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Correlation between Major Bioactive Compounds in Essential Oils from Wild and Cultivated Moroccan Plants and their Antibacterial Efficacy against Foodborne Pathogens

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ABSTRACT

Foodborne pathogens pose a significant risk due to surface contamination, often from inadequate hygiene or biofilm formation. While chemical disinfectants are commonly used, concerns about harmful by-products have led to the search for natural alternatives. This study aimed to explore the correlation between major compounds and the antibacterial effects of seven Moroccan essential oils (EOs) against common foodborne pathogens. Essential oils were extracted via hydro-distillation and analyzed using gas chromatography-mass spectrometry (GC-MS) and gas chromatography with flame ionization detection (GC-FID). The antibacterial activity was assessed using the disc diffusion method, and multivariate analyses, including principal component analysis (PCA) and hierarchical cluster analysis (HCA), were applied to evaluate correlations between chemical composition and antibacterial efficacy. Forty-five components were identified from the EOs and categorized into 10 chemical classes. Major components included carvacrol (31.93%) in *Origanum elongatum*, thymol (31.01%) in *Thymus vulgaris*, linalyl acetate (46.95%) in *Citrus aurantium*, 1,8-cineole (80.56%) in *Eucalyptus globulus*, citral (42.69%) in *Cymbopogon citratus*, borneol (28.10%) in *Thymus serpyllum*, and camphor (32.25%) in *Lavandula stoechas*. Strong antibacterial activity was observed in *O. elongatum*, *T. vulgaris*, and *T. serpyllum*, demonstrating bactericidal properties with low minimum inhibitory concentration (MIC) values. In contrast, *E. globulus* and *C. aurantium* had higher MIC values. Multivariate analyses revealed that phenols had the most substantial impact on antimicrobial activity, followed by terpenes and alcohols. These findings suggest that Moroccan EOs are promising natural disinfectants for food contact surfaces, reducing the risk of bacterial pathogen transmission.

Keywords: Essential oils, Chemical composition, Antibacterial activity, Natural disinfectant, Principal component analysis, Hierarchical cluster analysis.

Introduction

Food safety remains a critical global concern, impacting public health and economic stability. According to the World Health Organization (WHO; 2015), nearly 600 million people worldwide suffer from foodborne illnesses annually, resulting in approximately 420,000 deaths. These diseases particularly affect regions, such as Africa and South-East Asia.[1] In Morocco, annual reports from the national health surveillance system indicate between 1,000 to 1,600 cases of food poisoning, with hospitalization rates ranging from 30 to 45%. Moreover, 20 to 25% of food service and retail establishments inspected are deemed at risk due to inadequate hygiene practices.[2]

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Previous research underscores that a significant proportion of foodborne outbreaks occur in restaurant settings globally, attributed to various pathogens and inadequate sanitation of food contact surfaces and utensils. The persistence of pathogens, such as *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella* spp., *Campylobacter jejuni*, *Yersinia enterocolitica*, and pathogenic strains of *Escherichia coli* on surfaces underscores their ability to form biofilms, spread to food, and cause foodborne outbreaks.[3] Effective disinfection of these surfaces is crucial for eradicating or at least reducing microbial contamination. However, most industrial disinfectants are chemical-based, raising concerns about potential health and environmental impacts. Essential oils (EOs) could be a valuable alternative for decontaminating surfaces in contact with food.[4] These natural products offer effective antimicrobial properties, are biodegradable, and generally have fewer side effects and harmful residues that could affect human health. In contrast, chemical disinfectants can release reactive intermediates and by-products into indoor environments, and continuous exposure to these chemicals may pose health risks.[5–7] Additionally, the biological activity of EOs is primarily attributed to its major constituent, although synergies between multiple components often contribute to its overall efficacy.[5,8] These active compounds encompass diverse chemical groups including alcohols, esters, aldehydes, ketones, phenols, and phenolic ethers. Terpene compounds are the most prevalent, followed by terpenoids and other aromatic and aliphatic constituents. Notable plants containing these bioactive

compounds include thyme, lemongrass, eucalyptus, geranium, rosemary, lemon, citronella, orange, and, lavender.[5] Essential oils are emerging as environmentally friendly options with significant potential in the food industry.[9] Studies have shown that thyme and oregano EOs effectively eliminate pre-formed *Salmonella typhimurium* biofilms on surfaces, addressing a significant concern in foodborne illness prevention.[10] Additionally, oregano, thyme, lemongrass, citral, and clove EOs have demonstrated significant antimicrobial activity against various pathogenic bacteria responsible for foodborne illnesses, including *Listeria monocytogenes*, *E. coli*, and *Salmonella enterica*. Furthermore, EOs are promising antimicrobial agents against antibiotic-resistant bacteria such as *Enterococcus faecalis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*.[11]

Essential oils from Moroccan plants have been studied in the literature for their antibacterial potential with promising results against pathogens.[12–17] However, there is a gap in the literature regarding the comparative analysis of wild versus cultivated Moroccan EOs and their detailed chemical profiles. The integration of multivariate analyses, such as principal component analysis (PCA) and hierarchical cluster analysis (HCA), into the present study addresses this gap by providing a systematic approach to understanding how chemical variability influences antibacterial performance. The PCA and the MCA help users reduce the number of variables and minimize redundancy, particularly when analyzing correlations among many parameters and variables.[18] These analytical techniques enable a more nuanced understanding of how specific compounds contribute to antibacterial activity, and how the origin of the plant may influence this relationship. These EOs could represent a promising avenue for developing new antibacterial agents by leveraging their natural origins and diverse chemical compositions to disinfect food-contact surfaces and combat germs effectively. This research advances the understanding of EOs' antibacterial properties through innovative analytical techniques and offers valuable insights into the potential applications of Moroccan EOs in food safety, especially as bio-disinfectants.

The present study used multivariate analysis techniques (PCA and HCA) to investigate the correlation between the major bioactive compounds in seven EOs derived from wild and cultivated Moroccan plants and their antibacterial efficacy against bacteria isolated from food-contact surfaces.

Materials and Methods

Source and identification of plant materials

Following an extensive literature review,[5,11,12] seven plants were selected for the study: Four wild Moroccan species from the Lamiaceae family, including *Origanum elongatum* (Bonnet), *Thymus vulgaris* L., *Thymus serpyllum* L., and *Lavandula stoechas* L., as well as three cultivated species: *Citrus aurantium* L. subsp. *amara* (Rutaceae), *Eucalyptus globulus* Labill. (Myrtaceae), and *Cymbopogon citratus* (DC.) (Poaceae). These plants were chosen based on their availability and documented disinfectant properties in the literature. The plants were harvested from various locations in northwestern and north-central Morocco (Table 1). Botanical identification was conducted by Abdeslam Ennabili, a Botanist and Head of the Department of Phytology, National Agency of Medicinal and Aromatic Plants, Taounate, Morocco. Authenticated voucher specimens were deposited in the agency's herbarium.

Source of bacterial reference strains

Escherichia coli ATCC 25922 and *Staphylococcus aureus* ATCC 25923, as reference strains for quality control in disk diffusion and minimum inhibitory concentration (MIC) determination, were provided by the Regional Laboratory of Epidemiological Diagnosis and Environmental Hygiene (RLEDEH) laboratory in Fez. All strains were stored at -21°C in brain–heart infusion broth (BHI; Oxoid, Basingstoke, UK) containing 20% (v/v) glycerol and were propagated in the same medium at 37°C before use.

Preparation of essential oils from plant samples

The fresh aerial parts of the test plants were subjected to hydro-distillation at the Biopam Company, to extract the EOs according to standard procedures. The company is located in the Tahar Souk commune of the Taounate province (34° 39′ 03″N. 4° 16′ 40″W). EO samples were offered by this company and were stored at 4°C until analysis.

Characterization of the essential oils by gas chromatography-mass spectrometry

The gas chromatography-mass spectrometry (GC-MS) technique precisely determines the composition of EOs by identifying specific compounds based on their mass-to-charge ratio. Gas chromatographic analyses were conducted using Shimadzu GC (Trace GC Ultra, HP 6890, Hewlett-Packard), coupled with an MS (MS, 5973) supplied with HP-5 capillary column (60 m × 0.32 mm ID × 0.25 µm).

Table 1: Sources and date of collection of plant samples for preparing essential oils

Species	Date	Site	GPS coordinates	Altitude (m)
O. elongatum	May 2022	Rif region, Ketama	34° 52′ 35″N. 4° 37′ 10″W	1115
T. vulgaris	May 2022	Taounate, Mernissa	34° 40′ 48″N. 4° 15′ 0″W	905
C. aurantium	April 2022	Taounate, prefecture	34° 32′ 09″N. 4° 38′ 24″W	592
T. sepyllum	June 2022	Rif region, Ketama	34° 52′ 35″N. 4° 37′ 10″W	1115
E. globulus	June 2022	Taounate, Mernissa	34° 40′ 48″N. 4° 15′ 0″W	905
L. stoechas	June 2022	Taounate, Timezgana	34° 33′ 02.7″N. 4° 40′ 49.3″W	800
C.citratus	May 2022	Taounate prefecture	34° 32′ 09″N. 4° 38′ 24″W	592

GPS: Global Positioning System

Operating conditions were as follows: an initial temperature of 40°C for 2 minutes, increased to 160°C for 2 minutes, and then to 280°C for 2 minutes. At 280°C, the Polaris QMS mass spectrometer was engaged. Helium (99.99% purity; Sigma-Aldrich, USA) served as the carrier gas, with hexane (99.99% purity; Sigma-Aldrich, USA) as the solvent. The injection temperature was maintained at 250°C, and the pressure was set to 37.1 kPa. Identification of various phytochemical components within the EO was accomplished by determining their retention indices and comparing them with the NIST MS Search 2012 database and literature data.[19]

Characterization of the essential oils by gas chromatograph-flame ionization analysis

Samples were analyzed using a Shimadzu GC (Trace GC Ultra, HP 6890, Hewlett-Packard) equipped with an HP-5 capillary column (60 m x 0.32 mm, 0.25 m film thickness), an FID detector, and a 275°C injector. After an initial 5-minute hold, the oven temperature was increased to 250°C at 4°C/min. Nitrogen was used as the carrier gas, flowing at 1.8 mL/min. Samples, diluted 1:50 in methanol (99.99% purity; Sigma-Aldrich, USA), were injected in a 1 µL volume using a split mode with a ratio of 1:50 and a flow rate of 72.1 mL/min. Peak area normalization facilitated the determination of the relative proportions of the components. Retention indices (RIs) were calculated using a homologous alkane series on an HP-5 MS column (C8-C28).

Isolation of bacterial strains

The strains tested were collected from food contact surfaces between July 2021 and January 2022, in selected restaurants in Fez, central Morocco. Food samples were collected aseptically using sterile spoons and placed into sterile bags. Samples were transported to the laboratory in ice boxes (4°C). All samples were analyzed in an ISO/International Electrotechnical Commission (IEC) 17025:2005 accredited laboratory of the Regional Laboratory of Epidemiological Diagnosis and Environmental Hygiene (RLEDEH), Fez. Isolation of microbes from food contact surfaces was performed using the spread plating method on both general-purpose medium (nutrient agar; Biokar Diagnostics, France) and selective/differential media (mannitol salt agar (MSA; Biokar Diagnostics, France), *Pseudomonas* base agar (PBA; Biokar Diagnostics, France), Mac Conkey agar (MA; Biokar Diagnostics, France), eosin methylene blue (EMB; Biokar Diagnostics, France), Rappaport-Vassiliadis (RV; Biokar Diagnostics, France) broth, Hektoen agar (HA; Biokar Diagnostics, France), and gélose PALCAM (Biokar Diagnostics, France) for 48 hours at 37°C. Pure colonies were obtained and identified using standard biochemical tests, with confirmation utilizing the API kit (BioMérieux, France).

Evaluation of the antibacterial activity of essential oils using the disc diffusion assay

The preliminary evaluation of the antibacterial potential of the test EOs was carried out by disc diffusion assay, following a previously published protocol. A bacterial inoculum adjusted to 0.5 McFarland standard was spread on Mueller Hinton agar plates (Oxoid, Basingstoke, England). Sterile Whatman paper discs (6 mm in diameter) impregnated with 10 µL of each EO were then placed on the surface of the inoculated agar. Chloramphenicol (30 µg; OXOID) was used as a positive control, while sterile distilled water was employed as a negative control. The plates were incubated at 37°C for 24 hours. After incubation, the diameters of inhibition zones were measured in millimeters, and the results were expressed as means ± standard deviation of three replicates.[20]

According to the diameter of the inhibition zone (IZD) expressed in millimeters, antibacterial activity was categorized as follows: low for a diameter equal to or below 8 mm; medium for a diameter between 8 and 14 mm; high for a diameter between 14 and 20 mm; and extremely high for a diameter equal to or greater than 20 mm.[21]

Determination of the minimum inhibitory concentration (MIC)

The minimum inhibitory concentrations of *O. elongatum, T. vulgaris, C. aurantium, E. globulus, C. citratus, T. serpyllum, and L. Stoechas* were determined using the broth microdilution method.[22] Agar at a concentration of 0.15% (w/v) was employed to stabilize the extract-water mixture, while resazurin served as an indicator of bacterial growth. A 96-well polypropylene microtitre plate was filled with 50 µL of Mueller–Hinton broth (MHB; Oxoid, Basingstoke, England) supplemented with agar (Oxoid, Basingstoke, England) at 0.15% (w/v). Each EO, at a final concentration of 8% (v/v), was introduced into the first well, followed by serial $^1/_2$ dilutions. These dilutions were carried out by pipetting 50 µL from the first well and transferred to the next one, until reaching the 12th well, with the last 50 µL mixture discarded. Subsequently, 50 µL of bacterial suspension, approximately 10^6 cfu/mL, was added to each well. The final concentrations of the EOs ranged from 8 to 0.007812% (v/v). The plate was covered, sealed with parafilm, and then incubated at 37°C for 24 hours. Subsequently, 10 µL of resazurin was added to each well, followed by incubation for an additional 2 hours at 37°C. The minimum inhibitory concentration (MIC) was determined as the lowest concentration of EO that inhibited bacterial growth, indicated by the absence of colour change in the resazurin dye. Positive growth was identified by reducing the blue dye resazurin to pink resorufin.[23]

Determination of the minimum bactericidal concentration

The minimum bactericidal concentration (MBC) corresponded to the lowest concentration of the EO yielding negative subcultures after 24 hours of incubation at 37°C. It is determined in broth dilution tests by subculturing 10 µL from negative wells on plate count agar (PCA; Biokar Diagnostics, France) medium.[22] The estimation of the MBC/MIC ratio describes the bactericidal effect (MBC/MIC < 4) or bacteriostatic (MBC/MIC ≥ 4) of the test EO.[24]

Statistical analysis

Statistical analysis was performed using analysis of variance (ANOVA). Fifteen compounds from the EO samples, each with an average concentration exceeding 7.0%, were selected to evaluate their potential in reflecting chemotaxonomic and biological activity relationships. Antibacterial activity and component data were subjected to PCA and HCA. Statistical analyses were conducted using JMP Pro 14 software (JMP®, Version 14. SAS Institute Inc., Cary, NC, 1989–2023) and R Statistical Software (version 4.0.2; R Foundation for Statistical Computing, Vienna, Austria). A p-value of < 0.05 was considered statistically significant.

Results and Discussion

Chemical composition of the essential oils

The EOs of *E. globulus*, *O. elongatum*, *L. stoechas*, *T. serpyllum*, *T. vulgaris*, *C. citratus*, and *C. aurantium* were subjected to chromatographic analyses (GC/FID and GC/MS). Forty-five components, accounting for 96.88 to 100% of the total oil content, were identified. The identified components were categorized into ten chemical classes (Table 2). Monoterpene oxides (0–80.56±3.6%) were the most abundant, with 1,8-cineole as the major constituent. The second most prevalent class, monoterpene aldehydes (0–77.44±0.3%), featured neral (*β*-citral) and geranial (*α*-Citral) as the primary compounds. Monoterpene esters (0–57.36±2.9%) were the third most common, with linalyl acetate as the predominant compound. Monoterpene alcohols (0–50.33±2.8%) were the fourth major class, dominated by borneol, followed by linalool and terpineol. Monoterpene phenols (0–49.07±2.9%) were the fifth class, with carvacrol and thymol as the main constituents. The sixth class, monoterpene ketones (0–47.30±2.7%), was primarily composed of camphor and fenchone. Monoterpene hydrocarbons (1.81±0.2%–45.19±2.6%) ranked seventh, with *o*-cymene, *α*-pinene, *γ*-terpinene, and camphene as key components. Sesquiterpene hydrocarbons (0–8.16±0.9%) were the eighth class, with (E)-caryophyllene as the main compound. Sesquiterpene alcohols (0–6.30±0.4%), primarily cubebol, and sesquiterpene oxides (0–0.99±0.08%), primarily caryophyllene oxide, were the ninth and tenth classes, respectively. Figure 1 shows the chemical structures of the main components.

The chemical profiles of the seven test EOs have been previously documented. In the present study, ten constituents were identified in *O. elongatum* EO, representing 100% of its composition.

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Table 2: Chemical composition of selected essential oils obtained by gas chromatography-mass spectrometry.

Nº	Compounds	RI*	RI**	Content (%) Plant						
				E. globulus	*O. elongatum*	*L. stoechas*	*T. serpyllum*	*T. vulgaris*	*C. citratus*	*C. aurantium*
1	*α* -Pinene	948	934	4.58 ± 0.40	-	15.59 ± 1.10	3.70 ± 0.40	2.78 ± 0.20	-	-
2	Camphene	943	946	-	-	11.27± 0.90	6.69± 0.50	0.43± 0.01	1.13 ± 0.10	-
3	*o*-Cymene	1042	1041	14.86± 1.10	15.45 ± 1.11	0.65± 0.20	4.61± 0.40	26.21± 1.90	-	-
4	*γ*-Terpinene	998	1050	-	22.82± 1.8	-	1.42± 0.10	-	-	-
5	*α*-Terpinene	998	1010	-	3.65± 0.20	-	-	-	-	-
6	1,8-cinéole	1059	1057	80.56± 3.60	-	-	2.12± 0.20	0.55± 0.010	-	-
7	Thymol	1262	1271	-	17.14± 1.40	-	3.74± 0.40	31.01± 2.20	-	-
8	Carvacrol	1262	1282	-	31.93± 2.10	-	9,03± 0.90	10.15± 1.10	-	-
9	Neral (*β*-Citral)	1174	1220	-	-	-	-	-	33.67± 2.20	-
10	Geranial (α-Citral)	1174	1247	-	-	-	-	-	42.69± 2.80	-
11	*α*-Terpineol	1143	1175	-	-	-	15.33± 1.20	13.85± 1.00	-	8.48 ± 0.60
12	Borneol	1138	1136	-	-	3.51± 0.30	28.10± 1.80	1.35± 0.10	-	-
13	Geraniol	1228	1238	-	-	-	-	-	3.73± 0.20	1.29± 0.10
14	Linalool	1082	1086	-	2.64± 0.20	4.37± 0.30	4.34± 0.40	6.65± 0.50	1.76± 0.10	24.72± 1.70
15	Geranyl acetate	1352	1361	-	-	-	-	-	5.53± 0.40	6.40± 0.50
16	Linalyl acetate	1272	1240	-	-	-	-	-	-	46.95± 2.70
17	Neryl acetate	1352	1343	-	-	-	-	-	-	4.01± 0.30
18	Caryophyllene	1494	1419	-	2.15± 0.10	-	6.95± 0.60	1.66± 0.10	2.66± 0.20	1.33± 0.10
19	Selina-3,7(11)-diene	1507	1537	-	-	3.70± 0.30	-	-	-	-
20	Fenchone	1121	1072	-	-	15.05± 1.20	-	-	-	-

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21	Camphor	1121	1125	-	-	32.25± 2.20	1.91± 0.20	-	-	-
22	D-Limonene	1018	1023	-	-	1.63± 0.2	1.09± 0.09	0.89± 0.03	0.68± 0.01	1.72± 0.20
23	*β* -Myrcene	958	983	-	2.15± 0.20	-	-	0.88± 0.02	-	1.89± 0.20
24	*β* -Pinene	943	973	-	-	-	0.68± 0.020	-		1.23± 0.10
25	Terpinen-4-ol	1137	1164	-	-	-	2.56± 0.20	-	-	-
26	Bornyl acetate	1277	1271	-	-	-	2.68± 0.30	-	-	-
27	*γ*-Cadinene	1435	1505	-	-	-	0.63± 0.02	-	-	-
28	*δ*-Cadinene	1469	1513	-	-	-	0.58± 0.01	-	-	-
29	Bornyl formate	1275	1208	-	-	-	0.72± 0.03	-	-	-
30	Eugenol	1392	1339	-	-	-	-	-	0.82± 0.04	-
31	Caryophyllene oxide	1507	1570	-	-	-	-	-	0.99± 0.08	-
32	*γ*-Muurolene	1435	1473	-	-	-	-	-	2.92± 0.20	-
33	cis-Calamenene	1537	1509	-	-	0.91± 0.07	-	-	-	-
34	*β*-Selinene	1480	1469	-	-	0.68± 0.04	-	-	-	-
35	Myrtenyl formate	1312	1238	-	-	2.14± 0.20	-	-	-	-
36	Longifolene	1398	1400	-	-	-	-	0.85± 0.06	-	-
37	*γ*-Terpineol	1191	1146	-	-	-	-	1.96± 0.20	-	-
38	*α*-Thujene	912	925	-	1.12± 0.10	-	-	-	-	-
39	*β*-Ocimene	976	1038	-	-	-	-	-	-	1.98± 0.20
40	*β* -Terpineol, cis-	1158	1129	-	-	-	-	0.68± 0.04	-	-
41	Terpinen-4-ol acetate	1137	1283	-	0.95± 0.07	-	-	-	-	-
42	Isoneral	1174	1170	-	-	-	-	-	1.05± 0.10	-
43	4-Nonanone	1052	1030	-	-	-	-	-	1.35± 0.20	-
44	Cubebol	1484	1504	-	-	6.30± 0.40	-	-	-	-

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45	Tricyclene	729	921	-	-	0.89± 0.06	-	-	-	-
Chemical classes	Monoterpene hydrocarbons			19.44± 1.50	45.19± 2.60	30.03± 2.10	18.19± 1.40	31.19± 2.1	1.81± 0.20	6.82± 0.60
	Monoterpene oxides			80.56± 3.60	-	-	2.12± 0.20	0.55± 0.01	-	-
	Monoterpene aldehydes			-	-	-	-	-	77.41± 3.0	-
	Monoterpene alcohols			-	2.64± 0.20	7.88± 0.8	50.33± 2.8	24.49± 0.2	5.49± 0.20	34.49± 2.20
	Monoterpene esters			-	0.95± 0.08	2.14± 0.20	3.40± 0.30	-	5.53± 0.20	57.36± 2.90
	Monoterpene ketones			-	-	47.30± 2.70	1.91± 0.20	-	1.35± 0.20	-
	Monoterpene phenols			-	49.07± 2.90	-	12.77± 1.10	41.16± 2.0	0.82± 0.04	-
	Sesquiterpene hydrocarbons			-	2.15± 0.20	5.29± 0.90	8.16± 0.90	2.51± 0.3	5.58± 0.60	1.33± 0.20
	Sesquiterpene oxides			-	-	-	-	-	0.99± 0.08	-
	Sesquiterpene alcohols			-	-	6.30± 0.40	-	-	-	-
	Total			100± 0.00	100± 0.00	98.94± 1.20	96.88± 1.60	99.9± 0.07	98.98± 0.90	100± 0.00

Retention indices (RI)* are experimentally calculated retention indices for a homologous series of C8-C28 alkanes.
Literature-based retention indices (RI Lit) **[19].

The principal component was carvacrol (31.93%), followed by *γ*-terpinene (22.88%), thymol (17.14%), and *o*-cymene (15.45%). Similar findings were reported by Oualili *et al*. for EOs from Targuist, Morocco, with carvacrol (60.42%) as the major compound.[25] El Harsal *et al*. noted a predominance of oxygenated (65.14%) and hydrocarbon (28.02%) compounds, with thymol (63.44%) and *γ*-terpinene (14.63%) being prominent.[12] Ramzi *et al*. observed minor variations in chemical composition across five populations, with carvacrol (67.34–81.72%) being predominant[26]. Bakha *et al*. confirmed the dominance of carvacrol (10.52–77.45%) and thymol (0.98–62.40%) in EOs from the Rif and Middle Atlas Mountains.[27] Abdelaali *et al*. indicated that thymol, carvacrol, p-cymene, and *γ*-terpinene are consistently present in the EOs of all parts of *O. elongatum.*[28]

Concerning *T. vulgaris*, the EO was analyzed to contain fifteen compounds, with thymol (31.01%), *o*-cymene (26.21 ± 1.9%), *α*-terpineol (13.85%), and carvacrol (10.15%) being predominant. Imelouane *et al*. reported camphor (38.54%) and camphene (17.19%) as major constituents in EO from Taforalt, Morocco.[29] In contrast, thymol (35.87%) and carvacrol (18.62%) were major in *T. vulgaris* from Errachidia.[30] Sadiki *et al*. found thymol (40.0%) and γ-terpinene (12.0%) as primary components in Taounate.[31] Carvacrol was the major compound in EO from Beni Idder and Errachidia.[32] Moroccan *Thymus* species EOs are rich in oxygenated monoterpenes like carvacrol, thymol, borneol, camphor, and *α*-terpineol, while hydrocarbon monoterpenes, such as p-cymene, *γ*-terpinene, *α*-terpinene, camphene, and *α*-pinene are also prominent.[17] For *T. serpyllum*, a total of 19 compounds were identified, with dominant components being borneol (28.10%), *α*-terpineol (15.33%), and carvacrol (9.03%). Contrasting findings include linalyl acetate (52.2%) and (E)-nerolidol (15.1%) as the main components in EO from Oukaimden (High Atlas).[33]

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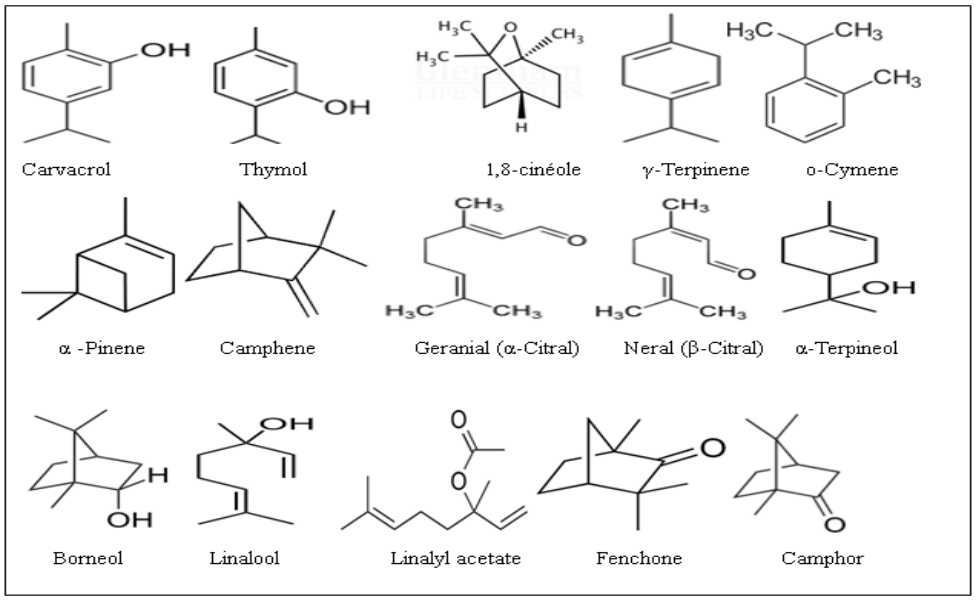

Figure 1: Chemical structures of major compounds isolated from *O. elongatum*, *T. vulgaris*, *T. serpyllum, C. citratus*, *L. stoechas*, *E. globulus*, and *C. aurantium* essential oils.

The EO of *L. stoechas* was dominated by camphor (32.25%), *α*-pinene (15.59%), fenchone (15.05%), and camphene (11.27%). This relatively contrasts with the findings of Benali *et al*., who reported fenchone (14.89%) and camphor (23.80%) as major constituents in Aknol, North Central, Morocco.[16] Bouyahya *et al.* reported fenchone (31.81%), camphor (29.60%), terpineol (13.14%), menthone (8.96%), and eucalyptol (5.88%) in EO from Ouezzane, North-West of Morocco.[15] Cherrat *et al*. identified cubenol and other compounds as prominent in their study.[34] Radi *et al*. reported camphor (24.35%) and cubenol (15.54%) as major components, categorized into oxygenated monoterpenes (49.93%), oxygenated sesquiterpenes (26.42%), monoterpenes (16.71%), and sesquiterpenes (6.94%).[13] For *E. globulus*, the EO mainly comprises 1,8-cineole (80.56%) and *o*-cymene (14.86%), differing from other reports. Mekkaoui *et al*. found 1,8-cineole (90.14%) and *α*-pinene (3.85%) as primary components.[35] Čmiková *et al*. reported 1,8-cineole (63.1%), p-cymene (7.7%), *α*-pinene (7.3%), and *α*-limonene (6.9%) in Slovakian samples. Monoterpenes make up to 99.2% of the oil.[36] El Guerrouj *et al*. reported eucalyptol (24.92%), pinocarveol (16.94%), 4,6-di-t-butylpyrogallol (14.40%), *β*-eudesmol (12.50%), and *α*-pinene (11.30%).[14] Previous studies consistently identify *Eucalyptus* species as belonging to the 1,8-cineole chemotype, with content ranging from 17.2% to 90.0%.[36,37]

Regarding *C. citratus*, the EO comprises thirteen compounds totaling 98.98%, with neral (*β*-citral) (33.67%) and geranial (*α*-citral; 42.69%) as the major constituents. Bassole *et al*. found similar results, with geranial (48.1%) and neral (34.6%) as primary components.[38] Another study identified neral (29.2%) and geranial (18.2%) as major components, along with *α*-pinene (4.8%) and myrcene (3.9%).[39] The biochemical properties of *C. citratus* are attributed to these constituents. Lemongrass EOs exhibit significant chemical diversity, with up to 72 bioactive molecules, including geranial, neral, geraniol, limonene, and *β*-myrcene. Variations are influenced by climate, geographical location, and plant parts.[40] For *C. aurantium*, chromatographic analysis identified eleven compounds, nearly covering 100% of the EO. Major constituents include linalyl acetate (46.95%), linalool (24.72%), and *α*-terpineol (8.48%). Ainane *et al*. reported linalool (27.68%), *α*-terpineol (14.05%), and *γ*-terpinene (7.33%) as major components.[41] Gniewosz *et al*. in Poland identified linalyl acetate (48.06%), linalool (26.88%), *α*-terpineol (5.74%), geranyl acetate (3.92%), geraniol (3.05%), and geranial (2.44%) as major components.[42] Regional variations in *C. aurantium* oils are attributed to genotype, climate, and soil conditions. Tunisian oils have high linalool content (22.35−62.57%), while Turkish oils contain high levels of oxygenated compounds (89.6%), with linalyl acetate (50.1%) and linalool (24.8%) predominating.[42] Figure 1 shows the chemical structures of the main components.

Antimicrobial potential of the essential oils

The antibacterial activity of the EOs was evaluated using the disc diffusion assay, and the micro-dilution method. The results of the qualitative antibacterial activities are summarized in Table 3.

The highest inhibition diameters were observed with the EOs of *O. elongatum*, *T. vulgaris*, and *T. serpyllum* against all eight test strains. Other EOs exhibited high inhibition diameters only against the SCN bacterial strain and showed medium to low inhibition diameters against the remaining seven bacterial strains. The MIC and MBC values of the EOs against the test bacteria are shown in Table 4, supporting the results of the disc diffusion assay. The lowest MIC values were for *O. elongatum*, *T. vulgaris*, and *T. serpyllum*. Minimal inhibitory concentration/ MBC values below 4 for these oils indicate bactericidal action. High MICs were observed for *E. globulus* and *C. aurantium*. The EO of *O. elongatum* exhibited significant antibacterial activity against all test bacteria, with inhibition zones ranging from 46.33±0.58 to 63.67±1.53 mm and MICs ranging from 0.0078 to 0.0625% (v/v). The MBC/MIC ratio (= 2) indicated a bactericidal effect against all test bacteria. Previous studies corroborate these findings, with variations in antibacterial activity attributed to compound concentration and synergistic effects. Tagnaout *et al*. reported considerable antibacterial activity of *O. elongatum* against *S. aureus, K. oxytoca, E. faecalis, , E. coli*, and *K. pneumoniae* with inhibition zones ranging from 9±0.00 to 48.05±0.95 mm and MICs of 2 to 32 µL/mL.[24] Moussaoui *et al*. reported that this EO exhibited MIC and MBC values ranging between 0.125 and 0.5% against *E. coli* O157.[43] El Harsal's fractions of EO from 141 to 160 min of hydro-distillation time showed the strongest antibacterial activity, with MIC values between 0.0312 and 0.125% (v/v) and MBC values from 0.0312 to 0.25 % (v/v) against *E. coli* K12 MBLA, *E. coli* ATCC 25922, and *S. aureus* ATCC 25923.[12] The EO's antibacterial activity is primarily attributed to its phenolic compounds, particularly carvacrol and thymol, which disrupt bacterial membranes. Synergistic effects of minor EO components may also enhance overall antibacterial efficacy.[44]

Concerning *T. vulgaris*, the results of the present study showed that the eight test bacterial strains were susceptible to the EO, with inhibition zones ranging from 40.57±1.16 to 49.67±1.16 mm and MICs from 0.0156 to 0.0625 % (v/v). Imelouane *et al*. reported that the EO obtained from thyme exhibits antimicrobial activity against all test microorganisms, with MIC values ranging from 0.33 to 2.67 mg/mL. Maximum activity was observed against the Gram-negative bacterium *E. coli*, while the oil showed poor activity against Gram-positive bacteria, such as *S. aureus* and *S. epidermidis.*[29]

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Table 3: Inhibition zone diameter (mm) of test essential oils

Bacterial strain	EO of the studied plant							
	O. elongatum	***T. vulgaris***	***C. aurantium***	***E. globulus***	***C. citratus***	***T. serpyllum***	***L. Stoechas***	**C *(30 µg)***
SCN	63.67 ± 1.53	47.67 ±1.16	16.33 ±1.0	19.67 ±1.16	27.33 ±1.16	42.00 ±1.73	18.67 ±0.58	24.00 ±0.6
P. aeruginosa	49.33 ± 0.58	44.67 ±1.16	12.00 ±1.73	13.67 ±1.16	11.33 ±1.16	29.33 ±0.58	15.33 ±1.16	18.00 ±0.6
S. aureus	60.33 ± 0.58	47.00 ±1.73	12.00 ±1.73	14.67 ±1.16	17.67 ±1.16	41.33 ±1.15	14.67 ±1.16	22.00 ±0.6
E. faecalis	46.33 ± 0.58	49.67 ±1.16	11.33 ±1.16	12.67 ± 1.16	14.33 ±0.58	44.33 ±0.58	12.00 ±1.73	20.00 ±0.4
***Salmonella* spp.**	62.00 ± 0.00	40.57 ±1.16	11.67 ±1.16	12.00 ±1.73	16.33 ± 0.58	33.33±1.16	10.67 ±1.16	21.00 ±0.6
K. pneumoniae	50.33 ± 0.58	45.33 ±1.16	15.33 ±0.58	11.67 ±1.16	13.33 ±1.16	39.00 ±1.73	12.67 ±1.16	19.00 ±0.6
P. mirabilis	45.67 ± 1.16	43.33 ±0.58	10.33 ±1.16	11.67 ±1.16	14.33 ±1.16	29.67 ±1.16	12.00 ±1.73	18.00 ±0.6
E. coli	46.33 ± 0.58	45.00 ±0.00	18.33 ±1.16	11.33 ±1.16	16.00 ±1.73	39.67 ±0.58	12.33 ±0.58	19.00 ±0.4
***E. coli* ATCC 25922**	47.33 ± 0.58	48.33 ±0.58	18.00±1.73	14.33 ± 0.58	17.33 ±1.16	42.66 ± 1.15	13.33 ±1.16	21.00 ±0.6
***S. aureus* ATCC 29213**	62.33 ± 0.58	49.00 ±0.00	15.33±0.58	17.67± 1.43	20.00 ±1.73	42.33 ± 1.15	15.33 ±0.58	24.00 ±0.4

SCN: *Staphylococcus* coagulase negative; C: Chloramphenicol: EO: Essential oil; Values are means (mm ± SD) of the triplicate determination.

Table 4: Antibacterial activity of selected essential oils against the test bacterial strains

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Bacterial strain	**Minimum inhibitory concentration (MIC) (%)**						
	EO of the studied plant						
	O.elongatum	***T.vulgaris***	***C.aurantium***	***E.globulus***	***C.citratus***	***T.serpyllum***	***L.Stoechas***
SCN	0.0078	0.0312	4.000	2.000	0.0312	0.0625	0.500
P. aeruginosa	0.0156	0.0312	8.000	4.000	1.000	0.250	1.000
S. aureus	0.0078	0.0312	8.000	4.000	0.250	0.0625	1.000
E. faecalis	0.0312	0.0156	8.000	8.000	0.500	0.0312	4.000
***Salmonella* spp.**	0.0078	0.0625	8.000	8.000	1.000	0.500	8.000
K. pneumoniae	0.0156	0.0312	4.000	8.000	0.500	0.0625	4.000
P. mirabilis	0.0625	0.0312	8.000	8.000	0.500	0.250	4.000
E. coli	0.0625	0.0312	2.000	8.000	0.500	0.0625	4.000
***E. coli* ATCC 25922**	0.0312	0.0156	2.000	4.000	0.250	0.0312	2.000
***S. aureus* ATCC 29213**	0.0078	0.0156	4.000	2.000	0.125	0.0625	1.000
	Minimum bactericidal concentration (MBC) (%)						
SCN	0,0156	0.0625	8.000	4.000	0.0625	0.125	2.000
P. aeruginosa	0.0312	0.0625	8.000	8.000	4.000	0.500	4.000
S. aureus	0.0156	0.0625	8.000	8.000	2.000	0,125	4.000
E. faecalis	0.0625	0.0312	8.000	8.000	4.000	0.0625	8.000
***Salmonella* spp.**	0.0156	0.125	8.000	8.000	4.000	1.000	8.000
K. pneumoniae	0.0312	0.0625	8.000	8.000	4.000	0.125	8.000
P. mirabilis	0.125	0.0625	8.000	8.000	4.000	0.500	8.000
E. coli	0.125	0.0625	4.000	8.000	4.000	0.125	8.000
***E. coli* ATCC 25922**	0.0626	0.0312	4.000	4.000	0.500	0.0625	4.000
***S. aureus* ATCC 29213**	0.156	0.0312	8.000	2.000	0.250	0.125	2.000
	MBC/MIC						
SCN	2	2	2	2	2	2	4
P. aeruginosa	2	2	1	2	4	2	4
S. aureus	2	2	1	2	8	2	4
E. faecalis	2	2	1	1	8	2	2
***Salmonella* spp.**	2	2	1	1	4	2	1
K. pneumoniae	2	2	2	1	8	2	2
P. mirabilis	2	2	1	1	8	2	2
E. coli	2	2	2	1	8	2	2
***E. coli* ATCC 25922**	2	2	2	1	2	2	2
***S. aureus* ATCC 29213**	2	2	2	1	2	2	2

SCN: *Staphylococcus* coagulase-negative.

The biological activity of EOs is influenced by their chemical composition, which depends on genotype and is affected by environmental and agronomic conditions.[45] Hattabi *et al.* found that Gram-positive bacteria, including *S. aureus* and *S. faecium* (MIC: 2.5 µL/mL), are more sensitive than Gram-negative bacteria like *E. coli* and *P. aeruginosa* (MIC: 5–10 µL/mL).[32] Sadiki *et al*. observed that the EO of *T. vulgaris* exhibited significant inhibitory activity against *S. aureus, E.coli, B.subtilis,* and *P.aeruginosa,* with inhibition occurring at very low concentrations, from 0.03 to 0.4% (v/v).[31] This antibacterial activity is primarily attributed to its chemical composition, rich in monoterpenes, including terpene alcohols (thymol, linalool, carvacrol), *α*-terpineol, and *o*-cymene. Zantar *et al*. reported that *T. vulgaris* EO showed strong activity against all test bacterial strains, with inhibition diameters ranging from 34 to 44 mm. The highest antimicrobial activity was observed against *S. aureus* (44 mm), while the weakest was against *S. senftenberg* and *E. coli* (34 and 37 mm, respectively).[46] The resistance of Gram-negative bacteria to EOs is partly attributable to the complexity of their cell wall, which includes an outer membrane, in contrast to the simpler cell wall structure of Gram-positive bacteria.[45]

For *T. serpyllum*, the results indicated that the eight test strains are susceptible to the EO, with inhibition zones ranging from 29.33±0.58 to 44.33±0.58 mm and MICs from 0.0312 to 0.5% (v/v). Similar results were reported by Amarti *et al*., who reported the considerable antibacterial activity of *T. serpyllum* against *S. aureus* and *E. coli* with MICs of 1/3000 (v/v) and 1/2000 (v/v).[47] Alaoui Jamali *et al*. reported that *S. aureus, E. coli, P. aeruginosa,* and *K. pneumoniae* are susceptible to the EO, with inhibition zones ranging from 23.5±0.5, 16.5±0.5, 10.0±0.0, and 22.0±0.0 mm, respectively, and MICs of 0.28, 1.14, 145.20, and 1.14 µL/mL, respectively.[48] The results demonstrated that the eight strains tested were susceptible to the essential oil of *L. stoechas*, with inhibition zones ranging from 10.67±1.16 to 18.67±0.58 mm, and minimum inhibitory concentrations (MICs) between 0.5 and 8% (v/v). These results are supported by findings reported in the literature. Bouyahya *et al*. reported that Gram-positive bacteria show more sensitivity toward EOs than Gram-negative bacteria, with *S. aureus, P. mirabilis,* and *P. aeruginosa* showing MIC values of 0.5, 0.5, 1, and >2% (v/v), respectively. *Pseudomonas. aeruginosa* was the most resistant strain to this oil.[15] Cherrat *et al*. showed bactericidal effects of this EO against *E. coli* O157.[34] In the study of Ben-Ali *et al*., they reported that *S. aureus, P. aeruginosa, P. mirabilis,* and *E. coli* were susceptible to *L. stoechas* EO harvested from three Moroccan sites, with significant antibacterial activity against *S. aureus, P. aeruginosa, P. mirabilis*, and *E. coli,* with inhibition zones of 6±0.00 to 7.66±0.57, 13.33±1.15 to 19±1.00, 18.66±1.15 to 22.66±0.57, and 6±0.00 to 10.66±0.57 mm, respectively.[16]. Radi *et al*. reported that the EO exhibited significant antibacterial activity against the test bacteria, inhibiting the growth of *E. coli* and *S. aureus* at concentrations of 1.56 and 12.5 µg/mL, respectively. Growth of *K. pneumoniae* and *S. epidermidis* was inhibited at 25 µg/mL.[13]

For *E. globulus*, the eight test strains were susceptible to the EO, with inhibition zones ranging from 11.33±1.16 to 19.67±1.16 mm and MIC values from 2 to 8% (v/v). Elguerrouj *et al*. demonstrated that the EO extracted from plants grown at an elevation of 950 m had inhibition zone diameters of 14.5, 11, 8.75, and 9.5 mm against *Staphylococcus aureus*, *Micrococcus luteus*, *Escherichia coli*, and *Pseudomonas aeruginosa*, respectively, with an MIC of 8% against *S. aureus*, indicating the most potent antibacterial activity compared to other EOs. The MICs were 2% for *E. coli* and 16% for *P. aeruginosa.*[14] Čmiková *et al*. showed stronger antimicrobial activity and larger inhibition zones against *Bacillus subtilis* (6.67±0.58 mm) than against *S. aureus* (5.67±0.58 mm). This may be due to the different chemical composition of *E. globulus* EO.[36]

All test bacteria were susceptible to the EO of *C. citratus*, with inhibition zones ranging from 11.33±1.16 to 27.33±1.16 mm, and MICs between 0.0312 and 1% (v/v). The results are relatively consistent with a previous study by Bassole *et al*., who reported that the EO of *C. citratus* showed the highest activity against *E. faecalis* (34±1.3 mm), *S. aureus* (24.3±0.4 mm), *E. coli* (15.3±1.1 mm), *S. enterica* (24±0.7 mm), *S. typhimurium* (31.7±0.4 mm), and *S. dysenteriae* (26±0.7 mm). *Pseudomonas aeruginosa* was the most resistant to the EO of *C. citratus.*[38] Concerning *C. aurantium*, the test eight strains were susceptible to the EO, with inhibition zones ranging from 10.33±1.16 to 18.33±1.16 mm and MICs from 2 to 8% (v/v). Gniewosz *et al*. reported that the MIC values of petitgrain oil for *S. aureus*, *E. coli*, and *S. enteritidis* bacteria were in the range of 1.25, 2.5, and 5 mg/mL, respectively, while the MBC values were 5 mg/mL. The Gram-negative bacterial strains, including *S. enteritidis*, were less susceptible to the effect of petitgrain oil. The MIC and MBC values ranged between 2.5 and 5.0 mg/mL.[42] These findings demonstrated that the seven test EOs exhibited significant antibacterial activity against food-contact surface pathogens, due to their antimicrobial properties. Notably, *O. elongatum* and *T. vulgaris* showed particularly strong antibacterial effects.

Correlation between the bioactive compounds in essential oils and their antibacterial activity

A correlation between major compounds and the antibacterial activity of EOs was detected using PCA and identified similar samples based on these activities and the major EO compounds. Principal component analysis also allowed for the explicit presentation of individuals and variables, highlighting differences in profiles between samples based on EOs. The seven essential oil samples represent the following species: *E. globulus*, *O. elongatum*, *L. stoechas*, *T. serpyllum*, *T. vulgaris*, *C. citratus*, and *C. aurantium.* The variables include the average of the main chemical composition components and antibacterial activity (MIC and MBC). The Kaiser criterion was applied to determine the number of components to retain, recommending the retention of components with eigenvalues greater than 1 in a normalized principal component analysis (PCA). The eigenvalues of the first four major components were greater than one and explained most of the variance, as shown in Table 5. The sum of these four components accounted for 85.79% of the variance in the data. The first component, consisting of carvacrol, thymol, *γ*-terpinene, *o*-cymene, and antibacterial activity, accounted for 42.63% of the variance. The second component, including camphor, fenchone, *α*-pinene, and camphene, explained 16.73% of the variance. The third component, represented by borneol, accounted for 15.66%, while the fourth component, comprising *α*-terpineol, *β*-citral, linalool, and linalyl acetate, explained 10.72% of the variance.

The loading plot (Figure 2), and correlation matrix (Table 6) demonstrated correlations between major compounds and antibacterial activity. A positive correlation was observed between antibacterial activity and the major compounds thymol, and *o*-cymene for the three strains *E. faecalis*, *E. coli,* and *P. mirabilis*. This suggests that these compounds have a pronounced impact on these strains and highlights their critical role in determining the antibacterial efficacy of the EOs. These findings are consistent with recent studies, such as that by Driouache *et al*., which emphasized the antibacterial potency of thymol and p-cymene in *O. elongatum* EO against resistant bacteria, such as *E. coli* and *E. faecalis*.[49]

Additionally, Sadiki *et al*. reported that *T. vulgaris* EO exhibited strong antibacterial activity against various strains of *E. coli*, including AL52, O128B12, CIP5412, and HB101, primarily due to its high content of thymol and p-cymene.[31] Another positive correlation was found between the major compounds carvacrol and *γ*-terpinene and the strains *S. coagulase-negative, P. aeruginosa, S. aureus, Salmonella* spp., and *K. pneumoniae*. This indicated that these five strains are particularly affected by carvacrol and *γ*-terpinene compared to other major compounds. However, *α*-pinene, camphene, fenchone, and camphor were negatively correlated with antibacterial activity and showed minimal influence on the test bacteria. These findings align with recent studies, such as those by Tagnaout *et al*., which highlighted the antibacterial effectiveness of carvacrol and *γ*-terpinene in *O. elongatum* EO against resistant bacteria, such as *K .pneumoniae,* resistant *S. aureus,* and *K. oxytoca*.[24] Figure 3 shows a heat map of correlations, confirming the correlations previously identified. A statistically significant positive correlation (approximately 1) exists between the predominant compounds carvacrol and *γ*-terpinene and the following five strains: *S. coagulase*-negative, *P. aeruginosa*, *S. aureus*, *Salmonella* spp., and *K. pneumoniae*. Similarly, a statistically significant positive correlation (about 1) exists between the major compounds' thymol and *o*-cymene and the antibacterial activity of *E. faecalis*, *E. coli,* and *P. mirabilis*.

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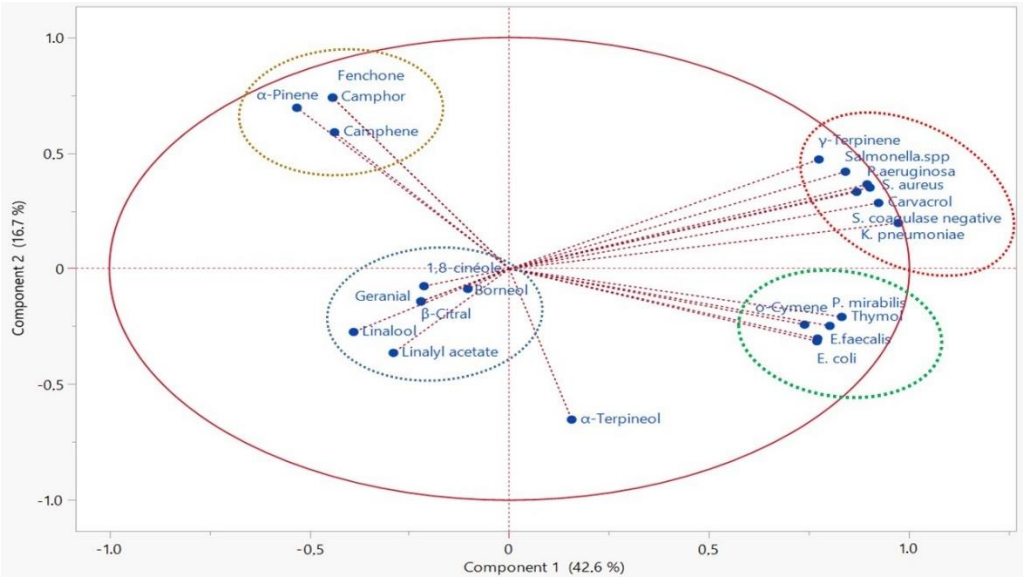

Figure 2: Variable loading plots or projections on the first (PC1) and second (PC2) principal components

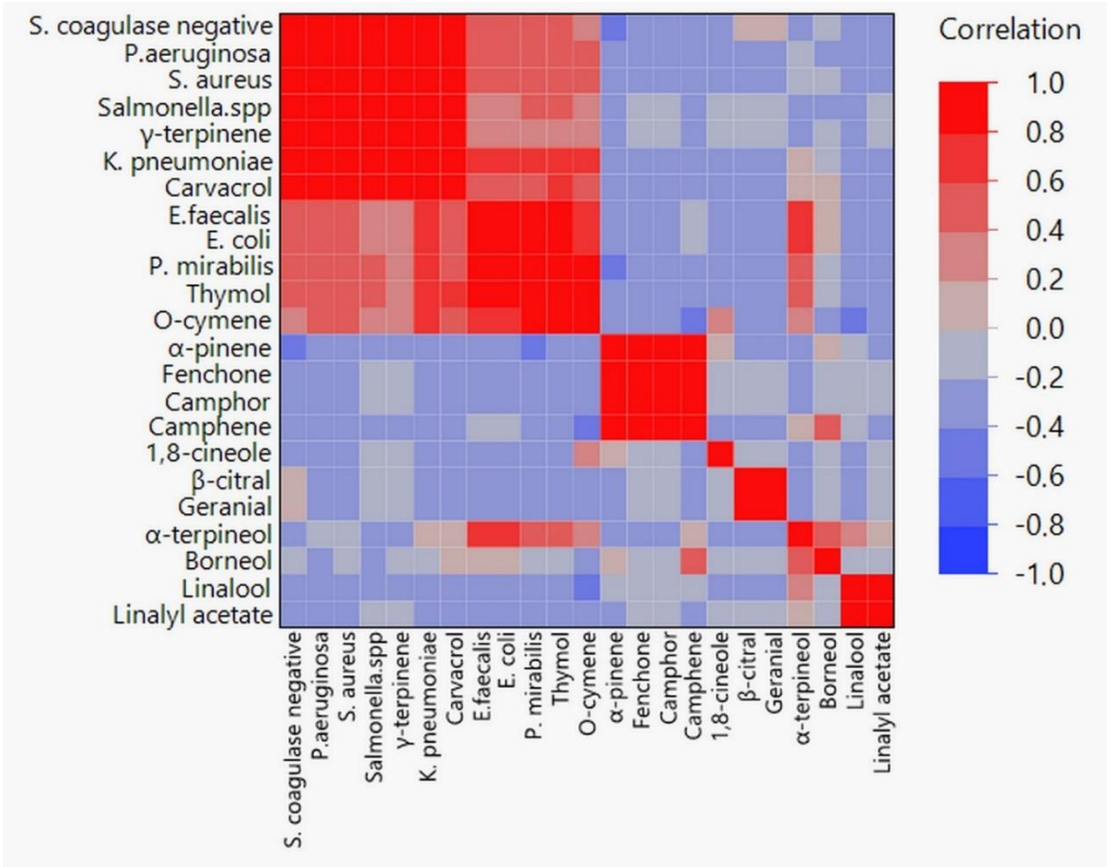

Figure 3: Heat map of correlations between antibacterial activity and the major compounds

The score plot (Figure 4a) showed that the samples were divided into four principal groups. The individual *T. vulgaris* represents the first group, the second group is composed of *O. elongatum,* the third group is composed of the individuals *C. aurantium, E. globulus, C. citratus*, and *T. serpyllum* and the individual *L. stoechas* represents the fourth group. The biplot (Figure 4b) synthesizes information from the previously presented variable and individual graphs, confirming consistent groupings of EOs and correlations between variables, including major compounds and antibacterial activity. Additionally, the graph revealed the following insights: The first cluster, characterized by the major compound's thymol and *o*-cymene, exhibits a positive correlation with the antibacterial activity against *E. faecalis*, *E. coli*, and *P. mirabilis*. This cluster prominently features *T. vulgaris* EO, known for its high thymol and *o*-cymene content and strong activity against these strains. The second cluster, composed of major compounds carvacrol and *γ*-terpinene, is associated with the strains *S. coagulase*-negative, *P. aeruginosa*, *S. aureus*, *Salmonella* spp., and *K. pneumoniae*. This cluster is characterized by *O. elongatum* EO, rich in carvacrol and *γ*-terpinene, and exhibits significant activity against these

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bacterial strains. The third cluster comprises major compounds 1,8-cineole, *β*-citral, geranial, borneol, linalool, and linalyl acetate. It includes oils from *C. aurantium*, *E. globulus*, *C. citratus*, and *T. serpyllum*, showing moderate activity against the test strains. The fourth cluster features major compounds *α*-pinene, camphene, fenchone, and camphor, associated with *L. stoechas* EO. Despite the abundance of these compounds in *L. stoechas* EO, their distance from the MICs of the test strains indicated a low effect of this oil against these bacteria.

To validate the PCA results, HCA was conducted to further classify the seven EOs. Figure 5 shows the classification of the EOs into four major clusters or groups: Cluster 1 includes *T. vulgaris*, while Cluster 2 contains *O. elongatum*; both exhibited high antibacterial activity. Cluster 3 includes *T. serpyllum*, *C. citratus*, *E. globulus*, and *C. aurantium*, which exhibited medium to low antibacterial activity.

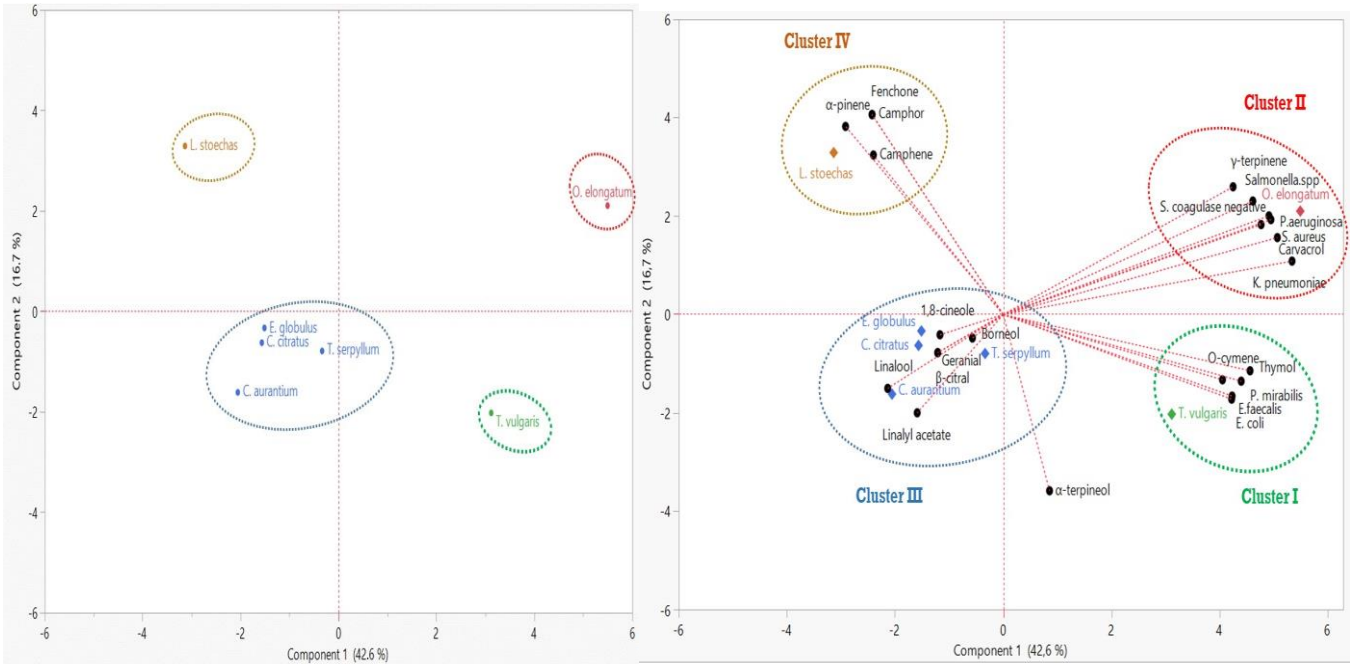

Figure 4 : **(a)** Plot of individual scores on the first (PC1) and second (PC2) principal components **(b)** Biplot of variables and individuals onto the first (PC1) second (PC2) principal components

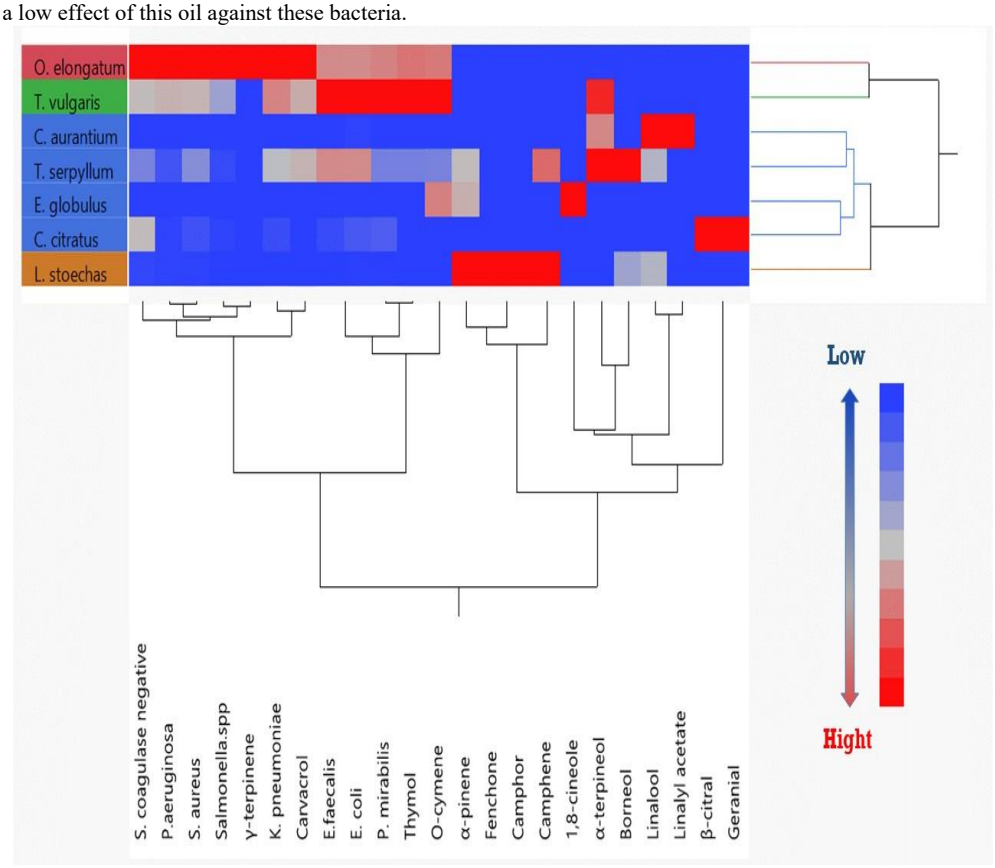

Figure 5: Dendrogram obtained from cluster analysis of the seven essential oils based on the main compounds and antibacterial activity.

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Table 5: The eigenvalues, loading matrix, proportion of variability clarified, and cumulative percentages of the tree's first principal components

	Component			
Bacterial strain	**CP1**	**CP2**	**CP3**	**CP4**
K. pneumoniae	0.972246	0.197743	0.007137	0.08654
Carvacrol	0.922818	0.284659	-0.051702	0.162988
S. aureus	0.901199	0.350972	-0.17719	0.158465
P .aeruginosa	0.89446	0.364908	-0.200704	0.162751
S. coagulase negative	0.868146	0.332614	-0.324946	0.032983
Salmonella spp	0.840192	0.419758	-0.27346	0.200808
Thymol	0.83089	-0.207829	0.356223	-0.160411
P. mirabilis	0.801086	-0.246494	0.35171	-0.211507
γ-Terpinene	0.773705	0.472556	-0.332939	0.23808
E. faecalis	0.770394	-0.301518	0.518614	-0.166186
E. coli	0.768899	-0.312903	0.505808	-0.17578
o-Cymene	0.738058	-0.241185	0.270662	-0.259111
Camphor	-0.441659	0.739665	0.392886	-0.051593
Fenchone	-0.441659	0.739665	0.392886	-0.051593
α-Pinene	-0.531047	0.695782	0.457992	-0.09897
α-Terpineol	0.155827	-0.651632	0.644097	0.195672
β-Citral	-0.220689	-0.140961	-0.625637	-0.554872
Geranial	-0.220689	-0.140961	-0.625637	-0.554872
Camphene	-0.437176	0.590071	0.606797	-0.012962
Linalool	-0.389153	-0.272975	-0.050226	0.843246
Linalyl acetate	-0.289768	-0.363364	-0.194634	0.813047
1,8-cineole	-0.213187	-0.074875	-0.176607	-0.202675
Borneol	-0.103828	-0.086715	0.49201	0.057763
Eigenvalues	9.80	3.85	3.61	2.47
Percentages of explained variability (%)	42.63	16.73	15.70	10.72
Cumulated percentages (%)	42.43	59.36	75.07	85.79

PC: principal component; CP1: First principal component; CP2: Second principal component; CP3: Third principal component.

Cluster 4, featuring *L. stoechas*, was characterized by low antibacterial activity. The results of the present study underscore the significant antibacterial potential of EO from Moroccan plants, particularly those rich in thymol, *o*-cymene, carvacrol, and *γ*-terpinene. These findings are consistent with recent research emphasizing the effectiveness of these compounds in combating bacterial pathogens. For instance, Driouche *et al*. conducted a study using PCA and HCA to examine correlations between the major components of endemic thymes, particularly *T. vulgaris* and *T. serpyllum* (zygis). Their study found that the phenols carvacrol/thymol exhibited the most significant positive effects against multi-resistant bacteria, such as *S. aureus* multi-resistant, *P. aeruginosa*, followed by the terpenes p-cymene and *γ*-terpinene. Both PCA and HCA indicated that the primary compounds had a significant effect on antibacterial activity.[45]

In the present study, origano and thym EOs exhibited remarkable antibacterial activity against the eight bacterial strains isolated from food-contact surfaces. This antibacterial activity of EOs would be attributed to the major compounds present, as well as the synergistic effects between these components and the additive effects of minor compounds, which can reinforce antibacterial action.[50]. Additionally, the study's findings underscore the significant antibacterial potential of EOs from Moroccan plants, particularly those rich in thymol, carvacrol, *o*-cymene, and *γ*-terpinene. The consistency between PCA and HCA validates the robustness of the present study's findings. Both analyses revealed that the major compounds in EOs significantly affect their antibacterial activity, confirming that the chemical profile of an EO is a critical determinant of its efficacy against test bacteria. These insights are pivotal for developing natural alternatives to synthetic chemicals, which can harm the environment and human health, and offer potential for disinfection applications. The differential antibacterial activity observed in EOs from wild and cultivated plants further highlights the importance of considering environmental factors in developing effective natural antimicrobial products.

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Table 6: Correlation matrix between antibacterial activity and the major compounds.

Bacterial strain	S .coagulase negative	*P. aeruginosa*	*S. aureus*	*E. faecalis*	*Salmonella.* spp	*K. pneumoniae*	*P. mirabilis*	*E. coli*	*α*-pinene	Camphene	*o*-Cymene	*γ*-Terpinene	1,8-cineole	Thymol	Carvacrol	*β*-citral	Geranial	*α*-Terpineol	Borneol	Linalool	Linalyl acetate	Fenchone	Camphor
S. coagulase negative	1																						
P. aeruginosa	0.97	1																					
S. aureus	0.97	0.99	1																				
E. faecalis	0.41	0.45	0.47	1																			
Salmonella. spp	0.97	0.99	0.99	0.34	1																		
K. pneumoniae	0.92	0.95	0.96	0.69	0.92	1																	
P. mirabilis	0.47	0.52	0.51	0.93	0.41	0.69	1																
E. coli	0.41	0.44	0.47	1.00	0.34	0.68	0.93	1															
α-pinene	-0.40	-0.33	-0.34	-0.38	-0.30	-0.39	-0.40	-0.39	1														
Camphene	-0.33	-0.29	-0.27	-0.17	-0.28	-0.28	-0.33	-0.18	0.87	1													
o-Cymene	0.38	0.47	0.46	0.77	0.38	0.60	0.85	0.76	-0.35	-0.45	1												
γ-Terpinene	0.95	0.97	0.97	0.24	0.99	0.87	0.29	0.23	-0.26	-0.25	0.29	1											
1,8-cineole	-0.29	-0.22	-0.24	-0.33	-0.19	-0.27	-0.28	-0.34	0.09	-0.25	0.26	-0.17	1										
Thymol	0.51	0.57	0.56	0.93	0.46	0.74	0.98	0.93	-0.39	-0.32	0.86	0.35	-0.27	1									
Carvacrol	0.94	0.97	0.98	0.57	0.95	0.98	0.55	0.57	-0.35	-0.22	0,49	0.92	-0,27	0.60	1								
β-citral	0.09	-0.21	-0.21	-0.29	-0.17	-0.26	-0.21	-0.27	-0.26	-0.25	-0.37	-0.17	-0.17	-0.27	-0.27	1							
Geranial	0.02	-0.21	-0.21	-0.29	-0.19	-0.26	-0.21	-0.27	-0.26	-0.25	-0.37	-0.17	-0.17	-0.27	-0.27	1.00	1						
α-Terpineol	-0.23	-0.19	-0.15	0.64	-0.27	0.07	0.42	0.64	-0.29	0.03	0.24	-0.34	-0.34	0.41	0.01	-0.34	-0.34	1					
Borneol	-0.17	-0.21	-0.12	0.19	-0.20	-0.04	-0.17	0.19	0.14	0.51	-0.23	-0.19	-0.19	-0.17	0.03	-0.19	-0.19	0.59	1				
Linalool	-0.37	-0.30	-0.31	-0.36	-0.27	-0.36	-0.36	-0.36	-0.09	-0.03	-0.49	-0.23	-0.23	-0.35	-0.32	-0.23	-0.23	0.25	-0.02	1			
Linalyl acetate	-0.29	-0.22	-0.24	-0.33	-0.19	-0.29	-0.28	-0.33	-0.26	-0.25	-0.37	-0.17	-0.17	-0.27	-0.27	-0.17	-0.17	0.19	-0.19	0.97	1		
Fenchone	-0.27	-0.21	-0.24	-0.33	-0.19	-0.29	-0.27	-0.33	0.94	0.84	-0.37	-0.17	-0.17	-0.27	-0.27	-0.17	-0.17	-0.34	-0.04	-0.02	-0.17	1	
Camphor	-0.27	-0.21	-0.24	-0.33	-0.19	-0.29	-0.27	-0.33	0.94	0.84	-0.37	-0.17	-0.17	-0.27	-0.27	-0.17	-0.17	-0.34	-0.04	-0.02	-0.17	1.00	1

Conclusion

This study demonstrated that the test EOs exhibited significant antibacterial activity against pathogens on food contact surfaces, attributable to their antimicrobial properties. The multivariate analysis (PCA and HCA) indicated that test EOs, rich in chemical compounds, such as carvacrol, thymol, *γ*-terpinene, and *o*-cymene, displayed effective antibacterial activity. Notably, *O. elongatum* and *T. vulgaris* showed exceptional antibacterial activity, suggesting their potential as natural disinfectants for these surfaces. Further research on plant extract synergy, safe disinfection formulations, and the effects of geography and climate on EO chemical profiles and antimicrobial activity is crucial for optimizing their use as disinfectants.

Conflict of Interests

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that they will assume any liability for claims related to the content of this article.

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