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ANTIMICROBIAL AND ANTIOXIDANT ACTIVITIES OF EXTRACTS OF *Euphorbia rigida* Bieb.

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ABSTRACT

The aim of this study was to determine antioxidant and antimicrobial activities of extracts of the *Euphorbia rigida* (Bieb.) plant which was extracted by using water, acetone, methanol, hexane and chloroform solution. Antimicrobial activity was determined by disc diffusion method against *Pseudomonas aeruginosa, Staphylococcus aureus* and *Candida albicans.* Antioxidant activity was determined by using β-carotene-linolenic acid, DPPH method, metal chelating activity, Cuprac and by evaluating the content of total phenolic compounds. Total antioxidant activities of *E. rigida* plant extracts at 50 and 100 µg/ml concentrations were compared against standard antioxidant activities such as BHT, α-tocopherol and quercetin activity. All extracts from *E. rigida,* except the chloroformic extract, showed antimicrobial activity against *P. aeruginosa*. However no activity was found against *S. aureus* and *C. albicans.* Antioxidant activities were found to be close to those of synthetic antioxidants such as BHT and α-tocopherol. The results indicate that *E. rigida* plant extracts can be used as a new and alternative antimicrobial agent against *P. aeruginosa*. It can also be argued that acetone and hexane extracts of *E. rigida* have the potential of becoming a natural antioxidant source. Moreover, all extracts from *E. rigida* might have important industrial, biotechnological, medical, agricultural, ecologial and economic potential.

Keywords: Antimicrobial activity, antioxidant activity, *Euphorbia rigida,* plant extracts.

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INTRODUCTION

Since ancient times, mankind has benefited from plants for different purposes such as feeding, sheltering, warming and especially for healing when they were sick. Today, plants or secondary metabolites produced from plants or drugs obtained from plants are used in the treatment of various diseases (Karuppusamy, 2009). It is known that the plant extracts and essential oils have antibacterial and antifungal activities and form the basis for many application areas such as pharmacy, food preservation, agricultural control (biopecticide, insecticide, herbicide) natural therapy and alternative medicine (Çalışkan *et al.,* 2019; Tiring *et al.,* 2021).

Euphorbia species containing white milk are among the largest genera of flowering plants in Euphorbiaceae family and about 100 species of the members of the Euphorbiaceae family (Tanker *et al.,* 1998), which has over 2000 species (Vasas and Hohmann, 2014; Kemboi *et al.,* 2020), are grown in Turkey. Most of the *Euphorbia* species are rich in resins, resinoids (Balabanlı *et al.,* 2006) phenolic and flavonoid compounds, aromatic esters, terpenes and diterpenes, triterpenoids (as tetracyclic or pentacyclic), steroller, cerebrosides, glycerols (Wu *et al.*, 2009; Gherraf *et al.,* 2010), alkanes, alkaloids, amino acids (Kumar *et al.*, 2010), myrsinane, resiniferatoxin like capsaicin (Vasas and Hohmann, 2014; Balpınar and Bardakcı, 2016), polyphenols, ascorbic acids, anthocyanins, volatile oil (Ghosh *et al.*, 2019), jatrophane, ingenane, daphnane, tigliane, lathyrane, essential oils, oxygenated sesquiterpenes, sesquiterpenes hydrocarbons, macrocyclic diterpenoids, lectins and as well as many bioactive compounds (Aleksandrov *et al.,* 2019; Kemboi *et al.,* 2020; Mishra and Parida, 2020 a,b). These bioactive compounds (diterpenoids and triterpenoids, steroller etc.) have biological activities such as antimicrobial, antipyretic, antineoplastic, antiproliferative, cytotoxic (Wu *et. al.,* 2009), sedative, antispasmodic, antifertile, antifungal, antimalarial (Kumar *et al.,* 2010), analgesic, antitumor, antiviral (Vasas and Hohmann, 2014),

antiasthmatic, antiarthritic, antitussives, antiallergic, antioxidant (Deveci *et al.,* 2018), antibacterial, anthelmintic, antidiarrheal, antimalarial, anxiolytic, antihypertensive, hepatoprotective, anti-inflammatory, gastroprotective (Aleksandrov *et al.,* 2019), antidiabetic, diuretic, larvicidal, antidysenteries (Ghosh *et al.,* 2019), antihepatitis B, antiseptic (Kemboi *et al.,* 2020) disinfectant, anticoagulan, antiherpetic and against polio, rhino, coxsackie viruses at *in vitro* (Mishra and Parida, 2020 a). They can also be used to inhibit HIV-1 viral infection (Wu *et. al.,* 2009), to treat bladder hyperreflexia and diabetic neuropathy (Vasas and Hohmann, 2014) and to stop accidentally bleeding wounds (Mishra and Parida, 2020 a).

Of the *Euphorbia* species, which have a skeletal structure of 14 Carbons (tetracyclic or pentacyclic), have constituents which are irritant, cytotoxic, toxic and cocarcinogen. That are jatrophane, ingenane and daphnane diterpene derivatives, lathyrane, rutin, ellagic acids, abietane, teraxerol, tigliane and betulin of the 300 terpenes (diterpenoids and triterpenoids) (Papp, 2004; Wu *et al*., 2009; Gherraf *et al.,* 2010; Vasas and Hohmann, 2014; Ghosh *et al.,* 2019). *Euphorbia* species are highly valuable sources of natural products that are biologically active and that could be used in the treatment of various diseases. Therefore, the *Euphorbia* genus is comprised of species having great economic value, which makes it a complex genus having great research potential (Gherraf *et al.,* 2010).

Phytochemical studies of the aerial parts afforded three triterpenes: betulin, cycloart-23Z-ene-3, 25-diol and cycloartan-3, 24, 25-triol, firstly isolated from *Euphorbia rigida* (Gherraf *et al.,* 2010). *E. rigida*, *E. myrsinites L*. and *E. nicaeensis* latexes have antihelmentic effects while *E. nicaeensis* ve *E. falcata* latexes have antifungal effects (Özbilgin and Çitoğlu-Saltan, 2012). Various extracts (water, ethanol, methanol, chloroform etc.) of *E. pulcherrima*, *E. fusiformis, E. characias*, *E. antiquorum, E. heterophylla, E. tirucalli, E. hirta* species obtained by using different plant tissues (aeral parts, leaves, seeds, all plant, roots, latex, dry fruit) were found to be effective against Gr (+) *S. aureus* and *Gr (-) E. coli.* Except for *E. characias*, *E. antiquorum,* they were found to be effective against *Gr (-) P. aeruginosa* and except for *E. fusiformis, E. antiquorum ve E. heterophylla,* they were found to be effective against *C. albicans. E. hirta* has anticarcinogenic agent, antioxidant, antiviral, biopesticide (nematicidal, antiprotozoal, antilarvacidal, antihelmentic), molloscoidal (*Lymnaea acuminata*), antivectorel (*Anopheles stephensis*), antimalerial (*Plasmodium falcifarum)* antiamoebic **(***Entamoeba histolytica)* genotoxic, toxicity **(**nephrotoxicity, hepatoxicity) effects. Roots of *E. nerifolia* (Mishra and Parida, 2020 a) and *E. formosana* (Aleksandrov *et al.,* 2019), latexes of *E. macroclada* and *E. coniosperma* are used against scorpion stings and snake bites, while the latex of *E. macroclada* also has an antimalarial effect (Özbilgin and Çitoğlu-Saltan, 2012). The species most commonly used for infections of skin, as anticeptic and disinfectant and due to their emollient properties are *E. maculata, E. peplus, E. orientalis*, *E. macroclada E. sessiliflora, E. apios*, and *E. hirta* (Avcı *et al.,* 2013; Kemboi *et al.,* 2020). *E. helioscopia, E. paralias, E. maschallian* and *E. myrsinites* species have been identified to have antiviral compounds. *E. hirta, E. pekinensis, E. peplus* are effective against virus infections (Gyurıs *et al.,* 2009; Özbilgin and Çitoğlu-Saltan, 2012; Mishra and Parida, 2020 a,b). *E. helioscopia* can be used effectively against the human cancer (Aleksandrov *et al.,* 2019). *E. graminea* plant showed a remarkable antioxidant and antiproliferative potentials against human breast (MCF-7) and lung (NCI-H460) cancer cell lines (Ikpefan *et al.,* 2020). *Euphorbia* (*E. tirucalli*, *E. formosana*, *E. helioscopia*, *E. fischeriana* and *E. humifusa*) extracts have been proved to exhibit cytotoxic activity mainly to human lung, bladder, leukemic cell, fibroblastoma cell line, pancreatic, hepatocelular, gastric and colorectal carcinoma cells (Wang *et al.,* 2006; Aleksandrov *et al.,* 2019; Ikpefan *et al.,* 2020).

Antimicrobial resistance has been a major problem facing the therapeutic use of different collections of antimicrobials, causing their efficacy to decline and fall continuously, creating a need for new antimicrobial and antioxidant drugs to be discovered (Ikpefan *et al.,* 2020). Essential oils having natural antioxidant effect in addition to antimicrobial effect make it possible to stop or delay lipid oxidation in foods and to prolong the shelf life of food products (Şengün and Öztürk, 2018). The research conducted in the past few years has confirmed that many of the common diseases (Alzhemer's and mental illness, diabetes, cataract, cancer, infertility) are connected with tissue deficiency and low intake of compound called antioxidant through diet (Avcı *et al.*, 2013). Natural drugs are more preferred because of the harmful and toxic effects of synthetic drugs (Deveci *et al.,* 2018) and pesticide residues can adeversely affect humans by causing important health problems such as cancer, infertilite and genetic disorder (Metin and Bürün, 2008, 2010; Tiryaki *et al.,* 2010). For this reason, researchers are increasingly interested in researching new sources of natural antimicrobial and antioxidant medicines and biopesticide.

In the current study, *Euphorbia rigida* (Bieb.) plant, which is widely found in Muğla province, was selected from the *Euphorbia* species that have the potential to be used for medical, industrial, agricultural and ecological purposes. Yet, although most of the *Euphorbia* species have been mentioned in folk medicine, there is a lack of clarity on their dosage, mechanisms, mode of actions, side effects, and efficacy. *E. rigida* is a natural and organic product, it has toxic, cytotoxic and genotoxic properties (Metin and Bürün, 2020). Therefore, more comprehensive research is needed to provide more clues about their safety and efficacy (Aleksandrov *et al.,* 2019;

Kemboi *et al.,* 2020; Metin and Bürün 2020). For this reason, it is necessary to test the plant extracts to be used in both medicine and industry. In this study, the antimicrobial and antioxidant effects of *E. rigida* were investigated for medical, industrial, agricultural and ecologial purposes.

MATERIALS AND METHODS

Plant collection: The aerial parts of *Euphorbia rigida* (Bieb.) in March 2009 at the flowering period were collected from Sıtkı Koçman Üniversity campus (GPS:37°09'40,1"N 28°22'34"E) in Muğla at Turkey. The taxanomic identification of plant materials was confirmed and they were deposited in the herbarium (Herbarium number: O0388). The samples were air-dried at room temperature and protected from direct sunlight. They were powdered and stored until use.

Preparation of plant extracts: Plants were extracted in September 2009 at the General Biology Laboratory of the Muğla Sıtkı Koçman Unviversity (MSKU), Faculty of Science, Biology Department (FSDB). Seventy five grams (g) of powdered plant materials were extracted successively using chloroform, methanol, hexane, acetone and water. Each solvent was used as 2.5 L at room temperature (25°C) and replicated for four times (24 h x 4). After filtrations, each extract was evaporated under vacuum until dry. Crude extracts were obtained with chloroform (CER=**C**hloroform **E**uphorbia **R**igida) 3.62 g, methanol (MER) 15.18 g, hexane (HER) 1.45 g, acetone (AER) 3.08 g. Additionally, 75 g of dried and powdered aeriel parts of *E. rigida* were extracted with 2.5 L boiling water for 60 min. Decoctions (aqueous phase) were filtered with a 2.5 µm fitler (Whatman No. 42) to remove suspended particles and the extract was kept at least 3 days at -20°C and later lyophilized to obtain crude (6.72 g) extract which was stored at -20°C (Table 1).

Antimicrobial Assay:

Microorganisms and Culture conditions: In this study, two bacterial strains and one fungus strain were used supplied of MSKU, FSDB, Microbiologi Laboratory: *Pseudomonas aeruginosa* MU152, (*P. aeruginosa* ATCC 27853), *Staphylococcus aureus* MU135 (*S. aureus* ATCC 25923) and *Candida albicans* MU1 (*C. albicans* ATCC 10239), which are multiple antibiotic resistant bacteria. *S. aureus* was cultured in Nutrient Broth (NB) (Difco) at 37 ± 0.1°C; *P. aeruginosa* was cultured in Nutrient Broth (NB) (Difco) at 30 ± 0.1°C; and *C. albicans* was cultured in Sabouraund Dextrose Broth (SDB) (Difco) at 30 ± 0.1°C (Bauer *et al.,* 1966).

Disc Diffusion Method: Disc diffusion method was used to determine antimicrobial activity of the *E. rigida* plant extracts. The diameters of the resulting inhibition zones on the MHA and SDA were assessed in millimetres at the end of the incubation periods. Studies were performed in triplicate and the averages of the results obtained from antimicrobial activity tests were recorded (Collins *et al.,* 1989) (Table 2).

Antioxidant Activity: Extracts are chemically complex, where even a mixture of compounds containing different functional groups, polarities and chemical behaviours, could lead to dispersed results, depending on the test used. Therefore, using multiple assays for determination the antioxidant potential of extracts are more useful and even essential. In this study, four methods have been used for determination of the antioxidant potential and calculation of total phenolic concentrations (Table 1) namely the metal chelating activity, the DPPH (2,2-Difenil-1- pikrilhidrazil) radical scavenging activity, the cupric reducing power and β-carotene bleaching method which were performed at the Analytical Chemistry Laboratory of the MSKU, Faculty of Science, Chemistry Department (Table 3 and 4). The total antioxidant activity of the *E. rigida* extracts was evaluated using β-carotene-linoleic acid test system as previously reported in the literature (Miller, 1971; Tel *et al.,* 2012). Radical scavenging activities were measured with DPPH free radical scavenging assays (Blois, 1958; Türkoglu *et al.,* 2007). Reducing powers were determined using CUPRAC assays (Apak *et al.,* 2004; Öztürk *et al.,* 2007). Metal chelating activity on ferrous ions was determined using the method described in the literature (Dinis *et al.,* 1994; Kolak *et al.,* 2006). The phenolic content of extracts was stated as microgram of pyrocatechol equivalents (PEs) (Slinkard and Singleton, 1977) (Tablo 1 and 4).

Statistical analysis: All the data from antioxidant and antimicrobial activity tests are the average of triplicate analyses and were recorded as mean ± SD. One-Way ANOVA procedures were performed for variance analysis. Duncan Multiple Range test was used for significant differences between means, p-values < 0.05 were regarded as significant (Harter, 1960; Bewic *et al*., 2004). All statistical analyses were performed using IBM SPSS 14.0 Software Package Program.

RESULTS

Antimicrobial and antifungal activities of 400 µg/ml of the extracts of *E. rigida* plant obtained by using water, acetone, methanol, hexane and chloroform solution and *E. rigida* plant juice (latex) against Gr (-) *P. aeruginosa,* Gr (+) *S. aureus* and *C. albicans* were determined through the disc diffusion method. All extracts studied of *E. rigida* had an effect on the Gram (-) (*P. aeruginosa* ATCC 27853*)* bacteria but had no effect on *C. albicans* (MU1=ATCC 10239) and Gram (+) test bacteria (*S. aureus* ATCC 25923). The inhibition zone diameter of the extracts is shown in Table 2. While an average of 26.75 mm zone formation was observed in Levofloxacin disk,

which is used for control purposes against *P. aeuroginosa*, an average of 20 mm in Gentamicin disk and a zone formation of 8 to 15 mm was observed in plant latex obtained from *E. rigida* plant and in all extracts. The maximum antibacterial activity on water extracts of this plant inhibited the growth of *P. aeruginosa* by a diamater of 15 mm. All the extracts except for **c**hloroform extract had antibacterial activities against *P. aeruginosa* ranging from 7 to 8.75 mm inhibition zones. The latex of *E. rigida* inhibited the growth of *P. aeruginosa* and *S. aureus* bacteria and the inhibition zones of 8 mm, but latex did not have any effect on the growth on the *C. albicans.* All extracts studied did not have an effect on the growth of the *S. aureus* and *C. albicans* (Table 2).

In this study, mainly five methods were used to determine antioxidant activity, which are β-carotene bleaching method, DPPH radical scavenging activity, metal chelating activity, cupric reducing power (Table 3 and 4) and the calculation of total phenolic concentrations for the extracts (Table 1). Total antioxidant activity of the extracts of the aerial parts of *E. rigida* in comparison with BHT and α-tokoferol, where the β-carotene bleaching method was used are shown in Table 3. Only acetone and hexane extracts showed an increase in the total antioxidant activity. Even acetone and hexane extracts of the *E. rigida* at the concentrations of 50 and 100 µg/ml (57.23%, 75.03% and 32.27%, 66.40%, respectively) were more active than the same concentrations of BHT and α-tokoferol (65.21%, 66.22% and 64.14%, 65.21%, respectively). Antioxidant activity was higher than that of BHT and α-tokoferol was not observed in none of the tested extracts, except for acetone and hexane extracts (Table 4). The increased amount of the acetone extracts of *E. rigida* increased the radical scavenging activity. The difference between the acetone extracts and control was statistically significant ($p<0.05$). No activity of free radical scavenging (DPPH) was observed in the tested extracts of *E. rigida,* except acetone. All the extracts of the aerial parts of *E. rigida* did not show activity of metal chelating and cupric reducing antioxidant activity (Table 3 and 4). The concentrations of phenolic content in the aerial parts of *E. rigida* extracts were found to be the higest in water extract (89.20 µg PEs/mg) and methanol extract (82.16 µg PEs/mg) compared to standart pyrocatehold. All the other extracts contain statistically low amounts of phenolic compounds (Table 1).

Table 1. Total phenolic contents of extracts of *E. rigida*

Sample Phenolic contents of extracts of *E. rigida*	µg PEs / mg extract ± SD	Yield of the extracts (g)
Water	**89.2073** ± 0.0022	6.72
Acetone	29.0853 ± 0.0005	3.08
Methanol	**82.1612** ± 0.0044	15.18
Hexane	23.8143 ± 0.0010	1.45
Chloroform	35.0609 ± 0.0210	3.62

SD: Standart deviation

Table 2. Zone diameters (mm) formed by water, acetone, methanol, hexane, chloroform extracts of the aerial parts of *E. rigida* and latex of *E. rigida* and standard antibiotic discs against *P. aeruginosa*, *S. aureus* and *C. albicans* after 24 hours.

	Zone diameters formed at the end of 24 hours (mm)*		
***Euphorbia rigida* extracts**	***P. aeuroginosa* (ATCC 27853) IZD ± SD**	***S. aureus* (ATCC 25923) IZD ± SD**	***C. albicans* (ATCC 10239) IZD ± SD**
Water (WER)	15 ± 2.28	0	0
Acetone (AER)	8 ± 0.5	0	0
Methanol (MER)	7 ± 0	0	0
Hexane (HER)	8.7± 0.95	0	0
Chloroform (CER)	0	0	0
Latex	8 ± 0	8 ± 0	0
Standard Antibiotic Discs			
Levofloxasin (LEV)	26.75± 1.25	NT	NT
Gentamicin (CN)	20± 0	NT	NT
Ciprofloxacin (CIP)	NT	26± 0	NT
Penicillin (P)	NT	18± 0	NT
Ampicillin/Sulbactam (SAM)	NT	37± 0	NT
Nystatin	NT	NT	14± 1,15

***** The values in the table are the averages of the 6-replicated disc zone diameters for all applications.
IZD: Inhibition Zone Diameter, SD: Standard deviation, NT: Not tested.

Table 3. Free radical removal activity results of *E. rigida* extractions and standards having antioxidant activity determined with the β-carotene/linoleic acid, DPPH system, Ferrozine-Fe^{+2} system and Cuprac system method

***Euphorbia rigida* extracts**	**Inhibition (%) in β-carotene/linoleic acid system (50 µg/ml) ± SD**	**IC_{50} (50 µg/ml) in DPPH system ± SD**	**Inhibition (%) Ferrozine-Fe^{+2} system (50 µg/ml) ± SD**	**Inhibition (%) in Cuprac system (50 µg/ml) ± SD**
Water	0.9345 ±0.0045	0.3721 ± 0.0239	0.4925 ± 0.0516	0.553 ± 0.01
Acetone	57.2376 ± 3,8695*	15.0181 ± 2.6374*	0.1440 ± 0.0048	0.144± 0.004
Methanol	-19.8833 ±1.4178	0.3532 ± 0.0393	0.1831 ± 0,0089	0.461 ±0.006
Hexane	32.2708 ± 4,0433*	2.1626 ± 0.7394	-31.2107 ± 2.5309	0.117 ±0.007
Chloroform	9.9548 ± 1.8006	0.3094 ± 0.1465	-8.9331 ± 3.4850	0.113 ±0.001
BHT	65.219 ± 0.91	38.431 ± 0.14	NT	3.513 ± 0.00
α-tokoferol	64.141 ± 0.21	95.233 ± 0.07	NT	1.852 ± 0.05
Quercetin	NT	NT	15.179 ± 0.88	NT

***** Active ($p<0.05$), SD: Standard deviation, NT: Not tested

Table 4. Results of scavenging activity determined by β-carotene/linoleic acid, DPPH system, Ferrozine-Fe^{+2} system and Cuprac system for all *E. rigida* extractions and standards.

***Euphorbia rigida* extractions**	**Inhibition (%) in β carotene/linoleic acid system (µg/ml) ± SD**				**Inhibition (%) in DPPH system (µg/ml) ± SD**				**Inhibition (%) Ferrozine-Fe^{+2} system (µg/ml) ± SD**				**Inhibition (%) in Cuprac system (µg/ml) ± SD**				
	50	**100**	**200**	**400**	**50**	**100**	**200**	**400**	**50**	**100**	**200**	**400**	**Control**	**50**	**100**	**200**	**400**
Water	0.93 ±0.0045	0.94 ± 0.0016	0.95 ± 0.015	0.96± 0.021	0.37 ± 0.02	0.41 ± 0.067	0.57 ± 0.028	0.63 ± 0.051	0.49 ± 0,051	0.52 ± 0.03	0.39 ± 0.08	0.34 ± 0.08	0.05±0.0007	0.55 ± 0.01	0.991 ± 0.03	**1.34 ± 0,05***	**2.17 ± 0.11***
Acetone	**57.23 ± 3,86***	**75.03 ± 4.44***	**92.95 ± 4.07***	**105.18 ± 5.20***	**15.01 ± 2.63***	**20.16 ± 1.05***	**40.84 ± 3.25***	**68.89 ± 1.176***	0.144 ± 0.004	0.11 ± 0.004	0.34 ± 0,006	0.63 ± 0,11	0.05±0.0007	0.14± 0.004	0.11 ± 0.004	0.34 ± 0.006	0.63 ± 0.11
Methanol	-19.88 ±1.41	-19.55 ±1.04	-14.87 ±0.70	-13 ± 0.65	0.35 ± 0.039	0.41 ± 0.033	0.65 ± 0.014	0.71 ± 0.04	0.183 ± 0,008	0.25 ± 0.04	0.18 ± 0.01	0.008 ± 0.004	0.05±0.0007	0.46± 0.006	0.86 ± 0.017	**1.27 ± 0.05***	**2.15 ± 0.10***
Hexane	**32.27 ± 4,04***	**66.40 ± 5.49***	**86.85 ± 5.53***	**117.41 ± 3.59***	2.16 ± 0,73	-0.96 ± 0.43	-0.78 ± 2.083	2.82 ± 1.18	-31.21 ± 2.53	-17.33 ± 3.11	-8.49 ± 1.74	-36.20 ± 4.73	0.05± 0.0007	0.11 ± 0.007	0.15 ± 0.045	0.24 ± 0.006	0.38 ± 0.052
Chloroform	9.95 ± 1.80	9.29 ± 2.65	11.95 ± 2.21	26.82 ± 2.33	0.30 ± 0.14	1.67 ± 1.17	3.39 ± 1.66	5.73 ± 0.66	-8.93 ± 3.48	-6.41 ± 0.85	-12.96 ± 1.24	-55.93 ± 8.56	0.05 ±0.0007	0.11 ± 0.001	0.15 ± 0.007	0.22 ± 0.016	0.35 ± 0.026
BHT	**65.21 ± 0.91**	**66.22 ± 1.00**	NT	NT	**38.43 ± 0.14**	**58.41 ± 0.45**	NT	NT	NT	NT	NT	NT	0.204 ± 0.01	**3.51 ± 0.00**	**3.72 ± 0.00**	NT	NT
α-tokoferol	**64.14 ± 0.21**	**65.21 ± 0.10**	NT	NT	**95.23 ± 0.07**	**96.33 ± 0.99**	NT	NT	NT	NT	NT	NT	0.204 ± 0.01	**1.85 ± 0.05**	**2.21± 0.28**	NT	NT
Quercetin	NT	NT	NT	NT	NT	NT	NT	NT	**15.179 ± 0.88**	**29.274 ± 0.35**	NT	NT	NT	NT	NT	NT	NT

*Active (p<0.05), SD: Standard deviation, NT: Not tested

DISCUSSION

The rising trend in antibiotic resistance has become a global health challenge; hence, there is a need for the discovery of new antibiotics which are inexpensive and effective for the treatment of microbial diseases (Ikpefan *et al*., 2020). Euphorbia species have been used in the traditional medicine in Turkey as well in the world to treat many diseases such as mouse leukaemia, various skin diseases, wart treatments, wounds, gonorrhoea, migraines, intestinal parasites, rheumatism, swelling and cancer (Barla *et al*., 2007; Wu *et al*., 2009; Gherraf *et al*., 2010; Mishra and Parida, 2020 a). Hence *E. rigida* wide range in ethnomedical use was the reason for this study.

The results of the present study are the first information on the antioxidant and antimicrobial activities of *E. rigida* with several methods. The ethanolic, metanolic and water extracts of Euphorbiaceae species such as *E. hirta, E. sequieriana, E. fusiformis, E. sessiliflora, E. orientalis, E. heterophylla, E. pulcherrima, E. characias, E. antiquarum and E. tirucalli* have antibacterial activities against Gram positive (+) bacteria, especially *S. aureus, Micrococcus sp., Bacillus subtilis and B. thuringensis* and Gram negative (-) bacteria especially *E. coli, Klebsiella pneumoniae, P. aeruginosa, S. typhi, Shigella dysenteriae and Proteus vulgaris, P. mirabilis.* They are among the most commonly encountered pathogens in clinical practice. Moreover, they exhibit antifungal activities against plant pathogens like *C. albicans*, *Aspergillus niger, A. fumigatus, A. flavus* and *Rhizopus oryza* and fungal plant patogens such as *Colletotricum capsici, Fusarium pallidoroseum, Botryodiplodia theobromae and Phomopsis caricae-papayae* and antimalerial activities against *Shigella spp.* (Kumar *et al.,* 2010; Özbilgin and Çitoğlu-Saltan 2012; Aleksandrov *et al.,* 2019; Raj *et al.,* 2019; Mishra and Parida, 2020 a,b). While many herbal extracts show antimicrobial activity for Gr (+) bacteria, the same herbal extracts do not have any antimicrobial activity for Gr (-) bacteria or against many actinomyces, yeast and fungal infections (Vural *et al.*, 2008; Karcıoğlu *et al.,* 2011). This is because Gr (-) bacteria have an outer membrane that is poorly permeable to hydrophobic or amphipathic molecules. The lipopolysaccharide layer on the outer membrane of Gr (-) bacteria limits the diffusion rate of hydrophobic compounds. Thus, they are least susceptible to antibiotics and herbal extracts. Lipoteichoic acids in the cell membrane of Gr (+) bacteria facilitate the penetration of hydrophobic compounds of essential oils and therefore Gr (+) bacteria are more sensitive to herbal extracts and essential oils than Gr (-) bacteria (Lawrey, 1989; Şengün and Öztürk, 2018). All extracts of *E. rigida* had antibacterial activities against especially Gr (-) *P. aeruginosa* except chloroform extract in the present study. Latex of *E. rigida* was found to have an antibacterial activity against Gr (-) *P. aeruginosa* and Gr (+) *S. aureus*, no antifungal activity was observed against *C. albicans* based on the results of the present study. This result may suggest that except chloroform extract, all the extracts and latex have antimicrobial activities expecially against Gr (-) bacteria and nosocomial infections. *P. aeruginosa* is a common and important infection agent causing morbidity, mortality and nosocomial infections, it can usually lead to treatment difficulties due to multi-drug resistance (Berktaş *et al.,* 2011; Atilla *et al.,* 2012). So, they can be used as antimicrobial agents in new drugs for the treatment of infectious diseases in human. *Euphorbia* species are effective against virus infections (Gyurıs *et al.,* 2009; Mishra and Parida, 2020 a) so *E. rigida* extract may also effective on viruses' capsid and DNA/RNA structures. Furthemore, the possibility of using *E. rigida* extract as an antiseptic for sterilization or disinfection of large and inanimate surfaces could be areas for further research (Metin and Bürün, 2020). *E. rigida* is an environmentally friendly natural resource as biopesticides to protect food stocks (Şahin *et al.,* 2006; Civelek and Weintraub 2004; Balpınar and Bardakçı, 2016) and from which liquid synthetic fuel and solid chemical raw material products can be obtained through biomass pyrolysis (Ateş and Pütün, 2003). Activated carbons obtained from *E. rigida* were determined to be an inexpensive, effective and environmentally friendly adsorbent for the removal of heavy metals in wastewater or aqueous solutions, and for the removal of textile dyes from textile wastewater (Gerçel and Gerçel, 2007; Kılıç *et al.,* 2012; Gerçel, 2015). A thermostable chymotrypsin-like serine protease was partially purified from *E. rigida* plant latex. Proteases are of great importance for industrial, biotechnological, medical and scientific applications. Industrial applications of proteases include leather processing, food processing etc. Medical applications are generally based on inhibitory drug design and direct *in vivo* applications for dissolving blood clots in cases such as strokes (Kocazorbaz *et al.,* 2017).

The antioxidant activities of *E. rigida* extracts were determined with β-carotene bleaching method, DPPH radical scavenging activity, metal chelating activity and cupric reducing power methods and concentrations of total phenolic compounds were also calculated for the extracts. Among the tested methods, the inhibition of lipid peroxidation in the β-carotene–linoleic acid system by acetone and hexane extracts of *E. rigida*, showed the highest activity Furthermore, the acetone extract was found to be the most active one, showing activity as that of the standard BHT and α-tokoferol. On the other hand, acetone extracts showed lesser DPPH scavenging activity than that of the standard BHT. Although, phenolic content of the water and methanol extracts were found to be more whereby their antioxidant activity was lower than that of BHA, α-tokoferol and quercetin at the concentration of 50 µg/ml. This should be dependent on the structures of phenolics. The role on stabilizing lipid peroxidation in

phenolic compounds in relation to their antioxidant activity are well known (Barla *et al*., 2007). So are the effects on oxidation of unsaturated lipids of free radicals (Kaur and Perkins, 1991), and in addition where DPPH radicals are used as a stable free radical to determine antioxidant activity of natural compounds (Shimada *et al*.,1992). However, a correlation was not found between total phenolic content and DPPH and β-carotene-linoleic acid assay in this study. Hence, it is believed that the constituents of the extracts should be studied even further to understand the different antioxidant activities in relation to phenolic content differences.

Barla *et al.* (2007), in their study with petroleum ether and ethanol extracts from *E. rigida* in DPPH system had the highest activity, while acetone extract showed an antioxidant activity above the standards. In the inhibition (%) β-carotene/linoleic acid system (50 µg/ml) test, *E. rigida* extracts with acetone > ethanol values, antioxidant activity was observed less than but close to the standards, while no antioxidant activity was observed in petroleum ether extracts. In the Inhibition (%) Ferrozine-Fe^{+2} system test, *E. rigida* extracts showed very little antioxidant activity in petroleum ether extracts and acetone, while antioxidant activity was not observed in ethanol extract. Phenolic content (µg PEs/mg extract) of *E. rigida* was found to be the highest in acetone (20.20 µg PEs/mg) followed by ethanol (5.14 µg PEs/mg) and petroleum ether (4.93 µg PEs/mg). Barla *et al.* (2007), in their study with *E. rigida*, the results obtained with acetone extract were highly consistent with the results we obtained in our study and confirmed the reliability of our results. Barla *et al.* (2007), found that the petroleum ether and ethanol extracts had antioxidant activities especially high in the DPPH system and close to the standards in the β-carotene/linoleic acid system, thus, they showed the antioxidant value of *E. rigida* and its potential to be drawn on. It was concluded that if *E. rigida* is used for antioxidant purposes, higher yields will be obtained from petroleum ether, ethanol, acetone, water and methanehole and acetone extracts should be preferred in case where the phenolic content of *E. rigida* will be used.

Conclusions: As a result of the present study, this extract can be considered as a new and alternative source that can be used in the treatment of Gr (-) bacteria, since the extracts and latex obtained from the *E. rigida* plant show antimicrobial activity for the Gr (-) bacteria *P. aeruginosa*. Petroleum ether, ethanol and acetone extracts of *E. rigida* have high antioxidant activity, especially in DPPH and β-carotene/linoleic acid system. Thus, *E. rigida* may help people to protect against lipid peroxidation and free radical damage. We can suggest that *E. rigida* extracts, with their antimicrobial and antioxidant properties, can have a good potential to produce safe food products and additives. In order to reach more information and certain conclusions on the use of *E. rigida* especially for industrial (antiseptic, disinfectant, leather processing, etc.) biotechnological and medical (a thermostable enzym is chymotrypsin-like serine protease, antitumor etc.,), agricultural (antimalarial, acaricidal, antiparasitic, larvicidal, molluscoidal, antifeedant etc.,) and ecologial purposes (adsorbent of heavy metal and textile waste dyes in water, biofuel by pyrolysis), further research is needed especially on petroleum ether, ethanole, acetone, water and methanol extracts of *E. rigida* and isolated constituents are needed or studies should be performed with different test systems. *E. rigida* plant can be an important ecological and economic potential for Turkey and other countries as a natural resource.

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