In vitro antimicrobial and antioxidant activities and GC/MS analysis of the essential oils of *Rumex crispus* and *Rumex cristatus*

Rumex crispus ve *Rumex cristatus*'un uçucu yağlarının in vitro antimikrobiyal ve antioksidan aktiviteleri ile GC/MS analizi

Research Article

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ABSTRACT

In this study, was investigated in vitro antimicrobial and antioxidant activities and GC/MS analysis of the essential oil of Rumex crispus and Rumex cristatus collected from Corum province and around. Total antioxidant status of essential oils prepared with ethanol and pure water were measured spectrophotometrically. *Staphylococcus aureus* ATCC 25923, *Eschrechia coli* ATCC 25922, *Enterecoccus faecalis* ATCC 29212 and *Candida albicans* ATCC 10231 were used to the antimicrobial studies. The antimicrobial properties of samples were determinate with the disk diffusion and agar-well method. The total antioxidant status in the essential oils of all *Rumex cristatus* contained high levels was determinate according to *Rumex crispus*. The developing of test microorganisms of the essential oils of all *Rumex crispus* and *Rumex cristatus* extracts have been observed to inhibit varying degrees on the antimicrobial activity test. Furthermore, extracts obtained with ethanol is more effective according to chloroform extracts and more efficient results which have been identified. *Enterecoccus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 *Staphylococcus aureus* ATCC 25923 and fungal culture of *Candida albicans* ATCC 10231. The diphenic acid derivatives were found the content of two Rumex sp. essential oils with GC/MS analysis.

Key Words

Rumex crispus, Rumex cristatus, essential oils, antioxidant activity, antimicrobial activity, GC-MS

ÖZET

Bu çalışmada Çorum ili ve çevresinden toplanan *Rumex crispus* ve *Rumex cristatus*'un uçucu yağlarının antimikrobiyal ve antioksidan aktiviteleri ile GC/MS analizleri araştırıldı. Etanol ve saf su ile hazırlanan örneklerin uçucu yağlarının toplam antioksidan statüsü spektrofotometrik olarak ölçüldü. Antimikrobiyal çalışmalarda *Staphylococcus aureus* ATCC 25923, *Eschrechia coli* ATCC 25922, *Enterecoccus faecalis* ATCC 29212 ve *Candida albicans* ATCC 10231 türleri kullanıldı. Örneklerin antimikrobiyal özellikleri disk ve agar-kuyu yöntemi ile belirlendi. Tüm *Rumex cristatus* uçucu yağlarının total antioksidan miktarı, *Rumex crispus*'a göre daha yüksek bulundu. Antimikrobiyal aktivite testlerinde *Rumex crispus* ve *Rumex cristatus* uçucu yağlarının test mikroorganizmalarının gelişimini farklı derecelerde inhibe ettiği gözlendi. Etanol ile elde edilen uçucu yağların kloroforma göre daha etkin olduğu tanımlandı.

Anahtar Kelimeler

Rumex crispus, Rumex cristatus, uçucu yağ, antioksidan aktivite, antimikrobiyal aktivite, GC-MS

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INTRODUCTION

Rumex (family: Polygonaceae) includes many edible plants which attracted the attention of many investigators because of their medicinal importance for the treatment of several diseases [1]. This genus is contains roughly 200 species, is widespread throughout the world and 23 species and 5 hybrids in Turkey [2]. This genus comprises several species that leaves and roots have been used in traditional medicine for inflammation, blood purification, constipation, purgative and tonic in Turkish [3].

Essential oils from medicinal and aromatic plants have been known to possess biological activity, antimicrobial and antioxidant activities [4]. Recently, much attention has been devoted to the essential oil and antimicrobial activities of plants since they have been used to extend the self-life of foods and folk medicine. Studies suggested that the antimicrobial and antioxidant activities of plants are especially related to main components of their essential oils [4, 5].

Reactive Oxygen Species (ROS) are considered to be associated with many cell disorders through their action on proteins, lipids, and DNA. Hence, they play an important role in the development of many diseases such as cardiovascular diseases, inflammatory disorders, cancer, and the ageing process [6, 7]. Plants are a good source of natural antioxidants such as phenolic compounds that protect organisms by preventing and associated diseases caused by ROS [8]. And also, they are rich in a wide variety of secondary metabolites and their antioxidant, anti-inflammatory, antimicrobial or cytotoxic properties are used to developed drugs, dietary supplements [9]. In recent years, both synthetic and natural antioxidants are used. But, synthetic antioxidants such as butylated hydroxyanisole/hydroxytoluene are considered to be responsible for damage on tissues and some organs and carcinogenesis [10]. Thus, it is essential to develop natural nontoxic antioxidants to protect human body from free radicals. Furthermore, researchers have been interested in biologically active compounds isolated from plant species for the elimination of pathogenic microorganisms because of the resistance that microorganisms have built against antibiotics [11, 12]. Recently, in several studies many medicinal plants have been demonstrated to possess potent antioxidant and antimicrobial activities as natural [13-16].

Until recently, relation with the biological activities of *R. crispus* and *R. cristatus* essential oils have few studies been reported. Therefore, in the present study, the antioxidant activities of *R. crispus* and *R. cristatus* essential oils with different extracts were determined. Also, it was of interest to determine the antimicrobial activities of these essential oils by the disk diffusion and agar-well diffusion methods. Additionally, the biological active compounds of these essential oils were analyzed using GC–MS technique.

MATERIAL AND METHODS

Plant Materials

Rumex crispus L. and *Rumex cristatus* L. plants were collected from six different localities in Corum/Turkey in April and July 2010. The identification of plant samples was confirmed by plant taxonomist Prof. Dr. Murat EKİCİ of the Department of Biology, Gazi University, Ankara, Turkey. After plant samples had collected, dried at room temperature (RT) for a week.

Isolation of Essential Oils

Five grams of the dried and powdered every plant materials (leaf) were extracted with different solvents including ethanol, chloroform and water (ultra pure). Air-dried plant materials were subjected to hydrodistillation for 4 h using a Clevenger-type apparatus. They were dried over anhydrous sodium sulphate, filtered and stored at +4°C in darkness until further studies.

Gas Chromatography/Mass Spectrometry (GC/MS) Analysis

The chemical compositions of the essential oils were analyzed by using GC–MS technique and the fragmentation analysis was performed (Figure 1). The mass spectrometer was Thermo Scientific DSQ II Single Quadrupole GC/MS in the electron impact (EI) ionisation mode (70 eV) and HP- 5MS (bonded and cross-linked 5% phenyl-methylpolysiloxane, 30 mm x 0.25 mm, coating thickness 0.25 μm) capillary column (Restek, Bellefonte, PA). Injector

and detector temperatures were set at 220°C. The oven temperature was held at 50°C for 30 min, then programmed to 240°C at rate of 3°C/min. Helium (99.99%) was the carrier gas at a flow rate of 1 mL/min. The molecular weight of decomposition products scan 60-200 g/mol range were performed. Diluted samples (1/100 in chloroform, v/v) of 1.0 μl were injected manually. The identification of the components was based on the comparison of their mass spectra with those of Wiley 7 N (contains 392.086 compounds spectra), Nist 2002 (contains 174.948 compounds spectra) and flavor (contains 419 compounds spectra) libraries and as well as by comparison of their retention times.

Determination of Antimicrobial Activity

Organisms and growth conditions

Bacterial cultures of *Enterecoccus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922 *Staphylococcus aureus* ATCC 25923 and fungal culture of *Candida albicans* ATCC 10231 were obtained from the culture collection at Hitit University, Faculty of Science and Arts, Departments of Biology. Nutrient broth and agar (Diffco) for *E. coli* cultures; Tryptone-Yeast extract-Cystine (TYC) broth and agar for *E. fecalis* and *S. aureus* cultures; Eosin Methylene Blue (EMB, Merck) broth and agar for *P. aeroginosa* cultures; and Sabouraud Dextrose broth and agar (SD, Merck) for *C. albicans* cultures were used growth of microorganisms in this study. All strains stored at -20°C in the appropriate medium containing 10 % glycerol and regenerated twice before use in the manipulations.

The determination of the antimicrobial activities of essential oils on test microorganisms was carried out by the disc diffusion and agar-well diffusion methods. The essential oils obtained from three solvents including ethanol, chloroform and water were used in the antimicrobial activity works.

Disc diffusion test

The culture suspensions were adjusted by comparing against 0.5 McFarland standard and 100 μl culture suspension were spread on Muller Hington Agar (MHA) media plates. After, sterile 6-mm in diameter filter paper discs (Whatman, no. 4) impregnated with 15 μl of essential oils diluted in ethanol (10 %), chloroform (10 %) and water (ultra pure) solution were placed on the inoculated agar. The same volumes (15 μl) of solvents were used a control. The inoculated plates were incubated at 37°C for 24 h for bacterial strains and 48 h for the yeast isolate. After incubation period the inhibition zones around the discs were measured as millimeters [7]. Each test was performed three times and the average of the results was taken.

Agar-well diffusion test

Microorganisms were cultured at 37°C for 4 h and prepared to turbidity equivalent to 0.5 McFarland standard. Petri dishes containing 10 mL MHA were prepared and inoculated with 100 μl of culture suspension. Wells (6 mm) were made in the petri dishes that prepared with MHA. Then, essential oils in different solvents were added to the wells (25 μl). The same volumes (25 μl) of solvents were used a control. The inoculated plates were incubated at 37°C for 24 h for bacterial strains and 48 h for the yeast isolate [17]. After incubation period the inhibition zones around the well were measured as millimeters. Each test was performed three times and the average of the results was taken.

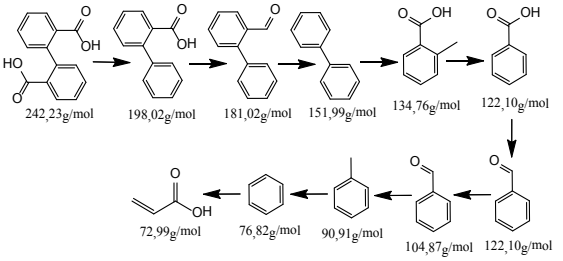
Determination of Total Antioxidant Status (TAS) of Essential Oils

The essential oils obtained from two solvents including ethanol and water were used in the total antioxidant status (TAS) works. The total antioxidant status (TAS) of the essential oil was determined using a novel automated colorimetric measurement method developed by Erel (2004) [18]. In this method, a hydroxyl radical was produced by the Fenton reaction and reacted with the colorless substrate o-dianisidine to produce the bright yellowish-brown dianisyl radical. The results were expressed as micromolar Trolox equivalents per liter (μmol Trolox Eq/L).

Statistical Analysis

The all experiments were done in triplicate. The results were expressed as average ± standard deviations (SD). Statistical analysis was performed on the data by SPSS 15.0 Bivariate Correlation Analysis (SPSS Inc., Chicago) with statistical significance determined at 0.05. The Pearson rank correlation test was used for

comparisons between the disc diffusion and agar-well diffusion methods used to determine the antimicrobial activity of essential oils. The inhibition zones of essential oils for two methods on test microorganisms not showed a significant correlation ($p > 0.05$). And also, the correlation is significant statistically at the 0.01 level between *Rumex cristatus* and *Rumex crispus* in TAS study.



Scheme 1. Mass spectral fragmentation pattern of diphenic acid.

RESULTS AND DISCUSSION

Gas Chromatography/Mass Spectrometry (GC/MS)

The extract cleavage products GC / MS spectrum was obtained according to the observed peaks (Figure 1). Structural formulas of the diagram are the appropriate degradation products are displayed on the peaks. The extract components were identified the aid of gas chromatography and decomposition products were characterized by mass analyzer detector GC-MS. The mass spectrum shows that the extract is diphenic acid and a schematic representation including the main fragmentation process for extract are given Scheme 1.

Determination of Antimicrobial Activity of Essential Oils

It is known that a lot of factors effect antibacterial activity. So, we think that the bacterial inhibition can vary with the essential oils, the solvents used for extraction, and the organisms tested. In this study, it was used three different soluble solutions as ethanol, chloroform and water (ultra pure) as different from previously studies are made by other researchers. Other researcher used mostly methanol as soluble solution for essential oil or extraction [19,20]. However, some researchers were used soluble solutions in our study [21-23]. The water from these solutions that used as soluble observed minimal effect. Antimicrobial activities of *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 25922, *Enterecoccus faecalis* ATCC 29212 and *Candida albicans* ATCC 10231 strains evaluated by both agar-well diffusion and disc diffusion methods. The antimicrobial activities (zone of growth inhibition) of the essential oils of *R. crispus and R. cristatus* with both disc diffusion and agar-well diffusion methods were given in Table 1 and Table 2. The results showed that especially the essential oils obtained from ethanol and chloroform of *R. crispus and R. cristatus* had antimicrobial activity against microorganisms. It was seen

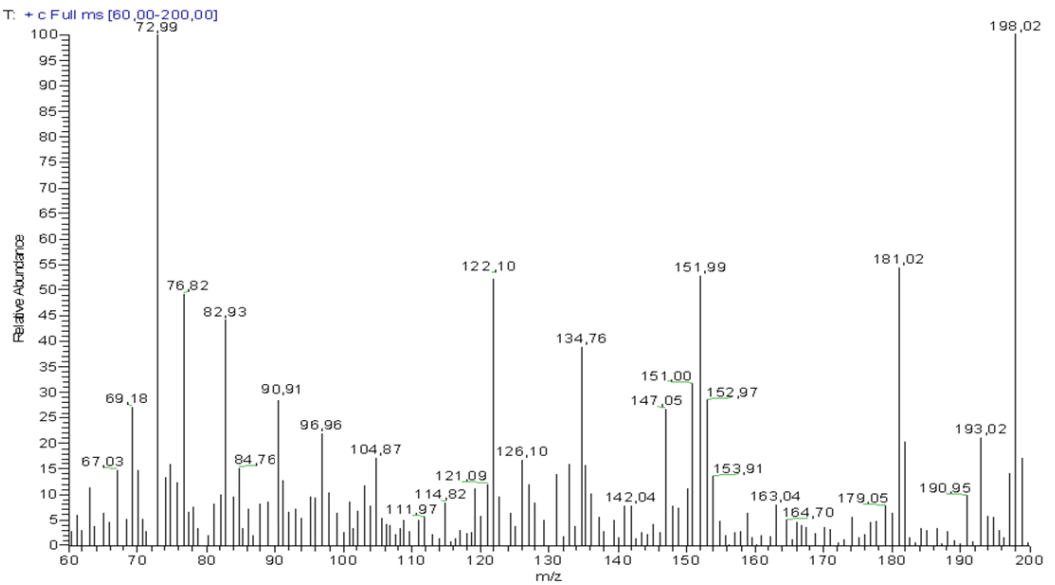


Figure 1. GC/MS spectra and fragmentation patterns of extract.

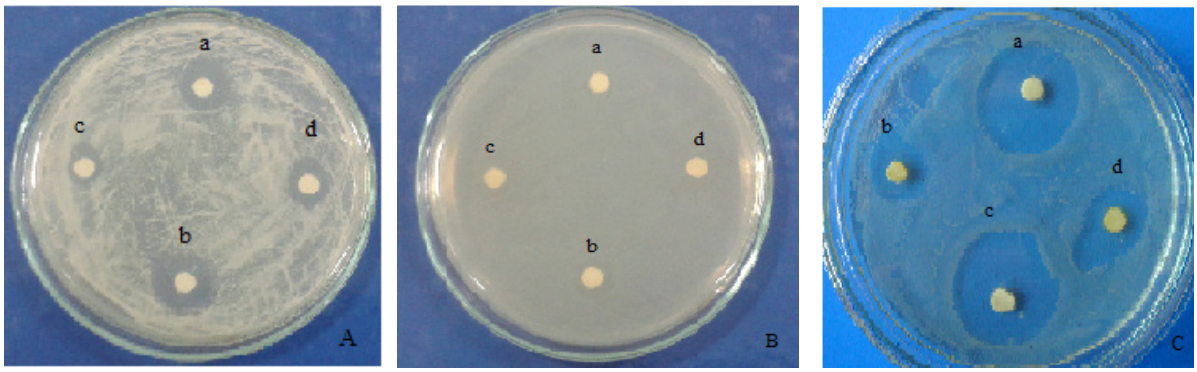


Figure 2. Examples from figures of the antimicrobial activity studies by disc diffusion method. A: effect of the essential oils of *Rumex crispus*-etanol on *C. albicans*; B: effect of the essential oils of *Rumex crispus*-water on *S. aureus*; C: effect of the essential oils of *Rumex crispus*-chloroform on *E. faecalis*.

that the inhibition zone of microorganisms by essential oils have changed between 14.5-32.7 mm. The agar-well diffusion results better than disc diffusion results because the amount of essential oils, which used to, is more. When compared with ethanol and chloroform solvents, chloroform is more effective. It was found that, *R. cristatus* was compared with *R. crispus,* essential oil made from *R. crispus*-ethanol was the most effective against *S. aureus* and *E. coli* (inhibition zones=17.2±1.8; 19.5±4.5, respectively) by agar-well diffusion method. Additionally, the essential oil made from *R. crispus*-chloroform was the most effective against *E. coli* and *E. faecalis* (inhibition zones=21.0±3.7; 25.1±2.8, respectively) by same method. Moreover, essential oil made from *R. crispus*-ethanol was the most effective against all microorganisms by disc diffusion method. The results are in confirmation with recent studies of Yıldırım (2001), Elzaawely (2005), Coruh (2008), Elzaawely (2012), in which they were shown that the antimicrobial activity of Rumex sp. inhibited the growth of all the bacterial and fungal test organisms, suggesting that the antimicrobial activity of the essential oils or extracts may be related to some phenolic components [19, 21-23].

Determination of Total Antioxidant Status (TAS) of Essential Oils

The total antioxidant status (TAS) values of essential oils are another important biological property of great interest fort hey may preserve foods from the toxic effects of oxidants. Furthermore, many studies point to strong antioxidant capacities of essential oils and plant extracts [7,19,20,23]. The TAS values of

Table 1. The antimicrobial activity of the essential oils of *Rumex crispus*

Methods	Strains	Antimicrobial activities of essential oils (mm ± SD)		
		*Ethanol[c]	*Chloroform[d]	Water
Agar-well diffusion[a]	*Staphylococcus aureus*	17.2±1.8	19.8±4.2	ND
	Escherichia coli	19.5±4.5	21.0±3.7	ND
	Enterecoccus faecalis	16.6±2.4	25.1±2.8	8.6±1.4
	Candida albicans	19.2±3.0	18.1±4.4	7.0±1.0
Disc diffusion[b]	*Staphylococcus aureus*	18.5±2.6	22.7±1.0	ND
	Escherichia coli	25.3±3,7	26.7±1.7	ND
	Enterecoccus faecalis	25.6±5.0	32.7±2.1	ND
	Candida albicans	31.5±4.5	30.2±2.0	ND

ND, not determined

*p > 0.05, Differences significant statistically not showed for two methods on test microorganisms

a*b for ethanol; p=0.456 ; a*b for chloroform; p=0.508

c*d for agar-well diffusion method; p=0.370 ; c*d for disc diffusion method; p=0.263

Table 2. The antimicrobial activity of the essential oils of *Rumex cristatus*

Methods	Strains	Antimicrobial activities of essential oils (mm ± SD)		
		*Ethanol[c]	*Chloroform[d]	Water
Agar-well diffusion[a]	*Staphylococcus aureus*	15.7±3.6	18.2±4.7	ND
	Escherichia coli	14.5±5.5	17.8±3.1	ND
	Enterecoccus faecalis	20.2±2.0	22.0±2.3	12.8±2.4
	Candida albicans	24.4±3.5	22.4±5.2	14.2±1.2
Disc diffusion[b]	*Staphylococcus aureus*	17.5±1.5	19.6±2.0	ND
	Escherichia coli	21.3±4.5	22.0±2.6	ND
	Enterecoccus faecalis	23.4±3.5	24.6±3.0	ND
	Candida albicans	29.0±6.5	27.1±4.0	ND

ND, not determined

*p > 0.05, Differences significant statistically not showed for two methods on test microorganisms.

a*b for ethanol; p=0.103 ; a*b for chloroform; p=0.104

c*d for agar-well diffusion method; p=0.052 ; c*d for disc diffusion method; p=0.017

Table 3. The total antioxidant status of the essential oils of *Rumex cristatus* and Rumex *crispus*.

	Total Antioxidant Status (TAS) (mmol Trolex equivalent /l)	
	Ethanol*	Water*
Rumex cristatus	1.47±0.13	1.92±0.48
Rumex crispus	1.50±0.30	2.16±0.84

*The correlation is significant at the 0.01 level between *Rumex cristatus* and Rumex *crispus*.

R. crispus and *R. cristatus* are shown in Table 3. The differences among ethanol and water were found statistically not important (p > 0.05). The TAS values of *R. crispus* and *R. cristatus* essential oils prepared with ethanol and water were found between 1.47±0.13-2.16±0.84. And also, The TAS values of the essential oils prepared with water higher than prepared with ethanol in this study.

CONCLUSION

Turkey is quite remarkable in terms of the diversity of existing plant and has a rich flora. Studies in the world on the whole, the use of various industrial fields of our country's the potential existing plant that there may be very important. The results showed that the essential oils of *R. crispus* and *R. cristatus* exhibited important antioxidant and antimicrobial activity.

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