International Journal of Biology and Life Sciences
ISSN: 2957-9511 | Vol. 5, No. 3, 2024

Review of the Antimicrobial Properties of Rosemary Extract for Preservative Functions

Shunyuan Zhang [1], Yong Wang [1], Huajun Shawn Fan [2], Rong Li [3, *]

[1] Tourism School of Kunming University, Kunming University, Kunming, Yunnan, 650000, China
[2] College of Chemical Engineering, Sichuan University of Science and Engineering, Zigong, Sichuan, 643002, China
[3] Xiangtan Institute of Technology, Xiangtan, Hunan, 411100, China

*** Corresponding author:** Rong Li

Abstract: Rosemary extract has physiological activities such as anti-cancer, anti-viral, anti-inflammatory, antiseptic, antibacterial and antioxidant, and has great economic and research value. The extraction methods of rosemary extract include solvent extraction and supercritical fluid extraction. Its purification methods include crystallization, solvent extraction, and macroporous resin adsorption. This article focuses on the analysis of rosemary extract, which mainly includes the key research results both at home and abroad in terms of its antibacterial and antiseptic, biological functions and extraction, purification and antiseptic mechanisms. The purpose of the study is to promote the application of rosemary extract in antibacterial and antiseptic and provide a theoretical basis.

Keywords: Rosemary; Extract; Activity; Antiseptic.

1. Introduction

1.1. Main Active Components of Rosemary

Rosemary, a widely utilized aromatic plant in culinary practices, is prominently distributed in regions including Yunnan, Hunan, and Guangxi in China. It encompasses an array of compounds such as phenolic acids, flavonoids, and terpenoids. Notably, its leaves contain a substantial amount of water-soluble antibacterial agents, primarily represented by rosmarinic acid. This attribute imparts significant economic and scientific research value to rosemary. The chemical formulas and concentrations of its primary active constituents are detailed in Table 1, with a graphical representation provided in Figure 1.

Table 1. Main Compounds in Rosemary

No.	Chinese Name	English Name	Chemical Formula
1	Carnosicacid	Carnosicacid	C20H28O4
2	Carnosol	Carnosol	C20H26O4
3	Rosmarinicacid	Rosmarinicacid	C18H16O8
4	Vanillicacid	Vanillicacid	C8H18O4
5	Caffeicacid	Caffeicacid	C9H8O4
6	Hispidulin	Hispidulin	C16H12O6
7	Cirsimaritin	Cirsimaritin	C17H14O6
8	Naringin	Naringin	C27H32O14
9	Rosmanol	Rosmanol	C20H26O5
10	Chlorogenicacid	Chlorogenicacid	C16H18O9
11	Rutin	Rutin	C27H30O16
12	Quercetin	Quercetin	C15H10O7
13	Kaempferol	Kaempferol	C15H10O6
14	Epirosmanol	Epirosmanol	C20H26O5
15	Rosmadial	Rosmadial	C20H24O5
16	Rosmaridiphenol	Rosmaridiphenol	C20H28O3

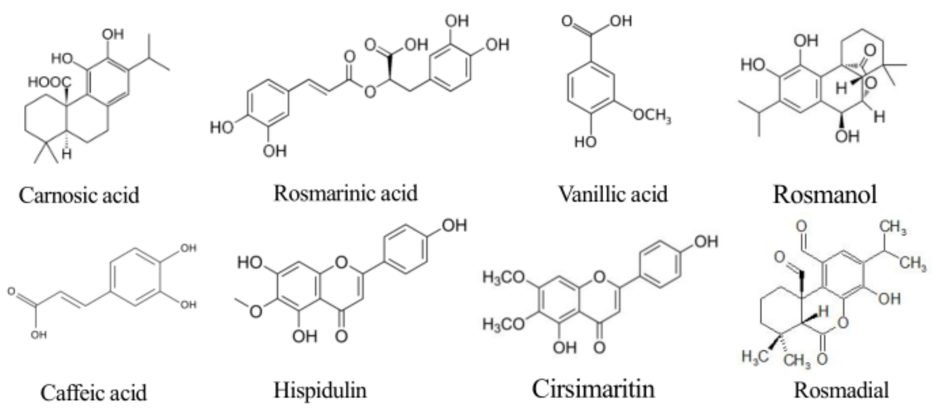

Figure 1. Main Compounds in Rosemary

1.2. Physiological Activities of Rosmarinic Acid in Rosemary

1.2.1. Antimicrobial and Preservative Effects

Numerous studies have confirmed the excellent antimicrobial properties of rosemary. Research has shown that methanol extracts of rosemary can effectively inhibit the growth of spoilage microorganisms and foodborne pathogens such as Brettanomyces, Saccharomyces cerevisiae, Candida albicans, Bacillus megaterium, Staphylococcus aureus, and Escherichia coli. Ojedasana et al. found that 1,8-cineole in rosemary essential oil also exhibits antimicrobial properties. Additionally, Bernarde's research indicates that it can inhibit the growth of bacteria such as Streptococcus pyogenes and Streptococcus salivarius.

1.2.2. Anti-Inflammatory Activity

Rosemary contains various active components, exhibiting significant anti-inflammatory activity. It has been reported that rosemary extracts have inhibitory effects on the synthesis of inflammatory cytokines, including tumor necrosis factor-alpha (TNF-α), interleukin-1 beta (IL-1β), and interleukin-6 (IL-6), induced by oxidized low-density lipoprotein and lipopolysaccharide in THP-1 monocyte-derived macrophages. This indicates its potential in alleviating inflammatory issues, primarily attributed to the presence of rosmarinic acid and carnosic acid. Research by Kuo suggests that rosemary extracts also inhibit the phosphorylation of factor α, exerting control over the translocation of p65. Furthermore, studies by Zhang Linlin in mouse experiments highlight the anti-inflammatory effects of rosemary essential oil, particularly in acute inflammation, including foot swelling in mice induced by carrageenan, ear swelling induced by xylene, and increased capillary permeability in the abdominal cavity.

1.2.3. Antioxidant Activity

Rosemary is widely utilized for its antioxidant properties, especially in the context of oil preservation, as illustrated in Table 2.

Table 2. Inhibition of Oil Oxidation Effects

Product Type	Target	Effect
Rosemary Extract and Rosemary Oil	Microalgae Oil	Both rosemary extract and rosemary oil effectively inhibit the elevation of TBARS and peroxide value in microalgae oil.
Rosemary Extract	Macadamia Nut Oil	Rosemary extract (0.08%, w/w) significantly extends the storage time of macadamia nut oil.
Rosemary Methanol Extract	Sardine Oil	Rosemary extract inhibits the oxidation of DHA and EPA in sardine oil under accelerated conditions (150°C, 30 min) and high-temperature storage (60°C, 0-5 days), with the best inhibitory effect observed at a concentration of 2.5%.
Rosemary Extract	Soybean Oil	Rosemary extract (1000mg/kg) inhibits the oxidation of soybean oil during heating at 180°C, extending the stability time from 7.52h to 13.5h.
Rosemary Extract	Soybean Oil	Rosemary extract (3000mg/kg) reduces the oxidation of soybean oil during heating at 180°C.
Rosemary Extract	Soybean Oil, Rice Bran Oil	Rosemary extract (400mg/kg) lowers the peroxide values of soybean oil and rice bran oil at 62°C, inhibiting lipid oxidation reactions.
Rosemary Oil	Sunflower Seed Oil	Rosemary oil effectively inhibits the increase in peroxide value, conjugated dienes, and conjugated trienes during the storage of sunflower seed oil (23°C, 0-115 days).
Rosemary Extract	Flaxseed Oil	At a storage temperature of 60°C, 400mg/kg rosemary extract inhibits the increase in peroxide value and anisidine value in flaxseed oil.
Rosemary Extract	Butter	Rosemary extract enhances the antioxidant capacity of butter, extending the shelf life from 45 days to 210 days at 37°C and from 4 days to 36 days at 60°C storage conditions.

1.2.4. Anti-tumor Activity

In recent years, malignant tumors have drawn widespread attention due to their detrimental impact on human health. On one hand, cell experiments indicate that rosemary extract can effectively inhibit the growth of various tumor cells, including Caco-2 colon cancer cells, MCF-7 human breast cancer cells, and HepG2 human liver cancer cells. For instance, Zhang Xiuying et al. discovered that rosemary acid can effectively inhibit the proliferation of human tongue cancer cells Tca8113. Additionally, rosemary exhibits inhibitory effects on breast cancer and colon cancer in SD rats and mice.

2. Current Research on Extraction, Purification, and Applications of Rosmarinic Acid

2.1. Extraction Methods for Rosmarinic Acid

The preparation of natural products generally involves two major steps: extraction and separation purification. The target compound is often found in different organs or tissues of various plants, and due to the compositional differences among plants, the extraction methods used can vary significantly. For instance, in the case of perilla, where rosmarinic acid is mainly concentrated in the leaves, Nakamura et al. (1998) first attempted an extraction method that involved crushing fresh perilla leaves. The process included initial methanol extraction followed by vacuum concentration, subsequent extraction using ethyl acetate-water (1:1) and n-butanol-water (1:1), and finally purification of the concentrated extract by high-performance liquid chromatography (HPLC) to obtain rosmarinic acid. Similarly, for danshen, where rosmarinic acid is present in the roots and stems, Gao Shurui et al. employed an ultrasound-assisted extraction method using 75% ethanol under a liquid-to-material ratio of 200 ml/g for extracting rosmarinic acid from the roots and stems of danshen.

Based on the differences in extracting solvents or methods, the extraction methods of rosmarinic acid from rosemary can be summarized as follows:

2.1.1. Solvent Extraction Method:

The solvent extraction method is based on the principle of "like dissolves like" to extract the target product. Due to the different polarities of solvents, different components are extracted. Yang Hailin and others conducted a detailed comparative study on solvents for extracting rosmarinic acid, testing various organic solvents such as ethanol, methanol, ether, hexane, and chloroform. The results showed that ethanol and methanol were more effective, with extraction yields of 3.6% and 5.3%, respectively. However, methanol is toxic and not suitable for use, while ethanol, being non-toxic, inexpensive, and easily recyclable, is the optimal solvent for extracting active components from rosemary. Bi Liangwu and others used a one-step solvent method, a mixture of petroleum ether, ethanol, ethyl acetate, and water in different ratios, to cyclically extract rosemary antioxidants at 80°C in a water bath. The best results were obtained by mixing petroleum ether and ethanol in a volume ratio of 5:1 to form a 1000 mL solvent, and adding 500 g of dried rosemary leaves. The resulting rosmarinic acid content was 1.08%. Bi Liangwu and others further improved the one-step extraction of rosemary antioxidants by adopting a two-step extraction method. The first step involved extracting with a more polar solvent, ethanol, to obtain an extract, and the second step used petroleum ether to remove impurities and obtain lipophilic antioxidants. The extract was then washed with water repeatedly, with the lipid-soluble antioxidants remaining in the water, mainly composed of water-soluble antioxidants, including rosmarinic acid. After repeated experiments, the optimal extraction conditions were determined to be using 95% ethanol at 90°C for 3-4 hours, obtaining an extract which was then subjected to boiling in ether at 60-90°C to remove impurities. The resulting lipophilic antioxidant content was 5.96%, and after washing with water, the water-soluble antioxidant content, rosmarinic acid, was 1.67%. To enhance extraction efficiency, ultrasound-assisted extraction can also be employed. Zhang Chunyan pointed out that by pre-treating rosemary leaves with ultrasound for 10 minutes, followed by reflux extraction for 1 hour using a 15% ethanol solution, the extraction rate of rosmarinic acid under these conditions reached 94.94%. Compared to solvent extraction and hot water extraction methods, ultrasound-assisted extraction can reduce extraction time and greatly improve efficiency.

2.1.2. Supercritical Fluid Extraction Method:

Supercritical fluid extraction is advantageous for selective separation, maintaining the natural properties of substances to the greatest extent, resistance to component destruction, no residual toxicity, and high extraction efficiency. Bi Liangwu believes that this method has a pressure difference issue, and the average yield of rosmarinic acid from rosemary is approximately 0.12, with a mass fraction of 5.13%. Lopez-Sebastin and others used orthogonal experimental design to systematically screen the influencing factors of supercritical fluid extraction, such as temperature, pressure, residual amount, and the amounts of three entrainers (ethanol, acetic acid, water). The optimal process conditions obtained were 60°C temperature,20 MPa pressure, and the highest content of rosemary antioxidants obtained without entrainers, with the ability to remove rosemary essential oil to the greatest extent.

2.2. Rosmarinic Acid Purification Methods

The crude extract needs to undergo purification. Currently, there is limited research on the purification of rosmarinic acid extracted from rosemary, with most studies focusing on extraction from plants such as perilla and peppermint. Common purification methods include:

2.2.1. Crystallization Method:

This method utilizes the principle that solubility of various solutes in the same solution varies significantly at different temperatures. By changing the system temperature, the solubility of the target substance or impurities decreases, leading to the crystallization of the target substance and achieving separation.

2.2.2. Solvent Extraction Method:

The solvent extraction method relies on the differences in solubility of the target substance in immiscible solvents to achieve concentration and purification. Advantages of solvent extraction include good selectivity, high separation degree, suitability for industrial production, fast speed, and ease of continuous operation. Factors affecting this method include the type and quantity of the extracting agent, pH value, time, and initial concentration. Solvents used for rosmarinic acid extraction include ethyl acetate, alcohols, and water. For instance, Zhai Ting extracted rosemary using ethyl acetate, chloroform, and petroleum ether, obtaining varying extraction rates. Luo Shengxu purified rosmarinic acid from peppermint using ethyl acetate as the extracting agent, achieving a content of 16.0%. Xue Jiao purified rosmarinic acid from perilla using a similar approach, obtaining a content of 28.7%. The extraction method is characterized by a short separation cycle, low energy consumption, high efficiency, and ease of scale-up, making it suitable for refining low-content components in natural products and showing great potential in purification.

2.2.3. Macroporous Resin Adsorption Method

Macroporous adsorption resins are often used for separating compounds in aqueous solutions, typically non-polar or low-polarity substances. According to Zhou Ping, the NK-109 type of macroporous adsorption resin has a better purification effect. Lin's research showed that after enrichment and purification with HP-20 resin, the rosmarinic acid content increased from 10.14% to 67.26%. Yuan Ganjun used various types of resins for purifying rosmarinic acid from perilla, with D101 resin showing superior results. Xie Fangfang utilized normal-phase silica gel chromatography for separation, increasing the acid mass fraction to approximately 0.18. Zhang Chunyan used X-5 resin for purification, achieving a purity of about 0.97. Li Ronggui used XAD-4 resin washed with ethanol for extraction, and through the extraction with petroleum ether and concentrated crystallization with ethanol-washed AB-8 resin, obtained rosmarinic acid with a purity of 0.95.

3. Mechanism and Application of Rosmarinic Acid in Antibacterial and Preservative Effects

The antibacterial properties of rosmarinic acid primarily stem from inducing changes in cell membrane permeability, leading to the leakage of proteins and reducing sugars, causing metabolic disruption within the cells. Additionally, it can inhibit DNA polymerase, preventing the chain reaction of polymerase and thereby hindering DNA replication. Both domestic and international studies have shown that rosmarinic acid can inhibit various bacteria, including Bacillus subtilis, Staphylococcus aureus, Pseudomonas syringae, and Escherichia coli. It exhibits inhibitory effects on Gram-positive Staphylococcus aureus and inhibits Escherichia coli. For instance, when the concentration of rosmarinic acid is 0.1%,there is no inhibition zone on plates coated with Escherichia coli, but there is an inhibition zone with a diameter of approximately 15 mm on plates coated with Staphylococcus aureus. Assuming a concentration of 0.006%, the diameter of the zone on Escherichia coli plates decreases to 11.5 mm, while on Staphylococcus aureus plates, it is 34.2 mm. Therefore, it is more effective against Staphylococcus aureus. Moreover, rosmarinic acid can inhibit various plant pathogenic fungi such as Venturia pirina, Penicillium italicum, Colletotrichum gloeosporioides, and Botrytis cinerea, showcasing good performance in low-temperature storage and exhibiting excellent heat stability. Additionally, studies by Neusal and others have found that rosmarinic acid can inhibit biofilm formation and the growth of oral bacteria, preventing and treating certain oral diseases. Considering existing research literature, it can be inferred that rosmarinic acid possesses antimicrobial activity, making it suitable for use in products like soaps, shampoos, shower gels, etc. Further research and development of the antibacterial properties of rosmarinic acid could effectively contribute to its utilization in fields such as medicine and agriculture.

4. Conclusion

As a aromatic plant, rosemary extracts are commonly used as food additives. Rosmarinic acid (RosA) exhibits various physiological activities, including anticancer, antiviral, anti-inflammatory, antibacterial, and antioxidant properties. The actions of rosemary and its extracts encompass liver protection, lipid-lowering, anti-inflammatory, anticancer, antibacterial, and antioxidant effects. Therefore, research on rosemary has been extensive and continues to deepen globally. This article provides a comprehensive review of rosemary extracts, focusing on applications such as antibacterial and preservative effects, biological functions, and extraction methods. The aim is to contribute theoretical foundations for the application of rosemary extracts in antibacterial and preservative contexts.

Acknowledgments

Authors would like to acknowledge the partial financial support from the Natural Science and Technology Foundation of Yunnan Province (202205 AP 130005) and 2023 Kunming University's scientific research team fund. Authors would like to thank the College of Chemical Engineering at Sichuan University of Science and Engineering for providing the lab facility and logistic support to this project.

References

[1] Xiao Liping, Li Linsheng. "Antibacterial Preservatives (I): History, Definition, and Classification of Antibacterial Preservatives." Daily Chemical Industry,2001, (05): 55-57.

[2] Li Mei, Wang Qingrui."Development and Application of Antibacterial Materials." New Chemical Materials, 1998, (05): 8-11.

[3] Wang H, Qiao X, Chen J, et al. Preparation of silver nanoparticles by chemical reduction method[J]. Colloids and Surfaces A:Physicochemical and Engineering Aspects, 2005 (2-3): 111-115.

[4] Bock C H, Hotchkiss M W, Shapiro-Ilan D I,et al.A comparison of organic fungicides:alternatives for reducing scab on pecan[J].Organic Agriculture, 2018(3): 305-314.

[5] Yu K H, Kim K N, Lee J H, et al. Comparative study on characteristics of lysozymes from the hemolymph of three lepidopteran larvae, Galleria mellonella, Bombyx mori, Agrius convolvuli[J]. Developmental & Comparative Immunology, 2002, 26(8): 707-713.

[6] Mei Congxiao, Fang Yuanchao, Zhao Jinfu. "Brief Introduction to the Application of Several Natural Food Preservatives." China Food Additives, 2000(04): 60-62.

[7] Lu Zhike, Xie Bixia. "Research Progress on Plant-Derived Natural Food Preservatives." Science and Technology of Food Industry, 2003(01): 94-96+93.

[8] Li Huiqin, Gong Jixian. "Natural Antibacterial Finishing Agents." Textile Journal, 2002, (02): 50-52+54.

[9] Porritt M J, Chen M, Rewell S S, et al. ACE inhibition reduces infarction in normotensive but not hypertensive rats: correlation with cortical ACE activity[J]. J Cereb Blood Flow Metab, 2010, 30(8): 1520-1526.

[10] Yin Guodong, Gao Zheng, Zhang Yanping. "Research Progress on Introduction, Cultivation, and Development and Utilization of Rosemary." Journal of Southwest Forestry University, 2010, 30(04): 82-88.

[11] Wang Hui. "Chemical Composition and Quality Evaluation of Rosemary." [Dissertation]. City: Liaoning University of Traditional Chinese Medicine, 2011.

[12] Zeng Yingnan, Gu Yuhang, Liu Jia, et al. "Research Progress of Natural Antioxidants in Oils." Anhui Agricultural Science Bulletin, 2019, 25(Z1): 21-23.

[13] Yang Yang, Wei Xiaoying, Ruan Zheng. "Research Progress on Natural Food Antioxidants at Home and Abroad." Food Science, 2002 (10): 137-140.

[14] Petersen M. Rosmarinic acid[J]. Phytochemistry, 2003, 62(2): 121-125.

[15] Trute A, Nahrstedt A. Separation of Rosmarinic Acid Enantiomers by Three Different Chromatographic Methods (HPLC, CE, GC) and the Determination of Rosmarinic Acid inHedera helix L[J]. Phytochemical Analysis, 1996, 7(4): 204-208.

[16] Chen Liya. "Research Overview of Rosmarinic Acid." China Pharmacy, 2007(11): 923-926+929.

[17] Cheng Xian, Bi Liangwu, Zhao Zhendong, et al. "Advances in Extraction, Purification, and Biological Activities of Rosmarinic Acid." Chemistry and Industry of Forest Products, 2015, 35(04): 151-158.

[18] Chen Ru, Weng Xinchu. "Introduction of Spices--Research on the Antioxidant Activity of Rosemary." Journal of Shanghai University (Natural Science Edition), 2006(01): 78-83.

[19] Cao Wen, Zhang Wenjuan, Pan Jinfeng, et al. "Research Progress on Pharmacological Effects of Rosmarinic Acid." Guangxi Traditional Chinese Medicine, 2019, (01): 54-58.

[20] Zhang Baoshun, Yuan Lvjian, Feng Min, et al. "Research and Application of Rosemary Natural Antioxidants." Jiangsu Food and Fermentation, 2006, (01): 20-22.

[21] Li Wanting, Wei Liqun, Li Qing, et al."Rosmarinic Acid Analogue-11 Inhibits Proliferation and Migration of Human Gastric Cancer MGC-830 Cells via the EGF-JNK Pathway." Chinese Pharmacological Bulletin, 2019(4): 504-508.