ORIGINAL RESEARCH

Chemical composition of Cinnamomum zeylanicum Blume essential oil traded in Tunisia and its effect against skin infection bacterial strains

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ABSTRACT
Background: Chronic wound infections, exacerbated by microbial pathogens such as bacteria and fungi, pose a significant contemporary healthcare challenge. These infections are further complicated by the escalating problem of antimicrobial resistance. Natural compounds, such as essential oils (EOs), comprised of a complex mixture of components, emerge as a potential solution. However, the landscape is clouded by concerns about the efficacy and safety of non-controlled commercial essential oils. **Methods:** In this study, the commercialized Cinnamomum zeylanicum EO in Tunisia were nalysed using GC/FID and GC/MS and tested aginst 13 bacterial strains responsible for skin infectionsusing the disc diffusion and microdilution broth methods. **Results**: Eighty components, representing 96.54% of the total oil, were identified. The predominant component was the aldehyde (E)-cinnamic aldehyde (66.23% ± 2.010%), followed by the sesquiterpene hydrocarbons β-caryophyllene (6.43 ± 0.73%), the monoterpenic alcohol linalool (3.58 ± 0.26%), and the monoterpenic ester cinnamyl acetate (3.14 ± 0.18%).The antibacterial activity of the EO varied significantly among microbial strains and methods used. The highest antibacterial activity was observed against the Gram-positive bacterium Staphylococcus aureus ATCC 2912, followed by the Gram-negative Proteus mirabilis P195/20, with an inhibition zone diameter higher than that produced by the antibiotic Gentamicin. **Conclusions:** Our findings suggest that C. zeylanicum EO could be considered as an alternative treatment for skin infections, given its promising antibacterial and activities.
Key Words: Cinnamomum zeylanicum, essential oil, gaz chromatoraphic analysis (GC), antibacterial activity.

INTRODUCTION
Cinnamomum zeylanicum Blume, a perennial tree species synonymous with Cinnamomum verum from the Lauraceae family, has been a part of international trade for culinary purposes for centuries.(1)Tunisia, for instance, has a rich tradition of using it to flavor various dishes, including the cake(Makroudh) and meat couscous. The bark of this tree is a valuable source of essential oils, primarily composed of cinnamic aldehyde(50,5%-71-50%) followed by α-copaene, benzaldehyde, β-Caryophyllene, (E)-cinnamyl acetate, δ-cadinene, 1,8-cineole, and eugenol.[2–5]However, the chemical composition varies according the environmental conditions, plant growth stage, specific part used, and processing methods.[6,7]These essential oils are globally commercialized for various applications, including confectionery, perfumery, food industry, pharmaceuticals, and therapeutic use. They are renowned for their effectiveness in treating

gastrointestinal disorders,[8,9]and have anti-infectious, anti-inflammatory[10], hypocholesterolemic, antidiabetic , warming, aphrodisiac[6,11,12], skin care and wound healing properties.[10,13]Numerous studies have also reported their immunomodulatory, antioxidant, antiviral, antibacterial, and antifungal activities and the stimulation of beneficial bacteria digestive tracts growth.[2,5,11,14,15]The skin is a barrier that limits invasion and growth of pathogenic bacteria.[16]Skin and soft-tissue infections are among the most common infections which may lead to serious local and systemic complications. Staphylococcus aureus and Sterptococcus pyogenes (groupe A Streptococcus) are the most prevalent bacterial strains responsible of skin diseases and superficial wound infections. For exeemple i)S. aureus it causes boils or abscesses as well as more serious postoperative wound infections,ii) S. pyogenes. causes infections in the superficial keratin layer (impetigo), the superficial epidermis (erysipelas), the subcutaneous tissue (cellulitis), the fascia (necrotizing fasciitis), or muscle (myositis and myonecrosis).(17,18)Pseudomonas aeruginosa, a Gram-negative bacteria wich was the most abundant bacteria in burn injury and chronic wounds.[19,20]Several authors reported that E. coli was found to be the causative agent of neonatal omphalitis,[21] cellulitis localized to lower or upper limbs[22,23], necrotizing fasciitis,[24–26]surgical site infections,[27]infections after burn injuries.[28]This bacteria was the third-most prevalent isolated species, preceded solely by S. aureus and Pseudomonas aeruginosa.[29]Klebtiella pneumoniae, a facultative Gram-negative bacteria was reported responsibe for the complication of skin and soft-tissue infections of extremities.[30]It is known to be a major colonizer of burn wound along with Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli.[31]Enterococcus faecalis is one of the most frequently isolated bacterial species in wounds, it modulates immune activation and slows healing during wound infection.[32]Proteus mirabilis, a Gram-negative bacillus of the axillary skin abscesses.[33]It is one of the most serious diabetic foot ulcers infectious agents.[34]S. epidermidis is a commensal Gram-positive bacteria, belonging of the skin and mucous membranes flora. However, if the host defenses are impaired, it is able to cause small abscess around stitches,[17,35]Bacillus subtilis is a Gram-positive bacterium present on skin as non pathogenic organism.[36]It plays an important role in preventing infection by promoting microbiota balance.[37]Additionally, it may exhibit a beneficial wound-healing effect through its antagonistic impact against pathogenic[38]Nevertheless, Bacillus spp. infections in soft tissue and bones have been linked to injuries and wounds. Additionally, it has been implicated in crepitant cellulitis.[39]

Chronic wound infections due to resistance of both bacterial and fungal strains can lead to prolonged patient debilitation and soaring healthcare expenses.[40–42]Moreover, despite the popularity of cinnamon essential oils in skincare products, there remains a significant gap in research concerning their impact on human skin infections. Therefore, the aim of our work was to identify the essential oil chemotype of Cinnamoumum zaylanicum traded in Tunisia and to explore its effective as natural alternative for treating skin antibacterial infections..

MATERIAL AND METHODS

GC Analysis

The EO extracts were analysed subsequently by GC and GC/MS in triplicates. GC analysis was carried out with a Hewlett-Packard 6890 apparatus equipped with FID and apolar HP5 cap. column. The remaining experiment parameters are as follow: the oven temperature (temp.) was programmed at 60°C for 1min, rising gradually from 60 °C to 250 °C at 3 °C/min, and then held isothermal at 250° for 3min; injector temp. at 250 °C; detector temp. at 280 °C, carrier gas, N_2 (1.2 mL/min). For each sample, 1□L (10% EO, in purified hexane) was injected for analysis. The relative concentration was calculated using software HP chemstation, which allows assimilating the percentages of the peak areas to the percentages of the various constituents. Retention indices (RI) were determined relatively to the retention time (t_R) of a series of n alkanes (C_9-C_{28}).

GC/MS Analysis

The EOs were analysed with a Hewlett-Packard 5890 series II apparatus equipped with a 5972 mass selective detector and an apolar HP5 column (30 m x 0.32 mm i.d., film thickness of 0.25 μm). Helium was used as a carrier gas. The mass spectrometer operating conditions were: ionisation voltage, 70eV; ion source, 230°. The GC analysis was carried out as described above (see GC Analysis).

Compound Identification

The identification of the compounds was based on the comparison of their RI (determined relatively to the t_R of n-alkanes (C_9-C_{28})) and their mass spectra with those of authentic compounds by means of NBS75K.L. and Wiley 275 databases, as well as with literature data.[43]

ANTIBACTERIAL TESTING

Bacterial strains

In this study, seven clinical bacterial isolates E. coli (A 11626), E. coli (C 2622), K. pneumoniae (A1237/2017915), K. pneumonae B2101/2018364, P. mirabilis (P195/20), P. mirabilis (C2524) and Streptococcus pyogenes (group A streptococci)were used, as well as six ATCC bacteria: P. aeruginosa (ATCC 27853), E. coli (ATCC 25922), S. aureus (ATCC 2912), Staphylococcus epidermidis (CIP 106510),Bacilus subtilis ATCC 6633Enterococcus faecalis**(**ATCC 29212) The Microbiology Laboratory

(EPS Fattoma Borguiba, Monastir, Tunisia) generously contributed all the strains exception for Staphylococcus epidermidis(CIP 106510)is obtained from the culture collection of the Laboratory of Analysis, Treatment and Valorization of Environmental Pollutants and Products, Faculty of Pharmacy, Monastir, Tunisia.

Disc diffusion method

Using bacterial inoculums of 0.5 McFarland and Mueller Hinton (MH) enriched with 5% sheep blood, the antibacterial activity of several EOs was assessed using a paper-disc agar diffusion method. The MH medium for P. aeruginosa, E. coli, and S. aureus, on the other hand, was not enriched. Briefly, 10 µL of each EO was impregnated into absorbent discs (Whatman disc N°3, 6 mm diameter) and then deposited on the surface of infected plates (90 mm). Gentamicine® (10 g/disc) positive control discs were included in each plate. The inhibition zone diameter (izd) was measured and represented in mm after 24h of incubation at 37 °C.

The results were interpreted as follows: i) not sensitive or no inhibitory effect (-) for izd less than 8 mm; ii) sensitive (+) or mild inhibitory effect for izd between 8 and 14 mm; iii) very sensitive or moderate inhibitory effect (++) for izd between 14 and 20 mm; iv) extremely sensitive or strong inhibitory effect (+++) for izd greater than 20 mm[44,45]. All of the tests were carried out in triplicate, and the results were expressed as mean±standard errors of mean.

Determination of MIC and MBC

The minimum inhibitory concentration (MIC) was determined using the micro-well dilution method according to the National Committee for Clinical Laboratory Standards.(46)An overnight incubated culture (37 °C) of each tested bacterial strain was prepared by adjusting the turbidity of each bacterial culture to reach an optical density of 0.5 McFarland standards. One hundred microliters from each EO diluted in DMSO (50%), initially prepared at a concentration of 0.5ml/mL, were added into the third well, followed by two-fold serial dilutions in MH broth medium until the 12th well. Subsequently, 80 µL of MH, 10 µL of the inoculum, and 10 µL of 0.02% resazurin solution were added into each well. The skipped first and the second wells were reserved for negative and positive controls, respectively. Negative control well contained bacteria in the MH broth medium whereas, positive control well contained bacteria in MH broth medium and 10 µg/ mL of Gentamicin® antibiotics.

After incubation for 24h at 37°C, the bacterial growth was characterized by color change from blue to pink. The MIC was defined as the lowest concentration that completely inhibits visible cell growth after incubation at 37°C (blue colored well) for 24h. To determine the minimum bactericidal concentration (MBC), 10 µL of each culture medium with no visible growth were removed and inoculated in MH plates. After incubation for 18-24h at 37 °C, the number of surviving organisms was determined. MBC was defined as the lowest concentration at which 99.9% of the bacteria culture were killed.[47]Based on the MBC/MIC ratio, the antibacterial activity was deemed bactericidal when the MBC/MIC ratio was ≤4; it was considered bacteriostatic when the MBC/MIC ratio exceeded 4.[48]As for all analyses, the experiments were performed in triplicate.

STATISTICAL ANALYSIS

We carried out the analysis of variance (ANOVA test) of the izd values obtained during the antimicrobial analysis. The significance of the difference between means was determined at $p < 0.05$ using Duncan's multiple range test. using IBM SPSS Statistics for Windows, Version 23.0 (Armonk, NY: IBM Corp).

RESULTS AND DISCUSSION

Chemical composition

The essential oils (EOs) were chromatographically analyzed using GC (RI) and GC (MS) (Figure1), resulting in the identification of 80 compounds (Table 1), accounting for 96.5% of the total oil content. These compounds were further divided into 11 classes (Table 1). The major class was constituted by the aldehydes (66.9%), with (E)-cinnamic aldehyde as the major constituent (C_1;66.23% ± 2.010%) (Figure 2). The sesquiterpene hydrocarbons were the second major class (9.35%) with β-caryophyllène (C_2;6.43%) as the major component, followed by α-humulene (C_3;1.26%), β-elemene (0.78%), α-calacorene (0.77%), α-copaene (0.74%). The monoterpenes hydrocarbons occupied the third position with a mean percentage of 5.8%. They were dominated by β-phellandrene (C_4;1.92% ± 0.75%) (Table2), p-cymene (C_5;1.43% ± 0.47%), α-thujene (0.96%), α-pinène (0.65% ± 0.15%), α-terpinene (0.59%), and □-3-carene (0.53%). The monoterpenic alcohols were the fourth major class (4.45%) with linalool as the major component (C_6;3.58%), followed by geraniol (0.79%), and cis-piperitol (0.72%). The monoterpenic esters occupied the fifth position (3.38%) with cinnamyl acetate as the major component (C_7;3.14%). The phenols with a mean percentage of 2.57% occupied the sixth position. They were dominated essentially by eugenol (C_8;2.45%). The other classes having a mean percentage inferior to 2.08% were not discussed.

Figure 1:Spectrogram of Cinnamomumzeylanicum Bark essential oil

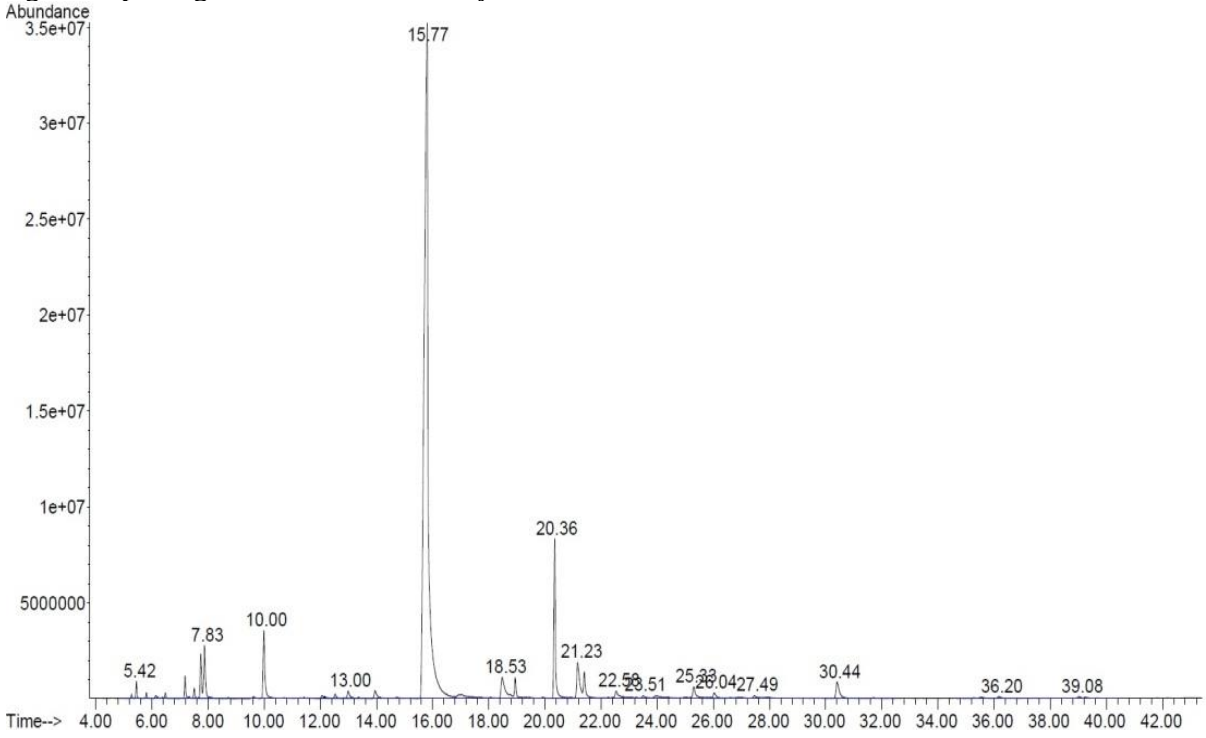

Figure 2: Chemical structure of some major components of Cinnamoumum zeylanicum oil Bark

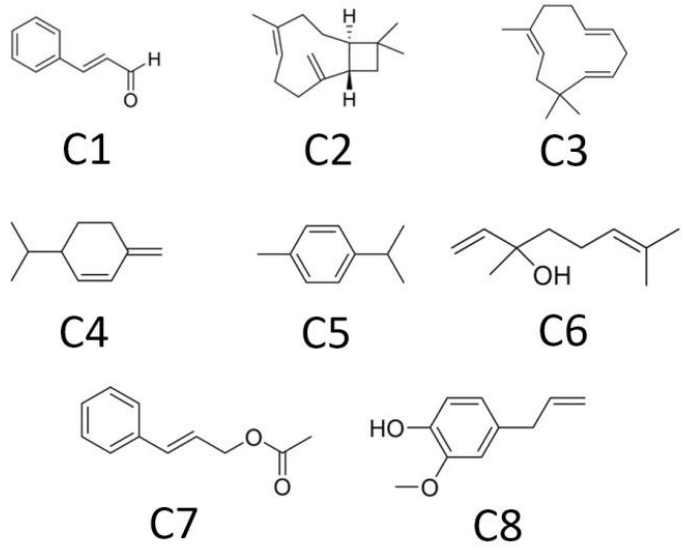

Table 1: Chemical composition of Cinnamum zyelanicum EO

RI_1*)	$RI_2$2**)	Classes and Compounds	Formula	Content (%)
		Monoterpenehydrocarbons		**5.77**
924	930	*α*-Thujene	C1H16	0.10
932	939	*α*-Pinene	C1H16	0.65
945	954	Camphéne	C1H16	0.18
970	975	Sabinene	C1H16	0.03
973	979	*β*-Pinene	C1H16	0.19
988	991	Myrcene	C1H16	0.01
1002	1003	*α*-Phellandrene	C1H16	0.50
1012	1012	*δ-3*-Carene	C1H16	0.06
1014	1017	*α-Terpinene*	C1H16	0.24
1021	1025	*p*-Cymene	C1H16	1.43

1024	1029	Limonene	C1H16	0.29
1028	1026	*β*–Phellandrene	C1H16	1.92
1034	1037	(*Z*)-*β*-Ocimene	C1H16	0.03
1043	1046	*cis*-*β*-Ocimene	C1H16	0.02
1053	1060	*γ*-Terpinene	C1H16	0.03
1083	1089	*α*-Terpinolene	C1H16	0.10
		Monoterpene esters		**3.37**
1065	1070	*cis*-Sabinene hydrate	C1H18O	0.01
1104	1108	Isoamylisovalerate	C1H2O2	0.07
1285	1289	Lavandulylacetate	C12H2O2	0.03
1293	1297	*trans*-Pinocarvylacetate	C12H2O2	0.11
1357	1365	Nerylacetate	C12H2O2	0.00
1378	1386	Gernaylacetate	C12H2O2	0.00
1439	1443	Cinnamylacetate	C11H12O2	3.14
		Monterpeneketons		**0.17**
1137		*L*-Camphor	C1H16O	0.06
1159	1160	*L*-Menthone	C1H18O	0.04
1165	1171	Umbellulone	C1H14O	0.01
1198	1195	*Z*-Dihydrocarvone	C1H16O	0.05
		Monoterpenealcohols		**4.45**
1095	1097	Linalool	C1H18O	3.58
1118	1122	*cis*-*p*-Menth2-en-1-ol	C1H18O	0.04
1136	1123	*trans*-p-Menth-2-en-1-ol		0.03
1159	1169	*endo*-bornéol	C1H18O	0.19
1169	1177	Terpinene-4-ol	C1H18O	0.27
1194	1193	*cis*-Piperitol	C1H18O	0.07
1204	1205	*trans*-Piperitol	C1H18O	0.06
1225	1229	*trans*-(+)-Carveol	C1H16O	0.11
1236	1238	Nerol	C1H18O	0.02
1253	1257	Geraniol	C1H18O	0.08
		Monterpenealdehydes		**0.10**
1237	1237	Neral	C1H16O	0.10
		Mehylphenol		0.01
1395	1401	Methyleugenol	C11H14O2	0.01
		Aldehydes		**66.94**
1213	1266	*cis*-Cinnamicaldehyde	C9H8O	0.29
1277	1277	*trans*-Cinnamicaldehyde	C9H8O	66.23
1520	1512	Ortho MethoxyCinnamicaldehyde	C1 H1 O2	0.42
		Phenols		2.57
1290	1289	Thymol	C1H14O	0.08
1297	1317	Carvacrol	C1H14O	0.00
1353		Eugenol	C1H12O2	2.38
1362	1364	Eugenol	C1H12O2	0.11
		Sesquiterpenehydrocarbons		9.35
1347	1350	*α*-Cubebene	C15H24	0.05
1367	1372	*α*- Copaene	C15H24	0.74
1381	1389	*β*- Elemene	C15H24	0.08
1403	1404	*α*-Gurjunene	C15H24	0.02
1408	1415	*β*-Caryophyllene	C15H24	6.43
1423	1436	*α*-Cedrene	C15H24	0.16
1432	1427	β-Gurjunene	C15H24	0.07
1448	1449	α-Humulene	C15H24	1.21
1457	1452	(*E*)- *β* -farnesene	C15H24	0.01
1465	1460	Alloaromadendrene	C15H24	0.04
1470	1470	(*Z*)-*β*-Farnsesen	C15H24	0.02
1473	1478	Germacrene D	C15H24	0.02
1477	1479	*γ*-Gurjunene	C15H24	0.14

1484	1495	α-amorphene	C15H24	0.02
1493	1494	bicyclogermacrene	C15H24	0.08
1516	1513	γ-Cadinene	C15H24	0.14
1530	1535	*trans*-γ-Bisabolene	C15H24	0.04
1530	1531	*trans*-γ-Bisabolene	C15H24	0.02
1533	1535	α-Calacorene(1538)	C15H24	0.08
		Sesquiterpenealcohols		0.54
1555	1565	Palustrol	C15H26O	0.13
1566	1572	Spathulenol	C15H26O	0.03
1596	1608	Ledol	C15H26O	0.04
1620	1632	γ-Eudesmol	C15H26O	0.05
1635	1645	α-Muurolol	C15H26O	0.03
1638	1647	β-Eudesmol	C15H26O	0.09
1649	1645	*T*-Murolol	C15H26O	0.15
1659	1654	α-Cadinol	C15H26O	0.02
		Sesquiterpeneoxides		**1.18**
1569	1581	Caryophylleneoxide	C15H24O	1.18
		Other		**2.08**
958	961	Benzaldehyde	C7H6O	0.19
1186	1190	Methyl salicylate	C8H8O3	0.46
1759	1750	Benzyl benzoate	C14H12O2	1.43
		Total identified		96.54

*) RI_1: calculated retention index; **) RI_2:Retention index according the bibiography

Table 2: Mean Percentage with Standard Deviation (SD) of Twenty Major Components of Three Injections of the Essential Oil of Cinnamomum zeylanicum

Compounds	Mean percentages
Terpinene-4-ol	0.27±0.5
Camphene	0.18±0.10
β-Pinene	0.19±0.08
cis-Cinnamicaldehyde	0.29±0.01
α–Terpinene	0.24±0.12
Ortho methoxycinnamicaldehyde	0.42±0.02
Methyl salicylate	0.46±0.04
α-Pinene	0.65±0.15
α-Phellandrene	0.50±0.26
α–Copaene	0.74±0.10
Caryophylleneoxide	1.18±0.12
α-Humulene	1.21±0.14
Benzyl benzoate	1.43±0.10
p-Cymene	1.43±0.47
Eugenol	2.38±0.07
β–Phellandrene	1.92±0.75
Cinnamylacetate	3.14±0.187
Linalool	3.58±.26
β-Caryophyllene	6.43±0.773
trans-cinnamicaldehyde	66.23±2.010

Table 3. Inhibition Zone Diameters (IZDs), Minimal Inhibitory Concentrations (MICs), Minimal Bactericidal Concentrations (MBCs), and MBC/MIC Ratios for Cinnamomum zeylanicum Bark Essential Oils and the Antibiotic (Gentamicin) Against Thirteen Bacterial Strains

	Cinnamoumumzeylanicum				Gentamicine			
Gram-Negative	IZD (mm)	MIC (µl/ml)	MBC (µl/ml)	MBC/ MIC	IZD	MIC (µl/ml)	MBC (µl/ml)	MBC/MIC ratio
Pseudomona aeruginosa (ATCC 27853)	15.3±0.6	0.250	0.500	2	30.3±0.6	19.5	19.5	1

Echerichia coli (ATCC 25922)	20.0±0.0	0,125	0,125	1	24.3±0.6	19.5	19.5	1
Echerichia coli A11626	22.0±2.0	NT**)	NT		23.0±2.6	NT	NT	
Echerichia coli C2622	23.0±8.7	NT	NT		20.0±1.0	NT	NT	
Proteus. mirabilis C2524	29.3±0.6	0.250	0.25	1	22.7±1.2	39.1	39.1	1
Proteus mirabilis P195/20	30.0±3.6	NT	NT		23.7±1.5	NT	NT	
Klebtiellapneumoniae B2101/2018364	22.7±2.1	NT	NT		23.7±1.5	NT	NT	
Klebtiellapneumoniae A1237/2017915	16.3±0.6	0,03125	0,0625	2	21.7±0.6	19.5	19.5	1
Streptococcus puyogenes (group A)	27.3±2.5	0,250	0,250	1	32.7±1.5	78.1	78.1	1
Gram-Positive								
Staphylococusepidermidis(CIP 106510)	21.0±1.7	0.0625	0.125	2	24.0±0.0	19.5	19.5	1
Staphylococcus aureus ATCC 2912	35.0±6.2	0,0625	0,125	2	30.0±0.0	19.5	19.5	1
Enterococcusfaecalis ATCC 29212	22.3±1.2	NT	NT		28.3±1.5	NT	NT	
BacilussubtilisATCC 6633	29.3±1.2	0.25	0.250	1	30.0±0.0	39.1	39.1	1

*) Values are means (mm±MSD) of triplicate determination; **)NT: Not tested

ANTIBACTERIA ACTIVY

Disc Diffusion method

The EOs were tested for their putative antibacterial activity against 13 bacterial strains (Table 2). The results showed that, all the bacteral strains were extremly senitive (+++) to the EO of Cinnamomum zeylanicum with the exception of the Gram-negative P aeruginosa and Klebtiella pneumoniae A1237/2017915 which were classified as very senitive (++). The highest activity which was better than that produced by the antibiotic gentamicine was observed againt Staphylococcus aureus ATCC 2912, (35.0±6.2 mm, izd) followed by Proteus mirabilis P195/20 (30.0±3.6 mm, izd) and Proteus mirabilis C2524 (29.3±0.6 mm, izd) via 30.0±0.0 mm, 23.7±1.5mm and 22.7±1.2mm for those produced by the gentamicin respectively. An almost equal activity was observed for the rest of the tested strains exception for the Gram-negativePseudomonas aeruginosa (ATCC 27853) and Klebtiellapneumoniae B2101/2018364 which were more resistant to the EO.

Microdilution Broth Method

According to the classification of Bury-Moné, the EO effect on all the tested bacterial strains was assessed as bactericidal, indicated by a ratio of MBC/MIC <4 (Table II)[48]. The most promising antimicrobial activity was observed against the Gram-positive bacteria, specifically Staphylococcus epidermidis (CIP 106510) and Staphylococcus aureus ATCC 2912, with MIC values of 0.0625 µl/ml and MBC values of 0.125 µl/ml. Additionally, notable activity was recorded against the Gram-negative bacteria Klebsiella pneumoniae A1237/2017915, exhibiting MIC and MBC values of 0.03125 µl/ml and 0.0625 µl/ml; respectively , as well as against Escherichia coli (ATCC 25922) with MIC and MBC values of 0.125 µl/ml.

Overall, the essential oil of Cinnamomum zeylanicum appears to have effective antibacterial activity, particularly against Gram-negative bacteria, with a predominance of bactericidal effects.

Comparison of Antibacterial Activity Using Disc Diffusion and Microdilution Broth Methods

The comparative analysis of the results obtained from both the disc diffusion and microdilution methods revealed agreement for Gram-negative bacterial strains Streptococcus pyogenes (group A) and Proteus mirabilis C2524, as well as for Gram-positive bacteria Staphylococcus aureus ATCC 2912 and Staphylococcus epidermidis CIP 106510, where the minimal inhibitory concentration (MIC) ranged from 0.25 µl/ml to 0.0625 µl/ml. However, some discrepancies were observed, particularly against Pseudomonas aeruginosa ATCC 27853 (0.25 µl/ml; MIC) and Klebsiella pneumoniae A1237/2017915 (0.03125 µl/ml; MIC).

DISCUSSION

Chemical composition

The comparative analysis of our results with those reported by various researchers reveals that the essential oil (EO) derived from Cinnamomumzeylanicum bark is predominantly composed of (E)-cinnamic aldehyde, consistent with

our findings. However, the average percentage varies, depending on the source and the extraction method, ranging from 42.2% to 89.31%.[2,3,6,11,49,50]Notably, Unlu et al. and Tepe and Ozaslan identified a higher average percentage of (E)-cinnamic aldehyde (68.95% and 81.39%, respectively) and cinnamyl acetate (7.48% and 4.2%, respectively) in commercially available Turkish Bark Cinnamomumzeylanicum EO compared to those traded in Tunisia. The latter were found to be richer in β-caryophyllene, benzyl benzoate, and α-humulene.[5,51]In contrast, benzaldehyde, absent in our oil, exhibited a relatively high content (9.94%) in the EO obtained from C. zeylanicum bark traded in Turkey.(5)Furthermore, a relatively higher average percentage of eugenol (4.4% and 7.09%) and limonene (13.2% and 8.31%) was reported in oils commercialized in Belgrade and Germany, respectively.[52,53]These oils shared similar (E)-cinnamic aldehyde content with our findings (62.79% and 68.4%, respectively). Additionally, it was noted that the C. zeylanicum EO from Malaysia and Iran differed from our oil due to significantly lower average percentages of (E)-cinnamic aldehyde (44.2% and 52.3%, respectively) and higher average percentages of α-copaene (4.8% and 11.4%),δ-cadinene, and β-phellandrene.[3,49]

Antibacterial activities

Our findings regarding the antibacterial activity using the disc diffusion method were quite similar to those obtained by Unlu et al. (2010) (5) against all the tested strains, with a small difference in their inhibition zone diameters, particularly against S. aureus ATCC 29213, Enterococcus faecalis ATCC 29212, E. coli ATCC 25922, and P. aeruginosa 27853 (>40, 30, 26, and 18 mm; izd for Turkey EO) via 35, 22.3, 20, and 15.3 mm, respectively, for the EO commercialized in Tunisia. The relatively high activity against all the strains could be attributed to the high mean percentage of trans-cinnamic aldehyde in both essential oils. However, the variation in activity might be due to the differences in the mean percentage of the terpenoidscinnamyl acetate and benzaldehyde, which were more abundant in the Turkey EO. Therefore, the synergistic effect of these two compounds could significantly enhance the activity.The commercialized C. zeylanicum EOs in Portugal (originating from Germany) characterized by a significantly higher mean percentage of limonene (13.2%) and eugenol (4.4%), lower content in cinnamyl acetate (2.8%) and benzyl benzoate (0.6%), and an almost equal high mean percentage in trans-cinnamic aldehyde exhibited lower activity against Gram-negative bacterial strains (Pseudomonas aeruginosa (9.5 mm; izd), E. coli (18.9 mm; izd), Klebsiellapneumoniae (14.8 mm; izd) and Gram-positive ones (S. aureus (18.9 mm; izd), B. subtilis (15.5 mm; izd).[52]This suggests that the decrease in activity could be attributed to the increasing level of the monoterpene hydrocarbons, especially limonene, exhibiting an antagonistic effect. Conversely, the increase in activity within our oil could be attributed to the higher mean percentage of esters, specifically cinnamyl acetate and benzyl benzoate, which could enhance the activity synergistically. This hypothesis is supported by Griffin et al., who reported that terpene acetate, with low water solubility and low hydrogen-bonding capacity, when tested alone, is not active.[54]The observed discordance of our results using the disc diffusion method and the micro dilution broth one could be attributed to the EO limited diffusion ability, influenced by water solubility and the capacity of its active components to to diffuse through the agar.[54,55]Overall, the antibacterial activity of C. zeylanicum EOs is linked to their major components containing potent functional groups (such as aldehydes, esters, alcohols, and phenols) and the presence of delocalized electrons.[56] However, the effects of minor compounds should also be considered.[57]

Mechanism of action of the Essential oil

Many studies have reported the antimicrobial activities of essential oils (EOs); however, the mechanisms underlying these actions have not been extensively studied. Existing literature indicates that the mechanism of action is closely linked to the hydrophobicity of essential oils and their components, as well as the nature of the microbial membrane strains and the interaction between them.[58]

Cinnamomum zeylanicum essential oil, as demonstrated by Behbahani et al. (2020), modifies bacterial cell membrane structures, penetrates deeply, and enhances the bacterial death rate. Furthermore, Cinnamomum verum essential oil, rich in cinnamaldehyde (73%), induces membrane depolarization, loss of integrity, reduced respiratory activity, and cytoplasmic material coagulation in P. aeruginosa.[59]Eugenol and trans-cinnamic aldehyde have been reported to enhance antibacterial activity by inhibiting essential enzymes and damaging bacterial cell walls.[60,61]Cinnamaldehyde alters the membrane lipid profile, inducing significant increases in saturated fatty acids, resulting in a more rigid membrane. This modification is likely a compensatory response to the fluidifying effect of cinnamaldehyde on E. coli cell structure. At varying concentrations, cinnamaldehyde interferes with enzymes crucial for cytokinesis and less critical cellular functions, acting as an ATPase inhibitor and disrupting cell membranes[62]. Specifically, its inhibition of cytokinesis in B. cereus involves binding to the FtsZ protein. Moreover, cinnamaldehyde alters the membrane lipid profile of E. coli, increasing saturated fatty acids to enhance membrane rigidity.(56,62)Trans-cinnamaldehyde, which enters the periplasm of the cell, disrupts cellular functions.[61,63]

Similarly, the hydroxyl group of eugenol, when binding to the cell membrane, modifies bacterial fatty

acid profiles, impacting cytoplasmic membrane permeability and inhibiting the activity of enzymes such as ATPase, amylase, histidine carboxylase, and proteases.[64,65]Eugenol down-regulates YidC, a crucial bacterial protein, and effectively eliminates biofilms in Staphylococcus aureus.[66]

CONCLUSION

The essential oil (EO) of Cinnamomum zeylanicum commercialized in Tunisia is characterized by the dominant aliphatic aldehyde, trans-cinnamic aldehyde. It exhibits higher antibacterial activity compared to reference antibiotics gentamicin againstStaphylococcus aureusATCC 2912 andProteus mirabilis P195/20. This oil may have an interresting propospect in therapitically application of some bacterial strains resposable for human skin infection.

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