# The antibacterial effect of *Artemisia Huguetii caball* essential oil against *Staphylococcus aureus* and *Enterococcus*

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**Abstract**. Antimicrobial resistance poses a significant global health threat because most microorganisms have become resistant to many antimicrobials. In fact, finding alternatives such as essential oils extracted from medicinal plants has become a necessity. These oils are recognized for their therapeutic properties and are utilized in various treatments. This study evaluates the antibacterial effect of the essential oil of *Artemisia Huguetii caball* against two pathogenic bacteria, *Staphylococcus aureus* and *Enterococcus spp*. The aerial parts of *Artemisia Huguetii caball* were collected from the Moroccan Hight Atlas. Essential oil was extracted by hydro-distillation, then analysed using gas chromatography mass spectrometry (GC_MS). The antibacterial effect was studied against the two bacteria strains studied that were isolated from human bronchial passages and identified using classical biochemical techniques. Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were determined using solid medium dilutions. Sixty constituents were identified in the essential oil with thujones (50.89 %) and camphor (14.36 %) as the major constituents. The bacteria whose resistance to antibiotics exceeded 80 %, were susceptible to the essential oil with an inhibition zone diameter of 12 mm in *Staphylococcus aureus* and 9 mm in *Enterococcus spp*. The MIC and MBC values were 2 mg/mL and 4 mg/mL, respectively.

## 1 Introduction :

Resistance to antimicrobials is a global public health challenge. In 2019, it contributed to approximately 4.95 million deaths worldwide [1]. Antimicrobial resistance naturally expressed in the species is called natural resistance. Resistance can also be adaptative if it is acquired in the presence of therapeutic antibiotics. Thus, the emergence of drug resistance is due to the application of antimicrobial agents to treat infections which has led to a scalable response in microorganisms. This resistance happens when microbes become insensitive to one or several classes of antimicrobial molecules [2]. Drug combinations can expend options for antimicrobial therapies but have not been systematically tested in Gram-positive species [3]. Gram-positive bacteria exhibit several resistance mechanisms that can be divided into four main groups:

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modification of the drug's target, enzymatic inactivation, limitation of drug absorption and increase in drug efflux [2]. In addition, gram-positive bacteria have a cell wall rich in peptidoglycan, which is covalently linked to teichoic acids, polymers of substituted glycerol units connected by phosphodiester bonds. Teichoic acids are covalently linked to glycolipids and are the main cell surface antigens [4]. *Staphylococcus aureus* and *Enterococcus* are among the most pathogenic gram-positive bacteria in the hospital environment [5]. These bacteria are opportunistic pathogens and can cause nosocomial infections, which may lead to bloodstream infections with a mortality rate of 20 % or higher, even if they are part of normal flora in living beings [6] [7] [8]. This situation requires efforts to combat these problems. For example, looking for alternative therapies such as using essential oils which can resolve the problem of antimicrobial resistance [9]. Essential oils are a group of volatile aromatic substances that are synthesised by different parts of medicinal plants. These plants have been used for many years in traditional medicines for therapeutic purposes. In fact, pharmaceutical and biological activities have been associated with essential oils. Therefore, due to all of these beneficial properties, they have attracted great interest in the scientific community [10]. This article presents the results of the study of the antibacterial effect of *Artemisia Huguetii caball* essential oil on two bacterial strains: *Staphylococcus aureus* and *Enterococcus spp*.

## 2 Materials and methods:

### 2.1 Extraction of essential oil of *Artemisia Huguetii caball*:

The essential oil of *Artemisia Huguetii caball* was extracted by hydro-distillation using Clevenger type device which has a two-litre flask. *Artemisia Huguetii caball* is a medicinal plant that grows primarily in the subtropical biome. The native range of this specie is Morocco [11]. *Artemisia Huguetii caball* belongs to the Artemisia genus, Asteraceae family [12]. A quantity of 1250 g of aerial parts of this plant was dried and put in a flask containing an adequate quantity of water. Then everything was heated.

### 2.2 Chemical analysis of essential oil of *Artemisia Huguetii caball*:

The Chemical composition of the essential oil of *Artemisia huguetii caball* was determined using gas chromatography coupled with mass spectrometry (GC-MS). The essential oil was diluted 1/10 (v/v) for analysis.

### 2.3 Isolation, culture and identification of bacterial strains:

Two Gram-positive strains were studied: *Staphylococcus aureus* and *Enterococcus spp*. All samples were isolated from patients under sterile conditions. Both bacterial strains were obtained by urine sampling and transported to the laboratory and cultured in petri dishes containing blood agar for 24 hours at 37 °C. Afterwards, bacteria were purified using selective culture media, such as Chapman medium. Identification of bacterial strains was performed by Gram-staining, as well as by biochemical tests including the catalase and oxidase tests.

### 2.4 Antibiogram:

The sensitivity of bacterial strains was determined using antibiograms. The inoculum of bacteria was prepared with a turbidity equivalent to 0.5 McFarland (1.5 x 108 CFU/mL), cultured on Mueller-Hinton agar, and left to dry. Antibiotic discs were applied to the Mueller-Hinton agar surface using sterile forceps. The tested antibiotics were: Colistin (50 µg), Streptomycin (25 µg),

Vancomycin (30 µg), Amoxicillin / Clavulanic acid (30 µg), Penicillin G (1 µg) et linezolid (10 µg).

### 2.5 Aromatogram, MIC and MBC:

Aromatogram was carried out by the disc diffusion method. Each disc has a diameter of 6 mm. These discs were impregnated with 6 µl of emulsified essential oil in a 2 % agar solution. Then, they were placed in petri dishes containing Muller Hinton agar medium spread by the bacterial inoculum at 0.5 McFarland. The bacterial cultures were incubated for 24 h at 37 ◦C, and then the diameters of inhibition zones were measured. A set of dilutions of the essential oil were prepared in agar solution: 1/10, 1/25, 1/50, 1/100, 1/200, 1/300, 1/500. 1mL of each precedent dilution was added in a test tube containing 9 mL of MH agar media sterilized in an autoclave (15 min, 121 ◦C), then, the tubes were cooled to 45 ◦C. The final concentrations become: 1/100, 1/250, 1/500, 1/1000, 1/2000, 1/3000, 1/5000 (v/v). The tubes were poured into Petri dishes. The control, containing the culture medium and 0.2 % agar solution, was used. The Petri dishes were seeded by streaks using a calibrated platinum handle to take the same volume of inoculum. The dishes were incubated at 37 ◦C in darkness for 24 hours.

## 3 Results and discussion:

### 3.1 Chemical compounds of essential oil of *Artemisia Huguetii caball*:

The result of chemical analysis of essential oil of *Artemisia Huguetii caball* showed that this oil contains sixty compounds of which the most representative one was thujone (50.9 %) followed by camphor (14.36 %) **(Fig 1)**.

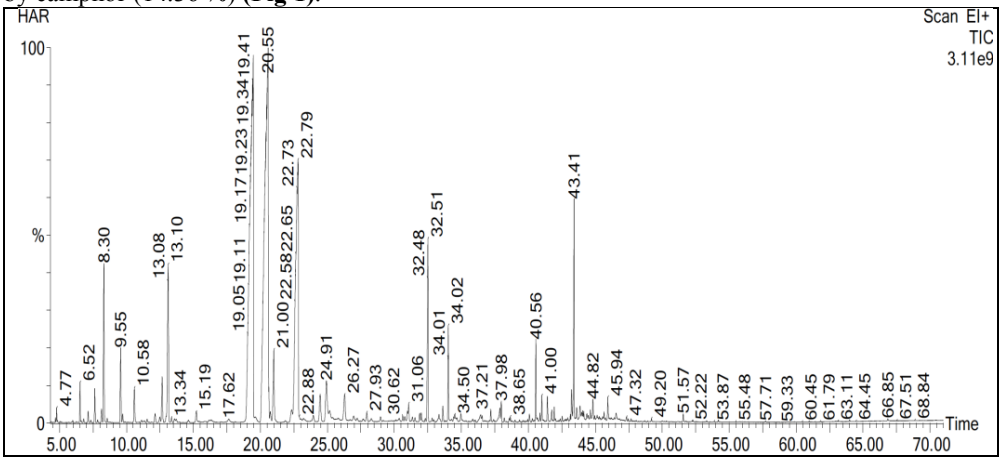


**Fig 1.** Chromatographic profile of essential oil of *Artemisia Huguetii caball* profiled by GC-MS analysis.

### 3.2 Antibiogram:

The results of the antibiogram were interpreted according to the standards indicated by the antibiogram committee of the French Society of Microbiology. In fact, both bacterial strains were resistant to all antibiotics tested except Streptomycin for *Staphylococcus aureus* and Linezolid for *Enterococcus spp* **(Table 1)**.

**Table 1**: Results of the antibiogram for the Gram-positive strains studied

| The name of the antibiotic | Diameter of the inhibition zone (mm) |
|---|---|

| | *Staphylococcus aureus* (human origin) | *Enterococcus spp* (human origin) |
|---|---|---|
| Amoxicillin/ Clavulanic Acid (30 µg) | 0 (Resistant) | 0 (Resistant) |
| Colistin (50 µg) | 12 (Resistant) | 14 (Resistant) |
| Penicillin G (1 µg) | 0 (Resistant) | 0 (Resistant) |
| Linezolid (10 µg) | 0 (Resistant) | 22 (sensitive) |
| Vancomycin (30 µg) | 7 (Resistant) | 0 (Resistant) |
| Streptomycin (25 µg) | 18 (sensitive) | 8(Resistant) |

### 3.3 Aromatogram, MIC and MBC:

The essential oil of *Artemisia Huguetii caball* exhibited activity against the two Gram-positive strains studied. The results of the inhibition zone measurements, MIC and MBC are shown in **Table 2**. The results showed that essential oil of *Artemisia Huguetii caball* inhibit studied strains with an inhibition zone of 12 mm in diameter in *Staphylococcus aureus* and 9 mm in *Enterococcus spp* **(Fig 2)**. The minimum inhibitory concentration against *Staphylococcus aureus* was 2 mg/mL and 4 mg/mL against *Enterococcus spp*. The minimum bactericidal concentration was also determined and was 4 mg/mL for both strains.


*Enterococcus spp* (human origin)


*Staphylococcus aureus* (human origin)

**Fig 2.** Results of the aromatogram of *Artemisia Huguetii caball* essential oil against *Enterococcus spp* and *Staphylococcus aureus*.

**Table 2.** Results of measurement of the inhibition zone, MIC and MBC of *Staphylococcus aureus* and *Enterococcus spp*

| | Inhibition zone (mm) | MIC (mg/mL) | MBC (mg/mL) |
|---|---|---|---|
| *Staphylococcus aureus* | 12 | 2 | 4 |
| *Enterococcus spp* | 9 | 4 | 19 |

### 3.4 Discussion:

Our study showed that *Artemisia Huguetii caball* essential oil may be used to inhibit Gram-positive bacteria, as it demonstrated antibacterial activity against *Staphylococcus aureus* and *Enterococcus*. The chemical composition showed predominance of thujones and camphor.

Literature showed the same results on the essential oil of *Artemisia Huguetii caball* from other regions, as like as on essential oils extracted from other species of *Artemisia* which are rich in oxygenated monoterpenes such as thujones [13]. In this article, the essential oil studied showed important antibacterial activity against the two bacterial strains studied. Another study that was carried out on *Artemisia Huguetii caball* native to Tata, region in Morocco, showed that the chemical composition of essential oil of this specie is almost the same as the specie studied in this article and which is from Moroccan high atlas origin. The study showed that the chemical composition is dominated by oxygenated monoterpenes, accounting for 88.30 % of the oil, whereas diterpenes, sesquiterpenes and hydrocarbon monoterpenes are present in lower proportions. The essential oil was mostly composed of camphor (37.57 %), cis-thujone (27.38 %), and trans-thujone (6.55 %). The obtained MIC results showed that the essential oil was active against both *Staphylococcus aureus* and *Enterobacter cloacae* with a value of MIC equal to 12.5 mg/mL. The MBC was also equal to 12.5 mg/mL [11]. According to studies carried out concerning antimicrobial resistance in *Staphylococcus aureus* and *Enterococcus*, these two bacterial strains showed important resistance to many antibiotics. *Enterococcus* was resistant to chloramphenicol, erythromycin, ampicillin, cotrimoxazole and teicoplanin, and susceptible to vancomycin and linezolid [14]. But in another studies, *Enterococcus* showed the highest resistance to vancomycin [15]. Another study showed that *Enterococcus faecalis* showed a resistance to ceftriaxone, amoxicillin, ciprofloxacin, cefuroxime, clindamycin, fluconazole and cefepime. While *Staphylococcus aureus* showed important resistance to clindamycin [16], amoxicillin clavulanic acid, ampicillin, amoxicillin, streptomycin, chloramphenicol, vancomycin [17], gentamycin, erythromycin, and penicillin [18]. Effectively, when the penicillin G was initially introduced in the early 1940s, all *Staphylococcus aureus* strains were susceptible to this antibiotic. However, by 1944, the first report of penicillin-resistant *Staphylococcus aureus* had already appeared. Today, all *Staphylococcus* strains have become resistant to penicillin, antipseudomonal penicillin and aminopenicillins [5]. Regarding the effect of essential oils on *Staphylococcus aureus* and *Enterococcus*, some studies gave the following results: essential oil of *Artemisia herba-alba* showed important activity against *Staphylococcus aureus* with a variable MIC between 0.5 µL/mL and 2 µL/mL according to harvest time but MBC was 10 µL/mL. So, chemical composition of plant and its antibacterial properties can be influenced by environmental factors and growth stages [18]. Synergism between several essential oils has also shown significant effects against Gram-positive bacteria. 1/CI values ranging from 0.50 to 0.83 in *Enterococcus faecalis* and from 0.60 to 0.85 in *Staphylococcus aureus* [19]. In another study, the MIC and MBC values reported for *Staphylococcus aureus* were: 2.5 mg/mL and 5 mg/mL, respectively [20]. In a concentration of 100 µL/mL of *Artemisia herba alba* essential oil, an inhibition zone of 8.33 ± 0.58 mm was observed in *Staphylococcus aureus*. The $MIC_{90}$ and MBC were 2.16 mg/mL and 15 mg/mL, respectively [21]. In another study, the inhibition zone was 17 mm [22]. Another study showed an inhibition zone of 13.36 ± 1.20 mm in *Enterococcus faecalis* and 12.46 ± 0.58 mm in *Staphylococcus aureus*. For MIC, it was 0.08 µL/mL in *Enterococcus faecalis* and 1.57 µL/mL in *Staphylococcus aureus* [23]. The results obtained in a study indicated that the inhibition zone was 7 ± 0.41 mm in *Staphylococcus aureus* and 6.5 ± 0.7 mm in *Enterococcus faecalis*. For MIC value, it was 0.46 mg/mL in both bacterial strains [24]. According to many studies, Gram-positive bacteria showed more sensitivity to the *Artemisia herba alba* essential oil than Gram-negative bacteria. The antibacterial effect of essential oil of *Artemisia herba alba* would be related to its oxygenated monoterpenes compounds which constitute about 50.53 % of the oil. In fact, it has been shown that monoterpenes, oxygenated monoterpenes and hydrocarbons in essential oils may destroy cellular integrity. However, it is difficult to link the effect of a complex mixture to a single or particular molecule. Major or trace constituents may contribute to the observed antibacterial effect. Thus, the molecules constituting the essential oil may have synergistic or antagonistic effects that can be crucial for microbial inhibition [25].

## 4 Conclusion:

In conclusion, *Artemisia Huguetii caball* essential oil has demonstrated an antibacterial effect against Gram-positive bacteria. This can push researchers to think about producing new antimicrobials of natural origin that can kill pathogenic bacteria or inhibit their growth, solve the problem of resistance to antibiotics in bacteria, and allow the use of products without side effects on health. We can also think of formulas resulting from the mixture between two or more essential oils if this will increase the antimicrobial effect of these oils.

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All information cited in the manuscript are taken from scientific articles which are cited in the bibliographic references. The figures showing the experimental results are photos taken by the author. The results of antibiogram are interpreted based on the Antibiogram Committee of the French Society of Microbiology which you can consult by using the following site: https://www.sfm-microbiologie.org/wp-content/uploads/2024/06/CASFM2024_V1.0.pdf

**Malika AIT OUADDI:** perform analyses, antibacterial tests, write and edit the manuscript.
**Meryem ELGARINI:** supervision of all the work and provision of information and corrections.
**Oualid El MEJJATI, Imane CHOUKRI:** contribution to the antibacterial activity and providing information.
**Ikhlass El BERBRI, Majid Mounir:** follow-up of the work and critical revision of the manuscript.
**Aouatif BENALI:** contribution to chromatographic analysis (CG-MS), interpretation, and Critical revision of the manuscript.
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**All authors** approved, after reading, the final version of manuscript and approved the submission.

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