Biological and Pharmacological Activities of Carvacrol and Carvacrol Bearing Essential Oils

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Abstract: Oregano essential oils obtained from the genera *Origanum, Thymus, Coridothymus, Thymbra, Satureja* and *Lippia* are rich in carvacrol, a monoterpenic phenol isomeric with thymol. Turkey is the biggest exporter of oregano herb and oil to the world markets. Oregano is mainly used in food, spice and pharmaceutical industries. Carvacrol is responsible for the biological activities of oregano. Many diverse activities of carvacrol such as antimicrobial, antitumor, antimutagenic, antigenotoxic, analgesic, antispasmodic, antiinflammatory, angiogenic, antiparasitic, antiplatelet, AChe inhibitory, antielastase, insecticidal, antihepatotoxic and hepatoprotective activities and uses such as feed additive, in honeybee breeding and in gastrointestinal ailments have been shown. This paper highlights these activities and attempts to explain the possible *in vivo* mechanism of action of carvacrol.

Key Words: Carvacrol, Oregano, biological activity, essential oil, monoterpenic phenols.

INTRODUCTION

Oregano is a collective term referring to members of several genera common feature being that they all contain carvacrol(1) as main constituent in their essential oils. Its isomer thymol (2) may also be present in lesser amount [1]. Such genera include *Origanum, Thymbra, Thymus, Satureja, Coridothymus* of the family Lamiaceae and *Lippia* of Verbenaceae among others. Plants smelling like oregano or thyme are called as "kekik" in Turkish [2]. *Origanum* species constitute the largest portion of oregano traded around the world [3].

ORIGANUM OF TURKEY

The genus *Origanum* consists of 43 species belonging to 10 sections. It also has 18 natural hybrids [1]. In the Flora of Turkey, there are 22 species and altogether 32 taxa. 21 taxa are endemic in Turkey. Since 60% of 52 known taxa of *Origanum* are known to grow in Turkey, she is regarded as a gene centre for the genus Origanum [4-6]. Carvacrol is a predominant constituent of the essential oils of Origanum species (Table **1**) [7,8].

Turkey is the biggest oregano producer in the world. It is estimated that over 10.000 tons of dried oregano are harvested in Turkey. About 1000 tons are consumed domestically, an unspecified portion is used in essential oil manufacturing and the rest is exported. Turkey also exports more than 20 tons of oregano oil annually. Cultivation of oregano has in recent years become a preferred practice. In the year 2004, agricultural areas reached 5250 hectares for the production of 7000 tons of dried oregano. In 2005, oregano exports of Turkey reached an all time high of 10.422 tons for a return of $ 17.874.450, bringing unit export price of oregano to $1.71 per kg.

Oregano oil is produced by steam distillation. Almost whole of this essential oil is exported. Oregano water, the hydrosol of oregano oil production, is aqueous portion of the distillate which separates from the oil. Oregano water contains ca. 0.1% totally dispersed essential oil in which the main constituents are carvacrol (1) (70%) and some rare p-menthen-diols (*ca*. 10%), namely *cis*-p-menth-4-en-1,2-diol (3) and *cis*-p-menth-3-en-1,2-diol (4) are also present (Fig. **1**) [1,25].

Oregano water (Kekik suyu in Turkish) is a popular herbal drink in Southern and Western Anatolia. It is produced using very simple make-shift distillation stills of ingenious design in house kitchens. This still consists of a large saucepan with a lid and a glass jar. Saucepan contains an elevation on which the jar sits. Oregano and water is placed at the bottom of the saucepan. Lid is turned upside down and the shallow part is filled with cold water. The still is placed on fire. As the distillation starts, water and oil vapours condense on the cold surface and gather in the jar. Finally, the oil which floats on top is scooped and kept in a separate bottle for rubbing on limps as a remedy for rheumatism. Oregano water is consumed as such or after dilution with water or fruit juice as antiulcer, digestive, antidiabetic and for the regulation of gastrointestinal system and for general prophylaxy. Pharmacological studies conducted on bileflow, barbiturate sleeping time, isolated ileum and aorta experiments on mice revealed choleretic activity, inhibition of gastrointestinal contractions (spasmolytic activity) and antihypertensive activity. No acute or chronic toxicity has been reported [25].

THYMUS OF TURKEY

Sixty taxa of Thymus which includes 39 species are known to grow in Turkey with 27 taxa being endemic consisting of 45% of all Thymus taxa in Turkey [4-6]. Thyme oils generally contain thymol as main constituent however

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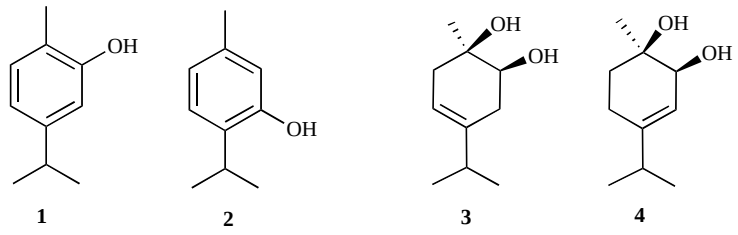
Table 1. Carvacrol Rich *Origanum* Taxa of Turkey

Taxon	Oil Yield (%)	Carvacrol (%)	Ref.
O. majorana	5.2-7.8	32 – 84	[9-11]
O. onites	1.1 – 4.7	67 – 82*	[12-14]
O. minutiflorum	1.1 – 3.8	42 – 82	[11,14]
O. vulgare subsp. *hirtum*	1.1 – 6.5	23 - 79[1]	[11,15,16]
O. syriacum var. *bevanii*	0.4 – 3.7	43 – 79	[11,17]
O. acutidens	1.4	63 - 66	[18,19]
O. bilgeri	1.3	66	[20]
O. hypericifolium	0.9 – 2.5	34 - 64[2]	[21]
O. x *intercedens* (natural hybrid of *O. onites* and *O. vulgare* subsp. *hirtum*)	4.3[3]	46	[1]
O. x *adanense* (natural hybrid of *O. laevigatum* and *O. bargyli*)	0.2	17	[23]
O. bargyli	1.1	15	[24]

[1]The ratios of carvacrol + thymol (A) were correlated with those of *p*-cymene + γ-terpinene (B) (A/B)against the yield of oil. The conclusion was *the larger the ratio, the higher the carvacrol (+ thymol) content, the better the oil yield*.
[2]Plants collected before flowering and budding stages yielded oils with high carvacrol content. Flowering plants had poor oil yields with *p*-cymene (36-48) and γ-terpinene (up to 40%) as main constituents. No wonder that people collect this species before flowering.
[3]A Greek material was reported to contain 85% carvacrol [22].



$C_{10}H_{14}O$
Mol. Wt.: 150,22

Fig. (1). Constituents of Oregano oil and Oregano water.

essential oils of several Thymus taxa of Turkey have been shown to contain carvacrol as main constituent (Table **2**).

Thymus kotschyanus var. *glabrescens* is known in commerce as Bitlis kekigi. It is traded among the Turkish oregano due to its relatively high content of oil and up to 70 % carvacrol. *T. migricus* which grows in Van province in Eastern Turkey also has potential for commercial exploitation due to high content of carvacrol in its oil.

CORIDOTHYMUS CAPITATUS

Also known as *Thymus capitatus* and Spanish oregano in trade, this bushy plant grows in South western corner of Turkey. Dried herb contains 0.5 – 5.2% essential oil with high content of carvacrol (44-78%) [38-40].

THYMBRA OF TURKEY

Thymbra is represented in Turkey by four taxa belonging to two species. Carvacrol was found as the main constituent in all the oil samples but one (Table **3**).

SATUREJA OF TURKEY

Satureja is represented in Turkey by 15 taxa. All of which but *S. amani* have been studied for essential oils (Table **4**).

ETHNOBOTANICAL USES

Oregano (kekik) is used as a condiment to flavour soups, pizza, olives, salad and meat dressings, especially grilled meat or fried beef steak and lamb chops. It is also an ingredient of several sauces used as seasoning [52]. Oregano is

Table 2. *Thymus* Species of Turkey with Essential Oils Rich in Carvacrol

Taxon	Oil Yield (%)	Carvacrol (%)	No. of Samples	Refs.
T. migricus	0.3 – 1.8	78 – 36	4	[26]
T. kotschyanus var. *glabrescens*	1.3 – 3.2	70 – 44	5	[27,28]
T. fallax	2.2	68	1	[29]
T. eigii	0.2 – 1.9	65 – 30	3	[30]
T. kotschyanus var. *kotschyanus*	1.5 – 1.9	60 – 54	3	[31]
T. zygioides var. *lycaonicus* (E)	1.0	48	1	[32]
T. sibthorpii	1.5 – 1.9	40 – 32	3	[33]
T. canoviridis	0.6 – 0.9	40 – 30	2	[34]
T. longicaulis subsp. *longicaulis* var. *subisophyllus*	0.3 – 1.0	32 – 15	4	[35]
T. leucostomus var. *leucostomus* (E)	0.5	22	1	[36]
T. pubescens var. *cratericola* (E)	0.4	18	1	[37]

(E): Endemic.

Table 3. Essential Oils of *Thymbra* Species Growing in Turkey

Taxon	Part*	Oil Yield (%)	Main Components (%)	No. of Samples	Refs.
T. spicata var. *spicata*	H	1.0 – 3.4	Carvacrol (56 – 71)	9	[2,41]
	L	1.6 – 5.2	Carvacrol (59 – 70)	2	[2,41]
	FW	4.1	Carvacrol (77)	1	[2,41]
T. spicata var. *intricata*	H	1.4 – 2.7	Carvacrol (49 – 71)	6	[2,42,43]
	H	2.0	Thymol (51)	1	[2]
T. sintenisii subsp. *sintenisii*	H	1.5	Carvacrol (43), Thymol (33), γ–Terpinene (9)	1	[44]
T. sintenisii subsp. *isaurica*	H	1.6	Carvacrol (39), *p*-cymene (26), γ–terpinene (13)	1	[45]

*H: Herba, L: Leaves, Fw: Flowers.

also a popular herbal tea in Turkey [53]. Oregano water rich in carvacrol is taken orally for gastro-intestinal disorders and especially the heartburn, to reduce blood cholesterol and glucose levels, is used as a painkiller in rheumatism by rubbing externally on the painful limbs and applied on decayed tooth as an antiseptic and analgesic. Origanum oil when applied on open wounds does not give a cause of burning sensation and expedites healing. It is good for mouth ulcers and inflamed gums [1].

Hippocrates (500 BC) knew about the antiseptic properties of Oregano and used it for stomachache and respiratory diseases [54]. Dioscorides (1st century AD) in his famous Materia Medica recommended drinking Oregano tea with wine for snake bites. Oregano herb mixed with honey is good for cold, cough and pneumonia. Herb decoction is recommended for stomach disorders and sea sickness. It is also good as a bath for psoriasis and jaundice. Pressed juice of the plant heals tonsillitis and aphthae [55]. Paracelsus (AD 16th century) used oregano for treating diarrhoea, psoriasis and fungal diseases.

Ancient Egyptians used to use oregano as a healing herb and as disinfectant. Ancient greeks respected it as the favorite herb of Aphrodites. They used to crown newlywed couples with oregano wreaths to symbolise love, honour and happiness. Artemis is also often shown with an oregano wreath on her head. They used to plant oregano on graves to give eternal peace and happiness to the deceased [56].

Origanum oil when applied on sensitive tissues like eyes and surroundings, mouth, vagina and thin skin may cause sensation. It may also cause skin irritation in certain cases. A cream made of oregano and Turkish sage oils for diabetic foot syndrome and sportsmen's massage in Germany.

MONOTERPENIC PHENOLS

The main constituents of oregano oil, carvacrol and thymol are monoterpenic phenols. They are biosynthesized from γ-terpinene (5) through p-cymene (6). Therefore, these two compounds are always present in oils containing carvacrol and thymol. Also, biosynthetic intermediates such as ter-

Table 4. ***Satureja*** **Oils of Turkey**

Taxon	Part	Yield (%)	Main Components (%)	Refs.
S.pilosa	H	1.1 – 2.7	carvacrol (38-53), *p*-cymene (7-17), γ-terpinene (1-14)	[46]
S.thymbra	H	0.9 - 1.0	carvacrol (44-45), γ-terpinene (18-25), *p*-cymene (7-13)	[2,42,43]
	L	2.9 – 4.3	carvacrol (30-40), γ-terpinene (23-25), *p*-cymene (14-16)	[2,42,43]
S.wiedemanniana (E)	H	0.8 – 3.0	carvacrol (21-62) [8 samples]	[47]
S.boissieri (E)	H	2.1	carvacrol (41)	[48]
S.cilicica (E)	H	0.9	carvacrol (38)	[49]
S.cuneifolia	H	0.3 – 3.6	carvacrol (26-72) [11 samples]	[50]
S.hortensis	H	1.3 – 2.7	carvacrol (42-63) [13 samples]	[51]
S.icarica	H	0.8 – 1.1	carvacrol (52-56)	[46]
S.macrantha	H	1.7	carvacrol (54)	[43]
S.montana	H	1.5	carvacrol (63)	[43]
	L	2.8	carvacrol (63)	[43]

*(E): Endemic H: Herba, L: Leaves.

pinen-4-ol (8), cumin alcohol (9), p-cymen-8-ol (10) may also be present as shown in Fig. (**2**) [57]. Carvacrol and thymol are often described as phenols in literature. This is somehow not correct since they are not biosynthetically derived from phenol. Phenol (12) is a dead molecule found only in fosil fuels. However, carvacrol and thymol are of monoterpenic nature containing methyl and isopropyl functions in para position to each other on a phenol ring. These volatile compounds are found in aromatic plants and essential oils. Although the only difference in their formula is the position of hydroxyl group, carvacrol is always liquid while thymol and phenol are crystalline. LD_{50} (p.o. rat) values indicate that carvacrol and thymol are much safer than phenol [58] (See Fig. **3**).

BIOLOGICAL ACTIVITIES

Gastrointestinal (GI) Activities of Oregano Oil and Carvacrol

According to an ethnobotanical information derived in Turkey, an infusion of oregano herb is taken for stomach

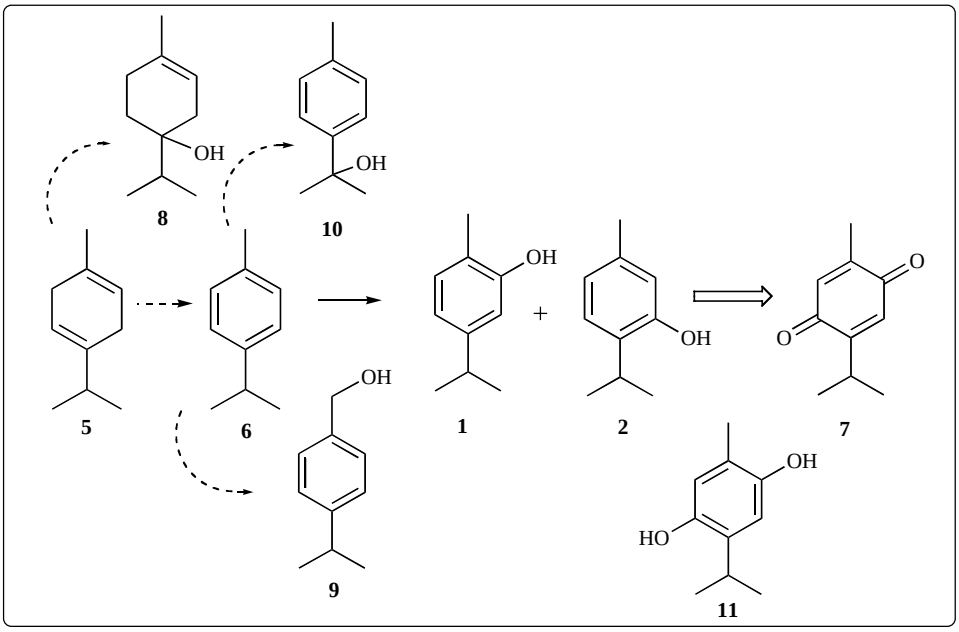


Fig. (2). Biosynthesis of carvacrol and related monoterpenes [57].

ache and common cold, and oregano oil is either rubbed on the abdomen or consumed orally against abdominal pain [59].

	Phenol	Carvacrol	Thymol
Physical occurrence	Crystalline	Liquid	Crystalline
MP	40-85°C	-	50-51°C
LD_{50} (p.o. rat)	530 mg/kg	810 mg/kg	980 mg/kg

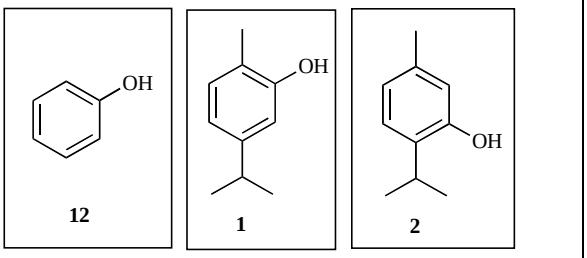


Fig. (3). Comparison of phenol, carvacrol and thymol [57].

Carvacrol (99.3%) isolated from Origanum oil tested on rat ileum was found to possess strong antispasmodic activity [25].

Hydrosol of oregano (Kekik water) showed choleretic activity and inhibited gastrointestinal contractions [25,60].

Oregano water exhibited hypertensive activity [61] while oregano oil showed hypotensive activity at 0.5 mL *i.v.* rat [62].

Analgesic activity of oregano oil has also been demonstrated [63].

Oregano oil reduced the tonus of rat stomach fundus, also reduced the carbachol-induced contractions on isolated rat ileum and inhibited the spontaneous activity of sheep ureter [64].

Antimicrobial Activities of Oregano Oil and Carvacrol

Antimicrobial activities of oregano oil and carvacrol has been shown on the following microorganisms [56,65-67].

- Food spoilage or pathogenic fungi, yeast and bacteria
- Human and animal pathogenic microorganisms
- Plant and crop pathogenic microorganisms

Such microorganisms include *Aspergillus flavus, Aspergillus niger, Bacillus cereus, Bacillus subtilis, Botrytis cinerea, Candida albicans, Candida tropicalis, Enterobacter aerogenes, Escherichia coli, Escherichia coli (O157:H7), Fusarium moniliforme, Fusarium solani, Geotrichum candidum, Hansenula anomala, Klebsiella pneumoniae, Kloeckera apiculata, Listeria monocytogenes, Micrococcus luteus, Microsporum canis, Mucor sp., Penicillium digitatum, Penicillium roquefortii, Pichia membranea, Proteus vulgaris, Pseudomonas aeruginosa, Pseudomonas vulgaris, Rhizoctonia solani, Rhizopus arrhizus, Rhodotorula glutinis, Saccharomyces cerevisia, Salmonella aureus, Salmonella typhimurium, Sclerotinia sclerotiorum, Shigella boydii, Staphylococcus aureus, Staphylococcus aureus (MRSA), Streptococcus pneumoniae, Trichoderma viride, Trichophyton mentagrophytes, etc.*

Effective concentration of the oil has been reported as over 0.01%. Similarly the activity has also been described as LC_{100}: 5-100 µl or MIC: 0.5-500 µg/ml [68].

Antimicrobial activities of oregano oil and carvacrol is well documented [56,69-74]. More examples will follow ahead.

Antimicrobial Mechanism of Action of Carvacrol

Carvacrol exhibits antimicrobial activity on the biological membranes of bacteria. It exerts its action by rapidly depleting intracellular ATP pool *via* reducing ATP synthesis and increasing ATP hydrolysis [75]. Reduction of transmembrane electric potential which is the driving force of ATP synthesis enhances proton permeability of the membrane. 1 mM carvacrol lowers internal pH of bacteria from 7.1 to 5.8 according to ion gradients of the cell membrane. Carvacrol (1 mM) decreases cell protein content from 12 mmol/mg to 0.99 mmol/mg by using potassium (K^+) of bacterial cells in a short time (5 min). Potassium (K^+) plays a role in the activation of cytoplasmic enzymes, in maintaining osmotic pressure and in the regulation of cytoplasmic pH. Leakage of K^+ out of the cell is a clear indication of membrane damage. At concentrations appropriate for flavouring of foods carvacrol exhibits its biological effects [e.g., nonalcoholic beverages [0.18 mM/28.54 ppm] and baked goods [15.75 ppm]) [76].

Ultee *et al.* hypothesized a scheme for the mechanism of action of carvacrol through the cytoplasmic membrane of bacteria. According to this hypothesis undissociated carvacrol diffuses through the cytoplasmic membrane and dissociates releasing its proton to the cytoplasm. It then returns undissociated through the membrane into the external environment carrying a potassium ion. Outside the cell carvacrol replaces potassium with proton and reenters the cell the same way [77].

Mechanism of action of oregano oils has been shown to be related, especially, to the synthesis of structural components and to the disruption of a series of energy systems. The leakage of ion, ATP and amino acid from bacterial cells explains this phenomenon. Potassium and phosphate ion concentrations were effected at a rate much lower than their MIC values [68].

Carvacrol and thymol increases overall permeability of the cytoplasmic membrane by disrupting outer membranes of gram negative bacteria leading to the leakage of ATP from the cell. Carvacrol also inhibits ATPase [78-80].

Changes in the fatty acid compositions of bacterial cell membranes were observed in bacteria at sublethal concentrations of carvacrol, resulting in an increase in unsaturated fatty acid contents [81].

Antimicrobial activities of carvacrol, carvacryl methylether. Carvacryl acetate, eugenol and menthol were tested on the following microorganisms in liquid and gas phases:

Bacteria: *Escherichia coli, Pseudomonas fluorescens, Staphylococcus aureus, Lactobacillus plantarum, Bacillus subtilis;* yeast: *Saccharomyces cerevisiae;* fungus: *Botrytis cinerea*

Eugenol and menthol showed weaker activity than the most hydrophobic carvacrol. Carvacrol derivatives did not show significant activity. It has been concluded that hydrophobicity is important and the presence of a free hydroxyl group and a delocalized system allows proton Exchange [82].

2-amino *p*-cymene (**13**), an amino analogue of carvacrol, while having the same hydrophobicity and exerting similar membrane disruption, showed 3-fold lower antimicrobial activity than carvacrol indicating a special role of hydroxyl group in killing microorganisms [83].

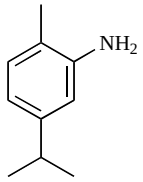


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Fig. (4). 2-amino *p*-cymene.

Carvacrol (1 mM) during overnight incubation stimulates *E. coli* O157:H7 to produce significant amounts of HSP60 (GroEL), but not HSP70 (DnaK), and prevents the synthesis of flagellin, causing cells to be aflagellate and therefore nonmotile. The speed of the flagellar rotary motor in *E. coli* is directly proportional to the proton motive force. These observations on motility appear to back up the proposal that carvacrol in some way causes dissipation of the proton motive force. The net advantage of carvacrol is that cells without flagella have been shown to be significantly less able to adhere to epithelial cells and to be less invasive than flagellated cells. This renders bacteria non-infective [84].

Antimicrobial Activity of Carvacrol

Carvacrol exhibited non-selective antifungal activity against strawberry anthracnose-causing fungal plant pathogens *Colletotrichum acutatum, Colletotrichum fragariae* and *Colletotrichum gloeosporioides* [85].

Carvacrol, thymol and *Lippia sidoides* essential oil were tested against mouth pathogens and showed strong antimicrobial activity against all the microorganisms at 0.625 and 10.0 mg/mL MIC values, the strongest activity was observed against *C. albicans* and *Streptococcus mutans* [86].

Wines macerated in *Origanum* and *Thymus* leaves or containing very low concentrations of essential oil and aromachemicals were reported to reduce the content of foodborne pathogens. Carvacrol showed better activity against *Salmonella enterica* than *E. coli* [87].

Carvacrol and thymol inhibits the growth of *Escherichia coli* O157:H7 in liquid food, especially when used in combination with a stabilizer or in an ethanol solution [88].

Carvacrol and thymol placed in the ear canal provided up to 81% effective treatment of acute otitis media in rats [89].

Origanum onites essential oil (79% carvacrol) was tested against *Aspergillus niger, Alternaria alternata, Penicillium rubrum* and *Trichoderma viride* formed during leather tanning as against commercial fungicides such as 2-thiocyanomethylthiobenzothiazole (TCMTB) and N-octyl-isothiazolinone (N-OITZ) and was found to possess strong fungicidal activity dose-dependantly. Origanum oil treated leather was found to be more resistant to fungal infections than those treated with commercial fungicides [90].

Carvacrol, thymol and cinnamaldehyde in vapour phase showed highly potent antimicrobial activity against Gram-negative bacteria (*Escherichia coli, Yersinia enterocolitica, Pseudomonas aeruginosa,* and *Salmonella choleraesuis*), Gram-positive bacteria (*Listeria monocytogenes, Staphylococcus aureus, Bacillus cereus,* and *Enterococcus faecalis*), molds (*Penicillium islandicum* and *Aspergillus flavus*), and yeast (*Candida albicans*) [91].

Carvacrol extended the shelf-life of table grapes through the inhibition of *Botrytis cinerea* in vapour-phase. Taste, odour and colour of the grapes kept in carvacrol atmosphere were not affected [92,93].

Carvacrol was the best inhibitor of mycelial growth (MIC=24.6 mL L^{-1}; ED_{50}= 9 mL L^{-1}) of *Penicillium expansum*, the cause of blue mould of pear. It was however, not as effective as trans-2-hexenal *in vivo* as fumigant [94].

Carvacrol-rich oregano oils (*Origanum syriacum* var. *bevanii* and *Thymbra spicata* subsp. *spicata*) completely inhibited the growth of *Phytophora infestans*, tomato late blight disease agent at 0.3 mg/ml concentration in vapour phase [95].

Antitumor Activity of Carvacrol

Carvacrol exhibited strong antitumor activity against DMBA-induced lung tumors in rats at 0.1 mg/kg *i.p.* dose. Although the mechanism of action of antitumor activity of carvacrol was not investigated in this study, evidences for an inhibitory effect on angiogenesis were observed [96].

In the tryphan blue exclusion assay, antitumor effects were observed for thymol and carvacrol. They did not show any dermal irritant and sensitizing or photosensitizing effects [97].

Carvacrol showed significant anticarcinogenic effect (30%) on 3,4-benzopyrene-induced rats. Antiproliferative activity of carvacrol (IC_{50}) was 90 μM and 67 μM for 24 h and 48 h of incubation of cells, respectively. Carvacrol possessed also mild antiplatelet effect, inducing the decrease of thromboxane A2 production in platelets and as a result - restrictive expression of the GPIIb/IIIa platelet receptor [98].

On a Human non-small cell lung cancer (NSCLC) cell line A549, carvacrol, dose dependantly (100, 250, 500 and 1000 μM), decreased the number of cancer cells, total protein content and degeneration of cell morphology as indication of its anticarcinogenic activity [99].

MTT assay with Hela cervix cancer cells of non-small lung cancer cells have showed that carvacrol had no proliferative effect on cancer cells at 0.01-10 μg/ml doses. However, at doses 75-200 μg/ml it reduced optical density and showed antiproliferative activity indicating anticancer activity.

Additional evidence for its antitumor activity was obtained when carvacrol was tested on CO25 myoblast cells bearing a mutant human N-ras oncogene during the transformation of differentiation processes. It was found to inhibit DNA synthesis while there was no change in protein synthesis. The findings suggested that the antitumor activity of carvacrol was not due to cytotoxicity but possibly due to prevention of prenylation of several proteins including ras [100].

When carvacrol was applied on NIH3T3 fibroblast cells at 0.01-100 μg/ml doses, MTT assay, Neutral Red Uptake Assay and cell count have clearly shown that carvacrol had antioxidant and proliferative actions on fibroblasts at doses starting from 0.01 μg/ml and reaching at a maximum at 10 μg/ml with no effect at 100μg/ml dose level.

Carvacrol has antioxidant and proliferative action on fibroblasts meaning anti-aging and wound healing activities at 0.01-10 μg/ml. It has selective action on cancer cells and is effective against lung and cervix cancer at 75-200 μg/ml dose levels [101].

Genotoxic and antigenotoxic activities of carvacrol were investigated by the *in-vitro* sister chromatid exchange (SCE) assay on human peripheral blood lymphosides. Inhibitory effect of carvacrol was tested in the presence of mitomycin C (MMC) in the same assay. At all doses carvacrol did not increase the formation of SCE, while it inhibited the rate of SCE induced by MMC.

The results show that carvacrol has "strong antimutagenic effects" supporting its antitumor activities. Its mechanism may be due to its antioxidant nature [102].

Origanum oil rich in carvacrol (74%) and carvacrol have been shown to have strong antimutagenic activity in TA98 and TA100 strains of *Salmonella typhimurium*. Inhibition of mutagenicity was around 50-60% for TA98 and 40% for TA100 in the presence of metabolic activity. These results also support antitumor activities of carvacrol [103].

Origanum compactum essential oil, carvacrol and thymol showed antimutagenic activity at somatic mutation and recombinant test (SMART) in *Drosophila melanogaster*. Carvacrol was the most active oil component [104].

Antimutagenic Effects

Carvacrol was shown to inhibit DNA damage induced by hydrogen peroxide, 2-amino-methimidazo[4,5-f]-quinoline (IQ) and mitomycin C (MMC) in lymphocytes at 0.05 mM dose, while causing DNA damage exceeding 0.1 mM [105,106].

Cell-Protective Effects of Carvacrol

In experiments with human hepatoma cells, human colon cells, Caco-2 and hamster liver cells V79 carvacrol and thymol caused no breakage on DNA. On the contrary, they provided protection against DNA damage induced by hydrogen peroxide [107].

AChE Inhibitory Activity of Carvacrol

Thymohydroquinone (12) showed the strongest acetylcholinesterase (AChE) inhibitory activity *in vitro*. Carvacrol (1) was the 2nd strongest while thymoquinone (7) took the 3rd rank. AChE inhibitory activity of carvacrol was 10-fold more than that of thymol(2) [108].

Antiplatelet Activity of Carvacrol

Carvacrol inhibited arachidonic acid-induced platelet (thrombocyte) aggregation and it was a much more potent inhibitor compared with aspirin [109].

Analgesic, Antiinflammatory and Antiangiogenic Activities of Carvacrol

Hot-plate and tail-flick tests have shown the analgesic activity of origanum oil [63]

Origanum onites oil and its main components: carvacrol, thymol, p-cymene, γ-terpinene were tested on *in vivo* Chorioallantoic Membrane Test (CAM). At 10-250 μg/pellet dosage neither the essential oil nor its components showed any antiinflammatory or antiangiogenic activity. However, the test has shown that thymol is responsible for the irritant effect of the oil dose-dependantly even at 10 μg/pellet dose [110].

Carvacrol, eugenol and thymol when applied to the tongue elicit a warm sensation. They are also known to be skin sensitizers and allergens. The transient receptor potential channel (TRPV3) is a warm-sensitive Ca^{2+}-permeable cation channel highly expressed in the skin, tongue and nose. Tests have shown that TRPV3 is strongly activated by carvacrol, thymol and eugenol. Tongue and skin epithelial cells respond to carvacrol and eugenol with an increase in intracellular Ca^{2+} levels. TRPV3 activity is strongly potentiated by phospholipase C-linked, G protein-coupled receptor stimulation. Furthermore, carvacrol activates and rapidly desensitizes TRPA1, which may explain the pungency of oregano [111].

Carvacrol showed strong analgesic activity by inhibiting prostaglandin synthesis [112].

Antioxidant Activity

Satureja montana and *Origanum vulgare* oils, and carvacrol and thymol inhibited the formation of 3-nitrotyrosin and malondialdehyde. p-Cymene and γ-terpinene did not show any activity. These results support, in particular for origanum, the nutraceutical value of these spices and the potential of thymol and carvacrol in preventing the formation of toxic products by the action of reactive nitrogen species [113].

Antielastase Activity of Carvacrol

The effects of essential oil extracted from *Nigella sativa* seeds and its main components on human neutrophil elastase (HNE) activity were investigated. The highest inhibitory concentration (HIC) of essential oil which caused total inhibition of HNE activity was 5.8 mg/ml. Microassays carried out to evaluate the inhibitory effect of major components of essential oil on HNE activity revealed that carvacrol (5-isopropyl-2-methylphenol) showed marked HNE inhibitory activity with a very low IC_{50} value (12 μM). Based on these results, the inhibitory effects of essential oil on HNE activity are due to the presence of bioactive molecules, mainly car-

vacrol. This compound is an inhibitor of HNE and could be considered as a natural antielastase agent and possible candidate for phytotherapy in the treatment of injuries that appear in some pathologic cases such as chronic obstructive pulmonary disease and emphysema [114].

Effects of Carvacrol on the Liver of Rats Following Partial Hepatectomy

In order to test its antihepatotoxic effects, carvacrol was tested in comparison with silymarin on male Wistar Albino rats which had undergone partial hepatectomy.

One dose of test materials was injected to control and test groups one hour before 68% partial hepatectomy. The liver regeneration ratio of the rats was calculated measuring the half weights of their liver before and after the hepatectomy.

H&E, IL-6 and PCNA treatments were applied to liver sections. AST, ALT, TNF-α and IL-6 levels were determined in serum samples. In AST, ALT, TNF-α and IL-6 levels, there were no statistically significant difference. Mitotic index and PCNA index comparisons displayed significant differences. Histological evaluations were also similar with these results of PCNA and Mitotic indexes. In conclusion, carvacrol increases the liver regeneration ratio similar to that of silymarin [115].

Effects of Carvacrol on Defects of Ischemia-Reperfusion in the Liver

Rats divided into 4 groups were injected a single dose of physiological serum, carvacrol and silymarin, resp. 60 min prior to the experiment. Except for the control group. The others were subjected to liver ischemia for 45 min followed by reperfusion for 60 min. Blood and tissue samples were collected after the experiment for biochemical and histological analyses. As a result, Carvacrol was shown to protect the liver against the defects of ischemia/reperfusion like silymarin, and was not hepatotoxic at the applied dose [116].

Antiparasitic Activities of Oregano Oil

As shown in Table **5**, essential oil of Turkish *Origanum onites* and its main components, carvacrol and thymol showed potent antiprotozoal activity. All test materials were devoid of cytotoxicity in mammalian L6 cells (IC_{50} > 50 mg/ml) [117].

Emulsified essential oil of *Origanum vulgare* (600 mg), when orally administered to 14 adult patients infected with enteric parasites, after 6 weeks there was a complete disappearance of *Blastocystis hominis* (in eight cases), *Entamoeba hartmanni* (four cases) and *Endolimax nana* (one case). Gastrointestinal symptoms improved in seven of the 11 patients with *B. hominis* infection [118]

Insecticidal Activities

Satureja oils (carvacrol 40%) were found lethal to adult turnip aphids, *Lipaphis pseudobrassicae*, at applied concentrations as low as 0.3 to 1.0 $mgml^{-1}$ [119].

Essential oils of *Origanum vulgare* subsp. *hirtum*, *Coridothymus capitatus*, *Satureja thymbra* with a carvacrol content of 75%, 82% and 3%, respectively, the latter containing 35% thymol as well as carvacrol, thymol and their various mixtures were tested on the larvae of *Drosophila melanogaster*. Carvacrol showed the strongest effect (LD_{50} 1.6), followed by thymol (LD_{50} 2.6). Carvacrol: thymol (105:1) mixture exhibited activity (LD_{50} 1.98) slightly lower than that of carvacrol. Increasing the amount of thmol in the mixture lowered the activity indicating an antagonistic effect. Among the oils *S. thymbra* oil gave stronger activity (LD_{50} 3.3) than *O. vulgare* (LD_{50} 5.6) and *C. capitatus* (LD_{50} 6.78) oils. Thymol was the only compound tested exhibiting genotoxic activity in the somatic mutation and recombination test on Drosophila [120].

Against common grain insect *Tribolium castaneum*, Oregano oil showed 100% mortality at 10% conc. [28].

The oil of *Origanum vulgare* showed strong activity against adults of *Ephestia kuehniella* and *Sitophilus granarius* grain insects in vapour phase exposure (0.5-2 μl/l). *Lasioderma serricorne* was also affected [121].

Carvacrol showed insecticidal activity against adults and eggs of the confused flour beetle, *Tribolium confusum* and eggs of the Mediterranean flour moth, *Ephestia kuehniella* at 46.2 mg/l air and an exposure of 24-96 h except for *E. kuehniella* larvae which required a higher dose, 184.8 mg/l [122].

Carvacrol, p-cymene, linalool, α-terpinene, and thymol were examined for their repellency against the mosquito *Culex pipiens* on a human forearm bioassay. α-Terpinene and carvacrol showed significantly greater repellency than a commercial formulation, N,N-diethyl-m-methylbenzamide (Deet), whereas thymol showed similar repellency to that of deet. The duration of repellency after application for all these monoterpenes was equal to or higher than that of deet [123].

Table 5. Antiprotozoal Activity of *Origanum onites*, Carvacrol and Thymol (IC_{50} Values)

Parasitic Protozoa	Essential oil	Carvacrol	Thymol	St. Drugs
Trypanosoma brucei rhodesiense	186 ng/ml	149 ng/ml	114 ng/ml	11 ng/ml Melarsoprol
Trypanosoma cruzii	> 30	> 30	> 30	74 ng/ml Benzimidazol
Leishmania donovani	17.8 mg/ml	17.8 mg/ml	17.8 mg/ml	0.14 mg/ml Miltefosine
Plasmodium falciparum	7.9 mg/ml	7.9 mg/ml	7.9 mg/ml	0.066 mg/ml Chloroquine

Origanum onites essential oil (carvacrol >%70).

Nootkaton-1,10-epoxide (RC_{50} %0.0858) ve carvacrol (RC_{50} %0.112) were found effective against nymphal *Ixodes scapularis* (Deer tick) in relatively low doses, although not as effective as deet (RC_{50} %0.0728). The ability of these natural products to repel ticks at relatively low concentrations may represent a potential alternative to synthetic commercial repellents [124].

Carvacrol and thymol showed strong nematicidal activity against the pine wood nematode *Bursaphelenchus xylophilus*. LC_{50} values of carvacrol and thymol were measured as 0.096 ve 0.099 mg ml^{-1} against juvenile nematodes [125].

Origanum onites oil, carvacrol, thymol, γ-terpinene and terpinen-4-ol were tested against the pine processionary moth (PPM), *Thaumetopoea wilkinsoni* Tams. (Lepidoptera: Thaumatopoeidae), which is an important forest pest in the Mediterranean area, and urticating hairs of the caterpillars of this species cause allergic reactions on skin of humans and animals. Carvacrol showed the strongest larvicidal effect (LD_{50} = 3100μL L^{-1}). Thymol ranked second (LD_{50} = 5500μL L^{-1}) while the other compounds showed weaker activities [126].

The Effect of Carvacrol on Honey Bee Diseases

The essential oil of *Origanum onites* and one of its ingredients, Carvacrol were found effective on American Foulbrood Disease [*Paenibacillus larvae* (the cause of American Foulbrood)] as well as on European Foulbrood Disease [*Melissococcus pluton* (the cause of European Foulbrood)] [127].

Oregano Oil as Feed Additive

Dietary addition of essential oils in a commercial blend (CRINA® Poultry) showed a decreased *E. coli* population in ileo-cecal digesta of broiler chickens. Furthermore, in high doses a significant increase in certain digestive enzyme activities of the pancreas and intestine was observed in broiler chickens. Main ingredient in CRINA® blends is oregano oil [128].

In another study, CRINA® Poultry was shown to control the colonization of the intestine of broilers with *Clostridium perfringens* and the stimulation of animal growth was put down to this development [129].

Commercial essential oil blends CRINA® Poultry and CRINA® Alternate were tested in broilers infected with viable oocysts of mixed *Eimeria* spp. It was concluded that these essential oil blends may serve as an alternative to antibiotics and/or ionophores in mixed *Eimeria* infections in non-cocci vaccinated broilers, but no benefit of essential oil supplementation was observed for vaccinated broilers against coccidia [130].

50 and 100 mg/kg of feed of oregano oil were tested on broilers. No growth promoting effect was observed. At 100 mg/kg of feed antioxidant effect was detected on chicken tissues. Oregano essential oil used in this study was in the form of a powder called Orego-Stim. This product contains 5% oregano essential oil (Ecopharm Hellas, SA, Kilkis, Greece) and 95% natural feed grade inert carrier. The oil of *Origanum vulgare* subsp. *hirtum* used in this product contains 85% carvacrol + thymol [131].

Positive results were reported for oregano oil added in poultry feed [132].

Inclusion of oregano oil at 0.005 and 0.01% in chicken diets for 38 days resulted in a significant antioxidant effect in raw and cooked breast and thigh muscle stored upto 9 days in refrigerator [133].

Oregano oil (55% carvacrol) exhibited a strong bactericidal effect against lactobacilli and following the oral administration of the oil MIC values of amicain, apramycin, streptomycin and neomycin against *E. coli* strains increased [134].

Essential oils from oregano herb (*Origanum onites* rich in carvacrol), Laurel leaf (*Laurus nobilis*), Sage leaf (*Salvia fruticosa*), fennel fruit (*Foeniculum vulgare*), Myrtle leaf (*Myrtus communis*) and citrus peel (rich in limonene) were mixed and formulated as feed additive. It is marketed in Turkey as poultry feed under the name Herbromix®. Main components of this mixture are carvacrol, thymol, 1,8-cineole and limonene. Our group has conducted three *in vivo* experiments with this product. The results are as follows:

1250 sexed one day-old, broiler chicks were subjected to a controlled clinical trial. Commercial essential oil combination at three different levels (24 mg, 48 mg and 72 mg) and antibiotic (10 mg avilamycin) per kg were added to the basal diet. Supplementation of 48 mg essential oil combination/kg to the broiler diet significantly improved the body weight, feed conversion ratio and carcass yield compared to other dietary treatments on 42 day of age. Death rate also decreased significantly [135-137].

In a 20 day clinical trial involving 480 54 day-old laying hens, supplementation of the essential oil mixture to the feed increased daily egg yield, feed consumption and feed efficiency, and decreased the number of broken eggs. However, did not have any effect on the bird death rate [138-140].

CRINA® Pigs was tested on pigs. The results for the first 21 day period showed that males grew faster, ate less and exhibited superior Feed Conversion Ratio compared to females. Although female carcass weight was higher, males had a significantly lower carcass fat than females [129].

The pigs appeared to prefer the garlic treated diet, and feed intake and average daily gain were significantly increased although no difference in feed efficiency was observed. Carcass and meat quality attributes were unchanged, although a slight reduction of lipid oxidation was noted in oregano fed pork. Since the composition of the oils is not clear, it is not possible to evaluate the results [141].

A study revealed that the inclusion of essential oil of oregano in a pigs' diet significantly improved the average daily weight gain and feed conversion ratio of the pigs. Pigs fed the essential oils had higher carcass weight, dressing percentage and carcass length than those fed the basal and antibiotic-supplemented diet. In the pigs, which received the essential oil supplementation had a significantly lower fat thickness. Also lean meat and ham portions from these pigs were significantly higher.

Therefore, the use of *Origanum* essential oil as feed additive improves the growth of pigs and has greater positive effects on carcass composition than antibiotics [142].

Ropadiar®, an essential oil of the oregano plant, was supplemented in the diet of weaning pigs as alternative for anti microbial growth promoters (AMGP), observing its efficacy on the performance of the piglets. Ropadiar ® liquid contains 10% Oregano oil and has been designed to be added to water. Compared to the negative control (without AMGP), Ropadiar® improved performance only during the first fourteen days after weaning. Based on the results of this trial it can not be argued the usefulness of Ropadiar® as an alternative for AMGP in diets of weanling pigs. However its addition in prestarter diets could improve performance of these animals [143]

The objective of another trial was to ascertain, if by adding oregano oil to the feed, there would be an effect on nutrient digestibilities, N-balance as well as on parameters of microbial activity in the gastrointestinal tract of weaned pigs. The apparent digestibility of crude nutrients (except fibre) and the N-balance of the weaned piglets in this study was not influenced by feeding piglets restrictively with this feed additive. By direct microbiological methods, no influence of the additive on the gut flora could be found [144].

The inclusion of essential oil of spices in the pigs' diet significantly improved the average daily weight gain and feed conversion ratio of the pigs in Groups 3, 4 and 5, as compared to Groups 1 and 2 ($P < 0.01$). Furthermore, pigs fed the essential oils had higher carcass weight ($P < 0.01$), dressing percentage ($P < 0.01$), and carcass length ($P < 0.01$) than those fed the basal and antibiotic-supplemented diet. In Groups 3, 4 and 5, back fat thickness was significantly lower than those in Groups 1 and 2. Moreover, lean meat and ham portions from pigs in Groups 3, 4 and 5 were significantly higher than those from pigs in Groups 1 and 2. In conclusion, the use of essential oils as feed additives improves the growth of pigs and has greater positive effects on carcass composition than antibiotics [142].

In a study to assess the effect of carvacrol supplement (250 and 500 mg/L) as a dietary additive to rumen fermentors, fed a barley seed : alfalfa hay (70:30) ration and to compare its effect with monensin supplementation (7.5 mg/L), carvacrol reduced *in vitro* dry matter (DM), crude protein (CP) and neutral-detergent fibre (NDF) digestion. Volatile fatty acid (VFA) profiles determined after 48 h of incubation showed 250 mg/L carvacrol increased butyrate and decreased acetate proportions, whereas M mainly stimulated propionate proportions, suggesting that the mechanism of action of carvacrol and M differs. 500 mg/L carvacrol significantly reduced total VFA production. Carvacrol could be of great interest for its usage as a potential modulator of ruminal fermentation [145].

Different doses of 12 plant extracts and 6 secondary plant metabolites were incubated for 24 h in diluted ruminal fluid with a 50:50 forage:concentrate diet. At 3,000 mg/L, capsicum oil, carvacrol, carvone, cinnamaldehyde, cinnamon oil, clove bud oil, eugenol, fenugreek, and oregano oil resulted in a 30 to 50% reduction in ammonia N concentration [146].

An *in vitro* assay measuring the antimicrobial activity of essential oils of *Coridothymus capitatus* (Spanish origanum), *Satureja montana*, *Thymus mastichina* (Spanish Origanum majorana), *Thymus zygis* (Spanish variety of *Thymus vulgaris*) and *Origanum vulgare* has been carried out against poultry origin strains of *Escherichia coli, Salmonella enteritidis* and *Salmonella essen*, and pig origin strains of enterotoxigenic *E. coli* (ETEC), *Salmonella choleraesuis* and *Salmonella typhimurium*. Using the broth microdilution method, all the essential oils showed an MIC $\geq$ 2% (v/v) for the two strains of *E. coli*. The essential oil that showed the highest antimicrobial activity against the four strains of Salmonella was *Origanum vulgare* (MIC $\leq$ 1% v/v), followed by *Thymus zygis* (MIC $\leq$2% v/v). *Thymus mastichina* inhibited all the microorganisms at the highest concentration, 4% (v/v), while the rest of the essential oils showed highly variable results. By chemotyping, higher inhibitory capacity was observed in the oils with a higher percentage of phenolic monoterpene components (carvacrol and thymol) in comparison with oils containing the monoterpenic alcohol linalool. The results of this work confirm the antimicrobial activity of some essential oils, as well as their potential application in the treatment and prevention of poultry and pig diseases caused by salmonella [147].

FINAL WORDS

In this review, I attempted to give an overview of the diversity of uses of oregano oil and carvacrol, its main constituent. I cannot claim that it is comprehensive but representative enough to show wide ranging uses and biological activities of carvacrol, a simple monoterpenic phenol and essential oils bearing this molecule. I hope, this paper stimulates interest into investigating this relatively safe volatile molecule for other activities.

ABBREVİATİONS

ALT	=	Alanine aminotransferase
AST	=	Aspartate aminotransferase
ATP	=	Adenosine triphosphate
DNA	=	Deoxyribonucleic acid
DMBA	=	7,12-dimethylbenz(a)anthracene
ED_{50}	=	Effective dose
H&E	=	Hematoxylin and eosin
IC_{50}	=	Inhibitory concentration
IL-6	=	Interleukin-6
i.v	=	Intravenous
LC100	=	Lethal concentration value
LD_{50}	=	Median lethal dose
MIC	=	Minimum Inhibitory Concentration
M.P.	=	Melting point
MTT	=	3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide
PCNA	=	Proliferating cell nuclear antigen
p.o	=	Per oral
RC_{50}	=	Chemical reactivity
TNF-α	=	Tumor necrosis factor-alpha

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