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Effect of essential oils of *Artemisia arborescens on Escherichia coli, Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Abstract: This study was carried out to investigate the potential use of *Artemisia arborescens* as a source of antimicrobial agents against pathogens. Essential oils of *A. arborescens* were collected by hydrodistillation. The antibacterial properties of *A. arborescens* essential oil was studied on the standard gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*), and *Staphylococcus aureus* (gram-positive bacteria), then agar disk diffusion, minimal inhibition concentration (MIC) and minimum bactericidal concentration (MBC) were detected. The results of agar disk diffusion tests showed the inhibition zones as follow: *S. aureus* 00-18 mm, *E. coli* 00-16 mm and 08-14 mm for *P. aeruginosa*. However, their antibacterial activities were lower than those of Gentamicin. The MIC for *S. aureus* and *P. aeruginosa* was between 33 and 66 mg/ml, and for Gram-negative bacteria of *E. coli* was between 66 and 132 mg/ml, while the MBC values of this oil against the tested bacterial strains were between 132 and 264 mg/ml.

Keywords: *Artemisia arborescens* Essential Oil, *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Antimicrobial Activity*

1. Introduction

In recent years, there has been considerable interest in assaying the composition and antibacterial activity of essential oils from *Asteraceae* species from around the world [1]. The genus *Artemisia* L. is one of the largest genera in the *Asteraceae* family consisting of more than 500 species [2, 3], which many of them are currently the subject of phytochemical attention because of their biological and chemical diversity and essential oil production [4]. *Artemisia* species, widespread throughout the world, are one of the most popular plants in traditional medicine to treat diseases such as malaria, hepatitis, cancer, diabetes mellitus, inflammation and infections by fungi, bacteria and viruses [5, 6, 7, 8]. Furthermore, the species of this genus are widely used in the pharmaceutics, cosmetics and food industry due to its antimicrobial, insecticidal, antioxidant, and anti-malarial properties [8, 4].

Data in the existing literature states that oils of *Artemisia* species are predominantly α-thujone, β-thujone, 1,8-cineole, germacrene-D, vulgarone-B, borneol, β-caryophyllene, caryophyllene oxide, davanone, artemisiaketone, and chrysanthenone [4].

Artemisia arborescens L. ("great mugwort", "arborescent mugwort") is a morphologically variable species (or mixture of species) with grey-green to silver leaves. It is native to the various habitats of the Mediterranean region, where it occurs as a shrub growing up to one metre in height. According to popular folklore, it is used as an anti-inflammatory remedy [9].

Considering that some plant extracts proved to have antibacterial effect and numerous microorganisms developed multiple resistance to antibiotics and chemotherapies used in therapy, in our study we aimed the evaluation of the antibacterial effect of *Artemisia arborescens* essential oil extracts on *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* strains.

2. Materials and Methods

2.1. Plant Material

Wild samples were collected around Mascara city

(Mamounia region), in northwest of Algeria between April and May 2013. The identity of the plants was confirmed by Dr. Belgharbi Benamar (Department of Biology, Mascara University, Algeria).

2.2. Isolation Procedure

The aerial parts (100 g of leaves, flowers and stems) were dried at room temperature (20-25°C), then subjected to hydrodistillation for 2 to 4 h, in 600-700 ml of distillated water, using a Clevenger-type apparatus. The essential oil was evaporated together with water vapour and finally collected after decantation. The distillate was isolated and dried in a Rota-vapor to giving blue oil. The oil was kept at 4 °C before testing the antimicrobial activity [10, 11]. The extraction yield of this essential oil was 0.256 % (w/w).

2.3. Microorganisms

Escherichia coli (ATCC 25922), *Staphylococcus aureus* (ATCC 25923), and *Pseudomonas aeruginosa* (ATCC 27853) were used as test microorganisms to determine the antimicrobial activity. Gentamicin (10 µg) (Pasteur institute, Algiers) was used as positive standards in order to control the sensitivity of the microorganisms.

2.4. Assessment of Antibacterial Activity

2.4.1. Agar Disc Diffusion Assay

To assess the antimicrobial activity of the oils a modified disc diffusion method was used [12]. Briefly, inocula were prepared by suspending overnight colonies from the pre-cultured test microorganisms *S. aureus*, *E. coli* and *P. aeruginosa*. The adjusted suspensions were swabbed onto the surfaces of Muller Hinton agar, then sterile paper discs (6 mm in diameter) were soaked with 5, 10, 20 and 30 µl of the essential oil which were placed on the inoculated dishes.

The antibacterial activity of essential oils was demonstrated by a clear zone of inhibition around the disc. After incubation for 24 h at 37°C, all plates were examined for any zones of growth inhibition, and the diameters of these zones were measured in millimeters.

2.4.2. Determination of Minimum Inhibitory Concentration and Minimal Bactericidal Concentration

Dilution method was used to measure MIC. The minimum inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) were determined for *A. arborescens* essential oil that showed antimicrobial activity against *S. aureus*, *E. coli*, and *P. aeruginosa*.

The *A. arborescens* essential oil dissolved in 3.2% dimethylsulfoxide (DMSO) were first diluted to the highest concentration (8 µl/ml) to be tested, and then serial dilutions were made in a concentration range from 16 to 529 mg/ml in 10 ml sterile test tubes containing nutrient broth. Then 1 ml of the microbial suspension was added to each tube. Tubes were incubated at 37 °C for 24 h. The MIC was defined as the lowest concentration of essential oil at which microorganisms show no visible growth. The minimum bactericidal concentration (MBC) was determined by removing 100 µl of bacterial suspension from subculture demonstrating no visible growth and inoculating this on Mueller Hinton agar plates. Plates were incubated at 37°C for 24 h. The MBC is defined as the lowest concentration of the essential oil at which ≥ 99.9 percent of the inoculated microorganisms were killed.

3. Results and Discussion

Results from the antimicrobial disc-diffusion assay are summarized in Table 1. The *A. arborescens* essential oil studied in this work showed antimicrobial activity against the test microorganisms, with inhibition zones ranging from 0 to 18 mm. Maximum inhibition (18 mm) was shown by 30 µl of essential oil against *S. aureus*, while the minimum (00 mm) was demonstrated by 5 µl against *S. aureus* and *E. coli*. The effectiveness of *A. arborescens* essential oil was greater against *S. aureus* than pathogens. The high inhibition zone diameter obtained by the oil against *E. coli* and *P. aeruginosa* were 16 and 14 mm respectively. No inhibition of growth of the tested *S. aureus* and *E. coli* was observed at 05µl of *A. arborescens* essential oil after 24 h incubation at 37°C. Also, the antibacterial property of the essential oil was compared to a synthetic antibiotic, Gentamicin.

Table 1. *Antibacterial activity of the essential oil from A. arborescens against S. aureus, E. coli and P. aeruginosa by the disc diffusion assay*

Strain	Zone of Inhibition (mm) produced by different amounts of oil dissolved				GN
	5µl	10µl	20µl	30µl	10µg
S. aureus	00	12	16	18	25
E. coli	00	10	12	16	21
P. aeruginosa	08	12	12	14	19

GN: Gentamicin

Gentamicin caused inhibition zones of 25, 21 and 19 mm against *S. aureus*, *E. coli* and *P. aeruginosa* respectively biggest than those of essential oil effect. The essential oil of *A. arborescens* had a lower inhibitory activity against all the microorganisms tested, compared with the antibiotic.

Table 2. *MIC and MBC (mg/ml) of A. arborescens essential oil against S. aureus, E. coli and P. aeruginosa*

Strain	MIC	MBC
S.aureus	33-66	132-264
E.coli	66-132	132-264
P.aeruginosa	33-66	132-264

The MIC and MBC values of *A. arborescens* essential oil tested against *S. aureus*, *E. coli* and *P. aeruginosa* are shown in Table 2. The MIC values for bacterial strains, which were sensitive to *A. arborescens* essential oil were between 33 and 66 mg/ml for *S. aureus* and *P. aeruginosa* and between 66 and 132 mg/ml for *E. coli*. All MBC values of this oil against the tested bacterial strains were between 132 and 264 mg/ml.

These results confirm previous reports of antibacterial activity for essential oils from *A. arborescens* against these bacteria [13, 14], but in contrast to the results of Saddi et al. (2007), who have reported no inhibition of this essential oil against twelve pathogens including *S. aureus*, *E. coli*, and *P. aeruginosa.*

Baykan et al (2012) have reported a 20-30 µl essential oil of *A. arborescens* inhibited the growth of *S. aureus* and *E. coli* (7-8 mm).

Younes et al. (2004) performed disc diffusion assays on essential oil of *A. arborescens*. The zones of inhibition at

10 µL volume are ranged between 8 to 15 mm for *S. aureus* and from 8 to 13.5 mm for *P. aeruginosa*. In their study, no activity was noted against *E.coli*.

The antibacterial activity of the *A. arborescens* essential oil observed in the present study can be attributed to the presence of a high concentration of camphor [14, 13], chamazulene, and β-thujone [16, 17], which have been reported to possess antimicrobial properties [18, 19, 20]. It is believed that monoterpene compounds, such as those found in *A. arborescens* essential oil, can accumulate in the bacterial membrane and cause a loss of integrity, leakage of the cytoplasmic contents, disruption of the proton motive force, lysis and cell death [21, 22].

In addition, the components present in lower amounts in *A. arborescens* essential oil, such as β-caryophyllene, α-thujone, p-Cymene and α-Terpineol which have been reported to be antibacterial activity [23, 24, 25, 26] could also contribute to antimicrobial activity of the oil. In fact, it is also possible that the minor components may be involved in some type of synergism with the other active compounds [27, 28].

5. Conclusion

Our study showed that the essential oil of aerial parts of *A.arborescens* L have antibacterial against the tested bacterial strains. The encouraging results validate the traditional use of this plant and indicate the possibility of its application as natural antibiotics to treat the infectious diseases caused by those pathogens.

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