UDC 582.284:543.061 **https://doi.org/10.15407/biotech11.03.069**

Ganoderma SPECIES EXTRACTS: ANTIOXIDANT ACTIVITY AND CHROMATOGRAPHY

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Received 19.02.2018

Research aimed to isolate biologically active compounds from mushrooms fruiting bodies of *Ganoderma lucidum*, *Ganoderma adspersum* and *Ganoderma applanatum* and to estimate their antioxidant activities. Various techniques were used to isolate biologically active compounds. Antioxidant properties were estimated with spectrophotometricaly measuring free radical scavenging activity. High performance liquid chromatography was applied to analyze the isolated extracts. Half maximal inhibitory concentration (IC_{50}) was 8.25±0.88 μg/ml and 1.70±1.13 μg/ml for *G. applanatum* and *G. adspersum*, respectively. However, petroleum ether and chloroform extracts of *G. lucidum* demonstrated highler antioxidant activity with an IC_{50} about 33.66 ± 3.69 μg/ml. Chromatograms of components of acetone and methanol extracts of *G. lucidum* were recorded. The main outcome of such chromatograms is the possibility to detect the presence of active components in various mushroom species without the usage of expensive standards.

Key words: Ganoderma species mushrooms, antioxidant activity, high performance liquid chromatography.

Various mushrooms species are the focus of researchers' interest. Hitherto, lectins, polysaccharides, polysaccharide-peptides, polysaccharide-protein complexes, lanostane-type triterpenoids, phenolics and flavonoids were isolated from some mushroom species [1]. Furthermore, various biological activities such as antioxidant, antibacterial, antifungal [2, 3], antitumor [4], anti-inflammatory [5], cytotoxic [6] and anti-cholinesterase [7] activities of the isolated compounds and/or complexes were investigated. In recent years, more variety of mushrooms were isolated and identified, and the number of mushrooms being cultivated for food or medicinal purposes were increasing rapidly.

Chemicals isolated from mushrooms have significant biological activity that may cause noticeable curative effects on human health and therefore could be used in medicine [8]. Hence, the comparison of mushroom components is highly desirable for the creation of drugs. Chromatography techniques are widely applied to separate biologically active components in extracts of mushrooms at first [1]. Then, chromatograms of such extracts could be compared to each other and peaks with identical retention times could be identified. Such approach would help to identify the extracts that possess biological activity and ease of their future purification on the way of drug formulation.

The aim of present research is developing of effective methods for preparation of samples containing biologically active compounds. Preliminary sample preparation was performed using solid-liquid, ultrasonic and Soxhlet extractions [9, 10]. Then, antioxidant activity and chromatograms were estimated for all extracts.

Materials and Methods

G. lucidum, G. adspersum, and G. applanatum fruiting bodies were obtained from Mula, Turkey (Table 1). Petroleum ether, methanol, chloroform and acetone of analytical and gradient grade were supplied by Merck.

G. lucidum (120 g), *G. adspersum* (385 g), and *G. applanatum* (1200 g) material were collected from Koycegiz, Mula, dried in the air and crushed into small particle (2–6 mm).

Solid-liquid extraction. Bioactive compounds of *G. lucidum* (50 g), *G. adspersum* (175 g) and *G. applanatum* (400 g) were extracted with a mixture of petroleum ether and chloroform (4:1, v/v, 400 ml, 1 l, 2 l respectively). For the extraction, all biological materials should be covered with these solvents. Then bioactive compounds were sequentially extracted with acetone (1 l), methanol (1 l), and water (1 l) at 25 °C. Each extraction experiment was performed until the solvent became colorless.

Ultrasonic extraction. Bioactive compounds of *G. lucidum* (10 g), *G. adspersum* (10 g) and *G. applanatum* (10 g) were extracted with a mixture of petroleum ether and chloroform (4:1, v/v, 100 ml) at 25 °C for 20 min in triplicates. Then, they were sequentially extracted with acetone (100 ml), methanol (100 ml) and water (100 ml).

Soxhlet extraction. Bioactive compounds of *G. lucidum* (50 g), *G. adspersum* (50 g), and *G. applanatum* (50 g) were extracted in a Soxhlet apparatus with a mixture of petroleum ether and chloroform (4:1, v/v, 1 l) for 4 h. Then, mushroom materials were sequentially extracted with acetone (1 l), methanol (1 l), and water (1 l).

Each extraction experiment was performed until the solvent became colorless. Sediments were filtered by means of filter paper. Filtrates were concentrated under vacuum (V = 0.5 ml) using a rotary evaporator and dried in the air. The extracts collected under different techniques started above were subjected to *in vitro* tests to confirm their antioxidant activities.

1. *Extraction of polysaccharides. Solid-liquid extraction*. After methanol extraction, mushroom materials were collected and extracted with distilled hot water. The polysaccharide extracts were obtained by hot water extraction and precipitation with ethanol.

Mushrooms material was extracted with 500–1000 ml of distilled hot water at +80 °C (until samples became cold). The crude hot water extracts were filtered and finally concentrated under vacuum (V = 50–100 ml) using a rotary evaporator. Then 200–400 ml of ethanol was added to concentrated hot water extracts. Polysaccharides were precipitated overnight at +4 °C. The precipitated polysaccharides were collected after centrifugation (N ve NF800) at 3100 × g for 2 min, and extraction yield was calculated.

Ultrasonic extraction. After methanol extraction, 10 g of material were extracted tree times with 100 ml of distilled water at 80 °C (until samples became cold) for 20 min with ultrasonication. Hot water extracts were filtered and combined (V = 300 ml). Finally, concentrated under vacuum (V = 30 ml) using a rotary evaporator. 120 ml of ethanol was added to concentrated hot water extracts and polysaccharides were precipitated overnight at +4 °C. The precipitated polysaccharides were collected after centrifugation (N ve NF800) at

Table 1. **Characteristics of mushroom species collecting through 2014**

Number	Mushroom species	Tree type	Region of collection	Time of collection
1	*G. lucidum*	Sweetgum	Mula, Fethiye	September
2	*G. adspersum*	Sweetgum	Mula, Fethiye	September
3	*G. applanatum*	Mulberry	Mula, Koycheiz	September
4	*G. lucidum*	Sweetgum	Mula, Koycheiz	September
5	*G. adspersum*	Walnut	Izmir, Balchova	October
6	*G. adspersum*	Peach	Mula, Ula	October
7	*G. adspersum*	Plum	Mula, Fethiye	September
8	*G. lucidum*	Sweetgum	Mula, Marmaris	November
9	*G. adspersum*	Sweetgum	Mula, Marmaris	November
10	*G. lucidum*	Sweetgum	Mula, Ula	November
11	*G. adspersum*	Mulberry	Mula, Koycheiz	November
12	*G. lucidum*	Mulberry	Mula, Koycheiz	November
13	*G. adspersum*	Mulberry	Karabalar, Mula	November

3100 × g for 2 min, and extraction yield was calculated.

Soxhlet extraction. After methanol extraction, 50 g of material were extracted in a Soxhlet apparatus with 1 l water for 4 h. Finally, concentrated under vacuum to V = 100 ml using a rotary evaporator. 400 ml of ethanol was added to concentrated hot water extracts. Polysaccharides were precipitated overnight at +4 °C. The precipitated polysaccharides were collected after centrifugation (Nüve NF800) at 3100 × g for 2 min, and extraction yield was calculated.

Determination of antioxidant activity. β-Carotene-linoleic acid assay

The procedure was done according to Ferreira et al. (2006) [7]. A stock solution of β-carotene and linoleic acid was prepared by dissolving 0.5 mg of β-carotene in 1 ml of chloroform and adding 25 µl of linoleic acid with 200 mg of Tween-40. The chloroform was evaporated at 40 °C under vacuum using a rotary evaporator. Aerated water (100 ml) was added to the residue.

4 ml of this mixture were transferred into different test tubes containing different concentrations of the sample in ethanol. The zero time-absorbance was measured at 470 nm. The samples were incubated for 2 h at 50 °C together with a blank solution, and four others containing the antioxidants Butylated hydroxyanisole (BHA), α-tocopherol. The absorbance was measured at 470 nm. The bleaching rate (R) of β-carotene was calculated according to the following equation:

$$R = (\ln a/b)/t,$$

where ln — natural log, a — absorbance at time zero and b — absorbance at time t (2 h). Antioxidant activity (AA) was calculated in terms of percent inhibition relative to the control, using following equation:

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

DPPH free radical scavenging activity

The free radical scavenging activity of extracts was determined by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay described by Blois [11] with slight modifications. In its radical form, DPPH absorbs at 517 nm, but on reduction by an antioxidant or a radical species its absorption decreases. 0.1 mmol·L^{-1} Solution of DPPH in methanol was prepared and 4 ml of this solution was added to 1 ml of sample solution in methanol at different concentrations. Thirty minutes in the dark later, the absorbance was measured at 517 nm. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. The capability to scavenge the DPPH radical of an antioxidant was calculated using the following equation:

$$\text{DPPH } \textit{scavenging effect (\%)} = [(A_{control} - A_{sample})/A_{control}] \times 100,$$

where $A_{control}$ is the absorbance of the DPPH solution and A_{sample} is the absorbance of the sample.

ABTS cation radical decolorization assay

The spectrophotometric analysis of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) scavenging activity was determined according to the method of Re et al. [12] with slight modifications. The ABTS was produced by the reaction between 7 mM ABTS in water and 2.45 mM potassium persulfate, stored in the dark at room temperature for 12 h. Oxidation of ABTS commenced immediately, but the absorbance was not maximal and stable until more than 6 h had elapsed. The radical cation was stable in this form for more than 2 days in storage in the dark at room temperature. Before usage, the ABTS solution was diluted to get an absorbance of 0.708 ± 0.025 at 734 nm with ethanol. Then, 160 µl of ABTS solution was added to 40 µl of sample solution in ethanol at different concentrations. After 10 min the absorbance was measured at 734 nm by using a 96-well microplate reader. The percentage inhibitions were calculated for each concentration relative to a blank absorbance (ethanol). The scavenging capability of ABTS was calculated using the following equation:

$$\text{ABTS } \textit{scavenging effect} = [(A_{control} - A_{sample})/A_{control}] \times 100,$$

where $A_{control}$ is the initial concentration of the ABTS and A_{sample} is the absorbance of the remaining concentration of ABTS in the presence of sample. The extract concentration providing 50% radical scavenging activity (EC_{50}) was calculated from the graph of ABTS scavenging effect percentage against extract concentration. BHT, α-tocopherol were used as antioxidant standards for comparison of the activity.

Cupric reducing antioxidant capacity (CUPRAC). The cupric reducing antioxidant capacity of the extracts was determined according to the CUPRAC method [13] with slight modifications. To each well, in a 96 well plate, 50 µl of 10 mM Cu (1 l), 50 µl of 7.5 mmol neocuproine, and 60 µl of NH_4Ac buffer (1 M, pH 7.0) solutions were added. 40 µl of extract at different concentrations was added to the

initial mixture so as to make the final volume 200 µl. After 1 h, the absorbance at 450 nm was recorded against a reagent blank by using a 96-well microplate reader. Results were given as absorbance and compared with BHA, a-tocopherol used as antioxidant standards.

HPLC analysis. A high performance liquid chromatographic system with multiwavelength spectrophotometer was used for measuring. Analytical RP-column Separon shim-pack VP-ODS (5 µm, 4.6 mm × 150 mm) was used for chromatographic separations. Chromatographic conditions used for methanol extracts were as follows. The mobile phase was a mixture of acetonitrile (A) and 0.1% CH_3COOH in water (B). 0 min — 2.0% A; 10 min — 2.0% A, 20 min — 5.0% A, 30 min — 20% A, 60 min — 100% A, 62 min — 100% A, 65 min — 2% A, 68 min — 2.0% A. The mobile phase was degassed in a sonicator, and pumped in gradient mode at a flow rate of 1.5 ml/min at 35 °C. The UV detection was accomplished at 245 nm. Samples of 20 µl were injected into column. The qualitative identification of the compounds present in the samples was based on comparison of retention time and UV spectrum with standards.

Chromatographic conditions used for acetone extracts were as follows.

The mobile phase was a mixture of methanol (A) and water (B). 0 min — 50.0% A; 10 min — 50.0% A, 65 min — 100.0% A, 67 min — 100% A, 70 min — 50% A, 75 min — 50% A. The mobile phase was degassed in a sonicator. The mobile phase was pumped in gradient mode at a flow rate of 1.5 ml/min at 35 °C. The UV detection was accomplished at 245 nm and samples of 20 µl were injected into column. The qualitative identification of the compounds present in the samples was based on comparison of retention time and UV spectrum with standards.

Statistical analysis. All the data on antioxidant activity tests were the average of triplicate analyses. The data were recorded as mean ± standard deviation. Significant differences between means were determined by Student's *t*-test, P values < 0.05 were regarded as significant.

Results and Discussion

The results for antioxidant activities of extracts collected under different sample preparation techniques are represented in the Tables 2–4. All extracts were tested in the range of their concentrations from 6.25 to 800 $mg \cdot l^{-1}$. Absorbance for those ones varies in the interval of 10÷90 absorbance units with the standard deviation 0.34÷4.

The antioxidant activity of mushroom extracts was compared with those of BHA and α-tocopherol that are used as standards in food and pharmaceutical industry. In β-carotene-linoleic acid assay, petroleum ether and choroform extracts of *G lucidum* demonstrated the best antioxidant activity with an IC_{50}: 33.66 ± 3.69 µg/ml, followed by acetone 36.97 ± 2.64 µg/ml, aqueous supernatant (IC_{50}: 76.03 ± 7.96 µg/ml), methanol (IC_{50}: 130.68 ± 28.05 µg/ml), and water extract (IC_{50}: 2966.67 ± 793.85). In DPPH assay, acetone extract of *G. lucidum* demonstrated the best antioxidant activity with an IC_{50}: 135.24 ± 8.94, followed by methanol 409.94 ± 10.09µg/ml, aqueous supernatant (IC_{50}: 586.51 ± 20.05 µg/ml), petroleum ether and choroform extracts (IC_{50}: 1195.25 ± 88.64). In general, the antioxidant activity of acetone extracts of all mushroom species was found as the highest. The DPPH free radical scavenging activity of the *G. lucidum* was better for solid-liquid extraction in acetone extracts (IC_{50}: 83.79 ± 1.37). The best activity was found to be in acetone extract of *G. lucidum* using Soxhlet extraction with β-carotene-linoleic acid assay (IC_{50}: 18.54 ± 2.38 µg/ml). The best ABTS scavenging activity was found in acetone extract of *G. lucidum* Soxhlet extraction with an IC_{50} of 25.07 ± 2.83 µg/ml. The best CUPRAC activity was found in acetone extract of *G. lucidum* using solid-liquid extraction (IC_{50}: 25.28 ± 0.14 µg/ml).

In β-carotene-linoleic acid assay, methanol extract of *G. adspersum* demonstrated the best antioxidant activity with an IC_{50}: 1.70 ± 1.13 µg/ml (Table 4), followed by acetone 9.79 ± 5.73 µg/ml, aqueous supernatant (IC_{50}: 69.19 ± 0.30 µg/ml), petroleum ether and choroform (IC_{50}: 157.17 ± 14.80 µg/ml), and water extracts (IC_{50}: 426.90 ± 24.27). In DPPH assay, acetone extract of *G. adspersum* demonstrated the best antioxidant activity with an IC_{50}: 10.36 ± 0.69 (Table 4), followed by methanol 36.54 ± 1.15 µg/ml, aqueous supernatant (IC_{50}: 282.85 ± 41.17 µg/ml), petroleum ether and choroform extracts (IC_{50}: 9950.60 ± 100.69). The DPPH free radical scavenging activity of the *G. adspersum* was better for ultrasonic extraction in acetone extract (IC_{50}: 10.36 ± 0.69). The best ABTS scavenging activity was found in acetone extract of *G. adspersum* in Soxhlet extraction with an IC_{50} of 3.18 ± 0.17 µg/ml. The best CUPRAC activity was found in acetone extract of *G. adspersum* using ultrasonic extraction (IC_{50}: 7.58 ± 1.33 µg/ml).

Table 2. **Antioxidant activity of the extracts of *G. lucidum, G. adspersum, G. applanatum* by the β-carotene-linoleic acid, DPPH, ABTS, and CUPRAC, obtained by solid-liquid extraction**

Mushrooms/ standards	Extracts	β-carotene-linoleic acid assay IC_{50a} (μg/ml)	DPPH assay IC_{50} (μg/ml)	ABTS assay IC_{50} (μg/ml)	CUPRAC IC_{50} (μg/ml)
G. lucidum	Petroleum ether and choroform	120.70±8.52* **	1448.76±42.06* **	890.02±199.93* **	477.50±14.85* **
	Acetone	22.84±1.78* **	83.79±1.37* **	27.14±2.24* **	25.28±0.14* **
	Methanol	24.94±0.07	249.09±7.23* **	35.78±20.56	199.25±48.44* **
	Water	260.18±10.00 * **	N.A.	150.70±17.62* **	26.15±0.68
	Aqueous supernatant	124.79±5.82* **	498.54±119.83	94.61±2.99* **	86.40±2.26* **
G. adspersum	Petroleum ether and choroform	367.35±0.41* **	6402.63±74.95* **	N.A.	436.33±12.90* **
	Methanol	41.20±2.42**	95.09±4.22**	23.23±1.05	31.89±0.77
	Water	24.97±3.34*	702.30±32.06* **	133.93±26.41* **	40.10±1.14**
G. applanatum	Petroleum ether and choroform	265.87±8.47* **	3653.56±242.66* **	N.A.	422.22±5.17* **
	Acetone	20.19±9.50**	3.50±1.68**	4.27±0.97**	1.16±0.17**
	Methanol	8.25±0.88	42.17±1.57* **	3.35±0.94	11.56±0.60*
	Water	41.79±1.28* **	231.62±0.25* **	79.36±10.54* **	47.50±2.12* **
Control α-Tocopherol b (standard)	Ethanol	0.81±0.01	28.99±0.87	15.37±0.50	64.50±3.94
Control BHA b (standard)	Ethanol	0.54±0.04	16.82±0.11		

Hereinafter: a — IC_{50} values represent the means ± standard deviation of three parallel measurements ($P < 0.05$); b — reference compounds; N.A. — not bioactive; * — level of confident probability between average value, statistical significance of the differences comparing to the first control (α-tocopherol), $P < 0.05$; ** — level of confident probability between average value, statistical significance of the differences comparing to the second control (BHA), $P < 0.05$.

In β-carotene-linoleic acid assay (Table 2), methanol extract of *G. applanatum* demonstrated the best antioxidant activity with an IC_{50}: 8.25 ± 0.88 μg/ml (Table 4), followed by acetone 20.19 ± 9.50 μg/ml, water (IC_{50}: 41.79 ± 1.28 μg/ml), petroleum ether and chloroform extracts (IC_{50}: 265.87±8.47 μg/ml). In DPPH assay, acetone extract of *G. applanatum* demonstrated the best antioxidant activity with an IC_{50}: 3.50 ± 1.68 (Table 2), followed by methanol 42.17 ± 1.57 μg/ml, water (IC_{50}: 231.62 ± 0.25 μg/ml), petroleum ether and choroform extracts (IC_{50}: 3653.56 ± 242.66). The best ABTS scavenging activity was found in methanol extract of *G. applanatum* in solid-liquid extraction with an IC_{50} of 3.35 ± 0.94 μg/ml. The best CUPRAC activity was found in acetone extract of *G. applanatum* using solid-liquid extraction (IC_{50}: 1.16 ± 0.17 μg/ml).

The acetone fraction of *G. adspersum* showed similar antioxidant activity in the

Table 3. **Antioxidant activity of the extracts of *G. lucidum, G. adspersum, G. applanatum* by the β-carotene-linoleic acid, DPPH, ABTS, and CUPRAC, obtained by Soxhlet extraction**

Mushrooms	Extracts	β-carotene-linoleic acid assay IC_{50} (μg/ml)	DPPH· assay IC_{50} (μg/ml)	ABTS·+ assay IC_{50} (μg/ml)	CUPRAC IC_{50} (μg/ml)
G. lucidum	Petroleum ether and choroform	88.57±0.67* **	1566.24±79.64* **	1045.57±342.46* **	356.00±5.57v
	Acetone	18.54±2.38*	94.72±1.18**	25.07±2.83**	27.68±1.11* **
	Methanol	29.08±1.94*	267.45±16.74**	191.22±5.38*	246.17±61.33* **
G. adspersum	Petroleum ether and choroform	59.27±4.06* **	4808.94±296.19* **	N.A.	399.50±0.71* **
	Acetone	17.50±2.41*	28.94±5.05*	3.18±0.17**	11.23±0.33*
	Methanol	31.35±4.71*	61.76±2.65**	15.53±1.86**	20.78±0.36*
G. applanatum	Petroleum ether and choroform	162.59±27.95* **	5108.02±376.58* **	N.A.	404.50±21.92* **
	Acetone	30.72±8.39*	11.94±0.60*	11.94±0.60*	9.40±0.25**
	Methanol	70.64±13.73* **	11.33±0.53**	15.09±0.14**	6.30±0.69*

Table 4. **Antioxidant activity of the extracts of *G. lucidum, G. adspersum, G. applanatum* by the β-carotene-linoleic acid, DPPH, ABTS, and CUPRAC, obtained by ultrasonic extraction**

Mushrooms	Extracts	β-carotene-linoleic acid assay IC_{50} (μg/ml)	DPPH· assay IC_{50} (μg/ml)	ABTS·+ assay IC_{50} (μg/ml)	CUPRAC IC_{50} (μg/ml)
G. lucidum	Petroleum ether and choroform	33.66±3.69**	1195.25±88.64*	N.A.	316.00±17.35**
	Acetone	36.97±2.64*	135.24±8.94**	39.66±1.89*	35.72±0.63*
	Methanol	130.68±28.05**	409.94±10.09* **	40.49±23.20**	370.00±12.53*
	Water	2966.67±793.85* **	N.A.	672.02±54.18* **	566.00±50.09* **
	Aqueous supernatant	76.03±7.96* **	586.51±20.05* **	119.58±1.34* **	101.88±11.68*
G. adspersum	Petroleum ether and choroform	157.17±14.80* **	9950.60±100.69* **	N.A.	379.67±3.79**
	Acetone	9.79±5.73*	10.36±0.69* **	17.72±1.60* **	7.58±1.33* **
	Methanol	1.70±1.13**	36.54±1.15* **	7.67±1.36* **	13.20±0.33*
	Water	426.90±24.27*	N.A.	66.38±4.93**	201.50±17.68* **
	Aqueous supernatant	69.19±0.30*	282.85±41.17	52.09±2.61*	26.15±0.68* **
G. applanatum	Petroleum ether and choroform	724.75±32.58	N.A.	N.A.	458.67±7.51**
	Acetone	N.A.	7.68±0.51* **	13.08±1.17**	6.08±1.31* **
	Methanol	67.84±0.25* **	5.42±0.83* **	11.15±2.48*	2.69±0.97* **
	Water	338.58±0.92* **	1566.68±615.75**	116.66±20.34**	52.00±0.45*
	Aqueous supernatant	267.85±22.91**	247.89±14.94*	42.67±3.32*	25.07±0.66**

β-carotene-linoleic acid assay in our (IC_{50} = 9.79 ± 5.73, Table 4) and other scientists research (IC_{50} = 7.89 ± 0.91 μg/ml, [10]). Authors of work [10] stated that among the extracts, the ethyl acetate fraction of *G. adspersum* demonstrated the highest activity in the β-carotene-linoleic acid assay (IC_{50} = 5.63 ± 0.66 μg/ml). In our research the highest antioxidant activity for *G. adspersum* was observed in methanol fraction (IC_{50} = 1.7 ± 1.13 μg/ml).

As an example, chromatograms of dry components of acetone extracts of *G. lucidum* ilustrated in the Fig. 1. Chromatogram in red color shows fingerprint for it. Chromatogram in black color shows the result of treatment of *G. lucidum* sample dissolved in methanol (40 000 ppm) with ABTS solution in ethanol. Solutions were mixed in ratio of 1 to 1. Concentration of *G. lucidum* in solution for both samples were the same (20 000 ppm).

Chromatogram of dry components of methanol extracts of *G. lucidum* ilustrated in the Fig. 2. Chromatogram shows fingerprint for it.

Thus, three-sample preparation techniques were used for obtaining mushrooms extracts. Antioxidant activity was estimated for all extracts. Antioxidant activities depend on investigated extract and method used for their measuring. Using solid-liquid extraction of *G. applanatum* and CUPRAC assay, optimal IC_{50} value is up to 1.16 ± 0.17 μg/ml. Soxhlet extraction of *G. adspersum* and ABTS assay gives the best IC_{50} value for this mushroom equal to 3.18 ± 0.17 μg/ml. Using ultrasonic

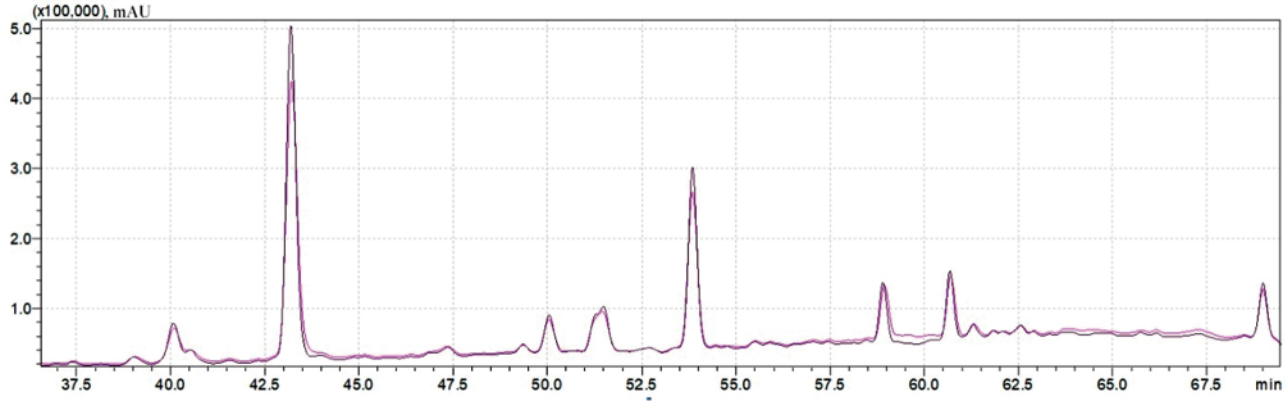

Fig. 1. **Chromatograms of dry components of acetone extracts of *G. lucidum***

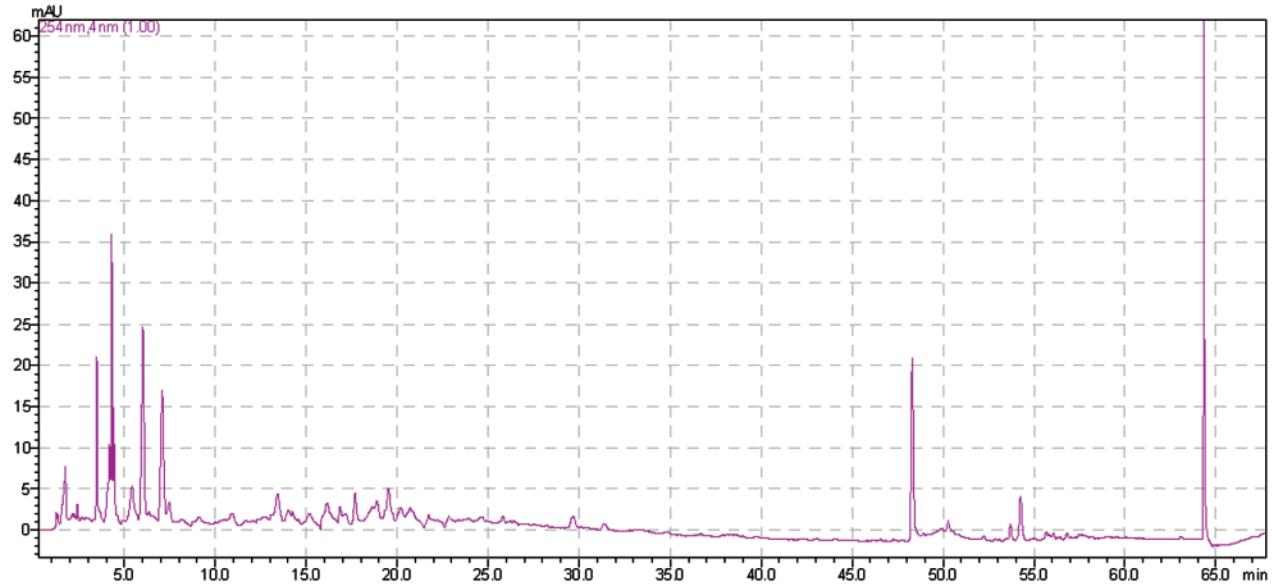

Fig. 2. **Chromatogram of dry components of methanol extracts of *G. lucidum***

extraction of *G. adspersum* and β-carotene-linoleic acid assay, methanol extract with the highest activity was found (IC_{50} 1.70 ± 1.13μg/ml).

HPLC conditions were developed for getting the chromatograms of extracts. Such chromatograms might be used to detect the presence of presence active components in various mushrooms species without usage of expensive standards.

Acknowledgements

The authors would like to acknowledge the technical help provided by Taras Shevchenko National University of Kyiv. One of the authors (*V. Raks*) would also like to thank to The Scientific and Technological Research Council of Turkey (TÜBİTAK) for supporting with the grant; namely, Fellowships for Visiting Scientists and Scientists on Sabbatical Leave (TUBITAK-BIDEB-2221).

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ХАРАКТЕРИСТИКА ЕКСТРАКТІВ ГРИБІВ РІЗНИХ ВИДІВ РОДУ *Ganoderma*: АНТИОКСИДАНТНА АКТИВНІСТЬ ТА ХРОМАТОГРАМИ

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Метою роботи було виділення біологічно активних сполук із плодових тіл грибів *Ganoderma lucidum*, *Ganoderma adspersum* і *Ganoderma applanatum* та оцінювання їхньої антиоксидантної активності. Для виділення біологічно активних сполук використовували різні методи. Антиоксидантні властивості визначали спектрофотометрично, вимірюючи активність захоплення вільних радикалів. Для аналізу хроматограм виділених екстрактів застосовували високоефективну рідинну хроматографію. В результаті аналізу з використанням β-каротин-лінолевої кислоти було визначено високу антиоксидантну активність метанольних екстрактів. Напівмаксимальне інгібування IC_{50} для *G. applanatum* і *G. adspersum* становило 8,25 ± 0,88 мкг/мл та 1,70 ± 1,13 мкг/мл відповідно. Водночас, екстракти петролейного ефіру і хлороформу *G. lucidum* мали вищу антиоксидантну активність: IC_{50} — близько 33,66 ± 3,69 мкг/мл. Отримано хроматограми компонентів ацетонових і метанольних екстрактів *G. lucidum*. Основною перевагою таких хроматограм є можливість виявлення активних компонентів різних видів грибів без використання високовартісних стандартів.

Ключові слова: гриби видів *Ganoderma*, антиоксидантна активність, високоефективна рідинна хроматографія.

ХАРАКТЕРИСТИКА ЭКСТРАКТОВ ГРИБОВ РАЗНЫХ ВИДОВ РОДА *Ganoderma*: АНТИОКСИДАНТНАЯ АКТИВНОСТЬ И ХРОМАТОГРАММЫ

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Целью работы было выделение биологически активных соединений из плодовых тел грибов *Ganoderma lucidum*, *Ganoderma adspersum* и *Ganoderma applanatum* и оценка их антиоксидантной активности. Для выделения биологически активных соединений использовали различные методы. Антиоксидантные свойства определяли спектрофотометрически, измеряя активность захвата свободных радикалов. Для анализа хроматограмм выделенных экстрактов применяли высокоэффективную жидкостную хроматографию. В результате анализа с использованием β-каротин-линолевой кислоты была определена высокая антиоксидантная активность метанольных экстрактов. Полумаксимальное ингибирование IC_{50} для *G. applanatum* и *G. adspersum* составило 8,25 ± 0,88 и 1,70 ± 1,13 мкг/мл соответственно. В то же время экстракты петролейного эфира и хлороформа *G. lucidum* имели большую антиоксидантную активность: IC_{50} — около 33,66 ± 3,69 мкг/мл. Были получены хроматограммы компонентов ацетоновых и метанольных экстрактов *G. lucidum*. Основным преимуществом таких хроматограмм является возможность выявления активных компонентов различных видов грибов без использования дорогостоящих стандартов.

Ключевые слова: грибы видов *Ganoderma*, антиоксидантная активность, высокоэффективная жидкостная хроматография.