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ANTIMICROBIAL ACTIVITY AND BIOACTIVE COMPOUND PROFILE OF EXTRACTS FROM *TANACETUM PARTHENIUM* AND *SALVIA GLUTINOSA* NATURALLY GROWING IN ARTVİN PROVINCE, TÜRKİYE

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Abstract

Medicinal and aromatic plants have gained prominence in pharmaceutical research due to the bioactive compounds they contain. The aim of this study is to determine the antimicrobial effects of methanol and ethanol extracts obtained from the aerial parts of *Tanacetum parthenium* and *Salvia glutinosa* plants, which naturally grow in the Artvin region, and to analyze the bioactive compounds present in these extracts. The antimicrobial activities of the extracts were evaluated by the disk diffusion method, and four bacterial strains *Staphylococcus aureus* (ATCC 25923), *Enterococcus faecalis* (ATCC 29212), *Escherichia coli* (ATCC 25922), *Bacillus spizizenii* (ATCC 6633), and the yeast *Candida albicans* (ATCC 10231) were used in the studies. The leaf extracts of *T. parthenium* showed activity against *S. aureus*, *B. spizizenii*, and *E. faecalis*, while the flower extracts exhibited antimicrobial activity only against *S. aureus* and *B. spizizenii*. The highest antimicrobial effect was observed in the leaf extract of *S. glutinosa* obtained with 96% ethanol, which was found to be more effective than the commercial antibiotic. Furthermore, HPLC analysis revealed that the main compound in both plant extracts was coumaric acid, and some phenolic compounds detected in these plants were reported for the first time in this study.

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Key words: *Tanacetum parthenium*, *Salvia glutinosa*, plant extracts, antimicrobial activity, bioactive compounds, HPLC analysis

Introduction. Medicinal and aromatic plants are emerging as a natural alternative in the prevention of diseases and the maintenance of health. The bioactive compounds found in these plants may not only enhance the effectiveness of existing treatment methods but also contribute to the development of new therapeutic options [1].

The Asteraceae and Lamiaceae families encompass numerous plant species with significant medicinal and aromatic properties. These two families are among the most widely used plant groups in both traditional medicine and modern pharmaceutical products. *Tanacetum parthenium*, a member of the Asteraceae family, is a perennial plant that resembles a daisy and is commonly found in gardens and along roadsides. *T. parthenium* is known for its various medicinal effects, including reducing fever and alleviating symptoms of arthritis and headaches [2]. Plants belonging to the Lamiaceae family are widely used around the world as aromatic spices, herbal teas, and traditional medicines [3]. *Salvia glutinosa*, a member of the Lamiaceae family, grows in moist areas such as deciduous forests, shrublands, and *Picea* forests in Northern and Southern Anatolia. Its flowering period extends from July to October [4]. The plant is covered with sticky glandular hairs, a characteristic that has led to the origin of its name, *"glutinosa"*.

In the literature, most studies have focused on the essential oils of *Tanacetum parthenium*, while research on the antibacterial effects of extracts obtained directly from the plant parts (such as flowers and leaves) remains limited [5, 6]. There are also studies investigating the antimicrobial activities of *Salvia glutinosa* extracts obtained from different geographical regions [7, 8]. However, the biological activity of plants is closely associated with the secondary metabolites they produce during specific stages of their life cycle. The production of secondary metabolites is influenced by both biotic factors (e.g., microorganisms) and abiotic factors (e.g., environmental conditions). Additionally, the chemical composition and concentration of compounds in the same plant species can vary depending on the geographical region in which they are grown. Therefore, studies on plants from different regions are crucial.

This study aims to determine the antimicrobial activities of extracts obtained from *Tanacetum parthenium* and *Salvia glutinosa*, two medicinal and aromatic plant species naturally growing in the Artvin province, against certain bacterial and yeast strains using the dry disc diffusion method. Furthermore, the bioactive compounds present in these plants will be identified.

Materials and methods. ***Collection of plant materials.*** This study utilized *Tanacetum parthenium* and *Salvia glutinosa* plants naturally growing in Artvin Province, Türkiye. These plants were collected during fieldwork conducted between June and October in 2022–2023 (Fig. 1). The identification of the plants

was carried out by Prof. Dr. Melahat Özcan. The coordinates are as follows: *T. parthenium* (1047 m, N41°14′20.2″, E041°51′24.8″) and *S. glutinosa* (1316 m, N41°14′33.9″, E041°51′04.2″).

a b

Fig. 1. Plant species used in the study: a. *Tanacetum parthenium*, b. *Salvia glutinosa*. (Photographs taken by Prof. Dr. Nurcan Albayrak İskender)

Preparation of plant extracts. After being brought to the laboratory, *T. parthenium* and *S. glutinosa* plants were washed with distilled water. The leaves and flowers were then separated and dried using a lyophilizer. The dried samples were ground into powder under aseptic conditions. Extraction was performed using the maceration method. For each sample, 1 g of plant material was combined with 9 ml of solvent and placed in an incubator with shaking for 24 hours. After the incubation period, the obtained extracts were first filtered through sterile four-layer gauze, then sterilized using filters with a pore size of 0.45 µm, and the filtrate was transferred into sterile flasks. The solvents were evaporated at 40–45 °C using a rotary evaporator, and the extracts were further dried in a lyophilizer for 2–3 days. Once dried, the extracts were carefully removed from the glass flasks, ground into powder, and dissolved in sterile physiological saline at a concentration of 0.5 g/ml. The extracts were then stored at 4 °C until use.

Evaluation of the antimicrobial activity of plant extracts. The antimicrobial activities of *Tanacetum parthenium* and *Salvia glutinosa* extracts against *Staphylococcus aureus* ATCC 25923, *Bacillus spizizenii* ATCC 6633, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, and *Candida albicans* ATCC 10231 strains were evaluated using the dry disc diffusion method. Lyophilized strains were activated according to the manufacturer's instructions. In this process, Nutrient Agar (NA) and Sabouraud Dextrose Agar (SDA) media were used. The density of 24-hour-old pure ATCC reference cultures was adjusted to approximately 1×10^8 cfu/ml according to the 0.5 McFarland standard. From each microorganism suspension, 200 µl was taken and spread onto Mueller-Hinton Agar (MHA), then homogenized over the surface using a sterile cotton swab. After this,

the plates were incubated for 15 min. Next, 20 µl of each plant extract was impregnated onto sterile 6 mm diameter discs (Bioanalyze), and these discs were placed onto the petri dishes. Ethanol and methanol were used as negative controls, while gentamicin and fluconazole were applied as standard antibiotics. The bacterial and yeast test microorganisms were incubated at 37 °C and 27 °C, respectively, for 24 to 48 h. At the end of the incubation period, the zones of inhibition (diameter of growth-inhibiting areas) around the discs were measured using a ruler, and the results were evaluated. All experiments were performed in triplicates. The data were analyzed from three replicates, and the average value for each sample was calculated.

Analysis of extracts by high-performance liquid chromatography (HPLC). The plant extracts were analyzed using the AGILENT 1260 INFINITY II HPLC-DAD system (Agilent Technologies, Santa Clara, CA, USA). The concentration of phenolic compounds was determined using an acetonitrile-based calibration method. Chromatographic separation was performed on a 250×4.6 mm ACE 5 C18 column. The mobile phase was composed of a mixture of acetonitrile (A) and 1.5% acetic acid (B), with the initial ratio set at 15% A and 85% B, changing to 40% A and 60% B at 29 min. The DAD detector was set to wavelengths of 250, 270, and 320 nm. The analysis was carried out at a flow rate of 0.7 mL/min, an injection volume of 10 µL, and a column temperature of 35 °C. Calibration curves were constructed using standards within the concentration range of 25–300 µg/mL, and samples were analyzed after appropriate dilution.

Results and discussion. ***Antimicrobial activity results of Tanacetum parthenium samples.*** The antimicrobial activity of *Tanacetum parthenium* leaf and flower extracts, prepared using 70% and 96% ethanol and methanol, was evaluated against *Candida albicans*, *Staphylococcus aureus*, *Bacillus spizizenii*, *Enterococcus faecalis*, and *Escherichia coli*.

Leaf extracts exhibited no activity against *C. albicans* or *E. coli*, but showed inhibition of *S. aureus* with 15 mm zones. The strongest effect on *B. spizizenii* (18.3 mm) was observed with the 96% ethanol extract, while the 70% ethanol extract demonstrated the highest activity against *E. faecalis* (14 mm). On the other hand, flower extracts demonstrated no activity against *C. albicans*, *E. coli*, or *E. faecalis*. However, the 70% methanol extract showed the most significant inhibition of *S. aureus* (17.3 mm), while other extracts produced inhibition zones ranging from 14 to 16 mm. The activity against *B. spizizenii* was lower (9–9.3 mm) (Table 1).

These findings are consistent with previous reports, such as Keleş et al. [9], who utilized 80% ethanol and observed selective activity against *S. aureus*. Kalodera et al. [10] also reported Gram-positive activity using 45% and 90% ethanol leaf extracts. In contrast, our study employed 70% and 96% concentrations, providing new insights into the solvent-dependent activity. This is the first report evaluating ethanol and methanol extracts of *T. parthenium* from Artvin,

Table 1

Antimicrobial activity of *T. parthenium* extracts against selected microorganisms (measured as inhibition zone diameters in mm)

Microorganism	96%EtOH	70%EtOH	70%MeOH	96%MeOH	PC	NC
Leaf extract						
S. aureus	15	15	15	15	21.4	–
B. spizizenii	18.3	15	16	15.6	25.5	–
E. faecalis	10.3	14	13	9.6	15.5	–
E. coli	–	–	–	–	21.3	–
C. albicans	–	–	–	–	27.8	–
Flower extract						
S. aureus	15.6	14	17.3	16	21.4	–
B. spizizenii	9.3	9	9.3	9.3	25.5	–
E. faecalis	–	–	–	–	15.5	–
E. coli	–	–	–	–	21.3	–
C. albicans	–	–	–	–	27.8	–

EtOH: Ethanol, MeOH: Methanol, PC: Positive control (Gentamicin for bacteria, Fluconazole for *C. albicans*), NC: Negative control (solvent only), (–): No inhibition zone

highlighting the plant's selective antimicrobial potential and the significance of extraction conditions.

Antimicrobial activity results of Salvia glutinosa samples. The antimicrobial activity of *Salvia glutinosa* leaf and flower extracts, prepared using 70% and 96% ethanol and methanol, was evaluated against *Staphylococcus aureus*, *Bacillus spizizenii*, *Enterococcus faecalis*, *Escherichia coli*, and *Candida albicans*. Activity was observed exclusively against *S. aureus*. The 96% ethanol leaf extract exhibited the strongest inhibition (26 mm), followed by other leaf extracts (23.3–24 mm). Flower extracts showed weaker effects, with inhibition zones of 12 mm (96% methanol) and 11.6 mm (96% ethanol) (Table 2).

Previous studies on the 96% ethanol extract of *S. glutinosa* from Serbia reported no antimicrobial activity against *S. aureus*, *E. coli*, or *C. albicans*, and only weak activity against *Aspergillus niger* [11]. In contrast, the 96% ethanol leaf extract of *S. glutinosa* collected from Artvin, Turkey, exhibited significant activity against *S. aureus*, surpassing the efficacy of some commercial antibiotics. While 70% ethanol extracts, including those from Romanian samples, have been reported to exhibit activity against both Gram-positive and Gram-negative bacteria [12], in our study, this extract inhibited only *S. aureus*. Another research has reported that a 100% methanol extract was most effective against *C. albicans* (23.8 mm) and showed moderate activity against *S. aureus* (13.3 mm) and *E. coli* (13.7 mm) [7]. The 96% methanol leaf extract, evaluated for the first time in this

Table 2

Antimicrobial activity of *S. glutinosa* extracts against selected microorganisms (measured as inhibition zone diameters in mm)

Microorganism	96%EtOH	70%EtOH	70%MeOH	96%MeOH	PC	NC
Leaf extract						
S. aureus	26	24	23.3	23.6	21.4	–
B. spizizenii	–	–	–	–	25.5	–
E. faecalis	–	–	–	–	15.5	–
E. coli	–	–	–	–	21.3	–
C. albicans	–	–	–	–	27.8	–
Flower extract						
S. aureus	11.6	nd	nd	12	21.4	–
B. spizizenii	–	–	–	–	25.5	–
E. faecalis	–	–	–	–	15.5	–
E. coli	–	–	–	–	21.3	–
C. albicans	–	–	–	–	27.8	–

EtOH: Ethanol, MeOH: Methanol, PC: Positive control (Gentamicin for bacteria, Fluconazole for *C. albicans*), NC: Negative control (solvent only), (–): No inhibition zone, nd: not determined

study, demonstrated higher activity against *S. aureus* versus the flower extract (23.6 mm versus 12 mm), confirming that solvent type and plant part significantly influence antimicrobial outcomes. Balaşoiu et al. [8] reported that 70% ethanol was more effective than 70% methanol; similarly, ethanol extracts in our study showed higher antimicrobial activity.

Differences between our findings and the literature may be attributed to environmental factors. Geographic location, climate, soil composition, and altitude can affect the production of secondary metabolites in plants. The high antimicrobial activity observed in *S. glutinosa* samples from Turkey highlights how regional variations can alter the pharmacological properties of the plant.

Bioactive compounds in antimicrobial active extracts. Crude plant extracts prepared using methanol or ethanol are widely employed in antimicrobial screening due to their efficiency in extracting bioactive compounds. In this study, *Tanacetum parthenium* and *Salvia glutinosa* extracts with notable antimicrobial activity were analyzed by HPLC-DAD. A range of phenolic compounds, flavonoids, and ascorbic acid were identified in these extracts. The retention times (RT) and quantitative values of all identified compounds are presented in Table 3.

In *T. parthenium* samples (extracts 1 and 2), major phenolic compounds included p-coumaric acid, apigenin, and gallic acid (absent in extract 2). This study is the first to report ascorbic acid, vanillic acid, rosmarinic acid, and rutin in these extracts. HPLC analysis identified coumaric acid as the dominant compound

in the ethanol extract of *T. parthenium* (151.06 mg/L) and in both ethanol (207.46 mg/L) and methanol (196.12 mg/L) extracts of *S. glutinosa* (Table 3). Given its abundance, the antimicrobial role of p-coumaric acid is notable; it increases membrane permeability and disrupts bacterial DNA function [13]. Rosmarinic acid and quercetin, present in all extracts, are known for antimicrobial activity with broad-spectrum effects, and quercetin with selective action against MRSA and *S. epidermidis* [14, 15]. Quercetin also induces surface-level morphological changes in *S. aureus*, including tufted structures and cell aggregation, without major cytoplasmic alterations [15].

Comparative studies from different regions have shown diverse phytochemical profiles. Peruvian *T. parthenium* samples, analyzed using 70% methanolic extracts, contained apigenin, luteolin, ferulic acid, and kaempferol [16]. In German *T. parthenium* samples, 80% methanolic extracts from leaves and flowers revealed lipophilic flavonoids, including methyl ether derivatives of 6-hydroxykaempferol and quercetagetin [17]. Ukrainian samples yielded eleven phenolic compounds, notably quercetin, kaempferol-3-rutinoside, the O-methylated flavonol santin, apigenin, chlorogenic acid, and several dicaffeoylquinic acid isomers [18]. Meanwhile, Turkish samples were rich in p-coumaric acid, apigenin, and gallic acid. The presence of apigenin in *T. parthenium* has been previously confirmed through HPLC analysis, as reported by Wu et al. [19], further corroborating these findings. These differences underscore the significant influence of geographic and environmental factors on the composition of bioactive compounds and their antimicrobial potential in plants from different regions.

Previous reports show the presence of kaempferol, apigenin, quercetin derivatives, caffeic acid, and rutin in *S. glutinosa* aerial parts [20–22]. In our study, vanillic acid rosmarinic acid, and epicatechin were identified in samples 3, 4, and 5 for the first time. Coumaric acid, rosmarinic acid, and epicatechin were dominant in extracts 3 and 4, while extract 5 was rich in quercetin (Table 3).

Conclusion. In this study, the antimicrobial activities of methanol and ethanol extracts obtained from *Tanacetum parthenium* and *Salvia glutinosa* plants, collected from the Artvin region, were investigated against various bacterial and fungal species. The extracts of *T. parthenium* exhibited significant antibacterial activity, particularly against Gram-positive bacteria such as *Staphylococcus aureus*, *Enterococcus faecalis*, and *Bacillus spizizenii*. On the other hand, *S. glutinosa* extracts showed activity only against *S. aureus*, with the 96% ethanol leaf extract displaying stronger efficacy than the standard antibiotic.

HPLC analysis revealed coumaric acid as the dominant compound in both plant extracts. Additionally, *T. parthenium* contained apigenin, gallic acid, rosmarinic acid, rutin, and quercetin, while *S. glutinosa* contained rosmarinic acid, quercetin, epicatechin, and caffeic acid. Given the antimicrobial properties of these compounds reported in the literature, they are believed to contribute to the observed antibacterial activities.

Table 3

Bioactive compounds and their retention times (RT) identified in the most active antimicrobial extracts (mg/L)

Compounds	1(A/RT)	2(A/RT)	3(A/RT)	4(A/RT)	5(A/RT)
Vitamin					
Ascorbic acid	0.0/3.710	4.11/3.704	0.0/3.784	0.0/3.652	0.0/3.764
Phenolics					
Caffeic acid	0.0/9.085	0.0/9.319	20.26/9.116	0.0/9.122	5.31/9.137
Coumaric acid	151.06/12.978	64.92/13.019	207.46/13.114	196.12/13.115	0.0/12.988
Ferulic acid	0.0/14.423	1.46/14.447	–	0.0/14.429	0.0/14.477
Gallic acid	47.94/5.091)	–	–	–	–
3,4–hydroxy benzoic acid	0.0/6.204)	–	0.0/6.278	0.0/6.277	0.0/6.402
Rosmarinic acid	15.00/17.276	9.11/17.300	14.26/17.554	25.62/17.553	9.90/17.580
Vanillic acid	0.27/9.641	0.70/9.637	0.0/9.622	0.81/9.450	10.95/9.651
Flavonoids					
Rutin	0.65/11.879	3.75/11.861	–	0.0 /11.917	0.0/11.814
Apigenin	26.81/28.861	66.92/28.876	0.0/28.863	0.0/28.867	0.0/28.890
Catechin	0.0/7.187)	–	–	–	–
Epicatechin	0.0/8.549)	0.0/8.411	17.42/8.448	31.62/8.477	0.0/8.440
Myricetin	0.0/18.068	0.0/18.052	0.0/17.829	0.0/18.047	0.0/17.864
Quercetin	14.62/24.344	14.57/24.355	14.84/24.119	14.67/24.075	15.06/24.059

1: *T. parthenium* leaf extract (96% EtOH), 2: *T. parthenium* flower extract (70% MeOH), 3: *S. glutinosa* leaf extract (96% EtOH), 4: *S. glutinosa* leaf extract (96% MeOH), 5: *S. glutinosa* flower extract (96% MeOH), A: Amount (mg/L), RT: Retention time (min), 0.0: Below detection limit, –: Not detected (Absent)

In conclusion, both *T. parthenium* and *S. glutinosa* species show promising antimicrobial potential, particularly against Gram-positive pathogens. Further investigation of the chemical structures and biological activities of their bioactive compounds is essential to enhance their pharmaceutical application potential.

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