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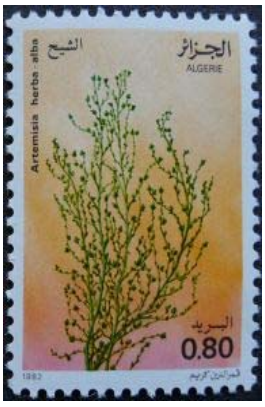

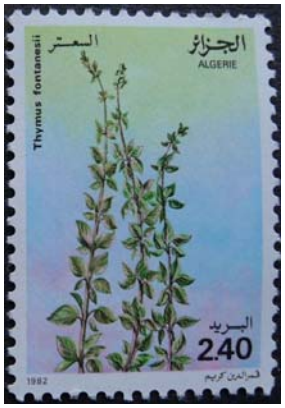

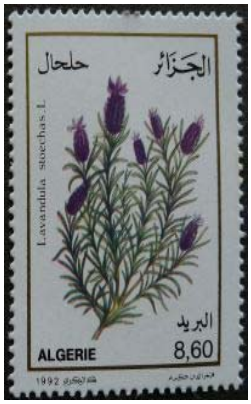

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Antimicrobial activity of essential oils of *Bubonium Graveolens* (Forssk.) and *Anvillea Radiata* (Coss.)

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Abstract- Essential oils are a group of secondary metabolites identified in several families of aromatic plants. These natural products are distinguished by their chemical characteristics and their interesting biological activities (inflammatory, antioxidant, antibacterial, insecticides,...). It is in this context articulates our work aims to study the antimicrobial activity of essential oils of two endemic medicinal plants of the Southwest Algerian: *Bubonium graveolens* Forssk (Tafs), *Anvillea radiata* Coss (Nogd), testing their antibacterial effect on four bacterial strains: *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacillus steorothermophilus* and *Staphylococcus aureus*. At the concentrations studied, both species manifested significant antibacterial property with a zone of inhibition more than 11 mm noted for *Pseudomonas aeruginosa* by the effect of the essential oil of *Bubonium graveolens*.

Key words: *Essential oil, Bubonium Graveolens, Anvillea Radiata , Biological activity, Bacterial strains.*

1. Introduction

The aromatogramme is a method for measuring the in vitro antibacterial activity of essential oils. This is the equivalent of a susceptibility where antibiotics are replaced by essential oils. [1] The use of essential oils in medicine was never abandoned despite the discovery of organic synthesis process and the birth of the pharmaceutical industry. They are considered a reservoir of basic molecules that are irreplaceable. [2] The essential oils which were utilized centuries ago in cosmetics usually show interesting biological features. The Asteraceae family contains many medicinal and aromatic plants. [3]

Bubonium graveolens (Forssk) and *Anvillea radiata* (Coss) belonging to the family Asteraceae, is an endemic herbaceous medicinal aromatic plant mainly distributed in south-western Algerian and south-eastern Morocco. [4]

Anvillea radiata is used in the folk medicine as excellent heating, for the treatment of dysentery, gastric–intestinal disorders and has been reported to have hypoglycemic activity. [5]

Bubonium graveolens has been used in Sahara folk medicine as a stomachic, for treating fever, gastrointestinal tract complaints, cephalic pains, bronchitis and as an intiinflammatory. [6]

2. Materials and methods

2.1. Plant material

Aerial parts of *B. graveolens* and *A. radiata* were collected during flowering stage, from the region between Bechar and Lahmar (over a distance of 30 km) in south-western Algeria (April 2012).

2.2. Extraction of essential oil

Samples of flowers and leaves were dried in shade. Both flowers and leaves are subjected to a steam distillation for 6 hours, in a montage developed by a pressure cooker, for increasing quantity of extracted oil. The oil was dried over anhydrous sodium sulphate and stored at 4°C until analysis.

2.3. Antimicrobial activity

2.3.1. Bacterial strains

For the determination of antibacterial activity of *B. graveolens* and *A. radiata* essential oils, standard and isolated strains of the following Gram-negative bacteria: *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853; Gram-positive bacteria: *Bacillus steorothermophilus* ATCC 12980, *Staphylococcus aureus* ATCC 25923, were used. The microorganisms were obtained from the "Pasteur Institute of Algiers, Algeria".

2.3.2. Preparation of dilutions

Due to the immiscibility of essential oils in water, different dilutions were prepared using DMSO eluent. [7]

2.3.3. Screening for antibacterial activity :

Antimicrobial activity was tested by the agar-well diffusion method. All bacterial cultures were first grown on Mueller Hinton agar at 37°C for 18–24 h prior to inoculation onto the nutrient agar. One or several colonies of similar morphology of the respective bacteria were transferred into API Suspension medium and adjusted to 0.5 McFarland turbidity standard (1-3 $\times 10^8$ bacteria /ml) with a photometer (UV lamp type Spectrolin, Model ENF-260/ FE).

The inoculums of the respective bacteria were streaked onto Mueller Hinton agar plates using a sterile swab. A sterile filter disc (diameter 6 mm, Whatman paper) was placed. The disc was impregnated by four (04) different concentrations of the tested essential oils (4 µL/disc). The treated Petri dishes were incubated at 37°C for 18–24 h.

Antimicrobial activity was evaluated by measuring the zone of growth inhibition around the discs after 24 h of incubation at 37°C. The diameter of the zones of inhibition around each of the discs was taken as measure of the antimicrobial activity. Each experiment was carried out in quintuplicate and the mean diameter of the inhibition zone was recorded.

3. Statistical Analysis:

The conventional statistical methods were used to calculate averages and standard deviations. All measurements were replicated five times, and data are presented as mean ± standard deviation.

4. Results and discussion:

The antimicrobial activities of *B. graveolens* and *A. radiata* were evaluated by a paper disc diffusion method against tested bacteria. The results showed that the essential oils were active against the microorganisms assayed. Related to the inhibition of growth, significant differences were detected among these cited oil types, since all of them showed an interesting activity for all tested strains.

The *Anvillea radiata* essential oil showed antimicrobial activity against all microbial strains tested. The concentration of 100 µg/ml showed a zone of inhibition against the bacterial strain

Bacillus steorothermophilus, then the concentration 50 mg / ml has an activity against *Pseudomonas aeruginosa*.
The essential oil of *Bubonium graveolens* shows a large zone of inhibition, which appeared with *P. aeruginosa* at the concentration of 25 µg/ml. This oil also has a large inhibition zone against *Escherichia coli* at the same concentration.
Bubonium graveolens essential oil is effective against gram-positive bacteria, with remarkable inhibitions zone for *S. aureus* and *B. steorothermophilus*. While *Anvillea radiata* essential oil proved an average antibacterial activity against *S. aureus* strains.
The results of the antibacterial activity for the two essential oils studied are shown in Tables 1 and 2.

Table 1: Antibacterial activity of *Anvillea radiata* essential oils against bacterial strains.

Bacterial strains	**Inhibition zone diameter (mm ± SD)**			
	C_1	**C_2**	**C_3**	**C_4**
Gram-negative bacteria				
E. coli ATCC 25922	7.8 ± 2.2	8.2 ± 1.8	9.6 ± 1.4	8.5 ± 1.5
P. aeruginosa ATCC 27853	8.4 ± 1.6	10.8 ± 1.2	7.0 ± 3.0	7.3 ± 1.7
Gram-positive bacteria				
B. steorothermophilus ATCC 12980	8.8 ± 1.2	6.7 ± 0.3	8.2 ± 2.8	11.0 ± 1.0
***S. aureus* ATCC 25923**	**7.6 ± 2.4**	**7.4 ± 1.6**	**6.5 ± 0.5**	**6.9 ± 1.1**

SD: standard deviation. C: significant concentration, C_1: 25, C_2: 50, C_3: 75, C_4:100.
Concentrations are expressed in µg/ml.

Table 2: Antibacterial activity of *Bubonium graveolens* essential oils against bacterial strains.

Bacterial strains	**Inhibition zone diameter (mm ± SD)**			
	C_1	**C_2**	**C_3**	**C_4**
Gram-negative bacteria				
E. coli ATCC 25922	11.0 ± 1.0	7.6 ± 2.4	8.4 ± 0.6	6.8 ± 1.2
P. aeruginosa ATCC 27853	11.8 ± 1.2	7.5 ± 2.5	11.4 ± 0.4	10.8 ± 1.2
Gram-positive bacteria				
B. steorothermophilus ATCC 12980	8.9 ± 2.1	6.7 ± 1.3	9.3 ± 1.7	9.0 ± 1.0
***S. aureus* ATCC 25923**	**9.4 ± 2.6**	**6.9 ± 1.1**	**7.0 ± 3.0**	**9.4 ± 1.6**

SD: standard deviation, C: significant concentration, C_1: 25, C_2: 50, C_3: 75, C_4:100.
Concentrations are expressed in µg/ml.

5. Conclusion:

The antimicrobial activity of different oils was tested using the diffusion method and by determining the inhibition zone. The results showed that all examined oil types had great potential of antimicrobial activity against strains.
These first results we have obtained allow a systematic study of many essential oils on pathogenic bacteria with increased resistance vis-à-vis conventional antibacterial agents, including inpatient samples.

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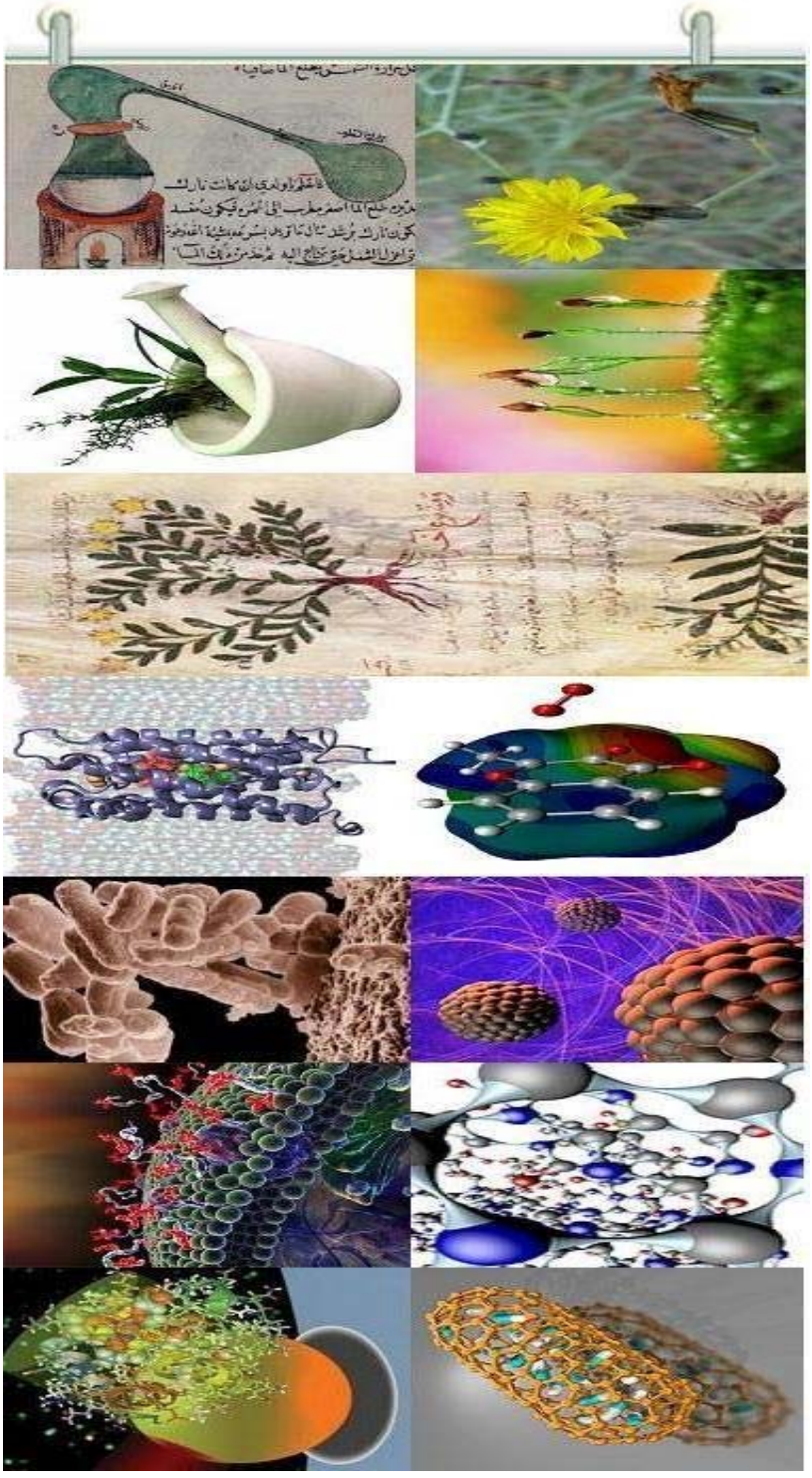

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