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RESEARCH ARTICLE

Antibacterial Activity of Essential Oils of *Rosmarinus officinalis*, *Salvia officinalis* and *Anthemis nobilis* Widespread in the Syrian Coast

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ABSTRACT:

We aimed in this study to determine the antibacterial efficacy of essential oils of *Rosmarinus officinalis*, *Salvia officinalis* leaves and *Anthemis nobilis* flowers widespread in the Syrian coast against several strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The essential oils were extracted and the yield was measured using Clevenger apparatus. In vitro investigation of antibacterial activity using agar disc diffusion method and minimal inhibitory concentration (MIC) of each essential oil was determined by microdilution method against studied bacteria. Results demonstrated that *Rosmarinus officinalis* essential oil had the highest activity (MIC for *Staphylococcus aureus* was 3.9µl/ml and MIC for *Pseudomonas aeruginosa* was 7.8 µl/ml). Salvia officinalis essential oil had moderate antibacterial effect with MIC of 31.2 µl/ml and 125µl/ml against *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively. On the other hand, *Anthemis nobilis* essential oil had no efficacy against tested bacterial strains. According to our study, *Rosmarinus officinalis* essential oil exhibited higher antibacterial activity against both *Staphylococcus aureus* and *Pseudomonas aeruginosa* than this of *Salvia officinalis*.

KEYWORDS: Rosemary, Sage, Chamomile, Essential oils, Antibacterial activity, MIC.

INTRODUCTION:

Development of natural antibacterial sources such as medical herbs for the treatment of infectious diseases is very necessary, in view of increasing incidence of drug-resistant bacteria. Rosemary (*Rosmarinus officinalis*), Sage (*Salvia officinalis*) and chamomile (*Anthemis nobilis)* are typical Mediterranean plants wide spread in the Syrian coast containing high concentrations of different biological active compounds such as essential oil, phenolic acids and flavonoids.[1,2] *Rosmarinus officinalis* and *Salvia officinalis* are two spices belonging to Lamiaceae family.

Several reports suggest strong antibacterial and antifungal activities of a wide range of essential oils, especially those belonging to the Lamiaceae family.[3,4]

Rosmarinus officinalis and *Salvia officinalis* are widely used in folk medicine, cosmetics, and flavoring of food products. Furthermore, they are defined as very powerful aromatic plants and their essential oils possess antimicrobial, antiviral, antifungal, antioxidant, hepatoprotective and anticarcinogenic properties.[5]

Anthemis nobilis belongs to Asteraceae family which consists of more than 210 species.[6] Its essential oil has anti-inflammatory antihistaminic and antispasmodic effects, in addition to sedative properties due to valerianic acid and cyanogenic glycosides. Two hydroperoxides compounds isolated from *Anthemis nobilis* showed a medium antibacterial activity.[7]

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MATERIAL AND METHODS:

Plant Collection:

The leaves of Rosmarinus officinalis, leaves of Salvia officinalis and flowers of *Anthemis nobilis* where collected in May 2017, from different regions in Tartous Syria. The study was carried out at the department of Pharmacognosy and department of Microbiology, faculty of pharmacy, Al Andalus University, Tartous, Syria.

All plants were air dried in the shade for two weeks at room temperature 20-25° C, then were dried in an oven at 40° C for 15 minutes every day for a week until the stability of weight and then were grinded to a fine powder in a mechanic grinder.

Essential oil extraction:

Samples of 20 g of the rosemary and sage dried leaves, chamomile dried flowers were subjected to hydrodistillation using 200 ml of distilled water for each, the process was obtained by Clevenger-type apparatus for 4 hours, the yield was recorded and the pure oil was saved in sealed glass vials at 4-5 C° until analysis.[1]

Culture Preparation:

Essential oils of rosemary, sage and chamomile were tested against *Staphylococcus aureus* (ATCC 6538) and *Pseudomonas aeruginosa* (ATCC 27853). These strains were obtained from Laboratory section of Tishreen hospital in Lattakia city and maintained on nutrient agar at 4℃.

Sensitivity test of the essential oils:

The agar disc diffusion method was employed to determine the sensitivity of the essential oils. The solid media plates were swabbed with the 0.5 McFarland bacterial suspensions.[8] Filter paper discs (6.0 mm in diameter) were infused with 5 µl essential oils and placed on the agar surface. All plates were incubated for 48 hours in 37° C.

Microdilution assay and MIC determination of the essential oils:

The estimation of the minimal inhibitory concentration (MIC) was performed by the broth micro-dilution method. The essential oils were dissolved in sterilized nutrient broth supplemented with Tween 80 at final concentration of 0.5% (v/v). Serial doubling dilutions of the oils in nutrient broth were prepared aseptically from 50% to 0.39% (v/v), by adding 1000 µL of 50% (v/v) diluted essential oil to 1000 µL nutrient broth, and eight serial 1:1 dilutions were made resulting in concentrations of 50%, 25%, 12.5%, 6.25%, 3.12%, 1.56%, 0.78% , 0.39% and 0.19%. [9]

Each test tube was then inoculated with 50 µl of 0.5 McFarland bacterial suspensions. Positive control tube containing nutrient broth with bacterial suspensions and negative control tube containing nutrient broth only were prepared concurrently. All test tubes were incubated in the incubator at 37° C temperature for 24 h.[10,11]

After incubation, all test tubes were examined for growth of microorganisms and MICs were determined. The MIC is defined as the lowest concentration of the essential oil at which the microorganism does not demonstrate visible growth.

RESULTS:

Yield of the essential oils:

The yield of essential oil extracted from each plant was recorded after extraction with Clevenger apparatus. *Rosmarinus officinalis* leaves had the richest content of essential oil with a yield of 12 ml/ 100 g of the dried leaves. *Rosmarinus officinalis* had a light yellow essential oil with a strong irritating odor. The essential oil of *Salvia officinalis* was dark yellow with a moderately heavy odor with a yield of 2.6 ml/100 g of the dried leaves. *Anthemis nobilis* essential oil was dark blue with heavy odor and the yield recorded was 4 ml of 100 grams of dried flowers.

Sensitivity of the essential oils:

The sensitivity of rosemary, sage and chamomile essential oils was examined against isolated bacteria by disc diffusion method. Both rosemary and sage essential oils exhibited significant susceptibility, with 18 mm inhibition diameter for rosemary essential oil and 13 mm inhibition diameter for sage essential oil against *Staphylococcus aureus*. The effect *on Pseudomonas aeruginosa* was weak (<10 mm inhibition diameter) for both essential oils. While chamomile essential oil had no activity against any bacterial strain and exhibited no inhibition zone. These results are demonstrated in Table (1)

MICs determination of essential oils:

As it can be seen in Table (2), rosemary essential oil exhibited higher antibacterial activity than that of sage essential oil. This finding was similar to the results of sensitivity test Table (1). The antimicrobial activity of rosemary essential oil and sage against *Staphylococcus aureus* was higher than against *Pseudomonas aeruginosa*.

Table (1): Inhibition zone diameters (mm) of rosemary, sage and chamomile essential oils

	Rosmarinus officinalis	*Salvia officinalis*	*Anthemis nobilis*
Staphylococcus aureus	20	12	No inhibition zone
Pseudomonas aeruginosa	9	7	No inhibition zone

Table (2): Minimal inhibitory concentration (MIC) of rosemary, sage and chamomile essential oils (µl/ml)

	MIC of *Rosmarinus officinalis*	**MIC of *Salvia officinalis***
Staphylococcus aureus	3.9	31.2
Pseudomonas aeruginosa	7.8	125

DISCUSSION:

According to our study, *Rosmarinus officinalis* essential oil exhibited higher antibacterial activity against both *Staphylococcus aureus* and *Pseudomonas aeruginosa* than this of *Salvia officinalis*.

MIC of *Rosmarinus officinalis* essential oil against *Staphylococcus aureus* was 3.9 µl/ml, which is twice lower than MIC against *Pseudomonas aeruginosa*, which is compatible to Hussain A. et al.[12], and Bozin et al.[13] findings, where *Rosmarinus officinalis* essential oil exhibited higher antibacterial activity against Gram-positive bacteria than against Gram-negative bacteria, and similar to Fu et al.[10] where they found that the antimicrobial activity of rosemary essential oil against *Pseudomonas aeruginosa* was less than against the other bacteria.

According to Fu et al., MICs for rosemary oil ranged from 0.125% (v/v) to 1.000 % (v/v)[8], which is corresponding to our results of rosemary essential oil (MIC for *S. aureus* was 0.39% and MIC for *P. aeruginosa* was 0.78% (v/v). The main compounds with antimicrobial effects in rosemary essential oil are 1,8-cineole, α-pinene, and camphor.[14]

CONCLUSION:

Many studies have been conducted to evaluate natural treatments for bacterial infections. We demonstrate here the antibacterial efficacy of essential oils extracted from some medicinal plants spread in the Syrian coast.

We found a high activity of rosemary essential oil against *Staphylococcus aureus* that can make it a good choice for preservative and curative purposes. The in vitro results of our study provide evidence that rosemary and sage essential oils represent a potentially rich source for medicine and food antibacterial compounds against the well-known resistant bacteria, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Further chemical and pharmacological investigations are required for rosemary and Sage essential oils to isolate active chemicals and perform additional in vitro and in vivo experiments.

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