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Investigation of antioxidant and antimicrobial activities of medicinal plants grown in the Eastern Blacksea region of Turkey

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Abstract

Objective: The aim of this study was to screen various extracts of plant of Gentian (*Gentiana pyrenaica* L.), Tarragon (*Artemisia dracunculus* L.), Persimmon (*Diospyros kaki*), Raspberry (*Rubus ideaus*) to display potent antimicrobial, antifungal and antioxidant activity in vitro, total phenolic and flavonoid contents in order to find possible sources for future novel antioxidants in food and pharmaceutical formulations.

Material and Methods: The antioxidant properties of 12 different samples of medicinal and aromatic plants such as leaves, flowers and scapus were investigated by DPPH, FRAP and CUPRAC assays. Total phenolic, total flavonoid content and the antimicrobial properties of extracts from these plants were also determined. Antibacterial and antifungal activities were investigated by microdilution method and agar diffusion method respectively.

Results: According to antioxidant results, dried leaves of Persimmon (*Diospyros kaki*) (obtained from Trabzon) plant had the best antioxidant activity that was carried out in all analyzes (except the analysis of total polyphenol). In accordance with analysis of total polyphenol, activity of purple flower of Gentian (*Gentiana pyrenaica* L.) plant was measured at 31,303±0,274 mg GAE /g dry sample and thus this plant had the highest total phenolic content. Antimicrobial activity tests were carried out by using disc diffusion methods with 12 microbial species and most of them displayed good-moderate antimicrobial activity.

Conclusion: Due to their antimicrobial, antifungal and antioxidant properties, the extracts some of these plants might be used as potential sources of natural antioxidant and antimicrobial agents.

Keywords: Gentian, Persimmon, Raspberry, Tarragon, Antioxidant, Antimicrobial

Introduction

Phenolic compounds are known as common plant secondary metabolites that have physiological functions in plants and positive effects for human health because they can act as antioxidants (1,2). Free radical scavengers/Antioxidants have vital effects on preventing chronic and degenerative illness such as cancer, autoimmune disorders, aging, cataract, rheumatoid arthritis, cardiovascular and neurodegenerative diseases and can enhance immune function. Antioxidant defenses protect the body from the harmful effects of free radicals produced by products of normal metabolism (3). Besides antioxidative properties, it was reported that phenolic compounds obtained from different plants had antimicrobial activities against different pathogenic microorganisms in literature (4–6).

Interest in medicinal plants as an alternative to synthetic drugs, especially against microbial agents owing to the development of antibiotic resistance, is increasing day by day (7). Thus, the need of finding new antimicrobial agents like phenolic compounds has become crucial. Medicinal plants are commonly used in daily life as a part of traditional remedies in Turkey. The flora in Turkey has a large variety and it is a good source for medicinal plants (8).

According to the investigations of WHO, approximately 9.000 species of 20.000 plants used for medicinal purposes in the world have been recorded from the flora of Turkey (9).

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Medicinal plants provide a wider source from which novel antibacterial and antifungal chemotherapeutic agents may be obtained (10). Plants can product certain bioactive molecules, such as phenols, phenolic acids, quinones, flavones, flavonoids, flavonols, tannins and coumarins (11). These constituents with phenolic structures can inhibit bacterial or fungal growth (12).

The genus Gentiana comprising 400 species, is mainly distributed in Southeast Asia, Europe and North America. Some Gentiana species have been employed as folk medicine since ancient times. The underground parts of various Gentiana species have been included in many herbal formulations as remedies for poor appetite, digestive problems and as hepatoprotective agents worldwide. As natural sources of food flavoring they are utilized in alcoholic and nonalcoholic beverages (13).

Artemisia dracunculus L. (Tarragon) is an important species in Artemisia genus and has approximately 800 species which are widely distributed throughout the world.Artemisia genus is industrially important due to its antifungal, insecticidal, allelopathic, antibacterial, and other characteristics. Furthermore, the plant is useful in Unani, Homeopathy, Ayurveda, and Siddha (14)

Diospyros kaki is the edible fruit of the persimmon tree which belongs to Ebenaceae family. The fruit is a seasonal fruit with important health benefits and consists of a berry, as large as an apple, orange in colour, with soft, sweet when it ripens. The persimmon tree (Diospyros kaki) is used in traditional medicine to treat apoplexy, arteriosclerosis, cough, and diarrhea. Many studies have addressed the antibacterial, antifeedant, antifungal, anti-dust miticidal, antimalarial, and cytotoxical activities of D. kaki root-derived materials (15).

Blackberries, raspberries (Rubus ideaus) and other small fruits are an excellent source of natural antioxidants, which is one of the major reasons for their increasing popularity in the human diet. Most of these fruits belong to the diverse Rubus genus, which consists of 250 species. Many Rubus fruits are consumed fresh or as processed products such as jams, jellies, syrups and wines. The leaves and roots have been used in various medicinal applications (16).

Medicinal plant species represent a large source of new compounds that help for the preparation of new drugs. The therapeutic activity of plants is due to their biologically active polyphenolic compounds. Thus, it is important to characterize different types of medicinal plants for their antioxidant and antimicrobial potential. The purpose of the present study was to investigate the antioxidant and antimicrobial properties of 12 different extracts of non-wood forest products, such as leaves, flowers, fruits, roots and scapus of some species of Gentian (Gentiana pyrenaica L.), Tarragon (Artemisia dracunculus L.), Persimmon (Diospyros kaki), Raspberry (Rubus ideaus) plant extracts used for medical purposes in the Eastern Anatolia Region of Turkey (Artvin, Trabzon and Bayburt).

Materials and methods

1. The Chemicals

Methanol, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid), 2,4,6-tripyridyl-s-triazine (TPTZ), Folin-Ciocalteu's phenol reagent and 2,2-diphenyl-1-picrylhydrazyl (DPPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA).

Sodium carbonate, acetic acid, neocuproine (2,9–dimethyl-1,10-phenanthroline), aluminium nitrate nonahydrate and ammonium acetate were purchased from Merck Chemical Co. (Darmstadt, Germany). The chemicals were analytical degree.

2. The Plant Material

Gentiana pyrenaica L. was collected in Murgul-Tiryal, *Artemisia dracunculus* L. was collected in Bayburt-Demirözü, *Diospyros kaki* and *Rubus ideaus* were collected from two different regions (Artvin-Hatila, Trabzon-Yeniköy). Collected plant materials were dried in the oven at 40°C before treatments. Approximately 10 g of dried sample of the fruits was used to prepare methanolic extracts for each species. These preparations were used to determine antioxidant activities, and the treatments were done three times. Spectrophotometric methods were used on total polyphenols, total flavonoids and antioxidant tests. Spectrophotometric methods are frequently used for the standardization of natural raw materials.

2.3. Total Phenolic Assay

The total phenolic content of plants has been determined by using the Folin-Ciocalteu assay (17). In this study, gallic acid (1; 0.5; 0.25; 0.125; 0.0625 and 0.03125 mg/ml) was used as a standard. Briefly, 20 µL of various concentrations of gallic acid and 20 µL methanolic samples (1 mg/ml), 400 µL of 0.5 N Folin-Ciocalteu reagents and 680 µL of distilled water were mixed and the mixture was vortexed. Following 3-minute incubation, 400 µL of Na_2CO_3 (10%) solution was added and after the process of vortexing, the mixture was incubated for 2 hours. After the incubation period at the room temperature, absorbances of the mixtures were measured at 760 nm. The concentrations of total phenolic compounds were calculated as mg of gallic acid equivalents per g of the dry weight of samples.

2.4. Total Flavonoid Assay

The total flavonoid content was measured by using the aluminum chloride assay (18). Quercetin was used as a standard. 0.5 ml of Quercetin (1; 0.5; 0.25; 0.125; 0.0625 and 0.03125 mg/ml), 4.3 ml methanol 0.1 ml 10% $Al(NO_3)_3$ and 0.1 ml 1 M NH_4CH_3COO were added in the test tubes and then they were mixed. Mixtures were incubated for 40 minutes. After incubation, absorbance was measured at 415 nm. The total flavonoid contents of plants were expressed as mg quercetin equivalents per g of dry weight sample.

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2.5. The Determination of Antioxidant Activity

The antioxidant activities of the samples were determined using by The ferric reducing ability of plasma (FRAP), cupric reducing antioxidant capacity (CUPRAC) and 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) assay methods. The FRAP method was used for the determination of total antioxidant capacity, based on the reduction of yellow Fe^{3+} -TPTZ complex to the blue Fe^{2+} -TPTZ complex by electron donating substance under acidic condition (19). The 3 ml of FRAP reagent (containing TPTZ, $FeCl_3$, and acetate buffer) and 100 µL of the test sample or the blank (solvents used for extraction) were added to the test tube and mixed. Maximum absorbance values at 593 nm were recorded for 4 min at 25°C. The final absorbance was compared with the standard curve (100-1000 µmol/L). The data were expressed as µmol $FeSO_4.7H_2O$ equivalents per gram of dry matter.

The CUPRAC method is comprised of mixing the antioxidant solution (directly or after acid hydrolysis) with a copper (II) chloride solution, a neocuproine alcoholic solution, and an ammonium acetate aqueous buffer at pH 7, and subsequently measuring the developed absorbance at 450 nm after 60 minutes (20). 1ml 10 mM $CuCl_2$, 1ml 7.5 mM Neocuproine and 1ml 1M NH_4Ac were added test tubes, than 0.2 ml sample and 0.9 ml H2O added and mixed. End volume was 4.1 ml. Then, the final absorbance was measured at 450 nm. The test results were evaluated by Trolox ® equivalent antioxidant capacity (TEAC).

The radical scavenging activity of methanolic extracts against 2,2-diphenyl-1-picrylhydrazyl ($DPPH^{\bullet}$) radical was measured at 517 nm in spectrophometer. The assay is based on the color change of the DPPH solution from purple to yellow as the radical is deactivated by the antioxidants (21). Briefly, various concentrations for 0.75 ml of each sample extracts were mixed with 0.75 ml of a 0.1 mM of DPPH in methanol.

The radical scavenging activity was measured by using Trolox as standards and the values are expressed as IC50 (mg or mg sample per ml), the concentration of the samples that causes 50% scavenging of $DPPH^{\bullet}$ radical.

6. The Biological Materials

The total of 12 bacteria strains has been used in this study (Table 1). All test microorganisms obtained from Karadeniz Technical University, Farabi Hospital, Trabzon, Turkey where the organisms were clinically isolated from patients.

The microorganisms were storaged at -80 °C in the Microbiology laboratory, Faculty of Science at the Karadeniz Technical University, Trabzon, Turkey where the antimicrobial tests were carried out. The strains were activated at 37°C for 24 h on muller hinton agar before use. The food-associated microorganisms were selected because they are frequently reported in food.

Table 1. The name and ATCC numbers of microorganisms used in the experiments

The Name	ATCC Numbers
Gram +	
Bacillus subtilis	ATCC 6633
Enterococcus faecalis	ATCC 29212
Staphylococcus aureus	ATCC 25923
Staphylococcus epidermidis	ATCC 12228
Gram -	
Escherichia coli	ATCC 25922
Klebsiella pneumonia	ATCC 13883
Pseudomonas aeruginosa	ATCC 27853
Proteus vulgaris	ATCC 13315
Salmonella typhimirium	ATCC 14028
Yersinia pseudotuberculosis	ATCC 911
Enterobacter cloaceae	ATCC 13047
Eukaryote	
Candida albicans	

7. The Antimicrobial Activity

7.1. Disc-diffusion assay

At first the antimicrobial activity of the extracts was determined by means of the disc diffusion method which is widely used for quick screening of natural products (22-24). All extracts were dissolved in solvent (methanol); the final concentration was 10 µg/disk. Cultures of each bacteria were inoculated to Muller-Hinton agar and incubated at 37° C for 16 hours, then their concentration adjusted to 0.5 McFarland standard turbidity (approximately 1 x 10^7 - 1 x 10^8 CFU/ml) with steril %0,09 isotonic solution. One hundred micro liter of each bacterial suspension was placed onto the surface of Mueller-Hinton agar in a 60-mm Petri dish and spread homogeneously with a Drigalski tip. The disc (6 mm in diameter) was embrued with extracts and placed on inoculated muller hinton agar. Negative controls were prepared using the same solvent (methanol) employed to obtain the extracts. Kanamycin were used as positive reference at 10 µg/disk (Sigma). The inoculated plates were incubated at 37 °C for 24 h for clinical bacterial strains and at 35 °C for 48 h for yeast. The inhibition zones were measured with a caliper considering the total diameters. Each experiment was performed in triplicate. The bacteria, inhibition zone in diameter ≥ 6 mm around the disks impregnated with methanol extract, were used for minimal inhibitory concentration (MIC).

7.2. Minimal inhibition concentration (MIC)

The MIC values were determined for the bacterial strains that were sensitive to the synthetic extract in the disk diffusion assay. The inoculum of the bacterial strains were prepared from 12 h agar cultures, and suspensions were adjusted to 0,5 McFarland standard turbidity. The extracts dissolved in methanol, were first diluted to the highest concentration (500 µg/ml) to be tested, and then serial 2-fold dilutions were made to obtain a concentration range from 500 µg/ml to 0,49 in 1 ml sterile test tubes containing Muller-Hinton broth.

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The MIC values of the synthetic extracts against bacterial strains were determined on the basis of a micro-well dilution method (22-24). Five hundred microliters from the stock solutions of synthetic extract prepared at the 5000 μg/ml concentration was added into the first sterile tube containing 4500 μl Muller-Hinton broth.

Then, 2500 μL from the serial dilutions was transferred into the eleven consecutive tubes. The last tube (twelve) containing 2500 µL of Muller-Hinton broth without compound.

The final volume in each tube was 2500 µL. Kanamycin at a concentration range of 500-0,49 µg/ml was prepared in Muller-Hinton broth and used as a standard drug for positive control and with the inoculum on each strip was used as a negative control. The 96-well plates were prepared by dispensing 200 µL of Muller-Hinton broth containing the diluted compound into each well, and 5 µL of 0,5 McFarland from 12 h agar cultures was added into each well.

The plate was covered with a sterile plate sealer. The contents of each well were incubated at 37°C temperatures for 24 h. The MIC was defined as the lowest concentration of extract to inhibit the growth of microorganisms. The extract tested in this study was screened twice against each organism.

Results

In general, phenolic acids and flavonoids are antioxidant molecules. High antioxidant value of these molecules indicates to high antioxidant properties of plants (25, 26). It is well-known that phenolic compounds contribute to quality and nutritional value in terms of modifying color, taste, aroma, and flavor and also in providing health benefical effects. They also serve in plant defense mechanisms to counteract reactive oxygen species (ROS) in order to survive and prevent molecular damage and destruction of microorganisms, insects, and herbivores (27).

The total phenolic and total flavonoid contents of plants, FRAP and CUPRAC values have been shown in Table 2.

Results showed that the highest phenolic content value obtained from G. pyr. fmt and G. pyr. rmt while D. kak. lah and D. kak. ltyshowed highest flavonoid contents. Between species the highest content of polyphenols and flavonoid were observed Gentian and Persimmon. In addition to these, D. kak. ltk and R. ide.lah showed maximum activity according to the FRAP whereas D. kak. ltk and G. pyr. rmt showed maximum activity according to the CUPRAC.

The IC50 values determined from analysis of DPPH were showed in Fig. 1.

Although D. kak. ltk and D. kak. lah had the highest DPPH radical cleaning, the lowest activity was obtained from A. dra. sbd.

Table 2. Results of phenolic contents, flavonoid contents, FRAP and CUPRAC for Gentian Tarragon, Persimmon and Raspberry species*

Samples*	Total phenolics (mg GAE/g)	Total flavonoid (mg QE/g)	FRAP (µmol Fe/g)	CUPRAC (mmol TEAC/g)
G. pyr. fmt	31.303 ±0.274	18.058±0.529	54.463±0.515	0.310 ±0.008
G. pyr. rmt	15.048 ±0.391	26.230±1.113	66.063 ±1.908	0.325 ±0.002
A. dra. lbd	2.681±0.120	10.975±0.270	18.844±1.165	0.178 ±0.012
A. dra. sbd	3.010 ±0.103	3.219±0.248	7.781±0.256	0.025 ±0.001
D. kak. fah	4.354±0.254	9.731±0.969	59.410±1.316	0.193 ± 0.051
D. kak. lah	10.989 ±1.257	84.236±2.461	89.108 ±2.609	0.251 ±0.031
D. kak. fty	2.008 ±0.045	0.457±0.053	30.064 ±0.653	0.229 ±0.050
D. kak. lty	11.182 ±1.874	64.512±4.153	115.526 ±3.932	0.559 ±0.063
R. ide. fah	6.047 ±0.615	10.975±0.270	62.289±0.466	0.193 ± 0.051
R. ide. lah	11.644±0.770	17.926±1.155	107.074±3.292	0.254 ±0.030
R. ide. fty	5.932±4.711	9.731±0.969	55.261 ±1.449	0.159 ±0.007
R. ide. lty	10.142 ±0.938	1.742±2.610	92.887±3.099	0.239±0.017

* G. pyr. fmt: Flowers of Gentiana pyrenaica L.(Murgul-Tiryal), G. pyr. rmt: roots of Gentiana pyrenaica L.(Murgul-Tiryal), A. dra. lbd: Leaves of Artemisia dracunculus L. (Bayburt-Demirozu), A. dra. sbd: Scapus of Artemisia dracunculus L. (Bayburt-Demirozu), D. kak. fah: Fruits of Diospyros kaki (Artvin-Hatila), D. kak. lah: Leaves of Diospyros kaki (Artvin-Hatila), D. kak. ft: Fruits of Diospyros kaki (Trabzon-Yenikoy), D. kak. ltk: Leaves of Diospyros kaki (Trabzon- Yenikoy), R. ide.fah: Fruits of Rubus ideaus (Artvin-Hatila), R. ide.lah: Leaves of Rubus ideaus (Artvin-Hatila), R. ide.fty Fruits of Rubus ideaus (Trabzon- Yenikoy), R. ide.lty Leaves of Rubus ideaus (Trabzon- Yenikoy).

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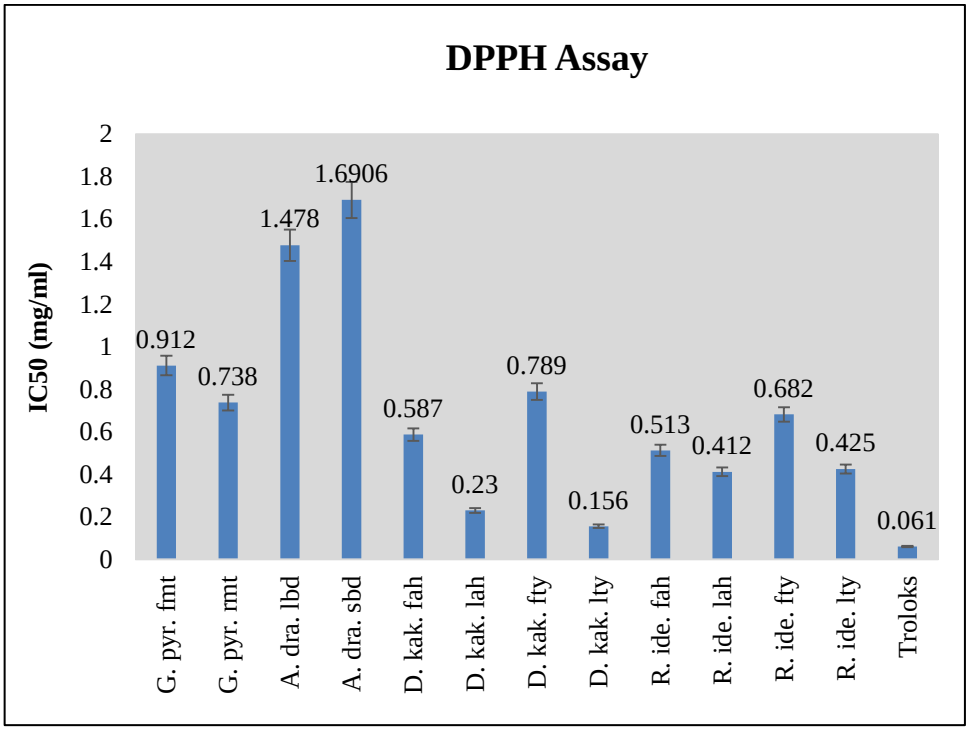

Figure 1: The radical scavenging activity of methanolic extracts against 2,2-diphenyl-1-picrylhydrazyl (DPPH●) radical was measured at 517 nm in spectrophometer. The results of DPPH for Gentian Tarragon, Persimmon and Raspberry species.

The antimicrobial activities of extracts assayed against the microorganisms in the present study were qualitatively and quantitatively assessed by evaluating the presence of inhibition zones, zone diameter, and MIC values. The results of antimicrobial activity of methanolic extracts are shown in Table 3.

Table 3. MIC values of compounds against the bacterial strains tested

Samples	Minimal Inhibition Concentration Values (µg/ml)											
	Bs	Ef	Sa	Se	Ec	Kp	Pa	Pv	St	Yp	Ec	Ca
G. pyr. fmt	0,392	-	-	50	-	-	-	-	-	-	-	0.392
G. pyr. rmt	0,196	-	-	50	-	-	-	-	-	-	-	0.392
A. dra. lbd	0,196	-	-	12.5	-	-	-	-	-	-	-	0.392
A. dra. sbd	0.196	-	-	-	-	-	-	-	-	-	--	0.392
D. kak. fah	0,392	-	25	50	-	-	-	50	-	-	-	1.562
D. kak. lah	-	-	-	-	-	-	-	-	-	-	-	-
D. kak. fty	0.196	-	-	100	-	-	-	100	-	-	-	0,196
D. kak. lty	0.196	-	-	25	-	-	-	-	-	-	-	0,196
R. ide. fah	0.392	-	50	100	50	-	-	-	-	-	-	0.780
R. ide. lah	0,780	-	50	100	100	-	-	-	-	-	-	0.780
R. ide. fty	0.196	-	1.25	50	50	-	-	-	-	-	-	0,196
R. ide. lty	0.392	-	25	100	100	-	-		-	-	-	0.392
Kanam.	0.196	6.25	0.782	0.392	1.562	0.392	-	0.196	1.562	0.782	1.562	-

Bs: *Bacillus subtilis* ATCC 6633, Ef: *Enterococcus faecalis* ATCC 29212, Sa: *Staphylococcus aureus* ATCC 25923, Se: *Staphylococcus epidermidis* ATCC 12228, Ec: *Escherichia coli* ATCC 25922, Kp: *Klebsiella pneumonia* ATCC 13883, Pa: *Pseudomonas aeruginosa* ATCC 27853, Pv: *Proteus vulgaris* ATCC 13315, St: *Salmonella typhimirium* ATCC 14028, Yp: *Yersinia pseudotuberculosis* ATCC 911, Ec: Enterobacter cloaceae ATCC 13047, Ca: Candida albicans ATCC 60193, Kanam.: Kanamycine, (—): no activity of test concentrations

Results obtained from disc diffusion method, followed by measurement of minimum inhibitory concentration (MIC), indicated that B. Subtilis and C. albicans were the most sensitive microorganisms showing lowest MIC values 0.196 µg/ml. Extracts of Gentiana pyrenaica L., Artemisia dracunculus L., Diospyros kaki, Rubus ideaus exhibited antimicrobial activity against the tested strains, but in variable degree. Results are comparable to the antibiotic kanamycine, used as a positive probe.

R. ide. fah, R. ide. lah, R. ide. fty, R. ide. lty and D. kak. fah showed antimicrobial activity against 5 out of 12 microorganisms, D. kak. fty, 4 out of 12 microorganisms, G. pyr. fmt, G. pyr. rmt, A. dra. lbd and D. kak. lty, 3 out of 12 and A. dra. sbd showed antimicrobial activity against 2 out of 12 microorganisms.

Gram positive bacteria were the most sensitive being inhibited by all the extracts except E. faecalis. Concerning Gram negative bacteria, extracts were able to inhibit the growth only E. Coli and P. vulgaris at the extract concentration tested (50-100 µg/ml). Also excellent antimicrobial activity results were observed on the test microorganism, yeast like fungy, Candida albicans (Ca) with the mic values between 0.196 -1.56 µg/ml is better than the standard drug of kanamycine except extract of D. kak. lah.

On the other hand, none of the extracts of plant exhibited the activity on the test microorganisms, E.faecalis, K. pneumonia, P. aeruginosa, S. typhimirium, Y.pseudotuberculosis, E. cloaceae. Extract of D. kak. lah did not shown any antimicrobial activity on the tested microorganisms.

Discussion

Medicinal plants are used in many diseases as reinforcement supplements. In this study, antioxidant and antimicrobial properties of some medicinal plants growing in the black sea region were investigated.

In a study extract on the root of Gentiana asclepiadea L. grown in Serbia, the total phenolic content of extract was 73.51 ± 1.51 mg GAE/g the total flavonoid content was 34.07 ± 0.19 mg QE/g and DPPH activity was 0.24 ± 0.018 mg/ml (28). According to our results extracts on the root of Gentiana pyrenaica L. (belongs to genus Gentiana that comprises about 400 species) grown in Murgul-Tiryal have less total phenolic content, total flavonoid content and DPPH activity.

In a study conducted in India, researches showed that the tested extracts of Gentiane kurroo roots and leaves possessed antibacterial activity against both Gram positive and Gram negative bacteria. The antibacterial activity of root extract was found to be comparatively higher than that of leaf extract. The MIC value of the root extract ranged from 0.15 ± 0.04 to 0.75 ± 0.05 mg/ml and that of leaf from 0.22 ± 0.08 to 0.90 ± 0.02 mg/ml (29). We found that the MIC value of the root extracts of Gentiana asclepiadea L. ranged from 0.196 to 50 µg/ml and that of flowers from 0.196 to 50 µg/ml.

Although several studies have been addressed on some Gentiana species (28, 29, 30) antioxidant and antimicrobial activities of Gentiana pyrenaica L. (roots, leave or flowers) have not been investigated.

In the literature, Artemisia essential oils exhibited weak antioxidant abilities with DDPH radical scavenging activities (31). In the present study, Artemisia dracunculus have showed low DDPH radical scavenging activities according to Trolox standard. While in a study conducted on Artemisia campestris the ability of the extracts to reduce Fe^{3+} was determined 110 ± 2.01 µg/ml according to FRAP assay (32), our result was 18.84 ± 1.16 µmol Fe/g (leave extracts of Artemisia dracunculus L.) and 7.78 ± 026 µmol Fe/g (scapus extracts of Artemisia dracunculus L.). Despite some studies on Artemisia species (31, 32, 33, 34) there has been no research about antioxidant properties of Artemisia dracunculus L. in detailed.

In an article on Artemisia dracunculus, it is reported that whereas Trichophyton rubrum showed the most susceptibility to Artemisia dracunculus extract with growth inhibition zone (20 ± 2.1 mm), Escherichia coli showed the least susceptibility with growth inhibition zone (8 ± 0.0 mm) (31). According to our result, while Artemisia dracunculus extract do not have inhibitory effect on E.coli, it inhibits the growth of S. epidermidis microorganism with MIC value 12.5 µg/ml.

The leaves of Diospyros kaki are commonly used for tea in Asia. Previous studies have shown that persimmon leaves have beneficial effects on the treatment of paralysis, frostbite, and burns, and to stop bleeding (35). It was reported that the extract of Diospyros kaki leaves contained 192 ± 9.6 mg/g total flavonoids and its DPPH radical scavenging activity was 96.36 ± 2.63 µg/ml (36). We found that total flavonoids of the extract of Diospyros kaki leaves is 84.2±2.5 mg/g and its DPPH radical scavenging activity is 230 µg/ml . In addition to that, D. The flavonoid contents of the other samples named as kak. fah, D. kak. fty and D. kak. lty are 9.731±0.969, 0.457±0.053 and 64.512±4.153 mg/g and their DPPH radical scavenging activities are 587, 789 and 156 µg/ml, respectively.

In an article on Diospyros kaki, the extract of persimmon peel did not exhihit potent anti-Helicobacter pylori activity (MIC>100 µg/ml) (37). In this research, it is found that various samples of Diospyros kaki exhibit inhibitory effect on Bacillus subtilis, Staphylococcus aureus, Proteus vulgaris with MIC values ranging from 0.196 to 100 µg/ml.

In the literature extract on the pomaces of Rubus idaeus, researches determined that the total phenolic content of extract was 26.3 ± 1.28 mg GAE/g the total flavonoid content was 25.2 ± 1.20 mg QE/g and DPPH activity was 3.86 ± 0.18 mg/ml (38). In our study extract on the fruits of Rubus idaeus grown in Artvin, the total phenolic content of extract was 6.05 ± 0.61 mg GAE/g the total flavonoid content was 10.98 ± 0.27 mg QE/g and DPPH activity was 0.51 mg/ml. In addition extract on the fruits of Rubus idaeus grown in Trabzon, the total phenolic content of extract was 5.93 ± 4.71 mg GAE/g the total flavonoid content was 9.73 ± 0.97 mg QE/g and DPPH activity was

0.68 mg/ml. There are several studies in the literature about antioxidant activity on fruits of Rubus idaeus species (38, 39, 40) however there are a few studies on the leaves of Rubus idaeus. Therefore, antioxidant activities of the leaves of Rubus idaeus was also investigated in this study.

In a research pomace extract of Rubus idaeus showed significantly higher activity towards reference E.coli and wild L.monocytogenes showing MIC values 0.29 mg/ml and 0.39 mg/ml, respectively (38). According to our study extract of Rubus idaeus both fruits and leaves exhibited higher activity towards effect on B. subtilis and C. albicans with MIC values ranging from 0.196 to 0.78 µg/ml.

The observed differences between our study and the study of Dragana et al. (38) are certainly caused by the activity is due to the composition and amount of active components and is dependent on genetic (i.e. genus, species, cultivar/genotype) and environmental factors, such as geographic areas, growth conditions of plant material, seasonal variations, climatic factors, ripening stage, harvesting time, storage condition and postharvest management (41, 42, 43). Solar radiation, temperature, virus status, and other biotic and abiotic stresses also affect phenolic content (43).

Based on these results, it is possible to conclude that methanolic extracts of Gentiana pyrenaica L., Artemisia dracunculus L., Diospyros kaki, Rubus ideaus had different levels of antioxidant and antimicrobial activity. The obtained results might be considered sufficient to further studies for the isolation and identification of the active principles and to evaluate of possible synergism among extract components for their antioxidant and antimicrobial activity. Investigations are in progress to determine the degree of toxicity of these extracts.

Conclusion

The findings of this study indicate that the plant extracts of Gentian, Tarragon, Persimmon and Raspberry contain compounds with antioxidant activity, antimicrobial and antifungal activity. The replacement of synthetic with natural antioxidants may be advantageous. Based on these results, it is possible to conclude that methanolic extracts of these plants can be the potent source of natural antioxidants.

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