TÜSEB
TÜRKİYE SAĞLIK ENSTİTÜLERİ BAŞKANLIĞI

JHIT
JOURNAL OF HEALTH INSTITUTES OF TURKEY
TÜRKİYE SAĞLIK ENSTİTÜLERİ BAŞKANLIĞI DERGİSİ

Urtica dioica, *Silybum marianum* ve *Cynara scolymus* Ekstraktlarının Antikanser, Antibakteriyel ve Antioksidan Aktivitesi

Rabia Yılmaz[1], Hilal Çalık[2], Hatice Feyzan Ay[3], Fatih Erci[4], Rabia Çakır Koç[5]

Özet

Mide kanseri, dünya çapında en yaygın kanser türlerinden biridir ve kansere bağlı ölümlerin önde gelen nedenlerindendir. Bu kanser türü, moleküler düzeyde yeterince anlaşılamamış bir karsinogeneze sahip, agresif ve heterojen bir hastalıktır. Bu nedenle etkili ilaç tedavi stratejilerine yönelik yapılan araştırmalar, hastalığın tedavisinde önemli bir rol oynamaktadır. Bu etkili tedavi stratejilerinden biri, yan etkileri düşük ve içeriğinde biyolojik olarak aktif birçok bileşik bulunan bitkisel bazlı terapötiklerdir. Bu çalışmada *U. dioica*, S. marianum ve *C. scolymus* bitkisel ekstraktlarının antikanser aktivitelerinin değerlendirilmesi için L929, AGS ve SH-SY5Y hücre hatlarındaki hücre canlılığına etkisi XTT testi ile analiz edilmiştir. Ekstraktların antibakteriyel ve antioksidan aktiviteleri için sırasıyla agar kuyusu difüzyon testi ve CUPRAC yöntemi kullanılmıştır. *U. dioica* ve *S. marianum* ekstraktlarının AGS ve SH-SY5Y kanser hücrelerinin canlılığı üzerinde önemli bir etki göstermediği görülmüştür. *C. scolymus* ekstraktı ise tüm konsantrasyonlarda AGS kanser hücreleri üzerinde yüksek antikanser etki göstermiş ancak SH-SY5Y hücreleri üzerinde hiçbir etkiye neden olmamıştır. *U. dioica* ve *C. scolymus* ekstraktları sırasıyla *S. aureus* ve *B. cereus* bakterilerine karşı antibakteriyel aktivite sergilemiştir. *S. marianum* ekstraktı herhangi bir antibakteriyel etki göstermemiştir. CUPRAC testi sonucunda *U. dioica* ve *S. marianum* ekstraktlarının güçlü antioksidan aktivite gösterdiği bulunmuştur. Sonuç olarak, elde edilen sonuçlar enginar olarak bilinen *C. scolymus* eksraktının antibakteriyel ve mide kanseri hücrelerinde antikanser terapötik potansiyelini ortaya koymuştur. Ancak, bu ekstraktın terapötik özelliklerinin daha iyi anlaşılması için daha fazla araştırmaya ihtiyaç vardır.

Anticancer, Antibacterial and Antioxidant Activity of Urtica dioica, Silybum marianum and Cynara scolymus Extracts

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Abstract

Gastric cancer (GC) is one of the most prevalent cancer types worldwide and one of the leading causes of cancer-related deaths. Gastric cancer is an aggressive and heterogeneous disease with a poorly understood carcinogenesis at the molecular level. Therefore, the research for effective drug therapy strategies plays a significant role in treating the disease. One of these effective treatment strategies is herbal-based therapeutics, which have low side effects and contain many biologically active compounds. In this study, the effect of U. dioica, S. marianum and C. scolymus herbal extracts on cell viability in L929, AGS and SH-SY5Y cell lines was analyzed by XTT test to evaluate the anticancer activities. Antibacterial and antioxidant activities of extracts were determined by the agar well diffusion test and CUPRAC method, respectively. We found that U. dioica and S. marianum extracts showed no significant effect on the viability of AGS and SH-SY5Y cancer cells. C. scolymus extract demonstrated strong anticancer activity on AGS cancer cells at all concentrations but had no effect on SH-SY5Y cells. U. dioica and C. scolymus exhibited antibacterial activity against S. aureus and B. cereus, respectively. No antibacterial activity was found in S. marianum extract. U. dioica and S. marianum extracts have shown strong antioxidant activity in CUPRAC assay. In conclusion, the obtained results revealed the antibacterial and anticancer therapeutic potential of C. scolymus extract known as artichoke in gastric cancer cells. However, more research is required to better explain the therapeutic properties of these extracts.

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Geliş Tarihi: 20.03.2022
Kabul Tarihi: 26.04.2022

Received Date: *20.03.2022*
Accepted Date: *26.04.2022*

Anahtar Kelimeler:
Urtica dioica,*Silybum marianum*,*Cynara scolymus*,Antikanser aktivite,Antibakteriyel aktivite

Keywords:
Urtica dioica, Silybum marianum, Cynara scolymus, Anticancer activity, Antibacterial activity

DOI:10.54537/tusebdergisi.1089792

INTRODUCTION

Cancer is a worldwide health problem and is the leading cause of death of all deaths estimated to the World Health Organization (WHO) 2019 (Siegel et al., 2022). Gastric cancer is a major disease worldwide that is responsible for more than one million new cases and an estimated 769,000 deaths (equal to one in 13 deaths globally) in 2020. With these numerical data, it ranks 5th in terms of incidence and 4th in terms of mortality worldwide. According to GLOBOCAN 2020 Turkey data, gastric cancer ranks 5th in incidence with 13,075 cases compared to other cancer types and 2nd in mortality rate with 10,789 deaths. The reason for the high incidence and mortality of gastric cancer is the recognition of the prognosis at a late stage and the high cellular and molecular heterogeneity of gastric cancer (Seeneevassen et al., 2021). One reason for the heterogeneity of gastric cancer is several histological classifications (Cisło et al., 2018). In addition, many different regulatory genes play a role in gastric cancer molecularly and may cause variable prognosis and heterogeneity.

Although gastric cancer is generally caused by environmental risk factors, genetic factors, and epigenetic factors, the main factor is *Helicobacter pylori* (*H. pylori*) infection, which is an environmental factor. *H. pylori* infects almost 50% of the world's population and induces chronic inflammation of the gastric mucosa. However, only a certain percentage of the infection rate induces gastric cancer. The reason for this; in addition to other risk factors such as the genetics of the host, *H. pylori* strain characteristics, environmental factors such as alcohol consumption and salty eating are also viable factors in the formation of gastric cancer. Many DNA mutations have been identified as the genetic factor causing gastric cancer. The gene most frequently mutated in common in all gastric cancer subtypes is the tumor suppressor gene TP53, which plays a significant role as a key regulator of cell genomic stability (Guo et al., 2017).

The use of herbal medicinal in cancer treatment is one of the best options to treat and/or prevent the disease. The most important reason for this, they can be effective against many cancer types owing to the diversity of active substances in plants that may interact with many signal pathway mechanisms. Another important reason is natural agents derived from plants show high therapeutic efficacy with reduced side effects (Dehelean et al., 2021). Herbal agents can be extracted and used alone or in combination with other anticancer treatments. They can be obtained naturally and inexpensively, and at the same time, biologically active compounds are more abundant in herbal-based drugs than synthetic drugs (Hassan, 2020). Approximately 50% of the Food and Drug Administration (FDA) approved anticancer therapeutic agents are derived from natural products or their derivatives (Newman & Cragg, 2016). For these reasons, the identification of effective herbal anticancer agents and molecules is very important for cancer treatment.

Urtica dioica (*U. dioica*), commonly known as dead nettle, is the most common species of the Urticaceae family. It is one of the most studied herbs worldwide and has a long history of a variety of health problems. Many studies have revealed that *U. dioica* has various pharmacological effects such as antiviral, antimicrobial, antioxidant, anti-inflammatory, antiaging, analgesic, anti-inflammatory, antidiabetic, immunomodulator, antimutagenic, anticancer (cervical, breast, epidermoid, colon, gastric, prostate and lung cancer) activity and nephroprotective (Taheri et al., 2022). These properties of *U. dioica* are assumed to result from a wide variety of bioactive natural compounds found in various parts of the plants, such as phenolic compounds (including flavonoids, tannins, coumarins and lignans), sterols, isolectins (Esposito et al., 2019).

Silybum marianum (*S. marianum*), commonly known as milk thistle, has been used for therapeutic purposes for many years due to its hepatoprotective, anti-diabetic, anti-inflammatory, analgesic, neuroprotective, antioxidant and antitumor effects (Marmouzi et al., 2020). The active ingredients of *S. marianum* have been investigated for the prevention and treatment of cancer. These ingredients have been reported to have anticarcinogenic effects for colon, breast, prostate, bladder and skin cancers. The main active ingredient of *S. marianum* is a flavonolignan complex silymarin. Most of the anticancer activity of *S. marianum* is attributed to the silybin molecule in the silymarin (Bittencourt et al., 2020). It has been reported that silymarin exerts its anticancer effect by interfering with cell cycle regulators and the expression of apoptosis-related proteins, thereby inducing apoptosis (Fallah et al., 2021).

Cynara cardunculus Scolymus L. (*C. scolymus*), belonging to the Asteraceae family, is an essential food for the Mediterranean population known as an artichoke. In studies, it has been reported that extracts obtained from artichoke flower leaves have hepatoprotective, anti-hypercholesterolemic, hypoglycemic, anti-microbial, anti-cancer and antioxidant effects (Abdel-Moneim et al., 2021). *C. scolymus* and its components have been recognized as potential phytotherapeutic agents for a variety of conditions, particularly cardiovascular, hepatic and gastric diseases. Active components of the *C. scolymus* are monocafeoylquinic and dicaffeoylquinic acids (e.g., chlorogenic acid and cynarin) and polyphenolic compounds such as flavonoids (e.g., luteolin, apigenin, and their glycosides and rutinosides) (Ben Salem et al., 2015). *C. scolymus* contain many powerful polyphenol-type antioxidants that may contribute to the prevention and treatment of prostate cancer, breast cancer and leukemia. It has been shown that *C. scolymus* thanks to its polyphenolic compounds such as rutin, quercetin and gallic acid causes a decrease in cell viability, inhibition of cell growth and initiation of apoptotic mechanisms in various cancer cells (Hassabou et al., 2020). In addition, it has been reported that artichoke extract does not show any significant side effects after continuous drug use for several months and is very safe for the human body (Tang et al., 2017).

With all this information, the identification of medicinal plants and their compounds as cancer treatment strategies is very significant to develop effective treatments. To the best of our knowledge, the anticancer activities of *C. scolymus*, *S. marianum* and *U. dioica* plant extracts in SH-SY5Y neuroblastoma cells, and *C. scolymus* in AGS gastric cancer cells have not been investigated before. This study aims to examine the cytotoxic activity of *C. scolymus*, *S. marianum* and *U. dioica* plant extract for anticancer activity in AGS gastric cancer, SH-SY5Y neuroblastoma and L929 fibroblast cells; to examine the antibacterial activities of these plant extracts in *Bacillus cereus*, *Staphylococcus aureus*, *Escherichia coli*, and *Salmonella Typhimurium* and finally to examine their antioxidant activities.

MATERIALS AND METHODS

Materials and Reagents

L929 mouse fibroblast (CRL-6364), AGS human gastric cancer (CRL-1739), and SH-SY5Y human neuroblastoma cells (CRL-2266) were obtained from American Type Culture Collection (ATCC). Dulbecco's Modified Eagle's Medium/Nutrient Mixture F-12 (DMEM-F12), fetal bovine serum (FBS), Trypsin-EDTA, and Trypan Blue solution were purchased from Gibco® (MT, USA), the penicillin-streptomycin antibiotic solution from PAN Biotech. Phosphate buffered saline (PBS), phenazine methosulfate (PMS), and XTT salt from Santa Cruz Biotechnology® (Texas, USA), and the plant extracts were gifted from Immunat Herbal Pharmaceuticals (Mugla, Turkiye).

Cell Culture

The L929 mouse fibroblast cells, AGS human gastric cancer cells, and SH-SY5Y human neuroblastoma cells were cultured in DMEM/F12 containing 10% FBS and 1% penicillin/streptomycin solution at 37°C in 5% CO_2. At confluency, cells were trypsinized and centrifuged at 1000 rpm for 5 min. Then, the supernatant was removed, the cells were counted and used for the experiments (Karavelioglu & Cakir Koc, 2021).

Cell Viability Assay

The *in vitro* cytotoxicity effects of plant extracts of *U. dioica, S. marianum* and *C. scolymus* on L929, AGS, and SH-SY5Y cell lines were determined using the XTT method. The cells were seeded into 96-well plates ($1x10^3$ cells/well) and incubated for 24 hours at 37°C in 5% CO_2 for cell attachment. Then, the cells were treated with different concentrations (0, 2.5, 5, 10, and 20 µg/ml) of plant extracts for 24 hours. The culture medium was removed and replaced with a fresh medium containing XTT salt. The cells were incubated for 4 hours at 37 °C in 5% CO_2, and optical density was measured at 450 nm wavelength. Cell culture medium was used as a negative control and the experiment was conducted in at least triplicate. The percentage of cell viability was calculated with the obtained data (Sungu Misirlioglu et al., 2020).

Determination of Antibacterial Activity

Lyophilized cultures of both gram-positive *Bacillus cereus* (ATCC 11778) and *Staphylococcus aureus* (ATCC 25923) and gram-negative *Escherichia coli* (ATCC 25922) and *Salmonella typhimurium* (ATCC 14028) bacteria used in the study were obtained from Microbiologics Inc. (Saint Cloud, MN, United States of America). Before the experiment, bacteria were kept in Nutrient Broth containing 20% glycerol at −18°C. Antibacterial activity was determined by the agar well diffusion test. The microorganisms obtained from stock cultures were transferred to tubes with Mueller Hinton Broth medium and incubated for 18-24 hours at 37°C. The bacterial suspension was adjusted to 0.5 Macfarland standard (1.5 x 10^8) colony-forming units KOB (CFU)/mL and spread on cation-adjusted Mueller Hinton agar (Lab M, UK) plates. 100 µl of the suspension was placed into the wells formed on an agar plate with a 6 mm diameter. Then, the agar plates were incubated at 37°C for 24 hours. The zone of inhibition formed around the wells was calculated in millimeters. All experiments were carried out in triplicate (Jahan et al., 2021).

Measurement of Total Antioxidant Capacity

The CUPRAC method was used to examine the antioxidant activities of the herbal extracts used. Solutions of copper (II) chloride ($CuCl_2$), ammonium acetate (NH_4Ac), and neocuproin (Nc) were used for the CUPRAC method. Trolox was used as a standard and the herbal extracts in different concentrations were added to 1 mL of $CuCl_2$ solution, Nc solution, and NH_4Ac buffer solutions. Then the solutions were incubated for 30 min at room temperature and in the dark. The absorbance values were measured in a microplate reader at a wavelength of 450 nm, and graphs were created with the extracts corresponding to the Trolox calibration curve (Apak et al., 2006).

Statistical Analysis

The GraphPad software was used to perform statistical analysis on the obtained data. Mean values were evaluated statistically significant at $p<0.05$ and $p<0.001$ by comparing experimental and control means and data were reported as mean ± standard deviation.

RESULTS

Antiproliferative Effects of Plant Extracts

The cytotoxic effects of *U. dioica*, *S. marianum*, and *C. scolymus* extracts on L929, AGS, and SH-SY5Y cells were examined by XTT cell viability assay and the results were compared with the untreated control group (Figure 1). *U. dioica* plant extract showed no effect on the viability of AGS, SH-SY5Y, or L929 cells after 24 h incubation in comparison to the control. It decreased the viability of SH-SY5Y cells by 20% at 20 µg/ml concentration ($p<0.001$). *S. marianum* extract had no significant effect on the viability of AGS and SH-SY5Y, however, it reduced the viability of L929 to 45% ($p<0.05$) and 37% ($p<0.001$) at 10 µg/ml and 20 µg/ml doses, respectively. *C. scolymus* extract demonstrated anticancer effects on AGS at all concentrations ($p<0.001$) but had no effect on SH-SY5Y ($p<0.05$). Also, it showed dose-dependent toxicity on non-cancerous L929 ($p<0.05$).

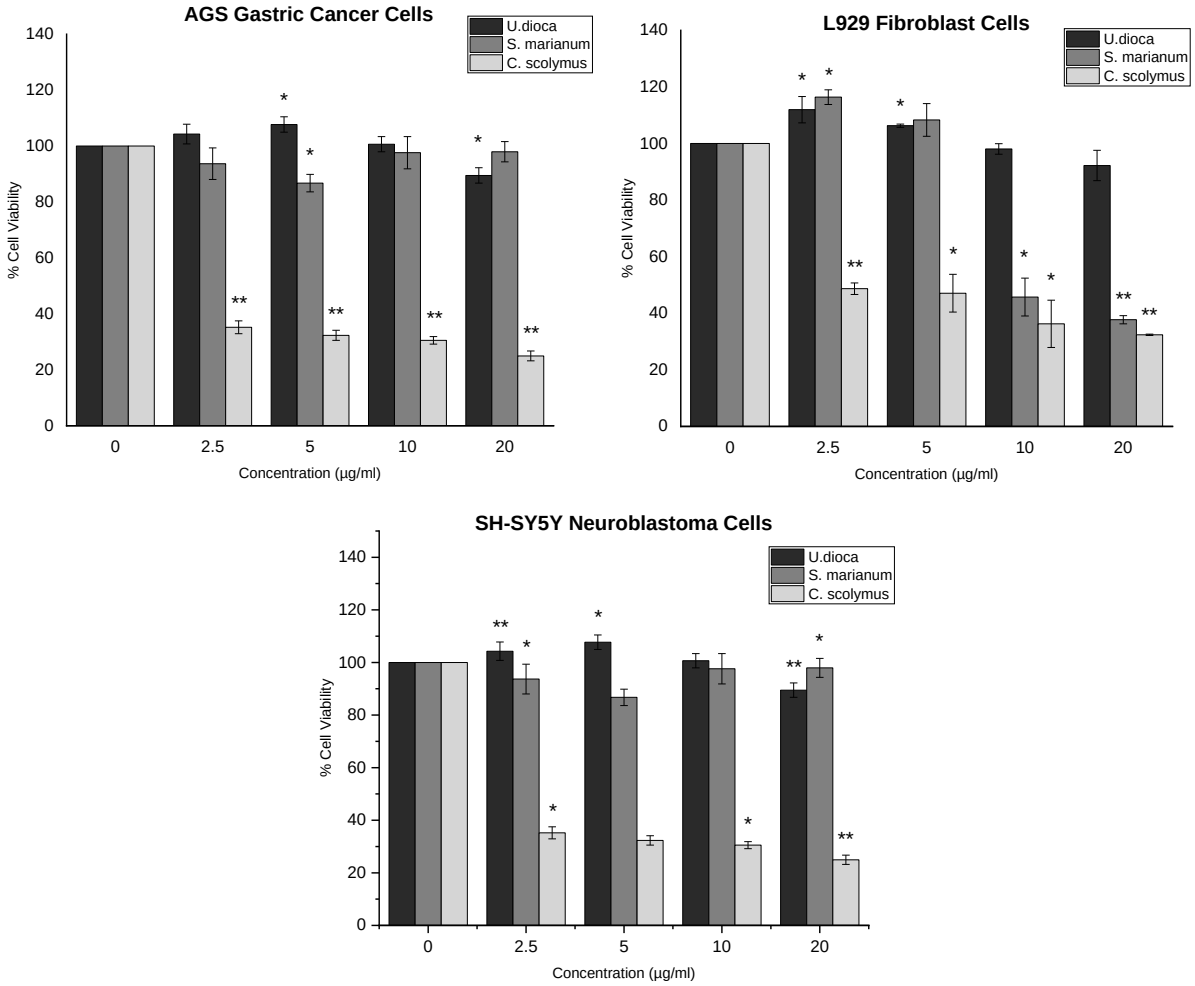

Figure 1. The cell viability of L929, AGS, and SH-SY5Y after 24 h incubation with *U. dioica, S. marianum, and C. scolymus* plant extracts at various concentrations. *$p<0.05$ and **$p<0.001$ compared with the control.

Antibacterial Activities of Plant Extracts

In vitro susceptibilities of the selected microorganisms against the samples were determined by the agar well diffusion method. The antibacterial activity of plant extracts was evaluated using the diameter of the inhibition zone (mm) around the discs and summarized in Table 1. The results indicated that *U. dioica* extract exhibited activity against *S. aureus*, but did not show any activity against *B. cereus, S. typhimurium,* and *E. coli*. Surprisingly, no antibacterial activity was found in *S. marianum* extract. *C. scolymus* extract only showed antibacterial activity against *B. cereus*.

Table 1: Inhibition zones (diameter) in mm of *U. dioica*, *S. marianum*, and *C. scolymus* plant extracts on tested bacteria by agar well diffusion method. The individual data points were expressed in the form of mean ± standard deviation (mean ± SD). ND denotes no antibacterial activity.

Table 1. Inhibition zones (diameter) in mm of *U. dioica, S. marianum, and C. scolymus* plant extracts on tested bacteria by agar well diffusion method. The individual data points were expressed in the form of mean ± standard deviation (mean ± SD). ND denotes no antibacterial activity.

Plant extracts	*S. aureus*	*B. cereus*	*E. coli*	*S. typhimurium*
U. dioica	7.97 ± 0.20	ND	ND	
S. marianum	ND	ND	ND	ND
C. scolymus	ND	7.95 ± 046	ND	ND

Total Antioxidant Capacities of Plant Extracts

Trolox equivalents for 2.5 µg/mL, 5 µg/mL, 10 µg/mL, and 20 µg/mL concentrations of *U. dioica* extract were determined to be 585 µM, 1031 µM, 1725 µM, and 2540 µM, respectively. *S. marianum* extract concentrations of 5 µg/mL, 10 µg/mL, and 20 µg/mL were found to have Trolox equivalents of 610 µM, 1009 µM, and 1640 µM, respectively. *C. scolymus* extract concentrations of 2.5 µg/mL, 5 µg/mL, and 10 µg/mL were found to have Trolox equivalents of 90 µM, 144 µM, and 208 µM, respectively. When the extract concentrations of 5 µg/ml and 10 µg/ml are compared, it is clear that the *U. dioica* plant extract has the highest total antioxidant capacity and the *C. scolymus* plant extract has the lowest antioxidant capacity (Figure 2).

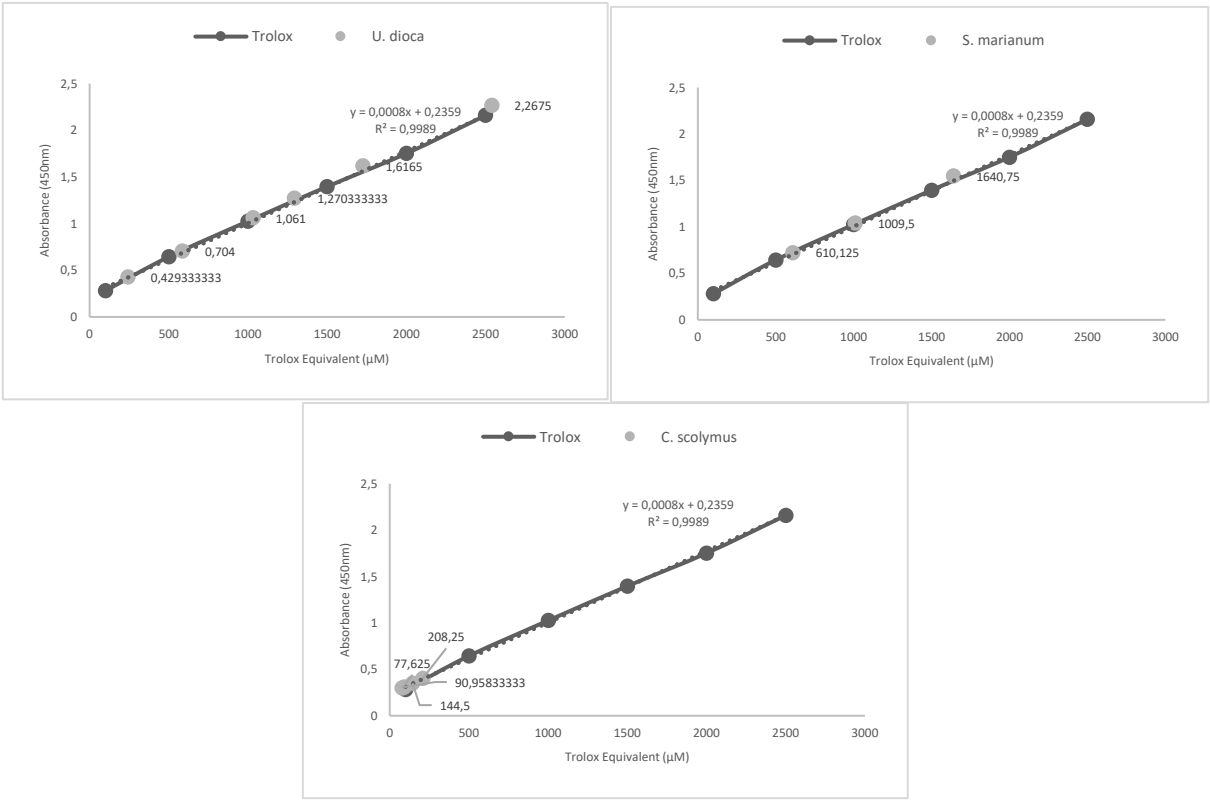

Figure 2. Total antioxidant capacity of *U. dioica, S. marianum,* and *C. scolymus* extracts.

DISCUSSION

Gastric cancer is the fourth leading cause of cancer death in the world with the incidence of more than 1 million cases every year and it remains to be a global health problem. Therefore, developing new therapeutic agents against gastric cancer is essential.

Herbal medicines are gaining attention as therapeutic approaches for the prevention and treatment of many cancers, including gastric cancer because many active ingredients in these natural therapeutics create a synergistic effect and gain the potential to interact with many different cancer-related signalling pathways (Pezzani et al., 2019). Therefore, several researchers have focused on exploring new effecive natural therapeutics rather than synthetic drugs. Although the anticancer activity of *U. dioica*, *C. scolymus*, and *S. marianum* has been studied on several different cancer types, no report on their anticancer activity against AGS and SH-SY5Y cells has yet been published.

Our results showed that *U. dioica* extract at 5 to 20 µg/ml concentrations had no significant effect on the viability of these cancer cells after 24 h incubation. Mohammadi et al. investigated the cytotoxic effects of 10-60 µg/ml concentrations of *U. dioica* extract on MDA-MB-468 human breast cancer and L929 fibroblast cells, and after 24- and 48-hours incubation, they found that *U. dioica* extract was found to have a cytotoxic effect on MDA-MB-468 cells while not causing damage to L929 cells (Mohammadi et al., 2016). Ghasemi et al. demonstrated that *U. dioica* extract at different concentrations ranging from 500 to 2000 µg/ml decreased the viability of human gastric (MKN45) and colon (HT29) cancer cells after 48- and 72-hours incubation while having cytotoxic effects on normal cells at 1000 and 2000 µg/ml dosages (Ghasemi et al., 2016). Therefore, in order to clarify the anticancer activity of *U. dioica* extract on AGS and SH-SY5Y cancer cells, it is necessary to

treat the extract with cells at higher concentrations and longer incubation times.

Different studies in the literature investigated the anticancer effects of *C. scolymus* extract on MDA-MB231 human breast cancer cells and it was found that *C. scolymus* extract inhibited breast cancer cell proliferation in a dose-dependent manner (Mileo et al., 2012; Mileo et al., 2015). Our study is the first to investigate the antiproliferative effects of *C. scolymus* extract on L929, AGS, and SH-SY5Y cells. We found that *C. scolymus* extract showed anticancer activity on AGS cells after 24 h incubation. In addition, the antiproliferative effect of *S. marianum* extract on the L929, AGS, and SH-SY5Y cell lines has never been studied. Silymarin, one of the active ingredients of *S. marianum*, was found to significantly reduce the AGS cell viability in a dose-dependent manner (Kim et al., 2019). However, our findings demonstrated that *S. marianum* extract with doses ranging from 2.5 to 20 µg/ml exhibited no cytotoxic effects on AGS and SH-SY5Y cancer cells after 24 hours. This indicates that the concentration of silymarin in the extract is insufficient to have an anticancer effect.

According to research findings, antibiotics can promote cancer apoptosis, inhibit cancer cell growth and prevent cancer metastasis (Gao et al, 2020). For this reason, the antibacterial effect of herbal extracts is significant for better effectiveness in cancer treatment. The bacterial activity experiments using both gram-positive and gram-negative bacteria demonstrated that *U. dioica* and *C. scolymus* extracts exhibited activity against *S. aureus* and *B. cereus*, respectively. However, *S. marianum* extract showed no bacterial activity. According to Motamedi et al., *U. dioica* extract has antibacterial activity against *E. coli*, *S. epidermidis*, *B. cereus*, and *S. aureus* (Motamedi et al., 2014). The highest activity against *S. epidermidis* was found in a methanolic extract of *U. dioica* leaves, whereas the ethanolic extract had the lowest activity against *B. cereus* and *S. aureus*, and the methanolic extract had the lowest activity against *B. cereus*. Mirtaghi et al. discovered that the ethanolic extract of *U. dioica* leaves inhibited the growth of *S. aureus*, S. *epidermidis*, and *S. saprophyticus* (Mirtaghi et al., 2016). There are very few studies in the literature on the antibacterial activity of *C. scolymus* extract. For instance, Zhu et al. found that the leaf extract of *C. scolymus* exhibited antibacterial activity against *B. subtilis*, *S. aureus*, *A. tumefaciens*, *M. luteus*, *E. coli*, and *S. typhimurium* (Zhu et al., 2004). In contrast to our findings, Bajwa et al. found that the dimethylformamide extract of *S. marianum* extract suppressed bacterial growth of methicillin-resistant *S. aureus*, the other extracts of methanol and dichloromethane showed no antibacterial activity. The isopropyl alcohol extract of the *S. marianum* extract showed minimal activity against resistant *E. coli* (Bajwa et al., 2016). As a result, the antibacterial effect of the extracts depends greatly on the solvent type, indicating that extracts prepared with various solvents should be studied to fully understand antibacterial activity.

Free radicals produced continuously in the cell are destroyed by the antioxidant defence systems produced during normal metabolism in the body. It has been reported that cancer cells are characterized by a higher amount of reactive oxygen species than healthy cells, and reactive oxygenated species are responsible for maintaining the cancer phenotype (Okon et al., 2015). Since oxidative stress is a considerable factor in many types of cancer, the antioxidant activity of herbal extracts may be beneficial in the prevention or treatment of cancer, although randomized controlled clinical trials did not provide evidence that antioxidant supplements are effective in cancer prevention (Wright et al., 2007; Neuhouser et al., 2009; Ezzedine et al., 2010). Antioxidant activity experiment showed that *U. dioica* extract has the highest total antioxidant capacity, whereas *C. scolymus* extract has the lowest at the concentrations of 2.5, 5, 10, and 20 µg/ml. Several reports have noted that *U.*

dioica extract shows modest antioxidant activity, which could be attributed to the presence of phenolic components in the extract. Phenolic compounds are a type of antioxidant agent that acts through scavenging or chelating free radicals (Khare et al., 2012; Kukric et al., 2012). Although *C. scolymus* extract has been found to have a lower antioxidant capacity than other extracts, Ben Salem et al. reported that *C. scolymus* extract contains bioactive molecules such as cynarin and chlorogenic acid that contribute to antioxidant activity (Ben Salem et al., 2017). In order to better understand the antioxidant activity of our *C. scolymus* extract, it is necessary to examine the total phenolic content of the extract. In addition, the fact that the antioxidant effect of this extract is low and the anticancer effect is high indicates that many different mechanisms are effective in the treatment of cancer. Therefore, anticancer therapeutics do not necessarily exhibit antioxidant properties. For instance, herbal therapeutics can exert anticancer effects by inducing molecules and signalling pathways that stimulate apoptosis (Gulfishan et al., 2018). We found that *S. marianum* extract has moderate antioxidant activity when compared to other extracts. Serçe et al. investigated the antioxidant potential of *S. marianum* extract using a variety of methods, including lipid peroxidation, 1,1–diphenyl–2–picrylhydrazyl (DPPH), and ferric reducing power assays and discovered that *S. marianum* has strong antioxidant activity, which they attribute to the presence of high phenolic and flavonoid content (Serçe et al., 2016).

As a result, the main explanation for certain inconsistencies between our findings and the literature is that the content of herbal extracts changes depending on the environment in which they are cultivated. Altitude, temperature, moisture, and illumination are significant factors to impact the metabolism and accumulation of herbal secondary metabolites. Environmental differences in different growth locations (such as altitude, temperature, lighting, precipitation, humidity, soils) contribute to the differences in the active ingredient contents of the plant extracts and thus in the anticancer, antibacterial and antioxidant activities (Liu et al., 2016).

CONCLUSION

In conclusion, *C. scolymus* extract may be used as a potential source of cancer therapeutic drugs for gastric cancer due to its antibacterial and anticancer activity. In addition, the extract could be a potential candidate for further investigation.

ACKNOWLEDGMENTS

We thank Immunat Herbal Pharmaceuticals (Mugla, Turkiye) for providing *U. dioica*, *S. marianum,* and *C. scolymus* plant extracts.

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