Original Research Article

Investigation of Antioxidant, Antimicrobial, Tyrosinase Inhibitor, Cytotoxic ActivitiesPhenolic Profile of *Isatis cappadocica*subsp. *alyssifoli*and Evaluation of The Plant's Applicability in The Pharmaceutical Field

Running Title: Biological Activity of ***Isatis cappadocica***

ABSTRACT

Isatis species, which are endemic across most of Turkey, exhibit antibacterial, anticancer and antiviral effects. The aim of this study was to determine of antioxidant, antimicrobial, tyrosinase inhibitor, cytotoxic activities, and phenolic profile of polar extracts of *Isatis cappadocica.* We analyzed the antioxidant properties of extracts using ferric reducing antioxidant power (FRAP), cupric reducing antioxidant capacity (CUPRAC), and 2,2-diphenylpicrylhydrazyl (DPPH) radical scavenging activity. The phenolic composition of methanolic extract of *Isatis cappadocica* was analyzed by reverse phase high performance liquid chromatography (RP-HPLC). Extracts' antimicrobial properties were evaluated based on the agar well diffusion technique. Tyrosinase inhibitor activity was measured colorimetrically. Methanolic extract yielded better FRAP and CUPRAC results and aqueous extract yielded better DPPH activity. Benzoic acid and sinapic acid were detected as major phenolic compounds. Methanolic extract was particularly effective against all the bacteria investigated, apart from *Yersinia pseudotuberculosis.* Methanol extract was exhibited tyrosinase inhibitory activity. Extract has caused to death of cells in the high concentrations on the PC-3 ve 3T3 celllines. The results showed that *Isatis cappadocica* could be used in the food, cosmetic, and drug industries due to their rich antioxidant, antimicrobial, cytotoxic and tyrosinase inhibitor activities.

Key Words: *Isatis cappadocica*, Antioxidant, Antimicrobial, Antityrosinase, Cytotoxicity,

INTRODUCTION

Very large numbers of modern drugs and medicines have been obtained from natural sources, often as a result of their employment in traditional remedies. Numerous medicinal plants are known to exhibit therapeutic properties [1]. At least 80% of the world population, largely in developing countries, is still thought to employ traditional remedies based on plant extracts [2]. Plants are of particular interest as possible sources of natural antioxidants. They contain a range of antioxidant compounds that provide resistance against reactive oxygen species (ROS) [3]. Radicals are implicated in molecular transformations and genetic mutations in numerous organisms. Oxidative stress is well-known to cause various degenerative and chronic diseases, such as cancer, diabetes and cardiovascular disease [4]. Antioxidant-based medications play major roles in the prevention and treatment of such diseases as atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer [5]. Despite the growing application of antibiotics and other chemicals, factors such as the emergence of drug-resistant forms and undesirable ecosystem impacts are restricting their use [6]. Increasing research is therefore being conducted into appropriate alternatives. Brassicaceae (Cruciferea) is a cosmopolitan family containing approximately 350 genera and 3000 species. Species from this family are employed for antidiabetic, antibacterial [7], anticancer [8], antiarthritic [9], and antirheumatic [10] purposes, as well as being powerful insecticides [11]. Isatis, particularly common plant species, consist of biennial, herbaceous shrubs from the family Brassicaceae. The genus consists of approximately 40 taxa, 24 being endemic to Turkey [12,13].*Isatis* species exhibit antibacterial, anticancer, antiviral, and astringent activities. The compounds in these species are known to be effective against numerous disorders, such as meningitis, encephalitis, mumps, influenza, erysipelas, and heat rash. The roots of these plants are particularly rich in antibacterial and anticancer chemical substances [14].

The objectives of this study were (i) to investigate antioxidant activities using different tests, (ii) to quantify the main phenolic content and (iii) to estimate the antimicrobial capacities against various bacteria and yeast in *Isatis cappadocica* subsp. *alyssifoli* extracts (iv) to investigate tyrosinase inhibitor activity and (v) to determine the content of phenolic acid compounds (HPLC) and the cytotoxic effect on PC-3, and 3T3 cells of the methanolic extract.

MATERİALS AND METHODS

Chemicals and Instrumentation

The following chemicals and reagents were employed in this study: 2,2-Diphenyl-1-picrylhydrazyl (DPPH), and 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) (Sigma-Aldrich); butylated hydroxytoluene (BHT) (Supelco); galanthamine (Sigma); methanol, ethanol, acetic acid, dimethyl sulfoxide, and acetonitrile (Merck); 6-hydroxy–2,5,7,8-tetramethylchroman–2-carboxylic acid (Trolox), 2,4,6-tripyridyl-s-triazine (TPTZ), and Folin–Ciocalteau reagent (Fluka), and polytetrafluoroethylene membranes (Sartorius).

A Spectro UV-Vis Double PC–8 auto cell spectrophotometer (Labomed Inc.) was used to measure absorbance values. Deionized water purified in an Elgacan® C114 Ultra Pure Water System Deioniser (The Elga Group, Buckinghamshire, England) was used in the preparation of all solutions.

Evaporation was performed with an IKA® RV 05 Basic (IKA®, Werke, USA) rotary evaporator system, and extraction procedures were performed with a Heidolph promax 2020 shaker. Dissolution procedures were carried out using a Heidolph Reax top vortex and Elma® Transsonic Digital ultrasonic water bath (Germany). A Hanna Instruments microprocessor pH meter was also employed as necessary.

Plant Material and Preparation of Extracts

Isatis cappadocica specimens were collected in 2016 from Erzincan (Turkey), and were identified by Prof. Ali Kandemir. Voucher specimens were deposited in the herbarium of the

Erzincan University Science Faculty (herbarium number: 10861). Fifty gram (50 g) of dried plant powder and then mixed this with 500 mL methanol. That mixture was then stirred over a 24-h period at room temperature, and subsequently filtered. The filtrate obtained was evaporated using a rotary evaporator (IKA-Werke RV05 Basic, Staufen, Germany). Finally, antioxidant, antimicrobial, cytotoxicity, anticholinesterase, and antityrosinase activities were studies using the extract obtained at the end of these procedures. For HPLC analysis, the extract was subjected to further dissolution in HPLC grade methanol and was additionally filtered through 0.45-µm membranes.

HPLC Conditions

The standards adopted for HPLC analysis consisted of vanillic acid, *p*-hydroxybenzoic acid, syringaldehyde, *p*-coumaric acid, sinapic acid, benzoic acid and quercetin. Stock solutions of the prepared standards were diluted at a concentration range of 5-100 µg mL^{-1} to elicit the calibration curve. HPLC analysis of phenolic compounds involved a reverse phase column (150 × 4.6 mm i.d, 5 µm) (Waters Spherisorb, Milfort, MA, USA), on a gradient program with a two-solvents system [A: 100% methanol; B: 2% acetic acid in water (pH 2.8)], and a constant solvent flow rate set to 1.5 mL min^{-1} on a HPLC system (Shimadzu Corporation, LC 20 AT, Kyoto, Japan) (Table 1). The injection volume was set to 20 µL. Signals were determined at 232, 246, 260, 270, 280, 290, 308, and 328 nm based on DAD detection with a column temperature of 25ºC.

Determination of Antioxidant Capacity

Total phenolic quantities in extracts were calculated using the method described by Folin-Ciocalteu [15]. Gallic acid was used as a standard. Total phenolic content was expressed as mg of gallic acid equivalents per gram of 100 g sample. To summarize, 0.01, 0.02, 0.03, 0.04 and 0.05 mg/mL concentrations of gallic acid were made ready in methanol, together with 0.1 and 1 mg/mL concentrations, also in methanol. In the following stage, 0.5 mL of each sample

104 was placed into test tubes and then mixed with 0.5 mL of 0.2 N Folin-Ciocalteu reagent and
105 with 1.5 mL of 2% sodium carbonate. The tubes were next sealed using parafilm and
106 incubated for 2 h at 20 °C. Finally, absorbance values were read spectrophotometrically at
107 760 nm. All measurements were conducted in triplicate.
108 The ferric reducing antioxidant power (FRAP) assay involves calculation of the iron-reducing
109 capacities of a specific extract [16]. Following exposure to 2,4,6-tripyridyl-S-triazine (TPTZ),
110 the Fe^{2+}-TPTZ complex forms a blue color that can be read at 593 nm. In summary, we added
111 3.0 mL of working FRAP reagent to an appropriate volume/concentration of extract. This was
112 next incubated for 4 min at 37 ^{0}C, and the absorbance was finally measured against a ferrous
113 sulfate standard at 593 nm. Trolox was also calculated under identical conditions as a standard
114 antioxidant compound for comparative analyses. The results were expressed as μM Trolox
115 equivalent of g sample.
116 DPPH radical-scavenging activity is linked to the DPPH cation radical scavenging capacity of
117 the antioxidant [17]. Briefly, we combined 0.75 mL of DPPH reagent (0.1 mM in methanol)
118 with 0.75 mL of tea extract or standard. This was then exposed to vigorous vortexing and then
119 allowed to stand for 30 min in the dark at room temperature. Discoloration occurring in DPPH
120 was calculated using the spectrophometric method at 517 nm. The percentage inhibition of the
121 discoloration resulting from the plant extract was calculated using Trolox as standard. Values
122 were expressed as SC_{50} (mg sample per mL), representing the concentration causing 50%
123 scavenging of DPPH radical.
124 Extracts' cupric reducing antioxidant power (CUPRAC) levels were measured by means of
125 the spectrophotometric method [18]. We first mixed 1 mL of $CuCl_2$ solution ($1.0x10^{-2}$ M), 1
126 mL of neocuproine solution ($7.5x10^{-3}$ M) and 1 mL NH_4Ac buffer solution inside a test tube.
127 Various different extract concentrations were combined with the initial mixture for a final
128 volume of 4.1 mL. All test tubes were next incubated for 30 mins. Absorbance was measured

at 450 nm against a reagent blank. CUPRAC values were expressed as µM Trolox equivalent per gram of sample.

Cytotoxicity Potential Evaluation

MTT assay was used to evaluate the cytotoxic potential of *I.cappadocic a*aqueous and methanolic extracts. In this test the viable cells metabolize the yellow water soluble 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) to purple hydrophobic formazan crystals. For this, human prostate cancer cells (PC-3, ATCC CRL-1435) and Mouse fibroblast cells (3T3, ATCC CRL-1658) were cultured in suitable medium (F12K DMEM for PC-3 and DMEM for 3T3 cells) supplemented with fetal bovine serum (10%) and antibiotic (100 U/mL penicillin and 100 mg/mL streptomycin) for 24 hours before exposure to different concentrations (180-5000 µg/mL for aqueous and 500 – 1500 µg/mL for methanolic extracts) of herbal extracts. After 24 hours exposure period 25 µL of 5 mg/mL MTT were added for every well andincubated for farther 2 hours. The supernatants were discarded and 100 µL of DMSO was added for each well to dissolve the formazan crystals. Microplate spectrophotometer system (Epoch, Erlangen, Germany) at 590 nm (wavelength 670 nm) was used to measure the Optical densities (OD). Cells exposed to 1% DMSO were evaluated as solvent control group while non-exposed cells accepted to growth control group. The inhibition of enzyme activity (cell death) was calculated compared to the solvent group, the concentration of extracts that caused a 50% inhibition of enzyme activity in the cells IC50 was used to express the cytotoxic potential[19].

Antimicrobial Activity Assessment

Escherichia coli ATCC 25922, *Yersinia pseudotuberculosis* ATCC 911, *Pseudomonas aeruginosa* ATCC 43288, *Staphylococcus aureus* ATCC 25923, *Enterococcus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 43251, *Bacillus cereus* 709 ROMA, *Mycobacterium smegmatis* ATCC607, *Candida albicans* ATCC 60193, and *Saccharomyces cerevisiae* RSKK

251 were obtained from the Hifzissihha Institute of Refik Saydam (Ankara, Turkey). For material preparation, we dissolved the extract in dimethyl sulfoxide (DMSO) in a stock solution of 178–256 mg/mL.

The agar-well diffusion method [20], including a number of modifications previously reported by [21] was applied for susceptibility screening. Bacteria were suspended in Mueller Hinton (MH) broth (Difco, Detroit, MI), and yeast-like fungi in yeast extracts broth. All micro-organisms were next diluted to a level of approximately 106 colony-forming units (cfus) per mL. Sabouraud Dextrose Agar (SDA) (Difco, Detriot, MI) was employed in the case of yeast-like fungi, while we used brain heart infusion agar (BHA) for *M. smegmatis* [22]. These were applied to the surface of MH and SD agars using the 'flood inoculation' technique, and then dried. Wells with a diameter of 5 mm were next created from the agar using a sterile cork-borer. Next, 8900- 12800 µg/50 µL of the extract materials was added to the wells. The plates were subsequently incubated for 18 h at 35 ^{o}C. *M. smegmatis* was cultured over the course of 3-5 days on BHA plates at 35 ^{o}C. Zones of inhibition were calculated against the test organism to measure antimicrobial activity. Ampicillin (10 µg), streptomycin (10 µg), and fluconazole (5 µg) were applied as standard drugs, with dimethylsulfoxide being employed as the control. Finally, minimal inhibition concentration values (µg mL^{-1}) were determined for *I. cappadocica*.

Antityrosinase Activity

Tyrosinase inhibitory activity (TIA) (EC 1.14.1.8.1, 30 U, mushroom tyrosinase, Sigma) measurements were performed with varying concentrations of kojic acid solutions as standard [23]. Reaction mixture absorbance was read at 490 nm using the spectrophotometric method on a microplate reader (VersaMax Molecular Devices, USA). TIA percentages were determined using the formula % inhibition = [[(A-B)-(C-D)] / (A-B)] x 100

RESULTS

Total Phenolic Content (TPC)

TPC values determined for the aqueous and methanolic extracts were 10.1 ± 0.005 and 10 ± 0.268 mg of GAE/g, respectively (Table 1).

Identification of Phenolic Compounds by RP-HPLC

Chromatograms of the various phenolic standards and methanolic extract employed are shown in Figures 1-2. Amounts of phenolic compounds determined in the different samples are shown in Table 2. The results indicated the presence in the methanolic extract of the plant of sinapic acid (12.23 mg/g) and benzoic acid (48.98 mg/g).

Antioxidant Activity

DPPH scavenging activity values determined for aqueous and methanolic extracts in this study were0.0425 ± 0.0017, 0.0661 ± 0.0014 mg/mL, respectively (Table 1). The extracts exhibited a lower radical scavenging capacity than BHT (0.0074 ± 0.0004 mg/mL). FRAP values for aqueous and methanolic extracts were398 ± 0.057, 666 ± 3.210 μM Trolox/g sample, respectively (Table 1). The CUPRAC activity of the aqueous and methanolic extracts in this study was determined as 2189 ± 1.141, 4377 ± 2.156 μM Trolox/g sample, respectively (Table 1).

Cytotoxicity Potential Evaluation

In order to determine the cytotoxicity potentialof the aqueous and methanolic extracts of *I. cappadocica* on PC-3 and 3T3 cell lines MTT assay was used after 24 hour exposure period. Our results show that the aqueous extracts did not cause significant cytotoxicity in both cell lines (maximum cell death was less than 7 %) at the tested concentrations whereas methanol extracts caused dose-dependent cell death in high concentrations(Figure 3,4); the IC_{50} values were calculated to be 1188.73 and 1075.99 in PC-3 and 3T3 cell lines, respectively.

Antimicrobial Activitiesof *I. cappadocica* Extracts

The antimicrobial activities of *I. cappadocica*extracts against the bacteria tested were assessed in terms of the presence of inhibition diameters (Table 3), and minimal inhibition concentrations (Table 4). The methanolic extract exhibited antimicrobial effect against *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Enterococcus faecalis and Mycobacterium smegmatis,Listeria monocytogenes, Bacillus cereus,*but not *Yersinia pseudotuberculosis, Candida albicans, Saccharomyces cerevisiae.*

Tyrosinase Inhibitor Activities of *I. cappadocica*Extracts

Tyrosinase inhibition in our study involved the use of L-DOPA as substrate and kojic acid as a positive control. It was found that only methanol extract inhibited tyrosinase enzyme. The half-maximal inhibitory concentration (IC_{50}) of the methanolic extract (891.2509 µg mL^{-1}) on tyrosinase were higher than the kojic acid (97.13 ± 0.3 µg mL^{-1}) (Table 5).

DISCUSSION

Phenolic compounds have been identified as potent chain-breaking antioxidants [24]. They are important components of plants with radical-scavenging capacities resulting from their hydroxyl groups [25]. Tanaka et al. suggested that polyphenolic compounds ingested at levels of 1.0 g per day from a stem- and vegetable-rich diet may exhibit suppressive effects on mutagenesis and carcinogenesis in humans [26]. Similar to our findings, Karakoca et al. (2013) determined the total content of phenolic compounds varied from 1.64 to 98.23 mg GAE/g extract in the root extracts of *Isatis floribunda* Boiss. ex Bornm [27]. Miceli et al. (2017) determined that the total phenolic content was 191.05 ± 2.94 mg GAE/g in extract of *Isatis tinctoria* L. [28]. The phenolic composition of plant extracts is affected by different factors such as variety, climate, and storage, processing [29].

Comparing our results with those of Mohn et al. only six phenolic compounds were already detected, i.e., peaks 8, 9, and 22, namely sinapic acid, ferulic acid, and isoscoparin were reported as constituents of dichloromethane extract whereas peaks 5, 6, namely sinapic acid,

229 and benzoic acid were found in the methanol extract. Emam & El-Moaty (2009) identified the
230 phenolic acid compounds of aqueous ethanol extracts obtained from *Isatis microcarpa* Boiss.
231 and *Pseuderucaria clavate* Boiss. & Reut. from the family Brassicaceae [30]. Karakoca et al.
232 (2013) identified the phenolic acid compounds of *I. microcarpa* and *P. clavata* as ferulic acid
233 and gallic acid. However, caffeic acid was only determined in *I. microcarpa* [27].As a result
234 of this study, the phenolic acid compounds of *I. cappadocica* have been identified as sinapic
235 acid, and benzoic acid. The sinapic acid is a bioactive phenolic acid and has the potential to
236 attenuate various chemically induced toxicities. Sinapic acid and its derivatives, particularly
237 4-vinylsyringol, are natural compounds with a diverse range of reported health benefits,
238 including antioxidant, anti-inflammatory, anticancer, antimutagenic, antiglycemic,
239 neuroprotective, and antibacterial activities [31].Benzoic acid is employed as a preservative in
240 the food industry since it suppresses mold, yeast [32] and a number of bacteria. It is also one
241 component of Whitfield's ointment, used as a remedy for fungal skin diseases such
242 as tinea, ringworm, and athlete's foot [33].
243 Various different mechanisms may be responsible for antioxidant activity, including the
244 inhibition of chain initiation and decreased peroxide capacity, radical scavenging and
245 decomposition. However, no single test is available for investigating the antioxidant profile of
246 a specific sample. Various techniques involving different approaches and mechanisms must
247 therefore be applied to analyze the antioxidant capacity of phytocomplexes or isolated
248 compounds obtained from plants [34].
249 Various methods are available for measuring antioxidant activities. Extracts are highly
250 complex entities, frequently consisting of large numbers of chemical compounds with
251 different functional groups, polarities and chemical behaviors. Results may therefore be
252 scattered results, depending on the assay used. Multiple assays will therefore yield greater
253 information concerning the antioxidant potential of an extract. In this study, mainly three

methods, DPPH, FRAP, and CUPRAC were used. Free radicals play a major role in the oxidation of unsaturated lipids [35]. DPPH radical has been employed as a stable free radical for measuring the antioxidant activity of natural compounds [36]. This relies on the reduction of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant deriving from the formation of the non-radical DPPH–H. Wang (2012) determined DPPH scavenging activity in the methanol, ethanol and water extract at 2.0 mg dw/mL of the *I. indigotica* Fort. and found only 34.85% in the water extract [37]. Karakoca et al. (2013) reported that DPPH scavenging activity was found in *Isatis floribunda* Boiss. ex Bornm. flower and root methanol, ethanol and water extracts at 2.0 mg/mL concentration and the highest DPPH scavenging activity was determined in the flower water extract as 89.58 % [27]. DPPH scavenging activity values determined for aqueous extract in this study were0.0425 ± 0.0017.From the present results it may be postulated that even if *I. cappadocica* extracts than BHT have less DPPH scavenging activity, it reduce the DPPH radical to corresponding hydrazine when they react with hydrogen donors in antioxidant principles.

We used the ferric reducing antioxidant assay in this study, a simple and accurate test based on the reduction of ferric 2, 4, 6– tripyridyl-S-triazine [Fe ( )–TPTZ] to the ferrous 2, 4, 6– tripyridyl-S-triazine [Fe ( )–TPTZ] complex by a reductant at low pH. The assay can be easily standardized, and has often been used to determine the antioxidant activity of various plants and fruits and a number of biological samples. The antioxidant ability of fractions and compounds was estimated on the basis of FRAP values, with greater absorbance indicating greater ferric reducing power.In this study, FRAP values for methanolic extract were666 ± 3.210 µM Trolox/g sample. Karakoca et al. (2013) reported that the highest result for ferric ion reducing power (A700 nm 0.50) was found in the water extract while the lowest values for ferric ion reducing power were found in the n-hexane extract (A700nm 0.11) in the flower extracts [27].

279 Various previous studies have applied the CUPRAC assay in order to determine the cupric-
280 reducing power activities of plant extracts or antioxidant compounds [38]. This assay is based
281 on the reduction of Cu^{2+} to Cu^{1+} by antioxidant compounds in the presence of neocuproine.
282 The method offers a number of significant advantages, such as simplicity of operation, the
283 transparency of the endpoint and mechanism, the fact that instrumentation is easily accessible,
284 and optimal intra- and inter-assay reproducibility [39]. The CUPRAC activity of the
285 methanolic extract in this study was determined as 4377 ± 2.156 µM Trolox/g sample.
286 Karakoca et al. (2013) reported that the highest Cu^{2+} reducing power were found in the flower
287 water extract (A450nm 0.39) and in the root methanol extract (A450nm 0.61) of*Isatis*
288 *floribunda* Boiss. ex Bornm.. Also, they found that BHT, at 31.25 µg/mL the concentration,
289 exhibited remarkably higher cupric ion reducing power (A450nm 0.39) than the extracts [27].
290 In a study by Karakoca et al., (2013) methanol extract of *I. floribunda* herb was observed to
291 exhibit cytotoxic effects of 5.88%, 6.27 and 13.25 respectively in MCF12A cells at
292 concentrations of 250, 500 and 1000 µg / mL [27]. Miceli et al., (2017) in their study
293 evaluated the cytotoxic effect of *I. tinctoria* by MTT test and observed that the leaf part of *I.*
294 *tinctoria* decreased anaplastic human thyroid carcinoma (CAL-62) cell line by 80% [28]. The
295 cytotoxic activity of dichloromethane, ethyl acetate, methanol extracts of microcarpa I. were
296 investigated in hepatic (HEPG2) and breast carcinoma (T47D) cell lines, dichloromethane
297 extract was found to be the most active ones with IC_{50} values 20.8 µg / mL and 34.3 µg / mL
298 in T47D and HEPG2 cell line respectively [40].The cytotoxic effects of *I. cappadocica*was
299 not evaluated previously, The non-cytotoxicity of water extract and the relatively high IC_{50}
300 values of methanolic extracts of *I. cappadocica* could be evaluated as a sign of low toxicity.
301 Further studies are warranted to evaluate the toxicity of *I. cappadocica* before any
302 commercial application.

Increasing numbers of reports concerning the antimicrobial properties of medicinal plants are emerging from various parts of the world. Faiyaz et al. (2012) adopted the agar well diffusion method and reported that the methanol and ethanol seed extracts (at the concentration of 40 mg) of the *Raphanus sativus Linn. (Brassicacea*) plant exhibited a significant antimicrobial effect on *Staphylococcus aureus (*ATCC25923, 13.50 and 19.00 mm), *Escherichia coli* (ATCC 25922, 12.50 and 14.50 mm) and *Pseudomonas aeruginosa* (ATCC 27853, 14.60 and 21.3 mm) [41]. Karakoca et al. (2013) found that the methanol and ethanol flower extracts (at the concentration of 2.5 mg/disc) showed antimicrobial activity against the pathogens of *S. aureus* (ATCC 25923, 10.49 and 12.92 mm), *E. coli* (ATCC 35218, 12.74 mm for only the ethanol extract) and *P. aeruginosa* (ATCC 27853, 11.60 mm for the methanol extract) [27]. Emam & El Moaty (2009) investigated the antimicrobial activity of ethanol and water extracts of *Isatis microcarpa* J. Gay ex Boiss. against a range of pathogen micro-organisms (*E. coli, S. aureus, Bacillus subtilis, Pseudomonasspp., and Salmonella spp.*) [30]. The extracts studies exhibited various degrees of antimicrobial activity. However, water extract exhibited no activity against the pathogens investigated in our study. Karakoca investigated antimicrobial activity recorded from flower and root ethanol extracts against *S. aureus* (ATCC 25923; 12.92 mm and 12.95 mm, respectively) and *E. coli* (O157:H7; 13.51 mm and 11.93 mm, respectively) [27]. Kıvanç & Kunduhoğlu (1997) investigated fresh juice of *Raphanus sativus* L. var. *radicula* (*Brassicaceae*) and *R. sativus* L. (*Brassicaceae*) in terms of antimicrobial activity against *B. cereus, E. coli, S. aureus*, and *P. aeruginosa* [42]. The extracts only exhibited antimicrobial activity against *B. cereus* (13 mm and 12 mm, respectively). In our study, methanolic extracts showed various antimicrobial activities against the same microorganisms. The results indicated that the methanolic extract showed various antibacterial activities against different pathogenic bacteria. The extract could therefore be used to source antibiotic substances for possible treatment of bacterial infections.

The enzyme tyrosinase plays a major role in mammalian melanin synthesis. Melanin is known to protect the skin against ultraviolet (UV) damage by absorbing UV sunlight and through the eradication of reactive oxygen species. Excessive production or abnormal melanin pigmentation results in esthetic anxieties. There is therefore a need for potent tyrosinase suppressors [43]. Our results seem to suggest that *I. cappadocica* extract may be a potential candidate for use in the design and development of novel tyrosinase inhibitors as anti-browning agents. *I. cappadocica* may be effective as an anti-browning agent in the search for novel tyrosinase inhibitors in cosmetic products.

CONCLUSIONS

The results presented in this study are the first information on the biological activities of *I. cappadocica*. In conclusion, the results showed the biological importance of *I.cappadocica*, one of commonly used edible and medicinal plants in Turkey. Thus, *I. cappadocica*may have been helped people to protect against lipid peroxidation and free radical damage, and its extracts will probably use for the development of safe food products and additives. *I. cappadocica* can be used to source antibiotic substances for possible treatment of bacterial infections. *I. cappadocica*has caused to death of cells dose-depending in the high concentration cytotoxic activity on the PC-3 and 3T3 cell lines. It may be effective as anti-browning agents for explore of novel tyrosinase inhibitors in cosmetic. However, further studies, especially antioxidant activity tests on extracts of *I. cappadocica*and isolated constituents are needed. Further studies are now needed to confirm the bioactive compounds involved in the antioxidant, antimicrobial, cytotoxic, and tyrosinase.

Conflict of Interest: The authors declare that no potential conflict of interest.

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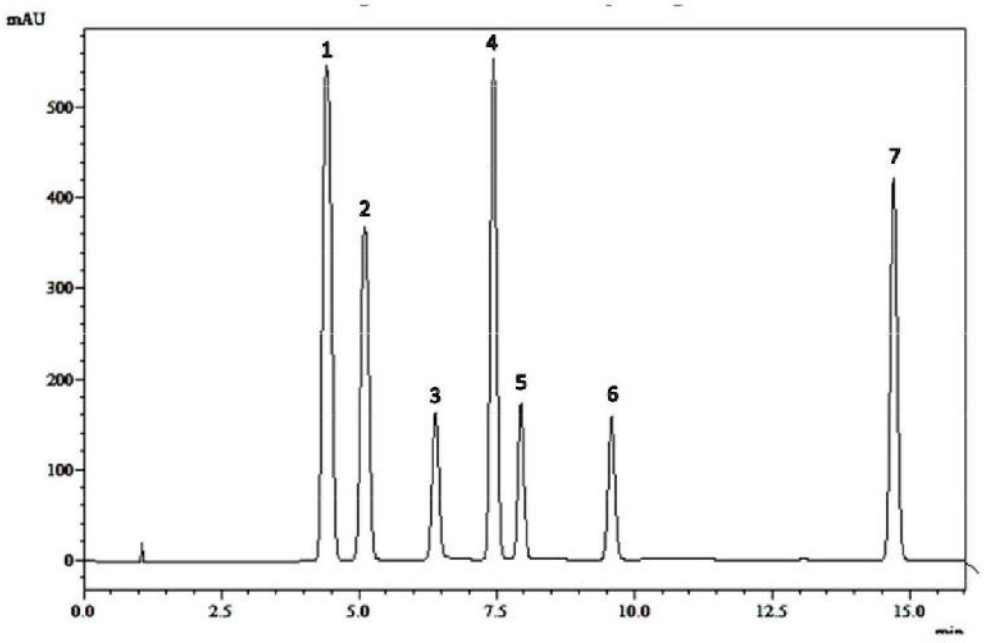

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465 **Fig. 1. RP-HPLC chromatogram of phenolic standards (50µM) searched in *Isatis***
466 ***cappadocica* samples detected at 270 nm by DAD. Peak identification: (1) *p*-hydroxy**
467 **benzoic acid, (2) vanillic acid, (3) syring aldehyde, (4) *p*-coumaric acid, (5) sinapic acid,**
468 **(6) benzoic acid, (7) quercetin.**
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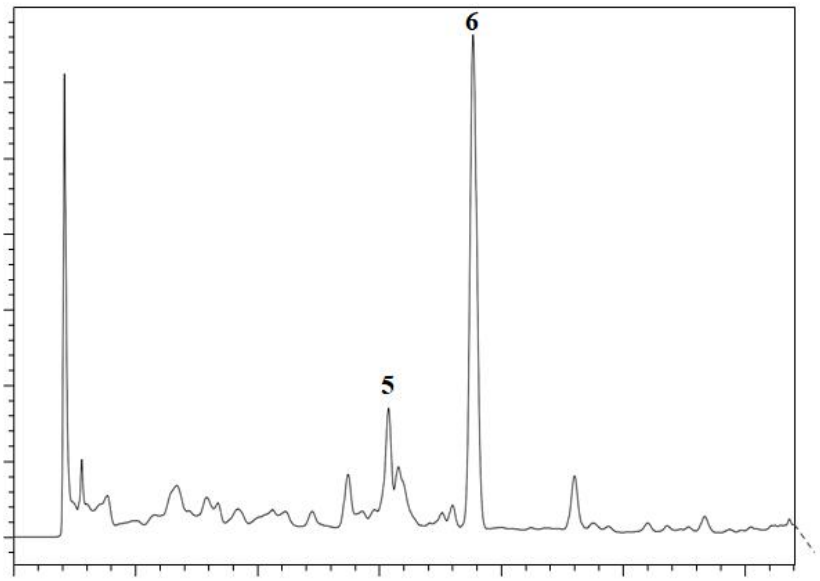

Fig. 2. RP-HPLC DAD chromatogram of *Isatis cappadocica* methanol extract (50 mg/mL) at 270 nm. Peak identification: (5) sinapic acid, (6) benzoic acid.

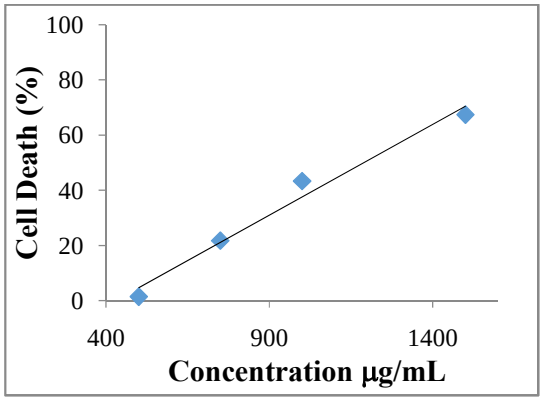

Fig. 3. The cytotoxic potential of *I. cappadocica* methanolic extracts in PC-3 cell line.

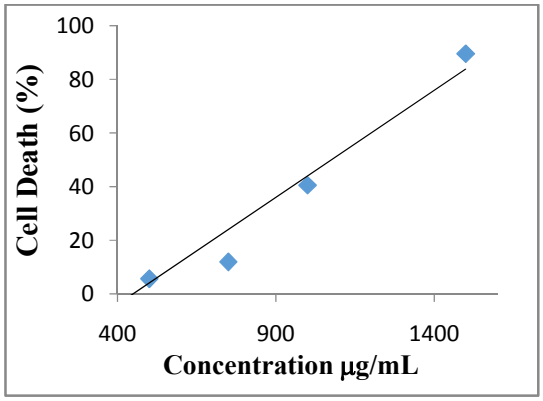

Fig. 4. The cytotoxic potential of *I. cappadocica* methanolic extracts in 3T3 cell line.

Table 1. Theantioxidantactivities of *Isatiscappadocica*extracts

Test Compounds	TPC[1]	FRAP[2]	CUPRAC[3]	DPPH[4]
Aqueousextract	10.1 ± 0.005	398 ± 0.057	2189 ± 1.141	0.0425 ± 0.0017
Methanolicextract	10 ± 0.268	666 ± 3.210	4377 ± 2.156	0.0661 ± 0.0014
BHT				0.0074 ± 0.0004

[1]Total phenoliccontentexpressed in mg of gallicacidequivalent (GAE) per gram of dryplantweight.

[2] FRAP valueexpressed as µMtroloxequivalents (TE) per gram of dryplantweight.

[3]Trolox equivalentantioxidantcapacity (TEAC) valueexpressed in µMtroloxequivalents (TE) per gram of dryplantweight.

[4]Concentration of test sample (mg/mL) requiredtoproduce 50% inhibition of the DPPH radical.

Table2. Phenoliccomposition of the methanolic extract of *Isatiscappadocica*

Phenoliccompounds	Retention time (min)	Amount (mg g^{-1})
p-hydroxybenzoicacid	4.197	-
Vanillicacid	5.318	-
Syringaldehyde	6.132	-
p-coumaricacid	7.407	-
Sinapicacid	7.894	12.23
Benzoicacid	9.432	48.08
Quercetin	14.688	-

Table 3.Inhibitiondiametersvalues of *Isatiscappadocica*extracts (mm)

TestedCompounds	MicroorganismsandInhibitionDiameters (µg/mL)										
	Quantity (µg/mL)	Gram negativebacteria			Gram positivebacteria				No grambacteria	YeastLikeFungi	
		Ec	Yp	Pa	Sa	Ef	Lm	Bc	Ms	Ca	Sc
MethanolicExtract	10000	8	-	6	6	8	6	8	15	-	-
AqueousExtract	10000	-	-	-	-	-	-	-	-	-	-
Ampicillin	10	10	10	18	35	10	10	15	-	-	-
Streptomycin	10								35		
Fluconazole	5									25	25

Ec: *Escherichiacoli* ATCC 25922, Yp: *YersiniapseudotuberculosisATCC* 911, Pa: *Pseudomonasaeruginosa*ATCC 27853, Sa: *Staphylococcusaureus* ATCC 25923, Ef: *Enterococcusfaecalis* ATCC 29212, Lm: *Listeriamonocytogenes* ATCC 43251, Bc: *Bacilluscereus* 702 Roma, Ms: *Mycobacteriumsmegmatis* ATCC607, Ca: *Candidaalbicans* ATCC 60193, Sc: *Saccharomycescerevisiae* RSKK 251, (-): noactivity of test concentrations (10 000 µg/mL).

Table 4.Minimal inhibitionconcentrationvaluesof *Isatiscappadocica*extracts (µg/mL)

TestedCompounds	Quantity (µg/mL)	Microorganismsand Minimal InhibitionConcentration (µg/mL)									
		Gram negativebacteria			Gram positivebacteria				No grambacteria	YeastLikeFungi	
		Ec	Yp	Pa	Sa	Ef	Lm	Bc	Ms	Ca	Sc
MethanolicExtract	10000	350	-	700	700	350	700	350	62.25	-	-
AqueousExtract	10000	-	-	-	-	-	-	-	-	-	-
Ampicillin	10	10	18	>128	35	10	10	15	-	-	-
Streptomycin	10								4		
Fluconazole	5									<8	<8

Ec: *Escherichiacoli* ATCC 25922, Yp: *Yersiniapseudotuberculosis*ATCC 911, Pa: *Pseudomonasaeruginosa*ATCC 27853, Sa: *Staphylococcusaureus* ATCC 25923, Ef: *Enterococcusfaecalis* ATCC 29212, Lm: *Listeriamonocytogenes* ATCC 43251, Bc: *Bacilluscereus* 702 Roma, Ms: *Mycobacteriumsmegmatis* ATCC607, Ca: *Candidaalbicans* ATCC 60193, Sc: *Saccharomycescerevisiae* RSKK 251, (-): noactivity of test concentrations (10 000 µg/mL).

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566 **Table5. Tyrosinaseinhibitoractivities (IC_{50}values) of *Isatiscappadocica*extracts**

Test Compounds	IC_{50} (µg/mL)
Aqueousextract	> 1000
Methanolicextract	891.2509
KojikAcid	97.13 ± 0.3

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