

Pharmacological Properties of *Genista sagittalis* L. (Fabaceae) Grown in Turkey

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Abstract: The genus *Genista* L. (Family: Fabaceae) is a plant having several traditional uses for treating common ailments such as diabetes, ulcer, and respiratory diseases. In this current study, the composition of essential oil and the biological activities of *Genista sagittalis* L. (Fabaceae) from Kocaeli: Yuvacık Dam Basin have been studied. A total of fourteen components were identified in the essential oil. The identified compounds belonged to straight-chain alkane, aromatic ether, and terpenoid derivatives. The antibacterial activity analyses demonstrated that *G. sagittalis* flower extract only had low activity against *P. mirabilis* and *P. aeruginosa* with MICs 1 to 2 mg/mL, as the peduncle extract showed strong anti-QS activity at 1.3 mg/mL. To the best of our knowledge, the current work is the first to report the antimicrobial and anti-quorum sensing activity of *G. sagittalis* growing in Turkey. Double-stranded DNA binding affinity investigations of the flower and peduncle ethanol extracts indicate that there are interactions with double-stranded DNA and related binding constants (K_b) were found as $1.97 \times 10^3 \pm 0.37$ and $3.68 \times 10^2 \pm 0.44$ for the flower and peduncle extract, respectively.

Keywords: antibacterial; anti-quorum sensing; DNA binding; Fabaceae; *Genista sagittalis*; plant extracts

■ INTRODUCTION

The genus *Genista* L., being a genus of the Fabaceae family, contains about 100 species (such as *G. anglica* L., *G. pilosa* L., *G. sagittalis* L., *G. tenera* (Jacq. ex Murray) Kuntze, *G. tinctoria* L., etc.) and has been used generally as folk medicine in Mediterranean area in the treatment of diabetes, ulcer, respiratory diseases and also in rheumatic disorders [1]. Apart from these kinds of medicinal uses, the genus *Genista* L. is also known for its yellow pigment property [2]. *Genista* species are also characterized by their flavonoid and isoflavonoid content [3], acting as primary antioxidants or free radical scavengers [4-5]. One of its species, *Genista sagittalis* L. (Syn. *Chamaespartium sagittale* (L.) P.E. Gibbs, *Genistella sagittalis* (L.) Gams) is native to West, South of Europe, and Northwestern Turkey [6-9]. It is a naturalized species

in the Czech Republic [10-11], Poland [12], and Lithuania [13]. The distribution of this species in Turkey has emerged with its population determined in the Yuvacık Dam Basin of Kocaeli province by Efe et al. [8]. *Genista sagittalis* usually grows in maquis habitats, in oak or conifer forests, shrub fields, dry grasslands, slopes, and mountain slopes at 1050–1600 m (rarely 2360 m) in South and Central Europe, in open woodland, maquis scrub, and rocky slopes at 450–850 m in Kocaeli. It was recommended by the International Union for Conservation of Nature (IUCN) [14] as the Red data category EN due to road construction works and livestock grazing in Turkey [8].

Genista sagittalis plant, which can be grown to a height of 10–50 cm from the soil surface, has leaf-like branches, semi-shrub, procumbent, rhizome or

caespitose. Young shoots with straight and silky hairs, later glabrous. Leaves are $0.5\text{--}2.2 \times 0.5\text{--}1.0$ cm, linear to elliptic, entire, pubescent beneath, and subglabrous above. It blooms in April–May. The number of flowers in the terminal inflorescence is variable between 4–25. Calyx hairy, 0.5–0.7 cm; Corolla yellow, glabrous, standard 1–1.2 cm; wings 0.9–1 cm; keel 0.9–1.1 cm; and legume fruit, hairy, $1.5\text{--}2.0 \times \text{ca. } 0.5$ cm. The number of seeds in the legume fruit ranges between 1 and 5. Olive green or blackish. *G. sagittalis* differs from other *Genista* species in that its shoots are winged and thornless. Illustrations of the plant's habitus, stem-wing, flower, calyx, corolla parts, fruit, seed, and root are given in detail by Efe et al. [8].

According to the literature reviewed, the investigations related to *G. sagittalis* are limited. The investigations have been mainly done on the flavonoid and isoflavonoid contents of *G. sagittalis* blooming aerial parts performed by liquid chromatography [15-17] and it is an antioxidant property [5]. Callus cultures of *G. sagittalis* have been established with the objective of producing high amounts of isoflavones of phytoestrogen activity [18]. And some other investigations are related to their morphology [19-20].

Plant extracts have their own importance as they are considered as most urgent biomolecule sources that can be screened due to the fact that not only various active pharmaceutical compounds have been isolated and characterized but also used as bioactive compounds in drug initiatives [21-22]. For this purpose, the extraction of such biomolecules by medicinal plants has been carried out using various solvents and different types of extraction methods. The plant extracts, which are widely known as therapeutic among the public, have attracted the attention of some researchers today, and they have been evaluated in a number of bio-analytical experiments in order to take the research to a further level. Today, many drugs targeting deoxyribonucleic acid (DNA) are both in use and in phase. There are many different types of these drugs that treat by different mechanisms. However, due to the serious side effects of these types of drugs, scientists continue their research to develop different types of drugs (with less toxic side effects).

As it is known, DNA, playing an important role in the transmission of genetic information, is one of the clear targets for drug development research. The majority of structure-specific molecules, such as intercalators and groove binders currently available on the market and in the literature, bind to DNA by non-covalent interaction. The first of these non-covalent types of attachment is intercalation, in which an insertion occurs between the base pairs of double-stranded DNA (dsDNA) and the small molecule through strong intermolecular forces, causing the DNA bases to rupture irreversibly in hydrogen bonds. The second is the groove binding, where the small molecule binds to the small or large groove of dsDNA. The third is external binding on the dsDNA surface, primarily through hydrogen-bonding interactions with the phosphodiester backbone and small molecules. Our research group has so far examined the interaction of different chemical structures (for example, drug molecules, some newly synthesized ligands, bioactive metal complexes and structures isolated from plants) with fish sperm and/or calf thymus dsDNA by spectroscopic, voltammetric, viscosimetric, and theoretical ways [23-27].

In the present work, investigations are done on the essential oil part while alcoholic extracts of the flower and

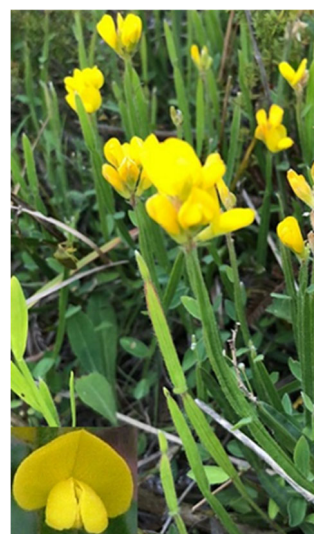


Fig 1. *Genista sagittalis* near Serinlik locality in Kocaeli-Turkey

peduncle parts of *G. sagittalis* collected from Kocaeli province-Turkey (Fig. 1). We analyzed the essential oil chemical profilings by using gas chromatography coupled to mass spectrometry (GC-MS) and, we tried for the first time the interaction of the flower and peduncle extracts of *G. sagittalis* with dsDNA by using UV-Vis spectroscopy and cyclic voltammetry (CV) techniques. In both techniques, we obtained the binding constants (K_b) of these extracts to dsDNA with reproducible results. Furthermore, to the best of our knowledge, the current work is the first to report the antimicrobial and anti-quorum sensing (anti-QS) activity of *G. sagittalis* extracts growing in Turkey.

■ EXPERIMENTAL SECTION

Materials

The materials used in this study were, sodium chloride (NaCl) (99.9% purity, Merck), synthetic double-stranded DNA (99.9% purity, Merck, Germany), and tris(hydroxymethyl) aminomethane hydrochloride ($C_4H_{12}ClNO_3$) (99.9% purity, Merck, Germany), used without further purification. Solvents used in this study, dichloromethane, ethyl alcohol, *n*-hexane, and methanol, were distilled before use.

Two Gram-positive (*Staphylococcus aureus* 29213, *Staphylococcus epidermidis* 12228) and three Gram-negative (*Chromobacterium violaceum* 12472, *Proteus mirabilis* 14153, *Pseudomonas aeruginosa* 27853) bacteria were used for antibacterial analysis. *C. violaceum* 12472 was also used to determine anti-QS activity. Production of the purple violacein pigment in wild type strain of *C. violaceum* (CV12472) relies on an intact quorum-sensing mechanism [28]. For strains' growth Luria-Bertani (LB) broth (1% tryptone, 0.5% yeast extract, 0.5% NaCl) at 28 °C, and 200 rpm was used.

A stock solution of 230 μ M dsDNA containing 0.2 M Tris-HCl and 150 mM NaCl (pH 7.4), is stable for one week and should be stored at 4 °C. The ratio of absorbance at 260 and 280 nm is used to assess the purity of the related solution, which gives $A_{260}/A_{280} = 1.92$ value. The molarity of dsDNA was calculated using $\epsilon = 6600 \text{ M}^{-1} \text{ cm}^{-1}$ [29]. In the UV-Vis spectroscopic studies, extracts 1 and 2 (same amounts for both extracts

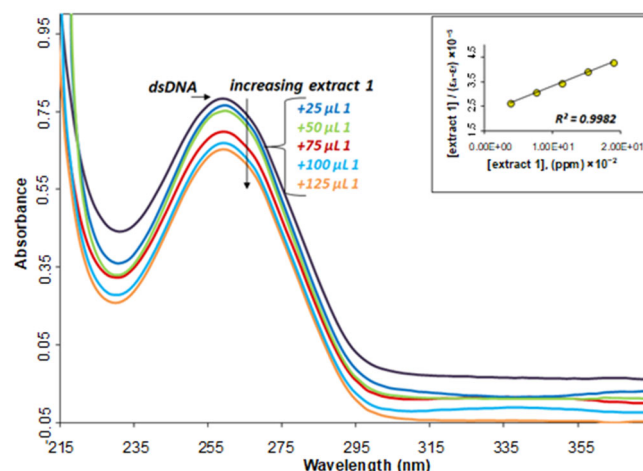


Fig 2. UV absorption titration of dsDNA (30 ppm) with extract 1 (25–125 μ L) in Tris-HCl buffer. (The arrow shows how absorbance decreased and not shifted ($\lambda_{\text{max}} = 260 \text{ nm}$) as the concentration of extract 1 increased, and binding constants (K_b) were determined from the inset)

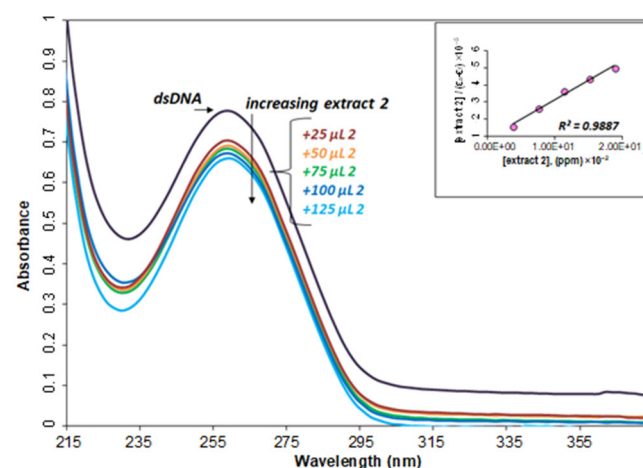


Fig 3. UV absorption titration of dsDNA (30 ppm) with extract 2 (25–125 μ L) in Tris-HCl buffer. (The arrow shows how absorbance decreased and not shifted ($\lambda_{\text{max}} = 260 \text{ nm}$) as the concentration of extract 2 increased, and binding constants (K_b) were determined from the inset)

in the range of 25–125 μ L) were added separately (with different experiments) at increasing concentrations onto the 30 ppm dsDNA solution. The absorption spectra of dsDNA + extract 1 or 2 solutions were recorded (Fig. 2 and 3). All experimental methods were repeated three times, and the binding constants based on the results are reported as relative standard deviation (RSD) values from the mean. In the voltammetric experiments, the

cyclic voltammograms of the prepared dsDNA + extract **1** or **2** solutions were recorded in the direction of oxidation by adding extract **1** or **2** (in the range of 12.5–75 µL) solution at increasing concentrations onto the 10 ppm dsDNA solution. In voltammetric measurements, dsDNA diluted with the acetate buffer solution with a pH of 4.8 has been used as dsDNA stock solution.

Instrumentation

The instruments used in this study were UV-VIS spectrophotometer (A T80 + UV-Vis spectrophotometer) and GC-MS (Thermo Trace GC Ultra with an HP Innwax column (30 m–0.25 mm–0.25 µm) and Thermo Trace DSQ). The prepared solutions were recorded against the blank and Tris-HCl (pH: 7.4) buffer solution. The GC-MS studies were performed at TUBITAK Marmara Research Center. Column conditions were as follows: 60 °C (3 min isothermal), heated to 220 °C at a rate of 3 °C/min, and 5 min (isothermal) at 220 °C. Inlet temperature: 175 °C; injection mode: splitless; carrier flow: 1 mL/min; transfer line temperature: 200 °C; with a mass range of 40–450 *m/z*, ion source temperature: 200 °C, incubation time: 30 min, and incubation temperature: 60 °C.

The voltammetric recordings were performed using an Autolab potentiostat/galvanostat (PGSTAT 302 N, Eco Chemie, the Netherlands), a BAS 100 W (BioanalyticalSystem, USA) potentiometer, and a glassy carbon electrode (BAS; U: 3 mm diameter). The General Purpose Electrochemical System (GPES) and Nova 2.2 software packages were used to control experimental conditions. An Ag/AgCl reference electrode (BAS; 3 M KCl in all experiments), a platinum wire counter electrode, and a 10 mL single-chamber standard cell with three electrodes were the triple electrode systems used.

Procedure

Fieldwork

Flower and peduncle samples of *G. sagittalis* (Fig. 1) were collected from Kocaeli province in May 2018. The location information where the samples were collected is given below.

Kocaeli: Yuvacık Dam Basin, between Camidüzü-Örnekköy, near Serinlik locality, on the upper side of the

road, on the side of the path, south, shrubland, 510 m, 40°39'02"N, 30°00'16' E.

In this study, the following taxa were accompanied at the location where the plant samples were collected: *Quercus petraea* (Mattuschka) Liebl. subsp. *iberica* (Steven ex M.Bieb.) Krassiln., *Tilia tomentosa* Moench, *Arbutus unedo* L., *Rosa canina* L., *Cistus creticus* L., *Erica arborea* L., and *Genista tinctoria* L. The study area was visited during the flowering period of *Genista sagittalis*. Individuals of this plant in the location detailed above were determined and identified in the area, and in order to be used in this study, inflorescence samples in the terminal part of the branches were collected in paper bags.

Extraction procedure

The collected inflorescence samples of the plant *G. sagittalis* were separated into two groups flowers and peduncles. Both groups were air dried in the shade indoors and ground into powder in 50 mesh size. The samples were extensively extracted with 300 mL of methanol in a Soxhlet apparatus. The yield percentages of the ethanol extracts were 10.78 and 7.38% as dry matter, respectively. The obtained extracts are then re-extracted with a separatory funnel containing 300 mL of cyclohexane. The crude flower and peduncle extracts were concentrated in vacuo to dryness at 40 °C.

For DNA binding experiments: the dry matter percentages of the flowers and peduncle of the ground samples were approximately 1.87 and 1.87%, respectively. Extractions of the flower and peduncle parts were performed with 20 mL of 70% ethanol, for 30 min in a water bath, at 60 °C. The ethanol extracts were then cooled and centrifuged at 4,500 rpm for 15 min to obtain an overhead liquid [5]. The yield percentages of ethanol extracts were 9.35 and 5.75% as dry matter, respectively. In this study, extracts **1** and **2** are denoted as the flower and peduncle extracts, respectively.

Isolation of essential oil

The essential oil of air-dried powdered 300 g *G. sagittalis* was obtained by hydrodistillation using a Clevenger-type apparatus.

Minimal inhibition concentration (MIC) analysis

A modified broth dilution assay in 96 well-microplate, described in detail in a previous paper of our group [30], has been used in the antibacterial activity analysis. Briefly, the stock solutions were prepared at 50 mg/mL in methanol from methanolic extracts of flower and peduncle parts of *G. sagittalis*. The extracts were added to each well in a total volume of 200 μ L with final concentrations of 0.25–2 mg/mL from the stock solution for assay. The bacterial suspensions were adjusted to optic density 600 0.1. Ampicillin (200 μ g/mL) and kanamycin (100 μ g/mL) were used as standard antibiotics. Covered plates were mixed carefully and incubated at 28 °C, 200 rpm for 24 h. The absorbance of each well was measured at 600 nm using an Epoch microplate spectrophotometer (Biotek, USA). All tests were performed in triplicate. The MIC was defined as the lowest concentration that inhibits the growth of microorganisms.

Anti-QS analysis

Anti-QS activity was analyzed by a modified broth dilution assay [30] in 96 well-microplate previously described using sub-MIC concentrations (1.0–1.9 mg/mL) with CV12472 biosensor strain. Kanamycin (100 μ g/mL) was used as a standard antibiotic. Covered plates were mixed thoroughly and incubated at 28 °C, 200 rpm for 24 h. The absorbance of each well was measured at 600 nm using an Epoch microplate spectrophotometer (Biotek, USA) to confirm the growth of bacterial cultures. QS activity was measured by quantitative analyses of violacein production [31]. A 200 μ L CV1472 culture sample from each well was

transferred to a microcentrifuge tube and centrifuged at 13,000 rpm for 10 min to precipitate insoluble violacein. Supernatants were discarded, and 200 μ L DMSO was added to the pellets and vortexed vigorously until a uniform resuspension was achieved. Removing of cell lysates from these resuspensions was immediately done with centrifugation at 13,000 rpm for 10 min. After centrifugation, the absorbance of the supernatant, which is added to a 96-well microplate, was measured at 585 nm with a microplate spectrophotometer.

Statistical analysis

For antibacterial and anti-QS analyses, values were calculated with Excel software (Microsoft Office). One-way ANOVA and Tukey's test were used for statistical analyses, and the results are expressed as the mean \pm SD. Statistical significance levels were shown as * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$.

RESULTS AND DISCUSSION

Chemical Composition of the Essential Oil

The GC-MS analysis of *G. sagittalis* oil detected 14 components, accounting for 9.88% of the total oil which is listed in Table 1. The main identified compounds were straight-chain alkanes ethers (7.51%), aromatic ethers and esters (1.43%) and terpenoids (0.94%). The representative gas chromatogram of *G. sagittalis* essential oil is given in Fig. 4.

Interaction with dsDNA

It is known that the mechanisms of action of some antitumor and antimalarial drugs include interactions with dsDNA. Alkylating agents (e.g., bendamustine,

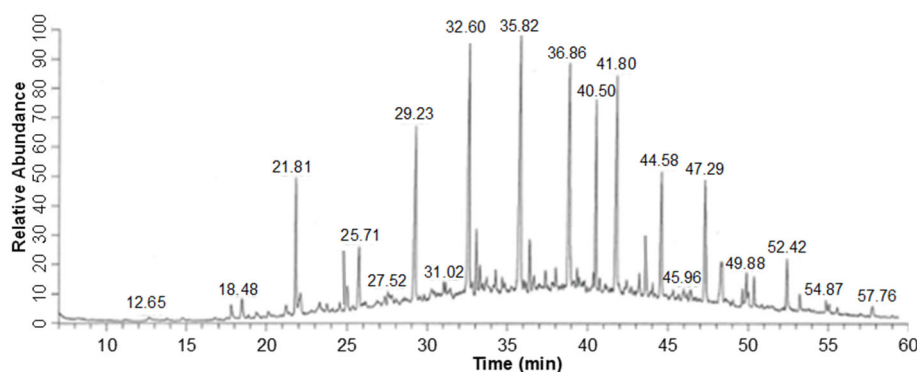


Fig 4. Gas chromatogram of *G. sagittalis* essential oil part

Table 1. Composition of the essential oil of *Genista sagittalis* from Kocaeli (Turkey)

Peak number	Classification	Component	Retention time/min	Area %	SI/RSI ^a
1	p-menthane monoterpene	α -terpinenyl acetate	21.81	0.78	911/938
2	straight-chain alkane	octadecane	25.71	0.31	850/858
3	straight-chain alkane	nonadecane	29.22	0.86	879/904
4	straight-chain alkane	eicosane	32.59	1.45	889/897
5	aromatic ether	1,2-dimethoxy-4-(1-propenyl)-(Isoeugenyl methyl ether)benzene	33.06	0.45	798/824
6	straight-chain alkane	heneicosane	35.79	1.50	874/904
7	sesquiterpene	hexahydrofarnesyl acetone (6,10,14-trimethyl-2-pentadecanone)	36.41	0.16	785/887
8	straight-chain alkane	hexatriacontane	38.83	1.11	880/926
9	aromatic ether	4-methoxy-6-(2-propenyl)-1,3-benzodioxole (Myristicin)	40.50	0.67	902/933
10	straight-chain alkane	tricosane	41.77	1.06	872/941
11	straight-chain alkane	heptacosane	44.56	0.53	845/887
12	straight-chain alkane	nonacosane	47.29	0.50	810/838
13	aromatic ester	2-cyclohexylethyl isobutylphthalate	48.30	0.31	690/837
14	straight-chain alkane	pentatriacontane	52.42	0.19	795/860

^aSI/RSI Search index/Reverse search index

carmustine, chlorambucil, cyclophosphamide, chlormethine, dacarbazine, fotemustine, lomustine, melphalan, streptozocin, temozolomide), platinum compounds (e.g., carboplatin, cisplatin, nedaplatin, oxaliplatin), antitumor antibiotics (e.g., bleomycin, doxorubicin, mithramycin), and various other substances (e.g., chloroquine) have been reported. Based on the interaction of small molecular weight ligand molecules with DNA, several short-term methods have been developed that can be applied to the discovery of naturally occurring agents that function through this mechanism. Doxorubicin, which is among the drug molecules known to interact with DNA through intercalation, decreases the peak current of DNA in the study conducted with High-Performance Liquid Chromatography (HPLC). Daunomycin and ethidium bromide, which are known to interact strongly with DNA, also showed similar properties [32-33]. In this context, the wide diversity in the binding mode between plant extracts and dsDNA has taken its place in the literature due to the following factors, with the idea of obtaining similar analytical signals [34]. These are $[DNA]/[plant\ extract]$ concentration ratio and buffer medium. This study is based

on $[DNA] = [plant\ extract]$ at pH 7.4 media.

DNA-Binding Study with UV Spectroscopy

UV-Vis absorption titration was used to determine the binding constants (K_b) and interaction of extracts **1** and **2** with dsDNA. In fact, the interaction of dsDNA with any molecule depends on the structure of that molecule. In studies conducted by some scientific groups, DNA interaction percentages with different plant extracts are given [21,35-37]. For example, the interaction of 52 methanolic and 51 dichloromethane crude plant extracts with herring sperm DNA has been made according to the HPLC method and was evaluated in terms of decreasing the peak absorbance values in HPLC by Correa et al. [35]. In the UV-Vis spectroscopic studies, the interaction of extracts **1** and **2** with dsDNA was investigated by examining the absorption spectra of the mixture solutions prepared at the appropriate concentration. Some solutions were prepared by adding increasing amounts of extract solutions (25–125 μ L) to dsDNA solution at a certain concentration prepared from a stock solution. The UV-Vis spectra obtained from these data are given in Fig. 2 and 3. The data

obtained from the spectrum clearly revealed that the information about the extract **1** or **2** - DNA interaction type and the K_b , which indicates the binding strength of the extracts to dsDNA, were calculated based on the titration data. The K_b also known as the intrinsic binding constant for two extracts with dsDNA can be obtained by monitoring the changes in absorbance between 215–375 nm and displayed as the slope of the graph, where $[\text{extract}]/(\epsilon_a - \epsilon_f)$ plotted against the molarity of extract according to the following “Benesi-Hildebrand” equation (Eq. 1) [38];

$$\frac{[\text{extract}]}{(\epsilon_a - \epsilon_f)} = \frac{[\text{extract}]}{(\epsilon_b - \epsilon_f)} + \frac{1}{K_b \times (\epsilon_a - \epsilon_f)} \quad (1)$$

where ϵ_a is the apparent extinction coefficient obtained by the calculation of $A_{\text{obsd}}/[\text{DNA}]$, ϵ_f is extinction coefficient of the DNA in its free form, ϵ_b is extinction coefficient for the DNA in the fully bound form and $[\text{extract}]$ is the increasing extract concentration. The high value of K_b obtained for extracts suggests a strong binding of two compounds to dsDNA. K_b values were found as $1.97 \times 10^3 \pm 0.37$ and $3.68 \times 10^2 \pm 0.44$ for extracts **1** and **2**, respectively. As a result, when extract **1** was added at increasing concentrations on 30 ppm dsDNA solution, a decrease in the absorbance value (hypochromic effect) of dsDNA was observed (% Hypo \pm RSDa = 16.73 ± 0.46). No red or blue shift was observed at the maximum wave value of dsDNA ($\lambda_{\text{max}} = 260$ nm). This situation was the same in the experiments performed with extract **2** solutions and the % Hypo \pm RSD for extract **2** was found to be 14.54 ± 0.39 . The formula “(% Hypo = % Hypochromism = $(A_0 - A)/A_0$)” was used in percent hypo calculations. All tests were performed in triplicate and are expressed as mean and relative standard deviation.

Voltammetric Studies

Voltammetry is an advantageous technique that provides high sensitivity, inexpensive, simple usage, rapid results, and compatibility with microfabrication technology. Due to the small sample requirement, it has been widely used in biological and chemical analyses. Electrochemical methods of DNA interaction of small molecules such as drugs are possible in two ways: (i) the signal change of the electroactive bases (guanine or

adenine, or both) is measured and interpreted according to these changes; (ii) necessary calculations are made by measuring the intensity of the peak currents in the oxidation and/or reduction direction of small molecules (before and after the interaction with DNA) [23-24,39]. In this study, extract **1** or **2** was separately added to the dsDNA solution of a certain concentration, and the changes in the peak current and peak potential of dsDNA were interpreted. The cyclic voltammograms of 10 ppm dsDNA (black) with increasing amounts of extract **1** and extract **2** in pH 4.8 acetate buffer have been given in Fig. 5 and 6.

The typical cyclic voltammograms (anodic direction) of 10 ppm dsDNA at GCE in pH 4.8 acetate buffer have two sharp oxidation that belongs to the dGuo and dAdo at 1.05 and 1.33 V, respectively. The interaction between extract **1** or extract **2** and dsDNA was evaluated after applying different incubation times from 3 to 15 min and cyclic voltammograms were recorded. The decrease in peak current intensities of both guanine and adenine, after activation with extract **1** or extract **2**, is clear evidence that these extracts interact over both bases. On the other hand, no oxidation peak was

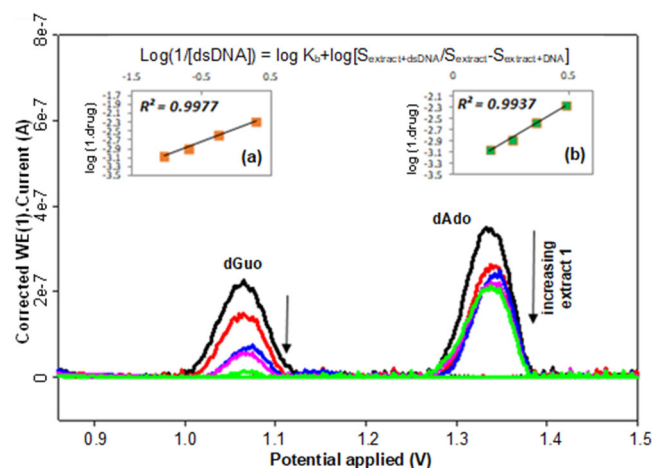


Fig 5. Cyclic voltammograms of 10 ppm dsDNA (black) with increasing amounts of extract **1** (12.5–75 μL) in pH 4.8 acetate buffer. (The arrow shows how the peak current decreased as the concentration of extract **1** increased, and binding constants (K_b) for dGuo (a) and dAdo (b) bases were determined from the slopes of the graphs)

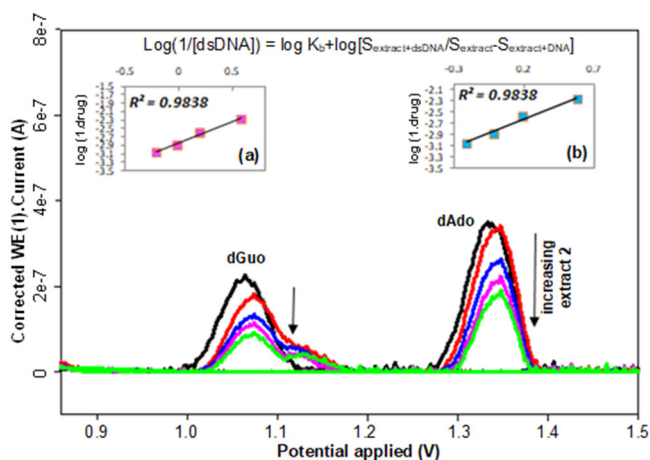


Fig 6. Cyclic voltammograms of 10 ppm dsDNA (black) with increasing amounts of extract 2 (12.5–75 μL) in pH 4.8 acetate buffer. (The arrow shows how the peak current decreased as the concentration of extract 2 increased, and binding constants (K_b) for dGuo (a) and dAdo (b) bases were determined from the slopes of the graphs)

observed in either extract 1 or extract 2 under these conditions. Based on these data, the K_b of each compound was calculated by Eq. 2 [40].

$$\log \frac{1}{[\text{extract}]} = \log K_b + \log \frac{S_{\text{dsDNA}} + \text{extract}}{S_{\text{dsDNA}} - (S_{\text{dsDNA}} + \text{extract})} \quad (2)$$

The terms used in the equation are as follows; [extract]: increasing extract concentration, $S_{\text{dsDNA-extract}}$: current signal received from dsDNA after interaction with

extract, and S_{dsDNA} : current signal received from the dsDNA alone extract [40]. The K_b and $\log K_b$ values calculated from these data are shown in Table 2. The two techniques have K_b values close to each other.

Antibacterial and Anti-QS Activities

The antimicrobial activity of alkaloid extract of *G. vuralii* A. Duran & Dural has been previously reported against *S. aureus*, *B. subtilis* and *Candida krusei* [41]. Antibacterial activities of genistin, isosalipurpol and koaburaside isolated from *G. numidica* Spach aerial parts were investigated in a recent study [42]. They were reported that these compounds have antibacterial activity against *S. aureus*, *S. epidermidis*, *P. aeruginosa* and *Enterococcus faecalis* with MICs ranging from 31.2 to 125 $\mu\text{g/mL}$, while 80% MeOH extract of the plant did not exhibit antibacterial activity. In our study, the growth of any of the bacteria species was not inhibited by *G. sagittalis* peduncle extract. However, the antibacterial activity of *G. sagittalis* flower extract was determined against *P. aeruginosa* 27853 and *P. mirabilis* 14153 with MICs 1 to 2 mg/mL (Table 3). Additionally, anti-QS activity was observed for peduncle extract at 1.3 mg/mL. Violacein production was reduced to $68.53 \pm 0.14\%$ (** $p < 0.01$) by the concentration of the extract. According to our results, *G. sagittalis* flower extract has low antibacterial activity against certain pathogens, while

Table 2. Voltammetric binding constant data for extracts

25 °C Compound 1	$K_b \pm \text{RSD}^a$		$\log K_b \pm \text{RSD}^a$	
	dGuo	dAdo	dGuo	dAdo
extract 1	$2.75 \times 10^2 \pm 0.41$	$2.82 \times 10^3 \pm 0.27$	2.44 ± 0.39	3.45 ± 0.35
extract 2	$6.76 \times 10^2 \pm 0.21$	$7.24 \times 10^2 \pm 0.33$	2.83 ± 0.43	2.86 ± 0.19

^a All tests were performed in triplicate and are expressed as mean and relative standard deviation

Table 3. Determination of the antibacterial activity of *G. sagittalis* methanol extracts (mg/mL)

	Flower extract (extract 1)	Peduncle extract (extract 2)	Growth inhibition %	Amp (200 $\mu\text{g/mL}$)	Kn (100 $\mu\text{g/mL}$)
<i>S. aureus</i> 29213	ND	ND	ND	+	+
<i>S. epidermidis</i> 12228	ND	ND	ND	+	+
<i>P. aeruginosa</i> 27853	2	ND	$17.33 \pm 0.94^{***}$	+	-
<i>P. mirabilis</i> 14153	1	ND	$11.17 \pm 2.32^*$	+	+
<i>C. violaceum</i> 12472	ND	ND	ND	-	+

ND: Not detected, Amp: Ampicillin, Kn: Kanamycine, +: Inhibition effect determined, -: Inhibition effect not determined,

* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

the peduncle extract showed strong anti-QS activity. However, to fully comprehend the processes underlying its antibacterial and anti-QS actions, more research is required.

■ CONCLUSION

Knowing that plants are a vital part of the world's natural heritage, and they have an important contribution to primary healthcare, this study presents the biological activities of *Genista sagittalis* L. (Fabaceae) from Kocaeli: Yuvacık Dam Basin in Turkey with the essential oil composition. The results demonstrated that a total of fourteen components were identified in the essential oil part. The antibacterial activity analyses demonstrated that *G. sagittalis* flower extract only had low activity against *P. mirabilis* and *P. aeruginosa* with MICs 1 to 2 mg/mL, as the peduncle extract showed strong anti-QS activity at 1.3 mg/mL. Additionally, dsDNA binding affinity investigations of the flower and peduncle ethanol extracts indicate that there are interactions with adenine and guanine bases. These results may offer new perspectives for pharmaceutical applications of *Genista sagittalis* and that further studies will enlighten the different applications and properties of this plant to scientists.

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■ AUTHOR CONTRIBUTIONS

Pelin Şenel and Elif Çepni Yüzbaşıoğlu conducted bioanalytical and microbiological studies, respectively. Bleda Can Sadıkoğulları and Gülnur Mertoğlu Elmas supervised experimental studies. Dilek Oral collected plant material. Ayşegül Gölçü and Ayşe Daut Özdemir

supervised, wrote and revised the manuscript. All authors agreed to the final version of this manuscript.

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