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Evaluation of Antimicrobial Activities of Various Herbal Oils Against *Helicobacter pylori* and their Cytotoxic Effects on HUVEC Cell Line

Çeşitli Bitkisel Yağların *Helicobacter pylori*'ye Karşı Antimikrobiyal Etkinlikleri ve HUVEC Hücre Hattı Üzerindeki Sitotoksik Etkilerinin Değerlendirilmesi

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ABSTRACT

Helicobacter pylori (H. pylori) infection is accepted as the most important chronic bacterial infection. In recent years, it is reported that the bacteria are developing resistance against the applied antibiotics. In order to increase the success rate, decrease recurrence and achieve eradication, it is very important to investigate nontoxic biocompatible herbal resources to be used in addition to antibiotic therapy. Herbal oils, obtained from plants are being used for various purposes for a long time, particularly in commercial and scientific fields. Therefore, in our study, we chose various herbal oils *(Eucalyptus globulus, Juniperus communis, Rosmarinus officinalis, Thymus vulgaris)* that are known to be effective against gastric and gastrointestinal tract diseases which do not have adequate investigations over *H. pylori* in the literature, and we aimed to investigate their antimicrobial activity over *H.pylori* and cytotoxic activity on Human Umbilical Vein Endothelial Cells (HUVEC) cell line. The antimicrobial activity is investigated by microdilution assay (MIC, MBC), and cytotoxic activity is investigated by MTT and LDH assays. As a result, it was found that *Eucalyptus globulus* (MIC: 2,81 %v/v, MBC: 5,62 %v/v), *Juniperus communis* (MIC: 0,35 %v/v, MBC: 0,70 %v/v), *Rosmarinus officinalis* (MIC: 2,81 %v/v, MBC: 5,62 %v/v), *Thymus vulgaris* (MIC: 0,70 %v/v, MBC: 1,40 %v/v) oils were effective against *H. pylori*. Besides, it was determined that *Thymus vulgaris* herbal oil had the highest cytotoxic effect, and *Eucalyptus globulus* herbal oil had the lowest cytotoxic effect on the HUVEC cell line.

Key Words

Helicobacter pylori, herbal oil, antibacterial activity, cytotoxicity.

ÖZ

Helicobacter pylori (*H. pylori*) enfeksiyonu en önemli kronik bakteriyel enfeksiyon olarak kabul edilmektedir. Son yıllarda tedavide kullanılan antibiyotiklere karşı bakterinin direnç geliştirdiği rapor edilmiştir. Tedavide başarı oranının yükselmesi ve rekürrensin azalması için, toksik olmayan biyouyumlu bitkisel kaynakların araştırılarak sistemik antibiyotik tedavisine ek olarak kullanılması ve eradikasyonun sağlanması son derece önemlidir. Bitkilerden elde edilen yağlar uzun yıllardan beri çeşitli amaçlara yönelik, özellikle ticari ve bilimsel alanlarda kullanılmaktadır. Bu nedenle çalışmamızda; genellikle mide ve gastrointestinal sistem rahatsızlıklarına iyi geldiği bilinen ve literatürde *H. pylori* üzerinde yapılmış yeterli çalışması bulunmayan çeşitli bitkisel yağlar (*Eucalyptus globulus, Juniperus communis, Rosmarinus officinalis, Thymus vulgaris*) seçilmiş ve *H. pylori*'ye karşı antimikrobiyal etkinlikleri ile Human Umbilical Vein Endothelial Cells (HUVEC) hücre soyu üzerindeki sitotoksik etkilerinin araştırılması amaçlanmıştır. Bitkisel yağların antimikrobiyal etkinlikleri mikrodilüsyon yöntemi ile (MIC, MBC), sitotoksik etkinlikleri ise MTT ve LDH yöntemleri ile incelenmiştir. *H. pylori*'ye karşı antimikrobiyal etkisini araştırdığımız, *Eucalyptus globulus* (MIC: 2,81 %v/v, MBC: 5,62 %v/v), *Juniperus communis* (MIC: 0,35 %v/v, MBC: 0,70 %v/v), *Rosmarinus officinalis* (MIC: 2,81 %v/v, MBC: 5,62 %v/v) ve *Thymus vulgaris* (MIC: 0,70 %v/v, MBC: 1,40 %v/v) 'in etkili olduğu gözlemlenmiştir. Aynı zamanda HUVEC hücre soyu üzerinde *Thymus vulgaris* bitkisel yağının en yüksek, *Eucalyptus globulus* bitkisel yağının ise en düşük sitotoksik etkiye sahip olduğu tespit edilmiştir.

Anahtar Kelimeler

Helicobacter pylori, bitkisel yağ, antibakteriyel aktivite, sitotoksitite.

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INTRODUCTION

It is thought that *H.pylori* and other helicobacteria found in the digestive system are as old as the first living organisms and settled as the main flora bacteria in the digestive systems of large mammals and the first primates [1]. *H. pylori*, which was produced in the laboratory for the first time in 1982, was thought to belong to this genus due to its similarities to the genus *Campylobacter* and was defined as *Campylobacter pyloridis* [2]. However, in later studies, it was revealed that many phenotypic and genotypic characteristics were different from the Campylobacter genus, and therefore this new genus was named *Helicobacter* [3]. *H. pylori*, which is highly motile, gram-negative, and spiral-shaped, has been found to colonize the mucus layer on the gastric mucosal epithelium in individuals with ulcer or gastritis disease [4]. Marshall and Warren, who discovered colonies formed by spiral-shaped microorganisms on the surface of the medium in their study, and then succeeded in producing spiral microorganisms in biopsy samples from more than 11 patients, concluded that the formation of some gastroduodenal diseases may be associated with these bacteria [5]. Proving the relationship between this spiral-shaped microorganism and gastroduodenal diseases is considered as one of the important developments that left its mark on medicine in the 20th century.

Today, *H. pylori* infection is accepted as the most important chronic bacterial infection and the most important cause of chronic gastritis [6]. It is known that *H. pylori*, which can survive in the stomach for life if left untreated, is associated with diseases such as peptic ulcer, gastritis, gastric cancer, and mucosal-associated lymphoid tissue (MALT) lymphoma [7]. According to the 2005 III. Maastricht Consensus reports of the European *H.pylori*study group, it is less common in developed countries than in developing countries, while this rate is 20%-30% in developed countries, it is 85%-95% in developing countries, and more than 50% of the world population is reported to be infected with this bacterium. *H.pylori* was first described as a group 1 carcinogen by IARC (International Agency for Research on Cancer) in 1994. Therefore, the treatment of *H.pylori* is very important. The primary reservoir of *H.pylori* is the human gastric mucosa. However, the passageway to this region, the factors affecting the passageway, and the rates of passage are still being investigated. Treatment of the infection is combined antibiotic therapy known as "triple therapy", which is administered to patients for 7-10 days. Clarithromycin and amoxicillin (or metronidazole) are administered in combination with proton pump inhibitors, but this treatment does not provide 100% eradication. This treatment protocol was determined by the 1997 report of the European *Helicobacter pylori* Working Group. Initially, the success of this combination was very high, but in recent years success rates have decreased, and recurrences have increased. It is thought that there are many factors such as antibiotic resistance, patient compliance, bacterial virulence and density, geographical features, and genetic differences [8,9]. *H.pylori* was first isolated from the oral flora by Krajden and Shames in 1989 [10], then in dental plaque by Majmudar et al. in 1990 [11], Dowsett et al. in 1999 on the dorsum of the tongue [12], and Oshowo et al. in saliva in 1998 [13], and they thought that the oral cavity could be a reservoir like the gastric mucosa. Although the current systemic antibiotic therapy is effective on the stomach, eradication is not successful because antibiotics cannot penetrate the dental plaque biofilm [14]. This situation has been reported in developed western countries that the treatment success has decreased at unacceptable rates. For this reason, it is thought that the drugs used in the treatment of *H.pylori* are insufficient. These problems encountered in treatment have led researchers to develop new antibacterial drugs to support treatment. More attention is paid to the search for new antibacterial drugs, especially herbal medicines, against *H. pylori*. Therefore, to increase the success rate of the treatment and decrease its recurrence, it is extremely important to search for non-toxic biocompatible herbal sources and to use them in addition to systemic antibiotic treatment and to ensure eradication. Essential oils obtained from plants have been used for various purposes for many years, especially in commercial and scientific fields [15,16]. Herbal oils, which have a wide range of uses, have been the subject of interest of many scientists in recent years and their biological activities have been investigated by examining their structures. Today, the research of medicinal plants and their essential oils is very important both scientifically and economically. Herbal oils are among the drugs used in the treatment from ancient times to the present day. Considering the purpose of use in folk medicine, some biological activities have been scientifically explained as a result of pharmacological studies on these herbal oils [17-19]. Therefore, in our study; various herbal oils, which are known to be good for stomach and gastrointestinal system disorders in folk medicine and for which

there are no adequate studies on *H.pylori* in the literature, were selected and it was aimed to investigate their antimicrobial activities against *H.pylori* as well as their cytotoxicity on human endothelial cells. For this purpose, antimicrobial activities of herbal oils against *H.pylori* were investigated by microdilution method and their cytotoxic properties were evaluated on HUVEC cell line by MTT and LDH methods.

MATERIALS and METHODS

H.pyloriculture

Cells were extracted from cryobank and incubated overnight in 37 °C CO_2 containing incubator, using solid medium. After incubation, petri dishes were evaluated morphologically for the production of different colonies. Cells, collected by inoculation loop were evaluated for motility and contamination with 40X magnification. The cells cultivated in the petri dish were collected by sterile cotton bud which was mixed in medium containing eppendorf and homogenous spreading cultivation was performed to a new petri dish with the same cotton bud for cell passaging and incubated for a night long. Following incubation, bacteria reproduced in the petri dish were collected by steril cotton bud and transferred to a FBS and antibiotic containing liquid medium and left in the mixing incubator (37 °C, 100 rpm) for a night long. Following incubation, cell count was performed in the spectrophotometry device to determine the number of bacteria to be used in the antimicrobial activity tests and cell characterization was also performed.

Gram staining

Ten µl of the medium was dropped to one side of the slide. A little amount of cells were collected from the cells in the petri dish by inoculation loop and dropped over the medium on the slide. After drying, the slide was fixed by crossing over the flame three times. Afterwards, the slide was consecutively dyed with crystal violet for 1 minute, washed with water, dyed with lugol for 1 minute, washed and decolorized with alcohol and lastly washed with aqueous fuchsine for 1 minute, washed and left for drying. After drying, immersion oil was dropped over the preparate, evaluated with 100X magnifying objective and photopgraphs were obtained.

Evaluation of properties of *H.pylori* with urease and cathalase tests

The bacteria obtained from fresh culture were cultivated over urea medium prepared in 1.5 ml eppendorf tubes and left for incubation in 37 °C. Turning of the medium color from yellow to pink was accepted as positivity of the presence of *H.pylori* in the medium. For the cathalase test, colony obtained from fresh culture was placed over the slide and 3% hydrogen peroxide was dropped. Bubble formation was accepted as the positivity fro the presence of *H. pylori*.

H.pyloricell count

In order to produce a time-growth curve of the cells, cell counts were made every 2 hours for 48 hours to create a growth curve of cells. At the end of the 2nd hour from bacteria passaged from solid medium to liquid medium, 20 µL was collected from the bacteria passaged from solid medium to aqueous medium, 980 µL of medium was added and measurement was performed in UV-VIS spectroscopy in 600 nm wave form.

Antimicrobial effects of the herbal oils against *H. pylori*

Minimal Inhibitory Concentration (MIC) test was performed in 96 well plates by microdilution method which was based on reference method recommended by the NCCLS. After determination of inhibition concentrations, minimal bactericidal concentrations were determined by MBC test [20-22].

MIC and MBC tests

MIC values were determined by broth dilution method. To each well of the 96 well plate, 100 µl, 10% FBS containing Brucella broth medium was added for testing concentrations. 90 µl oil and 10 µl DMSO were mixed in sterile eppendorf tube by vortexing and added to the first well that contained 100 µl Brucella broth medium. Two times serial dilutions of the herbal oils, starting from 45% in the first well and including 8 different concentrations were prepared. Then, 10^6/100 µl CFU bacteria suspension was added to each well. Amoxycillin and DMSO were used as positive and negative control, consecutively. At the same time, growth of *H.pylori* in a well without addition of any herbal oil or DMSO oil mixture over medium in another well was evaluated as control group. 25 µg in 8 µl of 250 mg 80 ml Amoxina suspension was added to positive control well. Also 10 µl DMSO was added to negative control well and tested. Plate was left for incubation in 37 °C for 24 hours. After incubation, % oil concentration in the first well without growth was identified as MIC value. For MBC, 10 µl was obtained from wells and cultivated to Brucella agar containing petri dishes with separate compartments for

each concentration and left for 24 hours incubation in 37 °C. After incubation, % oil concentration in the first well without growth was identified as MBC concentration.

Chemicals & Treatment

The herbal oils were obtained from Aksuvital Natural Products Corporation. Essential oils were produced from leaves or flowers by hydrodistillation method according to the type of plant and fixed oils were obtained from squeezing of the oily seeds of the plants by cold press machine. Cytotoxicity of herbal oils were evaluated on the human umbilical vein endothelium cell line (HUVEC ATCC® CRL-1730™).

Determination of cytotoxicity

For assessment of cytotoxicity, HUVEC cell line, cultured with DMEM/F12 culture medium (Thermo Fisher Scientific, Catalog No: 11320033) supplemented with 10% fetal bovine serum (Sigma-Aldrich, Catalog No: F7524) and 1% antibiotic solution (Thermo Fisher Scientific, Catalog No: 15140122) was used. 5 x 10^3 cells per well were seeded into 96-well cell culture plates as triplicates and incubated under humid environment containing 5% CO_2 at 37 °C tissue culture incubator overnight to allow attachment. Stock solutions of herbal oils were prepared by mixing with DMSO at 500 mg/mL concentration, and diluted to 1, 50, 100, 250 ve 500 ug/ml final concentrations by mixing with cell culture medium. Cells were incubated with the media containing herbal oils for 48 hours, and cytotoxicity was measured via LDH and MTT assays. For the evaluation of LDH release, commercial LDH cytotoxicity assay kit was used according to manufacturer's instructions (Cell Bio Labs CytoSelect LDH Cytotoxicity Assay Kit (Catalog No: CBA241)). For negative control, cell culture medium was used whereas for positive control, cells lysed by the addition of Triton X-100 solution provided by the kit was used. The absorbance was measured at 450 nm by a microplate spectrophotometer (BioTek Epoch Microplate Spectrophotometer) [23].

Relative cytotoxicity was calculated according to manufacturer's protocols:

$$\text{Relative Cytotoxicity (\%)} = \frac{OD\,Compound\,treated\,sample \;\; OD\,negative\,control}{OD\,positive\,control \;\; OD\,negative\,control} x100$$

Toxicity was also evaluated by MTT assay, which relies on the reduction of tetrazolium dye MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) to insoluble formazan by the activity of mitochondrial enzyme succinate dehydrogenase. For the determination of toxicity by MTT assay, cells were incubated with herbal oils as defined as in the LDH assay; at the end of the incubation period, the culture medium was replaced with 200 µl medium containing 10% MTT reagent (Sigma-Aldrich, Catalog No:TOX1-1KT). The non-treated group was served as positive control while plain cell culture medium was used as negative control. At the end of incubation time, formazan crystals were dissolved by the addition of solubilization solution provided with the kit. The resulting solutions (violet in color) were transferred to another 96 well plate as triplicates. The absorbance was measured by a microplate spectrophotometer at a wavelength of 570 nm [24-26]. Viability was calculated as given below.

$$\text{Relative Cellular Viability (\%)} = \frac{OD\,Compound\,treated\,sample \;\; OD\,negative\,control}{OD\,positive\,control \;\; OD\,negative\,control} x100$$

Statistical Analysis

The statistical analysis of dose and percentage cytotoxicity analysis were performed using "SPSS software for Windows version 17,0 (Statistical Package for the Social Sciences Inc, Chicago, IL, USA)" software. Continuous variables were defined by "Mean ± Standart Deviation". The 24th and 48th hour values within their own groups were evaluated using Friedman test ($p< 0,05$ was accepted as statistically significant) and comparison of 24th and 48th hour values were performed using Mann Whitney-U test ($p< 0,05$ was accepted as statistically significant). Multiple comparison of the groups were performed by Kruskal-Wallis test and dual comparison of the groups were performed by Mann Whitney-U test.

RESULTS and DISCUSSION

Antimicrobial effects of herbal oils

The MIC and MBC values were found as follows: *Eucalyptus globulus* (MIC: 2.81% v/v, MBK: 5.62% v/v), *Juniperus communis* (MIC: 0.35% v/v). v/ v, MBC: 0.70% v/v), *Rosmarinus officinalis* (MIC: 2.81% h/v, MBC: 5.62% v/v), and *Thymus vulgaris* (MIC: 0.70% h/v, MBC: 1.40% v/ v) (Table 1). As seen in the literature, Esmaili et al. [27] evaluated the anti-*H.pylori*activities of essential oils of *Thymus vulgaris* and *Eucalyptus globulus* by the agar diffusion method and found the anti-*H. pylori* activities for *T. Vulgaris* and *E. globulus* to be 10.8 and 46.4 µg/ mL, respectively. Therefore, *T. Vulgaris* had a better inhibitory effect against *H.pylori* than *E. globulus*, in

Table 1. MIC and MBC values of herbal oils against H.pylori (% v/v).

Herbal Oils	Tested Concentrations of Herbal Oils (% v/v)	MIC (% v/v)	MBC (% v/v)
Eucalyptus globules	45-0.35	2.81	5.62
Juniperus communis		0.35	0.70
Rosmarinus officinalis		2.81	5.62
Thymus vulgaris		0.70	1.40

agreement with our study. In the study of Mahady et al. [28], it was reported *Rosmarinus officinalis* as MIC: 25 µg/mL. The activity of *Juniperus communis* extract was also found to be weak (MIC: 100 µg/mL). Antimicrobial effects of *Rosmarinus officinalis* and *Juniperus communis* against *H. pylori* were observed in our study, which is in accordance with the study. However, *Juniperus communis* was found to be more effective than *Rosmarinus officinalis* in our study.

Cytotoxic effects of herbal oils

It is a desirable feature for a drug that will have the potential to be used in medicine, not only showing the expected effect but also not showing any toxic effects. In this context, as the next step, the cytotoxic effects of 4 herbal oils, which we found antimicrobial effect against *H. pylori*, on the HUVEC cell line were also investigated in our study. The viability of the HUVEC cell line was over 80% as a result of the application of *Eucalyptus globulus* at all concentrations. While the viability rate of *Rosmarinus officinalis* was above 100% at concentrations up to 500 µl/mL, it was observed that the viability rate decreased below 20% at 500 µl/mL concentration. It was observed that the viability of *Juniperus communis* decreased significantly in response to increasing concentrations, while the viability of the HUVEC cell line was observed to be significantly lower at all concentrations of *Thymus vulgaris* . In this situation; on HUVEC cell lines treated with 1, 5, 50, 100, 250 µl/ml concentrations of *Rosmarinus officinalis* and 500 µl/ml *Eucalyptus globulus*, the 24th hour viability rates were found to be the highest. When 48 hour the viability values of cells were compared (Table 2), the viability of cells were were above 80% up to 50 µl/mL concentration of *Eucalyptus globulus*, whereas the viability decreased below %50 significantly at higher doses of oils. Similar results were obtained with the increasing concentrations of *Rosmarinus officinalis* extract oil. It was observed that the viability of *Juniperus communis* was between 60-80% up to a concentration of 50 µl/mL, while at higher doses, the viability rate decreased significantly below 20%. Although the viability of the HUVEC cell line was significantly lower at all concentrations of *Thymus vulgaris* , it was noted that the viability rates increased from 20% to 40% with the increase in concentration. In this situation; The highest viability rates at the 48th hour were detected on HUVEC cell line treated with *Eucalyptus globulus* at 1, 5, 50, 100, 250 µl/ml concentrations and *Thymus vulgaris* at 500 µl/ml concentration.

Cabral et al. [29],. investigated the cytotoxicity of the essential oil of *Juniperus communis* subsp. alpina (Suter) čelak needles by MTT method, and they reported the essential oil decreased the human keratinocyte HaCaT cell viability as 65.65% ± 1.195 (at 1.25 µl/mL concentration); 21.47 ± 4.078 (at 0.64 µl/mL), and 1.59% ± 1.206 (at 0.32 µl/mL concentration) compared to the control. Similarly, in our study, it was observed that the viability rate of the HUVEC cell line that we studied in response to the increasing concentrations of J.communis decreased.

Döll-Boscardin et al. [30] investigated the cytotoxic effects of the essential oils of young and mature leaves of *Eucalyptus benthamii* on various cancer cells and reported that no cytotoxic effect was observed on the HeLa cell line, but a very weak cytotoxic effect on Jurkat cells. In our study, as a result of the application of E.globulus on the HUVEC cell line at all concentrations, the viability rate was above 80% at the end of the 24th hour. When the exposure time was prolonged, it was observed that the viability rate decreased below 50% at concentrations above 50 µl/mL at the end of the 48th hour.

Cattaneo et al. [31] investigated the efficacy of hydroalcoholic extracts of *Rosmarinus officinalis* on human

Table 2. Statistical values of the viability of herbal oils on HUVEC cell line measured by MTT method.

C	1 µl/ml (n:3)	5 µl/ml (n:3)	50 µl/ml (n:3)	100 µl/ml (n:3)	250 µl/ml (n:3)	500 µl/ml (n:3)	p*
			Eucalyptus globules				
24. hr	0.90 ± 0.01	0.87 ± 0.02	0.94 ± 0.02	0.96 ± 0.02	0.98 ± 0.01	0.98 ± 0.02	0.021
48. hr	0.97 ± 0.01	0.85 ± 0.01	0.81 ± 0.06	0.53 ± 0.01	0.44 ± 0.01	0.12 ± 0.01	0.012
p**	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
			Juniperus communis				
24. hr	1.21 ± 0.06	1.10 ± 0.03	0.90 ± 0.24	0.70 ± 0.15	0.25 ± 0.04	0.14 ± 0.02	0.012
48. hr	0.75 ± 0.01	0.60 ± 0.01	0.75 ± 0.01	0.15 ± 0.02	0.03 ± 0.01	0.06 ± 0.01	0.010
p**	< 0.001	< 0.001	> 0.05	< 0.001	< 0.001	< 0.001	
			Rosmarinus officinalis				
24. hr	1.33 ± 0.08	1.18 ± 0.06	1.15 ± 0.06	1.15 ± 0.07	1.15 ± 0.02	0.12 ± 0.01	0.079
48. hr	0.86 ± 0.02	0.73 ± 0.01	0.53 ± 0.01	0.51 ± 0.01	0.04 ± 0.02	0.07 ± 0.01	0.010
*p***	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
			Thymus vulgaris				
24. hr	0.26 ± 0.13	0.13 ± 0.10	0.35 ± 0.06	0.38 ± 0.03	0.41 ± 0.02	0.46 ±0.07	< 0.001
48. hr	0.11 ± 0.08	0.13± 0.08	0.13± 0.08	0.26 ± 0.20	0.41 ± 0.20	0.46 ± 0.11	< 0.001
p**	0.005	0.005	< 0.001	0.23	0.35	0.89	

C: Concentrations

* Friedman test

** Mann-Whitney U test

melanoma A375 cell line viability by MTT and Trypan blue methods. The results showed that *R.officinalis* extract decreased cell proliferation in a time and dose-dependent manner. In our study, it was observed that the viability rate was above 100% despite the application of R.officinalis essential oil at very high doses (up to 500 µl/mL) at the end of the 24th hour, and when the exposure time was extended to 48 hours, the viability rate gradually decreased with increasing doses.

Ayesh et al. [32] investigated the effects of *Thymus vulgaris* L. ethanol extract on cellular viability and cytotoxicity by MTT and LDH methods on THP-1 leukemia cell line and freshly isolated peripheral blood mononuclear cells (PBMCs). As a result, they reported that the extract significantly reduced the number of viable THP-1 and PBMCs in a concentration-dependent manner. In our study, it was observed that *T. vulgaris* herbal oil significantly reduced the viability of the HUVEC cell line at all concentrations.

When the % cytotoxicity rates of the oils measured by the LDH method on the HUVEC cell line were compared at the 24th hour (Table 3), *Thymus vulgaris* had the highest cytotoxicity at all concentrations, and it was observed the cytotoxicity rates increased from 60% to over 75% with the increase in dose. The cytotoxicity of *Juniperus communis* and *Rosmarinus officinalis* increased with increasing concentration, but the cytotoxicity of *Eucalyptus globulus* was observed at the lowest level at all concentrations and did not exceed %10-15. Thus, at all concentrations, *Thymus vulgaris* was detected as the herbal oil with the highest cytotoxicity at the 24th hour on the HUVEC cell line. When the % cytotoxicity rates of the oils measured by the LDH method on the HUVEC cell line were compared at the 48th hour (Table 3), it was shown that the cytotoxic effects of all oils increased significantly at increasing concentrations. In this situation; *Eucalyptus globulus* at concentrations of 1,100 *µl*/ml; *Thymus vulgaris* at concentrations of 5,500 *µl*/ml; and *Rosmarinus officinalis* at concentrations of 50, 250 *µl*/ml on HUVEC cell line were found to have the highest toxicity at the 48th hour.

Table 3. Statistical values of cytotoxic activities of herbal oils measured by LDH method on HUVEC cell line.

C	1 µl/ml (n: 3)	5 µl/ml (n: 3)	50 µl/ml (n: 3)	100 µl/ml (n: 3)	250 µl/ml (n: 3)	500 µl/ml (n: 3)	p*
			Eucalyptus globules				
24. hr	0.11 ± 0.01	0.11 ± 0.01	0.14 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.12 ± 0.01	0.024
48. hr	0.25 ± 0.01	0.26 ± 0.01	0.35 ± 0.01	0.68 ± 0.17	0.44 ± 0.01	0.65 ± 0.01	0.019
p**	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	
			Juniperus communis				
24. hr	0.15 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.21 ± 0.01	0.23 ± 0.01	0.60 ± 0.01	0.012
48. hr	0.10 ± 0.01	0.16 ± 0.01	0.19 ± 0.01	0.29 ± 0.01	0.30 ± 0.01	0.57 ± 0.01	0.010
p**	0.05	0.5	0.05	0.05	0.05	0.12	
			Rosmarinus officinalis				
24. hr	0.18 ± 0.01	0.21 ± 0.01	0.22 ± 0.01	0.23 ± 0.20	0.48 ± 0.01	0.63 ± 0.01	0.026
48. hr	0.14 ± 0.01	0.25 ± 0.01	0.37 ± 0.01	0.50 ± 0.01	0.64 ± 0.02	0.73 ± 0.02	0.010
p**	0.05	0.05	0.05	0.05	0.05	0.05	
			Thymus vulgaris				
24. hr	0.60 ± 0.01	0.57 ± 0.01	0.65 ± 0.01	0.65 ± 0.01	0.65 ± 0.01	0.74 ± 0.01	0.017
48. hr	0.04 ± 0.01	0.43± 0.10	0.36 ± 0.01	0.66 ± 0.01	0.46 ± 0.40	0.82± 0.02	0.055
p**	0.05	0.05	0.05	0.27	0.50	0.05	

K: Concentrations

* Friedman test

** Mann-Whitney U test

Comparisons of the efficacy of herbal oils on the HUVEC cell line evaluated by MTT and LDH methods are given in Figures 1-4.

In Figure 1; the highest viability rates at 24 hours were determined on the HUVEC cell line treated with *Rosmarinus officinalis* at a concentration of 1, 5, 50, 100, 250 µl/ml and *Eucalyptus globulus* at 500 µl/ml.

In Figure 2; the highest viability rates at 48 hours were determined on the HUVEC cell line treated with *Eucalyptus globulus* at concentrations of 1, 5, 50, 100, 250 µl/ml and *Thymus vulgaris* at 500 µl/ml concentration.

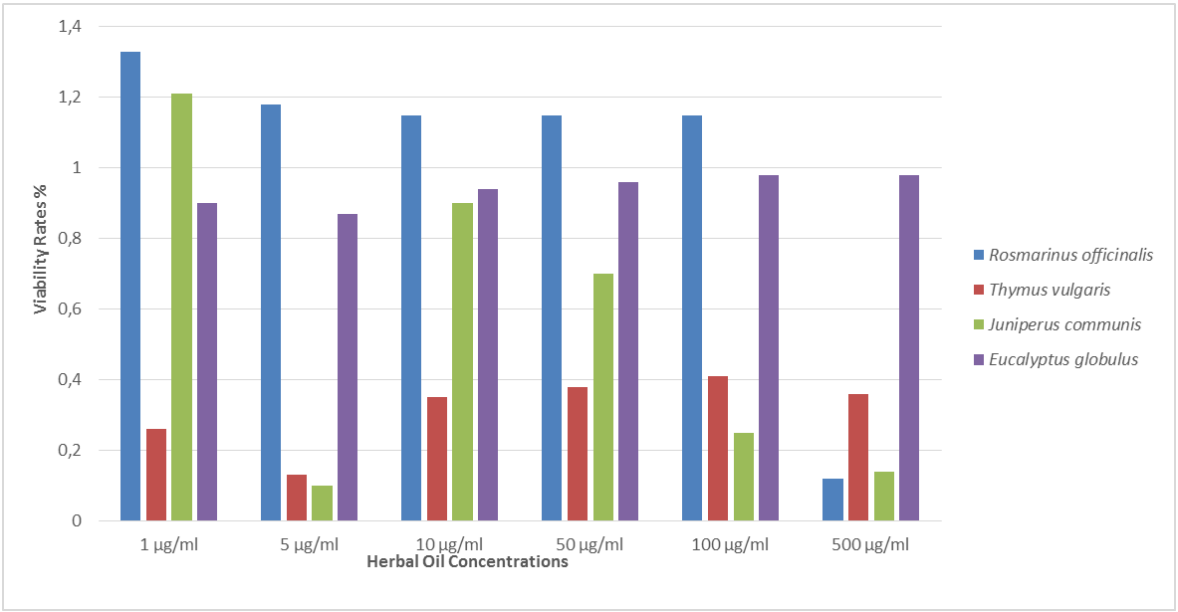

Figure 1. Comparison of the % viability of vegetable oils on HUVEC cell line at the 24th hour measured by MTT method.

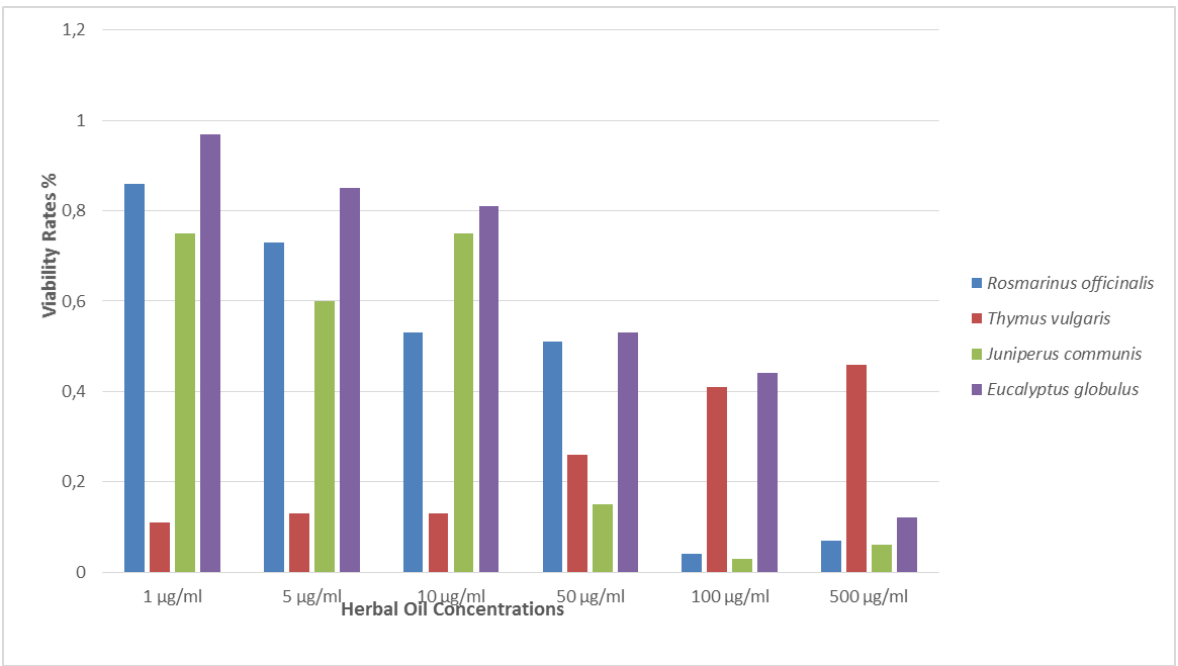

Figure 2. Comparison of the % viability of vegetable oils on HUVEC cell line at the 48th hour measured by MTT method.

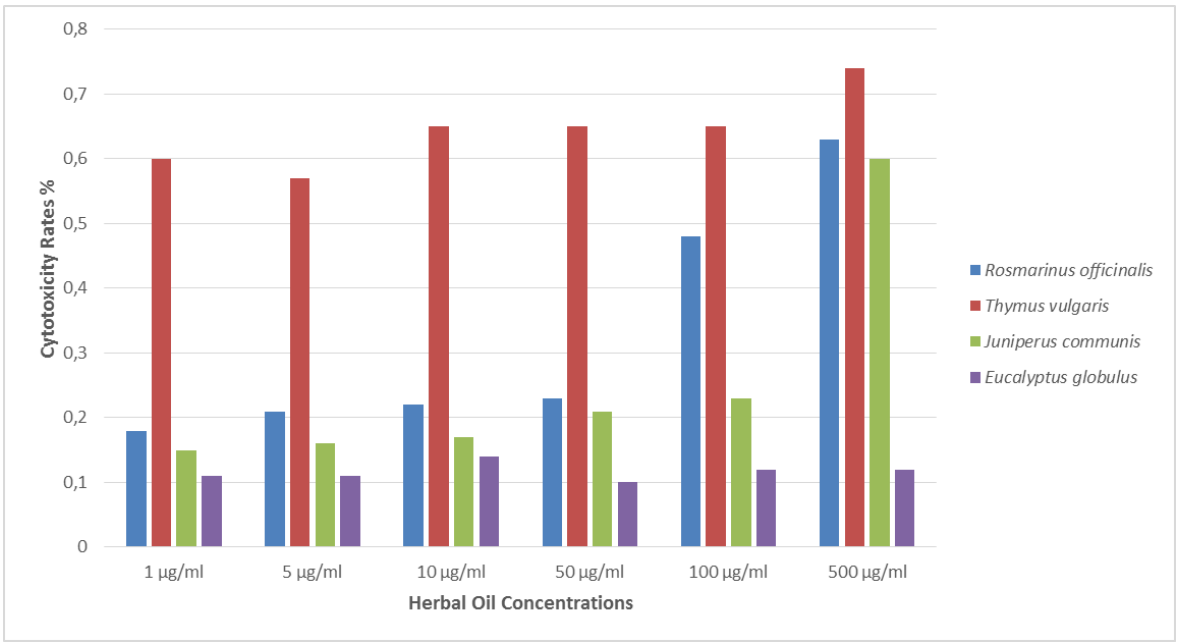

Figure 3. Comparison of % cytotoxicity rates of vegetable oils on HUVEC cell line at the 24th hour measured by LDH method.

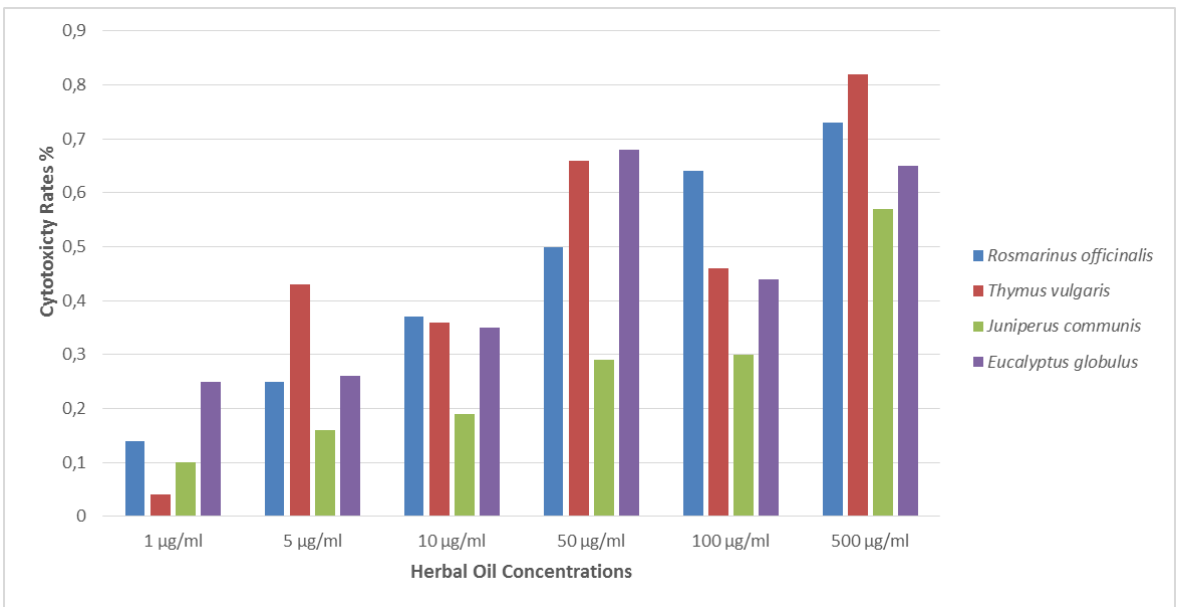

Figure 4. Comparison of % cytotoxicity rates of vegetable oils on HUVEC cell line at the 48th hour measured by LDH method.

In figure 3; at all concentrations, *Thymus vulgaris* was detected as the highest cytotoxic herbal oil against Huvec cell line at 24. hour.

In Figure 4, *Eucalyptus globulus* at concentrations of 1,100 µl/ml, *Thymus vulgaris* at concentrations of 5,500 µl/ml, and *Rosmarinus officinalis* at concentrations of 50, 250 µl/ml on HUVEC cell line were found to have the highest toxicity at 48 hours.

CONCLUSION

Vegetable oils have been used for thousands of years in food preservation, medicine, alternative medicine, and natural treatments. As a result of pharmacological studies on these vegetable oils, considering their intended use, some of their biological activities have been scientifically explained. Studies show that vegetable oils, which have a wide range of uses, have antimicrobial activities against various microorganisms. In our study, 4 different vegetable oils, which are generally known to be good for stomach and gastrointestinal system disorders and for which there are no adequate studies in the literature, were selected, and their antimicrobial activities against *H.pylori* and their cytotoxic effects on HUVEC cell line were examined. As a result, we believe that the findings of our study will contribute to the literature. The fact that *Eucalyptus globulus*, *Juniperus communis*, *Rosmarinus officinalis*, and *Thymus vulgaris*, which have been used in the treatment of gastrointestinal diseases in folk medicine for many years, have shown significant antibacterial activity against *H.pylori*constitutes very valuable findings for further research. Unlike our study, the cytotoxic effects of selected vegetable oils on healthy cell lines have not been adequately addressed in the literature.

In the light of the information we presented in our study, can be concluded that further in vitro and in vivo studies aiming to determine the efficacy and toxicity of the active chemical compounds of vegetable oils, which was found to have anti H pylori activity, on strains with different virulence, will contribute significantly to the development of adjuvant herbal medicines that will increase the eradication rates.

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