Investigation of Applicability Instead of Chemical Disinfectants of *Euphorbia orientalis* L. Extracts

Euphorbia Orientalis L. Özütlerinin Kimyasal Dezenfektanlar Yerine Uygulanabilirliğinin Araştırılması

Research Article

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ABSTRACT

In our study, disinfectant effects of *Euphorbia orientalis* L. were investigated that found as free in nature and collected from Corum/Turkey and environment. It was used three different soluble solutions (ethanol, chloroform and water) for extracts. *Staphylococcus aureus* ATCC 25923, *Candida albicans* ATCC 10231, *Escherichia coli* ATCC 25922, *Enterecoccus faecalis* ATCC 29212 and *Pseudomonas aeroginosa* ATCC 27853 strains used by disc diffusion method in determinate of antimicrobial activities of extracts. Water and ethanol extracts of plant were examined for antioxidant activities. And also, GC/MS analyses were carried out by chromatographic. On all microorganisms of ethanol and chloroform-plant extracts observed inhibition effects in different rates. The most sensitive *Candida albicans* strain and the most resistance strain *Staphylococcus aureus* were determinate in antimicrobial activity studies. An antioxidant activity of ethanol and water-plant extracts has observed in our studies. Antioxidant activities of water extract were higher than ethanol extract. At the same time, diphenic acid derivatives were found nearly the content of all extracts with GC/MS.

Key Words
Euphorbia orientalis L., chemical disinfectants, antimicrobial effect, antioxidant activity, GC/MS

ÖZET

Çalışmamızda, Çorum/Türkiye ve çevresinden toplanan ve doğada serbest olarak bulunan *Euphorbia orientalis* L.'in dezenfektan etkisi araştırıldı. Ekstraksiyonlar için üç farklı çözücü solüsyon (etanol, kloroform ve su) kullanıldı. Örneklerin antimikrobiyal aktivitesinin belirlenmesinde disk difüzyon yöntemi ile *Staphylococcus aureus* ATCC 25923, *Candida albicans* ATCC 10231, *Escherichia coli* ATCC 25922, *Enterecoccus faecalis* ATCC 29212 ve *Pseudomonas aeroginosa* ATCC 27853 suşları kullanıldı. Antioksidan aktivite için su ve etanol özütleri incelendi. Aynı zamanda kromotografi aracılığıyla GC/MS analizi yapıldı. Bitkinin etanol ve kloroform özütlerinin tüm mikroorganizmalar üzerinde farklı oranlarda inhibisyon etkisi gözlendi. Antimikrobiyal aktivite çalışmalarında en duyarlı suş *C. albicans* ve en dirençli suş *S. aureus* olarak belirlendi. Çalışmamızda bitkinin etanol ve su özütlerinin antioksidan aktiviteye sahip olduğu gözlendi. Bitkinin su özütlerinin antioksidan aktivitesinin etanol özütlerinden daha iyi olduğu belirlendi. Aynı zamanda, GC/MS ile hemen hemen tüm özütlerin içeriğinde difenik asit türevleri tespit edildi.

Anahtar Kelimeler
Euphorbia orientalis L., kimyasal dezenfektanlar, antimikrobiyal etki, antioksidan aktivite, GS/MS

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INTRODUCTION

Disinfectant and antiseptics are antimicrobial agents that used in hospitals to obtain the standard hygienic conditions and to prevent the risk of infections which caused by pathogen microorganisms. Many activities of these products should be known prior to use in the hospital. These chemical products to have some parameters important factors that affect health such as the pH spectrum (bacteria, viruses, fungi, etc.), the recommended contact time, storage [1]. Therefore, examination of the many effect before the acquisition of these substances is quite important. For this reason, the use of natural herbs should be investigated instead of disinfectants. Turkey is regarded as an important centre for much plant family. *Euphorbia* genus have been investigated for a long time in view of different specialties, like more energy content as alternative source of hydrocarbons, laticifers, phytochemicals and systematic [2]. Mediterranean *Euphorbia* species have been the object of various studies and they have been proposed as potential renewable sources of unsaturated. These plants which shown negative impact on the environment instead of chemical disinfectants used [3]. The contribution of oxidative stability together with the antimicrobial properties of the product to be used instead of disinfectant must also know.

Reactive oxygen radicals are the most common free radicals present in biological systems. These reactive oxygen species damage the biological system and causes different chronic diseases like cancer and heart diseases [4]. The modern research claims that oxidative stress is the cause of various disorders and diseases, therefore, the researcher focus on the role of antioxidants in the maintenance of biological system (human health), its remedy and treatment [5].

In our study, disinfectant effects of plant extract (*Euphorbia orientalis* L.) were investigated that found as free in nature. This study investigated the antimicrobial effect and total antioxidant activity of plant extract. And also the chemical compositions of the plant extracts were analyzed using GC–MS technique.

MATERIAL AND METHODS

Collection and identification of plant samples

Euphorbia orientalis L. plants were collected in August and September 2012 from Tolamehmet-Corum in Turkey. The identification of plant materials was confirmed by plant taxonomist in the Department of Biology, Dicle University, and Diyarbakır, Turkey.

Preparation of plant extracts

Five grams of the dried and powdered every plant materials (root, shoot, and leaf) were extracted with ethanol, chloroform and water (ultra pure) by using Soxhlet apparatus at 50°C for 8 h. The extract was filtered and concentrated under vacuum at 50°C by using a rotary evaporator (Heidolph, Laborota 4000, Schwabach, Germany), yielding a waxy material (18.20% w/w). Finally, the extract stored in the dark and in sterile glass bottles at 4°C until used within a maximum period of one week.

Measure of Antimicrobial Effect

Organisms and growth conditions

Bacterial cultures of *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeroginosa* ATCC 27853, *Escherichia coli* ATCC 25922 *Staphylococcus aureus* ATCC 6538 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. Different media used to growth of microorganisms in this study. Nutrient broth and agar (Diffco) were used 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. All strains stored at -20°C in the appropriate medium containing 10% glycerol and regenerated twice before use in the manipulations.

Determination of Antimicrobial Effect on Plant Extracts

The antimicrobial and antifungal effects of root, shoot, and leaf extracts of plant in different solvents were determined by the disc diffusion

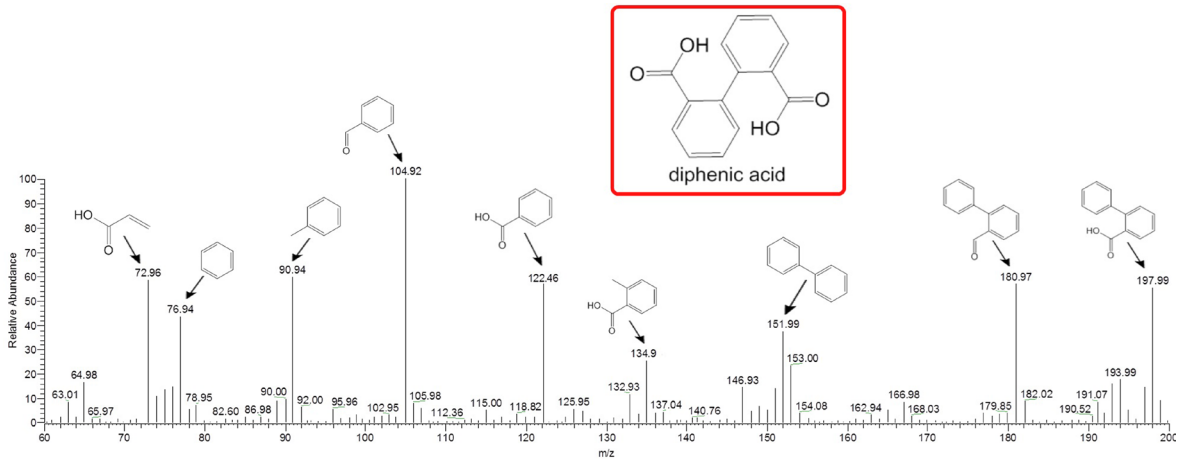

Figure 1. GC/MS spectra and fragmentation patterns of diphenic acid.

method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. The culture suspensions were adjusted by comparing against 5.0 McFarland. One hundred microlitres of suspension of the test microorganisms were spread on solid media plates.

The microorganisms were grown in a suitable broth at 37±1°C for 24 h. All the agar plates were prepared with Muller Hinton agar medium which a final depth of 4 mm. Next 0.1 ml suspension of tested microorganisms (10^8 cells/mL^{-1}; turbidity = McFarland barium sulfate standard 0.5) was spread on the agar plates. After, sterile 6-mm in diameter filter paper discs (Whatman, no. 4) were placed on the inoculated plates, which were stored at 4°C for 2 h and then incubated for 24 h. Distilled water (10 µL / disc), sodium hypochloride 3% (10 µL / disc), and hydrogen peroxide 1% (10µL/disc) were also used as positive control. At the end of the incubation period the inhibition zones around the discs were measured as millimeters. And also, activity index calculated for plant extracts with formula (IZ of test sample / IZ of Standard).

Measurement of the TAS

Principle of Assay

Antioxidants in the sample reduce dark blue-green colored ABTS radical to colorless reduced ABTS form. The change of absorbance at 660 nm is related with total antioxidant level of the sample. The assay is calibrated with a stable antioxidant standard solution which is traditionally named as Trolox Equivalent that is a vitamin E analog.

Determination of the TAS on plant extracts

The TAS was determined using a TAS Assay Kit (Rel Assay Diagnostics®, Gaziantep, Turkey) according to a novel automated measurement method developed by Erel (2004) [6]. 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. For this procedure, 500 µL of reagent 1 was placed in the cell, and 30 µL of sample (standard) was added. The initial absorbance at 660 nm for the first absorbance point was measured in a spectrophotometer (Shimadzu UV mini-1240, Kyoto, Japan). Subsequently, 75 µL of reagent 2 was added to the cell and incubated for 10 min at room temperature. The absorbance at 660 nm was read for a second time. The result obtained from this process was calculated with the following formula:

$$\text{Result}=[(\Delta\text{Abs Std1})-(\Delta\text{Abs Sample})]/[(\Delta\text{Abs Std1})-(\Delta\text{Abs Std2})]$$

ΔAbsorbance Standard1= (Second Absorbance of Std1- First Absorbance of Std1)

Δ Absorbance Standard2 = (Second Absorbance of Std2- First Absorbance of Std2)

Δ Sample Absorbance = (Second Absorbance of Sample- First Absorbance of Sample)

The results were expressed as micromolar Trolox equivalents per liter (µmol Trolox Eq/L).

Gas chromatography/mass spectrometry (GC/MS)

The chemical compositions of the plant extracts 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, 30mm 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

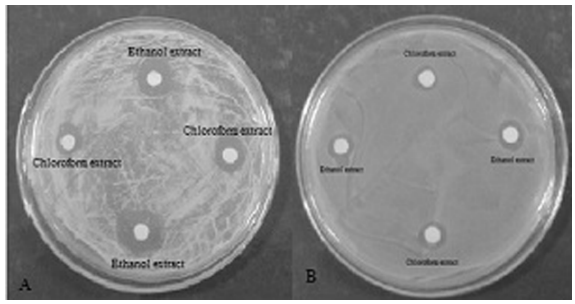

Figure 2. Antimicrobial effects (zone of inhibition) on C. albicans ATCC 10231 and *S.aureus* ATCC 25923 of root extracts. A) *C. albicans* ATCC 10231; B) *S.aureus* ATCC 25923

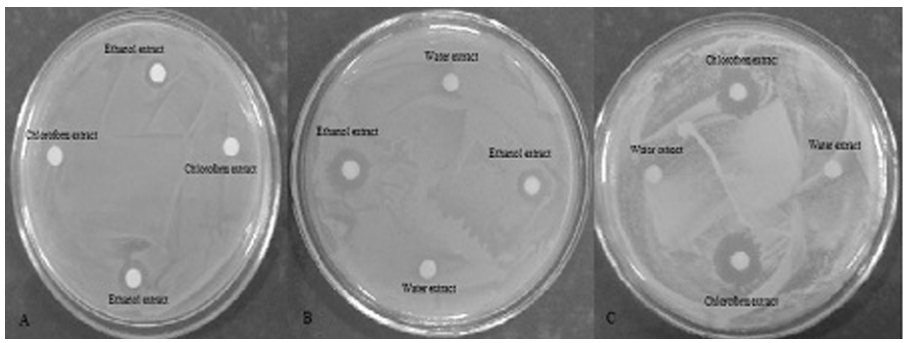

Figure 3. Antimicrobial effects (zone of inhibition) on *P. aeroginosa* ATCC 27853, *S. aureus* ATCC 25923 and *E. faecali*s ATCC 29212 of shoot extracts.
A) *P.aeroginosa* ATCC 27853; B) *S.aureus* ATCC 25923; C) *E. faecalis* ATCC 29212

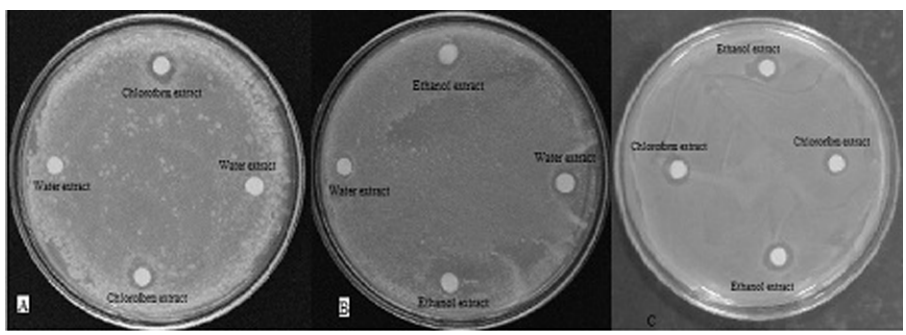

Figure 4. Antimicrobial effects (zone of inhibition) on *E. coli* ATCC 25922, *P. aeroginosa* ATCC 27853 and *C. albicans* ATCC 10231 of leaf extracts.
A) *E. coli* ATCC 25922; B) *P. aeroginosa* ATCC 27853; C) *C. albicans* ATCC 10231.

Table 1. Antimicrobial effects (zone of inhibition, mm±S.D.) of root, shoot, and leaf extracts of plant in different solvents.

		S. aureus ATCC 25923	E. faecalis ATCC 29212	P. aeroginosa ATCC 27853	E. coli ATCC 25922	C. albicans ATCC 10231
Root						
Ethanol extract	IZ	17.3±1.5	17.7±1.9	19.4±2.0	18.2±1.1	21.2±1.7
	AI	2.10±1.0	2.24±1.2	2.06±0.6	0.10±0.1	2.86±1.2
Chloroform extract	IZ	15.2±0.5	15.8±0.5	15.2±1.5	16.5±1.5	19.4±2.3
	AI	1.85±0.2	2.0±0.3	1.32±0.8	1.79±0.8	2.62±0.9
Water extract		ND	ND	ND	ND	ND
Shoot						
Ethanol extract	IZ	16.5±2.0	19.2±0.6	17.8±2.5	17.7±1.5	18.5±3.2
	AI	2.01±0.4	2.43±1.0	1.89±0.8	1.92±1.2	2.5±0.4
Chloroform extract	IZ	15.7±1.3	17.6±1.1	14.5±1.8	15.3±2.7	16.5±1.5
	AI	1.91±1.0	2.22±0.8	1.54±0.4	1.66±1.0	2.22±1.3
Water extract		ND	ND	ND	ND	ND
Leaf						
Ethanol extract	IZ	15.5±1.9	15.8±2.4	18.5±3.3	15.1±0.8	17.7±2.3
	AI	1.89±1.1	2.0±0.5	1.96±0.6	1.64±0.5	2.39±1.3
Chloroform extract	IZ	15.1±1.4	14.5±0.7	15.6±2.1	13.6±0.7	16.4±1.4
	AI	1.84±0.5	1.83±1.1	1.65±1.3	1.47±0.6	2.21±0.5
Water extract		ND	ND	ND	ND	ND

ND: Not determined
Values are mean of triplicate readings (mean ± S.D).
IZ= Inhibition zone (in mm) includes the diameter of disc (6 mm); Standards: Distilled Water (10µl/disc), Sodium hypochloride 3% (10µl/disc), Hydrogen peroxide 1% (10µl/disc); AI (activity index) = IZ of test sample / IZ of standard.

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.

Statistical analysis

Statistical analysis was performed on the data by SPSS 11.0 Bivariate Correlation Analysis (SPSS Inc., Chicago, III) with statistical significance determined at $P < 0.05$. All experiments were done in triplicate, and mean values are presented. The results were expressed as means ± standard deviations (SD).

RESULTS AND DISCUSSION

The diphenic acid was separated by gas chromatography and decomposition products were characterized by mass analyzer detector that the acrylic acid was determined as the product of the last decomposition. Diphenic acid cleavage products GC / MS spectrum was obtained according to the observed peaks (Figure 1). Structural formulas of the diagram is the appropriate degradation products are displayed on the peaks. Much plant extracts have been used as topical antiseptics, or have been reported to have antimicrobial properties [7]. It is important to investigate scientifically those plants which have been used in traditional medicines as potential sources of novel antimicrobial compounds [8]. It was used three different soluble solutions as ethanol, chloroform and water (ultra pure) in our study. The ethanol extracts are more effective than chloroform and water extracts. While water not observed effect from these soluble solutions, ethanol and chloroform were found quite effect on antimicrobial effect works. Antimicrobial activities of *Staphylococcus aureus* ATCC 25923, *Candida albicans* ATCC 10231, *Escherichia coli* ATCC 25922, *Enterecoccus faecalis* ATCC 29212 and *Pseudomonas aeroginosa* ATCC 27853 strains evaluated by disc diffusion method. On all microorganisms of ethanol and chloroform-plant extracts observed inhibition effects in different rates (Table 1). Looking at all antimicrobial activities results, ethanol extracts observed

Table 2. Antioxidant activity of shoot and leaf extracts of plant in water and ethanol solvents.

	Shoot		Leaf	
	Water extract	Ethanol extract	Water extract	Ethanol extract
Total Antioxidant Status*	4.71	2.34	13.6	7.51

*Total Antioxidant Status were calculate as µmol Trolox Eqv./L.

better than chloroform extracts. When root extracts examined, an antimicrobial effect on all microorganisms detected in both ethanol and chloroform extracts. Similar results showing that the alcoholic extract having the best antimicrobial activity is also reported by Preethi et al. (2010) [9]. Seyydnejad et al. (2010) also studied the effect of different alcoholic solvents such as ethanol and methanol for antimicrobial activity and observed that this difference in the activity between different alcoholic extract is due to the difference between extract compounds in this two extract [10]. The most sensitive strain *C. albicans* ATCC 10231 (21.2±1.7 mm) and the most resistance strain *S. aureus* ATCC 25923 (17.3±1.5 mm) were determinate in antimicrobial activity studies with ethanol extracts of root. And also, the most sensitive strain *C. albicans* ATCC 10231 (19.4±2.3 mm) and the most resistance strain *S. aureus* ATCC 25923 (15.2±0.5 mm) were determinate in antimicrobial activity studies with chloroform extracts of root (Figure 2). The most sensitive strain *E. faecalis* ATCC 29212 (19.4±2.3 mm, 17.6±1.1 mm, respectively) found in antimicrobial activity studies with both ethanol extracts and chloroform extracts of shoot. While *S. aureus* ATCC 25923 (16.5±2.0 mm) determine as the most resistance strain in studies with ethanol extracts, *P. aeroginosa* ATCC 27853 (14.5±1.8 mm) found as the most resistance strain in studies with chloroform extracts of shoot (Figure 3). The most resistance strain *E. coli* ATCC 25922 (15.1±0.8 mm, 13.6±0.7 mm, respectively) found in antimicrobial activity studies with both ethanol extracts and chloroform extracts of leaf. Macrae et al. (1988) the absence of microbial inhibitory effects of Euphorbiaceae was observed in a study [11]. The most sensitive strain *P. aeroginosa* ATCC 27853 (18.5±3.3 mm) found in ethanol extracts, while *C. albicans* ATCC 10231 (16.4±1.4 mm, respectively) was in chloroform extracts of leaf (Figure 4). Antibacterial activity of extracts of *E. hirta* was evaluated by measuring the diameters of zones of growth inhibition on some members of the enterobacteriaceae by El-Mahmood et al. (2009) [12]. Factors like the age of the plant and the polarity of the solvent used affect the yield. Thus in this studies, water seems to be the best solvent for this plant material, thus supporting the use of water as solvent of choice in traditional practice. *E.coli* strain determine as the most resistance strain in study [12]. Karou et al. (2006) reported that the susceptibility of bacteria to plant extracts, on the basis of inhibition zone diameters varied according to strains and species, similar to the data obtained in this study [13].

Antioxidants are integral part of the nutraceutical market. Last few years of research has confirmed that many of the common diseases (Cardiovascular diseases, diabetes, cataracts, blood pressure, infertility, respiratory infections, rheumatoid arthritis, Alzhemer's disease, several types of cancer, mental illness, including tumour promotions and AIDS) are associated with tissue deficiency and low dietary level of compound called antioxidant [14]. Therefore, the total antioxidant activity of shoot and leaf extracts of plant investigated in water and ethanol solvents. The antioxidant activities of shoot and leaf extracts are given in Table 2. The total antioxidant status (TAS) value of water extracts better than ethanol extracts compared with each other solvents. Moreover, TAS values of leaf extracts higher than TAS value of shoot extracts. While TAS values of leaf-water extract found as 13.6 μmol Trolox Eqv./L, TAS values of leaf-ethanol extract determined as 7.51 μmol Trolox Eqv./L. However, TAS values of shoot-water extract found as 4.71 μmol Trolox Eqv./L, TAS values of shoot-ethanol extract determined as 2.34 μmol Trolox Eqv./L.

The efficacy of the extracts, probably due to the identified secondary metabolites, further confirm its use as an antibacterial agent in medicine and may thus be useful in the treatment of infections [12]. The plant can be used to source for antibacterial Disinfectant and antiseptics that can treat infections caused by susceptible microorganisms. Some of these bioactive compounds which are synthesized as secondary metabolites as the plant grows also serve to protect the plant against microbial attacks and predation by animals [15]. In our studies analyzed content of plant extract with GS/MS technique by chromatographic. The diphenic acid derivatives were found nearly the content of all extracts.

In conclusion, although E. orientalis L. was found to contain bioactive compound that has

essential antibacterial and antioxidant activities, further anticarcinogenic studies will be needed to isolate the active constituent and evaluate the antimicrobial activities against a wide range of microbial pathogens

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