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Phytochemical Components, Antioxidant, Antibacterial, and Synergistic Effects of Endemic *Sideritis trojana* Extract in Combination with Antibiotics on Human Pathogens

Mehzat ALTUN[1]*

Abstract

In recent years, dramatically rising multidrug-resistant bacteria (MDR) and side effects of antibiotics lead ethnopharmacology to discover novel antimicrobials derived from plants for bacterial infections. Traditionally, decoction or infusion of the species of *Sideritis* has been used for the treatment of diseases such as cough, stomach, gastrointestinal, and kidney disorders. In this study, we aimed to investigate the antioxidant and antibacterial activity of the ethanolic extract of *Sideritis trojana* (*S.trojana*) and its synergistic potential with antibiotics against human pathogens. Gas chromatography-mass spectrometry (GC-MS), Folin- Ciocalteu, 1-diphenyl-2-picrylhydrazyl radical (DPPH), disc diffusion, and minimum inhibitory concentration (MIC) assays were used for identifying phytochemicals, total phenolic content (TPC), antioxidant, and antibacterial activity of ethanolic extract of *S.trojana* alone and binary combination with selected antibiotics against Gram-positive and Gram-negative bacterial strains, respectively. Furthermore, the combined effects of the extract with classical antibiotics were evaluated by measuring the inhibition zone diameter (IZD). The *S.trojana* extract showed moderate antibacterial activity with the MIC ranging between 15.625 to 500 µg/mL against human pathogens and the synergistic effect was detected in a dual combination of extract and antibiotics. The extract exhibited high antioxidant activity with a low IC_{50} value (0.138±0.010 mg/mL). The TPC value was 47.95±0.24 (mg GAE/g extract). After measurements of cytotoxicity were performed, *S.trojana* can be used alone or combined with antibiotics as an alternative therapy to eliminate pathogens and can be preferred as an antioxidant agent in the pharmaceutical industry.

Keywords: *S.trojana*, antioxidant capacity, antibacterial activity, synergism

1. INTRODUCTION

Antibiotics play an important role in the prevention and treatment of bacterial diseases. Nowadays, the excessive and inappropriate consumption of antibiotics, coupled with the lack of new effective antibiotics, and resistance mechanism developed by bacteria leads to treatment failures [1-3]. Antibiotic-resistant bacteria are reported by the World

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Health Organization (WHO) as a major global health issue due to increased morbidity, mortality, and healthcare costs [4, 5].

Difficulty to eliminate bacteria has led to research on natural antibacterial agents like extracts of plants with pharmacological activity. Among plants, the *Lamiaceae* family contains natural therapeutically bioactive compounds and has been used in traditional medicine since ancient times [6]. The extract of the genus *Sideritis* (belonging to *Lamiaceae*) includes 46 species in the flora of Turkey and 25 of them are endemic. *S.trojana* is used as an herbal tea for the treatment of colds and is endemic to Kazdagları (Ida Mountains) [7, 8].

Aerial parts of the *Sideritis* plant extracts are used in traditional medicine as an anti-inflammatory, antioxidant, and antimicrobial agent due to their variety content of secondary metabolites such as essential oils, flavonoids, iridoids, sterols, and terpenes [9]. Plant extracts combined with antibiotics may act synergistically and enhance their therapeutic effects against bacteria [10].

The objective of this study was to determine the phytochemical components, TPC, antioxidant, and antimicrobial activity of ethanolic extract of *S.trojana* alone and combined with classical antibiotics against 9 bacterial strains.

2. MATERIALS and METHODS

2.1. Plant Material

Aerial parts of *S.trojana* were collected from Kazdagları (Bayramic, Canakkale) in the Marmara Region of Turkey on August 2021. The plant was identified by Canakkale Onsekiz Mart University, Herbarium of the Department of Biology, and the voucher specimen was 002865. The plants were dried at 37°C in an oven.

2.2. Preparation of Ethanolic Extract

Aerial parts (stems, leaves, and flowers) of the *S.trojana* (20 gr) were milled into powder using an electric blender and extracted with 70% ethanol (250 mL) using a Soxhlet extractor for 6 h. Then, the filtrate was evaporated at 45°C by a vacuum evaporator. The crude extract was weighed and stored at 4°C in a dark until bioassays.

2.3.Gas Chromatography-mass Spectrometry

The chemical composition of *S.trojana* ethanolic extract was determined by GC-MS using Shimadzu GCMS QP 2010 ULTRA (USA) containing an RXI-5MS capillary column (30 m; 0.25 mm; 0.25 μm). Helium was used as a carrier gas (1.0 mL/min). The split ratio was 1:30. The injector temperature was set at 250°C. The initial oven temperature was set at 50°C for 5 min. Then it was programmed to increase from 50 to 270°C at the rate of 5°C/min and held at 270°C for 5 min. The achieved chromatographic mass spectra of the peaks were identified by comparison of their mass spectra with Wiley 9 (Wiley, New York, NY, USA) and NIST 11 (National Institute of Standards and Technology) (Gaithersburg, MD, USA) libraries.

2.4. Total Phenolic Content and Antioxidant Activity of the Ethanolic Extract

The Folin-Ciocalteu assay determines the total phenolic content by reacting phenolic compounds with the Folin-Ciocalteu reagent, resulting in the formation of a measurable blue complex detected spectrophotometrically. This method was used to determine the TPC of the ethanolic extract of *S.trojana*. The antioxidant activity of the extract was performed using a DPPH assay. Briefly, 0.0024 g DPPH was dissolved in 100 mL ethanol (0.6 mmol/L). 250 µL plant extract was added to 2.5 mL DPPH solution.

Gallic acid was used as a standard solution. Then, their antioxidant capacity was measured at 517 nm spectrophotometrically, and half maximal inhibitory concentration (IC_{50}) values were calculated [11].

2.5. Antibacterial Activity Assays

2.5.1. Bacterial Strains and Culture Condition

The strains were obtained from American Type Culture Collection (ATCC). Ethanolic extract of *S.trojana* was tested for antibacterial activity against *Proteus vulgaris* ATCC 13315, *Escherichia coli* ATCC 25922, *Staphylococcus epidermidis* ATCC 12228, *Staphylococcus aureus* ATCC 25923, S*treptococcus pyogenes* ATCC 19615, *Bacillus subtilis* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 10145, and *Streptococcus agalactiae* ATCC 12386. All strains were stored at -20°C in Brain Heart Infusion broth (BHI) (Biokar, France) with 20% glycerol (Sigma Aldrich).

2.5.2. Agar Disk Diffusion Assay

The antimicrobial activity of the extract of *S.trojana* was determined by using the disc diffusion method [12]. The strains were refreshed in BHI at 37°C for 24h. The bacterial suspension was adjusted to a concentration of 1.5×10^6 CFU/mL, and then, spread on Brain Heart Agar (BHA) (Biokar, France) plates. The crude extract was resuspended in 2.5% dimethyl sulfoxide (DMSO) and filtered through a 0.45 µm syringe filter. 2 mg/mL of 20 µL of extract-DMSO was impregnated to the 6 mm diameter paper discs under aseptic condition and incubated at room temperature for 20 minutes. After incubation at 37°C for 20 h, the inhibition zone diameters (IZDs) were measured. Gentamicin, ampicillin, and vancomycin were used as positive controls. DMSO (solvent) was used as a negative control.

2.5.3. Synergistic Activity of Ethanolic Extract of the Aerial Parts of *S.Trojana* in Combination with Selected Antibiotics

Gentamicin (for Gr negative strains), ampicillin (for Gr positive strains), and vancomycin (for *S.aureus*, and *S.epidermidis*) antibiotics were used in combination with aerial parts of the extract to evaluate the synergistic antimicrobial activity using double disc diffusion assay. 100 µL of each inoculum (1.5×10^6 CFU/mL) was spread on BHA plates. 10 µL of extract (at a concentration of 2 mg/mL) was added to antibiotic discs placed on the surface of the BHA plates. Followed by incubation at 37°C for 20 h, IZDs were measured in mm. To detect the combined effect between antimicrobials interpreted following the formula:

Growth inhibitory indices (GIIs): IZD in combination/IZD of the extract + IZD of antibiotic
If the result was GIIs> 0.5, 0.5, and < 0.5, the interaction was considered synergistic, additive, and antagonistic, respectively [13].

2.5.4. Determination of Minimum Inhibitory Concentration

The MIC of the extract was determined by microdilution broth using 96 well plates. Briefly, 100 µL of extract (2 mg/mL) was dissolved in 2.5% DMSO, then serially diluted in 100 µL of BHI in wells. Concentrations of ethanolic extract of *S.trojana* ranged from 1000 to 3.91 µg/mL. Each bacterial inoculum (20 µL) was added to all wells except sterility control [14]. Then, incubated at 37°C for 20 h, 10 µL of 0.2 mg/mL growth indicator (iodonitrotetrazolium chloride dye, Sigma-Aldrich) was added to each well to determine MIC. 5 µL of inoculum from negative wells was transferred on BHA and incubated at the same condition. The extract concentration, in which bacteria did not grow, was defined as MBC [15].

2.5.5. Statistical Analysis

Statistical analysis was performed using SPSS 19 version. One-way ANOVA and post hoc Tukey test were used to compare data (*p < 0.05).

3. RESULTS

3.1. Phytochemical Analysis of *S.trojana* Extract

Ethanolic extract of *S.trojana* was prepared by Soxhlet extractor. The GC-MS chromatogram of the extract is shown in Figure 1 and a total of 67 compounds were identified and shown in Table 1. Hydrocarbons 20.28% (Tridecane, tetradecane, dodecane, and trimethyl-tetrahydronaphthalene), sesquiterpenes 10.38% (caryophyllene oxide, β-caryophyllene, germacrene-D, bibisabolol oxide, cyclogermacrene, and farnesene), monoterpenes 8.31% (carvacrol, eugenol, geranyl-α-terpinene, 1.8-cineole, linalool, and myrtenol), maltol 3.68%, and sesquiterpenoids 3.14% (α-bisabolol, and β-bisabolene) were the main components of the extract and responsible for its biological properties.

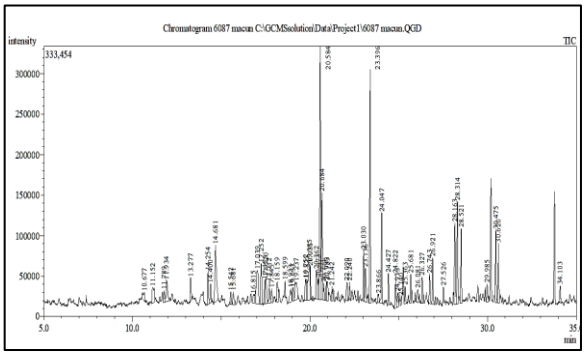

Figure 1 GC-MS chromatogram files of the aerial parts of the *S.trojana* extract with the Retention times (Rt)

Table 1 Composition (%) of ethanolic extract of *S.trojana* aerial parts

Peak	Rt	Area (%)	Components	Classification
1	1.445	1.75	Formic acid	Carboxylic acid
2	1.515	0.48	Propanal, 2-methyl-	Aldehyde.
3	1.694	2.76	Acetic acid (CAS)	Carboxylic acid
4	3.620	0.52	2,3-Butanediol (CAS)	Diol
5	4.704	2.57	Dimethylsulfoxonium formylmethylide	Ylide
6	10.677	0.52	Hexanoic acid (CAS)	Carboxylic acid
7	11.152	1.38	3-Hydroxy-4-pyrone	Pyrone
8	11.789	0.52	1,8-Cineole	Monoterpene oxide
9	11.934	1.19	Benzyl alcohol	Aromatic alcohol
10	13.277	0.82	Capryl alcohol	Fatty alcohol
11	14.254	1.25	Linalool	Terpene alcohol
12	14.400	0.75	Pelargonaldehyde	Aldehyde
13	14.681	3.68	Maltol	Pyrone
14	15.542	0.50	Bicyclo[3.1.1]heptan-3-ol, 6,6-dimethyl-2-methylene-, [1S-(1.alpha.,3.alpha.,5.alpha.)]-	Bicyclic alcohol
15	15.681	0.69	2,3-Dihydro-3,5-dihydroxy-6-methyl-4H-pyran-4-one	Pyranone
16	16.815	0.66	Geranyl butyrate	Ester
17	17.039	3.00	Undecanoic acid, ethyl ester (CAS)	Ester
18	17.252	2.08	3-Cyclohexene-1-methanol, .alpha.,.alpha.,4-trimethyl-, (S)- (CAS)	Cyclohexene alcohol
19	17.455	0.91	Myrtenol	Terpene alcohol
20	17.520	1.32	Butane, 1,1'-[methylenebis(oxy)]bis[3-methyl-	Bisether
21	17.712	0.83	Decanal (CAS)	Aldehyde
22	17.845	0.72	Bicyclo[3.1.1]hept-3-en-2-one, 4,6,6-trimethyl-, (1S)-	Bicyclic ketone

Table 1 Composition (%) of ethanolic extract of *S.trojana* aerial parts (Continue)

Peak	**Rt**	**Area (%)**	**Components**	Classification
23	18.159	1.11	Ethanol, 2-phenoxy- (CAS)	Ether
24	18.599	0.46	1H-Pyrrole-2,5-dione, 3-ethyl-4-methyl- (CAS)	Pyrrolidinedione
25	18.923	0.90	Benzeneacetic acid, ethyl ester (CAS)	Ester
26	19.037	0.53	3-Octanol (CAS)	Alcohol
27	19.237	1.60	Geranyl phenylacetate	Ester
28	19.752	1.10	Benzaldehyde, 4-propyl-	Aldehyde
29	19.825	1.02	cis-Pinonic acid	Carboxylic acid.
30	19.985	2.42	Dodecane, 4-methyl-	Alkane
31	20.035	0.72	Nonanoic acid (CAS)	Carboxylic acid
32	20.362	1.61	Propan-2-ol, 1-(3,4-dimethoxyphenyl)-2-methyl-	Alcohol
33	20.473	0.78	2-Propanol, 1-[2-(2-methoxy-1-methylethoxy)-1-methylethoxy]- (CAS)	Alcohol
34	20.584	9.16	Tridecane	Alkane
35	20.684	4.34	Carvacrol	Monoterpene phenol
36	20.771	0.58	Formamide, N,N-dibutyl-	Amide
37	20.930	0.83	Tri(1,2-propyleneglycol), monomethyl ether	Glycol ether
38	20.983	0.64	Guaiacol <4-vinyl->	Phenolic compound
39	21.242	0.56	Cyclohexane, 1,1,4,4-tetramethyl-2,6-bis(methylene)-	Substituted cycloalkane
40	22.090	0.87	trimethyl-tetrahydronaphthalene	Polycyclic hydrocarbon
41	22.240	0.48	Eugenol	Phenolic compound
42	23.030	1.47	2-Buten-1-one, 1-(2,6,6-trimethyl-1,3-cyclohexadien-1-yl)-, (E)-	Enone
43	23.136	1.48	trans-Z-.alpha.-Bisabolene epoxide	Sesquiterpene
44	23.396	7.83	Tetradecane (CAS)	Alkane
45	23.866	0.47	6,8-Nonadien-2-one, 6-methyl-5-(1-methylethylidene)-	Unsaturated ketone
46	24.047	3.00	Trans-β-Caryophyllene	Sesquiterpene
47	24.427	0.91	op[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene-, [1ar-(1a.alpha.,4a.alpha.,7.beta.,7a.beta.,	Tricyclic sesquiterpene alcohol
48	24.822	1.20	Diisopropyl adipate	Ester
49	24.933	0.73	Farnesene <(E)-, beta->	Sesquiterpene
50	25.140	0.45	Methyl-(2-hydoxy-3-ethoxy-benzyl)ether	Ether
51	25.363	0.62	Lauryl alcohol	Fatty alcohol
52	25.681	1.08	Germacrene-D	Sesquiterpene hydrocarbon
53	26.081	0.84	Bicyclogermacrene	Sesquiterpene hydrocarbon
54	26.327	1.00	beta.-Bisabolene	Sesquiterpene hydrocarbon.
55	26.744	0.93	Naphthalene, 1,2,3,5,6,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-, (1S-cis)-	Bicyclic hydrocarbon
56	26.921	1.48	2(4H)-Benzofuranone, 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-, (R)-	Benzofuranone derivative
57	27.526	0.45	Caryophyllene oxide	Sesquiterpene oxide
58	28.163	3.20	op[e]azulen-7-ol, decahydro-1,1,7-trimethyl-4-methylene	Opazulenoid alcohol
59	28.315	3.54	Caryophyllene oxide	Sesquiterpene oxide
60	28.521	2.43	Pentanoic acid, 2,2,4-trimethyl-3-carboxyisopropyl, isobutyl ester	Ester
61	29.985	0.74	Bisabolol oxide B <alpha->	Sesquiterpene oxide

Table 1 Composition (%) of ethanolic extract of *S.trojana* aerial parts (Continue)

Peak	Rt	Area (%)	Components	Classification
62	30.475	2.34	2H)-Naphthalenone, octahydro-4a,8a-dimethyl-7-(1-methylethyl)-, [4aR-(4a.alpha.,7.beta.,8a.alpha.	Octahydro-naphthalenone
63	30.620	2.14	Alpha-Bisabolol	Sesquiterpene alcohol
64	34.103	0.57	2-Pentadecanone, 6,10,14-trimethyl-	Ketone.
65	36.455	0.81	geranyl-.alpha.-terpinene	Terpene
66	37.125	1.03	18-Norabietane	Triterpene hydrocarbon
67	37.867	0.66	4b,8-Dimethyl-2-isopropylphenanthrene, 4b,5,6,7,8,8a,9,10-octahydro-	Phenanthrene derivative
		100		

Rt: Retention time

3.2. Antioxidant Properties of *S.trojana* Extract

The extract yields, TPC, and IC_{50} values for the extract are given in Table 2. According to the DPPH assay, the extract showed a strong radical scavenging effect with a low IC_{50} value of 0.138±0.010 mg/mL.

Table 2 Extract yield, and antioxidant capacity of *S.trojana* extract

The yield of extract (%)	TPC (mg GAE/g extract)	DPPH (IC_{50} mg/mL)
23%	47.95±0.24	0.138±0.010

3.3. Antibacterial Activity

The endemic plant extract was tested for its inhibitory activity alone and in combination with antibiotics on Gram-positive (*S.epidermidis, S.aureus, S.pyogenes, B.subtilis, E.faecalis,* and *S.agalactiae*) and Gram-negative (*P.aeruginosa*, *E.coli*, and *P.vulgaris*) bacterial strains using disc diffusion assay. The IZDs and MIC/MBC results were given in Table 3. The extract showed excellent antibacterial activity on strains ($p< 0.05$) except *P.aeruginosa* (p: 0.910) compared to antibiotics. The synergistic effect of the ethanolic extract of *S.trojana* was detected in combination with antibiotics against all strains except *P.aeruginosa*. The highest antibacterial effect of *S.trojana* extract against *B.subtilis*, and *P.aeruginosa* with a MIC value of 15.625 µg/mL.

4. DISCUSSION

Medicinal plants like *Sideritis* species and their active components have been used as antioxidant, antibacterial, anti-inflammatory, and antifungal alternative agents in pharmacology [16-20]. In a study conducted by Tunalier et al., the extract yields (%) of 27 *Sideriti*s species were found between 15.8 to 31.2% as same as our result (23%) [21]. In this study, hydrocarbons and the group of terpene which is responsible for its antioxidant and antimicrobial properties were detected in the *S.trojana* extract. Many studies have reported that common components of *Sideritis* species were monoterpenoids, monoterpenes, diterpenes, hydrocarbon, oxygenated monoterpene, and sesquiterpenoids [22, 23]. The differences in the compositions of the ethanolic extract are due to the climatic and experimental conditions, parts of the plant, the solvent type, and components that may affect its pharmacological activity [9, 24].

The antioxidant activity of *Sideritis* taxa [25-27], the TPC value of the methanolic extract of *Sideritis perfoliata* L. (41.64±0.99 mg GAEs/g extract) [28], and the ethanolic extract of *Salvia officinalis* (*Lamiaceae* family; 43.55 mg GAE/g) [29] was previously reported were consistent with our data. Different TPC and DPPH results of *Sideritis raeseri* ssp. *attica* [30] and *Sideritis cypria* [31] extracts were observed. The differences between results may be due to the extraction

method, species type, climate, and geographical location [32].

The experimental results of this study showed the MIC/MBC values ranged from 15.625 to 500 µg/mL. González-burgos et al. reported that the *Sideritis* spp. extracts exhibited strong antibacterial activity with MIC values ranging from 0.03 to 0.38 mg/mL against *B.subtilis*, *E.coli*, and *P.aeruginosa* [9]. The extract of *S.italica* showed antibacterial activity against *P.aeruginosa*, *P.mirabilis*, *S.typhi*, and *P.vulgaris* [33]. In a study conducted by Askun et al., antibacterial activity was detected the methanolic extract of *S.leptoclada* with a MIC of 640 µg/mL against *Enterobacter aerogenes* and *Salmonella typhimurium* [34]. The extract of *S.perfoliata* exhibited an antibacterial effect on *Cutibacterium acnes* [35]; *Streptococcus mutans* and *Prevotella intermedia* (Lall) with MIC values of 500 µg/mL, 6.25 mg/mL, and 3.125 mg/mL, respectively [14]. Moreover, the
methanolic extract of *S.inca* represented an antibacterial effect against Gram-positive and Gram-negative bacteria with MICs ranging from 2.60 to 20.863 mg/mL [36]. The antibacterial properties of the ethanolic extract of *S.trojana* are attributed to the presence of terpenes, some flavonoids, iridoids, sterols, and essential oils [8, 10, 37, 38].

In this study, synergistic interactions between *S.trojana* extract and commercial antibiotics on *P.vulgaris*, *E.coli*, *S.epidermidis*, *S.aureus*, *S.pyogenes*, *B.subtilis*, *E.faecalis*, and *S.agalactiae* were detected. These findings are in accordance with the results of the study conducted by Abullais et al., the ethanolic *P.granatum*, *Commiphora molmol*, and *Azadirachta indica* extracts in combination with amoxicillin, tetracycline, metronidazole, and azithromycin antibiotics showed a synergistic inhibitory effect on periodontopathic bacteria [10]. Another study reported that the ethanolic extract of *Ocimum sanctum* Linn. (*Lamiaceae*) showed synergistic activity in combination with chloramphenicol and trimethoprim against *Salmonella typhi* isolates [39].

Table 3 Antibacterial activity of *S.trojana* ethanolic extract alone and in combination with antibiotics used for the treatment of bacterial infections

Bacteria	Antibiotics	IZD Extract (mm)	IZD Antibiotics (mm)	IZD Antibiotics+ Extract (mm)	Outcome	p value	MIC (µg/mL)	MBC (µg/mL)
***S.epidermidis* ATCC 12228**	VA	25.50 ±1.290	18.5 ±0.577	22.50 ±0.577	0.511 S		62.5	125
***S.aureus* ATCC 25923**	VA	22.25 ±1.258	24.50 ±0.577	27.50 ±0.577	0.588 S		31.25	62.5
***S.pyogenes* ATCC 19615**	AMP	12.00 ±0.816	10.50 ±0.577	17.25 ±0.50	0.766 S	p< 0.05	500	Growth
***B.subtilis* ATCC 6633**	AMP	23.25 ±2.50	11.25 ±0.957	25.25 ±0.50	0.644 S		15.625	62.5
***E.faecalis* ATCC 29212**	AMP	15.00 ±0.816	14.75 ±0.50	15.75 ±1.50	0.529 S		250	Growth
***S.agalactiae* ATCC 12386**	AMP	21.00 ±0.816	18.25 ±1.258	27.25 ±0.25	0.694 S		62.5	500
***E.coli* ATCC 25922**	GEN	26.25 ±0.957	18.75 ±1.50	22.75 ±0.957	0.505 S		62.5	250
***P.vulgaris* ATCC 13315**	GEN	22.25 ±0.957	18.75 ±1.258	26.00 ±0.816	0.634 S		31.25	125
***P.aeruginosa* ATCC 10145**	GEN	29.25 ±0.957	20.50 ±0.577	20.75 ±0.957	0.417 A	p:0.910	15.625	62.5

*p < 0.05 = significant difference; S: Synergism, Ad: Additive, A: Antagonism. VA: Vancomycin, AMP: Ampicillin, GEN: Gentamycin

5. CONCLUSION

The ethanolic extract of aerial parts of *S.trojana* showed strong antioxidant activity and exhibited antibacterial activity both alone and combined with antibiotics against 8 human pathogens. The extract has a huge potential to develop new antibiotic formulations for the treatment of bacterial diseases. The combined use of antibiotics and plant extract can increase the antibacterial effect on resistant bacteria and may decrease their costs and side effects.

However, *in vitro* and *in vivo* toxicity assays and clinical trials are required for its use in therapy.

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Authors' Contribution
Laboratory studies, statistical analysis, writing article were performed by MA.

The Declaration of Conflict of Interest/ Common Interest
No conflict of interest.

The Declaration of Ethics Committee Approval
This study does not require ethics committee permission or any special permission.

The Declaration of Research and Publication Ethics
The authors of the paper declare that they comply with the scientific, ethical and quotation rules of SAUJS in all processes of the paper and that they do not make any falsification on the data collected. In addition, they declare that Sakarya University Journal of Science and its editorial board have no responsibility for any ethical violations that may be encountered, and that this study has not been evaluated in any academic publication environment other than Sakarya University Journal of Science.

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