

# Turkish Journal of Agriculture - Food Science and Technology

Available online, ISSN: 2148-127X | www.agrifoodscience.com | Turkish Science and Technology Publishing (TURSTEP)

## Studies on Antimicrobial, Antifungal and Antioxidant Properties of Rosemary: **A Review**

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ABSTRACT		
Rosemary has played an important role from past to present and has antimicrobial, antifungal and antioxidant properties. With these features, it is used in many sectors, especially food and pharmacy. Rosemary essential oils have a positive effect on biological activity. In addition, this oil prevents		
lipid oxidation on foods, providing a long and fresh shelf life. Due to the high antioxidant properties of rosemary, it eliminates the harmful effect of reactive oxygen species. Since the main components of rosemary such as 1,8-cineol, camphor, $\alpha$ -pinene, carnosic acid, and carnosol are being antimicrobial and antifungal, it is effective against many pathogens. With this review, it is aimed to provide comprehensive information on the biological activities of rosemary and its extracts to shed		
light on future research.		



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### Introduction

Demand for plants rich in valuable secondary metabolites is increasing day by day. One of these herbs is rosemary. Rosemary (Rosmarinus officinalis L.) is a valuable essential oil and spice plant from the Lamiaceae According to the evidence found by anthropologists and archaeologists, rosemary, which was used in medicine, food, and cosmetics in Ancient Egypt, China, Mesopotamia, and India, still maintains its importance in these areas today (Luqman et al., 2007). R. officinalis were used especially as pharmacological purpose of different geographical areas. Peoples were looks remedy for a lot of kind diseases then began to use of rosemary plant as cure of various illness such as stomach ache (Girón et al., 1991; Vieira, 2012; Benarba, 2016), sick throat (Gaspar et al., 2002; Montesano et al., 2012), strengthen to memory (Perry et al., 1999; Vieira, 2012; Benarba, 2016). It was emphasized conducted study that rosemary has some beneficial effects on the nervous system works and lipid metabolism. (Pawłowska et al., 2020). There were around 150 different compounds identified in rosemary essential oil (REO). Some of major compounds of these essential oils were used as various prevent various diseases (Table 1) (Borges et al., 2019).

Rosemary is an evergreen plant with a developed root and stem system and a lot of branching. These branches frequently have needle-shaped and bright green leaves with very short stems, each about 3 cm long. There are glandular hairs that carry plenty of essential oil on the lower surface of the leaves. This plant, which has blue flowers towards the ends of the stem, has round, slippery and dark colored fruits (Baydar, 2019).

Among the rosemary species in the world, R. officinalis, R. erriocaly Mill., R. laxiflorus (de Noé) and R. lavandulaceus (de Noé) are the most common species. Although it is one of the most characteristic plants of the Mediterranean, it grows naturally in the countries neighboring the Mediterranean, especially on the coast and mountain slopes facing the coast. Only R. officinalis species grows naturally and is cultivated in Mediterranean countries. The Aegean and Mediterranean coastline in Turkey shows the spread up to 1000 m altitude. However, economically, it is mostly collected wild in Mersin and Adana provinces at altitudes of 100 to 250 m from the coast and mountain slopes facing the coast (Baydar, 2019).

The economically utilized parts of the rosemary plant are its leaves and flowers, and its leaves contain 0.3-2.5% essential oil. The most important essential oil components

in rosemary plant are 1.8-cineol (15-30%), camphor (5-25%), and borneol (10-20%). These essential oils obtained from rosemary are especially valuable in perfumes, cosmetics and aromatherapy. Rosemary juice also has an antiseptic effect and accelerates blood circulation in the skin. For this reason, fresh or dried leaves of rosemary are also added to dishes to give flavor and taste (Baydar, 2019).

In addition to the essential oils that rosemary contains, it also contains many compounds such as caffeic acid, ursolic acid, rosmanol, rosmeridiphenol, which have anti-inflammatory, anti-obesity, antispasmodic, antiproliferative, neuro protective, antidepressant, antimutagenic, anticancer, antimicrobial and antiviral effects, especially carnosic acid, rosmarinic acid, camphor, and carnosol (Figure 1) (Pariš et al., 1993; Cuvelier et al., 1996; Lemonica et al., 1996; Kosaka and Yokoi, 2003; Oluwatuyi et al., 2004; Ibarra et al., 2011; Machado et al., 2012; Elansary and Mahmoud, 2015). Due to its antioxidant effects, extracts from rosemary are also widely used as stabilizers and natural antioxidants, as they prevent oxidative degradation in foods containing oil and fat (Hall and Cuppett, 1993; Pokorny; 1997; Miladi et al., 2013). For instance through REOs, flavoured cheese

shelf life were extended by refrain from lipid oxidation (Olmedo et al., 2013). Besides rosemary were provide that protect sulphited-lamb product long shelf life (Ortuño et al., 2015). Rosemary oils were also decreased heat stress negative effects in japanese quails (Ciftci et al., 2013). Consequently it is indicating that rosemary has great potential to ensure along food storage (Ojeda-Sana et al., 2013; Ribeiro-Santos et al., 2015).

In addition, due to its antioxidant compounds, there has been an increasing interest in rosemary related applications in the pharmaceutical industry in recent years (Kuhlmann and Röhl, 2006). REOs were indicated vigorous cytotoxicity against tumor cells (Bel-7402, SK-OV-3 and HO-8910) and importantly slowed down mitosis (Stojanović-Radić et al., 2010; Wang et al., 2012). In addition to the benefits of REO on human health, studies have shown that it can be an alternative to insecticide. Some of these are acaricidal (Martinez-Velazquez et al., 2011), insecticidal (Papachristos et al., 2004; Waliwitiya et al., 2009), larvicidal (Duarte et al., 2015), antiparasitic (Cabuk et al., 2003) effects.

Table 1. R. officinalis essential oils on biological activity.

Positive effect on biological activity	References		
Antioxidant	Pérez et al., 2007; Bozin et al., 2007; Rašković et al., 2014; Takayama et al., 2016; Lin et al., 2016; Mekonnen et al., 2016; Bajalan et al., 2017b; Borges et al., 2018		
Prevent liver injury	Rašković et al., 2014		
Anti-inflammatory effect	Juhás et al., 2009; Nam et al., 2014; Silva-Filho et al., 2014; Borges et al., 2019		
Inhibiton of lipid peroxidation	Bozin et al., 2007; Takayama et al., 2016		
Inhibition of mucosal injury	Takayama et al., 2016		
Anti-depressive	Faria et al., 2011; Machado et al.,2013		
Antialgic	Vilela et al., 2016; Takayama et al., 2016		
Anticancer	Al-Sereiti et al., 1999; Wang et al., 2012		



Figure 1. R. officinalis essential oil chemical structure a) carnosol b) camphor

#### **Antioxidant Activity of Rosemary Plants**

Medicinal plants contain valuable essential oils with antibacterial, antimicrobial, and antioxidant effects (Sevindik et al., 2017; Pehlivan and Sevindik, 2018; Sevindik et al., 2018; Pehlivan et al., 2018; Mohammed et al., 2019; Sevindik, 2019). Antioxidants are important to our daily life to reduce detrimental effects to reactive oxygen species, which can appear to results of exercise or rhythm of the life. A free radical is generally described as a molecule consists of unpaired electrons. There is not completely known that natural antioxidant defense system adequately resist the rising free radicals (Clarkson and Thompson, 2000). Thus it is important to understand emerge antioxidant activity as a result of some beneficial plants. Rosemary is type of plants that has antioxidant

activity to protect cells from detrimental free radicals (Rašković et al., 2014). The research conducted by different group signify that essential oil from various populations were evaluated to DPPH radical inhibition assay, displayed relatively good antioxidant capacity (Wang et al., 2008; Zaouali et al., 2010; Rašković et al., 2014; Bajalan et al., 2017b).

Perez and research groups were expressed that when dry rosemary leaf exposed to radiation, antioxidant activity generally enhanced according to unapplied samples. Gamma radiated dry leaf antioxidant activities were changed to depend on extracts, methanol extracts didn't show any important change while ethanol and water extracts improve activity substantially. However, methanol

based extracts demonstrated a superior antioxidant activity and higher phenolic content whether subject to gamma radiation or not. It was also clearly expressed that extraction solvent play main role to total phenolic content and antioxidant activity (Pérez et al., 2007).

Medicinal-Aromatic plants have acquired antifungal, antibacterial and antioxidant properties thanks to their secondary metabolites (Adrar et al., 2016; Bajalan et al., 2017a). Rašković and friends stated that REO antioxidant activities were noticed to reproduced partly from existence of phenolic groups. REO has free radical scavenging activity which has potential alleviate liver injury (Viuda-Martos et al., 2010; Rašković et al., 2014). REO has also anti-inflammatory effect, moreover block of AA-metabolites nascency and barrier to NF-κB transcription which inhibition may provide contribution impact on anti-inflammatory effects. Reactive oxygen species were responsible for noxious effect of inflammation. Which were alleviated due to essential oil high antioxidant activity (Arulselvan et al., 2016; Borges et al., 2019).

Investigation conducted by different group were clearly stated that lipid peroxidation importantly inhibited by antioxidant activity of REO (Bozin et al., 2007; Takayama et al., 2016). Rosemary salinity stress were reduced some essential oils such as  $\alpha$ -pinene,  $\beta$ -pinene. Although there was strong increase in verbenone, linalool, camphor, borneol, under salts stress. Salicylic acid was reduced harmful salt stress effect and enhanced to total phenolic, proline. Researches were signify that salicylic acid probably activate antioxidant enzymatic mechanism as well as enhance non-enzymatic antioxidants In same investigation quantitative real-time PCR were used for APX and various SOD genes expression which show higher expression level in salicylic acid applicated plants under salt stress (El-Esawi et al., 2017).

The studies about rosemary tissue culture which were conducted by Coskun (2019) and research group express that melatonin induced seconder metabolite content as elicitor. Another investigations were also determined that antioxidant enzymes become functional with phytomelatonin application (Li et al., 2012). Besides increase to mitochondrial electron transport chain performance, melatonin were played substantial role as defending plants against oxidative damages (Martinez et al., 2018). Interesting investigation by using tissue culture technic rosmarinic acid and total phenolic content stimulated through *Pseudomonas* using the as elicitor (Yang et al., 1997).

### **Antibacterial-Antimicrobial Activity of Rosemary Plants**

In recent years, the use of medicinal aromatic plants has increased significantly in the food, pharmaceutical, and agricultural industries. In particular, rosemary extracts have been reported to exhibit strong antibacterial properties due to their chemical composition. Abdallah et al. (2019) reported that rosemary extract has strong antibacterial activity against the Xoo of GZ 0005 strain, which is the pathogen of rice bacterial blight. In several studies applying rosemary extract, it has been reported that gram (+) bacteria are more sensitive than negative ones (Pintore et al., 2002; Abu-Shanab et al., 2005).

Many studies have been conducted with extracts extracted from various plants to determine antimicrobial

activity. In one of these, Abu-Shanab et al. (2004) founded that 3.125 and 1.5 mg/mL dose of rosemary extract was effective on *B. subtilis* and *S. aureus* gram positive bacteria. In a study conducted, it was shown that the oilbased formulations of rosemary have highly effective antimicrobial properties on colistin-resistant *Klebsiella pneumonia* clinical isolates (Abozahra et al., 2020). Baratta et al. (1998) showed significant activity against 7 of the 25 bacterial species used in the study.

It was found by Erdoğrul (2002) that rosemary has an antibacterial effect with ethyl acetate, methanol, and chloroform extracts *Bacillus brevis* FMC 3, *Bacillus megaterium* DSM 32, *B. subtilis* IMG 22, *B. subtilis* var. *niger* ATCC 10, *Micrococcus luteus* LA 2971, *Mycobacterium smegmatus* RUT, *E. coli* DM, *Listeria monocytogenes* SCOTT A, *Staphylococcus aureus* ATCC 25923, *Streptococcus thermophilus*, *Pseudomonas fluorescens*, and *Yersinia enterocolitica* O:3 P 41797. Thanks to the chemical compounds of the rosemary plant, its inhibitory effect on many different types of bacteria continue to be investigated.

It has been reported that the antimicrobial effect of REOs on bacterial species varies depending on the regions, season and months, but is quite high. The use of REOs was been found to give very good results against every bacterial strain (Celiktas et al., 2007; Gachkar et al., 2007). Bosnic et al. (2006) investigated the antibacterial properties of REO against gram (-) and (+) bacteria and reported that the most resistant microorganism was P. aeruginosa (11.5 mm) strain and the most sensitive was B. subtilis (23.0 mm). This was because they attributed it to the main component of thymol in rosemary. Bouyahya et al. (2017) examined REOs and their antibacterial, anti-leishmania, and antioxidant activities. GC / MS analysis of its essential oil detected 29 components, mainly oxygenated monoterpenes (63.743%) and hydrocarbon monoterpenes (21.231%). Its antibacterial activity was tested against ten pathogenic strains, and the susceptibility of *Listeria monocytogenes*, *B. subtilis* and *E.* coli strains of these was considerably higher than the commercialized antibiotic. Bajalan et al. (2017b), in their research using REO, found that it created an inhibition zone against mainly E. coli (18.51 mm), S. aureus (14.58 mm), S. agalactiae (13.12 mm), and K. pneumoniae (13.97 mm) bacteria, respectively. In a study investigating the changes in REOs under salinity stress, it was found that while the amount of linalool, camphor, borneol, and verbenone increased, it caused a decrease in α-pinene, β-pinene, and cineol. In the same study, it was determined that the antibacterial effect of REO obtained from stressed plants leaves was higher than antibiotics (El-Sawi et al., 2017).

In a study comparing the antimicrobial activity of rosemary extracts with grapevine leaf extracts, it was found that rosemary extracts showed better inhibition against gram positive (S. aureus, B. cereus, L. monocytogenes) and gram negative bacteria (C. jejuni, S. infantis, E. coli). Stronger antibacterial activity was determined especially against gram positive bacteria and C. jejuni (Abramovič et al., 2012). Bernardes et al. (2010) determined the ethanolic extracts of rosemary on Streptococcus mutans, S. salivarius, S. sobrinus, S. mitis, S. sanguinis and Enterococcus faecalis bacteria by liquid microdilution method. Accordingly, they determined that rosemary extract has antimicrobial activity against oral pathogens and this is caused by carnosic acid and carnosol (Table 2).

Table 2. *R. officinalis* major components and type of against pathogen (Gram negative bacteria (G), Gram positive bacteria (G+), Fungal species (F))

(G+), Fungal species (F)) Major components	Type of pathogen against	References
rajoi components	(G+) Micrococcus luteus, Staphylococcus aureus, (G-	Telefonees
1,8-cineole (13%); α-pinene (19.60%); Camphor (12.60%); α-terpineol (12.20%)	) Acinetobacter calcoaceticus, Beneckea natriegens, Citrobacter freundii, Erwinia carotovora, Lactobacillus plantarum; (F) Aspergillus niger	Baratta et al., 1998
1,8-cineol (31.12%); Camphor (30.12%); α- pinene (18.18%)	(G+) Streptococcus Pyogenes; (G-) Acinetobacter lwoffi, Shigella flexneri	Mangena and Muyima, 1999
Camphor (22.20%); 1,8-cineole (22%); Myrcene (15.20%)	(F) Ascosphaera apis	Larrán et al., 2001
Bornyl acetate (11.30-17%); 1,8-cineole (3.50-11.3%); α-pinene (13.70-24.60%); Verbenone (4.40-24.90%)	(G-) Escherichia coli, Pseudomonas aeruginosa; (G+) Staphylococcus aureus, ;Staphylococcus epidermidis	Pintore et al., 2002
1,8-cineol (18.03-22.23%); Camphor (17.88-24.57%); α-pinene (7.07-70.60%); Verbenone (1.50-16.00%)	(G+) B. subtilis, S. aureus; (G-) E. coli, P. aeruginosa; (F) C. albicans, A. niger	Abu-Shanab et al.; 2004; Santoyo et al., 2005; Bosnic et al. 2006
Limonene (21.70%); α -pinene (13.50%); Camphor (21.60%); Z-linalool oxide (10.80%)	(F) C. albicans, Trichophyton tonsurans, T. rubrum	Bozin et al., 2007
1,8-cineole (12.10-61.40%); Camphor (5.80-24.10%); α-pinene 0.40-14.20%)	(G+) S. aureus, Enterococcus feacalis,; S. epidermidis, B. subtilis; (G-) E. coli, Proteus vulgaris, Pseudomonas aureginosa, Klebsiella pneumonia; (F) C. albicans	Celiktas et al., 2007
α-pinene (14.90%); 1,8-cineole (7.43%); Linalool (14.90%)	(G+) Brevibacillus brevis, B. megaterium, B. subtilis, B.subtilis var. niger, S. aureus, Mycobacterium smegmatis, L. monocytogenes, S. thermophiles; (G-) E. coli, P. fluorescens, Y. enterocolitica	Erdoğrul, 2002; Gachkar et al., 2007
Isocamosol (19.77-65.55%); Dihydronormorphinone (3.36-14.34%); Verbenone (2.442-13.02%)	(G+) S. aureus, Bacillus cereus; (G-) E. coli, Pseudomonas aeruginosa	Genena et al., 2008
-	(G+) S. aureus, S. epidermidis, Streptococcus mutants, Mycobactcrium smegmalis	Luqman et al., 2007
Carnosic acid; Carnosol; Rosmarinic acid	(G+) Streptococcus mutans, S. salivarius, S. sobrinus, S. mitis, S. sanguinis, Enterococcus faecalis	Bernardes et al., 2010
1,8-cineol (38.50%) Camphor (17.10%); α-pinene (12.30%) Limonene (6.23%) Camphene (6.00%); Linalool (5.70%)	subtilis, Bacillus pumilis; (G-) Salmonella poona, E. coli, Pseudomonas aeruginosa	Hussain et al., 2010
Carnosic acid	(G+) S. aureus, B. cereus, L. monocytogenes; (G-) C. jejuni, S infantis, E. coli	Abramovič et al., 2012
-	(G+) Bacillus cereus, Micrococcus luteus, S. aureus	Chan et al., 2012
α-pinene (21.50%);1,8-cineol 15.20%); Borneol (8.60%); Verbenone (8.60%)	(G+) S. aureus S. intermedius S. hyicus S. epidermidis S. saprophyticus, S. aureus ssp. anaerobious, S. capare S. gallinarum S. arlettae S. lentus S. equorum S. simulans S. delphini S. chromogenes	Issabeagloo et al., 2012
1,8-cineol (13.66-49.05%); Camphor (12.62-19.20%); α-pinene (10.08-21.03%); Camphene (4.09-9.15%)	(G+) S. epidermidis, S. aureus, B. subtilis; (G-) Pseudomonas aeruginosa, E.coli,; Proteus vulgaris (F) C. albicans, A. niger	Fu et al., 2007; Wang et al., 2012; Matsuzaki et al., 2013
1,8-cineol (46.40%); Camphor (11.40%); α-pinene (11%); β-pinene (9.20%); Camphene (5.20%)	(G+) B. subtilis, S. aureus; (G-) E.coli, P. aeruginosa	Sienkiewicz et al., 2013; Ghaedi et al., 2015
α-pinene (50.80%); 1,8-Cineole (24.40%)	(G+) Staphylococcus epidermis; (G-) Pseudomonas aeruginosa, ;(F) Aspergillus spp., Trichophyton spp	Mekonnen et al., 2016
1,8-cineole (5.63-6.89%); Camphor (1.66-4.82%); α-pinene (14.69-0.81%)	(G+) S. aureus,S. agalactiae,; (G-) E. coli, K. pneumonae	Bajalan et al., 2017b
α-pinene (14.08%); 1,8-cineole (23.67%); Camphor (18.74%)	(G+) Listeria monocytogenes, B. subtilis; (G-) E. coli	Gouveia et al., 2016, Bouyahya et al., 2017
Linalool (5.43%); Camphor (16.24); Borneol (15.90%); Verbenone (16.25%)	(G+) L. monocytogenes, S. aureus, B. cereus, M. flavus; (G-) P. aeruginosa, E. coli (G-) Xanthomonas oryzae	El-Esawi et al., 2017 Abdallah et al., 2019
Eucalyptol (48.72%); Camphor (11.72%); α-pinene (9.86%); β-pinene (8.35%)	(G+) S. aureus, B. subtilis; (G-) E. coli, K. pneumoniae	Stojanović-Radić et al., 2010; Park et al., 2019; Risaliti et al., 2019; Abozahra et al., 2020
1,8-cineol (31.12%); Camphor (12.04%); α- pinene (47.31%)	(G+) Vagococcus salmoninarum	Metin and Biçer, 2020
Camphor (32.50%); 1,8-cineol (13.60%); α-pinene (9.80%)	(G+) S. aureus, S. enteritidis; (G-) P. aeruginosa, E. coli	Paulus et al., 2020
1,8-cineole (52.20%); Camphor (15.20%); α-pinene (12.40%)	(F) Aspergillus flavus	Silva Bomfim et al., 2020

Chan et al. (2012) compared dried and fresh samples of aromatic herbs such as rosemary, sage, thyme, basil, oregano, marjoram, peppermint, and spearmint in terms of antibacterial activity. While the plants used in the study did not show any effect against gram (-) bacteria, all of them showed antibacterial activity against gram (+) ones. It has been reported that the plant that shows the highest antibacterial activity among them is fresh rosemary.

#### **Antifungal Activity of Rosemary Plants**

Increasing health problems due to various pathologies such as various cancers, liver diseases, and hormonal disorders caused by chemical residues in agricultural products brought the use of essential oils to the agenda for the development of natural and environmentally-friendly alternative fungicides (Carnesecchi et al., 2001; Rouabhi, 2010; Najar et al., 2020).

Silva Bomfim et al. (2020) evaluated the antifungal and antiaphylatoxigenic activity of REO against Aspergillus flavus with their study. They founded that, A. flavus reduced mycelial growth at a concentration of 250 µg/mL (15.3%). In addition, it has been reported that ergosterol production and mycelium biomass decrease as the treatment concentration increases. Bozin et al. (2007) on the antifungal activity of REO on Trichophyton and Microsporum species for the first time in the literature stated that it was a highly effective agent against hydroxyl radicals and peroxidation processes of lipids. In addition, confirmed previously published data on its antibacterial and antifungal activity. Candida albicans is a polymorphic type of fungus that, under certain conditions, can cause infections ranging from skin infections to systemic infections that lead to death (Bozin et al., 2007; Celiktas et al., 2007; Mayer et al., 2013). There are studies showing that the essential oil of rosemary inhibits this type of fungus that causes many diseases in humans (Fu et al., 2007; Akroum, 2020). It is reported that essential oil of rosemary has fungus inhibiting properties against Aspergillus niger which is an important disease factor in humans, plants, and animals (Table 2) (Santoyo et al., 2005; Fu et al., 2007; Türe et al., 2008; Zhao-ming, 2009; De Sousa et al., 2013).

As a result of in vitro studies, it was determined that it has antifungal activity. Larrán et al. (2001) found in their study that  $800~\mu L$  / L dose of rosemary showed strong fungistatic activity against *Ascosphaera apis* which causes Chalkbrood disease in honey bees. Baratta et al. (1998) reported that rosemary is an effective inhibitor on *Fusarium graminearum*, however, Angioni et al. (2004) reported that it does not have an inhibitory effect and increases the growth of the fungus. *Trichophyton* spp. was determined to be inhibited by REO. It showed moderate activity against *Aspergillus spp*. (Table 2) (Mekonnen et al., 2016).

### **Conclusions**

Some of the chemical components contained in the rosemary plant have many beneficial properties such as anticancer, anti-depressive and anti-inflammatory effects. REO has also been used for this purpose from ancient times until now a day. In this regard, current studies focus on the properties (antimicrobial, antifungal, and antioxidant) of

rosemary that elicits on its pharmacological effects. Thanks to the new studies put forward, the potential for use as an alternative to harmful chemicals in the pharmaceutical industry has increased. REO by combating cytotoxic substances plays an important role in dealing with cancer. In addition REO was used many favorable treatments for detrimental diseases and also quite effective on antimicrobial usage.

It is seen that there were many investigations showing that rosemary extracts, which were also used in the food industry, provide the inhibition of lipid oxidation that will ensure the long-term preservation of fresh foods. Overall, past studies show us that , in the near future the harmless applications of rosemary will increase its widespread use in food, agriculture, cosmetics, dentistry, medicine and many more field.

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