Original article

Evaluation of Antioxidant and Antimicrobial Activities of *Tamus communis* L. ssp. *cretica* (L.) Kit Tan and Its Mineral Composition

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The aim of the present work was to examine antioxidant and antimicrobial properties of *Tamus communis* L. ssp. *cretica* (L.) Kit Tan (Dioscoreaceae). The applied methods for the antioxidant activity of aqueous extract from the aerial parts of the plant were 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging, flow injection analysis-luminol chemiluminescence (FIA-CL) and thiobarbituric acid (TBA) assays. Total phenolic content of the aqueous extract was determined with spectrophotometric method. The antimicrobial activity of the *n*-hexane, chloroform, ethyl acetate, and ethanol extracts of the aerial parts of the plant was assessed towards selected bacteria. In addition, mineral composition of the raw plant was examined by atomic absorption spectroscopy. Aqueous extract of *T. communis* ssp. *cretica* was found to possess DPPH free radical scavenging activity (IC_{50}= 2.85±1.30 mg/mL), inhibitory effect on H_2O_2- and HOCl-luminol chemiluminescence (-log IC_{50}=3.8±0.09 and IC_{50}=1.3x10^{-3}±4.9x10^{-4} mg/mL), and inhibitory activity toward lipid peroxidation (IC_{50}=3.82±1.67 µg/mL) compared to the references. Total phenolic content of the aqueous extract was found to be 56.66±0.21 mg gallic acid/g extract. The *n*-hexane, chloroform, ethyl acetate, and ethanol extracts showed moderate activity having MIC values of 250-500 µg/mL against selected microorganisms. According to our results of mineral composition, the raw plant has low amount of toxic elements (Pb, Cd and Al), macro (Ca and Mg), and microelements (Cu, Fe, Mn and Zn).

Key words: *Tamus communis* ssp. *cretica*, Dioscoreaceae, Antioxidant activity, Antimicrobial activity, Mineral analysis

Tamus communis L. ssp. *cretica* (L.) Kit Tan'ın Antioksidan ve Antimikrobiyal Aktivitelerinin Değerlendirilmesi ve Mineral Kompozisyonu

Bu çalışmanın amacı *Tamus communis* L. ssp. *cretica* (L.) Kit Tan'ın (Dioscoreaceae) antioksidan ve antimikrobiyal özelliklerinin incelenmesidir. Bitkinin toprak üstü kısımlarının sulu ekstresinin antioksidan aktivitesi için uygulanan metotlar 1,1-difenil-2-pikrilhidrazil (DPPH) süpürücü, akışa injeksiyon analiz-luminol kemiluminesens (FIA-CL) ve tiyobarbitürik asit (TBA) test sistemleridir. Sulu ekstrenin total fenolik içeriği spektrofotometrik metotla tayin edilmiştir. Bitkinin toprak üstü kısımlarının *n*-hekzan, kloroform, etil asetat ve etanol ekstrelerinin antimikrobiyal aktivitesi seçilen bakterilere karşı incelenmiştir. Ayrıca taze bitkinin mineral bileşimi atomik absorbsiyon spektroskopisi ile belirlenmiştir. *T. communis* ssp. *cretica*'nın sulu ekstresi referanslarla karşılaştırıldığında DPPH serbest radikal süpürücü aktiviteye (IC_{50}= 2.85±1.30 mg/mL), H_2O_2- ve HOCl-luminol kemiluminesens üzerine inhibitör etkiye (-log IC_{50}=3.8±0.09 ve IC_{50}=1.3x10^{-3}±4.9x10^{-4} mg/mL) ve lipid peroksidasyona karşı inhibitör etkiye (IC_{50}=3.82±1.67 µg/mL) sahip bulunmuştur. Sulu ekstrenin total fenolik içeriği 56.66±0.21 mg gallik asit/g ekstre olarak belirlenmiştir. *n*-Hekzan, kloroform, etil asetat ve etanol ekstreleri seçilen mikroorganizmalara karşı 250-500 µg/mL MIC değeri ile orta derecede aktiviteye sahip bulunmuştur. Mineral bileşimi sonuçlarına göre, taze bitki düşük miktarda toksik elementler (Pb, Cg ve Al), makro (Ca ve Mg) ve mikroelementleri (Cu, Fe, Mn ve Zn) içermektedir.

Anahtar kelimeler: *Tamus communis* ssp. *cretica*, Dioscoreaceae, Antioksidan aktivite, Antimikrobiyal aktivite, Mineral analizi

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INTRODUCTION

Tamus communis L. ssp. *cretica* (L.) Kit Tan (Dioscoreaceae), commonly known as black bryony, is a perennial herbaceous climbing plant with large fleshy tubers. It is distributed all over the tropical and warm temperate regions of the world, and is found under cliffs, rocky lime stone slopes, and steep grassy slopes. *T. communis* are represented two subspecies in Turkey, namely ssp. *communis* and subsp. *cretica* (1). Both the rhizomes and the berries of the plant have been traditionally used as effective rubefacient and for the treatment of rheumatism, artrosis, lumbago and dermatosis (2). In Turkish folk medicine, *T. communis* ssp. *cretica*, known as "kedi kuyruğu", is externally used for the treatment of rheumatism (3). *T. communis* is also called as "sarmaşık, tilkişen, dövülmüş avrat otu, gavur tilkişeni and kara asma" in the West of Turkey and the fleshy aerial parts of the plant are consumed as food (4,5).

Previous phytochemical studies on *T. communis* have resulted in the isolation of a series of phenanthrene derivatives (6-11), spirostane and furostane glycosides (12-14), flavonoids (15) sterols (16), and glucans (17). It was reported that the phenanthrene derivative compounds possessed cytotoxic and antiviral activities (9,18) and flavonoids showed significant antioxidant activity (15). In a previous report, the ethanol extract from *T. communis* roots was shown to have significant anti-inflammatory and analgesic activities (19,20). However, in other previous study, neither aqueous nor ethanol extracts prepared from both roots and aerial parts of *T. communis* was reported to have any remarkable anti-inflammatory or antinociceptive activities (21).

The aim of the present work was to examine antioxidant and antimicrobial properties of *T. communis* ssp. *cretica*. Antioxidant activity of the aqueous extract from the aerial parts of the plant was investigated in different *in vitro* antioxidant models such as 1,1-diphenyl-2-picrylhydrazyl (DPPH) scavenging, HOCl- or H_2O_2-induced luminol chemiluminesence (CL) and thiobarbituric acid (TBA) assays. Furthermore, the total phenolic content in the aqueous extract was determined. The antimicrobial activity of the *n*-hexane, chloroform, ethyl acetate and ethanol extracts from the aerial parts of the plant was tested against *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Enterobacter aerogenes, Proteus vulgaris, Salmonella typhimurium* and *Candida albicans* using the microdilution broth susceptibility assay. In addition, mineral composition of the raw plant was examined by atomic absorption method.

MATERIAL AND METHODS

Chemicals

All chemicals were of analytical reagent grade and obtained from the following sources: Luminol (5-amino-2,3-dihydro-1,4-phthalazinedione), ascorbic acid, sodium hydroxide, hexadecyltrimethylammonium bromide (HTAB), cobalt (II) chloride hexahydrate, 1,1-diphenyl-2-picrylhydrazyl (DPPH), Folin-Ciocalteu reagent, Bovine brain extract, Na_2CO_3, $FeCl_3$, butylated hydroxytoluene (BHT), quercetin, and propyl gallate (Sigma-Aldrich Chemical Co., St. Louis, MO, USA); hydrogen peroxide, sodium chloride, potassium dihydrogen phosphade, and methanol (Merck Co. Darmstadt, Germany); sodium hypochloride (Sigma-Aldrich). All other reagents were of analytical grade.

A luminol solution, hydrogen peroxide and HOCl solutions were freshly prepared before the experiment. A 10^{-3} M luminol stock solution was prepared by dissolving 17.7 mg of luminol in NaOH and, phosphate-buffered saline (PBS: 10 mM KH_2PO_4 and 150 mM NaCl, pH 7.4) was added up to 100.0 mL. PBS was used to control the acidity of the interacting system. HTAB (as a surfactant, final concentration was 10^{-5} M) was added into the working solution of 10^{-4} M luminol before adding PBS for maintaining luminol in basic environment (and 10^{-5} M in Co^{2+} when the oxidant in use was H_2O_2). It was stored at 4 ^{o}C and luminol solution was protected from light by a foil wrapper.

Hydrogen peroxide solutions were prepared daily by serial dilution of 100-volume hydrogen peroxide and protected from light by a foil wrapper. HOCl was prepared as

described previously by Vissers et al. (1994). NaOCl was diluted with PBS and the pH of the solution readjusted to 7.4. At this pH, the solution contains approximately 1:1 HOCl and NaOCl.

Spectrophotometric measurements

Spectrophotometric measurements were performed by a Perkin Elmer Lambda 40 (Boston, MA, USA) UV/VIS spectrophotometer.

Plant material

Tamus communis L. ssp. *cretica* (L.) Kit Tan (Dioscoriaceae) was collected from the vicinity of Avcılar, Aydin, Turkey in April 2006.

The plant was identified by Prof. Dr. Mecit Vural from the Department of Biology, Faculty of Science, Gazi University, Ankara, Turkey. A voucher specimen (GUE 2303) was kept in the Department of Pharmacognosy, Faculty of Pharmacy, Gazi University, Ankara, Turkey.

Preparation of plant extracts

The aerial parts of *T. communis* ssp. *cretica* were cutted into small pieces and air-dried until dryness at room temperature, and then powdered to a fine grade by using a laboratory scale mill.

Plant material (10 g) was extracted with distilled H_2O at room temperature for two times (x 50 mL). The combined aqueous extract was lyophilized to give the crude dry extract (4.44 %, w/w) and this extract was used for the determination of antioxidant activity.

For testing the antimicrobial activity, the aerial parts of plant (10 g) were sequentially extracted with *n*-hexane, chloroform, ethyl acetate and ethanol at room temperature for two times (x 50 mL). The extracts were filtered and evaporated to dryness in vacuo at 40 °C. The crude *n*-hexane, $CHCl_3$, EtOAc and EtOH extracts were obtained and the yields (w/w) are given respectively for the extracts as follows: 12.4, 9.6, 5.4 and 22.8 %. All the extracts were stored at -20 °C until used. Before analysis, an aliquot of each extract was dissolved and used in all experiments.

Determination of total phenolic compounds

Total phenolic content of *T. communis* ssp. *cretica* aqueous extract was determined with Folin-Ciocalteu reagent, according to the method of Singleton et al. (22), using gallic acid as a standard. An amount of 50 µL sample was added to 250 µL of undiluted Folin-Ciocalteu reagent. After 1 min, 750 µL of 20 % (w/v) aqueous Na_2CO_3 was added, and the volume was made up to 5.0 mL with H_2O. After 2 h incubation at 25 ºC, the absorbance was measured at 760 nm and compared to a gallic acid calibration curve. Total phenolics were determined as gallic acid equivalents (mg gallic acid/g extract), and the values were presented as means of triplicate analyses (mean±SEM).

Antioxidant activity

DPPH free radical-scavenging assay

The ability of the aqueous extract to scavenge DPPH free radicals was estimated to the method of Brand-Williams et al. (23). An amount of 0.75 mL of methanolic solution of the extract at different concentrations was mixed with 1.5 mL of a DPPH methanolic solution (20 mg/L). The controls contained all the reaction reagents except the extract or positive control substance. After 20 min incubation in darkness and at ambient temperature, the absorbance was recorded at 517 nm.

The percent of DPPH decolorization of the sample was calculated according to the equation % Decolorization = $[1 - (ABS_{sample}/ABS_{control})] \times 100$. BHT, quercetin and ascorbic acid were used as positive controls. The decolorization was plotted against the sample extract concentration, and a linear regression curve was established in order to calculate the IC_{50} (mg/mL) which is the amount of sample necessary to decrease by 50 % the absorbance of DPPH. All the analyses were carried out in triplicate and results were expressed mean±SEM.

HOCl- or H_2O_2-induced luminol chemiluminescence (CL) assay

The inhibitory effect of the aqueous extract of *T. communis* ssp. *cretica* on the peak chemiluminescence of hydrogen peroxide

(H_2O_2) and hypochlorous (HOCl) (derived from NaOCl) was evaluated by using flow injection analysis (FIA)-luminol chemiluminescence (CL) method (24). A peristaltic pump was a Gilson Minipuls 3 and the injection valve was a Rheodyne RH-5020, obtained from Anachem (Luton, Bedfordshire, UK). The pump tubing has a suitable internal diameter, to deliver the required flow-rate. The remainder of the flow-injection manifold was constructed from PTFE tubing joined with low-pressure fittings from Anachem (UK). CL detection was carried out using a luminometer (Model:Lumi-Flo, Chrono-log, USA). Results were recorded on a chart recorder, (Model 706-707, Chrono-log, USA).

The oxidant stream was merged with a luminol/buffer reagent immediately before the luminometer. The total flow rate was 1 mL/min, shared equally between the luminol and the oxidant channel; the oxidant channel includes an injection valve in the middle, which allows making successive nominally 20 μL injections of the extracts. Mixed flow of oxidant/antioxidant matches with luminol/buffer before the entrance to the flow cell.

The CL is measured as the photomultiplier output in mV; the effects of antioxidants were measured by the depression of the signal from its uninhibited level and were expressed as a percentage attenuation of the maximum CL due to the antioxidant. The sensitivity of extract was expressed as the inhibitor concentrations that elicited 50 % of the maximal responses (IC_{50}, mg/mL). IC_{50} values (mg/mL) of inhibitor effects of extracts and ascorbic acid were expressed as negative log M, using the Prism 3 Graph Pad program.

Thiobarbituric acid (TBA) assay

The lipid peroxidation activity was measured by the use of thiobarbituric acid (TBA) test described by Güvenç et al. (25). Different concentrations of the lyophilized aqueous extract of *T. communis* ssp. *cretica* (0.016-1 mg/mL) and propyl gallate (0.000064-1 mg/mL) as reference were tested for their lipid peroxidation activity against liposomes prepared from bovine brain extract in phosphate-buffered saline. Peroxidation was started by adding 0.1 mL $FeCl_3$ (1mM) and 0.1 mL ascorbic acid (1 mM), followed by incubation at 37 ^oC for 20 min. The absorbance was measured at 532 nm by a Shimadzu UV-1800 spectrophotometer. The inhibition of lipid peroxidation was calculated as follows:

$$\% \text{ inhibition} = 100 \times \frac{(\text{FRM-B}) - (\text{ET-B-EA})}{(\text{FRM-B})}$$

where FRM is the absorbance of control reaction, B is the absorbance of the blank mixture (liposomes only), ET is absorbance of the sample, EA is the absorbance due to the extract alone.

The IC_{50} value of the extract was calculated by linear regression analysis. Four replicate experiments were performed for each extract and results were given as mean values ± SEM.

Antimicrobial assay

Microorganisms were stored at + 4 ºC on agar slants. Standard strains of the following bacteria, namely *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 6538), *Pseudomonas aeruginosa* (ATCC 27853), *Enterobacter aerogenes* (NRRL 3567), *Proteus vulgaris* (NRRL B-123) and *Salmonella typhimurium* (NRRL B-4420) for the determination of antibacterial activity, and standard strains of *Candida albicans* (Clinical Isolate, Osmangazi University, Faculty of Medicine, Eskisehir, Turkey) for the determination of antifungal activity were used.

The antimicrobial activity of the *n*-hexane, chloroform, ethyl acetate and ethanol extracts obtained from *T. communis* ssp. *cretica* was tested by microdilution broth susceptibility assay (26,27). Stock solution was prepared in DMSO. Dilution series using sterile distilled water were prepared from 4 mg/mL to 0.007 mg/mL in micro-test tubes (Eppendorf), which were transferred to 96-well microtiter plates. Overnight grown bacterial and *C. albicans* suspensions in Mueller-Hinton broth were standardized to (for bacteria and *C. albicans* app. 10^8 and 10^6 cfu/mL respectively) using McFarland No: 0.5 standard solutions. Each microorganism suspension was then added into the wells. The last well column with medium and microorganism served as a positive growth control. After incubation at 37 ºC for 18-24 h,

the first well without turbidity was determined as the minimal inhibitory concentration (MIC). Chloramphenicol was used as standard antibacterial agent whereas ketoconazole was used as antifungal.

Mineral analysis

Plant material was digested in Microwave acid digestion system. An amount of 0.5 g raw plant material was subjected to acid digestion with HNO_3 65 % and H_2O_2 30 % mixture in microwave digestion unit and analyzed using a Varian 30/40 model atomic absorption spectrophotometer.

Pb, Cd, and Mn levels were determined by electrothermal atomization techniques (Graphite Furnace System) (Varian, GTA-96). Cu, Fe, Al, Ca, Mg, and Zn levels were determined by flame atomic absorption system (Varian, PSC-56).

Atomic absorption parameters were given in Tables 1 and 2.

RESULTS AND DISCUSSION

Antioxidant activity

In the present study, three different assays, namely, DPPH scavenging, HOCl- or H_2O_2-luminol chemiluminescence by flow injection analysis and thiobarbituric acid assays, were used to evaluate the antioxidant activity of the aqueous extracts of *T. communis* ssp. *cretica* growing in Turkey. DPPH and CL were often used to evaluate the free radical scavenging activity of pure compounds and extracts obtained from medicinal plants (28-30). Flow injection analysis is a rapid and quantitative method which can be used coupled with CL. TBA method was extensively used to determine the inhibition of lipid peroxidation. TBA reaction has been used for the detection of oxidative deterioration in lipids (25,31).

Table 1. Graphite furnace atomic absorption system

Elements	Wavelength (nm)	Ash temperature (ºC)	Atomisation temperature (ºC)
Pb	217.0	400	2000
Cd	228.8	300	1800
Mn	279.5	800	2400

Table 2. Flame atomic absorption system

Elements	Wavelength (nm)	Gas mixture
Cu	327.4	Air-acetylene
Fe	372.0	Air-acetylene
Ca	422.7	Nitrous oxide-acetylene
Mg	285.2	Air-acetylene
Zn	213.9	Air-acetylene
Al	396.1	Air-acetylene

In this study, DPPH scavenger capacity of the aqueous extract of *T. communis* ssp. *cretica* was compared with known antioxidant substances such as BHT, quercetin and ascorbic acid. The DPPH radical-scavenging activities of the reference substances and the extract are shown in Table 3. The aqueous extract of the plant was capable of scavenging DPPH radicals in a concentration-dependent manner. IC_{50} value was estimated as 2.85±0.30 mg/mL for the aqueous extract of the plant. It was evident that the extracts did show the hydrogen donating ability to act as antioxidants. The effectiveness of antioxidants as DPPH radical scavenger was ranged in the following descending order: quercetin (0.059±0.01 mg/mL) > ascorbic acid (0.09±0.01 mg/mL) > BHT (0.51±0.01 mg/mL) > aqueous extract of *T. communis* ssp. *cretica* (2.85±1.30 mg/mL).

In our FIA-CL study, a continuous CL signal from H_2O_2 (10^{-2} M) (in the presence of 10^{-4} M luminol and 10^{-5} M Co^{2+} in PBS at pH 7.4) was obtained. The H_2O_2-dependent CL signal was inhibited by the aqueous extract of the plant (10^{-2}-10 M) (n=6). Ascorbic acid (chain-breaking reference antioxidant) (10^{-8}-10^{-3}M) (n=6) also inhibited the CL signal in a concentration-dependent manner. The -log IC_{50} values were 3.82±0.09 and $1.4x10^{-4}$±$2.9x10^{-5}$ mg/mL for *T. communis* ssp. *cretica* and ascorbic acid, respectively. The continuous CL signal obtained from NaOCl (10^{-4}M), (in the presence of 10^{-4} M luminol in PBS at pH 7.4) was obtained. The HOCl-dependent CL signal was inhibited by the aqueous extract of the plant (10^{-6}-10^{-1} M) (n=6). Ascorbic acid (10^{-4}-10^{-1} M) (n=6) also inhibited the CL signal in a concentration-dependent manner. The -log IC_{50} values were $1.3x10^{-3}$±$4.9x10^{-4}$ and $1.8x10^{-5}$±$2.0x10^{-6}$ mg/mL for *T. communis* ssp. *cretica* and ascorbic acid, respectively (Table 3).

By using FIA-CL method, we have demonstrated that the aqueous extract of *T. communis* ssp. *cretica* has moderate inhibitory effects on the peak chemiluminescence signal produced by luminol-H_2O_2 or luminol-HOCl systems. According to the results in FIA-CL assay, the potency order was found to be ascorbic acid > the aqueous extract for H_2O_2-dependent CL and ascorbic acid > the aqueous extract for HOCl-dependent CL.

TBA test has been extensively used for the measurement of lipid oxidation in the foods, plant extracts, and pure compounds. The TBA reaction is based on the fact that peroxidation of most membrane systems leads to formation of small amounts of free malonaldehyde (MDA). One molecule of MDA reacts with two molecules of TBA to yield a colored product, which in an acid environment absorbs light at 532 nm, and it is readily extractable by organic solvents. Thus, it can be measured spectrophotometrically, and the intensity of color is a measure of MDA concentration. The incorporation of any antioxidant compound in the mixture will lead to a reduction of the extent of peroxidation and hence a reduction in color formation and absorbance (25,32). In the TBA assay, IC_{50} value was calculated as 3.82±1.67 μg/mL for the aqueous extract of *T. communis ssp. cretica*. Propyl gallate was used as reference and the IC_{50} value was determined as 0.21±0.01 μg/mL (Table 3).

Table 3. Antioxidant activities of aqueous extract of *T. communis* ssp. *cretica*

Extract/References	**DPPH [a]**	**HOCl-luminol CL [b]**	**H_2O_2-luminol CL [b]**	**TBA [c]**
Aqueous extract	2.85±1.30	$1.3x10^{-3}$±$4.9x10^{-4}$	3.82±0.09	3.82±1.67
BHT	0.51±0.01	-	-	-
Quercetin	0.059±0.01	-	-	-
Ascorbic acid	0.09±0.01	$1.8x10^{-5}$±$2.0x10^{-6}$	$1.4x10^{-4}$±$2.9x10^{-5}$	-
Propyl gallate	-	-	-	0.21±0.01

Data are presented as mean values ± standard error of mean (±SEM), BHT, quercetin, propyl gallate and ascorbic acid were used as positive control, [a]values expressed as IC_{50} (mg/mL), [b]values expressed as -log IC_{50} (mg/mL), [c]values expressed as IC_{50} (μg/mL)

In addition, the content of total phenolic compounds in the aqueous extract of *T. communis* ssp. *cretica* were determined from regression equation of calibration curve and expressed in gallic acid equivalents (GAE) using by Folin-Ciocalteu method. It was observed that aerial parts of the plant contained 56.66±0.21 mg/g total phenolics determined as mg gallic acid/g extract. It was reported that the phenolic compounds have been shown to be responsible for the antioxidant potency of plant materials. It was reported that some biological activities of polyphenolic compounds in plants, like flavonoids, may be attributed to their antioxidant potency (33).

In a previous study, methanol, ethyl acetate and chloroform extracts of selective Crotaian plants, including *T. communis*, were tested for their acetylcholinesterase inhibition and antioxidant activity. Antioxidant activities were determined by DPPH radical scavenging test and ferric reducing/antioxidant power assay (FRAP). In addition, total phenolic content of extracts was determined using Folin-Ciocalteu colorimetric method. As a result, ethyl acetate extract of *T. communis* showed the best antioxidant activity in FRAP method with 1362 µmol/L. Total phenolic content of the ethyl acetate, methanol and chloroform extracts of *T. communis* was found to be 5.99, 87.43 and 17.20, respectively (34). Boumerfeg et al. (35) have investigated the potential of *T. communis* root extracts to inhibit xanthine oxidoreductase (XOR) and to act as an antioxidant and free radical scavenging material. In their study, it was observed that *T. communis* is an efficient inhibitor of xanthine oxidase and has a significant antioxidant and free radical scavenging properties, which could be attributed to phenolic compounds.

In other previous study, phenolic profiles of traditional edible wild greens from some Mediterranean areas were determined by using HPLC-DAD-ESI/MS. Black bryony was the wild green that possessed the highest content of phenolic compounds (2200 mg/kg) (36).

Antimicrobial activity

The antimicrobial activity of *n*-hexane, chloroform, ethyl acetate and ethanol extracts of the aerial parts of *T. communis* ssp. *cretica* were tested against one species of Gram-positive bacterium, five species of Gram-negative bacteria, and one species of fungus. As summarized in Table 4, the microorganisms were also inhibited with moderate activity having MIC values of 250-500 µg/mL, lower than those of the antimicrobial standard agents. However, none of the extracts showed any significant antimicrobial activity against microorganisms.

Table 4. Antibacterial and antifungal activities of *Tamus communis* ssp. *cretica* extracts

Microorganisms	**Source**	**MIC (µg/mL)**				
Bacteria		EtOH	$CHCl_3$	EtOAc	*n*-Hexane	Std
Escherichia coli	NRRL B-3008	-	-	250	250	31.25*
Staphylococcus aureus	ATCC 6538	-	500	-	-	3.90*
Pseudomonas aeruginosa	ATCC 27853	-	500	-	-	62.50*
Enterobacter aerogenes	NRLL B-3567	-	-	-	250	62.50*
Proteus vulgaris	NRLL B-123	500	250	500	-	15.60*
Salmonella typhimurium	NRRL B-4420	-	-	500	-	31.25*
Fungi						
Candida albicans	Clinically isolated	-	500	-	500	62.50**

Tests were done in triplicate. Test concentration of extracts 2 mg/mL in DMSO.
MIC, minimum inhibitory concentration; - : no inhibition; Std: *Chloramphenicol ; **Ketoconazole

Mineral analysis

A lot of medicinal plants and foods could have toxic effects, particularly if they contain trace elements such as lead (Pb), cadmium (Cd) and arsenic (As) even at extremely low concentrations (37,38). For this reasons, we have determined both macro and microelements in the aerial parts of *T. communis* ssp. *cretica* which is consumed as food in Turkey.

According to the results, the raw plant has low amounts of toxic elements (Pb, Cd and Al), macro (Ca and Mg) and microelements (Cu, Fe, Mn and Zn) (Table 5).

Table 5. Content of elements in raw plant materials of *Tamus communis* ssp. *cretica*

Element	**Content of elements (µg/g)**	**Recommended/permissible quantity (mg/day)** (37,39)
Cu	36	1.5-3
Fe	57	10-15
Mn	19	2.0-5.0
Ca	2000	1.0-1.2
Mg	2000	310-320
Zn	34	12-15
Pb	0.007	10
Cd	0.79	0.3
Al	99	-

In the present study, it was observed that the extracts of *T. communis* ssp. *cretica* exhibited antioxidant and antimicrobial activities. This study also firstly identified the direct antioxidant potential of *T. communis* ssp. *cretica* against a spectrum of oxidants (H_2O_2 or HOCl) by using FIA coupled to luminol chemiluminescence. The experimental results pointed out that the antioxidant activities of the aqueous extract of the plant primarily due to the phenolic components such as flavonoids reported (15) in the plant. As a conclusion, this study reveals that *T. communis* ssp. *cretica* is a good candidate for a rich source of natural antioxidant compounds and further studies based on the present results will help to develop the new drugs for antioxidant therapy.

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REFERENCES

1. Kit Tan, *Tamus* L. In: Flora of Turkey and the East Aegean Islands, Vol 8, PH Davis, ed, University Press, Edinburgh, pp 552-554, 1984.
2. Grieve M, A Modern Herbal (Leyel, CF, ed), Tiger Books International, London, 1994.
3. Yeşilada E, Honda G, Sezik E, Tabata M, Fujita T, Tanaka T, Takeda Y, Takaishi Y, Traditional medicine in Turkey. V. Folk medicine in the inner Taurus Mountains, J Ethnopharmacol 46, 133-152, 1995.
4. Baytop T, A Dictionary of Vernacular Names of Wild Plants of Turkey, Publication of the Turkish Language Society, No 578, Ankara, pp 238, 1994.
5. Dogan Y, Traditionally used wild edible greens in the Aegean region of Turkey, Acta Societatis Botanicorum Poloniae, 81(4), 329-342, 2012.
6. Reisch J, Bathory M, Szendrei K, Minker E, Novak, I, Stickstoff-freie phenanthren-derivate aus dem rhizom von *Tamus communis* L., Tetrahedron Lett 2, 67-68, 1969.
7. Reisch J, Bathory M, Szendrei K, Novak I, Minker E, Weitere phenanthrene aus dem rhizom von *Tamus communis*, Phytochemistry 12, 228-229, 1973.
8. Aquino R, Behar I, De Simone F, Pizza C, Senatore F, Phenanthrene derivatives from

Tamus communis, Biochem Syst Ecol 13(3), 251-252, 1985.
9. Rethy B, Kovacs A, Zupko I, Forgo P, Vasas A, Falkay G, Hohmann J, Cytotoxic phenanthrenes from the rhizomes of *Tamus communis*, Planta Med 72(8), 767-770, 2006.
10. Kovacs A, Forgo P, Zupko I, Rethy B, Falkay G, Szabo P, Hohmann J, Phenanthrenes and a dihydrophenanthrene from *Tamus communis* and their cytotoxic activity, Phytochemistry 68(5), 687-691, 2007.
11. Kovacs A, Vasas A, Hohmann J, Natural phenanthrenes and their biological activity, Phytochemistry 69(5), 1084-1110, 2008.
12. Aquino R, Behar I, De Simone F, Pizza C, Steroidal glycosides of *Tamus communis*, Bollettino-Societa Italiana di Biologia Sperimentale 60(12), 2229-2235, 1984.
13. Aquino R, Behar I, De Simone F, D'Agostino M, Pizza C, Furostanol oligosides from *Tamus communis*, J Nat Prod 49(6), 1096-1101, 1986.
14. Aquino R, Conti C, De Simone F, Orsi N, Pizza C, Stein ML, Antiviral activity of constituents of *Tamus communis*, J Chemother 3(5), 305-309, 1991.
15. Shaheen F, Ali L, Ali S, Erdemoglu N, Şener B, Antioxidant Flavonoids from *Tamus communis* ssp. *cretica*, Chem Nat Comp 45(3), 346-349, 2009.
16. Capasso F, De Simone F, Senatore F, Sterol constituents of *Tamus communis* L., J Ethnopharmacol 8(3), 327-329, 1983.
17. Barbakadze VV, Kemertelidze EP, Dekanosidze HE, Usov AI, Isolation and characterization of glucans from roots of *Tamus communis* L. (Dioscoreaceae), Bioorganicheskaya Khimiya 19(7), 752-755, 1993.
18. Kovács A, Forgo P, Zupkó I, Réthy B, Falkay Gy, Szabó P, Hohmann J Phenanthrenes and a dihydrophenanthrene from *Tamus communis* and their cytotoxic activity, Phytochemistry 68, 687-691, 2007.
19. Capasso F, Mascolo N, Autore G, De Simone F, Senatore F, Anti-inflammatory and analgesic activity in alcoholic extract of *Tamus communis* L., J Ethnopharmacol 8(3), 321-325, 1983.
20. Mascolo N, Autore G, Capasso F, Local anti-inflammatory activity of *Tamus communis*, J Ethnopharmacol 19(1), 81-84, 1987.
21. Kupeli E, Orhan I, Yesilada, E, Evaluation of some plants used in Turkish folk medicine for their anti-inflammatory and antinociceptive activities, Pharm Biol 45(7), 547-555, 2007.
22. Singleton V, Orthofer R, Lamuela-Raventos R, Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent, Ed: Packer L, Oxidants and antioxidants, part A, methods in enzimology, vol 299, pp 152-178, Academic Press, New York 1999.
23. Brand-Williams W, Cuvelier ME, Berset C, Use of a free radical method to evaluate antioxidant activity, Lebensm-Wiss Technol 28, 25-30, 1995.
24. Sarıahmetoglu M, Wheatley RA, Cakici I, Kanzik I, Townshend A. Flow injection analysis for monitoring antioxidant effects on luminol chemiluminescence of reactive oxygen species, Anal Lett 36, 749-765, 2003.
25. Güvenc A, Houghton PJ, Duman H, Coskun M, Sahin P, Antioxidant activity studies on selected *Sideritis* species native to Turkey, Pharm Biol 43(2), 173-177, 2005.
26. Koneman EW, Allen SD, Janda WM, Schreckenberger PC, Winn WC, Color Atlas and Textbook of Diagnostic Microbiology, Lippincott-Raven Publ., Philadelphia, pp. 785, 1997.
27. Zgoda JR, Porter JR, A convenient microdilution method for screening natural products against bacteria and fungi, Pharm Biol 39, 221-225, 2001.
28. Choi SJ, Kang SH, Jung HA, Jung JH, Kang SS, A new cyclic phenyllactamide from *Salvia miltiorrhiza*, Fitoterapia 72, 30-34, 2001.
29. Küçükboyacı N, Güvenç A, Turan NN, Aydın A, Antioxidant activity and total phenolic content of aqueous extract from *Raphanus raphanistrum* L., Turk J Pharm Sci, 9(1), 93-100, 2012.
30. Fletcher P, Andrew KN, Calokerinos AC, Forbes S, Worsfold PJ, Analytical applications of flow injection with chemiluminescence detection-a review, Luminescence 16, 1-23, 2001.
31. Halliwell B, Chirica S, Lipid peroxidation: Its mechanism, measurement, and significance, Am J Clin Nutr 57, 715-725, 1993.
32. Conforti F, Statti GA, Tundis R, Menichini F, Houghton P, Antioxidant activity of methanolic extract of *Hypericum triquetrifolium* Turra aerial part, Fitoterapia 73(6), 479-483, 2002.
33. Rice-Evans C, Miller N, Paganga G, Structure-antioxidant activity relationships of flavonoids and phenolic acids, Free Radical Biology and Medicine 20, 933-956, 1996.
34. Jukic M, Burcul F, Carev I, Politeo O, Milos M, Screening for acetylcholinesterase inhibition and antioxidant activity of selected plants from Croatia, Nat Prod Res 26(18), 1703-1707, 2012.
35. Boumerfeg S, Baghiani A, Messaoudi D, Khennouf S, Arrar L, Antioxidant properties and xanthine oxidase inhibitory effects of

Tamus communis L. root extracts, Phytother Res 23(2), 283-288, 2009.
36. Barros L, Dueñas M, Ferreira ICFR, Maria Carvalho A, Santos-Buelga C, Use of HPLC-DAD-ESI/MS to profile phenolic compounds in edible wild greens from Portugal, Food Chem 127(1), 169-173, 2011.
37. Lozak A, Soltyk K, Ostapczuk P, Fijalek Z, Determination of selected trace elements in herbs and their infusions, Sci Total Environ 289(1-3), 33-40, 2002.
38. Zuliani T, Kralj Bl, Stibilj V, Milacic R, Minerals and trace elements in food commonly consumed in Slovenia, Italian J Food Sci 17(2), 155-166, 2005.
39. Hendler SS, Fleming T, Deutsch M, PDR for Nutritional Supplements, first edition, The information standart for nutritional supplements, Thomson PDR at Montvale, 2001.

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