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ORIGINAL ARTICLE

Antioxidant activity, phytochemical composition of *Andricus tomentosus* and its antiproliferative effect on Mia-Paca2 cell line

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Abstract

Plant derived products are widely used in cancer treatment. Gall species has been preferred for treatment various diseases in folk medicine, but there are few studies on the anticancer effects of gall species. The present study reports the antioxidant activity and total secondary metabolites of extracts of *A. tomentosus* galls. The antioxidant potency of galls was carried out using different *in-vitro* model systems. Their cytotoxic efficacy on Mia-Paca cell line was also explored. Gall extract was found to contain a large amount of phenolic acids. The extract potently scavenged free radicals including DPPH (IC_{50} 9.56 ± 1.08 µg/mL), ABTS (IC_{50} 18.51 ± 0.25 µg/mL). It can be seen as a potential source of antioxidants according to β-carotene/linoleic acid method (%92.58 ± 0.92) and Phosphomolybdenum assays (104.36 ± 4.95 mgAE/g). Gall extract also posses ability of metal chelating (%40.07 ± 2.30) and Fe^{3+} (184.01 ± 2.83 mgTE/g) and Cu^{2+} (89.81 ± 0.96 mgTE/g) reducing activity. According to total secondary metabolites tests, gall extract showed high total phenolic, total flavonoid and total tannin amount. HPLC analysis of the extract suggested it to contain caffeic acid (424.068.479 µg/g), ellagic acid (187.696.132 µg/g). XTT assay revealed gall extract to enhance percent survival of Mia-Paca2 cell line exposed *A. tomentosus* extracts. The best cytotoxic effect was determined in acetone extracts (IC_{50}: 124.7 µM). Expression of some genes (*Bax, Bcl-2, FAS, BID, caspase-3, caspase-8, caspase-9, caspase-10, FADD, TRADD*) in the apoptosis pathway was determined to invastigate apoptosis inducing activity. These results indicate that *A. tomentosus* galls possess potent antioxidant activity, when tested both in chemical as well as biological models.

Keywords *Andricus tomentosus* · Galls · Antioxidant · XTT · HPLC

Introduction

In recent years, the use of herbs for the prevention and treatment of cancer has gained more attention, due to various phytochemical components and fewer side effects. Cytotoxic potentials of four different medicinal plants against six different cancer cell lines (A-549, CCC-222, DU-145, MCF-7, K-562 and PC-3) and one normal cell line (Beas-2B) were investigated [1]. In another study was revealed that three different plant extracts have cytotoxic effect on MCF-7 cell line [2].

In one of the studies, phenolic compounds found in *Ocimum basilicum* and *Thymus algeriensis* essential oils were analyzed by High Pressure Liquid Chromatography (HPLC) and a rich flavonoid content was revealed. According to this research, the essential oil analysis of *Ocimum basilicum* and *Thymus algeriensis* shows that they respectively contains 26 and 29 different components. These two phytochemically rich species were found to have moderate antioxidant activity, a weak antimicrobial effect and cytotoxic effects on different cell lines [3].

Coriander (*Coriandrum sativum* L.) is a plant from the Apiaceae family and has been used for both medicinal and nutritional properties for centuries. In a study investigating the effects of *C. sativum* extract on gene expression, viability, colony formation, migration and invasion of human prostate cancer cell line (PC-3) and Lymph Node Carcinoma

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of the Prostate cancer cell lines (LNCaP), the effects of *C. sativum* extracts on prostate cancer are presented [4].

Pancreatic cancer is one of the leading causes of cancer-related death in World [5]. The incidence rate of pancreatic cancer worldwide was shared as 2.5 in the data of Globocan in 2018. Pancreas cancer is the 9th common type of cancer in Turkey. Mortality rate was recorded at %99.1 in Turkey and %94.2 in the world [6]. It has been suggested that traditional chemotherapy is not sufficient in the treatment of pancreatic cancer and some studies that investigate the effects of natural products on treatments attract attention [7]. Some studies investigating the anticancer properties of plant derived natural components show that phenolic components such as quercetin, myricetin, apigenin, naringenin, epigallocatechin-3-gallate have antipancreatic cancer [8]. Tannic acid (TA) is an another polyphenolic compound obtained from plant origin. Previous studies have shown multiple human health benefits of TA including the anti-cancer ability [9, 10]. In a research have been reported that efficient pancreatic cancer therapy was developed with tannic acid-pectine nano-complexes [11]. Some galls of *Quercus* species possess hydrolyzable tannin is an antioxidant compound and is produced by gall wasp [12]. The main component of oak gall is tannin (50–70%) especially the galicine acid that is formed of tannin acid, gallic acid, ellagic acid etc. [13]. *Quercus* galls are widely used in folkmedicine due to their multiple therapeutic properties [14]. In some traditional medicine, gall samples were used for treatment of some disease and pharmacological studies were shown that oak galls possess some biological activities such as antibacterial, antioxidant, local anaesthetic, anti-inflammatory, anticancer, analgesic, Angiotensin converting enzyme (ACE) inhibitory and antivenom activities [15, 16].

In the present study, we purposed to revealed the antioxidant capacity, phytochemical ingredient and cytotoxic activity of extracts prepared from *Andricus tomentosus* is an oak gall.

Materials and methods

Plant material

The galls result from the tumors provoked by the bite of female cynipid wasps in the buds of this plant [17]. *A. tomentosus* (Trotter, 1901) was collected from Seydikemer, Muğla (September 2018, May 2019), is an asexual oak gall. *A. tomentosus* prefers *Q. cerris*, *Q. frainetto, Q. infectoria, Q. lusitanica, Q. petraea, Q. pubescens, Q. robur* [18, 19] such as host. We collected from *Q. infectoria* Oliver in this study.

A. tomentosus has a single chamber. Its best distinguishing feature is that it is covered with a velvety cover. It is usually in the form of a cone. The mature gall is 12–18 mm tall and the base is 10–15 mm in diameter. This gall develops in late summer and matures in autumn [20]. The collected gall samples were identified by Prof.Dr. Yusuf Katılmış in Entomology Laboratory, Pamukkale University, Turkey.

Preparation of extracts

The pulverised gall (30 g) were separately subjected to solvent extraction in a Water Bath with acetone, ethanol, methanol and water (50 °C, 6 h). Then solvents were evaporated with IKA RV 10 Digital Rotary Evaporator, samples were lyophilised (Labconco Freezone 6). Lyophilized samples were stored at − 20 °C. The dried extracts were weighed to determine the percent of yield [21].

The percentage yield was obtained using this formula W1/W0 × 100. Where W1 is the final weight of the extract and W0 is the initial weight of sample.

Determination of total antioxidant activity

β-carotene-linoleic acid assay

This method is based on the monitoring of the color opening of β-carotene by alkyl peroxides formed by free radical chain reaction by heat and air oxidation of linoleic acid. β-carotene-linoleic acid assay was evaluated by Amin et al. [22]. The results are calculated with the formula using the initial and final absorbances of samples and control group.

$$\left[1-\left(A_C-As/A_C^{\circ}-A_S^{\circ}\right)\right]\times 100$$

where A_C and A_C° are absorbance values initial and final measurement of contol group ; As and A_S° are absorbance values of samples or standards, respectively.

Phosphomolybdenum assay

This assay is based on the reduction of Mo (IV) to Mo (V) with antioxidant agents. The green color resulting from the reduction is measured at 695 nm. Phosphomolydenum assay of gall extracts carried out Prieto et al. [23]. Results are given as equivalent to the ascorbic acid (AE) standard.

Determination of radical scavenging activity

DPPH radical scavenging activity

The free radical removal activities of the extracts were determined using 1,1-diphenyl-2-picrilhydrazil (DPPH) free radical. DPPH analysis of plant extracts prepared at different concentrations (0.005–0.025 mg/ml) was performed according to Wu et al. [24]. %50 effect concentrations (IC_{50}) values

were also evaluated after the results were calculated according to the following equation. Butylated Hydroxytoluene (BHT) was used as positive control.

$$\left[\text{Acontrol} - \text{Asample}/\text{Acontrol}\right] \times 100.$$

ABTS radical cation activity

2,2'-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) radical removal activity assays according to the Re et al. [25] method. It is based on the discoloration of the blue solution prepared with ABTS with potassium persulfite with antioxidants. IC_{50} values were given after % inhibitions were calculated according to the above formula. Positive control is BHT in this assay.

Reducing power activity

Cupric reducing antioxidant capacity (CUPRAC)

In this assay, Cu (II)-neokuproine complex becomes Cu (I)-neocuproine as a result of redox reaction with samples. Apak et al. [26]' s spectrophotometric method was based and absorbances were measured 450 nm. Trolox (TE) was used as standard.

Ferric reducing antioxidant power (FRAP)

The principle of this method is based on the reduction of a Fe(III)-tripyridyltriazine (TPTZ) complex to Fe(II)-TPTZ in the presence of antioxidants. The results measured at 593 nm are given as equivalent to trolox. This assay was carried out according to Apak et al. [27].

Metal chealating activity

The ferrous chealating capacity of gall extracts was determined with Dinis et al. [28]' s method with slight modification. According to this method, extracs inhibit ferrozine complexing with Fe^{2+} and color expansion is observed spectrophotometrically (562 nm). Ethylenediaminetetraacetic acid (EDTA) was used as standard and results were given equivalent to it.

Determination of total secondary metabolites amount

Total phenolic content

Total phenolic content of extracts was determined as equivalent to gallic acid using Folin-Ciocalteu Reagent [29]. The formation of the colored mixture is determined spectrophotometrically at 760 nm.

Total flavonoid content

The total amount of flavonoids is determined by using the colorimetric method of Aluminum chloride ($AlCl_3$) based on the formation of stable complexes of flavon and flavonols with C-4 keto group, C-3 or C-5 hydroxyl groups and aluminum chloride in acid. Results are presented as equivalent to quercetine [30].

Total tannin content

The vanillin-sulfuric acid method is widely used for the quantitative determination of the amount of condensed tannin in plants. The basic principle of this method is based on spectrophotometric measurement of red color formed by reacting sulfuric acid oxidized tannins with vanillin in the range of 500 nm. Total tannin amount of gall extracts were determined to Bekir et al. [31]'s method and equvelant to catechin (CE).

Phenolic compound characterization by HPLC

High-Performance Liquid Chromatography with Diode-Array Detection (HPLC-DAD) analysis of phenolic compounds was carried out at Mehmet Akif Ersoy University Scientific and Technology Application and Research Center. For the determination of phenolic compounds by HPLC, the method of Caponio et al. [32] was used with some modification. Gallic acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, caffeic acid, cinnamic acid, 2,5-dihydroxy benzoic acid, chlorogenic acid, vanillic acid, p-coumaric acid, ferulic acid, epicatechin, naringin, rutin, quercetin, ellagic acid were used as standard.

Determination of cytotoxic activity and anti-apopototic activity

Cell culture and XTT assay

Pancreatic cancer cell line, MiaPaca-2 (ATCC Cat# CRL-1420, RRID:CVCL_0428) were used in this study. The cells were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with %10 fetal bovine serum ((FBS) 04-121-18, Biological Industries), 0.1 mM Aminoacid solution (01-340-1B, Biological Industries), 100U/0.1 mg penicillin/streptomycin (03-031-1C, Biological Industries) in a humidified incubator with %5 CO2 at 37 °C. Briefly, MIA PaCa-2 cells were seeded at a density of 2×10^4 cells/well in 96-well plates and incubated for 24 h (37 C and 5% CO2) without extract. After incubation, the cells were treated with

a mixture of Dimethyl Sulfoxide (DMSO) (at a concentration of 0.5%) and the cell culture medium or with different concentrations of *Andricus tomentosus* extracts (5, 10, 20, 30, 40, 50, 75, 100, 250, 500 µg/mL) extracts during in the mixture of DMSO (at 0.5%) and the cell culture medium 24, 48 and 72 h, considering a time and dose dependent manner.

Cell viability and IC_{50} doses of *A. tomentosus* extracts in Mia-Paca2 were performed by using trypan blue dye exclusion test and sodium 3,3′-[1(phenylamino)carbonyl]-3,4-tetrazolium]-3is(4-methoxy-6-nitro) benzene sulfonic acid hydrate (XTT) assay was carried out with Cell Proliferation Assay with XTT Reagent—Cell Proliferation Kit (Cat # 30007-1000-BIOTIUM) according to manufacturers' instruction. Viability was calculated using as follows:

% Viability = (Absorbance value of experiment well/Absorbance value of control well) × 100

RNA isolation and real-time PCR

Total RNA was isolated from the cells after exposed to IC_{50} dose of *A. tomentosus* extracts and control cells with Trizol reagent (Invitrogen, USA) according to manufacturer instructions. Complementary DNA (cDNA) synthesis was performed on the total RNAs using cDNA Synthesis Kit (WIZBIO- Cat. No W2211-1, USA).

Bax, Bcl-2, FAS, Bid, Caspase-3, Caspase-8, Caspase-9, Caspase-10, FADD, TRADD gene expression was performed by Real Time Online RT-PCR according to the WizPure™ qPCR Master (SYBR) Mix protocol (WIZBIO-Cat.No. W1711-5, USA). The expression results were proportioned to the Beta- actine gene (housekeeping gene) expressions to calculate relative expression rations. Primer sequences are given in Table 1 [33].

Statistical analysis

Data were analyzed by using ΔΔCT method and quantified by computer program. VolcanoPlot analyses were used in the web-based "RT² Profiler ™ PCR Array Data Analysis" program. The aim of the method is based on the comparison of two expression results with ± 3SD. Thus, in cases where mRNA expression was compared, expression values of mRNAs in the groups were relatively determined. Comparison of groups was statistically evaluated by ANOVA and Tukey analysis in SPSS Analysis program.

Table 1 Primer sequences of the genes used in this study

Name	Definition	Primer sequence
ACTB	Beta Actin, house-keeping gene	F: TCCTCCTGAGCGCAAGTACTC
		R: CTGCTTGCTGATCCACATCTG
Bax	Bcl-2 associated X protein	F: AGAGGATGATTGCCGCCGT
		R: CAACCACCCTGGTCTTGGATC
Bcl-2	B-cell CLL/lymphoma 2	F: TTGGCCCCCGTTGCTT
		R: CGGTTATCGTACCCCGTTCTC
FAS	TNF receptor superfamily, member 6	F: CACTTCGGAGGATTGCTCAACA
		R: TATGTTGGCTCTTCAGCGCTA
Bid	BH3 interacting domain death agonist	F: CCTACCCTAGAGACATGGAGAAG
		R: TTTCTGGCTAAGCTCCTCACG
Caspase-3	Apoptosis related cysteine peptidase	F: GCAGCAAACCTCAGGGAAAC
		R: TGTCGGCATACTGTTTCAGCA
Caspase-8	Apoptosis related cysteine peptidase	F: CAGTAACCATGCCCGCATAGA
		R: AAGTTTCCCGAGGTTTCTCAGA
Caspase-9	Apoptosis related cysteine peptidase	R: CCGTTGACTCCGACCTTCAC
		F: GGCTGTCTACGGCACAGATGGA
Caspase-10	Apoptosis related cysteine peptidase	F: TAGGATTGGTCCCCAACAAGA
		R: GAGAAACCCTTTGTCGGGTGG
FADD	FAS (TNFRSF6) associated via death domain	F: GCTGGCTCGTCAGCTCAAA
		R: ACTGTTGCGTTCTCCTTCTCT
TRADD	TNFRSF1A associated cia death domain	F: GCTGTTTGAGTTGCATCCTAGC
		R: CCGCACTTCAGATTTCGCA

Results

Antioxidant activity

To determined antioxidant activity of *A. tomentosus* gall extracts, we preferred seven different methods to compare the results with each other and provide more reliable data (Table 2). β-carotene-linoleic acid and phosphomolybdenum assay were used to evaluated total antioxidant capacity of extracts. Methanol extracts (%92.58 ± 0.92) have highest antioxidant activity according to β-carotene-linoleic acid assay, but ethanol extracts (104.36 ± 4.95 mgAE/g) posses most effective activity in phosphomolybdenum assay.

DPPH and ABTS radical scavenging assays are commonly carried out for fast evaluation of antioxidant activity because of them stability in the radical form and simplicity of the assay. In both DPPH (IC50: 9.56 ± 1.08 µg/mL) and ABTS (IC50: 18.51 ± 0.25 µg/mL) tests, water extracts showed the highest radical scavenging activity. FRAP and CUPRAC assays were carried out to determine the reduction power of the extracts. Results which are equivalent to trolox standard, show that extracts have high reducing power activity ($CUPRAC_{methanol}$: 89.81 ± 0.96 mgTE/g; $FRAP_{ethanol}$: 184.01 ± 2.83 mgTE/g). And also, ferrious metal chelating capacity of extracts were evaluated and results were given in comparison with EDTA standard. According to this test, acetone extract had highest chelating capacity (%40.07 ± 2.30).

Extract yield and total secondary metabolites amount

The efficiency of extracts prepared with solvents with different polarities was calculated. As seen in Table 3, the highest extract amount obtained from water extract (%24,138). This result can be related to that water have the highest polarity.

Total phenolic content of extracts was calculated equivalent to gallic acid and the highest content was observed in ethanol extract (297.47 ± 2.52 mgGAE/g). Our results showed that water extract have the highest total flavonoid amount (46.88 ± 0.21 mgQE/g), while acetone extract have the highest total tannin amount (48.22 ± 1.09 mgCE/g).

Phenolic compound characterisation by HPLC

Phenolic compounds of extracted gall samples were analysed by HPLC method. The results have been listed in Table 4. According to this results, caffeic acid (424.068.479 µg/g), ellagic acid (187.696.132 µg/g) and 2,5-dihydroxy benzoic acid (69.399.147 µg/g) are most common phenolic

Table 2 Antioxidant activity of *A.tomentosus* Gall extracts

	Acetone	Ethanol	Methanol	Water	BHT	EDTA
β-carotene-linoleic acid assay (% inhibition)	73.56 ± 1.46^{a}	88.20 ± 2.91^{b}	92.58 ± 0.92^{b}	91.57 ± 0.27^{b}	93.78 ± 1.93^{b}	nt
Phosphomolybdenum (mgAE/g)	$71.24 \pm 1.92^{a,b}$	104.36 ± 4.95^{c}	84.74 ± 3.74^{b}	61.41 ± 1.37^{a}	nt	nt
DPPH (IC50 value)	18.89 ± 0.11^{b}	9.60 ± 0.60^{a}	9.56 ± 1.08^{a}	9.95 ± 0.52^{a}	8.71 ± 0.01^{a}	nt
ABTS(IC50 value)	32.17 ± 0.53^{b}	48.70 ± 1.02^{c}	19.58 ± 0.64^{a}	18.51 ± 0.25^{a}	17.03 ± 0.35^{a}	nt
CUPRAC (mgTE/g)	84.81 ± 0.52^{a}	88.45 ± 0.52^{b}	89.81 ± 0.96^{b}	85.11 ± 0.17^{a}	nt	nt
FRAP (mgTE/g)	132.42 ± 1.23^{b}	184.01 ± 2.83^{d}	107.66 ± 2.27^{a}	155.28 ± 0.82^{c}	nt	nt
Metal chelating activity (%)	40.07 ± 2.30^{a}	30.93 ± 3.71^{a}	28.42 ± 4.48^{a}	35.25 ± 2.01^{a}	nt	$96,18 \pm 0,95^{b}$

Data represent mean values ± standard error (n = 3). In the same extract, data marked with different letters indicate significant difference ($p < 0.05$)

AEs ascorbic acid equivalents, *TEs* trolox equivalents, *nd* not detected

Table 3 Extract yield and total secondary metabolites amount of *A. tomentous* according to different solvent

	Acetone	Ethanol	Methanol	Water
Extraction yield (%)	8547	9,0856	17,867	24,138
Total phenolic amount (mgGAE/g)	280.60 ± 1.38^{a}	297.47 ± 2.52^{a}	284.14 ± 1.18^{a}	287.89 ± 0.64^{a}
Total flavonoid amount (mgQE/g)	31.12 ± 0.67^{a}	29.97 ± 0.59^{a}	30.51 ± 0.46^{a}	46.88 ± 0.21^{b}
Total tannin amount (mgCE/g)	48.22 ± 1.09^{d}	23.00 ± 0.15^{c}	14.72 ± 0.14^{b}	6.63 ± 0.15^{a}

Data represent mean values ± standard error (n = 3). In the same extract, data marked with different letters indicate significant difference ($p < 0.05$)

GAEs gallic acid equivalents, *QEs* quercetin equivalents, *CEs* catechine equivalents

Table 4 Phenolic compounds characterization of methanolic gall extract by HPLC

Standards	Quantity of phenolic compounds (µg/g)	Retention time (min)
[1]Gallic acid	7218.09	6.8
[2]3,4-dihydroxy benzoic acid	3.109.659	10.7
[3]Chlorogenic acid	1.013.789	15.7
[4]4-hydroxybenzoic	3.859.173	17,2
[5]2,5-dihydroxy benzoic acid	69.399.147	18.2
[6]Vanillic acid	6.572.271	19.2
[7]Caffeic acid	424.068.479	21.3
[8]Epicatechin	53430.17	22.7
[9]p-coumaric acid	151.081	26.1
[10]Ferulic acid	100.731	30.1
[11]Naringin	315.325	45.6
[12]Rutin	337.586	47.7
[13]Ellagic acid	187.696.132	49.7
[14]Cinnamic acid	423.675	71.1
[15]Quercetin	1.141.256	70.4

compounds of methanol extract of *A. tomentosus*. It is seen that the other 12 components are also detected in high amounts.

Cytotoxic and anti-apoptic activity

The dose (5–500 µM) and time (24, 48 and 72 h) dependent cytotoxic effects of the extracts were investigated with XTT analysis. For determining the lowest and most effective dose rate 24, 48 and 72 h changes were also observed. A decrease in cell viability was detected at each of the 24, 48 and 72 h, but the lowest doses were obtained at the 24th h. This study showed that in the presence of acetone, ethanol, methanol and water extracts of *A. tomentosus*, 50% growth inhibition concentrations (IC_{50}) occur in Mia-Paca2 cell line after 24 h at concentrations 124.7 µg/mL, 158.3 µg/mL, 187.4 µg/mL and 169.8 µg/mL respectively (Table 5). Results showed that, cellular proliferation decreased by concentration dependent manner.

We performed the expression of selected genes (*Bax, Bcl-2, FAS, Bid, Caspase-3, Caspase-8, Capspase-9, Caspase-10, FADD* and *TRADD*) in cells exposed to the extract using RT- PCR method. According to this method, mRNA levels were analyzed in extract-treated cells and untreated control group cells. In the expression level changes of the cell cycle and apoptosis related genes in dose groups comparing to control group, which are statistically significant and absent, are shown in the Table 5.

Expression results of acetonic extract showed that *Bax, caspase-10, FADD and TRADD* gene expression levels in Mia-Paca2 cells were increased in dose group cells compared to the control cells in a time-dependent manner. While *Bax*, *Bid*, *caspase-3*, *caspase-8* and *TRADD* gene expression was significantly increased in the ethanolic dose group, the expression changes were not detected in other genes compared with the control cells. In methanol extract treated dose group, while expression of *Bid* and *caspase-3* were significantly increased, expression of *caspase-10* was decreased. All gene expression without Bax and Bid, were decreased in water extract dose group. The reason why the expression levels of genes have different effects in different solvents may be related to the fact that each solvent dissolves different components in the extract in different amounts. Since this affects cell signaling pathways differently, it can change the outcome of apoptosis.

Table 5 Fold regulation of genes comparing to control group

	Acetone (IC_{50}: 124.7 µM–24 h)		Ethanol (IC_{50}: 158.3 µM–24 h)		Methanol (IC_{50}: 187.4 µM–24 h)		Water (IC_{50}: 169.8 µM–24 h)	
Gene	FC	P_{value}	FC	P_{value}	FC	P_{value}	FC	P_{value}
Bax	25.05	0.001058*	26.08	0.000016*	8.13	0.150856	6.93	0.000001*
Bcl-2	− 2.6	0.030786*	− 1.44	0.820964	1.01	0.869223	− 12.75	0.008912*
FAS	− 11.96	0.1093	1.03	0.687722	− 1.07	0.674186	− 3.07	0.272017
Bid	10	0.009956*	6.4	0.011776*	4.05	0.003156*	1.42	0.127859
Caspase-3	− 5.67	0.085642	5.54	0.040855*	3.32	0.0001*	− 1.79	0.023235*
Caspase-8	− 1.8	0.000584*	1.78	0.007222*	3.17	0.179143	− 2.74	0.010501*
Caspase-9	− 4.32	0.116836	− 1.82	0.250015	− 1.16	0.535897	− 1.85	0.27676
Caspase-10	1.17	0.016329*	− 1.27	0.662722	− 1.71	0.00751*	− 5.44	0.000378*
FADD	5.73	0.02677*	5.46	0.058487	2.65	0.402193	− 3.17	0.270822
TRADD	3.91	0.000003*	1.56	0.020745*	− 1.11	0.255985	− 9.83	0.000138*

*$P < 0.05$ values was marked with *

Discussion

Quercus species are distributed in Iran, Iraq, and Turkey and then spread to Asia Minor, Europe, and Northern Africa [15, 34]. *Quercus infectoria* (Family: Fagaceae) commonly known as gall oak, *mazu* or *Manjakani*. Phytochemical analysis of galls investigated the presence of saponins, alkaloids, tannins, glycosides, triterpenes, sterols, phenolic compounds, carbohydrates, and flavonoids [35, 36]. It is known that the gall extracts have some biological activity such as antimicrobial, analgesic, anticarcinogenic, antioxidant and antioxidant activity thanks to their chemical composition [37].

In present study, some phytochemical analysis and biological activity of *A. tomentosus* extracts was determined. Our studies were revealed that *A. tomentosus* gall extracts possess the high total antioxidant capacity, radical scavenging activity, metal reducing and chealating power. In our previous study, DPPH (8.67 ± 0.58 µg/mL), ABTS (IC50: 44.97 ± 2.56 µg/mL) radical scavenging activity of *Andricus quercustozae* asexual gall extract was determined. And also, we were examined β–carotene/linoleic acid (%87.49 ± 1.27), Phosphomolybdenum (78.20 ± 1.63 mgAE/g) antioxidant capacity, CUPRAC (245.82 ± 1.06 mgTE/g) reducing power activity and the high total secondary metabolites of *A. quercustozae* extracts. Another studies showed that oak galls have the high antioxidant activity of using DPPH and ABTS method [38, 39]. With some research, reducing power activity of some gall species detected by CUPRAC and FRAP assays [40, 41].

Sukkor et al. [42] was carried out extraction of phenolic acids from oak galls and they were observed that gallic acid (497.34 mg/g), tannic acid (2430.48 mg/g) was the maximum amount phenolic acids. It was found that the most common phenolic acid in the *A. quercustozae* extract is caffeic acid, which has antioxidant, anti-aging properties [15]. Plant extracts with components such as caffeic acid, 2,5-dihydroxy benzoic acid and ellagic acid are used for pharmaceutical purposes because they have antioxidant, anticarcinogenic and anti-inflammatory properties [43].

Previous study was carried out to determine the potential of galls of *Q.infectoria* as an antiproliferative agent towards cervical cancer cells (HeLa, IC_{50}: 2.82 ± 0.21 µg/mL) and ovarian cancer cells (Caov-3, IC_{50}:6.50 ± 0.24 µg/mL). In their study, it was stated that galls can be recommended as an anticancer agent [44]. In another research showed that Galla Chinensis that is an oak gall, has important cytotoxic activity with IC50 = 4.339 µg/mL [45]. It was showed that oak gall had high reduction to mice mammary carcino cell line 2003 AMN3 cancer cell line with 2 µg/mL IC50 value [46]. The results of a study investigating the anticancer effect of extract of *Quercus infectoria* in colon cancer HT29 cell line show that aqueous extract increases the expression of the *Bax* and *Bcl-2* genes compared to *GAPDH* reference gene [47].

The antioxidant and anticancer properties of *A. tomentosus* extracts are strong because of their high content of caffeic acid (424.068.479), ellagic acid (187.696.132, 2,5-dihydroxy benzoic acid (69.399.147) and This is due to the fact that it contains phenolic, which has a lot of OH groups. It is possible to say that antioxidants may have an anticancer effect by inactivating certain transcription factors that oxidative stress can activate [48].

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Compliance with ethical standards

Conflict of interest All authors declare that they have no conflict of interest.

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