The Pharma Innovation Journal 2022; 11(9): 984-987

www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2022; 11(9): 984-987

www.thepharmajournal.com
Received: 20-07-2022
Accepted: 29-08-2022

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Antimicrobial activity of *Rosmarinus officinalis* essential oil against *Staphylococcus aureus* and *Bacillus subtilis*

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Abstract
In the present study, antimicrobial activity of *Rosmarinus officinalis Linn.* essential oil was evaluated against food-borne bacteria *Staphylococcus aureus and Bacillus Subtilis*. For *in vitro* antimicrobial tests, *Rosmarinus officinalis* essential oil exhibited moderate antibacterial activity against the test bacteria *S. aureus* and *B. subtilis*. *Rosmarinus officinalis* L. essential oil showed a significant reduction effect on the growth rate of surviving bacteria with inhibition zone diameter 10 ± 0.50 mm and 9.83 ± 0.58 mm respectively. Therefore, it can be suggested that rosemary oil has a potential to act as antimicrobial substitute in combating the Multi-drug resistant organisms (MDRO) which is a major global concern nowadays.

Keywords: Essential oils, antimicrobial, IZD, food spoilage

Introduction

Food spoilage caused by microbes is a major issue in food industries that is responsible for food borne illness, an increasingly major public health problem all over the world. Many side effects including headache, nausea, weakness, mental retardation, seizures, cancer and anorexia are associated with synthetic food preservatives used in preventing microbial contamination (Heymich *et al*., 2021) [14]. The oxidation of lipids in food, cosmetic, and pharmaceutical products, together with the growth of undesirable micro-organisms results in the development of spoilage, off-flavor, rancidity, and deterioration, rendering such products unacceptable for human consumption. It has been estimated that each year as many as 30% of people in industrialized countries suffer from food borne diseases. Thus, in the year 2000, at least two million people died from diarrheal disease worldwide. Furthermore, overproduction of free radicals in organisms and lipid peroxidation in cell membranes has been implicated in various pathophysiological dissorders, including cardiovascular diseases, mutagenesis, diabetes, ischemia-reperfusion injury, coronary atherosclerosis, Alzheimer's disease, and cancer genesis, as well as the aging process. The bacterial groups that have been mainly associated with food spoilage include psychrotolerant spore-forming bacteria such as (*Bacillus* spp., *Staphylococci* spp.), lactic acid bacteria as well as psychrotrophic gram-negative rods (*Pseudomonas* spp. and *Serratia* spp.) (Machado *et al*., 2017; Techer *et al*., 2020; Ibrahim *et al*., 2021) [32, 33, 15]. Admist, *Staphylococcus aureus* and *Bacillus subtilis* have been recognized as most prominent miscreant in food poisoning (Qiao *et al*., 2021; Moschonas *et al*., 2021; Soodsawaeng *et al*, 2022) [21, 18, 24]. In decades, synthetic additives have been widely used. However, the addition of synthetic preservatives is more restricted because of their toxic effects. Therefore, a strong interest in using natural substances for food preservation as an alternative has appeared (Singh *et al*., 2021; Gupta *et al*., 2021) [22, 13]. Essential oils are a complex mixture of natural, volatile, and aromatic compounds synthesized by aromatic plants and are classified as monoterpenes and sesquiterpenes, according to the number of isoprene units, monoterpenes being the most abundant in essential oils. These volatiles have attracted lot of scientific interests because they exhibit a wide spectrum of bioactivities, such as antibacterial, antifungal, antiviral, antioxidant, and insecticidal activities (Chen *et al*., 2021; Zhang *et al*., 2022; Tariq *et al*., 2019) [7, 28, 25]. Rosemary (*Rosmarinus officinalis* L.), which belongs to mint family, is a common dense, evergreen, aromatic shrub grown in many parts of the world. The fresh and dried leaves are frequently used in traditional Mediterranean cuisine as an additive. They have a bitter, astringent taste, which complements a wide variety of foods.

They are extensively used in cooking, therefore, they often are used to flavor foods while barbecuing. Historically, rosemary has been used as a medicinal agent to treat renal colic, dysmenorrheal, respiratory disorders Rosemary (*Rosmarinus officinalis* L.) plants grow worldwide and have been cultivated since long ago for its strong antioxidant and antimicrobial activities. This plant species also has many other beneficial activities such as antiviral, anti-inflammatory and anticarcinogenic (Aherne *et al*., 2007; Barni *et al*., 2012; Mengoni *et al*., 2011) [29, 30, 31] activity.

The present work was undertaken to determine the antimicrobial potential of *Rosmarinus officinalis* L. essential oil against two gram positive pathogenic bacteria namely, *Staphylococcus aureus* and *Bacillus subtilis*.

Experimental materials and chemicals

The *Rosmarinus officinalis Linn*. Essential oil was procured from CSIR-IIIM, Canal Road, Jammu. The test bacterial strains employed in this investigation were *S. aureus* MTCC 7443 and *B. subtilis* MTCC 2389, purchased from IMTECH, Chandigarh, India. Antibiotic (chloramphenicol) was procured from Hi-Media Laboratories, Mumbai, India.

Preparation of Inoculum

The selected test bacterial strains were inoculated in Muller Hinton broth (MHB) and incubated for 24 hours at 37 °C. Turbidity of the resulting bacterial suspension of active cultures was measured by taking absorbance at 600 nm using spectrophotometer and the optical density was adjusted equivalent to 0.5 Mcfarland standard by dilution with MHB.

Screening of antibacterial activity

Antibacterial activity of *Rosmarinus officinalis* L. Essential oil was carried out by agar disc diffusion assay Andrews, 2001. The sterilized nutrient agar (NA) medium about 20 ml was poured into the petriplates and allowed to solidify. Then 100 µl of bacterial suspension (10^8 CFU/ml) was uniformly spread over with a sterile glass spreader and then NA plates were kept to dry for 5 minutes. Whatman No. 1 sterile filter paper discs (6 mm diameter) were impregnated with 3 µl of the essential oil. The positive control used was chloramphenicol (2 µg) and negative control (DMSO). The discs were then placed on the inoculated nutrient agar surface and the plates were left at room temperature for about 30 min in order to allow the diffusion of oils into the agar. After that the plates were incubated at 37 °C for 24 hours. The plates were then examined for the measurement of inhibition zone diameters using transparent ruler after 24 hours of incubation.

Results and Discussion

Antibacterial activity of *Rosmarinus officinalis* essential oil

The results of the antibacterial activity of the *Rosmarinus officinalis* L. and conventional antibiotic (chloramphenicol) against two Gram positive (*Bacillus subtilis* and *Staphylococcus aureus*) bacterial strains revealed that *Rosmarinus officinalis* L. exhibited the antibacterial activity against the test strains with inhibition zone diameter (IZD) ranging from 9.83 ± 0.58 mm to 10 ± 0.50 mm at 1.90 mg/ml. Whereas, the inhibition zone diameter (IZD) of positive control (Chloramphenicol) was ranging from 15.50±0.5 to 16.83±1.04 against *B. subtilis* and *S. aureus* at the concentration of 2 µg/ml as depicted in Plate 1 and Figure 1. Some previous studies have demonstrated the antimicrobial efficacy of *Rosmarinus officinalis L.* against *S. aureus* and *B. Subtilis.* Zuhairi, *et al*., 2020 [33] observed the antimicrobial activity of *Rosmarinus officinalis L*. essential oil by virtue of inhibition zone diameter (9.5 ± 0.14) against *S. aureus.* Another study revealed the in vitro evaluation of antibacterial activity of essential oil of *Rosmarinus officinalis* L. against these contagious bacteria *viz*., *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas spp* (Burt, (2004) [4]; Eilyad *et al*., 2012; Gayatri *et al*., 2014; Walid *et al*., 2022) [9, 11, 26]. Similarly various reports investigated that incorporation of rosemary essential oil into meat, beef meatballs, cooked beef, pork sausage, lamb meat demonstrated inhibitory effect on bacteria contributing to food spoilage as compared to control meat (Sirocchi *et al*., 2013; Fernandez-Lopez *et al*., 2005; Ahn *et al*., 2007; Pandit *et al*., 1994; Gomez-Estaca *et al*., 2010; Camo *et al*., 2008; Ouattara *et al*., 2001) [23, 10, 3, 20, 12, 6, 19] The inhibitory effect of rosemary is the result of the action of rosmarinic acid, rosmaridiphenol, carnosol, epirosmanol, carnosic acid, rosmanol, isorosmanol, 1,8-cineole, α-pinene, verbenone, camphor, β-caryophyllene, and myrcene which interact with the cell membrane, resulting in leakage of cellular components and alter cell permeability by disrupting the phospholipid bilayer membrane and cause changes to membrane functions and properties (Stojanovic-*et al*., 2010, Djenane *et al*., 2011; Zuzarte *et al*., 2009; Ugur *et al*., 2009; Sikkema *et al*., 1995; Carson *et al*., 2002) [27, 8, 17, 1, 16, 5]. The antimicrobial properties of *R. officinalis L.* essential oil can be attributed to some major constituents 1, 8-cineole, α- pinene, borneol, and camphor. However, the minor constituents of the essential oil may also have a potentiating influence or a synergistic effect (Gill *et al*., 2002) [2].

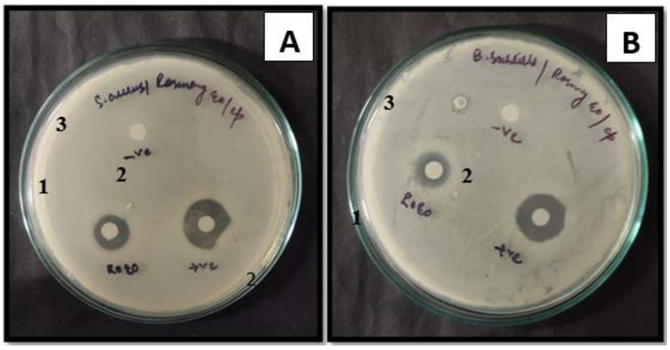

Plate 1: Antibacterial activity of *Rosmarinus officinalis* L. essential oil against selected test bacteria using agar disc diffusion assay: (A) *S. aureus* (B) *B. subtilis.* (1) Essential oil (1.90 mg/ml) (2) Chloramphenicol (2 µg/ml) (3) DMSO

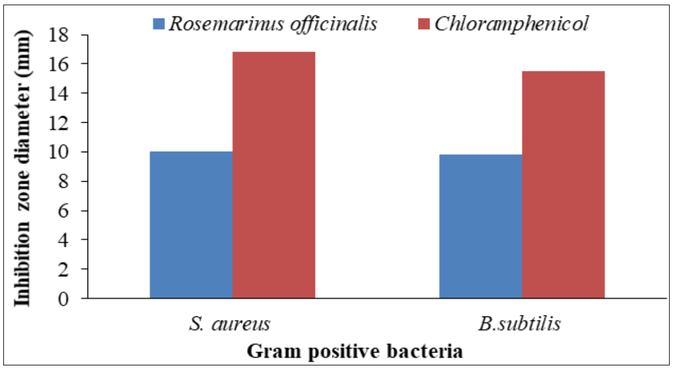

Fig 1: Antimicrobial activity of *Rosmarinus officinalis* L. against *S. aureus* and *B. subtilis* in terms of Inhibition Zone Diameter (mm)

Conclusion

The current study gives us insight about the antimicrobial potential of *R. officinalis L.* essential oil. The use of essential oil mixtures that are designed in accordance with the characteristics of the food can be considered as a new perspective in terms of the organoleptic properties of the food. However, the emergence of antibiotic resistance clearly demonstrates the dynamic evolution of microorganisms and significant clinical and economic impact. Therefore, use of natural products would be the better option with higher efficacy and decreased toxicity. Our data highlighted the ability of *R. officinalis* L. essential oil to act as antimicrobial with significant outcome. Lastly, new strategies for improving the stability of essential oils are needed in order to augment their applications and to exploit these volatiles as a potential replacement therapy and to target drug resistant microbes.

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