



A new possible antibacterial compound isolated from *Prangos aricakensis*, Daucosterol: Antibacterial activity and molecular docking approaches via DD-peptidase and gyrase

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ABSTRACT

Prangos species have been used for years to treat various ailments due to their chemical components. Eastern Türkiye is home to the locally endemic species *Prangos aricakensis*. This work is the first to isolate the daucosterol molecule from *P. aricakensis* stems. Daucosterol's structure was ascertained by 1D and 2D NMR analysis. Daucosterol has been studied to have many effects in many areas, such as antibacterial, immune system strengthening, and anti-cancer. Studies have been conducted on the molecule's antibacterial activity *in vitro* and its predicted molecular docking with antibiotic target proteins *in silico*. Compared to standard antibiotics, daucosterol had better effects against *Bacillus cereus* (512 µg/mL) and *Listeria monocytogenes* (1024 µg/mL). Additionally, daucosterol (512 µg/mL) and amoxicillin (512 µg/mL) demonstrated comparable efficacy against *Enterococcus faecalis* and *Klebsiella pneumoniae*. To predict the antibacterial effect of daucosterol in a computer environment, molecular docking interactions of daucosterol with DD-peptidase and gyrase (antibiotic target proteins) were studied and compared with tetracycline as a control antibiotic. Daucosterol and DD-peptidase showed perfect binding affinity, as -9.00, and daucosterol and gyrase had -8.90 kcal/mol. Results showed that the daucosterol molecule interacted with antibiotic target proteins and can be used as an *in vitro* antibacterial agent.

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Keywords

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Prangos aricakensis'ten izole edilen yeni olası antibakteriyel molekül, Daukosterol: Antibakteriyel aktivite ve DD-peptidaz ve giraz üzerinden moleküler yerleştirme yaklaşımları

ÖZET

Prangos türleri kimyasal bileşenlerinden dolayı yıllardır çeşitli rahatsızlıkların tedavisinde kullanılmıştır. Türkiye'nin doğusu, yerel olarak endemik tür olan *Prangos aricakensis*'e ev sahipliği yapmaktadır. Bu çalışma, daukosterol molekülünün *P. aricakensis* gövde kısmından izole edildiği ilk çalışmadır. Daukosterol'ün yapısı 1D ve 2D NMR analiziyle belirlenmiştir. Daukosterol'ün antibakteriyel, immün sistemi güçlendirici ve anti-kanser gibi birçok alanda etkisinin araştırıldığı bilinmektedir. Molekülün *in vitro* antibakteriyel aktivitesi ve *in silico* antibiyotik hedef proteinlerle öngörülen moleküler yerleşimi üzerine çalışmalar yürütülmüştür. Standart antibiyotiklerle karşılaştırıldığında, daukosterol *Bacillus cereus*'a (512 µg/mL) ve *Listeria monocytogenes*'e (1024 µg/mL) karşı iyi etkilere sahip çıkmıştır. Ayrıca, daukosterol (512 µg/mL) ve amoksisilin (512 µg/mL), *Enterococcus faecalis* ve *Klebsiella pneumoniae*'ye karşı karşılaştırılabilir etkinlik göstermiştir. Bilgisayar ortamında Daukosterol'ün antibakteriyel etkisini tahmin etmek için daukosterol'ün DD-peptidaz ve giraz (antibiyotik hedef proteinleri) ile moleküler yerleştirme etkileşimleri incelendi ve kontrol antibiyotik olarak tetrasiklinin bu hedef proteinlerle olan etkileşimleri ile karşılaştırıldı. Daukosterol ve DD-peptidaz -9.00 kcal/mol ile çok iyi

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bağlanma afinitesi gösterirken daukosterol ve giraz ise -8.90 kcal/mol ile iyi bir etkileşime sahip görünmektedir. Sonuçlar daukosterol molekülünün antibiyotik hedef proteinlerle etkileyici etkileşimlere sahip olduğunu ve *in vitro* antibakteriyel reaktif olarak kullanılabileceğini gösterdi.

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INTRODUCTION

Human bacteremia and other infectious disorders are linked to various pathogenic bacteria often resistant to aminopenicillins like ampicillin and amoxicillin (Landgren et al., 2005). Multidrug-resistant bacteria threaten the therapeutic effectiveness of several current antibiotics (Bandow et al., 2003). Antibiotic resistance has become one of the most urgent public health issues today. The swift and extensive emergence of resistance in pathogenic bacteria makes many conventional antibiotics ineffective, making once manageable infections increasingly challenging and, in some cases, impossible to treat. (Toner et al., 2015). In this study, daucoosterol isolated from *Prangos aricakensis*, an endemic species specific to Türkiye, may be important in terms of antibiotic resistance because it is a natural product.

The last transpeptidation step of the bacterial cell wall (peptidoglycan) production is catalyzed by the DD-peptidase enzymes (penicillin-binding proteins). Representatives of two low-molecular-mass groups of DD-peptidases have been shown to identify peptidoglycan structural components and react quickly with substrates and inhibitors that include these elements. There are still mysteries surrounding the substrate selectivity and quantitative features of the catalysis of bacterial DD-peptidases, even though their structures and overall reactivity are now well understood (Pratt, 2008).

One of the crucial and commonly targeted nodes is DNA gyrase, which is found in bacterial systems and antibacterial medications, such as the fluoroquinolone class of medicines. Gyrase is a desirable target for antibacterial strategies because of its crucial core function. Gyrase activity would be naturally controlled in cells, which would aid in coordinating DNA replication with doubling times. Many proteins have been found to inhibit DNA gyrase, albeit at lower concentrations. This activity may seem reversible and has regulatory significance (Ruan et al., 2024).

Plant materials, whether standardized extracts or pure chemicals, provide encouraging prospects for novel anti-infective medications. It is imperative to find new antimicrobial agents with various chemical structures and unique modes of action to treat emerging and reemerging infectious diseases. Thus, to create more effective antimicrobial medications, scientists are focusing more on natural ingredients (Cho et al., 2012). Increased yields of bioactive components have led to the cultivation of medicinal plants. The domestication of agricultural crops has increased the amount of certain bioactive chemicals, changing the phytochemical makeup of numerous plants over time (Schmidt et al., 2008).

The *Prangos* plant, which belongs to the Apiaceae family, was once used in Türkiye as an anthelmintic to stop external bleeding, a carminative for stomach or digestive issues, to heal wounds, to close scars, to treat leukoplakia, as a natural fertilizer, stimulant, and aphrodisiac (Bruno et al., 2019; Özek et al., 2018). *Prangos ferulacea*, often known as "Heliz" in Turkish, is an antimicrobial added to traditionally prepared herbaceous cheese. The young shoots of this plant, known as "Çasir," are consumed fried, pickled, or boiled (Bruno et al., 2019). *P. aricakensis*, one of the *Prangos* species, is a recently described plant that is locally endemic in the Arıcak locality of Elazığ province in Türkiye's Eastern Anatolia region. This plant thrives on rocky slopes between 2000 and 2050 meters above sea level and in areas smaller than 10 km² (Behçet et al., 2019).

Plant sterols or phytosterols, which have similar structural and biological functions to cholesterol, have been reported to have a variety of biological activities (Ramprasath & Awad, 2015). Daucoosterol [(3 β)-stigmast-5-en-3-yl β -D-glucopyranoside], a natural β -sitosterol glycoside, is a saponin phytosterol belonging to different families and genera found in many countries (El Omari et al., 2022). Daucoosterol, a β -sitosterol glycoside, is one of the main phytosterols in higher plants (Mozaffarian, 1996).

Daucoosterol has been reported to be promising in treating various conditions, including bacterial diseases (Cho et al., 2012). It has been found that the daucoosterol enhances immunological function by promoting the proliferation

of human peripheral blood leukocytes. Daucosterol has anti-cancer and anti-seizure properties and boosts the activity of natural killer cells, cytokines, interleukin 2, γ -interferon, and helper T-cells. Additionally, it has been shown to block the enzymes succinic semialdehyde reductase and succinic semialdehyde dehydrogenase, which break down gamma-aminobutyric acid (GABA). This suggests that it may have an anticonvulsant effect by increasing GABA levels in the central nervous system (Cho et al., 2012; Lee et al., 2007). According to the literature, daucosterol has different pharmacological characteristics and has been the subject of numerous investigations, such as its immunomodulator (Lee et al., 2007), chemopreventive (Nguedia et al., 2020), antioxidant (Abdollahnezhad et al., 2021), neuroprotective (Zhang et al., 2020), antidiabetic (Gu et al., 2021b), anti-inflammatory (Jang et al., 2019), and inhibition of α -amylase (Gu et al., 2021a) effects.

Daucosterol has previously been isolated in *Prangos* species only from the leaves and stems of *P. ferulacea* by maceration extraction with *n*-hexane, dichloromethane, ethyl acetate, and methanol (Abdollahnezhad et al., 2021). It has also been isolated from the Apiaceae family, the whole *Heracleum persicum* plant (Dehghan, 2019), and the aerial parts of *Dorema glabrum* Fisch. and C.A. Mey. (Delnavazi et al., 2015), and from the roots of *Sphallerocarpus gracilis* (Gao et al., 2011).

The literature investigates the antibacterial properties of compounds derived from natural sources and the *in silico* molecular docking interactions of these compounds with antibiotic target proteins for supporting *in vitro* studies (Abdullah et al., 2024; Dhamodiran et al., 2024; Nayel et al., 2024). In this study, a phytochemical compound, daucosterol, was isolated and characterized for the first time from the stems of *Prangos aricakensis*, and its *in vitro* antibacterial activity was investigated. In addition, the molecule's interactions with antibiotic target proteins were investigated by *in silico* molecular docking techniques.

MATERIAL and METHOD

Plant collection

At an elevation of roughly 2000–2050 m, with a herbarium number of BIN-6256, *P. aricakensis* Behçet & Yapar was found in Elazığ (B8: Arıcak, Akdağ Mountain, Cuber Plateau, 38°36'01" N 40°07'58" E, on stony slopes), collected and identified by Prof. Dr. Lutfi Behçet. *Prangos aricakensis* is introduced and depicted as a novel species originating from eastern Anatolia (Turkiye), identified from a unique site in the Arıcak (Elazığ) district. *Prangos aricakensis* bears resemblance to *Prangos turcica*, *Prangos hulusii*, *Prangos ilanae*, and *Prangos trifida*, yet it is distinct in terms of hair, leaf, bract, bracteole, and fruit traits (Behçet et al., 2019). The plant material was dried indoors with air conditioning in a dry, cold setting.

Extraction and chromatographic methods for the isolation of the compound

The maceration procedure was used to extract 100 g of the dried stem portion of the *P. aricakensis* plant using a CH₃OH: CHCl₃ (1:1) mixture, for 1400 mL. The process was repeated until the color change became visible 3 times at room temperature. A rotary evaporator was used at 40°C to remove the solvent. After, the raw extract was lyophilized and dried. Fractions were obtained from the crude extract (10 g) using silica gel column chromatography (50mm x 800 mm) and an ascending polarity system (*n*-hexane, CHCl₃, EtOAc, and CH₃OH, absolute solvents). (Marah et al., 2025). Fractions were obtained using an increasing polarity system from *n*-hexane to CH₃OH (100%:0% → 90%:10% → 0%:100%), under atmospheric pressure and 3 mL/min flow rate. Similar fractions were mixed using thin-layer chromatography (Gökçimen et al., 2024). An Agilent-600 MHz spectrometer and CDCl₃ were used to elucidate the structure of daucosterol by 1D and 2D NMR analysis (COSY, HSQC, and HMBC spectra were given as supplementary file, Figures S1-S3). Daucosterol was isolated from the fourth fraction (13th-17th tubes, approximately 100 mL) of the *P. aricakensis* stem CH₃OH: CHCl₃ (1:1) extract.

Antibacterial activity assay

The antibacterial properties of the molecule were determined using the Minimum Inhibitory Concentration (MIC) approach (Andrews, 2001). Tetracycline and amoxicillin were used as standard antibiotics (as a positive control, and we applied the MHB-solvent as a negative control) due to their frequent application in the literature. The tested bacterial strains included both Gram-positive and Gram-negative species: Gram-positive bacteria comprised *Bacillus cereus* (CCM 99), *Enterococcus faecalis* (ATCC 29212), *Listeria monocytogenes* (ATCC 35152), and *Staphylococcus aureus* (ATCC 25213), while Gram-negative bacteria included *Klebsiella pneumoniae* (ATCC 10031), *Escherichia coli* (ATCC 25922), *Salmonella enterica* (ATCC 13311), and *Pseudomonas aeruginosa* (ATCC 15442). 100 mL of Mueller Hinton Broth (MHB) was mixed with 1000 μ L of 2 mg/mL CaCl₂ and 400 μ L of 2 mg/mL MgCl₂ solutions to create cationic MHB. After adjusting the McFarland value of 100 μ L of bacteria, 900 μ L of cationic MHB was applied. The wells were filled with 100 μ L of cationic MHB, 100 μ L of sample, and 100 μ L of bacteria. For two hours, the plate was kept at 4°C. After incubation at 37°C for 16-18 hours. Samples were diluted

by half as a result of serial dilutions starting from a concentration of 1024 µg/mL. When the turbidity or precipitation was observed, the previous well's concentration value was recorded as MIC (µg/mL).

Molecular docking

The structures of the antibiotic targets were retrieved from the RCSB PDB at an appropriate resolution: PDB ID: 2XCS for gyrase and PDB ID: 3PTE for DD-peptidase (Berman et al., 2000). The Chem-Draw Ultra program 18.0 was used to draw the daucosterol and tetracycline molecules, and their minimal energy form were obtained in the Chem3D program. To ascertain the interactions between the proteins and daucosterol, molecular docking was carried out using the AutoDock Vina 1.5.7 program to determine the binding affinities between proteins and daucosterol (Trott & Olson, 2010). Ten rounds of the docking process were conducted using the grid box coordinates of the x, y, and z axes as follows: 13.712, -4.711, and 39.139 for 3PTE receptors, and 20.192, 44.731, and 45.356 for 2XCS. To determine the whole structure of the antibacterial target proteins and the molecule, and to obtain the 2D and 3D image shapes of the molecular docking product, BIOVIA Discovery Studio Visualizer 2019 was utilized (BIOVIA, 2019).

RESULTS and DISCUSSION

Characterization of the molecule

Initially, 18.55 grams of crude extract were obtained from 100 grams of dry plant. After separation into 7 fractions by column chromatography, 14 mg of daucosterol (0.075% yield) was isolated from the 4th fraction of *P. aricakensis* stem extract (Figure 1). Half of this amount was used in NMR analysis, and the other half in *in vitro* tests. ¹H and ¹³C NMR spectra of the molecule were given in Figure 2, data of the molecule and comparison of the data with literature (Li et al., 2010; Peshin & Kar, 2017) were given in Table 1. Also, the molecule's 2D (COSY, HSQC, and HMBC) spectra were given in Figures S1-S3.

When the ¹H NMR spectrum was examined, the signals in the range of 2.5-5 ppm, indicating the presence of a sugar group and the presence of aliphatic groups, are remarkable (Figure 2a). The presence of an olefinic double bond and anomeric carbon atoms in the structure was determined from the ¹³C NMR spectrum (Figure 2.b). The ¹H and ¹³C density in the aliphatic region and the presence of anomeric and olefinic carbon atoms suggested that the structure was a steroid containing a sugar group. The interactions of the specific H6 olefinic proton with H7 protons were observed from the COSY spectrum (Figure S1). Also, H3 and H2, H4 interactions, and H1' H2' sugar protons interaction were determined clearly. Detailed studies followed other overlapping COSY interactions, and the main steroid structure was obtained. The carbon atoms bound to the corresponding proton were determined from the HSQC spectrum (Figure S2). The interaction of the H3 proton directly with the anomeric sugar C1' carbon atom was determined from the HMBC spectrum (Figure S3). Also, anomeric C1' carbon atom interactions with the sugar H2' and OH protons were determined. The interaction of olefinic carbon atoms with H4 proton and H4 proton interaction with C3 carbon and H6 olefinic proton interaction with C4, C7, and C8 carbons was observed from the spectrum. Detailed studies followed other aliphatic region interactions, and the steroid structure was corrected and obtained from the COSY spectrum. All conducted spectrums and ¹³C and ¹H values showed that the molecule was daucosterol, the *o*-glycoside form of *β*-sitosterol, abundant in plant sources.

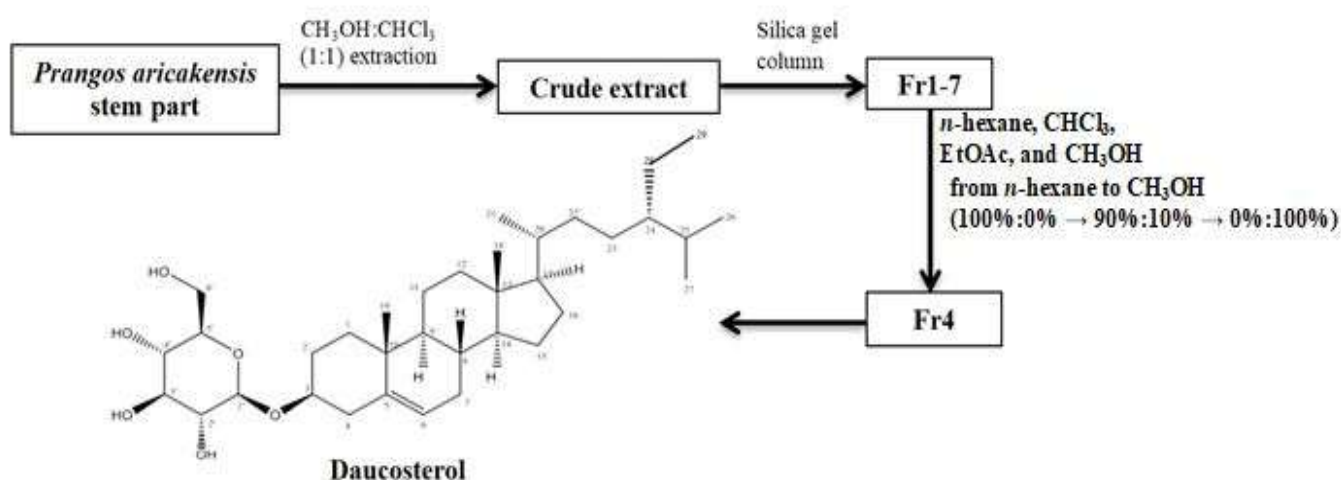


Figure 1. Isolation process scheme of daucosterol from the *P. aricakensis* stem part

Şekil 1. *P. aricakensis* gövde kısmından daukosterol'ün izolasyon proses şeması

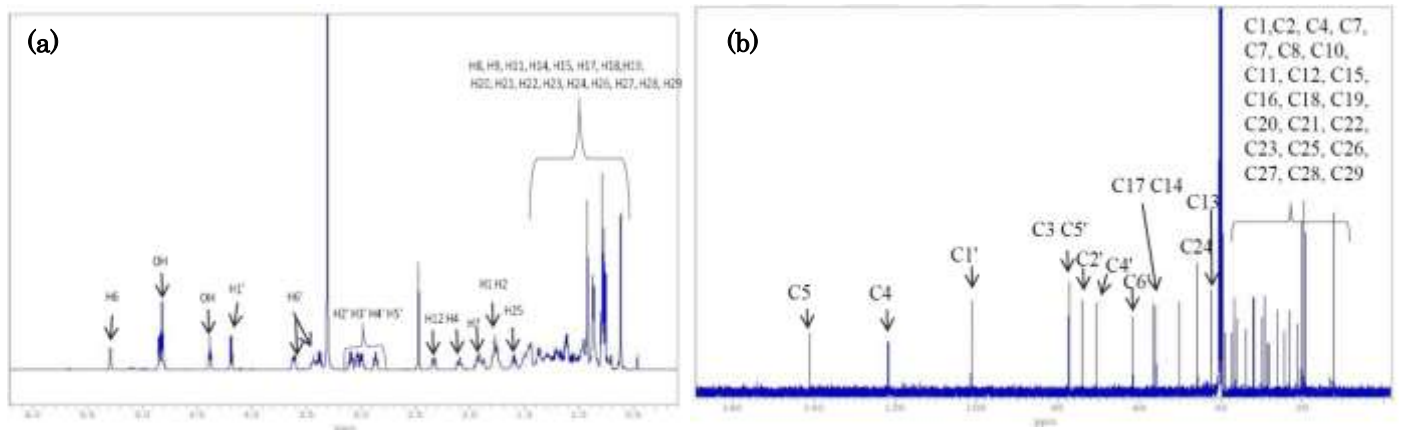


Figure 2. ¹H-NMR (DMSO *d*₆ 600 MHz) (a) and ¹³C-NMR (DMSO *d*₆ 150 MHz) (b) spectra of Daucosterol
Şekil 2. Daukosterol'ün (a) ¹H-NMR (DMSO *d*₆ 600 MHz) ve (b) ¹³C-NMR (DMSO *d*₆ 150 MHz) (b) spektrumları

Table 1. ¹H (DMSO *d*₆ 600 MHz) and ¹³C NMR (DMSO *d*₆ 150 MHz) data of daucosterol
Çizelge 1. Daukosterol'ün ¹H (DMSO *d*₆ 600 MHz) ve ¹³C NMR (DMSO *d*₆ 150 MHz) verileri

No	¹ H NMR data	¹³ C NMR data	¹ H NMR (Li et al., 2010)	¹³ C NMR (Peshin & Kar, 2017)
1	0.96-1.77 (d)	37.27	1.09 (d)	36.79
2	1.44-1.78 (d)	29.69	1.36 (d)	29.23
3	3.43 (s)	77.30	3.53 (s)	76.85
4	2.10-2.35 (d)	38.72	2.29 (d)	39.28
5	-	140.10	-	140.40
6	5.30 (d, <i>J</i> =3.3 Hz)	121.67	5.30 (s)	121.20
7	1.90 (s)	31.85	1.56 (d)	31.38
8	1.37 (s)	31.82	1.45 (s)	31.26
9	0.87 (s)	50.04	0.95 (s)	49.55
10	-	36.80	-	36.18
11	1.37-1.45 (d)	21.04	1.48 (d)	20.56
12	2.35-2.09 (d)	39.90	1.17 (d)	38.26
13	-	42.50	-	41.82
14	0.95 (s)	56.61	1.01 (s)	56.13
15	1.50 (s)	24.32	1.10 (d)	23.83
16	-	28.01	1.85 (s)	27.76
17	1.04 (s)	55.81	1.15 (s)	55.38
18	0.67 (s)	12.11	0.61 (s)	11.64
19	0.78 (s)	19.55	0.95 (s)	19.07
20	1.32 (s)	35.94	1.36 (s)	35.45
21	0.93 (s)	19.06	0.85 (s)	18.58
22	0.97 (s)	33.76	1.04 (d)	33.29
23	1.11 (s)	25.81	1.12 (s)	25.36
24	0.88 (s)	45.56	0.98 (s)	45.09
25	1.60 (s)	29.10	1.67 (s)	28.64
26	0.87 (s)	19.56	0.75 (s)	19.69
27	0.87 (s)	19.37	0.76 (s)	18.89
28	1.22-1.16 (d)	23.02	1.23 (d)	22.06
29	0.79 (s)	12.22	0.80 (s)	11.75
1'	4.18 (d, <i>J</i> =7.3 Hz)	101.10	4.35 (s)	100.75
2'	2.86 (s)	73.86	3.19 (s)	73.42
3'	3.04 (s)	77.32	3.38 (s)	76.86
4'	2.99 (s)	70.45	3.35 (s)	70.04
5'	3.04 (s)	77.20	3.23 (s)	76.72
6'	3.38-3.61 (d)	61.46	3.70 (d)	62.82

(s: singlet, d: doublet)

Antibacterial activities

The least dilution of a test culture that stops microbial growth is found using the minimum inhibitory concentration (MIC) assay. This test prevents organism development and is widely used in pharmaceutical regulatory testing (Vassallo et al., 2016).

As seen in Table 2 and Figure 3, while the daucosterol molecule (512 µg/mL) shows a similar effect to amoxicillin (512 µg/mL) against *K. pneumoniae* and *E. faecalis*, it seems to be more effective than amoxicillin (> 1024 µg/mL) against *S. enterica* (1024 µg/mL). In addition, daucosterol seems to be more effective than antibiotics against *B. cereus* (512 µg/mL) and *L. monocytogenes* (1024 µg/mL).

According to literature research, Stefani et al. (2019) isolated the daucosterol molecule from *Hechtia glomerata* leaves and determined the MIC values as 200 µg/mL against *P. aeruginosa* and > 250 µg/mL against *K. pneumoniae*, *E. coli*, and *S. aureus*. Lai et al. (2003) isolated the daucosterol molecule from the aerial parts of *Helwingia chinensis* and determined that it showed an antibacterial effect against *S. aureus*.

Also, Wang et al. (2016) showed daucosterol has anti-cancer properties and causes human colon cancer cells (HCT-116) to undergo apoptosis, inhibiting these cells' ability to migrate and invade. Along with altering the expression levels of proteins that are crucial for apoptosis, daucosterol also caused sub-G1 phase arrest. Esmaili and Farimani (2014) demonstrated that daucosterol affected MCF-7 cells and revealed that it decreased cell proliferation by triggering apoptosis, and this was brought on by oxidative stress linked to mitochondria and subsequently led to PI3K/Aktin. Daucoosterol could be one of the potential pharmacological therapy approaches for MCF-7 cells. Gao et al. (2019) showed that daucosterol induced cell cycle arrest, decreased cell growth, and facilitated autophagy-dependent apoptosis in prostate cancer. Zhao et al. (2015) showed that the daucosterol molecule prevents the formation of tumors both *in vivo* and *in vitro*. It causes ROS-triggered autophagy in place of apoptosis, which stops the growth of cancer cells.

Table 2. MIC results of Daucoosterol and antibiotics against gram-negative and gram-positive bacteria
Çizelge 2. Daucoosterol ve antibiyotiklerin gram negatif ve gram pozitif bakterilere karşı MİK sonuçları

Samples	Gram-negative bacteria			
	<i>E. coli</i>	<i>P. aeruginosa</i>	<i>K. pneumoniae</i>	<i>S. enterica</i>
Daucoosterol	512	1024	512	1024
Amoxicillin	2	256	512	> 1024
Tetracycline	8	128	256	32
Samples	Gram-positive bacteria			
	<i>E. faecalis</i>	<i>B. cereus</i>	<i>S. aureus</i>	<i>L. monocytogenes</i>
Daucoosterol	512	512	1024	1024
Amoxicillin	512	> 1024	512	> 1024
Tetracycline	512	> 1024	8	> 1024

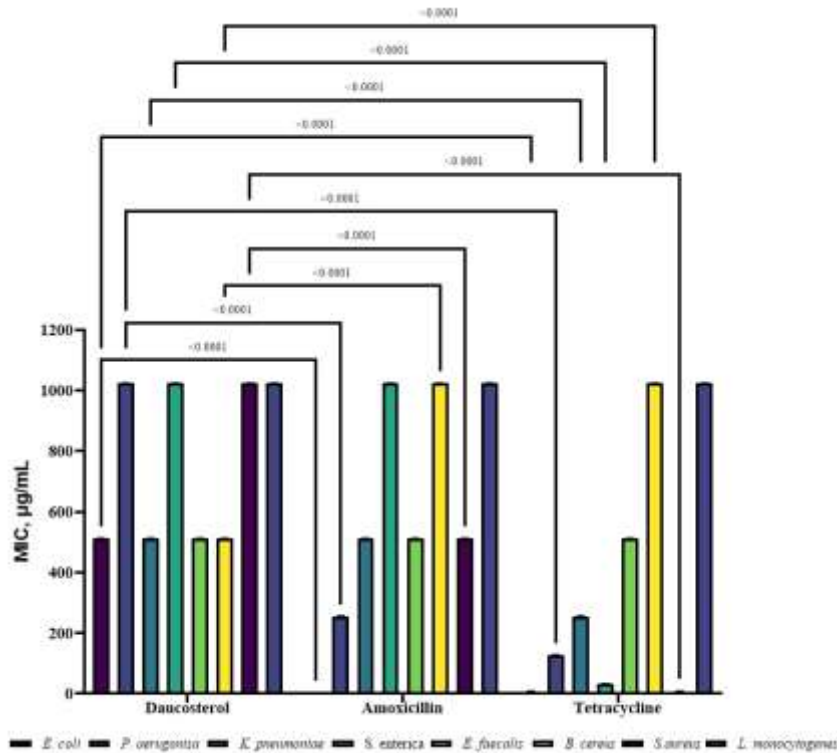


Figure 3. MIC results scheme of Daucoosterol and antibiotics against gram-negative and gram-positive bacteria
Şekil 3. Daucoosterol ve antibiyotiklerin gram negatif ve gram pozitif bakterilere karşı MİK sonuçlarının şeması

Molecular docking

Molecular docking is an essential technique for developing drugs based on receptor structure. To find as many complicated conformations as possible, molecular docking processing must properly evaluate the conformations that were looked for in the previous step. The current molecular docking methods typically reduce the molecular

between daucosterol and DD-peptidase was -9.00 kcal/mol. These results are more effective than interactions between tetracycline-gyrase (-8.20 kcal/mol) and tetracycline-DD-peptidase (-6.60 kcal/mol). The daucosterol molecule showed more effective results than the control antibiotic (tetracycline) with the antibiotic target proteins. Therefore, the daucosterol molecule showed quite strong *in silico* antibacterial activity. These results support the results of the *in vitro* MIC assay.

Table 3. Binding affinities and best pose interactions between gyrase-daucosterol, DD-peptidase-daucosterol, gyrase-tetracycline, and DD-peptidase-tetracycline

Çizelge 3. Giraz-daukosterol, DD-peptidaz-daukosterol, giraz-tetrasiklin ve DD-peptidaz-tetrasiklin arasındaki etkileşimlerin en iyi pozlarının bağlanma afiniteleri

Interactions	Aminoacid zone	Distance	Bond type	Binding affinity
Gyrase- daucosterol	B:GLN1267:O	2.15010	Conventional hydrogen bond	-8.90 kcal/mol
	B:GLN1267:O	1.69902	Conventional hydrogen bond	
	B:GLN1267:HA	2.35332	Carbon-hydrogen bond	
	B:ALA1118	3.29973	Alkyl	
	B:ALA1120	4.19222	Alkyl	
	B:VAL1091	3.32667	Alkyl	
	B:MET1121	4.85269	Alkyl	
	B:TYR1087	4.40309	Pi-Alkyl	
	B:TYR1087	4.26691	Pi-Alkyl	
	B:PHE1097	4.79960	Pi-Alkyl	
DD-peptidase- daucosterol	A:TYR159:HH	2.14508	Conventional hydrogen bond	-9.00 kcal/mol
	A:ASN161:HD22	2.01986	Conventional hydrogen bond	
	A:THR301:O	2.09578	Conventional hydrogen bond	
	A:ASN161:OD1	1.62295	Conventional hydrogen bond	
	A:SER62:OG	2.36572	Conventional hydrogen bond	
	A:GLY238:HA1	2.87777	Carbon-hydrogen bond	
	A:ASN161:OD1	2.54153	Carbon-hydrogen bond	
	A:PHE120	5.00314	Pi-Alkyl	
	A:PHE120	4.78580	Pi-Alkyl	
	A:TRP233	4.43550	Pi-Alkyl	
Gyrase- tetracycline	B:ARG1092:HE	2.86186	Conventional hydrogen bond	-8.20 kcal/mol
	B:GLN1267:HN	2.25921	Conventional hydrogen bond	
	B:VAL1268:HN	2.61484	Conventional hydrogen bond	
	B:SER1112:O	2.30673	Conventional hydrogen bond	
	B:SER1112:O	2.45312	Conventional hydrogen bond	
	B:GLN1095:OE1	1.98390	Conventional hydrogen bond	
	B:SER1098:HA	2.71730	Carbon-hydrogen bond	
	B:GLY1111:HA2	2.52624	Carbon-hydrogen bond	
	B:ASN1269:HA	2.45912	Carbon-hydrogen bond	
	B:SER1098:OG	2.52968	Carbon-hydrogen bond	
	B:GLN1095:HE21	2.08914	Pi-Donor hydrogen bond	
	B:ARG1092	4.43506	Alkyl	
	B:ARG1092	5.25543	Pi-Alkyl	
DD-peptidase- tetracycline	A:SER62:HG	1.83906	Conventional hydrogen bond	-6.60 kcal/mol
	A:TYR159:HH	2.25894	Conventional hydrogen bond	
	A:THR299:HG1	2.85249	Conventional hydrogen bond	
	A:THR301:HN	2.63362	Conventional hydrogen bond	
	A:THR301:HG1	2.60331	Conventional hydrogen bond	
	A:SER62:HB1	2.88460	Carbon-hydrogen bond	
	A:GLY300:HA2	2.67480	Carbon-hydrogen bond	
	A:VAL302:HA	2.90658	Carbon-hydrogen bond	
	A:PHE120	4.32081	Pi-Pi stacked	

There were ten interactions between daucosterol and gyrase: A conventional hydrogen bond interaction with GLN1267:O, a carbon-hydrogen bond with GLN1267: HA, an alkyl with ALA1118, ALA1120, VAL1091, and MET1121, and a pi-alkyl with TYR1087 and PHE1097. The ten interactions between daucosterol and DD-peptidase were: A conventional hydrogen bond interaction with TYR159:HH, ASN161:HD22, THR301:O, ASN161:OD1, and SER62:OG, a carbon-hydrogen bond with GLY238:HA1 and ASN161:OD1, and a pi-alkyl with PHE120 and TRP233. There were thirteen interactions between tetracycline and gyrase: A conventional hydrogen bond interaction with ARG1092:HE, GLN1267:HN, VAL1268:HN, SER1112:O, and GLN1095:OE1, a carbon-hydrogen bond with SER1098:HA, GLY1111:HA2, ASN1269:HA, and SER1098:OG, a pi-donor hydrogen bond with GLN1095:HE21, and alkyl and pi-alkyl with ARG1092. The nine interactions between tetracycline and DD-peptidase were: A conventional hydrogen bond interaction with SER62:HG, TYR159:HH, THR299:HG1, THR301:HN, and THR301:HG1, a carbon-hydrogen bond with SER62:HB1, GLY300:HA2, and VAL302:HA, and a pi-pi stacked with PHE120.

CONCLUSIONS

Computer modeling could provide novel insights into how antimicrobial drugs function, thereby preventing the spread of illness. Molecular docking can be used to predict which chemicals could bind selectively to the active portions of the protein that induce bacterial infections (Toubi et al., 2019).

The antibacterial properties of the isolated molecule were examined *in vitro* and *in silico*. The compound and the antibiotic target proteins interacted strongly, according to the *in silico* studies. Even the results were stronger than the interactions of the tetracycline with these target proteins. In the future, extensive expansion and further research are required in the use of this technology in clinical applications and in the development of new antibiotics. The simulations are found to assist and improve the experimental tests. Based on the *in vitro* MIC test outcomes, it was concluded that this molecule interacts strongly with the target proteins and may have antibacterial properties.

These results may provide a strong basis for the positive applications of *P. aricakensis*. As a result, the daucosterol molecule isolated from *P. aricakensis* may be useful in treating some antimicrobial illnesses. This paper was considered a useful resource for the potential upcoming *in vivo* research. Daucosterol, a substance found in the stems of the *P. aricakensis* plant, may have antibacterial properties. It was also shown that this molecule exhibited strong connections between gyrase and DD-peptidase, which are antibiotic target proteins. Despite all this, there are no *in vivo* tests, and it is necessary to test in animal models or investigate synergistic effects. Further, the main goals of future research should clarify its therapeutic efficacy in various cancer types and inflammatory diseases, enhancing bioavailability through drug delivery systems like nanoparticles, and bolstering its pharmacological potential with in-depth molecular dynamics simulations that target particular proteins. These studies will help clarify the clinical potential of daucosterol and might lead to the creation of novel treatment approaches.

Contribution Rate Statement Summary of Researchers

The authors declare that they have contributed equally to the article.

Conflict of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supporting Information

Some results of the current article are provided in the Supporting Information (Figures S1, S2, and S3).

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