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REVIEW

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Carvacrol and human health: A comprehensive review

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Carvacrol (CV) is a phenolic monoterpenoid found in essential oils of oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), pepperwort (*Lepidium flavum*), wild bergamot (*Citrus aurantium bergamia*), and other plants. Carvacrol possesses a wide range of bioactivities putatively useful for clinical applications such antimicrobial, antioxidant, and anticancer activities. Carvacrol antimicrobial activity is higher than that of other volatile compounds present in essential oils due to the presence of the free hydroxyl group, hydrophobicity, and the phenol moiety. The present review illustrates the state-of-the-art studies on the antimicrobial, antioxidant, and anticancer properties of CV. It is particularly effective against food-borne pathogens, including *Escherichia coli*, *Salmonella*, and *Bacillus cereus*. Carvacrol has high antioxidant activity and has been successfully used, mainly associated with thymol, as dietary phytoadditive to improve animal antioxidant status. The anticancer properties of CV have been reported in preclinical models of breast, liver, and lung carcinomas, acting on proapoptotic processes. Besides the interesting properties of CV and the toxicological profile becoming definite, to date, human trials on CV are still lacking, and this largely impedes any conclusions of clinical relevance.

KEYWORDS

anticancer, antimicrobial, antioxidant, carvacrol, essential oil

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1 | INTRODUCTION

The rate of development of new antimicrobial agents for clinical applications has declined dramatically during the last decades (Bassetti, Merelli, Temperoni, & Astilean, 2013; J. Sharifi-Rad, 2016; J. Sharifi-Rad, Myaner, et al., 2016). Natural plant-derived compounds could represent interesting alternatives to synthetic molecules as recently reported by several studies and reviews (Bagheri, Mirzaei, Mehrabi, & Sharifi-Rad, 2016; Burt, Ahad, Kinjet, & Santos, 2013; Butler, Robertson, & Cooper, 2014; Harvey, Edrada-Ebel, & Quinn, 2015; Sahraie-Rad, Izadyari, Rakizadeh, & Sharifi-Rad, 2015; Stojanović-Radić, Pejčić, Stojanović, Sharifi-Rad, & Stanković, 2016; J. Sharifi-Rad, Salehi, Stojanović-Radić, et al., 2017; Salehi et al., 2017). Essential oils (EO) have a great potential for the treatment of infections, especially related to antioxidant activity (Azzimonti et al., 2015; Krishnaiah, Sarbatly, & Nithyanandam, 2011; Rojas, Ochoa, Ocampo, & Mu oz, 2006; J. Sharifi-Rad, 2016; J. Sharifi-Rad, Ayatollahi, et al., 2017; J. Sharifi-Rad, Soufi, et al., 2016; J. Sharifi-Rad, Sureda, et al., 2017; M. Sharifi-Rad, Varoni, et al., 2017). Essential oils are liquid, volatile, limpid, and rarely colored; can be lipid soluble and soluble in organic solvents; and generally have densities lower than that of water (Bakkali, Averbeck, Averbeck, & Idaomar, 2008; J. Sharifi-Rad, Sureda, et al., 2017).

Carvacrol (CV; $C_{10}H_{14}O$) is a liquid phenolic monoterpenoid, 2-methyl-5-(1-methylethyl)phenol, present in EO of oregano (*Origanum vulgare*), thyme (*Thymus vulgaris*), pepperwort (*Lepidium flavum*), wild bergamot (*Citrus aurantium* var. *bergamia* Loisel), and other plants (Fachini-Queiroz et al., 2012; Kintzios, 2002; Lisin, Safiyev, & Craker, 1997; Tang, Chen, & Wang, 2011). Commercial CV is synthesized by chemical and biotechnological methods (Yadav & Kamble, 2009). It is also named 5-isopropyl-2-methylphenol by the International Union of Pure and Applied Chemistry. Carvacrol has lipophilic properties and a density of 0.976 g/ml at room temperature (25 °C); it is insoluble in water but highly soluble in ethanol, acetone, and diethyl ether (Yadav & Kamble, 2009). As recently reviewed by several authors (Baser, 2008; Suntres, Coccimiglio, & Alipour, 2015), this compound possesses a wide range of biological activities, including antibacterial and antifungal (Chavan & Tupe, 2014; Du et al., 2008; Nostro et al., 2004), antiviral (Gilling, Kitajima, Torrey, & Bright, 2014; Sánchez, Aznar, & Sánchez, 2015), antioxidant (Aristatile, Al-Numair, Al-Assaf, & Pugalendi, 2011; Guimarães et al., 2010; Milos & Makota, 2012), and anticarcinogenic properties (Arunasree, 2010; Ozkan & Erdogan, 2011; Q. H. Yin et al., 2012; Figure 1). The main findings have been summarized in Table 1. Due to the flavoring and antimicrobial activities, in particular, CV has been proposed as a natural food preservative for the food industry (Daferera, Ziogas, & Polissiou, 2003; Solórzano-Santos & Miranda-Novales, 2012; Salehi, Mishra, et al., 2018). In addition, CV, as a main constituent of plant extracts derived from *Zataria multiflora* Boiss, *Satureja hortensis*, and *O. vulgare*, improves the immune response (Boskabady & Gholami Mahtaj, 2015; Boskabady & Jalali, 2013; Boskabady, Tabatabaee, & Jalali, 2014; Khazdair, Ghorani, Alavinezhad, & Boskabady, 2018), mediating the antimicrobial, antioxidant, anti-inflammatory, and anticancer properties of these plants (Burt et al., 2016; Fitsiou et al., 2016; Hashemipour et al., 2013; Kim, Lillehoj, Lee, Jang, & Bravo, 2010; Lesjak et al., 2016; Mahtaj, Feizpour, Kianmehr, Soukhtanloo, & Boskabady, 2015; Oca a-Fuentes, Arranz-Gutiérrez, Se orans, & Reglero, 2010; Rajput et al., 2017; Silva et al., 2012). The major aim of this review was to provide the most updated evidence, including clinical trials, of CV in human health, with a focus on its pharmacokinetics and safety profile and antibacterial, antioxidant, and anticancer activities.

2 | METHODS

This narrative review was performed by consulting the database of PubMed, Web of Science, Embase, and Google Scholar (as a search engine) to retrieve the most updated articles on the topic under investigation (bioactivity of CV for human health). The strategy of the search included the use of the following keywords: "carvacrol" and

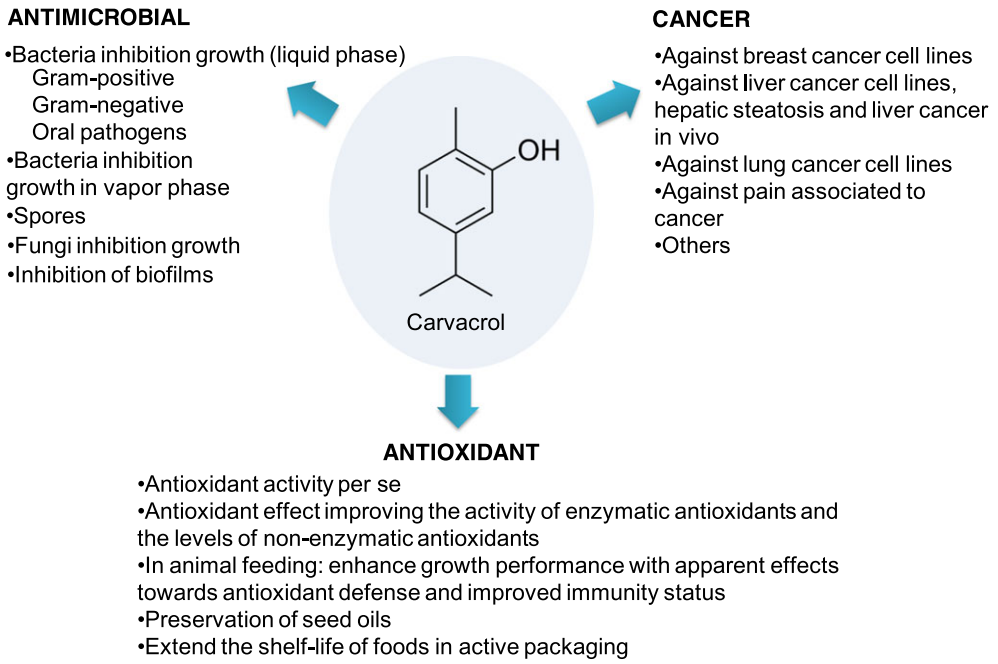

FIGURE 1 Bioactivity of carvacrol focuses on its antimicrobial, antioxidant and anticancer properties [Colour figure can be viewed at wileyonlinelibrary.com]

TABLE 1 Antimicrobial, antioxidant, and anticancer properties of carvacrol, when administered as a unique active ingredient

Biological activity		References
Antimicrobial activity	*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumonia*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Enterobacter* spp., and *Serratia* spp.	Bnyan, Abid, and Obied (2014)
	Aspergillus niger, *Aspergillus flavus*, *Alternaria alternata*, *Penicillium rubrum*, *Trichoderma viride*, *Candida* spp., and dermatophytes	Pina-Vaz et al. (2004)
	Bacillus cereus	Ultee, Slump, Steging, and Smid (2000)
	E. coli O157:H7 and *Salmonella*	Obaidat and Frank (2009)
	Listeria monocytogenes	Karatzas, Kets, Smid, and Bennik (2001)
	S. aureus	de Oliveira, Stamford, Gomes Neto, and de Souza (2010)
	Candida albicans	Čabarkapa et al. (2015); Dalleau, Cateau, Bergès, Berjeaud, and Imbert (2008)
	Pseudomonas aeruginosa	Soumya et al. (2011)
	Haemophilus influenzae in rats	Kristinsson, Magnusdottir, Petersen, and Hermansson (2005)
	A. flavus and *Aspergillus parasiticus* in poultry (animal feed)	Sridhar, Thammaiah, and Suganthi, (2016); H. B. Yin, Chen, Kollanoor-Johny, Darre, & Venkitanarayanan (2015)
Antioxidant activity	Hepatoprotective effects	Alma, Mavi, Yildirim, Digrak, & Hirata (2003); Aristatile, Al-Numair, Veeramani, and Pugalendi (2009)
	DNA protection	Slamenová, Horváthová, Sramková, and Marsálková (2007); Undeğer, Başaran, Degen, and Başaran (2009)
	Antioxidant activity mediates anticancer effects	Baranauskaite et al. (2017)
	Increase in antioxidant defense leads to improved immune system response	Giannenas et al. (2012); Hashemipour, Kermanshahi, Golian, and Veldkamp (2013)
	Management of pancreatitis in animal models	Bakır et al. (2016)
	Reduction of oxidative stress damage in the brain, liver, and kidney of rats	Samarghandian, Farkhondeh, Samini, and Borji (2016)
	Amelioration of intestinal dysfunction due to oxidative stress in piglets (animal feed)	Wei, Xue, Zhou, and Peng (2017)
	Improved parameters associated with mycotoxin toxicity in broiler chickens: growth performance, liver weight, transaminases, antioxidant enzymes, total antioxidant capacity, and malondialdehyde (animal feed)	Sridhar et al. (2016)
Anticancer activity	Cytotoxic, genotoxic, and proapoptotic activities with effects on cell invasion by decreasing the expression of matrix metalloprotease 2 and 9 (melanoma cell, larynx, colon, gastric, leiomyosarcoma cells, and chronic myeloid leukemia cells), K562, A549 non-small-cell lung cancer cells, MCF-7, and MDA-MB-231 human metastatic breast cancer cells	Arunasree (2010); Koparal and Zeytinoglu (2003); Fan et al. (2015); Günes-Bayir et al. (2017); He, Mo, Hadisusilo, Qureshi, and Elson (1997); Karkabounas et al. (2006); Lampronti, Saab, and Gambari (2006); Stammati et al. (1999); Q. H. Yin et al. (2012)
	Reactive-oxygen-species-induced apoptosis in hepatocarcinoma cells and prostate carcinoma cells	Alma et al. (2003); Elshafie et al. (2017); Khan, Khan, Farooqui, & Ansari (2017); Suntres et al. (2015); Q. H. Yin et al. (2012)
	Chemoprevention of liver and lung carcinoma in animal models	Jayakumar et al. (2012); Zeytinoğlu, Aydin, Oztürk, and Baser (1998)

"anticancer" or "antimicrobial" or "antioxidant" or "toxicity." Authors carefully examined articles in order to identify their strengths and weaknesses (West et al., 2002) and selected the more useful ones for the review, prioritizing the articles published from 2012 to 2017. Only English articles having full text were considered.

3 | RESULTS

3.1 | Pharmacokinetics—absorption, distribution, metabolism, and excretion of CV

According to studies performed in rabbits, CV is slowly absorbed in the intestine after oral administration (1.5 g), with more than 30% remaining in the gastrointestinal tract and about 25% of the total dose excreted in urine 22 hr later (Suntres et al., 2015). A further study, where different doses of CV derived from sesame oil were orally administered to rats (500 mg) and rabbits (1,500 and 5,000 mg), showed this compound to be mainly distributed in the stomach, intestines, and urine, with small amounts in the lung, liver, and muscle tissues (Schroder & Vollmer, 1932). In pigs, intestinal delivery of CV was greatly improved by alginate–whey protein microcapsules, that is, 250 and 800 μm in diameter, containing 72 and 76 g/kg of CV, respectively (Q. Wang, Gong, Huang, Yu, & Xue, 2009). Results showed over 95% of unencapsulated CV was absorbed or metabolized in the stomach and the duodenum, whereas microcapsules completely released the compound in the intestinal tract; larger microcapsules, in particular, showed a better recovery in the small intestine than smaller ones (Q. Wang et al., 2009). Therefore, orally ingested unencapsulated CV was mostly absorbed or metabolized in the upper gastrointestinal tract of pigs, whereas alginate–whey protein microcapsules effectively minimized the absorption of CV in the stomach and proximal intestine and increased the percentage of CV delivered to the distal small intestine (Zhang et al., 2016).

Carvacrol metabolism occurs, according to Austgulen, Solheim, and Scheline (1987), following two types of pathways. The main metabolic route is the conjugation of the phenolic group with glucuronic acid ($C_6H_{10}O_7$) and sulphate (SO_4^{2-}), but when administered at low levels, the metabolism of CV includes the oxidation of the terminal methyl groups to primary alcohols (Austgulen et al., 1987). These authors also showed that CV (1 mmol/kg) is excreted in urine, as such or as its glucuronide and sulphate conjugates in Albino rats (Austgulen et al., 1987); consistently, CV is a substrate of the uridine 5′-

diphospho-glucuronosyltransferase isoform 1A4 substrate (Smith, Sorich, McKinnon, & Miners, 2003). The extensive oxidation of CV methyl groups, however, resulted in the production of some derivatives such as benzyl alcohol and 2-phenylpropanol with their corresponding carboxylic acids (Suntres et al., 2015). A minor metabolite produced by ring hydroxylation was also detected (Alagawany, El-Hack, Farag, Tiwari, & Dhama, 2015).

In particular, Dong et al. investigated the role of cytochrome P450 in the metabolism of CV and its isomer thymol (TH), using human liver microsomes. They found that CYP2A6 was the main drug-metabolizing enzyme and detected new metabolites that resulted from the oxidation of CV (Dong et al., 2012). Through gastric fermentation simulation experiments in piglets, 29% degradation of CV occurred in the cecum, and not in the jejunum, but when the animals were treated with oral doses of 13.0, 13.2, 12.5, and 12.7 mg/kg of body weight of the compound, they showed a half-life between 1.84 and 2.05 hr throughout the digestive tract (Michiels et al., 2008). These results suggested an almost complete absorption of CV in the stomach and the proximal small intestine. Plasma levels peaked at 1.39 hr followed by a peak in urine (time of maximum response = 2 hr; Michiels et al., 2008). The cumulative absorption of CV and TH in the proximal small intestine was higher than 90%, without being affected by dose and formulation administered to piglets (Michiels et al., 2010).

To date, no in human evidences are available; thus interspecies differences in bioavailability, metabolism, and distribution should be taken into account.

3.2 | Safety profile of CV

Essential oils may have beneficial effects such antimicrobial, antioxidant, and antimutagenic or antigenotoxic effects, but besides these properties, EOs may also have potential toxic effects such as mutagenicity and genotoxicity (Llana-Ruiz-Cabello, Pichardo, et al., 2015). Mutagenicity and genotoxicity of CV have been demonstrated, using intestinal cell line Caco-2, when applied at high concentrations (460 μM), as it produced DNA damage at the level of purine bases (Llana-Ruiz-Cabello et al., 2014), although no effects could be found in Chinese hamster lung fibroblast, human hepatocytes, and human lymphocytes (Llana-Ruiz-Cabello et al., 2014; Maisanaba et al., 2015).

Suntres et al. (2015) reviewed data on CV toxicology, reporting the median lethal doses of CV: In rats, the dose was 810 mg/kg when orally administered and 80 and 73 mg/kg when intravenously or intraperitoneally injected, respectively; in mice, 110–233.3 mg/kg CV led to death, after inducing ataxia and somnolence. The median lethal dose in rabbits has been reported to be 2,700 mg/kg following dermal application, whereas it has reported to be 680 mg/kg following subcutaneous administration in mice and 310 mg/kg following intravenous administration in dogs (Suntres et al., 2015).

3.3 | Antimicrobial activity of CV

3.3.1 | In vitro studies

Antibacterial activity of CV against numerous strains, including *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pneumonia*, *Escherichia coli*, *Klebsiella pneumonia*, *Proteus mirabilis*, *Enterobacter* spp., and *Serratia* spp., has been studied. Carvacrol has a high inhibitory effect against both Gram-positive and Gram-negative bacteria, except *Pseudomonas aeruginosa* (Bnyan et al., 2014). In another study, the antibacterial activity of oregano EO against *Clostridium perfringens*, *P. aeruginosa*, and *S. aureus* has been reported (Andreou et al., 2011; Lambert, Skandamis, Coote, & Nychas, 2001) and it was associated with the presence of CV and TH (Rodriguez-Garcia et al., 2016).

Botelho et al. (2007) investigated the antibacterial activity of the *Lippia sidoides* EO and its major compounds (TH and CV; Guimarães, da Silva, Reis, Costa, & Alves, 2015), using four strains of cariogenic bacteria (*Streptococcus mutans*, *Streptococcus sanguinis*, *Streptococcus salivarius*, and *Streptococcus mitis*) and one yeast strain (*Candida albicans*), indicating strong antibacterial and antifungal activity. This was the first report supporting antimicrobial activity of this EO and its constituents against oral pathogens; in particular, the yeast *C. albicans*, frequently associated with infections in HIV(+) patients, and *S. mutans* were the most sensitive among all tested microorganisms (Botelho et al., 2007).

Carvacrol is also effective against various fungi such as *Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternata*, *Penicillium rubrum*, *Trichoderma viride*, *Candida* spp., and dermatophytes (Pina-Vaz et al., 2004). This activity is extended in the case of fungal plant pathogens such as *Colletotrichum acutatum*, *Colletotrichum fragariae*, and *Colletotrichum gloeosporioides* as indicated by using the direct overlay bioautography assay (Nostro & Papalia, 2012).

The food-borne pathogen *Bacillus cereus* has also been used to test the antimicrobial activity of CV. *Bacillus cereus* is a spore-forming, motile and facultative anaerobic, Gram-positive rod. Vegetative cells of *B. cereus* can be inactivated easily by heating, but spores can survive and cause food intoxication. Carvacrol can inhibit the growth of *B. cereus* at concentrations ≤0.4 mM, although without bactericidal effects (Ultee et al., 2000), and at a concentration of 0.06 mg/ml, it can cause a sharp decline (80%) in diarrheal toxin production. The mechanism of inhibition of toxin production is still unclear and could be associated with gene regulation, transcription, or translation or transport and excretion of the toxin. Excretion of the toxin, in particular, has been suggested to be a relevant target for CV: Being an active process and therefore energy dependent, CV would inhibit adenosine triphosphate (ATP) synthesis by dissipating the proton motive force (Ultee, Kets, & Smid, 1999). Along these lines, one study suggested the use of CV in food products, at concentrations below the minimum inhibitory concentration (MIC) value, against toxin production by *B. cereus* and to enhance food safety (Ultee & Smid, 2001). Similarly, the efficacy of CV vapor was successfully tested against the food-borne bacteria *E. coli* O157: H7 and *Salmonella* on the surface of freshly produced vegetables such as lettuce, spinach, and tomatoes (Obaidat & Frank, 2009).

The sensitivity of bacteria to CV may be influenced by factors such as pH, proteins, fats, salts, temperature, and preservative conditions. Therefore, various methods and treatments are required to reduce the adverse effects and reduce required doses (Veldhuizen, Creutzberg, Burt, & Haagsman, 2007). Karatzas et al. (2001) reported the effect of CV, cinnamaldehyde, and TH, along with mild heat treatment against *Listeria monocytogenes*. In another study, de Oliveira et al. (2010) examined the potential effects of combinations of CV with weak organic acids such as lactic and acetic acids against *S. aureus*.

The relation between the chemical structure and the antimicrobial activity of CV was investigated by comparing the antimicrobial activity of CV, eugenol, menthol, and two synthesized CV derivative compounds, CV methyl ether and carvacryl acetate, against several bacteria, *E. coli*, *Pseudomonas fluorescens*, *S. aureus*, *Lactobacillus plantarum*, *Bacillus subtilis*; a yeast, *Saccharomyces cerevisiae*; and one fungus, *Botrytis cinerea*. Carvacrol, being the most hydrophobic compound, showed stronger antibacterial activity than eugenol and menthol. The two synthesized compounds, carvacryl acetate and CV methyl ether, were not effective, suggesting that the specific structural features of the CV molecule are key to its bioactivity and the presence of a free phenolic hydroxyl group coupled with its hydrophobicity is essential for antimicrobial activity (Nostro & Papalia, 2012; Ben Arfa, Combes, Preziosi-Belloy, Gontard, & Chalier, 2006).

In addition, CV has been tested against bacteria growing in biofilm, a well-organized, bacterial structure able to reduce the efficacy towards antibiotic. Carvacrol could inhibit the specific processes involved in the initial phase of biofilm formation and prevent the establishment of mature biofilms and reduce the risk of antibiotic drug resistance. In order to investigate the antibiofilm action of EO containing CV and TH, their effect was tested on both the initial cell attachment by planktonic cells as well as on preformed biofilms of *C. albicans*. *Candida* biofilms are remarkably less sensitive to commonly used antifungals (Dalleau et al., 2008). Čabarkapa et al. (2015) have shown a dose-dependent inhibition of CV in the initial cell attachment. Concerning the potential mechanism of action, CV could target the ergosterol biosynthesis pathway by perforating the cell membrane and therefore disturbing its biosynthesis, besides reacting with the membrane itself by means of the reactive hydroxyl moiety (Čabarkapa et al., 2015). Among bacteria, *P. aeruginosa* has a high propensity to develop biofilms. *Pseudomonas aeruginosa* is a Gram-negative microorganism involved in respiratory infections, urinary tract infections, gastrointestinal infections, keratitis, and otitis media and responsible for an estimated 10–20% of all hospital-acquired infections. Carvacrol and TH can interfere with the starting phases of adherence as well as with *P. aeruginosa* biofilms. Carvacrol (2 × MIC) inhibition exceeds 90% for *P. aeruginosa* (ATCC 27853) and *P. aeruginosa* (IL5) biofilm, as the relative hydrophilicity of CV allows its diffusion through the polar polysaccharide matrix (Soumya et al., 2011). A further relevant nosocomial pathogen is staphylococci. The activity of oregano EO, CV, and TH on biofilm-grown *S. aureus* and *S. epidermidis* strains has been investigated. The in vitro activity of the oils on biofilms was only slightly lower than that on planktonic culture: For most of the strains, the concentration of CV required to inhibit biofilm formation was twofold or fourfold greater than the value needed in suspension; bacterial cell growth was inhibited by the presence of CV, and biofilm formation was reduced (Nostro et al., 2007).

Carvacrol and TH, being hydrophobic, can interfere with the lipid bilayer of cytoplasmic membranes of bacteria, bringing loss of integrity and increasing its fluidity and permeability and leakage of cellular material such as ions, ATP, and nucleic acids (Helander et al., 1998; Lambert et al., 2001; Ultee et al., 1999). The hydrophobicity of a compound is a criterion related to the extent of membrane damage. This can be examined by its partition coefficient in octanol–water (P). The logP of CV and TH are 3.64 and 3.30, respectively (Ultee, Bennik, & Moezelaar, 2002). Compounds with a logP value higher than 3 will partition deeply in the cell membrane (Nostro et al., 2007). However, a relative hydrophilicity of CV may favor its diffusion through the polar polysaccharide matrix of biofilms (Soumya et al., 2011). Carvacrol can also be responsible for accelerating the depletion of the intracellular ATP pool (Ultee et al., 1999), either by reduction of ATP synthesis or by an increase in ATP hydrolysis (Nostro & Papalia, 2012).

Lambert et al. (2001) propose that the main mechanism of action is to disintegrate the outer membrane of bacterial cells, by releasing the lipopolysaccharides and increasing the permeability of the cytoplasmic membrane to ATP (Bnyan et al., 2014). On the other hand, Bakkali et al. (2008) demonstrated that in eukaryotic cells, EO with CV as one of its component act as prooxidants affecting inner cell membranes and organelles such as mitochondria. They present cytotoxic effects on living cells but are usually nongenotoxic (Bakkali et al., 2008).

Recent research has been carried out on CV as a potential codrug against bacterial biofilm. Ten CV codrugs have been synthesized by linking the CV hydroxyl group to the carboxyl moiety of sulphur-containing amino acids via an ester bond (Figure 2), developing novel compounds with improved antimicrobial and antibiofilm activities and reduced toxicity compared with CV alone (Cacciatore et al., 2015). Among these derivatives, all CV codrugs revealed successful antifungal activity against *C. albicans* ATCC 10231, but generally with lower potency than CV. The novel CV codrugs showed no evidence of human blood hemolysis at their MIC values (below 50%) in cytotoxicity assay, except two codrugs (codrugs 8 and 9; MIC values over 50%). Further experiments performed using the most active CV codrug, that is, Ac-Cys(allyl)-CV, demonstrated higher antibacterial effect against the mature biofilm of *E. coli* ATCC 8739 compared with CV (Cacciatore et al., 2015).

3.3.2 | In vivo studies

Kristinsson et al. (2005) studied vapors of *Ocimum basilicum* EO and CV, TH, and salicylaldehyde as its components: It was effective against acute otitis media caused by pneumococci or *Haemophilus influenzae*, healing 56–81% of rats infected with *H. influenzae* and 6–75% of rats infected with pneumococci, compared with 5.6–6% of rats in the placebo group.

Carvacrol has also been suggested as a feed additive to control aflatoxin contamination in poultry feed. Indeed, CV (from 0.02% to 0.08%) significantly reduced *A. flavus* and *Aspergillus parasiticus* growth and aflatoxin production in broth culture (H. B. Yin et al., 2015). It is noteworthy that CV (from 0.4% to 1.0%) significantly decreased aflatoxin production in poultry feed (200-g portions) inoculated with both fungi by 60%, compared with controls. In particular, CV significantly downregulated the expression of major genes involved in aflatoxin biosynthesis in fungi (H. B. Yin et al., 2015).

3.4 | Antioxidant effect of CV

3.4.1 | In vitro studies

An imbalance between the reactive oxygen species and detoxifying the reactive intermediates via the biological system's ability causes oxidative

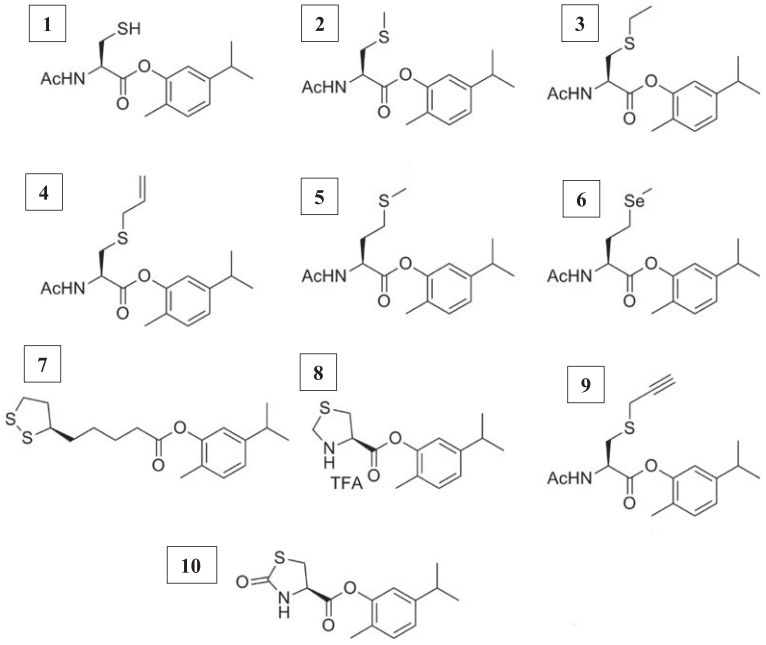

FIGURE 2 Chemical structures of carvacrol codrugs **1**–**10** obtained by linking the carvacrol hydroxyl group to the carboxyl moiety of sulphur-containing amino acids via an ester bond (Cacciatore et al., 2015)

stress (Apel & Hirt, 2004; Finkel & Holbrook, 2000). Free radical species lead to oxidative damage of different molecules in cells, such as proteins, lipids, and nucleic acids. Essential oils as natural antioxidants found in many plants can reduce oxidative damage and prevent mutagenesis, carcinogenesis, and aging due to their radical-scavenging activities (Alma et al., 2003; Coccimiglio, Alipour, Jiang, Gottardo, & Suntres, 2016; J. Sharifi-Rad, Salehi, Varoni, et al., 2017; J. Sharifi-Rad, Salehi, Schnitzler, et al., 2017; M. Sharifi-Rad, Mnayer, et al., 2018). Remarkably, CV presents higher antioxidant activity than other common volatile constituents of EO, with the exception of TH and eugenol (Dorman, Surai, & Deans, 2000). Moreover, an antioxidant synergism between TH and CV exists (Milos & Makota, 2012), as found in some Lamiaceae plants. In addition, CV can induce a significant hepatoprotective and antioxidant effect improving the activity of enzymatic antioxidants (superoxide dismutase, catalase, and glutathione peroxidase) and the levels of nonenzymatic antioxidants (vitamin C, vitamin E, and reduced glutathione), as demonstrated in the plasma of rats with D-galactosamine-induced hepatotoxicity (Aristatile et al., 2009).

Other studies suggested a lack of clastogenic activity for CV and TH at biologically relevant concentrations, a moderate antioxidant activity, and DNA protective effects in vitro (Slamenová et al., 2007; Undeğer et al., 2009). Alternatively, studies on drug-resistant H1299 cells have shown that CV and TH showed high cytotoxic effects (Llana-Ruiz-Cabello et al., 2015; Ozkan & Erdogan, 2012). The high amount of CV in *Origanum onites* L. showed antioxidant and anticancer activity more against the triple-negative breast cancer MDA-MB-231 cell line than the human glioblastoma U87 cell line (Baranauskaite et al., 2017).

Interestingly, as an alternative to synthetic antioxidants, CV can preserve the quality of seed oils, for example, inhibiting the formation of oxidative deterioration products and undesirable off-flavors (Quiroga, Asensio, & Nepote, 2015). It also has potential in polypropylene active packaging to extend the shelf-life of food products (Ramos, Beltran, Peltzer, Valente, & Garrigos, 2014). Another possibility is the use of CV in animal feed to improve health and production. In this sense, the use of TH and CV in poultry and fish feeding enhanced growth performance with apparent effects towards antioxidant defense and improved immune system (Giannenas et al., 2012; Hashemipour et al., 2013).

3.4.2 | In vivo studies

A study in animal models has shown that CV may improve acute pancreatitis through its antioxidative mechanisms (Bakır et al., 2016). A recent study on rats revealed that CV treatment ameliorated the oxidative stress damage in the brain, liver, and kidney (Samarghandian et al., 2016).

As animal feed, dietary supplementation with 100 mg/kg of a CV–TH (1:1) blend for 14 days reduced weaning-induced intestinal oxidative stress and inflammation in piglets, by decreasing mRNA levels of tumor necrosis factor α. Indeed, during weaning, piglets suffer intestinal dysfunction that compromises their performance. In addition, weaning stress decreases *Lactobacillus* population and increases *Enterococcus* spp. and *E. coli*. It is noteworthy that the CV–TH blend increased *Lactobacillus* spp. while decreasing the *Enterococcus* and *E. coli* populations (Wei et al., 2017).

Male broiler chickens fed with basal maize–soy diet contaminated with 1.0 ppm of aflatoxin B1 and supplemented with 1.0% CV showed improved parameters associated with mycotoxin toxicity, compared with controls, that is, growth performance, liver weight, transaminases, antioxidant enzymes, total antioxidant capacity, and malondialdehyde (Sridhar et al., 2016). An equal mixture of CV and TH (at 0, 60, 100, and 200 mg/kg of diet) was administered to broiler chickens for 42 days (Hashemipour et al., 2013). Feed supplementation with the highest concentration of phytogenic product significantly enhanced animal performance, increased superoxide dismutase and glutathione peroxidase activities, and decreased malondialdehyde level in the muscle, serum, and liver. Carvacrol + thymol also significantly reduced total saturated fatty acids and raised total unsaturated fatty acids in serum and muscle, compared with the control diet. In addition, intestinal and pancreatic trypsin, lipase, and proteinase activities were

significantly improved in the group supplemented with the phytogenic product, compared with the control group; also, the immune function of broilers was enhanced by increasing hypersensitivity response and total and IgG antisheep red blood cell titers and decreasing the heterophil-to-lymphocyte ratio. Therefore, the authors concluded that feed supplementation with CV + TH enhanced performance, increased antioxidant enzyme activities, retarded lipid oxidation, raised digestive enzyme activities, and improved immune response of broiler chickens (Hashemipour et al., 2013). The same authors also demonstrated that CV + TH, at 100 and 200 mg/kg of diet, administered to broilers up to 42 days, significantly increased body weight gain and improved feed conversion ratio. Furthermore, the animal diet with this blend included significantly decreased digesta viscosity and serum total cholesterol and increased plasma aspartate amino transferase, total protein, albumin, and globulin, compared with the control diet. The authors concluded that the addition of CV + TH to viscose-based diet might alleviate the detrimental effects of viscous compounds in poultry diets (Hashemipour, Kermanshahi, Golian, & Khaksar, 2014).

Some EO constituents, including CV, have also been investigated in fish nutrition. Similar results previously reported on poultry were obtained in this field, that is, improved fish resistance to diseases, growth, and feed utilization (Sutili, Gatlin, Heinzmann, & Baldisserotto, 2017). Rainbow trout (*Oncorhynchus mykiss*) juveniles fed diets containing from 1.0 to 3.0 g/kg of CV–TH showed significantly higher final weight and growth, food conversion ratio, lymphocyte number, whole-body lipid and protein contents than the control group (Ahmadifar, Falahatkar, & Akrami, 2011). In the same fish species, CV (12 g/kg) inclusion in aquafeeds improved growth performance, gut microbiota, and antioxidant status (Giannenas et al., 2012).

In summary, despite the lack of any information on CV in human nutrition, its application in animal science is well documented. In particular, CV has been successfully used as a component (phytoadditive) of feed supplements to improve animal health and performance.

3.5 | Anticancer effect of CV

3.5.1 | In vitro studies

Several reports have shown that CV exhibits strong cytotoxic, genotoxic, and proapoptotic activity against cancer cells, in a dose-dependent manner, also with effects on cell invasion by reducing the expression of matrix metalloprotease 2 and 9 (Fan et al., 2015). A plethora of cancer cells, indeed, have been tested, including mouse B16 melanoma (He et al., 1997), Hep-2 human larynx carcinoma cells (Stammati et al., 1999), gastric carcinoma cells (Günes-Bayir et al., 2017) leiomyosarcoma cells (Karkabounas et al., 2006), chronic myeloid leukemia cells, K562, A549 non-small-cell lung cancer cells, MDA-MB-231 human metastatic breast cancer cells (Arunasree, 2010; Baranauskaite et al., 2017; He et al., 1997; Koparal & Zeytinoglu, 2003; Lampronti et al., 2006), and human colon cancer cells (Fan et al., 2015).

Effects of CV on breast cancer have been reported in several studies (Arunasree, 2010; Q. H. Yin et al., 2012). The translational product of caspase-9 gene is an indicator of initiation of apoptosis, while caspase-3 is the downstream caspase that plays a crucial role in the terminal phase of apoptosis (Y. J. Wang, Niu, Yang, Han, & Ma, 2013). Moreover, the p53 gene plays a role in regulating apoptosis by interacting with the Bcl-2 family and upregulating the expression of the Bax gene via direct transcriptional activation of the Bax promoter with concomitant downregulation of the Bcl-2 gene. In a report by Al-Fatlawi, Irshad, Zafaryab, Rizvi, and Ahmad (2014), a downregulation of the Bcl-2 gene and dose-dependent upregulation of the Bax gene was observed in CV-treated MCF-7 cells, a chemosensitive breast cancer cell line. A similar result was reported in B16 melanoma cells in mouse when treated with CV (He et al., 1997). The mechanism of apoptosis has been explained in terms of alteration of membrane stability, causing the release of mitochondrial apoptosis initiation factors, apoptosis protease-activating factor (Apaf1), and cytochrome c into the cytosol, to activate caspase-9, caspase-3, and caspase-7 enzymes. Carvacrol-treated MCF-7 cells caused upregulation of caspase-3, caspase-6, and caspase-9 genes, compared with the untreated controls. This study illustrates that a possible mechanism of apoptosis induced by CV is through p53 and the mitochondrial pathway (Al-Fatlawi et al., 2014).

Arunasree did a similar study in 2010 to investigate the molecular mechanism involved in the antitumor activity of CV against metastatic breast cancer cells, MDA-MB-231 (Arunasree, 2010). Carvacrol clearly induced apoptosis in MDA-MB-231 cells in a dose-dependent manner with a half maximal inhibitory concentration of 100 μM; this resulted in a decrease in the mitochondrial membrane potential of the cells, causing the release of cytochrome c from mitochondria, caspase activation, and finally cleavage of poly-ADP-ribose polymerase (PARP; Arunasree, 2010). Flow cytometric analysis of CV-treated cells showed an increase in the sub-G0/G1 phase (apoptotic peak) of the cell cycle and a decrease of cells at the S phase, indicating the induction of apoptosis and inhibition of DNA synthesis in the S phase (Zeytinoglu, Incesu, & Baser, 2003).

The chemopreventive role of CV can be ascribed also to the effect of CV on hepatic steatosis, a condition that may cause steatohepatitis (hepatic steatosis with inflammation), fibrosis, and ultimately cirrhosis, a well-known risk factor for hepatocellular carcinoma (HCC). Hepatocellular carcinoma accounts for about 90% of all liver cancer terms, the most frequent primary malignancy of the liver. Antioxidant property is one of the major properties playing a key role in HCC tumorogenesis. Carvacrol and those EO that are rich in CV have shown strong antioxidant properties parallel to those of ascorbic acid, butyl hydroxytoluene, and vitamin E (Alma et al., 2003). Evidence exists about the activity of CV against Hep G2 HCC cells, inducing cell apoptosis by means of activation of caspase-3, cleavage of PARP, and decreased Bcl-2 gene expression (Q. H. Yin et al., 2012). In addition, CV has been shown to affect cell proliferation, by selectively altering the phosphorylation state of members of the MAPK superfamily and decreasing the phosphorylation of ERK1/2 in a dose-dependent manner, as well as activating phosphorylation of p38 (Elshafie et al., 2017; Suntres et al., 2015; Q. H. Yin et al., 2012). Similar effects were found in human prostate cancer cells, where CV induced reactive-oxygen-species-mediated apoptosis along with cell cycle arrest at G0/G1 in human prostate cancer cells (Khan et al., 2017).

Interestingly, CV showed stronger effects on HCC cells compared with normal human fetal liver cells, with no cytotoxicity to normal cells (Q. H. Yin et al., 2012), which similarly occurred with lung carcinoma and normal cells (Koparal & Zeytinoglu, 2003).

The non-small-cell lung carcinoma includes approximately 75% of lung cancers (Fossella et al., 2000). In recent years, the interest towards induction of apoptosis as a strategy to produce antitumor drugs has increased (Mishra et al., 2018). Finding an antitumor drug with an apoptosis-inducing agent and specificity for tumor cells may be ideal (Salehi et al., 2018). Cytotoxicity of CV towards a human non-small-cell lung carcinoma cell line (A549) was reported by Koparal and Zeytinoglu (2003). Carvacrol-treated cells showed some apoptotic characteristics as well as morphological changes such as cytoplasmic shrinkage and loss of cell–cell contacts, with dose dependence at 500 and 1,000 μM (Koparal & Zeytinoglu, 2003). Another report indicated these morphological changes as condensation and fragmentation of the cytoplasm and nuclear chromatin. The number of cells as well as protein content was decreased by CV (Koparal & Zeytinoglu, 2003).

3.5.2 | In vivo studies

The chemopreventive nature of CV during diethylnitrosamine-induced liver cancer in male Wistar albino rats was investigated by Jayakumar et al. (2012) who found CV pretreatment prevented significantly the appearance of liver foci and nodules. Results of this study showed CV to have potent free radical scavenging and antioxidant activities, to be able to modify the levels of lipid peroxidase, and to significantly elevate the endogenous antioxidant defense mechanism in diethylnitrosamine-induced hepatocellular carcinogenesis (Jayakumar et al., 2012). Mice with hepatitis fed with a CV-supplemented, high-fat diet for 10 weeks were resistant to high-fat diet-induced hepatic lipid accumulation, as evaluated by histological analysis (Kim, Choi, Jang, & Park, 2013). Also, the level of gene expression involved in cholesterol homeostasis in the liver, such as sterol regulatory element-binding protein 2, was higher in CV-fed mice than in mice fed with a high-fat diet (Kim et al., 2013).

Carvacrol was also effective in animal model of carcinogenesis, such as against 7,12-dimethylbenzanthracene-induced lung tumors in rats at 0.1-mg/kg intraperitoneal dose (Zeytinoğlu et al, 1998).

Besides anticancer activities, CV may also have a role in reducing adverse effects of chemotherapy. The anticancer drug irinotecan hydrochloride can induce intestinal mucositis, triggering inflammation and cell damage via the transient receptor potential cation channel, subfamily A, member 1 receptor. Carvacrol is an agonist of the transient receptor potential cation channel, subfamily A, member 1 receptor and was effective, in a mice model, in reducing inflammation biomarkers, such as nuclear factor κB and cyclooxygenase-2, and oxidative stress in terms of glutathione, malondialdehyde, and NO_x levels (Alvarenga et al., 2016). The intestinal architecture of the small intestine was restored, which improved survival (Alvarenga et al., 2016).

3.6 | Pain management using CV

Pain is one of the most distressing symptoms in over half of all cancer patients (Schmidt, Hamamoto, Simone, & Wilcox, 2010). Carvacrol possesses analgesic and anti-inflammatory effects and is shown to be a modulator of central neurotransmitter pathways, such as dopaminergic, serotoninergic, and GABAergic systems, as well as releasing inflammatory mediators (Cavalcante Melo et al., 2012).

3.6.1 | In vivo studies

In a study on mice, CV showed 66.4% reduction in hyperalgesia, besides significantly reducing the paw volume with average inhibition percentages of 42.0% (Guimarães et al., 2014). Carvacrol has several mechanisms of action, especially in neuromodulation, acting on the brain nuclei such as the periaqueductal gray, nucleus raphe magnus, and locus coeruleus, via γ-aminobutyric acid receptors. So CV is a suitable candidate in the treatment of cancer pain (Gilbert et al., 2014).

3.6.2 | Clinical trial

Carvacrol is an agonist of the TRP subfamily V3, implicated in pain transmission related to heat. Using a half-tongue method in human volunteers, a study demonstrated that the irritant sensation triggered by CV was reduced by repeated applications, thus producing self-desensitization (Klein, Carstens, & Carstens, 2013).

4 | CONCLUSIONS

The present review has focused on the antimicrobial, antioxidant, and anticancer potentials of CV. The antimicrobial potential of CV is higher than that of other volatile compounds present in EO, and the presence of a free hydroxyl group, hydrophobicity, and the phenol moiety is crucial. Among microorganisms, it is effective against food-borne pathogens, including *E. coli*, *Salmonella*, and *B. cereus*, inhibiting their growth and the production of toxins. Moreover, it is also active against fungi and inhibits the formation of biofilms, for example, *Candida*. This antimicrobial activity has been demonstrated in both liquid and vapor phases. Concerning the antioxidant properties of CV, it has been shown not only in vitro but also in animal models of pancreatitis, hepatotoxicity, and liver cancer. Numerous reports have shown that CV can also exhibit a strong anticancer activity mainly in vitro, in which caspase-3, PARP, and Bcl-2 gene expression may be implied in the apoptosis mechanisms. However, there are few reports concerning animal models, so that more studies are required. Human studies on CV to evaluate its bioavailability and to understand its active forms and target tissues are needed. In this sense, evidence suggests that the metabolism of CV produces glucuronide and sulphate metabolites, and, at minor levels, oxidation products, but the bioavailability of CV seems to depend largely on the animal model chosen.

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