



In vitro antibacterial activity of pineapple peel extracts and chemical composition of dimethyl sulfoxide extract

Gülçin ÖZCAN ATEŞ^{1*}, Rumeysa Asude GÖKALP²

¹Çanakkale Onsekiz Mart University, Vocational School of Health Services, Department of Medical Services and Techniques, Çanakkale, Türkiye

²Çanakkale Onsekiz Mart University, Engineering Faculty, Department of Bioengineering, Çanakkale, Türkiye
*gulcinozcan@comu.edu.tr, ²asude.gklp@gmail.com

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Ananas kabuğu ekstraktlarının in vitro antibakteriyel aktivitesi ve dimetil sülfoksit ekstraktının kimyasal kompozisyonu

Abstract: In this study, ethanol, methanol, and dimethyl sulfoxide (DMSO) extracts were obtained from pineapple (*Ananas comosus* L.) peel by ultrasonication, and their antibacterial activity was evaluated in vitro using the agar well diffusion and microdilution methods. Pineapple peel extracts obtained by the agar well diffusion method did not form any inhibitory zones against the tested bacteria (*Escherichia coli* (ATCC 1301, ATCC 25922, ATCC 8739, and NRRL B-3704), *Klebsiella pneumoniae* ATCC 700603, *Salmonella enterica* subsp. *enterica* ATCC 13076, and *Salmonella enterica* subsp. *typhimurium* ATCC 51812, *Pseudomonas aeruginosa* (ATCC 10145 and ATCC 27853), *Bacillus cereus*, *Bacillus subtilis*, *Enterococcus faecalis* ATCC 29212, and *Staphylococcus aureus* ATCC 29213). Furthermore, MIC values were determined to be >125 µL/mL by microdilution. Moreover, the chemical composition of the DMSO extract from pineapple peel was determined by headspace solid-phase microextraction–gas chromatography–mass spectrometry (HS-SPME-GC-MS), and the main component was identified as pinostrobin (44.66%), a flavanone. In conclusion, the extracts obtained from pineapple peel do not exhibit antibacterial activity, and the results are consistent with the chemical composition. This study demonstrates the importance of evaluating chemical composition together with in vitro biological activities in determining the biological potential of plant and food wastes.

Key words: Pineapple peel, extract, waste utilization, chemical composition, in vitro antibacterial activity

Özet: Bu çalışmada, ananas (*Ananas comosus* L.) kabuğundan ultrasonikasyonla elde edilen etanol, metanol ve dimetilsülfoksit (DMSO) ekstraktlarının antibakteriyel aktivitesi in vitro agar kuyu difüzyon ve mikrodilüsyon yöntemleriyle değerlendirildi. Agar kuyu difüzyon yöntemiyle elde edilen ananas kabuğu ekstraktlarının test edilen bakterilere (*Escherichia coli* (ATCC 1301, ATCC 25922, ATCC 8739 ve NRRL B-3704), *Klebsiella pneumoniae* ATCC 700603, *Salmonella enterica* subsp. *enterica* ATCC 13076, *Salmonella enterica* subsp. *typhimurium* ATCC 51812, *Pseudomonas aeruginosa* (ATCC 10145 ve ATCC 27853), *Bacillus cereus*, *Bacillus subtilis*, *Enterococcus faecalis* ATCC 29212 ve *Staphylococcus aureus* ATCC 29213 karşı herhangi bir inhibisyon bölgesi oluşturmadığı belirlendi. Ayrıca, mikrodilüsyon yöntemi kullanılarak MİK değerlerinin >125 µL/mL olduğu tespit edildi. Bununla birlikte, ananas kabuğundan elde edilen DMSO ekstraktının kimyasal kompozisyonu headspace katı faz mikroeekstraksiyon–gaz kromatografisi–kütle spektrometresi (HS-SPME-GC-MS) yöntemi ile belirlendi ve sonuç olarak ana bileşenin flavanon yapısındaki pinostrobin (%44,66) olduğu belirlendi. Sonuç olarak, ananas kabuğundan elde edilen ekstraktların antibakteriyel aktiviteye sahip olmadığı ve kimyasal kompozisyonu ile elde edilen sonuçların tutarlı olduğu belirlendi. Bu çalışma, bitki ve gıda atıklarının biyolojik potansiyellerinin belirlenmesinde kimyasal kompozisyon ile in vitro biyolojik aktivitelerin birlikte değerlendirilmesinin önemini göstermektedir.

Anahtar Kelimeler: Ananas kabuğu, ekstrakt, atıkların değerlendirilmesi, kimyasal kompozisyon, in vitro antibakteriyel aktivite

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1. Introduction

Widely cultivated in tropical and subtropical regions, pineapple (*Ananas comosus* L.) is the third most consumed fruit worldwide after citrus fruits and bananas, with an annual production reaching 27.9 million tons (FAO, 2023; Zhou et al., 2023). The fruit portion consumed by humans constitutes a maximum of 60% of the total weight of the pineapple; the remaining percentage consists of the crown, stem, peel, and clippings, which are considered waste (Banerjee et al., 2017; Meena et al., 2022; Zhou et al., 2023). If this pineapple waste is not properly disposed of, it causes environmental pollution. Pineapple waste leads to leachate formation, landfill gas emissions, and, when burned, the formation of dioxins, furans, acid gases, and particulate matter air pollutants (Zhou et al., 2023). Studies

on pineapple peel have shown that, in addition to cellulose, hemicellulose, and lignin, it is also rich in phytochemicals, possessing various bioactive components such as phenolic compounds (epicatechin, gallic acid, catechin), flavonoids (quercetin), bromelain enzyme, organic acids, and polysaccharides (Ali et al., 2020; Zhou et al., 2023; Mala et al., 2024). Bioactive components obtained from food waste, in particular, are of great importance for both sustainability and economic value (Yadav et al., 2024).

Studies on the conversion and extraction of pineapple peel into various components are reported in the literature, and it is considered an economical raw material. Bromelain (Ketnawa et al., 2012; Zaki et al., 2017), phenolic compounds (Saraswaty et al., 2017), fiber production (Huang et al., 2011; Sah et al., 2016), organic acids through

fermentation of sugars and other nutrients (Imandi et al., 2008; Cheok et al., 2018), and enzymes (Zaki et al., 2017; Sepúlveda et al., 2018) have been obtained from pineapple peel. In addition, vanillin (Cheok et al., 2018) and vinegar production (Roda et al., 2017a,b) have also been carried out from pineapple waste matrix. Studies have shown that pineapple waste can be used to produce functional food components with therapeutic properties (Roda & Lambri, 2019). Furthermore, it may be a potential source of natural antimicrobial and antioxidant compounds (Gunwantrao et al., 2016; Lubaina et al., 2019; Roda & Lambri, 2019).

Extraction is the most important step in obtaining bioactive compounds from fruit and vegetable waste, as it determines the type and quantity of these compounds, which play a significant role in biological activities (Khoddami et al., 2013; Baiano, 2014; Sagar et al., 2018). While bioactive compounds vary depending on the part of the fruit or vegetable used, they also vary with many factors, such as preparation and extraction conditions, temperature, and solvent (Hernández et al., 2009; Sagar et al., 2018). Extraction of bioactive compounds from plant waste can be performed using various methods, including traditional (Soxhlet, maceration, and hydrodistillation) and new techniques (Microwave-assisted extraction, Pulsed electric field extraction, Enzyme-assisted extraction, Liquid-liquid extraction, Solid-liquid extraction, Supercritical fluid extraction, Pressurized liquid extraction, Ultrasound-assisted extraction, and high-voltage electrical discharge extraction) (Sagar et al., 2018).

Although ultrasonic-assisted extraction, a new technique, is widely used today due to its advantages, literature on its functionality is difficult to find, particularly regarding different parameters such as temperature, frequency, power, solvent type, and solvent-to-material ratio. In thermal extraction processes, temperatures exceeding 50°C can degrade polyphenols, which are bioactive compounds. Therefore, it has been determined that biological activity is better preserved in UAE, especially at frequencies below 40 kHz, compared to maceration and Soxhlet extraction (Dzah et al., 2020).

While the literature on pineapple peel includes studies evaluating the antibacterial activity of extracts obtained by heat-treated extraction with different solvents (Gunwantrao et al., 2016; Lubaina et al., 2019), studies that interpret antibacterial activity alongside the chemical composition of the extracts are limited. The unique aspect of this study is that it addresses the relationship between the ultrasonic extraction method—a novel technique—the chemical composition, and antibacterial activity from a holistic perspective. For this reason, this study aims to determine the antibacterial activity of ethanol, methanol, and dimethyl sulfoxide (DMSO) extracts of pineapple peel obtained by ultrasonication and to determine the chemical composition of the DMSO extract.

2. Materials and Method

2.1. Extraction of pineapple peels

Pineapples obtained from a market in Çanakkale, Türkiye, were peeled. Afterward, they were pureed using a high-speed coffee spice grinder (150 watts, Kiwi KSPG-4820, China). For extraction, following the recommendations of Liu et al. (2018), a 1:1 ratio of puree: solvent (ethanol ($\geq 99.9\%$), methanol ($\geq 99.8\%$), dimethyl sulfoxide

($\geq 99.9\%$), Isolab, Germany) was used in an ultrasonic bath (Isolab, Germany) with a frequency of 40 kHz and an ultrasonic power of 120 W for 60 minutes. Afterward, the mixture was shaken (Nüve, Türkiye) at 150 rpm for 1 hour at room temperature. The resulting extracts were then passed through a Whatman No. 1 filter paper and a sterile cellulose acetate syringe-tip filter with a pore diameter of 0.45 μm (Isolab, Germany), collected in a sterile, colored bottle, and stored at +4°C for analysis.

2.2. Determination of antibacterial activity of pineapple peel extracts

The antibacterial activity of the pineapple peel extracts was determined using the agar well diffusion method described by Özcan Ateş and Topal (2025). First, bacterial cultures were incubated overnight at 37 °C on Tryptic Soy Agar (Biolife, Italy). The revived fresh cultures were adjusted to a cell density of 0.5 McFarland using physiological saline (0.85% w/v NaCl, Isolab, Türkiye) and a McFarland densitometer (Biosan DEN-1, Latvia). The cell suspension prepared for the agar well diffusion method was inoculated onto a dry Mueller-Hinton Agar (MHA) (Biolife, Italy) surface using a sterile cotton swab. Then, wells were drilled into the medium using a 6 mm-diameter cork borer. 20 μL of 100% extract was added to each well. Petri dishes were then incubated at 37 °C for 18-24 hours, and zone diameters were measured using a digital caliper (KMP150, OEM, China). Ethanol, methanol, and DMSO were used as negative controls, and a Levofloxacin (LEV) antibiotic disc (5 μg , Bioanalyse, Türkiye) was used as a positive control. The study was conducted three times on different days. Thus, the inhibition zone diameters were reported as mean $\pm$ standard deviation using IBM SPSS Statistics (version 23.0).

The minimum inhibitory concentration was determined by microdilution in U-based 96-well microplates, as described by Özcan Ateş and Topal (2025). First, Muller-Hinton Broth (MHB) media containing double-layer medium and double-layer extract were prepared, and 100 μL was added to each well. Then, 100 μL of 0.5 McFarland cell suspension, prepared as in the agar well diffusion method, was added to each well. The final concentrations were 125, 100, 50, 25, 20, 10, and 5 $\mu\text{L}/\text{mL}$. The microplates were incubated at 37 $\pm$ 2°C for 24-48 hours, and after incubation, color change was observed by adding 10 μL of sterile 1% tetrazolium chloride solution to the plates. The first well that showed no color change was identified as having the MIC value.

2.4. Determination of chemical composition of pineapple peel DMSO extracts

The DMSO extract was chosen for chemical characterization. DMSO is a solvent capable of dissolving a wide variety of polar and semi-polar organic compounds due to its strong polar aprotic structure. The presence of both polar sulfoxide groups and hydrophobic methyl groups in its molecular structure enables the dissolution of a broad range of compounds (Clark et al., 2008). Therefore, the DMSO extract was preferred for GC-MS analysis, as it was thought to reveal the bioactive compounds in pineapple peel more comprehensively.

The chemical composition of DMSO extract obtained from pineapple peel was determined using gas chromatography–mass spectrometry (HS-SPME-GC-MS) with headspace

solid-phase microextraction. For this purpose, a manual SPME device (Supelco, USA) coated with DVB/CAR/PDMS (50/30 μm) fiber was used. 3 g of DMSO extract was taken and weighed into a suitable headspace vial. The headspace of the sample tubes was purged with nitrogen gas to minimize oxidation and contamination from external sources. Magnetic stirring was used to enhance the transfer of volatile compounds into the headspace of liquid samples. Volatile compounds adsorbed onto the SPME fiber were then analyzed by thermal desorption in a GC-MS system. The SPME fiber injection time was 1 minute. The sample was first equilibrated in a sealed vial at 50 °C and exposed to the headspace phase of the SPME fiber for 60 minutes. Then, the fiber was transferred directly to the GC-MS injection port. For this purpose, an RXI-5MS capillary column (30 m $\times$ 0.25 mm $\times$ 0.25 μm) was used in a Shimadzu GCMS-QP2010 Ultra device. Helium was used as the carrier gas at a flow rate of 1 mL/min. The oven temperature was initially held at 50°C for 5 minutes, then increased to 270°C at 5°C/min. The samples were held at 270°C for 5 minutes. The injection temperature was set to 250°C, the ion source temperature to 200°C, and the interface temperature to 250°C. Compound identification was performed by comparing them with library data (Mass spectral library: Wiley9Nist11 (W9N11)).

3. Results

The antibacterial activity of ethanol, methanol, and DMSO extracts from ultrasonicated pineapple peel was evaluated against 13 bacterial strains using the agar well diffusion method. The 6.00 mm value represents the diameter of the agar well and indicates that no inhibition zone extending beyond the well has formed. Therefore, a value of 6.00 mm indicates the absence of antibacterial activity. The inhibition zone diameters of the tested extracts are given in Table 1.

The MIC values of the pineapple peel extracts, determined by the microdilution method, were >125 $\mu\text{L/mL}$. No

concentration that completely inhibited bacterial growth was determined within the tested concentration range.

HS-SPME-GC-MS determined the chemical composition of the DMSO extract from pineapple peel, and the component data are given in Table 2. HS-SPME-GC-MS revealed that the main component of the DMSO extract of pineapple peel is pinostrobin (44.66%), a flavanone. γ -selinene was identified as 9.52% among the sesquiterpenes. In addition, long-chain fatty acids were detected in the pineapple peel DMSO extract, including 9.26% palmitic acid, 2.08% oleic acid, and 0.05% myristic acid. Furthermore, based on GC-MS data, the concentration of the compound identified in the library as "sclerosol" was 10.54%. The peak reported as "sclerosol" at 10.54% in the GC-MS analysis is based solely on a match to the FFNSC1.2 library. However, it is thought that the molecular weight (Mol wt:78) and formula information ($\text{C}_2\text{H}_8\text{OS}$) of this compound may be related to a DMSO/solvent-induced signal. Therefore, the relevant peak has not been evaluated as a definite phytochemical component and is considered a "temporarily identified/solvent-associated peak".

4. Discussions

In this study, the antibacterial activity of ethanol, methanol, and DMSO extracts of ultrasonicated pineapple peel was evaluated using the agar well diffusion method; no antibacterial activity was detected. Consistent with the results of this study, Ogwu et al. (2019) reported that they could not detect inhibition zones against *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus faecalis*, and *Pseudomonas aeruginosa* in the ethanol extract of pineapple peel obtained by magnetic stirring at room temperature for 48 hours. In contrast, Gunwantrao et al. (2016) evaluated the antibacterial activity of ethanol and methanol extracts obtained from pineapple peel using the Soxhlet method against *Klebsiella pneumoniae* K2044, *Pseudomonas aeruginosa* MTCC4676, *Bacillus subtilis* Py79, and *Xanthomonas axonopodis* pv. *malvacearum* LMG859 bacteria using the agar well diffusion method and

Table 1. Inhibition zone diameters of pineapple peel extracts on agar well diffusion (in mm)

Microorganisms	Ethanol extract	Methanol Extract	DMSO Extract	Ethanol	Methanol	DMSO	LEV5
<i>Escherichia coli</i> ATCC 1301	6.00 $\pm$ 0.01*	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	40.78 $\pm$ 3.52
<i>Escherichia coli</i> ATCC 25922	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	20.76 $\pm$ 1.75
<i>Escherichia coli</i> ATCC 8739	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	25.38 $\pm$ 0.53
<i>Escherichia coli</i> NRRL B-3704	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	36.73 $\pm$ 0.81
<i>Klebsiella pneumoniae</i> ATCC 700603	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	25.20 $\pm$ 0.84
<i>Salmonella enterica</i> subsp. <i>enterica</i> ATCC 13076	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	22.95 $\pm$ 0.82
<i>Salmonella enterica</i> subsp. <i>typhimurium</i> ATCC 51812	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	22.00 $\pm$ 0.72
<i>Pseudomonas aeruginosa</i> ATCC 10145	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	39.77 $\pm$ 1.94
<i>Pseudomonas aeruginosa</i> ATCC 27853	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	23.81 $\pm$ 1.14
<i>Bacillus cereus</i> (isolate 1)	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	30.02 $\pm$ 0.76
<i>Bacillus subtilis</i> (isolate 2)	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	31.34 $\pm$ 1.27
<i>Enterococcus faecalis</i> ATCC 29212	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	18.46 $\pm$ 0.57
<i>Staphylococcus aureus</i> ATCC 29213	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	44.28 $\pm$ 2.02
<i>Staphylococcus aureus</i> ATCC 29213	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	6.00 $\pm$ 0.01	44.28 $\pm$ 2.02

* - No inhibition zone

Table 2. Chemical composition of pineapple peel DMSO extracts

R.Time	Area%	Name	IUPAC Name	Chemical class	Common name
4.910	0.00	3-Hexanone	hexan-3-one	Dialkyl Ketone	-
5.619	0.01	Hexanal (CAS)	hexane	Solvent	-
9.955	0.01	Pentanol <1-methyl->	-	-	-
21.621	10.54	Sclerosol	-	-	-
29.940	0.03	Phenol, 2-methoxy- (CAS)	2-methoxyphenol	Phenol	Guaiacol
31.318	0.03	Phenethyl alcohol	2-phenylethanol	Alcohol	-
33.913	0.01	Oxybenzene	phenol	Phenol	-
38.513	0.03	Guaiacol <4-vinyl->	4-ethenyl-2-methoxyphenol	Phenol	-
42.429	0.01	Diethyl Phthalate	diethyl benzene-1,2-dicarboxylate	Diethyl Ester	-
43.111	0.02	2,3-DIHYDRO-BENZOFURAN	2,3-dihydro-1-benzofuran	Benzofuran	-
4.910	0.00	3-Hexanone	hexan-3-one	Dialkyl Ketone	-
5.619	0.01	Hexanal (CAS)	hexane	Solvent	-
9.955	0.01	Pentanol <1-methyl->	-	-	-
21.621	10.54	Sclerosol	-	-	-
29.940	0.03	Phenol, 2-methoxy- (CAS)	2-methoxyphenol	Phenol	Guaiacol
31.318	0.03	Phenethyl alcohol	2-phenylethanol	Alcohol	-
33.913	0.01	Oxybenzene	phenol	Phenol	-
38.513	0.03	Guaiacol <4-vinyl->	4-ethenyl-2-methoxyphenol	Phenol	-
42.429	0.01	Diethyl Phthalate	diethyl benzene-1,2-dicarboxylate	Diethyl Ester	-
43.111	0.02	2,3-DIHYDRO-BENZOFURAN	2,3-dihydro-1-benzofuran	Benzofuran	-
4.910	0.00	3-Hexanone	hexan-3-one	Dialkyl Ketone	-
5.619	0.01	Hexanal (CAS)	hexane	Solvent	-
9.955	0.01	Pentanol <1-methyl->	-	-	-
21.621	10.54	Sclerosol	-	-	-
29.940	0.03	Phenol, 2-methoxy- (CAS)	2-methoxyphenol	Phenol	Guaiacol
31.318	0.03	Phenethyl alcohol	2-phenylethanol	Alcohol	-
33.913	0.01	Oxybenzene	phenol	Phenol	-
38.513	0.03	Guaiacol <4-vinyl->	4-ethenyl-2-methoxyphenol	Phenol	-
42.429	0.01	Diethyl Phthalate	diethyl benzene-1,2-dicarboxylate	Diethyl Ester	-
43.111	0.02	2,3-DIHYDRO-BENZOFURAN	2,3-dihydro-1-benzofuran	Benzofuran	-
43.460	0.20				
43.511	0.25				
43.950	0.27				
44.175	0.45				
44.502	0.33				
44.924	1.12				
45.246	1.55	4H-1-Benzopyran-4-one, 5-	(2S)-5-hydroxy-7-methoxy-2-phenyl-	(2S)-flavanone.	
45.496	1.04	hydroxy-7-methoxy-2-phenyl-	2,3-dihydrochromen-4-one	Pinostrobin	
45.836	2.03				
46.359	5.97				
46.968	2.98				
47.203	4.28				
47.420	2.39				
47.670	3.99				
48.110	9.12				
48.314	3.66				
48.570	5.03				
49.345	0.05	Myristic acid	tetradecanoic acid	long-chain saturated fatty acid	-
49.970	1.27	(3S,4R,5S,6R)-4,5-Bis(hydroxymethyl)-3,6-dimethylcyclohexene	-	-	-
50.645	4.43	Isocyclocitral (CAS)	2,4,6-trimethylcyclohex-3-ene-1-carbaldehyde;3,5,6-trimethylcyclohex-3-ene-1-carbaldehyde	organooxygen	-
51.020	5.38	9,10-Anthracenedimethanol, diacetate	(10-acetyloxyanthracen-9-yl) acetate	acene diester	-
52.295	3.32	7-epi-trans-sesquisabinene hydrate	(2R,5R)-2-methyl-5-(6-methylhept-5-en-2-yl)bicyclo[3.1.0]hexan-2-ol	sesquiterpenoid	-

53.390	9.26	Palmitic acid	hexadecanoic acid	long-chain saturated fatty acid	-
54.120	2.28	cis-13,16-Docosadienoic acid	(13E,16E)-docosa-13,16-dienoic acid	very long-chain fatty acid	-
56.813	9.52	Naphthalene, decahydro-4a-methyl-1-methylene-7-(1-methylethylidene)-, (4aR-trans)-	(8aR)-8a-methyl-4-methylidene-6-propan-2-ylidene-2,3,4a,5,7,8-hexahydro-1H-naphthalene	bicyclic sesquiterpene	γ -Selinene
58.335	0.57	Octadecanoic acid	octadecanoic acid	saturated fatty acid	-
59.246	2.08	9-Octadecenoic acid, (E)-	(E)-octadec-9-enoic acid	Oleic acid	Elaidic acid
60.351	2.34	6.beta.Bicyclo[4.3.0]nonane, 5.beta.-iodomethyl-1.beta.-isopropenyl-4.alpha.,5.alpha.-dimethyl-,	4-(iodomethyl)-4,5-dimethyl-7a-prop-1-en-2-yl-2,3,3a,5,6,7-hexahydro-1H-indene	-	-
61.012	2.92	9,12-Octadecadienoic acid (Z,Z)-	(9Z,12Z)-octadeca-9,12-dienoic acid	polyunsaturated fatty acid	Linoleic acid
62.081	1.20	Lanosta-8,24-dien-3-ol, acetate, (3.beta.)-	[(3S)-4,4,10,13,14-pentamethyl-17-(6-methylhept-5-en-2-yl)-2,3,5,6,7,11,12,15,16,17-decahydro-1H-cyclopenta[a]phenanthren-3-yl] acetate	-	-

determined that the smallest zone diameter was 17 ± 0.48 mm and the largest zone diameter was 27 ± 0.65 mm. Lubaina et al. (2019) evaluated the inhibition zones of *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Vibrio cholerae* MCV09, and *Klebsiella pneumoniae* ATCC 700603 using the agar well diffusion method with Soxhlet analysis. They found that the ethyl acetate extract produced the largest inhibition zone, measuring 16-20 mm in diameter. Studies in the literature have shown that antibacterial activity is particularly evident in Soxhlet extracts and that the extraction method and solvent used are important factors in determining the antibacterial activity of pineapple peel.

Studies in the literature regarding the antibacterial activity of pineapple peel extracts show varying results, with antibacterial activity being particularly detected in Soxhlet extracts. The recovery of bioactive substances in plant components, which play a significant role in antibacterial activity, depends on many different factors such as the extraction method used, the extraction power of the solvents used, the molecular affinity between the solvent and the solute, mass transfer, the use of auxiliary solvents, and heat (Azmir et al., 2013; Sagar et al., 2018). Especially in traditional extraction methods such as Soxhlet extraction, the recovery of bioactive substances depends on the solvent (Cowan, 1999; Azmir et al., 2013). However, traditional extraction presents several challenges, including long extraction times, high and costly solvent requirements, solvent evaporation due to heat, and decomposition of thermally unstable compounds (De Castro and Garcia-Ayuso, 1998; Azmir et al., 2013). Therefore, new extraction techniques have been developed. For effective extraction by ultrasonication, factors such as degree of grinding, moisture content, particle size, and solvent choice are important. Furthermore, ultrasonication time, frequency, temperature, and pressure are factors affecting extraction (Vinatoru et al., 1998; Azmir et al., 2013). In the extraction of bioactive compounds, solvent selection is crucial, considering factors such as selectivity, solubility, and safety. A solvent with a polarity close to that of the solute is likely to perform better according to the laws of similarity and miscibility (Zhang et al., 2018; Lee et al., 2024). Ethanol is a polar solvent that efficiently extracts both polar and nonpolar compounds. It is particularly

effective for extracting bioactive substances such as fatty acids, lipids, phenolic compounds, and terpenoids. It is also a safe substance for use in pharmaceuticals and food (Azwanida, 2015; Lee et al., 2024). Methanol is a polar solvent with high extraction efficiency and high solubility, used to extract various polar compounds. In addition to fatty acids, lipids, and phenolic compounds, it is used to extract amino acids, anthocyanins, lignans, polysaccharides, proteins, and terpenoids. However, it is toxic (Spigno et al., 2007; Carrera et al., 2012; Lee et al., 2024). Dimethyl sulfoxide (DMSO), on the other hand, is a non-toxic, polar, aprotic, and amphiphilic solvent that can dissolve both polar and non-polar components without degrading phenolic compounds; it is soluble in water and various organic solvents (Sricharoen et al., 2015; Selvakumar and Sivashanmugam, 2020; Luaces et al., 2021; Khodov et al., 2023; Eroğlu, 2025). DMSO also readily penetrates the cell membrane, enhancing the transport of non-ionized molecules (Selvakumar and Sivashanmugam, 2020). However, DMSO, found in trace amounts in some beverages, fruits, and vegetables, is approved by the Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for use in human and veterinary medicines (Khodov et al., 2023; Eroğlu, 2025). Compared to the most commonly used extraction solvents such as acetone, acetonitrile, ethyl acetate, ethanol, methanol, tetrahydrofuran, and water, DMSO has demonstrated the best performance in various antioxidant tests (Sricharoen et al., 2015; Eroğlu, 2025). Therefore, differences in antibacterial activity reported in the literature may be due to variations in extraction methods and solvents.

The microbicidal effect of ethanol depends on protein denaturation; 100% ethanol lacks reliable microbicidal activity because protein denaturation is difficult in the absence of water (Sauerbrei, 2020; Gold et al., 2023). Therefore, it has been determined that solvents do not form an inhibition zone.

The study is finding that pineapple peel extracts did not exhibit antibacterial activity is consistent with the chemical composition data for the DMSO extract. HS-SPME-GC-MS analysis identified pinostrobin (44.66%) as the main component of the DMSO extract. Pinostrobin is an active flavonoid with a flavanone structure and is naturally found in a wide variety of plants (Hernandez Tasco et al., 2020;

Kanchanapiboon et al., 2020; Shamsudin et al., 2022; Wang et al., 2023; Yıldız and Gürgöze, 2026). Hernandez Tasco et al. (2020) determined that pinostrobin obtained from *Lychnophora markgravii* dichloromethane extracts was ineffective against Gram-negative bacteria and *Staphylococcus epidermidis* (6epi), while the MIC value was 0.05 mg/mL against *Streptococcus mutans* (11.1), 0.1 mg/mL against *Streptococcus mutans* (ATCC 25175), and 0.5 mg/mL against *Staphylococcus aureus* penicillinase + (7+) and *Streptococcus sobrinus* (180.3). However, it has been reported that the antibacterial activity of flavanone compounds is structure-dependent and has a more limited effect, especially against Gram-negative bacteria (Shamsudin et al., 2022). Therefore, it is thought that the presence of pinostrobin alone does not determine antibacterial activity. In this study, the high pinostrobin content of the DMSO extract, despite the lack of detected antibacterial activity, can be explained by pinostrobin bioavailability in the extract, potential antagonistic interactions with other compounds, and differences in the susceptibility of the tested microorganisms. Furthermore, a significant portion of studies demonstrating antimicrobial activity in the literature have used purified pinostrobin or chemically modified pinostrobin derivatives (Kong et al., 2010; Massaro et al., 2014; Hernandez Tasco et al., 2020; Kanchanapiboon et al., 2020), which may lead to differences in results compared with those obtained from complex plant extracts.

The DMSO extract obtained from pineapple peel contains 14.31% fatty acids. Although studies on the antimicrobial activity of fatty acids have shown their presence, this depends on the fatty acids and their concentrations tested in pure form (Casillas-Vargas et al., 2021). However, our extract, being a mixture, contains not only fatty acids but also other components. Therefore, the antimicrobial activity of components within a matrix may be reduced compared to their pure forms. Consequently, their biological activity may be limited.

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Moreover, the compound identifications in this study are based on spectral library matching from GC-MS analyses. Library-based identifications, however, should not be considered definitive identifications, especially when dealing with compounds with low similarity scores or those rarely reported in natural products. In this study, sclerosol was identified only tentatively using the FFNSC1.2 library. Therefore, complementary methods such as comparison with standard substances, retention index analysis, or NMR are needed to confirm the identities of these compounds. Consequently, some compounds listed in Table 2 are not definitively identified as natural product components, but rather tentative identifications based on library matching.

In this study, the antibacterial activity of ethanol, methanol, and DMSO extracts of ultrasonicated pineapple peel could not be determined. However, this result does not indicate that pineapple peel has no antimicrobial potential. This is because the bioactive components, which are important for antimicrobial activity, may not have been obtained by this method. Therefore, future studies should use different raw material ratios, different solvents, different extraction methods, and increase the diversity of microorganisms studied.

Conflict of Interest

The authors declare no competing interests.

Authors' Contribution

Concept, design, control, data collection and/or processing, literature review, manuscript writing and critical review: G.Ö.A; Sources, materials, analysis and/or interpretation: G.Ö.A and R.A.G

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