

# **Incredible Rosemary (*Rosmarinus officinalis* L.): An Updated Review of its Phytochemistry, Anti-inflammatory Activity, and Mechanisms of Action Involved**

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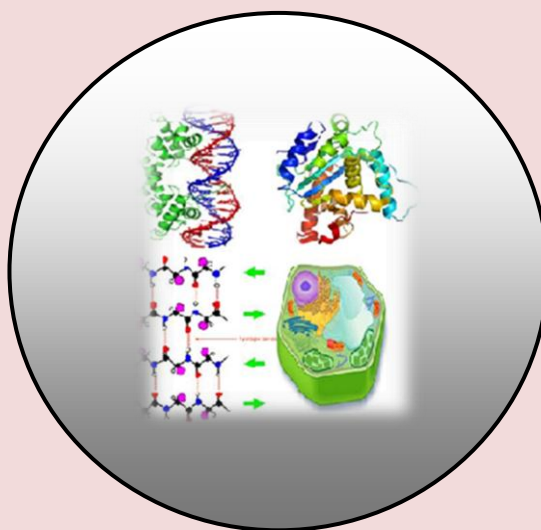
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**Incredible Rosemary (*Rosmarinus officinalis* L.): An Updated Review of its Phytochemistry, Anti-inflammatory Activity, and Mechanisms of Action Involved**

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**ABSTRACT**

The culinary, medicinal, and fragrance uses of rosemary are attributed to the vast arrays of chemical constituents collectively known as plant secondary metabolites. Of these, one group are small molecular weight aromatic compounds called essential oils which play vital role in the fragrance and culinary properties of the plant. Essential oils of rosemary dominated by 1,8-cineole,  $\alpha$ -pinene, camphene,  $\alpha$ -terpineol, and borneol as principal constituents are also responsible for various pharmacological effects (such as anti-inflammatory, antioxidant, antimicrobial, antiproliferative, antitumor, anticarcinogenic, protective, inhibitory and attenuating activities). The other group of secondary metabolites of rosemary are polyphenolic compounds including the flavonoids viz homoplantagin, cirsimaritin, genkwanin, galloocatechin, nepetrin, hesperidin, and luteolin derivatives and phenolic acid derivatives viz. rosmarinic acid. By far the most important group of rosemary compounds that gain significant attention in recent years, however, are the unique class of polyphenolic diterpenes. Various oxidized derivatives of carnosic acid we have been detected in rosemary leaves in low light, indicating chronic oxidation of this compound, and accumulated in plants exposed to stress conditions, in parallel with a loss of carnosic acid, confirming that chemical quenching of ROS by carnosic acid takes place in plant. Rosemary tea contains compounds that may have anti-inflammatory and antimicrobial properties. In this review paper, a literature gathering on rosemary, to identify main bioactive compounds, extracts and essential oils and their connection with pharmacological activities, has been precisely delineated.

**Keywords:** Carnosic Acid, Carnosol, Anti-inflammatory, Antimicrobial effects, Rosmarinic acid, Carnosic acid. Bioactive compounds, Pharmacological activities and Rosemary Tea.

## INTRODUCTION

Medicinal plants have been used worldwide by indigenous populations, playing an important role in the treatment of human and animal diseases [Uritu et al., 2008]. More recently, the majority of modern drugs have been developed from isolated compounds of medicinal plants, based on their ethnopharmacological uses/applications [Uritu et al., 2008, Pham-Huy et al., 2014, Rašković et al., 2014, Rašković et al., 2017, Rani B. and Maheshwari 2013]. The role of natural products on drug development has been increasing, not only when the bioactive compounds are directly used as therapeutic agents but also when they are used as raw material for drug synthesis, or as a base model for new biologically active compounds [Bozin et al., 2007, Casarotti and Jorge, 2014]. However, validating and using plants as a phytopharmaceutical requires a great deal of basic and applied research, in order to set this resource at the same level of importance of conventional pharmaceutical products. Rosemary, member of the Labiatae (Lamiaceae) family of plants (Fig 1), is a hardy evergreen perennial aromatic shrub that grows inherently in the Mediterranean region; however, it is cultivated around the globe due to its widespread use as a spice/seasoning, flavoring agent and for medicinal purposes. In addition, due to its inherent antioxidant properties, rosemary extract has widely been used in the food industry for shelf-life enhancement of various food products. Rosemary herb is typically propagated via seeds, cuttings, layering or division of roots. Rosemary leaves can be harvested 3 to 4x/ year, depending on the geographical region and whether the harvest is for plant material or essential oil. The crop is cut frequently before flowering commences, as the dried product contains only leaves.



**Figure 1. Rosemary Shrub.**

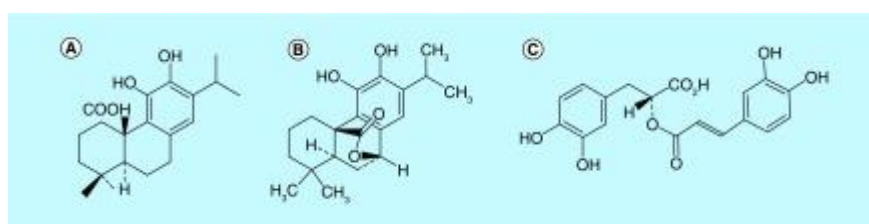
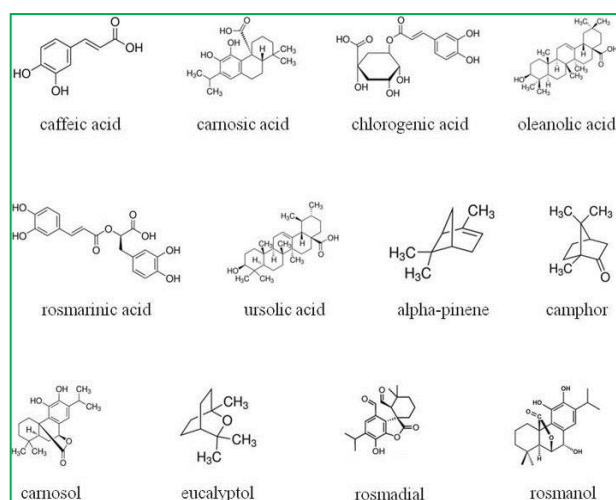
Rosemary is a rich source of antioxidants and anti-inflammatory compounds, which are thought to help boost the immune system and improve blood circulation. Laboratory studies have shown rosemary to be rich in antioxidants, which play an important role in neutralizing harmful particles called free radicals. The two most studied compounds in rosemary are rosmarinic acid and carnosic acid. Phytochemicals mainly present in *R. officinalis* are rosmarinic acid, camphor, caffeic acid, ursolic acid, betulinic acid, carnosic acid and carnosol. Therefore, *R. officinalis* is mainly composed of phenolic compounds, di- and triterpenes and essential oils. In *R. officinalis*, the most common polyphenols are apigenin, diosmin, luteolin, genkwanina and phenolic acids (>3%), especially rosmarinic acid, chlorogenic acid and caffeic acid. Other major compounds common in rosemary are terpenes, usually present in essential oils and resins, which include over 10,000 compounds divided into mono-, di-, tri- and sesquiterpenes, depending on the number of carbon atoms and isoprene groups (C<sub>5</sub>H<sub>8</sub>). It is possible to find in rosemary terpenes such as epirosmanol, carnosol, carnosic acid, ursolic acid and oleanolic acid (triterpenes).

## Phytochemicals and pharmacological activities

Carnosic acid, a phenolic diterpene specific to the Lamiaceae family, is highly abundant in rosemary (*Rosmarinus officinalis*). Despite numerous industrial and medicinal/pharmaceutical applications of its antioxidative features, this compound in planta and its antioxidant mechanism have received little attention, except a few studies of rosemary plants under natural conditions. In vitro analyses, using HPLC (high-performance liquid chromatography)-ultraviolet and luminescence imaging, revealed that carnosic acid and its major oxidized derivative, carnosol, protect lipids from oxidation. Both compounds preserved linolenic acid and monogalactosyldiacylglycerol from singlet oxygen and from hydroxyl radical. When applied exogenously, they were both able to protect thylakoid membranes prepared from *Arabidopsis thaliana* leaves against lipid peroxidation. Different levels of carnosic acid and carnosol in two contrasting rosemary varieties correlated with tolerance to lipid peroxidation. Upon reactive oxygen species (ROS) oxidation of lipids, carnosic acid was consumed and oxidized into various derivatives, including into carnosol, while carnosol resisted, suggesting that carnosic acid is a chemical quencher of ROS.

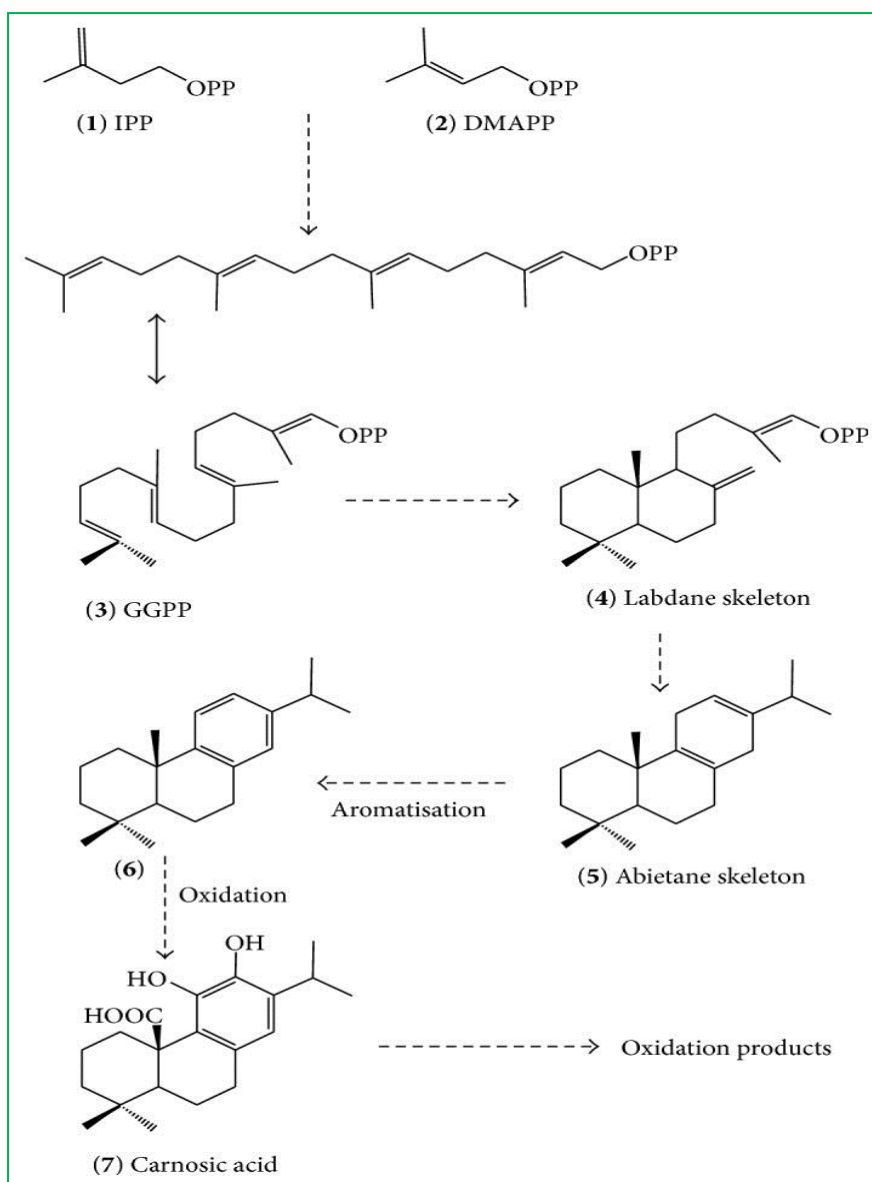


Figure 2. Rosemary Flowers.



The antioxidative function of carnosol relies on another mechanism, occurring directly in the lipid oxidation process. Under oxidative conditions that did not involve ROS generation, carnosol inhibited lipid peroxidation, contrary to carnosic acid. Using spin probes and electron paramagnetic resonance detection, we confirmed that carnosic acid, rather than carnosol, is a ROS quencher.

The antioxidant and anti-inflammatory activity of rosemary is largely attributed to its polyphenolic compounds like rosmarinic acid and carnosic acid. Due to its antioxidant capability, rosmarinic acid is often used as a natural preservative to increase the shelf life of perishable foods.



Several phytocompounds presenting pharmacological activities may be isolated from essential oils and extracts of *R. officinalis* L., varying the concentration of these molecules in each specimen of the plant. The phytocompounds most reported include caffeic acid, carnosic acid, chlorogenic acid, monomeric acid, oleanolic acid, rosmarinic acid, ursolic acid, alpha-pinene, camphor, carnosol, eucalyptol, rosmadial, rosmanol, rosmaquinones A and B, secohinokio, and derivatives of eugenol and luteolin [Casarotti and Jorge, 2014].

In order to obtain the biologically active compounds from rosemary, it is necessary to obtain the plant's extracts and/or essential oils, and perform a phytochemical characterization. The extraction methods are applied to the plant most active portions viz. leaves, roots, stems or flowers (Fig. 2), using selective solvents and standard procedures. These techniques result in complex mixtures in liquid and semisolid forms or, after removal of the solvent, in the form of dry powder. The essential oil (Fig 3) of rosemary obtained by steam distillation from the leaves (up to 2.5%) is colorless to light yellow, water-insoluble and with a characteristic aroma of camphor (31-37). The main constituents of the rosemary essential oil are camphor (5.0-21%), 1,8-cineole (15-55%),  $\alpha$ -pinene (9.0-26%), borneol (1.5-5.0%), camphene (2.5-12%),  $\beta$ -pinene (2.0-9.0%) and limonene (1.5-5.0%) in proportions that vary according to the vegetative stage and bioclimatic conditions.

### Rose Antioxidant properties

Of the natural antioxidants, rosemary extract has been widely recognized as one of the spices/seasoning that exhibits high antioxidant activity in numerous food applications. According to Moreno et al. (2006), both antioxidant and antimicrobial activities of rosemary extract are linked to its polyphenol composition. A good correlation existed between the antioxidant activities and total phenol content in the extracts. Although rosemary extract showed a high radical scavenging activity, a different efficacy as antimicrobial agent was observed. Methanol extract containing 30% of carnosic acid, 16% of carnosol and 5% of rosmarinic acid was the most effective antimicrobial against gram positive bacteria, gram negative bacteria and yeast. By contrast, water extract containing only 15% of rosmarinic acid showed a narrow activity range. Therefore, their results suggest that the antimicrobial activity of rosemary extracts was associated with their specific phenolic composition.

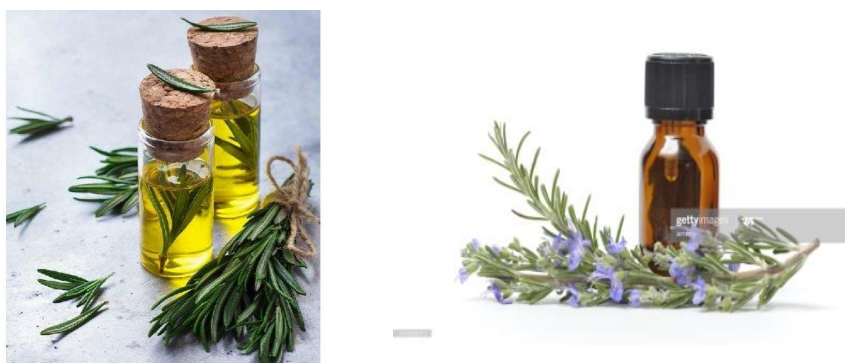


Figure 3. Rosemary Oil.

For beverage applications, rosemary extracts containing hydrophilic antioxidant molecules (i.e., rosmarinic acid) are commonly used. In most food applications, the actual usage rates of rosemary extract will vary depending on the total fat content of the food as well as the nature of the fat and intended use Table 1. Main features and benefits of commercially available rosemary extracts I alternative to synthetic antioxidants Clean label option Ease of handling, distribution and application Standardized antioxidant activity Low odor and flavor intensity Relative heat stability Availability of optimized tailored solutions Shelf-life extension in food Reduce formation of rancid flavors and off-odors in food Promotes longer food product freshness by protecting flavor, aroma, and/or color Minimize food product loss

## Rosemary Phenolic Compounds

Rosemary extract contains diverse groups of polyphenol compounds including phenolic terpenes, flavonoids and some phenolic acids. The composition of rosemary extracts varies depending on the type of extraction procedure used. Although several antioxidant components have been identified, the most potent antioxidant compounds are the phenolic diterpenes, carnosic acid and carnosol, which account for over 90% of the antioxidant activity of rosemary extract. Other phenolic compounds and minor constituents found in rosemary extract such as rosmanol, epirosmanol, isorosmanol, rosmadial, rosmaridiphenol, and rosmariquinone also exert some antioxidative functions. In addition, triterpenes and triterpenic acids such as betulin, amyrrin, betulinic acid, oleanic acid, and ursolic acid may also be present in rosemary extracts. Flavonoids present in rosemary extract include the flavones and flavonols such as apigenin, genkwanin, luteolin, hispidulin, rutin, kaempferol, naringin, hesperetin, apigenin-7-O-glucoside, and quercetin, among others. Phenolic acids reported in rosemary extract include rosmarinic acid, caffeic acid, chlorogenic acid, coumaric acid, p-coumaric acid, ferulic acid, vanillic acid, syringic acid, homovanillic acid and p-hydroxybenzoic acid, among many others.

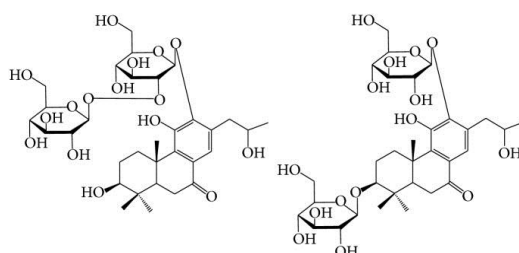
The main volatile compounds found in rosemary extract essential oil fraction include camphor, borneol, bornyl acetate, eucalyptol (1,8-cineol), verbenone,  $\alpha$ -terpineol,  $\alpha$ -pinene and limonene, among others (Senanayake, 2013). The essential oil fraction of rosemary extract is a colorless or pale-yellow liquid that contains these volatile compounds. The essential oils derived from rosemary herb are reported to exhibit antibacterial, antifungal and anticancer properties. The main compounds responsible for the antimicrobial activity are camphor, bornyl acetate,  $\alpha$ -pinene and eucalyptol. Bozin et al. (2007) investigated the antibacterial activity of essential oils derived from rosemary and sage against a number of food spoilage microorganisms. They concluded that the essential oils of both rosemary and sage were effective against *Escherichia coli*, *Salmonella typhi*, *S. Enteritidis*, and *Shigella sonnei*. In addition, rosemary essential oil exhibited some antifungal activity. Similar results were obtained by Fu et al. (2007) for the antibacterial activity of essential oils from rosemary and sage against some bacterial strains. Although rosemary extract contains both volatile compounds and antioxidant components, it may be produced such that its antioxidant functionality is enhanced. This can be done via steam distillation to remove some of the volatile oil fraction from the extract. However, residual quantities of volatile flavor compounds may remain in the final extract which may exert some flavor effects.

## Antioxidation Action of Rosemary Extract

Lipid oxidation is one of the most critical aspects of quality deterioration in foods which also affect their functional properties, nutritional value, safety, color, flavor as well as consumer acceptability. The control of lipid oxidation in food remains to be a continuing challenge as most foods are made up of complex matrices. Oxidation of unsaturated lipids in food involves a free radical chain reaction that is generally initiated by the presence of ultraviolet and/or visible light, heat, air (oxygen) and prooxidant metal ions. Lipid oxidation produces various primary and secondary oxidation by-products that influence quality of food. The classical mechanism of autoxidation, free radical chain reaction, includes initiation, propagation, and termination steps. Lipid oxidation is initiated when a hydrogen atom from a site in the fatty acyl chain of unsaturated lipids is abstracted and lipid alkyl radicals are formed. The resultant lipid free radical reacts swiftly with triplet oxygen ( $^3O_2$ ) and produces peroxy radical as well as another reactive lipid free radical.

The lipid peroxy radical abstracts a hydrogen atom from another lipid molecule resulting in the formation of hydroperoxide (LOOH) and a new lipid alkyl radical which can disseminate the oxidation chain reaction. The primary oxidation products, lipid hydroperoxides (ROOH), so produced are typically flavorless and odorless compounds. The decomposition of lipid hydroperoxides, which occurs in the presence of metals or at high temperature, leads to the formation of aldehydes, ketones, alcohols, acids, esters, furans, lactones and hydrocarbons. These compounds contribute to off-odors and off-flavors in food. The presence of naturally occurring antioxidants such as those derived from rosemary extract may help control these degradative reactions. Rosemary extract contains several bioactive compounds, which have proven to exert antioxidative properties, belonging mainly to the classes of phenolic acids, flavonoids, diterpenoids and triterpenes.

Reminiscent of other phenolic antioxidants, the presence of phenolic diterpenes such as carnosic acid and its derivative carnosol in rosemary extract are capable of interfering with lipid oxidation by donating hydrogen atoms to lipid free radicals. Synthetic phenolic antioxidants such as butylated hydroxyanisole (BHA) or butylated hydroxytoluene (BHT) each have a single aromatic ring with one hydroxyl group that can serve as a hydrogen donor. Similarly, carnosic acid and carnosol each have a single aromatic ring; however, each molecule has two O-phenolic hydroxyl groups, located at C 11 and C 12 positions of catechol moiety, capable of donating hydrogen atoms ( $H\bullet$ ) to lipid free radicals. Intrinsically, carnosic acid and carnosol have better antioxidant activity than BHA or BHT. In addition, the resultant radical intermediates of carnosic acid and carnosol are relatively stable due to resonance delocalization of unpaired electrons around the aromatic rings and lack of suitable sites in the phenoxyl radical for attack by the molecular oxygen.



Moreover, the hydroxyl groups situated in carnosic acid and carnosol molecules have the ability to chelate prooxidant metal ions, and thus help delay lipid oxidation via a secondary mechanism.

Research also suggests that rosemary phenolic diterpenes are capable of scavenging various types of free radicals including peroxy, hydroxyl and DPPH radicals. Rosemary phenolic diterpenes, carnosic acid and carnosol, can be further oxidized on exposure to elevated temperatures, oxygen, and light; however, their ability to delay lipid oxidation is maintained (Senanayake, 2013). When carnosic acid reacts with lipid free radicals, it converts into a carnosol molecule. Carnosol also reacts with lipid free radicals and converts into rosmanol. Hence, the principal decomposition products of carnosic acid are carnosol and rosmanol. Meanwhile, a minor degradation product of carnosic acid is epirosmanol. It has been postulated that rosmanol continues the free radical scavenging process until it becomes galdosol (Fig. 2). Liu et al. (2013) studied the degradation pathway of carnosic acid in a methanolic solution through isolation and structural identification of its degradation products. They reported that carnosic acid can decompose into carnosic acid quinone in methanol.

Carnosic acid quinone is the quinone intermediate of carnosic acid that can be reduced into carnosic acid or can be further converted into carnosol. Carnosol can further decompose to form epirosmanol, rosmanol, 7-methoxy-rosmanol, and 7-methoxyepirosmanol. The latter three compounds were identified as the final degradation products of carnosic acid in methanol because they appeared to be rather stable in the study. Volatile flavor compounds In addition to active antioxidant components, rosemary extract contains several volatile components that are responsible for its flavor Figure 1. Phenolic diterpenes identified as principal antioxidative components of rosemary extract (*Rosmarinus officinalis*, L.).

### Traditional Uses

In traditional medicine, the leaves of *R. officinalis* L. are used based on their antibacterial activities, carminative and as analgesic in muscles and joints/ Also, rosemary's essential oils and extracts obtained from flowers and leaves are used to treat minor wounds, rashes, headache, dyspepsia, circulation problems, but also as an expectorant, diuretic and antispasmodic in renal colic. Polyphenols are antioxidant chemical compounds primarily responsible for the fruit coloring, which are classified as phenolic acids, flavonoids and nonflavonoids. In addition to their antioxidant properties, they play a very important role in the plant defenses against herbivores, pathogens and predators; therefore, they have an application in the control of infectious agents in humans. The compounds in rosemary tea (Fig 4) may also have antimicrobial properties, which may help fight infections. Rosemary leaves are employed in traditional medicine for their antibacterial and wound healing effects. Studies have also investigated the effects of rosmarinic and carnosic acid on cancer. They have found that the two acids may have antitumor properties and even slow the growth of leukemia, breast, and prostate cancer cells. Rosemary extract has been well recognized in the old industry as a natural alternative to synthetic antioxidants such as BHA or BHT. Rosemary extracts, which are considered Generally Recognized as Safe (GRAS), have diverse applications in various food and beverage product categories. They have been widely used in several food products such as meat, poultry and seafood, dressings and sauces, potato chips, and baked goods.



Figure 4. Rosemary Tea.

### CONCLUSION

*Rosemary* is a medicinal plant of immense therapeutic applications. Besides the culinary uses due to the characteristic aroma, this plant is also widely employed by indigenous populations, where it grows wild. Rosemary extract derived from rosemary leaves contains several polyphenolic components which exhibit antioxidant properties, but the predominant active components are the phenolic diterpenes, namely, carnosic acid and carnosol.

Over 90% antioxidant activity of rosemary extract is attributed primarily to high content of lipophilic antioxidant molecules such as carnosic acid and carnosol. In addition to antioxidant components, rosemary extract also contains some volatile compounds. Rosemary extract bioactive compounds are also responsible for their distinctive aroma, color and flavor attributes. Rosemary extract can be applied in a wide range of oils and fats, and lipid-containing foods to delay lipid oxidation and to enhance the shelf-life of various food, feed and pet food products. This review delineates the chemistry, antioxidant mechanism, volatile flavor compounds, regulatory position, food applications, and stability of rosemary extract in foods. The extracts obtained from rosemary are used as a natural antioxidant, improving the shelf life of perishable foods. Rosemary tea contains compounds shown to have antioxidant, anti-inflammatory, and antimicrobial effects.

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