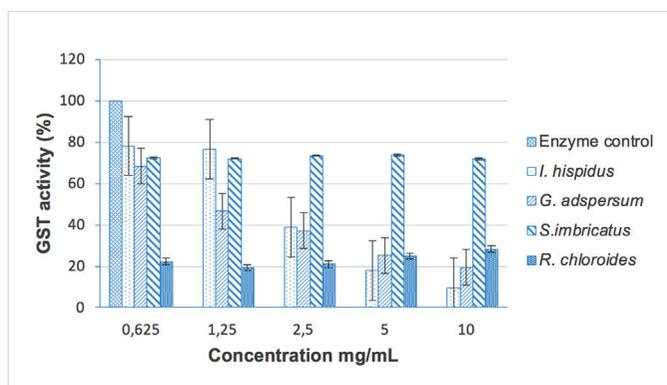
The phenolic profiles of the ethanol extracts from *G. adspersum*, *I. hispidus*, *R. chloroides*, and *S. imbricatus* were studied by HPLC assay. In this assay, myricetin, quercetin hydrate, ferulic, gallic, vanillic, caffeic, chlorogenic, and *p*-coumaric acids were used as references. According to these results, *R. chloroides* included ferulic acid, GA, and myricetin compounds, with 4.6020±0.23, 0.3027±0.02, and 1.7460±0.09 mg/g values, respectively. Moreover, myricetin was found in all of the ethanol extract solutions. However, none of the extract solutions contained vanillic, caffeic, chlorogenic, or *p*-coumaric acids. The results are given in Table 2.

With this study, it was shown that the *G. adspersum*, *I. hispidus*, *R. chloroides*, and *S. imbricatus* extracts had effects on GST enzyme activity. The extracts were used within the range of 0.625-10 mg/mL concentration while measuring the activity on GST. The best activity profile for GST was observed with the crude ethanol extracts of *I. hispidus* and *S. imbricatus* (Figure 2). The activities of the ethanol extracts of mushrooms are presented in Figure 2.

Moreover, we also demonstrated the antimicrobial activity of *G. adspersum*, *I. hispidus*, *R. chloroides*, and *S. imbricatus* extracts against the *S. aureus*, *E. coli*, and *P. aeruginosa* strains by disc diffusion assay. However, in comparison with the positive



**Figure 1. Percent free radical scavenging activity of mushroom extracts. The data represent the mean ± standard deviation (n=3)**



**Figure 2.** Effects of mushroom extracts on glutathione-S-transferase enzyme activity (%). The data represent the mean  $\pm$  standard deviation (n=3)

controls, none of the mushroom extracts showed significant inhibitory effects on the bacterial strains. The results of the disc diffusion assay of mushroom extracts are presented in Table 3.

**Table 3. Results of disc diffusion of mushroom extracts**

Mushroom	<i>Escherichia coli</i> ATCC 25922	<i>Staphylococcus aureus</i> ATCC 25923	<i>Pseudomonas aeruginosa</i> ATCC 27853
	Inhibition zone (mm)		
<i>Inonotus hispidus</i>	10	10	10
<i>Ganoderma adspersum</i>	10	10	8
<i>Sarcodon imbricatus</i>	9	9	9
<i>Russula chloroides</i>	8	8	8
Streptomycin	20	23	15
Tetracycline	13	35	20

## DISCUSSION

In the present study, we found that the ethanol extract of *G. adspersum* contained significant amounts of phenolic and flavonoid compounds. It was also shown that *G. adspersum* extract exhibited significant free radical scavenging effect. Moreover, the ethanol extract from *G. adspersum* included remarkable amounts of myricetin and quercetin hydrate compounds, which have benefits to health. These compounds possess antioxidant and anticancer properties.<sup>29-31</sup> It should also be noted that *G. adspersum* extract revealed weak antimicrobial activity against *S. aureus*, *E. coli*, and *P. aeruginosa* strains. Kuruni et al.<sup>32</sup> evaluated the antioxidant activity of methanol extracts of *G. applanatum* using *in vitro* models. They reported that high amounts of phenolic and flavonoid compounds in the extract were determined and, therefore, the extract exhibited a significant antioxidant capacity in the DPPH radical scavenging assay.

Furthermore, we demonstrated that the ethanol extract from *S. imbricatus* included small amounts of phenolic and flavonoid

compounds; therefore, it showed lower antioxidant and antimicrobial activity profiles. Using the HPLC assay we found that the ethanol extract of *S. imbricatus* also included GA and myricetin compounds. It was highly effective on GST activity at all doses. Marcotullio et al.<sup>33</sup> reported that the methanol extract of *S. imbricatus* contained high amounts of polyphenol contents, which can explain the radical scavenging activity.

In the present study, it was found that the ethanol extract of *I. hispidus* contained high amounts of phenolic and flavonoid contents, which suggests that the high potential effects of free radical scavenging activity may be raised due to the large amounts of polyphenolic profiles. The low dosage of *I. hispidus* extracts showed an elevated effect on GST activity. The ethanol extract of *I. hispidus* also demonstrated a slight antimicrobial effect on the bacterial strains. In another study, the inhibitory effect of phenolic compounds and alkaloids of *I. hispidus* was investigated on the lipase of *Candida rugosa*.<sup>34</sup> It was observed that the phenolic and alkaloid extracts were efficient inhibitors of the lipase of *C. rugosa*. Therefore, it was suggested that these compounds could be used in the treatment of candidiasis. The obtained results also indicated that the phenolic extracts showed stronger radical scavenging activity than the alkaloids extracts.<sup>34</sup>

In our study, *R. chloroides* included very low amounts of polyphenolic contents and showed antioxidant and antimicrobial activities. On the other hand, HPLC analysis showed that the ethanol extract of *R. chloroides* contained remarkable amounts of ferulic acid, myricetin, and GA compounds. However, the effect of this extract on GST enzyme activity was negligible.

## CONCLUSIONS

In the present research, the biological activity and detoxification potential of the polyphenol contents isolated from *G. adspersum*, *I. hispidus*, *R. chloroides*, and *S. imbricatus* species were investigated for their free radical scavenging and GST enzyme activities. It was found that the ethanol extract of *I. hispidus* had large amounts of phenolic and flavonoid contents. It was also observed that this ethanol extract had a high level of free radical scavenging potential. This activity may be attributed to the high concentration of polyphenol compounds in the ethanol extract of *I. hispidus*. Moreover, the ethanol extract of *I. hispidus* had a significant potential to increase GST enzyme activity, which plays a critical role in detoxification pathways. Therefore, *I. hispidus* was suggested to be a new potential source of natural medicine.

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*Conflict of Interest:* No conflict of interest was declared by the authors.

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