Original Research Article

Evaluation of Antioxidant, Antimicrobial, Antityrosinase and Cytotoxic Potentials of *Isatis cappadocica* subsp. *alyssifoli as a Potent Pharmaceutical Resource*

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* Desv. We analyzed the antioxidant properties of extracts using total phenolic content (TPC), 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 *I. 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 inhibitory 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. The methanol extract has caused to death of cells by dosage in the high concentrations cytotoxic activity on the PC-3 and 3T3 cell lines. The results showed that *I. cappadocica* could be used as a natural source 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 [1,2]. At least 80% of the world population, largely in developing countries, is still thought to employ traditional remedies based on plant extracts [3,4]. 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) [5,6]. 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 [7,8]. Antioxidant-based medications play major roles in the prevention and treatment of such diseases as atherosclerosis, stroke, diabetes, Alzheimer's disease and cancer [9]. 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 [10]. 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 [11], anticancer [12], antiarthritic [13], and antirheumatic [14] purposes, as well as being powerful insecticides [15]. 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 [16,17]. *Isatis* species exhibit antibacterial, anticancer, and antiviral 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 [18].

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 inhibitory activity and (v) to determine the content of phenolic acid compounds by high performance liquid chromatography (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); gallic acid, sodium carbonate, sodium hydroxide, iron (III) chloride, copper (II) chloride ($CuCl_2$) , ammonium acetate (NH_4Ac), neocuproine, mushroom tyrosinase (EC 1.14.1.8.1, 30 U), levodopa (L-DOPA), disodium phosphate, sodium dihydrogen phosphate (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. Microplate spectrophotometer system (Epoch, Erlangen, Germany) was used to measure the Optical densities (OD). 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. To weighing chemicals, powders and other samples were used Precision Balance (The Pioneer PX, Ohaus). HPLC studies were carried using Shimadzu Corporation, LC 20 AT, Kyoto, Japan.

Plant Material and Preparation of Extracts

I. 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. Finally, antioxidant, antimicrobial, cytotoxicity, 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 (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 a diode array detector (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 [19]. Gallic acid was used as a standard. TPC 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 was placed into test tubes and then mixed with 0.5 mL of 0.2 N Folin-Ciocalteu reagent and with 1.5 mL of 2% sodium carbonate. The tubes were next sealed using parafilm and incubated for 2 h at 20 °C. Finally, absorbance values were read spectrophotometrically at 760 nm. All measurements were conducted in triplicate. TPC value was calculated with the following equation using the calibration graph:

TPC = (The sample absorbance corresponds to [Gallic acid]) μM

The quantity of polyphenolic compounds were indicated as mg of gallic acid equivalents (GAE)/g sample.

The ferric reducing antioxidant power (FRAP) assay involves calculation of the iron-reducing capacities of a specific extract [20]. Following exposure to 2,4,6-tripyridyl-S-triazine (TPTZ), the Fe^{2+}-TPTZ complex forms a blue color that can be read at 593 nm. In summary, we added 3.0 mL of working FRAP reagent to an appropriate volume/concentration of extract. This was next incubated for 4 min at 37 ^{0}C, and the absorbance was finally measured against a ferrous sulfate standard at 593 nm. Trolox was also calculated under identical conditions as a standard antioxidant compound for comparative analyses. A calibration graph was plotted according to results. The FRAP value was calculated as follows:

FRAP = (The sample absorbance corresponds to [Trolox]) μM

The results were expressed as μM Trolox equivalent of g sample.

DPPH radical-scavenging activity is linked to the DPPH cation radical scavenging capacity of the antioxidant [21]. Briefly, we combined 0.75 mL of DPPH reagent (0.1 mM in methanol) with 0.75 mL of tea extract or standard. This was then exposed to vigorous vortexing and then allowed to stand for 30 min in the dark at room temperature. Discoloration occurring in DPPH was calculated using the spectrophometric method at 517 nm. The percentage inhibition of the discoloration resulting from the plant extract was calculated using BHT as standard.

The percent reduction of the DPPH radical was calculated using the following equation:

DPPH inhibition (%) = 100 - (Asample/Acontrol) × 100

SC_{50} value (representing the concentration causing 50% scavenging of DPPH radical) of extract was stated as mg/mL.

Extracts' cupric reducing antioxidant power (CUPRAC) levels were measured by means of the spectrophotometric method [22]. We first mixed 1 mL of $CuCl_2$ solution ($1.0x10^{-2}$ M), 1 mL of neocuproine solution ($7.5x10^{-3}$ M) and 1 mL NH_4Ac buffer solution inside a test tube. Various different extract concentrations were combined with the initial mixture for a final 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 calculated with the following equation using the calibration graph:

CUPRAC = (The sample absorbance corresponds to [Trolox]) µM

CUPRAC values were expressed as µM Trolox equivalent per gram of sample.

Cytotoxicity Potential Evaluation

3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay was used to evaluate the cytotoxic potential of *I.cappadocica* aqueous and methanolic extracts. In this test the viable cells metabolize the yellow water soluble 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 Dimethyl sulfoxide (DMSO) was added for each well to dissolve the formazan crystals. Microplate spectrophotometer system at 590 nm (wavelength 670 nm) was used to measure the 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 IC_{50} was used to express the cytotoxic potential [23].

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 DMSO in a stock solution of 178–256 mg/mL.

The agar-well diffusion method [24], including a number of modifications previously reported by [25] 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 (BHI) agar for *M. smegmatis* [26]. These were

179 applied to the surface of MH and SD agars using the 'flood inoculation' technique, and then
180 dried. Wells with a diameter of 5 mm were next created from the agar using a sterile cork-
181 borer. Next, 8900- 12800 µg/50 µL of the extract materials was added to the wells. The plates
182 were subsequently incubated for 18 h at 35 ^{o}C. *M. smegmatis* was cultured over the course of
183 3-5 days on BHI plates at 35 ^{o}C. Zones of inhibition were calculated against the test organism
184 to measure antimicrobial activity. Ampicillin (10 µg), streptomycin (10 µg), and fluconazole
185 (5 µg) were applied as standard drugs, with dimethylsulfoxide being employed as the control.
186 Finally, minimal inhibition concentration values (µg mL^{-1}) were determined for *I.*
187 *cappadocica*.
188 **Antityrosinase Activity**
189 Tyrosinase inhibitory activity (TIA) measurements were performed with various
190 concentrations (500, 100, 50 and 25 µg/mL) of kojic acid solutions as standard [27].
191 Tyrosinase solution (46 U/mL), different concentrations (500, 100, 50 and 25 µg/mL) of
192 methonolic plant extract solutions were prepared. 120 µL of phosphate buffer (0,2 M, pH 7.0),
193 and 40 µL tyrosinase solution for A wells; 160 µL of phosphate buffer (0,2 M, pH 6.8) for B
194 wells; 80 µL of phosphate buffer (0,2 M, pH 6.8), 40 µL tyrosinase solution and 40 µL sample
195 solution for C wells; 120 µL of phosphate buffer (0,2 M, pH 7.0) and 40 µL sample solution
196 for D wells were added and mixed in a 96-well plate and incubated for 10 min at 23°C. L-
197 DOPA solution (2,5 mM, 40 µL) was added to all wells and incubated for 10 min at 23°C.
198 Reaction mixture absorbance was read at 490 nm using the spectrophotometric method on a
199 microplate reader.
200 TIA percentages were determined using the formula % inhibition = [[(A-B)-(C-D)] / (A-B)] x
201 100

Then, the logarithm of the sample concentration was calculated and graph were plotted with these results. Using the equation of the graph, the value of IC_{50} (half-maximal inhibitory concentrations) was calculated.

RESULTS

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 1. 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

TPC values determined for the aqueous and methanolic extracts were 22.1 ± 0.0144 and 10.1 ± 0.0056 mg GAE/g sample, respectively (Table 2).

DPPH scavenging activity values determined for aqueous and methanolic extracts in this study were 0.0425 ± 0.0017, 0.0661 ± 0.0014 mg/mL, respectively (Table 2). The extracts exhibited a lower radical scavenging capacity than BHT (0.0074 ± 0.0004 mg/mL). FRAP values for aqueous and methanolic extracts were 848 ± 0.053, 398 ± 0.057 µM Trolox/g sample, respectively (Table 2). 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 2).

Cytotoxicity Potential Evaluation

In order to determine the cytotoxicity potential of 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 Activities of *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 *E. coli, P. aeruginosa, S. aureus, E. faecalis, L. monocytogenes, B. cereus, and M. smegmatis,* but not *Y. pseudotuberculosis, C. albicans, S. cerevisiae.*

Tyrosinase Inhibitory 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 ± 1.48 µg mL^{-1}) on tyrosinase were higher than the kojic acid (63.0957 ± 0.32 µg mL^{-1}) (Table 5).

DISCUSSION

Phenolic compounds have been identified as potent chain-breaking antioxidants [28]. They are important components of plants with radical-scavenging capacities resulting from their hydroxyl groups [29]. An earlier study 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 [30]. Similar to our findings, Karakoca K, et al. 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 [31]. Miceli N, et al. determined that the total phenolic content was 191.05 ± 2.94 mg GAE/g in extract of *Isatis tinctoria* L. [32]. The phenolic composition of plant extracts is affected by different factors such as variety, climate, and storage, processing [33].

252 Comparing our results with those of Mohn et al. only six phenolic compounds were already
253 detected, i.e., peaks 8, 9, and 22, namely sinapic acid, ferulic acid, and isoscoparin were
254 reported as constituents of dichloromethane extract whereas peaks 5, 6, namely sinapic acid,
255 and benzoic acid were found in the methanol extract. In another study they identified the
256 phenolic acid compounds of aqueous ethanol extracts obtained from *Isatis microcarpa* Boiss.
257 and *Pseuderucaria clavate* Boiss. & Reut. from the family Brassicaceae [34]. Karakoca K, et
258 al. identified the phenolic acid compounds of *I. microcarpa* and *P. clavata* as ferulic acid and
259 gallic acid. However, caffeic acid was only determined in *I. microcarpa* [31]. As a result of
260 this study, the phenolic acid compounds of *I. cappadocica* have been identified as sinapic
261 acid, and benzoic acid. The sinapic acid is a bioactive phenolic acid and has the potential to
262 attenuate various chemically induced toxicities. Sinapic acid and its derivatives, particularly
263 4-vinylsyringol, are natural compounds with a diverse range of reported health benefits,
264 including antioxidant, anti-inflammatory, anticancer, antimutagenic, antiglycemic,
265 neuroprotective, and antibacterial activities [35]. Benzoic acid is employed as a preservative
266 in the food industry since it suppresses mold, yeast [36] and a number of bacteria. It is also
267 one component of Whitfield's ointment, used as a remedy for fungal skin diseases such
268 as tinea, ringworm, and athlete's foot [37].
269 Various different mechanisms may be responsible for antioxidant activity, including the
270 inhibition of chain initiation and decreased peroxide capacity, radical scavenging and
271 decomposition. However, no single test is available for investigating the antioxidant profile of
272 a specific sample. Various techniques involving different approaches and mechanisms must
273 therefore be applied to analyze the antioxidant capacity of phytocomplexes or isolated
274 compounds obtained from plants [38].
275 Various methods are available for measuring antioxidant activities. Extracts are highly
276 complex entities, frequently consisting of large numbers of chemical compounds with

277 different functional groups, polarities and chemical behaviors. Results may therefore be
278 scattered results, depending on the assay used. Multiple assays will therefore yield greater
279 information concerning the antioxidant potential of an extract. In this study, mainly three
280 methods, DPPH, FRAP, and CUPRAC were used. Free radicals play a major role in the
281 oxidation of unsaturated lipids [39]. DPPH radical has been employed as a stable free radical
282 for measuring the antioxidant activity of natural compounds [40]. This relies on the reduction
283 of alcoholic DPPH solution in the presence of a hydrogen-donating antioxidant deriving from
284 the formation of the non-radical DPPH–H. Wang XH, determined DPPH scavenging activity
285 in the methanol, ethanol and water extract at 2.0 mg dw/mL of the *I. indigotica* Fort. and
286 found only 34.85% in the water extract [41]. Karakoca K, et al. reported that DPPH
287 scavenging activity was found in *Isatis floribunda* Boiss. ex Bornm. flower and root
288 methanol, ethanol and water extracts at 2.0 mg/mL concentration and the highest DPPH
289 scavenging activity was determined in the flower water extract as 89.58 % [31]. DPPH
290 scavenging activity values determined for aqueous extract in this study were 0.0425 ± 0.0017
291 mg/mL. From the present results it may be postulated that even if *I. cappadocica* extracts than
292 BHT have less DPPH scavenging activity, it reduce the DPPH radical to corresponding
293 hydrazine when they react with hydrogen donors in antioxidant principles.
294 We used the ferric reducing antioxidant assay in this study, a simple and accurate test based
295 on the reduction of ferric 2, 4, 6– tripyridyl-S-triazine [Fe (III) –TPTZ] to the ferrous 2, 4, 6–
296 tripyridyl-S-triazine [Fe (II) – TPTZ] complex by a reductant at low pH. The assay can be
297 easily standardized, and has often been used to determine the antioxidant activity of various
298 plants and fruits and a number of biological samples. The antioxidant ability of fractions and
299 compounds was estimated on the basis of FRAP values, with greater absorbance indicating
300 greater ferric reducing power. In this study, FRAP values for methanolic extract were 398 ±
301 0.057 μM Trolox/g sample. Karakoca K, et al. reported that the highest result for ferric ion

302 reducing power (A700 nm 0.50) was found in the water extract while the lowest values for
303 ferric ion reducing power were found in the n-hexane extract (A700nm 0.11) in the flower
304 extracts [31].
305 Various previous studies have applied the CUPRAC assay in order to determine the cupric-
306 reducing power activities of plant extracts or antioxidant compounds [42]. This assay is based
307 on the reduction of Cu^{2+} to Cu^{1+} by antioxidant compounds in the presence of neocuproine.
308 The method offers a number of significant advantages, such as simplicity of operation, the
309 transparency of the endpoint and mechanism, the fact that instrumentation is easily accessible,
310 and optimal intra- and inter-assay reproducibility [43]. The CUPRAC activity of the
311 methanolic extract in this study was determined as 4377 ± 2.156 µM Trolox/g sample.
312 Karakoca K, et al. reported that the highest Cu^{2+} reducing power were found in the flower
313 water extract (A450nm 0.39) and in the root methanol extract (A450nm 0.61) of *Isatis*
314 *floribunda* Boiss. ex Bornm.. Also, they found that BHT, at 31.25 µg/mL the concentration,
315 exhibited remarkably higher cupric ion reducing power (A450nm 0.39) than the extracts [31].
316 In a study by Karakoca K, et al., methanol extract of *I. floribunda* herb was observed to
317 exhibit cytotoxic effects of 5.88%, 6.27 and 13.25 respectively in MCF12A cells at
318 concentrations of 250, 500 and 1000 µg / mL [31]. Miceli N, et al. evaluated the cytotoxic
319 effect of *I. tinctoria* by MTT test and observed that the leaf part of *I. tinctoria* decreased
320 anaplastic human thyroid carcinoma (CAL-62) cell line by 80% [32]. The cytotoxic activity
321 of dichloromethane, ethyl acetate, methanol extracts of microcarpa I. were investigated in
322 hepatic (HEPG2) and breast carcinoma (T47D) cell lines, dichloromethane extract was found
323 to be the most active ones with IC_{50} values 20.8 µg / mL and 34.3 µg / mL in T47D and
324 HEPG2 cell line respectively [40]. The cytotoxic effects of *I. cappadocica*was not evaluated
325 previously, The non-cytotoxicity of water extract and the relatively high IC_{50} values of
326 methanolic extracts of *I. cappadocica* could be evaluated as a sign of low toxicity. Further

studies are warranted evaluate the toxicity of *I. cappadocica* before any commercial application. Increasing numbers of reports concerning the antimicrobial properties of medicinal plants are emerging from various parts of the world. Faiyaz A, et al. 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 *S. aureus (*ATCC25923, 13.50 and 19.00 mm), *E. coli* (ATCC 25922, 12.50 and 14.50 mm) and *P. aeruginosa* (ATCC 27853, 14.60 and 21.3 mm) [41]. Karakoca K, et al. 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) [31]. In another study they 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, B. subtilis, Pseudomonasspp., and Salmonella spp.*) [34]. The extracts studies exhibited various degrees of antimicrobial activity. However, water extract exhibited no activity against the pathogens investigated in our study. Karakoca K, et al. 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) [31]. In another study they 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* [46]. 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 [47]. 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

In conclusion, the results of this study indicated that extracts of *I. cappadocica* may be use as raw material by the pharmaceutical, cosmetic, and food industries because of their antioxidant, antimicrobial, antityrosinase, and cytotoxic potentials.

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

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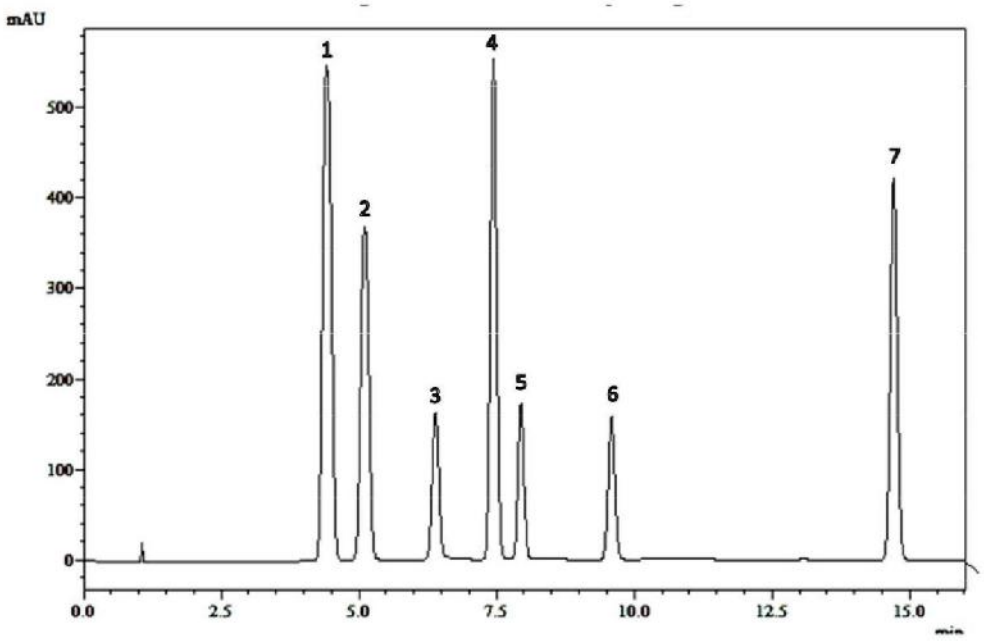

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489 **Fig. 1. RP-HPLC chromatogram of phenolic standards (50µM) searched in *I.***
490 ***cappadocica* samples detected at 270 nm by DAD. Peak identification: (1) *p*-hydroxy**
491 **benzoic acid, (2) vanillic acid, (3) syring aldehyde, (4) *p*-coumaric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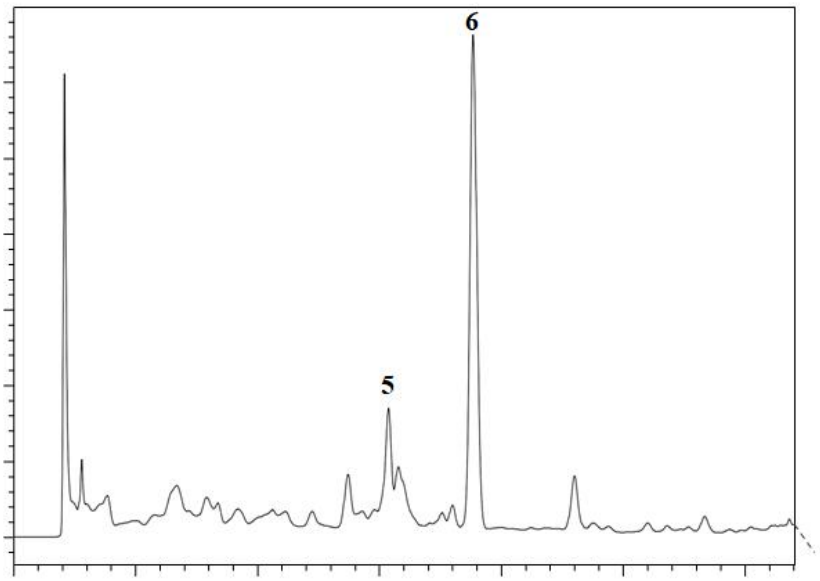

Fig. 2. RP-HPLC DAD chromatogram of *I. cappadocica* methanol extract (50 mg/mL) at 270 nm. Peak identification: (5) sinapic acid, (6) benzoic acid.

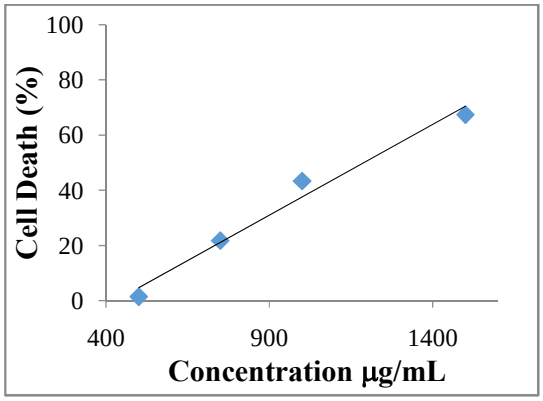

Fig. 3. The cytotoxic potential of *I. cappadocica* methanolic extracts in PC-3 cell line.

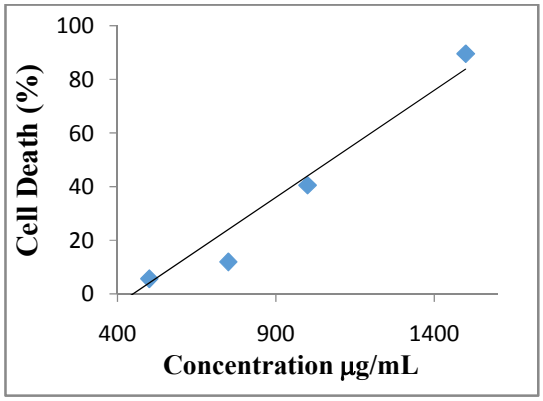

Fig. 4. The cytotoxic potential of *I. cappadocica* methanolic extracts in 3T3 cell line.

562 **Table 1. Phenolic composition of the methanolic extract of *I. cappadocica***

Phenolic compounds	Retention time (min)	Amount (mg g^{-1})
p-hydroxy benzoic acid	4.197	-
Vanillicacid	5.318	-
Syringaldehyde	6.132	-
p-coumaric acid	7.407	-
Sinapic acid	7.894	12.23
Benzoic acid	9.432	48.08
Quercetin	14.688	-

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Table 2. The antioxidant activities of *I. cappadocica* extracts

Test Compounds	TPC[1]	FRAP[2]	CUPRAC[3]	DPPH[4]
Aqueous extract	22.1 ± 0.0144	848 ± 0.053	2189 ± 1.141	0.0425 ± 0.0017
Methanolic extract	10.1 ± 0.0056	398 ± 0.057	4377 ± 2.156	0.0661 ± 0.0014
BHT				0.0074 ± 0.0004

Results were expressed as mean ± standard deviation.

[1]Total phenolic content expressed in mg of gallic acid equivalent (GAE) per gram of dry plant weight.

[2] FRAP value expressed as µM trolox equivalents (TE) per gram of dry plant weight.

[3]Trolox equivalent antioxidant capacity (TEAC) value expressed in µM trolox equivalents (TE) per gram of dry plant weight.

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

Table 3. Inhibition diameters values of *I. cappadocica* extracts

Tested Compounds	Quantity (µg/mL)	Microorganisms and Inhibition Diameters (mm)									
		Gram negative bacteria			Gram positive bacteria				No gram bacteria	Yeast Like Fungi	
		Ec	Yp	Pa	Sa	Ef	Lm	Bc	Ms	Ca	Sc
Methanolic Extract	10000	8	-	6	6	8	6	8	15	-	-
Aqueous Extract	10000	-	-	-	-	-	-	-	-	-	-
Ampicillin	10	10	10	18	35	10	10	15	-	-	-
Streptomycin	10								35		
Fluconazole	5									25	25

Ec: *E. coli* ATCC 25922, Yp: *Y. Pseudotuberculosis* ATCC 911, Pa: *P. Aeruginosa* ATCC 27853, Sa: *S. aureus* ATCC 25923, Ef: *E. faecalis* ATCC 29212, Lm: *L. monocytogenes* ATCC 43251, Bc: *B. cereus* 702 Roma, Ms: *M. smegmatis* ATCC607, Ca: *C. albicans* ATCC 60193, Sc: *S. cerevisiae* RSKK 251, (-): noactivity of test concentrations (10 000 µg/mL).

Table 4. Minimal inhibition concentration (MIC) values of *I. cappadocica* extracts

Test Compounds	Quantity (µg/mL)	Microorganisms and MIC values (µg/mL)									
		Gram negative bacteria			Gram positive bacteria				No gram bacteria	Yeast Like Fungi	
		Ec	Yp	Pa	Sa	Ef	Lm	Bc	Ms	Ca	Sc
Methanolic Extract	10000	350	-	700	700	350	700	350	62.25	-	-
Aqueous Extract	10000	-	-	-	-	-	-	-	-	-	-
Ampicillin	10	10	18	>128	35	10	10	15	-	-	-
Streptomycin	10								4		
Fluconazole	5									<8	<8

Ec: *E. coli* ATCC 25922, Yp: *Y. pseudotuberculosis* ATCC 911, Pa: *P. aeruginosa* ATCC 27853, Sa: *S. aureus* ATCC 25923, Ef: *E. faecalis* ATCC 29212, Lm: *L. monocytogenes* ATCC 43251, Bc: *B. cereus* 702 Roma, Ms: *M. smegmatis* ATCC607, Ca: *C. albicans* ATCC 60193, Sc: *S. cerevisiae* RSKK 251, (-): noactivity of test concentrations (10 000 µg/mL).

644 **Table 5. Tyrosinase inhibitor activities of *I. cappadocica* extracts**

Test Compounds	IC_{50} values (µg/mL)
Aqueous extract	> 1000
Methanolic extract	891.2509 ± 1.48
Kojic Acid	63.0957 ± 0.32

645 Results were expressed as mean ± standard deviation. IC_{50} : Half-maximal inhibitory concentrations