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ORIGINAL ARTICLE / ÖZGÜN MAKALE

ANTIOXIDANT, ANTIMICROBIAL AND ANTIPROLIFERATIVE ACTIVITIES OF *GALIUM APARINE*

GALIUM APARINE'NİN ANTİOKSİDAN, ANTİMİKROBİYAL VE ANTİPROLİFERATİF AKTİVİTELERİ

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ABSTRACT

Objective: *Plants are known to have many biological activities. In this study, the antioxidant, oxidant, antimicrobial and antiproliferative activities of Galium aparine L. were investigated.*

Material and Method: *The antioxidant and oxidant potentials of the plant, the aerial parts of which were extracted with ethanol in a Soxhlet device, were measured using Rel assay kits. Antimicrobial activity against bacteria and fungi was determined using the agar dilution method. Lung carcinoma cell line (A549) was used to determine the antiproliferative activity.*

Result and Discussion: *As a result of the studies, total antioxidant status (TAS) value of plant extracts was determined as 5.147 ± 0.237, total oxidant status (TOS) value as 18.679 ± 0.245 and oxidative stress index (OSI) value as 0.346 ± 0.018. Plant extracts were found to be effective against test microorganisms at concentrations of 50-200 µg/mL. In addition, it was determined that the antiproliferative activity of the plant extract showed potent effects depending on the increase in the extract concentration. Finally, it was determined that Galium aparine has high biological activity and can be used as a natural pharmacological agent in this context.*

Keywords: *Galium aparine, medicinal plants, antioxidant, antiproliferative*

ÖZ

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Amaç: *Bitkilerin birçok biyolojik aktiviteye sahip olduğu bilinmektedir. Bu çalışmada Galium aparine L.'nin antioksidan, oksidan, antimikrobiyal ve antiproliferatif aktiviteleri araştırıldı.*

Gereç ve Yöntem: *Toprak üstü kısımları Soxhlet cihazında etanol ile ekstrakte edilen bitkinin antioksidan ve oksidan potansiyelleri Rel test kitleri kullanılarak ölçüldü. Bakteri ve mantarlara karşı antimikrobiyal aktivite, agar seyreltme yöntemi kullanılarak belirlendi. Antiproliferatif aktiviteyi belirlemek için Lung Carcinoma Cell Line (A549) kullanıldı.*

Sonuç ve Tartışma: *Çalışmalar sonucunda bitki ekstraktlarının toplam antioksidan seviyesi (TAS) değeri 5.147 ± 0.237, toplam oksidan seviyesi (TOS) değeri 18.679 ± 0.245 ve oksidatif stres indeksi (OSI) değeri 0.346 ± 0.018 olarak belirlenmiştir. Bitki ekstraktlarının 50-200 µg/mL konsantrasyonlarında test mikroorganizmalarına karşı etkili olduğu bulundu. Ayrıca bitki ekstraktının antiproliferatif aktivitesinin ekstrakt konsantrasyonundaki artışa bağlı olarak güçlü etkiler gösterdiği belirlendi. Sonuç olarak Galium aparinin yüksek biyolojik aktiviteye sahip olduğu ve bu bağlamda doğal bir farmakolojik ajan olarak kullanılabileceği belirlendi.*

Anahtar Kelimeler: *Galium aparine, tıbbi bitkiler, antioksidan, antiproliferatif*

INTRODUCTION

The use of herbs in alternative medicine for different purposes goes back to ancient times. Plants are natural resources that contain compounds that have many different biological effects. These compounds have been found to have many different effects such as antibacterial, antiviral, antioxidant, antimutagenic, anticarcinogenic, antidepressant and anti tumor. Therefore, it has been stated that those who regularly consume herbs suffer less from cancer and cardiological disorders [1, 2].

Galium aparine L.(Rubiaceae), which is common in temperate regions, is a climbing plant native to Asia, Europe, and North America [3]. *G. aparine*, which draws attention with its detrimental effect on potential yield, is an undesirable plant species in cereal, rapeseed and sugar beet fields [4]. However, it has traditionally been used in numerous medical applications for a variety of health conditions. All plant parts (stem, leaves, flowers and seeds) have been used in fever and urinary tract infections, eczema or skin diseases, ulcers, chronic wounds. It has also been used to reduce swelling, infection, and inflammation, increase lymphatic flow, and stop bleeding [5]. It is also used as a local tea by boiling the herb. According to some reports, the active ingredients of G. aparine are flavonoids, polyphenolic acids, tannins, anthraquinones, iridoids, alkanes, fatty acids, aromatic compounds, chlorophylls, carotenoids, iridoids, sesquiterpenoids, squalene, polyphenol, phytosterols and vitamin C. These active ingredients contribute to the intake of natural antioxidants [6-12]. Researchers reported that *G. aparine* was used in the treatment of cancer, fever, leukemia, jaundice, lymph swelling, tonsillitis, wounds, scurvy and hypertension [13]. Also, roasted seeds of *G. aparine* were used as a coffee substitute in Sweden [4]. Roasted young shoots are eaten [14] and used to coagulate milk in Turkey, being known as "yogurt herb" [7,8]. Although it has a long history in phytotherapy and is used as a diuretic by modern herbalists, there are few pharmacological studies to support this efficacy.

Oxidation provides energy to the organism for vital activities. There is a balance between the amount of free radicals produced in the organism and the antiradicals that protect and remove the body from their harmful effects [15]. Free radicals, including hydrogen peroxide, superoxide anions and

hydroxyl radicals, play an important role in oxidative damage at the cellular level. Studies show that biochemical changes induced by reactive oxygen species (ROS) are determinants of various diseases such as diabetes mellitus, atherosclerosis, cancer, arthritis, inflammation and neurodegeneration [16]. The human body has developed many mechanisms, both enzymatic and non-enzymatic, to inactivate ROS, but these may not be sufficient to struggle severe oxidative stress conditions. Many studies have been carried out to prevent oxidative stress diseases. The most studied subjects in the fight against oxidative stress are to reduce the effect of oxidative stress on the body by providing more natural antioxidants that can be obtained by increasing the use of vegetables and fruits. Natural antioxidants are safe and also exhibit biological and pharmacological activities. Therefore, extensive studies have been carried out recently to identify herbs with antiradical ability that humans can consume [17].

Plants such as medicinal, aromatic and spice plants contain a wide variety of free radical scavenging molecules with antioxidant properties. For this reason, medicinal and aromatic plants have been evaluated as an alternative treatment method in recent years and studies in this field have been increasing. Most of the herbal extracts have antioxidant properties that prevent oxidative stress. Natural antioxidants that increase the power of antioxidants in plasma reduce the incidence of certain diseases such as heart disease, cancer and stroke. The aim of our study is to determine the antioxidant, antimicrobial and antiproliferative activities of *Galium aparine* L. plant collected in Gaziantep, Turkey.

MATERIAL AND METHOD

Plant Material and Extraction

The plant samples of *G. aparine* were collected in Gaziantep province. After aerial parts were reduced to powder. 30 gr of dry samples were weighed and extracted with EtOH at 50 ^{o}C for approximately 6 hours (Gerhardt EV 14). The extracts obtained are concentrated with a rotary evaporator (Heidolph Laborota 4000 Rotary Evaporator).

Antioxidant, Oxidant and Oxidative Stress Index Tests

The total antioxidant status (TAS), total oxidant status (TOS) and oxidative stress index (OSI) of the plant extracts we used in our study were determined using Rel Assay Kits (Rel Assay Kit Diagnostics, Turkey). TAS value was expressed as mmol Trolox equiv./L and Trolox was used as the calibrator [18]. The TOS value was expressed as μmol H_2O_2 equiv./L and hydrogen peroxide was used as the calibrator [19]. The OSI (Arbitrary Unit) was calculated with the formula below [20].

$$\text{OSI (AU)} = \frac{\text{TOS (}\mu\text{mol } H_2O_2 \text{ equiv./L)}}{\text{TAS (mmolTrolox equiv./L) X 10}}$$

Antimicrobial Activity

The most recognized standards are provided by the Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). Antimicrobial activity tests of plant EtOH extracts were performed using the agar dilution method recommended by CLSI and EUCAST. The minimum inhibitory concentrations (MIC) of the extract against standard bacterial and fungal strains were determined. The following microorganisms were used for this purpose: *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* (MRSA) ATCC 43300, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853 and *Acinetobacter baumannii* ATCC 19606 were used as gram-negative bacteria. Furthermore, *Candida albicans* ATCC 10231, *C. krusei* ATCC 34135 and *C. glabrata* ATCC 90030 were used as fungi and were obtained from the American culture collection. Bacteria strains were pre-cultured in Muller Hinton Broth medium and fungal strains were pre-cultured in RPMI 1640 Broth medium. To obtain standard inoculum, the turbidity of the bacteria and fungi was set based on the McFarland 0.5 scale. All extracts were tested at 800-12.5 µg/mL concentrations and distilled water was used in all dilutions. Solvents used in the extracts were individually tested for antimicrobial activity. Amphotericin B and Fluconazole were used as reference drugs against fungi. Amikacin, Ampicillin and Ciprofloxacin were used as reference drugs for bacteria. The lowest concentration that inhibits the growth of fungi and bacteria was determined as the minimum inhibitory concentration (MIC) [21-26].

Determination of Antiproliferative Effects

The MTT assay (3-[4,5-dimethylthiazol-2-yl] -2,5-diphenyl-tetrazolium bromide) was conducted on the Lung Carcinoma Cell Line (A549) of the EtOH extract obtained from plant samples to determine cell viability. Cells were separated with 3.0 mL Trypsin-EDTA solution (Sigma-Aldrich, MO, USA) after 70-80% coalescence. The product was cultured in plates after separation and incubated for 24 hours. Then the extracts were diluted at different concentrations (25, 50, 100, 200 µg/mL) and the cells were incubated for 24 hours. Controls were administered in growth medium not supplemented with fetal calf serum (FCS). After 48 hours of incubation, the supernatants were dissolved in growth medium and replaced with 1 mg/mL MTT (Sigma) and incubated at 37°C until purple precipitate formed. The extracted supernatants were then solubilized by adding dimethyl sulfoxide (DMSO) to MTT (Sigma-Aldrich, MO, USA) absorbed by the cells. Plates were then read at 570 nm with an Epoch spectrophotometer (BioTek Instruments, Winooska, VT) [27].

RESULT AND DISCUSSION

Antioxidant and Oxidant Status

In recent years, there has been an increasing interest among researchers towards antimicrobial

compounds and antioxidants that are natural and safe to use. Numerous herbs and spices are described as sources of antioxidants and antimicrobial substances. In recent studies, it has been reported that polyphenols in diet and herbal products prevent oxidative stress. The preventive ingredients of these foods are especially anthocyanins, polyphenols and flavonoids. An antioxidant compound is a substance that at low concentrations delays or prevents oxidation of a substrate. Living organisms produce reactive oxygen species as a result of environmental influences and metabolic activities. ROS produced at high levels can be harmful to living organisms. Antioxidant compounds suppress the negative effects of ROS. Oxidative stress occurs when antioxidant compounds are insufficient. Parkinson's, Alzheimer's, cancer or cardiological disorders occur in humans as a result of oxidative stress. Complementary antioxidants can be used to reduce the effects of oxidative stress [28- 30]. In this study, the antioxidant and oxidant potential of *G. aparine* was determined. The findings obtained are shown in Table 1.

Table 1. TAS, TOS and OSI Values of *Galium aparine*

	TAS (mmol/L)	**TOS (µmol/L)**	**OSI**
G. aparine	5.147±0.237	18.679±0.245	0.346±0.018

* Values are presented as mean±S.D.

TAS, TOS and OSI values of *G. aparine* were determined for the first time in this study. In studies conducted on different plant species, the TAS value of *Rhus coriaria* var. *zebaria* was reported as 7.342, TOS value 5.170 and OSI value 0.071 [31]. TAS value of *Mentha longifolia* subsp. *longifolia* was reported as 3.628, TOS value as 4.046 and OSI value as 0.112[32]. The TAS value of *Calendula officinalis* was reported as 5.55 [33]. The TAS value of *Allium calocephalum* has been reported as 5.853, the TOS value as 16.288 and the OSI value as 0.278 [34]. TAS value of *Scorzonera papposa* has been reported as 5.314, TOS value as 24.199 and OSI value as 0.456 [35]. The TAS value of *Ferulago platycarpa* was reported to be 5.688, TOS value 15.552 and OSI value 0.273 [36]. TAS value of *Thymbra spicata* was 8.399, TOS value was 6.530 and OSI value was 0.078 [37]. TAS value of *Gundelia tournefortii* was reported as 6.831, TOS value as 3.712 and OSI value as 0.054 [38]. The TAS value of *Rumex crispus* was reported as 6.758, TOS value as 5.802 and OSI value as 0.086 [39]. Compared to these studies, the TAS value of *G. aparine* was higher than *Mentha longifolia* subsp. *longifolia*, and lower than *Rhus coriaria* var. *zebaria*, *Calendula officinalis*, *Allium calocephalum*, *Scorzonera papposa*, *Ferulago platycarpa*, *Thymbra spicata*, *Gundelia tournefortii* and *Rumex crispus*. TAS value shows the whole of antioxidant effective compounds produced in the body of the organism. In this context, the higher TAS value, the higher antioxidant property of the plant. As a result, it was determined that *G. aparine* has significant antioxidant activity.

TOS value shows the whole of oxidant compounds produced as a result of environmental effects and metabolic activities in living organisms. As the TOS value increases, the oxidative stress of the plant

increases. OSI value shows how much oxidant compounds produced by environmental effects in living organisms are suppressed by endogenous antioxidants. As the OSI value increases, plant oxidant compounds are insufficient to suppress and oxidative stress occurs.

The TOS and OSI values of *G. aparine* were higher than *Rhuscoriaria* var. *zebaria*, *Menthalongifolia* subsp. *longifolia*, *Allium calocephalum*, *Ferulago platycarpa*, *Thymbra spicata*, *Gundelia tournefortii* and *Rumex crispus*, and lower than *Scorzoner apapposa*. In this context, it is seen that *G. aparine* is at high levels in terms of oxidant compound. In addition, it was determined that the oxidative stress levels of *G. aparine* were at normal levels.

In previous studies, the antioxidant activities of methanol extract of *G. aparine* and its n-hexane, ethyl acetate, butanol and aqueous extracts were examined in vitro. Studies revealed that all fractions exhibited remarkable antioxidant activity, but clearly demonstrated that the aqueous fraction of *G. aparine* strongly scavenged DPPH, ABTS, hydroxyl, hydrogen peroxide and superoxide radicals [6, 8]. In our study, it was determined that EtOH extract of *G. aparine* has antioxidant activity using Rel Assay kits. As a result, it was determined that *G. aparine* has significant antioxidant activity.

Antimicrobial Activity

Living organisms interact with many microorganisms depending on their environmental conditions. In their natural environment, they provide resistance against microorganisms with the secondary metabolites they produce as a result of this interaction. In this context, these natural products can be used by humans as an antimicrobial source. In our study, the effects of *G. aparine* against standard bacteria and fungi were investigated. The results obtained are shown in Table 2.

Table 2. Antimicrobial Activity of *G. aparine*

	A	**B**	**C**	**D**	**E**	**F**	**G**	**H**	**J**
G.aparine	100	100	200	100	200	100	50	50	50

The MIC values are presented in units of µg/mL
A: *S. aureus*, B: *S. aureus* MRSA, C: *E. faecalis*, D: *E. coli*, E: *P. aeruginosa*, F: *A. baumannii*, G: C. albicans, H: *C. glabrata*, I: *C. krusei*
*200, 100, 50 and 25 µg/mL extract concentrations

In previous studies, it was reported that hydromethanolic extract of *Galium aparine* has antimicrobial activity against *Enterobacter cloacae*, *Escherichia coli*, *Listeria monocytogenes*, *Micrococcus flavus*, *Salmonella enteritidis*, *Salmonella typhimurium*, *Staphylococcus aureus*, *Aspergillus versicolor*, *Aspergillus ochraceus*, *A. niger*, *Candida krusei*, *Penicillium funiculosum*, *P. ochrochloron* and *P. verrucosum* var. *cyclopium* [40].

It has been reported that analysis of ethanol and chloroform extracts of *Galium verum* L., *Galium salicifolium* Klokov, *Galium dasypodum* Klokov, *Galium aparine* L, *Galium carpaticum* Klokov and *Galium pseudomollugo* Klokov are effective at different concentrations against *Escherichia coli*, *Bacillus subtilis*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Candida*

albicans [41]. It has been reported that chloroform, methanol and hexane extracts of *Galium mexicanum* have antimicrobial activity against *Bacillus subtilis*, *Staphylococcus aureus*, *Staphylococcus aureus* MRSA, *Streptococcus pyogenes*, *Candida albicans*, *Cryptococcus neoformans* and *Trichophyton rubrum* [42]. In our study, it was determined that EtOH extract of *Galium aparine* was effective against *S. aureus*, *S. aureus* MRSA, *E. coli* and *A. baumannii* at concentrations of 100 µg/mL, *E. faecalis* and *P. aeruginosa* at 200 µg/mL, *C. albicans*, *C. glabrata* and *C. krusei* at 50 µg/mL. The result of the study showed that *Galium aparine* EtOH extract is more effective against fungal strains. Finally, it was determined that plant extracts can be an important natural resource in preventing the growth of microorganisms.

Antiproliferative activity

In parallel with the history of humanity, plants have been used by all cultures due to their health-supporting and disease-curing properties. It is estimated that approximately 80-85% of the human population trust on traditional medicine. A large part of traditional treatment goes through the use of herbal extracts. Many studies have reported that plants have anticancer properties. In addition, it has been reported that plants have achieved very important results especially in supportive treatments applied in cancer treatment. In our study, the effects of EtOH extract of *G. aparine* on lung carcinoma cell line (A549) were investigated. The findings obtained are shown in Figure 1.

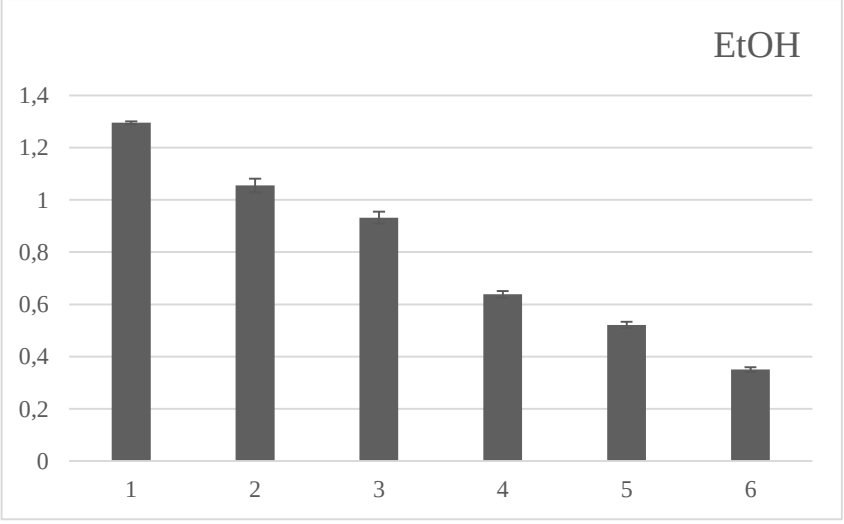

Figure 2. Antiproliferative activity of Galium aparine

In our study, it was determined that EtOH extracts of *G. aparine*exhibited strong cytotoxic effects on the A549 cell line depending on the dose increase. In previous studies, it was reported that methanol and ethyl acetate extracts of *G. aparine* were effective against Human breast cancer cells and Human colon cancer cells [10]. In a different study, *G. aparine*'s MeOH extract was reported to have a cytotoxic effect against breast cancer cell lines MCF-7 and MDA-MB-23 [43]. In this study, A549 cell line was

used and it was determined that it has strong cytotoxic effects. As a result, it was determined that *G. aparine* can be used as an anticarcinogenic.

In conlusion, medicinal plants are used in the treatment of many diseases. The biological activities of the Galium aparine plant were determined in our study. As a result of the studies, it was determined that the plant extract has an important antioxidant potential. In addition, it was found that it has antimicrobial activity against microorganisms and especially high antifungal activity. In addition, it was determined that the plant exhibited significant anticancer effect against lung carcinoma cell line. As a result, it was determined that the plant has a high biological activity and can be used as a natural agent in pharmacological studies.

AUTHOR CONTRIBUTIONS

Concept: *N.K., A.D., M.S.*; Design: *N.K., A.D., M.S.*; Control: *N.K., A.D., M.S.*; Sources: *F.S.M.*.; Materials: *N.K., A.D., M.S.*; Data Collection and/or processing: *N.K., A.D., M.S.*; Analysis and/or interpretation: *N.K., A.D., M.S.*; Literature review: *N.K., A.D., M.S.*; Manuscript writing: *F.S.M., N.K.*; Critical review: *N.K., A.D., M.S.*; Other: *N.K., A.D., M.S.*

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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