

In vitro antimicrobial activity of *Rose marium officinalis* essential oil cultivated in Balochistan, Pakistan

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Abstract

Rosmarinus officinalis L. is a perennial herb that belongs to the Lamiaceae family. It is used as a food flavoring agent, and well known medicinally for its powerful antibacterial and chemo preventive properties. Essential oils were extracted by hydro distillation at Arid Zone research Centre, Quetta, and antimicrobial effect of the oil were evaluated by applying on four different bacterial isolates using disc diffusion assay and measuring the diameter of zone inhibition (mm). The results exhibited that the essential oil of Rosemarium had effective antimicrobial potency against both gram positive and gram-negative bacteria.

Key words. Essential oil, Zone inhibition, Antimicrobial.

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INTRODUCTION

Rosmarinus officinalis is a woody, perennial herb with fragranced evergreen needle-like leaves belongs to the family Lamiaceae. "dew" (*roes*) and "sea" (*marinus*), or "dew of the sea" because it is frequently originate growing near the sea. The leaves volatile oil, the major components of the oil include monoterpene hydrocarbons (alpha and beta-pinene) camphene, limonene, camphor borneol, cineole and linalool (Okoh et al., 2010).

Rosemary plants extracts have been reported to possess various medicinal stuffs viz antioxidant, choleric, hepatoprotective, and antitumorogenic activities (Oluwatuyi et al., 2004, Ramirez et al., 2004). As a medicinal plant it has been used as an external stimulant or as a relaxant for nervousness, muscle spasms and headaches. Together with Coltsfoot leaves when scrubbed together and smoked for asthma and other affections of the throat and lungs. Rosemary contains a wide variety of impulsive and aromatic components against certain bacteria including *Staphylococcus aureus*, *S. albus*, *Vibrio cholerae*, *Escherichia coli* and *Coryne* bacteria has

been observed (Panthi et al., 2006). It was found to be most active against meat spoiling with *Pseudomonas* and *Lactobacillus* bacteria. The key compounds responsible for the antimicrobial activity are α -pinene, bornyl acetate, camphor and 1, 8-cineole (Pintore et al., 2002).

Plants have played a weighty role in protection human health and improving the value of human life for thousands of years. The World Health Organization assessed that 80% of the earth's inhabitants trust on traditional medicine for their primary health care needs and most of this therapy involves the use of plant extracts or their active components. Customers perceive those plants and their components as "natural" and "safe". Such mixtures are already established as flavorings in human and animal feeds. The dried rosemary leaves, whole or ground are used as seasonings for soups, stews, sausages, meat, fish, and poultry. Moreover many Western drugs had their origin in a plant extract (Craig, 1999). Aromatic plants have been used traditionally in the therapy of some diseases for a long time in the world. In addition to their antimicrobial activity they possess biological

activities such as that of antioxidants (Singh, 2007).

Pakistan has a great biodiversity of medicinal plants due to its varied climatic conditions. Commonly medicinal plants are originated more abundantly in the mountainous areas than in plains due to naturally favorable habitat and suitable climatic conditions (Anwar and Masood, 1998). Balochistan is also a natural home of many medicinal plants. In Balochistan several medicinal plants have been collected and sold in the local market by local community (Ahmad et al., 2008). However, very limited scientific knowledge is available on the potential herbs, which can be cultivated and utilized for different purposes. Moreover, over-exploitation of medicinal plants in Balochistan caused serious threat to the survival and re-generation of many medicinal plant species. At present, most of the medicinal plants are confined only in protected areas.

This study was designed to know about the cultivate characters and to find out the biological activities of essential oil of rose marium against certain microbes.

MATERIALS AND METHODS

Essential oil of Rosmarium officinalis:

Essential oil of Rosmarinus officinalis was obtained from the Medicinal Herbs Laboratory Arid Zone Research Centre, Brewery Road, Quetta.



Figure 1: *Rosmarinus officinalis* Plants at Botanical Garden AZRI, Quetta.

Preparation of Media and Reagents:

Mueller-Hinton agar (MHA) (Merck) was used as base medium and Normal saline for the preparation of inoculum. The media was

prepared in 1000 ml flask, autoclaved at 121 °C at 15 lb pressure for 20 minutes. After cooling at 45 °C media were poured in plates in order to prepare the agar plates. After solidification, the plates were incubated at 37°C or 24 hrs in order to check the sterility. Similarly, discs of various sizes were also cut with 06 mm diameter from hetman paper and autoclaved to ensure the sterility.

Collection of test Samples

The bacterial culture was obtained from different sources including Bolan Medical College Microbiological LAB. (Table-1)

Table 1: Sources of bacterial isolates tested for antimicrobial activity of Rose marium oil.

Sr No	Source/Site of collection	Name of Organism
1	Meat	<i>Salmonella spp</i>
2	Milk	<i>Staph* aureus</i>
3	Water	<i>Escherichia coli</i>
4	BMC**, Quetta	<i>Pseudomonas aeruginosa</i>

*Staph** *Staphylococcus*

BMC** bolan medical complex

Table 2: Zone of inhibition exhibited by Oil with DMSO Using Disc Diffusion Method

Sr No	Source/Site of collection	Name of Organism
1	Meat	<i>Salmonella spp</i>
2	Milk	<i>Staph* aureus</i>
3	Water	<i>Escherichia coli</i>
4	BMC**, Quetta	<i>Pseudomonas aeruginosa</i>

DSMO* Dimethyl sulphoxide



Figure 2: The zone of inhibition shown by rose marium plant essential oil.

Inoculum Preparation

Colonies of tested organisms were picked by sterile inoculating loop in a test tube containing normal Saline and matched with 0.5 McFarland turbidity standards. A sterile cotton swab was dipped into the standardized bacterial test suspension to inoculate the entire surface of MHA plate.

Disc diffusion Method

The antimicrobial activities of essential oil were evaluated against different microbial isolates using disc diffusion method. The oil was applied as pure and with different dilutions (1;1,1;2, 1;3, 1;4) in order to check the MIC. The oil was applied after mixing with Dimethyl sulphoxide (DMSO). All the discs were applied with 15 ul quantity using Kirby-Bauer method. The plates were left for 30 minutes to ensure the absorbance of oil.

After incubation at 37°C for 24 hrs the plate were observed for any inhibition zone formation

RESULTS AND DISCUSSION

The essential oil of Rose marium was collected from Arid Zone research centre, Quetta. Different isolates were collected from various sources and were subjected to evaluate the antimicrobial activity of oil.

The data indicated the antimicrobial impacts of rosemary essential oil against Staph aureus, which exhibited the inhibitory zones of 25, 12, 7 and 5 (mm) by applying pure, 1:1, 1:2 and 1:4 respective dilutions, against staphylococcus auerus (Table2, Fig-2).

Our these findings are in line with, Weckesser et al., (2007), who also evaluated the Rosemarium essential oil against Staph aureus with 35mm Zones by applying pure oil. The oil exhibited inhibitory zones against Salmonella 10, 8, 6 and 0 mm with pure, 1:1, 1:2 and 1: 4 dilutions. Such results were different from data reported in literature, (Moyosoluwa et al., 2004). Used the Rosemarium essential oil against. Salmonella where Rosemarium essential oil did not present antimicrobial effect against Salmonella. *by applying pure oil* Using a different dilution Method of oil. However, The zone of Inhibition was 30, 25, 20 and 15 mm by the application of Pure;1:1, 1;2 and 1;4 respectively against Escherichia coli. Such results were similar from data reported by the study of Kokoska et al., 2002 used extracts of Rosemarium essential oil that presented antimicrobial effect with 37mm exhibited inhibitory zones against *E. coli*. On pure oil.

The result of the this study of antimicrobial activity impact of the essential oil of Rosemarium plant on Pseudomonas with

diameter of zone of inhabition was 20, 15 ,8 and 7mm by the application of Pure;1:1, 1;2 and 1;4 respectively This data corroborate with (Moghtader and Afazli 2009),who conducted the Rosemarium essential oil against Pseudomonas with 25mm Zones reported by applying pure oil .

Plant extracts have some of the compounds that possess great potential as antimicrobial Compounds against microorganisms. Thus, they can be used in the treatment of infectious diseases caused by resistant microbes. To promote proper conservation and sustainable use of such plant resources, awareness of local communities should be enhanced incorporating the traditional knowledge with scientific findings

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