

Comparison of the Contents and Antimicrobial Activities of Commercial and Natural Cinnamon Oils

B. KASKATEPE*, M. E. KIYMACI, D. SIMSEK, H. B. EROL AND S. A. ERDEM¹

Department of Pharmaceutical Microbiology, ¹Department of Pharmacognosy, Ankara University Faculty of Pharmacy, Ankara, Turkey

Kaskatepe, *et al.*: Comparison of Antimicrobial Activities of Cinnamon Oils

There has been an increased interest in essential oils in recent years in accordance with new treatments against pathogens. The aim of the present study was to investigate the contents and to compare the antimicrobial activity of different brands of commercial oils with two natural cinnamon oils. Antibacterial and antifungal activities of cinnamon oils were estimated using disc diffusion and macro dilution methods against *Enterococcus faecalis* ATCC 19433, *Enterococcus faecalis* ATCC 29212, *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218, *Staphylococcus aureus* ATCC 29213, *Staphylococcus aureus* ATCC 25923, methicillin resistant *Staphylococcus aureus* ATCC 43300, *Pseudomonas aeruginosa* ATCC 9027, *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Klebsiella pneumoniae* RSKK 574, *Candida albicans* ATCC 10231, *Candida albicans* ATCC 033. The essential oil compositions were illuminated by gas chromatography-mass spectroscopy. Trans-cinnamaldehyde was the major compound of the essential oils obtained from the bark (C4 and C5; 92.3 and 90.1% respectively). The results of the commercial oils have revealed that these oils can be accepted as artificial oils. All the oils showed antimicrobial activity in a range of doses thought to be from cinnamaldehyde content.

Key words: Antimicrobial activity, cinnamon, essential oils

*Address for correspondence

E-mail: bkaskatepe@ankara.edu.tr

Essential oils have been used in medical, cosmetic and food industries for a long time. In recent years there has been an extensive research to explore and determine the antimicrobial activity of essential oils. Certain studies tilt to natural antimicrobial substances in recent decades because of the resistance to antibiotics. Besides, consumer preference for natural products or products including fewer chemicals in health and food sector has directed the scientific attention towards that area. Lots of essential oils were found to be effective against both standard and clinical bacterial strains. Researchers show that when combined with an antibiotic, essential oils increase their effect besides being alone^[1-3]. However, little quantitative data is available on the antimicrobial activity of commercial essential oils and antimicrobial effects are seldom mentioned in their product advertisements. Dussault *et al.* indicated that shelf life may have effect on the antimicrobial potential of essential oils^[4]. But, not enough study has been done in this regard. Moreover purity grades are not specified on commercial packages of many brands.

Cinnamon oil is one of the most studied essential oils because of its high affectivity and redolence. The genus *Cinnamomum* which belongs to Lauraceae family is represented by approximately 250 species. Among these species *C. verum* (syn. *C. zeylanicum*, true cinnamon) and *C. cassia* (Chinese cinnamon, cassia) are known and mostly used as a spice. Both of these species are also used to provide essential oils. Cinnamon is known as spice since 4000 BC from Chinese writings^[5] and also used in herbal medicine for treatment of some diseases like respiratory problems and diabetes. Various biologically active compounds have been identified in cinnamon such as cinnamaldehyde, cinnamic acid, cinnamyl acetate, eugenol and others. Due to their characteristic and biological properties, these chemical compounds have some antimicrobial, antiulcer, antidiabetic, antiinflammatory and antioxidant properties. Cinnamaldehyde is one of the main components of cinnamon and an aromatic organic compound that gives specific taste and odor^[6-8]. It is also less toxic (toxic for dividing cells at high concentrations), fat soluble, permeable across living cell membranes, easily undergoes degradation and accepted as biocompatible^[9].

Cinnamaldehyde is suggested as a potential candidate for chemoprevention against *Helicobacter pylori* related gastric pathogenic problems^[10]. It is possible to use this compound in several areas like regenerative

medicine and tissue engineering in low concentrations, and in neoplastic growth inhibition based on its various medicinal effects at high concentrations^[9]. Cinnamaldehyde also affects the structure of the yeast cells by inhibiting mycelial growth and has fungicidal activities by membrane damage against *Candida albicans*^[11]. Moreover, it was reported to have dose-dependent anti-*Candida albicans* effect. In some concentrations, cinnamaldehyde was found more effective and in some other concentrations similar to fluconazole and nystatine^[12]. Besides, *trans*-cinnamaldehyde shows the ability to inhibit uropathogenic *Escherichia coli* (UPEC) biofilm formation of UPEC on catheters. Some studies observed that cinnamaldehyde damages membrane integrity and permeability of *Staphylococcus aureus* (ATCC 3101) and *E. coli* (ATCC 8735)^[13].

Being known as “Generally Recognized as Safe” (GRAS) compounds, nowadays some components of essential oils have been thought as postharvest preservatives against microorganisms^[14]. Composition, structure, as well as functional groups of the compounds found in the oils play an important role in determining their antimicrobial activity^[15]. Antunes *et al.* indicated that commercial essential oils have lower biological activity due to the yield process^[16]. But there are no studies to verify or contradict these findings worldwide. For this reason, with this study, we aimed to investigate antimicrobial activity of cinnamon oils against to the most common human infectious bacteria. Besides the composition of the selected oils was determined by gas chromatography (GC) and gas chromatography-mass spectrometry (GC/MS) analysis.

Two cinnamon bark and three cinnamon oil samples belonging to different commercial brands were purchased from a local market in Turkey. Cinnamon barks were coded as C IV and C V. Essential oils of the barks were obtained by hydrodistillation using Clevenger type apparatus.

Analyses of the cinnamon oils were done simultaneously by GC and GC/MS. GC analysis was performed on

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an Agilent 6890N Network GC system and GC/MS analysis was performed on Agilent 5973 Network Mass Selective Detector integrated with the GC system. The analysis was performed using HP-Innowax column (60.0 m×0.25 mm×0.25 mm) and helium as carrier gas (1.2 ml/min). The oven temperature was set to 60° for 10 min after injection, then increased to 220° with 4°/min heating ramp for 10 min and increased to 240° with 1°/min heating ramp without hold. Both injector and detector (FID) temperatures were 250°; split ratio was adjusted to 50:1. Injection volume was 2.0 µl. MS conditions were as follows: ionization energy, 70 eV; ion source temperature, 280°; interface temperature, 250°; mass range, 34-450 atomic mass units.

Identification of the components was done by comparison of their relative retention indices and mass spectra with corresponding data^[17] and by comparison of their mass spectra with Wiley and NIST Standard Reference Database. The percentages of the components were calculated from the GC peak areas, using the normalization method.

Microorganisms that were selected for this study are *Enterococcus faecalis* ATCC 19433, *E. faecalis* ATCC 29212, *E. coli* ATCC 25922, *E. coli* ATCC 35218, *S. aureus* ATCC 29213, *S. aureus* ATCC 25923, methicillin resistant *S. aureus* ATCC 43300, *Pseudomonas aeruginosa* ATCC 9027, *P. aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Klebsiella pneumonia* RSKK 574, *C. albicans* ATCC 10231, *C. albicans* ATCC 033.

The bacteria were grown in Mueller Hinton Broth (MHB, Merck) and Mueller-Hinton Agar (MHA, Merck) media. Sabouraud Dextrose Agar (SDA, Merck) was used as growing medium for *C. albicans*.

Agar disc diffusion and broth dilution method were performed for determination of antimicrobial activity of oils. For agar disc diffusion method, microorganisms were cultured at 37° for 24 h and bacterial and fungal suspensions were prepared from these fresh cultures and adjusted the turbidity equivalent to McFarland 0.5 standard with nephelometer (Biosan). Microorganism suspension was spread on the MHA plate with sterile swab. Sterile discs (6 mm diameter) were impregnated with 15 µl of the essential oils and placed on the surface of the plate. Plates were subsequently incubated at the appropriate temperature for 16-20 h and zones of inhibition were calculated by measuring the diameter in mm.

Minimum inhibitory concentrations (MIC) of oils were

determined by the broth macrodilution method. For this purpose two-fold dilutions of essential oils were obtained in tubes including MHB and fresh bacterial and fungal suspension were added. Before analysis, essential oils were homogenized in dimethyl sulfoxide (DMSO). The tubes were incubated 16-20 h at 37°. The lowest concentration that inhibits growth was determined as MIC value. All the experiments were carried out in duplicate and the results were given as mean value. The results were analyzed statistically by SPSS packet program with one way ANOVA test. At 95% confidence interval, P<0.05 was considered statistically significant.

The composition of the obtained and commercial cinnamon oils which were identified by GC/MS are given in Table 1. Essential oils obtained from the cinnamon barks by hydrodistillation with the yield of 1.5% for C4 and 2.0% for C5. Thirteen and twenty-five compounds were detected representing 97.4% (C4) and 98.2% (C5) of the essential oils obtained from cinnamon barks. Both of the oils were dominated by aromatic hydrocarbons 95.2% for C4 and 89.3% for C5. Other than this, C4 was found to contain oxygen containing monoterpenes (1.9%) and monoterpene hydrocarbons (0.3%), while C5 was found to contain oxygen containing monoterpenes (3.9%) and sesquiterpene hydrocarbons (0.9%) and hydrocarbons (4.3%). The main component was *trans*-cinnamaldehyde for C4 and C5 with the amount of 92.3% and 86.5%, respectively. C4 and C5 have 1,8-cineole (0.4 and 0.3%, respectively), benzaldehyde (0.7 and 0.2%, respectively), bornyl acetate (0.3 and 0.9%, respectively), terpinen-4-ol (0.3 and 0.7%, respectively), α -terpineol (0.7 and 1.0%, respectively), hydroxyl cinnamic aldehyde (1.0 and 0.7%, respectively), *cis*-cinnamaldehyde (0.9 and 0.7%, respectively), cinnamyl acetate (2.5 and 0.8%, respectively), carvacrol (both 0.2%), and coumarin (0.3 and 0.4%, respectively) in common. As remarkable difference; monoterpene hydrocarbon content of C4 (α -pinene and limonene) and sesquiterpene hydrocarbon (α -copaene, β -caryophyllene, α -humulene, α -muurolene, δ -cadinenecalamenene and α -calacorene) and hexadecanoic acid with a notable amount (4.1%) for C5 can be observed.

Li *et al.*^[18] reported essential oil analyses of 6 different *C. cassia* and a *C. verum* samples; the yield of the essential oils were ranged from 0.72 to 3.08%, due to the variations in the climates, ecological environments and growth conditions in the different cultivation sites; these results are similar with our yields. *Trans*-

TABLE 1: PERCENTAGE COMPOSITION OF THE ESSENTIAL OILS OBTAINED FROM CINNAMON BARKS AND COMMERCIAL OILS

Compounds	RI ^a	Cinnamon I	Cinnamon II	Cinnamon III	Cinnamon IV	Cinnamon V
α -pinene	992	-	-	-	0.1	-
Limonene	1148	-	-	-	0.2	-
1,8-cineole	1154	-	-	-	0.4	0.3
Cyclohexanone	1226	-	-	-	-	0.1
α -copaene	1476	-	-	-	-	0.1
Benzaldehyde	1481	1.0	-	0.2	0.7	0.2
Linalool	1487	-	1.5	-	-	0.1
Bornyl acetate	1546	-	-	-	0.3	1.0
Terpinen-4-ol	1558	-	-	-	0.3	0.7
β -caryophyllene	1583	-	-	-	-	0.3
α -terpineol	1647	-	-	-	0.7	1.0
Borneol	1677	-	-	-	-	0.4
α -muurolene	1703	-	-	-	-	0.1
δ -cadinene	1733	-	-	-	-	0.2
Hydroxycinnamic aldehyde	1738	-	-	-	1.0	0.8
2,4-decadienal	1763	-	-	-	-	0.1
Anethole	1780	-	-	-	-	0.2
Calamenene	1801	-	-	-	-	0.2
Benzyl alcohol	1820	-	4.0	-	-	-
cis-cinnamaldehyde	1854	-	-	-	0.9	0.7
α -calacorene	1887	-	-	-	-	tr ^b
trans-cinnamaldehyde	2007	76.9	10.3	99.5	92.3	90.1
Triacetin	2100	22.1	-	-	-	-
Cinnamylacetate	2099	-	-	-	2.5	0.8
Eugenol	2111	-	-	-	-	0.1
Carvacrol	2147	-	-	-	0.2	0.2
Cinnamaldehyde propylene glycol acetal isomer	2210	-	39.0	-	-	-
Cinnamaldehyde propylene glycol acetal isomer	2226	-	31.0	-	-	-
Cinnamyl alcohol	2241	-	-	-	-	0.1
Coumarine	2423	-	-	-	0.3	0.4
Monoterpenehydrocarbons	-	-	-	-	0.3	-
Oxygen containing monoterpenes	-	-	1.5	-	1.9	4.0
Sesquiterpene hydrocarbons	-	-	-	-	-	0.9
Oxygen containing sesquiterpenes	-	-	-	-	-	-
Aromatic hydrocarbons	-	77.9	84.3	99.7	95.2	93.1
Hydrocarbons	-	22.1	-	-	-	0.2
Total identified	-	100	85.8	99.7	97.4	98.2

cinnamaldehyde was found between 66.28-77.21% for *C. cassia* whereas it was found 74.49% for *C. verum*. Eugenol amount was very low for *C. cassia* samples (tr-0.21%), but *C. verum* was found to contain in noticeable amount (7.29%). In another study, GC/MS analysis of essential oils from the barks of *C. zeylanicum* (*C. verum*) were resulted with an amount of 97.7% *trans*-cinnamaldehyde together with monoterpenes such as α -pinene, limonene, 1,8-cineole and sesquiterpenes α -copaene, α -amorphene and δ -cadinene^[6].

In literature, some studies determined that cinnamon has remarkable antimicrobial activity against some skin infection agents, and food pathogens^[4,19-21]. Moreira *et al.* found that essential oil of *C. zeylanicum* has a synergistic activity with b-pinene and suggested that these compounds may be an alternative to be inserted in pharmaceutical products as antimold agents^[22]. Ooi *et al.* found that both oil and pure cinnamaldehyde of *C. cassia* were equally effective in inhibiting the growth of bacteria including Gram positive and Gram negative and fungi. They concluded that broad-

spectrum antibiotic activities of *C. cassia* oils are due to cinnamaldehyde^[23]. Siddiqua *et al.* found that cinnamaldehyde and clove oils showed effective antibacterial activity and when combined, the activity increased^[24]. Yossa *et al.* reported that cinnamaldehyde was highly effective against *E. coli* O157:H7 and *Salmonella*^[25].

Commercial cinnamon oils were previously discussed^[26]. As a brief evaluation, cinnamon 2 was found to contain cinnamaldehyde propylene glycol acetal which is a synthetic compound used as a flavor especially in chewing gums^[27]. Cinnamon 3 consists of cinnamic aldehyde (99.5%) and cinnamon 1 contains triacetin (22.1%) addition to cinnamic aldehyde (76.9%). Triacetin is not a natural component of essential oils; it is used as diluent or carrier of flavors^[28]. Commercial oils were found to contain cinnamaldehyde as major compound, the other compounds were limited with benzaldehyde, benzyl alcohol or linalool, whereas composition of the oils obtained from the barks consist of cinnamaldehyde as major compound like the commercial oils, additionally they are found to contain monoterpenes or sesquiterpenes. Thus, commercial oils can be accepted as artificial oil.

In this work, commercial cinnamon oil 1 and 3, natural cinnamon oil 4 and 5 showed effective antimicrobial activity. Our GC/MS study revealed that cinnamaldehyde was the predominant active compound found in commercial and natural cinnamon oils. Similar to this study, other studies showed that the antibacterial activity of cinnamon oil was due to cinnamaldehyde which was found as major component^[29].

Three brands of cinnamon oil had antibacterial and

antifungal activity. C2 was the less active essential oil against tested microorganisms. Two of three commercial oils and two natural cinnamon oils were the most active. The antimicrobial activity results of the commercial and natural cinnamon oils are given as zone diameter and MIC values in Table 2.

In our study, we investigated the antimicrobial activity of different commercial brands of oils. The most effective of all the three was cinnamon oil 3 (C3). Maximum zone diameter for this oil was observed on *C. albicans* strains and methicillin resistant *S. aureus* ATCC 43300 (>50), minimum was on the *P. aeruginosa* ATCC 27853 (21 mm). Second most effective essential oil was cinnamon oil 1 (C1). It showed maximum zone diameter on *C. albicans* strains (>50 mm), MRSA 43300, *S. aureus* ATCC 25923 (50 mm) and minimum zone diameter on *P. aeruginosa* ATCC 27853 (18 mm). When compared these two oils in terms of content, both of the oils contains cinnamic aldehyde as major component while benzaldehyde was the common minor component. The antimicrobial effects of these oils are thought to be from cinnamaldehyde. The results are consistent with the studies in the literature^[18]. C2 comes in the third place in terms of activity level and it is quite different from the other two. Referring to a content analysis, cinnamaldehyde propylene glycol acetal which is a synthetic resin component especially used as a flavoring agent in chewing gums was observed. This oil is believed to be artificial oil. Containing the lower amount of cinnamic acid (10.3%) than C1 and C3 (76.9% and 99.5%, respectively) is believed to be the reason of weaker antimicrobial activity. Also C1 which has lower cinnamaldehyde than C3, C4 and C5

TABLE 2: INHIBITION ZONE DIAMETER AND MIC VALUES OF CINNAMON OIL

Microorganisms	I		II		III		IV		V	
	ZD	MIC ^a	ZD	MIC ^a	ZD	MIC ^a	ZD	MIC ^a	ZD	MIC ^a
<i>E. faecalis</i> ATCC 19433	20	0.78	17	12.5	33	1.56	28	0.39	32	<0.09
<i>E. faecalis</i> ATCC 29212	30	0.39	15	12.5	34	0.78	32	<0.09	35	<0.09
<i>E. coli</i> ATCC 25922	38	0.09	20	12.5	39	0.09	33	<0.09	25	0,39
<i>E. coli</i> ATCC 35218	33	0.19	14	12.5	36	0.09	26	<0.09	35	0,39
<i>S. aureus</i> ATCC 29213	37	0.19	13	6.25	40	0.19	34	<0.09	37	<0.09
<i>S. aureus</i> ATCC 25923	50	<0.09	15	6.25	45	0.09	35	0.19	42	0.19
<i>S. aureus</i> ATCC 43300	50	<0.19	24	6.25	>50	<0.09	42	<0.09	41	<0.09
<i>P. aeruginosa</i> ATCC 9027	20	0.78	15	12.5	22	0.78	20	1.56	22	0.78
<i>P. aeruginosa</i> ATCC 27853	18	1.56	18	12.5	21	0.78	21	1.56	24	0.78
<i>B. subtilis</i> ATCC 6633	40	0.09	19	6.25	40	<0.09	31	<0.09	36	<0.09
<i>K. pneumoniae</i> RSKK 574	35	0.09	19	6.25	39	<0.09	34	<0.09	43	<0.09
<i>C. albicans</i> ATCC 10231	>50	<0.09	>50	3.12	>50	<0.09	>50	<0.09	>50	<0.09
<i>C. albicans</i> ATCC 033	>50	<0.09	>50	1.56	>50	<0.09	>50	<0.09	>50	<0.09

^aMIC values are given in µl/ml; ZD: Zone diameter

has lower antimicrobial activity. When compared the results of two obtained cinnamon oils, similar results were obtained for both.

When the antimicrobial susceptibility test results were analyzed statistically, there was significant difference between cinnamon oil brands 1 and 2, 2 and 3 (P values were P=0.007 and P=0.003 respectively). There were no statistical differences between C1, C3 and C4, C5.

Also, these commercial available essential oil contents have been found to vary from brand to brand and this situation affects the antimicrobial activity. Being subjected to specific standards and stating contents on the label of these commercial preparations when used as a food additive and cosmetic product by external use, will provide more detailed information in terms of the quality, efficacy and safety of products. This will ensure the more conscious use of these products.

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There are no conflicts of interest.

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