

Research Article

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Physicochemical and biological properties of carvacrol

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Abstract: Carvacrol is a major component of many essential oils of the genus *Thymus*, *Satureja*, and *Origanum*,

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determining their antimicrobial, antioxidant, and other properties. The aim of the present study was to investigate some physicochemical and biological properties of pure carvacrol. The surface tension and density were measured at six different temperatures (30, 40, 50, 60, 70, and 80°C). The surface tension values were between 53.11 and 60.38 mN/m, while density varied from 0.978 to 0.99 kg/m³. The antibacterial activity against seven pathogenic and conditionally pathogenic bacteria was investigated. The diameter of the inhibition zones was determined in the range of 3.9–4.9 mm. The antioxidant activity was determined by the oxygen radical absorbance capacity (1687.0 µmol TE/mL) method.

Keywords: carvacrol, physicochemical properties, antibacterial and antioxidant activities

1 Introduction

Carvacrol (2-methyl-5-isopropyl phenol, 2-*p*-cymenol, 2-hydroxy-*p*-cymene, isopropyl-*o*-cresol, isothymol: molecular weight of 150.22 [CAS 499-75-2]). Its properties are as follows: thick colorless to pale yellow liquid with pungent, spicy odor; relative density (d_{20}^{20}) 0.974–0.979; refractive index (n_D^{20}), 1.5210–1.5260; melting point, 1°C; boiling point, 237–238°C; slightly soluble in water; soluble in ethanol, ether, alkalis; and very soluble in water [1,2].

This aromatic substance is mainly found in genera *Thymus*, *Satureja*, and *Origanum* plants, which belong

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to the family Lamiaceae. In case of improper storage of these essential oils (high temperature, presence of water, light, etc.), color changes occur due to separating precipitates from phenolates. It is known that in the presence of iron, phenols are oxidized in order to form red-colored products. It has been shown that hydroquinone is initially produced and oxidized to quinone, followed by polymerization processes. With prolonged exposure, the color changes to red-brown may occur. Oxidation is accompanied not only by a change in color but also by a change in smell. Initially, hydroperoxides are formed. Later, they undergo various transformations, turning into terpineol, carvone, polyhydroxy compounds, and finally into high-molecular polymer compounds [1,2].

Carvacrol is used in perfumery and cosmetics [1,2], as well as for the preparation of racemic menthol [1].

As a phenol, carvacrol possesses highly pronounced antimicrobial [3–10], antioxidant [11–13], and other biological properties [14,15].

Density, viscosity, refractive index, and surface tension in water/alcohol systems were measured and found that pure alcohols or alcohols mixed with water were used as solvents in industrial applications of pharmaceutical, perfumery, and cosmetic products [16]. Surface and interfacial tension against water for mint, lemon, and oregano oils were investigated as a function of time and temperature [17]. A mix of linalool + propan-1-ol was investigated in a temperature range between 283.15 and 328.15 K. Refractive index of the mixture was measured and determined that the essential oils are sensitive to light [18]. Density, surface tension, and refractive index were determined for investigation of equilibrium in the system 1-pentanol-*R*-(+)-limonene in temperature range [19]. Linear dependence between density and refractive indexes of different aromatic compounds down to the temperature of 273 K was investigated [20].

The aim of the present study was to investigate carvacrol as an aromatic substance by determining some physicochemical parameters, antibacterial and antioxidant activities, looking for possibilities for its application, as well as the essential oils containing it in food and cosmetic products.

2 Materials and methods

2.1 Material

Carvacrol was delivered from Sigma Aldrich, USA.

2.2 Physical and chemical indexes

The physical indicators of the refractive index [21] and relative density [22] have been determined. The infrared spectrum of carvacrol was recorded using a Nicolet iS 50 Thermo Scientific FT-IR spectrometer in the frequency region of 4,000–400 cm^{-1} , with the sample embedded in KBr matrixes.

Surface tension is determined by equation (1) [23].

$$\gamma = \frac{rg}{2}(\Delta H\rho_0 - r\rho), \quad (1)$$

where r is the radius of the capillary (m), g is the acceleration of gravity (m/s^2), ΔH is the maximum difference in the two gauges of the gauge, and ρ_0, ρ is the density of the manometric (water) and test liquid (kg/m^3).

The density of the test liquid is determined by equation (2) [23].

$$\rho = \frac{m_1 - m}{V} = \frac{m_1 - m}{\frac{m_1 - m}{\rho_1}} = \frac{m_1 - m}{m_2 - m}\rho_1, \quad (2)$$

where ρ is the density of the carvacrol (kg/m^3), ρ_1 is the density of distilled water (kg/m^3), m is the mass of the pycnometer (g), m_1 is the mass of the pycnometer with the carvacrol (g), and m_2 is the mass of the pycnometer with distilled water (g).

These two physicochemical indicators were measured at six temperatures (30, 40, 50, 60, 70, and 80°C), which were most often used in applying aromatic substances in various food and cosmetic products.

2.3 Antibacterial activity

Test microorganisms. Strains of pathogenic bacteria, reported as causing infections, toxicoinfections, and toxicosis, were used as test microorganisms. The investigated test cultures were some of the most common, both on the surface of food and cosmetic preparations, on work surfaces in companies, and on the hands of staff. Test microorganisms strains were supplied by the National Bank for Industrial Microorganisms and Cell Cultures, except for two that are clinical isolates. The following Gram-positive bacteria were used in this study: *Listeria monocytogenes* NCTC 11994, *Staphylococcus aureus* ATCC 25093, *Bacillus subtilis* ATCC 6633, and Gram-negative bacteria – *Escherichia coli* ATCC 8739, *Salmonella enterica* subsp. *Enterica* serovar *Abony* NCTC 6017.

The Gram-positive bacterium *Bacillus cereus* and the Gram-negative bacterium *Klebsiella* sp. are clinical isolates from the University of Food Technologies collection.

Antibacterial activity was determined by modifying the agar diffusion method by measuring the inhibition zones of the pathogen (1.10^4 cfu/mL) growth around metal rings ($\varnothing = 6$ mm) [24].

2.4 Antioxidant activity

The lipophilic oxygen radical absorbance capacity assay measures the antioxidant scavenging function of lipophilic antioxidants against peroxy radical induced by 2,2'-azobis(2-amidinopropane) dihydrochloride at 37°C [25].

2.5 Statistical analysis

The measurements were performed in triplicate. The results are presented as a mean value of the individual measurements with the corresponding standard deviation (SD).

3 Results and discussion

3.1 Physical and chemical indexes

Carvacrol is a clear yellowish liquid with a specific odor and the following physical indicators: relative density (d_{20}^{20}) 0.980 ± 0.0 and refractive index (n_D^{20}) 1.524 ± 0.01 . The values of the physical parameters of the studied carvacrol sample do not differ from data in the literature [1,2].

The IR spectrum of carvacrol is shown in Table 1 and Figure 1. In the carvacrol spectrum, clear characteristic bands of absorption were observed at $3,385\text{ cm}^{-1}$, which was characteristic of γOH group associated with the aromatic ring. The band at $3,021\text{ cm}^{-1}$ was characteristic of the $\gamma\text{C-H}$ relationship characteristic of an aromatic ring. The band at $2,961\text{ cm}^{-1}$ was characteristic of the γ as CH_3 group, which was confirmed by an additional band at $2,870\text{ cm}^{-1}$. Also noticeable was an absorption band at $2,927\text{ cm}^{-1}$, which was characteristic of the γ as $-\text{CH}_2-$ group. The absorption band at $1,621\text{ cm}^{-1}$ was characteristic of an absorption band of a conjugate double $\text{C}=\text{C}$ bond.

Table 1: IR Spectrum of carvacrol

Characteristic bands (cm^{-1})		Group type
Carvacrol	Reference data	
3,385	3,470–3,322	γOH group to aromatic ring (phenol)
3,021	3,040–3,010	$\gamma\text{C-H}$ average absorption band, characteristic of aromatics
2,961	2,970–2,950	Linear $\gamma_{\text{as}}\text{CH}_3$
2,927	2,940–2,915	$\gamma_{\text{as}}-\text{CH}_2-$
2,870	2,885–2,860	Presence of CH_3 group
—	2,870–2,845	Highly pronounced band $\gamma^{\text{as}}\text{CH}_2$
1,621	1,680–1,620	A little intense band of absorption, conjugated $\text{C}=\text{C}$
1,589	1,600–1,575	Pulsation oscillations of the carbon skeleton at an aromatic structure more intense about $1,500\text{ cm}^{-1}$ (in this case $1,502\text{ cm}^{-1}$)
1,522	1,525–1,475	Oscillations of substituents at aromatic ring
1,502	intense about $1,500\text{ cm}^{-1}$	Pulsation oscillations of the carbon skeleton at aromatic ring
1,459	1,465–1,440	Characteristic band of aromatic ring
1,421	1,435–1,415	$-\text{C}-\text{O}-\text{H}$ characteristic band of phenol
1,362	1,370–1,365	Two bands of approximately the same intensity corresponding to a structural fragment of the type $\text{C}(\text{CH}_3)_2$
1,302	1,310–1,290	Fluctuations $\delta\text{C-n}$ of connection C-H at the end vinyl group or associated with an aromatic ring
1,175	1,175–1,165	A characteristic band of the type $\text{CH}_3-\text{CH}-\text{CH}_3$
1,117	1,125–1,090	This is 1, 2, 4 substituted aromatics
995	1,000–960	This is 1, 2, 4 substituted aromatics
937	950–860	Antisymmetric valence oscillations of a ring
866	900–865	Three substituted aromatic ring at position 1, 2, 4
813	855–800	<i>p</i> -Substituted aromatic ring
757	770–735	<i>o</i> -Substituted aromatic ring

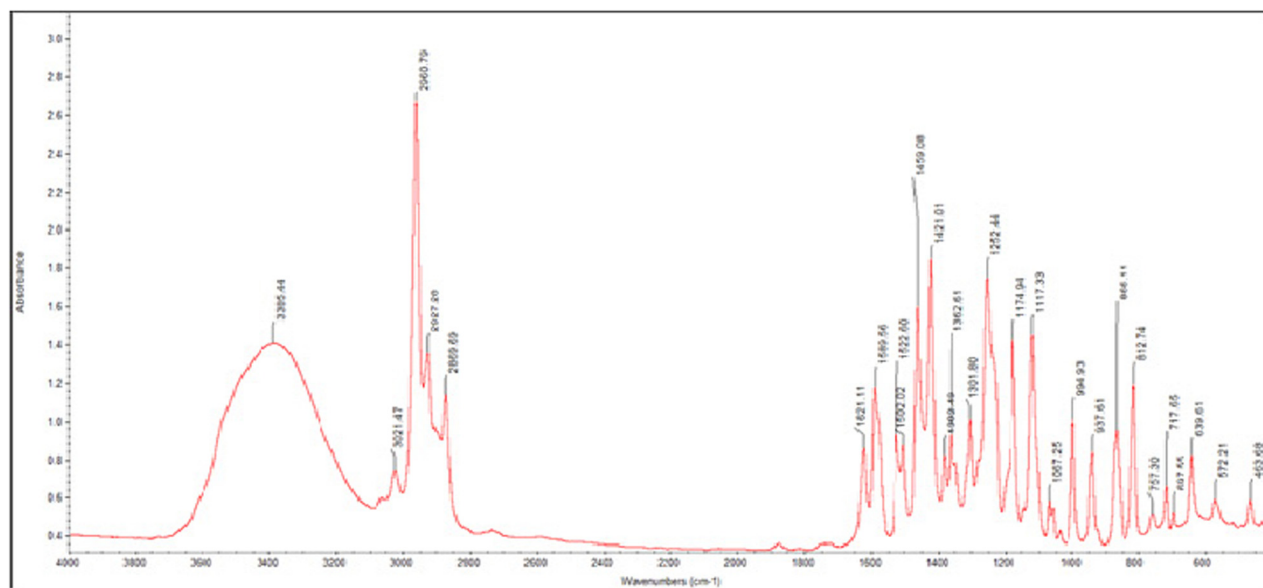


Figure 1: FT-IR Spectrum of carvacrol.

The intense absorption band at 1589 cm^{-1} was characteristic of the carbon skeleton with an aromatic structure that was more intense about 1500 cm^{-1} (in this case 1502 cm^{-1}). The bands 1589 , 1522 , 1502 , 1459 , and 1421 cm^{-1} were characteristic of the aromatic ring, with the strip 1421 cm^{-1} being characteristic of the C-O-H bond in phenols. The bands 1117 , 995 , and 866 cm^{-1} were characteristic of aromatic rings with substituents in positions 1, 2, and 4. The band at 813 cm^{-1} was typical for a *p*-substituted aromatic ring and that at 757 cm^{-1} for an *o*-substituted ring. From the conclusions drawn, it could be concluded that this compound belonged to the phenol group which had a CH_3 group in the *o*-position and a group of the type $\text{C}(\text{CH}_3)_2$ in the *p*-position, which was confirmed by the structural formula of carvacrol [26,27].

The surface tensions and density of pure carvacrol were measured. The results are presented in Table 2. The data showed that as the temperature increased, the values of surface tension and density decreased is a classical thermodynamic dependence and does not contradict the

literature for other substances [28]. The carvacrol usually had less tension than the water (72.75 mN/m) [29]. The decrease in the value of surface tension with increasing temperature is smooth, as at 40°C it is only 0.04% , at 50°C – by 2.42% , at 60°C – by 7.24% , at 70°C – by 9.64% , and at 80°C – by 12.04% , and the dependence is considered linear. A similar trend is found in the density, as the temperature dependence is also linear – at 40°C , the decrease is by 0.30% , at 50°C – by 0.50% , at 60°C – by 0.80% , at 70°C – by 1.00% , and at 80°C – with 1.31% .

When increasing the temperature from 30 to 80°C , no change in the color and smell of carvacrol was detected, indicating that oxidation and polymerization processes did not occur. Our results did not differ from the data reported by Fonseca *et al.* [27], according to which changes in the structure of carvacrol may occur towards 160°C . The temperature stability of carvacrol was explained by the presence of the aromatic ring [26,27].

In the literature, there are data on values of these physicochemical parameters at different temperatures. Still, for solutions of linalool [18,30] and other aromatic substances [19,20,31] in ethanol, propanol, and other solvents, it is not possible to make a comparison.

Table 2: Surface tension and density at different temperatures

Temperature, $^\circ\text{C}$	Surface tension, mN/m	Density, kg/m^3
30	60.38 ± 0.08	0.991 ± 0.11
40	60.35 ± 0.11	0.988 ± 0.08
50	58.92 ± 0.12	0.986 ± 0.17
60	56.01 ± 0.06	0.983 ± 0.03
70	54.56 ± 0.16	0.981 ± 0.11
80	53.11 ± 0.03	0.978 ± 0.08

3.2 Antibacterial activity

The antibacterial activity of carvacrol is presented in Table 3. The data showed that the diameter of the zones of inhibition was in the range of $3.9\text{--}4.9\text{ mm}$. The antimicrobial action of carvacrol has been found to be due to:

Table 3: Antimicrobial activity of carvacrol

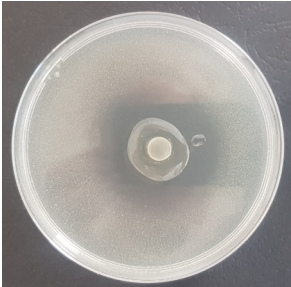
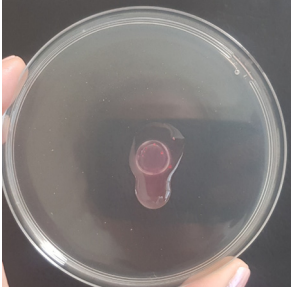
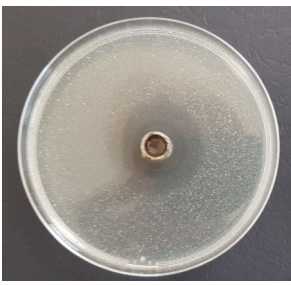
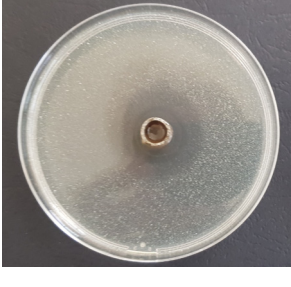
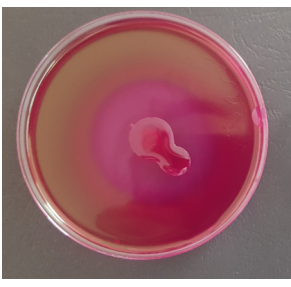
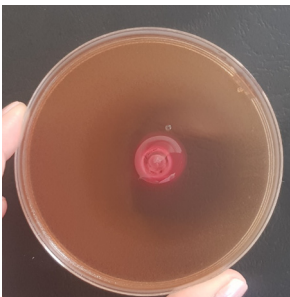
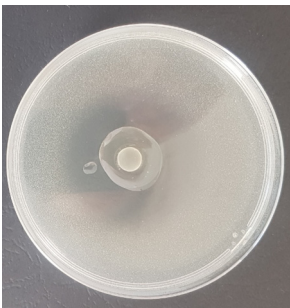
Test-microorganisms	Diameter of zone of inhibition, mm	
Gram-positive bacteria		
<i>Listeria monocytogenes</i> NCTC 11994	4.2 ± 0.04	
<i>Staphylococcus aureus</i> ATCC 25093	4.0 ± 0.03	
<i>Bacillus subtilis</i> ATCC 6633	3.9 ± 0.03	
<i>Bacillus cereus</i> (clinical isolate)	3.7 ± 0.03	
Gram-negative bacteria		
<i>Escherichia coli</i> ATCC 8739	4.5 ± 0.04	

Table 3: Continued

Test-microorganisms	Diameter of zone of inhibition, mm	
<i>Salmonella enterica</i> subsp. <i>Enterica</i> serovar Abony NCTC 6017	4.9 ± 0.04	
<i>Klebsiella</i> (clinical isolate)	3.9 ± 0.03	

depletion of intracellular ATF [10], induction of reactive oxygen species [32], inhibition of efflux pump [33], and inhibition of bacterial biofilm [34].

The data presented in Table 3 showed that no difference in antibacterial activity was reported against Gram-positive and Gram-negative bacteria. The established differences in the studied antibacterial activity and data from the literature, both for the pure substance and for the essential oils that contain it, could be explained by the analysis methods used in our study. In our research, “wells” were used into which carvacrol was dripped. In other studies published in the literature, “filter discs” were used, onto which the carvacrol was put. The aromatic substance’s concentration and the tested bacteria’s suspension should also be considered.

For example, Sarrazin et al. [35] reported that carvacrol was active against Gram-positive *Bacillus cereus*, *B. subtilis*, and Gram-negative bacteria *Salmonella typhimurium*, which was highly related to its chemical structure and composition. In another study, Du et al. [36] reported strong antibacterial activity of the carvacrol against pathogenic Gram-negative bacteria *Escherichia coli*, *Clostridium perfringens*, *Salmonella* strains, and Gram-positive *Lactobacillus* strains. Our results were lower than that reported by Bnyan et al. [37] who investigated the antimicrobial

effects of carvacrol against nine microbial species. As a result, they reported that carvacrol showed maximum inhibition against *E. coli* (26 mm), *Klebsiella pneumonia* (23 mm), and *S. aureus* (20 mm). The differences in the results could be due to the used species and the differences in the applied bacterial concentrations.

3.3 Antioxidant activity

The value for the antioxidant effect of carvacrol, determined by the oxygen radical absorbance capacity (ORAC) method described in the study was $1687.0 \pm 102.90 \mu\text{mol TE/mL}$. ORAC has some advantages that made it one of the most widely used methods for estimating total antioxidant capacity. It is based on the generation of peroxy radicals that are relevant in food and biological systems. Additionally, it is performed under physiological pH (7.4) and temperature (37°C) and most importantly, the assay has a modification for lyphophilic antiradical activity, in which lypophilic components are dissolved in water/buffer media with randomly methylated β -cyclodextrins. Essential oils containing carvacrol were known to exhibit antioxidant activity [12,14,38,39], which the authors attribute to its presence. The antioxidant activity of carvacrol was determined by different methods by a number of authors: ORAC (2.8 $\mu\text{mol Trolox equivalent}/\mu\text{L}$), ABTS (7804.4 $\mu\text{mol Trolox equivalent}/\mu\text{L}$), DPPH (activity lower than 0.05 $\mu\text{mol Trolox equivalent}/\mu\text{L}$), chelating power (activity lower than 0.05 $\mu\text{mol ethylenediaminetetraacetic acid equivalent/mL}$), and potassium ferricyanide (3239.6 $\mu\text{mol ascorbic acid equivalent/L}$) [40], DPPH (IC50 448.05 (lg/mL [41], DPPH (79.75 $\mu\text{g/mL}$) [42] and ORAC (152.23 $\mu\text{mol Trolox equivalent/g}$) and DPPH (51.09 %) [43]. The different values could be explained by the analysis methods used.

Other data on the antioxidant activity of essential oils containing carvacrol, determined by the described method, were not found in the literature. It is not easy to make a more detailed comparison.

4 Conclusions

Some physicochemical parameters at six different temperatures and two biological properties of pure carvacrol were investigated. Weak antimicrobial activity was determined against seven pathogenic and conditionally pathogenic microorganisms, and the ORAC method was used in order to determine the antioxidant activity. The results showed that carvacrol, as well as essential oils containing

it in larger quantities, could be used as an independent ingredient in food and cosmetic formulations, which could be a subject of further studies.

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Ethical approval: The conducted research is not related to either human or animal use.

Data availability statement: The data presented in this study are available on request from the authors.

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