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RESEARCH ARTICLE

GC-MS analysis of chemical composition and determination of antimicrobial activity of Laurel Leaf Extracts prepared by different methods and solvents

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A B S T R A C T

This study aimed to investigate the chemical composition and antimicrobial activity of laurel leaf extracts obtained using different methods and solvents. Extraction was performed using the Soxhlet, ultrasound-assisted, and orbital shaker methods with water, ethanol, methanol, ethyl acetate, acetone, hexane and chloroform. The chemical compositions of the extracts were analyzed with a gas chromatograph coupled to a mass spectrometer and a flame ionization detector (GC-MS-FID). The GC-MS-FID results were compared to data of the Wiley and National Institute of Standards and Technology (NIST) libraries to identify the chemical composition of the analytes. The antimicrobial activities of the extracts against *Staphylococcus aureus* NCTC10788, *Bacillus cereus* NCTC7464, *Salmonella typhi* NCTC11994, *Listeria monocytogenes* ATCC11994 and *Escherichia coli* NCTC2001 were determined with *in-house* disc diffusion testing. The highest efficiency was achieved using the Soxhlet method and methanol (49.11%). Among the solvents tested, hexane, and among the methods used, ultrasound-assisted extraction exhibited the lowest efficiency ($P<0.05$). The extracts showed a stronger inhibitory effect on Gram-positive bacteria ($P<0.05$). The highest level of antimicrobial activity was achieved against *S. aureus* with the use of the stock solution concentrations of the extracts obtained with the combined use of the ultrasound-assisted method and the solvents ethanol, ethyl acetate, acetone and hexane. The bacteria most resistant to almost all concentrations of the laurel leaf extracts were *S. typhi*, *E. coli* and *L. monocytogenes*. The method and solvent for extraction should be chosen carefully, depending on the targeted molecules and desired activity.

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INTRODUCTION

The unregulated and prolonged use of antibiotics are the two primary causes of the development of antimicrobial resistance. Increased antimicrobial resistance has led to a search for alternative sources and the development of novel pharmaceuticals in the field of human medicine. Today, both increased antimicrobial resistance and reduced access to synthetic pharmaceuticals highlight the importance of herbal pharmaceuticals. Research has shown that most of the global human population uses herbal products for treatment purposes (Abah and Egwari, 2011). It is reported that the leaves, fruits, skin and/or extracts of various plants, the chemical compositions of which are unknown, continue to be used for the treatment of several diseases in developing countries, including Nigeria, Ghana and Sudan (Agyare *et al*., 2006). This is mainly because they are thought to be very effective, cheaper than modern medicines, and readily available (Ahn, 2017).

Laurel (*Laurus nobilis*) is an aromatic evergreen shrub, which grows in all temperate zones of the world, particularly in the Mediterranean basin, and encompasses

32 genera and almost 2.000-25.000 species that vary in size and range from bushes to tall trees (Derwich *et al*., 2009). Since ancient times, the Mediterranean people use oil extracted from the laurel fruit for soap production, and laurel leaves both as a culinary aromatic herb and to prevent the growth of mold in food products (Özer *et al*., 2021). Laurel leaves are also reported to be used for the treatment of gastric diseases, epilepsy, neuralgia, rheumatism, sclerosis and Parkinson's disease (Derwich *et al*., 2009). In recent years, laurel has been indicated to show antifungal, antiviral and antibacterial activities (Caputo *et al*., 2017).

The present study aimed to investigate laurel (*Laurus nobilis*) leaf extracts, obtained with the use of different solvents and extraction methods, for their chemical composition by GC-MS-FID, as well as for their antibacterial activity.

MATERIALS AND METHODS

Chemicals: Analytical grade chemicals and solvents were used in this study. Ethyl acetate, ethanol, methanol, acetone, hexane, chloroform and potassium hydroxide were supplied from Sigma-Aldrich (Steinheim, Germany). Ultra-distilled water was obtained with the aid of an 18.3 MΩ Sartorius arium® comfort system.

Preparation of the plant materials and extracts: Laurel leaves were collected in the Tomruksuyu settlement of the Samandağı district of the Hatay province in Türkiye. The leaves were dried in a dry and cool environment by avoiding their direct exposure to sunlight. The dried plant material was ground into a fine powder using a laboratory grinder (Waring 8010G) and passed through a sieve (RETSCH, 250 µm, ISO 3310-1/ASTM E11) such that the powdered samples contained particles smaller than 250 µm. These samples were transferred into colored glass bottles, and stored in the dark, in a refrigerator at +4°C, until being used to produce laurel extracts. The extraction procedure of the powdered samples was performed using the conventional Soxhlet extraction method (SE) (Akdeniz, 2021), ultrasound-assisted extraction method (UAE) (Akdeniz, 2021), and orbital shaker extraction method (OSE) (Tan Mei Chin *et al*., 2021). Different solvents (ethanol, water, methanol, ethyl acetate, acetone, hexane and chloroform) were used for extraction. Extraction by the Soxhlet method was performed using 7.5g of laurel powder and 150mL of a solvent, and with the aid of a Soxhlet apparatus for 8h. Ultrasound-assisted extraction was performed by adding 7.5g of laurel powder with 150mL of a solvent in an Erlenmeyer flask, and immersing the flask in an ultrasonic bath (ELMAS) at a temperature range of 25-37°C for 45min. Extraction with the orbital shaker method was performed by adding 7.5g of laurel powder with 150mL of a solvent in an Erlenmeyer flask, and agitating the flask on an orbital shaker (GERHARD) at 200rpm at 25°C for 5h. All resulting extracts were passed through filter paper (Whatman Filter Paper No: 1). Each of the extracts, obtained using ethanol, methanol, ethyl acetate, acetone, hexane and chloroform, were concentrated using a rotary evaporator at 40°C under vacuum pressure. On the other hand, the extracts obtained using water were lyophilized at -80°C temperature, under 0.01mbar pressure for 48h (Telstar LyoQuest). All extracts were stored in the dark at +4°C until being used for analysis.

Esterification procedure for gas chromatography analyses: To determine the chemical composition of the extracts, 2mL of each extract was placed into a glass tube and added with 5mL of hexane. After being stirred, this mixture was added 2mL of 2M KOH in methanol and strongly agitated on a vortex shaker. After the mixture was centrifuged at 4500rpm for 10min, the supernatant was harvested, sieved through a 0.22-mm PTFE filter and transferred into 1.5mL-vials.

Gas chromatography-mass spectrometry/flame ionization detector (GC-MS/FID) analysis of the chemical components: Analyses for the determination of the chemical composition of the extracts were performed with gas chromatography (Agilent, 7890A)/mass spectrometry (5975C) coupled with an FID detector (Santa Clara, CA, USA). Analyses were conducted using a J&W 122-7061 column (60m length, 250µm id and 0.15µm thickness). The chemical composition analyses of the samples, injected in a volume of 1µL, in the splitless mode, were performed under the following conditions: the initial oven temperature was set to 50°C for 2min, and gradually increased up to 200°C at a rate of 20°C/min, followed by a gradual increase up to 230°C at a rate of 5°C/min, and was maintained at this final temperature for 30min. The total duration of the analysis of the extracts was 55.5min. Helium was used as a carrier gas (1mL/min). The MS results were compared to the Wiley and NIST library data and uploaded to the memory of the device to determine the chemical composition of the extracts.

Determination of antimicrobial activity: The antimicrobial activities of the laurel leaf extracts were tested against selected bacteria of clinical importance to human and animal health. For this purpose, the following standard bacterial strains were used: *Staphylococcus aureus* NCTC10788, *Bacillus cereus* NCTC7464, *Salmonella typhi* NCTC11994, *Listeria monocytogenes* ATCC11994, and *Escherichia coli* NCTC2001.

After fresh 24-48-h cultures of these standard strains were grown on solid media under aerobic/microaerophilic conditions, bacterial inocula were prepared in nutrient broth (CM0001B, ThermoFisher Sci.) at a concentration ($0.5x10^8$ bacteria/ml) standardized to McFarland No. 0.5. Twenty µl of each extract was impregnated to blank antibiotic assay discs and left to dry at room temperature for 5-10min. The bacterial inocula were inoculated onto Mueller Hinton (MH) agar (Thermofisher Sci., CM0337B), in volumes of 100µl, and spread over the surface of the agar with a glass rod. The extract-impregnated discs, which were prepared *in-house*, were placed onto the inoculated MH agar, and left for incubation under conditions specific to the inoculated bacteria. At the end of the incubation period, the diameters of the inhibition zones surrounding the discs were measured with a caliper to evaluate the antimicrobial activity of the extracts. The minimum inhibitory concentration (MIC) values of the extracts, which produced visible inhibition zones, were determined using the same method (disc diffusion) by soaking blank

antibiotic assay discs with 20µl of the two-fold sub-dilutions of the extracts prepared with the solvents used for their extraction.

Statistical analyses: All analyses and measurements were performed in triplicate. The results are expressed as the mean value of the individual measurements and the standard deviation (SD). Data analyses for the chemical composition and antimicrobial activity of the extracts obtained with different methods and solvents were performed with the SPSS software.

RESULTS

Percentage yields of the different extraction methods: The percentage yields of the extraction methods tested in the present study were calculated using the following formula:
"Percentage Yield (%) = (Amount of Dry Extract/Amount of Dry Laurel Leaf) x 100". The calculated values are shown in Fig.1.

The comparison of the three different extraction methods demonstrated that, for all the solvents tested, the Soxhlet method offered a higher percentage yield of extraction (P>0.05). The percentage yields of extraction achieved with the Soxhlet, ultrasound-assisted and orbital shaker methods using methanol as a solvent were 49.11, 24.02 and 31.94%, respectively, and were higher than the percentage yields achieved with the use of the other solvents tested (P>0.05). The highest percentage yield of extraction was achieved with the combined use of the Soxhlet method and methanol (49.11%). The solvent with the lowest extraction efficiency was hexane and the method offering the lowest percentage yield of extraction was the ultrasound-assisted method.

Chemical composition: chemical compositions of the laurel leaf extracts obtained with the Soxhlet, ultrasound-assisted and orbital shaker methods using ethanol, water, methanol, ethyl acetate, acetone, hexane and chloroform as solvents were determined with GC-MS-FID and expressed in "relative concentrations (%)". In total, 170 chemical compounds were detected in the extracts obtained with the use of the three selected methods and seven selected solvents. Of these compounds, 92 (54.12%) were detected at a relative concentration above 1%. The greatest varieties of chemical compounds were obtained with the use of the orbital shaker method (n= 145) and the Soxhlet method (n= 145). The total number of chemical compounds detected in the extracts obtained with the ultrasound-assisted extraction method was 135. Considering all solvents; the total number of chemical compounds recovered with all three of the extraction methods was 101. The number of chemical compounds recovered with only the ultrasound-assisted extraction method was 5 and these were 3-methoxy-4-[(trimethylsilyl)oxy] benzaldehyde O-methyl oxime, beta-terpineol, 4-[{4-(4-bromo-phenyl)-thiazol-2-yl}-methyl-amino]-butyric acid, cis-10-heptadecenoic acid, (3E.5E.8Z)-3.7.11-trimethyl-1.3.5.8.10-dodecapentanene. The number of chemical compounds recovered with only the use of the Soxhlet extraction method was also 4, and included 1.1.2.2-tetraethoxyethane, tetratetracontane, nonanedioic acid and pentadecanenitrile. On the other hand, the number of chemical compounds recovered with only the orbital shaker method was 7, and included tridecane, triacontane, 1-iodo-octacosane, carbonic acid-decyl-undecyl-ester, alpha-ocimene, butyl (tetradec-6-yl) sulfonate and carbonic acid hexadecyl 2.2.2-trichloroethyl ester. While the solvents offering the greatest variety of chemical compounds were ethyl acetate and hexane (n= 98), the smallest variety was obtained with the use of water (n= 59). Among the solvents tested, ethyl acetate yielded the highest number of chemical compounds with all three methods (n= 37), and ethanol yielded the lowest number of chemical compounds with all three methods (n= 7). The greatest individual variety of chemical compounds (n= 75) was obtained with the Soxhlet extraction method using ethyl acetate as a solvent. This was followed by the combined use of the orbital shaker method and hexane (n= 69), and the orbital shaker method and ethyl acetate (n= 67). The smallest variety of chemical compounds (n= 22) was obtained with the combined use of

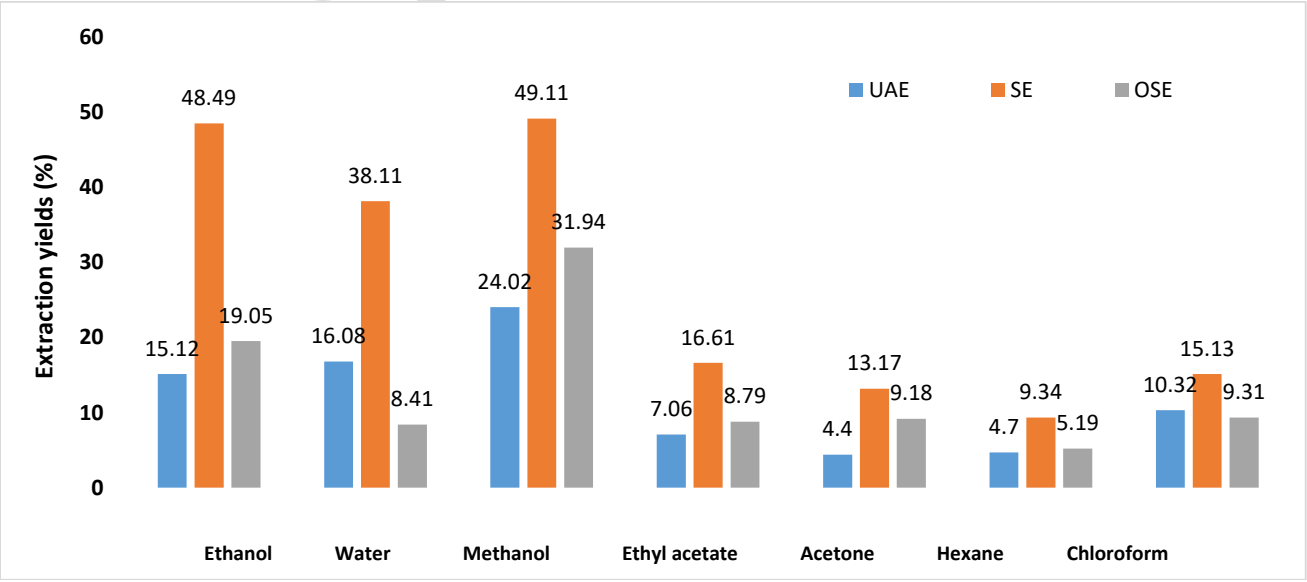

Fig. 1: Percentage yields of extractions. UAE: Ultrasound-assisted extraction; SE: Soxhlet extraction and OSE: Orbital shaker extraction.

the Soxhlet extraction method and ethanol. Tetratetracontane was recovered only with the use of the Soxhlet method and water, carbonic acid-decyl-undecyl-ester was recovered only with the orbital shaker method and acetone, alpha-ocimene was recovered only with the orbital shaker method and ethyl acetate, and nonanedioic acid and pentadecanenitrile were recovered only with the Soxhlet method and ethanol. 4-methyl-3-penten-2-one, 4-methoxy-4-methyl-2-pentanone, carbonic acid-decyl-undecyl-ester, 7-hexadecene (Z)- and 1-nonadecene were obtained only with acetone. Delta-selinene, alpha-ocimene, 1.4-benzenedicarboxylic acid and 3.7.11.15-tetramethylhexadec-2-en-1-yl acetate were obtained only with ethyl acetate. 2-methyloctacosane, carbonic acid-decyl-tetradecyl-ester, 3-ethyl-5-(2-ethylbutyl) octadecane, tetratetracontane, 1-iodotetracosane and dihydrodehydrocostus lactone were recovered only with water. 1-iodotriacontane and (9Z.12Z.15Z)-1-hydroxy-3-methoxypropan-2-yl octadeca-9.12.15-trienoate were obtained only with chloroform. Nonanedioic acid and pentadecanenitrile were obtained only with ethanol, and 4-(4-methoxyphenyl)-2-butanone and 4.6.6-trimethyl-2-(3-methylbuta-1.3-dienyl)-3-oxatricyclo [5.1.0.0(2.4)] octane were obtained only with methanol. The only chemical compound recovered at varying concentrations with all the tested methods and solvents was palmitic acid. Eucalyptol, methyl eugenol, and stearic acid were common chemical compounds obtained with all three of the tested methods and almost all the tested solvents. 4-methyl-3-penten-2-one, eucalyptol, 4-methoxy-4-methyl-2-pentanone, terpinolene, 1.1.2.2-tetraethoxyethane, docosane, 4-carvomenthenol, alpha-terpinene, palmitic acid and linolenic acid were the chemical compounds detected at the highest relative concentrations (>10%). Cyclodecene, (+)-ledene, valerenol, 1H-cycloprop[e]azulene 1a.2.3.5.6.7.7a.7b-octahydro-1.1.4.7-tetramethyl-(+)-, alpha-costol and hexacosanoic acid were detected at trace levels and were recovered with the use of only some of the tested methods and/or solvents (<0.5%).

While no significant difference was observed between the tested methods for the recovery of compounds belonging to the ketone group, the use of acetone as a solvent was determined to yield a high variety of chemical compounds at high relative concentrations. The use of the Soxhlet extraction method with ethyl acetate and chloroform yielded a wide variety of terpenes ($P<0.05$), such that terpinolene and alpha-terpinene were recovered at high concentrations. The extraction efficiencies of the different methods for the recovery of terpenoids were similar ($P>0.05$). The greatest variety of chemical compounds was obtained with the use of ethyl acetate (n= 98) and hexane (n= 98). Among the compounds belonging to the group of terpenoids, eucalyptol was the most common and was detected at the highest relative concentration. The use of the orbital shaker method with water yielded the greatest variety for alkane hydrocarbons. The extraction efficiencies of the methods and solvents differed significantly for this group of compounds ($P<0.05$). Sesquiterpenoids were generally detected at low relative concentrations and no significant difference was determined between the tested methods and solvents for the recovery of these compounds ($P>0.05$). The combined use of the orbital shaker method and acetone offered the greatest extraction yield for alkene hydrocarbons. Significant differences were determined between the tested methods and solvents for the recovery rates of sesquiterpenes ($P<0.05$). The most efficient method was the Soxhlet method, and the most efficient solvent was acetone. While the efficiencies of the different extraction methods were found to be similar for fatty acids, the greatest variety of fatty acids was achieved with the use of ethanol. The fatty acids detected at the highest relative concentrations were palmitic acid and linolenic acid. In the present study, 22 compounds, the chemical names of which were able to be identified, could not be classified under any known chemical group. The efficiencies of the tested methods and solvents were not able to be interpreted for the other few individual compounds and groups of chemicals detected in the extracts (Table 1).

Findings related to antimicrobial activity: The antimicrobial activities of the extracts obtained with the use of the different methods and solvents are given in Table 2. The testing of the laurel leaf extracts for their antibacterial activities against some Gram-negative and Gram-positive bacteria demonstrated varying activities as displayed by inhibition zones ranging from 7mm to 13mm in diameter. The extracts were determined to show a weaker inhibitory effect on Gram-negative bacteria, compared to Gram-positive bacteria ($P<0.05$). The strongest antibacterial activity (inhibition zone of 13mm diameter) was achieved against *S. aureus* with the use of the stock concentrations of the extracts obtained by ultrasound-assisted ethanol extraction (15.12% percentage yield of extraction and variety of 52 chemical compounds), ultrasound-assisted ethyl acetate extraction (7.06% percentage yield of extraction and variety of 56 chemical compounds), ultrasound-assisted acetone extraction (4.4% percentage yield of extraction and variety of 52 chemical compounds), and ultrasound-assisted hexane extraction (4.7% percentage yield of extraction and variety of 51 chemical compounds) (Table 2).

While the stock concentrations of the chemical compounds recovered with the use of the different methods and solvents showed varying activity against *S. aureus* and *B. cereus*, the bacteria most resistant to nearly all tested concentrations of the chemical compounds were *S. typhi*, *E. coli* and *L. monocytogenes*. Although at weak levels, the only extracts that showed antibacterial activity against *S. typhi*, *E. coli* and *L. monocytogenes* were obtained by the combined use of the Soxhlet method and ethanol (S (485µg/disc) and ½ dilution (242.5 µg/disc), the ultrasound-assisted method and chloroform (S (309.8µg/disc), and the Soxhlet method and ethanol (S (485µg/disc) and ½ dilution (242.5µg/disc), respectively. The strongest antibacterial activity against the tested bacteria, excluding *E. coli*, was achieved with the use of the extract obtained by the Soxhlet method and ethanol (48.49% percentage yield of extraction and variety of 22 chemical compounds). The stock concentrations of the extracts obtained with the Soxhlet method and methanol, the orbital shaker method and water, and the orbital shaker method and acetone did not show antibacterial activity against any of the selected bacteria.

Table 1: Relative concentration ranges (%) of the chemical compounds detected in the extracts with the different methods and solvents

Compound name	Ethanol			Water			Methanol			Ethyl Acetate			Acetone			Hexane			Chloroform		
	UAE	SE	OSE	UAE	SE	OSE	UAE	SE	OSE	UAE	SE	OSE	UAE	SE	OSE	UAE	SE	OSE	UAE	SE	OSE
ALKANE HYDROCARBONS																					
Dodecane/ Heneicosane/ 2-methyloctacosane/ Hexadecane/ Tetradecane/ 3.8-dimethyl-decane/ Tridecane/ Triacontane/ Pentadecane/ Docosane/ Eicosane/ Heptadecane/ 2-methylhexacosane/ Pentacosane/ Hexatriacontane/ Nonadecane/ Heptacosane/ Hentriacontane/ 3-ethyl-5-(2-ethylbutyl) octadecane/ Octadecane/ 9-octylheptadecane/ Cyclodecene/ Tetratetracontane/ Octacosane/ 1-iododocosane/ Tetratriacontane/ Hexacosane/ Tricosane/ 2-methyl-hexadecane/ Cyclohexadecane/ Tetracosane	0.28-0.81	0.52-3.56	0.06-0.29	0.15-7.11	0.14-10.11	0.17-13.34	0.03-0.32	0.03-0.13	0.07-0.83	0.11-0.25	0.09-1.2	0.1-1.82	0.03-0.93	0.03-1.4	0.17-1.78	0.16-1.1	0.10-0.60	0.09-2.96	0.11-0.23	0.08-0.34	0.19-3.20
HALOGENATED HYDROCARBONS																					
1-iodo-octacosane/ 1-iodoeicosane/ 1-iodohexacosane/ 1-iodotriacontane/ 1-iododocosane/ 1-iodotetracosane				1.08	0.85-2.89	0.35-1.83			0.36			2.02	0.28		0.87		0.05	0.09-0.71	0.23	0.17	0.19-0.58
ALKEN HYDROCARBON																					
1-hexadecene/ 7-hexadecene (Z)-/ Alpha-ocimene/ (E)-3-octadecene/ 1-octadecene/ 1-Nonadecene/ 1.4.8-dodecatriene. (E.E.E)-	0.56-0.86	1.24	0.67								0.57-1.56	0.59-1.11	0.28-1.61	0.45-1.85	0.3-3.95		0.85	0.42-0.66			
TERPEN																					
Sabinene/ (−)-β-pinene/ Limonene/ Eucalyptol/ Terpinolene/ Alpha-terpineol/ Beta-terpineol/ Delta-terpineol/ Delta-terpineol acetate/Linalol/4-carvomenthenol/ Alpha-terpinene/ Intermedeol/ Longipinocarveol trans-/ Phytol/ Neophytadiene/ 4-thujanol	0.82-15.78	0.46	0.57-13.77	1.56-8.96	0.26-10.44	2.09-5.49	0.15-16.33	0.08-12.28	0.24-12.52	0.19-17.78	0.18-15.99	0.14-18.69	0.22-4.71	0.15-13.58	0.17-6.37	0.5-20.74	0.35-19.96	0.05-19.46	0.34-15.3	0.34-20.4	0.39-17.7
SESQUITERPENOIDS																					
Ylangene/ Beta-selinene/ Delta-selinene/ Beta-elemene/ Beta-caryophyllene/ Alpha-elemene/ (+-)-Vetiselinene)/ Alpha-gurjunene/ Gamma-muurolene/ Aromandendrene/ Alpha-calacorene/ Beta-selinen/ (-)- alloaromadendrene/ (+)-gamma-gurjunene/ Alpha-selinene/ Spathulenol/ (+)-longifolene/ Valerenol/ Beta-gurjunene/ Alpha-eudesmol/ Beta-eudesmol/ (-)-alpha-panasinsen	0.13-4.08		0.03-3.39				0.14-2.88	0.1-1.57	0.17-1.8	0.04-2.23	0.02-2.16	0.09-2.68	0.08-0.96	0.09-2.67	0.17-2.91	0.28-2.74	0.49-4.7	0.09-4.28	0.23-1.6	0.08-1.92	0.19-2.72
SESQUITERPENE																					
Trans-beta-farnesene/ (-)-guaia-6.9-diene/ Beta-bisabolene/ Bisabolene/ Alpha-amorphene/ (-)-gamma-cadinene/ Alpha-muurolene/ Alpha-Calacorene/ Germacrene D/ (+)-ledene/ Valencene/ Valerena-4.7(11)-diene/ Alpha-costol/ Dihydrodehydrocostus lactone/ Dehydrocostus lactone/ Eremanthin	0.14-4.98		0.13-2.19	0.96		0.52	0.1-2.11	0.09-1.83	0.15-2.83	0.04-4.07	0.14-2.96	0.22-3.58	0.11-0.67	0.08-2.65	0.26-2.73	0.72-1.07	0.13-2.5	0.14-2.07	0.11-2.96	0.08-3.02	0.19-2.14
FATTY ACID																					
Lauric acid/ Myristic acid/ Pentadecanoic acid/ Nonanedioic acid/ Palmitic acid/ Palmitoleic acid/ Margaric acid/ cis-10-heptadecenoic acid/ Stearic acid/ Hexacosanoic acid/ Oleic acid/ Elaidic acid/ Linolelaidic acid/ Linoleic acid/ Linolenic acid/ Arachidic acid/ Behenic acid/ Lignoceric acid	0.14-1	0.41-35.26	0.1-14.77	0.59-5.73	0.29-3.51	0.52-2.88	0.36-13.53	0.07-18.06	0.1-17.97	0.1-9.29	0.1-7.62	0.19-8.3	0.08-0.24	0.19-1.54	0.13-0.91	0.38-5.01	0.23-10.6	0.14-6.82	0.11-8.1	0.08-7.62	0.19-10.12
FATTY ACID ESTER																					
Ethyl myristate/ Ethyl palmitate/ Ethyloctadecanoate/ Ethyl oleate	0.13-0.48	0.83-8.2	0.13-5.59							0.24-3.06	0.14-2.29	0.21-2.08									
ESTER																					
Carbonic acid decyl undecyl ester/ Carbonic acid decyl tetradecyl ester/ Carbonic acid hexadecyl 2.2.2-trichloroethyl ester/			0.38		0.32	0.26					0.54	0.24-1.23			3.08			1.36		0.75	0.97

(9Z.12Z.15Z)-1-hydroxy-3-methoxypropan-2-yl octadeca-9.12.15-trienoate/ 2-ethylhexyl acetate																				
KETONE																				
4-methyl-3-penten-2-one/ 4-methoxy-4-methyl-2-pentanone/ 2-tridecanone/ 2-Pentadecanone/ 4-(3'-thienyl)-1.5-dihydro-2H-pyrrol-5-one/ 4-(4-methoxyphenyl)-2-Butanone	0.14		0.1	0.9		1.13		0.07-0.37	0.53		0.15		0.07-51.74	0.3-17.66	0.52-28.79	0.18-1.53	0.12-0.83		0.11-0.23	0.17	
FENILPROPANOID																				
Beta-asarone/ Methyl eugenol/ Methyl isoeugenol	0.48-6.26		0.45-2.19	0.46-4.44	2.18	1.92	0.73-3.75	2.2	0.63-2.7	0.62-4.61	0.24-2.96	0.36-3.11	0.31-1.94	0.74-2.75	0.65-2.3	0.76-6.22	0.47-3.89	0.42-3.57	0.46-3.99	0.5-4.52	0.58-5.84
ALLYLBENZENE																				
Eugenol										1.24	0.55	0.5							0.23	0.34	0.97
ESSENTIAL OILS																				
Elemicin/ Isoelemicin	0.16		0.19	0.59		0.26	0.08	0.08	0.15	0.5	0.1	0.36	0.05	0.09-0.25	0.17	0.76	0.19-0.41	0.38	0.11	0.08-0.42	0.19-0.58
PHENOL																				
2.4-dimethylphenol/2.4-di-tert-butylphenol/2.5-ditert-butylphenol	0.47-0.66		0.32-2.22	0.31-6.65	3.3	2.09	0.72	0.02-0.12	1.29		0.69	0.73	0.42	0.29	1.03	0.77	0.24-0.49	0.24-1.18	0.46	0.25	1.56
ALCOHOL																				
1.3.3-trimethyl-2-oxabicyclo[2.2.2]octan-6-ol/ 1-nonadecanol	0.2		0.1				0.12-0.15	0.26			0.27		0.58	0.74	0.61	0.2-0.48	0.46		0.23	0.17-0.34	
CARBOXY ACID																				
4-[[4-(4-bromo-phenyl)-thiazol-2-yl]-methyl-amino]-butyric acid/ 1.4-benzenedicarboxylic acid/ 3.5-bis(1.1-dimethylethyl)-4-hydroxy-benzenepropanoic acid				2.15	2.41	1.39	0.23			0.32-1.32	0.47	0.14							2.5		
NITRILE																				
Pentadecanenitrile/ Oleanitrile		1.01-2.27	0.13			0.35	0.15		0.1					0.25					0.23	0.34	0.39
AMIDE																				
Oleamide/ Hexadecanamide		2.42	0.03-1.86	0.09	0.75	0.61		0.1-0.42	0.02	1.44	0.05-1.1	0.28	0.09	1.28	0.04		0.03	0.09		0.08-0.17	0.39
UNCLASSIFIED COMPOUNDS																				
9.10-dehydro-6-desoxy-indolinocodeine/ 3-methoxy-4- [(trimethylsilyl)oxy] benzaldehyde O-methyl oxime/ 6-Aza-5.7.12.14-tetrathiapentacene/ 1.1.2.2-tetraethoxyethane/ 2-(2-acetylamino-phenylazo)-2'-nitrostilbene/ 9-azatetracyclo [10.2.1.0(2.11).0(3.8)] pentadeca-3.5.7-triene-7-carboxylic acid. 10-(2.5-difluorophenyl)-/ Butyl (tetradec-6-yl)sulfonate/ Caryophyllene oxide/ N.N-diethyl-N'.N'-diphenyl-6-pyrrol-1-YL-[1.3.5]triazine-2.4-diamine/ Junenol/ 1.5-dimethyl-8-(1'-methylethenyl)bicyclo [4.4.0]dec-4-en-3-one/ 1.1.7.7a-tetramethyl-1a.2.6.7.7a.7 b-hexahydro-1H-cyclopropa[a]naphthalene/ (3aS.4R.7R)-1.4.9.9-tetramethyl-5.6.7.8-tetrahydro-4H-3a.7-methanoazulene/ 1H-cycloprop[e]azulene 1a.2.3.5.6.7.7a.7b-octahydro-1.1.4.7-tetramethyl-. (+)-/ 8-(1-methylethylidene)-bicyclo[5.1.0]octane/ 4.6.6-trimethyl-2-(3-methylbuta-1.3-dienyl)-3-oxatricyclo[5.1.0.0(2.4)]octane/ Bicyclo[7.2.0]undecan-5-ol. 10.10-dimethyl-2.6-bis (methylene)-. (1S.5S.9R)-/ 4-methylcyclohex-3-enecarbaldehyde/ (1R.7S.E)-7-isopropyl-4.10-dimethylenecyclodec-5-enol/ (3E.5E.8Z)-3.7.11-trimethyl-1.3.5.8.10-dodecapentanene/ 3.7.11.15-tetramethylhexadec-2-en-1-yl acetate/ Vulgarol A	0.34-4.92	1.27-20.22	0.16-1.24	2-4.9	4.47-10.01	0.52-3.66	0.15-0.78	0.08-0.64	0.19-0.8	0.1-3.21	0.16-2.33	0.19-0.99	0.11-0.65	0.15-2.84	0.17-0.39	0.36-2.34	0.12-4.13	0.05-2.49	0.34-5.7	0.17-0.75	0.39-1.17

UAE: ultrasound-assisted extraction; SE: Soxhlet extraction; OSE: orbital shaker extraction.

Table 2: The antibacterial activities of the laurel leaf extracts

Extraction methods and solvents	Extract dry weight (mg/mL)	Concentrations impregnated to the disc (µg/disc)			Zone of inhibition (mm)*														
					S. typhi			*S. aureus*			*E. coli*			*L. monocytogenes*			*B. cereus*		
		S	1/2	1/4	S	1/2	1/4	S	1/2	1/4	S	1/2	1/4	S	1/2	1/4	S	1/2	1/4
Ultrasound-assisted extraction (UAE)																			
Ethanol	22.68	453.6	226.8	113.4	R			13	R		R			R			8	R	
Water	25.47	509.4	254.7	127.4	R			8	R		R			R			R		
Methanol	36.04	720.8	360.4	180.2	R			9	R		R			R			7	R	
Ethyl acetate	10.59	211.8	105.9	53	R			13	R		R			R			8	R	
Acetone	6.59	131.8	65.9	33	R			13	R		R			R			R		
Hexane	7.05	141	70.5	35.3	R			13	R		R			R			7	R	
Chloroform	15.49	309.8	154.9	77.5	R			7	R		8	R		R			8	R	
Soxhlet extraction (SE)																			
Ethanol	24.25	485	242.5	121.3	7	7	R	10	9	7	R			8	7	R	11	R	
Water	38.11	762.2	381.1	190.6	R			7	R		R			R			R		
Methanol	49.11	982.2	491.1	245.6	R			R			R			R			R		
Ethyl acetate	16.61	332.2	166.1	83.1	R			R			R			R			7	R	
Acetone	13.17	263.4	131.7	65.9	R			R			R			R			7	R	
Hexane	9.34	186.8	93.4	46.7	R			R			R			R			7	R	
Chloroform	15.13	302.6	151.3	75.7	R			7	R		R			R			8	7	R
Orbital shaker extraction (OSE)																			
Ethanol	19.5	390	195	97.5	R			R			R			R			8	R	
Water	8.41	168.2	84.1	42.1	R			R			R			R			R		
Methanol	31.94	638.8	319.4	159.7	R			R			R			R			7	R	
Ethyl acetate	8.79	175.8	87.9	44	R			8	R		R			R			8	R	
Acetone	9.18	183.6	91.8	45.9	R			R			R			R			R		
Hexane	5.19	103.8	51.9	26	R			12	R		R			R			8	R	
Chloroform	9.31	186.2	93.1	46.6	R			8	R		R			R			R		

S: stock concentration, R: resistant, *: The effectiveness of a subdilution has not been tested when resistance was encountered at any certain concentration.

The extract obtained with the Soxhlet method and ethanol showed partial antibacterial activity against the selected bacteria (excluding *E. coli* and *B. cereus*) at a ½ dilution (242.5ug/disc). However, at a ¼ dilution (121.3ug/disc), this extract exhibited antibacterial activity only against *S. aureus* (Table 2).

DISCUSSION

Laurel, a plant native to the Mediterranean region, is used as a spice and an aromatic herb in the culinary and food industries and is preferred by the common people for its anti-rheumatic, diuretic and antidotal properties. The biological activities of laurel arise from the various phytochemicals it contains, including flavonoids, sesquiterpenoids and alkaloids (Özer *et al*., 2019). The recovery of these chemical compounds from laurel at adequate levels and without any harm depends on the selection of the appropriate extraction method. Several methods, including conventional and modern techniques, are used for the recovery of chemical compounds from different parts of laurel, including the leaves, roots and stem. Conventional methods such as the Soxhlet method, hydrodistillation, and solvent extraction have some disadvantages, including the degradation of some bioactive compounds due to these methods being performed at high temperatures, the loss of some essential oils due to these methods depending on the use of solvents, the requirement for the use of large volumes of solvents, and the long duration of extraction. Several modern alternative methods, including microwave-assisted extraction, ultrasound-assisted extraction, supercritical fluid extraction, pulsed electric field-assisted extraction and high electrostatic pressure-assisted extraction, have been developed for the recovery of chemical compounds from plants. These methods are environmentally friendly, offer high percentage yields of extraction and allow the production of high-quality extracts (Koçak and Pazır, 2018; Kapadia *et al*., 2022). All these methods offer different percentage yields of extraction and enable the recovery of different groups of chemicals. The Soxhlet method, described as the reference extraction method, owing to its high efficiency compared to other conventional solid and fluid extraction methods (Zhao and Zhang 2014, Koçak and Pazır, 2018), was determined to offer higher percentage yields of extraction, compared to the ultrasound-assisted and orbital shaker methods in the present study ($P>0.05$) (Fig. 1). Furthermore, after the orbital shaker extraction method, the greatest variety of chemical compounds was achieved with the use of the Soxhlet method. This method is the sole producer of the glyoxal derivative (1.1.2.2-tetraethoxyethane) and reveals one alkane hydrocarbon (tetratetracontane) with only water extract, and fatty acid (nonanedioic acid) and nitrile (pentadecanenitrile) with only ethanol extract. The Soxhlet method offered the greatest chemical compound variety (n= 75), when performed using ethyl acetate, in agreement with previous reports (Lohani *et al*., 2015; Karğılı and Aytaç, 2021). In agreement with previous research (Silalahi *et al*., 2021), the present study also showed that the efficiency of the Soxhlet method with respect to the recovery of alkane hydrocarbons was lower than that of the other methods (except for water extract). The orbital shaker extraction method also has some disadvantages, including the requirement for large volumes of plant material and solvents, and the relatively long duration of extraction (Souza *et al*., 2018). In the present study, the second method that offered the greatest variety of chemical compounds (n= 145) was the orbital shaker extraction method. Compared to the other solvents, methanol yielded

the highest efficiency with the orbital shaker method (31.94%) (Fig. 1). Similar to previous research (da Silva Haas *et al*., 2018), in the present study the greatest chemical compound variety (n= 75) on an individual basis was obtained with Soxhlet and ethyl acetate extraction. The performance of the orbital shaker extraction method with hexane and ethyl acetate gave the second greatest individual chemical compound varieties (n= 69 and n= 67, respectively). The orbital shaker method was determined to be the most efficient method in the recovery of alkane hydrocarbons, halogenated hydrocarbons and alkene hydrocarbons (Stankevičius *et al*., 2015). In our study, alkane hydrocarbons (tridecane, triacontane), a halogenated hydrocarbon (1-iodo-octacosane), an alkene hydrocarbon (alpha-ocimene), as well as a carbonic acid ester (carbonic acid-decyl-undecyl-ester) were determined to have been recovered only by this method. Also, carbonic acid hexadecyl 2.2.2-trichloroethyl ester and butyl (tetradec-6-yl) sulfonate were determined only by the orbital shaker method.

Ultrasound-assisted extraction is an inexpensive novel method offering efficient chemical compound recovery within a short period (Chen *et al*., 2021; Ünver and Çelik, 2022). This method is usually preferred for the extraction of compounds that are damaged at high temperatures. In the present study, different from previous research, the ultrasound-assisted method offered the lowest percentage yields of extraction and the smallest variety of chemical compounds. The highest percentage yield of extraction with this method was achieved with the use of methanol as a solvent (24.02%) (Fig. 1). Furthermore, a terpinol (beta-terpineol), a fatty acid (cis-10-heptadecenoic acid), a carboxylic acid (4-[{4-(4-bromo-phenyl)-thiazol-2-yl-methyl-amino}-butyric acid] as well as 3-methoxy-4-[(trimethylsilyl)oxy] benzaldehyde O-methyl oxime and (3E.5E.8Z)-3.7.11-trimethyl-1.3.5.8.10-dodecapentane) were determined to have been recovered only by the ultrasound-assisted extraction.

The selection of the solvents to be used for the extraction of chemicals from medicinal plants should be based on the species and part of use of the plant, as well as on the structure and polarity of the targeted group of chemicals. Polar compounds are generally extracted using polar solvents such as water, methanol and ethanol, whereas nonpolar compounds are extracted using nonpolar solvents such as hexane and dichloromethane (Zombe *et al*., 2022). Owing to its high polarity, methanol offers high extraction efficiency. However, it has no common use in the food and pharmaceutical industries due to its toxicity. Similar to previous research (Gahlot *et al*., 2018; Borges *et al*., 2020; Rimayani *et al*., 2022), in the present study, the highest extraction efficiency was achieved with the use of methanol as a solvent. Two-phase extraction procedures generally involve the combination of water with various solvents, and the use of water increases the efficiency of the recovery of phenolic compounds (Altıok *et al*., 2008). In the present study, although the use of water yielded a small variety of chemical compounds, the efficiency achieved with water was the second highest after methanol and ethanol. While the extraction efficiency achieved with water was in agreement with previous reports (Dhawan and Gupta, 2017; Sharma *et al*., 2020; Cao *et al*., 2022), this solvent was observed to fall short in the recovery of ketones, terpenes and sesquiterpenes. The use of acetone and ethyl acetate has been reported to increase the efficiency of the recovery of carotenoids (Borges *et al*., 2020). In the present study, while the use of ethyl acetate and acetone was associated with low extraction efficiency (Fig. 1), ethyl acetate (n= 98) yielded a greater variety of chemical compounds than acetone (n= 92). While ethyl acetate proved to be particularly efficient in the recovery of sesquiterpenoids (Wu *et al*., 2007), acetone yielded high efficiency in the recovery of ketones and alkene hydrocarbons (Borges *et al*., 2020). Ethanol is a polar solvent and requires low temperatures to obtain concentrated extracts. Ethanol has common use in the food industry, as it is safe for food production (Nunes *et al*., 2017). In this study, similar to the report of Alara *et al*. (2018), the extraction efficiency and chemical compound variety of ethanol were moderate. While ethanol offered high efficiency for the recovery of fatty acids, it fell short in the recovery of alkane hydrocarbons and sesquiterpenes. Chloroform, a nonpolar solvent, is highly efficient in the recovery of terpenoids. In the present study, chloroform offered low extraction efficiency, but yielded the third greatest variety of chemical compounds (n= 91). Chloroform is highly efficient in the recovery of terpenes, halogenated hydrocarbons and terpinol (Kınalıoğlu *et al*., 2016; Zombe *et al*., 2022). Hexane is a nonpolar solvent commonly used for lipid extraction (Borges *et al*., 2020; Gairola *et al*., 2022). In the present study, together with ethyl acetate (n= 98), hexane (n= 98) yielded the greatest variety of chemical compounds but offered a low extraction efficiency (Fig. 1).

In another study, the fatty acid composition and methanol extract of Tunisian laurel leaves were investigated. The dominant chemical class found in the essential oil of Tunisian laurel leaves was oxygenated monoterpentenes, which constituted 64.29% of the composition, and the highest chemical constituent was 1,8-cineole with a level of 46.8%. Oleic acid was also found to be dominant in the composition (Dhifi *et al*., 2018). In the present study, as shown in Table 1, palmitic acid was the highest chemical constituent of the extracts and was followed by oleic acid in the second place.

Dhifi *et al*. (2018) reported 92.88% of the chemical constituents of Tunisian laurel leaf essential oil as α-pinene, β-pinene, γ-terpinene, p-cymene, terpinolene, Δ-germacrene, sabinene, α-thujene, β-elemene, hydrocarbon monoterpenes, 1,8-cineole (eucalyptol), cis-linalool oxide, camphene, linalool, linalyl acetate, bornyl acetate, terpinene-4-ol, α-terpenyl acetate, myrtenyl acetate, α-terpineol, borneol, neryl acetate, geranyl acetate, geraniol, eugenol, methyl eugenol, thymol, oxygenated monoterpenes, β-caryophyllene, α-eudesmol, β-eusesmol, and sesquiterpenes. As Table 1 clearly shows, the chemical compounds obtained in both studies are mostly compatible.

Sangun *et al*. (2007) reported the constituents of essential oil prepared form laurel leaves collected from Antakya, Yayladağı and Samandağ as α-thujene, α-pinene, camphene, sabinene, myrcene, α-phellandrene, 1,8-cineole (Eucalyptol), trans-β-ocimene, γ-terpinene, trans-sabinene hydrate, cis-sabinene hydrate, α-terpinolene, linalool, terpinen-4-ol, α-terpinenol acetate, α-terpinyl acetate, eugenol, methyl eugenol, β-elemene, β-caryophyllene, α-humulene, caryophyllene oxide, calamenene α-eudesmol,

and β-eusesmol. As presented in Table 1, the chemical compounds obtained in both studies are compatible.

In a study by Sangun *et al*. (2007), it was reported that laurel leaves stand out with their 1.8-cineole (eucalyptol), linalool and α-terpinyl content. Pino *et al*. (1993) reported 1,8-cineole (eucalyptol) as the major constituent of laurel leaf essential oil, and indicated that eugenol and methyl eugenol, found in significant amounts, were very important factors in determining the odor and taste quality of laurel leaves. As shown in Table 1, in the present study, methyl isoeugenol and methyl eugenol were detected at the highest rate (6.26%) with the UAE method and ethanol, and at the lowest rate (0.23%) with the UAE method and chloroform.

Furthermore, Pino *et al*. (1993) detected 1,8-cineole (eucalyptol) within a range of 31.4-56%. In our study, the highest 1,8-cineole (eucalyptol) rate was detected as 20.4% in the extraction performed with the SE method and chloroform. The level detected in the present study being lower than that reported by Pino *et al*. (1993) was attributed to essential oil composition being greatly affected by climate and soil conditions.

Laurel extracts have been reported to show antimicrobial activity against *S. aureus* in several studies. Ouibrahim *et al*. (2013) have reported laurel extracts to show the highest antimicrobial activity against *Enterobacter* species, and have indicated antimicrobial activity against *S. aureus*, besides against *E. coli*. Merghni *et al*. (2016) indicated that laurel essential oil showed antimicrobial activity against oral *S. aureus* strains by preventing them from forming biofilms. Otsuka *et al*. (2008) determined that laurel leaf extracts showed antibacterial activity against methicillin-resistant *S. aureus* (MRSA). In agreement with these reports, in the present study, the strongest antibacterial activity (inhibition zone with a diameter of 13 mm) was achieved against *S. aureus* with the use of laurel extracts obtained with the ultrasound-assisted method and the solvents ethanol, ethyl acetate, acetone and hexane. This strong activity was attributed to the high content of terpenoids, terpinols, sesquiterpenoids, alkene hydrocarbons, sesquiterpens, fatty acids, epoxides and alkylbenzenes, for which an inhibitory effect has been previously proven (Guimaraes *et al*., 2019; Li *et al*., 2022; Salinas *et al*., 2022). In agreement with previous reports (Fidan *et al*., 2019; Tomar *et al*., 2020), the bacteria most resistant to the laurel extracts were determined as *S. typhi*, *E. coli* and *L. monocytogenes*. The synergy between terpenes, lactones, oxides gives laurel and monoterpenes essential oil a strong antibacterial activity (Sırıken *et al*., 2018). In the present study, the low antibacterial activity of the Soxhlet-ethanol extract against resistant bacteria such as *S. typhi* and *L. monocytogenes* was attributed to the presence of nonanedioic acid and pentadecanenitrile, which are chemical compounds obtained only by Soxhlet-ethanol extraction (Sırıken *et al*., 2018; Sakran *et al*., 2021). On the other hand, dehydro-6-desoxy-indolinocodeine, 1.1.2.2-tetraethoxyethane, lauric acid, myristic acid, pentadecanoic acid, palmitic acid, ethyl palmitate, oleanitrile and oleamide were obtained at higher levels with the other methods and solvents. Similarly, the activity of the extract obtained by ultrasound-assisted chloroform extraction against *E. coli* was attributed to it containing high levels of beta-terpineol, 4-[{4-(4-Bromo-phenyl)-thiazol-2-yl}-methyl-amino]-butyric acid, N.N-diethyl-N'.N'-diphenyl-6-pyrrol-1-YL-[1.3.5]triazine-2.4-diamine, alpha-selinene and (3E.5E.8Z)-3.7.11-trimethyl-1.3.5.8.10-dodecapentanene.

In the present study, no antibacterial activity was determined for the extracts obtained with the combined use of the Soxhlet method and methanol, the orbital shaker method and water, and the orbital shaker method and acetone. The extracts obtained with the combined use of the orbital shaker method and water (n= 43), and the orbital shaker method and acetone (n= 59) offering moderate varieties of chemical compounds, but low antimicrobial activity was attributed to their low extraction efficiencies (8.41 and 9.18%, respectively). The Soxhlet-methanol extract offering the highest extraction efficiency and a moderate chemical compound variety was attributed to its high polarity (Mehmood and Murtaza, 2018). On the other hand, the same extract not showing any antimicrobial activity was attributed to it not containing the major groups of chemicals associated with such activity (terpenes, halogenated hydrocarbons, alkene hydrocarbons, cycloalkenes etc. (Li *et al*., 2022; Nabi *et al*., 2022; Salinas *et al*., 2022).

Conclusion: This in vitro study, which was conducted using 3 different extraction methods, 7 different solvents and 5 different bacterial species demonstrated that: i) the percentage yield of extraction of the Soxhlet method was higher than the percentage yields of extraction of the ultrasound-assisted and orbital shaker extraction methods ii) the extraction efficiency achieved with methanol was higher than the extraction efficiencies achieved with the use of the other solvents iii) the laurel extracts showed a weaker inhibitory effect on Gram-negative bacteria, compared to Gram-positive bacteria, and the strongest antibacterial activity was determined against *S. aureus*. It is suggested that the method and solvent to be used for extraction should be selected according to the plant species from which extraction is intended, and the molecules and efficiency targeted for the plant extract.

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REFERENCES

Abah SE and Egwari LO, 2011. Methods of extraction and antimicrobial susceptibility testing of plant extracts. Aust J Basic Appl Sci 3:205-209.

Agyare C, Mensah AY and Osei-Asante S, 2006. Antimicrobial activity and phytochemical studies of some medicinal plants from Ghana. Boletín Latinoamericano Caribe Plantas Med Aromát 5(6):113-117.

Ahn K, 2017. The worldwide trend of using botanical drugs and strategies for developing global drugs. BMB Rep 50:111-116.

Alara OR, Abdurahman NH and Olalere OA, 2018. Ethanolic extraction of bioactive compounds from *Vernonia amygdalina* leaf using response surface methodology as an optimization tool. J Food Meas Charact 12:1107-1122.

Altıok E, Bayçın D, Bayraktar O, *et al*., 2008. Isolation of polyphenols from the extracts of olive leaves (*Olea europaea* L.) by adsorption on silk fibroin. Sep Purif Technol 62:342-348.

Akdeniz F, Dursun İ, Tepebaş K, *et al*., 2021. Comparison of different extraction methods for total phenolic content and antioxidant activity of dried *Diospyros lotus* L fruits. Turkish J Analytic Chem 3:64-69.

Borges A, José H, Homem V, *et al*., 2020. Comparison of techniques and solvents on the antimicrobial and antioxidant potential of extracts from *Acacia dealbata* and *Olea europaea*. Antibiotics (Basel) 9: 48.

Cao X, Zhang Y, Xun H, *et al.,* 2022. High-yield recovery of antioxidant compounds from *Bambusa chungii* culms using pressurized hot water extraction. Antioxidants 11:2231.

Caputo L, Nazzaro F, Souza LF, *et al*., 2017. *Laurus nobilis*: Composition of essential oil and its biological activities. Molecules 22:930.

Chen G, Sun F, Wang S, *et al*., 2021. Enhanced extraction of essential oil from *Cinnamomum cassia* bark by ultrasound assisted hydrodistillation. Chin J Chem Eng 36:38-46.

Da Silva Haas IC, Toaldo IM, Burin VM, *et al*., 2018. Extraction optimization for polyphenolic profiling and bioactive enrichment of extractives of non-pomace residue from grape processing. Ind Crops Prod 112:593-601.

Derwich E, Benziane Z and Boukir A, 2009. Chemical composition and antibacterial activity of leaves essential oil of *Laurus nobilis* from Morocco. Aust J Basic Appl Sci 3:3818-3824.

Dhawan D and Gupta J, 2017. Comparison of different solvents for phytochemical extraction potential from *Datura metel* plant leaves. Int J Biol Chem 11:17-22.

Dhifi W, Bellili S, Jazi, S, *et al*., 2018. Phytochemical composition and antioxidant activity of Tunisian Laurus nobilis. Pak J Pharm Sci 31:2397-2402.

Fidan H, Stefanova G, Kostova I, *et al.,* 2019. Chemical composition and antimicrobial activity of *Laurus nobilis* L. essential oils from Bulgaria. Molecules 24:804.

Gahlot M, Bhatt P and Joshi J, 2018. Study on yield of plant extracts using different solvents and methods. Bull Env Pharmacol Life Sci 7:65-67.

Gairola K, Gururani S, Kumar R, *et al*., 2022. Composition, antioxidant and anti-inflammatory activities of hexane and methanol extracts of *Acmella uliginosa* from Terai region of Uttarakhand. Braz J Pharm Sci 58:e20353.

Guimaraes AC, Meireles LM, Lemos MF, *et al*. 2019. Antibacterial activity of terpenes and terpenoids present in essential oils. Molecules 24:2471.

Kapadia P, Newell AS, Cunningham J, *et al*., 2022. Extraction of high-value chemicals from plants for technical and medical applications. Int J Mol Sci 23:10334.

Karğılı U and Aytaç E, 2021. Soxhlet extraction of Cannabinoids (CBD and THC) from four different strains of Cannabis grown in four different regions. Res Square 1-23.

Kınalıoğlu K, Aydın S, İlhan Ö, *et al*., 2016. Phenol and flavonoid amounts, antioxidant and antibacterial potencies of *Xanthoparmelia stenophylla* (Ach.) Ahti & D. Hawksw. Aku J Sci Eng 16:6-15.

Koçak E and Pazır F, 2018. Effect of extraction methods on bioactive compounds of plant origin. Turkish J Food Agri Sci 6:663-675.

Li H, Song X, Li H, *et al*., 2022. Sesquiterpenes and monoterpenes from the leaves and stems of *Illicium simonsii* and their antibacterial activity. Molecules 27:1115.

Lohani UC, Fallahi P and Muthukumarappan K, 2015. Comparison of ethyl acetate with hexane for oil extraction from various oilseeds. J Am Oil Chem Soc 92:743-754.

Mehmood A and Murtaza G, 2018. Phenolic contents, antimicrobial and antioxidant activity of *Olea ferruginea* Royle (Oleaceae). BMC Compl Altern Med 18:173.

Merghni A, Marzouki H, Hentati H, *et al*., M. 2015. Antibacterial and antibiofilm activities of *Laurus nobilis* L. essential oil against *Staphylococcus aureus* strains associated with oral infections. Curr Res Transl Med 64:29-34.

Nabi M, Tabassum N, Ganai BA, 2022. Phytochemical screening and antibacterial activity of *Skimmia anquetilia* N.P. Taylor and Airy Shaw: A first study from Kashmir Himalaya. Front Plant Sci 13:937946.

Nunes R, Pasko P, Tyszka-Czochara M, *et al*., 2017. Antibacterial, antioxidant and antiproliferative properties and zinc content of five South Portugal herbs. Pharmaceutical Biol 55:114-123.

Otsuka N, Liu MH, Shiota S, *et al*., 2008. Anti-methicillin resistant *Staphylococcus aureus* (MRSA) compounds isolated from *Laurus nobilis*. Biol Pharm Bull 31:1794-1797.

Ouibrahim A, Tlili-Ait-Kaki Y, Bennadja S, *et al*., 2013. Evaluation of antibacterial activity of *Laurus nobilis* L., *Rosmarinus officinalis* L. and *Ocimum basilicum* L. from Northeast of Algeria. African J Microbiol Res 7:4968-4973.

Özer T, Sert FZ and Öztürk Aİ, 2019. A research on laurel plant (Lauris nobilis) and oil. GBAD 8:25-34.

Özer T, Sert FZ and Öztürk Aİ, 2021. Natural laurel soap. Alanya Alaa Keyk Uni J Sci 3:29-37.

Pino J, Borges P and Roncal E, 1993. The chemical Composition of Bay Leaf oil from various origins. Die Nahrung 37:592-595.

Rimayani S, Noli ZA, Zulfikar Z, *et al*., 2022. Effect of seaweed extract from water, methanol, and ethanol extraction as biostimulant on growth and yield of Upland Rice (*Oryza sativa* L.) in Ultisol. Int J Progress Sci Technol 30:449-455.

Sakran KA, Raharjo D and Mertaniasih NM, 2021. Antimicrobial activities of *Laurus nobilis* leaves ethanol extract on *Staphylococcus aureus*, *Salmonellae typhi*, and *Escherichia coli*. Indonesian J Trop Infect Dis 9:120-125.

Salinas C, Florentín G, Rodríguez F, *et al*., 2022. Terpenes combinations inhibit biofilm formation in *Staphyloccocus aureus* by interfering with initial adhesion. Microorganisms 10:1527.

Sangun MK, Aydin E, Timur M, *et al*., 2007. Comparison of chemical composition of the essential oil of Laurus nobilis L. leaves and fruits from different regions of Hatay, Turkey. J Environ Biol 28: 731-733.

Sharma A, Thakur A, Kumari R, *et al*., 2020. Comparison of extraction yield, phytochemicals and *in vitro* antioxidant potential of different extracts of two *Ficus* species. Plant Archiv 20 (Supp 1):2669-2673.

Sırıken B, Yavuz C and Güler A, 2018. Antibacterial activity of *Laurus nobilis*: A review of literature. Med Sci Discov 5:374-379.

Silalahi E, Anita S and Teruna HY, 2021. Comparison of extraction techniques for the determination of polycyclic aromatic hydrocarbons (PAHs) in soil. J Phys Conf Ser 1819:012061.

Souza MM, Silva BD, Costa CSB, *et al*., 2018. Free phenolic compounds extraction from Brazilian halophytes, soybean and rice bran by ultrasound-assisted and orbital shaker methods. An Acad Bras Cienc 90:3363-3372.

Stankevičius M, Maruška A, Tiso N, *et al*., 2015. Gas chromatographic analysis of polycyclic aromatic hydrocarbons in the disposed creosote treated wooden railway sleepers collected from several storage sites in Lithuania. CHEMIJA 26:198-207.

Tan Mei Chin B, Ali A, Kamal H, *et al*., 2021. Optimizing parameters on the antioxidant capacity of watermelon pulp using conventional orbital shaker and ultrasound-assisted extraction methods. J Food Process Preserv 45:e15123.

Tomar O, Akarca G, Gök V, *et al*., 2020. Composition and antibacterial effects of Laurel (*Laurus nobilis* L.) leaves essential oil. J Essent Oil-Bear Plants 23:414-421.

Ünver N and Celik S, 2022. Optimization of ultrasound-assisted extraction of olive leaf (var. Halhalı) extracts. Hacettepe J Biol Chem 50:173-184.

Wu H, Su Z, Yang Y, *et al*., 2007. Isolation of three sesquiterpene lactones from the roots of *Cichorium glandulosum* Boiss. et Huet. by high-speed counter-current chromatography. J Chromatogr A 1176:217-222.

Zhao S and Zhang D, 2014. Supercritical CO_2 extraction of eucalyptus leaves oil and comparison with Soxhlet extraction and hydro-distillation methods. Sep Purif Technol 133:443-451.

Zombe K, Nyirenda J, Lumai A, *et al*., 2022. Impact of solvent type on total phenol and flavonoid content and sun protection factor of crude cashew nutshell liquid. Sustain Chem 3:334-344.